The Journal of American Science

ISSN 1545-1003

Volume 6 - Number 6 (Cumulated No. 27), June 1, 2010, ISSN 1545-1003

Marsland Press, Michigan, The United States

The Journal of American Science

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The Journal of American Science

ISSN 1545-1003

Volume 6, Issue 6, June 1, 2010

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CONTENTS

2 & 4. G.B. Pant Institute of Himalayan Environment and Development, Kosi – Katarmal- 263 643, Almora, Uttarakhand, India

Abstract: *Cordyceps sinensis,* belonging to the family Clavicipitaceae is a parasitic fungus on Lepidopteran larvae. Mainly it is found in subalpine regions from 3200 to 4000 m asl in grassy lands of Himalayas. It is very much valuable in Chinese and Tibetan medicine also. The residents of *Sutol* and *Kanol* villages (the most interior villages of Chamoli distt.) in Uttarakhand are extracting it. Every year the average collection of *Cordyceps* is about 140 kg from both villages. Near about 700 people were engaged in the collection of *Cordyceps* every year. Per head collection of *C. sinensis* was 200 gm per season. The collection period of this species is from May to July and the potential natural pockets are Bedini Bughyal, Homekund and Simbe. It is also track of famous religious "*Nanda Devi Raj Jat'' Yatra*. Basically the main collectors are men, women, young boys and girls which belong to the age group of 15 to 65 years. There is a drastic change in the economy of villagers and at the other hand some negative social impacts are also pertaining day by day in the last 3-4 years. [Journal of American Science 2010;6(6):5-9]. (ISSN: 1545-1003).
Keywords: *Cordyceps sinensis*, medicinal value, *keera ghaas*, interior villages, social impacts on rural economy, drastic change

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SYNTHESIS AND REACTION OF SOME NEW PYRIMIDINE THIONES

Abdelghani, E.; Sherif, M., H.; Assy, M., G. and Morsi, Gh., M.

Department of Chemistry, Faculty of Science, Zagazig University, Egypt

ABSTRACT: The addition of cyanomethylene derivative 2 to aroyl isothiocyanate 1 afforded mercaptopyrimidine derivative 5. Mercaptopyrimidine 11 was prepared and transformed upon alkylation with chloroacetamide to thienopyrimidine 13. Oxidation of 11 using I_2/AcOH yielded the disulphide 14, while oxidation using H_2O_2/AcOH gave pyrimidine derivative 15. Reaction of aldehydes with aminothiouracil 16 yielded 5-aroyl-2,8-di-thioxo-2,3,5,8,9,10-hexahydropyrimido[5',4':5,6]pyrido[2,3-d] pyrimidine derivative 19a,b. Addition of 16 to chalcone afforded pyridopyrimidine 20. Reaction of urea, α-naphthaldehyde and aminothiouracil afforded pyrimidopyrimidine 21. Reaction of 16 with NH_4SCN afforded compound 22 that oxidized to bis-isothiazolopyrimidine bisulphide 23. [Journal of American Science 2010;6(6):10-15]. (ISSN: 1545-1003).

KEY WORDS: mercaptopyrimidine, oxazine, thienopyrimidine, pyrimidine, pyridopyrimidine, pyrimidopyrimidine and isothiazolopyrimidine.

Full Text

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Neuroanatomical, Immunocytochemical and Electrophysiological Studies on Cercal Sensory Receptors in the Female Locust

Ehab Tousson and Afaf El-Atrash

Department of Zoology, Faculty of Science, Tanta University
toussonehab@yahoo.com afelatrash@yahoo.com

ABSTRACT: Relatively little is still known about the function, types and location of cercal sensory systems. This system detects and encodes different sensory modalities: wind, touch and gustatory. It is focused on fine structure and distribution of various types of cercal sensilla in the desert locust were investigated with scanning electron microscope and neuroanatomical techniques. Two types of sensory receptors were identified and classified as mechanoreceptors (filiform sensilla) and chemoreceptors (basiconic sensilla). Also, the morphology and the organization of individual sensory receptors (mechanoreceptors or chemoreceptors) in the CNS were examined by immunocytochemical characterization of single neuron. All afferent fibres from individual filiform and basiconic sensilla project in the tenth neuromere of the terminal abdominal ganglion. Projections from single multiply innervated hair sensilla do not segregate with the exception of one afferent of contact chemosensory hairs which terminate only in its segmental neuromere, as

Full Text

	was shown for other contact chemoreceptors of the abdomen. Another focus is on the electrophysiological response of individual mechanoreceptors or chemoreceptors to mechanical or chemical stimulation were analyzed. [Journal of American Science 2010;6(6):16-23]. (ISSN: 1545-1003). **Keywords:** Sensory neurons; Cerci; terminal segments; Innervation; Immunocytochemistry; Electrophysiological recording	
5	**Effect of H_2SO_4 on Seed Germination and Viability of *Canna indica* L. a Medicinal Plant** Sunil Chandra Joshi[1*] and S.C. Pant[2] 1 Division of Seed Science and Technology, Indian Agricultural Research Institute New Delhi-110012 India 2 Department of Horticulture, HNB Garhwal University Srinagar (Garhwal) Uttrakhand-246174 India scj.seed@gmail.com **Abstract:** *Canna indica* roots are used for medicinal purpose. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The seed of canna is extremely hard, and needs to be "scarified" before sowing. The aim of the present investigation is to determine the hardiness problem of the seed. The seed sample was collected from the IARI, New Delhi in 2008. The work consists of Physical purity, standard germination test, seed vigour test. Experimental results has shown that, seed sample recorded the purity of seed (97.55 %) and seed sample showed the maximum germination percentage 91% after three hrs. H_2SO_4 scarification. The maximum root length (7.51 cm), maximum shoot length (3.12 cm) and maximum seedling dry weight (0.203 gm) were observed at two hrs. H_2SO_4 scarification. The results indicated that H_2SO_4 scarification increase the germination percentage but it reduce the viability of the seed. [Journal of American Science 2010;6(6):24-25]. ISSN: 1545-1003). **Key words:** *Canna indica*, Germination, Scarification, Vigour	Full Text
6	**The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults** Faten S.Bayoumi [1] , Fatehya.M.Metwaly[2] ,Hind M.Rashd[2].and E.H.A. Abouel-Ezz..[3] [2]Professor in Environmental &Occupational Medicine Department, National Research Center [2]Assistant Professor in Environmental &Occupational Medicine Department, National Research Center [3]Professor in genetic orodental Dep. National Research Center **fatenbayoumi@yahoo.co.uk** **Abstract**: Aim : Elucidate the effect of genetic variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as a risk factors for periodontitis. Material &Methods: Sample of this study composed of 50 smokers & 50 non smoker volunteers (unrelated and of the same ethnic population) with 40-60 years old .Their periodontal status was estimated through periodontal examination (full mouth clinical attachment loss measurement ,probing depths ,plaque index scores, and bleeding on probing). Isolation and detection of certain oral pathogens; A.actinomycetemcomitans , Porphyromonas gingivalis ,and Provetella intermedia was performed . Genotype for bi-allelic IL-1A+4845, IL-lB+3954 gene polymorphisms using mouth wash was detected by PCR based methods. Results: There were a significant difference only between the two groups (smokers &non-smokers) as regards to colonization of A.actinomycetemcomitans & not among Porphyromonas gingivalis & Prevotella spp. There were no significant difference between the overall frequencies of carrying allele 2 of IL-l A, IL-1B among smoker and non-smokers. The percentage of non smokers having healthy periodontal status was much higher than smokers. On the other hand, smokers recorded much higher percentage for mild, moderate and severe periodontitis. The difference was statistically significant concerning the percentage of those with severe periodontitis. Conclusion: Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of	Full Text

	allele 2 of IL-l A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated A.actinomycetemcomitans growth. [Journal of American Science 2010;6(6):26-30]. (ISSN: 1545-1003). **Keywords:** genetic polymorphism, periodontitis, Interleukin -1, periodontal pathogens, smoking	
7	**Model for Computational Analysis of the Solution Temperature during Leaching of Iron Oxide Ore in Oxalic Acid Solution** Chukwuka Ikechukwu Nwoye Department of Materials and Metallurgical Engineering Federal University of Technology, P.M.B 1526 Owerri, Nigeria. chikeyn@yahoo.com **Abstract:** Model for computational analysis of the solution temperature (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived. The model; $T = e^{(14.9661/p)}$ is dependent depends on the value of the final pH of the leaching solution which varies with leaching time. It was observed that the validity of the model is rooted on the expression $\ln T = K_c/p$ where both sides of the equation are approximately equal to 3. The maximum deviation of the model-predicted solution temperature values from those of the experimental values were found to be insignificant hence establishing the validity and precision of the model. The correlation between mass of iron oxide ore and solution temperature as well as between final pH of leaching solution and solution temperature as obtained from experiment and derived model (0.9296 and 0.8911 as well as 0.9395 and 0.9988) respectively are quite close, indicating proximate agreement with values from actual experiment. [Journal of American Science 2010;6(6):31-37]. (ISSN: 1545-1003). **Keywords:** Model, Solution Temperature, Oxalic Acid, Iron Oxide Ore, Leaching	Full Text
8	**Model for Computational Analysis of the Quantity of Water Lost by Evaporation during Oven-Drying of Clay** Chukwuka Ikechukwu Nwoye Department of Materials and Metallurgical Engineering Federal University of Technology, P.M.B 1526, Owerri, Nigeria. chikeyn@yahoo.com **Abstract:** Model for computational analysis of the quantity of water lost by evaporation during oven drying of clay has been derived. The model; $\beta = \exp[(\ln t)^{0.998} - 2.9206]$ indicates that the quantity of evaporated water during the drying process is dependent on the drying time, the evaporating surface being constant. It was found that the validity of the model is rooted on the expression $(\text{Log}\alpha + \ln\beta)^N = \ln t$ where both sides of the expression are correspondingly almost equal. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 20% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. Water evaporation rate evaluated from experimental and model-predicted results are 0.0488 and 0.0530g/ min respectively, indicating proximate agreement. [Journal of American Science 2010;6(6):38-42]. (ISSN: 1545-1003). **Keywords:** Model, Water, Evaporation, Oven Drying, Clay	Full Text
9	**Bioaccumulation of Heavy Metals in *Pisum sativum* L. Growing in Fly Ash Amended Soil** Sudarshana Sharma[1], *Parmanand Sharma[2], Poonam Mehrotra [3] [1] Department of Biochemistry, Bundelkahnd University, Jhansi, India [2] School of Environmental Science, Jawaharlal Nehru University, New Delhi, India [3] Department of Botany, Bundelkahnd University, Jhansi, India pnsjnu@gmail.com; Sudarshana77@yahoo.com; mpunu@yahoo.co.uk	Full Text

Abstract: Presently, the crisis of enormous amounts of fly ash has been sorted out by using it significantly in stabilization and escalating crop growth. In present study pot-culture experiment was performed to observe the influence of fly ash amendments on the growth and accretion of heavy metal in pea plants. Fly ash utilized for this study with high alkalinity and metals was poor in N, P and humus comparable to garden soil. Fly ash and soil were mixed in different ratios i.e. 0, 5, 10, 15, 20 and 25% and used to fill earthen pots (2Kg/pot). Seven days old seedlings were transplanted (3 individual/ pot) in them at glass house. 25 ± 2^{0}C temperature and moisture at 50% of water holding capacity was maintained throughout the experiment. The results revealed that there was a significant increase in chlorophyll, carotenoids, proteins, biomass and overall growth of target plant up to 10% fly ash amendment. Whereas, phenols and ascorbic acid concentrations were maximum at 25% fly ash amendment. The heavy metals in growth media and plant were significantly augmented and found beneath the permissible limits up to 10% fly ash addition only. Pea seeds demonstrated fascinating results they were harboring the metal concentration in all amendments under permissible range and were safe to consume. Translocation factor was calculated and results illustrated that toxic heavy metals like Cd, Ni and Pb retained in the below ground while micronutrients like Cu, Zn and Fe translocated to above ground parts. Hence, it is evident that pea plants may be a good metal accumulator plant species that could use for restoration of waste land having high alkalinity and low nutrient values. [Journal of American Science 2010;6(6):43-50]. (ISSN: 1545-1003).

Key words: Crop yield; Heavy metals; Bioaccumulation; Translocation factor

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Evaluation of the Environmental Mitigation and Area Development (EMAD) component of the Bumbuna Hydroelectric Project (BHP) in Sierra Leone

Full Text

Alhaji Brima Gogra [a], Jun Yao [a,*], Edward Hinga Sandy [a], Gyula Zaray [b], Solomon Peter Gbanie [a], Celeste Tjobe [a], Tamba Samuel Bandagba [c]

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[b] Department of Chemical Technology and Environmental Chemistry, Eötvös University, H-1518 Budapest, P.O. Box 32, Hungary.

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Abstract: The most important development goal from the completion of the Bumbuna Hydroelectric Project (BHP) is to accelerate economic growth, and poverty reduction, through the development of affordable power generation for domestic use in an environmentally sustainable, and efficient manner. Besides mobilizing private capital, the proposed Project will promote private sector involvement in the management of the power sector, and sustainable sector reform. The first component includes Hydroelectric and Transmission Infrastructure; and the second component is the funding of the implementation of the Dam/Reservoir, and the Transmission Line Resettlement Action Plan, with livelihood restoration and agriculture stabilization subcomponents, in addition to a comprehensive Environmental Management and Mitigation Plan (EMP). And the Technical Assistance component will fund the management and supervision of activities under the second component, and in addition, provide support to the Project Implementation Unit (PIU), the Dam Review Panel (DRP), and the Environmental and Social Advisory Panel (ESAP). This paper discusses environmental sustainability vis-à-vis regulatory compliance and environmental policy issues as related to the challenges and benefits being experienced by the Bumbuna Hydroelectric Project (BHP) in Sierra Leone. Its goal is to present strategies by applying established theoretical concepts and frameworks to the BHP case and examines some critical success factors that could be integrated into best practice management, especially in the face of future environmental and socio-economic challenges. The paper focuses on the Environmental Mitigation and Area Development (EMAD) component of the project as opposed to project contracts and technical assistance. We (the authors) believe that the EMAD component has a direct influence on the

	livelihood of the people, and as such, it could be used to gain further insights into BHP. If effectively implemented, the EMAD component may become one of the most important strategic management initiatives taken by BHP in complying with environmental regulations, in reaping potential benefits, and in putting the project in a better position for future financial assistance. As such, this paper's main focus is on EMAD's activities and recommends the adoption of a competitive strategy like a focused low-cost strategy that will provide the project with a strategic advantage whilst capitalizing on the World Bank's Dam Planning/Management Action Plan (DAMAP). [Journal of American Science 2010;6(6):51-64]. (ISSN: 1545-1003). **Keywords:** Bumbuna Hydroelectric Project, Critical Success Factors, Environmental Sustainability, Sierra Leone, Strategies.	
11	**Physico-Chemical and Microbiological Study of Tehri Dam Reservoir, Garhwal Himalaya, India** Ashok K. Agarwal and Govind S. Rajwar Department of Botany, Government Post Graduate College, Rishikesh 249201, Uttarakhand, India. E-mail: rajwargs@hotmail.com **ABSTRACT:** In the present study physico-chemical and microbiological characteristics of the water of Tehri dam reservoir in the Garhwal Himalaya of India were determined during June 2003 through May 2005 when the reservoir was under construction, and was 5 km long and 40 m deep having an area of 2.2 sq km, and is located at 30º23' N latitude, 78º 29'E longitude and 635 m altitude at monthly intervals during June 2003 through May 2005 with an objective to estimate the impact of the reservoir on various physico-chemical and microbiological parameters of the water. Total solids, total suspended solids, total solids, turbidity and sulphate values were maximum on all the sites in rainy months, which may be due to the gradual disturbances in sedimentation of solids as well as dust particles deposited along with runoff rainwater. The alkalinity varied during different months. The values of pH, conductivity, hardness, calcium, dissolved oxygen and biological oxygen demand were higher during summer months. The chloride concentration was highest in the month of January and the nitrate increased in the summer months and early monsoon due to the higher phytoplanktonic production. The maximum number of total coliform, faecal coliform and total plate count was observed during summer and rainy seasons and minimum during winter. [Journal of American Science 2010;6(6):65-71]. (ISSN: 1545-1003). **Keywords:** Physico-chemical characteristics, Tehri Dam, Himalaya	Full Text
12	**Diversity, distribution and utilization of fodder species in sub-temperate, temperate and cold desert region of the Himachal Pradesh, north-western, Himalaya** Yashwant S. Rawat and Subash C.R. Vishvakarma G.B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263 643 Uttarakhar India yas_rawat@yahoo.com; yasrawat@gmail.com **Abstract:** Agriculture with animal husbandry is prevalent profession of rural people of Indian Himalayan Region. Livestock is considered one of the main sources of livelihood and integral part of livelihood, which rely mostly on fodder extracted from forests, grasslands, agriculture and agroforestry. The diversity, distribution and utilization pattern of the fodder species is important to prioritization of fodder species along an altitudinal gradient, and conservation and management practices of fodder species in both the Kullu and Lahaul valleys. Out of 67 fodder species, 43.28% were trees, 26.87% small trees and 29.85% shrubs, respectively. In general, maximum species were lopped annually, except *Olea ferruginea, Quercus floribunda, Q. leucotrichophora* and *Salix fragilis,* which were lopped an interval of 3 years. Majority of the fodder species are used as multipurpose and contributed to the high socioeconomic values. [Journal of American Science 2010;6(6):72-81]. (ISSN: 1545-1003).	Full Text

Keywords: Diversity; agroforestry; fodder; utilization; conservation and management; north-western Himalaya

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Light Hydrocarbon Correlation of Niger Delta Crude Oils

Full Text

*Mark O. Onyema and Patience N. Manilla
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ABSTRACT: The light hydrocarbon content of Niger Delta crude oils were studied with a view to providing a means of evaluating the Niger Delta petroleum system independent of higher molecular weight markers. Ultra high resolution gas chromatography was used in separation and analysis of the light hydrocarbons. Heptane ratio of oils ND-A3 (12.30), ND-A6 (12.07) and ND-B7 (10.33) were close and separate from ND-E5 (4.64). Invariance ratios and plot discriminated the oils into two groups. These apparent groups remained distinctly different in their graphical representation of ring preference. Star plots of oils ND-A3, ND-A6 and ND-B7 were shown to follow similar pattern, suggesting a strong similarity between them reflecting oil generation from same source rock, but followed different pattern from oil ND-E5 suggesting a negative correlation. These results strongly are consistent with two homologous sources for oils thus complementing the interpretations of higher molecular weight biomarkers and provide a quick and cost effective tool for correlation studies in Niger Delta, Nigeria. [Journal of American Science 2010;6(6):82-88]. (ISSN: 1545-1003).

Keyword: Niger Delta; Light Hydrocarbon; Invariance Ratio; Star Plot; Correlation

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Development of a Web Availability Analyzer Software Tool

Full Text

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Ferdowsi University of Mashhad, Department of Electrical Engineering, School of Engineering, Mashhad IRAN
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Abstract: In this study, results of the development of a web availability analyzer software tool that has been designed in order to measure internet availability from the end user's perspective are reported. The measured results of the availability of local and international sites along with a comparison of results indicate the successful operation of the software tool. The main objective of this paper is to present the approach used to measure the actual availability of internet sites through the development and use of a Web Availability Analyzer software Tool (WATT). [Journal of American Science 2010; 6(6):89-95]. (ISSN: 1545-1003).

Key words: Web, Internet Availability, Software package

15

Integrated Application of Cocoa Pod Ash and NPK Fertilizer: Effect on soil and Plant Nutrient Status and Maize Performance

Full Text

[1]L.S. AYENI[*], [1]M.T. ADETUNJI and [2]F.O. OLASANTAN
[1]Department of Soil and Land Management, University of Agriculture, Abeokuta, Nigeria
[2]Department of Horticulture, University of Agriculture, Abeokuta, Nigeria
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Abstract: Field experiment was conducted to study effect of application of cocoa pod ash and its integrated application with reduced levels of NPK 20:10:10 fertilizer (NPKF) on soil and plant nutrient, growth and grain yield of maize at Ondo in the rainforest zone of south west Nigeria. There were 10 treatments involving a control, ash applied at 5 and 10 t ha^{-1}, 100, 200, 400 kg ha^{-1} NPK fertilizer and combined use of ash with 100 or 200 kg ha^{-1} fertilizer. The treatments were replicated three times on field and the residual effect (one year later) on soil and plant macro and micro nutrient concentration, growth and grain yield of maize was

	studied. The soil in the experimental site was deficient in organic matter (OM), 2N, K and Mg. Application of cocoa pod ash, NPK fertilizer and their combinations significantly ($p<0.05$) increased soil organic matter, P, K, Ca, Mg, plant N, P and K, height, stover, root and grain yield on immediate and residual basis. NPKF also significantly ($p<005$) increased soil and plant Fe, Cu, Zn and Mn. Ash also increased plant Ca and Zn. Combined application of ash with 100 or 20 kg ha^{-1} NPKF, and NPKF (400 kg ha^{-1}) gave similar and highest cumulative grain yield varying between 5.4 to 5.9 t ha^{-1}. The control, cocoa pod ash at 10 t ha^{-1} and NPKF at100 kg ha^{-1} respectively gave least cumulative grain yield of between 3.3 and 4.2 t ha^{-1} for two years of study. The ash alone or combined with reduced NPKF gave highest residual effect on yield with increases of between 52 to 76% relative to control. [Journal of American Science 2010;6(6):96-102]. (ISSN: 1545-1003). **Key words:** integration, immediate and residual effect, yield	
16	**Estimate Biological Nitrogen Fixation in horse bean** Tayeb Saki Nejad Islamic Azad University, Ahvaz Branch Tayebsaki1350@yahoo.com; Saki1350@gmail.com **Abstract**: Research projects as split plot experiments in a randomized complete block design with four replications in field research in Islamic Azad University of Ahvaz 3 consecutive years (2006,2007,2008) implementation was the main plot assembly, four cultivar horse bean (*Vicia Faba*L.) plant: BARAKAT,ZOHRE,SHAMI and JAZAYERI, damascene the number of islands in the province have grown and sub-plots in the two years 2006 and 2007 three levels of nitrogen fertilizer (N1,N2 and N3 treatments, respectively 20 and 40 and 80 kg fertilizer N ha simultaneously planting) and the third year, 2008 values were doubled care. After the propagation earth, using cultivar with Rizobium bean plant (***Rh. Legominosarum***) inoculation and immediately cultured. Survey cultivar, BARAKAT highest percentage of mean total nitrogen plant 1.97 percent won. In sub-plots, with increasing amounts of nitrogen, accumulation of this element bean plants increased. Percent nitrogen treatments nodes N2 and N3 showed a significant difference, but the highest accumulation of nitrogen treatments N1 nodes with 1.67 percent won, thus whatever amount of fertilizer increased, the amount of biological nitrogen fixation nodes decreased. N3 treatment reduced accumulation of 40 to 50 percent nitrogen found in to other treatments. With increasing N rate, weight, number and size of the plant nodes decreased blessing average number of nodes 1250 nodes per plant among the highest number of cultivars grown offered. Number of nodes equal treatment and 1450 to increase the amount of fertilizer treatments 80 kg 998 nodes per plant decreased in all fertilizers in small amounts or how large gland enlargement process was observed. The mean largest tumor diameters in the treatment 1.98 cm were measured. Green and white non-effectiveness of enzyme Nitrogen's stated that usually the primary growth was achieved in pink and red and efficient biological nitrogen fixation, approximately 35 days after planting continued until after flowering and 10 days after flowering, gland Posts brown and black, showed the node representing aging and lack of nitrogen is established. [Journal of American Science 2010; 6(6):103-108]. (ISSN: 1545-1003). **Key words**: biological nitrogen fixation, horse bean	Full Text
17	**Effect of Different Types of Oral Iron Therapy Used for the Treatment of Iron Deficiency Anemia and Their Effects on Some Hormones and Minerals in Anemic Rats** Ghada, Z .A. Soliman 1 , Mohamed H. Mahfouz 2* and Ibrahim A. Emara2 1.Department of Biochemistry, National Institute of Nutrition (NNI), Cairo, Egypt. 2. Department of Biochemistry, National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt. *Corresponding author: mhesham5@yahoo.com **Abstract:** Iron deficiency anemia is the most common type of anemia related to malnutrition world wide. It represents a major problem in developing countries, especially in Egypt. The aim of this study was carried out to elucidate the effect of different types of oral iron therapy (used for the treatment of iron deficiency anemia) on some hormones and minerals in anemic rats. Forty weanling male Sprague-Dawley rats divided into 4	Full Text

	groups (10 rats each), G1; control group as negative control G2; anemic rats as positive control., G3; anemic rats receiving iron chelating amino acids (IDA+ICAA, 40 mg Fe/kg), G4; anemic rats receiving ferrous sulphate (IDA+FeSO4, 40 mg Fe/kg). Anemia was induced through feeding iron deficient diet (3-5 mg Fe/kg). At the end of the experiment, plasma, kidney and liver were used for determination of blood indices, tT3, tT4, Cu, Ca, Fe and MDA. Induction of iron in the diet improves body weight but still significantly lower than control group. Rats fed iron deficient diet had a significant lower Hb level, Hct value, RBCs count than normal controls. tT3 and tT4 levels of anemic rats were significantly lower than normal control (-15.16 & -30.59 % respectively). Treatment with ICAA gives better result than inorganic FeSO4. tT3/tT4 ratio was significantly higher in all treated groups than normal control group. A significant inverse correlation was found between tT3/tT4 ratio and liver Fe in anemic rats. Treatment of IDA rats with ICAA improves lipid peroxidation. Cu level of IDA group was significantly higher than normal control group, treatment with ICAA or FeSO4 returning Cu level to near normal. The plasma Ca level of ICAA treated groups was significantly higher than IDA groups. Plasma level of Fe or Fe/Cu ratio of IDA is significantly lower than normal control group, it reach less than half (58.3% decrease, $P < 0.0001$). A significant direct correlation was found between Ca level and kidney Fe in iron deficient anemia rats treated with iron chelating amino acids therapy. In Conclusion, the high bioavailability, easily tolerated doses of ferrous iron amino acid chelate allow lower doses to be used in IDA treatment than inorganic iron salts. [Journal of American Science 2010;6(6):109-118]. (ISSN: 1545- 1003). **Keywords:** Iron deficient anemia, iron chelating amino acids, inorganic iron	
18	***In vitro* Antimicrobial Assay and Phytochemical Analysis of Ethanolic Extracts of *Voacanga africana* Seeds** Christopher M. Duru[1] and Nkechi E.Onyedineke[2] [1,2]Department of Biology, Federal University of Technology, P. M. B.1526, Owerri, Imo State, Nigeria [1]Email: kristovad@yahoo.com; [2]Email: nonyedineke@yahoo.com **Abstract**: Dried and pulverized seeds of *Voacanga africana* were extracted with hot and cold absolute ethanol. The extracts were screened for their phytochemical composition and antimicrobial activities. The results revealed the presence of some bioactive compounds; alkaloids, anthranoids, anthraquinone, cardiac glycosides, phenols, phlobatanins, starch and tannins. The crude extracts exhibited antimicrobial activity against *Escherichia coli* (34.61 and 25%), *Serretia marcescens* (45.08 and 29.16%) and *Staphylococcus aureus* (42.10 and 34.21%). Others are *Alternaria solani* (33.33 and 25%), *Aspergilus flavus* (33.33 and 22%), *A. niger* (25 and 00%) *Candida albicans* (29.62 and 25.92 %) and *Rhizopus stolonifer* (22.58 and 19.35 %); relative to the standard antibiotics, Gentamicin and Clotrimazole; in the Agar Well. Diffusion sensitivity test. The efficacy of the hot extract was greater than the cold extracts in the test organisms, except in *Pseudomonas aeruginosa* where they appeared equipotent. [Journal of American Science 2010; 6(6):119-122]. (ISSN: 1545-1003). **Key words:** *Voacanga africana*, phytochemical, bioactive, equipotent	Full Text
19	**In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging ass** Gayatri Nahak and R.K. Sahu Department of Botany, B.J.B. Autonomous College, Bhubaneswar751014, Orissa, India. Email: sahurajani@yahoo.co.in **Abstract:** Medicinal plants are a major source of raw material for the traditional system like Ayurveda, Siddha & Unani. Even the modern system of medicine has more than 25 percent of drugs in use, which are either plant based or plant derived. Although several tree posses various medicinal properties, it has been ignored by indigenous & modern system of medicine. Among them ***Azadirachta indica* & *Melia azedarach*** belonging to family Meliaceae play a vital role in day to day usage of different indigenous communities due to its sacred and medicinal value. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants. In the course of finding potential antioxidant from plant source, two medicinal tree species belonging to family Meliaceae has been selected. Leaves were dried and extracted with	Full Text

	different solvent systems namely water, ethanol & methanol. Antioxidant activity using DPPH radical scavenging assay of six extracts from two genus of the family Meliaceae is reported & a comparison of the free radical scavenging ability of the extracts is emphasized. The result of the present study showed that the extract of Melia azedarach, which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparison to *Azadirachta indica* Neem. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger. [Journal of American Science 2010;6(6):123-128]. (ISSN: 1545-1003). **Key words:** Antioxidant activity, Azadirachta indica, Melia azedarach	
20	**Allocation of Spinning Reserve Cost Amongst Customers in Deregulated Power Systems** Ali Peiravi[1], Mehdi Hejazian[2] Ferdowsi University of Mashhad, Department of Electrical Engineering, School of Engineering, Mashhad IRAN Telephone number: (0098) 511-881-5100 [1]Ali_peiravi@yahoo.com, [2] mh_bgh@yahoo.com **Abstract:** In a deregulated power system, DISCOs are considered to be customers who can choose their desirable reliability levels and purchase their required reserve in an ancillary service market based on this reliability level. This paper presents a new approach for determining spinning reserve requirements considering customer's desired reliability level in a pool energy and reserve market. An approach is also developed to fairly allocate the cost associated with provision of spinning reserve amongst the customers. The effectiveness of the proposed approach is examined and the results are presented using the IEEE-RTS. [Journal of American Science 2010;6(6):129-138]. (ISSN: 1545-1003). **Key words**: Power market, system risk, spinning reserve, required reliability level, reserve allocation	Full Text
21	**Determination of Bauxite's phases by the bomb digest method at Kamsar laboratory ISO 9002 (Guinea)** Ibrahima Sory Cissé, Jiwen Ge* Institute of Ecology and Environmental Sciences, School of Environmental Studies China University of Geosciences. gejiwen2002@yahoo.com.cn **Abstract:** This paper presents the results of the experimental work done to find out the extraction percentage of alumina content in ore samples of bauxite from three mines of Guinea .So the knowledge of the chemical composition of a matter or a product directs us on its origins, its possible use and especially towards the technology which it will be necessary to apply for its transformation. This chemical composition is given at the laboratory which, to have reliable results uses adequate methods of analysis for each type of element to be proportioned in the matter. Thus for the analysis of bauxite exploited by the company of bauxites to Guinea (C.B.G.) and which currently comes from the plates of Sangaredi, Bidikoum and Silidara, the chemistry laboratory of Kamsar uses mainly two categories of methods which are instrumental and wet chemical method (volumetric). This study has relied on the chemical method due that it primarily rests on the quality of the matter to analyze and the concentration of the chemical elements which make it up. To this end, the Guinean bauxite exploited by the C.B.G having a high percentage in Al_2O_3 and a content of SiO_2 not exceeding 7%, for the determination of the various phases from this one, the section bomb digest of the laboratory at Kamsar uses a wet alkaline attack. Under high pressure and at variable temperatures according to the mineralogical phase to determine, this digestion is schematized as:Al_2O_3+2NaOH-2$NaAlO_2$+H_2O.Soluble aluminate. [Journal of American Science 2010;6(6):139-145]. (ISSN: 1545-1003). **Key words***:* Bauxite's phases, Gibbsite, Boehmite, Guinea and Bomb digests	Full Text
22	**Clinical utility of biochemical markers in ulcerative colitis among Egyptian patients**	Full Text

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Abstract: Biochemical markers are a non-invasive way of objectively measuring inflammation in ulcerative colitis and can play an adjunctive or primary role in the assessment of disease activity. **Aim** of this study was to A) evaluate serum levels of some biomarkers "leptin, adiponectin, resistin, and ghrelin" in ulcerative colitis (UC) patients, besides the ordinary inflammatory markers, B) to correlate the results with the disease activity, with the clinical characteristics of the disease C) and to examine the possible interaction between the estimated parameters values. Study was conducted on 56 UC patients from the Clinic of Internal Medicine Department and Endoscopy Unit of Alzahraa Hospital, Alazhar University, besides 30 healthy subjects served as control group. **Results:** Mean levels of ESR, CRP, TNF-α, resistin and ghrelin were significantly higher in active UC patients than the control group, while after the courses of treatment 47 patients achieved complete remission (inactive UC) mean values of these biochemical parameter deceased significantly than the original values at the active disease and the values reached nearly the normal ranges. While in patients (9 patients) who did not achieved complete remission, there were moderate decreased serum levels of these biochemical markers but still higher values than the control group and they still have manifestations of active UC. The mean level of leptin was significantly decreased in active UC patients compared to the control group, while after the course of treatment in patients achieved complete remission (inactive UC) the mean value increased significantly (with mean value 10.1 ng/ml). **Conclusion**: Our data indicate that, the increased plasma resistin, TNF-α and ghrelin levels correlated with activity of ulcerative colitis and so they could predict the response to therapy and possibly reflect an acute-phase response due to inflammation more than the ordinary inflammatory markers. Resistin, TNF-α and ghrelin levels could be considered as an independent predictor of disease activity in patients with UC and may represent link between inflammation and UC. [Journal of American Science 2010; 6(6):146-155]. (ISSN: 1545-1003).

Key words: ulcerative colitis, inflammatory markers, leptin, resistin, ghrelin, Tumor Necrosis factor alpha

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Degradation Hazard Assessment of Some Soils North Nile Delta, Egypt

Full Text

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[3]Prof. Dr. Refaat Abd El Kawey Youssef, Head of soils and water use Dept., Email: refatay1@yahoo.com

Abstract: This study aimed to identify and quantitatively evaluate land degradation processes in the northern Nile Delta region. Aerial photographs were used to follow the geo-indicators of different degradation processes. GIS is used to build up a database model including required parameters for obtaining inputs to the model implemented by FAO/UNEP for global assessment of land degradation. The obtained results reveal that the high risk of physical (i.e. soil compaction and water logging) and chemical vulnerability (i.e. salinization and alkalinization) cover an area of 18487 hectare and 11008 hectare, respectively. The human induced land degradation hazards due to soil compaction is slight to high, however moderate to high for water logging. The degree of salinization and alkalinization is slight to high. [Nature and Science 2010;6(6):156-161]. (ISSN: 1545-0740).

Keywords: soils degradation, remote sensing, GIS, North Nile Delta

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Calculation of Creeping Flow Past a Sphere Using Direct Boundary Element Method

Full Text

Ghulam Muhammad*, Nawazish Ali Shah
Department of Mathematics, University of Engineering & Technology Lahore – 54890, Pakistan.
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Abstract: In this paper, a steady, incompressible creeping flow past a sphere is calculated using direct

	boundary element method (DBEM). The surface of the sphere is discretized into quadrilateral elements over which the velocity distribution is calculated. The computed results are compared with analytical results. It is found that both these results are in good agreement. [Journal of American Science 2010;6(6):162-165], (ISSN: 1545-1003). Keywords**: Boundary element method , Creeping flow past a sphere**	
25	**Replacement Value of Urea Treated Corn with Cobs for Concentrate Feed Mixture in Pregnant Ewes Rations** Hamad M.R; Safaa Nadi Abed-Elazeem ; A.M. Aiad; S . A. Mohamed; and N. A. M. Soliman. Animal Production Research Institute. Agriculture Research Center Ministry of Agriculture, Dokki,Giza,Egypt. Dr_mona_zaki@yahoo.co.uk **Abstract:** Two trials were carried out to evaluate the effect of feeding urea treated corn with cobs (UCC) as 50% (T2) or total replacement (T3) of pelleted concentrate feed mixture (CFM) compared to the conventional diets (CFM) on its production and reproduction performance. Rice straw was offered separately from the concentrate. Evaluation criteria included DM intake and utilization, ruminal fermentation characteristics, milk yield, birth, weaning and marketing weight and feed efficiency. In the first trial, 27 Ossimi, ewes beginning 45 days before expected day of lambing were assigned to the control, T2 and T3 diets. The milk was measured on day 14 post partum and once every week up to the 12th week. The growth experimental periods were 137 day in duration using 15 weaned lambs. The selected lambs were allocated to the same three. In digestibility trial, 9 adult rams were allocated to three tested diets. In vivo digestibility, nutrients digestibility were different among diets. Feeding values (TDN) was greater for T3 followed by control diet whereas the highest DCP was recorded for T2. Feeding UCC had no effect on ruminal parameter in terms of pH, NH3 and total FVA`s across the sampling time except for NH3-N. The replacement of CFM by UCC resulted in insignificant higher ($p \leq 0.05$) lambs birth weight T3 (3.44 kg) but lower milk yield T3 (436 g /day). The lower birth weight lambs control group ($p \leq 0.05$) tended to grow faster and perform higher weaning as compared to the treated group. In growth trail, feeding UCC diets reduced ADG approximately 10% compared to control. The results indicated that DM, TDN and DCP needed produce 1 kg gain almost 5 to 10% better than the corresponding items from T2 and T3. Replacement of CFM in pregnant and growing lamps rations with UCC would be cost effective as cost UCC is only at 60% less than cost of CFM. [Journal of American Science 2010;6(6):166-178]. (ISSN: 1545-1003). **Keyword:** Sheep**,** feed**,** urea treated Corn-cobs**,** digestibility, nutritive value, growth, milk yield, performance	Full Text
26	**Application of multi-factorial experimental designs for optimization of biotin Production by a *Rhizopus nigricans* strain** Heba A. El-Refai[1], Ehab R.El-Helow[2], Magdy A. Amin[3], Lotfy A. Sallam[1], Hebat-Allah A. Salem[1] [1] Chemistry of Natural and Microbial Products Department, National Research Center, Dokki, Cairo, Egyp [2] Department of Botany and Microbiology, Faculty of Science, University of Alexandria, Alexandria, Egypt; [3] Department of Microbiology, Faculty of Pharmacy, University of Cairo, Egypt. dr_mona_zaki@yahoo.co.uk **Abstract:** The main objective of the present work is to demonstrate the efficiency of multi-factorial experimental designs to elucidate factors affecting the microbial production of biotin and to predict their optimum settings. A local *Rhizopus nigricans* strain was selected as a remarkable wild type biotin (vitamin H) producer. A preliminary medium formulation experiment suggested sucrose and peptone as appropriate donors of carbon, nitrogen and sulphur. An incomplete two level factorial experiment showed that concentrations of sucrose and peptone, as well as fungal growth stage are the most effective independent variables. A three level response surface methodology was then applied to accomplish a polynomial model which correlates the three key variables to biotin accumulation. When compared to the basal culture, the optimum condition predicted according to this model achieved about 10.4, 13.9, 5.7, 7.6 and 4.2-fold	Full Text

	increases in production, product yield coefficient, specific product yield coefficient, productivity and specific productivity, respectively. [Journal of American Science 2010;6(6):179-187]. (ISSN: 1545-1003). **Keywords:** Biotin, vitamin H, *Rhizopus nigricans*, experimental designs, response surface methodology	
27	**Some Physiological Factors Affecting Rapamycin Production by *Streptomyces hygroscopicus* ATCC 29253** Sallam[a], L.A.R.; El-Refai[a], A.F.; Osman[b], M.E.; Hamdy[a], A.A.; Ahmed[a], E.M. and Mohamed[a], M.A. [a] Natural and Microbial Products Chemistry Department, National Research Centre, Cairo, Egypt [b] Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt. dr_mona_zaki@yahoo.co.uk **Abstract:** The production of rapamycin, a potent antifungal, immunosuppressant and antitumor, by *Streptomyces hygroscopicus* ATCC 29253 has been studied in eight culture media. Rapamycin titer varied considerably in the tested media. The medium composed of soy meal, glucose, ammonium sulphate and KH_2PO_4 was the optimal for rapamycin production and so selected for further optimization. Studies for formulating the best carbon and nitrogen nutrition for rapamycin biosynthesis revealed that replacing glucose by D (+) mannose and excluding ammonium sulphate with decreasing soy meal concentration to 20 g/l led to four fold increase in rapamycin titer. Also, the effect of KH_2PO_4 concentration and medium initial pH were elucidated and the best requirements have been specified as 5 g/l KH_2PO_4 and pH 6. [Journal of American Science 2010;6(6):188-194]. (ISSN: 1545-1003). **Keywords:** Rapamycin**,** *Streptomyces hygroscopicus***,** Physiological studies	Full Text
28	**The Substance of the Universe A Philosophical Concept about the Origin of the Universe the Great Magnetic Mass and Velocity** Sami Al Taher Department of Agriculture, Cairo University, 13 Mohamed Tawfiq El Bakry, Heliopolis, Cairo, Egypt Tel. 202-26242045; seltaher@hotmail.com **Abstract:** The subject of atom and its components of electrons, and protons, have always occupied my mind since my scholar days studying agriculture at Cairo University -1952. Scientists suggested that the atom components are just particles. Although they could measure these particles, they didn't exactly define their nature. This paper depicts a philosophic concept of the nature. It is an invitation to reconsider the nature and the origin of the universe from a new perspective which might cause bewilderment to the reader. I realize that I don't need to run naked in the street, like what Archimedes did before and I certainly realize that it might take time before scientists would consider or accept my perspective. This article describes The Substance of the Universe A Philosophical Concept about the Origin of the Universe The Great Magnetic Mass and Velocity [Journal of American Science 2010;6(6):195-202]. (ISSN: 1545-1003). **Keywords:** Atoms, Big Bang, Electricity, Energy, Heat, Magnetic Mass, Matter Measurements, Mother Magnet, Photons, Quantum, Universe, Velocity	Full Text
29	**A trial for Induction of saprolegniosis in *Mugel cephalus* with special reference to biological control** Hussien, A.M. Osman; Ahmed, I.E. Noor El Deen; Waled, S.E. Solman Hydrobiology Department, National Research Center Dokki,Egypt. **dr.hussien_osman@yahoo.com** **Abstract:** A method was developed to experimentally induce saprolegniasis in *Mugel cephalus* fish exposed to physical stress, experimental descaling and descaling **with**	Full Text

	wounding in addition of sudden and graduall drop of water temperature. Fish which descaled and wounded were mostly affected with saprolegniasis than the other group. Thus combination of descaling with wounding and sudden drop of water temperature were more effective in inducing saprolegniasis in *Mugel cephalus* Present study also investigate biological treatment of *Mugel cephalus* natural infected with saprolegniasis using intestinal non pathogenic aeromonas strain for control saprolegniasis in vitro (plate) and in vivo (treatment tank) as a bath of aeromonas suspension 2 times for 3 days. [Journal of American Science 2010;6(6):203-209]. (ISSN: 1545-1003). **Keywords:** Saprolgniasis; Mugel cephalus; temperature; biological treatment	
30	**Effect of Annealing on DC Charge transport in Copper-Clay Cermets** O.A. Babalola*, A.B. Alabi and T. Akomolafe. Physics Department, University of Ilorin, Nigeria. babalolaOA@gmail.com **Abstract:** The influence of the annealing schedule on direct current charge transport of Copper-Clay based cermets is reported here. The cermets are cylindrical rods of constant 3.0mm diameter and varying lengths ranging between 5.0 mm and 25 mm. The cermets were fabricated by employing a compaction method that uses a mould at a constant pressure of 6.9×10^{8} N/m^2 on various Cu-Clay compositions ranging between 70 and 95 vol.% Cu. The cermets were subjected to varying peak annealing temperatures ranging between 100 and 1000 ^{0}C and for annealing time t_f ranging from 30 minutes to 180 minutes before being furnace-cooled to room temperature. Results showed that the annealing schedule greatly affects the resistivity, size-effect and Temperature coefficient of Resistance (TCR). The electrical properties showed that sintering is complete irrespective of the annealing temperature between 300 and 1000 ^{o}C when the annealing time t_f exceeds 120 minutes. [Journal of American Science 2010;6(6):210-216]. (ISSN: 1545-1003). **Keywords**: Cermet; Annealing; Composite; Clay; Size Effect	Full Text
31	**Genetic Analysis between and within Three Egyptian Water Buffalo Populations Using RAPD-PCR** Sekena H.Abdel-Aziem; Lamiaa M Salem.; Mohamed S Hassanane.; Karima F. Mahrous Cell Biology Department, National Research Center, Dokki, Egypt. dr_mona_zaki@yahoo.co.uk **Abstract:** The water buffalo represents an important part of animal production in Egypt It is economically a very important farm animal, genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and milk. Genetic similarity and polymorphisms among the three Egyptian water buffalo populations (El-Delta, Upper and Lower Egypt) were studied using random amplified polymorphic DNA (RAPD) technique. Out of fifteen primers screened using DNA samples of the three populations, thirteen primers generated reproducible and distinct to amplify DNA fragments in these three populations. RAPD patterns with a level of polymorphism were detected among populations. The results showed that a total of 126 loci were amplified and 106 polymorphic bands (84.13%) were produced. The genetic diversity had the highest value (0.2654) in El-Delta and the lowest value (0.2590) in Upper Egypt. This result confirms the closer between the three Egyptian population buffaloes. The dendrogram of genetic relationship based on overall RAPD primers confirmed the movement of Egyptian buffaloes between El-Delta and Upper, Lower Egypt. The results confirms that the Egyptian buffaloes are belongs to one breed. [Journal of American Science 2010;6(6):217-226]. (ISSN: 1545-1003). **Keywords:** water buffalo, RAPD-PCR, genetic diversity, Egypt	Full Text
32	**Women Physical Aggression (A Review)** Ali Edalati [1], Ma'rof Redzuan[2]	Full Text

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Abstract: Female aggression is a serious problem in most societies and is increasing these days in families. Female aggression has a negative effect on women as offender, their partners, children, and society in general. This paper aims to review the articles based on research that have been done on females' physical aggression. It attempts to show that females are also physically aggressive as males. According to the existing literatures, the rate of females' physical aggression is equal to those of males, and in some studies it is found to be higher than males. Based on these findings, it is concluded the rate of females' physical aggression is either equal to or higher than males, but not necessarily less than males. [Journal of American Science 2010;6(6):227-235]. (ISSN: 1545-1003).

Keywords: Wives Aggression, Female Aggression, Physical Aggression, Theories of Aggression

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Bioavailability of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in contr of induced Edwardsiellosis

Full Text

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Abstract: This study was conducted to investigate the Pharmacokinetics of Orbifloxacin in African sharptoo catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis caused by *Edwardsiella tar* (*E. tarda*), and to estimate its tissue distribution. Safety test, *in vitro* determination of the minimum inhibito concentration (MIC) of orbifloxacin against *E. tarda* isolate; in addition to; the *in vivo* efficacy of orbifloxac in treating Edwardsiellosis at 2 stages; the early stage 7 days and late stage 15 days post infection. The resul showed that orbifloxacin is safe for Catfish at concentrations up to 50 mg/L in water. The minimum inhibito concentration (MIC) of orbifloxacin against *E. tarda* isolate was 0.016 mg/L with MIC_{50} and MIC_{90} equal to 0 and 1.0 mg/L respectively. Almost 100% of the infected fish recovered after treatment with Orbifloxacin for hours in early stage of the disease with complete disappearance of clinical signs. No *Edwardsiella* could isolated from second group 96 hours post treatment; although the treated fish showed unhealed skin lesion results of liver dysfunction and tissue alterations were recorded. Orbifloxacin residues in Catfish musc decreased gradually after cessation of treatment and disappear by day 10 post-treatment in the first group. conclusion orbifloxacin can be awaited as effective antibacterial agent for control of edwardsiellosis caused *E. tarda*. The treatment is much more successful when initiated at the earliest time of infection. [Journal American Science 2010;6(6):236-244]. (ISSN: 1545-1003).

Keywords: Bioavailability, Edwardsiella tarda, African sharptooth catfish, Liver function tests, histopathology

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Optimization of microbial biomass production as biocontrol agent against root knot nematode on faba plants

Full Text

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Abstract: Our objective was to optimize nutritional and environmental conditions of the isolated *Serratia marcescens* Ba-2 and *Pseudomonas fluorescens* Ba-11 for biomass production and to evaluate the bio-control agents against the root knot disease caused by *Meloidogyne incognita* on Faba bean plants under greenhouse conditions. Glycerol at 10.2 g/L and peptone as a nitrogen source were the most suitable for biomass and antagonistic efficiency of *S. marcescens* or *P. fluorescens* against *Meloidogyne sp.* Cultures of *S. marcescens* and *P. fluorescens* supplemented with 10 g/L peptone, reduced larvae to 91% and 95% respectively. Optimum biomass and antagonistic activity of either bacteria against larvae was at pH 7.6, and incubation temperature at 30°C. 100% reduction of larval density was achieved when *S. marcescens* or *P. fluorescens* cultures were shaken at 120 and 160 rpm respectively. *S. marcescens* and *P. fluorescens* were very effective as biocontrol agaents to reduce the root – knot nematodes. Our data also indicate a marked effect of the biocontrol agents and Rhizobia on the growth response of faba plants. The obtained results showed that both bacterial treatments significantly increased the growth parameters as well as shoot and root dry weights and number of pods. [Journal of American Science 2010; 6(6):245-255]. (ISSN: 1545-1003).

Keywords: Biological control, *Serratia marcescens*, *Pseudomonas fluorescens*, root-knot nematode, rhizobia

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Resistin and Obesity- Associated Insulin Resistance in Children

Full Text

Abdelghaffar S[1], Hafez MH[1], Shaaban FA[2], Abou Esmail LA[3], Salama SI[4], Rashed RG [2]
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Abstract: Obesity, defined as excess body fat, is frequently accompanied by insulin resistance. It was hypothesized that resistin links obesity with insulin resistance and diabetes, however, debate exists about its possible role. The aim of this study was to measure serum resistin level in obese non diabetic children as well as to evaluate insulin resistance in them. It also aimed at exploring the possible correlation between serum resistin level, anthropometric, clinical and laboratory parameters in obese children. This study is a cross sectional study that comprised 45 children and adolescents with simple exogenous obesity and 30 apparently healthy non-obese age and sex matched children as control group. For each subject the following was performed: history taking, anthropometric measurements including body weight, height, BMI, waist circumference, hip circumference, waist hip ratio, skin folds thickness measurements (biceps, triceps, subscapular and suprailiac) and calculation of body fat. Clinical examination and pubertal assessment were performed. Laboratory investigations including fasting serum glucose, fasting serum insulin and resistin using ELISA technique. Insulin resistance was estimated by using the Homeostasis Model Assessment (HOMA). Serum resistin levels did not significantly differ between cases (6.7 ng/ml ±3.44) and (6.6 ng/ml ±2.47), ($p>0.05$). Fasting insulin and HOMA were significantly higher in obese children than controls, ($p < 0.001$ for both). About 78% of obese children had insulin resistance (high HOMA), 66.7% had high fasting insulin, 13.3% high resistin, 31.1 % had acanthosis nigricans and 8.9% had hypertension. A significant positive correlation was found between serum resistin levels and each of fasting insulin and HOMA, ($p<0.001$ for both). No significant correlation was found between serum resistin, HOMA and each of BMI, body fat percentage & waist circumference, ($p>0.05$). A significant positive correlation was found between BMI and each of waist circumference and systolic blood pressure, ($p< 0.001$ & < 0.05respectively). The present study confirm the link between resistin level and insulin resistance in obese children, however it couldn't prove whether high or low resistin level is more related to insulin resistance. A significant positive correlation was found between serum resistin levels and each of fasting insulin and HOMA .No significant correlation was found between serum resistin, HOMA and each of BMI, body fat percentage & waist circumference. HOMA was found to be a significant marker for early detection of insulin resistance in obese and overweight children. [Journal of American Science 2010;6(6):256-266]. (ISSN: 1545-1003).

	Keywords: Resistin- insulin- insulin resistance- HOMA- obesity- children- acanthosis nigricans	
36	**Adsorption Equilibrium, kinetics and thermodynamics of methylene blue from aqueous solutions using biopolymer oak sawdust composite** M.M. Abd El-Latif[1], Amal M. Ibrahim[2], M.F. El-Kady[1] [1.] Fabrication Technology Department, Institute of advanced technology and New Materials, Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt [2.] Surface Chemistry and Catalysis Laboratory, Physical Chemistry Department, National Research Center, Cairo, Egypt amona1911@yahoo.com **Abstract:** Oak sawdust (OSD), furniture industrial waste was chemically treated with 0.1N NaOH to give hydrolyzed oak sawdust (HOSD) which was immobilized on alginate biopolymer. Hydrolyzed oak sawdust composite (HOSDC) was utilized as low-cost adsorbent to remove basic dye (methylene blue, MB) from aqueous solution. HOSD and HOSDC were characterized by using Scanning electron microscope (SEM), thermo gravimetric analysis (TGA) and infrared spectrometer analysis (FTIR). The adsorption of (MB), whose isotherms are modeled according to Langmuir, Freundlich and Temkin, were studied at a variety of physical and chemical conditions. The data fitted very well with Freundlich isotherm. Batch adsorption models, based on the assumption of pseudo-first-order, pseudo-second-order and intraparticle diffusion mechanism, showed that kinetic data follow closely pseudo-second-order and intraparticle diffusion. In addition, various thermodynamic parameters, such as standard Gibbs free energy (ΔG°), standard enthalpy (ΔH°), standard entropy (ΔS°), and the activation energy (Ea) were calculated. The adsorption process of MB dye onto HOSDC was found to be spontaneous and endothermic process. Furthermore, a single-stage batch adsorber was designed for the removal of methylene blue by HOSDC based on the equilibrium data obtained. [Journal of American Science 2010;6(6):267-283]. (ISSN: 1545-1003). **Keywords:** Methylene blue; Sorption isotherms; Kinetics; thermodynamics; Sawdust; Binding polymers	Full Text

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Plant Water Relations and Osmotic Adjustment in *Brassica* Species under Salinity Stress

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Abstract: This investigation was carried out to compare the physiological behavior of two cultivars of *Brassica* grown under saline irrigations. The plants treated with saline water (ECe 15 dSm^{-1}) resulted in a quick development of water saturation deficit at 0.08 days after salinization (DAS) followed by a sharp decline in water potential at (0.25 DAS). Subsequently, a marked increase in diffusive resistance and a greater decrease in transpiration rate were noticed at one DAS. The response of *Brassica* at vegetative stage under salinization proved to be biphasic process. The first phase was characterized by rapid changes in turgor potential or volume change and the second phase represented the increase in solute concentration. Using the 'b' value (*ln* OP= a+b *ln* RWC) for judging the osmotic adjustment, both the species maintained turgor potential under salinization and thus exhibited osmotic adjustment, however, cv. HC 2 had an edge over its counterpart for higher osmotic adjustment as well as higher cell wall elasticity (less negative) during critical early phase of salinization. On the basis above findings it was concluded that both the *Brassica* species showed biphasic behavior during salinization, but during critical early phase of salinization cv. HC 2 showed some characters of better adaptation than cv. Kranti. [Journal of American Science 2010; 6(6):1-4]. (ISSN: 1545-1003).

Keywords: Brassica, osmotic adjustment, relative water content, salinity, transpiration, water potential

Abbreviations: CD- critical difference; cv- cultivar; DAS- days after salinization; DR- diffusive resistance; Ψ_s - osmotic potential (OP); RWC- relative water content; TP- turgor potential; TR- transpiration rate; WSD- water saturation deficit.

1. Introduction

Glycophytic plants have low salt tolerance and comprise the majority of cultivated species. When confronted with salinity, plants may undergo regulation of osmotic potential. Osmotic adjustment is a fundamental response of plants to salinity (Wyn Jones and Gorham 1983) and is necessary for survival and growth under saline conditions. Osmotic adjustment in response to salinity and drought is a result of solute accumulation which occurs through uptake of solutes and/ or synthesis of organic compounds. It results from the accumulation of solutes within cells which lowers the osmotic potential and helps to maintain turgor of both shoot and root. This allows turgor driven processes such as stomatal movement and expansion of growth to continue though at reduced rate to progressively lower water potential. Osmotic adjustment of salt adapted cells is mediated primarily through the accumulation of Na^+ and Cl^-, to generate sufficient turgor for survival and growth in the saline environment (Hasegava *et al.* 1990).

Adverse effects of low external water potential (Ψw) can be remedied by uptake of electrolytes but such uptake also creates the danger of ion excess, which could reduce cell turgor or volume. Thus, ion regulation and osmoregulation are the subject of intensive research into possible mechanism of salt tolerance (Greenway and Munns 1980). Turgor potential for stomatal movement and cell enlargement is governed by the process of osmotic adjustment and elasticity of tissue (Wright *et al.* 1997). Accumulation of ions (Na^+, K^+ and Cl^-) contributed to osmotic adjustment in *Accacia nilotica* and helped to maintain a positive water balance through osmotic adjustment. (Nabil and Coudret 1995).

One of the strategies for maintaining agricultural productivity in area affected by salinity or saline water irrigation, is the use of genotypes having

comparatively better osmotic adjustment and high salt tolerance. Hence this investigation was carried out to study the effect of salinity on osmotic adjustment, tissue elasticity and stomatal driven processes in two *Brassica* species to identify their variability for these traits, with the objective of improving crop performance under salinity stress.

2. Materials and Methods

This study was conducted as a short term experiment at vegetative stage of growth *i.e* 60 days after sowing by raising the cultivars Kranti and HC 2 of *Brassica* in china clay pots (15 cm diameter) under net house conditions. The pots were filled with 5 kg river sand each after thoroughly washing with distilled water. Two plants per pots were retained after thinning. The plants were supplied with Hoagland's solution at regular intervals. After sixty days of sowing, the plants were irrigated with saline water of ECe 15 dSm^{-1} prepared by using NaCl, $CaCl_2$ and Na_2SO_4 in the ratio of Na : Ca and Cl : SO_4 as 4:1 in Hoagland's solution. The sand medium of each pot was saturated with Hoagland's solution with ECe 2 dSm^{-1} and treated as control. The desired ECe level was maintained after observing the ECe of initial and final leachates. The sampling was done at 0.08, 0.25, 1, 2, 3, 6, 10 and 14 days after salinization (DAS). All the physiological observations were made on third fully expanded leaf from the top. Leaf diffusive resistance (DR) and transpiration rates (TR) were recorded by Steady State Porometer (Li-COR 1600, Lincoln, Nebraska, USA) at 11.00 h and were expressed in s cm^{-1} and µg cm^{-2} s^{-1} respectively from an average of eight replicates.

Leaf water potential (Ψ_w) was determined by using Plant Water Status Consol (Model 3000, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) and expressed in '-bars'. Osmotic potential (Ψ_s) of leaf given in '-bars' was measured with Vapour Pressure Osmometer (Model 3100 B, Wescor, Inc. Logan, Ultah, USA). Water saturation deficit (WSD) was determined according to Weatherly and Slatyer (1957). ΔTP/ ΔRWC was calculated according to the method described by Elston *et al.* (1976). The data was analyzed by calculating the critical difference (CD) and the significance was tested at 5% level.

3. Results and Discussion

Salt stress resulted in a marked reduction in transpiration rate (TR), water potential (Ψ_w), and osmotic potential (Ψ_s) of leaf, whereas the diffusive resistance (DR) and water saturation deficit (WSD) increased significantly (Fig. 1, 2, 3, 4, 5). Largest decrease in leaf Ψ_w and lowest WSD values were achieved at 0.25 DAS and 0.08 DAS, respectively, in both the cultivars. Subsequently a sharp decline in transpiration rate accompanied with increase in diffusive resistance was observed at one DAS. This points to rapid changes in turgor potential caused by saline water. The decrease in osmotic potential was progressive with passage of time. This points to second phase where increase in cellular concentration of the osmotically active solutes (osmolytes) which brings a new steady state i.e. enabled the plants to maintain turgor. Similarly, decrease in Ψ_w and Ψ_s was reported in *Brassica* (Burman *et al.* 2003). Among the cultivars, HC 2 exhibited high absolute value of TR and less DR under controlled and saline conditions. As a result of this there was greater decrease in Ψ_w and increase in WSD under salinity over control than in cv. Kranti.

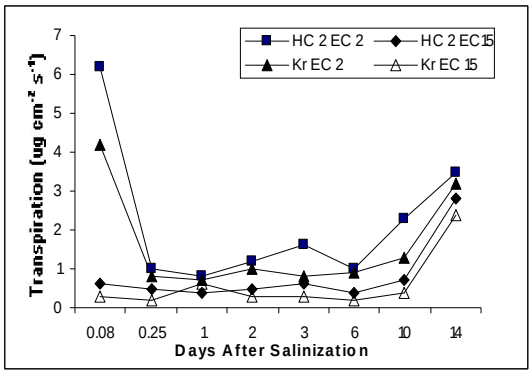

Figure. 1. Effect of salinity on Leaf transpiration rate in *Brassica* species (CD at 5% = 0.51)

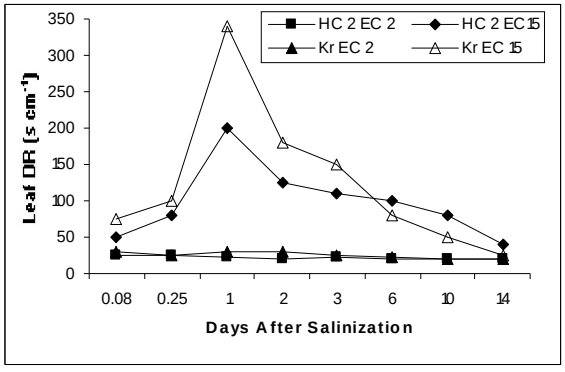

Figure. 2. Effect of salinity on Leaf Diffusive resistance in *Brassica* species (CD at 5% = 12.4**)**

Table 1. Effect of salinity on osmotic adjustment ('b' values) of leaf in *Brassica* species

Cultivars	*ln* OP = a+b *ln* RWC						
	Days after salinization						
	0.25	1	2	3	6	10	14
Kranti	1.14	1.16	1.10	1.25	1.36	1.28	1.39
HC 2	1.16	1.17	1.16	1.21	1.23	1.22	1.33

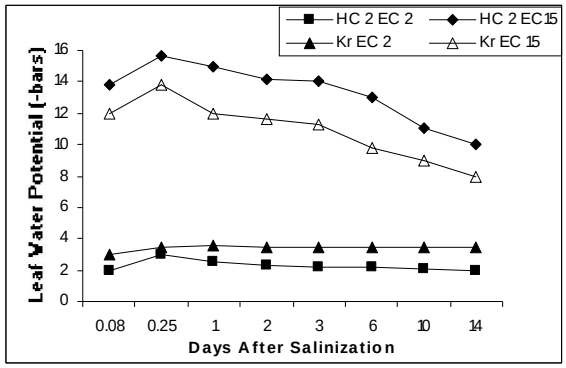

Figure. 3. Effect of salinity on Leaf water potential in *Brassica* species (CD at 5% = 0.52)

Table 2. Effect of salinity on cell wall elasticity (ΔTP/ ΔRWC) of leaf in *Brassica* species

Cultivars	ΔTP/ ΔRWC							
	Days after salinization							
	0.08	0.25	1	2	3	6	10	14
Kranti	1.95	0.8 0	0.4 6	0.3 6	0.2 4	0.0 9	0.0 2	0.0 8
HC 2	1.51	0.5 6	0.4 4	0.3 7	0.3 1	0.2 4	0.1 1	0.0 4

The 'b' value (*ln* OP= a+b *ln* RWC) is used for judging the osmotic adjustment (Singh *et al.*1996). Using this criterion, the osmotic adjustment was shown by both cultivars, but cv. HC 2 had edge over cv. Kranti during early phase of salinization (Table 1). Turgor maintenance by osmotic adjustment (Kumar *et al.* 1984, Li *et al*. 1993, Wright *et al*. 1997) had been dealt extensively under low water potential. The relationship between RWC and turgor potential (TP) showed that under salinization cv. HC 2 had less RWC as well as low TP compared to cv. Kranti; whereas under normal condition cv. Kranti possessed less RWC but higher TP (Table 2). The reason of discrepancies needs further investigations

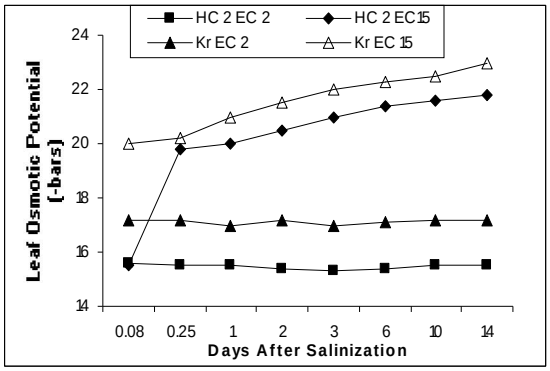

Figure. 4. Effect of salinity on Leaf osmotic potential in *Brassica* species (CD at 5% = 0.06**)**

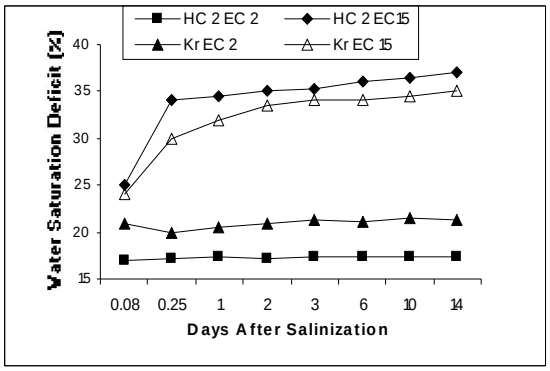

Figure. 5. Effect of salinity on Leaf water saturation deficit in *Brassica* species (CD at 5% = 0.33)

. The relationship between TP and transpiration rate showed that cv. HC 2 had less turgor but higher transpiration rate than cv. Kranti in normal and salinity treated plants. It indicates that cv. HC 2 comes under spender type, whereas cv. Kranti behaved as conservative type. The relationship between diffusive resistance and transpiration rate also support the above facts. This might probably be of cv. HC 2.

Differences in turgor maintenance may arise either from difference in solute accumulation and /or through differences in cell wall elasticity (Morgan 1984). Higher values of TP/RWC indicate the less cell wall elasticity (Table 2). Cell elasticity was greater in cv. Kranti than cv. HC 2 in later phase of salinization (3 to 14 DAS). However, during early phase of salinization (0.08 to 2 DAS), cv. HC 2 had high cell wall elasticity. A decline in Ψ_p/ RWC with decreasing Ψ_s in *Brassica juncea* for maintaining Ψ_p through maintenance of more elastic cell wall was reported by Kumar and Elston (1992).

The plant treated with saline water resulted in decreased turgor potential in both the cultivars (Table

3). Salinity induced reduction in turgor potential and water retention in *Brassica* (Wright *et al*. 1997) has been reported earlier. No doubt, both cultivars maintained turgor potential under saline condition by decreased Ψ_s (Table 3) due to accumulation of inorganic and organic solutes. The cv. Kranti had higher turgor potential because of increased DR and decreased transpiration and reverse was observed in cv. HC 2. As a result of this, cv HC 2 tried to maintain the hydrostatic pressure gradient and thus helped in regulation of various physiological processes which may lead to less reduction in yield. Gutknecht *et al*. (1978) explained that more hydrostatic pressure has no effect on turgor regulation and is the pressure gradient which is essential for maintaining various physiological processes under saline conditions.

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9/30/2009

Collection of *Cordyceps sinensis* (Berk.) Sacc. in the Interior Villages of Chamoli District in Garhwal Himalaya (Uttarakhand) and its Social Impacts

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Abstract: *Cordyceps sinensis*, belonging to the family Clavicipitaceae is a parasitic fungus on Lepidopteran larvae. Mainly it is found in subalpine regions from 3200 to 4000 m asl in grassy lands of Himalayas. It is very much valuable in Chinese and Tibetan medicine also. The residents of *Sutol* and *Kanol* villages (the most interior villages of Chamoli distt.) in Uttarakhand are extracting it. Every year the average collection of *Cordyceps* is about 140 kg from both villages. Near about 700 people were engaged in the collection of *Cordyceps* every year. Per head collection of *C. sinensis* was 200 gm per season. The collection period of this species is from May to July and the potential natural pockets are Bedini Bughyal, Homekund and Simbe. It is also track of famous religious "*Nanda Devi Raj Jat'' Yatra.* Basically the main collectors are men, women, young boys and girls which belong to the age group of 15 to 65 years. There is a drastic change in the economy of villagers and at the other hand some negative social impacts are also pertaining day by day in the last 3-4 years. [Journal of American Science 2010;6(6):5-9]. (ISSN: 1545-1003)..

Keywords: *Cordyceps sinensis*, medicinal value, *keera ghaas*, interior villages, social impacts on rural economy, drastic change

Introduction

Fungi having medicinal properties have been used as nutritional supplements for over 2000 years. Industries have strong interest in novel compounds, extracted from mycelium or fruiting body of fungi (Negi et al, 2006). In 1994, fungi represented a US$3.6 billion industry (Chang, 1996). Garhwal Himalayas are the main sources of the medicinal plants especially the upper region of the Himalays are the reservoir of the medicinal plants. *C. sinensis* is one of the main and highly valuable grasses like mushroom of this region. There are more than 310 species of *Cordyceps* but Webster 1980 and Sarbhoy, 1983 had reported that 150 species of *Cordyceps* are known. Out of these, three species have medicinal value and among them, *Cordyceps sinensis* is one of the highly valuable species of the world (Kobayasi, 1982).

About 1500 years ago in the Tibetan mountain pasture, herdsman observed that while grazing their cattle stumbled upon energetic, natural miracle plant (Zhu et. al.1998). They saw that their cattle and livestock became energetic after consuming a grass like mushroom and even older became vigorous. This miracle plant is known as *Yarsa gumba* or the caterpillar mushrooms in local Tibetan language and is a famous Chinese-Tibetan medicine. *Cordyceps sinensis* (Berk.) Sacc. belonging to family clavicipitaceae, is a mummified insect found is a result of fungal infection on Lepidopteran larvae. It is commonly known as Cordyceps, plantworm or caterpillar fungus. It is valued very highly in Tibetan and Chinese medicine and known as Yar- rsta- dgun- bu (Tibetan), Dong Chong Xia Cao – Chinese (Garbyal et. al, 2004).

1.1 Distribution

It is found in high mountains at an altitude above 4,000 m was noticed and reported in the year 2000. It is found in the alpine and subalpine zones between 3,600 – 4,200 meters above sea level. It is found in Nepal Himalaya, Tibet, Bhutan, Sikkim, Sichun, Qunghai, Xizang and Yunnan provinces of China. In India it is mainly found in the higher altitudes of Arunanchal Pradesh in the alpine medaws of Chiplakedar, Darma Vyas and Ralamdhura in Kumaun Himalaya where it is refered as "*Keera ghaas*" (insect herb) (Negi et al, 2006). Presently it is found in Chiplakot, Ultapara, Brahmkot, Najari and Nangnidhura – Munshyari region of district Pithoragarh. In some parts of Garhwal Himalaya, it is found in Niti and Mana valleys of Chamoli district and known as " *Keera jadi*".

General Features

Keera ghaas is a parasitic lepidopteran larva and these pyrenomyceptes belong to order Sphaeruales. It is found during May – June. They grow on caterpillars and pupae buried in soil of meadows. The caterpillar exploited in the Kumaun and Garhwal Himalaya has not been positively identified. However, caterpillars of *Hepialus oblifurcus* (Hepialidae) are known to be host of *C.sinensis* (Arora and Dhaliwal, 1997). The root has worm- like head, body and legs with numerous thin and fine transverse wrinkles. There are about eight pairs of legs on the body of the root and out of them four middle pairs are more prominent. Its lower part is thin while the upper part is slightly thicker. From the collar of this solitary root grows a dark brown grassy stalk which is thickened at the middle with slightly pointed tip and slender base. The larvae of *C.sinensis* get infected by the fungus at the end of autumn season. It infects the entire body and covers the whole larvae and kills it. The corpse of the insect enlarges and becomes resistant to decay due to a toxin, cordycepin, produced by the fungus (Dube 1983; Nair and Balakrishnan 1995). It remains buried under the ground during winter. It emerges in May on ground level (Garbyal et al, 2004). Its main associate species is *Rhododendron anthopogon* (*Sunpati*), it is one of the main indicators of *Cordyceps'* habitat. Sometimes the sign and pellet of musk deer, blue sheep, dung of yak etc. were observed (Aryal et. al ,2006). It is known as "*Keera jhar*" (insect herb) in the Indian mountains (Sharma, 2004).

Figure 1. *Cordyceps sinensis* in Dry Condition

Medicinal properties

Chinese practitioners of traditional medicine consider that *C.*sinensis is somewhat sweet in taste and warm in nature. Cordycepin, a boiactive metabolic contained in the fruit bodies of these fungi, also exhibits various biological activities (Trigg et al. 1971; Sugar and Mccaffery 1998; Zhou et al. 2002; Li et al. 2003; Yun et al. 2003). *C.sinensis* is effective against bacteria that have developed resistance to other antibiotics. It is also effective in respiratory infections, leprosy and leukemia. It inhibits active enzymes known as monoamine oxidase responsible for ageing. In Tibetan medicine system it is used to increase vitality and in restoring regenerative fluids – especially the fertility of sperms and kidney heat.

Materials and Methods

Study area

Chamoli district lies in the northeastern part of Uttarakhand state. It is bounded by North Latitude 29º 55' 00" & 31º 03' 45" and East Longitude 79º 02' 39" & 80º 03' 29" and falls in Survey of India toposheet nos. 53O, M and N. The geographical area of the district is 7820 km^2. Chamoli, the second largest district of Uttarakhand, is also important from strategic point of view as it shares its northern boundary with Tibet (China). Entire area is mountainous with agrarian economy. Forest cover (58.38%) is the main landuse. The total population, of the district, is 3, 70,359 out of the male and female population are 1, 83,745 and 1, 86,614, respectively (Census, 2001). The population density is 42 persons/km^2 and the male, female sex ratio is 1000:1017.The overall literacy rate is 76.23%.

Agriculture is the main occupation of the people. The agricultural activities are restricted to river terraces, gentle hill slopes and inter mountain valleys. The major crops are rice, wheat, potato, pulses, millets and seasonal vegetables. The net sown area, in the district, is 517.14 sq. km. out of which 173.37 sq. km. is sown more than once in a year. *Kanol and Sutol* are the last and interior villages of Ghat block. The average distance from Ghat is about 30 km. The nearest market of the villagers is Sital and Ghat. The economy of the both villages is based on traditional agriculture and animal husbandry. The total population of *Sutol* and *Kanol villages are* 650 and 900 respectively. There are 275 households in both the villages. *Sutol* and *Kanol* villages are situated on famous tourist track Lord Karjan's track. The biodiversity of this area is very rich. Mainly mixed forest and alpine meadows are present there. The main tree species are *Quercus semicarpipholia, Rhododendron arboreum, Taxus baccata, Cedrus deodara, Hippophae rhamnoides, Abies pindraw, Picea smithiana, Lyonia ovalifolia* etc.

Methods

Data collection and analysis

A random house hold sampling carried out for the records of information about the Yarsa gumba in interior village of Chamoli district. A questionnaire was used for

the information about different parameters of *Keera jhar*. A complete inventory was made about following objectives.

- To know the socio-economic impacts and future prospects of *Keera ghaas.*
- To know about natural collection pockets and states of *Keera ghaas* in study areas.
- To know the Govt. policies about collection of *Keera ghaas.*

Collection of *Cordyceps sinensis* in *Kanol* and *Sutol* villages

Kanol and *Sutol* are the last inhabited and most interior villages of Ghat block in Chamoli district. Basically the economy of the villages is traditional crop based. Potato, *Rajma, Ramdana* etc. are the main crops of the villages. Potato (*Solanum tuberosum*) and Rajma (*Phaseolus vulgaris*) are the cash crops of the farmers but not in wide scale because both villages are far from road head. Both the villages are situated at the average distance of 30 km. from road head. From last five to six years there are drastic changes in the economy of villagers due to collection of *Keera ghaas.* It fetches Rs. 60,000 to Rs. 80,000 per Kilogram in Pithoragarh, passes through Nepal and finally reaches markets in China to be sold at Rs. 1, 00,000 per kg. In 2001, it is believed 4-5 quintals of Yar Tsa Gumba found its way into Nepal from the border township of Dharchula alone (Taklakot in Tibet happens to be its largest market). Every year the average collection of *Keera ghaas* is near about 140 kg from both villages. Near about 700 people were engaged in the collection of *Keera ghaas* every year. Per head collection of *Keera ghaas* was 200 gm per season. The collection period of this species is May to July and the potential natural pockets are *Bedini Bughyal, Homekund* and *Simbe.* It is also found in the track of famous religious "*Nanda Devi Raj Jat Yatra*". Basically the main collectors are men, women, young boys and girls which belong to the age category of 15 to 65 years. The buyers come from Dharchula (mainly *Khampas* - schedule tribes) and Nepal but recently some educated localites and shopkeepers from adjacent areas are working as agents of big buyers. They are earning a good amount from these buyers. The average income of these agents is 50,000 rupees/kg. Based on the above data we can say they are the real beneficiary, because apart from this they are involving other works also. According to localites the average rate of *Keera ghaas* is Rs. 2, 50000 to 3, 00000 lakh.

Figure 2. Information Collection by a Researcher in the Study Area

Results and Discussion

Social Impacts

There are some drastic changes and impacts of *Keera ghaas* in the rural economy of *Sutol* and *Kanol* village. From last three to four years the villagers are quieting or decreasing their traditional crops and they are also not interested in Goat and Sheep rearing while this is the main traditional occupation of these areas. The seasonal migration of the villagers is also decreasing because the collection of *Keera ghaas*. Now they are moving or making the concrete houses instead of traditional stone and wood houses from the earning of *Keera ghaas.* Another positive change which is reflecting in field of education, in the past education of girls was not proper and the dropout rate of girls in compare to boys was too high from schools. Now the trend is changing day by day. The young boys and girls are going for education in adjacent towns and cities e.g. Ghat, Gopeshwar, Dehradun and Rishikesh etc. The drudgery system of local women is also decreasing day by day because collection of *Keera ghaas.* There are some of the negative impacts also. The traditional agricultural system as well as animal husbandry is also decreasing. Social relations between villagers and villages are spoiling because of its illegal trade and collection. There are some restrictions in adjacent villages for the collection of *C. sinensis*. For example there is great anxiety in *Sutol* and *Kanol* villages due to illegal extraction of *Keera ghaas* from nature.

Government Polices about *Cordyceps sinensis*

For ensuring the conservation of species in the wild, Uttarakhand Government issued guidelines for proper and

sustainable collection of *Keera ghaas in Uttarakhand*. The following guidelines are as follows.

- Collection season – May to July
- Issuing authority for the collection of *Keera ghaas- Van Panchyat/ Gram Panchyat.*
- *Van Panchyat* will issued collection authority to local villagers only and the local villagers will pay Rs. 1000 per head to *Van Panchyat*.
- Collector will deposit their collection to *Van Panchyat Sarpanch* then *Sarpanch* will verify the amount and approach to approved buying agencies through the forest department.
- The authorized buying agencies will pay Rs. 5000 per kg to the forest department as a royalty and the Govt. rate of *Keera ghaas* is Rs. 50,000 per kg. The Forest department will be responsible for issuing "*Ravanna*" or transition pass to buying agency.

Conclusion and Future Prospects

Keera ghaas or *Cordyceps sinensis* is unique and valuable for its medicinal properties, while it is not extracted sustainable in planed way in the study areas. There is a wide price gap in forest department and outside funding agencies, so it is supplied illegally. The social relations and customs are also affecting for the collection of *Keera ghaas*. The villagers are not much aware for its conservation priorities. The economic boom is easily seen in villages where the villagers are collecting *Keera ghaas*. There should be a proper understanding between collectors, forest departments and other agencies for the proper harvesting and conservation of this species. Awareness and scientific knowledge is very necessary for the future prospects regarding to conseve *Keera ghaas*.

Acknowledgments

Authors are thankful to local villagers of village Kanol and Sutol for providing necessary information and help. We are also very grateful to Mr. Daulat Singh Rawat, village – Ghat, practitioner of a Himalayan traditional medicine system.

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4/11/2009

SYNTHESIS AND REACTIONS OF SOME NEW PYRIMIDINE THIONES

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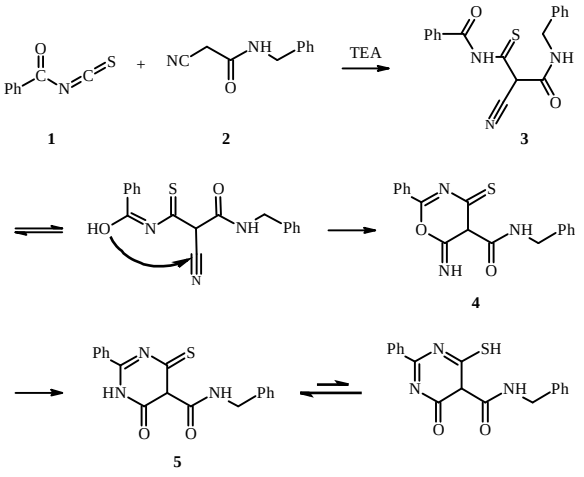
ABSTRACT: The addition of cyanomethylene derivative **2** to aroyl isothiocyanate **1** afforded mercaptopyrimidine derivative **5**. Mercaptopyrimidine **11** was prepared and transformed upon alkylation with chloroacetamide to thienopyrimidine **13**. Oxidation of **11** using I_2/AcOH yielded the disulphide **14**, while oxidation using H_2O_2/AcOH gave pyrimidine derivative **15**. Reaction of aldehydes with aminothiouracil **16** yielded 5-aroyl-2,8-dithioxo-2,3,5,8,9,10-hexahydropyrimido[5',4':5,6]pyrido[2,3-*d*]pyrimidine derivatives **19a,b**. Addition of **16** to chalcone afforded pyridopyrimidine **20**. Reaction of urea, 1-naphthaldehyde and aminothiouracil afforded pyrimidopyrimidine **21**. Reaction of **16** with NH_4SCN afforded compound **22** that oxidized to bis-isothiazolopyrimidine bisulphide **23**. [Journal of American Science 2010; 6 (6): 10-15]. (ISSN: 1545-1003).
KEY WORDS: mercaptopyrimidine, oxazine, thienopyrimidine, pyrimidine, pyridopyrimidine, pyrimidopyrimidine and isothiazolopyrimidine.

Introduction

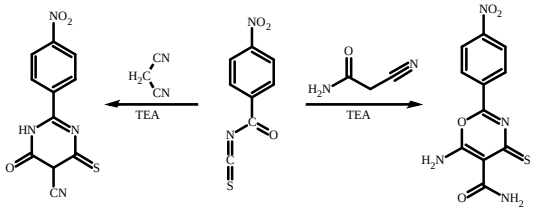
Pyrimidine derivatives comprise adverse and interested group of drugs **[Chabner et al., 2001]** and **[Hardman et al., 2001]**. Earlier a comprehensive review concerning pyrimidines had been published by Brown **[Brown et al., 1984]**. Pyrimidines in general are extremely important for their biological activities, for example, some are antiviral agents **[Nasr et al., 2002]**. The others, are selective cholecystokinine subtype receptor antagonists **[Bartolome-Nebreda et al., 2001]**, anti-inflammatory [**Santagati et al., 2002]**, **[Unangst et al., 1995**] and **[Tozkoparan et al., 1999]**, antihypertensive, diuretics, antimalarials, antithrombics, anticoagulants, antimicrobial **[Dubey et al., 2007]**, **[Learmonth et al., 2004]**, **[DeClercq et al., 2005]**, **[Demirayak et al., 2004]**, **[Ungureanu et al., 2006]**, **[Caprosu et al., 2005]** and **[Bahner et al., 1962]**.

As a part of a programme directed towards the synthesis of suitably functionalized heterocyclic systems of potential biological activity. [**Assy, et al., 1995]**, **[Assy et al., 2008]**, **[Sheriff et al., 2008]**, **[Sherif et al., 2008]**, **[Abdelghani., 2001]**, **[Abdelghani**., **1999]**. A new synthetic route for pyrimidine thione from aroyl isothiocyanate was undertaken.

The synthetic strategy towards the synthesis of pyrimidinethione involves the addition of cyanomethylene **2** to the electrophilic carbon of heteroallene **1** to give *N*-[3-(benzylamino)-2-cyano-3-oxopropanethioyl]benzamide **3** followed by intramolecular cyclization via the addition of enolic form to cyano function affording N-benzyl-6-imino-2-phenyl-4-thioxo-5,6-dihydro-4*H*-1,3-oxazine-5-carboxamide **4** which in turn undergoes ring transformation and rearrangement to give pyrimidinethione as the final product. But on base induced addition of *N*-benzyl-2-cyanoacetamide to benzoyl isothiocyanate, it afforded mercapto-pyrimidine **5**. The formation of **5** was potentiated by disappearance of CN group in its IR spectrum. The formation of **5** from addition of **2** to **1** may be proceeded presumably via the following mechanism:

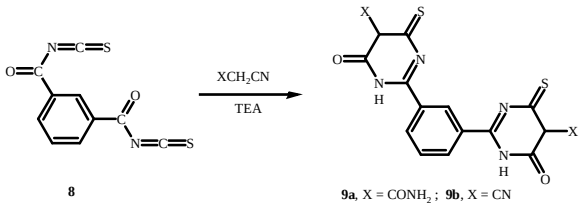


Depending on the reaction condition, p-nitrobenzoylisothiocyanate was reacted with malononitrile to give pyrimidinethione **6** upon heating. While, the addition of cyanoacetamide to p-nitrobenzoylisothiocyanate afforded 1,3-oxazine derivative **7** (Scheme I).



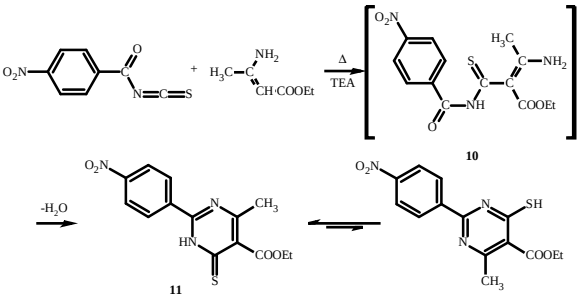
Scheme I

Addition of isophthaloyldiisothiocyanate **8** to cyanoacetamide and/or malononitrile derivative in the presence of TEA produced pyrimidine thione **9a** and **9b**, respectively (Scheme II).



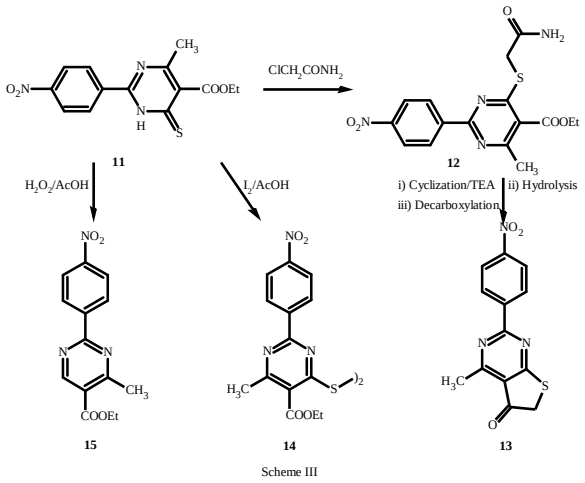
Scheme II

3-Aminocrotonate was added to p-nitrobenzoyl isothiocyanate to produce mercaptopyrimidine **11** presumably via the nonisolable intermediate **10** that undergo intramolecular cyclization followed by dehydration affording the final product **11**.



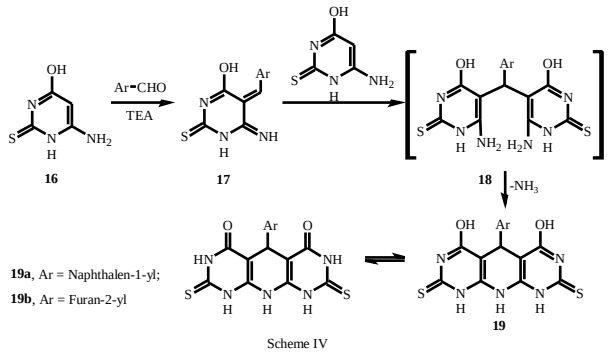
Compound **11** seemed to be of suitable located functionality for further functionalization and heterocyclization. Thus, alkylation of compound **11** using chloroacetamide in the presence of TEA afforded the alkylated derivative **12** that underwent intramolecular cyclization followed by hydrolysis and finally decarboxylated to give thienopyrimidine **13**.

Oxidation of compound **11** using I_2/AcOH it afforded the disulphide **14**. While, on oxidation using H_2O_2/AcOH it gave the desulphurized pyrimidine derivative **15** (Scheme III).



Scheme III

The synthesis of dihydropyridopyrimidine **19a,b** was achieved by refluxing of aminothiouracil **16** with aldehydes. The formation of **19** from **16** and aldehydes may be proceeded via the formation of Michael acceptors **17** followed by the addition of nucleophilic carbon of **16** and finally losing NH_3. Thus, reaction of pyrimidine derivative **16** and aldehydes namely 1-naphthaldehyde and/or furfural afforded the 2,3,5,8,9,10-hexahydropyrimido[5`,4`:5,6]pyrido[2,3-*d*]pyrimidine-4,6(1*H*,7*H*)-dione derivatives **19a,b** (Scheme IV). ^{1}H NMR spectra of **19a** showed complex spectra containing signals for each tautomeric form.

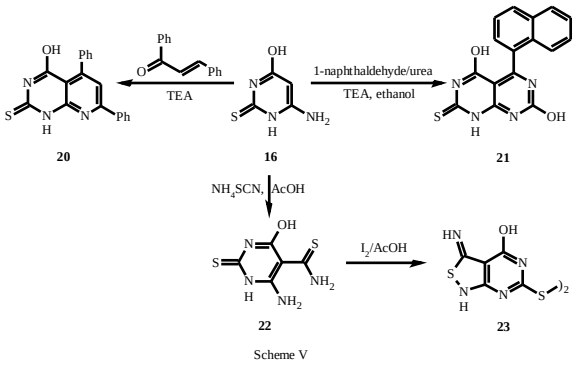


Scheme IV

Refluxing of compound **16** and 1,3-diphenylprop-2-en-1-one in the presence of TEA resulted in heterocyclization affording pyridopyrimidine **20**, while on refluxing with 1-naphthaldehyde and urea in presence of TEA, pyrimidopyrimidine **21** was obtained.

Finally, addition of enaminic carbon of aminothiouracil **16** to electrophilic carbon of isothiocyanate in acetic acid afforded pyrimidine

derivative **22**, which on treatment with iodine in acetic acid, it afforded the bis isothiazolopyrimidine disulphide **23** (Scheme V).



20 **16** **21** **22** **23**

Scheme V

Experimental

Mps are uncorrected. IR spectra (KBr discs) were recorded on a FT/IR-400 spectrophotometer (Perkin-Elmer). ^{1}H NMR spectra were recorded on a Varian 300 MHz (DMS-d_6) solutions. Chemical shifts are reported as δ values relative to tetramethylsilane (TMS) as internal reference. The elemental analysis were carried out at Micro analytical center, Cairo University.

N-[3-(benzylamino)-2-cyano-3-oxopropanethioyl]-benzamide (3):

A mixture of N-benzyl cyanoacetamide **2** (0.01 mole), benzoyl isothiocyanate **1** (0.01 mole) and TEA (3 drops) in (10 ml) acetone was stirred for 2 hours. The solid was filtered off, dried, to give **3**: yield 78%, as yellow crystals from benzene; m.p. 120-122 ^{o}C; its IR spectra: 3302, 3054 (NH), 1680 (C=O), 1648 (C=O), 2260 (CN), 1390 (C=S). Analysis for $C_{18}H_{15}N_3O_2S$ of mol. wt. 337.40, cal. C, 64.08; H, 4.48; N, 12.45; found C, 64.00; H, 4.44; N, 12.40.

***N*-benzyl-6-oxo-2-phenyl-4-thioxo-1,4,5,6-tetrahydropyrimidine-5-carboxamide (5):**

Compound **3** (0.01 mole) was dissolved in (20 ml) aqueous sodium hydroxide solution 10% and stirred for 1 hour at room temperature. The reaction mixture was neutralized by HCl, and the precipitated solid was filtered off, dried to give **5**: yield 76%, as white crystals from benzene; m.p. 170-172 ^{o}C; its IR spectra: 3302, 3034 (NH), 1690 (C=O), 1660 (C=O), 1350 (C=S); its ^{1}H NMR: δ = 4.47(d, 2H, J = 6.3 Hz, $PhCH_2$), 7.26-7.98(m, 11H, ArH's + CH methinyl), 9.06 (t, 1H, NH), 10.73(s, 1H, NH). Analysis for $C_{18}H_{15}N_3O_2S$ of mol. wt. 337.40, cal. C, 64.08; H, 4.48; N, 12.45; found C, 64.00; H, 4.44; N, 12.40.

Preparation of 6, 7, 9a and 9b.

General method:

A mixture of p-nitrobenzoylisothiocyanate (0.01 mole), cyanoacetamide and/or malononitrile (0.01 mole) and TEA (3 drops) in acetone (10 ml) was heated under reflux for 6-12 hours. The solid product obtained upon cooling, poured on ice and acidified by acetic acid, was filtered off, dried, and recrystallized from the proper solvent.

2-(4-nitrophenyl)-6-oxo-4-thioxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile (6): yield 86%, as white crystals from water; m.p. 235-237 ^{o}C; its IR spectra: 3268 (NH), 2278 (CN), 1696 (C=O), 1604 (C=N), 1350 (C=S); its ^{1}H NMR: δ = 7.70(s, 1H, CH), 8.38-8.17(m, 4H, ArH's), 13.64(s, 1H, NH). Analysis for $C_{11}H_6N_4O_3S$ of mol. wt. 274.26: cal. C, 48.17; H, 2.21; N, 20.43; found C, 48.10; 2.19; N, 20.40.

6-amino-2-(4-nitrophenyl)-4-thioxo-4*H*-1,3-oxazine-5-carboxamide (7): yield 86%, as white crystals from water; m.p. 190-192 ^{o}C; its IR spectra: 3168, 3308 (NH_2), 1712 (C=O), 1604 (C=N), 1344 (C=S); its ^{1}H NMR: δ = 7.69(s, 2H, NH_2), 8.07-8.33(m, 6H, ArH's + NH_2). Analysis for $C_{11}H_8N_4O_4S$ of mol. wt. 292.27: cal. C, 45.20; H, 2.76; N, 19.17; found C, 45.17; H, 2.71; N, 19.12.

2,2'-benzene-1,3-diylbis(6-oxo-4-thioxo-1,4,5,6-tetrahydropyrimidine-5-carboxamide) (9a): yield 77%, as yellow crystals from dimethyl formamide; m.p. 245-247 ^{o}C; its IR spectra: 3376, 3246 (NH_2), 3450 (NH) 1684 (C=O), 1608 (C=N), 1328 (C=S); ^{1}H NMR: δ = 2.09(s, 2H, 2CH), 7.64-8.44(m, 4H, ArH's), 9.65, 9.82(s, 4H, 2CON$\underline{H}_2$), 11.20(s, 2H, 2NH). Analysis for $C_{16}H_{12}N_6O_4S_2$ of mol. wt. 416.43: cal. C, 46.15, H, 2.90; N, 20.18; found c, 46.10; H, 2.88; N, 20.10.

2,2'-benzene-1,3-diylbis(6-oxo-4-thioxo-1,4,5,6-tetrahydropyrimidine-5-carbonitrile) (9b): yield 83%, as black crystals from methanol; m.p.265-267 ^{o}C; its IR spectra: 3246, 3378 (NH), 2284 (CN), 1682 (C=O), 1608 (C=N), 1242 (C=S). Analysis for $C_{16}H_8N_6O_2S_2$ of mol.wt. 380.40: C, 50.52; H, 2.12; N, 22.09; found C, 50.48; H, 2.10; N, 22.01.

Ethyl 4-methyl-2-(4-nitrophenyl)-6-thioxo-1,6-dihydropyrimidine-5-carboxylate (11):

A mixture of aminocrotonate (0.01 mole), p-nitrobenzoyl isothiocyanate (0.01 mole) and sodium carbonate (0.01 mole) in (20 ml) acetone was refluxed for one hour. The reaction mixture was cooled and neutralized with dilute HCl. The precipitated solid was filtered off, dried to give **11**:

yield 78%, as yellow crystals from aqueous methanol; m.p.170-172 ^{o}C; IR spectra: 3454 (NH), 1732 (C=O), 1608 (C=N), 1387 (C=S); ^{1}H NMR: δ = 1.30(t, 3H, J = 6.9 Hz, CH_3), 2.28(s, 3H, CH_3), 4.32(q, 2H, J = 7.2 Hz, CH_2), 7.64(s, 1H, NH), 8.07-8.38(m, 4H, ArH's). Analysis of $C_{14}H_{13}N_3O_4S$ of mol. wt. 319.34, cal: C, 52.66; H, 4.10; N, 13.16, found C, 52.60; H, 4.00; N, 13.11.

Ethyl 4-[(2-amino-2-oxoethyl)sulfanyl]-6-methyl-2-(4-nitrophenyl)pyrimidine-5-carboxylate (12) and 4-methyl-2-(4-nitrophenyl)thieno[2,3-*d*]-pyrimidin-5(6*H*)-one (13):

A mixture of **11** (0.01 mole), chloroacetamide (0.01 mole), and TEA (3 drops) in methanol (10 ml) was refluxed for 6 hours. The separated solid was filtered off, dissolved in water and the solid obtained after neutralization with HCl was dried and recrystallized from dimethylformamid to give **12**. The mother liquor was acidified by HCl, and the precipitated solid was filtered off, dried, and recrystallized from dimethylformamide/methanol mixture (1:1) to give **13**.

Compound 12: yield 70%, as white crystals; m.p. 253-255 ^{o}C; IR spectra: 3368, 3216 (NH_2), 1710(C=O), 1646 (C=O). Analysis for $C_{16}H_{16}N_4O_5S$ of mol. wt. 376.39, cal. C, 51.06; H, 4.28; N, 14.89, found C, 51.00; H, 4.20; N, 14.84.

Compound 13: yield 65%, as black crystals; m.p. 283-285 ^{o}C; IR spectra: 1674 (C=O); ^{1}H NMR spectrum δ = 2.89(s, 3H, CH_3), 2.95(s, 2H, CH_2), 8.35-8.69(m, 4H, ArH's). Analysis for $C_{13}H_9N_3O_3S$ of mol. wt. 287.29, cal. C, 54.35; H, 3.16; N, 14.63, found C, 54.31; H, 3.13; N, 14.60.

Preparation of 14 and 23.

General method:

Iodine (0.01 mole) was added to a suspension of **11** and/or **22** (0.01 mole) in acetic acid (20 ml) and left at room temperature with stirring for 4 hours. The resulted precipitate was poured on water and collected by filtration, washed with water, dried, and recrystallized from the proper solvent to give **14** and **23**, respectively.

Ethyl 4-{[5-(ethoxycarbonyl)-6-methyl-2-(4-nitrophenyl)-4-pyrimidinyl]disulfanyl}-6-methyl-2-(4-nitrophenyl)-5-pyrimidinecarboxylate (14): yield 85%, as yellow crystals from acetic acid, m.p. 236-238 ^{o}C; IR spectra: 1724 (C=O), 1684 (C=N); ^{1}H NMR: δ = 1.47(t, 6H, J = 6.9 Hz, $2CH_3$), 2.77(s, 6H, $2CH_3$), 4.59(q, 4H, J = 7.5 Hz, $2CH_2$), 8.34-8.20(m, 8H, 2ArH's). Analysis for $C_{28}H_{24}N_6O_8S_2$ of mol. wt. 636.66, cal. C, 52.82; H, 3.80; N, 13.20; found C, 52.78; H, 3.77; N, 13.17.

6,6'-disulfanediylbis(3-imino-1,3-dihydro-isothiazolo[3,4-*d*]pyrimidin-4-ol) (23): yield 86%, as yellow crystals from methanol, m.p. 359-360 ^{o}C; IR spectra: 3422 (OH), 3315, 3194 (NH), 1639 (C=N); ^{1}H NMR: δ = 4.69(s, 2H, 2NH), 6.35(s, 2H, 2NH), 11.57(s, 2H, 2OH). Analysis for $C_{10}H_6N_8O_2S_4$ of mol. wt. 398.47, cal. C, 30,14; H, 1.52; N, 28.12; found C, 30.10; H, 1.50; N, 28.09.

Ethyl 4-methyl-2-(4-nitrophenyl)pyrimidine-5-carboxylate (15):

To a solution of **11** (0.01 mole) in acetic acid (20 ml), H_2O_2 (0.02 mole) was added dropwise. The reaction mixture was stirred for one hour at room temperature. The separated solid was collected by filtration and dried to give **15**: yield 83%, as white crystals from acetic acid, m.p. 249-247 ^{o}C, IR spectra: 3120 (NH), 1724 (C=O), 1686 (C=N). Analysis for $C_{14}H_{13}N_3O_4$ of mol. wt. 287.27, cal. C, 58.53; H, 4.56; N,14.63; found C, 58.50; H, 4.50; N, 14.60.

Preparation of 19a and 19b.

General method:

A mixture of **16** (0.02 mole) and 1-naphthaldehyde and/or furfural (0.01 mole) in (20 ml) dimethyl formamide was refluxed for 30 hours. The solid product obtained upon cooling and pouring on ice was collected by filtration, dried, and recrystallized from the proper solvent to give **19a** and **19b**, respectively.

5-naphthalen-1-yl-2,8-dithioxo-2,3,5,8,9,10-hexa-hydropyrimido[5',4':5,6]pyrido[2,3-d]pyrimidine-4,6(1H,7H)-dione (19a): yield 75%, as yellow crystals from acetic, m.p. 350-352 ^{o}C, IR spectra: 3400 (OH enolic), 3165, 3134, 3070 (NH), 1686 (C=O), 1612(C=N), 1373 (C=S); ^{1}H NMR: δ = 7.43-8.92(m, 8H, ArH's + CH methinyl), 9.63(s, 2H, 2NH), 12.62(s, 2H, 2NH), 13.22(s, 1H, NH). Analysis for $C_{19}H_{13}N_5O_2S_2$ of mol. wt. 407.47, cal. C, 56.01; H, 3.22; N, 17.19; found C, 55.97; H, 3.19; N, 17.12 .

5-furan-2-yl-4,6-dihydroxy-5,10-dihydropyrimido-[5',4':5,6]pyrido[2,3-*d*]pyrimidine-2,8(1*H*,9*H*)-dithione (19b): yield 81%, as black crystals from methanol, m.p. 358-356 ^{o}C, IR spectra: 3397, 3323 (OH), 3180, 3089 (NH), 1622 (C=N), 1294 (C=S); Analysis for $C_{13}H_9N_5O_3S_2$ of mol. wt. 347.37, cal. C, 44.95; H, 2.61, N, 20.16; found C, 44.90; H, 2.60; N, 20.11.

4-hydroxy-5,7-diphenylpyrido[2,3-*d*]pyrimidine-2(1*H*)-thione (20):

A mixture of **16** (0.01 mole) and 1,3-diphenylprop-2-en-1-one (0.01 mole) and TEA (3 drops) in ethanol

(25 ml) was refluxed for 30 hours. The precipitated solid obtained upon cooling and neutralization with few drops of acetic acid was filtered off, dried, to give **20**: yield 77%, as yellow crystals from benzene/ethanol mixture (1:1), m.p. 200-202 ^{o}C, IR spectra: 3213 (OH), 3059 (NH), 1218 (C=S), ^{1}H NMR: δ = 7.16-8.16(m, 11H, ArH's + CH pyridine), 12.00(s, 1H, NH), 12.20(s, 1H, OH). Analysis for $C_{19}H_{13}N_3OS$ of mol. wt. 331.39, cal. C, 68.86; H, 3.95; N, 12.68, found C, 68.80; H, 3.93; N, 12.60.

4,7-dihydroxy-5-naphthalen-1-ylpyrimido[4,5-*d*]-pyrimidine-2(1*H*)-thione (21):

A mixture of **16** (0.01 mole) 1-naphthaldehyde (0.01 mole) and urea (0.01 mole) in dimethyl formamide (10 ml) was refluxed for 30 hours. The reaction mixture was cooled, poured on ice and the separated solid was collected by filtration, dried, to give **21**: yield 85%, as yellow crystals fom methanol, m.p. 330-332 ^{o}C; IR spectra: 3057 (OH), 1616(C=N), 1374 (C=S); ^{1}H NMR: δ = 7.35-9.01(m, 7H, ArH's), 9.64(s, 1H, NH), 12.00(s, 1H, OH), 12.86(s, 1H, OH). Analysis for $C_{16}H_{10}N_4O_2S$ of mol. wt. 322.34 cal. C, 59.62; H, 3.13; N, 17.38; found C, 59.55; H, 3.10; N, 17.30.

6-amino-4-hydroxy-2-thioxo-1,2-dihydro-pyrimidine-5-carbothioamide (22):

A mixture of **16** (0.01 mole) and ammonium thiocyanate (0.01 mole) in acetic acid (15 ml) was refluxed for 12 hours. The reaction mixture was cooled, poured on ice and the separated solid was collected by filtration, dried to give **22**: yield 85%, as yellow crystals from methanol, m.p. 310-312 ^{o}C; IR spectra: 3423, 3320 (NH_2), 3088 (NH), 1635 (C=N), 1292 (C=S); ^{1}H NMR: δ = 4.70(s, 2H, NH_2), 6.35(s, 2H, NH_2), 11.49(s, 1H, NH), 11.58(s, 1H, OH). Analysis for $C_5H_6N_4OS_2$ of mol. wt. 202.26, cal. C, 29.69; H, 2.99; N, 27.70; found C, 29.60; H, 2.94; N, 27.66.

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4/1/2010

Neuroanatomical, Immunocytochemical and Electrophysiological Studies on Cercal Sensory Receptors in the Female Locust

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ABSTRACT: Relatively little is still known about the function, types and location of cercal sensory systems. This system detects and encodes different sensory modalities: wind, touch and gustatory. It is focused on fine structure and distribution of various types of cercal sensilla in the desert locust were investigated with scanning electron microscope and neuroanatomical techniques. Two types of sensory receptors were identified and classified as mechanoreceptors (filiform sensilla) and chemoreceptors (basiconic sensilla). Also, the morphology and the organization of individual sensory receptors (mechanoreceptors or chemoreceptors) in the CNS were examined by immunocytochemical characterization of single neuron. All afferent fibres from individual filiform and basiconic sensilla project in the tenth neuromere of the terminal abdominal ganglion. Projections from single multiply innervated hair sensilla do not segregate with the exception of one afferent of contact chemosensory hairs which terminate only in its segmental neuromere, as was shown for other contact chemoreceptors of the abdomen. Another focus is on the electrophysiological response of individual mechanoreceptors or chemoreceptors to mechanical or chemical stimulation were analyzed. [Journal of American Science 2010;6(6):16-23]. (ISSN: 1545-1003).

Keywords: Sensory neurons; Cerci; terminal segments; Innervation; Immunocytochemistry; Electrophysiological recording

1. Introduction

Insect sensory receptors encode in their signals different types of information about the environment that can modify the animal's behaviour; these signals are integrated and distributed to several segmental ganglia by projection interneurones. Cercal receptor of insects mediate a range of behaviours such as running, jumping and flight (Camhi, 1980; Ritzmann, 1984; Boyan et al. 1989; Boyan and Ball, 1990; Kohstall, 1996), while these behaviours are expressed in a variety of context (Feeding, oviposition, mating, migration), each can be used to escape from a predator. Escape is one of the most dramatic episodes in an animal's life, and successful escape depends in large measure on the efficiency of the synaptic mechanisms and neuronal pathways linking sensory input to motor activity (Ritzmann, 1984). Both the clarity of its expression and its importance to the animal's survival has ensured that escape behaviour has attracted much attention from neurobiologists (Kohstall, 1996; Hörner and Heblich 2001; Rogers et al., 2003; Newland, 2004; Tousson and Gaaboub, 2004). In the insect nervous system the sensory afferents of cereal hairs have proved important for the study of presynaptic inhibition. The cerci project posteriorly from the last segment of the abdomen and are covered with two types of sensory hairs. Projection patterns of sensory neurons in the central nervous system, often follow topological rules that can be related the distribution of the sensilla on the body, (Pflüger et al., 1981; Newland and Burrows, 1994; Newland et al. 2000; Newland, 2004; Tousson, 2004 Tousson and Youssef, 2006). In the present study we give an account of the projections of single hair receptors into the terminal abdominal ganglion of locusts is given to reveal their differences in relation to the electrophysiological effects.

2. Materials and Methods

For the present study, adult females *Schistocerca gregaria* were taken 1-2 weeks after their final moult from crowded colony at the University of Tanta, Egypt. Animals were reared under a 12h light / 12h dark regime, and fed fresh wheat seedlings supplemented. Prior to dissection they were anaesthetized by cooling the preparation to 2-4°C. To identify the sensory receptors on the surface of cerci, scanning electron micrographs of the cuticle surface were taken. The terminal abdominal segments were usually rinsed in chloroform then critical point dried following dehydration in ethanol. After drying they were coated with gold-palladium, examined and photographed on a scanning electron microscope (SEM).

2.1. Neuroanatomical studies:

The distribution and peripheral innervation of sensory receptors on the cerci were revealed in whole-mount

preparations with the cobalt chloride backfilling technique (Pitman et al. 1972), and consecutive silver intensification (Bacon and Altman, 1977). Briefly, an intact insect was anaesthetized by chilling on ice and then mounted side down on a piece of non-toxic plasticine in Petri dish. The abdomen was dissected ventrally by an incision in the midline and the two sides of the body wall were pinned down laterally so that the abdominal cavity formed a pool which was filled with locust saline (Clements and May, 1974). The terminal abdominal ganglia, that innervate the cerci, were exposed by an incision along the ventral midline pins. In order to backfill the peripheral nerves and the sensory neurons of the receptors on the cerci, the cut ends of cercal nerve (Cer.N) was exposed, cut before their entrance into the cerci, and the distal stump was put in a small well of Vaseline containing 3 molar cobalt chloride, while the rest of the preparation was bathed in locust saline. The preparation was kept at 4-6°C for 36-48h, and then cobalt chloride in the neuronal structures was precipitated into black cobalt sulphite with 1-3 drops of ammonium sulphide in saline for 10-15 min. After rinsing with pure saline, the preparations were dehydrated and cleared in methyl salicylate. Subsequent silver intensification revealed in more details the cobalt precipitate in the nerve axons, the sensory somata and the sensory dendrites. Sensory neurons and the peripheral nerve distribution were drawn by using a camera Lucida attachment on a Zeiss compound microscope (Carl Zeiss, Germany).

2.2. Immunocytochemical characterization of single neuron:

The central projection of both contact chemoreceptor and mechanoreceptor neurons of cerci were visualized with neurobiotin (Vector Laboratories Inc.) in backfills (Tousson and Hustert, 1998) from single receptors in the periphery. The chemosensitive and the mechanosensitive sensilla from different areas of the cerci were stained by surrounding the receptor with a wall of vaseline. A droplet of distilled water was placed in this well and the sensillum was shaved off with a broken glass microelectrode, exposing the sensory dendrites. The distilled water was replaced with a droplet of 3% aqueous neurobiotin solution. Animals were then incubated for 72-96hr at 4°C or at room temperature for 48-72hr.

After incubation, the 7th and terminal abdominal ganglia were dissected out in insect saline and fixed in 4% Paraformaldehyde for 1 hr, and then dehydrated and cleared in xylene for 30 minutes. Then they were rehydrated and rinsed twice in phosphate-buffered saline (PBS; pH 7.2, 10 minutes each). The labelled ganglia were incubated for 1 h at 37°C in a solution of 1 mg collagenase, 1 mg hyaluronidase in 1 ml PBS, and then rinsed in PBS with two changes of 15 min then three changes of 15 min with 0.5% Triton X-100 added. Peroxidase binding to neurobiotin was achieved by using the avidin-biotin complex in buffer, incubated for 5-12 hr at room temperature. After incubation in the avidin-biotin complex, preparations were rinsed in two changes of buffer with 0.5% Triton X-100 and finally in PBS (each 15 min). Peroxidase bound to neurobiotin in the central afferent projection was localized with the black chromogen 3,3diaminobenzidine tetrahydrochloride (DAB) reaction. The preparations were incubated for 5-15 min in a solution of 30 mg DAB and 45µl hydrogen peroxide (30%) in 100 ml PBS. Then the reaction was stopped by two changes of PBS for 5 min. The preparations were then dehydrated in an ascending alcohol series and cleared in methyl salicylate for whole-mount viewing. The results were drawn by using a camera Lucida attachment on a Zeiss standard compound microscope and photographed by Canon digital Camera. At least five successful stains of afferents were made generally, and one was selected to be used for every representative figure in this study.

2.3. Electrophysiological studies:

The tip-recording technique (Hodgson et al. 1955) was used to record from the sensory neurons innervating both the mechanoreceptors (filiform sensilla) and chemoreceptors (basiconic sensilla) on the cerci. Before recording, filiform sensilla were cut to approximately half their length, but basiconic sensilla were left intact. Blunt glass recording microelectrodes containing 100 mM sodium chloride were then placed directly over the tips of the sensilla. The salt solution in the electrodes evoked spikes in some of the chemosensitive neurons, and movements of the electrode, which deflected the shafts of the sensilla, induced spikes in mechanosensory neurons (Newland and Burrows, 1994). The same electrode was therefore used to evoke and record simultaneously the spikes of both the mechano- and chemosensory afferents. Signals were fed to a standard high impedance D.C. amplifier and then A.C. coupled.

3. Results

The peripheral innervation of the cerci (Fig. 1) was revealed by whole-mount preparations after using the cobalt chloride backfill techniques (Figs. 2A & 2C). The terminal abdominal ganglionic mass in *Schistocerca gregaria* is an ovate structure lying in the 8th abdominal

segment and innervates the abdominal segments number 8, 9, 10 and 11; thus comprising the fused ganglia of last four segments (Fig. 2A). The cercal nerve precedes posterad from the common root passing lateral of the epiproct nerve. A considerable distance caudad of the ganglion, the large rectal nerve (Rec.N) emerges and passes dorsal dividing to innervate the muscles associated with the rectum. The rectal nerves is similar to that in the male as described in Bharadwaj and Banerjee (1971) except for the nerve to the dorsal dilator muscle of the rectum (286) which emerges close to the posterior ramification of the caudal branch and directly opposite to the branch to the lateral dilator muscle of the rectum (290). The remainder of the caudal branch ramifies to the intrinsic muscles of the rectum. The cephalad branch innervates only the intrinsic muscles of the rectum continuing posterad and crossing above the epiproct nerve, the cercal nerve divides into cercal and paraproct branches. The lateral or cercal branch (Figs. 2A-C) gives rise to a branch which turns lateral passing beneath the cercal nerve to innervate the transverse muscle (292) and a small sensory branch (S) is then received from the ventral region of the 10th tergum (Fig. 2A). The remainder of the cercal branch precedes posterad and divides to innervate the lateral and mesial surfaces of the circus (S).

SEM showed that the sensory receptors associated with the cerci (Fig. 1) could be divided into two main types, mechanoreceptors and chemoreceptors (Figs. 1C). Cobalt staining of the peripheral nerves and sensory neurons confirmed the identity of both mechanosensory receptors (filiform sensilla) with one neuron below their cuticular structure and contact chemoreceptors (basiconic sensilla) with five neurons (Figs. 2 & 3) gathered below the small and blunt hair with a terminal pore.

High resolution scanning electron micrographs revealed that basiconic sensilla are peg-like structures (Fig. 1C), 15-30 µm long, about 3-4 µm in basal diameter and showed a pore at its tip (1.2 µm in diameter) that provides access for contact with chemicals. The basiconic sensilla are supplied with groups of five deeply staining neurons (4-5 µm in diameter) that lie beneath each basiconic sensillum. Proximal to the somata each sensory neuron extends its axon, which joins those from the other cells in the group to form a small nerve that finally enters larger nerves (Fig. 3B).

The filiform sensilla act as mono mechanoreceptors that respond to air currents as the wind-sensitive type (Fig. 5). The filiform sensilla are arbitrarily divided by size, shape and type of socket into two main types, the long sensilla over 200 µm in length and about 4.5 µm in diameter at their base and the short sensilla 40-100 µm long and about 3.5 µm in basal diameter (Fig. 1C & 3A).

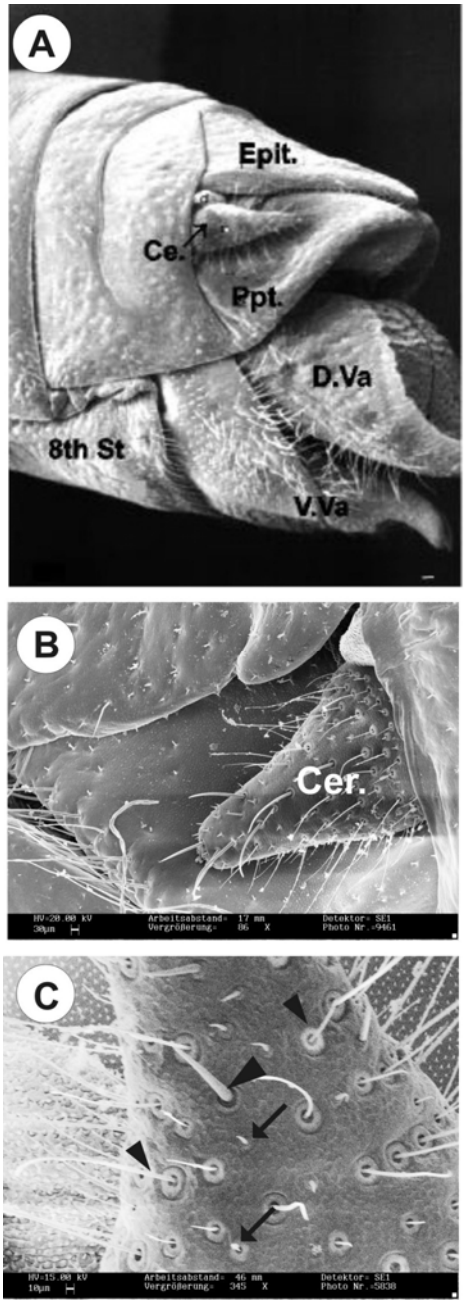

Fig.1 Scanning electron micrographs of the terminal abdominal segments in female locust. Fig. 1A: Lateral view of the terminal abdominal segment showing the cercus (Cer), the paraproct (Ppt), the epiproct (Ept), the dorsal ovipositor valve (D ov.) and the ventral ovipositor valve (V

ov.). Fig. 1B: Lateral view of the cerci with a large number of mechanosensory sensilla and chemosensory sensilla; Scale=30µm. Fig. 1C: Lateral margin of cerci fill with mechanoreceptors (filiform sensilla; arrow head) and contact chemoreceptors (basiconic sensilum; arrow); the basiconic sensilla scattered between the filiform sensilla; Scale=10 µm

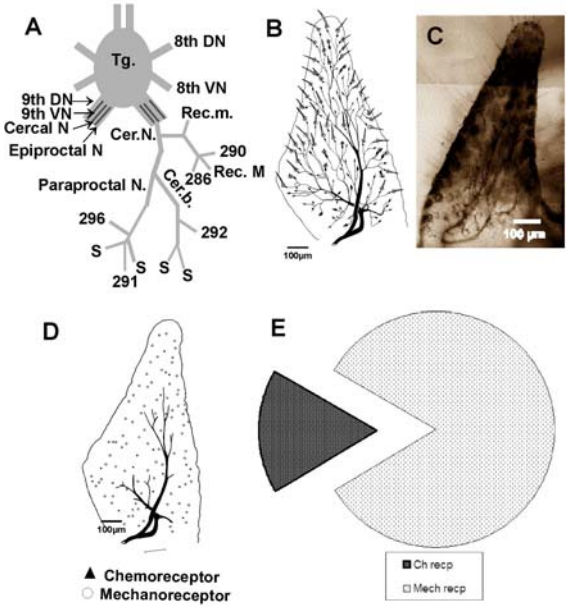

Fig. 2 A: Camera Lucida drawing of the terminal abdominal ganglion mass and the peripheral innervation of the terminal abdominal segments. Fig. 2B: Camera Lucida drawing of the peripheral innervation of the cerci female. Fig. 2C: Light micrograph of a whole mount stained with silver-intensified cobalt staining, showing the cerci sensilla and their innervation. Fig. 3D: Camera Lucida drawing shown the distribution of contact chemoreceptors (filled triangles) and mechanoreceptors (open circle) on the cerci of the female locust. Fig. 3E: Histogram showed the percentage of the contact chemoreceptors to the mechanoreceptors (open circle) on the cerci of the female locust.

As means of estimating the total number of receptors that were found on the locust subgenital plate, two basic types of receptors are present, contact chemoreceptors (Basiconic sensilla) and mechanoreceptors (filiform sensilla). It has been found that approximately 223 ± 5 receptors were identified and the average number of different types of receptors shows a ratio of 1:5 of chemosensory to mechanosensory sensilla (Figs. 2D & 2E).

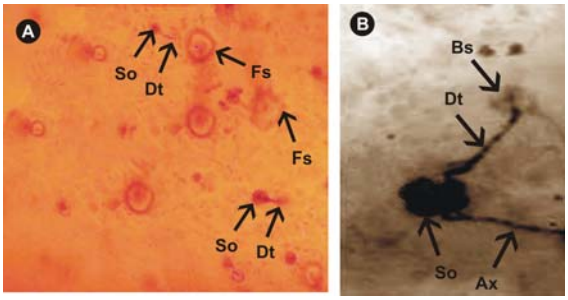

Fig. 3A: Light micrograph for individual filiform sensilla in the locust cerci innervated by single sensory neurons. Fig. 3B: Light micrograph for individual basiconic sensilla in the locust cerci innervated by five sensory neurons; (Fs, filiform sensilla; Bs, basiconic sensilla; So, sensory neuron; Dt, dendrites; Ax, axon).

Filiform sensilla on the cerci are each innervated by a single mechanosensory neuron (Fig. 3A), as indicated by the presence of spikes with single amplitude only in tip recordings from these sensilla following deflection of the hair-shaft (Figs. 5A-C). Similarly, only single sensory neurons were stained in the terminal ganglion in backfills from filiform sensilum using neurobiotin (Fig. 4A). These sensory neuron greater in diameter than the basiconic axon and entering the terminal ganglion via cercal nerve and have projection patterns in the 9th and 8th neuromere that is not resemble the neighboring basiconic afferents (Fig. 4C). The ipsilateral collateral ascends into the preceding ganglion.

Whenever a basiconic sensillum from the cerci was stained iontophoretically, five neurons (one mechanosensory and four presumably chemosensory) were revealed in the whole-mount (Figs. 4B.E). The axons enter the terminal abdominal ganglion via cercal nerve and proceed medially to the 9th and 8th abdominal neuromere. There, all axonal projections branch ventrally and send neuritis medially to the VAC at a median level within the VAC (Fig. 4D & 4E). From there, several branches extend into the contralateral neuropil and one turns posteriorly and only four collaterals proceed ventrally as a compact bundle through the anterior

ipsilateral connective and into the 7th abdominal ganglion (Figs. 4B & 4C). These axons then proceed to the medio-ventral contralateral area of the 7th abdominal neuromere where they terminate.

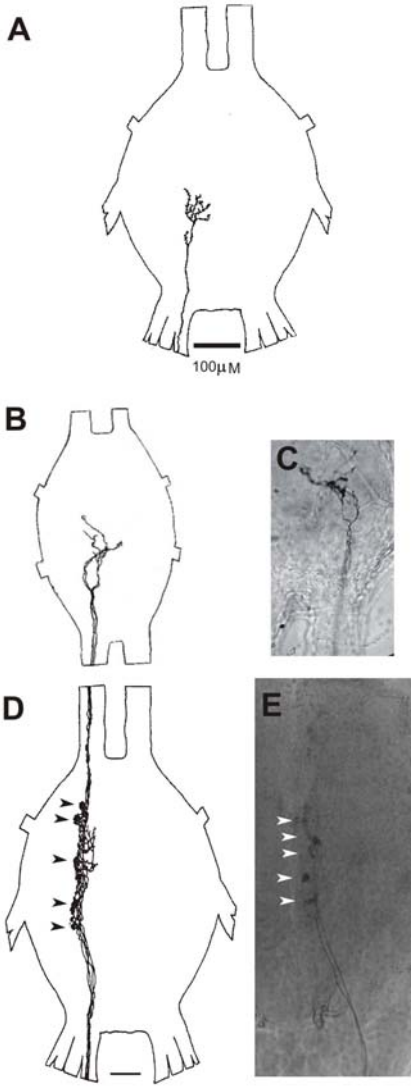

Fig. 4A: Central projections morphology in the terminal ganglion from individual filiform sensilla located on the cerci of the female locust. Fig. 4B-E: Central projections morphology in the terminal ganglion (D & E) and the seventh abdominal ganglion (B & C) from individual basiconic sensilla located on the cerci of the female locust, Five axons enter the terminal ganglion via the cercal nerve and proceed medially to the ninth and eighth abdominal neuromeres with glomeruli like structure (arrowheads) and only four lateral afferent collaterals proceed through the anterior ipsilateral connective on a ventral level into the seventh abdominal ganglion, where they terminate medio ventrally (B & C).

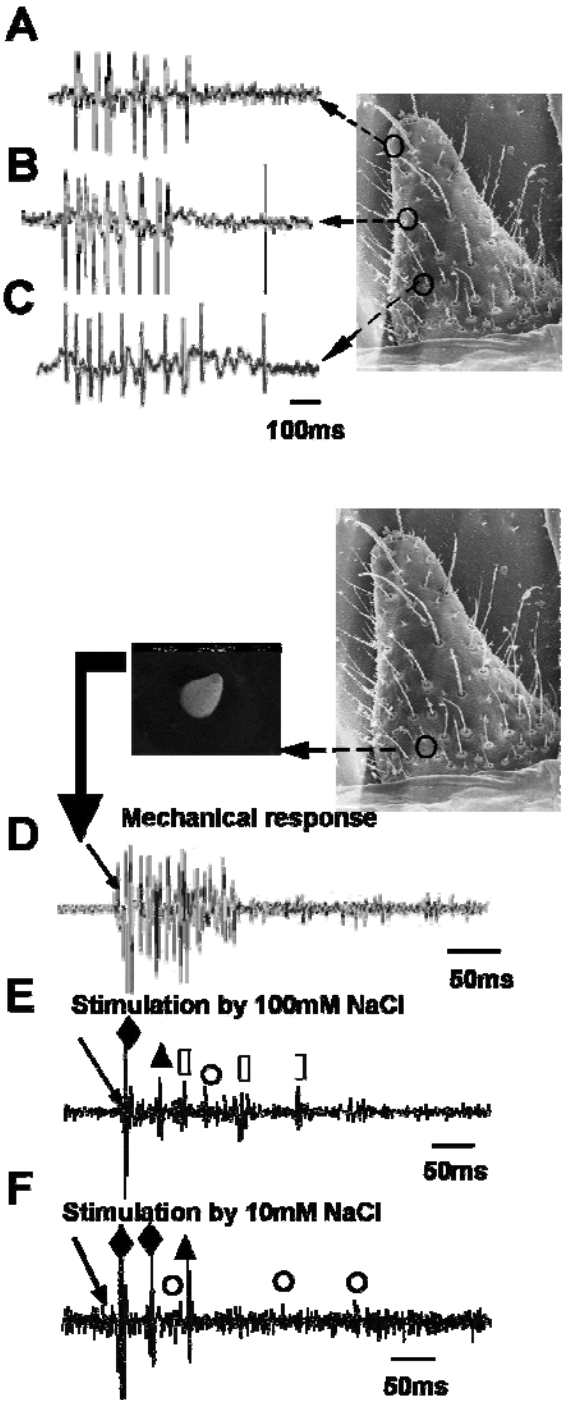

Fig. 5: Responses of individual filiform and

basiconic sensilla on the cerci to mechanical and chemical stimulants. Fig. 5A-5C: Response of individual cut shaft filiform sensilla in the locust cerci to deflecting evokes a burst of action potentials of single amplitude, indicating that the filiform sensilum is innervated by a single neuron. Fig. 5D: Deflecting the cut shaft of a basiconic sensilum (arrows) evokes a burst of action potentials of single amplitude, indicating that the basiconic sensilum is innervated by a single mechanosensory neuron. Fig. 5E: Response of individual basiconic sensilla in the locust cerci to 100 mM NaCl evokes a burst of action potentials of different amplitudes (dots indicating the smaller amplitude spikes). Fig. 5F: Spikes of three chemosensitive neurons evoked by stimulation with 10mM NaCl, decreasing the sodium chloride concentration (10mM NaCl) decrease the amplitude of the response without increasing the number of firing neurons.

Stimulation of basiconic sensilla by using the tip recording techniques showed that basiconic sensilla responded to chemical and mechanical stimuli. Spikes with several distinct amplitudes were elicited by placing electrodes containing sodium chloride (100 mM or 10 mM) over basiconic sensilla (Figs. 5E & 5F), indicating that more than one chemosensitive neuron was activated by the salt solution. The response to salt in a single basiconic sensillum is phasic with at least two main units. The characteristic feature of the chemosensory neuron responses to different concentrations of NaCl was a rapid reduction of their spike frequency during maintained chemical stimulation. This rapid adaptation resulted in an almost complete abolition of their responses to a chemical stimulant within one second of its application. Deflecting the shaft of the basiconic sensillum 3 seconds later evoked a further burst of larger amplitude action potentials from a mechanosensitive neuron (Fig. 5D).

Discussion

This work in its first part is focused on the different cerci sensory receptors of the female locust, using the SEM and the neuroanatomical studied by shown the peripheral innervation of cerci with cobalt chloride backfill technique. Another focused on the central projection of single sensory neuron from mechanoreceptors and chemoreceptors in the CNS with immunocytochemical techniques. In addition to the focus on the electrophysiological response of individual mechanoreceptors or chemoreceptors to mechanical or chemical stimulation were analyzed. In the insect nervous system, the sensory afferents of cercal sensilla have proved important for the study of presynaptic inhibition. The cerci project posteriorly from the last segment of the abdomen and are covered with sensory hairs. Filiform sensilla are very sensitive to air currents and even to low-frequency sound (Ritzmann, 1984). Their afferents run into the terminal ganglion where they synapse with giant interneurones that ascend the ventral nerve cord (Boyan et al*.* 1989; Kalogianni, 1995). Wind stimuli to the cerci can initiate running, jumping or flying in various insects as part of escape behaviour (Ritzmann, 1984; Boyan et al*.* 1986). For the sensory input evoking such vital behaviour to be interpreted unambiguously, it is necessary that hair displacement brought about by air currents is distinguishable from that caused by movement of the cercus. In the locust, filiform afferents are inhibited by presynaptic depolarization during passive displacement of the cercus (Ritzmann, 1984). This is thought to be evoked by the activity of a stretch receptor at the base of the cercus acting *via* an unidentified interneurone.

It is difficult to imagine that the female desert locust could perform the complex oviposition behaviour with only a central motor pattern in the absence of tuning by a peripheral sensory loop. The present study shows that the cerci is well endowed with sense organs that could be the source of information about position, movement and the chemical characters of oviposition substrate. The results showed that about 18% of receptors are basiconic chemoreceptors, which are typically contact chemosensory sensilla of the thick-walled type (Slifer, 1970, Zacharuck, 1980). Thus, it is not surprising to find a great number of chemoreceptors on the cerci as those found on the ovipositor valves (Kalogianni, 1996; Tousson, 2001, 2004; Tousson and Hustert, 2000, 2006), on the paraproct (Tousson and Gaaboub, 2004) and on the subgenital plate (Tousson and Youssef, 2006). The central projections and intersegmental interneurons with

chemosensory inputs from the contact chemoreceptors in *Schistocerca gregaria* have never been reported before.

Tousson and Hustert (1998) have shown, for the first time, how neurons of a single insect contact chemoreceptors project in the CNS. Previously, cobalt staining of single sensory neuron was performed successfully, mainly in insect mechanoreceptors (Hustert et al., 1981; Pflüger et al., 1981; Hustert, 1983; Tousson et al., 1999; Newland, 2004) but it did not work reliably for axon diameters of less than 1 µm that prevail for insect contact chemoreceptors. In the current study, we used the neurobiotin backfill technique (Tousson and Hustert, 1998) to identify the central projections of a single contact chemoreceptor. The present study also focused on the periphery and sensory innervation of the subgenital plate, in addition to the fine structure and distribution of various types of sensory sensilla investigated with cobalt chloride backfilling and scanning electron microscope. Another focus was on physiological responses and central nervous integration of basiconic sensilla to different concentration of sodium chloride solution. The central projections from cercal basiconic sensilla in the terminal ganglion resemble in outline the parproctal (Tousson and Gaaboub, 2004), but narrow and dense fields of arborization form in the ninth and the eighth neuromere, some of them shaped like glomeruli usually involving three different afferents.These sensilla are multimodal receptors which encode both mechanical and chemical cues. This means that these contact chemoreceptors may help the ovipositor on probing for suitable sites for oviposition and egg laying or may be play the some role in searching for food, similar to chemoreceptors on the tarsi of the fore and middle legs (Tousson et al., 1999; Gaaboub, 2000; Newland, 2004; Gaaboub et al., 2005).

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Effect of H_2SO_4 on Seed Germination and Viability of *Canna indica* L. a Medicinal Plant

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Abstract: *Canna indica* roots are used for medicinal purpose. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The seed of canna is extremely hard, and needs to be "scarified" before sowing. The aim of the present investigation is to determine the hardiness problem of the seed. The seed sample was collected from the IARI, New Delhi in 2008. The work consists of Physical purity, standard germination test, seed vigour test. Experimental results has shown that, seed sample recorded the purity of seed (97.55 %) and seed sample showed the maximum germination percentage 91% after three hrs. H_2SO_4 scarification. The maximum root length (7.51 cm), maximum shoot length (3.12 cm) and maximum seedling dry weight (0.203 gm) were observed at two hrs. H_2SO_4 scarification. The results indicated that H_2SO_4 scarification increase the germination percentage but it reduce the viability of the seed. [Journal of American Science 2010;6(6):24-25]. ISSN: 1545-1003).

Key words: *Canna indica*, Germination, Scarification, Vigour.

1. Introduction

Canna indica belongs to family cannaceae. *Canna indica* is a native of tropical America and is a very popular ornamental and Medicinal plant throughout the tropical world. *Canna indica* is an upright perennial rhizomatous herb. It having round, shiny black seeds**.** The seed of cannas is extremely hard, and needs to be "scarified" before sowing. Scarifying the seed can speed germination, especially if the seed has not swollen after being soaked. The seed are scarified generally with H_2SO_4. The seed usually germinates in 12days to 3 weeks.

The plant is used in the treatment of women's complaints. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The plant is also considered to be demulcent, diaphoretic and diuretic.

The main objective of the investigation was to determine the hard seed problem in canna and sulphuric acid scarification effect on germination.

2. Material and Methods

The investigation was conducted at the Seed Testing Laboratory of Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi.

The selected seed lot of *Canna indica* L. divided into four replication. The work consists of standard germination test, seed vigour test. Standard germination test was conducted on a 100 seeds per replicate at 25^0C for 16 days in germinator by using towel paper as a substratum.

Seedling length was taken after the completion of germination period (16 days) in randomly selected ten seedlings from each replication. The dry weight of the ten randomly selected seedlings for each replicate was measured after it was dried on oven at 80^0C for 18 hrs.

3. Result and Discussion:

Water impermeability of the testa is a physical exogenous dormancy according to **Nikolaeva (1969)**. Concentrated sulphuric acid has been used for many years for softening of hard seed coats. **(Hopkins, 1923)**. Germination test are based on pure seed components, this has been shown by the observations recorded and that purity analysis and germination tests compliment each other.

Table 1: Mean value of physical purity analysis of the *Canna indica* L. seed lot.

IM (gm)	OS (gm)	PS (gm)	P%
0.27	2.18	97.55	97.55

Acronym used: IM= Inert matter, OS = Other seed, PS= Pure seed, P= Purity

Table 2: Mean value of analysis by different tests methods of *Canna indica* L. seed germination, root length, shoot length and seedling dry weight. In the table seed lot recorded maximum germination in three hours H_2SO_4 scarification treatment.

Treatment	G%	RL (cm)	SL (cm)	SDW (gm)

Control	NE	-	-	-
One hr. H_2SO_4 scarification	76	6.61	2.53	0.173
Two hrs.	79	7.51	3.12	0.203
Three hrs.	91	7.21	2.62	0.190
Four hrs.	73	7.17	2.58	0.183

Acronym used: G= Germination, NE= No emergence, RL= Root length, SL= Shoot length, SWD= Seedling dry weight.

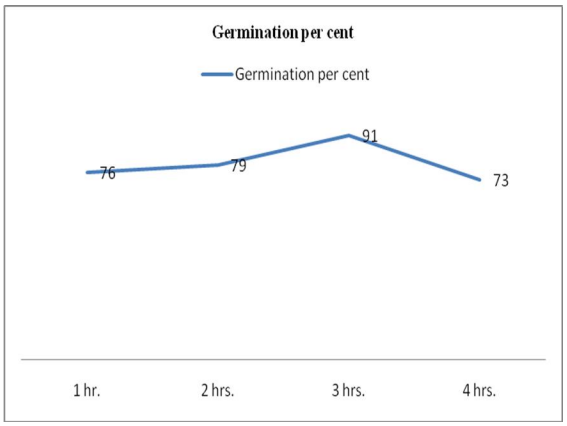

Fig.1. raphical representation of *Canna indica* seed germination after different duration of H_2SO_4 treatment.

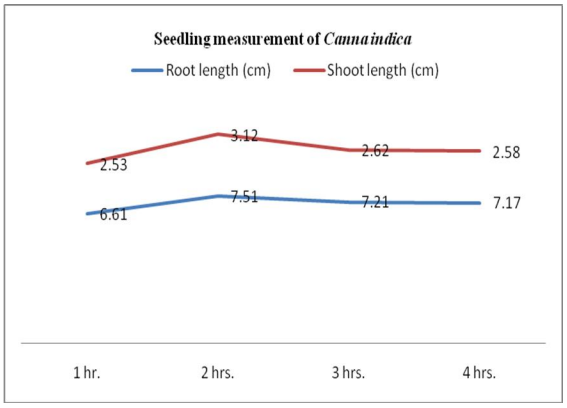

Fig.2.Seedling measurement of *Canna indica* after different duration of H_2SO_4 treatment.

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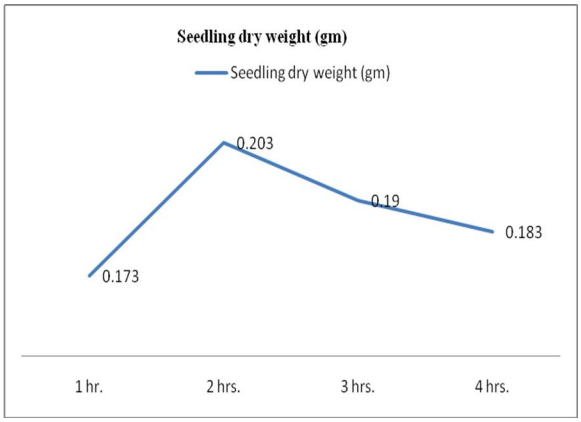

Fig.3.Graphical representation of *Canna indica* seedling dry weight.

Recorded experimental finding (Table1) showed that the purity percentage of the seed lot is 97.55% and (Table 2) showed that the germination percentage was observed maximum 91% in three hrs. H_2SO_4 scarification treatment. The root length (7.51cm), Shoot length (3.12 cm) and seedling dry weight (0.203 gm) was recorded maximum in two hrs. H_2SO_4 treatment.

Thus, from the discussion it may be concluded that the seed lot of *canna indica* L. showed good response in three hrs. H_2SO_4 treatment followed by two hrs. The results indicated that H_2SO_4 scarification increase the germination percentage but it reduce the viability of the seed.

Acknowledgement
The authors would like to thanks the staff members of Division of Seed Science and Technology, IARI New Delhi for their cooperation.

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The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults

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Abstract: Aim : Elucidate the effect of genetic variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as a risk factors for periodontitis. Material &Methods: Sample of this study composed of 50 smokers & 50 non smoker volunteers (unrelated and of the same ethnic population) with 40-60 years old .Their periodontal status was estimated through periodontal examination (full mouth clinical attachment loss measurement ,probing depths ,plaque index scores, and bleeding on probing). Isolation and detection of certain oral pathogens; A.actinomycetemcomitans , Porphyromonas gingivalis ,and Provetella intermedia was performed . Genotype for bi-allelic IL-1A+4845, IL-lB+3954 gene polymorphisms using mouth wash was detected by PCR based methods. Results: There were a significant difference only between the two groups (smokers &non-smokers) as regards to colonization of A.actinomycetemcomitans & not among Porphyromonas gingivalis & Prevotella spp. There were no significant difference between the overall frequencies of carrying allele 2 of IL-l A, IL-1B among smoker and non-smokers. The percentage of non smokers having healthy periodontal status was much higher than smokers. On the other hand, smokers recorded much higher percentage for mild, moderate and severe periodontitis. The difference was statistically significant concerning the percentage of those with severe periodontitis. Conclusion: Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-l A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated A.actinomycetemcomitans growth. [Journal of American Science 2010;6(6):26-30]. (ISSN: 1545-1003).

Keywords: genetic polymorphism, periodontitis, Interleukin -1, periodontal pathogens, smoking

1. Introduction

The oral cavity is vulnerable to external agents as cigarette smoking exposure which causes oral changes in both hard and soft tissues Susin,et al.,(2004).

Periodontitis is a chronic inflammatory disease initiated by specific bacteria that activate host mechanisms destroying bone and connective tissues which support the teeth. Substantial data supported the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (Page et al., 1997), but the rate of progression and disease severity are determined by host modifiers such as smoking (Bergstrom, 1989),diabetes, (Collin et al., 1998) and genetic influences.(Kornman,2006)

Smoking has major effects on the host response, but there are also a number of studies recorded some microbiological differences between smokers and non-smokers (Gomes ,et al., 2006).

The pro-inflammatory cytokine interleukin-1 (IL-1) is a key regulator of the host responses to microbial infection and a major modulator of extracellular matrix catabolism and bone desorption. It has been reported that variations in the IL-1 gene cluster on chromosome 2 are associated with increased susceptibility to severe periodontitis (Mc Devitt, et al., 2002).Therefore, a genetic test was being marketed to predict risk for periodontal disease progression (Higashi ,2002).

2.Aim of the work:

Elucidate the effect of genetic variability including the variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as
effects influencing risk for periodontitis.

3.Subjects and methods

Subjects:

Sample of this study composed of 50 smokers & 50 non smokers volunteers (unrelated and of the same ethnic population) with 40-60 years old. Both groups were interviewed and filled a detailed questionnaires for family history, dental, medical as well as smoking habits,. Cigarette consumption was calculated (i.e. mean numbers of packs/day× number of years smoked)

Methods:

1-Periodontal Examination:

Criteria for assessment of the severity of periodontitis:

1- Mild periodontitis: Mean CAL ≥0.6 mm to 1.5 mm,no. interproximal sites with CAL≥3mm.No more than 3 missing teeth with the exception of orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing.
2- Moderate periodontitis: Mean CAL ≥1.6 mm to 2.4 mm and≤ 8mm, interproximal sites with CAL≥3mm distributed through at least 3 quadrants or at 6 teeth. No more than 5 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing
3- Severe periodontitis: Mean CAL ≥ 2.5 mm and 1 or more sites in 3 out of 4 quadrants with interproximal sites with CAL≥5mm. No more than 14 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing

2-Microbiological Examination

Sampling : Paper-point samples were taken from the 4 deepest sub-gingival sites in each quadrant of the dentition (Mombelli et al., 1991, 1994).Samples were then placed in sterile pre-reduced anaerobically transport fluid(RTF) and transferred to the laboratory within 10 minutes.

Culturing: The samples were dispersed for 6 sec. with a vortex mixer and 10 fold serially diluted in RTF. Aliquots of 0.1 ml of appropriate dilutions were placed in duplicate onto specific media for different microorganisms in concern.

Samples were grown anaerobically (80% N2, 10% H2, 10% C02) at 37°C on 5% horse blood agar plates (Oxoid no. 2, Basingstoke, England) enriched with hemin (5 mg/L) and menadione (1 mg/L) for detection of Porphyromonas gingivalis and on Trypticase soyserum bacitracin-vancomysin (TSBV) medium in air with 5%CO2 at 37°C for the selective isolation of A.actinomycetemcomitans. KVLB-2 (kanamycin 75µ g/ml-Vancomycin 2 µ g/ml laked blood agar) for isolation of pigmented and non pigmented Prevotella spp.

Identification: Porphyromonas gingivalis was identified on the basis of Gram stain, anaerobic growth, and the inability to ferment glucose, the production of indole, and a positive hemagglutination test with 3% sheep erythrocytes. A.actinomycetemcomitans was identified on TSBV plates, based on typical colony morphology and positive catalase reaction. The percentage of the microorganisms of total colony-forming units (CFU) was counted.

3- PCR based methods:

a-DNA Isolation from Mouthwash

DNA from all subjects was isolated according to the method of de Vries et al. (1996) as modified and validated for the study of cytokine gene polymorphisms (Laine et al., 2000). In short, each individual rinsed out his/her mouth with 10 mL of 0.9% saline for 60 sec. Buccal epithelial cells were centrifuged at 300 x g for 10 min. The pellet was washed twice in 0.9% saline, re-suspended in 100 pL of 50 mM NaOH, and boiled for 10 min. Samples were neutralized with 14 pL of 1 M Tris (pH 7.5) and centrifuged at 14,000 x g for 3 min. Supernatants were collected and stored at 4°C until analysis.

b-Analysis of Polymorphisms in Genes of the IL- 1 A &B

The bi-allelic polymorphisms at position -889 within the promoter region of the IL-IA gene (McDowell et al., 1995) and at position +3954 (Taq I RFLP) within exon 5 of the IL-1B gene (Bioque et al., 1995), were determined according to previously described methods.

4.Results

Table (1): Percentage of bacterial species in plaque samples of smoker and non smoker individuals examined

Bacterial species	% of Non Smoker individuals	% of smokers individuals	Z.score	P
Gram (-) facultative rods A. actinomycetemcomitans	6% (3/50)	30% (15/50)*	1.96*	0.02
Gram (-) anaerobes Porphyrmonas gingivalis	12% (6/50)	24% (12/50)	1.3	0.09
Gram (-)anaerobes Provotella intermedia	8% (4/50)	16% (8/50)	0.92	0.17

The distribution of isolated microorganisms among examined groups was illustrated in table (1). Porphyromonas gingivalis , Prevotella spp and A.actinomycetemcomitans colonized 24% ,16%, and 30% of the smokers and 12% , 8% and 6% of non- smokers respectively.

There were significant difference between the two groups (smokers &non-smokers) as regards to colonization of Gram (-ve) facultative rods(A. actinomycetemcomitans)& no significant difference between the other two organisms.

Table (2): Distribution ples of smokers and non smokers of composite IL-1 genotype of allele 2 carriage of IL-1A(+4845) & IL-1B (+3953)among sam

IL-1 genotype of allele 2 carriage of Il-1A(+4845) &IL-1B(+3953)	Il-1A	IL-1B	Total carriers of allele 2
Non smokers	14/50(28%)	3/50(6%)	17/50(34%)
Smokers	15/50 (30)	5/50(10%)	20/50(40%)
Total studied	29/100 (29%)	8/100(8%)	37/100(37%)

P value ;0.7

The percentage of smokers & non smokers carried allele 2 of IL-1A was more than those carried IL-1B (30%,28%& 10%,6%) respectively, table (2) . The difference was statistically non- significant.

Table(3): Distribution of allele 2 frequency of IL-1A(+4845) & IL-1B (+3953)composite genotype among studied individuals in relation to severity of periodontitis

	Total carriers of IL-1 genotype of allele 2 (Il-1A(+4845) &IL-1B(+3953)			
	Non smokers (n=	Smokers N=20)	Z scc	P.valu
Healthy	(8/17) 47%*	0/20(0%)	3.0(	0.00
Mild	Periodontital status	15%(3/20)	0.2	0.42
Moderate	(2/17) 11.8%	20%(4/20)	0.2	0.4
Sever	(5/17) 29.4%	65%(13/20)*	1.8	0.03

Smokers and non- smoker individuals carried allele 2 of IL-1A and IL-1 B were further divided according to the severity of the periodontitis . The percentage of non smokers having healthy periodontal status (47%) was much higher compared to smokers and the difference was significant. On the other hand, smokers & non smokers recorded nearly a similar percentage among those complained from mild, moderate (15%, 20% & 11.8%,11.8%)with no statistical significant difference .Whereas, statistically significant difference was noted concerning the percentage of individuals in both groups complained from severe periodontitis (65%&29.4% respectively), table (3) agreed with Kornman et al., (1997) who reported the same correlation and explained this finding as genetic mechanism by which some individuals, if challenged by bacterial accumulations ,may have more vigorous immune-inflammatory response leading to more severe periodontitis..Moreover, Kornman(2006) added that monocytes from individuals homozygous for the IL-1 B +3953 allele 2produce four-fold more IL-1 β and heterozygous cells produce approximately two-folds more IL-1 β from individuals homozygous for allele1.
compared with roughly 11% of those who had only one of the risk factors.

Our results correlated the severity of periodontitis to presence of carriers of allele 2 genotype in the IL-1A and IL-1B genes. A data

Our results showed that the nature of the host response is determined primarily by genetic factors , environmental and acquired factors (smoking). The complex interactions that occur between host-response mechanisms and oral pathogens in periodontal disease have made elucidation of genetic factors in disease susceptibility more difficult (Hassell et al., 1995).

5.Dicussion

Periodontitis is a multifactorial chronic inflammatory disease. However, it is difficult to acertain the role of the different factors involved in its pathogenesis. Cigarette smoking is associated with increased prevalence and severity of destructive periodontitis in terms of periodontal pocketing, periodontal bone loss, and tooth loss (Gomes,2006).

In microbiological study we focused on a number of microbial species e.g.,Actinobacillus actinomycetemcomitans,Porphyromonas gingivalis, and Prevotella intermedia) which were proposed to be a useful tool for the identification of susceptible individuals (Slots and Listgarten, 1988, Maiden et al., 1990 and haffajee et al., 1994). Substantial data supported the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (Page et al., 1997), but the rate of progression and disease severity are determined by host modifiers such as smoking, diabetes, and genetic influences.

In the present study approximately half of the smokers and one fourth of the non smokers harbored these three microorganisms in their oral cavity. There were significant difference between the two groups (smokers &non-smokers) as regards to colonization of Gram (-ve) facultative rods (A. actinomycetemcomitans)& no significant difference between the other two organisms.

Genes which encode inflammatory cytokines were subject to polymorphisms in their regulatory regions that might affect both the level and the ratio of cytokines produced in response to exogenous stimuli. These variant alleles were observed in a large percentage of the population and were often associated with increased or decreased susceptibility or severity (modifiers) to infectious, immune or inflammatory diseases (yucesoy et al.,(2003).Axelsson (2002) reported that two factors, smoking and IL-1 genotype, significantly increased the risk of progression of alveolar bone loss and tooth loss due to progressive periodontitis. Moreover, the effect was synergistic: 41% of the IL-1 genotype-positive smokers lost 2 teeth,

6.Conclusion

Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-l A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated A.actinomycetemcomitans growth

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2/2/2010

Model for Computational Analysis of the Solution Temperature during Leaching of Iron Oxide Ore in Oxalic Acid Solution

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Abstract: Model for computational analysis of the solution temperature (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived. The model;

$$T = e^{(14.9661/p)}$$

is dependent depends on the value of the final pH of the leaching solution which varies with leaching time. It was observed that the validity of the model is rooted on the expression $\ln T = K_c/p$ where both sides of the equation are approximately equal to 3. The maximum deviation of the model-predicted solution temperature values from those of the experimental values were found to be less than 9% hence establishing the validity and precision of the model. The correlation between mass of iron oxide ore and solution temperature as well as between final pH of leaching solution and solution temperature as obtained from experiment and derived model (0.9296 and 0.8911 as well as 0.9395 and 0.9988) respectively are quite close, indicating proximate agreement with values from actual experiment. [Journal of American Science 2010;6(6):31-37]. (ISSN: 1545-1003).

Keywords: Model, Solution Temperature, Oxalic Acid, Iron Oxide Ore, Leaching.

1. Introduction

The dissolution of iron oxide is believed to take place through a photo-electro chemical reduction process, involving a complicated mechanism of charge transfer between the predominant oxalate species, namely ferric oxalate $Fe(C_2O_4)_3^{3-}$, ferrous oxalate $Fe(C_2O_4)_2^{2-}$ acting also as an auto catalyst, and the oxalate ligand on the iron oxide surface (Taxiarchour et al, 1997a). The dissolution of iron oxides in oxalic acid was found to be very slow at temperatures within the range 25-60^{o}C, but its rate increases rapidly above 90^{o}C (Lee et al, 2006). The dissolution rate also increases with increasing oxalate concentration at the constant pH values set within the optimum range of pH 2.5-3.0. At this optimum pH, the dissolution of fine pure haematite (Fe_2O_3) (105-140µm) follows a diffusion-controlled shrinking core model.

The presence of Fe^{2+} was found to significantly enhance the leaching of iron extraction from silica sand at a temperature even as low as 25^{o}C (Taxiarchour et al, 1997b). Ferrous oxalate however is oxidized quickly by air during the dissolution and in general an induction period of a few hours was observed to exist unless a strong acidic environment (<pH 1) or an inert atmosphere is maintained. Maintaining the high level of ferrous oxalate in the leach liquor using inert gas was found to enhance the reaction kinetics.

It has been reported (Lee et al, 1999) that the leaching of 3g/L pure haematite (98.2% purity, 105-140µm size range) using 0.048-0.48M oxalic acid at 80-100^{0}C passed through a maximum peak at pH 2.5. Dissolution of haematite was found to be slower than magnetite ($FeO.Fe_2O_3$) and other hydrated iron oxide such as goethite (α-FeOOH), lapidochrosite (γ-FeOOH) and iron hydroxide ($Fe(OH)_3$) (Lee et al, 1999).

The mixed potential model of leaching assumes that the charge transfer processes occurring at the mineral surfaces are those that control the rate of dissolution (Kanevskii et al, 1963).

Model for quantitative analysis of dissolved haematite (relative to the initial solution pH) during leaching of iron oxide ore in oxalic acid solution has been derived by Nwoye et al. (2009). The model;

$$\%Fe_2O_3 = \left(\frac{N}{N_c}\left(\frac{1}{\gamma}\right)\right) \qquad (1)$$

was found to calculate the concentration of dissolved haematite being dependent on the values of the initial leaching solution pH measured during the leaching process. The respective positive and negative deviation of the model-predicted values of $\%Fe_2O_3$ (dissolved) from the corresponding experimental values was found to be less than 11% which is quite within the acceptable range of deviation limit of experimental results. The values of the assumed coefficients of dilution (N) and dissolution of haematite (N_c) in oxalic acid solution were calculated to be 197.7503 and 700.0618 respectively.

Nwoye (2008) derived a model for evaluating the final pH of the leaching solution during leaching of iron oxide ore in oxalic acid solution. The model evaluates the pH value as the sum of two parts,

involving the % concentrations of Fe and Fe_2O_3 dissolved. The model can be expressed as;

$$\gamma = 0.5\left(\frac{K_1}{\%Fe} + \frac{K_2}{\%\ Fe_2O_3}\right) \qquad (2)$$

Where

K_1 and K_2 = dissolution constants of Fe and Fe_2O_3 respectively.

γ = final pH of leaching solution (after time t).

It was also found that the model (Nwoye, 2008) could predict the concentration of Fe or Fe_2O_3 dissolved in the oxalic acid solution at a particular final solution pH by taking Fe or Fe_2O_3 as the subject formular. The prevailing process conditions under which the model works include: leaching time of 30mins., constant leaching temperature of 30°C, average ore grain size; 150µm and 0.1M oxalic acid.

Nwoye (2009) has reported that the heat absorbed by oxalic acid solution during leaching of iron oxide ore can be predicted using the model he derived which works under the process condition; initial pH 6.9, average ore grain size; 150µm and leaching temperature; 30°C. The model (Nwoye, 2009) can be stated as

$$Q = K_N\left(\frac{\gamma}{\%Fe_2O_3}\right) \qquad (3)$$

Where

Q = Quantity of heat absorbed by oxalic acid solution during the leaching process. (J)

γ = Final pH of the leaching solution (at time t).

$\%Fe_2O_3$ = Concentration of haematite dissolved in oxalic acid solution during the leaching process.

K_N = 4.57(Haematite dissolution constant in oxalic acid solution) determined in the experiment (Nwoye, 2008).

Nwoye (2009) carried out further work on the model using the same process conditions and observed that on re-arranging the model as;

$$\%Fe_2O_3 = K_N\left(\frac{\gamma}{Q}\right) \qquad (4)$$

the concentrations of haematite predicted deviated very insignificantly from the corresponding experimental values. In this case, the value of Q was calculated by considering the specific heat capacity of oxalic acid. Values of heat absorbed by the oxalic acid solution during the leaching of iron oxide ore as predicted by the model (Nwoye, 2009) agree with the experimental values that the leaching process is endothermic. This is because all the predicted values of the heat absorbed by the oxalic acid solution were positive. The model shows that the quantity of heat absorbed by oxalic acid solution during the leaching process is directly proportional to the final pH of the solution and inversely proportional to the concentration of haematite dissolved.

Nwoye et al. (2009) derived a model for calculating the concentration of leached iron during leaching of iron oxide ore in sulphuric acid solution. The model is expressed as;

$$\%Fe = e^{-2.0421(\ln T)} \qquad (5)$$

The model was found to predict %Fe (leached) very close to the values obtained from the experiment, being dependent on the values of the final leaching solution temperature measured during the leaching process. It was observed that the validity of the model is rooted in the expression ln(%Fe) = N(InT) where both sides of the expression are correspondingly approximately equal. The positive or negative deviation of each of the model-predicted values of %Fe (leached) from those of the experimental values was found to be less than 37%.

Nwoye et al. (2009) derived a model for predicting the initial solution pH at determined final pH and leaching time during leaching of iron oxide ore in hydrogen peroxide solution. It was observed that the validity of the model is rooted in the mathematical expression; $(\ln t)^{1/2} = N(\beta^C/\alpha^C)$ where both sides of the relationship are approximately equal to 2. The model is expressed as;

$$\beta = \text{Antilog}[0.2439\text{Log}(\alpha^{4.1}(\ln t)^{1/2}/3.6)] \qquad (6)$$

The model shows that the initial solution pH is dependent on the values of the final solution pH and leaching time. The respective positive or negative deviation of the model-predicted final pH from its corresponding experimental value was found to be less than 8%, which is quite within the acceptable deviation limit of experimental results depicting the validity of the model.

Model for predictive analysis of the concentration of dissolved iron during leaching of iron oxide ore in sulphuric acid solution was derived by Nwoye et al. (2009). The model expressed as;

$$\%Fe = 0.987(\mu/T) \qquad (7)$$

was found to predict %Fe dissolved with high degree of precision being dependent on the values of the leaching temperature and weight of iron oxide ore added. It was observed that the validity of the model is rooted in the expression %Fe = N(µ/T) where both sides of the relationship are correspondingly approximately equal. The positive or negative deviation of each of the model-predicted values of %Fe (dissolved) from those of the

experimental values was found to be less than 19% which is quite within the acceptable range of deviation limit for experimental results, hence depicting the usefulness of the model as a tool for predictive analysis of the dissolved iron during the process.

Model for calculating the solution pH during hydrogen peroxide leaching of iron oxide ore has also been derived by Nwoye et al. (2009). It was observed that the validity of the model is rooted in the expression $\ln\gamma = K_C[(\%Fe_2O_3/\%Fe)^N]$ where both sides of the equation are correspondingly approximately equal to 2. The model expressed as;

$$\gamma = \exp\left(K_C[(\%Fe_2O_3/\%Fe)^N]\right) \qquad (8)$$

The final solution pH was found to depend on the values of the % concentrations of dissolved iron and haematite from experiment. The respective deviation of the model-predicted pH values from the corresponding experimental values was found to be less than 20% which is quite within the acceptable range of deviation limit of experimental results.

Model for evaluation of the concentration of dissolved phosphorus (relative to the final pH of the leaching solution) during leaching of iron oxide ore in oxalic acid solution has been derived by Nwoye (2009). The model is expressed as;

$$P = e^{(12.25/\alpha)} \qquad (9)$$

Where

P = Concentration of phosphorus removed during the leaching process (mg/Kg)

N = 12.25; (pH coefficient for phosphorus dissolution in oxalic acid solution) determined in the experiment (Nwoye, 2003).

α = Final pH of the leaching solution at the time t when the concentration of dissolved phosphorus is evaluated.

It was observed that the validity of the model is rooted in the relationship $\ln P = N/\alpha$ where both sides of the expression are approximately equal to 4. The model depends on the value of the final pH of the leaching solution which varies with leaching time. In all, the positive or negative deviation of the model-predicted phosphorus concentration from its corresponding value obtained from the experiment was found to be less than 22%, which is quite within the acceptable deviation limit of experimental results hence establishing the validity and precision of the model.

Nwoye et al. (2008) derived a model for evaluation of the concentration of dissolved iron (relative to the final solution pH and temperature) during leaching of iron oxide ore in sulphuric acid solution. It was observed that the validity of the model was rooted in expression $(\%Fe/N)^{1/3} = \alpha/T$ where both sides of the expression are approximately equal to 0.2. The model is expressed as;

$$\%Fe = 0.35(\alpha/T)^3 \qquad (10)$$

Where

T = Solution temperature at the time t when the concentration of dissolved iron is evaluated. (^{0}C)

N= 0.35(pH coefficient for sulphuric acid solution during leaching of iron oxide ore) determined in the experiment (Nwoye, 2007).

α = Final pH of the leaching solution at the time t when the concentration of dissolved iron is evaluated.

The aim of this work is to derive a model for computational analysis of the solution temperature relative to the final pH of the solution during leaching of Itakpe (Nigerian) iron oxide ore using oxalic acid solution.

2. Model

The solid phase (ore) is assumed to be stationary, contains the un-leached iron remaining in the ore. Hydrogen ions from the oxalic acid attack the ore within the liquid phase in the presence of oxygen.

2.1 Model Formulation

Experimental data obtained from research work (Nwoye, 2005) carried out at SynchroWell Research Laboratory, Enugu were used for this work.

Results of the experiment as presented in report (Nwoye, 2005) and used for the model formulation are as shown in Table 1.

Computational analysis of the experimental data (Nwoye, 2005) shown in Table 1, resulted to Table 2 which indicate that;

$$\ln T = K_c \quad \text{(approximately)} \qquad (11)$$

$$T = e^{Kc/p} \qquad (12)$$

Introducing the value of K_c into equation (12)

$$T = e^{(14.9661/p)} \qquad (13)$$

Where

T = Solution temperature during leaching of iron oxide ore using oxalic acid (^{0}C)

K_c = 14.9661; (pH coefficient for oxalic acid solution during leaching of iron oxide ore) determined in the experiment.(Nwoye, 2005)

p = Final pH of the leaching solution at the time t when the solution temperature is evaluated.

Equation (13) is the derived model.

Table1: Variation of final pH of solution with solution temperature. (Nwoye,2005)

M (g)	p	$T_{exp}(^0C)$
2	4.88	23.5
4	4.73	23.9
6	4.69	24.1
8	4.63	25.0
10	4.61	25.1
14	4.60	25.2
16	4.58	25.4

Where M = Mass of iron oxide ore used for the leaching process (g).

3. Boundary and Initial Condition

Consider iron ore in cylindrical flask 30cm high containing leaching solution of oxalic acid. The leaching solution is stationary i.e (non-flowing). The flask is assumed to be initially free of attach bacteria. Initially, atmospheric levels of oxygen are assumed. Varying weights (2-16g) of iron oxide ore were used as outlined in Table 1. The initial pH of leaching solution; 4.85 and leaching time of 30 minutes was used for all samples.

A constant leaching temperature of 25°C was used. Ore grain size; 150µm, volume of leaching solution; 0.1litre and oxalic acid concentration; 0.1mol/litre were used.These and other process conditions are as stated in the experimental technique (Nwoye, 2005).

The boundary conditions are: atmospheric levels of oxygen (since the cylinder was open at the top) at the top and bottom of the ore particles in the liquid and gas phases respectively. At the bottom of the particles, a zero gradient for the liquid scalar are assumed and also for the gas phase at the top of the particles. The leaching solution is stationary. The sides of the particles are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of T values from model data and those from the experimental data for equality or near equality. Analysis and comparison between these data reveal deviations of model data from experimental data. This is believed to be due to the fact that the surface properties of the ore and the physiochemical interactions between the ore and leaching solution which were found to have played vital roles during the leaching process (Nwoye, 2005) were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model data to that of the experimental values. (Table 3)

Deviation (Dv) of model T values from experimental T values is given by

$$Dv = \left(\frac{Dp - DE}{DE}\right) \times 100 \qquad (14)$$

Where Dp = Predicted data from model
DE = Experimental data

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \qquad (15)$$

Therefore

$$Cf = -100\left(\frac{Dp - DE}{DE}\right) \qquad (16)$$

Introduction of the corresponding values of Cf from equation (16) into the model gives exactly the corresponding experimental T values. (Nwoye, 2005)

5. Results and Discussion

The derived model is equation (13).

Computational analysis of values in Table 1 resulted to Table 2.

Table 2: Variation of lnT with K_c/p

M (g)	p	lnT	K_c/p
2	4.88	3.1570	3.0668
4	4.73	3.1739	3.1641
6	4.69	3.1822	3.1911
8	4.63	3.2189	3.2324
10	4.61	3.2229	3.2464
14	4.60	3.2268	3.2535
16	4.58	3.2347	3.2677

The derived model is equation (13). An ideal comparison of the T values as obtained from experiment (Nwoye, 2005) and as predicted by the model for the purpose of testing the validity of the model is achieved by considering the R^2 values (coefficient of determination). The values of the correlation coefficient, R calculated from the equation;

$$R = \sqrt{R^2} \qquad (17)$$

using the r-squared values (coefficient of determination) from Comparison between Figures 1-4 show that the correlation between mass of iron oxide ore and solution temperature as well as between final pH of leaching solution and solution temperature as obtained from experiment (Nwoye, 2005) and derived model; (0.9296 and 0.8911) as well as (0.9395 and 0.9988) respectively are quite close indicating proximate agreement with values from actual experiment.

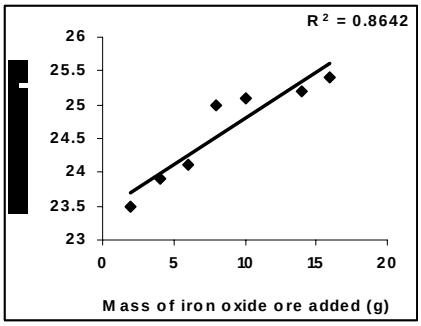

Figure 1- Effect of mass of iron oxide ore added on the solution temperature as obtained from the experiment (Nwoye, 2005)

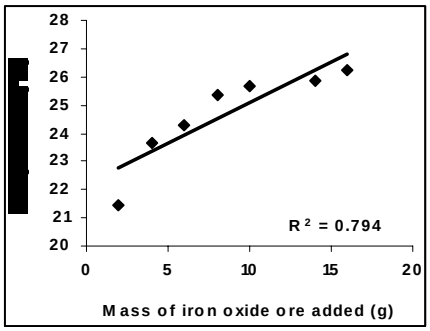

Figure 2- Effect of mass of iron oxide ore added on the solution temperature as obtained from derived model

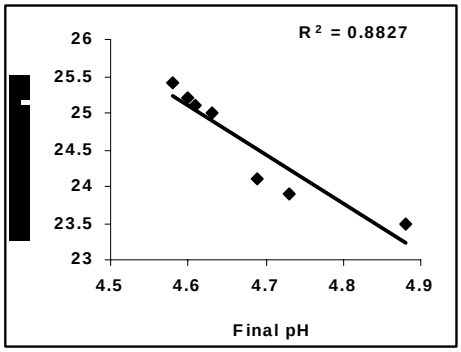

Figure 3- Effect of final pH of solution on the solution temperature as obtained from the experiment (Nwoye, 2005)

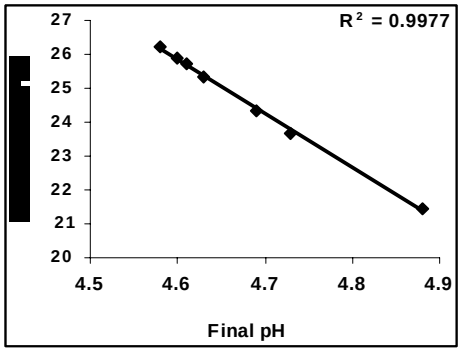

Figure 4- Effect of final pH on the solution temperature as obtained from derived model

Figures 5 and 6 show very close alignment of the curves from model-predicted values of T (MoD) and that from the corresponding experimental values (ExD). The degree of alignment of these curves is indicative of the proximate agreement between both experimental and model-predicted values T.

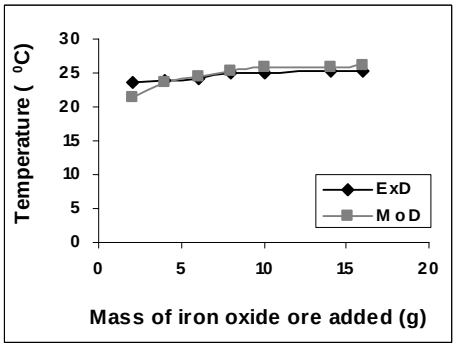

Figure 5- Comparison of solution temperatures resulting from the addition of iron oxide ore as obtained from experiment (Nwoye, 2005) and derived model

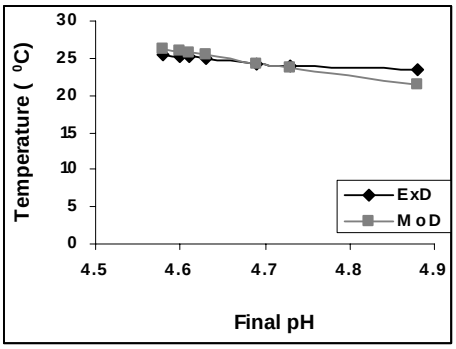

Figure 6- Comparison of solution temperatures relative to the final pH as obtained from experiment (Nwoye, 2005) and derived model

Table 3 shows that the maximum deviation of the model-predicted values of T from the corresponding experimental values (Nwoye, 2005) is less than 9% which is quite very insignificant and within the acceptable range of deviation limit of experimental results hence depicting the reliability and validity of the model. The validity of the model is believed to be rooted on equation (11) where both sides of the equation are approximately equal to 3. Table 2 also agrees with equation (11) following the values of lnT and K_c/p evaluated from Table 1 as a result of computational and statistical analysis.

The least and highest magnitude of deviation of the model-predicted T (from the corresponding experimental values) are + 0.01% and - 8.64% which correspond to solution temperature 25.34 and 21.47 respectively. Table 3 indicates that a correction factor of - 0.01% and + 8.64% make up for the least and highest deviation of + 0.01% and – 8.64% resulting from final pH of 4.63 and 4.88 due to addition of 8 and 2g of iron oxide ore

respectively. It is pertinent to state that the actual deviations are just the modulus of the values. The role of the sign attached to the values is just to show when the deviation is surplus or deficit.

Table:3 Variation of model-predicted solution temperature with the associated deviation and correction factors

T_M (^{0}C)	Dv (%)	Cf (%)
21.47	-8.64	+8.64
23.67	-0.96	+0.96
24.31	+0.87	-0.87
25.34	+0.01	-0.01
25.70	+0.02	-0.02
25.88	+0.03	-0.03
26.25	+0.03	-0.03

T_M = T values predicted by model.

6. Conclusion

The model computes the solution temperature relative to the final solution temperature during leaching of Itakpe iron oxide ore. The validity of the model is believed to be rooted on the expression $\ln T = K_c$ where both sides of the expression are approximately equal to 3. The maximum deviation of the model-predicted values of T from the corresponding experimental values is less than 9% which is quite very insignificant and within the acceptable range of deviation limit of experimental results.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model data from that of the experimental.

Acknowledgement

The authors thank Dr. Ekeme Udoh, a modelling expert at Linkwell Modelling Centre Calabar for his technical inputs. The management of SynchroWell Nig. Ltd. Enugu is also appreciated for permitting and providing the experimental data used in this work.

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Model for Computational Analysis of the Quantity of Water Lost by Evaporation during Oven-Drying of Clay

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Abstract: Model for computational analysis of the quantity of water lost by evaporation during oven drying of clay has been derived. The model;

$$\beta = \exp[(\ln t)^{0.998} - 2.9206]$$

indicates that the quantity of evaporated water during the drying process is dependent on the drying time, the evaporating surface being constant. It was found that the validity of the model is rooted on the expression $(\log\alpha + \ln\beta)^N = \ln t$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 20% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. Water evaporation rate evaluated from experimental and model-predicted results are 0.0488 and 0.0530g/ min respectively, indicating proximate agreement. [Journal of American Science 2010;6(6):38-42]. (ISSN: 1545-1003).

Keywords: Model, Water, Evaporation, Oven Drying, Clay

1. Introduction

Reed (1988) described firing as having three stages through which it proceeds; preliminary reactions which include binder burnout, elimination of gaseous product of decomposition and oxidation, sintering as well as cooling which may include thermal and chemical annealing.

Several works (Barsoum, 1999; Viewey and Larrly, 1978; Keey, 1978) have been carried out on shrinkage of clay during drying. In all these works, porosity has been shown to influence the swelling and shrinkage behaviour of clay products of different geometry. It has been reported (Reed, 1988) that drying occurs in three stages; increasing rate, constant and decreasing rate. He pointed out that during the increasing rate; evaporation rate is higher than evaporating surface hence more water is lost. At constant rate, the evaporation rate and evaporation surface are constant. He posited that shrinkage occurs at this stage. Keey (1978) also in a similar study suggested that at this stage, free water is removed between the particles and the inter-particle separation decreases, resulting in shrinkage. During the decreasing rate, particles make contacts as water is removed, which causes shrinkage to cease.

Model for calculating the volume shrinkage resulting from the initial air-drying of wet clay has been derived (Nwoye, 2008). The model;

$$\theta = \gamma^3 - 3\gamma^2 + 3\gamma \qquad (1)$$

calculates the volume shrinkage when the value of dried shrinkage γ, experienced during air-drying of wet clays is known. The model was found to be third-order polynomial in nature. Olokoro clay was found to have the highest shrinkage during the air drying condition, followed by Ukpor clay while Otamiri clay has the lowest shrinkage. Volume shrinkage was discovered to increase with increase in dried shrinkage until maximum volume shrinkage was reached, hence a direct relationship.

A mathematical model for evaluating internal volume shrinkage of fired clays has been derived by Nwoye (2008). The model;

$$\beta = \alpha^3 - 3\alpha^2 + 3\alpha \qquad (2)$$

evaluates the volume shrinkage β when the value of fired shrinkage α, resulting from intense firing (to a temperature of 1200^0C) . The model was found to be third-order polynomial in nature.

Nwoye et al. (2008) derived a model for the evaluation of overall volume shrinkage in molded clay products (from initial air-drying stage to completion of firing at a temperature of 1200^0C). It was observed that the overall volume shrinkage values predicted by the model were in agreement with those calculated using conventional equations. The model;

$$S_T = \alpha^3 + \gamma^3 - 3(\alpha^2 + \gamma^2) + 3(\alpha + \gamma) \qquad (3)$$

depends on direct values of the dried γ and fired shrinkage α for its precision. Overall volume shrinkage was found to increase with increase in dried

and fired shrinkages until overall volume shrinkage reaches maximum.

Nwoye (2009) derived a model for calculating the quantity of water lost by evaporation during oven drying of clay at 90^0C. The model;

$$\gamma = \exp[(\ln t)^{1.0638} - 2.9206] \quad (4)$$

indicated that the quantity of evaporated water, γ during the drying process is dependent on the drying time t, the evaporating surface being constant. The validity of the model was found to be rooted in the expression $(\text{Log}\beta + \ln\gamma)^N = \ln t$.

Model for predictive analysis of the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin has been derived by Nwoye et al. (2009). The model;

$$\alpha = e^{(\ln t/2.1992)} \quad (5)$$

shows that the quantity of water α, evaporated at 110^0C, during the drying process is also dependent on the drying time t, where the evaporating surface is constant. It was found that the validity of the model is rooted on the expression $(\ln t/\ln\alpha)^N = \text{Log}\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value was found to be less than 22% which is quite within the acceptable deviation range of experimental results.

Model for quantifying the extent and magnitude of water evaporated during time dependent drying of clay has been derived (Nwoye et al., 2009). The model;

$$\gamma = \exp((\ln t/2.9206)^{1.4}) \quad (6)$$

indicates that the quantity of evaporated water γ during the drying process (at 90^0C) is dependent on the drying time, t the evaporating surface being constant. It was found that the validity of the model is rooted in the expression $\ln\gamma = (\ln t/\text{Log}\beta)^N$ where both sides of the expression are correspondingly almost equal.

The present work is to derive a model for computational analysis of the quantity of water lost by evaporation during oven drying of Ukpor (Nigeria) clay at 80^0C.

2.1 Model Formulation

Experimental data obtained from research work (Nwoye, 2007) carried out at SynchroWell Research Laboratory, Enugu were used for this work. Results of the experiment used for the model formulation are as shown in Table1.Computational analysis of the experimental data (Nwoye, 2007) shown in Table 1, gave rise to Table 2 which indicate that;

$$(\text{Log}\alpha + \ln\beta)^N = \ln t \quad \text{(approximately)} \quad (7)$$

Multiplying the indices of both sides of equation (7) by 1/N

$$\text{Log}\,\alpha + \ln\beta = (\ln t)^{1/N} \quad (8)$$

Introducing the value of N into equation (8)

$$\text{Log}\,\alpha + \ln\beta = (\ln t)^{1/1.002} \quad (9)$$

$$\text{Log}\,\alpha + \ln\beta = (\ln t)^{0.998} \quad (10)$$

$$\ln\beta = (\ln t)^{0.998} - \text{Log}\,\alpha \quad (11)$$

$$\beta = \exp[(\ln t)^{0.998} - \text{Log}\,\alpha] \quad (12)$$

Introducing the value of α into equation (12) reduces it to;

$$\beta = \exp[(\ln t)^{0.998} - 2.9206] \quad (13)$$

Where

(β) = Weight of water lost by evaporation during the drying process (g)
(α) = Area of evaporating surface (mm^2)
N = 1.002; (Collapsibility coefficient of binder-clay particle boundary at the drying temperature of 80^0C) determined in the experiment (Nwoye, 2007).
(t) = Drying time (mins.).

Table 1: Variation of quantity of evaporated water with drying time. (Nwoye, 2007)

(t)	(α)	(β)
30	833	2.00
50	833	2.80
70	833	3.70
90	833	4.70
110	833	5.90
130	833	5.90

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 49mm, width 17mm, and breadth 9mm exposed to drying in the furnace while it was in wet condition. Initially, atmospheric levels of oxygen are assumed. Atmospheric pressure was assumed to be acting on the clay samples during the drying process (since the furnace is not air-tight). The grain size of clay particles used is 425µm, weight of clay and binder (bentonite) used (for each rectangular product); 100g and 10g respectively, quantity of water used for mixing; 2% (of total weight), drying temperature used; 80^0C, area of evaporating surface; $833mm^2$ and range of drying time used; (30-130 mins.). The boundary conditions are: atmospheric levels of oxygen at the top and bottom of the clay samples since they are dried under the atmospheric condition. No external force due to compression or tension was applied to the drying clays. The sides of the particles and the rectangular shaped clay products are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of the model-predicted β values and those from the experiment for equality or near equality. Analysis and comparison between these β values reveal deviations of model-predicted β from those of the experimental values. This is believed to be due to the fact that the surface properties of the clay and the physiochemical interactions between the clay and binder, which were found to have played vital role during the evaporation process (Nwoye, 2007) were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted β value to that of the corresponding experimental value (Table 3).

Deviation (Dv) (%) of model-predicted β values from the experimental β values is given by

$$Dv = \left(\frac{Pw - Ew}{Ew}\right) \times 100 \qquad (14)$$

Where

Pw = Quantity of water evaporated as predicted by model

Ew = Quantity of water evaporated as obtained from experiment (g) (Nwoye, 2007)

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \qquad (15)$$

Therefore

$$Cf = -100\left(\frac{Pw - Ew}{Ew}\right) \qquad (16)$$

Introduction of the value of Cf from equation (16) into the model gives exactly the corresponding experimental value of β (Nwoye, 2007).

5. Results and Discussion

The derived model is equation (13). Computational analysis of the experimental data (Nwoye, 2007) shown in Table 1, gave rise to Table 2

Table 2: Variation of lnt with $(Log\alpha + ln\beta)^N$

lnt	Logα	lnβ	$(Log\alpha + ln\beta)^N$
3.4012	2.9206	0.6931	3.6230
3.9120	2.9206	1.0296	3.9611
4.2485	2.9206	1.3083	4.2411
4.4998	2.9206	1.5476	4.4816
4.7005	2.9206	1.7750	4.7101
4.8675	2.9206	1.7750	4.7101

An ideal comparison of the quantities of water evaporated per unit rise in the drying time as obtained from experiment and as predicted by the model for the purpose of testing the validity of the model is achieved by considering the R^2 values. The values of the correlation coefficient, R calculated from the equation;

$$R = \sqrt{R^2} \qquad (17)$$

using the r-squared values (coefficient of determination) from Figures 1 and 2 which show a better correlation (1.0000) with model-predicted quantity of water evaporated per unit rise in the drying time compared to that obtained from experiment (0.9851).

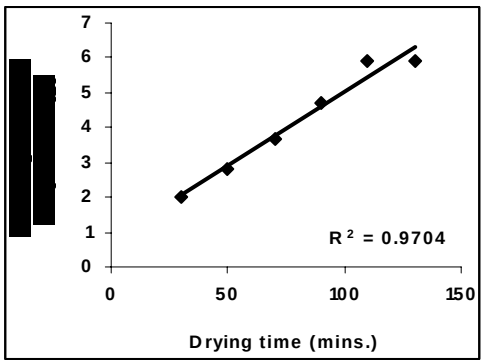

Figure 1: Variation of quantity of water evaporated with drying time as obtained from experiment (Nwoye, 2007)

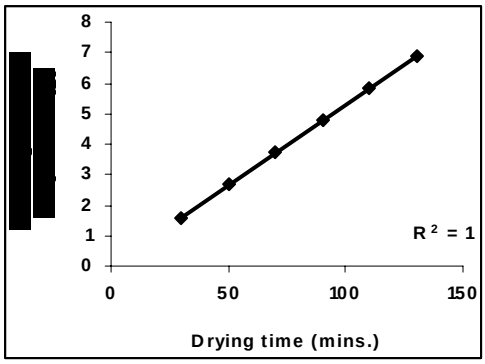

Figure 2: Variation of quantity of water evaporated with drying time as obtained from derived model

This suggests that the model predicts more accurate, reliable and ideal quantity of evaporated water than the actual experiment despite its deviations from the experimental values

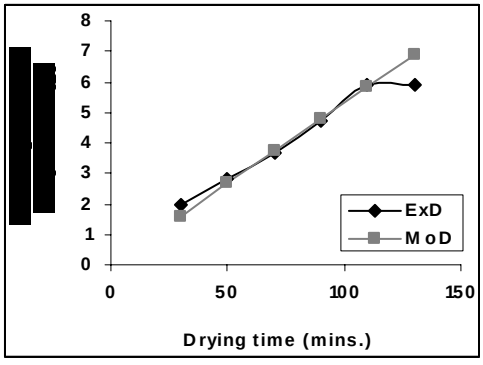

Figure 3: Comparison of the quantities of water evaporated as obtained from experiment (Nwoye, 2007) and derived model

Figure 3 shows that values of E obtained from the experiment and those from the model are generally quite close hence depicting the reliability and validity of the model. However, Figure 3 shows that the

quantities of water evaporated per unit rise in the drying time as obtained from the experiment (Nwoye, 2007), designated by the line ExD and as predicted by the model (line MoD) are in very good agreement within a drying time range 30-130mins.

5.1 Evaporation per unit rise in drying time

Water evaporated per unit rise in drying time resulting from drying of the clay at a temperature 80^0C for a time range 30-130mins. was determined following comparison of the evaporation per unit rise in drying time obtained by calculations involving experimental results, and model-predicted results obtained directly from the model.

Evaporation per unit rise in the drying time, E_d (g / min) was calculated from the equation;

$$E_d = E/t \qquad (18)$$

Therefore, a plot of mass of water evaporated E against drying time t, as in Figure 1 using experimental results in Table 1, gives a slope, S at points (2.0, 30) and (5.9, 110) and following their substitution into the mathematical expression;

$$S = \Delta E/\Delta T \qquad (19)$$

Eqn. (19) is detailed as

$$S = E_2 - E_1/ T_2 - T_1 \qquad (20)$$

Where

ΔE = Change in the quantities of water evaporated E_2, E_1 at two drying time values t_2, t_1. Considering the points (2.0, 30) and (5.9, 110) for (E_1,T_1) and (E_2,T_2) respectively, and substituting them into eqn. (20), gives the slope as 0.0488g / min which is the quantity of water evaporated per unit rise in the drying time during the actual experimental drying process. Also similar plot (as in Figure 2) using model-predicted results gives a slope. Considering points (1.6036, 30) and (5.8436, 110) for (E_1, T_1) and (E_2, T_2) respectively and substituting them into eqn. (20) gives the value of slope, S as 0.0530g/ min. This is the model-predicted quantity of water evaporated per unit rise in the drying time during the drying of the clay. A comparison of these two quantities of water evaporated per unit rise in the drying time shows proximate agreement. This indicates a very high degree of validity for the model as a reliable tool for predicting the quantity of water evaporated as well as the evaporation per unit rise in the drying time during drying of Ukpor clay at a temperature of 80^0C within a time range 30-110 mins.

A comparison of the values of β obtained from the experiment and those from the derived model (Table 3) shows maximum deviation less than 20% which is quite within the acceptable deviation range of experimental results, hence depicting the usefulness of the model. It was found that the validity of the model is rooted in the expression $(\text{Log}\alpha + \ln\beta)^N$ where both sides of the equation are correspondingly almost equal. Table 2 also agrees with equation (7) following the values of $(\text{Log}\alpha + \ln\beta)^N$ and lnt evaluated from Table 1 as a result of corresponding computational analysis.

Table 3: Variation of model-predicted quantities of evaporated water with the associated deviation and correction factor

β_M	Dv (%)	Cf (%)
1.6036	-19.82	+19.82
2.6665	-4.77	+4.77
3.7271	+0.73	-0.73
4.7860	+1.83	-1.83
5.8436	-0.96	+0.96
6.9002	+16.95	-16.95

β_M = Weight of water evaporated as predicted by the derived model

6. Conclusion

The model computes the quantity of water lost by evaporation during oven-drying of Ukpor (Nigeria) clay at 80^0C within the time range: 30-130 mins. It was found that the validity of the model is rooted in the expression $(\text{Log}\alpha + \ln\beta)^N$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of evaporated water from the corresponding experimental value is less than 20% which is quite within the acceptable deviation range of experimental results.

Further works should incorporate more process parameters into the model with the aim of reducing the deviations of the model-predicted β values from those of the experimental.

Acknowledgement

The authors thank Dr. Ekeme Udoh, a modelling expert at Linkwell Modelling Centre Calabar for his technical inputs. The management of SynchroWell Nig. Ltd. Enugu is also appreciated for permitting and providing the experimental data used in this work.

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3/3/2010

Bioaccumulation of Heavy Metals in *Pisum sativum* L. Growing in Fly Ash Amended Soil

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Abstract: Presently, the crisis of enormous amounts of fly ash has been sorted out by using it significantly in stabilization and escalating crop growth. In present study pot-culture experiment was performed to observe the influence of fly ash amendments on the growth and accretion of heavy metal in pea plants. Fly ash utilized for this study with high alkalinity and metals was poor in N, P and humus comparable to garden soil. Fly ash and soil were mixed in different ratios i.e. 0, 5, 10, 15, 20 and 25% and used to fill earthen pots (2Kg/pot). Seven days old seedlings were transplanted (3 individual/ pot) in them at glass house. 25 ± 2^0C temperature and moisture at 50% of water holding capacity was maintained throughout the experiment. The results revealed that there was a significant increase in chlorophyll, carotenoids, proteins, biomass and overall growth of target plant up to 10% fly ash amendment. Whereas, phenols and ascorbic acid concentrations were maximum at 25% fly ash amendment. The heavy metals in growth media and plant were significantly augmented and found beneath the permissible limits up to 10% fly ash addition only. Pea seeds demonstrated fascinating results they were harboring the metal concentration in all amendments under permissible range and were safe to consume. Translocation factor was calculated and results illustrated that toxic heavy metals like Cd, Ni and Pb retained in the below ground while micronutrients like Cu, Zn and Fe translocated to above ground parts. Hence, it is evident that pea plants may be a good metal accumulator plant species that could use for restoration of waste land having high alkalinity and low nutrient values. [Journal of American Science 2010;6(6):43-50]. (ISSN: 1545-1003).

Key words: Crop yield; Heavy metals; Bioaccumulation; Translocation factor

1 INTRODUCTION:

Today thermal power plants, based on coal combustion, are major producers of both electricity and fly ash. Fly ash, a finely divided residue, regarded as an amorphous ferroaluminosilicate mineral, containing the naturally elements i.e. similar to that of soil except humus and nitrogen (Wong and Wong, 1986).

In India, about 79% of the electricity is generated by coal based thermal power plants (Singh and Siddiqui, 2003), leads into 110 million tones fly ash per year (Jamwal, 2003) and it will surpass 140 million tones by year 2020 (Kalra et al., 1997). Currently the disposal of such massive amounts of fly ash; disposed off either by dry (dump in landfills and fly ash basins) or wet methods (artificial lagoons/ pond ash) has been become a major environmental quandary. All methods ultimately lead to the dumping of fly ash on open land causing deterioration of soil and environment (Jala and Goyal, 2006). On the other hand profitable uses of the fly ash have been also reported for various uses, raw material for building making and in agricultural land reclamation, quarry restoration, (Kriesel et al., 1994) a vast potential for use in agronomy as an amendment and etc.

Today, the steadily increasing input of xenobiotics into environment has elicited growing concerns regarding the impact of fly ash on several ecological aspects. In recent years, all metals present in fly ash viz. Zn, Cu, Cd, Hg and Fe have received much attention in ecotoxicological researches (Lee et al., 2006 and Tiwari et al., 2008). Although, some of them are considered to be essential elements except Hg, and As having no role in biological systems (Pahlsson, 1989). To overcome from this serious ecological threat, (Vajpayee et al., 2000) phytoremediation may be used to clean and revegetate fly ash landfills by various suitable plantation to check the dispersal of fly ash arising into the atmosphere and to develop a bioaesthetic environment. Because earlier Cha et al., (1999) observed that addition of alkaline fly ash (pH over 9.0), may diminish soil acidity to a level and may amplify the availability of trace metals, SO_2^{-4} and other nutrients except availability of nitrogen (N) in soil did not affected by the fly ash addition (Lee et al., 2006).

Although it is scarce in N and P it was observed that the response of plants to macro- and micro-nutrients of fly ash may vary from advantageous to detrimental in various concentrations (Singh et al.,

1997). Earlier Adrino et al., (1980) stated that successful revegetation of alkaline fly ash is limited by: (1) phytotoxicity due to Boron and Al; (2) restriction of root growth due to the fine particle size of the ash; (3) nutrient deficiency and; diminution in free living and symbiotic N fixing microorganisms. Therefore, disposal and utilization of fly ash is required careful assessment

So, keeping above views, the present study was designed with following objectives: to evaluate the effect of fly ash amended soil on heavy metals accretion, growth, biomass and some biochemical responses of pea plants. This study is vital because it deals with pea plant which is a leguminous plant (could compensate the N deficiency of fly ash) and also an imperative crop of north India.

MATERIAL AND METHODS:

2.1. Collection of samples and preparation

Unweathered fly ash sample was collected from a fly ash landfill of Parichcha thermal power plant, Jhansi, India. Fly ash and garden soil samples were stored in a transparent poly bags and fresh samples were used for pH (electrode), Electrical Conductivity (electrode), Moisture Content (%) and Water Holding Capacity (WHC), Organic carbon and CEC analyses. All the analyses were done by the standard methods given by Jackson (1958).

The processed fly ash and soil were mixed in six proportions i.e. 0, 5, 10, 15, 20 and 25% and used to fill earthen pots (2Kg/pot) one week before to planting. Pea seeds (*Pisum sativum* L.) were collected from National Seed Corporation, New Delhi (NSC) and seven days old seedlings were planted, 3 per pot, by pressed into soils to a depth of about 1cm under glass house condition (25±2^{0}C temperature, moisture at 50% of water holding capacity and humidity 40-50% throughout the experiment).

2.2 Soil and plant analysis

Soil samples were analyzed at two diverse stages during the experiment i.e. at the initial stage (seedling establishment stage) of experiment and at culmination of plants. Analyses were done for the potential bioavailable heavy metals by DTPA method (Lindsay and Norvell (1978) and total heavy metal concentration (EPA 3050 method) in both fly ash and soil. This method has been adopted by the USEPA as a standard method and recovers almost 100% of the metals from samples.

At culmination, plants were aloof from pots and plant components were alienated and washed with running tap water for few minutes. Plants were kept in oven at 70^{0}C till constant weight, ground and sieved (through 0.1mm sieve). Heavy metals in plant material (dried samples) were estimated by using method of Allen et al., (1986) with Atomic Absorption Spectrophotometer (Model AA 6800, Perkin–Elmer, Inc., Norwalk, CT, USA).

For chlorophyll, carotenoid, total phenols and ascorbic acid content in fresh leaves were quantified following Machlachlan and Zalik, (1963); Duxbury and Yentsch, (1956); Bray and Thorpe (1954) and Keller and Schwager (1977) respectively. Foliar protein contents were precise by method of Lowry et al., (1951) using bovine serum albumin (BSA) as standard.

2.3 Translocation factor (TF)

The accretion of metals in the plant parts was determined as *f* factor, also known as transfer coefficient (Smith, 1996).

2.4 Statistical analysis:

All the data were analyzed using one way ANOVA test using GPIS software (1.13) (Graphpad, California, USA) and different correlations and regressions has been done for statistical analysis of data by using SPSS version 11.5.

RESULTS:

3.1 Physicochemical properties of fly ash and soil

The physico-chemical properties of fly ash depend on the nature of parent coal, conditions of combustion, type of emission control devices, storage and handling methods.

In the study the garden soil used for experiments was slightly alkaline pH (7.6±0.04) with sufficient amount of N, P and organic carbon (Table 1). Soils metals were followed the trend Fe>Zn>Cu>Pb>Ni>Cd respectively. Whereas, fly ash illustrated a high value of pH (10.68±0.02) and heavy metals (Table-1). The trend of metals in fly ash was as followed Fe>Ni>Pb>Zn>Cu>Cd. Fly ash was containing insufficient quantity of nutrients like N (0.04±0.01), P (0.03±0.02) and also organic carbon (0.39±0.05). But occurrence of other micronutrients viz. Cu, Zn, Fe in a high enormity makes it apposite to use as manure to augment crop productivity.

3.2 Plant biomass production

Results exhibited a highly significant ($p<0.001$) rise in the growth of plants at lower ratio i.e. 5- 10% compare to control (Table 2). This may be explained by soil's high buffering capacity to the alkalinity of fly ash. At high application rates (20 and 25%), a significant diminution ($p<0.001\%$) was perceived in length and weight of root and shoot. It might be due to compactness of particles which, probably served as physical barrier to root elongations.

Similar pattern for the protein, cholorophyll and carotenoid content were noticed in plants and were depicted in Table 3. At 5-10% fly ash ratios ascorbic acid and phenols demonstrated a non-significant ($p>0.05$) augmentation. But at 20 and 25% fly ash treatment a significant augmentation were noticed.

The regression (r) analysis between soil available metal concentration at culmination to root, shoot biomass and weight of seeds were represented in Table 4. Results explained that roots and shoot length did not influenced by Cu and Cd availability in soil ($p<0.001$) while seeds weight and metal concentrations were significantly correlated. Root and shoot length were highly correlated ($p<0.001$) with the availability of Zn, Pb, Ni and Fe in soil at culmination. Seeds demonstrated a different pattern and it was noticed that Cu, Cd, Pb and Ni were positively and significantly correlated with weight of seed while Fe and Zn availability explained a non significant ($p<0.001$) correlation.

3.3 Plant elemental uptake

With increasing percent of fly ash, metal concentration in plants increased in each treatment. Cu, Zn and Ni demonstrated that their concentration traversed permissible limits at 20 and 25% of fly ash addition, although Cd and Pb concentration didn't reach up to phytotoxic level in any ratio but phtotoxicity symptoms were noticed at 20 and 25% of fly ash addition viz. decline in growth parameters.

Fig 1 portrayed the concentration of metals in roots, shoots, and seeds of pea plants. Micronutrients concentration were fallen within the permissible range for roots and shoots i.e. 20-100 and 100-400 µg/g for Cu and Zn respectively at 5-10% ratios (Kabata and Pendias, 2000). But they become elevated then acceptable limit at 20 and 25% fly ash amendments. In case of seeds metals were found to be within the dietary limit i.e. 20 and 50µg/g/day respectively at all ratios and hence safe to consume (Pahlsson, 1989).

Fly ash amendments raise the Cd, Pb and Ni availability in soil and hence its uptake by the pea plants (Fig 1). They were retained by the roots and not transferred to the shoots and seeds. This study was consistent with the Kabata and Pendias, 2000 and Cd and Pb concentrations were observed within 5-30 and 30-300 µg/g respectively. Up to 15% fly ash amendment it was observed that Ni concentration was beneath or within the limit (10-100 µg/g).

Seeds obtained from all treatments demonstrated that Cd, Pb and Ni concentrations were beneath the limit for daily intake of food i.e. 3-10, 25-85 and 250µg/g/day respectively (MacNicol and Beckett, 1985).

Table 5 illustrated the linear regression coefficient values (r) between soil available metals at maturity to metal concentration in root, shoot and seed of pea plant. All the metals showed an extremely significant relation ($p>0.001$) with uptake and availability of metals in all ratios at culmination.

3.4 Translocation of metals within plant body

Translocation factor for various metals from root to shoot and shoot to seed were represented in Table 6. It was noticed that translocation factors were almost less than one unit except Cu (in some cases). Ratio of root to shoot (S/R) for Cu varies 0.44-0.56, which again indicated that most of the Cu retains in the root tissues and not transferred to shoots, while ratio of shoot to seeds Se/S for Cu varies between 0.80-1.10 that shows a good translocation of Cu from shoot to seed. For Zn S/R varies between 0.53 to 0.86 and Se/S varies between 0.83 to 0.92, which again showed that pea plants tends to accumulate Zn in aerial parts than to below ground parts. Fe is also a very important metal for plant growth but translocation factor showed a different pattern for this metal. Most of the Fe remains in the shoots and not transferred to seeds (S/R 0.50 to 0.57 and Se/S is 0.22 to 0.31).

Translocation factor of S/R for Cd is more than Ni and Pb, which has almost same values but on the other hand it is also important to know that Se/S was found to lowest for Cd. It varies between 0.06-0.20 that shows most of the Cd retains by the roots and rest is restricted by shoots. Baker (1981) divides plants in three category i.e. accumulator, excluder and indicator. In accumulator plants the concentration ratio of the element in the plant to that in the soil is >1. In excluder plant metal concentrations in aerial parts are maintained low (<<1) and constant over a wide range of soil concentrations. In indicator plants the uptake and transport of metals were regulated in such a way that the ratio of the concentration of element in the plant to that in the soil is near 1.

Thus, *P. sativum* in this study was found to be accumulator for Cu, Zn and Fe while it was an excluder for Cd, Pb and Ni.

4. DISCUSSION:

According to researches, reduction in acidity by addition of a medium having pH over 9.0 is suitable for agriculture because it may increase the availability of trace metals, sulfates and other nutrients. On the other hand absence or low concentration of nitrogen, phosphorus and microorganisms makes this medium questionable.

In this study, the retarded root growth of *P. sativum*, grown in different fly ash amended soil was observed as the fly ash ratios increased. Gunse et al., (2000) reported that root growth inhibition was might be due to high contents of heavy metals like Cu, Cd, Zn, etc. which inhibited the root elongation by reducing cell division. Therefore the reduced growth of root in fly ash amended soil may be attributed to heavy metals, Boron and Al toxicity (Gunse et al., 2000). Adrino et al., (1980) stated that nutrient deficiency, reduction in free living and

symbiotic N fixing microorganisms and inhibition of root growth due to the fine particle size of the ash were also responsible for retarded growth of plants.

In present study retardation of over all performance of plant attributed to the toxicity caused by high metal concentrations in higher ratios of fly ash and this high concentration of such metals affect basic photosynthetic tools. The decrease in chlorophyll content may also be ascribed due to decreases in carotenoids contents, a non-enzymatic antioxidants playing a important role in protection of chlorophyll pigments against a stress (Krupa and Baszynski, 1995). Besides it low availability of N and P contents also responsible for poor growth and development (Jala and Goyal, 2006). It is interesting to observe that the addition of fly ash to the soil did not generate any statistically significant inhibition in the production of biomass. Here growth response is in line with results of other researchers (Moliner and Street 1982).

There was no any visible injury noticed (necrosis) due to fly ash addition during the growth and development of plants during experiment. In fact upto 15%, fly ash could be used as soil ameliorant to increase crop performance. No visible symptoms of nutrient deficiency or phytotoxicity were observed in plants grown in 5-15% but at 20 and 25% of fly ash amendment heavy metal toxicity was clearly visible in the form of reduction of shoot and root length. Although Adrino et al., (1980) suggested that application of fly ash on agricultural soils should not exceed the10% rate owing the adverse effect of fly ash on soil. Therefore, this study is consistent with the findings of above mentioned scientist. Besides this Singh et al. (2008), reported that fly ash amendments affected negatively the growth of *Beta vulgaris* at all treatments (0-20%). In our study all metals were highly correlated with its availability and uptake at culmination which was consistent with the finding of Oritz and Alcaniz, (2006).

Table 1: Physico-chemical characteristics of the soil and fly ash used in the study

Properties	Soil	Fly ash
pH	7.6±0.04	10.68±0.02
EC m mhos cm-1	0.12±0.01	5.73±0.05
CEC MEQ	4.12±0.02	0.45±0.04
Particle distribution (%)		
Sand	65.55±0.24	45.54±0.12
Silt	22.28±0.28	40.45±0.18
Clay	12.17±0.02	14.01±0.08
Total-N %	0.25±0.12	0.04±0.21
Av- N (g/Kg)	46.55±0.28	BDL
Toatl-P %	0.08±0.01	0.03±0.02
Av- P (g/Kg)	26±0.26	BDL
OC %	1.65±0.03	0.39±0.05
Cu (mg/Kg)	1.56±0.12	2.40±0.02
Zn (mg/Kg)	10.63±0.21	3.43±0.02
Cd (mg/Kg)	0.33±0.02	0.98±0.01
Pb (mg/Kg)	1.34±0.12	6.89±0.02
Ni (mg/Kg)	1.02±0.01	10.68±0.04
Fe (mg/Kg)	61.25±0.64	122.48±0.55

Notation: All the values are mean of three values (±SD); BDL: below detection limit

Table 2: Effect of fly ash amendments on crop growth and yield

Percentage of fly ash (%)	Root			Shoot			Seeds
	Length (Cm)	Fresh weight (g)	Dry weight (g)	Length (Cm)	Fresh weight(g)	Dry weight (g)	Dry weight (g/pot)
0	10.45	0.65	0.13	20.98	6.96	0.69	1.54
5	11.32[a]	0.78[c]	0.16	22.85[a]	7.65[a]	0.78[b]	1.76
10	11.98[a]	0.81[b]	0.18	24.04[a]	8.89[a]	0.89[a]	2.18

15	9.67[a]	0.61[ba]	0.11[c]	21.64[a]	7.18[a]	0.75a	2.69[ab]
20	7.85[a]	0.48[bac]	0.09[cb]	18.12[a]	6.72[a]	0.66[ab]	2.58[b]
25	7.01[a]	0.39[a]	0.07[ba]	16.01[a]	5.43[a]	0.60[ba]	2.27[c]

Notation: [a]‹ 0.001 [b]‹ 0.01 [c]‹ 0.05. Compare to control. Data are mean value ±SD. Means followed by the same letter are not significantly different (p<0.05).

Table 3: Effect of fly ash amendments on protein, photosynthetic pigments and antioxidants contents in leaves of pea plants

Percentage of fly ash (%)	Proteins (mg/g fresh leaf)	Photosynthetic pigments (mg/g fresh leaf)				Antioxidants (mg/g fresh leaf)	
		Chlorophyll a	Chlorophyll b	Total	Carotenoids	Ascorbic acid	Phenols
0	17.32	0.71	0.18	0.89	0.39	0.34	5.21
5	19.47	0.77[c]	0.20	0.97[a]	0.42	0.33	5.33
10	21.86[ac]	0.80[b]	0.22	1.02[ac]	0.45	0.35	5.36
15	15.67[a]	0.67[a]	0.15[cb]	0.82[ba]	0.35[b]	0.38	5.82[a]
20	12.54[ab]	0.59[a]	0.13[cba]	0.72[a]	0.31[cba]	0.41[cb]	6.22[a]
25	10.76[a]	0.51[a]	0.11[ba]	0.62[a]	0.26[bac]	0.47[abc]	6.65[a]

Notation: [a]‹ 0.001 [b]‹ 0.01 [c]‹ 0.05. Compare to control. Data are mean value ±SD. Means followed by the same letter are not significantly different (p<0.05).

Table 4: Linear regression coefficient values[*] (r) between soil available metals at maturity to length of root, shoot and wt of seeds of pea plant

Treatments	Root	Shoot	Seeds
Cu	-0.747[NS]	-0.614[NS]	0.777[c]
Zn	-0.918[a]	-0.897[a]	0.555[NS]
Fe	-0.847[b]	-0. 839[c]	0.647[NS]
Cd	-0.676[NS]	-0.568[NS]	0.832[b]
Pb	-0.760[c]	-0.6711[c]	0.817[b]
Ni	-0. 912[a]	-0.763[c]	0.779[c]

Notation: Significant at [a] highly significant [b] significant [c] less significant; [NS]: Non significant (p<0.001)

Table 5: Linear regression coefficient values[*] (r) between soil available metals at maturity to metal concentration in root, shoot and seed of pea plant

Treatments	Root	Shoot	Seeds
Cu	0.949[a]	0.948[a]	0.961[a]
Zn	0.962[a]	0.984[a]	0.984[a]
Fe	0.990[a]	0.995[a]	0.931[a]
Cd	0.923[a]	0.954[a]	0.960[a]
Pb	0.982[a]	0.973[a]	0.967[a]
Ni	0.997[a]	0.995[a]	0.988[a]

Notation: [a] Extremely significant (p<0.001)

Table 6: Translocation factors* between root to shoot (S/R) and shoot to seed (Se/S) of different heavy metals in pea plants

Percentage of fly ash (%)	Cu		Zn		Fe		Cd		Pb		Ni	
	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S	S/R	Se/S
0	0.56	0.84	0.53	0.90	0.56	0.29	0.49	0.06	0.37	0.11	0.36	0.14
5	0.56	1.06	0.56	0.83	0.57	0.31	0.39	0.10	0.36	0.13	0.33	0.16

10	0.48	1.10	0.63	0.84	0.53	0.22	0.48	0.15	0.34	0.16	0.34	0.21
15	0.44	0.80	0.71	0.83	0.54	0.26	0.42	0.07	0.36	0.18	0.33	0.25
20	0.46	0.86	0.77	0.92	0.50	0.27	0.33	0.20	0.38	0.18	0.34	0.23
25	0.51	0.80	0.86	0.88	0.50	0.30	0.36	0.19	0.38	0.17	0.32	0.28

Notation: *Translocation factors the ratio of metal concentration in shoot and metal concentration in plant root.

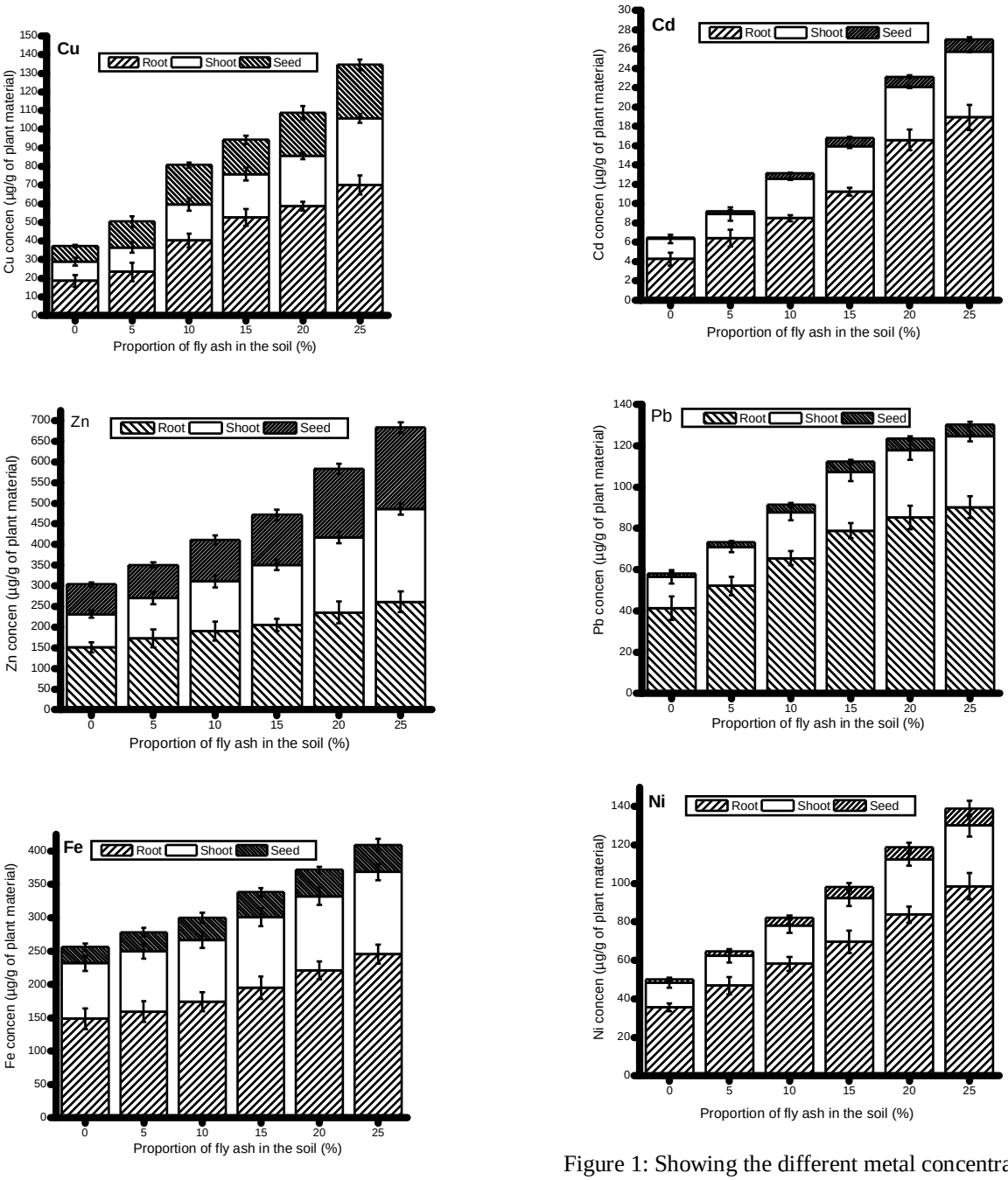

Figure 1: Showing the different metal concentration in root, shoot and seeds of pea plants at maturity. Data are mean of three replication ±SD.

5. CONCLUSIONS

It is well documented that fly ash generated by thermal power plants is similar in physicochemical properties to soil except nitrogen and of organic matter (humus). Application of fly ash to soil has been found to increase the bioavailability of heavy metals, and its low doses (up to 10%) did not cause significant increases in heavy metal concentration and could be used as soil manure. It is most interesting finding of this study that metals which transferred to aerial parts are micronutrients and important for plant growth and heavy metals which are considered to toxic, retains in the roots of pea plants only. Pea seeds showed interesting results they were harbouring the metal concentration in all amendments under permissible range and were safe to consume.

So, pea plants may be an alternative plant species for restoration of waste land having high acidity with low nutrients. However, extensive trials are prerequisite to find out a proper combination of fly ash with each soil type. But the care should be taken to access the level of metals at the time of consumption of seeds.

ACKNOWLEDGMENT

Authors like to give their thanks to Bundelkhand University, Jhansi and School of Environmental Science, Jawaharlal Nehru University, New Delhi to providing necessary facilities to complete this study.

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Evaluation of the Environmental Mitigation and Area Development (EMAD) component of the Bumbuna Hydroelectric Project (BHP) in Sierra Leone.

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Abstract: The most important development goals from the completion of the Bumbuna Hydroelectric Project (BHP) will be to accelerate economic growth, and poverty reduction, through the development of affordable power generation for domestic use in an environmentally sustainable, and efficient manner. Besides mobilizing private capital, the proposed Project will promote private sector involvement in the management of the power sector, and sustainable sector reform. The first component includes Hydroelectric and Transmission Infrastructure; and the second component is the funding of the implementation of the Dam/Reservoir, and the Transmission Line Resettlement Action Plan, with livelihood restoration and agriculture stabilization subcomponents, in addition to a comprehensive Environmental Management and Mitigation Plan (EMP). And the Technical Assistance component will fund the management and supervision of activities under the second component, and in addition, provide support to the Project Implementation Unit (PIU), the Dam Review Panel (DRP), and the Environmental and Social Advisory Panel (ESAP). This paper discusses environmental sustainability vis-à-vis regulatory compliance and environmental policy issues as related to the challenges and benefits being experienced by the Bumbuna Hydroelectric Project (BHP) in Sierra Leone. Its goal is to present strategies by applying established theoretical concepts and frameworks to the BHP case and examines some critical success factors that could be integrated into best practice management, especially in the face of future environmental and socio-economic challenges. The paper focuses on the Environmental Mitigation and Area Development (EMAD) component of the project as opposed to project contracts and technical assistance. We (the authors) believe that the EMAD component has a direct influence on the livelihood of the people, and as such, it could be used to gain further insights into BHP. If effectively implemented, the EMAD component may become one of the most important strategic management initiatives taken by BHP in complying with environmental regulations, in reaping potential benefits, and in putting the project in a better position for future financial assistance. As such, this paper's main focus is on EMAD's activities and recommends the adoption of a competitive strategy like a focused low-cost strategy that will provide the project with a strategic advantage whilst capitalizing on the World Bank's Dam Planning/Management Action Plan (DAMAP). [Journal of American Science 2010;6(6):51-64]. (ISSN: 1545-1003).

Keywords: Bumbuna Hydroelectric Project, Critical Success Factors, Environmental Sustainability, Sierra Leone, Strategies.

1. Introduction

The Bumbuna hydroelectric dam, a creation of the Bumbuna Hydroelectric Project (BHP), is found in the valleys of the Sula Mountains in Sierra Leone on the river Seli. The main objective of BHP, which was initiated in 1970, was to provide least-cost and sustainable electricity supply within the country, especially the capital city of Freetown. This objective is in line with that of the New Partnership for Africa's Development (NEPAD) which aims at the integration of Africa into the global energy economy and the reduction of poverty. However, for the past thirty-six years, BHP has still not achieved its objective, as the dam is yet to be operational. The delay in project completion has mainly been due to inadequate

finances, environmental constraints, and a ten year old civil strife (1991-2001) in the country.

Analysis revealed that the development of the Bumbuna Falls offered the most attractive option to expand and diversify power supply for the country including the capital, Freetown. A feasibility study prepared in 1980 and funded by the World Bank (WB) recommended though, a down-scale of the original design to match Sierra Leone's ability to absorb the generated capacity and therefore, repays the investors. Subsequent studies by the donor community throughout the late 1980s and 1990s have confirmed the present scaled-down version of Bumbuna as least-cost for hydroelectric development to supply the Freetown peninsula served by the Western grid as well as provincial centres along the transmission route. The delay in financing the project after the redesign of the scaled-down version in 1984 however, rendered the project impossible to start, not until 1990 on the one hand. The project was to be commissioned in 1998 but because of the civil war all works were abandoned in May 1997 but not before the project was 85 percent (%) completed, on the other hand. Discussions for the resumption of the project resumed after the 2002 national elections, and in 2003, the donor community pledged resources to complete the project.

Since then many stops have been pulled down on the way of the project with the view to completing the remaining 15 % of the entire project: Panels of experts or consultants including geologists, geological engineers, and water resource engineers, civil engineer specialist in hydraulics and hydraulic structures and dam engineer specialists continue to work overtime to conclude the project. There are environmental and social advisory experts both local and expatriate engaged in the project affected areas stretching from Freetown to the valleys and the Sula Mountains of Bumbuna and have been holding consultative meetings with the Project Affected People (PAP) other stakeholders including officials of government and non-governmental organizations, etc.

The project's initial plan was financed by the United Nations Development Program (UNDP). In 1984, after the completion of a feasibility study, the World Bank decided to invest in the project on the basis of its Dam Planning and Management Action Plan (DAMAP). Within the project's chequered history, the government of Italy also made substantial investment, and in 1993, the African Development Bank (ADB) joined forces with the other partners in view of providing funds to BHP. Other major international stakeholders include Salini Costruttori (Salcost), ABB Italia, and Studio Pietrangeli.

BHP is divided into three components, namely: Hydropower Project Contracts, Environmental Mitigation/Area Development (EMAD), and the Technical Assistance component. Although there would not have been the EMAD component without project contracts and technical assistance, yet the EMAD component is of great importance in relation to environmental sustainability vis-à-vis regulatory compliance and environmental policy issues. EMAD (comprising of community development initiatives and issues relating to land acquisition, compensation and rehabilitation) deals mainly with the effects of BHP on the environment, the economy, and society.

The project is envisioned as the first phase of the Bumbuna-Yiben hydropower development scheme with an ultimate potential of 270 MW. The subsequent phases of this development foresee the construction of a multi-annual regulation dam at Yiben, 28 kilometer (km) upstream of the existing Bumbuna dam, and the extension of the Bumbuna powerhouse with two additional turbine units to raise its output to 100 megawatts (MW) and further options include construction of additional powerhouses at the Yiben dam and downstream of Bumbuna falls. The completed project will consist of the 88m high asphalt faced rockfill dam and a crest-length of 440 meters (m) now in place (Figure 2); two freestanding bell-mouth spillways upstream of the dam, one leading to the primary spillway tunnel on the left bank of the dam and one to a combined power and auxiliary spillway tunnel on the right bank; a single inlet tower intake (Figure 3; giving an idea of how high the water will rise in the valley before the water level is high enough to power the turbines); a 50 MW surface power station and associated works; and 200 km of 161 kV transmission line linking the power station to the Western Area grid network at a substation in Freetown. The shield wires above the 161 kilovolts (kV) conductors will be energized at 35.5 kV to supply loads up to 5 MW at the provincial towns of Makeni and Lunsar along the transmission route. A narrow Y-shaped reservoir (Figure 4) 30 km long with width between 0.2 and 1 km, a surface area at the maximum operating level of 21 km^2, and a water volume of 445 x 10^6 m^3, with two upstream branches will be created at full impoundment.

The above-ground powerhouse set at the base of the dam will house 2 x 25 MW turbo-generator units. The hydro power plant will have a maximum operating capacity of 50 MW. The water level of the reservoir will be controlled by two “morning glory” spillways. Apart from power generation, the BHP will be capable of 35 x 10^6 m^3 regulation for downstream flood control. The

spillways discharging through left and right bank tunnels have a total design discharge of 3,000 $m^3 s^{-1}$.

Power and energy production from the Project is estimated as presented in Table 1.

Table 1. Certain characteristics of the BHP project.

Characters	Estimated potential
Installed Power	50 MW
Firm Power	18 MW
Total Average Annual Energy Production	315 GWh
Annual Firm Energy Production	158 GWh
Annual Average Secondary Energy Production	157 GWh

(Source: Mansaray and Khare, 2007)

Currently, the country's unreliable electricity supply, thermal in nature, is provided by the government-subsidized National Power Authority (NPA) upon which BHP is dependent. In effect, in order for BHP to succeed, NPA, like the Government of Sierra Leone (GoSL), will not only have to provide a clear sense of direction but also be more proactive in determining demand and supply features of sources of energy.

2. Description of study area.

The Bumbuna dam site is located on the upper reaches of the Seli (or Rokel) River, in the valleys of the Sula Mountains about 200 km northeast of the capital city Freetown (Figure 1). The dam site is 2.5 km upstream of Bumbuna Falls close to the town of Bumbuna (population of about 3,000) in the Kalansogia Chiefdom in Tonkolili District. The Seli River is the third largest of nine major river systems in Sierra Leone and has a drainage area of 10,620 km^2. A regional road from Freetown provides access to Bumbuna Town and the dam site.

2.1 Hydroelectric Component

Hydrology: The Bumbuna project is a run-of-river facility with a drainage area of 3,240 km^2 (2,635 mm average annual precipitation at Bumbuna). The Seli River has its sources in the northeast of Sierra Leone. For about 100 km, it flows across the Interior Plateau in a southwestern direction. About 30 km upstream of Bumbuna near the village of Yiben, the Seli river flows out of the interior plateau cutting through the Sula Mountains (Figure 3). After a fall of about 40m over Bumbuna Falls and the adjoining rapids it reaches the interior lowland floodplains and subsequently flows to a second fall, about 30km upstream of the estuary north of Freetown.

Figure 1 – A Map of Sierra Leone showing the Location of Bumbuna (in blue colour circle) (Source: Encyclopaedia Britannica, Inc.).

The hydrology reflects the seasonal rainfall pattern characterized by very wet periods followed by 4-5 months of markedly dry periods. Since 1970, the stages of the Seli River have been recorded at Badela and Bumbuna by two gauges installed under a UNDP Program. The intake flow regime of the Seli River recorded at Bumbuna for the period 1970 - 1979 shows a mean annual discharge o f 122.8 $m^3 s^{-1}$, with a maximum of 331 $m^3 s^{-1}$ in September and a minimum of 6.1 $m^3 s^{-1}$ in March and the highest peak flows are reached between August and October and normally range between 600 and 1200 $m^3 s^{-1}$, with the maximum-flow recorded in September 1970 at 1,052 $m^3 s^{-1}$. The design flood determined in the feasibility study through analysis of precipitation records for stations south of the catchment area and by infiltration measurements corresponds to a probable maximum flood with a peak flow of 2,970 $m^3 s^{-1}$.

Geology: The Seli River runs north to south through a relatively deep, narrow valley in the Sula Mountains, cut into pre-Cambrian, crystalline basement rock. The region is characterized primarily by gneissose granite and granodiorite of the West African Craton. The Bumbuna dam site itself lies on the western edge of a Precambrian curvilinear greenstone belt locally referred to as the Sula Group, which is surrounded by basement granitoids and late kinematic granites. Residues of weathered granite overlie most of the Precambrian rock formations.

Figure 2 – Illustration of the 88m high asphalt faced rockfill dam (Source: author's visit in 2006).

A comprehensive investigation was made of the geological and geotechnical conditions at the Yiben and Bumbuna dam sites for one and two years from 1972 and 1977, respectively. The Bumbuna dam site has firm bedrock at the surface in the center of the riverbed and at depths varying to 30m in the extremes of the flanks. On the right side, the rock is mainly granite and granodorite and the overburden is weathered granite. On the left side, the rock is mainly amphibolite with an overburden of weathered rock.

Figure 3 - One of the two towers where water will spill down over two-hundred feet onto turbines which will power the generators. (Source: author's visit in 2006).

Alluvium is nearly absent in the riverbed at the dam site, where bedrock outcrops are fresh and sound. A hard granodiorite shelf crosses the river about 2 km downstream of the dam site forming Bumbuna Falls. Most of the potential geotechnical risks associated with construction have been mitigated as the dam and the main civil works are in place.

Seismicity: The project area is considered to have very low natural seismicity. The crystalline rock of the West African Craton on which the Bumbuna project is located, have been geologically stable since the Pre-Cambrian, Faults identified in this basement complex are not considered to be planes of weakness that could reactivate due to seismic forces and there is no evidence of movement of any faults within the craton since the Cretaceous Period some 65 million years ago. There is a slight risk of induced seismicity. Considering experience of reservoirs impounded on Pre-Cambrian Cratons around the world an earthquake of 4.5 magnitude on the Richter scale with a hypocenter at a distance of 5 km from the site was incorporated in Project design criteria.

Sedimentation: There hasn't been any comprehensive, nationwide sediment survey of Sierra Leone's river systems. Sediment loads in the Seli River at the dam site, as determined by measurements taken from 1978 to 1979 as part of the 1980 Feasibility Study activities (World Bank, 2005), indicated monthly suspended concentrations, as at that time, varied from 1.1 milligram per liter (mg l^{-1}) in the dry season to 105.3 mg l^{-1} in the wet season. The mean annual transport was estimated at about 90,000 m^3. Recent measurements (Cemmats Group Limited, 2004) suggest that the amount of suspended sediment in the river may be increasing (over 100 mg l^{-1} was recorded in 2004 near the end of the wet season). The indication of higher levels in the recent survey likely reflects land use changes after 30 years a result of the slash and burn agriculture, where trees and shrubs are replaced by shallow-rooted crops (or grasses in areas left fallow).

The analysis of the International Hydropower Association (IHA)-supported Dam Review Panel (2004) indicates that a conservative estimate of bed load could be about 20 % of the suspended load, about 18,000 m^3. The total sediment inflow was thus estimated at about 108,000 m^3 (suspended plus bed load). As most of the suspended sediment would pass through the Dam the Review Panel (DRP) concluded that deposited sediment would be small compared to the reservoirs inactive storage of 95 million m^3. To address the potential risk accelerated soil erosion, a watershed management plan has been proposed as a component of the Project to improve vegetation and land management practices. The downstream channel consists of boulders, cobbles and gravel but its generally coarse superficial materials have produced a natural armored layer reducing erosion.

2.2 Project Description

2.2.1 Instruments

Two IDA instruments are used to finance the BHP:

(a) A US$ 38 million Partial Risk Guarantee (PRG), to mobilize a commercial loan for the completion of the BHP (two major physical

components of the project: (I) Contract A2/B comprising the BHP civil works and hydraulic structures; and (11) Contract C comprising the electromechanical equipment); and

(b) A technical assistance grant to finance the other project components including the Environmental Management Plan (EMP); the Resettlement Action Plans (RAPs) for the dam reservoir and the transmission line; supervision of the EMP and the RAPs; support for the Bumbuna Project Implementation Unit (PIU); the Dam Review Panel (DRP) of Experts; the Environment and Social Advisory Panel (ESAP) of Experts; Upper Seli Community Development Initiative (USCDI), the Communication Action Plan (CAP); and capacity building for relevant ministries and agencies for sustainable implementation of the project's safeguard measures.

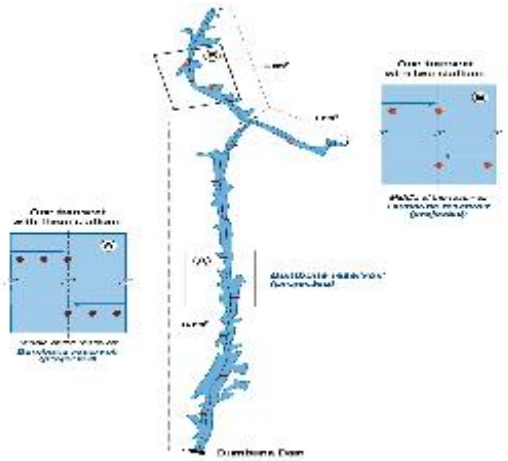

Figure 4 – Illustration of the Y-shaped Reservoir of the Bumbuna Hydroelectric Project dam. (Source: Encyclopaedia Britannica, Inc.).

2.2.2 Project Development Objective (PDO) and Key Indicators The PDO assumes that all energy generated by the BHP is sold to NPA and that NPA functions properly.

The main development objective of the proposed Project is to expand the capacity of Sierra Leone to increase the supply of electricity services at least-cost, and in an efficient and environmentally and socially sustainable manner. This would enable Sierra Leone to overcome one of the key constraints on growth, as well as on the effective delivery of social services.

The key outcome indicators include:

- Increase in electricity sales from 68.9 gigawatts per hour (GWh) in 2003 to 230 GWh in 2008;
- Percentage reduction of average electricity tariffs;
- A bio-diversity index to be established under the Additional Environmental Studies, acceptable to the Association, does not reduce by the end of the Project;
- 60 % increase of the targeted watershed area under improved agricultural conservation practice by the end of the Project;
- Sustainable fishery practices in 60 % of the reservoir and downstream of the dam;
- 100 % of Affected Persons resettled, as per definition in World Bank 2001 Operational Policy (OP) 4.12, by the end of the Project;
- Eighty percent increase of income of involved households with new income generating activities (forestry, fisheries, agriculture, soap making, carpentry, etc.) by the end of the Project;
- The Bumbuna Watershed Management Plan (BWMP) was to be developed by March 1,2007;
- Establishment of Environmental and Social Management Unit in the Ministry of Energy and Power (MoEP) and was to be operational by December 1, 2008;
- The Bumbuna Conservation Area was to be established by October 31, 2006 and management plan adopted by March 1, 2007; and Environmental management monitoring system developed by the date of effectiveness of the Grant.

3. Project Components, Environmental Sustainability, the Major Environmental and Social Impacts, etc.

3.1 Project Components

The three components of BHP are briefly described below:

3.1.1 Hydroelectric and Transmission Infrastructure (Component A)

The completed BHP will consist of an 88 m high rockfill dam with an asphalted concrete upstream face with a crest-length of 440 meters, two "morning glory" spillway intakes leading to a spillway tunnel and combined power/auxiliary spillway tunnel, a gated power intake incorporated in the spillway tower for the combined tunnel, and an above-ground powerhouse with two 25 MW turbo-generator units. A narrow Y-shaped reservoir between 0.2 and 1 km wide (Figure 4) and 30 km in length will form in the river behind the dam. It will have a capacity of 445 million m^3 and a surface area at the maximum operating level of 21 km^2. The energy from the hydroplant will be stepped up with a

13.4 kV/161 kV switchyard and transmitted to Freetown through a single-circuit 200 km-long transmission line on self-supporting steel towers (poles). In Freetown, the energy will be stepped down with a 161 kV/34.5 kV/11 kV substation, for distribution in the Western Area. Power service to Makeni, Lunsar, and Port Loko (all along the transmission line) will be provided by means of an insulated shield wire above the 161 kV line conductors that will be energized at 34.5 kV.

Although preliminary works have been undertaken as early as in the 80s, construction of the project began in the early 90s. Before the inception of the civil war, the major civil structures were in place (spillways, intake, tunnel, gates) and the rockfill dam had been finished. When the dam was about 85 % complete in May 1997, construction had to be suspended as a result of the ten-year old civil-armed conflict. The existing pre-civil war contracts cover the completion of civil works (Contract A2), the hydraulic steel structures (Contract B), the electro-mechanical equipment (Contract C), and the transmission line (Contract D).

3.1.2 Environmental Mitigation, Resettlement, Watershed Management and Benefit Sharing Environmental Management Plan, EMP (Component B)

The EMP and the RAP were prepared, approved and disclosed, both in-country and at the World Bank's Infoshop. The oversight arrangement for ensuring the implementation of these plans rests with the Bumbuna PIU. The PIU supervises the transmission and the main civil contractors, who are contractually obligated to implement and report on environmental and social mitigation measures for their respective construction activities. In parallel, the PIU is also responsible for coordinating with local government councils, traditional authorities and with line Ministries and agencies who are involved in specific EMP studies and activities funded by the project (particularly the Environment and Agriculture Departments), and for the hiring and supervision of consultants, non-governmental organizations (NGOs) and contractors for their implementation. Throughout the life of the project, this component provides assistance to develop an Environmental and Social Management Unit, within the Ministry of Energy and Power to take over the oversight function from the PIU upon completion of the BHP.

The PIU initiates all the legal and administrative activities needed to establish the Bumbuna Watershed Management Authority (BWMA) and the Bumbuna Conservation Area (BCA) as a Conservation Offset, which organizations will be established shortly after the test reservoir impoundment, and will undertake implementation of the EMP; and the RAPs will be implemented by the PIU under a separate contract. The BWMA will be responsible for the BWMP that aims to protect the reservoir from excessive sedimentation through improving agricultural practices in the Bumbuna catchment area, improving the livelihood of farmers, and protecting the remaining animal and plant biodiversity in the catchment, which includes the chimpanzee and other primate communities (World Bank, 2005).

The BWMP has several sub-programs for: (i) community awareness; (ii) land and soil management; (iii) agro-forestry and forestry; (iv) agricultural development; and (v) downstream and reservoir fisheries management. The PIU will also initiate and gradually transfer to the BWMA other EMP activities funded by the project, including actions for: (i) monitoring of public health impacts on diseases such as bilharzia and malaria; (ii) establishment of an environmental and social amenity flow; (iii) management of water quality in the reservoir area; and (iv) preparation and implementation of a comprehensive environmental monitoring program. All of these actions are necessary to protect the Bumbuna Reservoir from sedimentation, improve the livelihood of farmers in the catchment area, increase economic opportunities for communities in the area, protect biodiversity, ensure adequate water quality for users of the reservoir and downstream users, ensure sufficient water quantity for downstream uses, and protect the downstream environment. After commissioning of the BHP, a special-purpose project company, the Bumbuna Hydropower Company (BHC) will be contractually responsible for, among other obligations, and, pursuant to an Operations and Maintenance (O&M) management contract to be entered into between BHC and an operator ("the Operator") maintaining ongoing environment and management programs for the operation of the dam and reservoir and the transmission facilities and right-of-way (ROW). These responsibilities will be specified in the O&M management contract for the international operator for the dam/reservoir and the transmission facilities.

3.1.2.1 Land Acquisition, Compensation and Rehabilitation.

Reservoir Area: Laser images, GPS positioning and socio-economic impact surveys have determined varying degrees of impact in the dam/reservoir area. The Dam/Reservoir Resettlement Action Plan includes resettlement and compensation measures for: one village, which will be physically relocated; one village, which will lose almost all its farmland; and 31 villages which will lose a

substantial part of their farmland (approximately 40 %). In addition, four villages will be compensated, through community development compensation, for losses that occurred during the construction of the dam, the camp, access roads and the quarry in the 1980's and 1990's. Almost all project-affected people have started receiving appropriate compensation as per impact category, i.e. new houses and other structures, new farmland, assistance to move and to prepare new farmland, and compensation for other property such as shrines.

Technical assistance and funding will be provided under this project to ensure: (a) that farmers are able to reinstall themselves on their new farms and with better agricultural techniques, through the Stabilized Agriculture Program; and (b) that individual household incomes are restored to at least pre-resettlement levels through the Livelihood Assessment and Income Restoration Program.

Transmission Line: A Transmission Line Resettlement Action Plan has been developed, even though only very limited land acquisition and resettlement was required. In line with public consultations which expressed an unwillingness to resettle and conforming to accepted utility practice in many developed and developing countries, an approach was adopted which retains existing land use and permits transmission lines to overpass existing building and houses, provided that there was a minimum clearance between structures and the lowest conductor of 7.0 meters. Otherwise houses have to be moved and people compensated and resettled. One school near Makeni needed to be moved to an area adjacent to the present building. In addition, five dwellings also needed to be moved out of the ROW to adjacent areas. Provisions were made to control future building and land use in the ROW, with the guiding principle of maintaining the minimum clearance. The transmission line RAP will be implemented prior to the energizing of the transmission line.

Implementation of the Two RAPS: There is a common resettlement plan implementation arrangement for both parts of the project. The Dam/Reservoir RAP and the Transmission Line RAP is to be implemented by the PIU, by a unit of international and national resettlement consultants. The Resettlement Unit also provides continuous legal assistance to the project affected people and therefore included a legal counsel in the form of a local NGO – The Lawyers Centre for Legal Assistance (LAWCLA). The project-affected people are participating fully in the process and each village formed a Village Resettlement Committee (VRC). The overseeing and monitoring function is ensured by a Resettlement Advisory Group (RAG) in which all stakeholders at all levels are represented, and by an independent observer in the form of a Witness NGO.

Benefit Sharing: USCDI developed and tested an innovative institutional model for the proposed Bumbuna Trust, with the aim of sharing benefits with the indirectly affected population in the area around the reservoir and downstream of the dam. It consists of two activities:

(a) Ward and Community Sub-projects. Affected communities continue to receive development benefits, based on their demands for improved public services, through sub-projects implemented by them in collaboration with ward development committees and in harmony with overall district development plans. Public services include clearing and rehabilitation of smaller access/feeder roads, hand dug community wells and construction of latrines, management of organic waste, rehabilitation of existing school buildings and health centers, etc.

(b) Youth Capacity Building. Young women and men have been receiving training in marketable trade skills as well as business and life skills, and small grants given to community-based youth organizations. A Catchment Stakeholder Forum will enable a wider group of stakeholders to have a say in the formation of the Trust, and an Advisory Group will provide overall strategic direction for the Trust. USCDI is expected to provide technical assistance for the establishment of the Bumbuna Trust, in addition to its role in helping it develop approached and processes for benefit sharing.

3.1.3 Technical Assistance (Component C)

Component C is composed of management and supervision of the activities under Component B; and support for the Bumbuna PIU, the Dam Review Panel of Experts, the Environmental and Social Advisory Panel of Experts (ESAP), the RAP Implementation Team and the Communications Action Program. An international and national Environmental Management Technical Assistance (EMTA) team assist the PIU in implementing the EMP, in collaboration with the Department of Environment and other Sierra Leonean agencies. The PIU also expected to establish its own environmental management structure to oversee implementation of EMPs prepared by the contractors for the dam and transmission line.

3.2 Environmental Sustainability - Environmental Management and Mitigation Plan (EMP)

BHP's EMAD component, an overall EMP, is the major environmental sustainability initiative to

be funded by the World Bank. It ranges from land and soil management to agricultural development and fisheries management programs. Also, it is partly in the form of a trust fund, a community development initiative or safeguard measures revolving around capacity building and benefit sharing. Its financial sources will come from sales of electricity, and from funds provided to developing countries by the Organisation for Economic Co-operation and Development (OECD) through its carbon finance program. The EMAD component was established in response to the global demand for project-based greenhouse gas emission (GHG) reductions in the energy sector in relation to the rationale for environmental regulation, which initiative is an embodiment of innovative management practices based on environmental performance measures. It also serves to monitor social responsibility whilst capitalizing on business opportunities within the framework of the Kyoto Protocol's Clean Development Mechanism (CDM). Piasecki et al. (1999) observed that environmental performance measures are not only evaluative in nature, but they are also goals based on cost quantification and activity driver identification wherein environmental activities are divided into the following five cost pools:

- Strategic/tactical positioning
- Business risk management
- Program administration
- Impact minimization
- Penalty and injury

Using these cost pools, an attempt has been made below to evaluate the environmental sustainability of BHP's Environmental Mitigation/Area Development component.

3.2.1 Strategic or tactical positioning

Strategic positioning as a cost pool could be seen in the recommendation made in a study (sponsored by the United Nations Economic Commission for Africa, UNECA, on behalf of the Government of Sierra Leone, GoSL) done by Cemmats Group Ltd. (2004) which called for the need to enforce a governmental or non-governmental intervention policy in view of dealing with the environmental impacts of BHP; and it also came up with a strategic energy policy for the country. It called for government intervention in enforcing regulations so that fair play and consumer protectionism could be maintained and private investments and competition could be encouraged. The study, which was convincing in portraying strategic positioning as a cost pool wherein strategic policy statements are stated for implementation, was however, although short- and medium-term policy priority actions are mentioned, yet the study's Action Plan is not detailed enough to state specific actions to be taken in the long-term. Overall, it provides an understanding of the necessity to achieve environmental compliance through the enforcement of regulations in the hydropower sector of Sierra Leone and also encourages the GoSL to reaffirm its strategic commitment of addressing environmental issues and leads to the 2005 World Bank pledge to fund an EMP for Bumbuna Hydro within the framework of the Bank's-managed Public or Private Infrastructure Advisory Facility (PPIAF).

3.2.2 Business risk management

Business risk management as a cost pool is considered by BHP as partnership and contractual arrangements. The BHP became a private public partnership in 2003 with participatory bodies as the World Bank (WB), the IDA, the African Development Bank (ADB), the Government of Italy (GoI), and the Oil Producing Exporting Countries (OPEC) Fund. The project appraisal document of WB, report no. 31844-SL (World Bank, 2005) states that about 64% of the business risk will be borne by the public sector, mainly the GoSL, and 36% by the private sector.

Under an operations and maintenance agreement, the BHP will be incorporated as the Bumbuna Hydro Company (BHC) with the Italian firm, Salcost, holding 95% of its shares and the GoSL 5%.

According to the WB Report no. 31844-SL (World Bank, 2005), total project costs for BHP's Environmental Mitigation Area Development component was estimated at US$7.693 million to local investors and US$0.603 million to foreign investors, indicating that much more effort was needed from the public sector, especially the government in terms of good governance and security. Moreover, it also points to the fact that the government should not only rely on debt cancellations and donations from other countries, but to provide its people a sense of direction through wise leadership.

3.2.3 Program administration

In terms of identifying program administration as a cost pool for evaluating the cost effectiveness of BHP's EMAD component, it is observed that a Cabinet Sub-Committee has been serving as a policymaking body, consisting of the nation's vice-president and five other ministers, notably those for finance, energy and power, and works. The others are lands, and local government.

Under the Technical Assistance component, there is a technical committee which provides general

project coordination and supervises the PIU; which committee is constituted of a chief environmental officer, a member of the Professional Engineers Association, other professional heads, and government ministerial secretaries. In addition to the supervision of the Wrap up Agreement with Salcost, the PIU also serves as a communication link between both local and international stakeholders.

3.2.4 Impact minimization

The Azimut Consultants (2005) report prepared for the government of Sierra Leone discussed impact minimization as a cost pool for evaluating cost effectiveness of BHP'S EMAD, in terms of satisfying donors, and addressing public expectations. The methodology used to compare the legislations on involuntary resettlement in Sierra Leone and those found in the World Bank's Operation Manual OP 4.12 (World Bank, 2001) is descriptive. This report highlights two approaches in relation to resolving the issue of resettlement; the first was to disallow any land use within a 30-metre wide ROW of the electrical transmission line and the second, which was chosen, was to reduce the ROW to 7 metres thereby minimizing resettlements of people and making it more cost effective for the project.

Also the report convincingly addressed valuation methods for compensation, including the presentation of a clear perspective for calculated values of land, land improvement, and building construction. The least convincing though is the future implementation of the RAP. The report provided tools, in the form of valuation methods, to achieve its objective of providing a fair and equitable resettlement and compensation package to BHP affected people. This does not mean that the objective is already achieved, but it's entirely up to the implementation unit of the project to execute the necessary recommendations of the RAP. The report contributed to the general understanding of emerging environmental and social issues in the hydropower business in Sierra Leone.

3.2.5 Penalties and injury

Structures have been put in place by BHP that will adequately compensate project affected people (PAP) by addressing penalties and injury to human health and the environment as a cost pool. The Stabilized Agriculture Program (SAP), and the Livelihood Assessment and Income Restoration Program (LAIRP) are two social initiatives (World Bank, 2005) that will make sure that farmers are provided with the necessary technical assistance and that their incomes are restored appropriately. Hopefully, that part of the activities of the EMP will be to monitor public health within the project affected area for minimizing the spread of water-borne diseases such as malaria and bilharzias. In general, BHP's technical committee will be responsible for compensation of PAP, communications, and watershed and wildlife management issues. Another area in which penalties may arise is in the case of default by the NPA in terms of making timely Power Purchase Agreement (PPA) payments. Although measures are being taken to prevent default, yet it is seen that unpaid and overdue payments will endanger BHP's operations. To address potential risks of the project to human health and the environment in an attempt to mitigate penalties and injury, a great deal of preventive measures has being taken by BHP. For instance, it is expected that the endangered chimpanzee population will be protected, and an Emergency Preparedness Plan (EPP) has been put in place in terms of a warning and protection system for people downstream in case of emergency.

In conclusion, it could be said that by using the above-stated cost pools or environmental performance measures (Piasecki et al., 1999), it is found that BHP's Environmental Mitigation/Area Development program has made significant strife to address the issue of environmental sustainability.

3.3 The Major Environmental and Social Impacts of the BHP

Below are the summaries of the major environmental and social impacts. Both the 1996 and the 2005 EIAs identified the presence of some chimpanzee (considered an endangered species) communities in the wider reservoir area. There are no indications at present that the area to be inundated contains any other endemic or endangered plant or animal species. The area to be inundated has not been considered a critical natural habitat, according to the Natural Habitat Policy OP 4.04 (World Bank, 2001). The project is financing mitigation measures to protect the remaining chimpanzee communities, other primates, and other biodiversity in the Bumbuna catchment. Further biodiversity studies are underway, and reservoir filling was to start when recommendations of these studies are known. Impacts on fish as a consequence of the closure of the dam and the impoundment of the reservoir are expected to be minimal, as fish surveys were carried out during the 1996 and 2005 EIAs preparations; the results of which surveys indicate that the Seli River does not contain any endemic or endangered fish species.

Some changes in the hydrological regime of the Seli River downstream of the dam shall result from the annual filling of the reservoir, as reservoir

operation will regulate flow in the river, which will alter the hydrological regime downstream; an amenity or environmental flow will be maintained during all phases (pre-impoundment, impoundment, and operation). The amenity flow during the dry and rainy seasons will be greater than or equal to 6 m^3 and 100 m^3, respectively, and the operation of the reservoir will result in an increase in dry season flow.

The prevalence of bilharzia and malaria might increase; and the prevalence of river blindness (onchocerciasis) will likely decrease as a result of the inundation of a number of rapids in the reservoir area.

People and chimpanzees will likely be squeezed into a smaller area because of the impoundment of the reservoir. This habitat squeeze will further decrease the sustainability of the present agricultural-shifting cultivation system, and thereby increasing pressure on the chimpanzee habitat. The project's EMP includes appropriate mitigation measures, partly financed from the electricity tariff.

To warn and protect people downstream in case of an emergency, an EPP was to be prepared and operational before reservoir filling. And a benefit sharing mechanism will be provided with the establishment of the USCDI (World Bank, 2005), through which indirectly affected communities around the reservoir can benefit from social and economic development activities.

The GoSL is dedicated to implementing these mitigation measures.

3.4 Safeguard Policies

The project has got considerable environmental and social impacts, therefore, designated a category "A" project with safeguard classification of S2. The following safeguard policies (World Bank, 2001) are triggered by the BHP: Environmental Assessment (OPBP 4-01), Natural Habitats (OPBP 4-04), Forests (OPBP 4.36), Cultural Property (OPN 11-03), Involuntary Resettlement (OP/BP 4.12) and Safety of Dams (OPBP 4.37). The environmental impacts and resettlement issues raised by the BHP have been addressed in the 1996 and 2005 EIAs, in the Dam and Reservoir RAP, in the Transmission Line RAP, and by instituting a DRP and an ESAP. Since the Seli River is not an international river, the Safeguard Policy on Projects in International Waterways OPBP 7.50 is never activated.

The Safeguard Policy issues raised by the project are briefly discussed below:

Environmental Assessment. The GoSL's 2005 EIA includes a comprehensive EMP, which is been implemented during project construction and operation.

Analysis of Alternatives. The 2005 EL4 includes a comprehensive Retrospective Analysis of Alternatives (or Options Assessment/scoping). This analysis indicates clearly that the BHP was the preferred option to provide electricity to Freetown and intermediate towns at the least cost.

Natural Habitat. The 2005 EIA, the ESAP, and qualified Bank Safeguard Policy staff concluded that the area to be inundated was not a critical natural habitat under the definition in the Bank's Natural Habitat Policy OP 4.04 (World Bank, 2005) and that the reservoir area to be inundated fell under the definition of natural habitat in OP 4.04. The Bank can only finance projects that involve the significant conversion of natural habitat under certain conditions. In such cases, an environmental offset will be required.

Cultural Property. The 2005 EIA (which investigated the Cultural Property aspects of the Bumbuna reservoir area, as did that of the 1996) included an archeological survey (which did not reveal any evidence of cultural property in the area to be inundated) and interviews with the local population. An additional survey was to be carried out once the reservoir area was cleared, but before reservoir filling. Cultural property (including sacred sites, that belong to "secret societies"; and graveyards) in the dam/reservoir area may disappear due to the impoundment. The dam/reservoir RAP includes provision for relocation of and compensation for sacred sites.

Resettlement. The two RAPs prepared in 2005 include, one for the dam and reservoir area and one for the transmission line. Both RAPs have been approved by the Bank Safeguard Policy staff and were disclosed in country and in the Infoshop in Washington DC on January 26, 2005 (World Bank, 2005).

RAP for the Transmission Line. Practices in countries such as Canada, New Zealand, Spain, and Mexico have demonstrated that from a safety and public health point of view, it is an acceptable standard to build a high voltage power line over houses, as long as the required safety clearance is observed. An international expert has verified the acceptability of this practice in regard to safety and Electromagnetic Field (EMF) guidelines. Therefore, the resettlement along the transmission line was then minimized to include only six structures, which is in line with the results of public consultation, which demonstrated that PAP was unwilling to relocate.

3.5 Legacy Issues (Background and Approach)

About 450 households were affected by loss of land and property when the transmission line foundations were constructed in 1994-1995; the

impact was documented and a resettlement plan was prepared by the construction contractor (ABB). The resettlement plan, which included individual compensation contracts for each affected household, was been implemented by the PIU within a five-year period, effective 2007. Revenues from the project (3%), allocated to a special account, will be for the purpose of RAP implementation. The total cost, including escalation, was approximated to US$1.2 million.

Implementation of the RAPS. Both the RAPs for the Dam/reservoir and for the Transmission Line will be implemented by the Resettlement Unit (RU), which has been a unit under the responsibility of the PIU **(**composed of international and national consultants). The RU has been implementing the RAPS in close collaboration with the Village Resettlement Committees (VRCs). It's also been using the services of a legal association in Sierra Leone for the purpose of providing legal assistance to the PAP. A Witness NGO has ensured independent monitoring and evaluation and the Resettlement Advisory Group (RAG), composed of representatives from all stakeholder categories, has a general oversight function.

Forests. The riparian forest patches (which are under increasing human pressure in West Africa) in the reservoir area has to be cleared before the inundation of the reservoir and these patches in the reservoir area were not considered critical natural habitat areas by the 2005 EM (World Bank, 2005). The remaining riparian forests in the Bumbuna watershed are to be protected as described in the EMP (World Bank, 2005).

Safety of Dams. The GoSL constituted a DRP in 2004 to comply with the World Bank Dam Safety Policy OP/BP 4.37. Although the findings of the DRP indicated that the dam is safe, the review of the consultant's report, (World Bank, 2005), recommended that the Emergency Preparedness Plan (EPP) needed significant improvement. The findings of this panel indicated that the dam is safe and also indicated that the hydrology in the Seli River had not changed in the past decades, but recommended the collection of additional hydrological data. It (the panel) also indicated that at present the sediment loads in the Seli River do not pose a risk to the lifetime of the Bumbuna reservoir.

Borrower Capacity. Though the borrower capacity to implement the safeguard policy recommendations has been insufficient, the project has been financing environmental management and resettlement implementation as well as capacity building through technical assistance (TA). The TA, handled by the PIU, consists of on-the-job training and capacity building in environmental management and monitoring by international consultants and national experts within the responsible Sierra Leonean institution, the Department of Environment.

Implementation of the EMP. The TA is been financed through an IDA TA Grant and implemented in close collaboration with Sierra Leonean partners such as the Department of Environment, a team of international and national experts in the Bumbuna PIU, the Bumbuna Watershed Management Authority (BWMA) and others satisfactory to IDA, is engaged in monitoring the implementation of the EMP, the contractors EMP, and provide legal aid to PAPS.

Public Consultations. The terms of reference (TOR) for the 2005 EIA was submitted through public scoping sessions with primary and secondary stakeholders, to ensure that all relevant stakeholder concerns have been addressed in the 2005 EIA. Extensive stakeholder consultations have been carried out on the Draft Final 2005 EIA. The minutes of these public meetings have been included in the Final 2005 EL4 report. Public consultations carried out for the 1996 EIA influenced the design of the EMP, and elements from the 1996 EMP have been carried over into the 2005 EMP (World Bank, 2005).

Disclosure of the 2005 EIA Report. The Final 2005 EIA and EMP, including a budget, have been approved by the World Bank and subsequently disclosed in the project areas, nationally and in the above-mentioned Infoshop in Washington. The main findings of the EIA are presented in World Bank 2005.

3.6 Political and Other Risks

Large dam projects involving resettlement are very sensitive due to their possible negative social and environmental impacts, which may hinder multi-stakeholder support for the project if disclosure of information and transparency is not properly ensured. Given this potential risk, a communications needs assessment was carried out through extensive one-on-one meetings, interviews, and focus groups with the national media, NGOs operating in Sierra Leone, opinion leaders, and the general public. The assessment established that overall there was broad political support for the project among all groups interviewed. A smaller risk was associated with the lack of government communications, as well as adequate and timely information dissemination.

The assessment concluded that the political and other risks associated with the project could be mitigated through a communication program aimed at ensuring interaction with national and international NGOs and national media. The program explains the project's commitment to fair, transparent, and

proactive initiatives aimed at promoting the high standards of social inclusion, environmental protection, and poverty alleviation attached to the project objectives.

3.7 Policy Exceptions and Readiness

The exception to World Bank 2001 Operational Policy (OP) 4.12 (a World Bank policy on involuntary resettlement, which requires that, if adverse indirect social or economic impacts might result, a social assessment should be done and measures to minimize or mitigate such impacts implemented.) was approved by the chairman of the Operations Committee and also instructed that this decision be fully disclosed in the PAD. The threefold rationale for seeking the exception to OP 4.12 includes:

(a) Because the loss of property and farmland occurred when the transmission line foundations were constructed during the civil war, the situation is very different from the normal situation where involuntary resettlement occurs. The affected people have moved and are not in immediate need of compensation.

(b) Since Government is normally responsible for payment of compensation related to resettlement and the unique circumstances prevailing in Sierra Leone (post conflict), use of project revenues is appropriate to enable the compensation be paid over five years. Very similar procedures have been followed to pay the legacy compensation as are considered for the payment of compensation of people affected by the filling of the reservoir; which procedures included the hiring of a 'witness NGO' to verify that compensation has, in fact, been paid to the affected people.

(c) Given the unique nature of the project and country circumstances, the region feels that an exception to OP 4.12 was justifiable and that the proposed compensation structure for the legacy issue discussed in this work eventually met the objectives of OP 4.12.

Otherwise the project is said to be in full compliance with the requirements of all other triggered World Bank Safeguards.

3.8 Challenges and Benefits

The debates on the long-term sustainability of hydropower projects are becoming increasingly more significant, especially with regard to the challenges and benefits for developing countries like Sierra Leone. In the midst of all the tremendous socioeconomic benefits to be realized from BHP, the sustainability of the project is apparently plagued with an excess of environmental and socioeconomic challenges (not only linked to the nature of the project and the disruption of the environment, but also to the displacement, compensation/rehabilitation of PAP, and the difficulty in acquiring funds for project completion) that might have more adverse effects on the country. This might mainly be due to the inadequacy or lack of necessary resources, skills, and infrastructures to sustain the hydropower project. In effect, the maximum benefits of the project might not be realized.

In terms of environmental degradation, compensation/rehabilitation, conflicts with some local communities are common. The construction of the BHP dam has diverted the flow of the river Seli from its natural course thereby disrupting surrounding ecosystems, which effect has been felt upstream and downstream of the river system by both flora and fauna. While ecosystems upstream are flooded with water, those downstream are deprived of an essential for livelihood. Dams, such as the BHP, are known to change water quality in terms of increase in river water temperature and dissolved gases, consequently, negatively affecting aquatic habitats, ecosystems, and human communities. Fish migration, particularly, is hampered by the slow-flowing dam water and hydropower turbines; which effect might result in the decline of fish population and food shortage for humans. Similarly, there is a high possibility for the BHP dam to accumulate contaminated sediments which affect fish spawning and deprive downstream ecosystems from receiving sediments.

Moreover, the country's post-civil war environment is not conducive enough to attract private investments thereby failing to fill the gap left by dwindling public sources of finance. The short-term financially uncompetitive nature of the project is not only a challenge in accessing long-term finance, but also in bringing about delay in donor remittances. Under normal circumstance, the cash flow of the project is expected to be a source of revenue. The Bumbuna Hydropower Company (BHC) and the NPA will set tariffs in favour of BHC as a risk mitigation measure. Finally, the high construction risk and time, the lack of an effective regulatory framework and bad media exposure are also compounding the challenges of the capital and local cost-intensive BHP.

The benefits from the BHP are promising even though the challenges of the project are seemingly formidable; including the facts that a well-proven and simple technology was used to provide electricity supply to a country that has been deprived for decades of a reliable source of energy. This means that an increased impetus would be added to agriculture, trade and industry thereby creating and improving secondary economic opportunities like

youth capacity building, tourism, culture and sports. With anticipated carbon finance benefits associated with its greenhouse gas (GHG) emissions reduction, its low operating/maintenance (O&M) costs, and its long project life years, the BHP will be in a position to strengthen its link to Sierra Leone's development vision for 2025 and its participation in regional development programs of the Economic Committee of West African States (ECOWAS) and the New Partnership for Africa's Development (NEPAD).

The goal of Sierra Leone's energy policy is not only to produce and use energy efficiently but also to add value to sustainable social and economic development (Cemmats Group Ltd., 2004). It is hoped that this will help reduce poverty as reflected in the objectives of NEPAD (New Partnership for Africa's Development, 2006), and the Millennium Development Goals (MDGs).

In the April 28, 2006 edition of Awareness Times newspaper (Munu, 2006), a BHP consultant was quoted as saying that the project will need US$93.8 million for final completion. In comparison with the 2005 financing plan of estimated project cost it is crystal clear that the possibility of an increase in cost could not be unfounded. In this case, there has been a change in the amount of project cost; an increase in cost of US$2 million between 2005 and 2006. In terms of debt servicing and the time value of money, otherwise known as discounting, it could be calculated that if US$91.8 million were borrowed to finance BHP, and if for instance the investors require a rate of return of 10 percent per year, then the project should be generating US$9.18 million yearly in order to repay the debt. Most of these economic costs include total costs to complete hydropower and transmission structures, operations and maintenance costs (O&M), environmental and development costs, and costs emanating from technical assistance (World Bank, 2005). In effect, the economic benefits are mainly revenues from sale of electricity, and carbon credits. From the ongoing analysis, it could be observed that the project costs of Bumbuna Hydro are significantly influenced by the concept of innovation and change.

Poor access to electricity is a major challenge for West Africa (Davidson, 2006), suggesting the need to integrate both urban and rural electricity development programs within the context of the West African Power Pool (WAPP), which is especially true for a war-torn country like Sierra Leone where poverty reigns and the challenge is the lack of "affordability" by the people to purchase electricity when available.

Head (2000) made mention of the current trends in private financing based on the drive for run-of-river projects and avoidance of large dams that tend to be costly, plagued by lengthy construction time, and poor publicity. The second major trend in private financing is that towards smaller hydropower projects like BHP (50MW). This trend arises in view of mitigating the effects of environmental degradation caused by larger projects, although according to the author it might not be a best practice.

As stated earlier the report prepared by Azimut Consultants (2005) for the government of Sierra Leone is in response to a World Bank requirement related to involuntary resettlement and the corporate responsibility of BHP for providing a fair and equitable resettlement and compensation package. The challenge was to develop and use a cost-effective and an appropriate valuation method for compensation of PAP. A solution might be to perpetually maintain a balance between the interrelated challenges and benefits, entailing a series of changes like policy reforms, energy production, distribution and consumption, reflecting the shift towards the era of the sustainable energy economy.

In summary, the environmental and socio-economic challenges and benefits of BHP to PAP and the industry are based on its long-term sustainability, including, the restoration of the livelihood of PAP through youth capacity building, benefit sharing, and compliance to the power purchase agreement (PPA). In effect, BHP is forced to be socially responsible and environmentally accountable if it is to survive in today's competitive corporate world.

In recent times, key players in hydropower-production are becoming more concerned with the long-term sustainability of the hydropower business, especially where companies could combine sustainable practices with profit-making. In such cases, alternative intervention strategies are needed to address each situation in its own sense.

4. Concluding Remarks

The research study's problem was to identify the potential environmental challenges and benefits associated with BHP and to evaluate its Environmental Mitigation and Area Development (EMAD) component.

The findings were that in identifying challenges and benefits and determining strategies, BHP has the overall internal strength to capitalize on external opportunities in view of combating threats. Also, that BHP is faced with alternative strategies, albeit irreversible strategic commitments in the midst of innovation and change, making access to private finance more critical in achieving the goals of the project. Moreover, it was also discovered that because of a possible Power Purchase Agreement-default, and the country risks (poor infrastructures,

post-war environment, and improper governance) involved, the project might be ineffective and unviable for long-term private financing. And, because of some or all of these risks and challenges, BHP, at one time or the other, has been denied funding by some private financiers. In effect, these risks and challenges have made executing best practices, completion of BHP, and effective implementation a difficult task.

As a concluding remark, it should be stressed that apart from the WB-related documents on BHP, research information about the project is scanty. Among other issues, the future of energy and the environment is relatively bleak in this part of the world. However, Sierra Leone's population is still hoping to see the completion of BHP.

This study is limited to an analysis of the dimensions of the problems revolving around the uncompleted BHP in view of evaluating its Environmental Mitigation and Area Development (EMAD) component. Recommendations for further study may include an application of a set of sustainability guidelines developed by the International Hydropower Association (IHA), (2004) to the case of BHP. A potential objective might be to evaluate BHP on the basis of these guidelines as they aim to promote an awareness of the environmental, social and economic aspects of sustainability assessment in hydropower projects.

Acknowledgement

This work was supported in parts by grants from National Outstanding Youth Research Foundation of China (40925010), International Joint Key Project from National Natural Science Foundation of China (40920134003), National Natural Science Foundation of China (40873060, 40673065), and International Joint Key Project from Chinese Ministry of Science and Technology, and the 111 Project (B08030).

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3rd December, 2009.

Physico-Chemical and Microbiological Study of Tehri Dam Reservoir, Garhwal Himalaya, India

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ABSTRACT : In the present study physico-chemical and microbiological characteristics of the water of Tehri dam reservoir in the Garhwal Himalaya of India were determined during June 2003 through May 2005 when the reservoir was under construction, and was 5 km long and 40 m deep having an area of 2.2 sq km, and is located at 30º23' N latitude, 78º 29'E longitude and 635 m altitude at monthly intervals during June 2003 through May 2005 with an objective to estimate the impact of the reservoir on various physico-chemical and microbiological parameters of the water. Total solids, total suspended solids, total solids, turbidity and sulphate values were maximum on all the sites in rainy months, which may be due to the gradual disturbances in sedimentation of solids as well as dust particles deposited along with runoff rainwater. The alkalinity varied during different months. The values of pH, conductivity, hardness, calcium, dissolved oxygen and biological oxygen demand were higher during summer months. The chloride concentration was highest in the month of January and the nitrate increased in the summer months and early monsoon due to the higher phytoplanktonic production. The maximum number of total coliform, faecal coliform and total plate count was observed during summer and rainy seasons and minimum during winter. [Journal of American Science 2010;6(6):65-71]. (ISSN: 1545-1003).

Keywords: Physico-chemical characteristics, Tehri Dam, Himalaya.

1. INTRODUCTION

Water is the elixir of life and abounds on earth, but this vast natural resource has been depleted and turned into scarce commodity with increased usage catering to the needs of ever-expanding population. There is almost a global shortage of water and the world's most urgent and front rank problem today is supply and maintenance of clean drinking water. The climate change and spells of droughts have even stressed regional water tables. There are strides to fight the grim battle of acute shortages of water. The problems relating to water attract the attention to the urgency for investigating causes and suggest remedies in a bid to prepare future plan of action for maintenance of potable waters and related development issues.

The lakes are large or considerable body of water within land (Wetzel, 1983). The maintenance of a healthy aquatic ecosystem is dependent on the physico-chemical properties of water and the biological diversity. A large number of streams and rivers in India have been impounded to store the water for multipurpose beneficial uses like irrigation, fisheries, power generation and drinking water supply. Now-a-days, the ecology of reservoirs is under stressed condition due to fast pace of development, deforestation, cultural practices and agriculture. These activities trigger the rate of sedimentation of the reservoir bed characterised by silt and organic suspended material which initiates the process of eutrophication at a very early stage and show a deterioration of habitat quality.

The main purpose of analysing physical, chemical and microbiological characteristics of water is to determine its nutrient status. Since, the water contains dissolved and suspended materials in various proportions, its physical and chemical characteristics differ along with its biological characteristics. The water quality is also affected by pollutants which act on elements existing in water such as dissolved oxygen or produce substances such as ammonia, nitrates etc. It is not possible to understand biological phenomena fully without the knowledge of water chemistry as the limnobiological and limnochemical components of the ecosystem. If we can find some correlations among these numerous parameters, however, the task of periodic monitoring of water quality may be facilitated to a good extent (Tiwari, 1992). The physico-chemical means are useful in detecting effects of pollution on the water quality but changes in the trophic conditions of water are reflected in the biotic community-structure including species pattern, distribution and diversity (Kaushik and Saksena, 1995). Some ponds of India have been extensively studied by various workers (Michael, 1969; Saha *et al.*, 1971; Vasisht and Sharma, 1975).

The present study has provided detailed information on physico-chemical and microbiological parameters of the Dam reservoir water at three

different sites with an objective to indicate changes in the quality of waters at the beginning and lower end of the reservoir. The study will be helpful in estimating the impact of the reservoir on various physico-chemical and biological parameters of the water.

2. MATERIALS AND METHODS

Study Area

The large multipurpose Tehri Dam, during the present study was under final stage of construction near the confluence of the Bhagirathi and Bhilangana rivers near old Tehri Town, has two main catchments of these rivers draining into its reservoir. Both these rivers originate in the glaciers of the higher Himalayan region and flow through deep gorges, dense forests and habitation alike (Figure 1). The source of river Bhagirathi is Gaumukh, while the Bhilangana originates from the comparatively smaller Khatling glacier. The Tehri dam is the highest earth rockfill dam in Asia. During the period of present study the reservoir was 5 km long and 40 m Deep having 2.2 sq km area and is located at 30º 23' N latitude, 78º 29' E longitude and 635 m altitude. Three sites selected for the study along reservoir banks were as follows (Figure 2):

1. Old Tehri Iron Bridge
2. Padiyar Village
3. Kandal Village

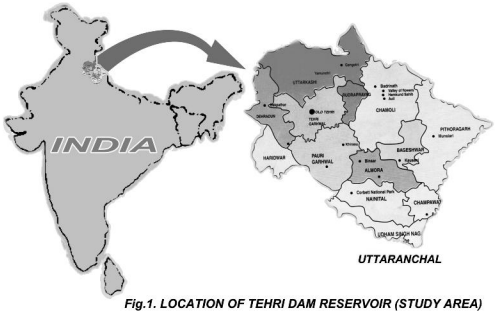

Fig.1. LOCATION OF TEHRI DAM RESERVOIR (STUDY AREA)

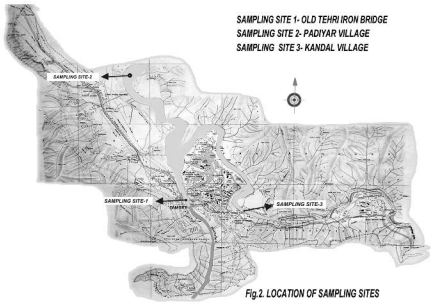

Fig.2. LOCATION OF SAMPLING SITES

First two study sites were situated on right side of the reservoir and third site was on the left side. The water of the reservoir is generally used for washing the clothes and drinking purposes near the settlements.

Climate

The climate of the study area is tropical monsoonic with three distinct seasons. The atmospheric temperature ranged between a minimum of 5ºC in December and January, and maximum of 37ºC in June during the study period. The maximum rainfall (230 mm) occurred in July, whereas the humidity ranged from 47% (May) to 92% (January).

Methods

Following characteristics were analysed in the study:

1. Physical Characteristics: The physical characteristics included temperature colour, conductivity, turbidity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS).

2. Chemical Characteristics: The chemical characteristics included alkalinity, hardness, pH, dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD), total kjeldahl nitrogen (TKN), ammonical nitrogen, chloride, nitrate, phosphate, sulphate and calcium.

4. Microbiological Parameters: The microbiological parameters included green algae, blue-green algae, diatoms, total coliforms (TC), faecal coliforms (FC) and total plate count (TPC).

The water and algal samples were collected, studied and analysed from three study sites at monthly intervals during June 2003 through May 2005. All water samples for the estimation of different parameters were collected in the early hours of morning on a specified date. The samples were pretreated in the field to fix the samples and immediately brought to the laboratory for an on spot physical, chemical and microbiological analysis of various parameters following the standard methods (APHA, 1998). The data were analysed and compared statistically.

3. RESULTS AND DISCUSION

The quality of natural water is generally governed by various physico-chemical and biological parameters. The maximum and minimum values for various parameters during the study period are presented in Tables 1-3.

Water temperature

The maximum and minimum temperatures of reservoir water were observed in the months of June and January respectively on all the sites. The

values ranged between 7.03-27.03 ^{0}C. Steady change in the atmospheric temperature with the change in the seasons results in the corresponding change in the water temperature. There is a very close similarity between the temperature of atmosphere and water due to the depth of reservoir. High summer temperature and bright sunshine accelerate the process of decay of organic matter resulting into the liberation of large quantities of CO_2 and nutrients.

Turbidity

The maximum value of turbidity was recorded during July to September (monsoon period) and minimum during winter period on all the sites. The increased turbidity during rainy months was attributed to soil erosion in the nearby catchment and massive contribution of suspended solids from sewage. Surface runoffs and domestic wastes mainly contribute to the increased turbidity of the reservoir. But in this region, the suspended solids play an important role in governing the turbidity, which enter the reservoir through land erosion.

Total dissolved solids (TDS)

The values of TDS were maximum in the months of April and May. The minimum values were observed in the months of June on sites 2 and 3, and in August on site 1 (Table). Sabata and Nayar (1995) in Ganga water showed wide variation in TDS in different months on different sites.

Table 1. Maximum and minimum values for various physico-chemical characteristics for site I of Tehri dam reservoir

.

PARAMETER	UNIT	MAXIMUM VALUE AND MONTH		MINIMUM VALUE AND MONTH	
Temperature	0C	26.06	June	7.03	January
Conductivity	µ mhos	0.162	May	0.099	October, November
Turbidity	NTU	159	July	14.1	January
TDS	mg/l	134	July	87.3	August
TSS	mg/l	116	July	13.8	January
TSS	mg/l	250.2	July	102	January
Alkalinity	mg/l	64.2	July	40.1	January
Hardness	mg/l	72.0	May	48.0	January
pH		7.98	May	6.79	June
DO	mg/l	8.2	June	7.06	September
COD	mg/l	24.1	May	8.9	December
TKN	mg/l	4.48	April	0.29	October
Ammonical nitrogen	mg/l	0.042	April	0.028	March
Chloride	mg/l	20.3	January	9.0	October
Nitrate	mg/l	1.19	June	0.25	February
Phosphate	mg/l	1.14	April	0.03	February
Sulphate	mg/l	37.7	July	18.7	June, October, February
Calcium	mg/l	25.7	April	14.4	February
BOD	mg/l	3.6	April, May	0.5	September

Table 2. Maximum and minimum values for various physico-chemical parameters for Site II of Tehri dam reservoir .

PARAMETER	UNIT	MAXIMUM VALUE AND MONTH		MINIMUM VALUE AND MONTH	
Temperature	0C	27.03	June	9.0	January
Conductivity	µ mhos	0.441	February	0.085	September
Turbidity	NTU	295.0	July	14.8	May
TDS	mg/l	118.0	April	80.4	June
TSS	mg/l	493.4	June	19.1	January
TSS	mg/l	573.8	June	104.2	January
Alkalinity	mg/l	70.1	May	37.2	January
Hardness	mg/l	72.2	May	35.2	January
pH		7.85	July	7.2	June
DO	mg/l	8.96	June	6.56	September
COD	mg/l	39.4	July	5.9	January
TKN	mg/l	3.92	February	0.27	December
Ammonical nitrogen	mg/l	0.062	March	0.06	July
Chloride	mg/l	15.9	May	9.9	July
Nitrate	mg/l	0.97	May	0.12	June
Phosphate	mg/l	0.85	April	0.05	October
Sulphate	mg/l	38.0	July	14.2	November
Calcium	mg/l	21.1	May	11.3	June
BOD	mg/l	3.4	May	0.5	June

Table 3. Maximum and minimum values for various physico-chemical parameters for Site III of Tehri dam reservoir.

PARAMETER	UNIT	MAXIMUM VALUE AND MONTH		MINIMUM VALUE AND MONTH	
Temperature	0C	27.0	June	9.03	January
Conductivity	µ mhos	0.176	May	0.097	June
Turbidity	NTU	278.0	August	18.0	January
TDS	mg/l	126.0	May	84.1	June
TSS	mg/l	513.9	June	28.9	January
TSS	mg/l	598.1	June	119.5	January
Alkalinity	mg/l	71.8	Feb	39.0	September
Hardness	mg/l	71.9	October	38.9	January
pH		7.91	May	7.25	June
DO	mg/l	8.56	June,July	7.5	September
COD	mg/l	32.2	September	8.2	January
TKN	mg/l	4.22	April	0.48	August
Ammonical nitrogen	mg/l	0.076	March	0.031	May
Chloride	mg/l	19.2	January	8.0	October
Nitrate	mg/l	0.8	May	0.08	June
Phosphate	mg/l	1.6	April	0.03	November
Sulphate	mg/l	38.0	July	16.3	February
Calcium	mg/l	25.6	April	11.2	October
BOD	mg/l	4.7	August	0.3	July

Total suspended solids (TSS)

The value of TSS ranged between 13.8 and 513.9 mg/l on different sites of the present reservoir. Suspended solids cause ecological imbalance in the aquatic ecosystem by mechanical abrasive action. Suspended solids may be in the form of coarse, floating, fine or colloidal particles as a floating film. Maximum values reported in the present study during monsoon months at all study sites were due to increased surface runoff from nearby catchments. Most of the Indian reservoirs and rivers showed a similar tendency with respect to fluctuations of suspended solids.

Total solids (TS)

TS values were maximum on all the sites in rainy months which may be due to the gradual disturbances in sedimentation of solids as well as dust particles deposited along with runoff rainwater. The high amount of TS on all sites affects the quality of running water and it is unsuitable for any other purpose including irrigation and drinking. The dissolved solids in a reservoir depend on various parameters such as geological character of the watershed, rainfall and amount of surface runoff.

Alkalinity

Alkalinity of water is a measure of weak acid present in it and of the cations balanced against them. Alkalinity plays an important role in controlling enzyme activities. Maximum and minimum values of alkalinity on different sites of the present study showed variations in different months. Venkateswarlu (1969) attributed that there is an indication to suggest that alkalinity concentration is affected directly by rainfall. Similar effect has been noticed in the present investigation immediately often the onset of rains. The alkalinity of this reservoir indicated the productive nature of water as also shown in findings of Banerjea (1979) in a man-made reservoir of India. Man-made water bodies usually show wide range of fluctuation in alkalinity values depending upon a number of factors. According to Michael (1969), alkalinity concentration is affected directly by rainfall. In the present investigation also, alkalinity level reduced in the post-rainy months. Higher level of alkalinity during summer months as observed in the most of the sites has also been reported by Singh and Saha (1987).

Hardness

The water hardness on all study sites of Tehri dam reservoir was higher during summer months which might have caused increased concentration of salts by excessive evaporation as also observed by Bhatt *et al*. (1999). The hardness of river increases in the polluted waters by the deposition of calcium and magnesium salts. Since the study area is free from industrial pollution, the hardness was observed quite low, which was because of several calcium and magnesium salts coming from the mountain area. The hardness was positively related with rainfall on all the sites (r=0.1762 to 0.3547).

pH

The pH is affected not only by the reaction of carbon dioxide but also by organic and inorganic solutes present in water. Any alteration in water pH is accompanied by the change in other physico-chemical parameters. pH maintenance (buffering capacity) is one of the most important attributes of any aquatic system since all the biochemical activities depend on pH of the surrounding water. In the present study, the range of pH on the study sites was between 6.79 to 7.98. pH increased during summer months and decreased during monsoon and winter months. Maximum values during summer may be due to increased photosynthesis of the algal blooms resulting into the precipitation of carbonates of calcium and magnesium from bicarbonates causing higher alkalinity. The decrease in pH during winter may be due to decrease in photosynthesis, while during monsoon it may be due to greater inflow of water.

Dissolved oxygen (DO)

DO is a very important parameter of water quality and an index of physical and biological process going on in water. In the present study, the maximum concentration of dissolved oxygen was observed in the month of June after the snow melting due to heavy rainfall, which favours solubility of oxygen among the study sites. The highest concentration (8.96 mg/l) was recorded on site 2 but the range was not narrow for other sites. A definite trend in DO concentration was observed on all the sites showing highest values in June and lowest in September. DO is of great importance to all living organisms. It may be present in water due to direct diffusion from air and photosynthetic activity of autotrophs. Concentration of DO is one of the most important parameters to indicate water purity and to determine the distribution and abundance of various algal groups.

Chemical oxygen demand (COD)

COD is a measure of pollution in aquatic ecosystems. It estimates carbonaceous factor of organic matter. The range of values of COD in the present study was 5.9 to 39.4 mg/l. The maximum values of COD at sites 2 and 3 indicated the higher

degree of pollution compared to that of site 1. Higher concentration of COD in summer and rainy months may be due to high temperature and higher concentration of suspended and dissolved solids.

Biochemical oxygen demand (BOD)

BOD is the amount of oxygen required by the living organisms engaged in the utilization and ultimate destruction or stabilization of organic water (Hawkes, 1963). It is a very important indicator of the pollution status of a water body. The values of BOD clearly showed higher concentration during most of the summer and rainy months and comparatively low during winter months. Many workers like John (1952), Robert (1969) and Richard (1966) showed higher BOD during summer due to low level at river discharge. This is supported by the results of present study (0.3 to 4.7 mg/l) as the river had low flow during the winter season.

Total kjeldahl nitrogen (TKN)

TKN is a measure of organic nitrogen plus ammonical nitrogen. It plays as important role in the eutrophication of water along with phosphates. The values for different sites ranged from 0.27 to 4.48 mg/l which indicated no organic pollution. The main material source of TKN in aquatic ecosystem is the death and decay of the plant and animal remains which contains nitrogenous substances and is incorporated into the soil.

Ammonical nitrogen

Ammonical nitrogen reaches reservoir through diverse sources, major contributor being domestic wastes. Significant amount of ammonical nitrogen was recorded during March, April and May on all the sites, and in February and July on site 2 ranging showing a range from 0.06-0.076 mg/l. This was low because of no sewage pollution. It was mostly not detected on the sampling sites in most months of the year except summer months. In many samples it could not be detected. Most of the Indian authors have not investigated ammonical nitrogen in rivers.

Chloride

Chloride is one of the important indicators of pollution. Chlorides are present in sewage, effluents and farm drainage. The value of chloride concentration in the present study was highest on site 1 (20.3 mg/l) and site 3 (19.2 mg/l). These values are usually in the lower range of values for different rivers of India (Sabata and Nayar, 1995). The low value in the present study may be attributed to the absence of major pollutants.

Nitrate

Nitrate concentration depends on the activity of nitrifying bacteria which in turn get influenced by presence of dissolved oxygen. In the present study the values of nitrate ranged from 0.08 to 0.97 mg/l showing highest values in summer months and early monsoon on all the sites. This may be due to the higher phytoplanktonic production, decaying macrophytes and concentration of nutrients owing to the evaporation of reservoir water with subsequent increase in nitrate value. These observations have also been stressed by Epstein (1972) in his observations. Decrease in nitrate content during winter months was probably due to its utilization as nutrient by the algal community as evidenced by the luxuriant growth of algae particularly in the winter months.

Phosphate

The amount of phosphate on all the sites of reservoir is observed probably due to the presence and decomposition of aquatic vegetation which releases phosphate. The phosphate is an important constituent not only for the aquatic vascular plants but also for the growth of phytoplankton. Phosphate was found only in smaller amount on all sites. The low concentration of phosphate affects the growth of aquatic flora as it is very essential plant nutrient. The concentration of phosphate was more in summer during which the blooms of algae were observed, while minimum value in winter months was possibly due to its immediate utilization by the overgrowth of phytoplankton.

Sulphate

Sulphur is utilized by all living organisms in the form of both mineral and organic sulphates. The highest concentration of sulphates was observed during rainy season from July to September, which was caused by the surface run-off bringing into the river more suspended solids along with organic matter and soluble salts from the catchment area. The concentration of sulphate was positively related with rainfall showing higher value of correlation coefficent.

Calcium

Calcium is essential for all organisms and regulates various physiological functions. The calcium ions contribute to the hardness of water. The concentration of calcium was highest in the month of April on sites 1 and 3, and in May on site 2. The highest values of calcium were obtained on sites 1 and 3 than on site 2. The lesser amount of calcium was due to more presence of macrophytic vegetation which utilizes calcium as one of the nutrient as also due to large size of phytoplankton.

Coliforms (total and faecal) and total plate count

The luxurious growth of bacterial population during summer and monsoon months is the outcome at the influx of washed organic matter in the reservoir from the surrounding forest areas. It is natural that the incoming nutrient load finds its way first to the surface, thereby encouraging bacterial proliferation during monsoon. Collins (1963) has suggested that the rains bring in particulate matter, which serves as sites of adsorption for bacteria, thereby increasing the bacterial load.

In the present study, the maximum number of total coliforms was in the month of April and June. The minimum number was recorded in the month of January. Total plate count ranged between 15.3 to 94.6 colonies on the study sites. The higher values were recorded on sites 2 and 3 during summer season.

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Diversity, distribution and utilization of fodder species in sub-temperate, temperate and cold desert region of the Himachal Pradesh, north-western, Himalaya

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Abstract: Agriculture with animal husbandry is prevalent profession of rural people of Indian Himalayan Region. Livestock is considered one of the main sources of livelihood and integral part of livelihood, which rely mostly on fodder extracted from forests, grasslands, agriculture and agroforestry. The diversity, distribution and utilization pattern of the fodder species is important to prioritization of fodder species along an altitudinal gradient, and conservation and management practices of fodder species in both the Kullu and Lahaul valleys. Out of 67 fodder species, 43.28% were trees, 26.87% small trees and 29.85% shrubs, respectively. In general, maximum species were lopped annually, except *Olea ferruginea, Quercus floribunda, Q. leucotrichophora* and *Salix fragilis,* which were lopped an interval of 3 years. Majority of the fodder species are used as multipurpose and contributed to the high socioeconomic values. [Journal of American Science 2010;6(6):72-81]. (ISSN: 1545-1003).

Keywords: Diversity; agroforestry; fodder; utilization; conservation and management; north-western Himalaya

1. Introduction

In the north-western Himalaya, farmers maintain naturally regenerating tree species, particularly on edges of terraced agriculture fields without any significant input of manpower. This system is called as indigenous agroforestry system (Ram and Singh, 1996; Vishvakarma et al., 1998). The indigenous agroforestry system is a model of ex-situ and in-situ conservation that reduces the pressure from forest resources in terms of fodder and fuelwood along with site improvement (Ram and Ramakrishnan, 1988; Pathak, 1991; Maikhuri and Semwal, 1997). Tree fodder is valuable for temperate climate (Singh and Kanstra, 1981; Roder, 1992), particularly during winter months when green fodder becomes scarcely available in quantity (Khanal and Subba, 2001; Subba et al., 1994) and quality (Vishvakarma et al., 1998, Roder et al., 2003). Nearly 279 fodder species has been reported from the West Himalaya (Samant, 1998), which have been used to feed the livestock. The livestock is the integral part of the rural people of mountains. The major portion of fodder comes from forests, though some requirements of fodder are also met from grassland, agriculture and agroforestry systems (Purohit and Samant, 1995; Singh et al., 1998). The poor quality of fodder is inadequate to maintain the body weight of livestock (Roder, 1992).

Several tree species are used for fodder purposes in both the Kullu and Lahaul valleys. *Grewia oppositifolia, Robinia pseudoacacia, Morus serrata, Bauhinia variegata, Quercus leucotrichophora, Olea ferruginea, Pyrus pashia, Celtis australis* are common species in indigenous agroforestry system in the Kullu valley (Vishvakarma et al., 1998), and *Salix fragilis, S. alba* tree (Rawat et al., 2006) and shrubs such as *Fraxinus xanthoxyloides, Prunus cornuta, P. prostrata,* particularly in the lower parts of the Lahaul valley are important fodder species. The present study deals with diversity, distribution and utilization pattern of fodder species.

2. Material and Methods

Study area

The Kullu valley starts from Larji (957 m) in south and extends towards north direction along river Beas up to Rohtang pass (3978 m). This valley is about 80 km long and maximum 3 km wide near Bajaura. In the north Pir-Panjal ranges demarcates it from cold desert of the Lahaul-Spiti. Agricultural zone in the valley falls in between 957 to 2200 m. The cold desert of the Lahaul-Spiti district extends between 31°44’34” N & 32°59’57” N latitude and 76°46’29” E & 78°41’34” E longitude. The Great Himalayan range in the north and the Pir Panjal range of the Lesser Himalaya in the south demarcated the northern and southern boundaries of the Lahaul valley. The valley is accessible only during summers after clearing of snow from the Rohtang Pass. The Lahaul valley extends from Khoksar in the southeast direction to Tindi, near Udaipur town, in the

northwest direction. The elevation of this geographical entity ranged between 2400 to 6400 m above mean sea level. Agriculture in the valley is done on terraced fields. In large parts of the valley, willow and occasionally poplar trees were cultivated along the margins of the terraces under the indigenous agroforestry system.

Climate

The Kullu valley receives 112.5 cm annual average rainfall, which is fairly distributed throughout the year. From December to March mercury dips below freezing point during night and in these months, the valley experiences snowfall; the higher reaches of the valley have deposits of fairly good amount of snow. Average snowfall is ~68 cm in the valley region. Maximum temperature ranges from 0.9°C in January to 18.1°C in July (Figure 1a). On the whole, Kullu valley represents mid-hills and sub-humid sub-temperate (915 to 1523 m) and high hills with temperate wet (1524 m to 2472 m) agro-climatic conditions of the northwestern Himalaya.

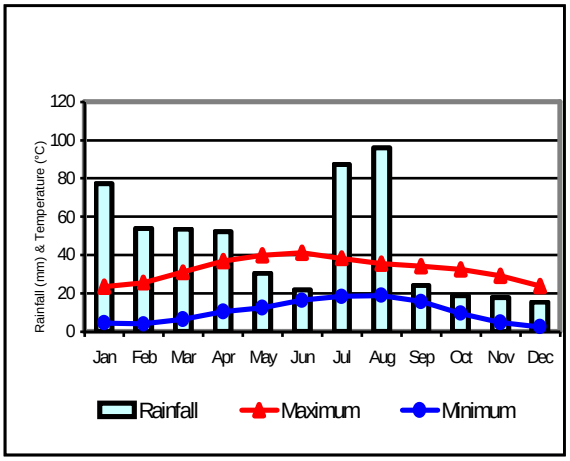

Figure 1a. Climate of the Kullu valley

Climatically, the Lahaul valley comes under cold arid zone with a very low rainfall and high snowfall, severe and prolonged winters. The region remains cut-off by high mountain ranges after heavy snowfall. The valley receives an average of 25 mm rainfall between June and July, and 3000 mm snowfall from November to May (Figure 1b). The valley has extremely harsh climatic conditions. The formation of soil in the region is sandy, alluvial and podsolic soils in the Kullu valley and sandy loam and moraine in nature in the Lahaul valley.

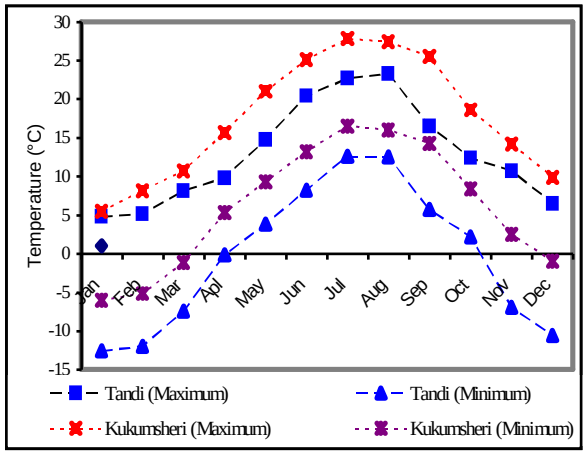

Figure 1b. Climate of the cold desert of the Lahaul valley

Study, data collection and analysis

The five indigenous agroforestry systems were selected for present study namely, Khokhan (1300 m), Bhosh (1700 m) and Bhanara (2020 m) in the Kullu valley, and Hinsa (2700 m) and Jahlma (3000 m) in cold desert of the Lahaul valley. The present study is based on the extensive and intensive surveys conducted in the representative parts of the Kullu and Lahaul valleys for documentation of all the fodder species found in Kullu and Lahaul valleys. The information on local names, altitudinal range, feeding season, life form and other uses was gathered with interviews held with local knowledgeable people (male and female). The collected bundles of fodder species were observed and species were identified with the help of local flora (Aswal and Mehrotra, 1994; Dhaliwal and Sharma, 1999). Fodder collection sites were also visited along with villagers during collection of fodder to identify the fodder species. The Nativity of the species was identified following Anonymous (1883-1970) and Samant (1998). Surveys were also carried out for seasonal lopping and utilization pattern of each species on the monthly basis. Species wise seasonal utilization pattern of fodder and mode of use (green and dry) were studied through direct observation.

3. Results and Discussion

Village systems

The altitudinal zonation of present study villages varied from 1300 m to 2020 m in the Kullu valley and 2700 m to 3000 m in cold desert of the Lahaul valleys (Table 1). The revenue areas under Bhosh and Hinsa villages were more or less equal about 33 to 34 ha, whereas, largest village in the area was Jahlma followed by Khokhan in Kullu valley.

The revenue of Bhanara was relatively smaller as compared to other villages. Number of households in the study villages were highest in Khokhan followed by Hinsa and lowest number of household were in Bhosh village.

Table 1. Location, revenue area, numbers of households and population of study villages in the Kullu and Lahaul valleys

	Village	Altitude (m)	Geographical locations		Area (ha)	House holds	Population (Person)	Land holding (ha/capita)
			Latitude	Longitude				
Kullu	Khokhan	1300	31°52 ’676 N	37°7’ 956 E	52.26	101	611	0.09
	Bhosh	1700	32°08 ’133 N	77°10’ 568 E	34.46	35	272	0.13
	Bhanara	2020	32°12 ’173 N	77°12 ’395 E	23.14	38	244	0.09
Lahaul	Hinsa	2700	32°41 ’367 N	76° 41’ 167E	33.28	52	386	0.09
	Jahlma	3000	32°38 ’226 N	76°52’ 009 E	58.27	41	479	0.12
	Total	-	-	-	-	267	1992	

Livestock

Agriculture with animal husbandry is prime occupation of native communities living in rural areas of the Kullu and Lahaul valleys. The oxen were reared for drought power for land tilling; however, yak was used for breeding purposes or transportation in the inaccessible areas (Singh et al., 1997). Cattle like jersey cow, desi cow, yak breeds, horse and ponies, mule, sheep and goat were common livestock in the study villages (Figure 2). Desi cow (local breed) is short in size and was lesser in number as compared to Jersey and *Brogar*. *Brogar* is a variety of cow originated after segregation of genes. *Brogar* originates after cross between jersey bull and desi cow.

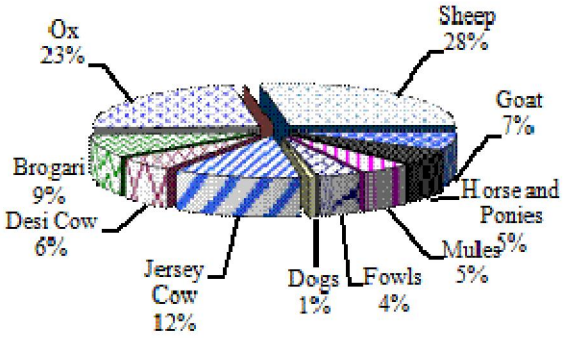

Figure 2. Composition of livestock in the study villages of the Kullu and Lahaul valleys

Brogar is slightly larger than desi cow and smaller than hybrid Jersey cow. Maximum number of *Brogar* was found in Khokhan followed by Bhanara village. Churi, a hybrid of yak and desi cow was fading in number due to introduction of Jersey cow in the Lahaul valley. Churies were nearly in half number of the desi cows. Oxen were relatively higher in number as compared to desi cow and churi. Sheep and goat were kept basically for wool and meat purposes in all the villages. These animals are supporting the livelihood and economy of the rural villages. In the entire Himachal as well as in Kullu valley, desi cow are being gradually replaced by Jersey cow due to their ability of higher milk production (Vishvakarma et al., 1998). Similar trend was found in cold desert of the Lahaul valley (Rawat et al., 2006).

Diversity, distribution, nativity and uses

An inventory of plant species of five indigenous agroforestry systems was prepared (Table 2). The distribution of fodder species along an altitudinal gradient ranged between 900-3800 m. The trees included deciduous and evergreen species and most of the fodder species are native to the Himalayan region and neighbouring countries. Out of 67 fodder species, 43.28% were trees, 26.87% small trees and 29.85% shrubs. Indigenous agroforestry system of Khokhan was dominated by *G. oppositifolia*. However, *Q. floribunda* was dominant species at Bhosh and Bhanara villages. The important fodder species in the Lahaul valley were *S. fragilis, Hippophae rhamnoides, Juglans regia, Populus nigra, P. armeniaca, P. cornuta* and *P. communis*. *S. fragilis* was a major contributor of fuelwood and fodder in cold desert of the Lahaul valley. *M. serrata, R. pseudoacasia* and *S. wallichiana* were common species found in all the three villages of Kullu valley, whereas, *S. fragilis, F. xanthoxyloides* and *P. cornuta* were common species at both villages of the Lahaul valley.

There was not any special tree plantation in the Kullu valley, exclusively for fuelwood and fodder purposes. However, in the Lahaul valley willow plantations are along the agroforestry and wasteland. Law established by the Department of Forest, Govt. of Himachal Pradesh, allow farmers for raising plantation on forest land with an aim to create greenery in the cold deserts. The farmers can use the produce of the plantation but ownership of the land will remains with department of Forest (Pandey, 1993-94 to 2006-07). However, such forest law does not bless people of the Kullu valley.

Utilization pattern

The utilization patterns of fodder species varied from season to season and from lower to higher elevations. The variation in utilization pattern is due to the availability of species in respective seasons. The most of the fodder species were used in summer due to availability of deciduous species; however, the availability of fodder was scarce during winter. There was specific seasonal lopping pattern for tree fodder species in the Kullu and Lahaul valleys (Table 3). In general, maximum species were lopped annually, except *O. ferruginea, Q. floribunda, Q. leucotrichophora* and *S. fragilis*, which were lopped an interval of 3 years. Shrubs were lopped on annual basis.

Nutritious tree fodder is important for oxen at time of field preparations, when, agricultural fields are tilled. The nutritive values showed that *C. australis* had 14.0%, crude protein, 50.8% nitrogen free extract and 11.9% crude fibre, G. oppositifolia had crude protein 10.1% nitrogen free extract 54.8%, calcium 4.2% and phosphorous 10.3%, *Q. leucotrichophora* had crude protein 9.5%, crude fibre 31.3% and nitrogen free extract 48.4% (Anonymous, 1970-1997; Purohit and Samant, 1995). The nutritive fodder is required to the animals for growth, maintenance, production and reproduction. It is depend on to intake, chemical composition and digestibility factors (Gutteridge, 1995). Therefore, these species are required priority attention for mass multiplication and conservation (Bisht et al., 1999). *Bombax ceiba, C. australis, Crataegus songarica, Malus baccata, Melia azedarach, P. cornuta, P. prostrata, P. pashia, R. pseudoacacia, S. wallichiana* and *Toona serrata* were used as fresh fodder during summer from June to November in the Kullu and Lahaul valleys (Table 3). Majority of the fodder species are used as multipurpose and contributed to the high socioeconomic values.

Tree species such as *Ficus palmata, M. serrata, Ulmus villosa* and *U. wallichiana*, were used both as fresh and dry in the Kullu valley; similarly *F. xanthoxyloides* is used both fresh and dry in the Lahaul valley (Table 3). Dry leaves of *Aesculus indica* and *Ficus palmata*, and fresh leaves of *G. oppositifolia, O. ferruginea, Pistacia integerrima, Q. floribunda, Q. leucotrichophora* in the Kullu and *S. fragilis, S. acmophylla* in the Lahaul valley were used during winter months from November to March. The bark of *S. fragilis* is peeled out from lopped branches and coppices and given to cattle as green fodder during winters. Shrubs were used as a fodder during summer. Fine twigs of horticultural crops are also used as fodder during winter. However, some farmers do not use apple twigs as fodder with a faith that their cattle may get cold diseases.

The leaves of *A. indica, C. songarica, Ficus palmate, F. xanthoxyloides, M. baccata, P. integerrima, P. prostrata* and *T. serrata* were fed to the sheep and goats, while, fodder obtained from the remaining species were given to all types of livestock. Shrubs like *Cotoneaster bacillaris, C. pruinosus, Prinsepia utilis, Rhamnus triqueter, Ribes grossularia, R. orientale* and *Rosa macrophylla* were also given to sheep and goat.

4. Recommendation and conclusions

1. The study indicated that *M. serrata* and *Q. floribunda* was key species for villages like Bhosh and Bhanara (temperate), where, pressure on forest resources was more due to conversion of grassland and agricultural land for horticulture. *S. fragilis* in the Lahaul valley (cold desert) and *S. wallichiana* in the Kullu valley (sub-temperate) were ecologically suitable species in their respective locality.
2. Studies on population, biomass, and identification of biotechnological measures to improve germination, propagation and dissemination of know-how to the farmers are required. In addition, analysis of nutritive value for fodder species is immediately required for assessment of quality fodder.
3. This study provides comprehensive information on diversity, distribution and utilization pattern of fodder species along an altitudinal gradient. Lopping and seasonal utilization pattern of fodder species is useful in understanding the mode of fodder use and lopping period. Capacity building and skill development of farmers are also required on mass multiplication, pollarding, coppicing, lopping and utilization pattern of fodder species.
4. Protected plantation of potential multipurpose fodder species are needed in wasteland, and need to initiate programme like afforestation, reforestation and forest rehabilitation with participatory approaches.

Table 2. Important plant species found under indigenous agroforestry systems of the Kullu and Lahaul valleys

S. No.	Name of species	Local name	Khokhan (1300 m)	Bhosh (1700 m)	Bhanara (2020 m)	Hinsa (2700 m)	Jahlma (3000 m)	Uses	Nativity	Altitude (m)
A. Trees										
1	*Abies pindrow* Royle	Rai	—	—	++	++	—	Fl., Ti.	Re. Himal.	2100-3500
2	*Aesculus indica* (Wall. ex Camb.) Hook.	Khanor	—	—	++	—	—	Fl., Fd., Med., Ed.	Reg. Himal.	1700-2500
3	*Alnus nitida* Endl.	Kosh	++	++	++	—	—	Fl, Ti., Agr. Imp., NF.	Reg. Himal.	1000-3000
4	*Bombax ceiba* L.	Semal	++	—	—	—	—	Fl., Fd., Med., Ed.	Am. Austr.	1200-2600
5	*Cedrus deodara* Don	Devdar	—	++	++	—	—	Fl., Ti.	Reg. Himal.	1800-3000
6	*Celtis australis* L.	Kharak	++	++	++	—	—	Fl., Fd., Ed.	Eu. As. Temp. Ind. Or.	800-2000
7	*Dalbergia sisoo* Rox	Shisham	++	—	—	—	—	Fl., Ti, NF	Ind. Or. Afghan.	900-1800
8	*Grewia oppositifolia* Roxb.	Beul	++	—	—	—	—	Fl., Fd., Fib., Ed.	Reg. Himal.	800-2000
9	*Juglans regia* L.	Akhrot	++	++	++	++	++	Fr., Ti.	As Occ. Reg. Himal.	1000-3000
10	*Juniperus macropoda* Boiss.	Shur	—	—	—	—	++	Inc., Aes.	Persia, Reg. Himal.	2600-4000
11	*Malus baccata* (L.) Borkh.	Lijo	—	—	—	++	++	Fl., Fd., Ed.	Reg. Himal. As Bor.	2400-3000
12	*Melia azedarach* L.	Drack	++	—	—	—	—	Fl, Fd., Med.	Reg. Himal. (alibicult)	1200-2600
13	*Morus serrata* Roxb.	Toot/Chenw/ Sahtoot	++	++	++	—	—	Fl., Fd., Med., Ed.	Reg. Himal.	1000-2200
14	*Olea ferruginea* Royle	Kahoo	++	—	—	—	—	Fl., Fd., Med.	Reg. Oriens	1200-2500
15	*Pinus wallichiana* A.B. Jackson	Kail	++	—	—	—	—	Fl., Ti.	Reg. Himal.	1700-3000
16	*Pistacia integerrima* (Stewart.)Rech.	Kakar Singi	++	—	—	—	—	Fl., Fd., Med.	China	1200-2500
17	*Populus ciliata* Royl	Poplar	++	++	++	—	—	Fl., Ti.	Reg. Himal. Illus	1800-3000
18	*Populus nigra* L.	Poplar	—	—	—	++	++	Fl., Ti.	Reg. Himal.	1500-3500
19	*Prunus cornuta* (Wall. ex Royle) Stued	Kurun/Jamu	—	—	++	++	++	Fl., Fd., Ed.	Europe As. Bor.	2400-3300
20	*Quercus floribunda* (Lindl.)	Mor	—	++	++	—	—	Fl., Fd., Ti., Agr. Imp.	Reg. Himal.	1200-3000
21	*Quercus leucotrichophora* A. Camus	Bon	++	++	—	—	—	Fl., Fd., Ti., Agr. Imp.	Reg. Himal.	1200-2500
22	*Robinia pseudoacacia* L.	Kikar	++	++	++	++	++	Fl., Fd., Rec.	Amer. Bor.	800-1500
23	*Salix wallichiana* Anderss.	Buins	++	++	++	—	—	Fl, Fd. Rec.	Reg. Himal.	1200-2000
24	*Salix fragilis* L.	Beli	—	—	—	++	++	Fl., Fd., Tim., Agr. Impl., Rec.	Europe As Bor.	2400-3600
25	*Sapindus mukoro* Gaertn.	Doda	++	—	—	—	—	Fl., Fd., Wa., Cl.	As. Trop.	Upto 1500
26	*Toona serrata* (Roy M. Roem.	Daral	++	++	++	—	—	Fl., Fd., Ti.	Malaya Australia	2000-2800
27	*Ulmus villosa* Brandis	Kashau/Hambe r	—	++	++	—	—	Fl., Fd., Ti.	Europe As Bor.	1200-2500
28	*Ulmus wallichiana* Planch.	Mahun	++	++	++	—	—	Fl, Fd.	Ind. or.	1000-2000
29	*Rhus punjabensis* Stewart ex Brandis	Karvi Copi	—	++	—	—	—	Fl., Med.	Reg. Himal.	1500-2200

B. Small trees										
1	*Citrus limonum* (RISSO)	Nimbu	++	++	—	—	—	Fr.	As Trop.	1000-2000
2	*Crataegus songarica* C.Koch.	Pingyat	—	—	—	++	++	Fl, Fd., Ed., Ti.	Europe as Temp.	2400-3500
3	*Ficus palmata* Forsk.	Phagra	++	++	++	—	—	Fl, Fd., Med., Ed.	Afr. Trop. Arab,; Ind. or.	800-2000
4	*Fraxinus xanthoxyloides* (D.Don) DC.	Chhum/Sanjal	—	—	—	++	++	Fl., Fd., Med. Agr. Imp.	Reg. Himal.	2400-3000
5	*Prunus amygda* Batsch	Badam	—	++	++	—	—	Fr.	Middle East	1500-2200
6	*Prunus armeniaca* L.[1]	Khumani	++	++	++	++	—	Fr.	Reg. Caucas	1500-3000
7	*Prunus armeniaca* L.[2]	Khumani karvi	++	++	++	—	++	Fr.		1500-3000
8	*Prunus avium* L.	Chery	++	++	++	—	++	Fr.	Reg. Himal.	2000-3000
9	*Prunus domestica* L.	Plum	++	++	++	—	++	Fr.	Europe.; Reg. Cauc.	1500-3000
10	*Prunus persica* (L.) Batsch	Aru	++	++	++	—	—	Fr.	Reg. Himal.	1500-3000
11	*Prunus prostrata* Labill.	Ralyo	—	—	—	++	—	Fl., Fd., Ed.	Reg. Mediterri; Oriens	2400-3300
12	*Punica granatum* L.	Anar	++	++	++	—	—	Fr.	Europe austr. Maurit	1000-2000
13	*Pyrus communis* L.	Nashpati	++	++	++	—	—	Fr.	Europe, As. Bor.; Reg. Himal.	1000-2000
14	*Pyrus malus* L.	Seb	++	++	++	++	++	Fr.	Europe, As. Bor.; Reg. Himal.	1300-3000
15	*Pyrus pashia* L.	Segal	++	++	++	—	—	Fl., Fd., Med., Ed.	Reg. Himal.	1000-2000
16	*Salix acmophylla* Boiss.	Jangli Beli	—	—	—	—	++	Fl., Fd.	Oriens; Ind. or	2400-3500
17	*Diospyros kaki* L.	Japani	++	++	++	—	—	Fr.	An. Bor.	1500-2500
18	*Cydonia oblonga* Mill.	Bee Dana	++	++	++	—	—	Fr.	Reg. Mediterr. Et Cauc	1500-2500
C. Shrubs										
1	*Berberis chitria* Lin	Kingor	++	++	++	—	—	Fl., Med., Ed.	Ind. or.	1000-2500
2	*Berberis jaeschkeana* Schneid.	Kyamali	—	—	—	—	++	Fl., Med., Ed.	Reg. Himal.	2400-3800
3	*Berberis pseudumbellata* Parker	Kyamali	—	—	—	++	—	Fl., Med., Ed.	Himal. bor. Occ	2400-3800
4	*Cotoneaster bacillaris* Wall. ex Lindl.	Ruins	++	++	++	—	—	Fl., Fd., Ti.	Reg. Himal.	2400-3800
5	*Cotoneaster pruinosus* Klotz.	Roktali	—	—	—	++	—	Fl., Fd.	Reg. Himal.	2400-3800
6	*Hippophae rhamnoides* L.	Sarla, Chharma	—	—	—	++	++	Fl., Fd., Ti., Med., Rec., Fen.	U.S.S.R, Afghanistan, India, W. Pakistan, Tibet, Mongolia	2400-3800
7	*Indigofera heterantha* Wall. ex Brandis	Kali Kathi	++	++	++	—	—	Fl., Fd.	Reg. Himal.	2400-3800
8	*Juniperus communis* L.var. saxatilis Pallas	Path/Bithar	—	—	—	++	—	Fl., Med.	Reg. Bor. Temp. et arit	2500-3800
9	*Lonicera hypoleuca* Decne.	Kharmu	—	—	—	++	++	Fl., Fd.	Reg. Himal.	2400-3500
10	*Prinsepia utilis* Royle	Bhenkul	++	++	++	—	—	Fl., Fd.	Reg. Himal.	900-2000

11	*Rhamnus triqueter* (Wall. ex Roxb.) Lason	Chamso	++	++	++	––	––	Fl., Fd., Ti.	Reg. Himal.	2400-3500
12	*Ribes alpestre* Decne.	Pilikcha	––	––	––	++	++	Fl., Fd.	Reg. Himal., Euop.; Afr. Bor.;	2400-3500
13	*Ribes orientale* Desf.	-	––	––	––	++	––	Fl., Fd.	Oriens; Reg. Himal.	2400-3500
14	*Rosa webbiana* Wall. ex Royle	Shyabala	––	––	––	++	++	Aes., Fl., Fd.	Reg. Himal.	2200-3500
15	*Rosa macroph* Lindl.	Kuja	++	++	++	––	––	Fl, Fd., Fen.	Reg. Himal., China	900-2200
16	*Rubus ellipticus* Smith	Aachha	++	++	++	––	––	Fl., Ed.	Ind. or.	1200-2200
17	*Salix daphnoides* Vill.	Jangli Beli	––	––	––	++	––	Fl., Fd., Ba.	Europe; As bor.	2400-3600
18	*Syringa emodi* Wall. ex Royle	Pashu	––	––	––	++	––	Fl., Fd.	Reg, Himal.	2400-3300
19	*Zanthoxylum arma* DC.	Timber	++	––	––	––	––	Fl., Med.	Reg. Himal. China	1500-2500
20	*Ziziphus oxyphylla* Edgew.	Ber	++	––	––	––	––	Fl., Ed.	Reg. Himal.	900-2000

1. Wild variety
2. Sweet variety grafted on wild variety
++= Presence of species
––= Absence of species

Abbreviations used: Reg. Himal.=Himalayan Region, Ind Or=Indian Oriental, Bor=Borealis, Temp=Temperate, Arct=Arctic, et=And, As=Asia, Centr=Central, Afr=Africa, Geront=Gerontia, Trop=Tropical, Amphig=Amphigaea, Austr=Australia, Amer=America, N. Zel.= New Zealand, Orient=Oriental, Cosmop=Cosmopolitan, Occ=Occidentalis, Afghan=Afghanistan, Turkist=Turkistan, Arab=Arabia, Subtrop=Subtropical, Hisp=Hispan, Min=Minor, Polynes=Polynesia, Madag=Madagascar, Alger=Algeria

Aes=Aesthetic, Agr. Imp. = Agricultural Implements, Ba. = Basket, Fen. =Fencing, Cl.=Cleaning, Ed.=Edible, Fd.=Fodder, Fl.=Fuel, Fib. =Fiber, Fr.=Fruit, Inc.= Incense, Med.=Medicinal, NF. =Nitrogen Fixing, Rec.= Reclamation, Ti=Timber, Was. =Washing

Table 3. Seasonal lopping period and utilization pattern of important plant species under agroforestry systems of the Kullu and Lahaul valleys

Name of Species	Vernacular name	Mode of use		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
A. Trees		Green	Dry												
1. *Aesculus indica* (Wall. ex Camb.) Hook.	Khanor		D	*	*								√	√	*
2. *Bombax ceiba* L.	Semal	G							√*	√*	√*	√*			
3. *Celtis australis* L.	Kharak	G								√*	√*	√*			
4. *Grewia oppositifolia* Roxb.	Beul	G		√*	√*									√*	√*
5. *Malus baccata* (L.) Borkh.	Lijo	G								√*	√*	√*	√*		
6. *Melia azedarach* L.	Drak	G								√*	√*	√*	√*		
7. *Morus serrata* Roxb.	Sahtoot	G	D	*	*				√*	√*	√*	√*	√*	√*	*
8. *Olea ferruginea* Royle	Kaw	G		√*	√*									√*	√*
9. Pistacia integerrima (Stewart) Rech.	Kakar Singi		D	*	*								√	√	*
10. *Prunus cornuta* (Wall. ex Royle) Stued	Kurun/Jamu	G									√*	√*	√*		

11.	*Quercus floribunda* (Lindl.)	Mor	G		√*	√*	√*								√*	√*
12.	*Quercus leucotrichophora* A. Camus	Bon	G		√*	√*	√*								√*	√*
13.	*Robinia pseudoacacia* L.	Kikar	G					√*	√*	√*	√*	√*	√*	√*		
14.	*Salix fragilis* L.	Beli	G		√*	√*	√*								√*	√*
15.	*Salix wallichiana* Anderss.	Buins	G						√*	√*	√*	√*	√*			
16.	*Toona serrata (Royle)* M. Roem.	Daral	G						√*	√*	√*	√*				
17.	*Ulmus villosa* Brandis	Kashau/Hamber	G	D	*	*					√*	√*	*	*		
18.	*Ulmus wallichiana* Planch	Mahun	G	D	*	*				√*	√*	√*	*	*		
B. Small trees																
1.	*Crataegus songarica* C.Koch.	Pingyat	G						√*	√*	√*	√*				
2.	*Ficus palmata* Forsk.	Phagra		D	*	*	*					√	√	*		
3.	*Fraxinus xanthoxyloides* (D. Don) DC.	Chhum/Sanjal	G	D	*	*	*		√*	√*	√*	√*	*	*		
4.	*Prunus armeniaca* L.[1]	Khumani	G		√*	√*	*							√*		
5.	*Prunus armeniaca* L.[2]	Khumani karvi	G		√*	√*	*							√*		
6.	*Prunus avium* L.	Chery	G		√*	√*	*							√*		
7.	*Prunus domestica* L.	Plum	G		√*	√*	*							√*		
8.	*Prunus persica* (L.) Batsch	Aru	G		√*	√*	*							√*		
9.	*Prunus prostrata* Labill.	Ralyo	G							√*	√*	√*				
10.	*Punica granatum* L.	Anar	G		√*	√*	*							√*		
11.	*Pyrus communis* L.	Nashpati	G		√*	√*	*							√*		
12.	*Pyrus malus* L.	Seb	G		√*	√*	*							√*		
13.	*Pyrus pashia* L.	Segal	G						√*	√*	√*					
14.	*Salix acmophylla* Boiss.	Jangli Beli	G		√*	√*							√*	√*		
C. Shrubs[a]																
1.	*Cotoneaster bacillaris* Wall. ex Lindl.	Ruins	G							√*	√*	√*				
2.	*Cotoneaster pruinosus* Klotz.	Roktali	G							√*	√*					

3.	*Hippophae rhamnoides* L.	Sarla, *Chharma*	G					√*	√*	√*	√*				
4.	*Indigofera heterantha* Wall. ex Brandis	Kali Kathi	G					√*	√*	√*	√*	√*			
5.	Lonicera hypoleuca Decne.	Kharmu	G					√*	√*	√*	√*				
6.	*Prinsepia utilis* Royle	Bhenkul	G								√*	√*	√*		
7.	*Rhamnus triqueter* (Wall. ex Roxb.) Lason	Chamso	G							√*	√*	√*	√*		
8.	*Ribes alpestre* Decne.	Pilikcha	G					√*	√*	√*	√*				
9.	*Ribes orientale* Desf.	-	G					√*	√*	√*	√*				
10.	*Rosa macrophylla* Lindl.	Kuja	G					√*	√*	√*	√*	√*			
11.	*Salix daphnoides* Vill.	Jangli Beli	G	√*	√*										√*
12.	*Syringa emodi* Wall. ex Royle	Pashu	G					√*	√*	√*	√*				

1 Wild variety
2 Sweet variety grafted on wild variety
√ = Lopping period
*= Seasonal utilization months
a= Most of shrubs are fodder of sheep and goat
G=green, D= dry

Acknowledgements:

We thanks to the Director G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora, Uttarakhand for providing necessary facilities to carry out this work. We are also thankful to Council of Scientific and Industrial Research (CSIR), New Delhi and Ministry of Environment and Forests, Govt. of India for financial support. Co-operation of villagers of the study area is highly acknowledged.

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3/8/2010

Light Hydrocarbon Correlation of Niger Delta Crude Oils

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ABSTRACT

The light hydrocarbon content of Niger Delta crude oils were studied with a view to providing a means of evaluating the Niger Delta petroleum system independent of higher molecular weight markers. Ultra high resolution gas chromatography was used in separation and analysis of the light hydrocarbons. Heptane ratio of oils ND-A3 (12.30), ND-A6 (12.07) and ND-B7 (10.33) were close and separate from ND-E5 (4.64). Invariance ratios and plot discriminated the oils into two groups. These apparent groups remained distinctly different in their graphical representation of ring preference. Star plots of oils ND-A3, ND-A6 and ND-B7 were shown to follow similar pattern, suggesting a strong similarity between them reflecting oil generation from same source rock, but followed different pattern from oil ND-E5 suggesting a negative correlation. These results strongly are consistent with two homologous sources for oils thus complementing the interpretations of higher molecular weight biomarkers and provide a quick and cost effective tool for correlation studies in Niger Delta, Nigeria. [Journal of American Science 2010;6(6):82-88]. (ISSN: 1545-1003).

Keyword: Niger Delta; Light Hydrocarbon; Invariance Ratio; Star Plot; Correlation.

1. Introduction

A good knowledge of petroleum system is usually an important factor in oil exploration in order to know whether one, two or more source rocks may have been responsible for crude oil already discovered. Since petroleum is generated from organic matter of fine grained rocks, it is possible to correlate crude oils having a common source but reservoired in different horizons (Osuji and Anita, 2005). Geochemists have used available technique to evaluate the Niger Delta Petroleum system. Part of the tools so far used include trace metal characterization of kerozen (Akinlua *et al*, 2007), bulk parameters and whole oil gas chromatographic (GC) fingerprints (Oyekunle and Famakin, 2004; Manilla and Eking, 2007), isotope and biological matter screening by gas chromatography / mass spectrometry (GC/MS) (Ekweozor *et al* 1979 a,b; Eneogwe and Ekundayo, 2003; Manilla and Eking, 2008). Ekweozor and Udo (1988) reported significant differences between Western and Eastern Niger Delta oils on the basis of the oleanane content, a pentacyclic triterpane. These same results were reported by Manilla and Eking (2008) using both saturates and aromatic biological markers and Onyema (2005) using multivariate plots of low molecular weight marker compounds.

Light hydrocarbons are an important component in petroleum and natural gas and they account for over 50% of the carbon in petroleum. It is widely believed that the light hydrocarbons are products of thermal cracking of higher hydrocarbons (Tissot and Welte, 1984), however Mango (1992) proposed transition metals as catalytic agents in the generation of light hydrocarbons. The light hydrocarbons have proven to be very effective in oil-oil and oil-source correlation (Mango, 1987, 1990, 1994 and 1997; Halpern, 1995; Ten Haven, 1996).

Mango (1990) showed an invariance in the ratios of the sum of concentrations of certain isoheptanes in crude oils regardless of their absolute concentration. These invariant ratios (k_1 and k_2) of isoheptanes are almost constant throughout hydrocarbon generation among homologous sets, namely sets of oils from a common source (Mango, 1997). Ten Haven (1996) however has shown differences in k_1 and used it in oil-oil correlation studies and particularly in oil-condensate and condensate-condensate correlations.

This paper examines the light hydrocarbon contents of some Niger Delta crude oils with a view to providing another means of evaluating its petroleum system independent of the higher hydrocarbon makers in order to provide a quick and inexpensive way of understanding its petroleum system.

2. Material and Methods

The Niger Delta is one of the world's largest tertiary delta system and an extremely prolific

hydrocarbon province. It is situated on the West African continental margin at the apex of the Gulf of Guinea (Doust, 1990). Rocks within the system are from paleocene to recent in age. The source rocks for crude oil in the Niger Delta are the marine shale Akata formation and the shale interbedded with paralic sandstone of the lower Agbada formation. One petroleum system has been identified in the Niger Delta province referred to as the tertiary Niger Delta (Akata-Agbada) petroleum system (Tuttle *et al.*, 1999).

Four crude oils were used for this study. The crude oil samples were collected from the Niger Delta region, Nigeria, by field technicians from the wellheads of producing wells. Two oil samples were collected from Akwa Ibom State, one from Rivers State and the fourth from Delta State and were labeled as ND-A3, ND-A6, ND-B7 and ND-E5 respectively. The light hydrocarbons were analyzed using the Hewlett Packard (HP) 6890 gas chromatography (GC) fitted to a fused silica capillary column (30m x 0.25µm) and equipped with a flame ionization detector (FID). Ultra high resolution gas chromatography oven temperature was programmed from 40°C to 140°C at 5°C/min with a 5min hold at 40°C and 20mins hold at 140°C. Light hydrocarbon peak identification was based on data presented by Mango (1987, 1990 and 1994) and area integration of each peak was processed by the HP chemstation software.

3. Results and Discussion

The normalized percent C_7 hydrocarbon distribution of the Niger Delta crude oil samples based on the area integration of the compound peaks are presented in figure 1. Methylcyclohexane was observed to be the most abundant light hydrocarbon in all the Niger Delta crude oils analysed. However the distribution profiles of the normalized percent C_7 hydrocarbons do not permit meaningful distinction between them. This led to the use of the C_7 hydrocarbon compounds. The basis for correlation of compounds is that these compounds should exhibit sample to sample variations that permit correlation and / or differentiation between the fluids.

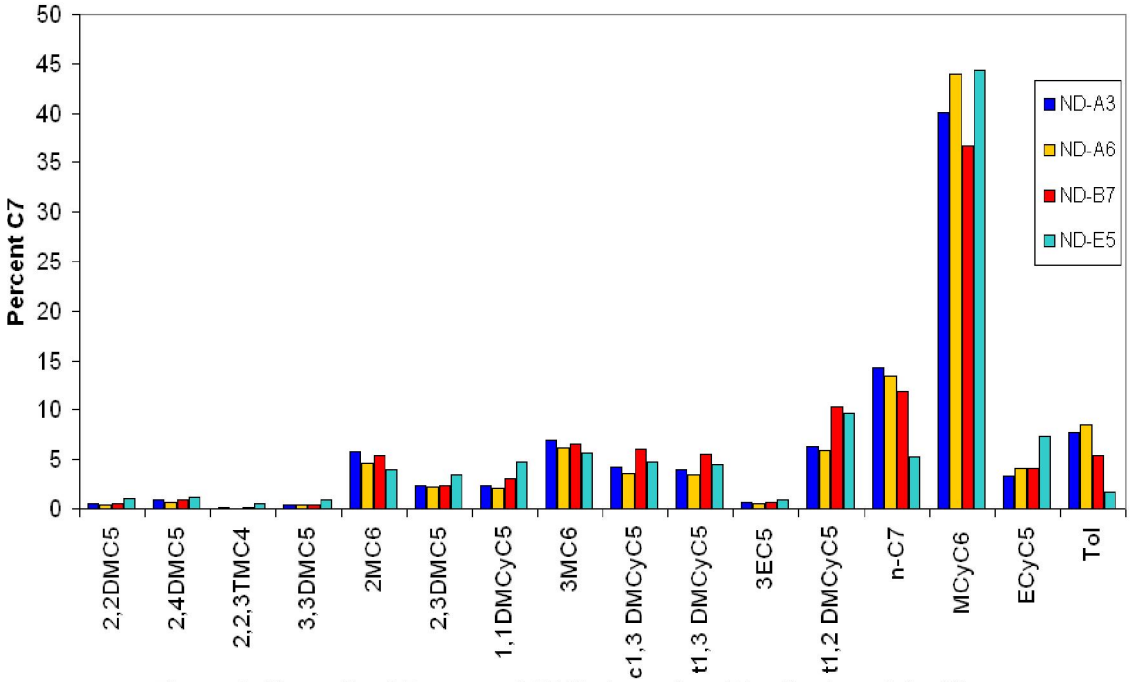

Figure 1: Normalized Percent of C7 Hydrocarbon Distribution of the Niger Delta Crude Oil Samples

Thompson (1983) used heptane ratio as indicator of source (kerogen type) of crude oil. Heptane ratios of Niger Delta crude oils range from 4.64 to 12.30 (Table 1). However, the heptane ratios of oils ND-A3 (12.30), ND-A6 (12.07) and ND-B7 (10.33) were close and separate from ND-E5 (4.64). These data show that crude oils from the Niger Delta are of at least two different sources.

The invariance of isoheptanes (k_1 and k_2) is very useful in oil correlation studies (Mango, 1990) as their ratio remains remarkably constant throughout hydrocarbon generation, regardless of their absolute concentration, for a set of oils from the same source.

$$k_1 = \frac{2MC6 + 2,3DMC5}{3MC6 + 2,4DMC5} \qquad k_2 = \frac{P_3}{P_2 + N_2}$$

Table 1: Summary of Light Hydrocarbon Characteristics of Crude Oils from the Niger Delta.

	Crude oil Sample ID			
Light Hydrocarbon Characteristics	**ND-A3**	**ND-A6**	**ND-B7**	**ND-E5**
Total C_7	6127.83	2981.80	10087.06	1194.35
n-C7	872.67	399.18	1195.51	63.22
MCyC6	2457.09	1309.08	3706.01	529.06
Tol	477.08	252.36	550.60	20.55
P_2	781.78	323.89	1209.04	116.26
P_3	305.47	124.98	496.03	94.39
N_2	640.06	271.89	1484.21	167.93
Heptane Ratio	12.30	12.07	10.33	4.64
k_1	1.02	1.00	1.03	1.08
k_2	0.21	0.21	0.18	0.33

Data is based on peak areas of the selected compound from the GC results.
P_2 = 2MC6 + 3MC6
P_3 = 2,2DMC5 + 2,4DMC5 + 2,2,3TMC4 + 3,3DMC5 + 2,3DMC5 + 3EC5
N_2 = 1,1DMCyC5 + c1,3 DMCyC5 + t1,3 DMCyC5

$$\text{Heptane Ratio} = \frac{n\text{-}C7}{(\sum CyC6 + C7\ HCs)} \times 100$$

The invariance ratio k_1 (table 1) for oils ND-A3, 1.02; ND-A6, 1.00 and ND-B7, 1.03 were close, but separate from ND-E5 which has a ratio of 1.08. These data suggests different sources responsible for the Niger Delta oils. The invariance ratio k_2 displays similar resolving power, consistent with different sources for the oils. Graphical representation of the invariance ratios (k_1 vs k_2) is presented in figure 2. The plot of invariance ratios discriminated clearly the studied oils into two distinctly different sources for crude oils in the Niger Delta.

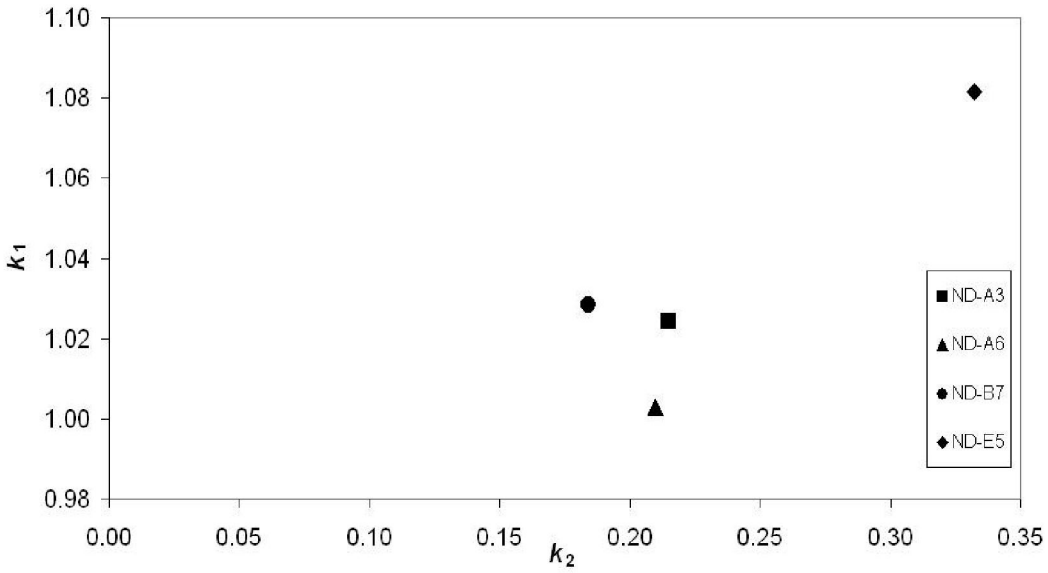

Figure 2: An Invariance Plot of k_1 and k_2 shows that Oils ND-A3, ND-A6 and ND-B7 constitute one distinct homologous oil set, which is different from ND-E5

This distinctly difference sources responsible for crude oil in the Niger Delta is also illustrated graphically by the ring preference plot of the oils (figures 3 and 4). The ring preference plots separate the oils clearly into two homologous sets thus supporting the fact that the invariance ratios in oils ND-A3, ND-A6 and ND-B7 remained tightly constrained and distinct form crude oil ND-E5.

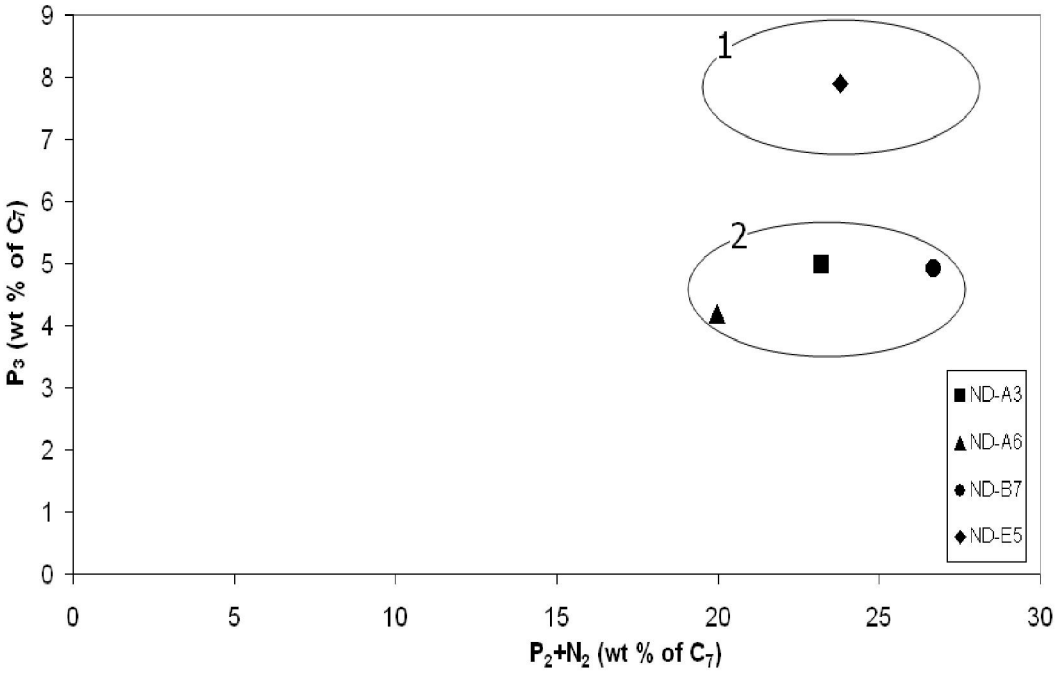

Figure 3: Ring Preference Plot of P_3 (wt %of C_7) and a sum of P_2 and N_2 (wt % of C_7)

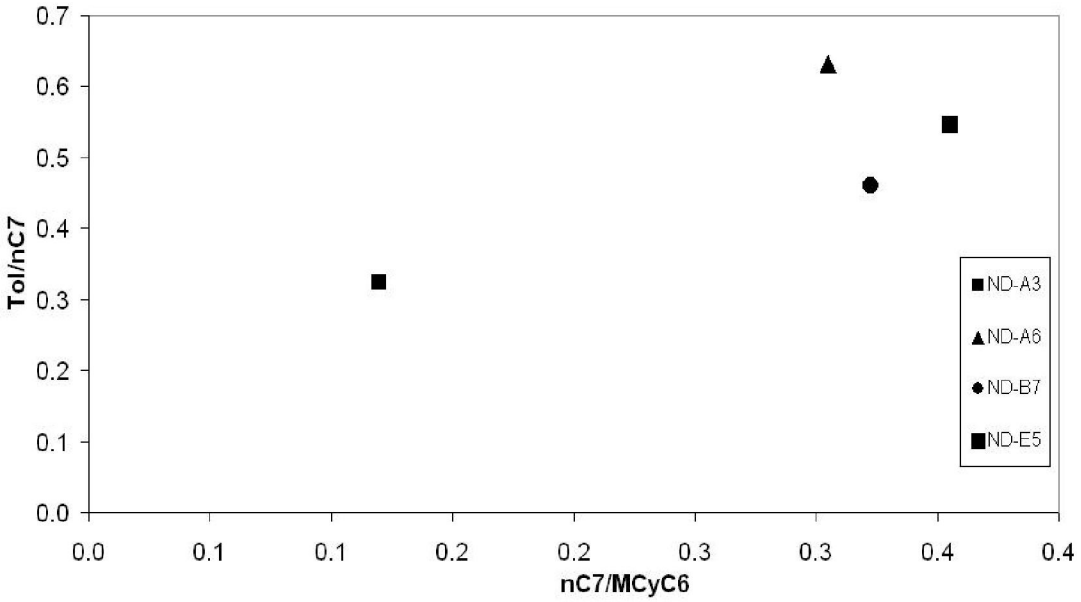

Figure 4: Cross Plot of Tol/nC7 and nC7/MCyC6 showing that Oil ND-E5 constitute a homologous set which is different from oils ND-A3, ND-A6 and ND-B7

Micro-scale correlation technique using gas chromatographic analysis of light hydrocarbons was used for the purpose of correlation and / or differentiation between the oils. The objective is to correlate the oils by comparing ratios of compounds. These comparisms are put in pictorial form of a star plot diagram to make correlation and / or differentiation of the Niger Delta oils easier. Star plots have been used to represent chemical compositions of oil and water samples from reservoirs, as well as correlation and / or differentiation (Halpern, 1995; Ali *et al*, 2002; Volk *et al;* 2005). For clearness, the star plot that will be used in this study will have nine (9) axis.

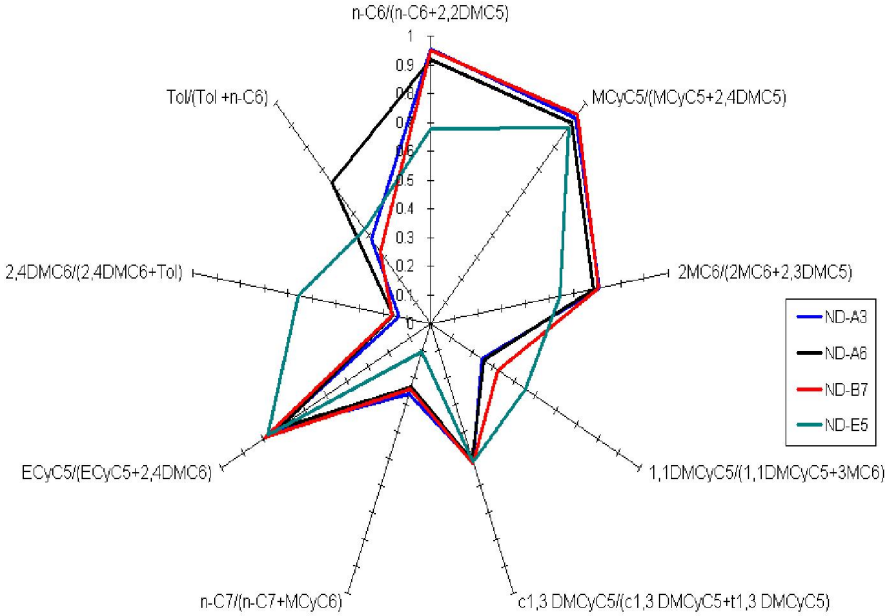

Figure 5: Star Plot of Selected Light Hydrocarbon Ratios showing the followed by the Niger Delta Crude Oil Samples

Figure 5 shows the star plot of all crude oils used in the study. Crude oils ND-A3, ND-A6 and ND-B7 were shown by their star plots to follow similar path. This suggests a strong similarity between the oils reflecting oil generation from the same source rock. However, differences were observed in oil ND-E5, which followed patterns that is different from other oils. This differentiation is in line with differences in source rock between the oils (Ali *et al*; 2002). The oils ND-A3, ND-A6 and ND-B7 correlated positively amongst themselves and negatively with oils ND-E5. The light hydrocarbons exhibited good resolution for the Niger Delta crude oils and thus useful in oil correlation studies of the Niger Delta.

4. Conclusion

Light hydrocarbon ratios and plots prove a powerful tool for correlating Niger Delta crude oils. The ratios and plots are based on the analysis of light hydrocarbons present and separated by ultra high resolution gas chromatography (UHRGC) without any pretreatment. The data presented here (invariance ratios, graphical representations and star plots) indicated two source rocks responsible for crude oils in Niger Delta, thereby complementing the interpretations based on the higher molecular weight hydrocarbons.

Acknowledgements:

I am grateful to the facilities and support provided by Exxon Mobil Co. I am equally grateful to Leo C. Osuji and Chidi I. Eneogwe for introducing me to geochemical markers and light hydrocarbon research.

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12/27/2009

Development of a Web Availability Analyzer Software Tool

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Abstract: In this study, results of the development of a web availability analyzer software tool that has been designed in order to measure internet availability from the end user's perspective are reported. The measured results of the availability of local and international sites along with a comparison of results indicate the successful operation of the software tool. The main objective of this paper is to present the approach used to measure the actual availability of internet sites through the development and use of a Web Availability Analyzer software Tool (WATT). [Journal of American Science 2010; 6(6):89-95]. (ISSN: 1545-1003).

Key words: Web, Internet Availability, Software package

Introduction

The use of the internet has become so wide-spread that it covers almost every aspect of human life today. Acts such as banking, payment of bills, shopping, personal and family affairs such as e-mail and community memberships, etc. are relying on computers and the internet more and more. Therefore, the internet has become very vital in man's economic and social life.

Hence, the various issues related to internet and its performance have become of great interest to researchers. One important issue is internet reliability measured by its availability. There are both theoretical and experimental approaches to compute or measure internet availability. The focus of this research is the steps taken in the development of a software package used for the measurement and statistical analysis of actual availability of web sites on the internet.

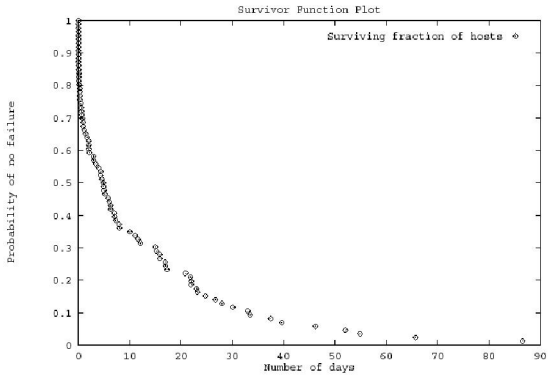

Fig 1 Time to failure extracted from wtmp file adopted from Sriram (1993)

Statistical approaches to measure the availability of the internet have been used since 1991. These approaches include analysis of wtmp file as shown in Fig.1, tattler, ICMP ping, etc.

Tattler System Monitoring Approach

Long (1992) proposed the Tattler network monitoring system as shown in Fig 2.

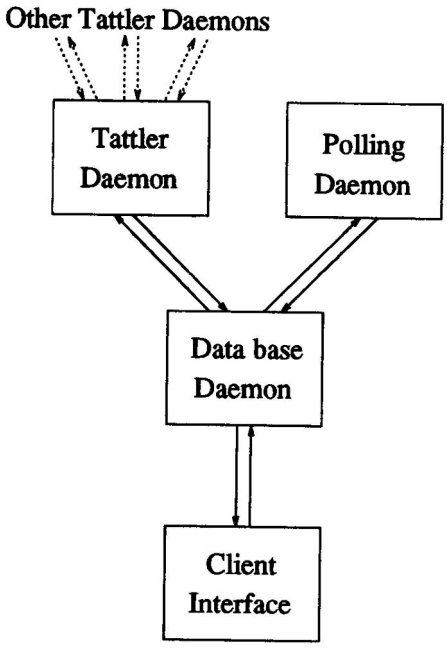

Fig 2 The structure of the Tattler system

Each tattler is composed of a client interface, a polling daemon, a data base daemon and a tattler daemon.

The tattlers are connected to hosts in several locations as shown in Fig 3. The tattlers making up a group are responsible for maintaining a list of hosts to be monitored plus preparing logs. The tattler daemon communicates the logged information to other tattlers so that a consistent log is kept across the network. The drawback of this approach is that the data obtained is invalid when the system itself or its communication lines fail.

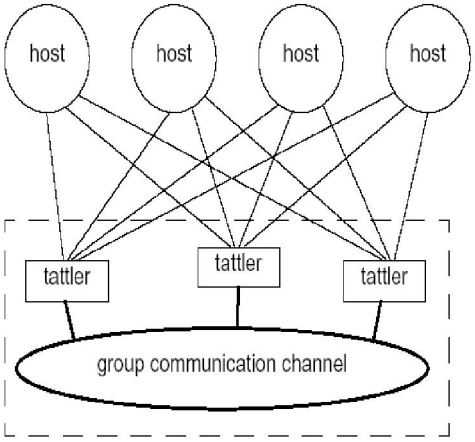

Fig 3 The structure of the Tattler system adopted from Long (1992)

ICMP Ping

ICMP ping may be used to check a remote host for its availability as shown in Fig 4. Local hosts should respond to such ping requests within a few milliseconds, but it may take them longer if there is heavy traffic on the network. Sending an ICMP Echo request and a consequent ICMP timeout may be used to indicate unavailability of the target site as perceived by the requesting system.

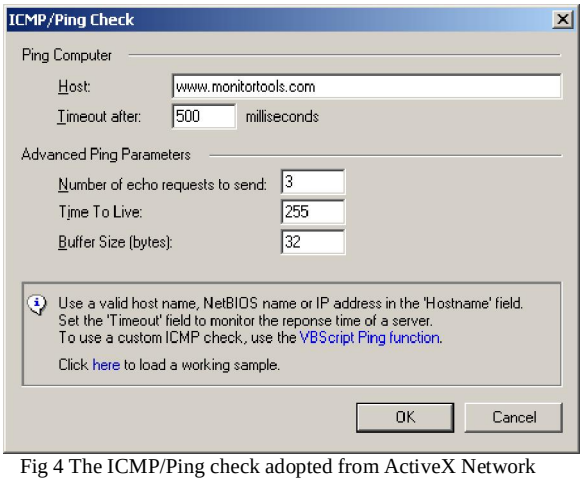

Fig 4 The ICMP/Ping check adopted from ActiveX Network Monitor

The Development of Web Availability Analyzer Tool Software

This software tool was developed using C# computer programming language. This tool is indeed a simulation of a browser that sends its requests to the various web sites and records the results. It analyzes the results at the end of each day. The main screen of the WAAT software tool is presented in Figure 5.

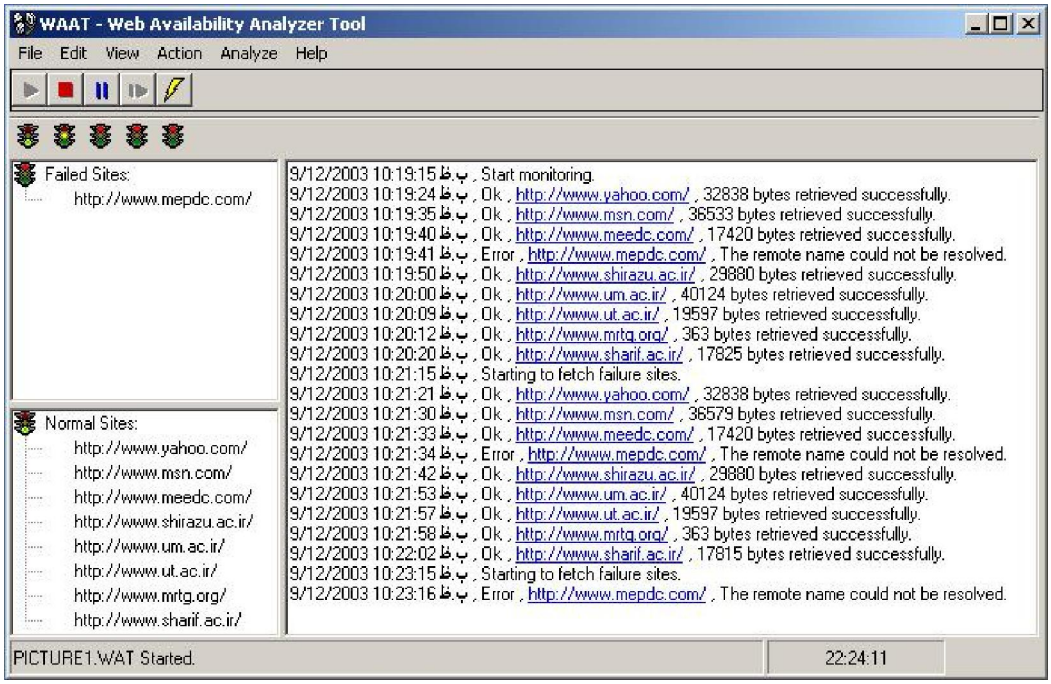

Fig 5 – The main screen of WAAT software

This software tool is composed of the following subsystems:
1) Main Menu
2) Action Bar
3) Internodes Bar
4) Status Windows
5) Log Window
6) Status Panel

The main menu consists of File, Edit, View, Action (Start, Stop, Pause, etc), Resume, Analyze, and Help submenus.

There is an optional internodes bar that shows the status of intermediate nodes being analyzed through the ICMP ECHO protocol. These nodes are analyzed 5 times in each ten minute period. If all the Ping operations are successful, then a green light is turned on. But if this operation is only successful one or two times, then a yellow light is turned on. Finally, a red light will be lit in case there is no response at all to the ping operations. The intermediate nodes are usually routers in the network.

Status Windows

Two status windows are designed in this tool to enable the user to recognize the condition of the current status of the internet sites under study. The upper window shows the functioning sites and the lower window shows the malfunctioning ones. These windows are updated once every time a response is obtained by the statistical engine.

Log Window

The actions taken by the software are constantly reported in the log window. Each line consists of date and time followed by a report of the action taken. This window is updated rapidly since the software package developed is multithread and does not depend on the traffic encountered by the statistical engine.

Status Panel

The name of the current project and its status is recorded on the left hand side of the status window. The time of the system used by the statistical engine is recorded on the right hand side.

Project definition may be started by choosing the New Project submenu in the File menu as shown in Fig 6.

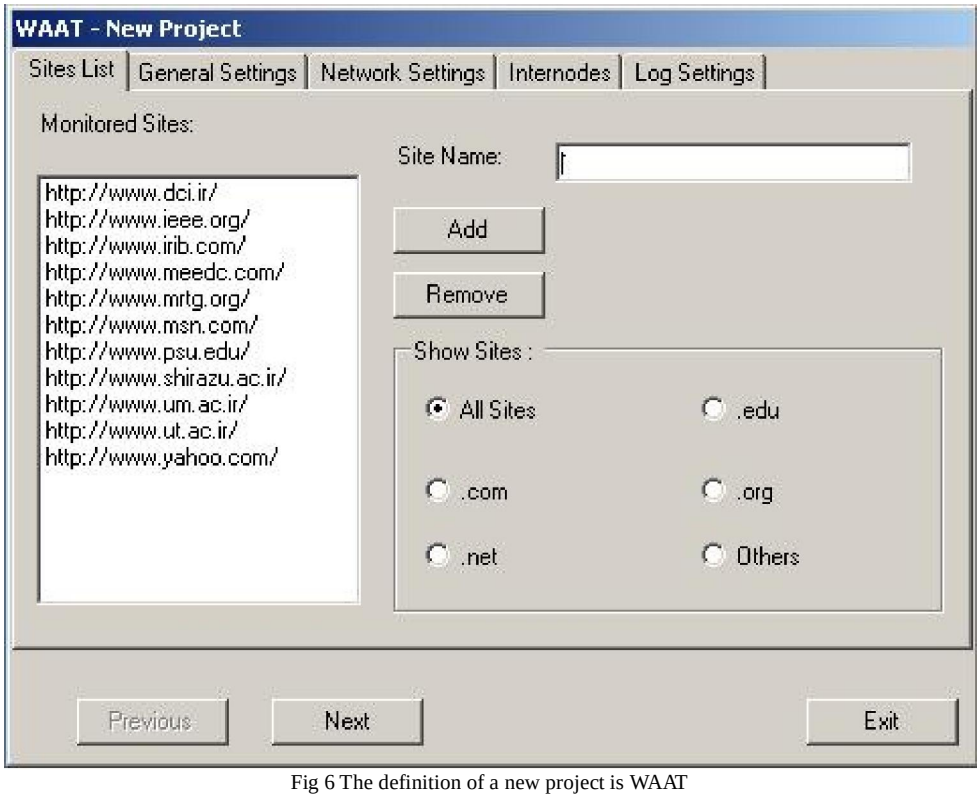

Fig 6 The definition of a new project is WAAT

This window consists of the following subsections:
1- Sites List
2- General Settings
3- Network Settings
4- Internodes
5- Log Settings

The general settings window may be used to

define the parameters that indicate the period between retrials for a malfunctioning site, the period between retrials for a functioning site and the number of successful trials to check a site to determine that it is available as shown in Fig. 7.

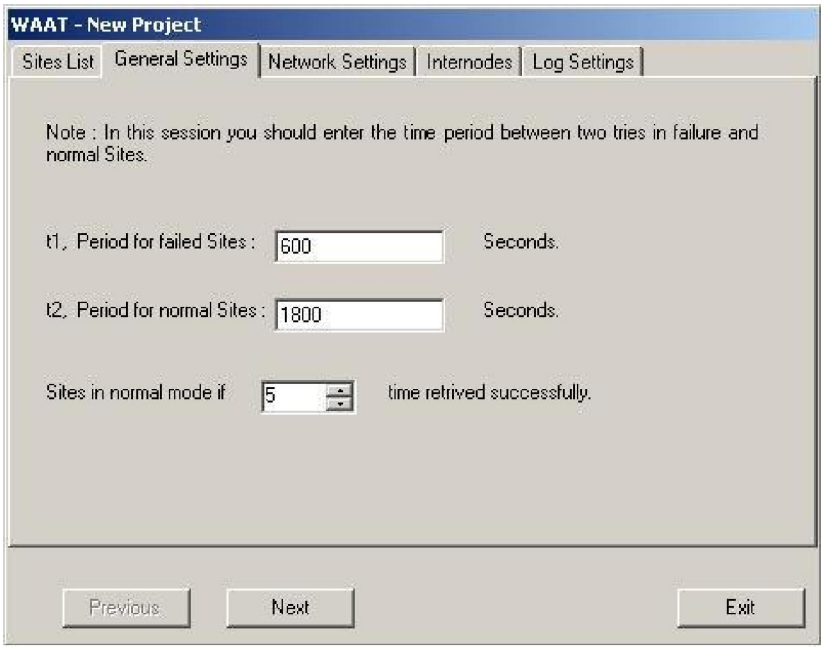

Fig 7 The General Settings window in WAAT

The Network Settings window may be used to set the output port used by the software in case there are several network adaptors in the system. The DNS server is also specified here so that the software does not use its TCP/IP settings as shown in Fig 8.

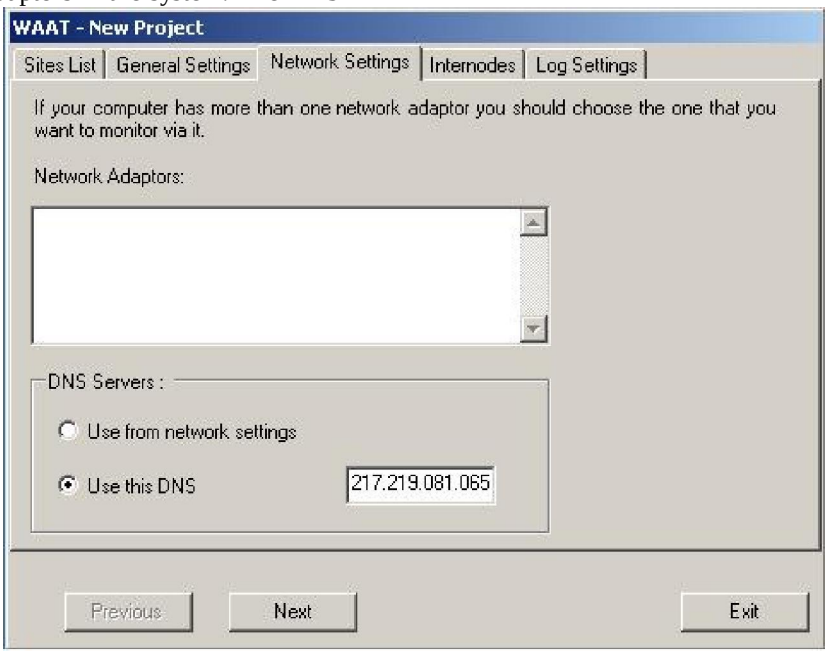

Fig 8 The Network Settings window

The WAAT software uses ping to check intermediate nodes. The maximum number of these nodes may be five and their IPs must be specified in the Internodes window as shown in Fig 9.

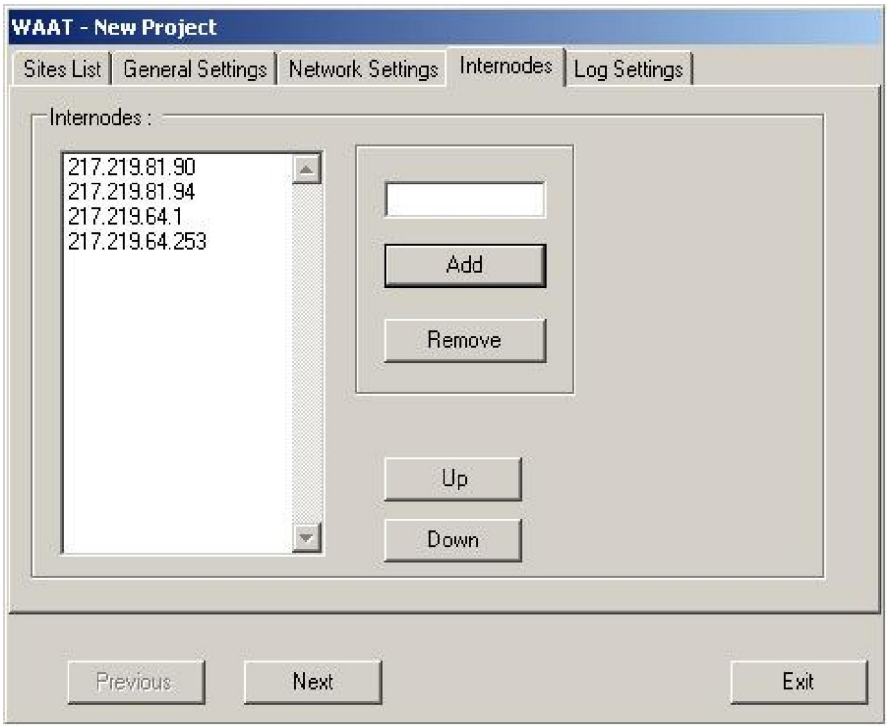

Fig 9 The Internodes window of WAAT

The type of desired log file may be entered into the software through the Log Settings window as shown in Fig 10. Possible options are Text Mode, MS Access Database and MS SQL Server Database. The time of day at which the contents of the log file should be analyzed is also stated in this window.

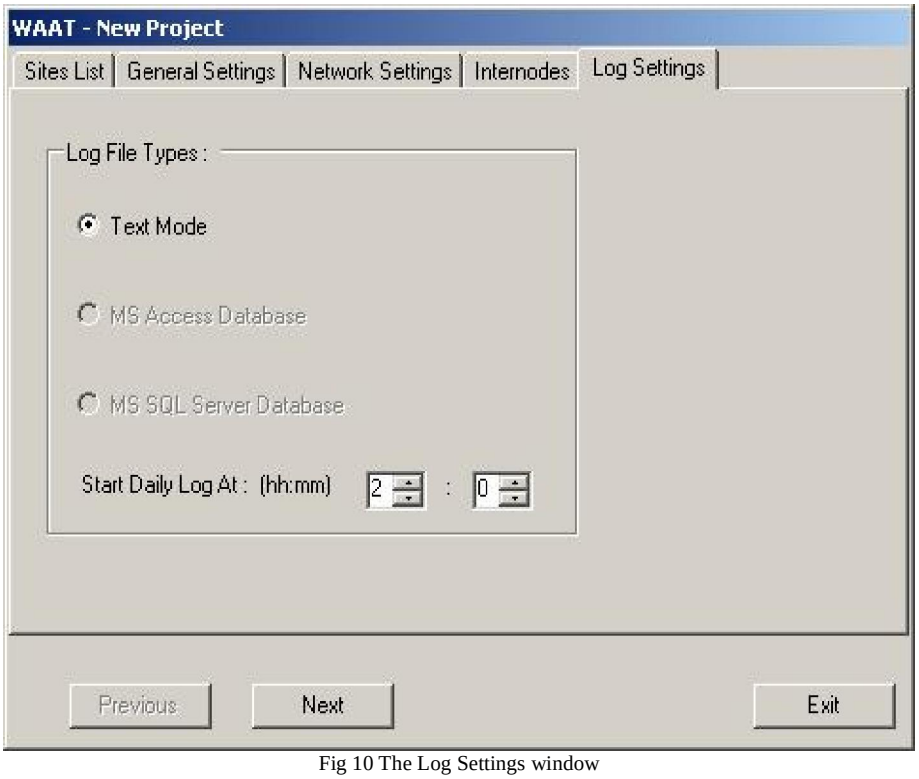

Fig 10 The Log Settings window

A project may be loaded and its data will appear as shown in Fig 11.

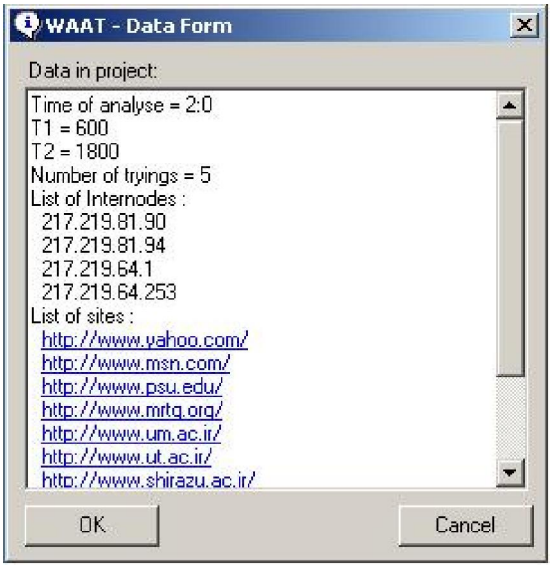

Fig 11 The data form of a loaded project

The software tool developed was tested and it generated a log file for each 24 hours. It was run for 90 days to measure reliability data for 159 Iranian hosts. The hosts chosen included 18 universities, 6 news agencies, 36 internet service providers, 29 government agencies and the rest were other public sites. This mix was chosen so as to obtain an average measure of intrinsic internet availability in Iran. The unavailability data measurement was executed from two different points of connection to the internet to remove any unavailability data related to the facilities of the measurement sites themselves so as to purely obtain the behavior of the hosts under study. We used ping to exclude failures related to intermediate lines and nodes, and thus eliminated any failures due to the internet backbone, too. Analysis of the log files revealed the following results as shown in Table 1.

Table 1 - The mean and median Availability values

Mean availability	0.865
	±0.007 (50% confidence)
	±0.012 (95% confidence)
	±0.016 (99% confidence)
Median availability	0.934
Number of hosts	159

Long et al. (1995) had reported a similar study with the following results after surveying 1170 hosts that were uniformly distributed over the name space and could respond to RPC polls for 90 days. Their results are shown in Table 2.

Table 2 - The mean and median Availability values

Mean MTTR	0.9260
	±0.002 (50% confidence)
	±0.007 (95% confidence)
	±0.009 (99% confidence)
Median MTTR	0.9723
Number of hosts	1162

Table 3 – A comparison of results for the survey on Iranian hosts and international hosts

Hosts	**MTTF (days)**	**MTTR (days)**	**Availability**
159 Iranian Hosts	21.56	3.375	0.865
1162 International Hosts	15.92	1.201	0.926

Table 3 shows a comparison of the results of the two studies. This indicates an average lower level of reliability for Iranian hosts, compared with international hosts indicating that a lot more work is needed for Iranian hosts to reach the average international availability levels.

Conclusions

The development of a Web Availability Analyzer software Tool is reported along with its real application to measurement of internet availability. The software developed simulates a browser and operates like a web client to make sure that the access to the desired host is truly measured. The program developed is reconfigurable and it performs statistical analysis of logged data to derive the desirable measures of internet reliability. The software developed uses multithreading and is suitable for the development of a tattler system. A comparison of results obtained for the availability of national web sites with that on international sites indicated a lower availability of national sites.

Acknowledgement

I would like to thank the Office of Vice Chancellor of Research and Technology of Ferdowsi University of Mashhad for the Grant Project No. 10448 dated 1388/10/2 to support the study that has resulted in the preparation of this manuscript.

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Integrated Application of Cocoa Pod Ash and NPK Fertilizer: Effect on soil and Plant Nutrient Status and Maize Performance – Field Experiment

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Abstract: Field experiment was conducted to study the effect of application of cocoa pod ash and its integrated application with reduced levels of NPK 20:10:10 fertilizer (NPKF) on soil and plant nutrient, growth and grain yield of maize at Ondo in the rainforest zone of south west Nigeria. There were 10 treatments involving a control, ash applied at 5 and 10 t ha^{-1}, 100, 200, 400 kg ha^{-1} NPK fertilizer and combined use of ash with 100 or 200 kg ha^{-1} fertilizer. The treatments were replicated three times on field and the residual effect (one year later) on soil and plant macro and micro nutrient concentration, growth and grain yield of maize was studied. The soil in the experimental site was deficient in organic matter (OM), N, K and Mg. Application of cocoa pod ash, NPK fertilizer and their combinations significantly ($p<0.05$) increased soil organic matter, P, K, Ca, Mg, plant N, P and K, height, stover, root and grain yield on immediate and residual basis. NPKF also significantly ($p<005$) increased soil and plant Fe, Cu, Zn and Mn. Ash also increased plant Ca and Zn. Combined application of ash with 100 or 200 kg ha^{-1} NPKF, and NPKF (400 kg ha^{-1}) gave similar and highest cumulative grain yield varying between 5.4 to 5.9 t ha^{-1}. The control, cocoa pod ash at 10 t ha^{-1} and NPKF at100 kg ha^{-1} respectively gave least cumulative grain yield of between 3.3 and 4.2t ha^{-1} for two years of study. The ash alone or combined with reduced NPKF gave highest residual effect on yield with increases of between 52 to 76% relative to control. [Journal of American Science 2010; 6(6):96-102]. (ISSN: 1545-1003).

Key words: integration, immediate and residual effect, yield

1 Introduction

In tropical countries, high cost and scarcity of fertilizer, nutrient imbalance and soil acidity are problems associated with use of inorganic fertilizers which led to recent interest in use of agricultural wastes as nutrient source in crop production. Also research has shifted to integrated application of organic and inorganic fertilizers. Some studies confirmed that combined application of these fertilizers gave superior effect in terms of balanced nutrient supply; improve soil fertility and maize yield compared with use of either of the fertilizers (Ajayi *et al*, 2007b). Other advantages of combined application of organic and inorganic fertilizers are that it would reduce the quantity of fertilizer required, and aid release of nutrient from organic sources.

Cocoa pod husk and its ash have not been adequately investigated in plant nutrition. Moyin Jesu (2007a, Ayeni, 2008a) after extensive literature search noted scarcity of report on use of cocoa husk in plant nutrition. Egunjobi (1976) found that ground cocoa husk applied to soil increased maize yield by 124%, also increased uptake of P, K and Mg. The effect of cocoa pod husk ash as nutrient source for kola seedlings was investigated (Ajayi *et al*, 2007a and 2007b). The cocoa pod ash increased growth and nutrient uptake by kola seedlings, and soil P, K, Ca and Mg were increased. Compared with NPK fertilizer, cocoa pod ash at 2, 4, 6, 8 and 10 t ha^{-1} increased root N, P, K, Ca and Mg with increased level of ash. The effect of animal manure amended cocoa pod husk on tomato was also studied (Ojeniyi, *et al*., 2007, Ayeni, 2008b). Amended husk significantly increased growth and yield of tomato.

About 800, 000 tonnes of cocoa pod husk are generated annually in Nigeria and often wasted. It is advised that the husk be burnt into ash as method of farm sanitation and for the control of black pod disease. The husk left on the farm harbours the fungus causing the black pod disease. This work studied the immediate and residual effect of integrated application of cocoa pod ash and NPK fertilizer in maize production with reference to effect on soil and plant nutrient uptake, growth and yield of maize.

2 Materials and methods

Field experiment was carried out on sandy clay Alfisol at Ondo (07^0 05^1 N, 04 55^1 N) in the rain forest zone of southwest Nigeria. The site was cultivated to maize, yam and cassava for more than ten years. The land was manually cleared and ridges with a spacing of 30cm x 75cm.

There were ten manurial treatments involving NPK 20:10:10 fertilizer (NPKF) at 100 (C0F100), 200 (C0F200), 400 (C0F400), kg ha^{-1}, NPK 20:10:10 fertilizer at 100 and 200 kg ha^{-1} were combined with 5 t

ha^{-1} of cocoa pod ash (C5F0, C5F100, CF200) and 10 t ha^{-1} (C10F0, C10F100, C10F200). There was a control without any manure or fertilizer (C0F0). The treatments were replicated three times on maize using a randomized complete block design. Each plot was 5m x 5m. Cocoa pod ash was incorporated using hoe at 2 weeks before planting in March 2005, and NPKF was applied in ring form immediately after hoeing at two weeks after planting. SUWAN maize type was planted.

Soil Analysis

Before commencement of experiment, surface (0 - 20 cm depth), the soil samples were collected inn zig - zag method over the site of experiment using auger, bulked, air – dried and 2 mm sieved for analysis. Samples collected over each treatment were also processed similarly. Chemical was done as described by Cater (1993). Organic matter (OM) was determined using wet dichromate method, total N by Kjedahl method. Available P as extracted using Bray -1- method, and determined by molybdenum blue colorimetry. Exchangeable K, Ca and Mg were extracted using ammonium acetate; K was read on flame photometer, Ca and Mg on atomic absorption spectrophotometer. The Mn, Fe, Zn and Cu were extracted using 0.1N HCl and read using atomic absorption spectrophotometer.

Leaf Analysis

Leaf samples collected from five randomly selected plants at 45 days after planting were dried at 65^{o} C to constant weight. The dried leaves were then ground to pass through 0.5mm sieve and chemically analyzed as described by Tel and Hagarty (1984). Total N was determined by Kjedahl method. For other nutrients, ground samples were subjected to wet digestion using 25 – 5 – 5 ml of HNO_3 – $H2SO_4$ – $HClO_4$ acids (AOAC, 1994). The digest was used for nutrient determination as mentioned for soil analysis. Cocoa pod ash was analyzed as described for leaf.

Yield components

At harvest (90days after planting), five plants were randomly selected and uprooted to determine plant height, roots separated from shoot to determine shoot and dry root matter. The parts were oven dried at 75 ^{o}C until constant weight. Forty maize plants were thereafter selected from the middle row and harvested. Cobs were air – dried, shelled and grain yield determined at 12% moisture content. Grain yield per hectare was calculated.

Residual Effects

In 2006, the experiment was repeated and the same treatments plots were maintained for maize planting without new treatments. Crop and soil analysis data were generated as for 2005 experiment in order to study effect of treatments at one year after the first experiment.

Statistical Analysis

The Duncan multiple range test was used to compare the mean data at 5% level.

3 RESULTS

The sandy clay soil at site the of experiment had 1.31% OM, 0.06% total N, 4.9 mg kg^{-1} available P, 0.16 c mol kg^{-1} exchangeable K, 2.32 c mol kg^{-1} Ca, 0.20c mol kg^{-1} Mg, pH (H_2O) 5.8, Fe 2.44 mg kg^{-1}, Cu 0.41 mg kg^{-1}, Zn 3.3 mg kg^{-1} and Mn 4.2 mg kg^{-1}. Using established critical levels for these nutrients (Kayode and Agboola, 1994, Agboola, 1994), the soil was deficient in OM, N, P, Mg, Cu, Fe and Mn with respect to maize production. This finding is in contrast with the widely held view that soils in the rainforest area were not deficient in these micronutrients. The deficiency in nutrient status is attributable to long term cropping of the land to staple crops such as yam, cassava and maize. Therefore, the test maize crop and the soil are expected to benefit significantly from cocoa pod ash and NPKF.

Cocoa pod ash had 14.52% OC, 0.68%N, 0.50%P, 11.9%K, 2.9%Ca and 0.40% Mg with C: N ratio of 12.3. This value of C: N is conducive for rapid degradation and dissolution of ash. Hence, it is expected that the nutrients in the ash would be released for uptake of maize. The ash is expected to release other nutrients such as micronutrients to the benefit of the soil and crop. Analytical data of cocoa pod husk given by Sobamiwa and Longe (1994) and cocoa pod ash (Ayeni *et al.,* 2008a and 2008b) showed that it contained, K, P, Mg and lower values of Zn, Fe, Cu and Mg.

Relative to control, cocoa pod ash, NPK fertilizer applied at 200 and 400 kg ha^{-1} and combination of ash with 100 and 200 kg ha^{-1} significantly ($p<0.05$) increased soil OM, K and Ca (Table 1 and 2).

Combined cocoa pod ash and NPKF significantly ($p<0.05$) increased soil N and Fe (Table 1& 2). One year after treatments application, all the treatments increased soil P and K relative to control. NPKF increased availability of Zn and Mn, which tended to increase with level of fertilizer. The fertilizer also significantly ($p<0.05$) increased soil Cu and Fe in 2005 and the elements increased with level of NPKF. Thus, NPKF tended to enhance presence of the micronutrients.

Addition of NPKF to cocoa pod ash significantly increased ($p<0.05$) soil OM, N, P, K, Mg, Zn and Fe in the fist year. The same observation applied to soil K and P one year after treatments

application especially with cocoa pod ash at 5t ha^{-1}.

Table 1: Effect of combined cocoa pod ash and NPK fertilizer on soil chemical properties in field experiment in 2005

Treatment	OM	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
	%	%	mg kg^{-1}	----------c mol kg^{-1}----------			----------mg kg^{-1} ----------			
C0F0	3.45bc	0.19b	8.21c	0.23c	3.82c	1.27ab	1.53b	1.12a	3.55d	21.23bc
C0F100	3.30c	0.18b	8.92b	0.28bc	3.82c	1.25b	1.33c	1.06b	3.90c	22.80b
C0F200	3.56b	0.19b	8.99b	0.28bc	4.00c	1.73a	1.72a	1.04b	4.31b	24.51b
C0F400	3.70b	0.19b	14.53a	0.31a	2.13d	0.93c	1.17d	0.62d	5.12a	36.72a
C5F0	3.38c	0.18b	9.10b	0.31a	4.26bc	1.13b	1.89a	0.62d	1.41f	18.78c
C5F100	3.62b	0.19b	10.52a	0.37a	4.08c	1.50a	1.18d	0.60d	2.70e	19.24c
C5F200	2.60b	0.21a	9.98ab	0.35a	3.68c	1.47a	1.17d	0.61d	2.85e	20.02c
C10F0	3.24c	0.17b	8.45bc	0.19c	5.23a	0.60d	1.21d	0.70c	0.75h	14.97d
C10F100	3.40c	0.18b	9.07b	0.36a	4.66b	1.03c	1.14d	0.25e	0.95h	15.89d
C10F200	4.08a	0.20a	10.05a	0.38a	4.50b	1.03c	1.16d	0.23e	1.64g	16.81d

Table 2: Residual effect of combined cocoa pod ash and NPK fertilizer on soil chemical properties in year 2005

Treatment	OM	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
	%	%	mg kg^{-1}	----------c mol kg^{-1}----------			----------mg kg^{-1}			
C0F0	2.24c	0.11b	7.91b	0.17c	1.95d	1.00b	1.04e	0.45c	6.16a	30.11a
C0F100	2.44c	0.11b	7.91b	0.17c	1.78d	1.03b	1.30c	0.84b	3.99c	20.32c
C0F200	2.32c	0.11b	8.00b	0.17c	2.01d	1.03b	1.19d	0.86b	3.25dc	18.88c
C0F400	2.94a	0.14a	9.20a	0.27b	1.47e	1.03b	1.61b	1.02a	4.00c	20.44c
C5F0	2.53c	0.12ab	8.60b	0.20c	2.64c	1.13a	1.64b	1.17a	5.04b	24.54b
C5F100	2.31c	0.11b	9.76a	0.21a	2.81b	1.10a	1.74b	1.14a	5.05b	22.88b
C5F200	2.60b	0.13a	7.68a	0.28a	2.45c	1.06b	1.64b	0.46c	3.09d	19.10c
C10F0	2.78b	0.14a	7.18a	0.26ab	3.03a	1.07b	1.96a	0.19e	2.94d	18.58c
C10F100	3.04a	0.15a	8.32b	0.34a	2.70b	1.07b	1.92a	0.16e	1.63e	16.05c
C10F200	2.76b	0.14a	8.05b	0.31a	3.05a	1.12a	1.91a	0.31d	3.09d	25.22b

Means with the same letters are not significantly different according to Duncan Multiple Range Test at 5% level

Cocoa pod ash, NPKF and their combinations significantly increased ($p<0.5$) plant N, P and K (tables 3 & 4). Ash at 5 t ha^{-1} alone combined with fertilizer significantly increased plant Ca and Zn. In the second year, cocoa pod ash applied alone or combined with NPK fertilizer increased N uptake. Addition of NPKF to ash increased plant P, K, Zn, Fe and Mn (at 10 t ha^{-1} only).

Table 3: Effect of combined cocoa pod ash and NPK fertilizer on maize nutrient concentration in 2005

Treatment	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
	---------- % ----------					---------- mg kg^{-1}--------			
C0F0	2.15c	0.36b	2.64h	0.26c	0.16ab	28.68f	5.33e	23.03c	26.80c
C0F100	2.61b	0.40ab	3.29g	0.27c	0.15b	30.15d	2.75i	22.57c	28.16b
C0F200	2.71b	0.46a	3.56e	0.35b	0.17a	25.43g	6.32a	25.20b	31.28a
C0F400	3.44a	0.47a	3.45e	0.30b	0.17a	21.72h	4.00f	27.80a	23.53d
C5F0	3.03a	0.38b	3.79d	0.49a	0.17a	30.70e	5.04b	19.97cd	26.40c
C5F100	2.94a	0.42a	4.27a	0.29c	0.16ab	33.30d	4.52d	22.57c	23.63d
C5F200	3.00a	0.42a	3.17g	0.52a	0.17a	30.20e	4.49e	23.10c	21.89e
C10F0	3.05a	0.33b	3.52e	0.40a	0.17a	39.67c	3.72g	15.40e	22.27de
C10F100	3.11a	0.38b	4.04c	0.26c	0.15b	49.82b	3.62h	18.53d	27.05c
C10F200	3.03a	0.43a	4.14b	0.29c	0.16ab	45.41a	3.68h	19.93d	27.65bc

Means with the same letters are not significantly different according to Duncan Multiple Range Test at 5% level

Table 4: Effect of combined cocoa pod ash and NPK fertilizer on maize nutrient concentration after one year of application

Treatment	N	P	K	Ca	Mg	Zn	Cu	Fe	Mn
	----------% ----------					----------mg kg^{-1}----------			
C0F0	1.43b	0.26a	1.42b	0.20a	0.14a	35.35b	4.68a	18.22a	16.92bc
C0F100	1.38d	0.25a	1.39c	0.18a	0.13a	23.54d	4.12a	17.07b	17.43a
C0F200	1.40b	0.25a	1.42b	0.19a	0.12a	29.75c	4.47a	16.68b	20.00a
C0F400	1.34b	0.25a	1.36a	0.17a	0.12a	19.69e	3.56a	18.00a	22.50a
C5F0	1.39c	0.24a	1.44b	0.19a	0.12a	32.42b	4.39a	20.04a	15.34c
C5F100	1.49a	0.23a	1.44b	0.19a	0.11a	31.75b	4.20a	19.70a	9.54d
C5F200	1.55a	0.23a	1.45b	0.19a	0.10a	34.21b	3.95a	19.65a	10.74d
C10F0	1.40b	0.23a	1.40b	0.17a	0.10a	30.95bc	3.18ab	17.62b	9.97d
C10F100	1.67a	0.21a	1.50a	0.18a	0.10a	43.37a	2.94b	18.65a	10.83d
C10F200	1.51a	0.22a	1.43b	0.17a	0.10a	32.13b	3.69a	16.36c	10.23d

Means with the same letters are not significantly different according to Duncan Multiple Range Test at 5%

Relative to control, cocoa pod ash, NPKF and combination of reduced level of NPKF with the ash increased significantly ($p<0.05$) plant height, stover yield, root yield and maize grain yield in first year and second year (Tables 5 and 6). However, the ash at 10 t ha^{-1} did not increase yield significantly. Also, the NPKF at 100 and 400 kg ha^{-1} did not increase yield significantly one year after treatments application.

On immediate basis, NPKF at 400 kg ha^{-1}, ash (5t ha^{-1}) + NPKF at 100kg ha^{-1}, ash (10t ha^{-1}) + NPKF 200kg ha^{-1} and NPKF 200 kg ha^{-1} respectively gave highest grain yield increases being 116, 91, 80 and 65% respectively over the control. One year after treatments application, ash (10t ha^{-1}) + NPKF 200kg ha^{-1}, ash 10t ha^{-1} + NPKF 100 kg ha^{-1}, ash 10 t ha^{-1} and ash 5 t ha^{-1} + NPKF 100 kg ha^{-1} gave highest increases respectively being 76, 63, 58 and 52% respectively. The ash 5t ha^{-1} + NPKF 100kg ha^{-1}, NPKF 400 kg ha^{-1}, ash 10 t ha^{-1} + NPKF 200 kg ha^{-1}

and ash 5 t ha^{-1} + NPKF 200 kg ha^{-1} a respectively gave highest and similar cumulative grain yield of 5.85, 5.60, 5.54 and 5.40 t ha^{-1} respectively. The control, ash at 10 t ha^{-1}, NPKF at 100 kg ha^{-1} and ash at 5 t ha^{-1} respectively gave least cumulative yield of 3.3, 4.2, 4.4 and 4.6 t ha^{-1} respectively. By combining cocoa pod ash at 5 t ha^{-1} with NPKF at 100 kg ha^{-1}, the requirement for the fertilizer in maize production is reduced by 75%.

Table 5: Effect of cocoa pod and NPK20:10:10 combinations on agronomic parameters of maize in 2005

Treatments	Height cm	Grain yield t ha^{-1}	Stover yield t ha^{-1}	Root Yield t ha^{-1}	increase in grain yield%
C0F0	162.12b	1.98d	3.83c	1.23e	0
C0F100	204.18a	2.95bc	4.61b	1.23e	25
C0F200	221.40a	3.27b	5.12a	1.81b	65
C0F400	204.67a	4.27a	5.42a	2.03a	116
C5F0	184.22c	2.84c	4.39b	1.33e	43
C5F100	201.68a	3.79b	4.68b	1.46d	91
C5F200	200.11a	3.67b	4.65b	1.69c	49
C10F0	178.70b	2.04d	3.07e	0.87g	39
C10F100	198.67b	2.76c	3.60d	0.90f	59
C10F200	207.00a	3.15b	4.15b	1.31e	80

Means with the same letters are not significantly different according to Duncan Multiple Range Test at 5%

Table 6: Residual effect of cocoa pod ash and NPK20:10:10 on agronomic parameters of maize after one year of application

Treatments	Height cm	Grain yield t ha^{-1}	Stover yield t ha^{-1}	Root Yield t ha^{-1}	increase in grain yield%
C0F0	143.44b	1.36c	2.30cd	0.20c	0
C0F100	154.17a	1.40c	2.08e	0.38c	3
C0F200	165.44a	1.63b	2.26d	0.34c	20
C0F400	168.33a	1.38c	2.08e	0.34c	2
C5F0	163.11a	1.73b	2.37c	0.24c	27
C5F100	178.22a	2.06ab	2.87b	0.30c	52
C5F200	168.56a	1.68b	2.49c	0.41b	19
C10F0	161.56a	2.15a	2.97a	0.44b	58
C10F100	173.22a	2.22a	2.91b	0.47b	63
C10F200	168.67a	2.39a	2.77b	0.79a	76

Means with the same letters are not significantly different according to Duncan Multiple Range Test at 5%

Although, the cocoa pod ash increased stover and grain yield significantly, NPKF at 400 kg ha^{-1} gave higher yield in first year. But one year after treatments application, cocoa pod ash gave higher stover and grain yield than NPKF the accumulative grain yield were 4.2 t ha^{-1} for 10t ha^{-1} ash, 4.6t /ha for 5t ha^{-1} ash, 4.4 t ha^{-1} for PKF 100 kg ha^{-1}, 5.0 t ha^{-1} for NPKF 200 kg ha^{-1} and 5.6t ha^{-1} for NPKF 400kg ha^{-1}. Therefore NPKF at 200 and 400 kg ha^{-1} gave higher yield than cocoa pod ash but the yield are similar with 5 t ha^{-1} ash especially when combined wit 100kg ha^{-1} NPKF

4 Discussion

Cocoa pod ash, NPK fertilizer and their combinations (with reduced level of fertilizer) increased soil OM, P, K and Ca significantly and the combination increased N. One year later, all the treatments increased soil P and K this indicates that

the ash had effect on availability of P, and Ca. It was also found that addition of NPK fertilizer to the ash increased nutrient released from ash as indicated by increase in soil OM, N, P, K, Mg, Zn and Fe especially if the ash supplied at 5 t ha^{-1}. The NPK fertilizer increased the Cu, Fe, Mn and Zn status of soil whereas cocoa pod ash tended to reduce it. This is attributable to the fact that NPK fertilizer is known to increase soil acidity (Moyin Jesu, 2007a), a situation that favours availability of these micronutrients (Brady and Weil, 1999). Cocoa pod ash has been found to reduce soil acidity significantly and increase soil OM, P, K, Ca and Mg (Ayeni and Adeleye 2009,Ajayi *et al*., 2007a, Ayeni *et al*., 2008a). Moyin Jesu (2007a) also found that cocoa husk increased soil OM, N, P, K, Ca, Mg and pH. Similar observation was made by Moyin Jesu (2007b) and Ojeniyi *et al*., (2007) therefore the present work and other recent ones affirmed hat cocoa pod and it ash released N, PK Ca and Mg into soil, when used alone or combined with NPK fertilizer.

Application of cocoa pod ash alone, NPK fertilizer and their combinations increased plant N, P and K. ash alone increased plant Ca and had residual effect on N. Hence, the ash increased nutrient uptake. Previous studies had found that cocoa husk increased uptake of N, P, K, Ca and Mg by okra (Moyin Jesu, 2007a) and Arabica coffee seedling (Moyin Jesu, 2007b) . Addition of NPKF to cocoa pod ash increased plan P, K, Zn, Fe and Mn. This affirms the observation that NPK fertilizer in addition to the supply of N, P and K enhanced the availability of micronutrients in the soil. The combination of the two materials would have ensured a more balanced nutrition. While the ash was particularly effective in increasing K and Ca, The NPK fertilizer was particularly effective in increasing the availability of N, P and the micronutrients. The combined application therefore is expected to influence maize yield better than use of the fertilizer or cocoa pod ash alone.

Hence, it was found that, although the ash and fertilizer significantly increased maize growth parameters and yield on immediate and one year later, addition of NPK fertilizer to cocoa pod ash (especially at 5 t ha^{-1})gave higher cumulative yield covering the immediate and residual cases. Addition of the fertilizer to the ash which increased plant P, K, Zn, Fe and Mn, and soil O, N, P, K, Mg, Zn and Fe and increased grain yield, stover and root yield. The combined use of the materials gave cumulative yield higher than NPK fertilizer. However, the NPK fertilizer gave higher yield than the ash or combined applications in the first year which is attributable to quicker release of N, P and K than from the organic ash source. The ash unlike the mineral fertilizer had residual effect n plant N and increased plant Ca. It also ensured availability of K, Ca and Mg on immediate and one year later. The increase performance of maize due to cocoa pod ash is consistent with earlier finings (Ajayi *et al*., 2007a, 20007b) that cocoa pod ash increased growth of kola seedling significantly. Moyin Jesu, (2007a, 2007b) also found that ground cocoa husk increased growth and yield of okra and growth of coffee seedling respectively. The more balanced nutrition due to combination of NPK fertilizer and cocoa pod ash ensured high cumulative yield than use of ash or NPK fertilizer alone. In fact the ash alone gave least yield although which were higher than the control. The combination of cocoa pod ash with NPK fertilizer reduced need for the fertilizer to 100 or 200 kg ha^{-1}. If the ash was applied at 5t ha^{-1}, the requirement for NPK fertilizer becomes 100 kg ha^{-1} instead of the recommended 400 kg ha^{-1}. With the use of ash at 10t ha^{-1}, it was found that Zn and Cu were below the critical level of 3 ad 1g kg^{-1} respectively. Hence, the 5t ha^{-1} ash recommended to be used with 100kg ha^{-1} NPK fertilizer. Also the plant N, P, K and Mg were below the critical levels of 2.8%, 0.25%%, 1.73% and 0.2 – 0.4% respectively (Agboola, 1994) for the second crop of maize. Hence the yield of untreated second plant fell relatively to the first crop. Therefore it is necessary that the second crop receive NPKF since fertilizer has less residual effect compared with cocoa pod ash in this experiment. The residual effect recorded for NPKF relative to control was between 2 to 20%, whereas it was between19 to 76% for treatments involving ash alone or combined with NPK fertilizer

CONCLUSION

Cocoa pod ash at 5t ha^{-1} increased availability in soil and uptake of N, P, K, Ca and Mg by maize plant. This led to significant increases in growth and grain yield of maize. The combined application of the ash with reduced level (100 or 200 kg ha^{-1}) of NPK fertilizer was more effective in increasing cumulative yield of maize than NPK fertilizer or ash alone. However, NPK fertilizer at 400kg ha^{-1} gave higher yield than the ash at 5 or 10t ha^{-1} or its combined use with fertilizer on immediate basis. The ash had more residual effect on yield than NPK fertilizer.

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Acknowledgement:
I wish to express my profound gratitude to Professor M.T. Adetunji, Professor S.O. Olasantan and Dr. C. O. Adejuyigbe of College of Plant Sciences, University of Agriculture, Abeokuta, Ogun State, Nigeria for their supervisory role during the conduct of this research

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Submission Date 28/12/2009

Estimate Biological Nitrogen Fixation in horse bean

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Abstract : Research projects as split plot experiments in a randomized complete block design with four replications in field research in Islamic Azad University of Ahvaz 3 consecutive years (2006,2007,2008) implementation was the main plot assembly, four cultivar horse bean (*Vicia Faba*L.) plant: BARAKAT,ZOHRE,SHAMI and JAZAYERI, damascene the number of islands in the province have grown and sub-plots in the two years 2006 and 2007 three levels of nitrogen fertilizer (N1,N2 and N3 treatments, respectively 20 and 40 and 80 kg fertilizer N ha simultaneously planting) and the third year, 2008 values were doubled care. After the propagation earth, using cultivar with Rizobium bean plant (***Rh.Legominosarum***) inoculation and immediately cultured. Survey cultivar, BARAKAT highest percentage of mean total nitrogen plant 1.97 percent won. In sub-plots, with increasing amounts of nitrogen, accumulation of this element bean plants increased. Percent nitrogen treatments nodes N2 and N3 showed a significant difference, but the highest accumulation of nitrogen treatments N1 nodes with 1.67 percent won, thus whatever amount of fertilizer increased, the amount of biological nitrogen fixation nodes decreased. N3 treatment reduced accumulation of 40 to 50 percent nitrogen found in to other treatments. With increasing N rate, weight, number and size of the plant nodes decreased blessing average number of nodes 1250 nodes per plant among the highest number of cultivars grown offered. Number of nodes equal treatment and 1450 to increase the amount of fertilizer treatments 80 kg 998 nodes per plant decreased in all fertilizers in small amounts or how large gland enlargement process was observed. The mean largest tumor diameters in the treatment 1.98 cm were measured. Green and white non-effectiveness of enzyme Nitrogen's stated that usually the primary growth was achieved in pink and red and efficient biological nitrogen fixation, approximately 35 days after planting continued until after flowering and 10 days after flowering, gland Posts brown and black, showed the node representing aging and lack of nitrogen is established. [Journal of American Science 2010; 6(6):103-108]. (ISSN: 1545-1003).

Key words: biological nitrogen fixation, horse bean

1. Introduction

During the recent years in the world, food production and consumption of fertilizers has increased gradually. Demand for nitrogen fixation as the chemical and irregular increase is nearly twice (Table 1) due to the current energy crisis situation will be difficult. In addition, chemical nitrogen fixation in the field, since the fundamental solution to reduce the energy required for the traditional method (Haber - Bush) in the production of ammonia is not recommended. Biological nitrogen fixation can produce the crisis and to modulate the nitrogen fertilizer application. Identifying factors influencing production efficiency of this process can be beneficial and highly stabilized nitrogen increased. Province with more than 7000 hectares under cultivation Bean (2007) one of the major producing provinces of the product is high and nitrogen fertilizer application, average 300-250 kilograms per hectare to increase performance is common among farmers These values increased cost of nitrogen fertilizer plant as well as severe pollution to the River that shed all of the search, so the necessity of expanding and increasing the efficiency of biological fixation system, it is felt the product, according to the necessity of this study was to implement appropriate amounts of fertilizers and nitrogen are introduced improved varieties can be used to stabilize natural systems use high nitrogen fertilizer nitrogen prevents said (Table 2).

Table 1. The global need for nitrogen (million tons) during the coming years

Region / Year	1985	1990	1995	2000	2010
Developed countries	47	58	71	88	98
Indeveloping countries	25	33	43	54	70
World	73	92	115	139	178

Table 2. Estimate biological fixation nitrogen in world

countries	legume	year	BNF(Kg/ha)
India	pea	1979-82	16.6
India	Alfalfa	2004	72
Greece	Lens	1997	45
Pakistan	bean	1998	37
Brazil	Horse B.	2002	101
Australia	Pea	2005	20

2. Material and methods

This research farm research - Research, Islamic Azad University of Ahvaz Southern city of Ahwaz in 3 years were, where experiment and semi-arid climate is dry and the 40-year Meteorological Data Ahvaz 94/213 mm average annual rainfall, mean annual temperature of 24/25, the average maximum 92/32 annual temperature, average minimum annual 4 / 18 ° C is. Planting date mid every 3 years was before this date, disk and plow the earth and fire trowel and calcium phosphate fertilizer menu and then the earth was based classification map plots in the field experiment was performed in every plot of 24 square meters the bed took up 10 lines and culture based on the amount of nitrogen fertilizer treatments the tape stack was added. 3 -2 on the test weed weeds was conducted for disposal. Test plan as split plot randomized complete block design with four replications that included four main treatment plant bean varieties that are: blessing V1, Z. V2, SHAMI V3 and V4 figure JAZAYERI and sub-plots in the first two years 1383 and 2006, Kvass levels (N0 = 20, N3 = 80, N1 = 40 kg per ha) and the third year was double 2008 values were studied. reviews root cylinder method was performed by the full scoop enough of the node number and diameter of root parameters of (BARAKAT) were measured and cut Posts tumor diagnosis was inside color. some plant gland intact shoots, including leaves and stems for the estimated amount of nitrogen using Kjldal was sent to the laboratory. and also using the root Newman and graduated cylinder method of water transport, root volume was measured.. before the implementation experiment to evaluate soil field sampling of the depth of 15-0, 30-15 and 60-30 cm was 15 and a total analysis of soil samples were sent to the laboratory that the final results of this analysis is given in Table 3

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

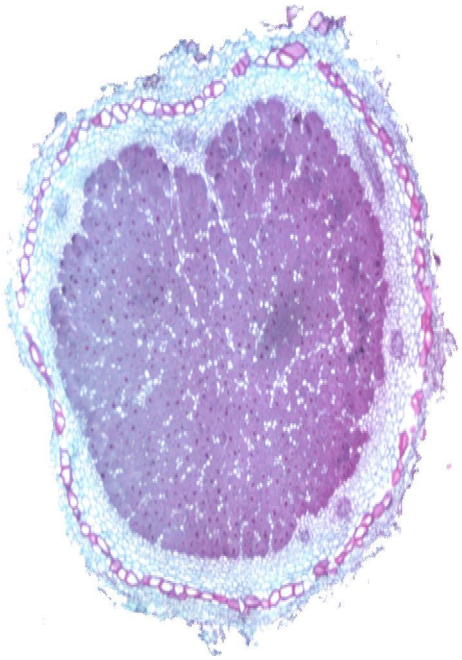

Figure 1. Full nodules

Table 3. Soil chemicals analysis

soil	Deep (cm)	EC	Organic matter (%)	PH	Nitrogen (ppm)
Silty	0-15	6.5	0.6	7.7	635
Silty	15-30	6.6	0.3	7.6	648
Clay loam	30-60	5.7	-	7.3	211

Figure 2. nodules on root

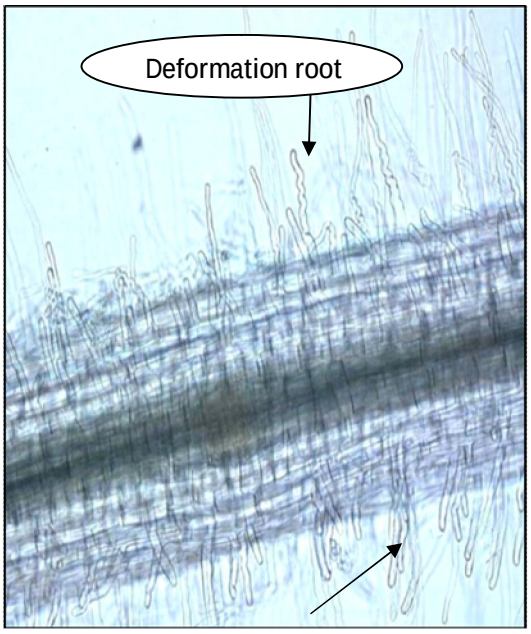

Figure 3. Inoculation on root

3. Results

3.1. Percentage of total plant nitrogen, Analysis of variance showed that treatments of bean cultivars and the amount of fertilizer nitrogen accumulation in plant-level% 1 in every 3 years were significant, cultivar BARAKAT highest percentage of mean total nitrogen plant 1.97 percent won. And cultivar JAZAYERI and ZOHRE. respectively 1.76 and 1.68 percent and Shami figure with 1.43 percent in the next categories were. For sub-plots that V3 with mean 2.73 percent of V1 with the highest and 1.83 percent of the lowest average accumulation of nitrogen element in bean plants showed other words, increasing the amount of nitrogen accumulation of this element increased the bean plant demonstrates the potential for plant uptake is the element that increased dry matter and number of branches is required. (Hard arson and Jetty 2004).

3.2. Percent nitrogen root gland, Percentage of nitrogen nodes to significant amounts of fertilizer and bean cultivars and their interaction showed a 1% level. N2 and N3 treatments statistically the same, but with the N10 treatment, significant differences were present, what amount of nitrogen increased, the amount of biological fixation of this element nodes decreased. N3 treatment reduced accumulation of 40 to 50 percent nitrogen found in to other treatments. Small amounts of soil nitrogen caused the gene is activated and stabilized biological Nitrogen's enzyme, glutathione synthesis of nitrogen into the air in the root of high quantities but do nitrogen, available nitrogen in soil Nitrogen's enzyme in glutathione synthesis is used (Aviv and Hardy 2003) . Percent nitrogen accumulation of blessing the figure was more than other varieties and Hay etal (2005) have stated that the figures that matter most called the root secretion and assembly, which is attracting more Rizobium bacteria in root level and increase the amount established is. Aviv and Hardy (2003) announced that the small amounts of soil nitrogen in the biological fixation gene that is activated enzyme nitrogen's nitrogen into the air Glutathione synthesis in the root and thus stabilize do take place but when large amounts of soil nitrogen by this act nitrogen's enzyme using the available nitrogen in soil occurs in glutathione synthesis (22).Hay etal (2005) in numbers that indicate their more established, have said that the figures that matter most called the root secretion and assembly, which is attracting more Rizobium bacteria in root level and increase the amount established gives (14). Cultivars grown in the review, figure blessing percent more nitrogen accumulation demonstrated that because of this, probably is a Matter of theory Hay and Yvtzy. Tumor characteristics Blessing figure that the highest percentage of nitrogen due to consolidation period from planting to flowering, flowering later than other cultivars (Nadir and Hay 2004), the highest weight (926 mg per plant) and number of nodes (1250 Nodes per plant per day) provided is. With increased fertilizer value, weight and number of nodes on the root node treatment decreased The Number equal to 1450 and increasing fertilizer treatment to 998 nodes per plant decreased.

4. Discussion Increasing amount of nitrogen accumulation of this element in the bean plant was increased and this demonstrates the potential for plant uptake is the element that increased dry matter and number of branches is required. (Hard arson and Jetty 2004) Small amounts of soil nitrogen caused the gene is activated and stabilized biological Nitrogenase enzyme, glutathione synthesis of nitrogen into the air in the root of high quantities but do nitrogen, available nitrogen in soil Nitrogenase enzyme in glutathione synthesis is used (Hardy 2003) . Percent of nitrogen accumulation in the root node blessing figure was more than other varieties. Nadir (2005) have stated that the figures that matter most Lectin called the root secretion and assembly, which is attracting more Rizobium bacteria in root level and increases the amount of consolidation. Hardy (2003) announced that small amounts of soil nitrogen caused the gene is activated and stabilized biological Nitrogenase enzyme, glutathione synthesis of nitrogen into the air and thus do roots stabilize the soil nitrogen is high value but when this act by enzyme Nitrogenase using nitrogen in the soil occurs in glutathione synthesis. Nadir (2004) as were the node weight function parameters such as effective during the growth period from planting to flowering increased the amount of nitrogen is established and also the effectiveness of inoculation of bacteria. Blessing figure that the highest percentage of nitrogen due to consolidation period from planting to flowering, flowering later than other cultivars highest weight (926 mg per plant) and number of nodes (1250 nodes per plant per day) can provide. Increasing amount of nitrogen, weight and number of nodes on the roots was reduced so the number of nodes equal to 1450 and attendance increased fertilizer treatment to 998 nodes per plant decreased. Treatment N1 node weight value 759 mg per plant were in a group that care N3 and N2 values of 654 and 644 mg per plant were the group b were statistically significant differences. Theory based on Thomas (1999) in the presence of high amounts of soil nitrogen due to lack of enzyme activity and accumulation of nitrogen in stabilizing node nodes will not be. What Nitrogen fertilizer increased the amount of tumor size was smaller than the other because the plant needs through existing fertilizer plant in soil their investment to reduce tumor development, thus has significantly reduced tumor size. Gland enlargement process (based on the largest diameter) That growth during the flowering period increased the diameter of the nodes is done after flowering in tumor size remains approximately constant and seed filling and maturity of the hand to large amounts of nitrogen and water, especially its smaller size show that sinha (2001) as can be after flowering, plant nutrition support Legume resilient to nodes is very limited, which causes shrinking and loss many node is inside (Table 4). Assessment of the number of nodes during flowering cultivars were observed when the maximum leaf area index in different bean cultivars reached, the number of root nodes decreased dramatically demonstrated. Sami Field (1996) announced that during the flowering plant metabolism due to Go and spend a lot of energy for its flowering, allocation of carbon hydrate Go to root values that it is used nodes will stop the result of Rizobium bacteria and plant suffered Symbiosis and nodes is impaired due to lack of carbon hydrate reach the plant roots started to make loss, this phenomenon during the flowering of maximum LAI was clearly tested cultivars were observed. Go to the amounts of nitrogen fertilizer increased the base and expanding leaf area index and been in the early stages of growth during the LAI decrease and faster plant growth stage is rapid LAI. Crop growth rate an indicator of production efficiency in the production of ground vegetation is live weight and an indicator of the ability of agricultural production that Watson provided it is calculated. But only for the plants that together, in the coating of packet crop or natural communities grow used to. Assessment values and product trend growth rate of different treatments applied in this experiment is the following corollary: Applied nitrogen fertilizer in the early stages growth increased growth rate and slope product has been early stages of growth (48 days after sowing) been harsh and fast speed growth earlier phase has been that due to high speed net photosynthesis and leaf area index in this period because the growth rate multiplied product is mentioned two parameters. Biological nitrogen fixation trend growth rate in the different levels of nitrogen products to each other has somewhat because the lack of nitrogen could with equal levels of nitrogen to some extent but this equality can be seen in the early stages of growth and to enter the phase of rapid growth and flowering differences in trend growth rate seen in the product.

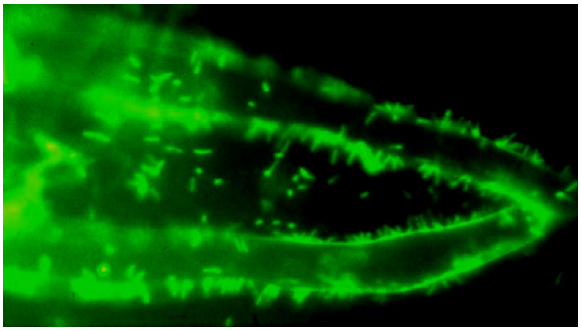

Figure 4. Rizobium on root

Table 4. Gland enlargement process (cm) during the growing season and its effect on nitrogen fertilizer

Days after planting / fertilizer (kg/ha)	33	45	57 flowering	68	88
20	0.44	0.98	1.78	1.39	1.66
40	0.24	0.78	1.46	1.56	1.8
80	0.1	0.12	0.51	0.53	0.48

Table 5. Mean nitrogen accumulation in plant **(Np)**, nitrogen in the root node **(Nr)**, node root weight **(Wn),** number of root nodes **(Nn)**, node size **(Sn)** (Duncan test at 1 % level)

cultivars	Np	Nr	Wn	Nn	Sn
BARAKAT	A1.68	A1250	A926	A1.2	A1.97
ZOHRE	B1.22	B1024	C625	B0.98	B1.68
SHAMI	C0.98	C942	C608	Bc0.8	BC1.4
JAZAYERI	B1.23	B1050	B875	B0.99	B1.76
FERTILIZER (Kg/ha)					
20	A1.98	A1450	A759	A1.73	B1.83
40	B1.22	B1020	B654	B1.01	A2.66
80	C0.78	B998	B644	B0.98	A2.73

Acknowledgements:

President of research the Islamic Azad University, Ahvaz branch, Dr. Zarrin Abadi

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Effect of Different Types of Oral Iron Therapy Used for the Treatment of Iron Deficiency Anemia and Their Effects on Some Hormones and Minerals in Anemic Rats

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Abstract: Iron deficiency anemia is the most common type of anemia related to malnutrition world wide. It represents a major problem in developing countries, especially in Egypt. The aim of this study was carried out to elucidate the effect of different types of oral iron therapy (used for the treatment of iron deficiency anemia) on some hormones and minerals in anemic rats. Forty weanling male Sprague-Dawley rats divided into 4 groups (10 rats each), G1; control group as negative control G2; anemic rats as positive control., G3; anemic rats receiving iron chelating amino acids (IDA+ICAA, 40 mg Fe/kg), G4; anemic rats receiving ferrous sulphate (IDA+$FeSO_4$, 40 mg Fe/kg). Anemia was induced through feeding iron deficient diet (3-5 mg Fe/kg). At the end of the experiment, plasma, kidney and liver were used for determination of blood indices, tT3, tT4, Cu, Ca, Fe and MDA. Induction of iron in the diet improves body weight but still significantly lower than control group. Rats fed iron deficient diet had a significant lower Hb level, Hct value, RBCs count than normal controls. tT3 and tT4 levels of anemic rats were significantly lower than normal control (-15.16 & -30.59 % respectively). Treatment with ICAA gives better result than inorganic $FeSO_4$. tT3/tT4 ratio was significantly higher in all treated groups than normal control group. A significant inverse correlation was found between tT3/tT4 ratio and liver Fe in anemic rats. Treatment of IDA rats with ICAA improves lipid peroxidation. Cu level of IDA group was significantly higher than normal control group, treatment with ICAA or $FeSO_4$ returning Cu level to near normal. The plasma Ca level of ICAA treated groups was significantly higher than IDA groups. Plasma level of Fe or Fe/Cu ratio of IDA is significantly lower than normal control group, it reach less than half (58.3% decrease, $P < 0.0001$). A significant direct correlation was found between Ca level and kidney Fe in iron deficient anemia rats treated with iron chelating amino acids therapy. In Conclusion, the high bioavailability, easily tolerated doses of ferrous iron amino acid chelate allow lower doses to be used in IDA treatment than inorganic iron salts. [Journal of American Science 2010;6(6):109-118]. (ISSN: 1545-1003).

Keywords: Iron deficient anemia, iron chelating amino acids, inorganic iron

1. Introduction

Iron deficiency anemia is the most prevalent nutritional deficiency worldwide and it is often associated with some trace elements change (iron, zinc, copper). It is a major public health problem with adverse consequences, especially for women and children. Globally, iron deficiency affects over 2 billion people, mainly young women and children are iron-deficient (WHO/UNICEF/UNU, 2001). Anemia affects one-quarter of the world's population and is concentrated in preschool-aged children and women, making it a global public health problem (McLean et al., 2009). An estimated 39% of preschool children are anemic. Over 90% of affected individuals live in developing countries. The main cause of iron deficiency is the low iron bioavailability of the diet. The consequences of iron deficiency are many and serious, affecting not only individuals health but also the development of societies and countries. The distinction between "iron deficiency" and "anemia" is important. They often go hand in hand, but people can be iron deficient without being anemic. Iron deficiency is a depletion of iron stores while anemia refers to the depletion of iron in the red blood cells. Only 50% of anemia is caused by iron deficiency, the remainder is caused by vitamin A, B12, folate deficiencies, malaria, HIV, other infectious diseases, sickle cell disease and other inherited anemia (Yip, 1994). Tradition treatment of iron deficiency anemia include treatment with ferrous iron as ferrous sulfate ($FeSO_4$) which is much better absorbed than ferric iron e.g. ferric citrate (Davidsson et al., 2000), but it is known to produce intestinal side effects such as constipation, nausea, and bloating. Other forms of iron supplements as iron amino acid chelate are readily absorbed and less likely to cause intestinal side effects. Amino acids are ideal, good chelators or

readily absorbed and less likely to cause intestinal side effects. Amino acids are ideal, good chelators or ligands from both chemical and nutritional points of view. The body is efficient at absorbing amino acids and dipeptide. Iron amino acid chelate has been shown to have an increased bioavailability and reduced irritability over inorganic sources of iron (Pineda et al., 1994).

Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism that associated with lower plasma thyroid hormone concentrations in rodents and, in some studies, in humans. Oxidant stress has been shown to play an important role in the pathogenesis of IDA and there are association between lymphocyte DNA damage, total antioxidant capacity and the degree of anemia in patients with IDA (Aslan et al., 2006). Amino acid–Fe(II)–chelator complexes exhibit strong antioxidant activity. Iron amino acid chelate is said to have more rapid effect with less GIT side effects so we tried to see if ICAA has more beneficial effects than the traditional iron therapy. The aim of this study was carried out to elucidate the effect of different types of oral iron therapy (used for the treatment of iron deficiency anemia) on some hormones and minerals in anemic rats.

2. Materials and Methods

Approval of the experimental protocol had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes (GOTHI), Cairo, Egypt.

Four types of diet were prepared:

- Standard diet (iron sufficient diet, ISD, 40 mg/kg diet): according to the AIN-93G formulation (Reeves et al. 1993 and National Research Council Committee on Animal Nutrition, 1978).
- Iron deficient diet (IDD): standard diet without Fe in the mineral mixture (Fe 3-5 mg/kg diet as assessed by atomic absorption Unicam).
- Standard diet supplemented with Fe chelating amino acids (ICAA, 40 mg/kg diet) supplied by Nerhadou international.
- Standard diet supplemented with Fe in the form of dietary $FeSO_4$ (40 mg/ kg).

Some modifications were done as: menadione was used instead of phylloquinone; corn starch instead of sucrose; corn starch instead of cellulose (cellulose was omitted as a fiber source because of its variable iron content) as recommended by the American Institute of Nutrition, 1980. The iron content of all diets at baseline was confirmed by atomic absorption analysis. Rats were given free access to food and deionized water.

Forty weanling male Sprague-Dawley rats weighing 68 - 86 g were purchased from the breeding unit of the Egyptian organization for biological products and vaccines (Helwan, Egypt). They were obtained at 21 day of age and housed individually in stainless steel cages. They were fed on standard diet for 10 days before experiments began (adaptation period). Thirty rats of them will be anemic by introducing basal diet without Fe source (iron deficient, ID, 3-5 mg Fe/kg) for 21 days. The animals were divided into 4 groups consisting of 10 rats each and were maintained as follows: Group 1 (control group); rats fed on basal diets. Group 2 (IDA; iron deficient anemia); anemic rats, it will be sacrificed acting as positive control. Group 3 (IDA+ICAA; iron deficient anemia treated with iron chelating amino acids therapy); rats fed basal diet containing ICAA as the only source of Fe for treatment. Group 4 (IDA+$FeSO_4$, iron deficient anemia treated with dietary $FeSO_4$); anemic rats treated with conventional iron therapy (rats fed basal diet containing dietary Fe as the only source of Fe for treatment).

At the end of the experimental period (6 weeks), rats were fasted over night before sacrificing, blood was collected, centrifuged; serum was stored at - 20°C until analysis. Part of the blood is collected on tubes coated with EDTA for hemoglobin (Hb) and hematocrite (Hct) determination. Some hormones as tT3 and tT4, some minerals as Fe, Cu and Ca were determined. After sacrificing, liver and kidney were removed, washed with saline. Parts of liver and kidney each alone were homogenized in 1.15% KCl and used for malonedialdehyde (MDA) determination. Another part of each liver and kidney were kept for Fe determination.

Biochemical Analysis:

Hemoglobin was measured using the cyanomethaemoglobin method using Randox kits, Randox: Laboratories, USA (Dacie and Lewis, 1975). Hematocrite was measured by centrifugation of blood collected into heparinized microcapillary tubes no. 563 supplied by Bio Merieux (Mciniory, 1954). Hematocrite was calculated using the equation: Hct = length of red cell column (mm) / length of total column (mm). Red blood cells count (RBCs) was counted manually (Monica, 2004). Mean cell hemoglobin concentration (MCHC) was calculated using the equation: MCHC = [(Hb*100) / Hct]. Mean cell hemoglobin (MCH) was calculated using the equation: MCHC = [(Hb*10) / RBC]. Mean red cell volume (MCV) was calculated using the equation: MCV = [(Hct*10) / RBC].

Plasma, liver and kidney MDA were used as marker of (*in vivo*) lipid peroxidation and measured according to the method of Yoshioka et al., 1979. Plasma iron and total iron binding capacity, (TIBC) were determined colorimetrically using SGM kits according to the methods of Ruutu, 1975, Ceriotti and Ceriotti, 1980 respectively. Nearly all the binding capacity is due to transferrin. For standards, iron, copper and calcium reference solutions (1g Fe/L, Ca/L, 1g Cu/L; Merck KGaA, Darmstadt, Germany) were used. A control serum sample (Iron, copper and calcium /Seriscann ® normal) Control, QCA, Amposta, Spain), was included in the analysis of the plasma iron, TIBC, copper and calcium determinations to verify accuracy of measurements. Liver and kidney non-heme iron was measured colorimetrically after acid digestion of tissues (Torrance and Bothwell, 1980). Calcium was measured according to the method of Moorehead and Biggs 1974 using QCA kits (Quimica Clinica Aplicada S. A., Amposta/Spain). Copper was measured using Greiner kits (Greiner Diagnostic G mbH-Unter Gereuth 10- Bahlingen-Germany) according to the method of Abe et al., 1989. Total triiodothyronine (tT3) and total thyroxine (tT4) were determined according to the method of Chopra (1977) and Chopra et al. (1971) respectively. using monobind, INC, Costa Mesa, CA 92627 (USA), AccuBind ELISA microwells, 125-300 and 225-300.

Statistical analysis:

All results were expressed as the mean ±SD. Statistical analysis was performed with statistical package for the social science for windows (SPSS, version 13.0, Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. Pearson's correlation analysis was used to determine the correlation among the parameters assessed. The p- value < 0.05 was considered statistically significant (Dawson and Trapp, 2001).

3. Results

The body weight of the experimental rats at the beginning showed no significant differences. Anemic rats showed significant decrease in body weight compared with normal control group. The mean body weight of anemic rats at the end of the study was 62% of normal rats. Induction of iron in the diet improves body weight but still significantly lower than control group (table 1) being more pronounced in groups receiving iron chelating amino acids. Mean body weight of ICAA treated rats was 93, 96 % of that of normal control rats. Daily food intake were significantly lower in anemic rats than normal control or treated groups.

Rats fed the iron deficient diet had a significant lower blood Hb concentration, Hct value, RBC count than normal controls (table 2). Red blood indices showed that MCHC of ICAA treated group was significantly higher than normal control or IDA groups. MCH and MCV of IDA group were significantly higher than normal control. The reticulocyte count of normal control (expressed as a percentage of the total number of RBC) was about twice as high as in IDA rats as in controls ($P < 0.0001$). It is believed that most of the Fe in plasma comes from continuous recycling of heme from senescent RBC through the reticuloendothelial system (Knutson and Wessling-Resnick 2003). In general treatment with ICAA improves level of IDA to normal or near normal better, faster than traditional treatment.

Table 1. Initial and final body weight, body weight gain and daily food intake in the different experimental studied groups (Means ± SD)

Parameters	**Experimental groups**			
	1	**2**	**3**	**4**
IBW (gm)	74.3 ± 4.75	75.6 ± 5.52	75.1 ± 5.64	74.7 ± 5.93
IBW2 (gm)	127.3 ± 8.05	-----	128 ± 7.04 [a]	126.2 ± 8.49 [a]
FBW (gm)	195.4 ± 12.27	121.3 ± 10.08 [a]	191.2 ± 12.04 [a,b,d]	187.80 ± 13.97 [a,b,c]
BWG (gm)	68.30 ± 9.97	45.7 ± 6.00 [a,c,d]	63.2±7.27 [a,b,d]	61.60 ± 8.34 [a,b,c]
Food intake(gm/day)	17.19 ± 2.09	10.82 ± 1.01 [a,c,d]	15.6 ± 0.79 [a,b,d]	13.97 ± 0.80 [a,b,c]

IBW: Initial Body weight; IBW2: Initial Body weight after adaptation period, FBW: Final Body weight;; BWG: Body Weight Gain; [a]p < 0.0001 Vs. Group (1), [b]p < 0.0001 Vs. Group (2),[c]p < 0.0001 Vs. Group (3),[d]p < 0.0001 Vs .Group (4),

Table 3 revealed that levels of tT3 and tT4 in anemic rats were significantly lower than normal control (-15.16% and -30.59% respectively). Treatment with ICAA gives better result then inorganic $FeSO_4$. tT3/tT4 ratio was significantly higher in all treated groups than control group. An inverse correlation was found between tT3/tT4 ratio and liver Fe in anemic rats ($r = -0.663$, $P = 0.037$) (Fig. 1). Also, there was a positive correlation between tT3/tT4 ratio and plasma Ca in ICAA treated group ($r = 0.662$, $P = 0.037$) (Fig. 2).

Table 4 showed the level of MDA in plasma, liver and kidney. They showed significant decrease than normal control group. Treatment of IDA rats with ICAA improves lipid peroxidation even exceed level of the normal control group

Table 5 showed the levels of Cu, Ca, and Fe in normal, IDA and treated groups. The level of Cu in IDA group was significantly higher than normal control group, treatment with ICAA or $FeSO_4$ returning Cu level to near normal. Both types of treatment gives the same effects with no significant difference. No statistically significant changes of plasma Ca level between iron deficient rats and normal control animals; on the other hand, the level of Ca in ICAA treated groups were significantly higher than IDA groups ($P < 0.05$). Both treatments for IDA showed no statistically significant changes when compared with normal control group. The plasma levels of Fe and Fe/Cu ratio of IDA were significantly lower than normal control group, it reach less than half (58.3% decrease, $P < 0.0001$). A positive correlation was found between plasma Ca and kidney Fe in ICAA treated group ($r = 0.743$, $P = 0.014$) (Fig. 3).

Table 6 showed the levels of non-heme iron in liver and kidney, TIBC and transferrin saturation in the studied groups. Plasma, liver and kidney Fe and transferrin were significantly lower. than control group. Treatment with ICAA or $FeSO_4$ improves these parameters, being more pronounced in groups treated with ICAA.

Table 2. Biological profile in the different experimental studied groups

Parameters	Experimental groups			
	1	2	3	4
Hb (g/dl)	13.49 ± 0.68	9.29 ± 0.69 ^{a,c,d}	12.56 ± 0.94 ^{b,d}	11.08 ± 0.79 ^{a,b,c}
Hct (%)	42.02 ± 2.08	29.06 ± 2.46 ^{a,c,d}	38.17 ± 3.15 ^{a,b,d}	34.18 ± 2.73 ^{a,b,c}
MCHC (g/dl)	32.11± 0.85	32.01 ± 0.68 ^{c}	32.94 ± 0.80 ^{a,b}	32.43 ± 0.55
MCH (Pg)	19.39 ± 1.01	23.57 ± 1.12 ^{a,c,d}	19.68 ± 0.93 ^{b,d}	18.36 ± 1.62 ^{b,c}
MCV (fl)	60.47 ± 4.31	73.62 ± 2.46 ^{a,c,d}	59.76 ± 2.72 ^{b,d}	56.65 ± 5.11 ^{b}
RBC ($x10^6$/µl)	6.98 ± 0.60	3.95 ± 0.34 ^{a,c,d}	6.40 ± 0.61 ^{a,b,d}	6.06 ± 0.53 ^{a,b,c}

$^{a}p < 0.0001$ Vs. Group (1), $^{b}p < 0.0001$ Vs. Group (2), $^{c}p < 0.0001$ Vs. Group (3), $^{d}p < 0.0001$ Vs. Group (4),

Table 3. Plasma total triiodothyronine (tT3); total thyroxine (tT4) and tT3/tT4 ratio in the studied groups

Parameters	Experimental groups			
	1	2	3	4
tT3 (ng/L)	4.40 ± 0.44	3.74 ± 0.32 ^{a,c,d}	5.02 ± 0.44 ^{a,b}	4.61 ± 0.24 ^{d}
tT4 (µg/L)	0.44 ± 0.085	0.31 ± 0.04 ^{a,c,d}	0.37 ± 0.04 ^{a,b,d}	0.34 ± 0.03 ^{a,b}
tT3/tT4 ratio	10.27 ± 0.71	12.34 ± 1.73 ^{a}	13.74 ± 1.63 ^{a}	13.87 ± 1.68 ^{a}

$^{a}p < 0.0001$ Vs. Group (1), $^{b}p < 0.0001$ Vs. Group (2), $^{c}p < 0.0001$ Vs. Group (3), $^{d}p < 0.0001$ Vs. Group (4).

Table 4. Plasma, liver and kidney MDA in experimental studied groups

Parameters	Experimental groups			
	1	2	3	4
Plasma MDA (nmol/L)	61.27 ± 4.10	55.97 ± 4.29 ^{a,c}	69.85 ± 3.46 ^{a,b,d}	58.17 ± 2.90 ^{c}
Liver MDA (nmol/g)	60.44 ± 3.95	40.78 ± 3.74 ^{a,c,d}	67.56 ± 4.28 ^{a,b,d}	58.67 ± 2.70 ^{b,c}
Kidney MDA (nmol/g)	184.27 ± 18.95	157.81 ± 14.08 ^{a,c,d}	236.45 ± 11.59 ^{a,b}	219.99 ± 23.66 ^{a,b}

$^{a}p < 0.0001$ Vs. Group (1), $^{b}p < 0.0001$ Vs. Group (2), $^{c}p < 0.0001$ Vs. Group (3), $^{d}p < 0.0001$ Vs. Group (4).

Table 5. Plasma levels of Cu, Ca, Fe and Fe/Cu ratio in the studied groups.

Parameters	Experimental groups			
	1	2	3	4
Cu (µg/dl)	36.28 ± 4.17	55.57 ± 6.37 [a,c,d]	39.47 ± 2.19 [b]	40.99 ± 2.45 [b]
Ca (mg/dl)	8.72 ± 0.57	8.51± 0.46 [c]	8.90 ± 0.35 [b]	8.61± 0.43
Fe (µg/dl)	158.12 ± 19.03	65.27 ± 7.88 [a,c,,d]	150.26 ± 19.10 [b,d]	135.08 ± 8.66 [b]
Fe/Cu ratio	4.40 ± 0.66	1.19 ± 0.22 [a,c,d]	3.78 ± 0.53 [a,,b]	3.45 ± 0.26 [a,b]

[a]p < 0.0001 Vs. Group (1), [b]p < 0.0001 Vs. Group (2),[c]p < 0.0001 Vs. Group (3),[d]p < 0.0001 Vs. Group (4).

Table 6. Iron status in plasma, liver and kidney in the studied groups

Parameters	Experimental groups			
	1	2	3	4
Plasma Fe (µg/dl)	158.12 ± 19.03	65.27 ± 7.88 [a,c,,d]	150.26 ± 19.10 [b,d]	135.08 ± 8.66 [b]
Liver Fe (µg/g)	104.27 ± 12.56	79.11 ± 10.29 [a,c,d]	97.04 ± 7.48 [b,d]	91.84 ± 5.17 [a,b]
Kidney Fe (µg/g)	139.79 ± 11.16	85.76 ± 8.42 [a,c,d]	114.25 ± 8.38 [a,b,d]	106.19 ± 4.48 [a,b]
TIBC (µg/dl)	309.03 ± 27.07	442.09 ± 16.72 [a,c,d]	292.21 ± 45.06 [b,d]	300.91± 27.03 [b]
Transferin Sat (%)	51.14 ± 4.02	14.76 ± 1.60 [a,c,d]	51.66 ± 2.70 [b,d]	45.07 ± 3.18 [a,b,c]

[a]p < 0.0001 Vs. Group (1), [b]p < 0.0001 Vs. Group (2),[c]p < 0.0001 Vs. Group (3),[d]p < 0.0001 Vs. Group (4).

4. Discussion

All iron supplements are not the same. Ferrous iron (e.g. ferrous sulfate) is much better absorbed than ferric iron (e.g. ferric citrate) (Davidsson et al., 2000). The most common form of iron supplement is ferrous sulfate, but it is known to produce intestinal side effects such as constipation, nausea, and bloating in many users (Hansen, 1994). Some forms of ferrous sulfate are enteric-coated to delay tablet dissolving and prevent some side effects, but enteric-coated iron may not absorb as well as iron from standard supplements (Rickettes, 1993). Other forms of iron supplements, such as ferrous fumarate, ferrous gluconate, and iron glycine amino acid chelate (Fox et al., 1998) are readily absorbed and less likely to cause intestinal side effects. In order to overcome the problems (side effects) created from using tradition treatment of anemia, we tried to use iron chelated amino acids. Iron amino acid chelate with the iron in the ferrous state to develop an organic mineral delivery system that is negatively charged which is believed to overcome the problems. The ferrous form of iron amino acid chelate belongs to the class of bicyclic chelates where each respective amino acid bonds the same iron atom through its carboxyl oxygen and α-amino groups. This iron amino acid chelate has been shown to have an increased bioavailability and reduced irritability over inorganic sources of iron (Pineda et al., 1994).

Ferrous iron is the form that is mostly used for correction of iron deficiency. About 3–5% of the iron present in alimentary canal in ferrous form is absorbed. Acidic milieu facilitates the absorption by keeping iron in the ferrous form. Ferrous iron is a central pro-oxidant that propagates free radical reactions through Fenton chemistry both locally in the gastrointestinal tract and systemically. An excess of pro-oxidants over antioxidants results in oxidative stress (Kurtoglu et al., 2003).

In this study, the primary cause of anemia was feeding iron deficient diet for a long period (3 weeks). Anemia is considered as one of the most common index of nutritional deficiency worldwide and is caused by iron deficiency store or iron deficiency erythropoiesis (Lin et al., 2003). Several authors have reported that IDA is mainly caused by some food constituents that may contribute to inhibition of iron absorption, hence contribute to the high prevalence of ID, and IDA (Lin et al., 2003).

The highly significant differences in blood indices response (increase of Hb level, Hct, MCHC, MCH, RBC count, returning to near normal level especially when using ICAA) could be explained by the increased assimilation by chelation process as indicated by Burns (2002). Forbes and Erdman (1983) found that increased assimilation by as much as 300%. It seems that the effect of the ingested doses of ICAA were enough to stimulate iron absorption in

experimental rat groups with highly significant increase. Insufficient body stores of iron lead to a

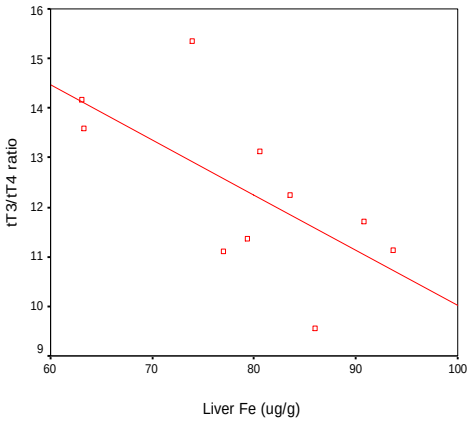

Fig. 1: Correlation between tT3/tT4 ratio and liver iron in Iron Deficient Anemia (IDA) rats (r = – 0.663, P = 0.037)

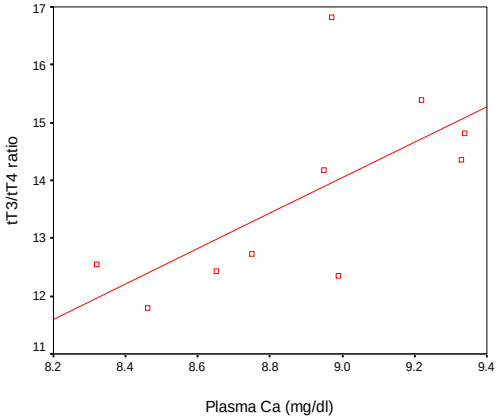

Fig. 2: Correlation between tT3/tT4 ratio and plasma Ca in Iron Deficient Anemia rats treated with Iron Chelating Amino Acids therapy) (IDA+ICAA) (r = 0.662, P = 0.037)

depleted RBC mass which, in turn, leads to a decreased hemoglobin concentration (hypochromia) and decreased oxygen-carrying capacity of the blood which agree with Campos et al. (1998). Also this decrease seems to indicate that the organism is unable to maintain haemoglobin levels, as it cannot obtain iron from its reserves without endangering the activity of the iron-dependent enzymatic mechanisms as Campos et al.(1998) stated. Such mechanisms are essential to the organism, although, as reported by Beutler (1988) some of these enzymes would already be depleted.

In our study, plasma tT4 and tT3 concentrations were significantly lower in Fe-deficient rats than controls which are in agreement with Chen et al. (1983), Martinez-Torres et al. (1984), Beard et al. (1990), Zimmermann and KÖhrle (2002). The decrease in tT3 and tT4 concentration of IDA reach 15.16%, 30.59% respectively when compared with normal control group. The two initial steps of thyroid hormone synthesis are catalyzed by thyroperoxidases (heme–containing thyroid peroxidase) and are dependent on iron. Studies in human and animals have shown that iron deficiency impairs thyroid metabolism, lower thyroperoxidase activity and interfere with the synthesis of thyroid hormones. Hess et al. (2002) have shown that thyroid peroxidase activity is significantly reduced in iron deficiency anemia. In rats, iron deficiency decreases plasma thyroid hormone concentration, impairs peripheral

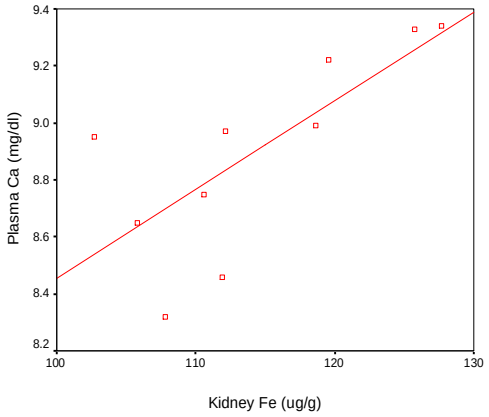

Fig. 3: Correlation between plasma Ca and kidney Fe in Iron Deficient Anemia rats treated with Iron Chelating Amino Acids therapy) (IDA+ICAA) (r = 0.743, P = 0.014)

conversion of T4 to T3 (Dillman et al., 1980), reduces the activity of hepatic thyroxine 5'-deiodinase, which catalyzes the conversion of T4 to T3, and blunts the thyrotropin (TSH) response to thyrotropin releasing hormone, TRH, (Beard et al.,1998 and Hess et al., 2002). These reasons might be the reason for the significantly decreased level of T3, T4 found in the present study. These results are in agreement with the previous studies. The T3 levels in IDA rats were only 85% of those in the control group.

In biological systems, the steady-state level of lipid peroxidation is often assessed by the measurement of lipid peroxidation breakdown products such as malondialdehyde, MDA (Draper and Hadley 1990 and Janero, 1990). Increased lipid peroxidation in IDA rats identifies adverse effect of

iron deficiency and further emphasizes the need for preventing and correcting it. Lipid peroxidation is initiated by reactive oxygen species, and also stimulated by iron ions (Braughler et al., 1986), which can catalyze the formation of te hydroxyl radical and accelerate the decomposition of lipid hydroperoxides (Davies and Slater 1987). Despite extensive literature on iron and lipid peroxidation, few studies have investigated the effects of oral iron supplements on lipid peroxidation. In our study, results showed that iron deficiency anemia is associated with decrease in lipid peroxidation which is indicated by significant decrease in liver and kidney MDA and slight decrease in plasma MDA which are in agreement with Rao and Jagadeesan (1996). Our study disagree with Sundaram et al. (2007) who found an elevated level of MDA in anemic patients and a significant decrease in MDA after treatment. Kurtoglu et al. (2003) observed a significant decrease of oxidative stress in IDA patients. Also Aslan et al. (2006) and DÂaz-Castro et al. (2008) has reported that thiobarbytric acid reactive substances (TBARs) production was similar in liver cytosols from iron deficiency anemia and control rats (IDA did not affect lipid peroxidation in rats), suggesting that hepatic antioxidant capacity is normal or there is enough compensatory capacity to keep antioxidant defenses high, probably due to the adequate antioxidant enzyme activities as glutathione peroxidase, Catalase and glutathione reductase.

The source of iron is either from food supply (heme) or free iron from other sources. Both kinds of iron are processed by the gut (stomach and intestine) where they are converted to a form of iron readily used by the body. Finally, the iron winds up in the intestinal epithelial cells, ready for export to red blood cells, muscle tissue and organs. It has to get out of the gut into the blood stream, but this is particularly difficult because the so-called "hydrophobic" intestinal membrane wants to reject the charged iron molecule. Fe is absorbed from the intestinal lumen into the circulation through a series of steps beginning with uptake into the enterocyte by the apical membrane transporter DMT1 "divalent metal transporter 1" (Gunshin et al., 1997). This step requires Fe in the reduced state which is carried out by a reductase, duodenal cytochrome b, located in the enterocyte apical membrane (McKie et al., 2001). The Fe taken into the cell likely equilibrates with various Fe pools. Fe is exported from the enterocyte to the circulation by the basolateral membrane transporter, ferroportin (McKie et al., 2000) which apparently can transport Fe only in the oxidized state. To accomplish this, a Cu-dependent ferroxidase protein, hephaestin (Hp) (Vulpe et al., 1999), in the intestinal enterocytes, is thought to oxidize Fe_2 to Fe_3 and may act as a helper molecule forming a complex with a yet unknown transport protein where it allows iron to make its way through the membrane (Vulpe et al., 1999). Fe_3 is then transported into the circulation and is bound to apotransferrin .

Many factors affect absorption of minerals and one of the most important ones is the interaction of one mineral with another to the extent that absorption and utilization are reduced or enhanced. There are nutrients which need to be present for iron absorption as B12, folic acid, vitamin C, vitamin A, copper, calcium, manganese, molybdenum and other of the B complex vitamins. According to Ebihara and Okano (1995), sufficient iron is absorbed via the large intestine for recovery from IDA. In this study IDA rats showed significant decrease in plasma Fe concentration when compared with the control group and after iron therapy, mean Fe level increase which agree with Sundaram et al. (2007). Results of this study shows that there is a significant decrease in the concentration of iron in the liver and serum; and this confirms the findings of Milne et al. (1990) who stated that in nutritional IDA all the reserves of iron in the organism were first depleted before hemoglobin levels were drastically affected, and this differ from hemolytic anemia which does not deplete the organism's reserves of iron in such a generalized manner (Flanagan et al., 1980 and Kalpalathika et al., 1991).

The study showed increased level of Cu in IDA rats when compared with the control normal group which agree with GÃrgÃze et al. (2006) and Yokoi et al. (1991) and disagree with Van et al. (2006).

Our results demonstrated a non significant change in Ca level of IDA rats when compared with the control normal group which disagree with Yokoi et al. (1991). Little information is available on the influence of the development of IDA on the metabolism of calcium. Campos et al., 1998 found that the digestive utilization of calcium was higher in IDA than in the control animals. The greater absorption of calcium might also be due to the phenomenon described by Hill and Matrone (1970) who showed that the deficiency of a divalent cation, such as iron, in the intestinal region produces an increase in the absorption of other divalent cations such as calcium. These interactions, which have been studied by Cook et al. (1992). Hallberg et al. (1991) showed that when dietary calcium content increases, the absorption of iron falls; in our study, the administration of an iron-deficient diet led to an

increase in calcium absorption, accompanied by a parallel increase in the absorption of phosphorus and magnesium. In this study our results for IDA rats also agree with Campos et al. (1998). Hallberg et al. (1991) speculated that calcium and iron might competitively bind to one or more substances that are important in the transcellular absorptive pathway, resulting in the inhibitory effect of calcium on iron absorption. Actually in rats some duodenal and intestinal proteins such as mobilferrin and calreticulin have affinity for both calcium and iron (Conrad et al., 1993).

This research describes an effective dietary intervention for the treatment of iron deficiency anemia through specific ferrous iron amino acid chelate. Due to its high bioavailability, relatively small, easily tolerated doses may be administered. The high bioavailability of iron as the ferrous amino acid chelate allows lower doses of iron on a daily basis than would be expected to allow repletion of iron stores if supplied as an inorganic iron salt.

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4/1/2010

In vitro Antimicrobial Assay and Phytochemical Analysis of Ethanolic Extracts of *Voacanga africana* Seeds

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Abstract: Dried and pulverized seeds of *Voacanga africana* were extracted with hot and cold absolute ethanol. The extracts were screened for their phytochemical composition and antimicrobial activities. The results revealed the presence of some bioactive compounds; alkaloids, anthranoids, anthraquinone, cardiac glycosides, phenols, phlobatanins, starch and tannins. The crude extracts exhibited antimicrobial activity against *Escherichia coli* (34.61 and 25%), *Serretia marcescens* (45.08 and 29.16%) and *Staphylococcus aureus* (42.10 and 34.21%). Others are *Alternaria solani* (33.33 and 25%), *Aspergilus flavus* (33.33 and 22%), *A. niger* (25 and 00%) *Candida albicans* (29.62 and 25.92 %) and *Rhizopus stolonifer* (22.58 and 19.35 %); relative to the standard antibiotics, Gentamicin and Clotrimazole; in the Agar Well. Diffusion sensitivity test. The efficacy of the hot extract was greater than the cold extracts in the test organisms, except in *Pseudomonas aeruginosa* where they appeared equipotent. [Journal of American Science 2010; 6(6):119-122]. (ISSN: 1545-1003).

Key words: *Voacanga africana*, phytochemical, bioactive, equipotent.

1. Introduction

Knowledge and application of ethno-medicinal properties of plants dates back to about 300 years BC. (Makhubu, 1998; Ogbonna *et al.* 2007). Plants therapeutic essence is secondary metabolites, known as phytochemicals. These organic chemical substances are stored in matured cells of the various organs, such as roots, stems, leaves, flowers, fruits and seeds.(Sofowora, 1982).Some of the phytochemicals implicated in this exercise; alkaloid, flavonoids, glycosides, phenols, phlobatanins, saponins, tannins, etc.., had been found in crude extracts of some plant species, called medicinal plants (Okwu, 2001; Ano and Ubochi, 2007). Among these plants is a tropical shrub called *Voacanga africana*

V. africana is a deciduous, mesophytic, sap-woody, perennial, aborescent shrub of the primary and secondary forest, within the Tropical Rain Forest and the Guinea Savannah woodland belt. A mature *V. africana* crop is not more than 10m tall, lowly branched, stem, with smooth, grayish white bark. Slash exudes milky latex. Leaves are simple, petiolate and decussately arranged. Inflorescence, terminal, lax, pedunculate, cyme. Flower, pedicellate and mildly scented; corolla lobe, with overlapping aestivation. Stamen, pentamerous and epipetalous. Ovary, superior and bicarpellary. Fruit, globose berry with brownish – white blotches. Seed, dark, bean –shape with denticulate ornamentation. (Duru, 2009).

The leaves and roots decoction of this plant had been implicated in folk medicine for the treatment of malaria, diarrhea, infant convulsion, insane persons and heart arches. (Burkill, 1995; Duru, 2009). This stimulated interest to further investigate this plant, with a view to determining the antimicrobial activity of the seed extracts in *in vitro* culture as well as the phytochemical composition of the crude extracts.

2. Materials And Methods

Collection of Plant Materials:

Matured fruits of the plant were harvested from the wild and identified as *Voacanga africana* Stapf by a plant Taxonomist, at the Department of Biology, Federal University of Technology, Owerri, Imo State, Nigeria. The fruits were slit open and seeds extricated. The seeds were oven- dried at 40^{o}C for seven (7) days, pulverized and stored in air-tight sterile bottle.

Test Organism:

Clinical isolates of the Bacteria- *Escherichia coli, Pseudomonas aeruginosa, Serretia marcescen, Staphylococcus aureus* and the test Fungi- *Candida albicans*, were collected from the Department of Microbiology, Federal Medical Centre, Owerri, Imo State, Nigeria; while the other test fungi- *Aspergilus flavus, A.niger, Alternaria solani* and *Rhizopus stolonifer*, were collected from the Plant Pathology Laboratory, National Root Crop Research Institute, Umudike, Abia State, Nigeria. They were separately sub-cultured and the pure culture re-subcultured on Nutrient Agar and Sabouraud Dextrose Agar media, respectively and stored at 4O^{o}C for further studies.

Extraction of Active Principles:

Cold and hot absolute ethanol was used in the extractions. The cold process followed the method of Boakye-Yiadom (1979). While the hot process, followed the methods of Harborne (1973) and Ogbonna *et al*. (2007). In the cold percolation, 20g of the dried, blended seeds were weighed out, transferred into a beaker, and 100ml of absolute alcohol added. The mixture were agitated and allowed to extract at laboratory temperature for 48hrs.The mixture was then filtered in a flask, using Whatman's No 1 filter paper. The filtrate was evaporated at 40 ^{o}C on a hot plate till supernatant. The concentrated extracts were allowed to cool and stored in a sterile bottle. The hot ethanol extraction (Soxhlet), 20g of the dried powdered seeds were Fed into the Soxhlet extractor and extracted for 24hrs at 80 ^{o}C in 200ml of absolute ethanol. The extracts were allowed to cool, and stored at 4 ^{o}C in a sterile bottle.

Phytochemical Screening:

The screening procedure adopted, followed the methods described by Trease and Evans (1983), Banso and Adeyemo (2007).

Microbial Susceptibility Test:

The agar well diffusion technique was used in the investigation, following the procedure described by Russell and Fur (1977), Boakye- Yiadom (1979), Banso and Adeyemo (2007), and Radhika, *et a*l. (2008). Five (5) wells, 8mm each were made on solidified nutrient agar and sabouraud dextrose agar media plates, respectively with the aid of a sterile cork borer. 0.2ml of the log phase culture of the test microbes: *E. coli, Pseudomonas aeruginosa, Serratia marcescen* and *Staphylococcus aureus* were seeded on the surface of the nutrient agar medium while *Candida albicans, Aspergillus flavus, A. niger, Alternaria solani* and *Rhizopus stolonifer* were seeded on the Sabouraud Dextrose Agar (SDA) medium, using swab stick. The cut agar discs were removed with the aid of sterile forceps. Concentrations of 25g/ml, 50g/ml, 100g/ml, 150g/ml, 200g/ml, 250g/ml, and 500g/ml of the extracts were separately introduced into separate cavities. Three (3) control holes were set up, one, empty, one filled with gentamicin and the other filled with clotrimazole, to serve as positive control for the bacteria and fungi, respectively.

The plates were incubated at 37 ^{o}C for 24hrs and 15days respectively for the bacterial and fungal cultures. The observed zones of inhibition were measured using transparent metric ruler.

Minimum Inhibitory Concentration of the Extracts:

Determination of the Minimum Inhibitory Concentrations (MIC) followed the methods of Egorov (1985), Brown (1994), and Radhika, *et al*. (2008). Extracts concentrations of 10g/ml, 15g/ml, 25g/ml, 50g/ml, 100g/ml, 125g/ml, 150g/ml, 200g/ml, 250g/ml and 500g/ml were used in the exercise. The lowest concentration of each of the extracts in each treatment, showing zero growth after 24hrs for the bacteria and 15 days for the fungi, were recorded as the MIC values.

Minimum Cidal/Static Concentration:

The determination of the minimum bactericidal (MBC) and fungicidal (MFC) concentrations of the extracts were done according to the procedure described by Rotimi, *et al*. (1988), Alade and Irobi (1993), and Banso and Adeyemo (2007). The inoculums from the pure culture tubes containing different concentrations of the extracts, showing no visible growth of the organisms from the MIC test, were subcultured in sterile nutrient agar and incubated at 37^{o}C for 24hrs and 15days, respectively for the bacteria and fungi. The lowest concentration of the extracts with out any growth was noted as the minimum cidal concentration (MBC / MFC).

3. Results

The results of the phytochemical screening are shown in Table 1

Table 1: Phytochemical Analysis of the Seeds Extracts of *Voacanga africana*

Test	Remarks
Alkaloid	+
Anthranoid	+
Anthraquinone	+
Cardiac glycoside	+
Phenol	+
Phlobatanin	+
Saponin	–
Starch	+
Tannin	+

Key: +ve = present ; -ve = absent

The phytochemical screening test, showed the presence of some active principles; Alkaloids, Anthranoids, Anthraquinone, Cardiac glycosides, phenol, phlobatanins, Starch and Tannins.

At the end of the incubation periods of 24 hours and 15 days respectively, for the bacteria and fungi sets. The zones of inhibition of *E. coli, P. aeruginosa, Serretia marcescen* and *Staphylococcus aureus; Candida albicans, Aspergilus flavus, A. niger, Alternaria solani* and *Rhizopus stolonifer* were determined, and the result was shown in Table 2.

Table 2: Sensitivity Test for The Bacterial and Fungal species on the seeds extracts of *Voacanga africana*

Test Organisms	Zones Of Inhibition (Mm)						
	HEE		CEE		EH	GH	CH
	100 mg/ ml	200 mg/ ml	100 mg/ ml	200 mg/ ml			
Escherichia. coli	9	12	6.5	8	00	26	00
Pseudomonas. aeruginosa,	6.5	8	6.5	7.5	00	24	00
Serretia marcescen	11	12	7	8	00	24	00
Staphylococcus aureus	8	8	6.5	6.5	00	19	00
Candida albicans,	8	10	7	8	00	00	27
Aspergillus flavus	9	9	6	7	00	00	27
Aspergillus. niger	7	7	-	-	00	00	28
Alternaria solani	10	11	75	8.5	00	00	30
Rhizopus stolonifer	7	9	6	6.5	00	00	31

.

Key:
HEE ------Hot Ethanol Extract
CEE ------Cold Ethanol Extract
EH ------ Empty Hole
GH ------ Gentamicin Hole
CH -------Clotrimazole Hole

The antimicrobial sensitivity test, using Agar Well Diffusion technique, showed that there was no inhibition on the growth of *Aspergilus niger* by the cold ethanolic extracts. However, all the test microbes were susceptible to the extracts. With mean inhibition diameter ranging from 6.5mm – 12mm in the hot ethanolic extract and 6.mm – 8.5 mm in the cold extract.(Table 2).

The minimum inhibitory concentration of the extracts against the test organisms susceptible to it range from 25g/ml – 100g/ml in hot ethanol extract and 50g/ml – 200g/ml in the cold extract. (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of The Seeds Extracts of *Voacanga africana*

Test Organism	Extracts Concentration (g/ml)	
	HEE	CEE
Escherichia coli	25	50
Pseudomonas aeruginosa	50	100
Serretia marcescen	25	50
Staphylococcus aureus	25	100
Aspergillus flavus	50	100
Aspergillus niger	50	-
Alternaria solani	100	100
Candida albicans,	100	100
Rhizopus stolonifer	100	200

4. Discussion

Absolute ethanol was used as the extraction agent because it wais readily available and cheap to procure. Some seeds contain oil and fatty acid that may not be soluble in water. The extracts had antibacterial activity against, *Escherichia coli, Pseudomonas aeruginosa, Serretia marcescen* and *Staphylococcus aureus.* It also demonstrated antifungal activity against *Alternaria solani, Aspergilus flavus, A. niger , Candida albican* and *Rhizopus stolonifer* as such suggesting that the seeds extracts of *V. africana* has a broad spectrum antimicrobial potency. The antibacterial and the antimycotic potency may be due to the presence of some active principles, like Alkaloids, Anthranoids, Anthraquinone, Cardiac glycosides, Phenols, Phlobatanins, Starch and Tannins. This result agrees with the report of Ebena, *et al.* (1991), Trease and Evans (2005), and Banso and Adeyemo (2007).

The sensitivity test result, showed that the extracts, were less potent than the standard antibiotics ;Gentamicin and Clotrimazole, used in the study. The hot and the cold fractions were apparently not equipotent. At 100g/ml concentration, we had 34.61% (HEE) and 25% (CEE), against *E. coli*. 27.08% (HEE) and 27.08% (CEE) against *Pseudomonas aeruginosa.* 45.08% (HEE) and 29.16% (CEE), against *Serretia marcescen.* 42.10% (HEE) and 34.21% (CEE), against *Staphylococcus aureus.* 33.33% (HEE) and 25% (CEE), against *Alternaria solani*. 33.33% (HEE) and 22.22% (CEE) against *Aspergilus flavus,* 25% (HEE) and 00% CEE against *A. niger* . 29.62% (HEE) and 25.92% against. *Candida albican*, 22.58% (HEE) and 19.35% (CEE), against *Rhizopus stolonifer*. However they were equipotent against *Pseudomonas aeruginosa* 27.08% for both HEE and CEE treatments. Generally, the reduced efficacy of the extracts, relative to the standard antibiotics, used in the study may be due to the fact that, they are still crude and require further purification.

The cold extracts did not elicit antimicrobial activity, against *A. niger*. These effects could be due to the fact that the concentration quotient was too minimal to elicit cidal activity on the test fungi.

Seeds of *Voacanga africana*, which hitherto, waste in our forest contain medicinally, useful phytochemicals, such as Alkaloids, anthranoids, anthraquinones, cardiac glycosides, phenols, phlobatanins, starch and tannins. These substances are antimicrobial and could be extracted for bacterial and fungal diseases management, pharmaceutical exploits, research in Microbiology, Biotechnology and general Medicine.

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Submitted: January 15, 2010

In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay

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Abstract: Medicinal plants are a major source of raw material for the traditional system like Ayurveda, Siddha & Unani. Even the modern system of medicine has more than 25 percent of drugs in use, which are either plant based or plant derived. Although several tree posses various medicinal properties, it has been ignored by indigenous & modern system of medicine. Among them ***Azadirachta indica* & *Melia azedarach*** belonging to family Meliaceae play a vital role in day to day usage of different indigenous communities due to its sacred and medicinal value. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants. In the course of finding potential antioxidant from plant source, two medicinal tree species belonging to family Meliaceae has been selected. Leaves were dried and extracted with different solvent systems namely water, ethanol & methanol. Antioxidant activity using DPPH radical scavenging assay of six extracts from two genus of the family Meliaceae is reported & a comparison of the free radical scavenging ability of the extracts is emphasized. The result of the present study showed that the extract of Melia azedarach, which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparison to *Azadirachta indica* Neem. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger. [Journal of American Science 2010;6(6):123-128]. (ISSN: 1545-1003).

Key words: Antioxidant activity, Azadirachta indica, Melia azedarach.

1. Introduction

India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. Medicinal plants constitute the major constituents of most indigenous medicines and a large number of Western medical preparations contain one or more ingredients of plant origin. Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries contribute continuously to the type, quality, presentation and concept of medicinal preparation. The therapeutic use of development of human knowledge, scientists endeavored to isolate different chemical constituents from plant, put them to biological and pharmacological tests and thus have been used to prepare modern medicines.

There is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industrial (dietary, pharmaceutical and cosmetics) purposes. This is mainly due to their strong biological activity, excluding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis. Therefore, the need exists for safe, economic, powerful and natural antioxidants to replace these synthetic ones. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts. (McClements & Decker, 2000). Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers (Zheng & Wang, 2001). A number of plants have been investigated for their biological activities and antioxidant principles (Baris et al., 2006; Saleem, et al, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Ito, et al, 1983). In addition, naturally antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers.

Recent works have highlighted the role of polyphenolic compounds of the higher plants (Hertog et al, 1993) such as flavonols (Salah et al, 1995), anthaquines (Yen, et al 2000), Xanthaninsms that contribute to their anticarcinogen or cardioprotective effects. Increasing experimental evidence has suggested that these compounds can affect a wide range of cell biological function by virtue of their radical scavenging properties (Aruoma, 1998). The intake of antioxidants such as polyphenols has been effective in the prevention of diseases (Cao et al 1997; Vinson et al 1995). In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the

last few decades (Singh et al, 2002).Some antioxidant compounds are extracted from easy sources, such as agricultural and horticultural crops, or medicinal plants. Among them the medicinal plants are taking the main role for providing a large no of pure antioxidants.

It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are present quite commonly in the plant family Meliaceae. ***A.indica*** is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. ***A. indica*** **and** ***M. azedarach*** are two closely related species of Meliaceae family. The former is popularly known as Indian Neem (Margosa tree) or India lilac, and the latter as Mahaneem or Persian lilac. All parts of the plant have been used for medicinal purposes including fruits, seeds, leaves, roots and barks (Anon 1985). Neem has been extensively used in Ayurveda,Unani and homoepathic medicine and has become a Synonym of modern medicine. The Neem tree contains more than 100 bioactive ingredients. The most important bioactive compound is azadirichtin.***Melia azedarach***, the Persian Lilac is popularly known as Maha neem tree and cultivated in all stations. It is a large evergreen tree found throughout India and very similar to Neem. It is native to upper Burmah region. It's Flowering time is May-June and Fruiting time is Nov-Dec. The inner bark contains a resinous alkaloid substance and is used as an anthelmintic. Various scientific studies reported the analgesic, anticancer, antiviral, antimalarial, antibacterial, and antifungal, antifeedent and antifertility activity of this plant. (Vishnukanta, 2008).

Leaf & bark extract of *A. indica* has been studied for its anti-oxidant activity (Ghimeray et al 2009; Sultane et al 2007). However anti-oxidant activity of *M.azedarach* another very important medicine plant has not been investigated. In present work leaves, extracted in water, ethanol & methanol of two trees, *A. indica* & *M. azedarach* belonging to family Meliaceae were investigated for the presence of phenol content & antioxidant activity in a comparative way.

2. Material and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India), Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH) and Ascorbic acid are obtained from (Himedia Lab., India). All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

2.1.2. Plant Materials

The leaves of the two species i.e. *A. indica* and *Melia azedarach* of Meliaceae family were collected from the Medicinal Garden of B.J.B (A) College, Bhubaneswar, Orissa. Fresh plant leaves were rinsed severally with clean tap water to make it dust and debris free. Then the leaves were spread evenly and dried in the shady condition for 3to4 days until they become crispy while still retaining the greenish coloration. Dried leaves were ground in electric chopper to get fine powder form for further use.

2.1.3. Instrumentations

Collection of multi-solvent extract was done by Soxhlet apparatus (J.S.G.W) with varying temperatures according to the B.P. of the solvents. The samples were evaporated through the Rotary vacuum evaporator at 60-100^0C according to the B.P. of supplied solvents. Absorbance spectrophotometery was carried out using a UV-vis spectrophotometer (EI, model-1371).Wavelength scans and absorbance measurements were in 1ml quartz cells of 1cm path length.

2.2 Preparation of plant extracts

The dried and powdered Neem and Maha-neem leaves (each 50g) were extracted successively with double distilled water, ethanol and methanol (each 400ml.) for 10-12 hrs.,using a Soxhlet apparatus. Then collected solutions were filtered through Whatman No-1 filer paper. The extracts were evaporated to dryness under reduced pressure at 90^0C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at -18^0C until used for further analysis. These extracts were designated as AW:]Aqueous Extract of Azadirachta, AE: Ethanolic Extract of Azadirachta, AM: Methanolic Extract of Azadirachta, MW: AqueousExtract of Melia, ME: Ethanolic Extract of Melia, MM: Methanolic Extract of Melia respectively.

2.3. Phenolic Estimation

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described by Kim et al (2007). Reading samples on a UV-vis spectrophotometer at 650 nm. Results were expressed as catechol equivalents (µg/mg).

2.4. Antioxidative activity

The antioxidant activity of the Neem and Mahaneem (Leaves) on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by Brand-Williams et al. (1995) with slight modification. The following concentrations of extracts were prepared 0.02mg/mL, 0.04mg/mL,0.06mg/mL, 0.08mg/mL and 0.1mg/mL. All the solutions were prepared with

methanol. 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = (1-As/Ac) ×100

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

3. Results and Discussion

3.1. The effect of different solvents on the yields of Azadirachta and Melia leaf extracts.

The significant variation in the yields of **Azadirachta** and **Melia** extracts were shown using various fraction solvents. The yield of extracts using Water, Methanol and Ethanol in case of **Azadirachta** were 4.93gm, 4.34gm and 6.36gm respectively. Likewise the **Melia** leaf extract also followed the same order as the **Azadirachta** extracts, and they were 5.92gm, 5.62gm and 5.95gm. The variation in yield may be due to the polarity of the solvents used in the extraction process. (Table-1)

3.1.2. Free radical and antioxidative activity

Table-2 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result revealed that the ethanol fraction of **Melia** exhibited the highest radical scavenging activity with 68.23±0.03 followed by its aqueous extract with 64.34±0.04 and methanol extract with 61.17±0.05. In comparison to **Melia** the **Azadirachta** extract shows less scavenging activity. The **Azadirachta** extract of obtained from ethanol shows 50.48±0.03. i.e. highest scavenging activity followed by its aqueous extract with 49.48±0.03 and methnolic extract with 41.17±0.04. In overall comparison the ethanolic extract of both **Azadirachta** and **Melia** show the highest scavenging activity followed by the aqueous and then methanol. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju & Becher, 2003). In the present study, the values of ethanolic and aqueous extracts were higher than those of methanolic ones. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity (Karadeniz, et al 2005). Fig. 1. Shows the comparative study of radical scavenging activity between **Melia** and **Azadirachta** with respect to Ascorbic acid as standard.

3.1.3. Phenol content & antioxidant activity:

It is reported that the phenolics are responsible for the variation in the antioxidant activity of the plant (Cai et al., 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorney, 2001; Pitchaon et al., 2007). The total phenolic content varied significantly between the two species of Maliaceae family i.e. ***Azadirachta indica*** **and** ***Melia azedarach***. The contents of total phenolic compounds in crude ethanolic extracts obtained from these two **Azadirachta** plants are presented in Table-1. The results were reported as catechol equivalents (µg/mg). The highest concentration of total phenolics was 360µg/mg present in the ethanolic extract of **Melia** plant where as lowest in aqueous extract of **Azadirachta** plant i.e. 120µg/mg. The aqueous and methanolic fractions of **Melia** showed 140µg/mg and 268µg/mg of phenlic contents respectively. Similarly the **Melia** ethanolic extract and **Azadirachta** methanolic extract exhiited highest phenol contents of i.e.300µg/mg and 258µg/mg.

3.1.4 *IC_{50} value*

IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. Results showed in table-1 reports no IC_{50} value in water and methanol extraction of Azadirachta indica. Only ethanolic extract of **Azadirachta** showed an IC_{50} value of 0.008µg/mg. In comparison of **Azadirachta**, all extracts of **Melia** showed lower IC_{50} value, however MnE being the lowest (Fig-2).The ethanolic extract of Mahaneem exhibited significant activity with low IC_{50} value in comparison to **Azadirachta**. The antioxidant activity of **Azadirachta** and **Melia** extracts rise with the rising of polyphenol content of the extract. A linear relationship between the reciprocal of IC_{50} value and the total polyphenol content of **Azadirachta** and Melia was observed in this study, indicating that increasing the polyphenol content strengths the antioxidant activity. This finding is similar to that reported by Katsube, et al 2004.

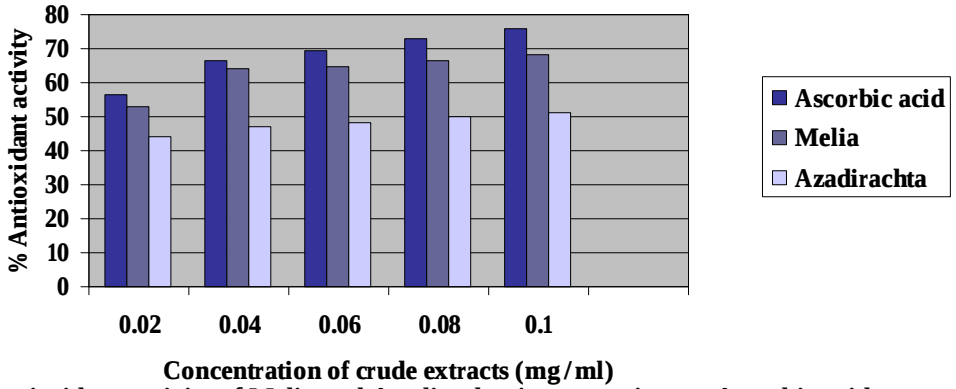

Fig. 1. Antioxidant activity of Melia and Azadirachta in comparison to Ascorbic acid.

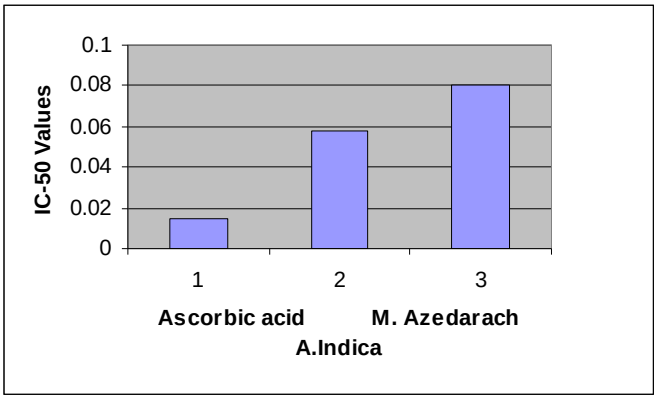

Fig. 2. IC_{50} value of Melia and Azadirachta leaf extracts obtained from ethanol.

Table-1-Crude extracts phenolic content & IC_{50} Value in Melia and Azadirachta leaves.

	Azadirachta indica			*Melia azedarach*		
Solvent used	Crude Extracts (gm)	Phenol content (µg/mg)	IC_{50} Value (µg/ml)	Crude Extracts (gm)	Phenol content (µg/mg)	IC_{50} Value (µg/ml)
Water	4.93	120	<50%	5.92	140	0.062
Methanol	4.34	258	<50%	5.62	268	0.066
Ethanol	6.36	300	0.080	5.95	360	0.058

Table-2-Antioxidant activities of Melia and Azadirachta in different solvents

Concentration of extracts (mg/ml)	Antioxidant activity (%)		Antioxidant activity (%)		Antioxidant activity (%)	
	AW	MW	AM	MM	ME	MW
0.02	45.24±0.04	53.69±0.03	34.11±0.04	55.29±0.03	44.10±0.01	52.94±0.05
0.04	44.18±0.03	55.75±0.05	35.84±0.06	56.47±0.06	47.05±0.03	64.11±0.03
0.06	47.48±0.02	60.43±0.03	39.43±0.06	59.98±0.09	48.20±0.06	64.70±0.04
0.08	48.47±0.05	63.14±0.04	40.00±0.10	60.59±0.04	50.02±0.13	66.47±0.03
0.1	49.48±0.03	64.34±0.04	41.17±0.04	61.17±0.05	50.48±0.03	68.23±0.03

AW: Aqueous Extract of Azadirachta, AE: Ethanolic Extract of Azadirachta, AM: Methanolic Extract of Azadirachta, MW: AqueousExtract of Melia, ME: Ethanolic Extract of Melia, MM: Methanolic Extract of Melia

3.1.5 Conclusion

The result of the present study showed that the extract of ***Melia azedarach***. which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparision to Azadirachta indica. The high scavenging property of **Melia** may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger.

Acknowledgment

The authors are thankful to University Grants Commission New Delhi, for Financial Assistance in form of major research project to one of the author (R.K.S) we are also thankful to Susanta Kumar sethy for typing the manuscript. Sabitri Nahak is also acknowledging for her timely help to the author (G.N).

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12/2/2009

Allocation of Spinning Reserve Cost Amongst Customers in Deregulated Power Systems

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Abstract: In a deregulated power system, DISCOs are considered to be customers who can choose their desirable reliability levels and purchase their required reserve in an ancillary service market based on this reliability level. This paper presents a new approach for determining spinning reserve requirements considering customer's desired reliability level in a pool energy and reserve market. An approach is also developed to fairly allocate the cost associated with provision of spinning reserve amongst the customers. The effectiveness of the proposed approach is examined and the results are presented using the IEEE-RTS. [Journal of American Science 2010;6(6):129-138]. (ISSN: 1545-1003).

Key words: Power market, system risk, spinning reserve, required reliability level, reserve allocation

1. Introduction

In a vertically integrated power system, spinning reserve is principally determined by system operators to maintain the entire system reliability at a favorable level. The main assumption here is that customers connected to different load points all benefit from the same level of reliability. However, this method is not optimal in a market-based system since it does not balance the value that consumers place on not being disconnected against the cost of providing enough reserve to prevent such disconnections. Thus, it may happen that the amount of spinning reserve planned during some periods exceeds what is economically viable, whereas it may be inadequate at some other times.

The question of determination of optimal reserve in a power system is an old one. The classic approach was to adopt reserve equal to the size of the largest generating unit. Anstine et al. (1963) proposed a method whereby the forced outage rates of the generating units are also considered such that the overall system reliability never falls below a certain predetermined value. Gooi et al. (1999) described the optimum scheduling of spinning reserve by using the Lagrangian relaxation method together with probabilistic reserve assessment in a conventional power system. In a conventional system, spinning-reserve allocation amongst the generating units generally has a significant bearing on unit commitment and dispatch decisions, since it comes at a price which should be kept to a minimum. As a result, spinning reserve requirements can be fitted to different generating units to maintain the whole start-up/back-down and operating costs at a minimum [Chattopadhyay and Baldick (2002)].

Marketing gives the customers different choices regarding what to buy. In a deregulated power system customers can choose the energy and transmission providers, as well as desirable reliability levels. This means that the reliability levels provided by different customers (or DISCOs) are different. In this context, reliability is mainly expressed by the amount of generation reserve. In a power market, customers can buy the spinning reserve from generation providers. Goel et al. (2004) indicated that customers can obtain their favorable reliability level by making various reserve bilateral agreements. It is obvious that a lower cost paid for spinning reserve results in less reliable electric service, and customers have to pay more for a higher level of reliability. Bouffard and Galiana (2004) used the mixed integer linear programming method to make the system operate at less than a definite EENS or loss of load probability (LOLP) requirement. The procedure also determines a greatest value for EENS to meet a predefined system reliability requirement.

Wang et al. (2005) proposed to rely on a cost-benefit analysis to determine the optimal reserve. The benefit gained from a higher reserve can be computed from the reduction of the amount of energy not served. Motamedi and Fotuhi-Firuzabad (2007) proposed the use of a hybrid deterministic/probabilistic approach to determine the spinning reserve in restructured power systems. Qi and Ding (2009) proposed the allocation of spinning reserve cost amongst all market partners involved in power generation, transmission and consumption having risk elements. The spinning reserve's value was evaluated by using the expectation of spinning reserve's gain or loss, and allocated in proportion according to the spinning reserve's value for all the market partners. Ahmadi-Khatir et al. (2009) addressed the problem of spinning reserve procurement in a pool-based market and the associated cost allocation using well-being analysis. This paper proposes a new approach for determining spinning reserve requirements based on desired reliability level in a pool

energy and reserve market. An approach is also presented to fairly allocate the cost associated with provision of spinning reserve amongst the customers. The IEEE Reliability Test System has been used to illustrate the effectiveness of the proposed method.

2. Energy and Reserve Market Model

The model of energy and reserve market that is defined in this paper is a pool model. The operating reserve market is independent of the reserve market and is cleared right after the energy market is cleared when the unit commitment problem has already been solved. As spinning reserve is provided by synchronous units, generating units only participate in the reserve market when they are accepted in the energy market. Sellers (GENCOs) send curves of energy and reserve generation price, their ramp-up rates and the amount of maximum generation capacity to ISO. DISCOs send amount of their needed demand and the desirable level of reliability to ISO. The load model in this market is inelastic with respect to price. In fact, the customers do not send any bids for price to the market for their required energy. Only generating units send their bids. The criterion used for clearing the energy market is included in the unit commitment (UC) problem. After UC, load economic dispatch is performed to determine the amount of accepted generation for each unit. The main objective in clearing the energy market is to minimize the payment to the generating units for purchasing energy. After clearing the energy market and determining the amount of generation for each unit, generation units submit the spinning reserve capacity which they can provide in ten minutes. Due to the limits imposed by ramping rate, each unit has a different capability to present for the spinning reserve market. The maximum amount of spinning reserve capability equals the minimum of ten times the ramping rate, in MW per minute, and the unit's capacity. Each unit has a limited capacity to provide spinning reserve due to its limited ramping rate [Zhu et al. (2000)]. The amount of capacity that generating units can present to the spinning reserve market is calculated as follows:

$$AC_i = MIN\ ((P_i^{MAX} - G_i), 10 \times RR_i) \quad (1)$$

where AC_i is the actual generating capacity of unit i for presenting to the spinning reserve market; P_i^{MAX} is the maximum generation capacity of unit i; G_i is the generation of unit i; and RR_i is the ramping rate limit of generating unit i.

ISO clears the spinning reserve market considering the results obtained from the energy market, and the requested reliability levels of the customers. The proposed model assumes that load forecasts have some uncertainty. The effect of transmission and distribution networks is also ignored. The reliability model of the generating units is considered to have only two states being either available or on outage. Rapid start units and load curtailment philosophies are also not taken into consideration.

3. The Algorithm for Clearing the Reserve Market

In this section, the algorithm for clearing the reserve market is presented. Customers send their energy requirements and the desired reliability levels to ISO. After receiving and collecting the information regarding desired reliability levels of the customers plus the information related to clearing the energy market, ISO starts to clear the reserve market. The procedure for clearing the reserve market is as follows:

1- A risk level should be defined for the entire system using the desired reliability levels.

2- Reserve capacity should be purchased based on a priority list in the reserve market until the desired overall system risk level is satisfied.

3- The generation reserve should be allocated to the customers in the market after the procurement of the required reserve in the system.

The flowchart of the procedure to clear the reserve market is shown in Figure 1.

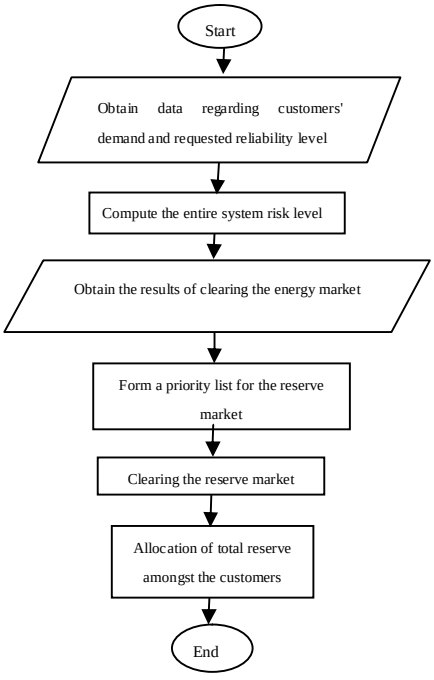

Fig. 1. The flowchart of clearing the reserve market and allocating the reserve to customers.

4. The Reliability of the Entire System

As the load level of the customers increases, the reliability level of the whole system decreases. In other words, when the load of a given customer is reduced, the

load level of the whole system goes down and the risk level of the system decreases. Thus, we use the following equation for computing the risk level of the overall system using the reliability level required by customers as in (2):

$$LOLPT = 1 - \frac{\sum_{i=1}^{n} R_i (1 - LOLPL_i)}{N - \sum_{i=1}^{n} LOLPL_i} \quad (2)$$

where R_i is the desired reliability level for customer i, $LOLP_i$ is the system risk level after shedding the load of customer i, N is the number of customers in the market, and $LOLPT$ is the reliability level of the entire system. For example, if three customers A, B and C participate in the reserve market with their demand, LOLPL and requested reliability levels as shown in Table 1, then the LOLPT will be:

$$LOLPT = 1 - \frac{R_A \times (1 - LOLPL_A) + R_B \times (1 - LOLPL_B) + R_C \times (1 - LOLPL_C)}{N - LOLPL_A - LOLPL_B - LOLPL_C} = 0.01045$$

Table 1. A sample of customer information sent to ISO

customer	Customer demand (MW)	Customer desirable reliability level	LOLPL
A	1600	0.999	0.0568217
B	500	0.98	0.0196308
C	500	0.99	0.0196308

5. Reserve Allocation Mechanism

The amount of required spinning reserve in the system is found according to the load risk imposed on the system because of consumers and also the total system reliability level. As mentioned before, the overall system reliability level is calculated according to the customers (or DISCOs) requested reliability levels.

The total reserve planned in the system should be allocated to the customers. Thus, a fair and rational method of allocating the reserve amongst the customers of reserve (or DISCOs) should be used. In this study, a reserve allocation mechanism is introduced to solve the problem of reserve allocation to the customers for reserve in a fair and rational manner.

The customer' imposed risk level and the required reliability level of DISCOs are factors that should be considered when allocating the reserve amongst the reserve customers. That is, the imposed risk level of a DISCO on a system increases when the required amount of energy consumption of the DISCO is high.

Also, the required reserve for a given DISCO is more when his requested reliability level is higher. Equation (3) clearly shows the relation between the DISCO's share of reserve, the customer's imposed risk level and the requested reliability level.

$$CSFR \propto \frac{RRL}{CILR} \quad (3)$$

where $CSFR$ is the customer's share of the reserve, RRL is the requested reliability level, and $CILR$ is the customer's imposed risk level.

The proposed method of allocating reserve amongst the various customers is presented next. A coefficient is obtained for each customer according to the reliability level requested by the DISCOs. The optimal reserve is divided amongst the customers according to this coefficient. The flowchart for the implementation of the proposed method is shown in Figure 2.

Step 1: The required reliability levels which the DISCOs have requested from the ISO are sorted in an ascending manner.

Step 2: Reserve is purchased for the system to the extent that the reliability level increases from R_{i-1} to R_i.

Step 3: Allocation of the reserve which is bought (BOR) is done according to the customer's imposed risk level to customers who have asked for a reliability level equal to or higher than R_i.

Step 4: Repeat steps 2 and 3 for all N customers.

Step 5: Each customer's share of reserve in the various periods are added up as follows:

$$RE_i = \sum_{K=1}^{n} RE_{ik} \quad (4)$$

where RE_i is the customer's share, and RE_{ik} is the i^{th} customer's share in period k.

The number of division steps is equal to the number of sent reliability levels to ISO. The customer with a higher level of requested reliability will participate in more periods of reserve allocation. For example, customer N (with the highest requested reliability level) participates in all periods of reserve allocation, while the first customer (with the lowest requested reliability level) participates in reserve allocation only in the first step.

Step 6: The coefficient RF_i to be used for the i^{th} customer will be computed from equation (5).

$$RF_i = \frac{RE_i}{\sum_{i=1}^{n} RE_i} \quad (5)$$

Step 7: The share of each customer from the reserve is calculated based on the bids of the generating units for the reserve market such as the one shown in Table 3, and the allocation coefficients RF_i obtained above as shown in (6):

$$AR_i = RF_i \times Reserve \quad (6)$$

where AR_i is the share of the i[th] customer from the reserve.

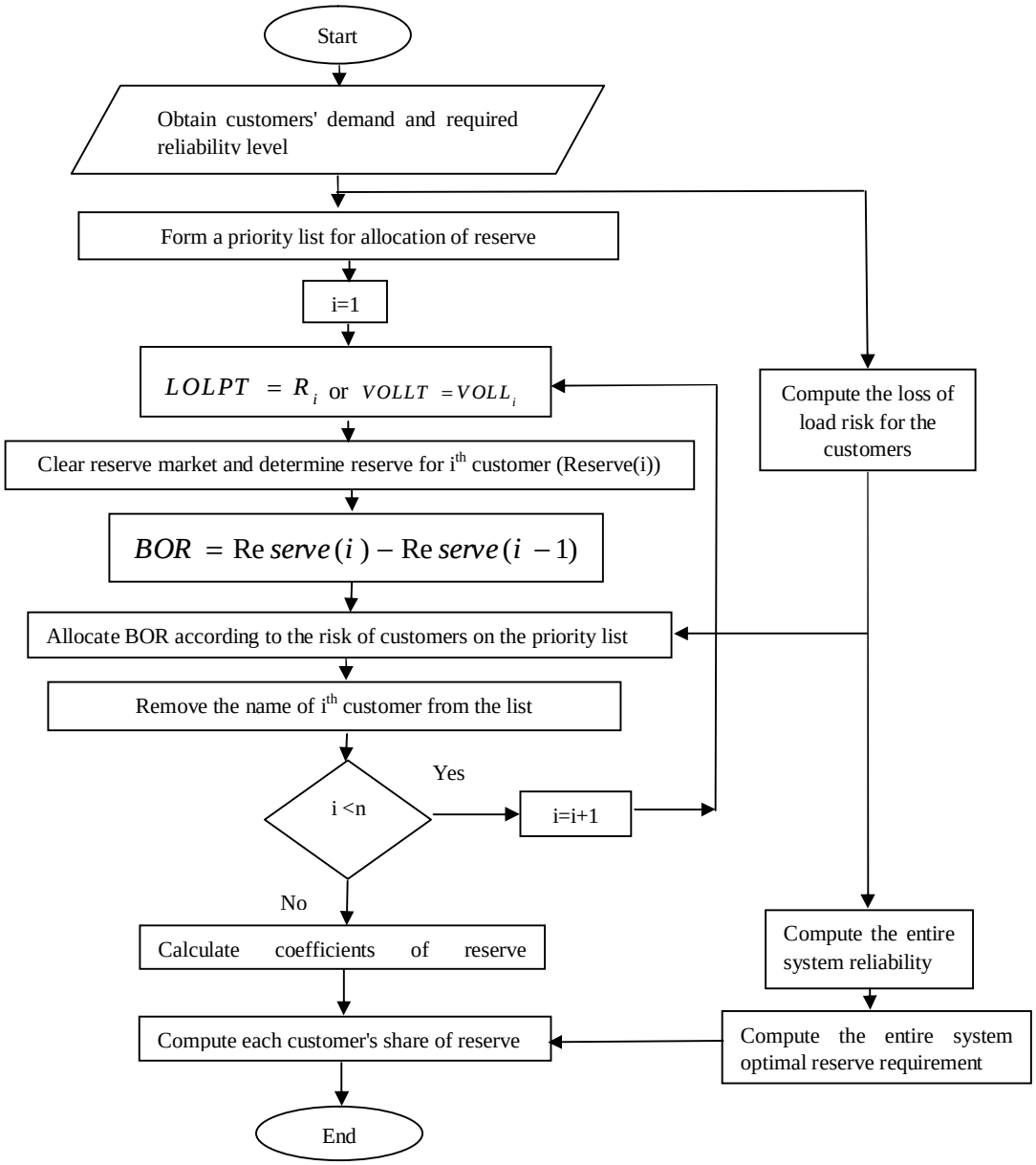

Fig 2 - The flowchart for clearing the reserve market

6. Results of Simulation

The proposed method of procurement of reserves needed for the system and the allocation of the cost of total reserve amongst the various consumers is tested on the IEEE reliability test system as presented by the Reliability Test System Task Force (1999) excluding the hydro units. Generation data for this system is shown in Table 2 and the one-line diagram is shown in Figure 3 [Reliability Test System Task Force (1999)].

The unit commitment data presented by Ouyang and Shahidehpour (1991) and Wang and Shahidehpour (1993)

are used in this study. Total generation in this system is 3405MW and annual maximum load is 2850MW. For simplicity, we consider that the load buses of the system are divided into three major distribution companies. DISCO A supplies load for consumers that are connected to buses 1 to 12. DISCO B does the same for consumers that are connected to buses 15, 18, 21 and 22. DISCO C supplies the load for consumers on buses 13, 14, 19, 20 and 23 as shown in Figure 4.

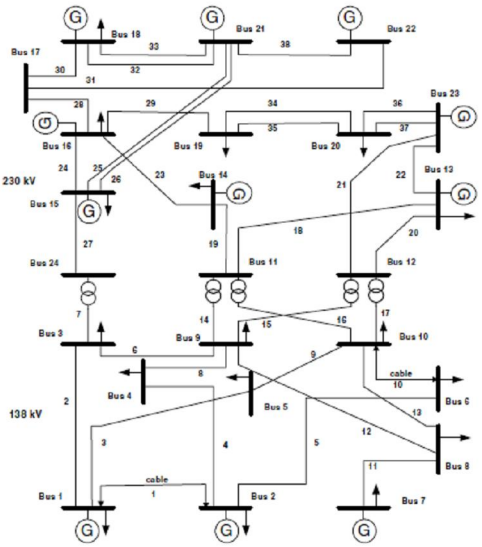

Fig 3 - The one line diagram of the IEEE-RTS system

[Reliability Test System Task Force (1999)]

By this method, the consumers in the market can request their needed energy and desired level of reliability from the ISO in order to buy their needed reserve.

Sellers (GENCOs) in the market also present their bids for selling electrical energy along with minimum and maximum production levels and ramp-up rate to ISO. It is considered that the suggested prices for energy in the power market are equal to the marginal costs of the generating units. We supposed that the GENCOs offer their bids as shown in Table 3 for the reserve market.

After clearing the energy market and defining the generation level of each unit, ISO clears the reserve market separately for each hour considering the list of priorities in the reserve market. Considering that clearing the reserve market is done on an hourly basis, the lead time of production units is equal to one.

The results obtained from the reserve market clearing for 1800MW load level are shown in the following Tables.

The total reliability level is the total risk level of system that is computed using equation (2). The information provided to ISO by the customers and the computed total risk level for the system are all shown in Table 4.

Considering the results obtained after clearing the energy market and identification of the amount of energy produced by each generating unit, we can compute the capacity that each generating units can present in the spinning reserve market by using equation (1).

Considering the expressed bids presented by sellers, as well as specifying the ability rate of generating units for participating in the reserve market, ISO uses the reserve market priority and purchases generating reserve in order to satisfy the total reliability level of the system. The result of reserve market liquidation and the total purchased reserve are shown in Table 5.

After clearing the reserve market, and procurement of total needed reserve in the system, ISO should allocate the purchased reserve to its customers using the proposed allocation mechanism. Table 6 shows the results obtained using the allocation coefficients for DISCOs as shown in Table 5. Also, the cost of this reserve is divided amongst these DISCOs using these coefficients.

Table 2 - Generation data for the IEEE-RTS system adopted from Reliability Test System Task Force (1999) excluding the hydro units

Generation Units	Unit Size (MW)	Unit Type	Bus No.	P_{min} (MW)	P_{max} (MW)
1,2	20	Oil/CT	1	6	20
3,4	76	Coal/Steam	1	25	76
5,6	20	Oil/CT	2	6	20
7,8	76	Coal/Steam	2	25	76
9,10,11	100	Oil/Steam	7	40	100
12,13,14	197	Oil/Steam	13	80	197
15,16,17, 18,19	12	Oil/Steam	15	5	12
20	155	Coal/Steam	15	3	155
21	155	Coal/Steam	16	60	155
22	400	Nuclear	18	200	400
23	400	Nuclear	21	200	155
24,25	155	Coal/Steam	23	60	155
26	350	Coal/Steam	23	150	350

7. Sensitivity Analysis

In this part, a sensitivity analysis is performed. Changes of the purchased reserve with respect to the alterations in load levels, the change in the required reliability level for DISCOs, uncertainties in load forecast and also changes in the ORR of generators are studied.

7.1 Changes in the load level

In order to study the effect of changes in load level of the DISCOs on the amount of spinning reserve required, we assumed that the requested load level ranges from 1200MW load level to2850 MW load level (peak load).

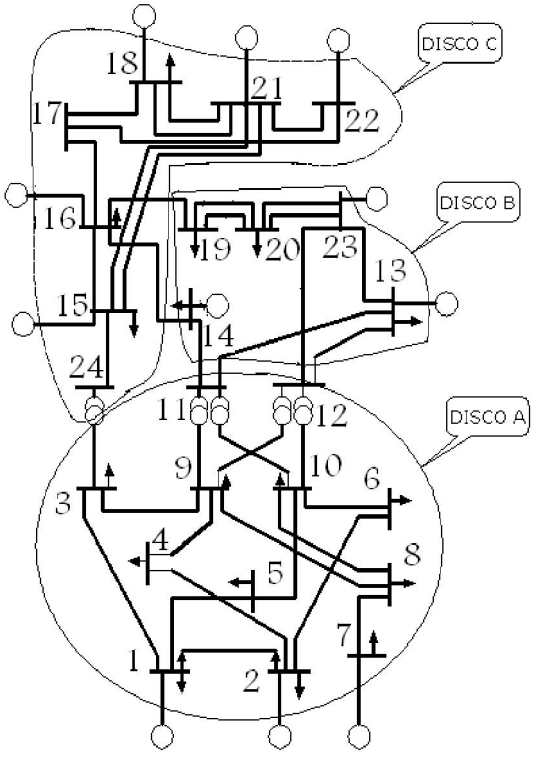

Fig 4 - The one line diagram of the IEEE-RTS system indicating how the customers are grouped into DISCO A, DISCA B and DISCO C.

The results obtained for the amount of spinning reserve as load level is changed are shown in Figure 5.

With the increase of the total purchased reserve in the system, the share of each DISCO for reserve increases as seen from Figure 6.

7.2 Changes in the required reliability level of the customers

The required reserve for the system is related to the reliability level of the system. In this part the effect of changes in the requested reliability level by the customers on the amount of reserve in the system is studied. Since the highest reliability level for the system is always less than one, the requested reliability level for DISCOs is increased from its initial value so that it is close to one.

For this purpose, the difference between one and the required reliability level for the DISCOs is calculated and a percentage of this difference is added to the requested reliability level in 25% increments. The results obtained are shown in Fig.7.

It can be seen from this Figure that the amount of needed reserve increases as a higher reliability level is requested. Moreover, as a higher amount of spinning reserve is provided, the risk of the system is decreased. We can also see from Figure 7 that when the reliability level of the customers is increased to 75%, the system risk level is reduced and as a result the purchased reserve in the system for all the load levels is fixed at about 400 megawatts.

Table 3 - Assumed bid for prices of generation units for the reserve market

U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14	U15	U16	U17
10	12	13	14	15	16	17	18	19	20	21	22	22	23	25	25	25

Table 4. Customer information in the market for total system load level of 1800MW

Total load of the system (MW)	The load and requested reliability level of each DISCO			Total level of system Risk(LOLP)
	DISCO	load (MW)	Requested Reliability level	
1800	A	830	0. 997	0. 006
	B	450	0. 9938	
	C	520	0. 9912	

Table 5 Spinning reserve market clearing for load level of 1800MW and system risk level

Customers	G_i Energy market	g_i reserve market	$g_i + G_i$
U1	310.5	89.5	400
U2	312.4	10.5	322.9
U3	350	0	350
U4	56	0	56
U5	101	0	101
U6	104	0	104
U7	110	0	100
U8	100	0	100
U9	100	0	100
U10	100	0	100
U11	20	0	20
U12	20	0	20
U13	20	0	20
U14	20	0	20
U15	76	0	76
	Total demand=1800MW	Total purchased reserve=100MW	
	Total System Risk (LOLP)=0.0185876		Total System Risk (LOLP)=0.005824

Table 6 - The steps of obtaining the allocation coefficients

Requested reliability levels		The share of purchased spinning reserve for customers for various courses			purchased reserve in each course of division
Entire system	DISCO	DISCO A	DISCO B	DISCO C	
LOLP=0.006	0.9912	20.05	17.45	18.5	56
	0. 9938	23.52	20.48	0	100
	0. 997	30	0	0	130
RE_I	customer's share	73.57	37.93	18.5	
RF_I	division coefficients	0.56	0.31	0.13	

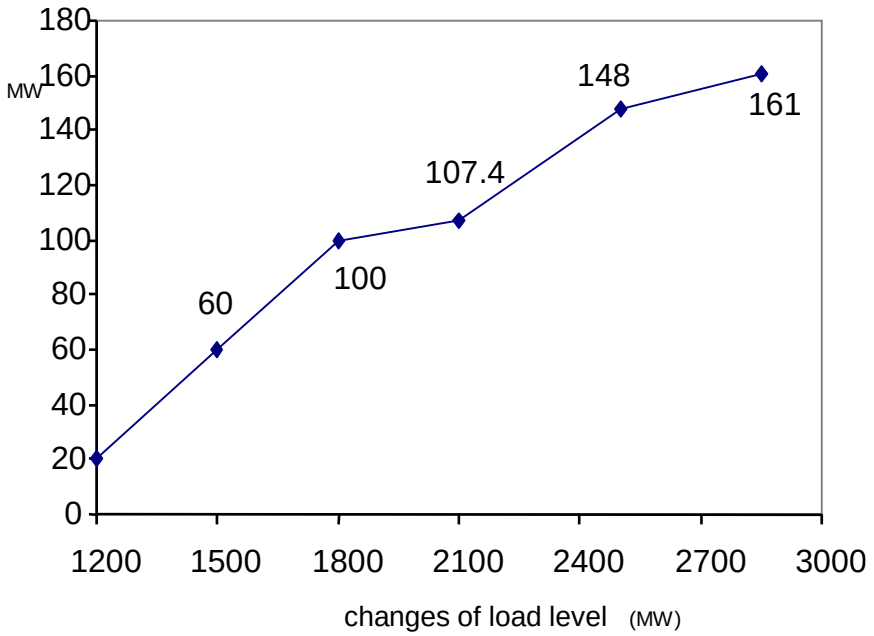

Fig 5 Changes in purchased reserve in the system with respect to the load level changes of DISCOs

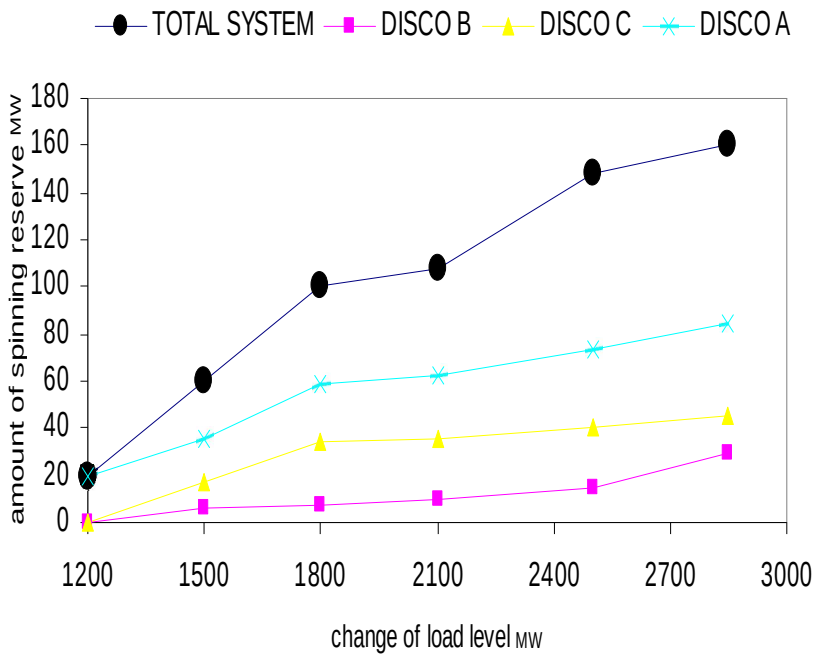

Fig 6 Change in spinning reserve and share of each DISCO from reserve

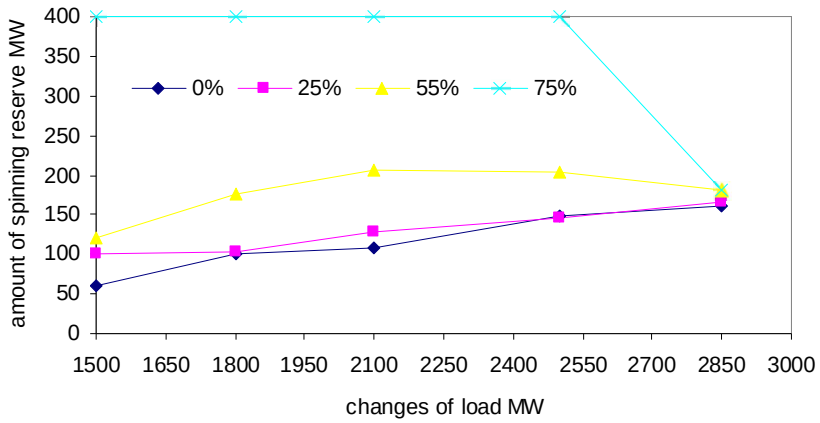

Fig 7 Change in spinning reserve with respect to risk level of the customers

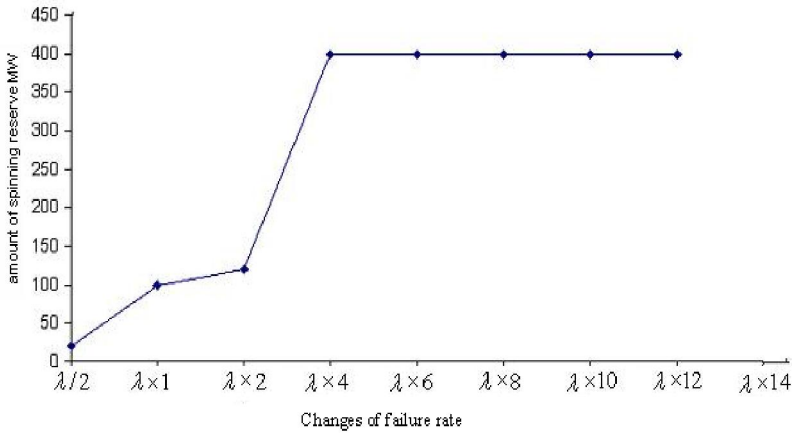

Fig 8 Changes of amount of spinning reserve with respect to changes of failure rate

7.3 Changes in the failure rate of generating units

The higher the failure rate of the generating units, the more is the risk of system and as a result system reliability level reduces. Hence ISO must consider larger amounts of reserve for system. This point is shown in Fig. 8 where the changes in the failure rate of generating units are shown up to fourteen times greater than the actual rate for a load of 1800MW.

8. Conclusions

In this paper, a new approach is proposed for clearing the reserve market and allocating the associated costs. In the proposed method, customers have the chance to choose their required risk levels. That is, the customers send their energy requirement and desired risk levels to the market. Reserve market is cleared such that the total risk level is satisfied. After clearing the reserve market, the cost of reserve is allocated amongst the various customers according to their requested reliability levels. Lower cost for spinning reserve leads to less reliable electric service whereas a higher reliability level requires that the customers pay more. All these choices are given to the customers. From the customers' perspective, the system reliability is not uniform any more. The proposed method has been applied to the IEEE-RTS and the simulation results which ascertain its effectiveness and efficiency have been presented.

Acknowledgement

We would like to express our gratitude for the Office of Vice Chancellor of Research and Technology of the Ferdowsi University of Mashhad for the Grant Project.

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3/9/2010

Determination of Bauxite's phases by the bomb digest method at Kamsar laboratory ISO 9002 (Guinea)

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Abstract: This paper presents the results of the experimental work done to find out the extraction percentage of alumina content in ore samples of bauxite from three mines of Guinea .So the knowledge of the chemical composition of a matter or a product directs us on its origins, its possible use and especially towards the technology which it will be necessary to apply for its transformation. This chemical composition is given at the laboratory which, to have reliable results uses adequate methods of analysis for each type of element to be proportioned in the matter. Thus for the analysis of bauxite exploited by the company of bauxites to Guinea (C.B.G.) and which currently comes from the plates of Sangaredi, Bidikoum and Silidara, the chemistry laboratory of Kamsar uses mainly two categories of methods which are instrumental and wet chemical method (volumetric). This study has relied on the chemical method due that it primarily rests on the quality of the matter to analyze and the concentration of the chemical elements which make it up. To this end, the Guinean bauxite exploited by the C.B.G having a high percentage in Al_2O_3 and a content of SiO_2 not exceeding 7%, for the determination of the various phases from this one, the section bomb digest of the laboratory at Kamsar uses a wet alkaline attack. Under high pressure and at variable temperatures according to the mineralogical phase to determine, this digestion is schematized as:Al_2O_3 + 2NaOH - $2NaAlO_2$ +H_2O.Soluble aluminate. [Journal of American Science 2010;6(6):139-145]. (ISSN: 1545-1003).

Key words*:* Bauxite's phases, Gibbsite, Boehmite, Guinea and Bomb digests

I. Introduction

Initially the bauxite term was introduced in 1821 by the French man Berthier to indicate a red ground deposit level on the ground, close to the village of the Baux - de - Province (valley of Rhone) in the Southern France (Sedat et al. 2006; J.Canérot et al 1999) The term bauxite is used to describe weathering products rich in alumina but low in alkali, alkaline earth and silica. It is composed principally of one or more hydrated aluminum minerals: gibbsite ($Al_2O_3·3H_2O$), boehmite ($Al_2O_3·3H_2O$) and diaspore ($Al_2O_3·H_2O$) with impurities of silica, iron oxide, titanium oxide and other elements in minor or trace amounts (Y. Idris et al.2004 .Shaffer, 1975). Valeton (1972) defined bauxite ore as bauxite that is economically mineable at present or in the foreseeable future, containing not less than 45–50% Al_2O_3, and not more than 20% Fe_2O_3 and 3–5% combined silica.

In terms of the geological field exploration for bauxite, the quantities and topological distribution of the main components and that of the contaminants are of great importance to be reckoned with in the search for new resources. Early international practices of bauxite analyses are usually done to determine loss of ignition, and alumina, silica, iron oxide and titanium contents. But recently, the range of bauxite components considered has been extended to the contaminants, substantially influencing bauxite processing (Authier-Martin et al., 2001; UNIDO, 1985). An idea of the contents of these contaminants is therefore very important as it is taken into account when determining the individual value and price of a given ore.

The world's prospective bauxite resources are located mainly in the developing countries, where one half of the world production comes from (Dash et al., 2007;Lotze, 1978). In Africa, the largest deposits are found in Guinea, which account for Africa's 83.7% proven and probable resources of high-grade bauxite (Y. Idris et al.2004) with Cameron coming second in terms of proven reserve (Clarke, 1987). Other African bauxite producers are Ghana, Mozambique, Sierra Leone and Zimbabwe.The bauxite is a no definite geological formation, it is a

mixture of oxides whose aluminum oxide is far more dominating, then comes the iron oxide which gives the reddish color to bauxite, the silica oxide and some compounds from metals such as: vanadium, titanium, lead, calcium, Zinc. Sometimes sulphur, copper, Nickel ,according by M.Karadag et al 2009.

The composition of bauxites generally varies in the following percentages (Santos et al 2004):
AL_2O_3 40-60%;H_2O 12-30%;Fe_2O_3 5-30%; SiO_2 1-20%

The bauxites quality initially depends on their content of Al_2O_3 and SiO_2. The more the bauxites contain Al_2O_3 and less SiO_2, the higher their economic and industrial values are significant (Tardy et al. 1991).

In Kamsar, it is the trihydrate which is most widespread whose characteristics make this bauxite most economically exploited. It is most soluble in alkalis and dissolves between 90 at 100 C. It comes in general from the deterioration of the sediments (schist, aleurolothes, argillites) and basic magmatic rocks (dolerites, gabbro-dolerites, konga-diabase) (Luke J. Kirwan 2009).

The bauxite of Guinea exploited by the CBG is in the open air very rich in alumina and a content of SiO2 lower than 7 %(Santos M.C, 2003**)**. For bauxites rich in Al_2 O_3 and low SiO_2, the wet alkaline process is best according by N. Zwingmann et al 2009 .The purpose of this study therefore is to ascertain the quality and the nature of bauxite often the customers' request. Compared to the other methods of decomposition and setting in solution such as the triacid method (HCI, HNO3 and H2SO4), this method uses little reagent, less expensive, less toxic, gives reliable and precise results (S.A. Hussain et al. 2000).One of the sections in the kamsar's laboratory use this method which calls section Bomb digest.

II - **Properties of alumina hydrates**

The properties of alumina hydrate (A. Asghar Calagari, 2007) as fallowing

Table 1.The Properties of alumina hydrates

N°	Designation	Gibbsite or Hydrargillite	Boehmite	Diaspore
1	Content alumina	65.4	85	85
2	System Crystallin	Monoclinic	Orthorhombic	Orthorhombic
3	Mohs hardness	2.5-3.5	3.5-4.0	6.5-7.0
4	Fast temperature of dehydration	150°C	350°C	450°C
5	Product of dehydration	X-Al2O3	Y-Al2O3	Z – Al2O3
6	Density	2.42	3.01	3.44
7	Solubility in Na2O 100g/l à 125°C, Al2O3 g/l	128	54	Unsoluble

III - Method and Materials

III.1- Materials and solutions needed

This study has used the following materials**.**

Table 2. materials and specifications

N°	***Materials***	***Specifications***
1	Thermometer	control the temperature
2	Whattman 40	filter the solution
3	Bombs	in which is the bauxite-soda mixture

4	Furnaces of 143C and 235C	allow heating the bomb containing the mixed solution (bauxite-soda)
5	Copper disc	for the bomb
6	Drying oven 105C	dry bauxite
7	Torque wrench	tighten the lid of the bomb firmly
8	Burette and stand	for the titration
9	Bomb pilot	for the control
10	Sound stop watch	for the time
11	Grip	leave the bombs in the furnaces
12	Bain-marie	cool the bombs
13	Policeman	: in which we put water to clean the bombs
14	Magnetic stirrer	make homogeneous the solution
15	Cone cup 500ml	in which the mixture occurs

Solutions Needed

1. NaOH standard solution (C_{NaOH}=0.500 mol/L)
2. HCl standard solution (C_{HCl}=0.500 mol/L)
3. NaOH.SiO2, 102 g/l
4. Starch and paper pulps
5. Gluconate of sodium ($NaC_6H_{11}O_7$)
6. NaCl 5 g/l
7. NaOH 102g/l
8. Phenolphthalein indicator
9. Buffer solution pH 8.0
10. HCl1:1 solution
11. KF solution

III.2- Methods used

The quantity (1,3±0,0001g and 0,65±0,0001g) of bauxite are heated, under pressure, in a solution of sodium hydroxide (102g/l) respectively at 143°C , 235°C (fig.1); the insoluble matters are separated by filtration. A alumina present in the filtrate is given by titrating the equivalent of the ions hydroxides (OH^-) released by fluorine in a chelating solution containing sodium gluconate (**Loh et al., 2005).**

The Chemical Processes involving the dissolution of Al_2O_3 by soda is done according to the reaction below:

$Al_2O_3 + 2NaOH - 2NaAlO_2 + H2O$ (eq.1)

A part of Al_2O_3 and SiO_2 combined in kaolinite reacts on soda according to the equation (eq.2)

$Al_2O_3.2SiO_2 + H_2O + 2NaOH - Na_2O.Al_2O_3.2SiO_2 + 2H_2O$ (2)

The starch and paper pulp make it possible to agglomerate the fine suspended particles in the bomb and to release the base with the policeman from it. The sodium gluconate ($NaC_6H_{11}O_7$ or $CH_2OH\text{-}(CHOH)_4C^{=o}\text{-}ONa$ in solution 25% combines with sodium aluminates to form a complex easily hydrolysable as shown in equation 3.

$NaAlO_2 + CH_2OH\text{-}(CHOH)_4\text{-}C^{=o}ONa + 2H_2 \longrightarrow Al(OH)_3 + CH_2OH\text{-}(CHOH)_4\ C^{=o}ONa \pm NaOH$ (3)

The KF solution moves sodium gluconate to fix aluminum in the form of cryolite (Santos et al 2004a) (eq.4).

$Al(OH)_3 + CH_2OH\ (CHOH)_4\text{-}C^{=o}ONa + 6KF \longrightarrow AlF_6\ K_3 + CH_2OH\text{-}(CHOH)_4\text{-}C^{=o}ONa + 3KOH$ and the potash releases which one titrates by HCI 0.5 normal by using at the same time the pHmeter and the indicator according to the equation 5.

$KOH + HCI \longrightarrow KCI + H_2O$ where $H^+ + OH^- \longrightarrow H_2O$ (eq.5)

Figure 1. The furnaces of 143°C and 235 °C

III.2.1- Determination of alumina by the equations of the reactions

Adding equations 1,3 and 4 by multiplying the two last by 2 to obtain the general equation of proportioning

$Al_2O_3 + 2NaOH \quad 2NaAlO_2 + H_2O$

$2NaAlO_2 + 2CH_2OH\text{-}(CHOH)4\text{-}C\text{-}ONa + 4H_2O \longrightarrow 2Al(OH)_3 + CH_2OH(CHOH)_4\text{-}C\text{-}ONa + 2NaOH$

$2Al(OH)_3 + CH_2OH(CHOH)_4\text{-}C\text{-}ONa + 12KF \longrightarrow 2K_3AlF_6 + 2CH_2OH\text{-}(CHOH)_4\text{-}C\text{-}ONa + 6KOH$

$$Al_2O_3 + 12KF + 3H_2O \longrightarrow 2K_3AlF_6 + 6KOH \text{ (eq.6)}$$

$$KOH + HCl \longrightarrow KCl + H_2O$$

where $OH^- + H^+ \leftrightarrow H_2O$

Equation (5) is a reaction of neutralization and at equivalence we have: quantity of ions OH^- released by the solution of KOH is equal to that of the ions H^+ brought by the solution of HCl 0.5 N.

$n_{OH^-} = n_{H^+} \rightarrow N_{OH-} \times V_{OH-} = N_{H+} \times V_{H+} - = meg$

According to the formula mass m = Eq.meg → meg = m / Eq = $N_{OH-} \times V_{OH-} = N_{H+} \times V_{H+}$

And the equation of reaction, we obtain

$N_{OH-} \times V_{OH-} = N_{H+} \times V_{H+} = m_{KOH} / Eq_{KOH} \rightarrow m_{KOH} = N_{H+} \times V_{H+} \times Eq_{KOH-}$

From equation (6)

$102g\ Al_2O_3 \rightarrow 6 \times 56g\ KOH$

$mAl_2O_3 \rightarrow m_{KOH}$

x m $m_{Al2O3} = 102g \chi m_{KOH} / 6 \times 56g = 102xN_H + V_H + \times Eq_{KOH} / 6x56g$

$Eq_{KOH} = 56/1 = 56$; $N_{H+} = 0.5N$

$m_{Al2O3} = 102g \times 56 \times N_{H+} \times V_H+ / 6 \times 56g = 102 \times 0.5 / 6 \times V_H +$

$mAl_2O_3 = 8.5\ V_H +$ en mg.

In practice, we checked if all the quantity of KOH is proportioned by adding 5ml HCl 0.5 N in excess which we titrated by soda having same normality.

Not proportioned KOH is equal to $5 - V_{NaOH}$ where $m_{Al2O3} = 8.5 \times 10^{-3}(V_H^+ + 5 - V_{NaOH})$ in gram but $V_{NaOH} + 5 = V_{THCl}$ therefore $m_{Al2O3} = 8.5 \times 10^{-3}(V_{THCl} - V_{NaOH})$.

Knowing the mass of Al_2O_3,we determined its percentage according to the relation

PE → 100

$m_{Al2O3} \rightarrow y$; $y = (m_{Al2O3} / PE) \times 100$

$y = \%\ Al_2O_3 = (8.5 \times 10^{-3}(V_{THCl} - V_{NaOH})/PE) \times 100$

In the furnace of 143°C PE = 1.3±0. 0001

$\%\ Al_2O_3 = 0.85\ (V_{THCl} - V_{NaOH}) / 1.3.$ $\%\ Al_2O_3 = 0.65(V_{THCl} - V_{NaOH})$

In the furnace of 235°C PE = 0.65± 0.0001

$\%\ Al_2O_3 = 0.85\ (V_{THCl} - V_{NaOH}) / 0.65.$ $\%\ Al_2O_3 = 1.3(V_{THCl} - V_{NaOH})$

III.2.2- Mass percentage of Al_2O_3

Formula: $\%\ Al_2O_3$ (gibbsite) = (axV±b/1.30) × *x*100

$\%\ Al_2O_3$ (gibbsite and boehmite) = (axV±b/0.65) × *x*100

Factor of the day: $y = 0.00891x - 0.0114$

IV- Results and Discusions.

The concentration of Al2O3 (143°C) and Al2O3 (235°C) in the samples as presented respectively in Table 3 ranges from 44.43% to 44.49% ;Table 4 ranges from 50.33% to 50.34% . For the standard bauxite sample N°021002040s the analyzed concentration of Al_2O_3 (143°C)as presented below in Table 3 is 44.83% as compared to the certified value of 44.77%; inTable 4 is 51.84% as compared also to the certified value 51.84 This shows that the error in terms of accuracy of the measurement is less than 1%. Also the error in terms of precision of the measurements defined as one standard deviation of the percentage of the mean is less than 2% for all the samples.

Table 3: Quantity of gibbsite contained in each 1,3±0,0001g of bauxite samples at 143°C

N° Bomb	N° of Laboratory	Mass samples	Calculation	%Mass	
06	021002035	1.3000	65.70 + 5 =70.70-4.50 = 66.20	44.49
12	021002036	1.3000	65.80 + 5 =70.80-4.50 = 66.30	44.56
13	021002037	1.3000	65.60 + 5 =70.60-4.50 = 66.10	44.43
14	021002038	1.3000	65.70 + 5 =70.70-4.50 = 66.20	44.49
15	021002040s	1.3000	66.10 + 5 =71.10-4.50 = 66.60	44.77
16	021002040s	1.3000	66.20 + 5 =71.10-4.50 = 66.70	44.83

Table 4: Quantity of gibbsite and boehmite contained in each 0,65±0,0001g of bauxite samples at 235°C

N° Bomb	N° of Laboratory	Mass samples	Calculation	%Mass
17	021002035	0.6500	37.60 + 5 =42.60-4.60 = 38.0	50.34
18	021002036	0.6500	37.40+ 5 =42.40-4.60 = 37.80	50.34
19	021002037	0.6500	37.60 + 5 =42.60-4.60 = 38.00	50.33
20	021002038	0.6500	37.60 + 5 =42.6 -4.60 = 38.00	50.34
24	021002040s	0.6500	38.60 + 5 =43.60-4.50 = 39.10	51.84
25	021002040s	0.6500	38.60 + 5 =43.60-4.50 = 39.10	51.84

From table (3) and (4),the experiment showed that trihydrate (gibbsite) is more soluble than monohydrate (boehmite). It dissolves at lower temperatures but in the solution, it is transformed into monohydrate at high temperature

The results show that solubility increases with the soda concentration used and the temperature. This is why in the bomb digest, trihydrate is attacked at 143°C and at the temperature of 235°C we have the two (2) phases which are attacked (**H**. Chuanbin et al 2008) .The advantage of these proportioning results from the request of the customers for whom the choice of quality depends on the nature of bauxite, otherwise if the bauxite is a monohydrate or a tri hydrate.

By P. Smith 2009**,** the bauxites with weak content of SiO_2 are the best on the market; because in the current industry of Al_2O_3, the most delicate operation is the separation of impurities containing silica.

V- Conclusion

This study briefly focused on the section bomb digest of chemistry laboratory , to define the types of bauxite and especially the contents of Al_2O_3 soluble.The determination of the bauxite mineralogical phases by the Bomb digest method makes it possible to know well the nature of bauxite and especially its content of soluble Al_2O_3, i.e. the decompositions at the temperature: 143°C pour le trihydrate (gibbsite) and 235°C for total alumina (trihydrate and monohydrate).The chemical composition in Al_2O_3 soluble is significant, but it is also necessary to lay a particular stress on the method of proportioning for the bomb digest which for the moment is used. The method of the calibration curve can show the impossibility of measurement in consequence of awkward substances, if a line is not obtained, in this case it is recommended to use the chemical reactions for the calculation of the percentage of Al_2O_3.

Acknowledgements : Authors are grateful to the Department of School of Environmental Studies China University of Geosciences for financial support to carry out this work.

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Submission date : 10/02/ 2010

Clinical utility of biochemical markers in ulcerative colitis among Egyptian patients

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Abstract

Biochemical markers are a non-invasive way of objectively measuring inflammation in ulcerative colitis and can play an adjunctive or primary role in the assessment of disease activity. **Aim** of this study was to A) evaluate serum levels of some biomarkers "leptin, adiponectin, resistin, and ghrelin" in ulcerative colitis (UC) patients, besides the ordinary inflammatory markers, B) to correlate the results with the disease activity, with the clinical characteristics of the disease C) and to examine the possible interaction between the estimated parameters values. Study was conducted on 56 UC patients from the Clinic of Internal Medicine Department and Endoscopy Unit of Alzahraa Hospital, Alazhar University, besides 30 healthy subjects served as control group. **Results:** Mean levels of ESR, CRP, TNF-α, resistin and ghrelin were significantly higher in active UC patients than the control group, while after the courses of treatment 47 patients achieved complete remission (inactive UC) mean values of these biochemical parameter deceased significantly than the original values at the active disease and the values reached nearly the normal ranges. While in patients (9 patients) who did not achieved complete remission, there were moderate decreased serum levels of these biochemical markers but still higher values than the control group and they still have manifestations of active UC. The mean level of leptin was significantly decreased in active UC patients compared to the control group, while after the course of treatment in patients achieved complete remission (inactive UC) the mean value increased significantly (with mean value 10.1 ng/ml). **Conclusion**: Our data indicate that, the increased plasma resistin, TNF-α and ghrelin levels correlated with activity of ulcerative colitis and so they could predict the response to therapy and possibly reflect an acute-phase response due to inflammation more than the ordinary inflammatory markers. Resistin, TNF-α and ghrelin levels could be considered as an independent predictor of disease activity in patients with UC and may represent link between inflammation and UC. [Journal of American Science 2010; 6(6):146-155]. (ISSN: 1545-1003).

Key words: ulcerative colitis, inflammatory markers, leptin, resistin, ghrelin, Tumor Necrosis factor alpha.

1. Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) usually affects only the innermost lining of your large intestine (colon) and rectum, with characteristic ulcers, or open sores, in the colon. Ulcerative colitis occurs in all age groups, with the most common age of diagnosis being 15–35 years of age with a second, lesser peak between 55 and 65 years. Men and women are affected equally. Whites are more frequently affected than other racial groups, and people of Jewish origin have 3–6 times greater likelihood of suffering from any IBD (Baumgart and Carding 2007).

Ulcerative colitis typically begins gradually, with abdominal pain and diarrhea that is sometimes bloody. In more serious cases, diarrhea is severe and frequent. Fever, loss of appetite, and weight loss occur. The severity of the disease depends on how much of the colon is involved. (Kasper DL et al. 2005).

Many aspects of the IBDs, Crohn's disease (CD) and UC, still present challenges for physicians treating this disorder: diagnosis, prognosis, assessment of disease activity and severity, as well as outcome of therapy. For each of these aspects, there is no single "gold standard" test or examination. Instead, physicians apply a combination of symptoms, clinical examination, laboratory indices, radiology, and endoscopy with histology to make the diagnosis, to assess severity, and to predict the outcome of disease. There are several reasons why laboratory markers have been studied in IBD in the past decades: firstly, to gain an objective measurement of disease activity as symptoms are often subjective; and secondly, to avoid invasive (endoscopic) procedures which are often a burden to the patient (Vermeire et al. 2006).

Some studies suggest that white adipose tissue (WAT), besides its ability to respond to afferent signals from traditional hormone systems and the central nervous system also expresses and secretes factors with important functions, collectively called adipocytokines. There is evidence that adipocytokines are involved in inflammatory and metabolic pathways in humans. Among the adipocytokines, leptin, adiponectin, and

resistin appear to play an important role (Konstantinos et al. 2005).

Human resistin is a 108-amino acid peptide hormone with a molecular weight of 12.5 kDa. Resistin was extensively studied in patients with both rheumatoid arthritis and osteoarthritis and significant correlation with inflammation and elevated C-reactive protein (CRP) was reported (Schaffler et al. 2003). There is evidence that resistin is involved in the inflammatory and metabolic pathways in humans and a possible role in IBD was recently postulated in certain IBD patients (Paul et al. 2005).

Leptin is a 16-kDa non-glycosylated protein, adipocytes secrete leptin in direct proportion to WAT mass and this secretion is greater from subcutaneous compared to visceral WAT. Leptin possesses a proinflammatory as well as anti-inflammatory properties according to the experimental conditions. Its role in IBD has been studied, but the results are conflicting, therefore further investigation is required (Otero et al. 2005).

Ghrelin is a recently discovered hormone, with a crucial role in the regulation of food intake and energy homeostasis. It is mainly produced at the stomach but is also expressed in WAT, albeit in trace amounts. Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) and it has been identified in T cells. Ghrelin can inhibit cytokine activation including interleukins, TNF-α, and most interestingly leptin. Recently, high levels of serum ghrelin were found in patients with celiac disease (Peracchi et al. 2003).

This study designed to study the utility of biomarkers (leptin, adiponectin, resistin, and ghrelin) besides the ordinary inflammatory markers in measuring disease activity and/or severity and predicting the response to therapy in patients with UC, and to examine the possible interaction between the estimated biomarkers values.

2. Subjects and methods

The present study was conducted on 56 patients (35 males and 21 females). They were selected from Inpatient and Outpatient Clinic of Internal Medicine Department and Endoscopy Unit of Alzahraa Hospital, Alazhar University. The Patients were newly diagnosed with acute active ulcerative colitis (AUC), besides 30 healthy subjects (18 males and 12 females) to serve as a control group. Patients with coexisting conditions that may influence the result of serum biomarkers, such as recurrent infections, malignancy, recent surgery, major systemic illnesses, and inflammatory arthritis were excluded from analysis. All patients and control subjects were non-smoker and gave their informed consent to participate in the study, which was approved by the Hospital's Scientific Committee.

2.1 The diagnosis of UC disease was based on:

(i) - History taking:

Include a history of diarrhoea and/or rectal bleeding for 6 weeks or more.

(ii) - Complete clinical examination:

During baseline evaluation, disease severity in patients with UC (active or inactive) was assessed clinically according to the practice guidelines of the American College of Gastroenterology. Mild UC was defined as having ≤3 stools daily with or without blood and with no systemic toxicity. Moderate UC was defined as having 4–6 stools daily with or without blood or with minimal signs of toxicity. Severe UC was defined as having ≥7 stools daily with or without blood, and with moderate to severe systemic toxicity (fever, ≥10% weight loss, orthostasis, haemoglobin ≤10 g/dL), or required hospitalization.

(iii) - Endoscopic findings:

- Colonoscopy: baseline colonoscopy with biopsy sampling performed in all patients with UC, in order to assess the endoscopic severity and extent of disease. Endoscopic severity measured by a modified endoscopic score with an 18-point scale involving nine parameters: erythema, vascular pattern, friability, granularity, spontaneous bleeding, occurrence and severity of ulcers, extent of ulcerated surface, and presence of mucopurulent exudates. All parameters were scored from 0 to 2 points. Four grades of activity were considered according to the sum of all parameters: inactive disease (0-3), mild disease (4-7), moderate disease (8-12), and severe disease (13-18). Grading of endoscopic severity was done from the most inflamed part of the bowel. The extent of disease was recorded as recto-sigmoiditis, left-sided colitis, and pancolitis.

- Characteristic microscopic changes of biopsy specimens reveal abnormal mucosal architecture and lamina propria cellularity, neutrophil polymorph infiltration and epithelial cell abnormality.

2.2 Treatment protocol

Patients with active UC were treated for attenuation of disease activity with high-dose corticosteroids (prednisone 40 mg/day) and mesalazine (3-4gm/day) orally and rectally. Patients were set into a follow-up program with regular visits every 2nd wk for 12 wks. Corticosteroids were tapered off with a weekly based schedule throughout the study period. At the end of the study (12th wk), complete clinical, endoscopic and laboratory evaluation, similar to baseline week, was performed in all patients with active colitis. Complete response to therapy (remission) was considered, if a SCCAI score (Simple Clinical Colitis Activity Index) of

≤2 and endoscopic remission was achieved after 12 wks of therapy. Partial response was considered if a 50% reduction of SCCAI score was noted together with a reduction of endoscopic activity by at least one grade.

2.3 Sampling:

Peripheral blood samples were drawn from the patients at diagnosis (acute active group) and after treatment (inactive group). Five ml of fasting venous blood samples were collected from each subject. The blood was left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r. p. m. Sera were divided into several aliquots and stored at –70°C until assay.

2.4 Laboratory tests

- Hemogram: included hemoglobin concentration, total leucocytic count, platelet count using Coulter counter and examination of Lishman or Wright-stained peripheral blood smears.
- Body mass index (BMI) was calculated as body weight in kilogram divided by the square of height in meter (kg/m^2).
- ESR was measured by standard laboratory technique (normal values <20 mm/h) according to Westergren (Westergren, 1921).
- Tumor Necrosis factor alpha (TNF-α) was determined by imunoenzymometeric assay using kit supplied by Bio-Sourse Europe S.A. (Rue de Industries 8-B-1400 Nivelles, Belgium) (Bienvenu A, 1993).
- Serum leptin concentration was determined using a direct enzyme-linked immunosorbent assay (ELISA) kit (Cat. No.DSL-10-23100, Med Diagn. Comp, Germany) (Agata et al, 1997).
- Serum resistin levels were measured by sandwich enzyme-linked immunesorbent assay (BioVendor Laboratory Medicine, Inc., Plackeho, Czech Republic) (Youn et al. 2004).
- Serum ghrelin levels were measured using DSL-10-33700 active total ghrelin ELISA kits, USA, using one-step sandwich-type immunoassay (Grochi et al. 2002).
- C reactive protein (CRP), C reactive protein (CRP), was determined by a high sensitive immunoassay for measuring human CRP which is a two step sandwich ELISA technique using kit supplied by diagnostic system laboratories (DSL-10-42100) Webster, Texas, USA (Rifai et al. 1999).
- Total cholesterol was determined by colorimetric method using Bio-Merieux test kit (Richmond W. 1973).
- High density lipoprotein cholesterol was measured after precipitation of LDL and VDL using phosphotungastate according to Henary et al. (1974).
- Low density lipoprotein cholesterol was measured by Friedwald method (Friedwald et al. 1972).
- Triglyceride level was determined by enzymatic colorimetric test with lipid clearing factor (Fossati and Prencipe, 1982**)**.

2.5 Statistical analysis

Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA). Data were presented as range, mean ±SD and number (%). Comparisons between the 3 groups (healthy controls, patients with active UC (before treatment) and patients with inactive UC after treatment) was performed using one way ANOVA as a parametric test for continuous variables as the biochemical markers, also, comparisons were performed between different subgroups of patients with active UC according to disease activity were made by the Kruskal-Wallis test (nonparametric ANOVA). Post hoc multiple comparisons tests were made by Dunn's test. Chi square test was used to compare between patients at diagnosis and after treatment regarding the clinical and endoscopic severity. Correlations between serum biomarkers and indices of disease activity were analyzed with the Pearson's correlation method. *P*-value of ≤ 0.05 was considered statistically significant.

3. Results

The study was conducted on 56 patients with active UC (35 males and 21 females, age range 26 - 45 years with a mean of 35.3 $\pm$ 2.1 years). besides 30 healthy subjects (18 males and 12 females, age range 25 - 46 years and mean of 36.05 $\pm$ 2.14 years) to serve as a control group. Colonoscopies were performed to all the patients; the active inflammation was confirmed by histological assessment. Among the 56 patients with acute colitis, there were 48 patients with non-extensive colitis and 8 patients with extensive colitis. The demographic, biochemical and clinical characteristic of the patients and the control groups are summarized in table (1).

All the patients and the control subjects were non-smokers, the patients received the treatment protocol for 12 weeks and then evaluated after therapy, 47 patients achieved a complete remission which is approved by the clinical, laboratory and endoscopic data. While, nine patients did not response well to the therapy as they did not achieve a complete remission (partial remission). So they received another therapy and excluded from the study.

Mean levels of ESR, CRP, TNF-α, resistin and ghrelin were significantly higher in active UC (56) patients (with mean values of 60 mm/l, 16.7 mg/l, 14.48 pg/ml, 18.86 ng/ml and 20 ng/ml respectively) than the

control group, while after the course of treatment 47 patients achieved complete remission (inactive UC) the mean values of these biochemical parameter deceased significantly (with mean values of 21.3 mm/l, 4.1 mg/l, 6.53 pg/ml, 10.93 ng/ml and 5.97 ng/ml respectively) than the original values at the active disease and the values reached nearly the normal ranges. While in patients (9 patients) who did not achieved complete remission there were moderate decreased (non-significant) serum levels of these biochemical markers (with mean values of 51 mm/l, 13.3 mg/l, 14.5 pg/ml, 16.3 ng/ml, and 8.9 ng/ml respectively) but still higher values than the control group because they still have manifestations of active UC (Table 2, figure 1).

The mean level of leptin was significantly decreased in active UC patients (with mean value 3.77 ng/ml) as compared to the control group, while after the course of treatment in patients achieved complete remission (inactive UC) the mean value increased significantly (with mean value 10.1 ng/ml). While in patients (9 patients) who did not achieved complete remission there were decreased serum level with mean value 5.8 ng/ml but still lower values than the control group because they have still manifestations of active UC (Table 2).

There were no significant differences in the levels of fasting blood glucose, total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride between control group, patients with active UC, patients with complete remission (inactive UC), and patients with partial remission (Table 2).

The correlation of serum biomarkers with clinical severity of UC is shown in Table 3. This demonstrates that most the commonly used biomarkers (ESR, CRP, TNF-α, leptin, resistin and ghrelin) were significantly associated with clinically severe disease. But there were no significant correlation in the levels of fasting blood glucose, total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride with the severity of UC, as well as with patient's personal characteristics such as age and gender.

Table 1: Demographic and clinical data of ulcerative colitis patients (mean and range)

	Control	**Active UC At diagnosis**	**Inactive UC After treatment**	**Partial remission During treatment**
number	30	56	47	9
Age (years)	36.05(25 – 46)	35.3(26 – 45)	37.6 (26 – 40)	40.6 (36 – 45)
Male	18 (60%)	31(55.36 %)	26 (55.3 %)	5 (55.5%)
female	12 (40%)	25 (44.64%)	21(44.7 %)	4 (44.5%)
BMI (kg/m^2)	22.5 (20 – 25)	19.3 (17 – 22) *****	18.9(17.5 – 21.1)*****	19.5(16.9 – 21.9)*****
Leuko.count (×10^9/L)	8.9 (4 – 10.5)	14.8 (6.9 – 24.5)*****	9.1 (5.6 – 11.4) ¶	12.3(6.5 – 18.7) ***¶**
Hemoglobin (g/dL)	13.7 (12 – 14.3)	10.8 (7.5 -11.7)*****	12.6 (8.9 – 13.5) ¶	10.5 (7.5 – 11.7)*****
Plalelet (×10^9/L)	224 (150-285)	375 (160 – 688)*****	232 (165 – 311) ¶	345 (125 – 564)*****
Clinical severity				
Mild	-	24 (42.9%)	4 (8.5%)	0
Moderate	-	26 (46.4%)	0	7 (77.7%)
Severe	-	6 (10.7%)	0	2 (22.3%)
SCCAI Score	-	7 ± 3 (4 -11)	0 ± 1 (0-2)	4 ± 2 (2-7)
Endoscopic severity				
Non extensive colitis	-	48 (85.8%)	45 (95.7 %)	3 (33.3%)
extensive colitis	-	8 (14.2%)	2 (4.3%)	6 (66.7%)

Leuko.count: leukocytic count SCCAI: simple clinical colitis activity index.
*Significant as compared to control group
¶ Significant as compared to active UC
SCCAI Score (Simple Clinical Colitis Activity Index) score (Walmsey et al. 1998)

Table 2: Biochemical parameters in patients with UC at diagnosis and after the treatment

Parameters	Control group (30)	At diagnosis	After treatment	
		Active UC (56)	Inactive UC (47)	Partial remission (9)
• **ESR (mm/h)**				
Range	16-19 mm/h	40-97	19 – 30	36 – 82
Mean ± SD	17 ± 1.2	60 ± 21*	21.3 ± 12	51 ± 17*
• **CRP (mg/l)**				
Range	3- 4.5 mg/L	9.1- 35.5	3.1- 7.3	11 – 29.2
Mean ± SD	4 ± 0.8	16.7± 2.2*	4.1±1.7	13.3 ± 1.5*
• **TNF-α (pg/ml)**				
Range	5 – 6.88	12.13 – 17.5	5.23 – 7.5	11 – 15.5
Mean ± SD	6.06 ± 0.28	14.486 ± 1.75*	6.53 ± 0.37	13.5 ± 1.8*
• **Resistin (ng/ml)**				
Range	9.5 -10.81	15.99 – 26.12	10.51-13.39	15.2 – 24.6
Mean ± SD	10.1 ± 0.32	18.86 ± 3.14*	10.93 ± 0.699	16.3 ± 2.5*
• **Leptin (ng/ml)**				
Range	9.59 – 10.92	3 – 4.5	8.98-11.39	3.4 – 4.5
Mean ± SD	10.24 ± 0.42	3.77 ± 0.45*	10.147 ± 0.68	5.8 ± 0.51*
• **Ghrelin (ng/ml)**				
Range	5.21- 7.23	18.9- 21.22	5.13- 6.53	6.75- 11.96
Mean ± SD	6.19 ± 0.49	20 ± 0.73*	5.97 ± 0.48	8.9 ± 0.52*
• **FBS (mg/dl)**				
Range	85 – 125	92 -132	95 – 135	95 – 125
Mean ± SD	109.5 ± 9.09	111.2 ± 10.45	116.6 ± 9.9334	117.5 ± 8.6
• **T-chol.(mg/dl)**				
Range	140 -165	145 – 168	150 – 167	146 – 165
Mean ± SD	152.85 ± 7.85	153.5 ± 5.24	155.05 ± 5.15	151.2 ± 4.7
• **LDL-chol. (mg/dl)**				
Range	68 – 85	68 -75	66.5 – 77.5	69 – 78
Mean ± SD	73.6 ± 5.99	70.2 ± 3.59	71.35 ± 3.66	72.2 ± 3.1
• **HDL-chol. (mg/dl)**				
Range	41 – 59	44.5 – 58	45.8 – 59.4	44 – 58
Mean ± SD	47.95 ± 4.850	51.97 ± 4.60	49.99 ±11.39	52.1 ± 3.9
• **Triglyceride (mg/dl)**				
Range	88 -110	85 – 115	83.5 – 109.5	88 – 112
Mean ± SD	94.6 ± 5.77	95.5 ± 7.61	93.96 ± 2.25	93.3 ± 8.21

* Significant P<0.05 compared to control group. Data are expressed as range (mean ± DS).
ESR: Erythrocytic sedimentation rate. CRP: C-reactive protein.
TNF-α: tumor necrosis factor alpha FBS: fasting blood sugar
T-chol.: total cholesterol DHL-chol: high density lipoprotein cholesterol
LDL-chol: low density lipoprotein cholesterol

Table (3): Correlation of serum biomarkers with clinical severity of ulcerative colitis

	Mild (24)	Moderate (26)	Sever (6)	p- value
Age	34 (31 – 40)	36 (26 – 41)	38 (30 – 45)	0.213§
Male	13 (54.2%)	14 (53.8%)	4 (66.7%)	0.126§
Female	11 (45.8%)	12 (46.2%)	2 (33.3%)	
ESR (mm/h)	42 (40- 50)	51 (41-80)	59 (45-97)	0.031 ¶*
CRP (mg/L)	16.5 (9.1- 38)	20 (9.5 – 52)	34.7 (10 – 65.5)	0.0044 ¶*
TNF-α (pg/ml)	13 (12.1 – 14)	14.1 (14.5 – 16)2	16.7 (12.5 – 17.5)	0.0443 ¶*
Resistin (ng/ml)	15.8 (15.9 – 19)	17.8 (16.6 – 23.5)	21.8(15.9 – 26.1)	0.034 ¶*
Leptin (ng/ml)	4.2 (3 – 4.5)	3.7 (3.1 – 4.1)	3.5 (3.2 – 3.9)	0.054 ¶
Ghrelin (ng/ml)	16 (18.2 – 19.5)	16.5 (18.9-20.7)	17.4 (18.5- 21.22)	0.044 ¶*
FBS (mg/dl)	101.5 (98 -120)	110.2 (100 -129)	111.2 (115 -132)	0.065
T-chol.(mg/dl)	148.1 (145 – 151)	153.5 (155 – 160)	162.5 (162 – 168)	0.121
HDL-chol.(mg/dl)	49.9 (44.5 – 49)	51.3 (45 – 53)	55.2 (46 – 58)	0.078
LDL-chol. (mg/dl)	69.2 (68 -70)	70.9 (69.2 -75)	72.7 (70 -75)	0.098
Triglyceride (mg/dl)	90.6 (85 - 96)	92.5 (88 - 110)	93 (90 - 115)	0.059

Data are expressed as median (range). § Chi-squared test; ¶ Kruskal–Wallis test.
ESR: erythrocytic sedimentation rate. CRP: C-reactive protein.
TNF-α, tumor necrosis factor alpha FBS: fasting blood sugar
T-chol.: total cholesterol DHL-chol.: high density lipoprotein cholesterol
LHL-chol: low density lipoprotein cholesterol * significant p<0.05

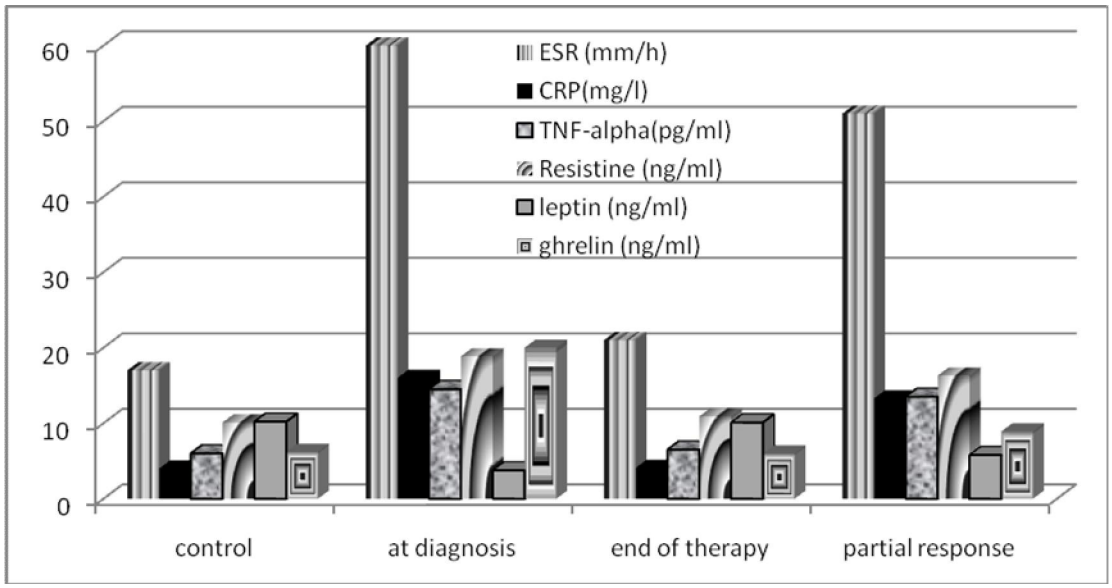

Figure (1): changes in serum values of biochemical markers in UC patients during therapy

4. Discussion

There are several reasons why laboratory markers have been studied in IBD in the past decades: firstly, to gain an objective measurement of disease activity as symptoms are often subjective; and secondly, to avoid invasive (endoscopic) procedures which are often a burden to the patient (Vermeire et al. 2006).

We studied serum levels of leptin, TNF-α, resistin, and ghrelin in patients with ulcerative colitis (UC) beside the traditional inflammatory markers to evaluate possible associations with the course and characteristics of the disease. Also, in order to provide objective assessment of the disease activity and decide which patient should undergo further assessment by colonoscopy.

The results of our study showed that there were significant decrement in the level of BMI and Hb%, while there were significant increase in the level of total leucocytic count and platelet count as compared to the normal control group. These levels were improved on the therapeutic protocol and reach nearly normal values after treatment, except in 9 patients who showed no improvement clinically and laboratory on therapy and we change the protocol of therapy to them and excluded from the study. Our results were in accordance with Vermeire et al. (2006) who stated that, more generally used laboratory markers include white blood cell count, platelets, and albumin. White blood cell count will increase as part of the acute phase response. Increased leucocytosis is therefore not a specific feature of IBD and may be seen in other inflammatory conditions and stressful events. Platelet count will also increase without being a specific marker of inflammation, given the wide range of normal values for platelet count, it has been less useful.

During an acute inflammatory episode, the leukocyte count and platelet count increase whereas the hemoglobin and albumin level decrease. Leucocytosis is not a useful marker of disease activity in clinical practice as there are many factors besides disease activity (systemic glucocorticosteroids, immunosuppressant, presence of abscess) that affect it. Platelet count correlates with disease activity in IBD but it is not used in clinical practice in IBD as there are other factors such as haemorrhage from other sites and iron deficiency anaemia which can cause elevation of platelet count. The role of these serum markers and their correlation with endoscopic and histological inflammation is not well established in UC patients (Desai et al. 2007).

Our results showed that, serum ESR and CRP levels were significantly increased in the acute active stage and these levels decreased following the therapy to nearly normal levels except in the 9 patients who did not respond to therapy. These results favour the results of Vermeire et al. (2006) who demonstrated that, established common serum biomarkers included CRP, ESR, hemoglobin, leukocyte count, platelet count and albumin level. The development of biological treatments has renewed interest in these biomarkers, given their potential to select responders to this therapy. CRP is found to be the most useful one in this respect. Whereas other acute phase reactants and markers of inflammation such as ESR also give reliable information on disease activity, their longer half life and interference with other factors make them less useful in clinical practice compared with CRP.

Lok et al. (2008) reported that Abnormal CRP, ESR, white cell count, haemoglobin, platelet count and albumin occurred in 42.3%, 55.1%, 23.1%, 21.8%, 32.1% and 25.6% of these mucosal inflammatory episodes, respectively. For the severity of the clinical disease, all serum biomarkers demonstrated a good correlation with the severity grading. On the other hand, the serum biomarkers correlated well with endoscopic extensive colitis but not with proctitis or left-sided colitis. Oruc et al. (2009) found that Ulcerative colitis patients had slightly higher procalcitonin levels and significantly higher C-reactive protein levels than controls and they conclude that Serum C-reactive protein is a reliable marker for disease activity in inflammatory bowel disease.

The results of our study showed the TNF-α, level was significantly increased in the active UC patients and this levels decreased gradually in response to therapy in 47 patients who showed inactive disease (complete remission) while in 9 patients the level of TNF-α, did not return to nearly normal values in response to therapy which called (partial remission). The results of our study enforced the results of Komatsu et al. (2001) who reported that the median serum concentration of TNF-α, in IBD patients overall was 1.7-fold higher in the active stage of UC than in the inactive stage ($P < 0.05$), and this difference could be detected in individual patients.

Inflammation is undoubtedly a key component in the pathogenesis of ulcerative colitis (Podolsky 2002), and proinflammatory cytokines (IL-1, IL-6, IL-8, TNF-α, and IFN-γ) operate as a cascade and network in stimulating the production of acute-phase proteins and induction of acute-phase manifestations (Gabay, 1999). On the other hand, Umehara (2006) found that serum levels of TNF-α,

were within the normal range in most of cases despite being in the active phase. Tumor necrosis factor-*α* is produced by activated macrophages and monocytes. Although the serum concentration of TNF-*α* is often increased in patients with active IBD, serum concentrations of TNF-*α* have not been consistently elevated and are thus of limited utility as markers of disease activity in these patients (Desai et al. 2007).

In our study, we measured the circulating levels of 4 hormones (adipocytokines) in patients with IBD. These hormones are produced by WAT either in large amounts (leptin, adiponectin, and resistin) or in trace amounts (ghrelin), and all of them are closely related to human metabolism and inflammation. Human metabolism dramatically changes in IBD, and chronic inflammation is the hallmark of the disease. Our results demonstrated that serum leptin levels are significantly decreased in patients during the active stage (acute) of UC than in those of healthy subjects and these levels return to nearly normal values in response to therapy except in 9 patients who showed partial improvement.

These results were in accordance to Kirchgessner et al. (1997) and Bruun et al. (2002) who found that UC patients had significantly lower leptin levels than control group. Leptin levels were significantly lower in IBD patients, mainly in UC, compared with control, suggesting that chronic intestinal inflammation may decrease circulating leptin levels. TNF-a is possibly the major cytokine involved in intestinal inflammation in IBD patients. Recently, a number of studies indicated that whereas TNF-α, transiently induces acute release of intracellular pools of leptin, it decreases leptin synthesis during chronic inflammation. On the other hand, although inflammatory gut diseases, through their associated cytokines, mediate energy loss and weight loss, it is not clear how the mechanisms work. It has been proposed by the investigators that proinflammatory cytokines release leptin from adipose tissue, which leads to increased plasma concentrations and this increase is inappropriately high for the percentage of fat mass (Ballinger 1999).

Barbier et al. (1998) reported that increased secretion of plasma leptin concentrations has been observed in the early stages of experimental intestinal inflammation in rats. Tuzun et al. (2004) reported that patients enrolled in their study were in the acute stage of UC. High leptin levels in these patients suggest that the acute inflammation associated with UC increases circulating leptin levels. Yet, the mechanism of the induction of leptin secretion in intestinal inflammation remains obscure. Recently, inflamed colonic epithelial cells were found to express and release leptin into the intestinal lumen, and the product appears to induce epithelial wall damage and neutrophil infiltration, a characteristic histological finding in IBD (Sitaraman et al. 2004).

The main production of resistin and ghrelin occurs in different sites, and in addition to their participation in the mechanisms of energy homeostasis, they seem to have in common a close association with inflammation, a fact that may implicate them in the pathogenesis of IBD (Konstantinos et al. 2006). In our study, the patients with active UC had significantly higher circulating levels of resistin and ghrelin as compared with healthy control. And these levels were decreased to nearly normal values as a response to therapy except the 9 patients who showed partial remission. Also, the results showed a significant increase of their levels with the progress of the disease.

The results of our study enforced the results of Ates et al. (2008) where serum ghrelin levels were significantly higher in patients with active UC than in those in remission. Their study demonstrates that patients with active IBD have higher serum ghrelin levels than patients in remission and high levels of circulating ghrelin correlate with the severity of disease and the activity markers. Finally, they arrived at the conclusion that ghrelin level may be important in determination of the activity in UC patients and evaluation of nutritional status. Also, Konrad et al. (2007) found that patients with IBD showed significantly higher resistin levels compared with controls. In patients with UC, resistin concentrations were significantly associated with elevated white blood cell count, C-reactive protein (CRP) and disease activity.

Ghrelin can inhibit cytokine activation including interleukin, TNF-α, and most interestingly leptin (Dixit et al. 2004). Our results for leptin levels agree with this observation because higher ghrelin levels were correlated with lower leptin levels in IBD patients compared with HC. Patients with celiac disease have higher ghrelin levels than BMI-matched controls and ghrelin decreases on a gluten-free diet. A correlation between the severity of the inflammation and ghrelin levels has been suggested (Peracchi et al. 2003).

5. Conclusion

Our data indicated that, the increased plasma resistin, TNF-α and ghrelin levels were positively correlated with activity of ulcerative colitis more than the ordinary inflammatory markers, so they could predict the response to therapy and early relapse of the disease, and possibly reflect an acute-phase response due to inflammation. The resistin, TNF-α and ghrelin levels could be considered as independent predictor of disease activity in patients with UC and may represent link between inflammation and UC.

Detection of adipocytokines level might decrease the need of repeated endoscopy to know the severity of the disease and to follow the response of therapy.

Further studies are needed to elucidate the role of adipocytokines in UC.

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Degradation Hazard Assessment of Some Soils North Nile Delta, Egypt

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Abstract: This study aimed to identify and quantitatively evaluate land degradation processes in the northern Nile Delta region. Aerial photographs were used to follow the geo-indicators of different degradation processes. GIS is used to build up a database model including required parameters for obtaining inputs to the model implemented by FAO/UNEP for global assessment of land degradation. The obtained results reveal that the high risk of physical (i.e. soil compaction and water logging) and chemical vulnerability (i.e. salinization and alkalinization) cover an area of 18487 hectare and 11008 hectare, respectively. The human induced land degradation hazards due to soil compaction is slight to high, however moderate to high for water logging. The degree of salinization and alkalinization is slight to high. [Nature and Science 2010;6(6):156-161]. (ISSN: 1545-0740).

Keywords: soils degradation, remote sensing, GIS, North Nile Delta

1. Introduction

The total cultivated area of the Nile Delta is 4, 354, 382 feddans (1741753 hectare) representing 55.5% of the cultivated land of Egypt. The Nile Delta, as well as arid land, is threatened by water logging, soil compaction, salinization and alkalinization **(El Gabaly, 1972**, **Gad & Abel Samei, 1998, and El Kassas, 1999).**

Land degradation was identified by different authors **(i.e. FAO/UNEP 1978, Warren, & Agnew 1988, Lal, & Stewart 1990 and Wim, & El-Hadji 2002)** as the processes which lower the current and / or the potential capability of the soils. **FAO/UNEP 1984, Dregene et al. 1995, and Condom et al., 1999** referred that soil degradation includes six types of processes (i.e. water erosion, wind erosion, excess of salts, chemical degradation, physical degradation and biological degradation.

The study area is located in the north of the Nile Delta (Figure, 1). This area belongs mainly to Kafr El-Sheikh governorate stretching between longitudes 31o 45′ and 31o 55′ east and latitudes 31o 12′ and 31o 30′ North, with a total area of 124044 hectare. According to **Egyptian Meteorological Authority (1996)** the mean annual temperature of the representative metrological station (i.e. Sakha) reaches its maximum (26.4 c°) in August and minimum (11.0 c°) in January, February and March. The amount of average annual rainfall is very low and mostly falls in winter, as it reaches 6.5 mm/year. and drainage network exist in the area since 1820. This irrigation system, caused natural drainage to be

The evaporation values show that it ranges between 34.3 and 81.7 mm. The relative humidity ranges between 54.2, in May, and 68.6 %, in December and January.

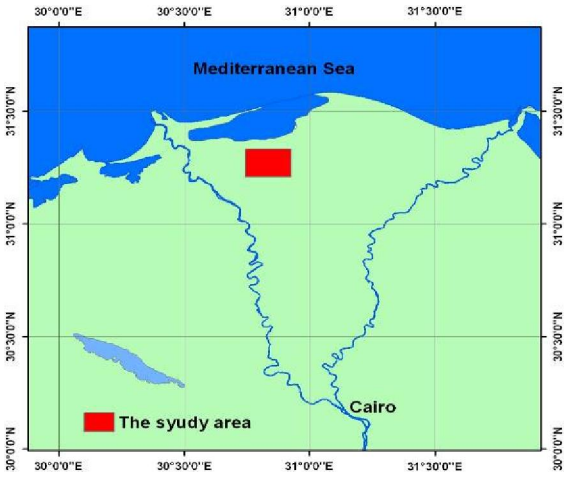

Figure 1. Location of the study area

Concerning the geology of the study area, **Shata, and El Fayoumy, (1969)** pointed out that the northern regional structure of the Nile Delta is a portion of a major downward and zone charactering the unstable shelf region of north Egypt. The geomorphic units of the area south El Borolus Lake are alluvial plain and fluvio lacustrine **(Abou El Enain, 1997)**. Irrigation insufficient to drain the soils, and an artificial drainage system was required.

The purpose of this study is to identify and quantitatively evaluate land degradation processes in the northern Nile Delta region.

2. Material and Methods

Panchromatic aerial photographs scale (1: 40.000) which were taken during the year (1991) consisting of 48 aerial photographs has been used to produce the physiographic map of the study area, using "the physiographic analysis" detailed by **Zinck, and Valenzuala (1990)**. The field work has been planned in two transects crossing different mapping units. A number of 15 soil profiles, representing different mapping units were studied. Forty four soils samples were collected, analyzed and classified, to the level of sub-great groups, according to **(USDA, 2003 & USDA, 2004)**. ArcGIS, version 9.2 has been used as the main GIS software for the purpose of producing geo-referenced maps to evaluated land degradation processes.

Measurements units and rating of degradation classes specific to each group of processes have been chosen according to **FAO/UNEP (1978)**. Natural vulnerability was calculated on basis of soil, topography and climatic factors adopted in the universal soil loss equation. Degradation hazard was also estimated using the current values of soil depth, bulk density, EC and ESP.

3. Results and discussions:

3.1- Physiography of the studied area

Compatible with **Abou El Enain, (1997)** the Physiographic analysis of panchromatic aerial photographs, made it possible to define two main landscapes types dominating the area (i.e. Flood plain and Fluvial-lacustrine plain). Moreover, it was possible to recognize the landforms of river terraces, over flow mantle, levees and basins within the flood plain. Also, a number of 6 landforms were identified in the Fluvial-lacustrine plain (i.e. Terraces of various elevations, over flow mantle, man-made terraces, overflow and decantation basins, levees and turtle backs). Figure (2) show the physiographic units of studied area.

3.2- Soils of the studied area

The morphological study and analytical data of 15 representative soil profiles made it possible to classify the soils in two orders (Aridisols and Entisols). Four great groups were identifies coinciding with the mapped land forms (**Abou El Enain, 1997)**. Table (1) shows some physico-chemical properties and soil taxonomy of mapping units.

Table 1. The main characteristics of different mapping units

landscape	landform	Symbol	Soil depth (cm)	Bulk density g/cm^3	EC dS/m	ESP %	Taxonomic unit
Alluvial plain	Decantation basin	APd	100	1.28	6.9	15.3	*Typic Torrifluvents*
	High terraces	Apt1	60	1.31	8.3	14.6	*Typic Torrifluvents*
	River levees	APl	90	1.28	4.7	15.2	*Vertic Torrifluvents*
	Low terraces	APt3	100	1.31	7.2	18.3	*Vertic Torrifluvents*
	Moderately high terraces	APt2	100	1.22	4.6	12.3	*Typic Haplargids*
	Overflow basin	APo	70	1.29	6.3	9.7	*Typic Haplargids*
	Overflow mantle	APm	75	1.21	13.1	14.6	*Vertic Torrifluvents*
Lacustrine plain	Decantation basin	LPd	60	1.32	16.5	13.2	*Typic Haplargids*
	High Terraces	LPt1	110	1.26	3.7	14.5	*Vertic Torrifluvents*
	River levees	LPl	110	1.33	5.1	12.9	*Vertic Torrifluvents*
	Low Terraces	LPt3	100	1.34	4.9	11.4	*Typic Torrifluvents*
	Moderately high terraces	LPt2	90	1.42	5.8	33.5	*Vertic Torrifluvents*
	Overflow basins	LPo	70	1.36	12.9	15.2	*Vertic Torrifluvents*
	Overflow mantle	LPm	90	1.22	6.7	11.5	*Typic Torrifluvents*
	Man-made terraces	LPmt	70	1.33	10.6	16.8	*Typic Torrifluvents*

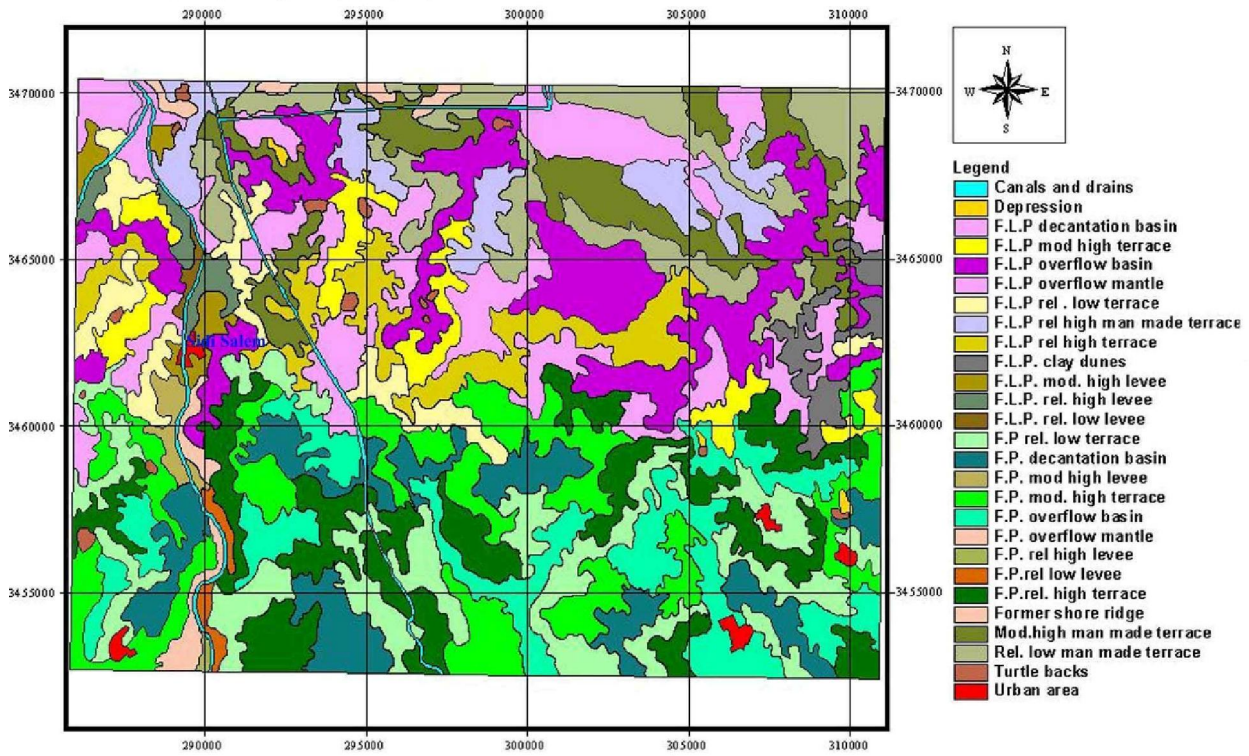

Figure 2. Physiographic units of the studied area

3.3- Natural vulnerability

Figure (3) and table (2) present the natural vulnerability in the north Nile Delta area for the different mapping units. The obtained data reveals that the soils of river terraces in the flood plain (Apt) have a slight to high risk of physical degradation and slight to moderate chemical degradation. The soils of over-flow mantle (APm) and decantation basin (LPD) are subjected to a moderate risk of physical vulnerability, while slight risk of chemical vulnerability. The soils of over-flow basin (APo), decantation, (APd), moderate and high river terraces of the fluvial-lacustrine plain (LPt2, and LPt3) and levees (LPl) have a moderate risk of both physical and chemical degradation. The soils of low river terraces in the fluvial-lacustrine plain (LPt1) have a high physical degradation and slight chemical degradation. In the fluvial-lacustrine, the soils of man made terraces (LPmt), overflow basin (LPo) and sodium percentage, bulk density and the depth of water table range between (3.7-16.5) d S /m, (9.7-33.5) %, (1.21-1.42) g/Cm³ and (60-110) Cm., respectively (table 1).

The soil degradation is mostly resulted from improper land management. The main types of human induced land degradation in the investigated areas are salinization, alkalinization, soil compaction and water logging. Human induced salinization and overflow mantle (LPm) are characterized by slight physical degradation risk and moderate chemical risk. Table (2) shows the input and out put values of calculating the natural vulnerability in the studied area. Table (3) shows that a significant area (45.1% of study area) is exhibited by a moderate physical degradation class and slight chemical degradation. The area that characterized by a slight class of both physical and chemical degradation is restricted to be only 8.5% of study area.

3.4- Degree of land degradation

The degree of four human induced land degradation processes (i.e. water logging, soil compaction, slainization and alkalinization) was estimated in relation to the depth of water table, bulk density, electric conductivity and exchangeable sodium percentage. In the north Nile Delta, the present values of electric conductivity, exchangeable alkalinization can be caused by poor management of irrigation schemes, usage of saline irrigation water and inefficient drainage. This type of salt accumulation mainly occurs under arid and semi-arid condition. Salinization and or alkalinization may be also caused by intrusion of sea water or fossil saline ground water bodies to the ground water reserves of good quality. Soil compaction mainly occurs in the soils with low structure stability, under the improper

human activities. In the studied areas soil compaction result from improperly timed use of heavy machinery, misuse of irrigation, absence of conservation measurements, shortening of the fallow period and the excessive use of chemical fertilizers. Water logging is caused by the misuse of irrigation techniques leading to flooding, especially in heavy clay soils. Also, inefficient drainages, and destruction of subsurface drainage networks by uncontrolled plough (in some parts) are causes of water logging in the studied areas.

Table 2. Factors of input elements, values and classes of natural vulnerability

Profile No.	Symbol	Physical Degradation				Chemical Degradation			
		Climate	Soil	Value	Class	Climate	Soil	Value	Class
1	APd	0.1	0.93	0.1	M	0.12	1	0.12	M
2	Apt1	0.1	0.76	0.2	H	0.12	0.5	0.0.6	S
3	APl	0.1	0.41	0.07	S	0.12	1	0.12	M
4	Apt3	0.1	0.71	0.04	S	0.12	1	0.12	M
5	Apt2	0.1	0.98	0.1	S	0.12	1	0.12	M
6	APo	0.1	1.08	0.1	M	0.12	1	0.12	M
7	APm	0.1	1.40	0.1	M	0.12	0.5	0.06	S
8	LPd	0.1	1.65	0.14	M	0.12	0.5	0.06	S
9	LPt1	0.1	1.15	0.2	H	0.12	0.5	0.06	S
10	LPl	0.1	1.13	0.11	M	0.12	1	0.12	M
11	LPt3	0.1	1.08	0.11	M	0.12	1	0.12	M
12	LPt2	0.1	0.95	0.11	M	0.12	1	0.12	M
13	LPo	0.1	0.86	0.09	S	0.12	1	0.12	M
14	LPm	0.1	0.81	0.08	S	0.12	1	0.12	M
15	LPmt	0.1	0.65	0.08	S	0.12	1	0.12	M

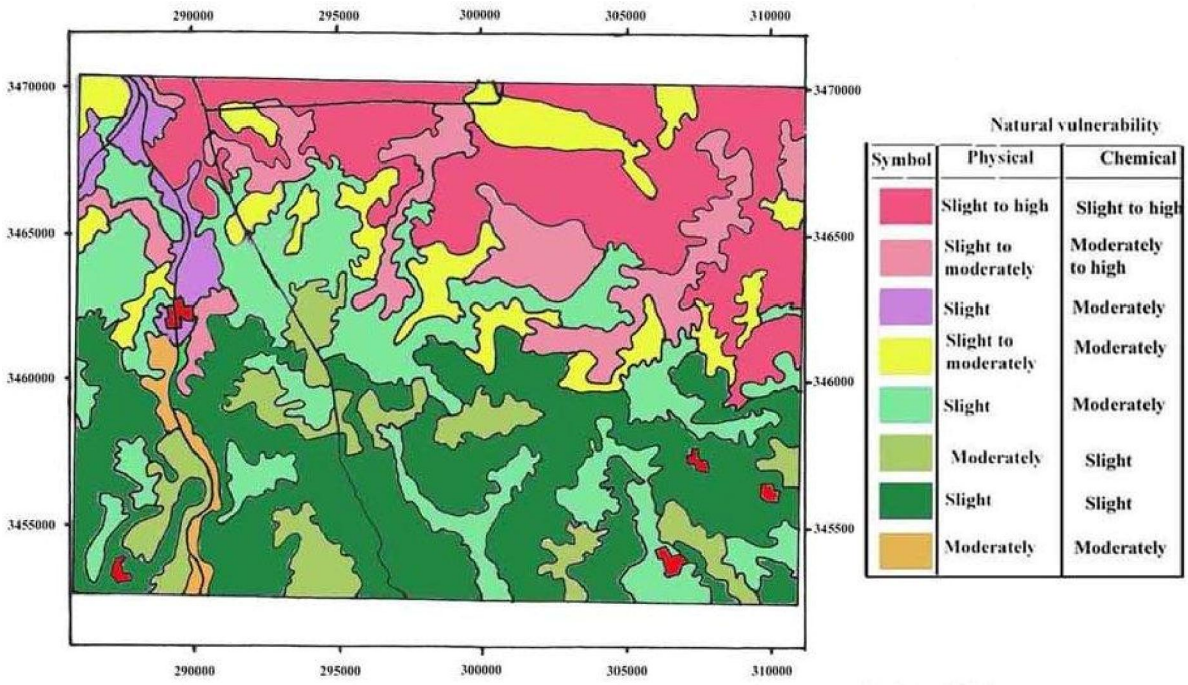

Figure 3. Natural vulnerability classes

Table 3. Classes of degradation hazard in the study area

landscape	landform	Symbol	Area (hectare)	Type of land degradation W	C	S	A
Alluvial plain	Decantation basin	APd	21951	1	2	2	1
	High terraces	Apt1	9797	3	2	3	1
	River levees	APl	801	2	2	2	1
	Low terraces	APt3	9051	1	2	2	2
	Moderately high terraces	APt2	6528	1	1	2	1
	Overflow basin	APo	11008	3	2	2	1
	Overflow mantle	APm	2504	2	1	3	2
Lacustrine plain	Decantation basin	LPd	10927	3	2	4	2
	High Terraces	LPt1	2536	1	2	1	1
	River levees	LPl	1488	1	2	2	1
	Low Terraces	LPt3	7559	1	2	2	1
	Moderately high terraces	LPt2	481	2	3	2	3
	Overflow basins	LPo	6018	3	3	3	1
	Overflow mantle	LPm	1875	2	2	2	1
	Man-made terraces	LPmt	11967	3	1	3	2

W: Water logging, C: Compaction, S: Salinization, A: Alkalinization. 1= Slight, 2= Moderate, 3= Hig

4. Conclusion

It can be concluded that a significant area in the northern Nile Delta is subjected a high risk of physical and chemical degradation. Moreover, processes of water logging, soil compaction, soil salinity and alkalinity are slight to high in different land units. GIS is very helpful tool to store, manipulate and quantitatively evaluate soil degradation. Remote sensing is a satisfactory source of ground truth information needed in parametric evaluation.

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Calculation of Creeping Flow Past a Sphere Using Direct Boundary Element Method

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Abstract:

In this paper, a steady, incompressible creeping flow past a sphere is calculated using direct boundary element method (DBEM). The surface of the sphere is discretized into quadrilateral elements over which the velocity distribution is calculated. The computed results are compared with analytical results. It is found that both these results are in good agreement. [Journal of American Science 2010;6(6):162-165], (ISSN: 1545-1003).

Keywords:

Boundary element method , Creeping flow past a sphere.

Introduction

In recent past, the well-known computational methods such as finite difference method (FDM), finite element method (FEM) and boundary element method (BEM) have been applied for the flow field calculations around objects and in such methods, the whole region of flow field is discretised. Whereas in boundary element method only the surface of the body under consideration is discretized into different types of boundary elements (C.A.Brebbia & S.Walker,1980). BEM is well–suited to two-and three-dimensional problems for which finite elements are not suitable or insufficient, especially for problems where domain is exterior to the boundary, as in the case of flow past bodies. The most important features of BEM are the much smaller system of equations and considerable reduction in data, which are essential to run a computer program efficiently. That is why; BEM is more accurate, efficient and economical than other competitive computational methods. Boundary element methods are further classified into direct and indirect methods. In the past, indirect method has been applied to calculate potential flow around arbitrary bodies (Hess & Smith, 1967, Mushtaq, 2010). In present paper, DBEM is applied to calculate creeping flow past a sphere. The study of flow past a sphere is of great practical importance in fluid dynamics. In creeping flow, the inertial effects become very small, whereas, the viscous effects become dominant. Therefore, the steady flow Navier-Stokes' equations are greatly simplified by neglecting the inertia terms (J.F. Dougles, J.. Gasiorek & J. A. Swaffield,1990). The direct boundary element method (DBEM) for potential flow calculations around objects was applied first in the past by Morino (1975). In recent past, the direct element method has been applied by the author for flow field calculations around two- and three-dimensional bodies.

Mathematical Formulation of Steady and Incompressible Creeping Flow

The differential equations governing the creeping flow are the continuity equation and the Navier – Stokes' equations (Milne–Thomson 1968)

$$\nabla \cdot \vec{V} = 0 \qquad (1)$$

and

$$\frac{\partial \vec{V}}{\partial t} + (\vec{V} \cdot \nabla)\vec{V} = -\frac{1}{\rho}\nabla p + \nu \nabla^2 \vec{V} \qquad (2)$$

In the case of very creeping motion or in the case of very highly viscous fluid , the Reynold's number will be small ($Re << 1$) . In such cases the inertia term or convective acceleration term $(\vec{V} \cdot \nabla)\vec{V}$ is approximately zero. Thus equations (1) and (2) reduce to

$$\nabla \cdot \vec{V} = 0 \qquad (3)$$

and

$$\frac{\partial \vec{V}}{\partial t} = -\frac{1}{\rho}\nabla p + \nu \nabla^2 \vec{V} \qquad (4)$$

These equations are known as Stokes' equations for very creeping motion . Flows which satisfy equation (4) are called creeping flows .

Equation (4) represents the following three scalar equations .

$$\left.\begin{aligned} \frac{\partial u}{\partial t} &= -\frac{1}{\rho}\frac{\partial p}{\partial x} + \nu \nabla^2 u \\ \frac{\partial v}{\partial t} &= -\frac{1}{\rho}\frac{\partial p}{\partial y} + \nu \nabla^2 v \\ \frac{\partial w}{\partial t} &= -\frac{1}{\rho}\frac{\partial p}{\partial z} + \nu \nabla^2 w \end{aligned}\right] \quad (5)$$

These equations together with the continuity equation (1) represent four scalar equations in four unknown u , v , w , and p . The great simplification in Stokes' equations is that these equations are now linear . In the case of steady flow , Stokes' equation (4) reduces to

$$\nabla p = \mu \nabla^2 \vec{V} \quad (6)$$

Equation (6) can be written in scalar form as

$$\left.\begin{aligned} \frac{\partial p}{\partial x} &= \mu \nabla^2 u \\ \frac{\partial p}{\partial y} &= \mu \nabla^2 v \\ \frac{\partial p}{\partial z} &= \mu \nabla^2 w \end{aligned}\right] \quad (7)$$

The Stokes' equations are considerably simple from mathematical point of view as they are linear differential equations . Moreover , their order remain the same as that of full Navier – Stokes' equations so that as many boundary conditions may be satisfied with the Stokes' equations as with the full Navier – Stokes' equations .

The Navier-Stoke equations for creeping incompressible viscous flow in the absence of body force is as follows:

$$\frac{\partial \vec{V}}{\partial t} = -\frac{1}{\rho}\nabla p + \nu \nabla^2 \vec{V} \quad (8)$$

To obtain the equation governing the pressure , take the divergence of both sides of equation (8), we get

$$\nabla . \frac{\partial \vec{V}}{\partial t} = -\frac{1}{\rho}\nabla . \nabla p + \nu \nabla . \nabla^2 \vec{V} \quad (9)$$

$$\text{or} \quad \frac{\partial}{\partial t}\left(\nabla . \vec{V}\right) = -\frac{1}{\rho}\nabla^2 p + \nu \nabla^2 \left(\nabla . \vec{V}\right) \quad (10)$$

Using continuity equation (1) , equation (10) becomes

$$\nabla^2 p = 0 \quad (11)$$

i.e. for very slow motion the pressure p satisfies Laplace's equation and is therefore a harmonic function .

Steady Creeping Flow Past a Sphere

This problem was first solved by Stokes' and is often referred to as **Stokes' flow** or **Stokes' law** . Stoke was the first who analytically solved the problem of creeping flow.

Let a solid sphere of radius 'a' be held fixed in a uniform stream U flowing steadily in the positive direction of the z – axis. Let the centre of the sphere be the origin of the coordinate system. Let z – axis be in the direction of the uniform stream in the coordinate system, as shown in figure (1). The streamlines are symmetrical around the sphere; therefore there is no wake on the rear of a sphere. The flow past a sphere varies with the Reynolds number. In general, the larger the Reynolds number, the smaller the region of flow field in which the viscous effects are paramount and vice versa.

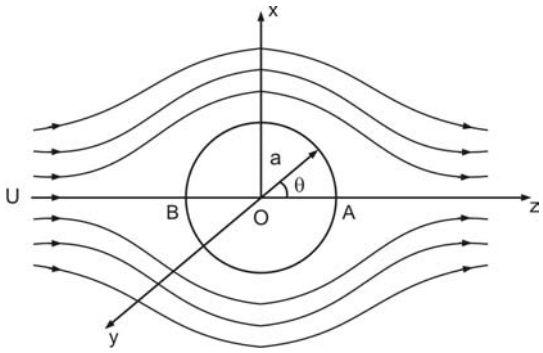


Figure (1)

Stream Function for Creeping Flow

$$\psi = -\frac{1}{4}\frac{U a^3}{r}\sin^2\theta + \frac{3}{4}U a r \sin^2\theta - \frac{1}{2}U r^2 \sin^2\theta$$

$$= \frac{3}{4}U a r\left(1 - \frac{1}{3}\frac{a^2}{r^2} - \frac{2}{3}\frac{r}{a}\right)\sin^2\theta \quad (12)$$

Velocity Distribution

The velocity components in terms of Stokes' stream function are (Milne–Thomson 1968, White 1991)

$$v_r = -\frac{1}{r^2 \sin\theta}\frac{\partial \psi}{\partial \theta} \text{ and } v_\theta = \frac{1}{r \sin\theta}\frac{\partial \psi}{\partial r} \quad (13)$$

The velocity components in this case are

$$v_r = -\frac{1}{r^2 \sin\theta}\frac{\partial \Psi}{\partial \theta}$$
$$= U\left(1 - \frac{3a}{2r} + \frac{a^3}{2r^3}\right)\cos\theta$$
$$v_\theta = \frac{1}{r \sin\theta}\frac{\partial \psi}{\partial r}$$
$$= U\left(-1 + \frac{3a}{4r} + \frac{a^3}{4r^3}\right)\sin\theta$$
$$V = \sqrt{v_r^2 + v_\theta^2}$$
$$= U \sqrt{\left(1 - \frac{3a}{2r} + \frac{a^3}{2r^3}\right)^2 \cos^2\theta + \left(-1 + \frac{3a}{4r} + \frac{a^3}{4r^3}\right)^2 \sin^2\theta} \quad (13)$$

The boundary conditions which must be satisfied by the flow are

$$v_r = 0, \quad v_\theta = 0 \quad \text{at} \quad r = a$$

and $\psi = -\frac{1}{2} U r^2 \sin^2\theta$ at $r = \infty$.

Equation of DBEM:

For three-dimensional exterior flow problems, the equation of direct boundary element method over the surface 'S'of the body is given by

$$c_i \phi_i = \phi_\infty - \frac{1}{4\pi}\iint_S \frac{1}{r}\frac{\partial \phi}{\partial n} dS + \frac{1}{4\pi}\iint_{S-i} \phi \frac{\partial}{\partial n}\left(\frac{1}{r}\right) dS \quad (14)$$

Discretization of Sphere:

The surface of the sphere is discretized into quadrilateral elements. The scheme of discretization is as shown in the figure (2).

The direct boundary element method is applied to calculate the creeping flow solution around the sphere for which the analytical solution is available

Consider the surface of the sphere in one octant to be divided into three quadrilateral elements by joining the centroid of the surface with the mid points of the curves in the coordinate planes as shown in figure (2) (Mustaq et al, 2009).

Then each element is divided further into four elements by joining the centroid of that element with the mid–point of each side of the element. Thus one octant of the surface of the sphere is divided into 12 elements and the whole surface of the body is divided into 96 boundary elements. The above mentioned method is adopted in order to produce a uniform distribution of element over the surface of the body .

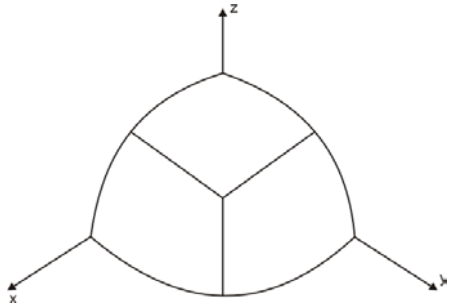


Figure (2)

Figure (3) shows the method for finding the coordinate (x_p, y_p, z_p) of any point P on the surface of the sphere.

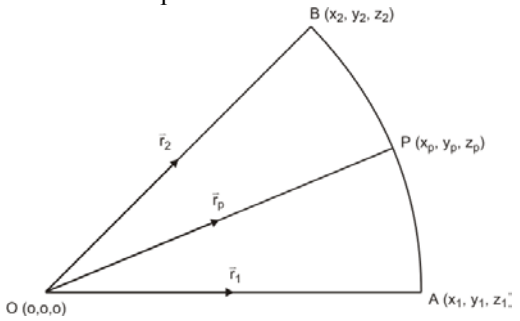


Figure (3)

From above figure, we have the following equation

$$|\vec{r}_p| = 1$$
$$\vec{r}_p \cdot \vec{r}_1 = \vec{r}_p \cdot \vec{r}_2$$
$$(\vec{r}_1 - \vec{r}_2) \cdot \vec{r}_p = 0$$

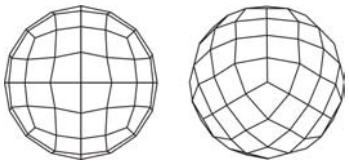
or in cartesian form

$$x_p^2 + y_p^2 + z_p^2 = 1$$
$$x_p (x_1 - x_2) + y_p (y_1 - y_2) + z_p (z_1 - z_2) = 0$$
$$x_p (y_1 z_2 - z_1 y_2) + y_p (x_2 z_1 - x_1 z_2) + z_p (x_1 y_2 - x_2 y_1) = 0$$

As the body possesses planes of symmetry, this fact may be used in the input to the program and only the non–redundant portion need be specified by input points. The other portions are automatically taken into account. The planes of symmetry are taken to be the coordinate planes of the reference coordinate system. The advantage of the use of symmetry is that it reduces the order of the resulting system of equations and consequently reduces the

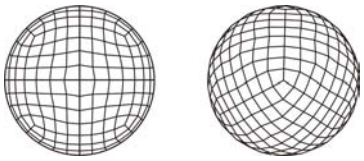
computing time in running a program. As a sphere is symmetric with respect to all three coordinate planes of the reference coordinate system, only one eighth of the body surface need be specified by the input points, while the other seven–eighth can be accounted for by symmetry.

The sphere is discretised into 96 and 384 boundary elements and the computed velocity distributions are compared with analytical solutions for the sphere using Fortran programming.



(a) (b)

Figure (4): Discretization of sphere into 96 boundary elements. The point of observation is (a) on the z-axis; (b) at 45º to all axes.



(a) (b)

Figure (5): Discretization of sphere into 384 boundary elements. The point of observation is (a) on the z-axis; (b) at 45º to all axes.

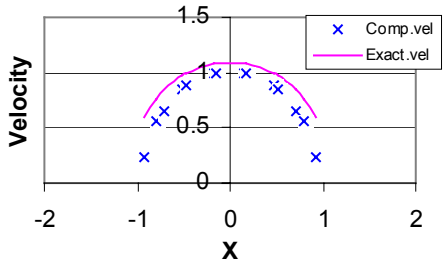


Figure (6): Comparison of computed and analytical velocity distributions over the surface of the sphere using 96 boundary elements.

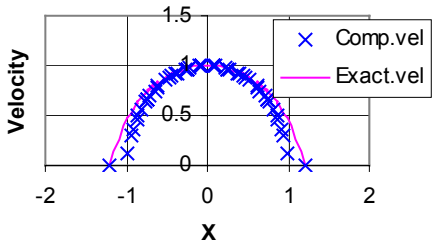


Figure (7): Comparison of computed and analytical velocity distributions over the surface of the sphere using 384 boundary elements.

Since the streamlines are symmetrical around the sphere, the graphs shown above are symmetrical on both sides. At the top of figure (7), the computed results are convergent with the exact results and as we come down, the computed results are slightly different with the analytical ones due to increase of viscous effects.

Conclusion:

Direct boundary element method has been used to calculate slow flow past a sphere using different number of boundary elements. The computed velocities obtained in this way are compared with exact velocities for this flow over the boundary of the sphere. From the above figures, it is concluded that the computed values are in good agreement with the exact values for the body of the sphere..

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Replacement Value of Urea Treated Corn with Cobs for Concentrate Feed Mixture in Pregnant Ewes Rations

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Abstract: Two trials were carried out to evaluate the effect of feeding urea treated corn with cobs (UCC) as 50% (T2) or total replacement (T3) of pelleted concentrate feed mixture (CFM) compared to the conventional diets (CFM) on its production and reproduction performance. Rice straw was offered separately from the concentrate. Evaluation criteria included DM intake and utilization, ruminal fermentation characteristics, milk yield, birth, weaning and marketing weight and feed efficiency. In the first trial, 27 Ossimi, ewes beginning 45 days before expected day of lambing were assigned to the control, T2 and T3 diets. The milk was measured on day 14 post partum and once every week up to the 12th week. The growth experimental periods were 137 day in duration using 15 weaned lambs. The selected lambs were allocated to the same three. In digestibility trial, 9 adult rams were allocated to three tested diets. In vivo digestibility, nutrients digestibility were different among diets. Feeding values (TDN) was greater for T3 followed by control diet whereas the highest DCP was recorded for T2. Feeding UCC had no effect on ruminal parameter in terms of pH, NH3 and total FVA`s across the sampling time except for NH3-N. The replacement of CFM by UCC resulted in insignificant higher ($p \leq 0.05$) lambs birth weight T3 (3.44 kg) but lower milk yield T3 (436 g /day). The lower birth weight lambs control group ($p \leq 0.05$) tended to grow faster and perform higher weaning as compared to the treated group. In growth trail, feeding UCC diets reduced ADG approximately 10% compared to control. The results indicated that DM, TDN and DCP needed produce 1 kg gain almost 5 to 10% better than the corresponding items from T2 and T3. Replacement of CFM in pregnant and growing lamps rations with UCC would be cost effective as cost UCC is only at 60% less than cost of CFM. [Journal of American Science 2010;6(6):166-178]. (ISSN: 1545-1003).

Keyword: Sheep**,** feed**,** urea treated Corn-cobs**,** digestibility, nutritive value, growth, milk yield, performance.

1. Introduction

Sheep populations in Egypt are almost 5 million, (MOA 2005). During the last two decade the importance of sheep production as a source of animal protein in Egypt has been increased. Meanwhile, the mutton price has also increased. In fact, the small ruminants are mainly associated with small farmers. Therefore there is need to research and develop stall-feeding systems for small ruminants based on crop by-products.

In Egypt, maize for grain is planted on approximately 1.68 million feddan producing 5.8 million metric tons averaged 3.47 ton per feddan**)**. Importation is 4.7 million tons. Almost 70% home production whole-crop maize is utilized by ruminants (MOA 2005). Cobs (as residues) were estimated to about 1.7 million tones (represent 25% of ear corn).

A major constraint facing livestock development is the lack of adequate supplies of feedstuffs at economic prices. Feeds represent the greatest proportional cost in livestock production and its availability is affected by seasonal variation in feed quantity and quality which causes fluctuations in animal nutrition and productivity throughout the year in particular during the summer season. Moreover, soybean meal and cottonseed meal are two important sources of protein used extensively in Egypt to feed ruminants and represent the most expensive ingredients in ruminant rations. There are large quantities of maize cobs which could be fed to ruminants instead of being wasted. Collection of maize cobs is easier than that of maize stalk which is left in the field where the maize is harvested while the cobs are gathered before dehusking and shelling.

In order to improve the low quality byproducts the most pragmatic and UCCeptable is chemical treatment. This treatment disrupts the cell wall by solubilizing hemi cellulose, lignin and silica, hydrolyzing uronic acid and acetic acid esters and swelling cellulose (Jackson 1977). The use of urea or ammonia to upgrade straws and other low quality has been world wide spread in the last three decades. Urea, the most commonly used an inexpensive NPN source are an attractive protein replacement compared with nowadays tremendously expensive natural proteins (Oji` *et al* 2007) stated that fertilizer grade urea can be used to improve the nutritional value of maize residues for small ruminant feeding during off season periods.

On the other hand, the relationship of birth weight to weaning and weans weight to slaughter weight is economically very important in lamb

production and is affected by genetic, physiological and feeding of ewes and fetal growth affect by feeding her mother during pregnancy stage (Wu.G 2006).

The experiments reported here studied the possibility of replacing concentrate feed mixture (CFM) in diets of pregnant ewes and growing lambs with ammonia treated corn with cobs**.**

2. Material and Methods:

The present work conducted at sedes Experimental Station and By-Product Utilization Department , Animal Production Research Institute (APRI) to study the effect of including urea treated corn with cobs in small ruminant diets on performance of Ossimi ewes (lactation and new born lambs performance) and considering a simple economical evaluation of urea treated corn with cobs supplemented rations . Nutrients in the CFM, UCC and RS were chemically measured before formulating the experimental rations.

Ewes feeding trails:

Twenty seven pregnant Ossimi ewes were selected 2-3 years old averaged 50.0 kg live body weight (LBW) in the last six week of gestation. The selected animals divided based on their live weight into three similar groups 9 ewes each and randomly allocated to diets of either control, T2 and T3.
Animals were group-housed and the diets were offered in two portions at 8 am and 16 pm and had free UCCess to water. Animals were weighed at the beginning and at the end of the trial. The animals were healthy during the experimental period.

Survival rate:

Live lambs per born lambs and live lambs per ewe were determined after parturition and 30, 60 and 84 days after lambing.

Milk recording:

The 24-h milk production of each ewe was measured on d 14 (2weeks post partum) and once every week by hand milking throughout a 70-d of lactation period at 7-d intervals. On the day of parturition, ewes and lambs were weighed. Lambs were weaned at 84 d of age and both the ewe and lambs were weighed at this time. Milk production was measured using procedures described Rusev and lazarov,(1967) and Farage,(1979). UCCording to this methods the ewes have been milked twice daily by milking one teat while the lamb suckle the other one. The morning and evening milked yield multiplied by 2 to calculate the daily output. The weight of the collected milk was recorded and used to determine 24-h milk production. Total milk production for each ewe was calculated as the sum of milk produced on each day of milking.

Digestibility trials:

The metabolism trial included 9, each lamb was placed in a separate metabolism cage designed to collect with a 2 wk adjustment period and a 7 days collection period. Three rams were randomly assigned to each of the same ration as in feeding trial. Feeds offered, output of feces was recorded daily during the last 7 days of the collection period. Fecal trays were placed for total fecal collection during the 7 days collection period. Feed was offered twice daily and water was refreshed at 0700 and 01500. Fecal output was weighed, sub sampled (10% of wet weight), and composted across 7days within lamb for each period. Samples were stored frozen (– 20°C) until dried in a forced-air oven for 48 h. The collections were made concurrently with the meals. Samples of feed were taken daily at 10% of the total offered and the residues were collected. A sub-sample (20%) of feces was composed, kept each day in plastic bags in the freezer (-20 °C) until the end of the experiment. Feed ingredients and dried feces were ground to pass a 1-mm screen in a hammer mill before analysis. The following chemical analyses were determined: dry matter (DM), Crude protein (CP), Crude fiber (CF), Ether Extract (EE), Ash and Nitrogen Free Extract (NFE) UCCording to AOAC (1990). The feed offered to each ram during the preliminary and collection period was set to 90% of average feed intake during the second week of the adjustment period. There were no feed refusals during the 7-d collection period.

Weaning weight:

The born lambs were weighed every two weeks up to the 84th days of age.

Growth trial:

The growth of 15 weaned lambs 84 days old was evaluated for 137days. The lambs were divided into 3 equal groups five each group with initial body weight 19.06, 18.87 and 19.18 kg given UCCess to the tested diets for control, T2 and T3, respectively similar to those used in ewes trial. Lambs were fed at 0700 and 01500 daily and the basal diet (CFM and UCC) and RS were offered separately at each feeding and were allowed free UCCess to lick mineral blocks and water. Animal weights were recorded at the beginning and in 15dayes interval throughout the 137 days growth period. To minimize variation due to drinking, feeding, and defecation, lambs were weighed full on the morning of the first day of the experiment and every 15 days before morning replenishment of feed.

The amount of feed provided for the late-gestation and lactating ewes was based on the guidelines put forth (APRI) to be applied y the experimental stations and was determined for per group based on BW measured biweekly. The standard practice for the sheep flock at the Animal production research institute to feed adult, no lactating, no pregnant ewes in confinement a maintenance ration of 2% of BW/d. During the ewes and growth trials the basal levels were adjusted so that diets were completely consumed each day; orts consisted solely of straw.

Rumen fluid:

Approximately 15 ml of ruminal fluid was collected using ruminal tube, and pH was measured immediately using a portable pH meter. Ruminal fluid samples representing 0, 3 and 6 h after feeding.
Ruminal parameter (pH, ammonia nitrogen and VFA's)
Ruminal kinetics (pH, Ammonia nitrogen and VFA's) were determined using liquor collected by rumen tube via esophagus three times before morning feeding (zero time, 3 and 6 hrs after feeding)
This ruminal fluid sample (15 ml) was acidified with 1.0 ml of 6 N H2SO4 and frozen (-10°C) ; this sample was later thawed at room temperature, centrifuged at 10,000 x g for 10 min, and a portion of the supernatant was analyzed for NH3- N UCCording to Broderick and Kang (1980). The resulting NH3 concentrations were converted to NH3 - N. for statistical analysis, and NH3 - N concentrations are reported in. Total VFA's concentrations were determined using.

The data were statistically analyzed using GLM produces of SAS (1990). Duncan's test (1955) was applied in experiment whenever to test differences.

The following model was used:

$Y_{ij} = \mu + Ti + e_{ij}$

Where : Y_{ij} = observed trait,
μ = overall mean,
Ti = effect of treatment,
e_{ij} = random error.

3. Results

Chemical Composition

The analyzed composition of dietary ingredients is reported in Table (1). The concentrate feed mixture (CFM) contained 87.11 OM, 14.04% CP, 18.95% CF, and 2.46.0% EE (DM basis). Treated Corn with cobs averaged 98.24%OM, 13.99% CP, 17.10% CF and 4.16% EE, and rice straw 81.5% OM, 3.92% CP, 35.24% CF and 0.46% EE on DM basis.

Urea treatment was effective in upgrading the nutritional value of corn with cobs. Treated corn with cobs had higher (N x 6.25) 13.99% as compared to7.59 to for untreated represent 85% increment. Meantime, ether extract and ash content decreased from 5.60 and 2.27 to 4.16 and 1.76% respectively (Table 1). The moisture content of the treated corn with cobs upon opening the stack was approximately 27% reduced to 14.85% after exposing to air for 24 hrs.

The formulated tested diets were isonitrogenous (almost 10% CP) containing 0.00, 16.86 and 32.4 gram nitrogen (NPN) originated from urea treated corn with cobs for control, T2 and T3, respectively. These values represent 19.2 and 38.5% for the total nitrogen for T2 and T3, respectively.

Digestibility trial

Data presented in (Table 2) revealed that the intake from the concentrate (CFM and / or UCC) for T2 and T3 during the digestibility trial was 3 and 7% lower as compared to the control ration. Also, there was tendency for straw intake to decrease (20 and 27%) by feeding 50% and 100% urea treated corn with cobs, respectively. These together resulted in 10 and 15% decrease in the total dry matter intake for T2 and T3, respectively. There was slight difference (3 and 6% lower for T2 and T3, respectively as compared to control) regarding the DMI of straw among the three groups. The fecal nitrogen excretion was 6.2, 6.8 and 5.3 g/d/h represent 38, 44 and 39% from the N intake for control, T2 and T3, respectively. The almost similar percentage of digested N retained (as percentage of intake) for control and 100% UCC group revealed that NPN urea source has no effect N efficiency.

The apparent digestibility coefficients are presented in (Table 2). Highest DM and OM digestibility (69.67 and 71.96%) were recorded for the control ration being 9% and 6% and 7 and 3% higher than T2 and T3, respectively. Crude protein exhibited almost similar digestibility for control and 100%UCC diet (61.42 and 60.60%, respectively) being 8% lower than 50% UCC group. The digestibility for crude fiber ranged between 69.94% (control) and 53.05 for T3. The CF in control diet was highly digestible (16 and 32%) than T2 and T3, respectively. control group. The EE digestibility for T3 was 9 and 5% higher than the values recorded for T1 and T2, respectively. The values for NFE showed similar coefficients for control and T3 diets being 7% higher than T2.

Table (1): Chemical composition of experimental concentrate feed mixture, rice straw, urea treated corn cobs with corn cobs

Item	Moisture %	DM composition					
		OM	CP	CF	EE	NFE	Ash
Concentrate feed mixture CFM	8.45	87.11	14.04	18.95	2.46	51.66	12.89
Rice straw RS	7.85	81.50	3.92	35.24	0.46	41.88	18.50
Urea treated corn cobs UCC	14.85	98.24	13.99	7.71	4.16	72.38	1.76
Corn cobs CC	9.75	97.73	7.59	7.70	5.60	76.84	2.27

CFM : 26%, undecortecated cotton seed meal , 44% wheat bran, 19% yellow corn, 5% rice bran, 1% salt mixture, 2% lime stone and 3% molasses.

Table (2): Dry matter intake, nutrient digestibility and nutritive values of different experimental diets.

Item	Experimental Diets			
	T1	T2 50 %UCC	T3 100%UCC	±SE
Animal weight , kg	40.50	39.51	38.18	±4.90
CFM	0.916	0.458	----	…..
UCC	------	0.426	0.852	……
RS	0. 627	0. 507	0.461	±2.42
Total DMI kg /head /day	1.543	1.391	1.313	±1.26
Nutrient Digestibility %				
DM	69.76 [a]	63.72 [b]	65.92 [b]	±0.61
OM	71.96	67.23	69.88	±0.68
CP	61.41 [b]	66.09 [a]	60.60 [b]	±0.39
CF	69.94 [a]	60.28 [b]	53.05 [c]	±1.42
EE	77.46 [b]	79..90 [b]	85.10 [a]	±1.26
NFE	75.06	69.93	76.37	±1.21
Nutrient Values				
TDN	62.78	60.81	64.92	± 0.25
DCP	6.12	6.46	5.80	±0.22
TDN intake g/h/day	968	846	852	±2.44
DCP intake g/h/day	94 [a]	90 [a]	76 [b]	±0.03

[a , b, c] Means in the some row having different superscripts are significantly different at, ($p< 0.05$).

Rumen fluid parameter:

Rumen pH values at zero time ranged between 7.42 and 7.43 (Table 3)and tended to slightly increase 3h after feeding to7.5, 7.7 and rose by 6 h to 7.9, 8.0 and 8.0 for T1, T2 and T3, respectively. This result revealed that the rumen pH values are not affected by the source of nitrogen.

Ruminal ammonia concentration was 16.4, 13.6 and 15 (mg/100 ml) for control, T2 and T3 at zero time tended to increase up to 22.9, 23.5 and 23.6 at 3 hrs after feeding (Table 3). At 6 hrs after feeding the concentration showed remarkable decrease up to 13.3, 9.5 and 11.5 for control, T2 and T3, respectively. The control group presented higher ammonia concentration compare to the treated group. Statistically, the differences were significant ($P < 0.05$) at 6 h.

Total VFA concentration in ruminal fluid (Table 3) was lower in the control ration at zero time being 6.07 as compared to T2 and T3 (7.26 and 7.34 respectively). Meanwhile, it tended to be greater up to 11.3 at 6h after feeding as compared to 10.7 and 10.80 for T2 and T3, respectively.

Statistically, neither pH nor total VFA concentration significantly differed among diets while ammonia concentration only displayed a weak tendency towards reduction with the 50% UCC (T2) diet.

Table (3): Rumen fluid parameter of lambs fed the experimental diets

Item		Experimental Diets			±SE
		T1 CFM	T2 50 %UCC	T3 100 %UCC	
pH	hrs				
	0	7.42	7.43	7.42	±0.06
	3	7.54	7.68	7.70	±0.07
	6	7.92	8.04	7.99	±0.05
Overall mean		7.63	7.72	7.70	±0.11
NH3-N(mg/dl)	hrs				
	0	16.36 [a]	13.58 [b]	14.95 [a]	±1.10
	3	22.87	23.53	23.64	±0.20
	6	13.27[a]	9.53[b]	11.50[a]	±0.49
Overall mean		17.50	15.55	16.70	±1.11
VFA`s (meq / dl)	hrs				
	0	6.07	7.26	7.34	±0.75
	3	10.25	8.91	9.70	±0.65
	6	11.30	10.70	10.80	±0.12
Overall mean		9.21	8.96	9.34	±0.42

[a , b, c] Means in the some row having different superscripts are significantly different at (P< 0.05).

Ewes feeding trial and milk yield:

Data presented in (Table 4) showed that intake from control ration and T2 were almost similar being 720 and 702 concentrate and 480 and 470g rice straw, respectively. The T3 ration presented much lower intake 677 and 451g concentrate and rice straw, respectively.

The calculated feeding values in terms of TDN and DCP resulted for the digestibility trial (Table 2) showed that feeding 100% UCC plus RS increased TDN content (64.90) by 3 and 7% compared with the control (62.78) and T2 (60.81), respectively. On the other hand, DCP for the same diet (5.80) was 5 and 10% lower than T1 and T2, respectively. However, feed intakes for the selected late pregnant ewes were in the range 3 and 3.2% of body weight indicating that the diets were palatable.

UCCording to the feeding values (in terms of TDN and DCP) of the tested diets extracted from the digestibility trial (Table2), the TDN intake for control group (968 g/h/d) was approximately 15% higher than the T2 and T3 groups, respectively. Corresponding values for DCP intake (94.4 g/h/d) was 5 and 24% higher for control group than T2 (89.9 g/h/d) and T3 76.2 g/h/d).

Milk yield:

Over the 70 - d lactation (begin from the third week post partum) , average estimated (7 days interval) milk production for the nine selected ewes rearing single lambs (Table 4) was 527, 497 and 436 g/h/d, for the control, T2 and T3, respectively.

The differences between control group and 50% UCC group were reduced by mid and late lactation. In fact, average estimated milk production in the first month of lactation for control group was slightly (almost 5%) higher than the other two tested groups. The level of milk production was declining and continued to decline after d 49.

Table (4): Ewes feeding and milk yield

Item	Experimental groups			
	T1 CFM	T2 50 %UCC	T3 100 %UCC	±SE
Ewes feeding g / h / d :				
Concentrate	720	702	677	14.69
Rice straw (RS)	480	470	451	16.36
Total dry matter intake (DMI)	1200	1172	1128	14.17
Average milk yield g/h /d	527	497	436	3.20

[a, b,c] Means in the some row having different superscripts are significantly different at (p< 0.05).

Survival rates

Lambs survival rates normally derived from the number of lambs/ewe present at 4 stages: born alive, 30 days after birth, from 30 up to 60 and end weaning (84 day). UCCording to the results obtained in this study (Table 5) lambs mortality rates for T1, T2 and T3 were 18.2, 0.0 and 11.1 % for the first stage, respectively, being nil for the other stages for the three groups.

Table (5): Effect of feeding the experimental diets on lambs survival rate %.

Item	Experimental groups			
	T1 CFM	T2 50 %UCC	T3 100 %UCC	±SE
At first day	81-------82	100.00	88--------89	--------
Form day 1 up to 30	81------82	100.00	88-------89	-------
Form day 30 up to 60	81------82	100.00	88--------89	--------
Form day 60 up to 90	81-----82	100.00	88-------89	--------
Lambs survived at weaning per 100.00 ewes	100.00	111.1	88-------89	--------

Growth trial - Lambs performance

Weaning period

Although lambs born from ewes fed on the control ration were significantly light in weight (2.48 kg/h) than those on tested rations (3.17 and 3.44 kg /h for T2 and T3, respectively) , it tended to grow faster than those suckled from ewes fed on T2 and T3 rations and had higher daily gain and weaning body weight of 18.13kg compared to 18.08 and 17.63kg for T2 and T3 (Table 6). Mean daily live weight gain (from birth up to weaning, 84 days) for the control and treatment groups T2 and T3 were found to be 172, 164 and 156 g, respectively. However, the differences were not significant.

Respective to the effect of sex on birth weight and daily gain up to weaning, regardless from the different treatments, born male lambs showed higher average birth weight for the three groups (3.80 kg) and lower average daily gain 156.04g/h as compared to female born lambs 3.08 kg and 155.8g/h, respectively).

Table (6): Average total weight gain for male and female lambs and some reproductive performance of ewes.

Item	Experimental groups					
	(T1) CFM		(T2) 50 %UCC		(T3) 100 %UCC	
	♂	♀	♂	♀	♂	♀
Birth weight BW kg	2.48	2.48	3.77	2.58	3.80	3.08
Average (M and F)	2.44		3.18		3.44	
Weaning weight WW kg	18.25	18.00	20.25	15.92	18.00	17.25
Average (M and F)	18.3		18.1		17.6	
Total gain TG kg	15.78	15.52	16.48	13.33	14.20	14.18
Average (M and F)	15.65		14.91		14.19	
Average daily gain ADG gm	173.41	170.55	181.10	146.48	156.04	155.82
Average (M and F)	171.98		163.79		155.93	
No .of ewes / treat .	9		9		9	
No. of lambs born / teat.	11.00		10.00		9.00	
Average litter size / ewe	1.22		1.11		1.00	
Average of lambs birth weight, kg	2.24		3.12		3.16	
Average litter weight, kg	2.73		3.46		3.16	

weaning period = 84 days BW = birth weight WW = weaning weight TG = total gain

Growth period

All weaned lambs used in the growth trial were fed almost at 3.0% of BW throughout the trial and the quantity of feed refusals (data not shown) was very minimal and did not differ among treatments.

Feeding cost based on*	per ton
CFM	900 LE
CC	500 LE
RS	100 LE
Urea treatment	50LE

For the period from weaning up to the end of growth period (137 d), the lambs fed the control ration consumed more concentrates and rice straw than those fed on treated groups. The data presented in (Table 7) showed that the rice straw and the basal diet (CFM and/or UCC) was for control group almost 3 and 8% higher than T2 (50%UCC) and T3 (100% UCC). Organic matter intake did not differ among the groups ranged between 772.5 and 776 g/h/d, whereas CPI varied between 84.0 and 87.8 g/h/d across treatments (Table 6).

The CP intake ranged between 90g/h/d (control) and 84g/h/d for T3. Because of their numerically higher DMI of CFM as well RS for control group, lambs consumed a greater quantity of crude fiber (231.8 compare to 194.5 and 158.0g/h/d for T2 and T3, respectively. These figures represent 15 and 33% higher for control than T2 and T3, respectively. Ether extract intake for the all urea treated ear corn group (T3) was 60 and 24% higher than control and T2, respectively. It could be observed from the recorded DMI figures that there was a tendency for straw DM intake to decrease as UCC in the diet increased. However, the concentrates in three tested diets represent almost 60% of the total dry matter intake.

Feeding the tested weaned lambs on the experimental rations for 137days resulted in slight differences in ADG between the control (9 and 12%) higher than T2 and T3, respectively. Based on initial and final BW of the tested animals (Table 7) during the 137 days growth period, the average daily gain was 156., 142 and 139 g/d/h. for control and 50% and 100% UCC groups, respectively.

The weaned lambs light in weight in particular those fed on control and 100% UCC diet exhibited higher growth rate than the heavier lambs. The lambs over 20kg weaning weight in the three tested groups were the lowest ADG across the growth period as compared to those less than 20 kg fed the same diets. It seems that the low weaning weight lambs have the capacity to grow at rates at least approaching, if not equivalent to, the high weaned weight during the growth period.

Feed efficiency ratio in terms of (Kg of DM intake need to produce 1 kg gain was comparable for T2 and T3 consuming 5% DM higher than the control (5.82, 6.20 and 6.1 Kg DMI/ 1Kg gain, respectively). The TDN conversion rate comparable for control and T2 groups being slightly better than T3 representing 3.7, 3.8 and 4.0 Kg TDN per kg gain weight. The conversion rate for DCP was similar for control and T3 being 11% lower than T2 in terms of kg gain /kg DCP intake. The DCP amount needed for 1kg gain for T2 was 356, 401 and 353 g/ kg gain for control, T2 and T3, respectively. However, the different were in significant among rations (Table 7).

Providing that the production cost are similar except the feed cost changed UCCording to variation in the price of its components, therefore the economical efficiency well calculated from the input (feeding cost) and output (gain per unit feed). The calculated feeding cost based on the price of CFM, UCC and Rice straw (year 2003) were in average of 0.473, 0.391 and 0.313 per head daily for control, T2 and T3, respectively. UCCordingly, the cost (of feed) for producing 1 Kg gain was in average of 3.02, 2.76 and 2.26 LE/h/d for control, T2 and T3, respectively. The calculated decrease in feed cost / kg gain relative to the control was 9 (50% AAC) and 33% (100% UCC).

4. Discussions

Using urea as an agent to improve the nutritional value of low quality by products still considered as the most favorable up till now. Oji *et al*, (2007) stated that feed grade urea or the equivalent weight of fertilizer grade urea can be used to improve the nutritional value of chopped cobs (approximately 1 cm length) in terms of N, DM, NDF, ADF and OM for small ruminant feeding during off season periods. Moreover, Koster *et al*, (2002) concluded that, urea could replace between 20 and 40% of the degradable intake protein (drawn from values presented by NRC 1996).Also, Sahoo *et al*, (2002) reported that treatment with urea (storage time 21 days) improve the nutritive values as compared to urea supplementation just prior to feeding. Concerning the treatment period and moisture level, it was found that at least two weeks and 25-45% moisture level is sufficient for maximum response during summer months (Hadjipanayiotou and Economides1997 and Lines *et al*,(1996). The authors added that covered urea treated straw (UTS) is superior to non-covered, and UTS is also superior to urea-spraying prior to feeding. Lines *et al*, (1996) added that most of the changes caused by ammoniation were completed by 21 d after ammoniation. In this study, the stack opened was open after 28 days after treatment.

Increase the nitrogen content in urea treated corn and cobs by almost 185%. Wanapat *et al*.,

(1985) reported significantly increased up to 7-fold by urine and urea treatments. It could be due to the lower nitrogen content in the treated material.

The tendency, in this study, for rice straw and concentrate) to decrease by feeding 100% UCC and hence total DM, OM, and N intake decreased was similar to the results observed by Matejovsky and Sanson (1995) using ear corn as basic diets. The decrease of DMI and CP digestibility for T3 could be due increasing energy (T3) without adequate protein availability which was associated with depressed intake and digestibility (Del Curto *et al*, 1990).

Ration formulation

Forage to concentrate ratio

The diets used in this study generally have about 60% concentrates during 137 d growth period. It should be mentioned that the forage concentrate ratio does not take into consideration the quality of the forage, particle size of the forage, the type and processing of the cereal grains, and the concentration of non forage fiber sources in the diet to affect dietary starch concentration as the case in using corn and cobs in the study.

Ludden and Cecava (1995) formulate diets contained 12.5% CP using cracked corn (70%), ground corn cobs (15%), and different source of protein supplement (included urea). The results suggest that corn-based diets may be limiting in ruminally degradable N, especially when high ruminal escape protein sources are fed as supplemental CP.

The digestibility data concerning the fibrous portion tended to decrease as the proportions of CF content decreased as described by Woodford *et al* (1986).

The lower DM and CF digestibility observed in this study for 50% and 100% UCC was reported by Sanson *et al*. (1990). The authors stated that reported a decrease in DM and hemicelluloses digestibility as dietary level of corn increased from 0.26 to 0.52% of BW in steers consuming low-quality meadow hay.

Feeding urea as protein supplement to starch-based energy diet (corn) has been shown to cause depressions in forage intake as well as negative associative effects on dry matter and fiber digestibility (Chase and Hibberd, 1987; Pordomingo *et al*, 1991 and Matejovsky and Sanson (1995).Similar DMD for T2 and T3 (Table 2) was found by Nelson *et al* (1984) . The authors used maize cobs containing 40% moisture reported 61.30, 61.69 and 65.94% DMD for 2, 3 and 4% ammonia treatment, respectively.

Contrary to the negative effect of ammoniation on nutritive digestibility mentioned previously, Cottyn and De Boever (1988) and Genin *et* al, (2007) reported upgrading of straw by ammoniation. Treatment of straw with 3% NH3 improved digestibility and energy value, the contents of crude protein (CP) and digestible crude proteins (DCP) by withers. Also, Zinn *et al*, (2000) found that total tract OM digestion was slightly greater for diets containing 20% of N as NPN (partial replacement of fish meal). Moreover, the tendency for CP digestibility for urea treated corn and cobs (as source of NPN) to be greater for T2 has been reported by Bohnert *et al*, (2002a)

The lower DMD of the T2 and T3 could be due, as explained by Tuah ¹ and Ørskov (1989), to that with the cobs, most of the material was cell wall while the cell content was about 6.04 and the hemicellulose very high (46.4%). They stated that the cellulose and the hemicellulose of the maize cobs may therefore not be made readily available for microbial degradation, thus decreasing its DMD and DCF values.

The lower DMI for T2 and T3 (in particular during digestibility trial) should be taken in UCCount when comparing the tested diets since intake has great effect on digestibility (Tyrrel and Moe 1975).

UCCording to Kauffmann *et al* (1980) and Hoover WH, (1986) and Galina *et al*., (2007) who stated that major factor appears to be responsible for the decrease in fiber digestion are the rumen pH. Moderate depression in pH, to approximately 6.0, results in a small decrease in fiber digestion and considered as the lowest limit to adequate activity of the celullolitic bacteria.

The lack of an effect of NPN source on fecal N excretion agrees with other research using urea as CP supplements to low-quality forage (Coleman and Wyatt, 1982; Bohnert *et al*., 2002b). Joy *et al*., (1992) and Hammadi (2007) observed that increasing urea dosages up to 8 % DM basis and different levels of moisture content (up to 40%) increased total N content as well as a significant effect on the DOM in low quality roughages and improvements in ADG and gain/feed (Brown *et al*., (1995)

The higher CP digestibility for T2 (50% UCC) compared with other two groups could be due to the associated effect the two sources of nitrogen (soybean in the CFM and urea in the treated corn and cobs. Ammerman et al. (1972), observed an increase in N balance and digested N retained (expressed as a percentage of N intake) in wethers consuming low-quality forage (2.6% CP) and supplemented with urea and soybean meal (50:50 N basis), or biuret and soybean meal (50:50 N basis) compared with withers receiving just forage.

The relative lower CP digestibility for T3 (100% UCC) have been explained by Oltjen *et al*, 1969; Ammerman *et al*, 1972; Bohnert *et al*, 2002b. The

authors stated that NPN source did not affect N balance or digested N retained suggest that urea or biuret can be effectively used as a source of supplemental N by ruminants consuming low-quality forage 7% CP.

Weaning and growth period

The effect urea treatment on lamb birth weight and on ADG during weaning and growth periods observed in this study are matched with the results of Koster *et al*, (2002). The authors concluded that Prepartum urea treatment did not affect pregnancy rate, calf birth weight, or ADG.Rapid Concerning the rapid growth of the lower birth weight born lambs for control group in this work (Table 6), Greenwood et al., (2002) concluded that low-birth-weight lambs are less mature than their high-birth-weight counterparts in some aspects of endocrine and metabolic development at birth which may enhance their capacity to utilize amino acids for energy production and to support gluconeogenesis during the immediate postpartum period. The authors added that under appropriate environmental and nutritional conditions, vital life support systems can mature sufficiently to allow extremely low birth weight lambs to survive and achieve growth.

Meanwhile, Sidwell *et al*, (1964) reported positive correlation between birth and weaning weights which contradict the results of this study. Also, Greenwood et al., (1998) stated that average daily gain tended to be greater in the high- than in the low birth-weight lambs given ad libitum UCCess to feed.

However, differences in weaning weight due to breed, sex, month of birth and litter size were reported by Bodisco et al (1973) and Gonzalez (1972). On other hand, Bodisco et al (1973) reported differences due to year and litter size but not to sex. Refer to the results presented in Table (6); sex of born lambs gave insignificant differences for both birth and weaning weight.

The higher ADG of male born lambs compared the female was in UCCordance with the finding of Yilmaz (2007) who reported that at birth, 90 and 180 days of age, ram lambs were heavier than ewe lambs. Zinn et al., (2000) reported that overall, ADG was 17% greater for cattle consuming diets containing 20 vs 40% NPN. Overall, gain efficiency was 6% greater for diets containing 20% NPN.

Rumen parameter

pH value

Ruminal fluid pH was not affected by dietary treatment and averaged 7.6 across treatments. It has been reported (Ørskov 1992) that feeding grain (corn) and forage decreased ruminal pH below 6.0 and reduce the activity of cellulolytic bacteria which could reduce forage fiber digestibility. In this study, although the T3 (100% UCC) diet has been formulated from corn and cobs and rice straw, the ruminal pH was comparable to the other tested diets averaged 7.7compared to 7.6and 7.7 for the control and T2. Therefore, the lack of effect of feeding corn on ruminal pH could be due urea treatment.

Ammonia- N

The concentration of ruminal NH3-N seems to be adequate and maintained fermentation of diets in this experiment, as long VFA concentrations was within the optimal range of 2.0 to 5.0 mg/dL ruminal NH3- N as suggested by Satter and Slyter (1974) to maintain microbial growth.

The statement of Ludden and Cecava (1995) could explain the CF digestibility by the treated groups. The author consider a 3.6 mg/dL for urea as supplemental protein sources for steers fed corn-based diets as evidence for possible shortage of ruminally degradable N, in the present work the rminal ammonia was far below this concentration.

The lack of effect feeding urea treated corn and cobs on ruminal ammonia nitrogen as compared to the control has been documented by Lines and Weiss (1996) who stated that cows fed the urea diet had higher concentrations of ruminal NH3 than did cows fed urea treated hay.

TFVA´S

The higher total VFA concentration for control ration at 3 and 6 hrs after feeding could be a result of a greater supply of fermentable material that have been made available than the other tested rations.

Breeding and lambing season:

UCCording to the breeding plan in Sids experimental station, the ewes averaged three lambings over two years. It was originally decided to synchronize breeding to takes place in January, Mai and September so lambing would occur in June, October and February , respectively and hence births group between October and February when there is green forage (berseem) provide sufficient nourishment for the ewes to have enough milk and for their lambs to develop normally. Births group between June and July when feed availability is low, reducing the chances of lamb survival (mortality rates are as high as 25%). Weaning percentages are low (under 70%). On the other hand, it should be taken in UCCount that the conception rate for ewes bred in January are higher than in Mai and September. Lambing in October could be early that the lambs may be born before the Berseem could provide sufficient nourishment for the ewes to have enough

milk and for their lambs to develop normally. However, considering lambing percentages and lamb survival and growth, breeding in March-April and August-September is preferable. Breeding takes place throughout the year, most breeding is linked with the highly seasonal availability of Berseem.

Survival rate

UCCording to Dwyer and Morgan (2006), the worldwide rate of mortality in newborn lambs is in excess of 15% of lambs born and represents a challenge to sheep production and welfare. Dwyer and Morgan (2006) added that especially in prolific ewes the mortality rates are high in lambs with low birth weights and that after birth the absolute growth rates are lower in the surviving light lambs than in the heavier lambs. However, in this study low birth weight lambs exhibit faster growth than the heavier born lambs.

Milk production

Respective the effect of ammoniation on the milk production, it was found that feeding lactating cows tended to gain more weight and produce more milk when fed dehydrated alfalfa meal than did than did cows supplemented ammonia treated corn cobs or soybean (Rock. *et al.*,1991). However, Hadjipanayiotou *et al*, (1993) found that feeding Awassi ewes on urea treated straw (AS) diet produced significantly less milk than those on the control diet (AS, 432 *vs* 462 g milk/ewe/day).
Meanwhile, Lines and Weiss (1996) stated that use diverse sources of dietary N (ammoniation, urea, soybean meal, or a commercial blend of animal protein meals) did not greatly influence N utilization by dairy cows.

Conclusion

Urea treatment improved the nutritive value of corn with cobs and made it at least equivalent to CFM respective CP content (14%) and when offered alone proved better efficiency compared to the control and the 50% UCC. The increased milk production of the ewes given CFM, before and during, lactation was limited in but type of feeding had an effect on the birth weight and weaning weight of lambs born and raised by these ewes.

In addition, treatment at the time of ensiling high-moisture grains may decrease mold growth and DM losses, especially in grains with less than 80% DM. Reducing particle size of these grains prior to ammoniation is important in UCCelerating anaerobic fermentation and improving feed stability during storage and may increase aerobic stability of these high-moisture grains at the time of feeding, especially during months of elevated environmental temperatures (Eastridge 2006).

The effectiveness of ammonia in inactivating aflatoxins in contaminated livestock feed stuffs has been investigated by several authors brekke (1977), Grove (1984), Fremy (1988), Bailey et. al., (1994), Hoogenboom et. al., (2001) The authors stated that ammoniation of contaminated ingredients of livestock feed resulted in efficient reduction of aflatoxin levels and abolished the detectable transfer of AFM1 or AFB1 into milk, and greatly reduced the carcinogenic risk posed by any carry-over of aflatoxins or their derivatives into milk. which most likely caused by a decreased bioavailability of the degradation products.

Further work is required to investigate other sources that could enhance the nutritive value of the residues in order to stimulate intake and production. Feeding Ossimi ewes on UCC around parturition during the summer season did not seem to enhance ewe or lamb production traits but the feed costs for lactating ewes and growing lambs can be minimized. Strategic timing of feeding urea treated by products for Ossimi sheep ewes may provide a method for increasing the weight of lambs weaned during periods of limited green forage availability.

Whether ammoniation of the by products is economical depends on relative costs of anhydrous NH3 , Urea and alternative feedstuffs, such as cereal grains. However, this system is only UCCeptable if the value of the response is higher than associated costs of processing and treatment. However, the use of urea is up till today is feasible.

There was little difference in average daily gain or feed efficiency between lambs fed the rations based on CFM and those included UCC but reduced feed cost per kg of weight gain by 15% (50% UCC) or 35% (100% UCC), suggesting that a crude protein level near 14% based on UCC would be optimal for 25 - 40kg growing Ossimi lambs. Replacement of CFM in pregnant and growing lams rations with UCC would be cost effective as cost UCC is only at 60% less than cost of CFM.

Moreover from feeding management such ruminant exposed once to urea treated any feed stuff performed better when exposed later on to treated feed stuffs. As stated by Wiedmeier *et al*, (2002). Thus, managers should consider previous exposure to treated material (in particular the low quality) when considering applying this technology to reduce food costs.

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3/8/2010

Application of multi-factorial experimental designs for optimization of biotin Production by a *Rhizopus nigricans* strain

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Abstract: The main objective of the present work is to demonstrate the efficiency of multi-factorial experimental designs to elucidate factors affecting the microbial production of biotin and to predict their optimum settings. A local *Rhizopus nigricans* strain was selected as a remarkable wild type biotin (vitamin H) producer. A preliminary medium formulation experiment suggested sucrose and peptone as appropriate donors of carbon, nitrogen and sulphur. An incomplete two level factorial experiment showed that concentrations of sucrose and peptone, as well as fungal growth stage are the most effective independent variables. A three level response surface methodology was then applied to accomplish a polynomial model which correlates the three key variables to biotin accumulation. When compared to the basal culture, the optimum condition predicted according to this model achieved about 10.4, 13.9, 5.7, 7.6 and 4.2-fold increases in production, product yield coefficient, specific product yield coefficient, productivity and specific productivity, respectively. [Journal of American Science 2010;6(6):179-187]. (ISSN: 1545-1003).

Keywords: Biotin, vitamin H, *Rhizopus nigricans*, experimental designs, response surface methodology

1. Introduction

Biotin ($C_{10}H_{16}N_2O_3S$), also known as vitamin H or B7, is a coenzyme essential for many cellular carboxylation and decarboxylation reactions, fatty acid biosynthesis, gluconeogenesis, and amino acid metabolism (Streit and Entcheva, 2003). It also affects gene expression through a diverse array of cell signaling pathways (Rodriguez-Melendez and Zempleni, 2009). Deficiency of biotin may cause, for example, seborrhea, dermatitis, loss of appetite and lassitude (Birch *et al*., 2000). Since its bioavailability is rather low, it is commonly added to many food, feed and cosmetic products.

While humans and animals require several hundred micrograms per day, most microorganisms and plants have the ability to synthesize biotin (Streit and Entcheva, 2003). Some wild isolates of *Saccharomyces cerevisiae* are biotin prototrophs and others are not (Hall and Dietrich, 2007). Research has led to understanding the complete biotin biosynthesis pathways in many different microorganisms. Among the most important microorganisms reported as biotin producers are *Rhizopus delmar* (Shchelkovo and Vorob'ev, 1982; Maksimov *et al.*, 1983), *Rhizobium sp.* (Sierra *et al.*, 1999) and *Kurthia sp* (Hoshino *et al*., 1999). Biotin over-production by fermentations of recombinant microorganisms such as *Sphingomonas sp.* (Saito *et al.*, 2000) and *Candida utilis* (Hong *et al*., 2006) has been reported.

Since its bioavailability is rather low, biotin is commonly added to many food, feed and cosmetic products. However, the majority of vitamin H in the market is synthesized chemically. To overcome the disadvantages faced by the chemical biotin industry, including high energy input and the production of considerable amounts of chemical waste, (Streit and Entcheva 2003), efficient fermentation processes to produce biotin are in great demand. Thus, the main goal of the present study is to contribute a model that can be applied for optimization of biotin production fermentations. Traditional optimization of fermentation factors (one at a time) is generally a time consuming and labor-intensive process. On the contrary, statistically designed multi-factorial experiments proved to be valuable tools for optimizing microbial culture conditions (Hooijkaas *et al.*, 1998; Lotfy *et al.*, 2007). One of the advantages of applying experimental designs is that, they consider the interaction between the non-linear natures of the response in short experiments (Gresham and Inamine, 1986).

2. Material and Methods

Microorganism:

The fungus used in this study is *Rhizopus nigricans* NRC strain FR105 which is a local isolate obtained from the cultures collection of the National Research Centre, Dokki, Cairo, Egypt. This strain had been

selected as a promising biotin producing fungus (Salem, 2009).

Growth media:

Maintenance medium: Potato-dextrose agar (PDA) medium was supplied as a dry powder preparation which has the following composition expressed as a percentage (g/100 ml): potato infusion 0.4 (infusion from 200 g potatoes); glucose, 2; agar 1.5. The pH was adjusted to 5.6. Prepared PDA slants were inoculated by the pure fungal cultures and incubated at 28°C. Slant cultures were transferred every month.

Preliminary biotin production medium: The medium chosen for investigating biotin production by the experimental *Rhizopus* strain contained (%): glucose, 5; peptone, 1; KH_2PO_4, 0.5 and KCl, 0.25. The pH value was adjusted to 5.5 before autoclaving (Mahato *et al.*, 1988).

Fungal Cultivation:

The inoculum was prepared by adding 3ml of sterile distilled water to each PDA slant followed by scratching with a sterile needle. This suspension was used to inoculate 50 ml of sterile biotin fermentation medium dispensed in a 250 ml Erlenmeyer flask. Flasks were then incubated on a rotary shaker with 150 rpm at 30°C for different periods of incubation. Thereafter, cultures were centrifuged at 5000 rpm for 10 min to separate the fungal mycelia from the culture filtrate. Final pH, fungal biomass (mass after being dried at 60°C till constant weights) and biotin content were then determined.

Isolation and determination of biotin:

Biotin was separated, purified, chemically characterized and compared to an authentic biotin sample based on the TLC technique described by Birch *et al.* (2000). Biotin concentration was estimated calorimetrically as described by Shimada *et al.* (1969).

Experimental designs:

The Plackett-Burman design: The Plackett-Burman experimental design, a fractional factorial design (Plackett and Burman, 1946; Yu *et al.*, 1997), was used to reflect the relative importance of various environmental factors on biotin production as well as dry weight and final pH in liquid cultures. Seven independent variables were screened in nine combinations organized according to the Plackett-Burman design matrix described in the Results section. For each variable, a high level (+) and low level (-) was tested. All trials were performed in duplicates and the averages of biotin production results were treated as the responses. The main effect of each variable was determined with the following equation:

$$E_{xi} = (\sum_{i+} M - \sum_{i-} M) / N$$

Where E_{xi} is the variable main effect, M_{i+} and M_{i-} are biotin production in trials where the independent variable (xi) was present in high and in low settings, respectively, and N is the number of trials divided by 2. For determination of variable significance, statistical t-values for equal unpaired samples were calculated with respect to observations.

Box-Behnken design: In the second phase of medium formulation for optimum biotin production, the box-behnken experimental design, which is a response surface methodology (Box and Behnken, 1960), was applied. In this model, the most significant independent variables, named (X_1), (X_2) and (X_3) are included and each factor can be examined at the three different levels, low (-), high (+) and central or basal (0).

Here, these factors included concentrations of sucrose (X_1) and peptone (X_2) and KH2PO4 (X_3) were treated as independent variables. Thirteen combinations and their observations (shown in the Results section) were fitted to the following second order polynomial mode:

$$Y=b_o+b_1X_1+b_2X_2+b_3X3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+$$

$$b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2$$

Where, Y is the dependent variable (biotin production); X_1, X_2 and X_3 are the independent variables; b_o is the regression coefficient at center point; b_1, b_2 and b_3 are linear coefficients; b_{12} b_{13} and b_{23} are second-order interaction coefficients; and b_{11} and b_{33} are quadratic coefficients.

The values of the coefficients were calculated and the optimum concentrations were predicted using Statistica software. The quality of the fit of the polynomial model equation was expressed by R^2, the coefficient of determination.

3. Results and Discussion

Screening for biotin production substrates

Biotin has the chemical formula $C_{10}H_{16}N_2O_3S$. Accordingly, a quick identification of carbon, nitrogen and sulphur sources suitable for biotin production by *R. nigricans* NRC, FR105 was aimed. This was performed by two simple screening experiments. In the former, substitution of the glucose content in the preliminary biotin production medium by equal amounts of fructose, sorbose,

sucrose, starch, lactose, maltose and glycerol was examined. Biotin content was determined after four days of incubation. As shown in Table (1), variation of carbon sources in the basal medium dramatically modulated the efficiency of the fungus to produce biotin as well as biomass.

Among the different tested carbon sources, sucrose afforded the highest biotin production record (2.81 mg/l) with a 1.8-fold increase when compared to glucose. The obtained results and calculations suggest that sucrose obviously enhanced biotin synthesis rather than mycelial production and consequently, attained the highest specific product yield (0.205 mg product/g fungal biomass). Considerable enhancements in biotin production were also recorded by fructose, sorbose and glycerol cultures. On the contrary, the experimental fungus failed to produce biotin in the presence of starch or lactose.

Sierra *et al.* (1999) demonstrated that the nature of the carbon source as well as the age of the fungus culture strongly influence the pattern of vitamins released by *Rhizopial* cultures. It has been also reported that glycerol (6%) is suitable as a carbon source for biotin formation by *Kurthia sp* (Furuichi *et al.*, 2000). Similar to our results, sucrose proved to be appropriate for growth and finally converted to biotin by *E. coli* (Brich *et al*., 2000).

For the evaluation of alternative donors of nitrogen and sulphur, representative organic sources (including yeast extract, baker's yeast, corn steep liquor and urea) and an inorganic source (ammonium sulphate) were examined as peptone substiuents in the fermentation medium. Each was employed at a nitrogen concentration equivalent to 1% peptone in the presence of the most favorable carbon source, sucrose. However, none of the examined nitrogenous compounds exceeded peptone with respect to biotin production.

Considerable biotin production results were also recorded by the cultures contained yeast extract and corn steep liquor (2.3 and 1.87 mg/l). However, corn steep liquor dramatically stimulated the experimental *R. nigricans* strain for mycelial growth rather than biotin production. It recorded a 2.2–fold increase in biomass production when compared to peptone (data not shown).

In the case of urea, no detectable biotin was accumulated in the medium. The inability of the fungus to synthesize biotin under such a condition is simply a consequence of the absence of sulphur, a component of the biotin molecule. This observation demonstrates the sensitivity of the applied biotin estimation method. On the other hand, the results showed that ammonium sulphate did not support biotin biosynthesis or even mycelial growth. Accordingly, it can be suggested that ammonium sulphate cannot serve as a nitrogen or sulfur contributor in cultures of *R. nigricans* NRC strain FR105. Similarly, Maksimov *et al*. (1983) has found that *R. delmar* does not efficiently assimilate ammonium sulphate and urea which offered negligible quantities of biotin.

Peptone, a peptic digest of fresh meat, has relatively high contents of nitrogen and sulphur (Atlas, 2004) which are elementary for building biotin molecules. Therefore, among the nitrogenous compounds examined in this work, peptone has been chosen as a component of the fermentation medium.

Optimization of biotin production by multi-factorial designs:

The medium formula containing sucrose and peptone as substrates for biotin synthesis was chosen as the basal medium for an optimization strategy that involved a two-phase experimental design. The first step was to evaluate the relative importance of various fermentation factors by applying a fractional factorial design. In the second phase, levels of the variables, which have significant influences on biotin formation, were further investigated.

Elucidation of fermentation factors affecting biotin production:

The relative importance of various environmental factors involved in the process of biotin production was explored using the Placket-Burman design (Plackett and Burman, 1946) described in the Material and Methods section. Examined levels of 7 culture variables are presented in Table (2). The design was applied with 9 different fermentation conditions as shown in Table (3). All experiments were performed in duplicates and the averages of the observations are presented in the table. Fungal growth, final pH and biotin production were determined after 3, 4 or 5 days according to the proposed design. The main effects of each variable upon biotin production, as well as fungal growth, were estimated and expressed graphically in Figure (1). The results showed clearly that the dry weights of the mycelia are positively affected by the presence of high levels of sucrose, peptone and inoculum size. On the other hand, high concentrations of sucrose and KH_2PO_4 showed negative effects on biotin production.

The calculated main effects of this experiment suggested also that increasing the level of peptone in the culture medium, decreasing sucrose

concentration and extending the incubation period stimulate biotin production. On agreement with our results, it has been previously reported that the rate of biotin synthesis by a *R. delemar* strain increased drastically when the major portion of sugar was consumed and the fungal growth was terminated (Shchelkovo and Vorob'ev, 1982). All these observations suggest that the genes responsible for expressing biotin synthesizing enzymes in *Rhizopus* are growth-phase dependent. It is likely that they are triggered post-exponentially or under limiting growth conditions. Moreover, biotin production by other microbial cultures including *Sphingomonas sp.* (Shaw *et al.*, 1999) and *Agrobacterium* (Saito *et al.*, 2000) were relatively high under limiting growth conditions.

Based on the results obtained from the Placket-Burman experiment, a formula of the following composition (%) is predicted to be near optimum for biotin production: sucrose, 4; peptone, 1.5; KH_2PO_4, 0.4; KCl, 0.15 and inoculum size, 3 with an initial pH of 4.5 and an incubation period of 5 days. In order to determine the accuracy of this experiment, a verification test was carried out in a triplicate. The predicted near optimum levels of independent variables were examined against the basal condition settings. The average of the achieved biotin content was 20.1 mg/l which represent about 7-fold increase when compared to the basal condition.

The results of this experiment suggested also that the most effective variables, concerning biotin production are the concentrations of peptone and sucrose in addition to the incubation period. Among those, statistical analyses of the data (t-test) demonstrated the significance of peptone and sucrose Table (4).

Optimization of biotin production by Box-Behnken design:

In order to approach the optimum response region of biotin production, the effective independent variables including sucrose concentration (X_1), peptone concentration (X_2) and incubation time (X_3) were further investigated, each at three levels according to the Box and Behnken design (Box and Behnken, 1960). However, KCl, initial pH, inoculum size and KH_2PO_4 were treated as constant factors: KCl (0.15%), KH_2PO_4 (0.4%), inoculum size (3%) and initial pH (4.5). Table (5) represents the design matrix of the coded variables together with the experimental results of final pH, growth and biotin production.

As shown in Table (5), the highest biotin accumulation records (28.2, 23.3 and 26.2 mg/l) were achieved by the trials number 3, 4 and 10, respectively, which contained peptone at its examined high level (3%). On the other hand, it is clear that the lowest biotin production records (18.1, 19.1 and 18.4 mg/l) were achieved by the trials number 2, 6 and 8, respectively, that contained sucrose at its examined high level (3%).

Expressing the experimental results in the form of surface plots reflects the interactive effects of examined variables. Figure (2) shows the influences of variations in sucrose concentration and incubation time on the experimental fungus with respect to biotin production. From this figure, it can be suggested that, up to approximately 5.5 hours, the more the incubation time the more the biotin accumulation in the medium. It seams likely that a longer incubation time would not allow more biotin synthesis. However, an optimum level of sucrose appears to be close to the mean of the examined concentrations.

For a precise prediction of the optimal point, a second order polynomial function was fitted to the biotin production results of the applied Box-Behnken experiment. According to the obtained statistical analysis results, the correlation between the response and the three independent variables can be described by the following model.

$$Z = 4.93 + 2.44\,X_1 + 1.07\,X_2 + 5X_3 - 0.22X_1X_2 - 0.15X_1X_3 - 0.37X_2X_3 - 0.56\,X_1^2 + 0.74\,X_2^2 - 0.37\,X_3^2.$$

Where Z is the product of biotin (dependent variable); X_1, X_2 and X_3 are levels of sucrose, peptone and incubation time, respectively. The degree of fitting of the model is relatively high as the calculated coefficient of determination (R^2) was 0.99. The closer the R^2 value to 1, the stronger the model is and the better the response predicted.

Solving the model according to the data obtained from Table (5) revealed an optimum response at $X_1 = 1.5$, $X_2 = 3$ and $X_3 = 5.5$ with a predicted Z (response) of 29.5 mg/l. Thus, according to the results of the two optimization experiments, an optimum response (biotin production) is predicted under the following fermentation condition: sucrose, 1.5%; peptone, 3%; KCl 0.15%; KH_2PO_4, 0.4% initial pH, 4.5; inoculum size 3% and an incubation period of 5.5 days.

In order to evaluate this proposition, a verification experiment was performed in which the predicted optimal condition was practically compared with the basal fermentation settings in triplicates. The optimized culture condition attained a biotin accumulation average of 28.2 mg/l which is relatively

close to the theoretically predicted value. When compared to the basal culture condition this achievement resulted in about 10.4, 13.9, 5.7, 7.6 and 4.2-fold increases in production, product yield coefficient, specific product yield coefficient, productivity and specific productivity, respectively Table (6).

Table 1: Effect of different carbon sources on biomass and biotin production by *Rhizopus nigricans* NRC, FR105

Carbon source	Final pH	Mycelial dry weight (g/l)	Biotin content (mg/l)	Specific product yield (mg product/g biomass)
Glucose	4.7	12.6	1.50	0.119
Fructose	5.0	16.4	2.30	0.140
Sorbose	5.1	14.8	2.21	0.149
Sucrose	4.9	13.2	2.70	0.205
Starch	5.0	18.0	0.00	0.000
Lactose	7.5	16.6	0.00	0.000
Maltose	4.3	10.6	0.08	0.008
Glycerol	5.6	17.2	2.10	0.122

Table 2: Factors examined as independent variables affecting biotin production by *R. nigricans* NRC, FR105 and their levels in the Plackett-Burman experiment

Factor	Symbol	Level		
		-1	0	1
Time (days)	T	3	4	5
Sucrose (%)	S	4	5.5	7
Peptone (%)	P	0.5	1	1.5
$KH_2 PO_4$ (%)	KH_2	0.4	0.5	0.6
Inoculum size (%)	1S	1	2	3
Initial pH	pH	4.5	5.5	6.5
KCl (%)	KCl	0.15	0.25	0.35

Table 3: The Plackett-Burman experimental design for 7 variables and its results

Trial	Independent Variables [1]							Response		
	T	S	P	KH_2	IS	pH	KCl	Final pH	Dry weight (g/l)	Biotin (mg/l)
1	-	-	-	+	+	+	-	3.50	09.1	5.7
2	+	-	-	-	-	+	+	3.51	08.2	10.7
3	-	+	-	-	+	-	+	3.37	16.2	2.6
4	+	+	-	+	-	-	-	3.59	12.1	2.9
5	-	-	+	+	-	-	+	3.78	13.3	15.6
6	+	-	+	-	+	-	-	4.10	16.4	20.1

7	-	+	+	-	-	+	-	3.84	21.2	10.1
8	+	+	+	+	+	+	+	4.16	16.1	11.3
9	0	0	0	0	0	0	0	3.89	11.2	16.5
10	0	0	0	0	0	0	0	3.87	11.0	16.3

[1] Factor symbols are shown in Table 5

Table 4: Statistical analysis of the Plackett-Burman experimental results

Variable	Growth		Biotin	
	Main effect	*t*-value	Main effect	*t*-value
Time	-0.09	-0.92	2.75	0.67
Sucrose	0.23	**2.47**	-6.30	**-1.53**
Peptone	0.27	**2.83**	8.80	**2.13**
KH_2PO_4	-0.14	**-1.51**	-2.00	-0.48
Inoculum size	0.04	0.39	0.10	0.02
pH	-0.04	-0.46	-0.85	-0.21
KCl	-0.06	-0.67	0.35	0.08

Critical t-values at α= 0.05 and 0.1 are 2.015 and 1.467, respectively.

Table 5: Examined concentrations of the key variables and results of the Box-Behnken experiment

Trial	Independent variable			Response		
	Sucrose % X_1	Peptone % X_2	Days X_3	Final pH	Dry weight (g/l)	Biotin (mg/l)
1	1 (-)	1 (-)	5.5(0)	3.5	07.2	20.4
2	3 (+)	1 (-)	5.5(0)	3.4	15.5	18.1
3	1 (-)	3 (+)	5.5(0)	4.2	24.0	28.2
4	3 (+)	3 (+)	5.5(0)	3.7	20.3	23.3
5	1 (-)	2 (0)	4(-)	3.7	09.4	20.5
6	3 (+)	2 (0)	4(-)	3.6	08.0	19.1
7	1 (-)	2 (0)	7(-)	3.8	12.6	22.5
8	3 (+)	2 (0)	7(+)	3.7	14.4	18.4
9	2 (0)	1 (-)	4(-)	3.5	13.8	20.1
10	2 (0)	3 (+)	4(-)	4.56	09.2	26.2
11	2 (0)	1 (-)	7(+)	3.5	15.6	21.3
12	2 (0)	3 (+)	7(+)	3.8	12.0	20.7
13	2 (0)	2 (0)	5.5(0)	3.64	12.2	22.2

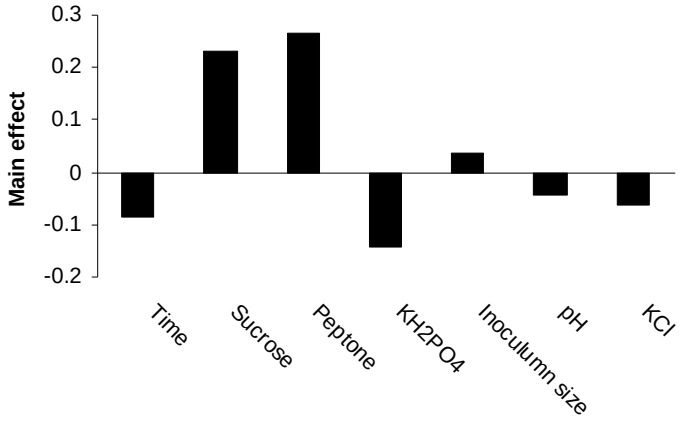
13	2 (0)	2 (0)	5.5(0)	3.63	12.0	22.3
13	2 (0)	2 (0)	5.5(0)	3.64	12.4	22.2

Table 6: Kinetic parameters and coefficients of biotin fermentation by FR105 under basal and optimized conditions

Parameter	Unit	Condition		Fold increase
		Basal	Optimum	
Production	(mg product/l)	2.700	28.20	10.4
Product yield coefficient	(mg product/g substrate[1])	0.045	0.63	13.9
Specific product yield coefficient	(mg product/g cells)	0.205	1.18	5.7
Productivity	(mg product/l/h)	0.675	5.13	7.6
Specific productivity	(mg product/g cells/h)	0.051	0.21	4.2

[1] Substrate = g sucrose + g peptone

(a)



(b)

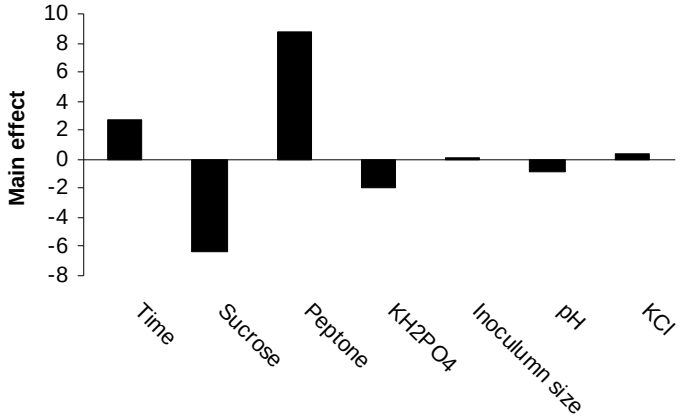


Figure 1: Main effects of independent variables upon growth (a) and biotin production (b) by *R. nigricans* NRC, FR105 according to the results of the Plackett-Burman experiment

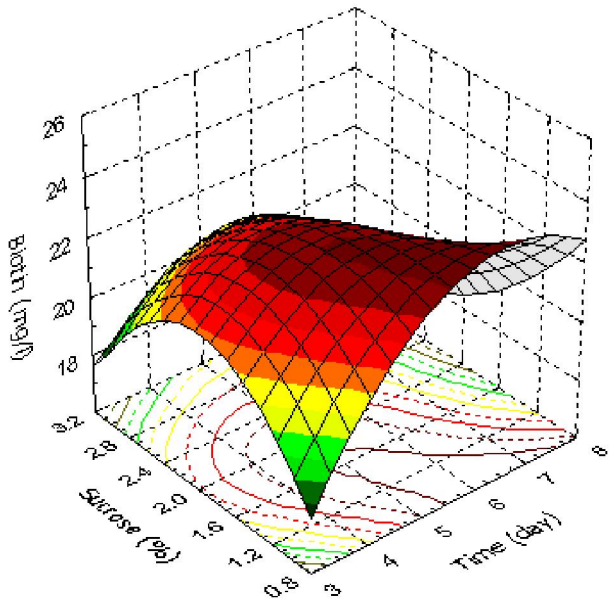


Figure 2: Sucrose and time as independent variables that affect the production of biotin by *R. nigricans* NRC, FR105 based on the results of the Box-Behnken experiment

Conclusion

It has been proposed that any fermentation process has to be able to produce more than 1 g biotin per liter in order to be cost-effective (Streit and Entcheva 2003). A recombinant strain of *Serratia marcescens* was able to produce biotin at the 500-mg/l level (Sakurai *et al.*, 1994). In addition, more than 1 g biotin per liter of culture medium was achieved by a *B. subtilis* strain that over-expresses the genes responsible for biotin synthesis (Bower *et al.*, 2001). For more cost-effective results, we suggest that the fermentation of such recombinant biotin over-producer strains could be considerably improved by a sequential economization and optimization approach which covers each of the following investigations: (1) screening for carbon, nitrogen and sulphur containing agro-industrial wastes that efficiently support the recombinant strain for growth, as well as biotin production, (2) elucidation of the fermentation factors the significantly regulate the synthesis of biotin and (3) application of a suitable multi-factorial experimental design for optimization of biotin production. In each optimization phase, a special attention should be given to other important biotechnological parameters including: product yield coefficient (g product/g substrate), specific product yield coefficient (g product/g cells) and productivity (g product/l/h).

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4/1/2010

Some Physiological Factors Affecting Rapamycin Production by *Streptomyces hygroscopicus* ATCC 29253

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Abstract: The production of rapamycin, a potent antifungal, immunosuppressant and antitumor, by *Streptomyces hygroscopicus* ATCC 29253 has been studied in eight culture media. Rapamycin titer varied considerably in the tested media. The medium composed of soy meal, glucose, ammonium sulphate and KH_2PO_4 was the optimal for rapamycin production and so selected for further optimization. Studies for formulating the best carbon and nitrogen nutrition for rapamycin biosynthesis revealed that replacing glucose by D (+) mannose and excluding ammonium sulphate with decreasing soy meal concentration to 20 g/l led to four fold increase in rapamycin titer. Also, the effect of KH_2PO_4 concentration and medium initial pH were elucidated and the best requirements have been specified as 5 g/l KH_2PO_4 and pH 6. [Journal of American Science 2010;6(6):188-194]. (ISSN: 1545-1003).

Keywords: Rapamycin**,** *Streptomyces hygroscopicus***,** Physiological studies

1. Introduction

Since it has been discovered, and along the last few decades, rapamycin (Rap) has showed a panel of interesting bioactivities which attracted many researchers overall the world and encouraged them to explore more of its activities and to expect a promising role waiting for this compound as a multi-function drug. Rap was firstly discovered in 1975 as an antifungal agent (Vezina *et al.*, 1975) having no any antibacterial activity (Baker *et al.*, 1978). Few years latter, other activities have been frequently discovered; it was shown to have an immunosuppressive activity (Martel *et al*., 1977) and it showed a good activity against mammary, colon and brain tumor model systems (Douros and Suffness, 1981). As an immunosuppressant, Rap acts via a mechanism that is completely different to that of cyclosporine A, and it has the fabulous advantage to be of greater activity which is 150 times as that of cyclosporine A with remarked lower toxicity (Kojima *et al*., 1995). Up to date, Rap has got two approvals from the American FDA, the first was in August 1999 for preventing host-rejection in kidney transplants (Cruz *et al.*, 2001) and the second was in 2003 for use in drug-eluting stent (Tsang *et al.*, 2007) to prevent restenosis of coronary arteries following angioplasty (Marx and Marks, 2001).

Considering the chemical structure of Rap, it is nitrogen containing macrolide of the molecular formula of $C_{51}H_{79}NO_{13}$ with a very large 31-membered lactone ring. It contains three conjugated double bonds and could be classified as a polyene compound.

The first Rap producing isolate was identified as *Streptomyces hygroscopicus* ATCC 29253 that has been isolated from soil sample collected from an island known as Rapa Nui (Vezina *et al.*, 1975).

While the vast majority of published works concentrated on clinical activities of Rap, limited number of investigations studied the production of Rap (Kojima *et al*. 1995; Lee *et al*. 1997). According to the available literatures, there are no published data about the optimum conditions of Rap production by the original strain *Streptomyces hygroscopicus* ATCC 29253. This justified the present efforts aiming to optimize the physiological conditions allowing maximum production of Rap by *Streptomyces hygroscopicus* ATCC 29253.

2. Material and Methods

Microorganism

The organism used in this investigation *Streptomyces hygroscopicus* ATCC 29253 was purchased from Microbiological Resources Centre in Cairo (Cairo MIRCEN), Egypt. It was grown on slants of oat meal medium (contained oat meal, 20 g/l; agar, 20 g/l; pH 7) for 10 days at 28 ºC after which spores were collected by addition of 4 ml of 10% (v/v) glycerol to each slant. Spore suspensions were then pooled together to get a suspension of 25.8 x 10^6 CFU/ml that was then dispersed in cryopreservation vials each contained 1 ml and stored at -20 ºC to be the source of organism during this study.

Inoculum

Inoculum culture was prepared by inoculating 1 ml of thawed spore suspension (25.8 x 10^6 CFU/ml) into 50 ml starch casein broth (contained in g/l: starch, 10; casein, 0.3; KNO_3, 2; NaCl, 2; K_2HPO_4 2; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01; pH 7) in 250-ml Erlenmeyer flask. The flask was then incubated at 28±2 ºC for 7 days at 150 rpm. Two milliliters of the grown culture were used to inoculate 50 ml of fermentation medium.

Fermentation

Fermentation was carried out in duplicate 250-ml Erlenmeyer flasks, each contained 50 ml production medium, and they have been incubated at 25 ºC ±2 for 7 days at 150 rpm. Production of Rap was initially tested in eight different media. The most suitable medium underwent further detailed studies for maximizing Rap production. The composition (g/l) of the eight tested media was as follow:

Medium I (modified Xu *et al*., 2005)

It contained: soluble starch, 10; yeast extract, 6; peptone, 6; N-Z amine type B, 1.5; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 1; pH 6.5.

Medium II (modified Sehgal *et al*., 1975)

It composed of: soybean meal, 30; glucose, 20; $(NH_4)_2SO_4$, 5; KH_2PO_4, 5. Components have been dissolved in tap water and pH was adjusted to 6.

Medium III

It is modified Bennette's agar medium (Atlas, 1997). It consisted of: glucose, 10: N-Z amine type B, 2; beef extract, 1; yeast extract, 1; pH 7.3.

Medium IV

It had the same composition of Krainsky's asparagine agar medium (Atlas, 1997) with elimination of agar. Its composition was: glucose, 10; L-asparagine, 0.5; K_2HPO_4, 0.5; pH 7.

Medium V

It had the same composition of starch casein agar (Atlas, 1997) with elimination of agar. It contained: soluble starch, 10; K_2HPO_4, 2; KNO_3, 2; NaCl, 2; casein 0.3; $MgSO_4.7H_2O$, 0.05; $FeSO_4.7H_2O$, 0.01; $CaCO_3$, 0.02; pH 7.

Medium VI (modified Starch-Casein medium)

It is modified than Starch-Casein medium mentioned before by replacing casein and nitrate with 2 g/l ammonium sulphate.

Medium VII

Its composition is the same like chitin agar medium (Hsu and Lockwood 1975) after elimination of agar. It contained: Colloidal chitin, 4; KH_2PO_4, 0.3; K_2HPO_4, 0.7; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; $ZnSO_4.7H_2O$, 0.001; $MnCl_2.4H_2O$, 0.001; pH 8.

Medium VIII

It is modified Benedict's medium (Porter *et al*., 1960). It composed of the following: glycerol, 20; L-arginine, 2.5; NaCl, 1; $CaCO_3$, 0.1; $FeSO_4.7H_2O$, 0.1; $MgSO_4.7H_2O$, 0.1; pH 7.

Analysis

The data obtained throughout the present investigation were the average of triplicate treatments.

Estimation of the microbial growth

At the end of fermentation, the microbial growth under the submerged conditions appeared as spherical pellets. Microbial dry cell weight was determined by placing a 10-ml sample of whole fermentation medium into a pre-weighed 15-ml tube and centrifuging at 3500 rpm for 5 minutes. The supernatant was decanted and the microbial residue present in the tube was dried at 80ºC for two days. The tubes were placed in a desiccator before reweighing to determine the growth yield expressed as gram dry weight per liter fermentation medium.

Extraction of Rap

At the end of fermentation, aliquots of 3 ml were taken where microbial growth was separated as mentioned before and extracted twice by shaking with 3 ml methanol for 30 minutes. Then the two extracts were pooled to be assayed for Rap concentration.

Estimation of Rap

Bioassay determination of Rap was achieved by paper-disc agar diffusion method as described by Kojima *et al.* (1995). The assay was conducted in agar plates of assay medium seeded with *Candida albicans* ATCC 10231 as the test organism. Assay medium composed of (g/l): peptone, 2; glucose, 5; agar, 11; pH 6. Five µl of cells methanol extract have been loaded onto paper discs (Whatman no. 3) of 6 mm diameter. The discs were then carefully placed on the surface of bioassay medium seeded with test organism. After incubation for 20 hr at 37 ºC, inhibition zone around each disc was recorded. Similarly, inhibition zones around standard concentrations of Rap were recorded. Plotting the relation between logarithms of Rap concentration against inhibition zone showed straight line whose linear equation was used to get Rap concentrations from inhibition zone readings. It was referred to milligrams of Rap produced in 1 liter of fermentation media as "volumetric production" whereas milligrams Rap produced per gram dry cell weight has been referred as "specific production".

3. Results and Discussion

1. Suitability of the fermentation media

This experiment was directed to test the ability of eight different fermentation media to support the production of Rap. The results illustrated in Table (1) showed great variation in Rap titer in different fermentation media. Medium II had remarkable superiority over all other tested media and its volumetric Rap titer was approximately 30 times as that of medium III which gave the secondary highest Rap yield. Minor concentrations of Rap were produced in media I, V and VI which were still comparable to yield obtained in medium III. Out of the media under the study, media IV, VII and VIII showed inability to support Rap production. Medium II which produced the highest yield contained soy meal that represents a good source of proteins, amino acids and vitamins. It was slightly modified than that used by Sehgal *et al*. (1975) in production of Rap. Although Xu *et al*. (2005) produced high concentrations of Rap in medium closely related to medium I, it showed here poor yield that was nearly1/40 of that in medium II. Each of the media IV, VII and VIII failed to satisfy nutritional requirements for Rap biosynthesis. The current results profoundly clarify the impact of the composition of nutritional media on the production of secondary metabolites; the productivity may remarkably raise many folds or completely suppressed under the influence of the used medium.
Also, the results showed noticeable incoherence between volumetric and specific titers of Rap; the profile revealed from one of them differed completely from that of the other. The results here gave a true example for contradiction between specific and volumetric Rap yields; medium VI produced the lowest volumetric titer comparing with other media that could support Rap production, and in the same time it had the highest specific titer. This discrepancy could be easily solved when considering biomass yield in medium VI which was the lowest than that at all tested media. Considering the economics, the volumetric titer represents the actual quantity of Rap that could be gained from fermentation and thus it is the realistic quantity that is reliable to compare between different fermentation variants. As such, medium II was the best suited medium for Rap production and it was selected to be optimized for maximizing Rap yield. Because of interference from insoluble soy meal with growth measurements, there was no ability to determine specific Rap titer in medium II and it was fully satisfied to depend on volumetric titer in subsequent investigations.

Table 1: Production of Rap by *Streptomyces hygroscopicus* ATCC 29253 in different fermentation

Production medium	Final pH	Growth (g/l)	Volumetric Rap production (mg/l)	Specific Rap production (mg/g dry cell weight)
I	7.90	2.34	0.25	0.11
II	4.75	Nd*	10.66	Nd
III	5.09	1.24	0.36	0.29
IV	7.09	0.10	0.00	0.00
V	7.68	1.03	0.20	0.19
VI	5.80	0.01	0.10	10
VII	5.52	1.77	0.00	0.00
VIII	7.18	0.05	0.00	0.00

* Not determined

2. Role of carbon nutrition

Glucose in medium II was individually replaced by one of the tested carbon sources on basis of carbon equivalent. Initial pH was adjusted to 6 and after 7 days final pH and volumetric Rap titer were determined. The data in Table (2) showed that replacing glucose by D (+) mannose caused three times increase in Rap titer which supported the investigation of Kojima *et al*. (1995) where mannose was one of the best carbon sources for Rap production. Lactose monohydrate produced nearly the same yield as that of glucose. With other tested carbon sources, Rap was produced in very small amounts. The medium having no additions of carbon sources showed detectable concentrations of Rap which refers to the ability of that medium to completely sustain growth basing on the carbon constituents of the soy meal. On the other hand, fructose, sucrose, cellulose, sodium acetate and citric acid could not exert considerable change in Rap titer although observable change in pH has occurred.

Speculations within results obtained in case of D-glucose, D-fructose and sucrose reveal some features about physiology of Rap production. Sucrose is a disaccharide that consists of glucose and fructose and these two monosaccharides have different effects on Rap production; D-glucose is the second best carbon source to be added for Rap production and where it was supplemented with D-fructose in the form of sucrose there was sharp depletion in Rap titer from 9.41 mg/l to 0.55 mg/l. This may point out to carbon metabolite repression effect of fructose on Rap production. On the other hand, when glucose supplemented with galactose in the form of lactose,

the yield of Rap was 10.35 mg/l which is close to that obtained with glucose (9.41 mg/l) and thus repression attributed to fructose disappeared if it was replaced with galactose.

Table 2: Effect of different carbon sources on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

Carbon Source	Final pH	volumetric Rap titer (mg/l)
None*	8.42	0.53
D-Glucose	4.72	9.41
D (-) Fructose	4.42	0.13
D (+) Mannose	6.47	30.71
Sucrose	7.86	0.55
Lactose Monohydrate	5.76	10.35
Maltose Monohydrate	7.46	2.53
Dextrin from potato**	5.56	2.05
Starch**	5.7	1.68
Cellulose**	7.7	0.80
Sodium Acetate	5.91	0.10
Citric Acid	5.88	0.11

None*: Medium II without glucose. It contained: soy meal, 30g; $(NH_4)_2SO_4$, 5g; KH_2PO_4, 5g; tap water 1L; pH 6

The work has extended to determine the optimum concentration of D (+) mannose, as the best carbon source for Rap production. The results depicted in Fig. (1) revealed that Rap yield increased linearly with increasing mannose concentration up to 20 g/l. Within that range Rap production was carbon source dependent due to availability of all nutrients other than carbon source. The balance between carbon source and other nutrients was obtained at 20 g/l mannose at which Rap produced in the highest yield. Further increase in mannose concentration to 30 g/l was not corresponded with increase in Rap titer. This is in agreement with results of Kojima *et al.* (1995) who found that the best concentration of carbon source was 20 g/l with no better yields at the higher concentrations. In addition, final medium pH decreased obviously with increasing mannose concentration and it was nearly around the initial value (pH 6) at 20 g/l mannose.

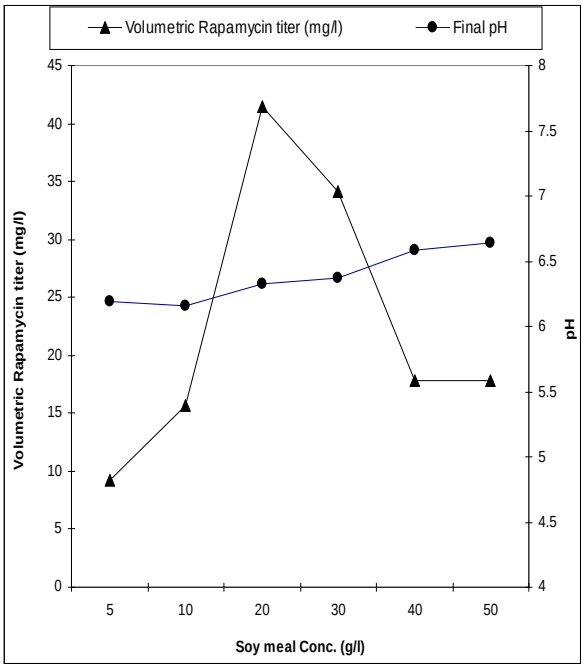

Fig. 1: Effect of different D (+) mannose concentrations on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

3. Role of nitrogen nutrition

To medium II containing D (+) mannose (20 g/l) instead of glucose, different nitrogen equivalent sources were added, one-at-a-time, as replacements of ammonium sulphate. Also, medium without ammonium sulphate was studied. As illustrated in Table (3), the medium having no additional nitrogen source was fully satisfied for Rap production basing on soy meal as a rich source for nitrogenous compounds. Moreover, nearly with all tested nitrogen sources, Rap titer has clearly dropped. This may be explained as nitrogen metabolite repression caused by easier nitrogenous compounds provided by the tested nitrogen sources.

As previously mentioned, soy meal, with no additional nitrogen source, was a good and sufficient source for nitrogenous compounds. So, it was of an importance to study Rap production at different soy mal concentrations. The results shown in Table (4) revealed that soy meal in 20 g/l was the optimal for Rap production. Concentrations lower and higher than the optimal showed markedly retarded productivities. Medium final pH varied slightly with change in soy meal concentration.

Table 3: Effect of addition of different nitrogen sources on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

Nitrogen Source	Final pH	volumetric Rap titer (mg/l)
None*	6.28	33.81
Ammonium sulphate	6.43	30.14
Ammonium Chloride	4.99	13.50
Diammonium Hydrogen Phosphate	6.03	19.62
Sodium Nitrate	7.23	7.27
Urea	7.11	9.02
Yeast Extract	6.63	3.80
Beef Extract	5.78	7.59
Peptone	7.31	11.58
Casein	6.35	6.38

None*: It contained: soy meal, 30g; D (+) mannose, 20 g; KH_2PO_4, 5g; tap water, 1L; pH 6

Table 4: Effect of different soy meal concentrations on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

Soy meal conc. (g/l)	Final pH	volumetric Rap titer (mg/l)
5	6.19	9.18
10	6.16	15.63
20	6.33	41.46
30	6.37	34.15
40	6.58	17.85
50	6.64	17.85

4. Effect of different KH_2PO_4 concentrations

The results demonstrated in Table (5) showed that increasing the level of KH_2PO_4 from 0 to 5 g/l was associated with a drop in the medium final pH value from 8.36 to 6.43 and accompanied with an increase in Rap titer up to the highest value of 40.16 mg/l. Above 5 g/l, KH_2PO_4 negatively interfered with Rap production although medium pH was kept around the initial value, indicating a possible buffering effect of the added phosphate. Increase of phosphate salt to10 g/l obviously inhibited Rap production. Inhibition of secondary metabolites biosynthesis by high inorganic phosphate concentration was reported in elsewhere. Aharonowitz and Demain (1977) found that production of cephalosporin increased with increase in phosphate concentration up to 25 mM after which further addition of phosphate led to sharp decrease in the production of antibiotic. Iwai and Omura (1982) stated that addition of relatively high concentration of inorganic phosphate increased the consumption of carbon and nitrogen sources and respiration resulting in good growth with reduced antibiotics titer. Also, phosphate inhibition of macrolide synthesis in different strains of *Streptomyces hygroscopicus* has been reported by Gersch *et al*. (1979). With respect to Rap biosynthesis by *Streptomyces hygroscopicus*, Cheng *et al.* (1995) reported on specific negative control of its production by elevated phosphate concentrations. Moreover, recently Rouf *et al.* (2007) studied the stability of Rap in different media and found that Rap was very unstable in phosphate buffer saline where it degraded in faster rates with increase in temperature and at 37 ºC almost all drug was destroyed in 24 hours. These findings are surprisingly pointing out to another effect by which phosphate interferes specifically with Rap production in addition to conventional nutritional role of phosphate.

Table 5: Effect of different KH_2PO_4 concentration on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

KH_2PO_4 Conc. (g/l)	Final pH	volumetric Rap titer (mg/l)
0.0	8.36	0.00
1.0	7.51	25.13
2.0	7.05	30.66
3.0	7.09	32.05
4.0	6.93	33.81
5.0	6.43	40.16
7.5	6.39	37.82
10.0	6.40	28.91

5. pH value relations

Production of Rap was affected by the initial pH value of the production medium. Referring to data presented in Table 6, highest Rap titer was obtained at initial medium pH of 6, and comparable yields have been produced around this pH i.e., pHs 5.5 and

6.5. Interestingly, Rap biosynthesis was very sensitive to rise in pH over 6.5; Rap titer at pH 7 was less than half of that recorded at pH 6.5. Further increase of pH was accompanied with remarkable decrease in Rap yield. Below the optimum pH, Rap production has also been suppressed and ultimately ceased at pH 4. These results are in agreement with many literatures reported on Rap production where it has been produced in media of pH 6 (Kojima *et al*., 1995; Cheng *et al*., 1995a&b; Fang and Demain, 1995; Lee *et al*., 1997). However, Xu *et al*. (2005) produced Rap in solid media of slightly increased pH (ranged from 6.3 to 6.8). Also, it is of an importance to refer to the advantageous choice of using pH 6 as the initial pH for Rap production at the present experimental conditions since the final medium pH value remained closely near the initial optimum value which maintains good Rap productivity with no need to use buffer solutions.

Table 6: Effect of initial pH of production medium on Rap production by *Streptomyces hygroscopicus* ATCC 29253.

Initial pH value	Final pH	volumetric Rap titer (mg/l)
4.0	4.28	0.00
5.0	6.19	23.27
5.5	6.22	30.75
6.0	6.30	38.14
6.5	6.91	34.06
7.0	7.39	15.34
7.5	8.11	6.35
8.0	8.20	2.35
9.0	8.20	2.86

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4/1/2010

The Substance of the Universe A Philosophical Concept about the Origin of the Universe the Great Magnetic Mass and Velocity

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Abstract: The subject of atom and its components of electrons, and protons, have always occupied my mind since my scholar days studying agriculture at Cairo University -1952. Scientists suggested that the atom components are just particles. Although they could measure these particles, they didn't exactly define their nature. This paper depicts a philosophic concept of the nature. It is an invitation to reconsider the nature and the origin of the universe from a new perspective which might cause bewilderment to the reader. I realize that I don't need to run in the street like Archimedes and I certainly realize that it might take time before scientists would consider or accept my perspective. This article describes The Substance of the Universe A Philosophical Concept about the Origin of the Universe The Great Magnetic Mass and Velocity [Journal of American Science 2010;6(6):195-202]. (ISSN: 1545-1003).

Keywords: Atoms, Big Bang, Electricity, Energy, Heat, Magnetic Mass, Matter Measurements, Mother Magnet, Photons, Quantum, Universe, Velocity, Gravity, Black Hole, Galaxies, Ether, Dark Matter, Dark Power, Void, Eternity, Infinity, Laser Beam, Confusion Energy, Attractive Charge, Compulsive Charge, Thunderbolt .

Introduction

The subject of atom and its components of electrons, and protons, have always occupied my mind since my scholar days studying agriculture at Cairo University -1952. Scientists suggested that the atom components are just particles. Although they could measure these particles, they didn't exactly define their nature.

This paper depicts a philosophic concept of the nature. It is an invitation to reconsider the nature and the origin of the universe from a new perspective which might cause bewilderment to the reader. I realize that I don't need to run like Archimedes and I certainly realize that it might take time before scientists would consider or accept my perspective. But, first of all, I would like to remind the reader of the philosophers past saying "Truth is impossible". With this, I suggest a way of thinking, which in essence is an invitation to review the existing scientific approach to explain nature phenomena.

A central premise to this paper is that human mind fell into several caveats in its attempts to understand the substance of universe. For example:

- Physicists and Mathematicians refused to accept the concepts of Eternity and Infinity in mathematical terms; and they cannot conceive their existence in the substance. In my opinion, the applied measurements to understand the nature phenomena have stood in the way of human mind to be free to conceive the concept of absolute infinite (abstract) as a real (substance).
- Human mind consistently looks for a reason behind every action and a source for any energy, at the time when there is no reason for a beginning point of the nature. There is no cause for magnetism to be referred to a dark matter. There is no limit of the existence and no limit of the time.
- Man feels heat and considers it a de-facto subject. I can suggest as well that nature is cool, and feeling heat is just a biologic sensation.
- Man never expected that light may kill people. He supposed that it is electricity when thunder and lightning destroys substances and kills people. While thunderbolt is just a beam of light that we can suppose as LASER BEAM.

Let us discuss how these concepts limited the way humanity strived to understand nature.

Part One: Scientists Lose Their Way To Determine The Origin Of The Universe.

1-1 Human Mind Confined by Measurements

It is surprising to see how the human mind, in its attempt to rationalize and make sense of everything around, is still confined by measurements. Although when Armstrong stepped on the moon surface, he said "This is one small step for (a) man, but one giant leap for mankind", the human mind is still unable to step out of its own confinements. Man invented tools and used mathematical formulas to carefully jump on the moon, but failed to

comprehend his own nature and the nature of his surroundings. He is not able to grasp the substance of the universe. The most dangerous thing is that, man sometime builds his thoughts on wrong readings for his observations.

Professor Stephen Hawking suggested in his book "The Universe in a Nutshell" that the majority of Physicists insist on the idea that the Universe began only few billion light years ago, and that it was very similar to how it is today. It is possible that the Universe has existed for an infinite time, because if the stars have been indefinitely shinning, the universe would have become hot to a great extent. that would have led to the heating of the Universe to a great extent, and the skies would be shinning like the sun even at night.

In my opinion, what Professor Stephen Hawking says is an example of how measurements constrain the man thinking by relying on the taken for granted assumptions.

Throughout history, anything fell outside the available man measurements -at the time- was rejected. Galileo Galilee was sentenced to death for claiming that the earth is not the center of the universe. The Italian astronaut GIORDANO BRUNO was burnt to death for his attempt to break free from the earth confinement to the heaven horizons by claiming that the universe is infinite and that the stars in the skies are suns similar to our sun.

1.2 Man Biologically Confined

It is even more intriguing that man has used his biologic sensation to envisage natural forces and deal with them as a de-facto subject, precisely heat and electricity. These are mere phenomena similar to ocean waves, storms, and earthquakes. Heat and electricity are neither part of the atom nor a part of the nature.

- ELECTRICITY is a man invention. When a generator moves turning around a bobbin inside a block of magnet, this causes excitation (confusion) to the matter components. ELECTRICITY expresses a confusion case.
- HEAT expresses velocity. Man feels danger from the accelerated velocity of the sun photons, and from the moving molecules around him. He called this danger as HEAT. Scientists expressed these events of kinetic energy, potential energy and others to simplify dealing with matter as daily occurrence. But for nature and the universe, it differs. The sun never radiates heat but it generates velocity in the form of rays of photons.

These points will be discussed later in part two of this paper when we present the new perception of nature.

1.3 Eternity and Infinity, are they realities?

Isaac Newton proposed an infinite universe where planets and stars are governed by gravity. But scientists of modern Mathematics and Quantum Mechanics challenged this concept. Instead, they proposed equations that led them to opposed theories. Some Cosmologists proposed one limited universe. Others proposed multiple universes. The French astronomic Jean Pierre Luminet proposed mirrors of universes in one universe.

For the atom, Quantum Mechanics physicists suppose the atom is composed of 300 bodies.

Simply, I suggest that talking about a limited universe means that it exists within void beyond it. Yes, it is nothingness, but for what extend? Since eternity and infinity are realities for the void, why couldn't they be realities for the universe? I hereby propose the acknowledgement of eternity and infinity as realities for void and as a reasonable starting point that can lead us to a balanced concept of the universe.

1.4 The Big Bang

Measurements have led physicists and cosmologists to the theory of the Big Bang. In the CERN Laboratory of France, physicists claimed that the universe started with complete nothingness. Then an infinite dense scalar field exploded. In the first few picoseconds, there was a very high temperature (like hell) and then strings were formed that excreted particles and forces including gravity.

The Italian Physicist Gabriele Veneziano added that, before the big explosion, there began an extremely high density space which caused the formation of a black hole. Inside that high density space, there were MAGNETIC WAVES which were trapped by the black hole. These waves interacted within forming strings, the base to gravity then the big bang which preceded the formation of particles and the universe.

John Ellis a Mathematician from Cape Town University claimed that we had clear proofs that the universe started at a very high speed from a fire ball. Ellis said that the universe went through a stage of extreme heat in the beginning of its formation (over a billion degrees centigrade). He suggested that from the very first second of the universe life, Protons, Neutrons and other Quarks existed. Within a few minutes of the universe start, the Quark particles united to form atomic nucleus. Addition to the dark matter, Professor Ellis presumed the existence of a DARK ENERGY.

On the other hand, the astrophysicist Andrei Linde, one of the Big Bang theorists, added there were extremely small scalar fields existed but did not explode. Rather, some other scalar fields grew

tremendously to the point that it exploded, and that we live on one of them (UNESCO, 2001).

According to the Big Bang theory, the universe expanded (and is still expanding) from its initial state due to the great explosion. Its lifetime is estimated at few billions of light years.

From my perspective, while it is possible that a great explosion has taken place, the human mind has overlooked the fact that the measurements that were used to reach the Big Bang are actually limited conceivable measurements that constitute only a part of the infinite universe. There is no proof whatsoever that there is a beginning point (a center) which can be used to measure the line of expansion in any straight direction. It is acceptable to explain the dynamics expansion of the universe as a balloon that is growing, but it should not be linked to the Big Bang. While some galaxies are getting apart, there may be others moving closer elsewhere. That may refer to the auto-motion of what Professor Ellis presumed as DARK ENERGY. Therefore, estimating the age of the universe on the observation that galaxies move apart seems doubtful.

1.5 Atoms as a Classical Approach to Explain Nature

Scientists of the 19th century established rules for the laboratory experiments. The great scientist, Joseph J. Thomson used the electrical discharge device to polarize a substance which he called ELECTRON, thus placing the first brick in building the atom concept. Thomson was followed by Ernest Rutherford, who conceptualized the idea of the nucleus.

Despite all the discoveries regarding the structure of the atom, the scientific domain had no answer to how an Electron separates free from an atom?

Professor Konrad B.Krauskopt; said in his book, "The Physical Universe" that electron, proton, and neutron are elementary particles, in a sense that they could not be broken down into anything else. However, scientists opposed that, saying in the same book that Chemical reaction in the battery keeps the electrons moving; that electron can be set free from atom by light and that in a television picture tube, a beam of electrons is directed at a fluorescent screen that glows where electrons strike it.

I suggest that the particle that can be set free from metals and moves in current or as beam, belongs to the rays group. If this particle is beta ray, we should leave the electrons happy in their orbits surrounding the nuclei of an atom.

While the scientist William Conrad Roentgen named the X rays, and the Scientist Albert A. Michelson precisely measured the light speed, Madam Marie Curie worked on the radiating element Uranium. The shirt of the scientist Henri Becquerel was burnt from a tiny Uranium particle which he carried from the laboratory of Madam Curie.

The contemporary science discoverers did not explain why Becquerel's shirt and Curie's fingers burnt from dealing with radiating elements while no thing got burnt from dealing with X rays. It is not reasonable to say that was because of radiation. Was that because of heat (kinetic energy) or because of the components that radiated free from radiating Uranium element?

It is reasonable that protons and electrons gain super speed when they got free radiating from Uranium element; and that was the cause of burning Curie's fingers and Becquerel's shirt. This opposes Albert Einstein saying that light is the most speed in nature? And; as the electron builds a stable main balance in the structure of the atom and hence, if what Thomson has polarized, should be just only rays?

Einstein introduced his theory about light as tiny bursts of energy in series of packets. He called them photons. I wonder why (burst)? This opposes Max Planck's theory of light as waves. Scientists approved both sayings. They approved that the wave theory of light and the quantum theory of light complement each other. ??

In any case, scientists have contributed with a great deal of information that better to be re-considered.

1.6 Quantum and the Collision Accelerators Explain the Atom

Mathematics is an interesting field where mathematicians enjoy dealing with algorithms.
Early in the 20th century, a new science of Mechanical Quantum was introduced by the scientist NIELS BOHR to express the rules of Physics. Many collision accelerators were built around the world. That led to ideas and correlated probabilities about the atom, the last of which were composed of 300 bodies that were mostly unstable.

The Quantum theory is controversial; it introduced the Positron which contrasts with the Electron, the contrasting Protons, and the mass-less material. Pheromones and Peons, as well as many others, were derived from quantum equations. Some scientists considered Quarks are just pillar items in the mathematical equations of atom. Others considered them real physical bodies that should be sought in the atom structure.

It is clear that smashing the particles of an atom in the collision accelerators led physicists and mathematicians to suggest complicated properties. They assigned names to a large number of infinitesimal bodies that were created in laboratories.

Sam Treiman, 2002, in his book "The Odd Quantum", mentioned that the possibility of the existence of bodies emitting from fields, led us to generalize the notion that electrons, protons, and other bodies are quantum for similar fields. These fields were not traditionally known for us, they were introduced as quantity fields to define required body quantities. Therefore, we would like to replace the following question: "What are the fundamental bodies of the universe and what are the working forces between them?". By this question: "What are the fundamental fields of the universe and how they do affect each other?"

That is the question; what is the nature of the components of an atom? Are they Material or non material? This long standing question will be discussed in part two of this paper.

1.7 Ether as a Medium to Transmit Waves

Michael Faraday defined the electric field and offered to the human kind his great discovery of the bobbin which turns around a magnet and generates electricity.

J. Clerk Maxwell introduced his mathematical equations about electric waves linked to magnetic waves which he called the Electromagnetic energy. He found that the speed of this energy waves is the same as the speed of light, and therefore Maxwell claimed that light could be a form of these electromagnetic waves.

Heinrich Hertz revised the wireless electromagnetic waves, and Guglielmo Marconi used these to invent the telegram.

There has been a need for a medium to transmit the electromagnetic waves, wireless waves, and light waves. The philosophers and scientists of the 19th century assumed the existence of a medium, a phenomenon which they lapelled ETHER. They wondered about the nature of this Ether. Is it a chaos or a void? Or is it a space-time continuum? They wondered if it is a material or a non-material. The Ether density was estimated to be ten fold million of lead density. They defined it as a medium which fills the void and occupies all the spaces between the material minutes. It transfers the energy waves like the universal rays and the wireless rays (Foad Sarrouf, 1932)

I should repeat here that electricity is a man invention, and what is generated from the mechanical motion of an electric generator disappears once the source stops. Hence Electromagnetism is better to be called Velomagnetism. That will be defined in the new look for nature in part two of this paper.

1.8 Dark Matter

In 1930s, the Swiss astrologist Fernz Zwicky claimed that in his observations on galaxies he noticed a huge variance in mass unknown in nature and does not emit any kind of radiation, which he called the LOST MASS.

In the 1980s, scientists observed an arch of light surrounding part of a group of galaxies; which confirmed what Einstein earlier mentioned about the polarization of light. They endorsed what Zwicky claimed about a huge mass of energy that does not transmit radiation. Scientists concluded that there exists an unrecognized form of a magnetic energy that caused this phenomenon. They wondered about the source of this huge mass of energy and named this source "Dark Matter". They said it was neither universal dust, nor gaseous balls, nor brown nor black dwarfs. Some scientists assumed the dark matter existed from strange particles that resulted from the Big Bang. Others assumed it a shadow matter (Wasfy, 1994).

Many assumptions explained the source of this huge mass of energy! But for this merely huge mass of energy, I was looking; for fifty years; to reconsider a new perception of the nature, that will be discussed in part two of this paper.

Part Two: A New Suggested Philosophical Concept of Nature

2.1 Two options to choose from:

When we think about the beginning of the universe, we will be faced by two options:

The commonly known beginning point of the universe, was a scalar field (material) limited in time and in space, within the theory of the Big Bang. This theory is agreed upon by the majority of scientists.

OR

An absolute energy, unlimited in time and in space which scientists explained its effects in the huge magnetic field which they thought comes from the Dark Matter.

The human mind always looks for an actor for every act, and for any energy he looks for a source for this energy. Let us free our minds from the constraints that the conventional intellectual dogma imposed on them. Let us assume that it is not a

magnetic field caused by a dark matter, but it is a magnetic energy with no cause. It is an infinite eternal magnet (a substance) surrounding us within which we live; just like the aquatic beings live in the oceans deep depths under the huge pressure of the water mass. This substance is just energy. It is a great magnetic compression which I would like to call Mother Magnet. This magnet would have no meaning whatever unless it evolves from within.

2.2 The Mother Magnet - Pre-Universe

In the beginning, I believe, there has been an eternal magnetic continuum, looks like a gel. It was light in its density and weak in its magnetic effect. It was and still moving in currents similar to the ocean currents. This magnetic phantasm had an auto-ability to magnetically increase in density. As this condensation increased with time, it led to what I can call the maximum mass of the Mother Magnet.

From the maximum mass point, the great magnetic compression moved to the next stage. That was the secretion and ejection of minutes (sols – particles) of extremely compressed magnets, like a gel that internally secrets sols.

We have to notice that, if Mother Magnet was completely compressed from the very beginning: it would have been balanced and would not have needed to maintain its balance by secretion. This would have meant that this universe would have never come to exist.

Movement is a sign of existence, and thus it seems more sensible to assume that the magnetic mass moves rather than it stay still.

- The shift in the earth magnetic direction which occurs every epoch: could be a proof that the Mother Magnet moves.
- The movement of the Galaxies apart from (or closer to each other, elsewhere) may be due to the Mother Magnet motion.

2.3 Beginning of the Universe

Now we have an extremely powerful magnetic mass - the Mother Magnet - which secreted particles (minutes - sols) that are extremely magnetic compressions. These magnets acquired the following characteristics which caused the manifested existence of the universe and gave a meaning to its existence.

- The magnets were secreted in different sizes.
- The secretion process from the Mother Magnet granted the magnets velocities. Like heated water, the molecules of water gain accelerated velocities, and when reach certain point they evaporate.
- Since the magnets had different sizes, they had different magnetic forces (fields), and accordingly different velocities.
- The equality between the velocity force of the the secreted magnets, and the Mother Magnet's self developed force capacity, led to nature balance.

2.4 Matter Formation

These particles that are extremely magnetic compressions and they gain different magnetic fields and different velocities, these are what will be known later as Electrons, Protons, Neutrons, Photons, and other different ray compressions.

The material formation of the universe came into being existed from the contradiction of the following two opposing forces and nothing else:

The Magnetism That Characterizes Force Of Attraction and The Velocity That Characterizes Force of Repulsion that we shall express as attraction charge and repulsion charge.

When the magnetic compressions gathered and reach a stable internal balance due to the balance between the different velocities and the different magnetic fields, they combined in a form that we consider Matter. This matter started with simple atoms, and then gathered in molecules, then to growing compounds of elements, and the way to organic formations. The formation of atom started with simple and gaseous elements: those were hydrogen and helium.

Opposite state takes also place when the velocities and the magnetic fields enter a phase of an imbalanced state, which results in immense reactions through either attraction or rejection, as seen in the Solar Reactions or Planets Explosions.

Magnetic compressions are divided into two groups:

- A group of basic components that make up the balanced and stabilized atom and molecule. This group has big mass, super speed when it is free, and strong magnetic field. It is the Protons, Neutrons, and Electrons and all other supposed heavy components. This group is what scientists expressed as, "elementary particles, in a sense that it cannot be broken down to any thing else".
- A group of magnetically compressed components with light mass, light magnetic field, and low speed When they are compared to the atom structure, they seem relatively look like fine dust (fluffy). Assuming a Proton is in the size of a football and an electron is in the size of a grain, the Photons and all other rays bodies will be relatively in the size of fine dust particles.
- The rays are light photons, X-rays, alpha rays, beta rays and others. These are also different in mass,

different in velocity, and different in their magnetic field.

- Gamma rays are the same. They are tiny particles, but cannot be sensed by ordinary tools.
- All rays are not of the main components of the atom. They are easily sucked in (absorbed), and easily released from the atoms and molecules. Scientists expressed that as gain or lose of energy.

2.5 Heat

- Considering what I explained before, it is clear that the universe would be based on a unity that is Magnetism. It exerted particles of severely compressed magnets. These particles carry two charges; Pulling charge (force) represented by their magnetic field and, repulsion charge (force) represented by their velocity, and nothing else. That is to say, there is no concept of heat or electricity in nature.
- I repeat that heat is a mere human biologic sensation. Man feels danger from accelerating velocity of particles around him. He calls this danger as heat.

- James Joule was the first to relate friction and velocity with heat. He accelerated the speed of water by turning paddle wheel in water. He supposed that water gained heat by friction. Did he measure the heat of water? Or did he measure the accelerating velocity of the mercury in the thermometer related to the accelerating velocity of water? He measured velocity concerned to velocity.

- Scientists claim that heat increases the velocity of the matter. On the contrary, heat is rather a man expression of matter velocity. The relations between the different velocities of the compound formations (the matter) are measured by man in the name of heat. All physical laws of heat are the same for velocity.

- It is very important to know that measuring heat as a velocity at 3000 degree Kelvin; the degree estimated by scientists for the universal explosions, we find that the photon speed is almost the speed of a turtle. In my view, there are two groups of velocities: The super high velocity of free Protons and Electrons, and the limited velocity of the groups of rays.

2.6 Electricity

2500 years ago in Greek, Thales experimented rubbing amber with fur, that caused sparks to occur and that attracted light bits of paper. As amber is called electron in Greece, Thales named the charge that occurred an electron charge instead of amber charge. Thales did not mean that this was electricity, because what he noticed was a magnetic charge. Scientist Thomson took the term electron and built upon it the story of electricity in the atom.

What was the rubbing effect? Rubbing confused the stable relations in the atoms of the material rubbed. This confusion affected either the attractive force in the atom which caused attracting the light objects, or the repulsion force that caused light photons to release.

This stimulation also appears in thunderstorms. The strong rubbing between clouds confuses the molecules of water. That causes photons of light to release confused molecules of water. The lightning is light photons which released confused molecules of water. Thunderbolt is a bundle of light similar to Laser beams.

Electricity is an example of how scientists were confined to measurements. They affirmed the existing of the electric current, in the time it is an electric energy that flows in waves. They invented extant tools to measure electricity. They made high efficient control over electricity that is now the most principle for our civilization. Nevertheless scientists failed to define the nature of electricity saying it is (not known till now).

Electricity is neither a part of the atom nor a part of the nature. Electricity is a man invention. The generator moves turning around a copper wire bobbin inside a block of magnet, by doing so, two external forces are affecting the bobbin. One of these external forces is velocity generated by turning around the bobbin, and the other external force which affects the copper wire is magnetism added by the magnet block. These two external forces excite (confuse) the stable relations between the magnetic fields and the velocities of the copper atoms and molecules components. This disturbance (caused by confusion) is transferred to the transmitting wires as waves that named Electricity. This confusion affects all the atoms and molecules components and not only the electron. Electrons never flew in current.

This proves my analysis that relations in the atom are between magnetism and velocity. This also shows the similarity relations between magnetism (gravity) and velocity in the universe.

2.7 Atomic Formation

In the book "The Odd Quantum", Sam Treiman suggested the possibility of the nonexistence at all of

material bodies but rather the existence of fields. He explained that it is possible that what we think as bodies could in fact be strong condensed pulling fields. He rejected later that concept, saying that it was a theoretical probability that bared no value.

I think that what Treiman refused is in fact closer to reality. That is, atom components are bodies of strong condensed pulling fields (and velocities). Thais is what I expressed as "sols of severely compressed magnets".

I believe that the atomic formation is governed by a group of factors:

- The unity of the universe is Magnetism that excreted bodies of magnetic compressions having velocities.
- As these excreted bodies take define place in the space, this means that they have masses; and since they are compressions, they must store energy
- There are only two energies produced by these magnetic compressions (substance): The pulling force of the magnetic field and the repulsion force presented in velocity.
- The universe is cold. Heat is a mere biological sensation.
- There is no electricity in the atom formation. Electricity is a human invention.
- Every particle in the atom has its own-two charges which indicate magnetic and velocity charges. This would be expressed as (velomagnet) instead of (electromagnet).
- The atom main structure is composed of basic magnetic compressions that have big masses. These are the Protons, Neutrons, Electrons, and others. Electron, proton, and neutron are elementary particles.
- It is of no mean to say that neutron has not any charge. I wonder if scientists supposed it is in the nuclei just to keep quantum equations balanced. They have to search after other quantum solution.
- Mathematicians would (likely) put equations to the balance points between the nuclei and the electrons surrounding it.
- Rays are a group of small weight magnetic compressions, which are intrusive components to the atom and not part of its basic composition. These components have limited magnetic fields, and limited velocities in comparison to the compressions that have heavy masses. That makes them easily trapped by the heavy components of atoms and molecules.
- Rays strongly reside in huge quantities, sticking inside the atomic, the molecular, and the biological compositions.
- There should be a revision of the Isotopes phenomenon in relation to the accumulation of the ray particles in different quantities in the atom.
- The rays save their velocities after being trapped by the heavy components of the atom. They acquire the velocities of the units they are stuck to. Once released from this trap for any reason, rays regain their own velocities.
- Rays release its trap in the atom due to external effects, which cause excitation (confusion) to the matter components. These reasons are of mechanical effect (electricity or rubbing), accelerating velocity (heat), chemical effect, solar reaction, or global explosion.
- Stars and planets explosions release huge amounts of rays which fill the universe and the excess is absorbed by the Mother Magnet.
- Accelerator Laboratories cause strong clashing which led to results and assumptions that are mostly unstable elements.

2.8 Light Photons

- Rays characteristics demand extensive research, specifically what concerns the light photons:
- The Photosynthesis process in plants is the presence of solar photons inside the botanical biological structures. Plants are the media which transmit the Solar Photons to all living beings. In the process of food assimilation, living beings gain their "heat" from the Photons released from molecules of carbohydrates and lipids. Oxygen is used for chemical assimilation process not for giving heat.
- Petrol is a huge reserve of Photons which accumulated from the plant Photosynthesis in the old times.
- The following soil studies equation is an indication of the phenomenon of light photons preservation in huge quantities in the soil:
- Humid soil + heat (photons accumulation) = dry soil + evaporating water

Hence

- Dry soil + added water = humid soil + heat (photon radiation)
- The question now is: where are the photons preserved?

2.9 Redefining Common Expressions

- Electrons and Protons are commonly defined as particles that carry respectively negative and positive electrical charge. They should rather be defined as: "Magnetic compressions that have Magnetic fields and velocities".
- For the universe, there is nothing either called positive neither negative magnetic field, nor called northern or southern magnetic field. It is just a universal magnetic flow without fixed northern or southern poles, which may be compared to water

currents in the oceans.

- In the range of the earth or even in the Milky Way galaxy, poles of the magnetic field of the earth changes its direction according to the galaxy changed situation in the universal magnetic flow.
- The tremendous atom explosions (nuclear fission) result only from splitting the components of the atom. What would be the result of expanding the compression of an electron? Is it possible? In CERN Laboratory, scientists seem they need two thousands time of the speed of light to explode two electrons or protons if crashed.
- The scientific notion that the matter does not vanish or renovate ought to be revised. This is because the Mother Magnet will always remain an infinite source of new compressions, while roaming condensations will disperse in it. Black holes could let the universe components return to the Mother Magnet in a way that keeps global balance.

It is this dispersion of light inside the Mother Magnet which explains why the stars could shine indefinitely without heating the universe up to the stars heat degree and didn't lighten the skies at night, as Dr Hawking assumes. (Remember that the light photons and other compressions are magnetic particles, and there is nothing weird about these melting in the surrounding they originated from.)

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17/03/2010

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A trial for Induction of saprolegniosis in *Mugel cephalus* with special reference to biological control

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Abstract: A method was developed to experimentally induce saprolegniasis in *Mugel cephalus* fish exposed to physical stress, experimental descaling and descaling with wounding in addition of sudden and graduall drop of water temperature. Fish which descaled and wounded were mostly affected with saprolegniasis than the other group . Thus combination of descaling with wounding and sudden drop of water temperature were more effective in inducing saprolegniasis in *Mugel cephalus* Present study also investigate biological treatment of *Mugel cephalus* natural infected with saprolegniasis using intestinal non pathogenic aeromonas strain for control saprolegniasis in vitro (plate) and in vivo (treatment tank) as a bath of aeromonas suspension 2 times for 3 days. [Journal of American Science 2010;6(6):203-209]. (ISSN: 1545-1003).

Keywords: Saprolgniasis; Mugel cephalus ; temperature ; biological treatment

1. Introduction

Saprolegniasis is a serious mycotic winter freshwater fish disease, often affects wild and cultured fishes. Its presence is correlated to stress factors such as abrasions, cutenous wounds sexual maturity, poor water quality, crowdness, malnutrition, handling and bacterial and /or parasitic infections (Noga,1993;Pickering 1994). Several authors have carried out experimental infections with various species of saprolegnia using some predisposing factors to increase susceptibility of fish to infection as coetaneous scarification (Howe and Stehly 1998), modification of water temperature (Howe *et al.*1998;Van West 2006), combination of scarification and drop of water temperature (Howe and Stehly1998). Saprolegniosis usually starts as a cotton wool-like white to dark grayish or brownish growth on the head region and dorsal fin then spread allover the body in the form of focal patches (Abdel-Aziz *et al.*, 2002;Bangyakhun *et al.*, 2003;Osman *et.al.*,2008).

Saprolegniosis causes high economic losses in intensive fish farming (Bly et al 1996;Delgado *et al.*,2003). Treatment of saprolegniosis using anti fungal agents *are vital for the maintenance of healthy fishes and their eggs (Bly et al.*,1997;Fornerisa *et al.*,2003). Although, the disadvantages of using chemical fungicides (malachite green and formalin) reprsented as low withdrawal affinity and high carcinogenic activity on human and fish, yet, they used by many veterinarians for the control of saprolegniasis. Biological control of saprolegniosis has received little attention in Egypt, therefore present study was aimed to induce experimental saprolegniosis and investigate potential biological agent for control of saprolegniosis in *Oreochromis niloticus* by using of intestinal non pathogenic aeromonas strain and to confirm the hypothesis that it could be used in treatment of saprolegniosis in field.

2. Material and Methods

2.1 Fish:

A. natural infected fish :

400 natural infected *Mugel cephalus* fingerlings fish with saprolegniasis were obtained from private fish farm from kafr El-sheikh Governorate.100 were used inisolation of spors and 300 were used in biological treatment

B. Experimental Fish :

Apparantly healthy alive sixty *Mugel cephalus* fish of (50±10g) body weight collected from private cement fish farm for experimental induction of saprolgniosis. Fish transported in plastic tanks aerated with battery air pumps.

subdivided into 6 groups of ten fish each in 6 glass aquaria of (50 x 50 x 100 cm^3) dimensions, supplied with the natural water of the farm, fishes were fed with commercial feed pellets daily 5% of body weight.

2.2.Induction of saprolegniosis : Fishes were acclimated at water temperature (22 ± 1^oC) using thermostatically adjusted heater for 7 days . the first three groups (1,2,3) were descaled only while the other groups (4,5,6) were descaled and wounded on the sides and peduncle of the tail using sharp scalple .

First and fourth groups were subjected to sharp drop of water temperture ($5^oC\pm1^oC$) within 5 h using ice pieces placed around the aquaria from outside to avoid direct contact of fish with ice .

2_{nd} and 5^{th} groups were subjected to graduall drop of water temperature to (5 ± 1^oC) within 10 days.

the 3^{rd} and 6^{th} groups subjected to ($22^oC \pm 1$) during the time of the experiment (control). Fish groups were observed for behavioral, clinical signs of infection and morbidity /mortality rate. spores of saprolegnia were placed in each tank with each group of fish (Willoughby1994;Hatai and Hoshiai 1994) . The spores according to (Bly et al.,1993;Howe and Stehly1998) counted to determine the mean number of spores / ml of holding water .

2.3. Identification of the involved saprolegnia: Wet mount preparations of fungal skin lesions were microscopically examined according to Hussein and Hatai (2001). materials from fungal skin lesions of naturally infected fish were cultured on Sabaroud's dextrose agar(SDA,Difco)

With adding chloramphenicol at the rate of 25mg/L, plates were incubated at 22^oC (temperature resembled to that of the experimental aquaria) and periodically examined and reisolation and cultivation of saprolegnia sp. on plates of Sabaroud's dextrose agar enriched with crushed hempseed for flourishing saprolegnian hyphae. .

Identification of recovered saprolegnia spp. Was carried out using cultural morphological and microscopic characteristics recorded by **(**Hatai 1990) .

2.4.Isolation of saprolagnia spores :

In test tubes containing sterilzed distilled water, one sterilize pierced hemp seeds in each tube with the cotton wool like hyphae and incubated for 24 at room temperature then the water centrifuged (3.000rpm/for 10 mint the spores setteled down discard supernatant) and the spores counted on the haemocytometer and used later in induction of saprolegniasis

2.5. Preparation of Non Pathogenic Aeromonas Strain (NPAS) :

Under complete aseptic condition intestinal swabs were taken from apparently healthy *Mugel cephalus* fish and cultured in tryptone soy broth (TSB $_{CM129Oxid}$) and incubated for 24 h at 27^oC subcultured of these samplas onto TSA for examination of their growth and colony character. Microscopical examination of such bacteria indicates gram negative bacteria, short bacilli. Confermatory biochemical identification of these bacteria was done. *Aeromonas* colonies were taken from the plates and subcultured into TSB for 24 h at 27^oC.(Mayer-Harting *et al.*,1972) .

2.6 .Experimental Checking the virulence of NPAS on healthy *O. niloticus*:

Alive healthy 15 *Mugel cephalus* fish were injected I/P with 0.2 ml of $1x10^7$cells/ml (NPAS)/fish for determination of the pathogensity of the bacterial strain to the fish and observed for 14 days for recording the clinical signs and the PM lesions were recorded.

2.7. Prepration of fungal material and inoculating technique (vitro) :

For testing (NPAS) in vitro, hyphal tips obtained from a culture of saprolegnia grown on sabroud's dextrose agar at 25 ^oC were inoculated onto the prepared (NPAS) plates. In the first half of the plate hyphal tips were inoculated onto the area containing (NPAS) while inoculation in the second half of the plate served as a control to observe the saprolegnian hyphae gowth.This

for confirmatory testing of the antagonistic activity of (NPAS) to saprolegnia in vitro (Fig 5).

2.8.Preparation of NPAS bath for controlling of saprolegnosis (vivo) :

20 natural infected fish with saprolegnosis subjected for treatment using 4 tanks provided with The prepared (NPAS) which grown in Tryptone Soy Broth (TSB) overnight and diluted in the tank water to give approximately 10^6-10^8 cells/mL in 10L of dechlorinated water (provided with air pumps) The suspension was added to the tanks , which contained natural infected fish with saprolegnosis, Fish were observed for behaviour and clinical signs of saprolegnosis. Tankwater was partialy replaced by 2.5L from each tank daily with addition of (NPAS) at conc.10^{3-} 10^4cell / mL (for presrvation the concentration. of NPAS in the Water of the treatment tank)

3.Results and Discussion

Saprolegniosis is an acute infection affecting *Mugel cephalus*, the natural infected fish revealed focal greyish white patches on the head regions as well as skin, fins and occasionally gills. In advanced stages of infection, saprolegniasis spread out to cover the whole body (Fig B). Identification of recovered saprolegnia spp. was carried out using cultural morphological and microscopic characteristics (Fig C)

In regard to experimental induction of saprolegniasis the results showed in (table 1) the 1st group (subjected to sudden drop of water temperature) 30% of the fish were infected with soprolegniasis (Fig A) the 2nd group (subjected to gradual drop of water temperature) 10% of the fish were infected on the other hand 4th group (subjected to sudden drop of water temperature) 70% of the fish were infected, the 5th group of fish (subjected to gradual drop of water temperature) 40% of fish infected with saprolegnia. The mortality rate in the 1st group was 10% while the 4th group was 60% on the other hand the mortality rate in the group was 0% while in the 5th group was 30% .

Regarding to checking of the virulence of NPAS on healthy *Mugel cephalus*, the investigated bacterial strain was I/P injected in apparently healthy fish and observed for 2 weeks, no clinical signs produced nor pathological signs was found on the fish.

In regard to antagonistic action of NPAS on saprolegneasis in (vitro). The top half of the plate (Fig D) which contains NPAS had not grown the hyphae of saprolegnia while the bottom half lacked NPAS and served as a control to monitor vegetative growth of saprolegnia after 72 h incubation at room temperature.

In regard to treatment of saprolegniasis with NPAS in (vivo) the study involved 15 *Mugel cephalus* fish naturally infected with saprolegniosis, fish was initially immersed in bath containing NPAS after which normal water of the bath changed (50%) daily. Hyphen masses were observed floating on the water column after overnight exposure to NPAS. The fish appeared to be recovered as judged by absence of saprolegnia growth although the wounds remain unhealed, three days after treatment however the fish began showing clinical signs of saprolegniasis in the inflamed wounds at this stage NPAS could not be isolated from the tank water after 3 days another treatment bath was applied using NPAS at the same concentration. Although the wound was free from saprolegnian growth, the wounds began to heal and the fish recovered from the infection.

Saprolegniasis is an acute infection affecting fishes it is world wide mycotic freshwater disease affects wild and cultured species the clinical signs of saprolegniasis on *M.cephalus* resembled the recorded sings and lesions which were recorded by (Shaheen 1986;Badran 1989;Marzouk *et al* 1990;Kamoun 2003;Van West *et al* 2003;Birch *et al* 2006;Osman et al., 2008).Regarding the experimental induction of saprolegniasis, from the results it is clear that the group of fish which descaled only, the rate of infection and the mortality rate were less than that of the other group which desalted and wounded, also water temperature play on important role in susceptibility to various

infections especially saprolegnia. Several authors induce saprolegniasis in fishes (Howe and Stehly 1998). in rainbow trout (Howe et al 1998). and catfish but the present study was aimed to investigate, the induction of saprolegniasis in *O.niloticus* using some physical predisposing factors (descaling, wounding, sudden and gradual drop of water temperature) saprolegniasis is disease promoted by physical stressors like, poor water quality, malnutrition, injuries during handling, and transportation also overcrowding, temperature shock, spawning or external parasitism (Yanong 2003;Gieseker et al 2006) .

Scales and skin act as physialbarrier against external pathogens especially mycotic agents. The stressors predisposed fishes to saprolegniasis in the present investigation were represented as descaling and/or wounding combined with gradual or sudden drop of water temperature (Howe and Stehly1998). who demonstrated that, handling, rough surfaces of tanks or cages, overcrowding, parasitic infestation, damage skin, fins and gills increasing infections susceptibility causing osmotic stress and mortality Several authors induce saprolegniasis in fishes (Howe and Stehly1998) in Rainbow trout, (Howe et al.,1998) in catfish and (Osman *et al*., 2008) in *Oreochromis niloticus* but the present study was aimed to investigate the induction of saprolegniasis in *Mugel cephalus* using some physical predisposing factors.

In the present study, the prevalence of saprolegniasis hence mortality rate in the group of fishes predisposed to saprolegniasis by (descaling) were lower than that of the other group (descaled and wounded) this indicate that the importance of the scales and skin as physical barrier this may be owed to disturbance of osmoregultion as infection of saprolegniasis generally occur in the epidermis and dermis and occasionally in the superficial musculature so the destruction of skin can disturbs the fish's osmoregulatory system and cause a lethal dilution of body fluids (Pickering and Willoughly 1988;Hatai and Hoshiai 1993;Willoughby 1994). Skin of a fish is the envelope for the body and the first line of defense against diseases it also affords protection from the environmental factors.

Regarding water temperature, fish are cold blooded animals primarily dependent upon water as a medium in which to live. Fish can tolerate wide range of water temperature they can distinguish a rise in temperature from a fall but the physiological mechanism for such discrimination is not known (Hatai and Hoshiai 1993;Grandes *et al*.,2001). Temperature stress, particularly cold temperatures can completely halt the activity of immune system eliminating this defense against invading disease organisms (Knights and Lasee 1996).

Furthermore, decreasing of water temperature especially the sudden drop compromise the immune system of the fish, increasing the susceptibility to pathogens especially mycotic agents. Temperature stress particularly rapid changes severely affect the ability of fishes to release antibodies, giving the invaders the chance to produce the disease to fish (Neish and Hughes 1980) .

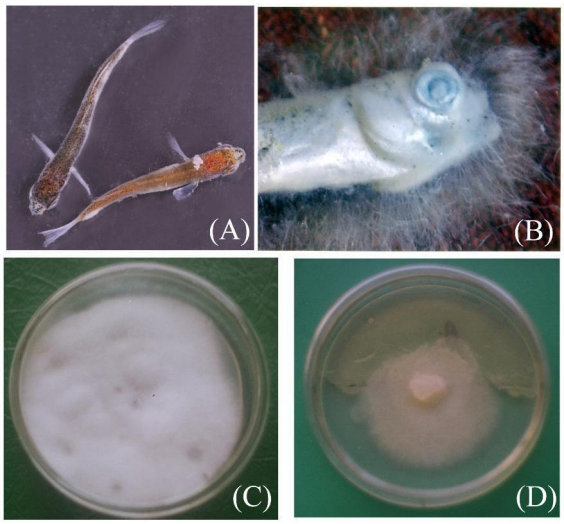
Regarding the antagonism of NPAS as biological control of saprolegniasis could play a significant role in the management of saprolegnia while the in vitro results demonstrated that NPAS was active antagonistic agent against saprolegnia. We can speculate that the presence of viable NPAS created conditions unfavorable for growth of saprolegnia after initial over night exposure to NPAS. It was clear that the growth of the saprolegnia has been retarded. Hyphal masses were also observed floating in the water after the first and second NPAS treatment baths. (3days each). The observations suggest that in these conditions, the pathogen detaches from the mucus and epidermal layer of the fish and released into the water. The ability of NPAS to inhibit saprolegnia appeared related to its ability to liquefy gelatin of such fungi. However the direct effect of gelatin hydrolase on saprolegnia growth. NPAS is considered as gelatinase positive (Holt et al.,1993). Parenthetically another candidate for the inhibitory activity for saprolegnia

is cellulase, an enzyme produced by NPAS (Hussein and Hatai 2001). The saprolegniacae have cellulose rather than chitin in their cell wall (Mullins 1973;Dick 1990). Using live bacteria for biological control may cause disease in fish. The investigated bacterial strain was non-pathogenic and safe for fish confirmed by I/p injection of this strain in apparently healthy fish and observed for 2 weeks. the result was no clinical signs produced nor pathological signs were found . There were reports discussed the in vitro inhibition of saprolegnia sp. by a gram negative rod, *Pseudo m onas fluorescens* by (Hatai and Willoughby 1988;B1y et al 1996;Delgado et al 2003). Reported that inhibition of saprolegnia by bacteria not related to the secretoray substance but rather the result of competition. (Hussein and Hatai 2001). showed in vitro antifungal activity by a number of Gram negative bacteria inclusive of the genus aeromonas, against pathogenic strains of saprolegnia parasitica. The discovery of existence of both in vitro and potential in vivo antifungal activity of NPAS increases its suitability as a probiotic and presents a possible approach to the management of saprolegniasis in M *cephalus.* In conclusion, *M.cephalus* were unable to withstand sharp or sudden drop of water temperature, accompanied with (physical stress) wounding or descaling. Such factors exclusively were the critical points for experimental induction of saprolegniosis in *Mugel cephalus* fish.

Table 1: showing the number of experimentally inducing saprolegniasis to *Mugel cephalus*

time Of exp												
	1st gp control *		dccatdMaphalu~rd 2nd gp gradual **		9P sudden drop ***		1st gp control *		2nd gp gradual **		3rd 9P sudden drop ***	
	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died
1st day	0	0	0	0	0	0	0	0	1	0	1	1
5th day	0	0	1	0	1	1	0	0	3	3	3	0
10 day	0	0	1	0	2	0	0	1	0	3	3	2
total	0	0	10	0	30	10	0	10	40	60	70	30

* ISt group+4th group= sudden drop of water temperature (22-5oc within5hours)
**2nd group+5th group= graduall drop of water temperature(22-5oc within 10 days)
*** 3rd group+6th group= room temperature (22+loc control)



A- *Mugel cephalus* **experimentally infected with saprolegniosis .**
B -*Mugel cephalus* fingerlings natural infected with saprolegniosis .
C- Saprolegnia growth on sabaroud's dextrose agar .
D-The upper half of plate with NPAS while lower have without NPAS showing growth of sapralegnia hyphae .

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3/15/2010

Effect of Annealing on DC Charge transport in Copper-Clay Cermets

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Abstract: The influence of the annealing schedule on direct current charge transport of Copper-Clay based cermets is reported here. The cermets are cylindrical rods of constant 3.0mm diameter and varying lengths ranging between 5.0 mm and 25 mm. The cermets were fabricated by employing a compaction method that uses a mould at a constant pressure of 6.9×10^{8} N/m^2 on various Cu-Clay compositions ranging between 70 and 95 vol.% Cu. The cermets were subjected to varying peak annealing temperatures ranging between 100 and 1000 ^{0}C and for annealing time t_f ranging from 30 minutes to 180 minutes before being furnace-cooled to room temperature. Results showed that the annealing schedule greatly affects the resistivity, size-effect and Temperature coefficient of Resistance (TCR). The electrical properties showed that sintering is complete irrespective of the annealing temperature between 300 and 1000 ^{o}C when the annealing time t_f exceeds 120 minutes. [Journal of American Science 2010;6(6):210-216]. (ISSN: 1545-1003).

Key words: Cermet; Annealing; Composite; Clay; Size Effect

1.0 Introduction:

A close control of the electrical properties of thick-film resistors is of primary importance in the development of passive network for hybrid microelectronics. It is well known that ohmic values and temperature coefficients of resistance (TCR) are correlated to the firing parameters (time and temperature) used in ink-processing (Harper, 1974, Hoffman and Popowich, 1971) so that a close control of the firing cycle is essential for reliable properties of thick film resistors.

Due to the very complex nature of clay materials, there had been few studies reported in characterizing qualitatively and quantitatively the electrical properties of cermets produced from a suitable metal powder and clay. Various investigators such as Wimmer et al (1974), Mizsei and Lantto (1991), Prudenziati et al (1991), Prudenziati and Acquab (1994), Morten et al (1994), Akomolafe and Oladipo (1996), Afronte et al (1997), Kuzy (1997) and Stein et al (1997) have sought to explain the factors contributing to the complex behaviour exhibited by thick film resistor systems. Some of these properties exhibited include temperature coefficient of resistance (TCR), size effects, ohmic and non-ohmic resistances, piezoelectric and pyroelectric effects, thermally induced variations, etc.

Below a certain volume fraction of metal in the composite defined as the critical threshold ϕ_c, the composite behaves as an insulator while above this composition it becomes an electrical conductor. The material so produced is known as cermet.

A steady-state model for the resistivity of composites is presented, based on the idea that the resistance offered by a composite is the resultant of large number of resistors combined in series and parallel. There are three separate contributions to the resulting resistance namely, constriction resistance at the contacts, tunnelling resistance at the contacts, and intrinsic filler resistance through each particle, with tunnelling resistance generally dominating the overall magnitude of the overall resistance (Ruschau et al, 1992).

This paper reports the effect of heat treatment on the electrical properties of copper-clay cermets. The clay used is obtained from Ilorin, Kwara State in Nigeria.

2.0 Experimental Procedure:

A mechanically operated high-pressure press capable of compressive force in excess of $5\times10^{3}\ N$ was fabricated for this experiment. The press is capable of producing one resistor at a time with the resistors having selected lengths between 5 mm to 25 mm but of a constant diameter of 3 mm.

The composite resistor is made up of conducting, insulating, and binding elements with attached terminals. The conducting element used for fabricating the cermet resistors is copper powder of about 99.95% purity. This was ground to a fine powder so as to remove lumps using a mortar and pestle. The insulating and binding elements were clay powder to which a few drops of sodium silicate was added as binder. The clay samples were obtained from Ilorin. They were carefully selected for a

homogeneous physical property and then, dried and processed to a fine powder of an average particle size of 250 μm. Graphite rods were used as the terminals of the Cu-clay composite resistors produced because the fired cermets were not solderable.

In the course of this research work, use of Cu-clay composite by volume ratio rather than by mass ratio was selected so that it will be easy to compare the result with similar experiments performed irrespective of the density of the conducting and insulating material used.

The copper and clay powders were mixed together in five different fixed ratios. The ratios were obtained in terms of volume such that clay occupied 5 %, 10 %, 15 %, 20 % and 25 % of the total Cu-clay powder mixture.

A constant pressure of $(6.9 \pm 0.05)\times 10^8$ N/m ^{2} was exerted to produce each resistor in the mould. Several resistors of varying lengths as mentioned above were produced for each cermet composition. These resistors were air-dried for several days. The resistors were put in a furnace regulated to 100 ^{o}C and fired at this temperature for two hours. It was observed that change of cermet resistance with annealing temperature becomes insignificant as the annealing time exceeds 120 minutes as observed in Fig. 5 when the annealing temperature exceeds 300 ^{o}C.

The resistors were then furnace–cooled to room temperature and the resistance and length measured. The average of several measurements was then recorded for the resistance of each selected lengths. This procedure was repeated for all the resistors, which were fired in steps of 100 ^{o}C up to a maximum of 1000 ^{o}C.

3.0 Results and Discussion:

Data on sheet resistivity and TCR changes due to variation in firing thermal cycle of resistors have been reported (Ayodele and Akomolafe,2005) but poor information in available on the phenomenon responsible for these changes in electrical properties.

In compacted composites, it is well established that, for a given composition, the electrical properties strongly depend on grain size, morphology and applied pressure (Thornmerel et al, 2002). Three major models have been developed previously to make predictions on the electrical behaviour of ideal composites.

i.) The effective medium approximation,
ii.) Percolation theory and
iii.) The micro-structural approach.

The properties of composite system are understood in terms of percolation phenomena, when a sufficient amount of conductive filler is loaded into an insulator matrix, the composite transforms from an insulator into a conductor as a result of continuous linkages of filler particles. As the volume fraction of filler increases, the probability of continuity increases until the critical volume fraction beyond which the electrical conduction is high and becomes, comparable to the conducting filler material.

Effective-media theory attempts to quantify the resistance of these systems, based on the idea that contribution of each phase to the conductivity depend not only on the relative amount of the phase present but also on the degree of conductivity offered by the phase. A number of effective-media equations have been derived to model the shape of this curve (Ruschau et al, 1992, Kirkpatric, 1973 and McLachlan et al, 1990). While these equations can successfully mimic this shape, they are not useful in describing the magnitude of the electrical resistivity of the composite. In this work, attempts were made to study variation of resistance with annealing temperatures, variation of resistance with annealing time and variation of TCR with annealing temperature.

Variation of Resistance with annealing temperature

The effect of the variation of resistance with annealing temperature T_f was studied. It was observed that the variation of resistance with firing temperature generally exhibits a trough-like form as shown in Fig. 1 which shows the variation of resistance with annealing temperatures for a resistor of length l =10 mm.

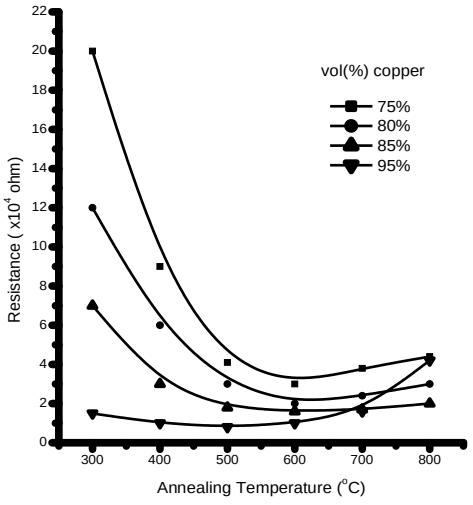

Fig. 1 Variation of resistance with annealing temperature (l = 10 mm)

Figure 2 shows the relationship between the cermets resistance, clay particle size and the annealing temperature. It can be seen that the curve produced is also trough-like in form. Clay of higher particle sizes generally produce resistors of higher resistivities than those made from the smaller particle sizes.

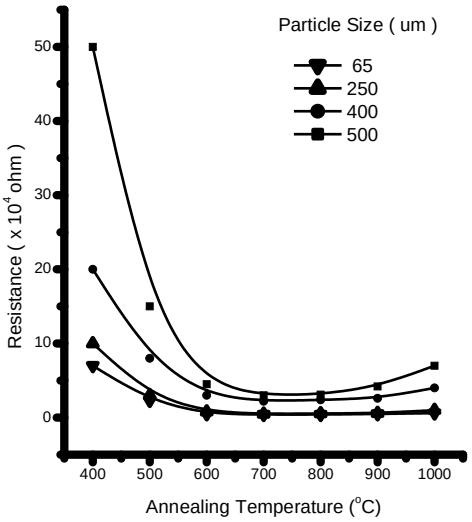

Fig. 2 Variation of resistance with annealing temperature (l = 25 mm)

For all the resistance studied, the resistance of the composite resistor containing 95 %(vol.) Cu was found to be lowest at all annealing temperatures for all the resistor length considered. It is expected that the particle size of the insulator phase would affect significantly the compactness of the cermets material, transmission of pressure, vacancies, grain boundaries of the cermet material (Akomolafe and Oladipo, 1996). This should in turn affect the final electrical property of the cermets material. The resistance/resistivity of the copper-clay cermets resistor were observed to increase non-linearly with the clay particle size for all the annealing temperatures considered; this variation is shown in Fig 3, which show the variation of resistance with average clay particle size at various firing temperatures.

Fig. 4 shows the variation of cermets resistance R with clay content for a resistor length of 10 mm and for various annealing temperatures. The graph shows an exponential increase in resistance with increasing clay powder concentrations at all firing temperatures.

Ligabue (1984) and Ruffi (1984) observed that factors which affect the final resistance of a cermets resistor depend on the nature of the glassy matrix and the substrate, reactions of the conductive grains with the matrix, sintering and ripening of the grains and glass, and partial crystallization of glass.

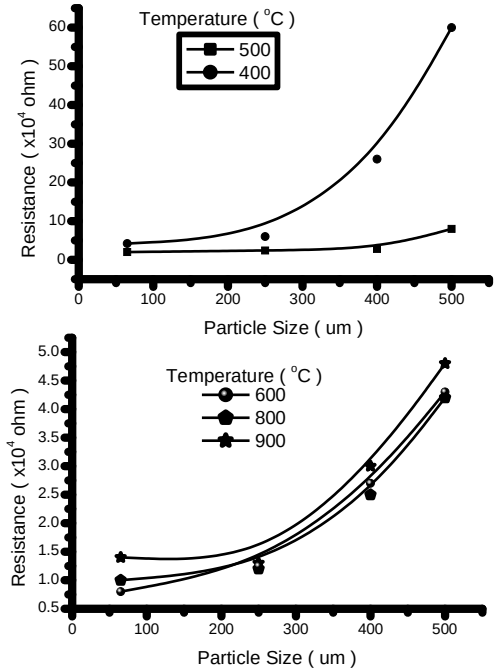

Fig. 3 Variation of resistance with clay particle size (l = 15 mm)

The variation of resistance R with peak annealing temperature T_f exhibits a trough-like form, which was observed to become more pronounced with increasing clay concentration. The comparatively high resistivity occurring for T_f < 400 ^{o}C could be attributed to the incomplete sintering of the copper-clay composite mixture as a result of the low T_f.

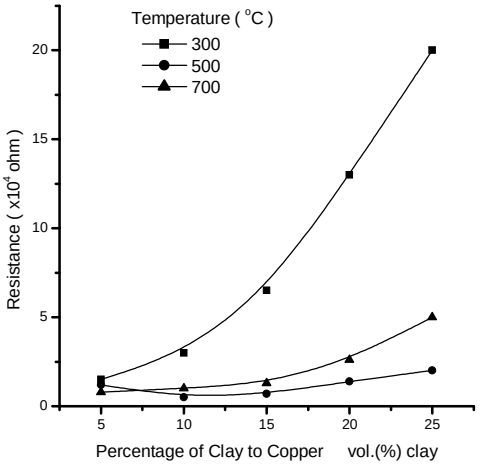

Fig. 4 Variation of resistance with clay content (l = 10 mm)

However, the high resistivity occurring for cermets fired at $T_f > 700\ ^oC$ is most likely the result of furnace atmosphere oxidation effect. Observation of the resistors under a low power optical microscope shows that the outer shell of the cermets contains end materials structurally different from those of the inner core. This may be responsible for the variation of resistivity of the resistors with length.

According to Ziman (1960) using the free election theory, the resistivity may be obtained in terms of the carrier mean free path such that

$$\rho = \frac{(3/8)^3 h}{q^2 n^{2/3} \lambda} \qquad 1.$$

where, h is the Plancks constant, λ is the carrier mean path and q is the charge of the electron. It is expected that narrowing the conduction path will reduce λ and thus increase the resistivity ρ. This explains the increase in resistance of the composite resistors with increasing annealing temperatures at annealing temperatures exceeding 700 ^{o}C.

Variation of resistance with annealing time

The average resistivities of the cermets were found to decrease for all cermet concentration with annealing time. This decrease in resistivity approaches a constant level as the annealing time t_f becomes greater than 120 minutes. Observation shows that the rate of sintering increased with increase in the annealing temperature as shown in Fig. 5 which shows that complete sintering takes place at all annealing temperatures considered between 300 and 1000 ^{0}C for $t_f \geq 120$ min.

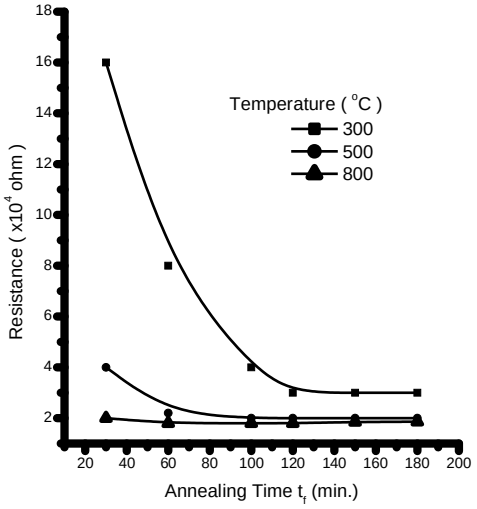

Fig. 5 Variation of Resistance with Annealing time (l = 10 mm., and 80% (vol.) Cu)

The change in resistivity obviously shows that re-arrangement of the internal structure of the cermets had taken place. This re-arrangement may have caused conductive grains to fuse in the cermet probably due to the composite being subjected to elevated temperatures. Other factors which may be responsible for the lower resistivity with increasing annealing temperature is the reduction in insulative phase by decomposition of carbonates and evaporation of the insulative phase. It is also possible that diffusion of copper ions into the insulative phase takes place hence, lowering the overall resistivity of the cermet.

Variation of Size-Effect with annealing temperatures

The variation of resistor length on the normalized resistance R_s is known as size effect. In this case, the normalized resistance as used by Akomolafe and Oladipo (1996) is

$$R_s = \frac{R_n}{R_{n\,(25\ mm)}} \qquad 2.$$

where,

R_n = Resistance of a given length of a resistor and

$R_{n(25\ mm)}$ = Resistance of the maximum length of Cermet resistor.

The effect of resistor length on the normalized sheet resistance is shown in fig. 6 for three copper concentrations of 95 %, 85 % and 75 %(vol.) . It was observed that direct size effect (namely, a lower effective sheet resistance for short resistors) is exhibited for composite resistors irrespective of the annealing temperature.

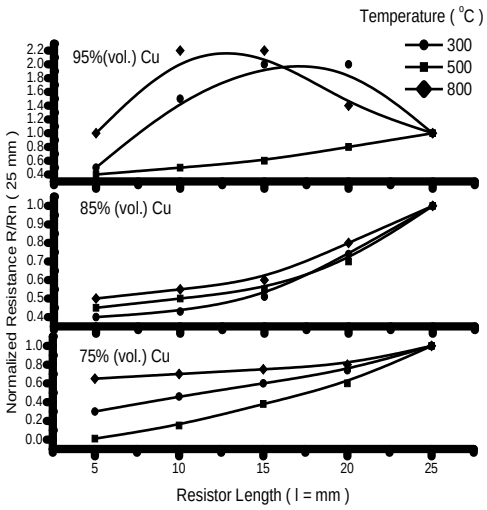

Fig. 6 Variation of Cermets Resistance with Resistor Length

This is apparent in Fig. 1 at copper concentrations of 85% and 75% (vol.) fired at T_f = 300 ^oC, 500 ^oC and 700 ^oC.

Ususally, a material exhibiting size effect will present either a direct size effect or an inverse size effect but certainly not both occurring in the same material. However, as a result of annealing, an anomalous size effect was observed for the 95%(vol.) copper-clay cermets annealed at T_f = 300 ^oC and 700 ^oC in that we showed that the same cermet resistors exhibited both direct and inverse size effects depending on its length. It is known that size effects in resistive phase are due to a change in shape when the length varies and/or a change in resistivity along the layer associated with a compositional change.

When the effective sheet resistance for short resistors is higher than that for longer resistors, a material is said to exhibit inverse effect: otherwise, it exhibits direct effect. Prudenziati et al (1991), Morten et al (1991), Hrovat et al (1986) and Akomolafe (1995) have reported these effects in various works.

The variation of cermet resistivity with change in resistor length observed in our cermets could be greatly influenced by a change of resistivity along the path of conduction rather than a change in thickness of the cermets along its length due to the in-homogeneity of the cermet composition along its length. According to Akomolafe and Oladipo(1995), the in-homogeneity in the composition of the cermets along its length and chemical reaction of terminations has been found to affect the size effect of cermets. It was concluded that the graphite terminal could not have caused any significant change in the size effect observed in the cermets because the termination were not attached to the cermet during annealing but only during the process of measurement such that significant diffusion or chemical reaction could not have taken place as to affect the overall size effect.

It was observed that all cermets of copper concentration less than 95 % (vol.) irrespective of their length exhibited a direct effect i.e. shorter resistors have lower resistance. The direct size effect observed may be due to a "Pinch-off-effect" analogous to that which occurs in the conduction channel in FETs. Thus as the length of the cermets increases significantly, chemical reaction which result in compounds of higher resistivities along the axial depth into the resistors increases thus producing a very narrow conduction channel. This causes the longer resistors to exhibit higher resistance i.e. direct size effect is exhibited.

The anomalous size effect observed is due to the transition of the cermet resistor from one exhibiting a direct size effect to one exhibiting an inverse size effect. It can be inferred from the rate of change of cermets resistance with annealing temperature that rapid chemical reactions take place within the resistors at two ranges of temperatures (Fig. 1 and Fig. 2.) i.e. between 300 ^oC and 400 ^oC and when annealing temperature becomes greater than 700 ^oC i.e. at the two high slopes of the trough. The direct size effect dominates throughout in the cermet except when the copper concentration exceeds 95% when the inverse size effect dominates the direct size effect. The inverse effect observed may be as a result of the diffusion of furnace gases into the cermets whose rate reduced because of the hard shell formed round the core of the cermets, thus diffusion of gases through the terminal surface alone becomes significant. Chemical reaction from these terminal surfaces causes the resistance at the ends of the cermets to increase significantly. This increase in resistance is not a function of the cermets length and seems to maintain a fairly constant value. Hence an inverse size effect results i.e. shorter resistance having higher resistance takes effect.

Variation of TCR with annealing temperature

Plots of the variation of cermets resistance with cermets temperatures ranging between 20 ^oC and 100 ^oC were obtained for cermets resistor annealed at T_f=400, 600, 800 and 1000 ^oC. The temperature coefficient of resistance was observed to be negative for all the copper-clay cermet studied. Results of the cermets TCR measured at 30 ^oC is presented in Fig. 7. The result showed that increasing annealing temperatures reduced the magnitude of the TCR as presented in Fig. 7.

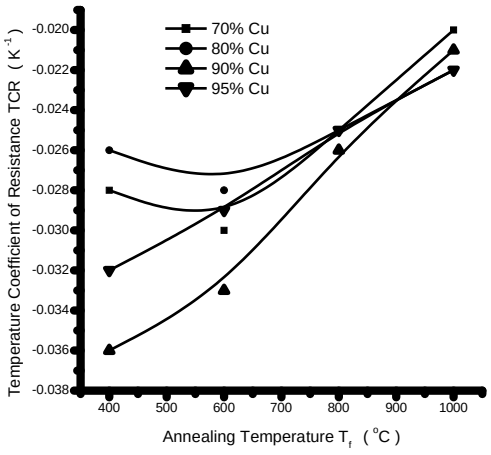

Fig. 7 Variation of TCR with annealing Temperature measured at 28 ^oC

The effects of the peak annealing temperature on the TCR and the rate of change of TCR of Cu-clay cermets are observed in figs. 7 and 8 respectively. It is expected that the TCR of a cermets will increase and tend toward positive values as the metallic content increase (since metals posses positive TCR).

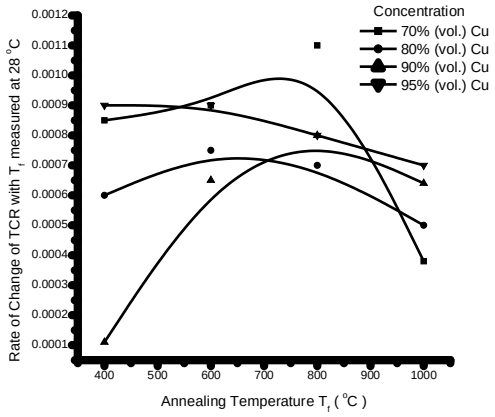

Fig. 8 Variation $\left.\frac{d(TCR)}{dT_f}\right|_{T=28\,^{\circ}C}$ of at 28 °C with Annealing Temperature T_f

However, fig. 7, which presents the result of the variation of TCR measured at 28 °C with copper concentration, shows that the TCR still remains negative with increase in the metallic content. The disagreement between the expected result and that obtained here show that even when the concentration of copper is 95 %, the cermet is still far from what can be modelled by the free election theory. It has been shown by Ruffi (1984) and Akomolafe (1995) that the mechanism of conduction in cermets in mainly due to tunnelling of electrons.

The basic assumption is that the cermets composition is made up of conducting grains embedded in a glassy matrix and these conductive grains can be pictured as a multi-valued resistor network through which charge carriers move by percolative tunnelling. This model assumes that the resistance of the resistor network is electrically equivalent to a thick film resistor where each resistor is insulated by a very thin dielectric layer through which electrons flow by tunnelling and by thermally activated ionic diffusion.

According to this model above, higher cermets temperatures will produce lower resistivity in the cermets because electrons will acquire higher energies, which will increase the probability of tunnelling through the insulating barrier.

The effect of the annealing temperature on the rate of change of TCR (Fig. 8) show that the magnitude of the rate of change of TCR decreased with increase in annealing temperature except for cermets with 95%(vol.) copper content. The variation of the derivative of TCR

$$\gamma = \left.\frac{d(TCR)}{dT_f}\right|_{T=28\ ^{\circ}C} \qquad 3.$$

with the annealing temperature (eqn. 3) expresses how sensitive the cermets is when used at room temperature as a thermistor. The result is presented as a function of the annealing temperature. It shows that maximum sensitivity of cermets resistance to temperature (at room temperature) is obtained for cermets annealed between 600 and 800 °C for 70%(vol.) copper cermets.

The explanations for this behaviour in terms of the annealing schedule is not yet clear and effort is still on to obtain the relationship between the annealing temperature and the TCR of copper-clay cermets.

4.0 Conclusion:

Copper-clay based cermets of varying copper content and length have been fabricated using a constant pressure of about 6.897×10^8 $Nm^{-2.}$ The fabricated resistors were all of average diameter 3mm and were made of various length ranging between 5 mm and 25 mm in steps of 5 mm.

Experimental results show that annealing greatly affects the overall electrical properties such as the resistivity, TCR and size effect phenomenon. The rate of change of resistance with annealing time become insignificant when the annealing time T_f exceeds 120 min., the overall resistivity of the cermets was found to exhibit a trough-like form; High resistivity occurring at the low annealing temperature and the high end of the peak annealing temperature T_f.

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Genetic Analysis between and within Three Egyptian Water Buffalo Populations Using RAPD-PCR

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Abstract: The water buffalo represents an important part of animal production in Egypt It is economically a very important farm animal, genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and milk. Genetic similarity and polymorphisms among the three Egyptian water buffalo populations (El-Delta, Upper and Lower Egypt) were studied using random amplified polymorphic DNA (RAPD) technique. Out of fifteen primers screened using DNA samples of the three populations, thirteen primers generated reproducible and distinct to amplify DNA fragments in these three populations. RAPD patterns with a level of polymorphism were detected among populations. The results showed that a total of 126 loci were amplified and 106 polymorphic bands (84.13%) were produced. The genetic diversity had the highest value (0.2654) in El-Delta and the lowest value (0.2590) in Upper Egypt. This result confirms the closer between the three Egyptian population buffaloes. The dendrogram of genetic relationship based on overall RAPD primers confirmed the movement of Egyptian buffaloes between El-Delta and Upper, Lower Egypt. The results confirms that the Egyptian buffaloes are belongs to one breed. [Journal of American Science 2010;6(6):217-226]. (ISSN: 1545-1003).

Keywords: water buffalo, RAPD-PCR, genetic diversity, Egypt.

1. Introduction

The water buffalo represents an important part of animal production in Egypt. The estimated herd number exceeds 3.6 million heads (FAO, 2002). It is economically a very important farm animal and genetic improvement of these animals is of economic importance, especially in reproductive performance and quantity of meat and milk as well as diseases and parasite resistance (El-Nahas et al., 1998).

Based on the phenotypic characters, Egyptian water buffaloes were classified to three populations: Beheiry, Minoufy and Saidy (El-Barbary and Abdel-Latif, 1985). To identify the genetic relationship among these three populations, random amplified polymorphic DNA (RAPD) technique was used in this study.

Genetic diversity may be measured through genetic markers. These have been used to estimating the genetic diversity of species, breeds and populations, as well as decisions related to selection of breeds/populations to be conserved (Zhang et al., 2006). However, breeders tend to concentrate on specific genotypes for determination of genetic diversity which combined traits of interest and may be used as progenitors in several breeding programs in order to introduce agronomical important traits (Rahman et al., 2006).

Random amplified polymorphic DNA (RAPD) developed by Welsh and McCelland (1990) and Williams et al., (1990), the methodology proved to be a powerful tool in different genetic analyses. This approach detects DNA polymorphisms based on amplification using a single primer of arbitrary nucleotide sequence of genomic DNA fragments. RAPD markers are attractive because they are specific and quick, nanograms of DNA are required, automation is feasible, and there is no requirement for previous DNA sequence information Williams *et al., (*1990), modest coast and ability to detect relatively small amounts of genetic variation (Ragot and Hoisington, 1993).

RAPD markers have been used successfully in estimating genetic relatedness among various breeds and populations of sheep, cattle, goat and chicken (Mahfouz et al., 2008; Hassen et al., 2007; Rahman et al., 2006; Okumus and Kaya, 2005) respectively.

The RAPD methodology proved to be efficient method in assessing genetic diversity, it is used extensively in genetic diversity studies for cattle in many countries including Uruguay (Rincón *et al.,* 2000), South Korea (Yeo et al., 2000), Turkey (GÜNEREN et al., 2010), Ethiopia (Hassen et al., 2007).

The objective of this study was to use the RAPD technique to evaluate genetic diversity and relatedness within and between three buffalo populations: Information from this work provides basic genetic knowledge that is critical for conservation and breeding programs.

2. Material and Methods

Animals: Fifteen water buffaloes representing three Egyptian populations (El-Delta and Upper, Lower Egypt) were carefully selected from three different regions in Egypt. For DNA extraction, five animals from each flock (north, middle and South) were chosen from Upper Egypt, El-Delta and Lower Egypt, respectively. The samples were taken from animals not related to each other.

DNA Extraction: For DNA extraction, blood samples from El-Delta and Upper, Lower Egypt (Beheiry and Saidy) buffaloes were collected on EDTA as a coagulant matter, blood samples were stored at -20°C until DNA extraction.. DNA extraction was carried out by method of Sharma *et al.,* (2000) as fellows: to an aliquot of 100 µl blood (after thawing), 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform- isoamylalcohol (25:24:1) then chloroform-isoamylalcohol (24:1). DNA was precipitated by adding two equal volumes of pre-chilled ethanol in the presence of a high concentration of salts (10% from 3 M sodium acetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of TE buffer according to Pfeiffer et al., (2004).

PCR and Gel Electrophoresis: RAPD-PCR was carried out with the pooled and the individual genomic DNA samples. A total of 15 random decamer primers of arbitrary sequences with 60-70 GC% content were used as listed in table (1). The amplification conditions and PCR mixture were set according to Williams et al., (1990) and Kuske et al., (1998) , PCR was performed in reaction volume of 25 µl using 25 ng of genomic DNA from pooled samples, 25 pmol of each primer, 10X PCR buffer, 0.75 unit from Taq DNA polymerase buffer including MgCl , 25 pmol dNTPs and 0.8 U *Taq* DNA polymerase (Finnzyme). Thermal cycling (ABI 9700) was carried out by initial denaturation at 94°C for 2-5 min, followed by 34-45 cycles each at 94°C for 30-60s, annealing temperature at 28-54 for 30-60s, polymerization temperature at 72°C for 30-60s and final extension at 72°C for 10 min. The samples were cooled at 4°C. The amplified DNA fragments were separated on 3% agarose gel, stained with ethidium bromide, visualized on a UV transilluminator and photographed by Gel Documentation system Gel Pro (version 3.1 for window 3).

Scoring and Data Analysis of RAPDs: The sizes of amplified bands were determined using Gel-Pro analyzer and RAPD banding patterns were scored for the presence (1) and the absence (0) of bands for each sample. The scores were then pooled for constructing a single data matrix. The statistical analysis of the data was performed using the free software "Popgene version 1.31" computer program (Yeh et al., 1999) including the calculation of allele frequencies according to Nei (1987).

The genetic distances are designed to express the genetic differences between two populations as a single number. If there is no difference, the distances could be set to zero, whereas if the populations have no allele in common at any locus, the distance may be set equal to maximum value, i.e., the genetic identity is expressed as the genetic similarity between individuals of same or different populations. The genetic distances (D) and genetic identity (I) were calculated by POPGENE software (Yeh et al., 1999) using standard genetic distance and identity equations (Nei, 1972)

This program estimated the number and percentage of polymorphic loci and the genetic diversity according to Nei (1973). UPGMA dendrogram showed the genetic distance among breeds and was constructed according to Nei (1972).

3. Results and Discussion

RAPD amplification of polymorphic DNA-PCR is a powerful molecular genetic technique for detection of genetic variability in the different breeds/populations of the livestock (Cushwa et al., 1996). The use of RAPD markers is not limited to genetic diversity studies but it is also extended to other studies such as estimation of breeding coefficient in cattle (Bhattacharya et al., 2003).

Out of 15 primers screened using DNA samples of 3 buffalo populations, only 13 primers generated reproducible and distinct RAPD profile. All the primers detected polymorphic bands among three populations. In this study, from the thirteen primers produced 126 loci were amplified, 106 of them were polymorphic (84.13 %). The number of bands varied from 4 to 15 with molecular size of the amplification was in the range of 64 to 1675 bp length. The maximum numbers of bands were observed in OPB12 primer (15), while minimum number of bands was recorded with OPB09 primer (4) in all populations (Table 2). It has been suggested that the sequence of OPB12 primer may occur frequently in all populations and scored maximum number of bands, whereas primer OP09 was found less polymorphic within and between the populations. Sharma *et al.,* (2001) found that RAPD technique detects sufficient polymorphism within and between

populations. In the present study, an individual primer failed to produce any specific population or population specific marker in any of the three populations studied. Similarly, Kumar et al., (2004; 2008) also did not find any breed specific RAPD marker in Indian breeds of sheep.

Five of the 106 polymorphic bands had significantly different frequency distributions across breeds ($P \leq 0.05$, Table 3). These bands could be investigated further by first cloning and conversion to sequence characterized regions (Gu et al., 1995).

The percentage of the polymorphic loci represented in Table (4) which ranged from 59.52% in upper buffalo population to 63.49 % in lower Egypt buffalo population. According to Nei (1987), similar results were obtained; Abdel-Rahman and Elsayed (2007) found a high genetic similarity among three Egyptian water buffalo populations using RAPD-PCR technique. Also, the same results found in another Egyptian Native breeds, The use of RAPDS methodology in Egypt for assessing genetic diversity is not limited to buffalo but it is also extended to other farm animals species, Ali (2003) showed closer proximity in Egyptian Native sheep breeds Barki to Rahmani and Baladi (95.7 and 91.3%), respectively that was detected by random amplified polymorphic DNA markers.

The present and earlier studies (Wei, et al., 1994; Bailey and Lear, 1994; Smith et al., 1996 and Egito et al., 2007) indicate that RAPD analysis requires screening of a large number of random primers in order to detect polymorphism, because the amplification from the arbitrary primers depends on the presence or absence of the corresponding primer binding sites in the genome. Hence, comparatively large numbers of random primers are required to detect sufficient polymorphism to be utilized for genetic analysis.

The characteristics of amplification profiles generated by primer OPB14, OPC15, OPD05, OPD01 in three populations of buffalo are presented in Figs. (1, 2, 3 and 4). Table (3) showed that chi-square test of each studied primer

The genetic identity (I) and genetic distance (D) between the three populations were calculated using Nei (1972) equations through POPGENE software (table 5) in the present study the genetic identity ranged from 0.0713 to 0.1461 and 0.0849 to 0.9186, respectively (Table 5). These two measures of genetic relatedness revealed similar trend of relationship among three populations of buffaloes. Similar ranges of genetic identity and genetic distance have also been obtained by other workers (Atta ,et al., 2009;. Abdel-Rahman and **Elsayed** 2007).

The UPGMA dendrogram, based on genetic distance, was constructed to show phylogenetic relationships among the buffalo population Figure (5). The lower Egypt population appeared to be most distant from the other population whereas the upper Egypt and El-Delta populations were closely related with the highest genetic similarity as shown by an UPGMA dendrogram based on Nei's standard genetic distance.

The RAPD technique has also been used for constructing phylogenetic relationships in other farm animals such as; cattle (Kemp and Teale, 1994; Gwakisa et al., 1994; Glazko et al., 1999; Horng and Huang, 2000; Rincon et al., 2000; Zubets et al., 2001 and Jarina Joshi et al., 2007) , goat (Ahmed, 2004; Chen Xiang et al., 2004 and Li et al., 2006) , horse (Baily and Lear, 1994) and sheep (Mel'nikova et al., 1995; Cushwa et al., 1996; Stephen et al., 2000; Gong et al., 2002; Ali, 2003; Paiva et al., 2005 and Mahfouz et al., 2008) .

Conclusion

In conclusion, this work has revealed that genetic diversity exists among the three Egyptian water buffalo populations studied. With further experimentations, the RAPD profile generated for each flock can be effectively used as a supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution (Allard et al., 1992; Dinesh et al., 1993; Rao, 1996).

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Table (1): Primer codes, sequences and CG content used to variation in Egyptian buffalo populations

Primers	Sequence 5`- 3`	CG%
A12	TCGGCGATAG	60
B03	CAT CCC CCT G	70
B07	GGT GAC GCA G	70
B09	TGG GGG ACT C	70
B10	CTG CTG GGA C	70
B11	GTAGACCCGT	60
B12	CCT TGA CGC A	60
B14	TCC GCT CTG G	70
B18	CCA CAG CAG T	60
B20	GGA CCC TTA C	60
C02	GTG AGG CGT C	70
C05	GAT GAC CGCC	70
C15	GACGGATCAG	60
D01	ACCGCGAAGG	70
D05	TGA GCG GAC A	60

Table (2): Total number of bands, polymorphic bands, % of polymorphic loci and their size ranges from the random primers.

Primer	Total No of bands	No. of polymorphic bands	% of polymorphic loci	Size range (in bp)	
				min	max
OPA12	13	12	92.31%	64	1389
OPB03	12	10	83.33%	91	1493
OPB07	6	5	83.33%	458	1454
OPB09	4	4	100%	288	1444
OPB10	10	9	90%	152	1675
OPB11	10	7	70%	72	1349
OPB12	15	10	66.67%	76	1416
OPB14	12	9	75%	190	1646
OPB18	9	7	77.78%	203	1479
OPB20	8	8	100%	139	1407
OPC15	6	5	77.78%	310	1371
OPD01	10	10	100%	134	1358
OPD05	11	10	90.91%	118	1129

Table (3): Chi square significance of differences revealed by random primers in Egyptian buffalo population

Primer	OPA12	OPB03	OPB07	OPB09	OPB10	OPB11	OPB12	OPB14	OPB18	OPB20	OPC15	OPD01	OPD05
Fragment (bp)	888 752 734 354 303 299 271 245 138 127 112 85 64	589 447 390 304 280 271 242 206 175 149 101 91	682 458 145 143 134 133	303 288 144 133	999 780 520 339 290 286 271 167 152 142	517 400 361 307 303 231 134 104 85 72	1358 1114 641 280 249 231 211 191 167 144 134 133 101 85 76	1646 1078 1015 789 690 603 540 373 334 278 252 190	1479 1397 1387 585 555 453 233 218 203	1407 1397 939 420 327 233 216 139	1371 1106 903 810 511 310	1358 1078 771 564 528 432 354 331 214 134	1129 919 631 432 303 271 247 240 234 224 118
P-value	0.34 1.00 0.56 0.03* 0.74 0.00* * 0.56 0.01* * 0.03* 0.25 0.74 0.76 0.25	0.34 0.28 1.00 0.12 0.12 0.25 0.09 0.28 0.28 0.34 1.00 0.25	1.00 0.34 0.34 0.56 0.76 0.34	0.34 0.76 0.28 0.12	0.76 1.00 0.25 0.71 0.76 0.25 0.56 0.71 0.12 1.00	1.00 0.25 1.00 0.56 0.28 0.12 0.74 1.00 0.09 0.76	0.02* 0.09 0.09 0.09 0.02* 0.09 0.34 0.34 0.34 0.34 1.00 1.00 1.00 1.00 1.00	1.00 0.15 0.34 0.56 0.76 1.00 0.09 1.00 0.76 0.74 0.74 0.28	0.43 0.25 0.25 0.74 0.43 1.00 0.74 0.25 1.00 0.43	0.25 0.25 0.34 0.56 0.74 0.74 0.74	1.00 0.34 0.76 0.34 0.76 0.76	0.25 0.74 0.43 0.74 0.30 0.74 0.74 0.74 0.34 1.00	0.43 0.34 1.00 0.76 0.56 0.12 0.74 0.76 0.43 0.74 1.00

*Number of bands significantly different ($P \leq 0.05$) among three Egyptian population of buffalo

Table (4): Genetic diversity in investigated buffalo populations breeds based on RAPD markers.

Buffalo population	No. of polymorphic loci	% of polymorphic loci	Genetic diversity Nei ,s (1973)	Shannon's diversity (Lewontin 1972)
Upper Egypt	75	59.52	0.2590	0.3718
El-Delta	78	61.90	0.2654	0.3824
Lower Egypt	80	63.49	0.2603	0.3794
All	106	84.13	0.3086	0.4574

Table (5): Genetic identity (above diagonal) and genetic distance (below diagonal) between the investigated buffalo populations (Nei's 1972)

Pop	Upper Egypt	El-Delta	Lower Egypt
Upper Egypt	****	0.9312	0.8641
El-Delta	0.0713	****	0.9186
Lower Egypt	0.1461	0.0849	*****

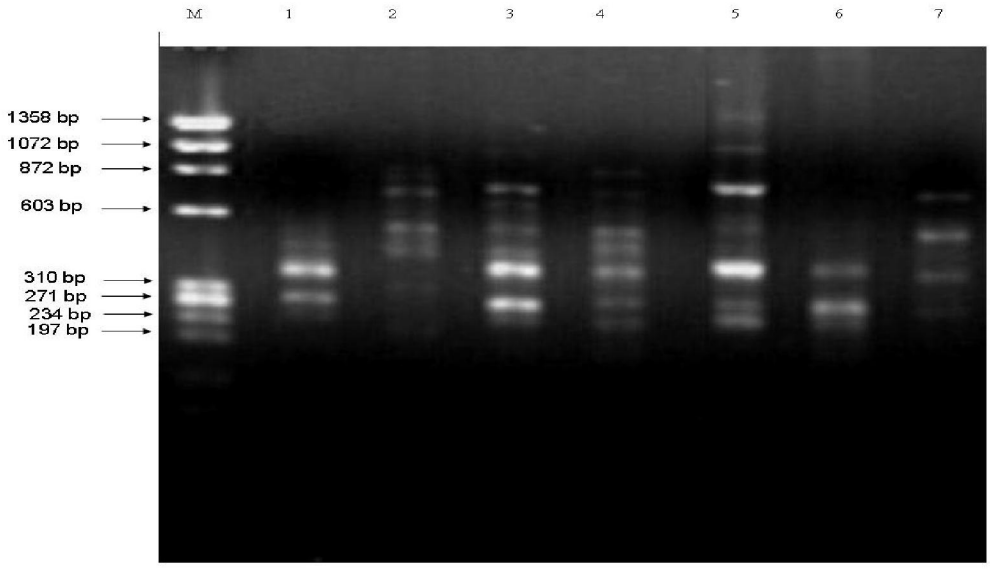

Fig. (1): Random amplified polymorphic DNA (RAPD) profile generated by primer OPB-14 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,7) represents DNA of pop3.

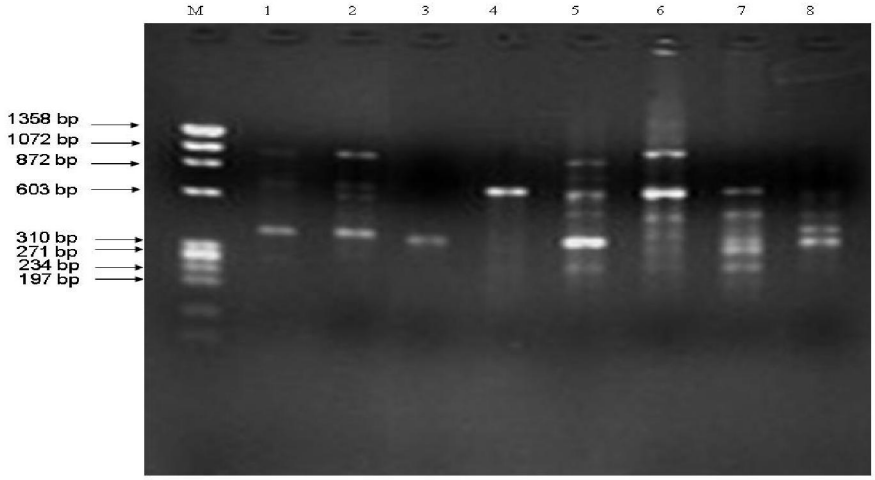

Fig. (2): Random amplified polymorphic DNA (RAPD) profile generated by primer OPC-15 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2,3) represents DNA of pop1, lane (4,5) represents DNA of pop2 and lane (6,7,8) represents DNA of pop3.

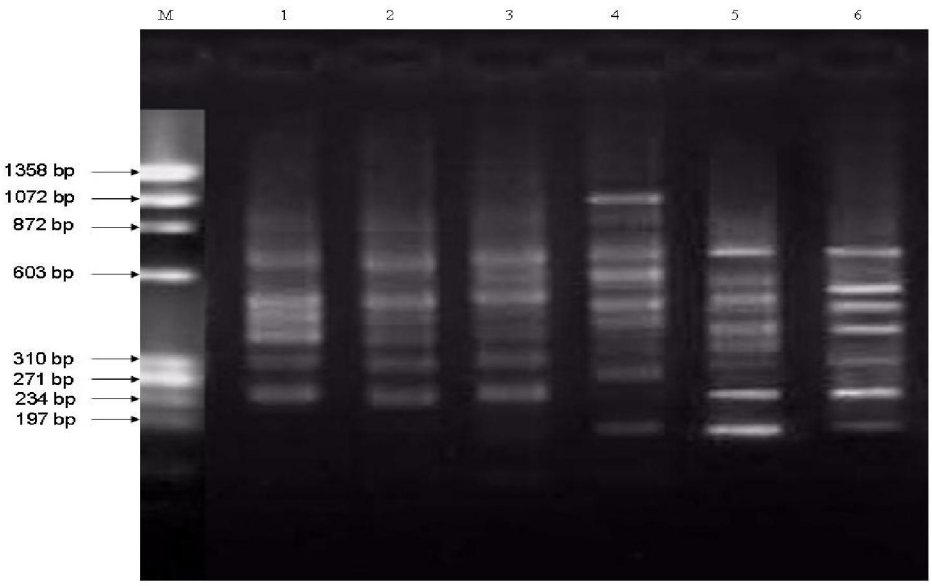

Fig. (3): Random amplified polymorphic DNA (RAPD) profile generated by primer OPD-05 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,) represents DNA of pop3.

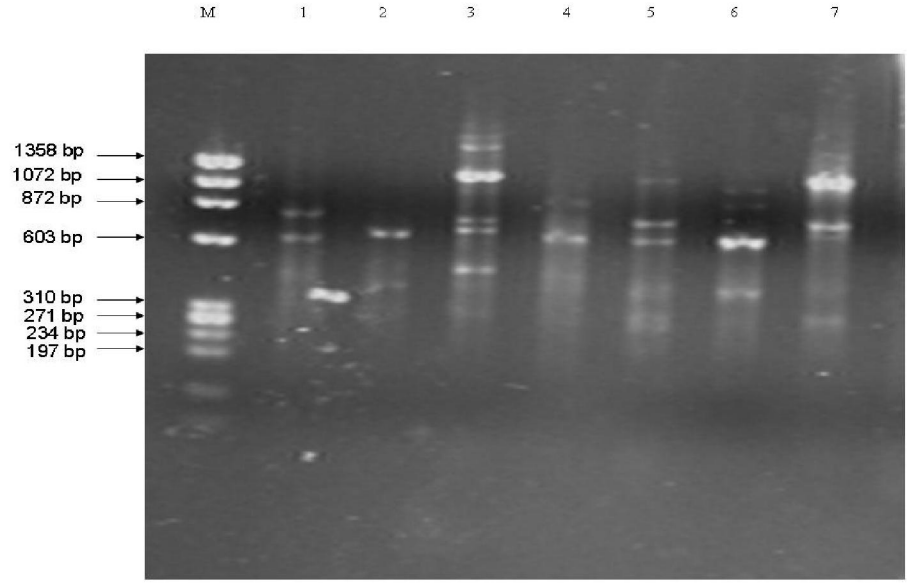

Fig.(4): Random amplified polymorphic DNA (RAPD) profile generated by primer OPD-01 in individual buffalo of different populations, Lane M= molecular marker(ϕ x 174 DNA Hae III digest. lane (1,2) represents DNA of pop1, lane (3,4) represents DNA of pop2 and lane (5,6,) represents DNA of pop3.

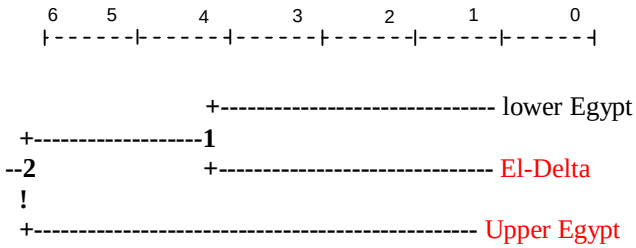

Fig. (5): Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA
--Modified from NEIGHBOR procedure of PHYLIP Version 3.5

6/18/2009

Women Physical Aggression (A Review)

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Abstract: Female aggression is a serious problem in most societies and is increasing these days in families. Female aggression has a negative effect on women as offender, their partners, children, and society in general. This paper aims to review the articles based on research that have been done on females' physical aggression. It attempts to show that females are also physically aggressive as males. According to the existing literatures, the rate of females' physical aggression is equal to those of males, and in some studies it is found to be higher than males. Based on these findings, it is concluded the rate of females' physical aggression is either equal to or higher than males, but not necessarily less than males. [Journal of American Science 2010;6(6):227-235]. (ISSN: 1545-1003).

Keywords: Wives Aggression, Female Aggression, Physical Aggression, Theories of Aggression

1. Introduction

There is a common belief in almost every culture in this world that men are more aggressive than women. As stated by Eagly and Steffen (Franzoi, 1996) men are more likely than women to engage in aggression that produces pain or physical injury. Thus, aggressive behavior by women has been neglected by people and the society at large (Straus, 2006). Hitherto, aggression which happens within the context of the family was mainly attributed to males. However, findings from several research reveal that females are as aggressive as their male counterparts (Hamel, 2005; Brown, 2004; George, 2003; Cercone, Beach & Arias, 2005; Straus, 2004; Katz, Kuffel & Coblentz, 2002; Dutton, 2007; Felson, 2006). Although female aggression and conflict have always been a part of human society, however, it has rather remained under researched until in the early of 1970s (Dutton & Nicholls, 2005).

In 1980, Straus carried out a large-scale national survey of family aggression in the United States, where he found that 23% females reported engaging in aggression against their partners. Since then, researchers have shown an increased interest in female aggression, and have heightened the need for investigating this phenomenon across the world's societies (Perry & Fromuth, 2005; Straus, 2006; Cook, 1997; Capaldi & Owen, 2001; Kaura & Allen, 2004; Nicholls & Dutton, 2001; Magdol, Moffitt, Caspi & Newman, 1997; Straus, 1997). Although different sources have reported aggression between spouses (Perry & Fromuth, 2005; O'Leary and Slep, 2006; Hood & Carruthers, 2002), these have led to the recognition of the fact that the female aggression is a common phenomenon in the society (Brown 2004; Swan & Snow, 2002). However, there are mixed reports on the

amount and type of observed aggressive behaviours with respect to gender. Jenkins and Aube (2002) revealed that women as compared with their male counterparts were more aggressive in certain conditions, and more likely to kick, hit or physically assault their husband. Although some researchers argue that female aggression is a relatively new problem, it has largely remained under reported (Straus, 2006). In general, the complicated nature of human behavior, these include different cultures and family values do not allow for a general consensus on the issue. This paper attempts to review several literatures that deal with women aggression, particularly physical aggression.

2. The Concept of Aggression and Its Causes

Baron and Byrne (2000) define aggression as any behavior directed toward the goal of harming another people who is motivated to avoid such treatment. In other words, aggression is related to the intentional infliction of some form of harm to others. Meanwhile, Franzoi (1996) categorizes aggression into two main types - instrumental aggression and hostile aggression. Instrumental aggression is the intentional use of harmful behavior so that one can achieve some other goal; while hostile aggression is the intentional use of harmful behavior in which the goal is simply to cause injury or death to the victim. Aggression can also be categorized into legal aggression and illegal aggression (Ma'rof, 2001). Legal aggression refers to the aggressive behaviors that are legally accepted by the norm (laws) of particular society (e.g., a policeman kills a criminal); meanwhile, illegal aggression refers to the aggressive behaviors that are not accepted by the norm.

There are several theories that discuss the causes of aggression. The instinct perspective sees aggression stems form from innate urges and tendencies. The major proponents of this perspective were Sigmund Freud and Konrad Lorenz. Sigmund Freud believed that aggression stems mainly from a death wish (*thanatos*) acquire by all human being. This theory stresses that death instinct is initially aimed at self-destruction, but is soon redirected outward, toward others. Meanwhile, Lorenz suggested that aggression develops mainly from an inherited fighting instinct that human beings share with many other species. However, social psychologists reject these instinct theories, where they believe that aggression stems mainly from an externally elicited drive to harm others. Their theories are called drive theories of aggression. Among the proponents is Berkowitz (1989). The theories suggest that external conditions, especially frustration, disappointment or any interference with goal-directed behavior, can rouse a strong motive to harm others. The aggressive drive, in turn, leads to overt acts of aggression. On the other hand, there are modern theories that discuss the causes of aggression. These new theories take into consideration of many factors that contribute to aggressive behavior. One of the theories is called general affective aggression model (GAAM) which was proposed by Anderson (1997). This model suggests that aggression is triggered or elicited by a wide range of inputs variables. Inputs variables are aspects of the current situations and/or tendencies individuals bring with them to a given situation. According to Baron and Byrne (2000), there are two main groups of input variables. The first group of input variables include: frustration, some kind of attack from other person (e.g., an assault), exposure to aggressive models (other people behaving aggressively), the present of cues associated with aggression (e.g., gun and

other weapons), and virtually anything that causes individuals to experience discomfort. The other group of input variables is individual differences, which include traits that predispose individual toward aggression (e.g., irritability), certain belief and attitudes violence (e.g., believing that it is acceptable and appropriate), values about violence (e.g., the view that it is a "good" thing), and specific skills related to aggression (e.g., knowing how to fight and to use weapons). These situational and individual differences variables may lead to overt aggression - first through increasing the physiological arousal or excitement (arousal); second through arousing the hostile feeling (affective stage); and third, through inducing individuals to think hostile thoughts or bring hostile memories to mind (cognitions). Depending on individuals' interpretation s (appraisals) of the current situation and possible restraining factors, aggression either occurs or does not occur.

Aggression can take several forms – verbal, emotional and physical. The focus of this paper is on physical aggression. Tremblay et, al (1999) argued that physical aggression includes biting, hitting, kicking, assault, getting involved in fight, encounter with a weapon, rape, homicide, and threatening to use physical force. Physical aggression occur when there is any non-accidental form of physical and assault injury. It can involve burning, pushing, shoving, shaking, kicking on a person or on property (Kar, 2000).

3. Literatures Related to Female Physical Aggression

Female aggression has existed almost in all cultures and countries. Most of the literature presented in the field of female aggression has linked male and female aggression and for this reason, in this paper the writers bring the literature that compares the two groups of male and female. These literatures aim to show the fact that female physical aggression is prevalent like male physical aggression in which the level of female physical is not lower than men, and there is a requisite to study female physical aggression as male physical aggression. The high level of development, knowledge and welfare in some developed countries has triggered the study of female aggression. However, in undeveloped countries, due to shortage of financial budget for research and the attitude toward female aggression, the study of female aggression is believed as no longer required. Moreover, the presence of patriarchal opinion in the university and government has ignored cases of female aggression.

According to Moyer (1977), female aggression is a recent topic. Physical aggression after marriage has been studied by several researches. Physical force is a common way of resolving problems and fights in any marriage life and couple relations. Although the aggressive behaviors are different from culture to culture, however, there are some similarities where in general aggressive behaviors can be predicted. For the purpose of having a comprehensive picture of female aggression, we need to search several countries that have carried out studies related to female aggression. Although the contexts are different and we can not generalize these findings to other cultures, however, there are still some similarities between cultures in terms of causes and nature of female aggression.

This incidence of violence has been studied within the context of families as well. For instance, Kim and Emery (2003) have studied 1500 South Koreans couples. The results indicated that the ratio of male to female aggression was 27.8%, while the female to male was observed to be 15.8%.

In Caetano, Schafter, Field, & Nelson (2002), about 1635 couples performed on Conflict Tactics Scale (CTS). The result indicated that the agreement concerning mutual partner violence was about 40%. In this study, wives reported that they were committing more partner violence than their spouses across the three ethnic groups. This is assumed to support the race-free and ethnicity-free profile of violence.

The same findings of studies, bearing more or less similarities, were observed across other studies. Simonelli, Mullis, Elliot, & Pierce (2002), for example, in an their experimental study, had investigated 120 students, of whom 61 were boys, and 59 were girl. In their study, the researchers used the CTS. It was realized that, from both genders, 10% of boys and 33% of girls have committed at least one type of physical aggressive behaviors. Moreover, it was found that 18% of boys and 15% of girls had experience physical aggression from their partners. The other study was conducted by Schumacher and Leonard (2005), in which they had a sample of 634 newly married couples. From this sample, 60% were Euro-American and 30% were African-American, who completed the CTS2 on 3 situations for a period of three years. The percentage of wife to husband aggression in this study was estimated to be 48%, 45%, and 41%; whereas, the husband to wife aggression was observed to be 37%, 38%, and 37% across the years of investigation.

There are some other researchers who have analyzed the available research reports in order to present a collective account of the issue of female and wives aggression. Mallory, McCloskey, Griggsby and Gardner (2003), in a review research which examines women's use of violence in intimate relationships have presented a cumulative account of the issue. In another study, Lewis and Fremouw (2001), in an examination of the literature cited that there are many evidences that females initiate more violence than males. However, the current writers believe that such studies need to be based on a meta-analysis of either descriptive research or surveys so far have been conducted with respect to the role and nature of female aggression, which is definitely absent in the reviewed literature, except for few cases. For example, Archer (2000) in a meta-analyses of sex differences in physical aggression indicated that women were more likely than men to use one or more acts of physical aggression and to use such acts more frequently. Looking at the possibility of getting injuries, women were found to be injured, and research shows that 62% of women are injured as the result of these abuses (Archer, 2000). Moreover, conducting a meta-analysis study entails certain methodological steps among which, similarity of the scope of the researches included in meta-analysis and similar objectives are just a few. This seems not have been possible due to the wide scope of research in aggression by now.

The issue of female aggression has been investigated yet from another perspective, i.e. gender parity of partners. Leisring, Dowd and Rosenbaum (2003), discussed information regarding gender equality in partner aggression. They provided a working reason for the study of female offenders and describe characteristics of partner of these aggressive women. In their study, they presented the treatment program for partners of aggressive women at University of Massachusetts Medical School.

Meanwhile, Katz, Kuffel and Coblentz (2002) reported two studies, in which there were dating men and women experiencing violence at comparable levels. It was found

that men have experienced more frequent moderate violence compared to women. In the first study from a sample of 183 women and 103 men, 55% of the women had reported no case of violence with their partners, while 50% of men only had nonviolent mates. In the second study, it was reported that from 78 women and 45 men who were eligible for this research, 73% of women had nonviolent partners. This figure is reported against the 58% of men who have reported no violence from their female mates.

Graham, Plant, & Plant (2004), in a study which was conducted as a cross-sectional study, adopted a sample of 2027. The sample consisted of 1052 women and 975 men. In this research both groups were interviewed looking for their experience of partner aggression. The findings showed that 16% of women were physically aggressing their male mates within a period of two years as reported to the interviewers. For the male participants only 13% had experienced some physical aggression towards their female mates.

George (2003), and an analysis of female initiated aggression reported some historical, as well as empirical case evidences to prove the reality of "battered husband syndrome". This review is however, re-confirmed by other researchers, for example, Felson (2006) who reported that while men were eight times more likely to commit overall violence than women, there was a gender equality in partner violence.

In another study which was conducted as a longitudinal study, Fergusson, Horwood and Ridder (2005) examined that the scope of the extent of domestic violence experience. This study had a sample of 828 of whom 437 were women and 391 were men. These participants were all young adults around 25 years old. The participants were selected for a long term longitudinal study and they were asked to take the CTS2. The results revealed that there were more men exposed to severe domestic violence compared to women. Moreover, it was found that, mild and moderate rates of violence were similar for men and women. In the study, 39.4% of women and 30.9% of men reported violence scores of 3 or higher. However, in terms of initiation of partner assaults, it was found that 34% of women and 12% of men have reported initiating physical assaults.

As an evidence of treating these figures with caution, Felton & Pare (2005), has analyzed the data from The National Violence Against Women Survey, and they have found that male victims are particularly reluctant to report assaults by their female partners. The possible reasons for non-reporting include: fear of reprisal, or they have this perception that police could do nothing to help and charges would not be believed.

The other relatively recent study was conducted by Dutton (2007). He has researched female intimate partner violence and developmental trajectories of abusive families. He realized that female violence towards intimate male partners was just as severe and has similar consequences as male violence towards women. This report supports the mixed results as the findings in this regard are mixed across the available research literature. For example, Cercone, Beach and Arias (2005), in their study of 414 students, from whom 189 were men and 225 were women, revealed that male and female subjects were equally committing acts of minor violence in intimate dating relationships. However, it was realized that women were twice more likely compared to men to commit severe acts of violence.

From among other comprehensive researches, Brown (2004), has summarized the available partner violence data from the 1999 Canadian General Social Survey (GSS). The GSS was based on a representative sample of 25,876 persons. In a period of one year of the research period, an estimated of 3% of Canadian women and 2% of Canadian men were reported experiencing violence from their partners. The report continues to report the 5 year period from 1995-1999, in which an estimated 8% of Canadian women and 7% of Canadian men reported violence from their partners. The researcher has reviewed the available police and legal responses to domestic violence in Edmonton, Canada. He has concluded that men who were involved in disputes with their partners were disadvantaged by the police or the entertainers and were treated less favorably.

In terms of kinds of aggressive behaviors, Archer (2002) reported that women were more likely than men to throw thing at their partners. Among other possible reactions, he stated that slapping, kicking, biting, punching and hitting with an object were more common. On the other hand, he reported that men were more likely than women to strangle, choke, or beat up their partners.

4. Conclusion

Female aggression is a serious problem in most societies and is increasing these days in families all around the world. Female aggression has a negative effect on women as offenders, their partners, children, and society in general. Thus more researches on female aggression are needed. This paper reviews the female physical aggression. According to the existing literatures, the rate of female physical aggression is equal to those of men, and in some studies, the rates of physical aggression among women are found to be higher than men. Some researches show that men and women are physically abusing each other at the same rates. Based on these findings, the rate of women physical aggression not to be lower than men, instead it is either equal to or higher than men.

There are consequences of family aggression. The negative attributes of family aggression on the child and the society are undeniable, and have marked detrimental consequences for both victims and aggressors (Andrews & Brewin, 1990; Campbell, 2002; Hood & Carruthers, 2002; Kanoy, Ulku-Sreiner, Cox, & Burchinal, 2003; Stark & Flitcraft, 1996; Walker, 2000). These latter effects made it worthy of attention, from the viewpoint of female aggression (Strauss, 2006). Moreover, the development of research institutions, allocation of budgets and the accessibility to scientific resources in some countries have allowed researchers to present a clear picture of the phenomenon (Felson, 2007; Horwood et al. 2005). Although the rate of female aggression in some studies is higher, women are still physically weaker than men, thus the rate of injuries for women is higher. In all situations, if women use aggression or their husband reacts or uses aggression, the injuries for women is higher than men. Thus more study is needed on female aggression.

Acknowledgements:
The authors would like to thank Alireza Akbarpoor, and Nicol, for reviewing this paper. The comments greatly improved the manuscript.

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6/18/2009

Bioavailability of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis

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Abstract: This study was conducted to investigate the Pharmacokinetics of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis caused by *Edwardsiella tarda* (*E. tarda*), and to estimate its tissue distribution. Safety test, *in vitro* determination of the minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate; in addition to; the *in vivo* efficacy of orbifloxacin in treating Edwardsiellosis at 2 stages; the early stage 7 days and late stage 15 days post infection. The results showed that orbifloxacin is safe for Catfish at concentrations up to 50 mg/L in water. The minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate was 0.016 mg/L with MIC_{50} and MIC_{90} equal to 0.5 and 1.0 mg/L respectively. Almost 100% of the infected fish recovered after treatment with Orbifloxacin for 72 hours in early stage of the disease with complete disappearance of clinical signs. No *Edwardsiella* could be isolated from second group 96 hours post treatment; although the treated fish showed unhealed skin lesions, results of liver dysfunction and tissue alterations were recorded. Orbifloxacin residues in Catfish muscles decreased gradually after cessation of treatment and disappear by day 10 post-treatment in the first group. In conclusion orbifloxacin can be awaited as effective antibacterial agent for control of edwardsiellosis caused by *E. tarda*. The treatment is much more successful when initiated at the earliest time of infection. [Journal of American Science 2010;6(6):236-244]. (ISSN: 1545-1003).

Keywords: Bioavailability, Edwardsiella tarda, African sharptooth catfish, Liver function tests, histopathology.

1. Introduction

Bacterial diseases are significant setback for successful aquaculture (Austin and Austin2007). *Edwardsiella* is among the most important pathogens in aquatic environment (Mohanty and Sahoo 2007). Human intestinal and extra intestinal infections with *Edwardsiella tarda* are well documented (Walton et al., 1993, Coutlee et al., 1992, Castro et. al., 2006 and Mohanty and Sahoo 2007). *Edwardsiella tarda* has been infrequently isolated from variety of warm blooded animals including birds (Thune et al., 1993). Edwardsiellosis is an important disease in various fish species; clinical manifestation of *Edwardsiella tarda* infections was first and well described by Meyer and Bullock (1973) in channel catfish, in tilapia (Kubota et al., 1981), in turbot, *Scophthalmus maximus (L.)* (Padros et al., 2006), Korean catfish (Yu et al., 2009 & 2010), Mullets (*Mugil cephalus*) (Kusuda et al., 1976). Among the most important strategies for control of aquaculture infectious diseases is to alleviate the predisposing causes of the disease (Noga, 2000), and to improve health management practices. For successful treatment of fish exhibiting signs of edwardsiellosis; immediate diagnosis and treatment are recommended while the majority of the fish are still feeding (Samuelsen et. al., 1998 and McGinnis et al., 2003; in this respect the use of antibacterial agents of low inhibitory concentrations and effective systemic distribution is the best choice (Samuelsen 2006). Currently, the antibiotics approved for use with fish food according to the Food and Drug Administration (FDA) in USA were oxytetracycline and sulphadimethoxine and ormetoprim combination. However, there are reports of bacterial resistance to these antibiotics (Plumb et al., 1995 and Smith et al., 1994 & Xiao et al., 2009). In addition, palatability problems have been reported with sulphadimethoxine and ormetoprim combinations (Poe and Wilson 1989). Therefore, search for an alternative, effective and save remedy for edwardsiellosis is essential. Quinolones are important group of antibacterial agents used to treat bacterial diseases in fish (Samuelsen, 2006). Fluoroquinolones are antimicrobial drugs that generally have very good activities against a broad spectrum of bacteria and are used for the treatment and prevention of diseases in fish (Hannan et al., 1997; Samanidou and Evaggelopoulou 2007 & Zhu et al., 2009). Some

Fluoroquinolones were licensed in Europe and United states for use in fish and companion animals to control gram-negative and gram-positive bacteria, in addition to Mycoplasma species (Schrieder et al., 1996, Schrieder et al., 2004 and Albarellos et al., 2005). Enterofloxacin, the first veterinary fluoroquinolone in the market, soon was joined by orbifloxacin, Marbofloxacin and others. Marbofloxacin, a fluoroquinolone, showed effectiveness in aquaculture of carp (Fungke et al., 2006 and Zhu et al., 2009).The main target site for their bactericidal action is the DNA-gyrase, an enzyme required for uncoiling of DNA to allow spatial arrangement of DNA in the bacterial cell. (Samanidou and Evaggelopoulou 2007). Orbifloxacin is a new synthetic fluoroquinolone antimicrobial drug that has been developed especially for use in veterinary medicine (Nakamura, 1995). Sensitivity of *Edwardsiella* to antimicrobial agents was reported by Muyerube et al., 1973, Reinhardt et al., 1985, Waltman et al., 1986, Clark et al., 1991. Although there is a conclusion that strains of *E. tarda* have uniform susceptibility to about 22 antibiotics, there are differences linked to the source of strains. The presence of various R- plasmids that mediate antibiotic resistance were reported in *Edwardsiella* by Aoki et al., 1977, Lobb and Rhoades 1987, Libb et al., 1993. Because little data is available about registration of orbifloxacin as a drug to control Edwardsiellosis in catfish, the current study aimed to investigate the Pharmacokinetics of Orbifloxacin in African sharptooth catfish, *Clarias gariepinus*, and its efficacy in control of induced Edwardsiellosis at two levels; the early stage of infection (7 days post infection) and the late stage of infection (15 days post infection).

2. Material and Methods

2.1. Orbifloxacin

A fluoroquinolone antibiotic, 1-cyclopropyle-7-[(C3s,5r)-3,5-dimethylpiperazin-1-y1]–5,6,8-trifluro-4-oxo-1,4-dihydroquinoline-3 carboxylic acid (IUPAC), $C_{19}H_{20}F_3N_3O_3$, of molecular mass 395.37g/mol.,was obtained as water soluble white powder in pure form; supplied by Dainippon Pharmaceutical Co, Ltd., Osaka, Japan.

2.2. Fish

A total of 720 sharptooth Catfish (*Clarias gariepinus*) weighting 50±2g were obtained alive from semi intensive fish farm. The fish were apparently healthy; transferred and maintained for acclimatization in glass aquaria (30× 40×80 cm^3); according to the recommended biomass for each aquarium; supplied with dechlorinated tap water, according to Best et al. (2002). Samples from the catfish were used for isolation of *E. tarda* infection and found negative for isolation. Both sexes of fish were used and no attempt was made to determine gender or sexual maturity.

2.3. Edwardsiella tarda strain

Edwardsiella tarda strain was locally isolated and identified from clinically diseased African sharptooth Catfish (*Clarias gariepinus*) using MacConky's agar, Edwardsiella isolation agar (Shotts, and Waltman 1990) and the biochemical reactions according to Quinn et al. (2002). The selected isolate was tested for its pathogenicity and was found to be pathogenic after intra-peritoneal injection (IP) in a concentration of (2.4×10^4 CFU /ml).

2.4. Assay procedures

2.4.1. Safety testing

To investigate the safety of orbifloxacin to catfish, a total of 210 apparently healthy catfish were used; two groups each of 40 in 3 replicates were treated by application of one of two doses of (0.0 mg^{-1} and 50 mg^{-1} /day) of orbifloxacin in water according to Paget and Barnes (1964). The treated groups were kept under observation for 7 consecutive days. The safety of orbifloxacin was justified by recording mortalities; alteration on the behavioral patterns and clinical picture in the two groups following the Code of American Federal Regulation (1985).

2.4.2. In vitro determination of minimum inhibitory concentration of orbifloxacin versus E. tarda:

The minimum inhibitory concentrations (MICs) testing against the E. tarda was performed in accordance with the NCCLS approved performance standards guideline for the agar dilution susceptibility test, with adjustments necessary for testing E. tarda. A stock solution containing 1,280 mg/ ml of orbifloxacin was prepared. Orbifloxacin was then serially diluted in sterile distilled water at concentrations of 640, 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/ml. Each dilution was then poured into Mueller–Hinton agar (MHA) with 5% sheep blood to final concentrations of 0, 0.002, 0.004, 0.008, 0.016, 0.03, 0.06, 0.125, 0.250, 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/ml. Two plates of each concentration were poured. Edwardsiella tarda colonies were suspended in sterile broth media to the density of McFarland 0.5 barium sulfate turbidity standard (approximately 1×10^8 to 2×10^8 colony-forming units/ml) with a sterile cotton swab. One to two microliters of the inoculated broth was placed on the surface of duplicate blood agar plates from the lowest concentration (0 mg/ml on a control plate) to the highest concentration (64 mg/ml). A second control agar plate (0 mg/ml) was inoculated last to ensure that there was no contamination or antimicrobial agent carryover from the inoculation. Plates were then incubated at 25 ± 2 C for 2 days and observed to determine which concentration completely inhibited the growth of E. tarda. The control bacteria used were E. coli ATCC 25922. Controls were incubated and read at 16 to 20 hours according to the NCCLS guidelines. Results were determined as (++) no visual inhibition of bacterial growth, (+)scant bacterial growth observed,

and (-) no growth observed Korsholm and Sogaard (1987).

2.4.3. In vivo orbifloxacin

A total of 600 apparently healthy catfish were grouped in to 5 groups each of 40 fish (I, II, III, IV and V); used for the treatment trials by orbifloxacin as follows; all treatment trials were repeated in triplicate.

2.4.3. 1. Group I

Forty catfish were individually inoculated intraperitoneally (I.P.) with 0.2 ml of Edwardsiella tarda (2.4×10^4 CFU /ml) (Austin and Austin, 2007). The onset of treatment with orbifloxacin 50 mg^{-1} water /day started 7 days post infection.

2.4.3. 1. Group II

A second 40 catfish were assigned for the second group, inoculated intraperitoneally (I.P.) with 0.2 ml of E. tarda (2.4×10^4 CFU /ml). The onset of treatment with orbifloxacin 50 mg^{-1} water /day started 15 days post infection.

2.4.3. 1. Group III

Fish of group III consists of 40 catfish were kept without infection and treated daily with orbifloxacin in water in the same dose and method as the previous groups.

2.4.3. 1. Group IV

Forty catfish were experimentally infected by E. tarda and not treated by orbifloxacin.

2.4.3. 1. Group V

Forty catfish were kept none infected and none treated.

2.5.1. Aquaria and management

The temperature in the experimental aquaria was 24 ±1 C when measured by digital thermometer; dissolved oxygen was maintained at a range from 6.0 to 8.0 mg/L during treatment. The pH of experimental aquarium water ranged from 7.70 to 8.32 during dosing. Alkalinity and hardness were measured titrimetrically; alkalinity ranged from 112 to 132 mg/L as CaCO3 and hardness ranged from 152 to 168 mg/L as CaCO3. Fish were kept under an approximately 12-hour light: 12-hour dark photoperiod regime throughout the study. A commercial diet of 35% protein was offered in a feeding rate of 2% body weight daily.

2.5.3. Anaethesia and sampling

Tricaine methane sulphonate (MS222) was used to anesthetize fish in a dose of 50 mg/ml buffered by 100 mg/ml sodium bicarbonate to adjust water alkalinity to exceed 50 mg/L as CaCO3 according to Davis et al,, (2008).

For blood sampling, blood were collected from the caudal vein; using a 2 ml syringe. Blood was stored in heparinized tubes and centrifuged at 4000 g for 20 min at 15 C. The supernatant (plasma) was frozen and stored at -80 C.

Tissue samples of muscles and kidney were taken daily from Two Catfish/ group for re-isolation of E. tarda.

For histopathological examination; by the end of the treatment trials; five catfish from each treated group was sacrificed; liver, spleen, skeletal muscles, cardiac muscle and gills were preserved using formalin buffered saline for tissue alteration studies.

2.5.4. Orbifloxacin -Tissue distribution

The residue of orbifloxacin in Catfish muscles was accomplished by a modified agar diffusion bioassay method reported previously by Bennett et al. (1966), Bo¨ttcher et al. (2001) and Albarellos et al. (2005) and Althaus et al., (2009) using Escherichia coli (ATCC 10536) as the reference organism. The medium was prepared by dissolving 9.5 g Mueller–Hinton agar in 250 mL distilled water in a 0.5 L flat-bottomed flask, which was autoclaved for 20 min. After cooling to 50 C in a water bath, 0.4 mL of the diluted suspension of reference organism was added to the media. After the medium was poured (25 mL) and solidified, six wells were cut at equal distances in the solidified bioassay plates. Triplicate tissue samples and a known standard concentration of the drug in tissue (0.01 to 50 mg/kg) were placed directly into the wells without any clean-up step. The standard (in tissue) was included in each assay plate in order to compensate for any plate-to-plate variations. The plates were kept at room temperature for 2 h before being incubated at 37 C for 18 h. Zones of inhibition were measured using micrometers, and the results from the standards were used to calculate the concentration in each sample. A linear relationship existed between the zone of inhibition and the logarithm of orbifloxacin concentrations 0.015 and 50mg/kg, with a correlation coefficient of 0.995. The concentration of Orbifloxacin in tissue samples was calculated by linear regression analysis of the corresponding zones of inhibition to the standard curve.

2.5.5. Liver function tests:

Plasmatic activity of AspAT and AlaAT were determined in the collected plasma at l ¼ 340 nm (37 C) with Bio Merieux kits –France according to the manufacturer's instructions. Results were expressed in U l/1, where 1 U is the amount of enzyme that converts 1 m mol substrate per min under the specific conditions of the procedure.

2.5.6. Histopathological study:

Tissue samples were taken from liver, spleen, skeletal muscles, cardiac muscle and gills of the 4 fish groups. The samples were prepared following the method of Bancroft, et-al., (1996).

2.6.1. Data recording

The tested Catfish under experimentation as well as control ones were clinically examined daily for morbidity, mortality according to Kimberley (2004), the behavioral patterns during the treatment periods were daily observed and recorded according to Tsubokawa et al. (2009). The plasmatic activities of AspAT and

AlaAT were recorded**;** in addition to the results of the tissue alterations.

2.6.2. Statistical analysis

Data were expressed as mean ± S.D. Statistical analysis was carried out using computerized SPSS program (version 8.0, Chicago, IL, USA) with one way ANOVA test for significance according to Snedecor and Cochran (1986).

2.6.3. The conflict of interest

The study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Cairo University. No conflict of interest is known. All phases of this study were conducted in compliance with US Food and Drug Administration guidelines for Good Laboratory Practice Standards.

3. Results

3.1. Safety test

Orbifloxacin proved to be safe when applied in water for Catfish at a concentration of 50 mg^{-1} /day for 7 days in comparison to the control group (0.0 mg^{-1} concentration of orbifloxacin). The treated fish did not display any concentration-related changes regarding mortalities, clinical and behavioral abnormalities,

3.2. In vitro evaluation of Orbifloxacin versus E. tarda

The minimum inhibitory concentration (MIC) of orbifloxacin against E. tarda isolate was 0.016 µ/ml with MIC50 equal to 0.5 and MIC90 equal to 1.0 µ/ml.

3.3. The in vivo efficacy:

Treatment of the induced edwardsiellosis by orbifloxacin in Group I resulted in complete recovery, the results was proved by the negative isolation for the E. tarda from the liver kidney and muscles of the infected fish 72 hour post-treatment. The treated Catfish held in appropriate management and water quality expressed complete recovery from the clinical signs within one week post -treatment. In the Group II the onset of treatment with orbifloxacin was 15 days post induced infection, no bacterial growth could be detected 96 hours post –treatment; although the treated fish showed unhealed skin lesions, despite the fact that the treated fish were kept in appropriate management conditions.

3.3.1. Behavioral Observations during treatment trials

The experimentally induced infected fish Group III; expressed severe behavioral changes manifested by lethargy, podding, and accumulation on one side of the aquaria. Fish exhibited abnormal flight/fright response to the stimulus in comparison to Group V. In Group I; a marked improvement in the behaviours was recorded, in Group II; no clear changes in the adverse behavioral alterations were seen as a result of treatment.

3.3.2. Clinical picture, Morbidity and mortality rates

During the early stage of experimental infection with *E. tarda*, (Group I), revealed loss of appetite, skin depigmentation with some external petechial hemorrhage and small cutaneous ulcerative lesions. Internally, the infected fish organs showed general signs of septicemia with some edematous fluids in the abdominal cavity. During the time of induced infection by *E. tarda* and before the onset of treatment, a total of 20% mortality rates were recorded in the infected groups (Group IV) starting from day 10 tell the 15 days post infection. In the experimentally infected and treated groups; no dead fish were recorded in Group I and in Group II. Groups IV and V; no mortalities were recorded along the corse of the experiments.

3.3.3. The tissue distribution

In the first 2 days post treatment; the level of orbifloxacin in muscles reached up to 0.03 mg/g tissues. At the 7^{th} day post treatment traces of orbifloxacin (0.015 mg/g tissue) were detected. At the 10^{th} day post treatment, no tissue residues of orbifloxacin were found in the muscles sampled.

3.3.4. The liver function tests

In the present study; the recorded values for AspAT and AlaAT activities in group V were (143±0.2 and 17±0.2 U/L). Induced infection in catfish with *E. tarda* resulted in significant increase in plasma AspAT and AlaAT activity (230±0.11and 35±0.11 respectively); after 15 days post infection the values of plasma AspAT and AlaAT increased to reach 220±0.2 and 60±0.2 respectively. The values of the liver function tests decreased upon treatment with orbifloxacin nearly to the normal values 6-8 days from the start of treatment. In Group III; the values of AspAT and AlaAT were 353±0.1 and 30±0.5 as shown in table (1).

Table 1: Effect of orbifloxacin on plasma liver enzymes of African sharptooth Catfish.

3.3.5. Histopathological examination

The results of histopathological examination revealed that; in Group I, liver sections showed apparent normal histological picture, the spleen showed slight lymphocytic depletion (Fig 4). The cardiac muscle showed intra cellular vacuolation and myositis as well as some leucocytic infiltrations (Fig. 6). No pathological changes were observed in skeletal muscles sections. The examined gill sections revealed slight necrosis of gill lamellae associated with slight leucocytic infiltration (Fig. 8). In the second group; liver showed vacuolar degeneration of the hepatocytes and dilatation with congestion of the hepatic sinasoids (Fig. 1 and 2). The spleen cleared a marked hemorrhages associated with lymphocytic depletion (Fig. 3). The heart muscle showed focal necrosis of myocytes completely replaced by leucocytic infiltrations (Fig. 5), meanwhile the muscles showed focal myolysis replaced by fibrinous connective tissue proliferation (Fig. 7). The gills showed clear lamellar oedema, focal necrosis associated with massive leucocytic infiltration (Fig. 9). Group III, On histopathological examination, E. tarda infection causes

hypertrophy of the liver cells and enlargement of their nuclei. Bacteria-laden phagocytes are found in the sinusoids of the anterior kidney, liver and spleen. Liquefaction and gaseous necrosis is seen in body musculature leading to ulcer formation. The gills show hyperplastic changes, the spleen accumulates haemosiderin pigments along with the presence of hyperaemia and necrotic changes. Group V exhibited normal histological sections in all the organs.

4. Discussions

It is important that therapeutic regimes are designed to maximize efficacy, and minimize the risk of development of resistant pathogens. In this respect, the study of the pharmacokinetic properties of drugs, in combination with susceptibility test, is an important tool for the establishment of optimal dosage regimes and thus the promotion of their correct use Samuelsen, (2006). Bath treatment, was the method of choice in the present study as it is suitable to carry out with agents of high solubility in water in addition to its privilege in treatment of fish suffering from systemic and skin infections (Samuelsen and Lunestad, 1996; Samuelsen, 2003 and Samuelsen, 2006).; which is the case in edwardsiellosis. Orbifloxacin proved to be safe when applied in water for Catfish at a concentration of 50 mg/L for 15 days. Fish, (2001) reported improved pharmacokinetic properties and more acceptable safety profile of fluoroquinolones, as norfloxacin and ciprofloxacin in fish. Immediate diagnosis and treatment with antibiotics has previously been recommended (McGinnis et al., 2003). The minimum inhibitory concentration (MIC) of orbifloxacin against *E. tarda* isolate was 0.016 µ/ml with MIC50 and MIC90 equal to 0.5 and 1.0 µ/ml respectively. The MIC for orbifloxacin against *E. tarda* was comparable to other fluoroqinolones as no available literature concerning MIC of orbifloxacin in fish could be obtained; against Enterobacteriaceae (0.03-0.5), Pseudomonas aeruginosa (0.12-2.0), Aeromonas hydrophilia (0.03-1.0), Acinetobacter calcoaceticus (1.0-2.0), Brucella melitensis (0.5-2.0), staphylococci (0.06-1.0) and enterococci (1.0-2.0) (Qadri et al., 1993). On studying the *in vivo* efficacy; during the experimental infection with *E. tarda*, mortalities started at the 10th day post infection and reached 20% of the total infected fish, Catfish revealed loss of appetite, skin depigmentation with some external petechial hemorrhage and small cutaneous ulcerative lesions. Internally, the infected fish organs showed general signs of septicemia with some edematous fluids in the abdominal cavity. As the disease progress; the clinical signs were severe loss of skin colour with extensive external petechial hemorrhages. Pyogenic reaction and/or ulcerations reached the muscles of the flanks and caudal peduncle, the PM examination showed severe fibrinous peritonitis and organs appeared as one homogenous necrotic mass with white nodules in the liver. The clinical signs of infection reported in the current study were similar to those seen in *Clarias batrachus* (Sahoo et. al. 1998), the Japanese flounder Paralicthys Olivaceus (Miwa and Mana, 2000) and in Indian major carp, *Labeo rohita* (Mohanty et. al. 2007). Similar findings were previously reported by Muratori *et. al.* (2000), Padros *et. al.* (2006) and Mohanty *et. al.* (2007). E. tarda was re-isolated from the infected fish. Treatment of the induced edwardsiellosis by orbifloxacin; Group I resulted in complete recovery, the results was proved by negative isolation for the *E. tarda* from liver, kidney and muscles of the infected fish 72 hour post-treatment. The treated catfish expressed complete recovery from the clinical signs within one week post exposure to orbifloxacin when accompanied by good hygienic practices and good water quality. In Group II, no bacterial isolation could be detected 96 hours post – treatment; although the treated fish showed unhealed skin lesions. The mortalities stopped in the experimentally induced infected fish post treatment. The results are concedes with results of Elliott and Shotts., (1980) who stated that initiation of treatment in the earliest time possible is much more successful than in the late stages of the disease.

This study is among the first studies to measure the in vitro and in vivo activity of orbifloxacin against *E. tarda* in Catfish; its application in water. In mammals, orbifloxacin was an effective, safe, and convenient antibiotic for the treatment of superficial and deep staphylococcal pyoderma in dogs (Scott et al., 2006), and for the treatment of a variety of infections, including skin infections, urinary tract infections, respiratory infections and wound infections caused by susceptible bacteria (Nakamura, 1995). Regarding behavioral observations during treatment trials; the improved responses of catfish in the first post treatment with orbifloxacin could be attributed to the over all improvement of health condition as the fish was in the recovery stage. In the second group, the infected fish expressed severe behavioral changes; this may be explained by the decreased amount of orbifloxacin accidentally absorbed orally since fish could not eat as the disease progress (Samuelsen et. al. 1998). Immediate diagnosis and treatment with antibiotics while the majority of the fish are still feeding has been previously recommended (McGinnis et al., 2003).

The tissue distribution in muscles was measured; knowing the fact that muscles are the end of the systemic distribution and it is the edible part of the fish; so it is important to evaluate the drug in muscles and its withdrawal time for public health safety. In the first 2 days post treatment; the level of orbifloxacin in muscles reached to 0.03 mg/g tissues. At the 7th day post treatment traces of orbifloxacin (0.015 mg/g tissue)

were detected. At the 10th day post treatment, no tissue residues of orbifloxacin were found in the muscles sampled. The limits of *in vitro* detection of orbifloxacin were 10 to 30µg/kg in the tissue. Blood chemistry is the mirror for the internal reaction of the body to certain stimulus and it largely explains the external clinical signs and the internal tissue alterations related to the intended stimuli. Plasma chemistry studies of specific diseases in fishes are scanty. This makes interpretation of fish blood chemistries from clinical patients very difficult, Stoskope, (1993). Two plasma enzymes, AspAT and AlaAT, are frequently used to determine the toxic effects of varied pollutants (Oruc and Uner, 1998; Malbrouck et al., 2003). An increase of their activity in plasma indicates the development of tissue lesions and is observed primarily when hepatic lesions occur.

In the present study; the recorded values for AspAT and AlaAT activities in control fish were (17±0.2 and 143±0.2 U/L) comparable to those previously reported (17.5 and 95 U/L for AspAT and AlaAT respectively in channel catfish; Stoskope, 1993), AspAT and AlaAT are present in low concentrations in plasma, probably as a result of normal cell degradation (Schmidt and Schmidt, 1974).

Our data shows that injection of *E. tarda* in a dose of 0.2 ml of 2.4×10^4 CFU/ml induces significant increase in plasma AspAT and AlaAT activity in Catfish . Yu et al., (2010) reported also a significant increase in AspAT and AlaAT in plasma of the Korean catfish, Silurus asotus, after infection with E. tarda. This can be attributed to the induced hepatic damage as cleared by significance elevation of plasma liver enzymes and histopathological picture.Treatment with orbifloxacin had returned plasma liver enzymes nearly to the normal values 6-8 days from the start of treatment. This effect may be due to eradication of infection. In catfish received orbifloxacin alone a marked increase in liver enzymes was recorded, this result may suggest that orbifloxacin may have a hepatotoxic effect in catfish when used in prevention. In this respect in mammals, other fluroquinolones caused no additional hepatotoxicity when they were used by patients with hepatitis (Ho et al., 2009) and did not present a risk to patients receiving DX-619 (a new fluroqinolone) in clinical trials (Sarapa et al., 2007). The different predilection of body organs and tissues could be an important aid for following up treatment and prognosis of diseases. From this point of view, experimentally inoculated and treated Catfish was subjected to histopathological examination 72 hour post treatment. It is clear that catfish in the first group expressed very mild and non significant histopathological changes in comparison to those seen in the second group. In this concern, some workers have recently revealed the relationship between the kinetic change of apoptosis in lymphoid organs and the inflammatory process. The extensive apoptosis in the thymus and spleen suggests that *Edwardsiella* septicemia generates systemic immunosuppression via lymphocyte apoptosis (Pirarat *et al*, 2007). Moreover, Wilson and Macmillan, (1989) reported that fluoroquinolones therapy in channel Catfish may induce a minimal to mild dose-dependent decrease in hematopoietic/ lymphopoietic tissue of spleen.

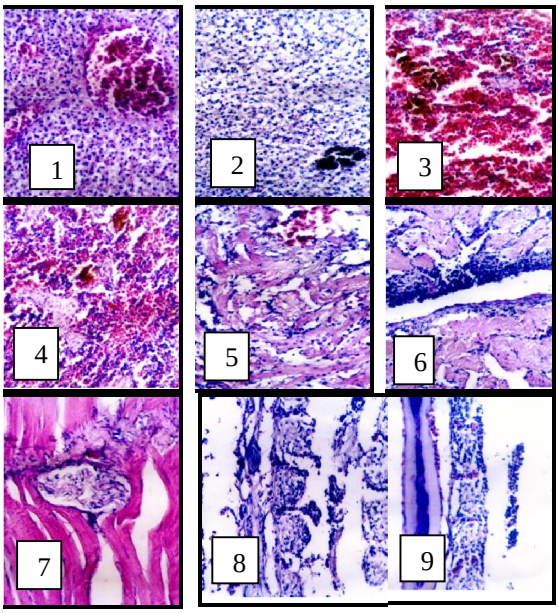

FIGURE 1. Liver from group 2 showing congestion of central veins and vacuolar degeneration of hepatocytes (H&E ×200).

FIGURE 2. Liver from group 2 showing vacuolar degeneration of hepatocytes (H&E ×200).

FIGURE 3. Spleen from group 1 showing marked hemorrhages and slight lymphocytic depletion (H&E ×200).

FIGURE 4. Spleen from group 2 showed marked heamorrhages associated with lymphocytic depletion (H&E×200).

FIGURE 5. Heart muscle from group 1 showed vacculation of some cardiac myositis as well as some leucocytic infiltrations (H&E ×200).

FIGURE 6. Heart muscle from group 2 showed focal necrosis of myocytes completely replaced by leucocytic infiltrations (H&E ×200).

FIGURE 7. Muscles of fish from group 2 showed focal myolysis replaced by fibronous connective tissue proliferation (H&E ×200).

FIGURE 8. Gills from group 2 showed necrosis of gill lamellae associated with leucocytic cell infiltration (H&E ×200).

FIGURE 9. Gills from group 2 showed clear lamellar oedema, focal necrosis associated with massive leucocytic infiltration (H&E ×200).

Table 1: Effect of orbifloxacin (50mg/ml) treatment on some plasmatic activities (AspAT and AlaAT) of catfish.

Treatment	Time post treatment	AlaAT	AspAT
	Infected	35±0.11	230±0.11
First stage	2days	30±0.2	126±0.1
(7 days)	4 days	31±0.11	230±0.3
	6 days	30±0.2	335±0.3
	8 days	28±0.1	231±0.1
	Infected	60±0.2	220±0.2
Second stage	2days	67±0.11	294±0.4
(15 days)	4days	126±0.1	1009±0.2
	6days	33±0.3	151±0.1
	8days	37±0.2	71±0.3
Control (Non infected treated)	-	30±0.5	353±0.1
Control (Non infected non treated)	-	17±0.2	143±0.2

*Significant at <0.05

5. Conclusion Orbifloxacin is potentially an effective antibacterial agent for the treatment of edwardsiellosis caused by *E. tarda* by water born method. In addition, control of orbifloxacin is much more successful when initiated at the earliest time of infection.

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4/1/2010

Optimization of microbial biomass production as biocontrol agent against root knot nematode on faba plants

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Abstract: Our objective was to optimize nutritional and environmental conditions of the isolated *Serratia marcescens* Ba-2 and *Pseudomonas fluorescens* Ba-11 for biomass production and to evaluate the bio-control agents against the root knot disease caused by *Meloidogyne incognita* on Faba bean plants under greenhouse conditions. Glycerol at 10.2 g/L and peptone as a nitrogen source were the most suitable for biomass and antagonistic efficiency of *S. marcescens* or *P. fluorescens* against *Meloidogyne sp*. Cultures of *S. marcescens* and *P. fluorescens* supplemented with 10 g/L peptone, reduced larvae to 91% and 95% respectively. Optimum biomass and antagonistic activity of either bacteria against larvae was at pH 7.6, and incubation temperature at 30°C. 100% reduction of larval density was achieved when *S. marcescens* or *P. fluorescens* cultures were shaken at 120 and 160 rpm respectively. *S. marcescens* and *P. fluorescens* were very effective as biocontrol agaents to reduce the root – knot nematodes. Our data also indicate a marked effect of the biocontrol agents and Rhizobia on the growth response of faba plants. The obtained results showed that both bacterial treatments significantly increased the growth parameters as well as shoot and root dry weights and number of pods. [Journal of American Science 2010; 6(6):245-255]. (ISSN: 1545-1003).

Keywords: Biological control, *Serratia marcescens*, *Pseudomonas fluorescens*, root-knot nematode, rhizobia.

1. Introduction

Root-knot nematodes are major pests that cause serious root damage of fruits and vegetables in temperate and tropical regions. Biological control of plant-parasitic nematodes with microbial agents is an alternative approach that received greater interest among nematologists in last decades, providing effective control against the target nematodes and avoiding environmental pollution.

In their review, Tian et al. (2007) stated that nematophagous bacteria exhibit diverse modes of action: these include parasitizing, producing toxins, antibiotics, or enzymes, competing for nutrients, inducing systemic resistance of plants, and promoting plant health.

Rhizobacteria are a subset of total rhizosphere bacteria which have the capacity, upon reintroduction to seed or vegetative plant parts (such as potato seed pieces), to colonize the developing root system in the presence of competing soil microflora (Kloepper et al. 1999). Fluorescent *Pseudomonas spp*. are among the most effective rhizosphere bacteria in reducing soil-borne diseases in disease – suppressive soils (Weller, 1988), where disease incidence is low, despite the presence of pathogens and environmental conditions conductive to disease prevalence. *Pseudomonas* isolates caused greater inhibitory effect on hatching and penetration of *M. incognita* than caused by isolates of *Bacillus* (Siddiqui et al., 2009). It was reported by Siddiqui et al. (2005) that *Pseudomonas fluorescens* CHAO mutant resulted in reduced biocontrol activity against the root-knot nematode *Meloidogyne incognita* during tomato and soybean infection. Exposure of root-knot nematode to culture filtrates of *P. fluorescens* under *in vitro* conditions significantly reduced egg hatch and caused substantial mortality of *M. javanica* juveniles (Siddiqui and Shaukat 2003). Ali (1996) found that the population density of nematode species was reduced by application of five bacterial isolates (*Arthrobacterium sp.*, *Bacillus sp.*, *Corynebacterium sp.*, *Serratia sp.*, and *Streptomyces sp.*). Reductions of nematode populations were ranged between 46% and 100%. Youssef et al. (1998) studied the potential of *Azotobacter chroococcum*, *Bacillus megatherium* and *Rhizobium lupine* for the control of *Meloidogyne incognita* infecting cowpea and tomato plants. They noticed number of both root galls and egg masses of *M. incognita* were decreased in soil treated with *Bacillus megatherium* and *Azotobacter chroococcum* except *Rhizobium lupine* treated soil. El-Sherif et al. (1999) studied the effect of culture filtrates of 5 isolates for their nematotoxic effect against plant parasitic nematode (*Bacillus sp. Corynebacterium sp., Serratia sp., Arthrobacterium sp.*, and *Streptomyces sp.*). The

authors determined the culture filtrate concentration as 0.1% to inhibit the hatching of the eggs and 0.6% to be highly toxic to the juveniles. The toxic effect of the filtrate varied with the different nematode species.

Our objectives were to investigate optimizing of nutritional and environmental conditions of the local isolates (*Pseudomonas fluorescens* Ba-11 and *Serratia marcescens* Ba-2) for biomass production. We aimed also to use the biomass greenhouse experiments as safe biological control agents of root-knot nematode disease.

2. Material and Methods

Bacterial strains, pathogen and plant:

Antagonists *Serratia marsescens* and *Pseudomones fluorescens* were previously isolated from a rhizosphere soil from a farm, in Cairo, Egypt. The isolates were identified by Kamel et al. (2009) and it were used as potential sources of antagonistic bacteria. *P. fluorescens* was cultured on King's medium (King et al., 1954), whereas *S. marsescens* was grown on nutrient broth media. Faba bean seeds (Giza 714) were obtained from the Agriculture Research Center, Giza, Egypt.

Nematode larvae extraction: Using Oosten brinks elutritor method (Goody, 1963).

Propagation of the selected bacterial isolates in shake flasks:

Conical flasks (250 ml capacity) containing 100 ml of nutrient broth medium were inoculated by one ml of the selected bacterial isolates, then incubated on GFL rotatory shaker (120 rpm) at 28°C for 48 h. Oxygen absorption rate (OAR) was 0.22 mMO_2/L/min. In these experiments different carbon and nitrogen sources were tested to study their effect on the growth of bacterial isolates and reduction percentage of population density of root-knot nematode larvae. The amount of nitrogenous and carbon compounds added to the propagation broth medium were calculated to give the final nitrogen and carbon concentration equal to 0.8 and 0.4 g/L respectively.

Potential antagonism of *Serratia marcescens* and *Pseudomonas fluorescens* against *Meloidogyne incognita* under greenhouse conditions.

Pot experiment was conducted to explore effectiveness of both *Serratia marcescens* and *Pseudomonas fluorescens* to reduce population density of root-knot nematodes larvae, under greenhouse conditions. Seeds of faba bean (Giza, 714) were sown in 30 cm pots containing autoclaved sandy loam soil (1:1). Five seeds were sown in each pot, then thinned to two plants/pot just 10 days after germination. Pots were divided into nine groups, each contained four replicates. Treatments were bio-agents of *Serratia marcescens* and *Pseudomonas fluorescens*, which were individually incorporated into the soil at dose rate of 20 ml/pot (10^9 cells/ml) every 10 days for four applications. *Rhizobium leguminosarum bv*. vaceae was used to inoculate faba bean seeds before planting using seed coating technique.

Meloidogyne incognita belongs to a group of nematodes that cause important crop losses in developing countries (Luc et al., (1990) and Sasser and Freckmann (1987). The present study as well as those of previous studies (Overbeek et al., 1997; Marschner et al., 1999; Tian et al., 2000; Shapiro Ilan et al., 2006

Pots were arranged in a complete randomized block design, watered and received the normal agricultural practices. Pots nematodes infested were received newly hatched second stage larvae of *M. incognita* at dose rate of 20 ml/pot (50 larvae/ml) after 10 days of planting.

Two months later, the plants in each pot were uprooted and the roots were gently separated from soil, washed with flow water and dried by pressing lightly between blotting paper. Average numbers of galls and rhizobia nodules were counted. Nematode larvae population density after harvest (Pf) were extracted from soil and counted using Oosten brink's elutritor technique. Reduction of nematodes population density in soil sample was calculated according to Tilton formula, as follows: Tilton formula =

$$\left(1-\frac{\text{Population density in the treated pot after application}}{\text{Population density in the treated pot before application}}\times\frac{\text{Population density of the control pot before application}}{\text{Population density of the control pot after application}}\right)\times 100$$

The growth response of faba bean (roots, shoot dry wt and number of pods/plant) were also recorded. Data were subjected to statistical analysis and means compared using the least significant difference (L.S.D. at P = 0.01).

Determinations of bacterial biomass dry weight: (White, 1954).

Determination of oxygen absorption rate (OAR): Cooper et al., (1944).

Determination of reducing sugars: Flood and Priestely method (1973).

Determination of glycerol: Was determined enzymatically in the fermented liquor using special kits according to Fossati & Prencipe (1982).

Statistical analysis calculations: Were achieved according to Gomez and Gomez (1984)

Results and Discussion

and Siddiqui et al., 2009) demonstrated that nutritional factors have great impact on growth and on the antagonistic activity of the antagonist against the pathogen. In the present study, *Serratia marcescens* and *Pseudomonas fluorescens* are capable of using different carbohydrates as a sole carbon source (Table 1). Maximum biomass and antagonistic efficiency were obtained when glycerol was used as a carbon source for both organisms. The percentage reduction in population density of nematodes larvae ranged from 91% to 93% when *S. marcescens* and *P. fluorescens* were grown on glycerol concentration of 10.2% and 12.2% respectively (Table 2). Our results agree with those reported by Daffy and Defago (1999) who found that the antagonistic agent *P. fluorescens* was stimulated by glycerol but were inhibited by glucose.

In the present study, organic nitrogen sources gave higher growth of both tested bacteria than the inorganic nitrogen sources (Table 3). Peptone and tryptone were the most effective sources, whereas, weak growth was obtained on ammonium acetate. Peptone also supported good level of antagonistic activity of *S. marcescens* and *P. fluorescens*, the reduction in population density of root-knot nematodes larvae of *Meloidogyne sp*. reached 90% and 94% respectively with peptone and 85% and 93.9% with tryptone-containing medium, respectively. These results could be attributed to NH^{3+} produced during the decomposition of peptone or tryptone which was the principal element responsible for the population density reduction of nematodes larvae. Similar results were reported by Walker, (1971) and Zavaleta et al., (1989), who reported that volatile substances produced by *S. marcescens* have capability to inactivate root-knot nematodes larvae. These nematotoxic volatile substances were produced by *S. marcescens* when the nitrogen source in the growth medium was organic in the form of amino group. Table 4 shows that the optimum biomass yields for both bacterial species were detected at 10 g/l peptone concentration. Increasing peptone concentration more than 10 g/l resulted in decreasing bacterial growth of *S. marcescens* and *P. fluorescens*. The highest percentage in nematode larvae by *S. marcescens* was obtained in peptone-containing medium at 6 g/1 whereas the nematicidal activity of *P. fluorescens* was recorded at 10 – 12 g/l peptone concentration. Increasing or decreasing the medium peptone concentration than 10 or 12 g/l, resulted in decreasing the reduction efficiency of the cells for larvae. This observation could be attributed to the number of viable cells as previously reported by Racke and Sikora, (1992), who found that the antagonistic activity of *Agrobacterium radiobacter* and *Bacillus sphaericus* against the nematode *Globodera pallida* was shown to be directly correlated with the number of colony forming units. Similarly results obtained by Weidenborner and Kunz (1993) revealed that the reduction of the concentration of peptone and yeast extract in the broth to 50 % increased the nematicidal activity of *P. fluorescens* to 70.8 %.

The data represented in Tables 5 and 6, show that the highest growth of the selected two strains was achieved in media buffered at pH 7.6. The same pH was also optimal for nematicidal activity of both tested bacterial strains. This is in accordance with the previous findings of Slininger and Shea - Wilbur (1995) who found that the antagonistic activity of *P. fluorescens* was very sensitive to the culture pH, and pH 7 was the optimum. Data in tables 5 and 6 show also that changing the pH value of the growth medium than pH 7.6 caused slight decrease in the efficiency of both tested strains to reduce nematodes larvae. These results could be attributed to the bacterial cells count, which was the principal element responsible for reducing the population density of nematodes larvae. Stirling and Sharma (1990) reported similar results, and they documented that increasing of bacterial cells number resulted in increasing numbers of cells attached to nematodes larvae.

Many authors demonstrated the importance of incubation temperature on growth and metabolic activities of *P. fluorescens* (Tu, 1994; Slininger et al., 1995) and *S. marcescens* (Pearson et al., 1997; Daffy and Defago, 1999). In this study, it has been found that good growth of either *S. marcescens* and *P. fluorescens* was obtained at incubation temperature ranged between 25°C to 35°C and the temperature 30°C could be optimal (Table 7). The results also clearly indicate that the efficacy of the bio-control agents was closely correlated with the suitable incubation temperature for the optimal bacterial growth. Sharp decrease in biomass yield and nematicidal activity was detected when either bacterial strains were incubated at 20°C or 40°C. These results are in line with those reported by Racke and Sikora (1992) who found that the antagonistic activities of *Agrobacterium radiobacter* or *Bacilltis sphericus* against *Globodera pallida* were directly correlated with the number of colony forming units. Significant reduction of root infection was recorded with bacterial density of $9.7x\ 10^9$.

Our study also represent that maximum biomass and nematicidal activity yield efficiencies of *S. marcescens* (Ba-2) and *P. fluorescens* (Ba-11) were achieved when medium volume to air ratio was 1:4 (Table 8). The increase in medium volume to air ratio has led to a decrease in cultural growth. This result agrees with that obtained by Yousten and Wallis (1987). Increasing of biomass dry weight and biomass yield efficiency with decreasing the medium volume

could be attributed to increase rate of oxygen absorption within the medium as reported by Karim et al., (1993). Similar results were obtained by Jaspe et al., (2000) on the effect of extra aeration on enzymatic activities and growth of *P. fluorescens*.

Table 9 elucidate the effect of oxygen absorption rate (OAR) on the biomass production of both *S. marcescens* and *P. fluorescens*. The reached data reveal that high efficiency (100%) of the tested strains to reduce the population density of nematodes larvae was achieved when *S. marcescens* (Ba-2) or *P. fluorescens* (Ba-11) were shaked on rotary shaker at 120 rpm (0.31 mMO_2/L/min.) and 160 rpm (0.52 mMO_2/L/min.), respectively. Accordingly, nutritional and environmental factors are needed to secure the optimal growth of both isolates, by culturing them on modified growth medium containing glycerol (10.2 g/1), peptone (10 g/1) and monobasic potassium phosphate (0.4 g/1) with pH value of 7.60 at 30°C for 48 h. To achieve maximum growth of *S. marcescens*, it should be aerated with 0.31 mMO_2/L/min. (OAR) within the growth medium (rotary shaker at 120 rpm, with working volume 50 ml) while, the optimal growth of *P. fluorescens* required 0.52 mMO_2/L/min on a rotary shaker at 160 rpm and the medium occupied 20 % of the flask.

Studies were conducted under greenhouse conditions to evaluate the biological control potential of *P. fluorescens* and *S. marcescens* as a soil treatment against *Meloidogyne incognita* infesting faba bean. Results in Table 10 indicate that populations of the nematode *Meloidogyne incognita* were affected by application of both bacterial isolates. The suppressive effect on the number of juveniles ranged between 77.2 % and 84.4 % during the growing season. The infectivity of the nematode was greatly affected in the presence of any of the bioagents, the number of galls per root system were significantly decreased, more prominently in pots inoculated with *Rhizobium leguminosarum*. The tested biocontrol agents not only reduced the infectivity of nematodes but also increased the number of rhizobia nodules on the root system.

These results are in a line with those reported by Zavaleta et al. (1989), who reported potentiality of *S. marcescens* to suppress root-knot larvae of *M. incognita*, they attributed this effect to the volatile substances produced during its metabolic activity. Eklund (1970) and Tian et al. (2007) confirmed that Pseudomonads, are natural inhabitants on the root surface and primary consumers of root exudates rich in amino acids which are converted to ammonia along the root to maintain a micro-zone around the growing roots that would be suppressive to pathogens. The reduction of root galls number may be due to that the majority of the encumbered juveniles were not able to penetrate the host root. Davis et al. (1988); Stirling and Sharma, (1990) and El-Nagar et al. (1998), reported that *Bacillus penetrans* not only prevents reproduction of the root-knot nematodes but also reduces the infectivity of the juveniles. Zaki et al. (2009) reported that *Pseudomonas* isolates caused greater effect on hatching and penetration of *M. incognita* that caused by isolates of *Bacillus*.

A limited number of bacterial spp. have been reported as biocontrol agents for nematode diseases. The bacterium *Bacillus penetrans* (Sayre, (1988); Brown et al. (1985a); Davis et al. (1988); Stirling and Sharma, (1990); Abou-Eid et al. (1997) and El-Nagar et al. (1998) and *Pasteura penetrans* Daudi et al. (1990); Oostendorp et al. (1991) and Liu et al. (1995) were recognized as antagonistic to phyto-nematodes including root-knot nematodes. Entomopathogenic strains of *S. entomophila* were used as a bio-control in New Zealand as they induce inhibition of larval feeding activity and larval death 1-3 months from the on set of infection (O'Callaghan and Jackson, (1993) and Villalobos et al. (1997)).

Results in Table 10 show an increase in the number of root galls in the pots group of *Meloidogyne + Rhizobia* treatment in comparison with the control treatment. El-Bahrawy and Salem (1989), concluded that *Rhizobia* had stimulatory effect on *M. incognita* infecting broad bean. On the other hand, our results showed a 54.35 % reduction in rhizobia nodules in comparison with control. This reduction could be attributed to the deleterious effect of *M. incognita* on the development of rhizobia nodules, or to the interference of root-knot nematodes with nitrogen fixation in legume hosts treated with *Rhizobium* (Sharma and Sethi, 1976 and Chahal and Chahal, (1987)).

Treating pepper seedlings, wheat plants, vitis vinifera, rice, potato roots or tomato plants with isolates of *P. fluorescens* reduced the nematode damage caused by *M. incognita* (Eapen et al. (1997); Brimercombe et al. (1999); Shanthi et al. (1998); Ramakrishnan et al. (1999); Mani et al. (1998) and Duponnois et al. (1999)).

Ali, (1996) and Mercer et al. (1992) suggested that chitinase of *S. marcescens* or *P. fluorescens* strains caused premature hatch of nematode eggs and could be used as an aid in the control of nematodes on sunflower plant.

Regarding the effect of bacterial agents on faba bean growth, the obtained results in Table 11 showed that all bacterial treatments significally ($P < 0.05$ and 0.01), increased the plant growth parameters

as compared with the control treatment (Mi, treatment) while. Mi + Rh treatment failed to cause significant increase. Results also, indicate that there was a marked effect of biocontrol agents (*S. marcescens* (Ba-2) or *P. fluorescens* (Ba-11)) and Rhizobia on the growth response of faba bean in either nematodes free pots or nematodes infested pots compared with that of control (Rh-treatment). Thus the application of *S. marcescens* resulted in increasing shoot, root dry weights and number of pods up to 28.40, 43.64 and 32.14 % over Rh-treatment, respectively. The corresponding figures for *P. fluorescens* were 46.80, 77.27 and 45.24 %, respectively.

The growth promotion of faba bean observed in the present study (Table 11) may be attributed to those bio-agents may benefit plant growth by providing growth factors or regulators or by producing toxic metabolites which may inhibit nematodes and exclude other deleterious microorganisms. The ability of *S. marcescens* and *P. fluorescens* isolated from soil and rhizosphere to produce biologically active compounds have been reported by several investigators (Daffy and Defago 1999; Siddiqui and Shaukat, 2003; Shapira-Ilan *et al*. 2006 and Burkett-Cadena *et al*. 2008 and others).

Consequently, the introduction of such bacteria in soils, or cultural practices aimed to increase the activity of native strains of these bacteria could greatly contribute to the efficiency of nematode biocontrol with *S. marcescens* and *P. fluorescens*.

Table 1. Effect of different carbon sources on the bacterial biomass of *S. marcescens* or *P. fluorescens* on *Meloidogyne sp. Larvae* (I , *S. marcescens*; II, *P. fluorescens*).

Carbon sources	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
Glucose	2.20	2.10	7.58	7.56	9.20	9.40	23.90	22.30	89	90
Fructose	1.70	2.10	7.10	7.57	9.00	9.20	18.90	22.80	89	90
Sucrose	2.00	2.40	7.57	8.12	8.90	8.90	22.47	26.90	89	93
Galactose	2.00	2.10	7.50	7.57	8.50	8.60	23.50	24.40	89	83
Xylose	0.70	1.20	6.89	6.30	7.30	7.30	09.58	16.40	79	83
Manitol	2.00	1.60	7.62	6.91	8.50	8.90	23.52	17.90	89	83
Maltose	1.60	2.00	7.00	7.45	7.90	9.50	22.80	24.20	89	90
Mannose	2.10	2.00	7.57	7.31	9.00	9.60	23.30	20.80	89	90
Raffinose	0.60	0.80	6.658	6.10	7.10	7.30	08.46	21.90	70	79
Glycerol	2.60	2.70	8.72	8.73	9.30	9.40	27.90	28.70	89	93

Table 2. Effect of different concentrations of glycerol on the bacterial biomass and antagonist of *S. marcescens* or *P. fluorescens* on *Meloidogyne sp. Larvae* (I , *S. marcescens*; II, *P. fluorescens*).

Glycerol conc. (g/L)	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
2.20	0.20	0.40	5.35	5.44	2.00	2.20	10.00	18.20	80	80
4.20	0.60	0.80	5.35	6.16	4.00	4.00	15.00	20.00	80	80
6.20	1.00	1.30	6.25	7.35	6.00	6.10	16.60	21.30	80	90
8.20	2.00	1.85	7.43	7.95	8.00	7.90	22.20	23.40	89	90
10.20	2.65	2.55	8.62	8.73	9.90	8.70	26.80	29.30	91	93
12.20	2.60	2.60	8.62	8.72	11.88	11.20	14.16	22.30	91	93
14.20	1.96	2.60	7.22	8.64	13.83	11.70	12.80	21.40	89	93

16.20	1.80	2.40	7.15	8.63	14.00	13.70	11.24	17.50	89	93
18.20	2.00	2.40	7.45	8.63	17.80	16.20	9.570	15.70	89	93
20.20	1.90	2.40	7.25	8.64	19.85	16.60	9.572	14.50	89	93

Table 3. Effect of different organic and inorganic nitrogen sources on the bacterial biomass and antagonistic effect of *S. marcescens* or *P. fluorescens* against larvae of *Meloidogyne sp* (I , *S. marcescens*; II, *P. fluorescens*).

Nitrogen source	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
Organic sources										
Urea	1.44	1.90	7.45	7.752	9.96	9.20	14.46	20.65	13.20	23.00
Peptone	2.54	2.88	8.81	8.712	10.00	10.00	25.40	28.80	90.00	94.00
Yeast extract	1.12	0.85	7.18	6.151	9.63	8.35	11.63	10.08	29.00	15.80
Tryptone	1.90	2.55	7.75	8.011	9.76	9.91	19.40	25.73	85.00	93.90
Amm. acetate	0.05	0.06	4.12	3.544	3.40	3.00	01.47	2.00	5.00	3.00
Inorganic sources										
Amm. chloride	0.60	0.70	6.650	6.892	8.04	7.30	7.40	9.60	14.00	10.00
Amm. sulphate	0.50	0.57	6.215	6.541	8.35	8.35	6.00	6.82	10.00	13.00
Amm. phosphate	0.62	0.80	6.711	6.910	9.85	8.00	6.29	10.00	13.90	11.00
Amm. nitrate	0.70	0.70	6.875	6.890	7.70	9.00	9.09	7.77	7.00	9.80
Potassium nitrate	1.20	0.65	7.115	6.710	9.85	8.85	12.18	7.34	15.00	26.00

Table 4. Effect of different peptone concentrations on the bacterial biomass and antagonistic effect of *S. marcescens* or *P. fluorescens* against larvae of *Meloidogyne sp*, (I , *S. marcescens*; II, *P. fluorescens*).

Peptone concentrations (g/L)	Biomass dry wt. (g/L)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
2	0.790	0.880	6.810	6.961	8.350	9.850	9.460	8.930	80	80
4	0.880	1.500	6.950	7.430	8.290	8.980	10.610	16.700	80	93
6	0.980	1.900	7.121	7.830	9.000	8.390	10.890	22.650	90	90
8	1.590	1.900	7.610	7.850	9.500	8.050	16.740	23.600	90	90
10	2.520	2.700	8.810	8.680	10.000	9.040	25.200	29.800	90	95
12	2.520	2.300	8.810	8.540	10.000	9.700	25.200	23.710	89	95
14	2.350	1.700	8.711	7.462	9.900	9.790	23.740	17.360	90	90
16	2.480	1.900	8.730	7.753	9.800	9.770	25.310	19.450	91	91
18	2.530	1.650	8.810	7.431	10.000	9.900	25.300	16.670	91	90
20	2.500	1.850	8.771	7.621	10.000	9.800	25.000	18.880	91	90

Table 5. Effect of the growth medium pH values on the biomass production of *S. marcescens* and antagonistic effect on *Meloidogyne* larvae.

Medium pH value		Biomass dry wt. g/l	Bacterial cells count (log no./ml)	Sugar consumption g/l	Biomass yield efficiency %	Nematode larvae reduction %
Initial	Final					
6.00	7.80	1.29	6.35	9.78	13.19	80
6.20	8.22	1.38	6.41	9.80	14.08	80
6.80	8.00	1.85	7.32	9.78	18.92	85
7.20	7.30	2.42	8.62	9.78	24.74	90
7.60	7.80	2.65	9.09	9.81	27.01	95
7.80	7.71	2.00	7.32	9.94	23.14	85
8.20	8.30	1.65	7.21	8.50	19.41	85
8.40	7.80	1.31	6.71	8.00	16.25	80
8.60	7.70	1.45	6.43	7.70	18.83	80

Table 6. Effect of the growth medium pH values on the biomass production of *P. fluorescens* and antagonistic effect on *Meloidogyne* larvae.

Medium pH value		Biomass dry wt. g/l	Bacterial cells count (log no./ml)	Sugar consumption g/l	Biomass yield efficiency %	Nematode larvae reduction %
Initial	Final					
6.00	7.40	7.32	6.55	8.30	15.90	80
6.20	7.13	1.39	6.45	8.40	11.50	80
6.80	7.51	2.03	7.85	8.30	24.45	88
7.20	7.58	2.62	8.71	9.00	27.58	95
7.60	7.42	2.85	9.21	9.00	31.67	100
7.80	7.68	2.35	8.32	8.30	27.65	92
8.20	7.66	2.00	7.41	8.30	24.09	88
8.40	7.78	1.05	6.32	8.00	13.13	81
8.60	7.80	1.00	6.21	7.60	13.16	82

Table 7. Effect of different incubation temperatures on the biomass production of *S. marcescens* or *P. fluorescens.* (I , *S. marcescens*; II, *P. fluorescens*).

Incubation temperature °C	Biomass dry wt. (g/l)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
20	0.82	0.75	6.92	6.74	8.95	8.78	9.16	8.54	80	80
25	2.45	2.65	8.63	8.79	9.80	9.42	25.00	28.13	90	90
30	2.65	2.85	9.09	9.31	9.00	10.00	29.40	28.50	95	100
35	2.55	2.31	8.74	8.55	9.75	9.45	26.15	24.44	86	89
40	1.23	1.57	6.12	6.61	8.50	8.75	14.47	17.26	70	76

Table 8. Effect of different medium working volumes on the biomass production of *S. marcescens* or *P. fluorescens.* . (I , *S. marcescens*; II, *P. fluorescens*).

Medium working volume (ml)	Biomass dry wt. (g/l)		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II
25	2.03	2.50	7.85	8.74	9.10	9.00	22.31	27.78	88	95
50	2.95	3.20	9.22	9.42	9.35	9.40	31.55	34.04	100	100
100	2.66	2.81	9.09	9.21	9.81	9.31	27.01	30.18	95	100
150	1.84	1.80	7.33	7.32	7.85	7.25	23.44	24.83	84	81

Table 9. Effect of different speed of shaking on the biomass production of *S. marcescens* or *P. fluorescens.* (OAR, Oxygen Absorption Rate ; I , *S. marcescens*; II, *P. fluorescens*).

Shaking speed (rpm)	(OAR) $mMO_2/L/min$		Biomass dry wt. g/l		Bacterial cells count (log no./ml)		Sugar consumption (g/L)		Biomass yield efficiency (%)		Nematodes larvae reduction (%)	
	I	II	I	II	I	II	I	II	I	II	I	II
80	0.18	0.18	2.75	2.74	8.82	8.80	9.04	9.50	30.42	28.84	90	90
120	0.31	0.31	3.45	2.91	9.73	9.51	9.15	10.00	37.70	29.10	100	100
160	0.52	0.52	1.95	3.60	7.45	10.22	8.25	10.00	23.64	36.00	90	100

Table 10. Extended effect of *S. marcescens* or *P. fluorescens* on the root-knot nematode *Meloidogyne incognita* infecting faba bean, under greenhouse conditions. (Mi, *Meloidogyne incognita;* S., *Serratia marcescens*; P., *Pseudomonas fluorescens*; Rh, *Rhizobium leguminosarum;* Pi, initial population density of nematodes in siol,; Pf, nematodes population density at harvest).

Treatments	**Nematodes population density in soil**			**No. of galls/roots system**		**No. of rhizobia nodules/roots**		
	Pi	**Pf**	**Reduction %**		**Reduction %**		**Reduction %**	**Increase %**
Mi+S.+Rh	1000	950	77.2	27	74.04	53	0	15.22
Mi+P.+Rh	1000	830	80.1	23	77.9	61	0	32.61
Mi+S.	1000	750	82.0	18	82.7	0	0	0
Mi+P.	1000	650	84.4	10	90.4	0	0	0
Mi+Rh	1000	3900	0	115	0	21	54.35	0
S.+Rh	0	0	0	0	0	56	0	21.75
P.+Rh	0	0	0	0	0	67	0	45.7
Mi	1000	4100	0	104	0	0	0	0
Rh	0	0	0	0	0	46	0	0
LSD 0.01				16.6		13.65		
0.05				12.2		9.87		

Table 11. The growth response of faba bean as affected by different treatments of *S. marcescens* or *P. fluorescens* on the root-knot nematodes. (Mi, *Meloidogyne incognita;* S., *Serratia marcescens*; P., *Pseudomonas fluorescens*; Rh, *Rhizobium leguminosarum*).

Treatments	Nematodes population density in soil			No. of galls/roots system		No. of rhizobia nodules/roots		
	Pi	Pf	Reduction %		Reduction %		Reduction %	Increase %
Mi+S.+Rh	1000	950	77.2	27	74.04	53	0	15.22
Mi+P.+Rh	1000	830	80.1	23	77.9	61	0	32.61
Mi+S.	1000	750	82.0	18	82.7	0	0	0
Mi+P.	1000	650	84.4	10	90.4	0	0	0
Mi+Rh	1000	3900	0	115	0	21	54.35	0
S.+Rh	0	0	0	0	0	56	0	21.75
P.+Rh	0	0	0	0	0	67	0	45.7
Mi	1000	4100	0	104	0	0	0	0
Rh	0	0	0	0	0	46	0	0
LSD 0.01				16.6		13.65		
0.05				12.2		9.87		

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Resistin and Obesity- Associated Insulin Resistance in Children

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Abstract: Obesity, defined as excess body fat, is frequently accompanied by insulin resistance. It was hypothesized that resistin links obesity with insulin resistance and diabetes, however, debate exists about its possible role. The aim of this study was to measure serum resistin level in obese non diabetic children as well as to evaluate insulin resistance in them. It also aimed at exploring the possible correlation between serum resistin level, anthropometric, clinical and laboratory parameters in obese children. This study is a cross sectional study that comprised 45 children and adolescents with simple exogenous obesity and 30 apparently healthy non-obese age and sex matched children as control group. For each subject the following was performed: history taking, anthropometric measurements including body weight, height, BMI, waist circumference, hip circumference, waist hip ratio, skin folds thickness measurements (biceps, triceps, subscapular and suprailiac) and calculation of body fat. Clinical examination and pubertal assessment were performed. Laboratory investigations including fasting serum glucose, fasting serum insulin and resistin using ELISA technique. Insulin resistance was estimated by using the Homeostasis Model Assessment (HOMA). Serum resistin levels did not significantly differ between cases (6.7 ng/ml ±3.44) and (6.6 ng/ml ±2.47), ($p>0.05$). Fasting insulin and HOMA were significantly higher in obese children than controls, ($p < 0.001$ for both). About 78% of obese children had insulin resistance (high HOMA), 66.7% had high fasting insulin, 13.3% high resistin, 31.1 % had acanthosis nigricans and 8.9% had hypertension. A significant positive correlation was found between serum resistin levels and each of fasting insulin and HOMA, ($p<0.001$ for both). No significant correlation was found between serum resistin, HOMA and each of BMI, body fat percentage & waist circumference, ($p>0.05$). A significant positive correlation was found between BMI and each of waist circumference and systolic blood pressure, ($p< 0.001$ & < 0.05respectively). The present study confirm the link between resistin level and insulin resistance in obese children, however it couldn't prove whether high or low resistin level is more related to insulin resistance. A significant positive correlation was found between serum resistin levels and each of fasting insulin and HOMA .No significant correlation was found between serum resistin, HOMA and each of BMI, body fat percentage & waist circumference. HOMA was found to be a significant marker for early detection of insulin resistance in obese and overweight children. [Journal of American Science 2010;6(6):256-266]. (ISSN: 1545-1003).

Keywords: Resistin- insulin- insulin resistance- HOMA- obesity- children- acanthosis nigricans.

1. Introduction

Obesity is a substantial public health crisis in the developed world and the prevalence is increasing rapidly worldwide in numerous developing nations. This growing rate represents a pandemic that needs urgent attention if its potential mortality and economic tolls are to be avoided. (1). Obesity is particularly alarming in children and adolescents, thus passing the epidemic into adulthood and creating a growing health burden for the next generation. (2) Childhood obesity is associated with substantial co-morbidity and late sequelae, including type 2 diabetes, hypertension, liver disease and cardiovascular complications. (3, 4)

The most common underlying cause of insulin resistance is central obesity. (5) Excess abdominal adipose tissue has been shown to release increased amounts of free fatty acids which directly affect insulin signaling, diminish glucose uptake in muscle, drive exaggerated triglyceride synthesis and induce gluconeogenesis in the liver. (6) Although an accelerated atherogenic process is present, the clinical cardiovascular lesions appear later. (7)

Insulin resistance may be implicated in the development of many pathological states, such as hypertension, type 2 diabetes mellitus, lipodystrophies, polycystic ovary syndrome and chronic infection. (8, 9)

Resistin, discovered in 2001, is a novel adipocyte-secreted factor that has been proposed to be the link between obesity and insulin resistance. (10) The association between resistin and obesity induced insulin resistance could be supported by the fact that: resistin expression is 15 folds higher in visceral fat than subcutaneous fat, visceral fat is considered as the major risk factor for insulin resistance and decreased insulin sensitivity. (11)

However, the role of resistin in the pathophysiology of obesity and insulin resistance in humans is still controversial. Several studies have shown positive

correlations of circulating resistin levels with BMI and waist circumference, (12, 13) as well as insulin resistance. (14, 15). However, other studies found no such relationship. (16, 17, 18) These controversial results may reflect variations in the study design and the lack of adjustment for potential confounding factors. Therefore, further studies are needed to define the relationship of resistin to obesity associated insulin resistance. (19**)**

The aim of this study was to evaluate resistin levels in obese non diabetic children. It also aimed at exploring the possible correlation between serum resistin level, anthropometric, clinical and laboratory parameters in them.

2. Material and Methods

This study is a cross sectional study that comprised 45 obese children and adolescents (20 males and 25 females) in the age range (7-15 years), attending the outpatient clinic of the Diabetic, Endocrine and Metabolic Pediatric Unit (DEMPU), Abo-Elrish Hospital, Cairo University during the period from January to May 2007. They fulfilled the inclusion criteria of having a BMI exceeding the 95th percentile of the same gender and age according to the Egyptian Growth Charts, (2002) and simple exogenous obesity. All cases had high waist circumference exceeding the 95th percentile according to the British percentiles. Thirty apparently healthy non-obese age and sex matched children (17 males and 13 females) were recruited, as control group, from the general pediatric outpatient clinic, Abo-Elrish Hospital, Cairo University. The protocol was approved by the ethics committee in the National Research Center and Abo-Elrish children Hospital, Cairo University and a written informed consent was obtained from each child's parents.

For each subject the following was performed:

- History taking including personal history, past history for systemic diseases, drug administration (corticosteroids), and family history (obesity, diabetes& hypertension).
- Anthropometric measurements including body weight, height, BMI calculation and evaluation according to the Egyptian Growth Charts (2002), [BMI = Weight (kg)/ Height (m^2)], waist circumference, hip circumference, calculation of waist hip ratio and skin folds thickness measurements (biceps, triceps, subscapular and suprailiac).Waist circumference values were plotted on the waist circumference percentile curves for British children (which are used for Caucasian children). (20) Calculation of body fat was done by plotting values of skin fold thickness on the British sex-specific percentile curves for body fat. (21)
- Clinical examination to confirm the diagnosis of simple obesity and to exclude signs & symptoms of acute or chronic inflammation and systemic diseases (bronchial asthma, autoimmune diseases, Cushing disease and hypothyroidism) as well as the presence of Acanthosis Nigricans. Pubertal assessment according to Tanner Staging was performed.

Laboratory Investigations:

Five cc of venous blood were drawn aseptically then left to clot. The separated serum was stored at -80^{o}C until analytic measurement of serum insulin and resistin were performed, except for glucose which was determined immediately after blood was drawn.

Fasting serum glucose:

Serum glucose was measured with glucose oxidase using a Hitachi autoanalyzer.

Stanbio Enzymatic glucose procedure No.1075; a single reagent glucose method based on a technique described by Trinder, (1959). (22)

Fasting serum insulin using ELISA technique:

The BioSource INS-EASIA (manufactured by Europe S.A. - Rue de l'Industrie, 8 - B-1400 Nivelles – Belgium) is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplates. It is an Immunoenzymetric assay for the in vitro quantitative measurement of human Insulin (INS) in serum and plasma. Results of the samples are determined using the standard curves. Normal range 5-19µIU/ml. (23)

Serum resistin level using ELISA technique:

Resistin was measured by an enzyme linked immunoassay kit obtained from the BioVendor'laboratory Medicine,Inc ,Palackeho(Czech Republic). Normal range is 6.6-12 ng/ml. (24)

Insulin resistance was estimated by using the Homeostasis Model Assessment (HOMA) which will be calculated according to the formula:

HOMA=

$$\frac{\text{Fasting serum insulin(IU/ml) X Fasting serum glucose (m mol/L)}}{22.5}$$

(Insulin resistance being defined as a HOMA index > 3.16) The greater the HOMA value the greater the level of insulin resistance. (25)

Statistical Analysis:

Data analysis was done using SPSS version 15. For comparing between two means, Student t-test of significance was done while one way analysis of variance was used to compare between more than two means. The Chi-square test of significance was used to compare frequency between two categorical variables. Correlation analysis using Pearson test was performed between different quantitative variables. P value less than 0.05 was considered significant.

3. Results

This study included 45 obese children: 20 males (44.4%), 25 females (56.6%) with a mean age of 9.5 years and 30 non obese control children: 17 males (56.7%), 13 females (43.3%) with a mean age of 8.6 years. There was no significant statistical difference in between cases and controls as regards sex and age. All cases had BMI, percentage of body fat and waist circumference exceeding the 95th percentile.

There was no significant statistical difference in between cases with +ve family history of obesity, diabetes and hypertension and those with –ve family history as regards anthropometric measurements, clinical data and laboratory parameters.

A statistically significant difference was found between cases and controls as regards anthropometric, clinical and laboratory parameters except for systolic blood pressure and resistin levels (Table 1). The percentages of high fasting insulin, high HOMA and high resistin levels were significantly higher in cases compared to controls (Figure 1).

A comparison among cases according to different anthropometric, clinical and laboratory parameters is displayed in Table (2). This study revealed that resistin and insulin resistance were independent of age and pubertal stage.

The correlation between the different parameters in cases revealed a significant positive correlation between resistin level and each of fasting insulin and HOMA as well as between BMI and each of waist circumference and systolic blood pressure (Table 3)

Figure 2 & 3 show the correlation between resistin and each of fasting insulin and HOMA in cases.

Only 4 cases (8.9%), 3 females and one male showed high systolic and diastolic blood pressure for age and sex according to the age-specific percentiles of blood pressure (BP) measurements for boys and girls, while none of the controls were hypertensive. The four hypertensive cases had high HOMA and low serum resistin level, 3 of them had high fasting insulin and positive Acanthosis Nigricans. Only one patient gave positive family history of obesity and diabetes.

By grouping cases according to presence of high HOMA, Acanthosis Nigricans and altered serum resistin levels we found that:

- Twenty cases had high HOMA and low serum resistin level, among them, 8 cases had Acanthosis Nigricans.
- Six cases had high HOMA and high serum resistin level, among them, 2 cases had Acanthosis Nigricans.

Table 1. Anthropometric, clinical and laboratory data among cases and controls (*, Significant p value; †BMI, Body Mass Index; ‡BP, Blood pressure; †HOMA, Homeostasis Model Assessment)

	Cases n=45		Controls n=30		p value
	Mean ± SD		Mean ± SD		
BMI (Kg /m^2)†	31.32	5.59	16.9	2.34	0.000*
Waist circumference (cm)	89.54	12.22	55.65	5.43	0.000*
Waist hip ratio	0.93	0.50	0.8	0.06	0.000*
Body fat %	40.6	3.84	18.4	3.39	0.000*
Systolic BP (mmHg)‡	109.0	11.41	107.5	4.31	0.494
Diastolic BP (mmHg)‡	69.9	7.19	64.83	4.64	0.001*
Fasting glucose (mg/dl)	90.93	9.10	86.40	9.38	0.039*
Fasting Insulin(µIU/ml)	23.98	10.61	15.96	4.61	0.000*
HOMA†	5.40	2.54	3.4	0.99	0.000*
Resistin(ng/ml)	6.74	3.44	6.62	2.47	0.871
Puberty (No-%)					0.002*
• Pubertal	34	75.6	12	40	
• Pre-Pubertal	11	24.4	18	60	
Acanthosis nigricans (No-%)					0.001*
• Present	14	31.1	0	0	
• Absent	31	68.9	30	100	

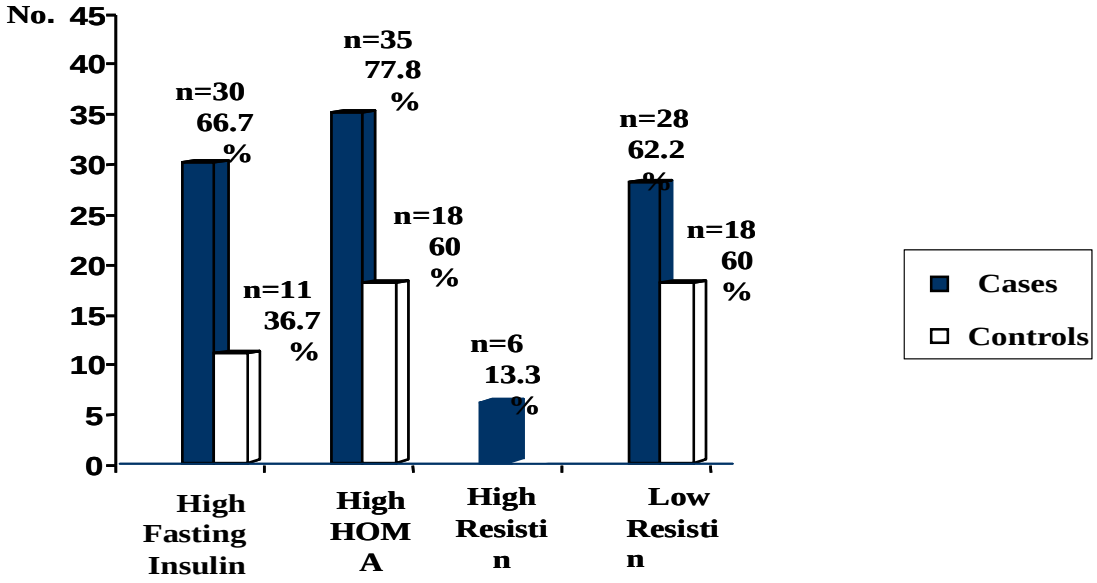

Figure 1. Frequency of abnormal laboratory parameter values among cases and controls (High HOMA > 3.16; Low resistin level < 6.6ng/ml; High fasting insulin level > 19µIU/ml; High resistin level >12ng/ml)

Table 2. A comparison among cases according to different anthropometric, clinical and laboratory parameters (**, p< 0.001; *, p< 0.05; € , p< 0.05 between low & normal resistin; © , p< 0.05 between high & normal resistin; †BMI, Body Mass Index; ‡ BP, Blood pressure; ||HOMA, Homeostasis Model Assessment)

	Insulin		HOMA		Resistin			Acanthosis nigricans	
	> 19µIU/ml n=30	< 19µIU/ml n=15	>3.16 n=35	<3.16 n=10	Low <6.6ng/ml n=28	High >12ng/ml n=6	Normal 6.6-12ng/ml n=11	Present n=14	Absent n=31
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
BMI (Kg/m^2)†	32.09±6.39	29.77±3.15	31.9±6.13	29.3±2.31	31.72±6.67	31.95±4.47	29.93±2.16	35.21± 7.24	29.55±3.59**
Waist circumference(cm)	89.2±13.38	90.23±9.89	89.7±13.08	89.1±9.15	89.75±13.89	90.75±12.05	88.36±7.81	96.64±13.05	86.33±10.53**
Waist / hip ratio	0.93±0.55	0.95±0.34	0.93±0.05	0.94±0.04	0.93±0.05	0.93±0.39	0.94±0.06	0.92±0.060	0.93±0.044
Body fat %	39.59±4.1	42.78±2.08*	40.13±4.12	42.46±1.85	40.81±3.98	41.15±2.06	39.97±4.39	38.95±3.40	41.41±3.83
Systolic BP(mmHg)‡	108.17±10.8	110.67±12.8	109.5±12.45	107.0±6.75	110.36±13.39	107.5±9.87	106.36±5.05	112.5±13.69	107.41±10.07
Diastolic BP(mmHg)‡	69.33±7.28	71.0±7.12	70.1±7.52	69.0±6.15	70.35±8.26	69.16±7.35	69.09±3.75	72.50±8.26	68.70±6.45
Fasting glucose(mg/dl)	92.43±8.39	87.93±10.0	91.7±8.01	88.1±12.28	90.46±6.77	93.83±6.21	90.54±14.67	94.21±8.45	89.45±9.12
Fasting insulin(µIU/ml)			27.7±8.91*	11.1±3.71**	34.03±8.98€	30.26±12.38©	21.32±10.88	24.73±8.42	23.64±11.57
Resistin(ng/ml)	7.3±3.78	5.63±2.36	7.21±3.72	5.1±1.26				7.05±4.08	6.60±3.17
HOMA ||	6.64±2.08	2.8±0.86**			7.82±2.00€	6.70±2.86©	4.69±2.90	5.67±1.93	5.22±2.79

Table 3. r values of the correlation between the different parameters among cases and controls (*, p<0.05; **, *, p<0.001; r, Corrélation coefficient)

	HOMA [r]		Resistin [r]		Fasting insulin [r]		BMI [r]	
	Cases n=45	**Controls n=30**	**Cases n=45**	**Controls N=30**	**Cases N=45**	**Controls N=30**	**Cases N=45**	**Controls N=30**
BMI	0.20	0.07	-0.02	-0.16	0.19	0.36		
Body fat%	-0.28	-0.01	0.10	-0.07	-0.26	**0.52****	-0.21	0.32
Waist circumference(cm)	-0.25	0.12	-0.03	-0.12	0.06	0.18	**0.84****	**0.74****
Waist /hip	-0.25	-0.17	-0.01	0.21	-0.29	-0.13	-0.09	**-0.49****
Systolic BP (mmHg)†	-0.01	0.27	-0.16	-0.05	-0.05	0.29	**0.30***	0.30
Diastolic BP (mmHg)	-0.09	0.04	-0.13	-0.03	-0.13	0.01	0.25	**0.43***
Fasting glucose(mg/dl)	0.32*	0.34	0.21	-0.13	0.14	-0.12	0.12	0.23
Fasting insulin(µIU/ml)	**0.98****	**0.57***	**0.42****	0.09			0.19	0.36
Resistin(ng/ml)	**0.45****	**0.43***			**0.42****	0.09	-0.02	-0.16
HOMA‡			**0.45****	0.04	**0.98****	a	0.20	0.10

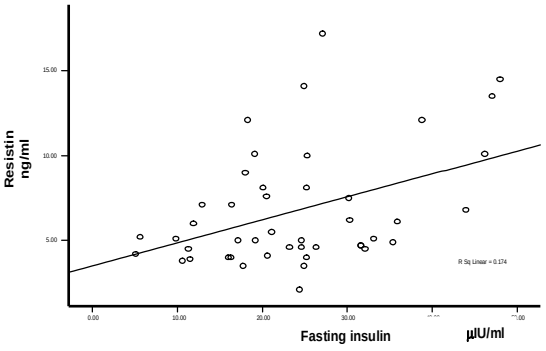

Figure 2. Correlation between serum resistin level and fasting insulin in cases (r = 0.42; p< 0.001)

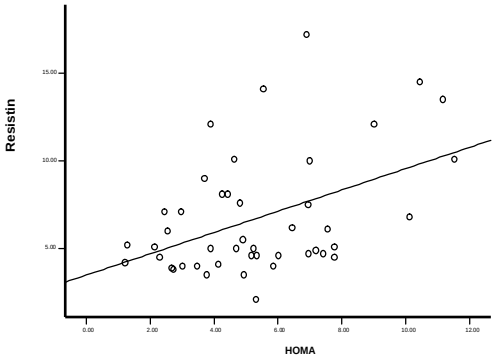

Figure 3: Correlation between serum resistin level and HOMA in cases. (r = .45 P< 0.001)

Discussion

Although obesity, defined as excess body fat, is frequently accompanied by insulin resistance, the molecular basis for the link between obesity and insulin resistance has not yet been clarified. (9) Identifying IR in children may be of substantial clinical importance and has been proposed as a strategy for identifying high-risk children for targeted diabetes prevention interventions. (26)

The 95th percentile of BMI was chosen as a cut off level for obesity as this is the agreed upon level incriminated with insulin resistance and cardiovascular risk. (27) Although waist circumference, being an index for central or visceral obesity, is more related to insulin resistance and its health consequences, (28, 29) we could not use it as an inclusion criterion for enrollment of cases because we don't have norms for our country.

Many studies stressed the importance of family history of diabetes, hypertension and obesity in assessment of health risks in obese children. (30) However, in the present study, despite of having +ve family history of obesity (53.3%), type-2 diabetes (35.6%) and of hypertension (28.9%) among obese children there was no significant statistical difference in between cases with +ve family history and those with –ve family history as regards anthropometric measurements, clinical data and laboratory parameters. Similarly, Goran et al., (2003) showed no influence of positive family history of type 2 diabetes mellitus on fasting glucose , insulin level and insulin resistance in a group of pre-pubertal obese children. (31)

It was hypothesized that resistin links obesity with insulin resistance and diabetes, but this has not been studied in children and adolescents to date. (18) In the present study the resistin levels did not significantly differ between obese children and controls. This agrees with the findings of some investigators (9, 18, 32). On the contrary, other investigators had found significantly higher resistin levels in obese compared to non obese controls. (13, 33-35)

We found no statistical difference in serum resistin level between girls and boys in both cases and controls and this agrees with the finding of Schaffler et al, (2004).(36) Other studies, on the other hand, reported significantly higher serum resistin levels in girls than in boys. (13, 18, 32) However Li et al., (2009) reported that the gender related difference in serum resistin levels was quite significant only when comparing pubertal groups suggesting a link with development.(13) However these relations with gender and age in children have not always been found. (37-39)

In the present study as well as in some other studies waist circumference and BMI did not show any significant correlation with resistin level. (16, 17, 26) On the contrary , some other studies have found that obesity markers were positively correlated with resistin levels (12, 13). However, Li et al., (2009) emphasized that none of these associations was found when exclusively analyzing the prepubertal group, thus pointing to a role played by puberty on the serum level of resistin (13). On the other hand, Gambino et al., (2005) found a significant correlation between serum resistin level and waist circumference in healthy non obese control group.(17)

In the present study a link between resistin level and insulin resistance in obese children was confirmed by finding a significant positive correlation between serum resistin levels and each of fasting insulin and HOMA which agrees with the findings of Silha et al., (2003) and Koebnick et al., (2006). (14,15) On the other hand Li et al., (2009) reported that only few indices of insulin resistance were linked with plasma resistin in either gender (13). On the contrary, other studies found no significant correlation between serums resistin levels and insulin resistance (18, 33, 40, 41). However, Gambino et al., (2005) found a correlation between serum resistin and fasting insulin only in normal subjects. (17)

In this study, 24.5% of cases had normal resistin level, 13.3% had high level and 62.2% had low level. A significant difference between cases with either high or low resistin level on one hand and cases with normal resistin on the other hand was found regarding fasting insulin level and HOMA. These observations suggest that altered resistin level whether high or low can be related to insulin resistance associated obesity.

The pediatric metabolic syndrome is defined as the presence of at least three of the following: abdominal obesity, (waist circumference ≥ 90th percentile), low HDL-C level (≤40 mg/dl), hypertriglyceridemia (>90th percentile), hypertension (> 90th percentile), and/ or impaired glucose tolerance (42). In this study, 4 out of 45 cases (8.9%) were hypertensive, showing high systolic and diastolic blood pressure for age and sex. Hypertensive children had high waist circumference and body fat percentage (>95th percentile); also they had high HOMA and three of them had acanthosis nigricans. The presence of these risk factors makes those children more vulnerable to the development of the metabolic syndrome later on. Moreover, the association of childhood obesity with features of metabolic syndrome has been demonstrated in this study by the positive correlation found between BMI and systolic blood pressure in obese children which agrees with the findings of other investigators (29,

43, 44). In accordance to the results of Weiss et al., 2004 and Vardi et al., 2007, this study found a positive correlation between BMI and waist circumference in obese children. (29, 43)

Our results are consistent with previous studies that demonstrated that obesity is one of the most important risk factors for insulin resistance. In the present study, HOMA was used for assessment of insulin resistance. Fasting insulin and HOMA levels were significantly higher in obese children compared to non-obese controls reflecting the relation between obesity & insulin resistance. These results agree with the results of other investigators (9, 44, 45). In consistent with that, Salbe et al., (2002) found that insulin concentrations increased with increasing adiposity. (46) Similarly Rudzka-Kocjan et al., (2006) and Zou et al., (2007) found a significant correlation between BMI and insulin resistance. (9, 45)

It is interesting to notice that up to 60% of normal controls had high HOMA compared to 77.8% of obese children. This could be explained in the healthy controls by the fact that puberty is associated with temporary increases in insulin resistance with a peak reduction in insulin sensitivity by 25–30% during Tanner stage 3 with complete recovery by pubertal completion (47, 48). Regarding the effect of gender, we found no significant difference in HOMA value between males and females in both cases and controls and this agrees with Zou et al., (2007) who found no correlation between HOMA and gender. (9) On the other hand it disagrees with the findings of other investigators, (26,49, 50) who found that girls had significantly higher mean HOMA than boys after adjustment for race, age, and weight. This may reflect the effect of puberty on insulin resistance, as girls experience puberty at an earlier age than boys. The absence of sex difference in the present study could be attributed to the small sample size.

This study revealed that resistin and insulin resistance were independent of age and pubertal stage and this was in accordance to the finding of Reinehr et al., (2006) (32). Interestingly, Gerber et al., (2005) reported that in both obese and lean children resistin correlated with age and Tanner stage. (18) On the other hand, Zou et al, (2007) found a significant correlation between insulin resistance parameters, age and sexual development (9). Recently, Li et al., (2009), stated that in both boys and girls resistin tended to decrease with age. (13)

Almost 67% of obese children in this study had high fasting insulin level which is more or less in the same range as that recorded by Freedman et al., (1999) who found that 58% of the obese individuals studied in the age group of 5–17 years showed elevated insulin levels.(51) A much higher value of 83.02% was reported recently by Rudzka- kocjan et al., (2006) (45). This difference could be attributed to the effect of puberty as Klein et al., (2004) found that fasting insulin values were greater in pubertal than prepubertal girls. (48) In the present study hyperinsulinemic children showed higher HOMA and body fat percentage than obese children with normal fasting insulin thus confirming the association between obesity, hyperinsulinemia and insulin resistance. Similarly Lee et al., (2006) reported a high correlation between HOMA-IR and insulin levels. (26)

Acanthosis nigricans is pathognomonic for insulin resistance. (52) In the present study, it was observed that 14 out of 45 cases (31%) had acanthosis nigricans which is more or less similar to that found by Vardi et al., (2007), (35%) in a study on obese pre pubertal and adolescent children (43). It was observed that children with acanthosis nigricans showed significantly higher BMI and waist circumference than cases without acanthosis nigricans, again confirming the relation between adiposity& insulin resistance.

The present study confirm the link between resistin level and insulin resistance in obese children, however it couldn't prove whether high or low resistin level is more related to insulin resistance. A significant positive correlation was found between serum resistin levels and each of fasting insulin and HOMA .No significant correlation was found between serum resistin, HOMA and each of BMI, body fat percentage & waist circumference. HOMA was found to be a significant marker for early detection of insulin resistance in obese and overweight children.

Future studies with a larger sample size are recommended to explain the link between resistin and obesity associated insulin resistance. Regular monitoring for blood pressure, fasting glucose, fasting insulin and HOMA as well as presence of acanthosis negricans are highly recommended for early detection of insulin resistance in obese and overweight children. Also norms of waist circumference and waist hip ratio age and sex specific percentiles should be determined for every country or every ethnic and racial group. More concern should be given to the role of schools in treatment of obesity including psychological therapy, healthy food education, healthy dietary habits, exercise programs and medical follow up.

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4/1/2010

Adsorption Equilibrium, kinetics and thermodynamics of methylene blue from aqueous solutions using biopolymer oak sawdust composite

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Abstract: Oak sawdust (OSD), furniture industrial waste was chemically treated with 0.1N NaOH to give hydrolyzed oak sawdust (HOSD) which was immobilized on alginate biopolymer. Hydrolyzed oak sawdust composite (HOSDC) was utilized as low-cost adsorbent to remove basic dye (methylene blue, MB) from aqueous solution. HOSD and HOSDC were characterized by using Scanning electron microscope (SEM), thermo gravimetric analysis (TGA) and infrared spectrometer analysis (FTIR). The adsorption of (MB), whose isotherms are modeled according to Langmuir, Freundlich and Temkin, were studied at a variety of physical and chemical conditions. The data fitted very well with Freundlich isotherm. Batch adsorption models, based on the assumption of pseudo-first-order, pseudo-second-order and intraparticle diffusion mechanism, showed that kinetic data follow closely pseudo-second-order and intraparticle diffusion. In addition, various thermodynamic parameters, such as standard Gibbs free energy (ΔG°), standard enthalpy (ΔH°), standard entropy (ΔS°), and the activation energy (Ea) were calculated. The adsorption process of MB dye onto HOSDC was found to be spontaneous and endothermic process. Furthermore, a single-stage batch adsorber was designed for the removal of methylene blue by HOSDC based on the equilibrium data obtained. [Journal of American Science 2010;6(6):267-283]. (ISSN: 1545-1003).

Keywords: Methylene blue; Sorption isotherms; Kinetics; thermodynamics; Sawdust; Binding polymers.

1. Introduction

The discharge of dyes in the environment is worrying for both toxicological and esthetical reasons [1]. Industries such as textile, leather, paper, plastics, etc., are some of the sources for dye effluents [2]. It is estimated that more than 100,000 commercially available dyes with over 7×10^5 tones of dyestuff produced annually [3]. Although MB is used in some medical treatments, and in dying textile, it can cause eye injury for both human and animals. On inhalation, it can give rise to short periods of rapid or difficult breathing while ingestion through the mouth produces a burning sensation and may cause nausea, vomiting, profuse sweating, diarrhea, gastritis, mental confusion and methemoglobinemia [4, 5]. Thus, the removal of MB from industrial effluents has become one of the major environmental concerns.

A range of conventional treatment technologies for dye removal have been investigated extensively, such as biological treatment, adsorption, chemical oxidation, coagulation, and reverse osmosis [6, 7]. However, most of the above methods suffer from one or more limitations and none of them are successful in completely removing the color from wastewater. The removal of dyes and organics in an economic way remains an important problem, although a number of systems have been developed with adsorption techniques. Adsorption was found to be superior to other techniques for water re-use in terms of initial cost, simplicity of design, ease of operation and insensitivity to toxic substances [8, 9]. Activated carbon adsorption is highly effective for removing dyes and pigments [10], it is often too expensive to be used in developing countries; the use of low-cost adsorbents, such as clay minerals [11], bottom and fly ash [12–14], fungi [15], waste materials from agriculture representing an essential target for these countries [16–20]. Also a number of studies in the last years focused on the adsorption of some dyes (acid, basic, reactive, and metal complex) on sawdust of different woods: beech [21–24], rubber wood [25], walnut and cherry tree, pine [26–28], cedar [29], clay–wood sawdust mixture [30], Indian rosewood [31], treated sawdust[32],spruce sawdust magnetically modified [33,34], charred [35] and oak [36,37]. Oak sawdust, alkali treated oak sawdust and acid treated oak sawdust were utilized successfully for the removal of basic dye from aqueous solutions [36] but still some difficulties in its removal from the wastewater.

In order to overcome the handling problem, biopolymer binding polymers for the granulation of (HOSD) have a number of advantages such as simplicity of preparation procedure and excellent physicochemical properties [38]. Alginic acid is a biopolymer having carboxyl groups capable of forming complexes with divalent cations such as Ca^{2+} and Ba^{2+}. Thus the prominent immobilizing ability of alginic acid and alginates seems to be effective for the granulation of (HOSD), and this granulation method has a number of advantages such as simplicity for preparation, high content of active component (HOSD), high porosity, easy to be separated from the wastewater [38].

The objective of this study is to immobilize the HOSD into the porous matrices of alginate gel polymer in order to employ the granulated composite (HOSDC) for the removal of methylene blue (MB) from aqueous solution. The effects of various operating parameters such as solution pH, adsorbent dose, binding polymer, initial dye concentration, contact time, agitation speed and temperature on MB adsorption were investigated. Adsorption isotherms and kinetics of the sorption process were studied. Also various thermodynamic parameters were calculated. Further, a single-stage batch adsorber was designed for the removal of methylene blue by HOSDC based on the equilibrium data obtained.

2. Materials and methods

2.1. Materials

The structure of a cationic MB dye, $C_{16}H_{18}N_3SCl.3H_2O$, supplied from (NICE CHEMICALS Pvt. Ltd., COCHIN) is shown in Fig.1. The stock dye solution was prepared by dissolving 1g of methylene blue in 1000ml distilled water to obtain 1000mg/L dye used for preparing different initial dye concentrations. For hydrolyzed sawdust throughout the experiment, a sodium hydroxide solution was used. For pH adjustment throughout the experiment, hydrochloric acid and/or sodium hydroxide solutions were used as necessary.

Sodium alginate (NaALG) with a high viscosity (2% solution= 14000cP) was supplied from (Sigma-Aldrich company). Sodium alginate linear unbranched polymers containing β-(1⟶4)-linked D-mannuronic acid (**M**) and α-(1⟶4)-linked L-guluronic acid (**G**) is shown in Fig.(2). Calcium Chloride ($CaCl_2$) was supplied from Riedel-de Hean.

2.2. Adsorbent preparation and characterization

Oak sawdust, was used as an adsorbent for the removal of methylene blue, was obtained from a local furniture manufacturing company, as a suitable source for full-scale/industrial applications. OSD was washed with distilled water to remove the water-soluble impurities and surface adhered particles, dried in a digital dryer of (Carbolite, Aston lane, Hope Sheffield, 5302RP, England) for twenty four hours at 105°C to get rid of the moisture and other volatile impurities and sieved using sieve analyzer (AS200 Retsch, Germany) to different particle size ranges 45- 500µm. The material after sieving in the range 125-250 µm was isolated and placed in a conical flask contains 0.1N NaOH solution at a liquid to solid ratio of 10:1 with 200 rpm agitation speed using orbital shaker (yellow line Os10 Control, Germany) for four hours at room temperature. The excess alkaline solutions were decanted, and the alkaline HOSD was washed continuously with distilled water until the pH of the washing water became less than 8 using pH meter (Denver Instrument Co., U.S.A.). HOSD was characterized using FTIR, SEM and TGA techniques.

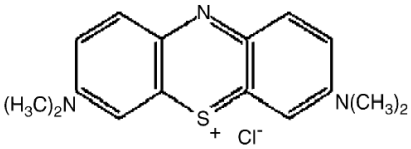


Figure 1. Structure of Methlyene Blue

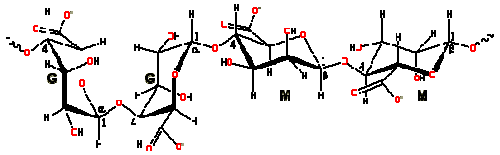


Figure 2. Sodium alginate structure

2.3. Preparation of HOSDC

The preparation procedure for hydrolyzed oak sawdust –calcium alginate composite was carried out as follow (Fig.3): a viscous NaALG weighted amount solution was dissolved in distilled water to form 1.25 wt% solutions; the hydrolyzed oak sawdust was added to the alginate to form a mixture of 2.5:1 HOSD-alginate with good mixing by vigorous stirring. Here the mixing ratio was defined as mass (g) of HOSD to the mass (g) of NaALG. The formed mixture was added drop wise by peristaltic pump at flow rate 0.5 ml/s with tube 2.79 mm internal diameter to 3% $CaCl_2$ solution with stirring at room temperature to form spherical shape after overnight standing. They were separated from calcium chloride solution, washed with distilled water, dried at 50ºC and finally stored in sealed vessel. The dried composite was used for detailed studies. The prepared composite was characterized using FTIR,

SEM and TGA techniques and also tested for swelling and turbidity.

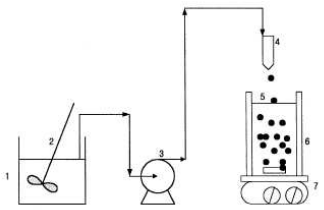


Figure 3. Schematic diagram of hydrolyzed Oak sawdust immobilization to form beads: 1, alginate & hydrolyzed Oak sawdust; 2, mechanical stirrer; 3, peristaltic pump; 4, out let tubing; 5$CaCl_2$; 6, glass beaker; 7, magnetic stirrer.

2.4. Adsorption studies

Batch adsorption experiments were carried out at room temperature (22°C ± 2). Exactly 100 ml of cationic dye solution of known initial concentration (200mg/L) was shaken at the certain agitation speed (250rpm) with a required dose of adsorbents (5.0g/L) for a specific period of contact time (120 min) in an orbital shaker, after noting down the initial pH of the solution to the optimum pH. The pH of the solutions were adjusted to the required value by adding either 0.1NHCl or 0.1N NaOH solution. The final concentration (C_t) was measured. The percentage removal of dye was calculated using the following relationship:

$$\% \text{ Removal} = ((C_o - C_t)/ C_o) * 100) \quad (1)$$

where, C_o and C_t (both in mg/L) are the initial dye concentration and the dye concentration at any time respectively.

The adsorption capacity q_t (mg/g) at any time was calculated using the mass balance relationship equation as follows:

$$q_t = (C_o - C_t) * (V/W) \quad (2)$$

where, V is the volume of the solution (L), W is the mass of the dry adsorbent used (g).

- **Adsorption parameters studied**

1. Solution pH (1, 3, 5, 7, 8, 9 and 12)
2. Adsorbent dose (0.5, 1, 2.5, 5, 7.5 and 10 g/L)
3. Agitation speed (0, 50, 100, 150, 250,300 and 400 rpm)
4. Effect of binding polymer
5. Initial dye concentration (25, 50, 75, 100, 150 and200 mg/L)
6. Contact time up to 330 min.
7. Temperature (22, 40, 50 and 60°C)

2.5. Batch kinetic studies

Batch adsorption experiments were carried out at room temperature (22± 2°C). Exactly 100 ml of cationic dye solution of known initial concentration (25– 200 mg/L) was shaken at the agitation speed (250 rpm) with a required dose of adsorbents 5 g/L (OSDC) for a specific period of contact time 330 min in an orbital shaker, after noting down the initial pH of the solution to the optimum pH (12). Samples were withdrawn at different time intervals.

2.6. Equilibrium studies

Adsorption experiments were carried out by adding a fixed amount of sorbent (0.5g) into 250-ml Erlenmeyer flasks containing 100 ml of different initial concentrations (25, 50, 75, 100, 150 and 200 mg/L) of dye solution. The temperature was controlled at 22°±2°C. Agitation was provided at 250 rpm for 270 min. The equilibrium contact time was previously determined by kinetic studies using the same conditions. The amount of dye adsorption at equilibrium, q_e (mg/g), was calculated using the following equation

$$q_e = (C_o - C_e) * (V/W) \quad (3)$$

where, C_e (mg/L) is the liquid-phase concentration of dye at equilibrium and q_t is the adsorption capacity (mg/g) at equilibrium.

2.7. Analytical methods

The concentration of MB remaining in the supernatant after and before adsorption was determined with a 1.0 cm light path quartz cells using spectrophotometer ((Perkin Elmer model GBC 902)) at λ max of 665 nm. Prior to the measurement, a calibration curve was obtained by using the standard MB solution with known concentrations.

2.8 Theory of adsorption isotherm, kinetics and thermodynamics

2.8.1. Adsorption Isotherm models

Adsorption isotherm is basically important to describe how solutes interact with adsorbents, and is critical in optimizing the use of adsorbents. The Langmuir [39], the Freundlich [40] and the Temkin [41] isotherms were employed in the present study. The linearized forms of the three isotherms are

$$C_e / q_e = C_e /q_m + 1/ (K_a. q_m) \quad (4)$$

$$\log q_e = \log KF + 1/n \log C_e \quad (5)$$

$$q_e = B \ln A + B \ln C_e \quad (6)$$

where B = RT/b.

where q_m (mg/g) and K_a (L/mg) are Langmuir constants related to adsorption capacity and energy of adsorption, respectively. The constants q_m and K_a can be calculated from the plot between C_e/q_e versus C_e (Eq. (4)). C_e (mg/L) and q_e (mg/g) are the equilibrium concentration, and the amount of dye adsorbed at equilibrium, respectively. Similarly the Freundlich isotherm constants KF and 1/n can be calculated from the plot of log (q_e) versus log (C_e) (Eq. (5)). KF ($mg/g(L/g)^{1/n}$) and n are the Freundlich constants,

which are indicators of adsorption capacity and adsorption intensity, respectively [42]. The Temkin isotherm [41] has generally been applied in the form given by Eq. (6). Therefore, by plotting q_e versus ln C_e (Eq. (6)), enables the determination of the constants A and B. B is the Temkin constant related to heat of sorption (J/mol), A is the Temkin isotherm constant (L/g), R the gas constant (8.314 J/mol K), b is Temkin isotherm constant and T the absolute temperature (K).

2.8.2. Kinetics models

The most common models used to fit the kinetic sorption experiments are Lagergren's pseudo-first-order model (Eq. (7)) [43] and pseudo- second-order model (Eq. (8)) [44] were used:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (7)$$

$$t/q_t = 1/k_2 q_e^2 + t/q_e \quad (8)$$

where q_e (mg/g) and q_t (mg/g) are the amount of dye adsorbed at equilibrium and at time t, respectively. k_1 (min^{-1}) and k_2 (gmg^{-1} min) are the pseudo-first-order and pseudo-second order adsorption rate constants, respectively.

2.8.3. Intraparticle diffusion model

In order to investigate the mechanism of the MB adsorption onto HOSDC, intraparticle diffusion-based mechanism was studied. The most commonly used technique for identifying the mechanism involved in the adsorption process is by fitting an intraparticle diffusion plot. It is an empirically found functional relationship, common to the most adsorption processes, where uptake varies almost proportionally with $t^{1/2}$ rather than with the contact time t. According to the theory proposed by Weber and Morris [45]:

$$q_t = k_{id} t^{1/2} + C_i \quad (9)$$

where k_{id} ($mg\ g^{-1}\ min^{1/2}$), the rate parameter of stage i, is obtained from the slope of the straight line of q_t versus $t^{1/2}$. C_i is the intercept which are proportional to the extent of boundary layer thickness [46].

2.8.4. Thermodynamics studies

Thermodynamic parameters were evaluated to confirm the adsorption nature of the present study. The thermodynamic constants, Gibbs free energy change (ΔG°), enthalpy change (ΔH°) and, entropy change (ΔS°) were calculated to evaluate the thermodynamic feasibility and the spontaneous nature of the process. The change in enthalpy (ΔH°) and entropy (ΔS°) are calculated using the van't Hoff [47, 48]:

$$\ln kc = \Delta S^\circ/R - \Delta H^\circ/RT \quad (10)$$

where kc = Fe/(1 - Fe), and Fe = $(C_o - C_e)/C_o$; is the fraction adsorbed at equilibrium, while T is the temperature in degree K and R is the gas constant [8.314 J/mol K)]. From the slope and the intercept of van't Hoff plots, the values of ΔH° and ΔS° were computed

The Gibbs free energy change (ΔG°) was calculated using the following equation [49]:

$$\Delta G^\circ = -RT \ln kc \quad (11)$$

The magnitude of activation energy (Ea) gives an idea about the type of adsorption which is mainly diffusion-controlled processes or chemical reaction processes. Energies of activation below 42 kJ mol^{-1} generally indicate diffusion-controlled processes and higher values represent chemical reaction processes [50–53]. This is because the temperature dependence of the pore diffusivity is relatively weak. Here, the diffusion process refers to the movement of the solute to an external surface of adsorbent and not diffusivity of material along micropore wall surfaces in a particle [54].

Energy of activation, Ea, was calculated according to a relationship between Ea and ΔH° for reactions in solution using the following equation [55]:

$$Ea = \Delta H^\circ + RT \quad (12)$$

3. Results and discussion

3.1. Characterization of adsorbing material.

3.1.1. Thermogravimetric analysis TGA

The thermal stability of HOSD and HOSDC were evaluated by Thermo Gravimetric Analysis TGA using Thermo Gravimetric Analyzer Shimadzu TGA-50 Japan. Fig**.** (4-a) showed the TGA of HOSD, the main step began at 32°C and ended at 98°C is attributed to the loss of surface adsorbed water in HOSD structure and the weight loss is 6.12% the second main step began at 220°C and ended at 774°C is corresponding to loss of all the organic constituents in the HOSD. Fig**.** (4-b) showed the TGA pattern of the prepared HOSDC which contained three main steps. The first step began at 23°C and ended at 132°C which was explained by the removal of external water molecules together with degradation of alginate chain and it is interpreted with second degradation temperature due to elimination of side-groups of polymer [56]. The third step is obviously due to the complete loss of the organic components and it started at 395°C and ended at 657°C [57].

3.1.2. FTIR analysis

FTIR analysis was performed on both HOSD and HOSDC using Fourier transfer for infrared spectrophotometer FTIR-8400 S Shimadzu, Japan. The saponification process of the wood fiber using base cause increase of the cation sorption capacity of wood fiber which could be explained by production of carboxylate groups instead of ester groups which can bind cations as shown in the following Eq. (13) [58]:

$R'COOR + H_2O^+ OH^- \rightarrow RCOO^- + R'OH + H_2O$ (13)

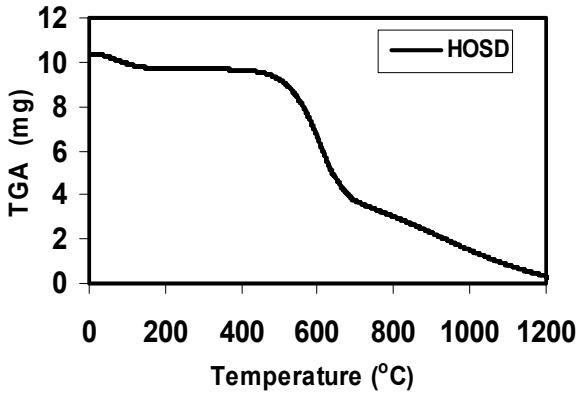
The IR bands consisted of four regions: the broad hydrogen band (3200–3600 cm^{-1}), C–H stretching region (2800–3000 cm^{-1}), carbonyl group stretching region (1550–1750 cm^{-1}), and fingerprint bands (below1550 cm^{-1}). In finger print region absorption cannot clearly be assigned to any particular vibration because they correspond to complex interacting vibration systems. It can be observed from Fig.5 that the region between 1800 and 3500 cm^{-1} presents two major band centered at about 3420 cm^{-1} (the H-bonded OH group) and at 2921 cm^{-1} (the C–H stretching of the CH_2 groups).this band shows markedly decrease in composite pattern which may be devoted to the restriction of stretching vibration of CH_2 groups due to composite formation. The region between 1500 and 1800 cm^{-1} is a special range to evaluate the degree of saponification since this represents the carbonyl and double bond region [59–61]. As had been published by many authors [56, 57, 59–61], the wave number of the carboxyl acid groups in the organic compounds is approximately 1740 cm^{-1}. While the band wave number of the carboxylate ion groups is about 1620 cm^{-1}. In this range, there are two bands centered at 1654 and 1749 cm^{-1}, one of them at 1749 cm^{-1} and this band disappeared in composite and this could be explained by masking of carboxylic group by the presence of alginate structure, the other band exists in both HOSD and HOSDC at 1654 cm^{-1}.

3.1.3. Scanning Electron Microscope SEM

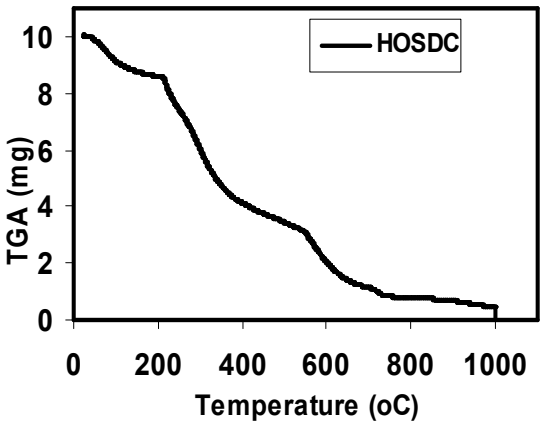
The morphology of HOSD and the prepared composite were investigated using Jeol JSM-6360 LA analytical Scanning Electron Microscope SEM. The samples were stocked over a holder and sprayed with gold. The samples were scanned to identify the structure and estimate the diameter of the composite. SEM photographs (Fig.6.a) show thin plant boundary walls due to the treatment and also a widening in the cavities which may explain the high efficiency of the treated Oak sawdust. It was shown in Fig. 6.b. that the composite prepared from HOSD and alginate mixture has almost uniform porosity with average pore diameter range 135 µm. The diameter of the composite beads ranged from 3-3.5 mm.

3.1.4. Turbidity measurements as a factor in mechanical strength

The beads produced from oak sawdust with alginate exhibited rubber like elastic properties, alginate improved the surface properties, reduced the tendency to agglomerate [62]. Although it is well known that alginate has bad mechanical properties, the mechanical properties of the composite were studied to determine its availability for the column operations. It is obvious, from water turbidity measurements after stirring for different time intervals, that the beads of the prepared composite have excellent mechanical strength under stirring for 65 hours at 1000 rpm.



(a)



(b)

Figure 4. Thermogravemetric analysis of HOSD (a) and HOSDC (b)

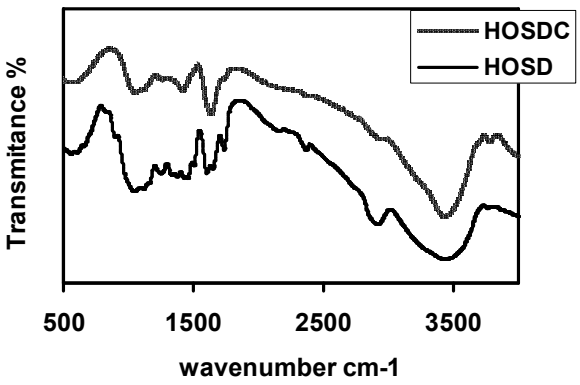
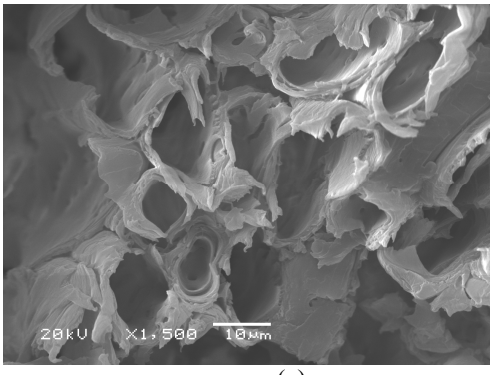
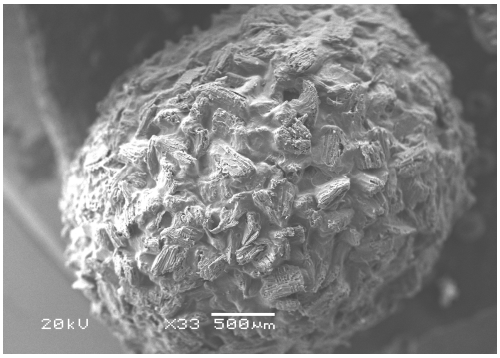
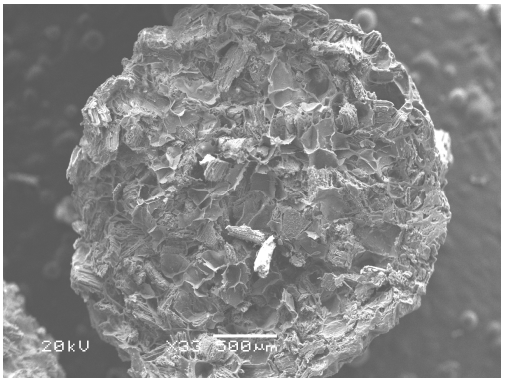


Figure 5. FTIR Spectra of HOSD and HOSDC



(a)





(b)

Figure 6. Scanning electron micrograph of HOSD (a) and HOSDC (b)

3.1.5. Swelling measurements

In order to test the suitability of the beads for column operation, the swelling behavior has been studied. It is concluded that the composite beads swelling in water increase with time till reached equilibrium swelling value after 3 hours. So the produced HOSDC suitable to be used in column operation after being swollen in water for 3 hours to avoid column clogging during the treatment process.

3.2. Investigation of adsorption parameters

The adsorption process of MB on HOSDC Characterization have been discussed considering the effect of different factors

3.2.1. Effect of initial solution pH

pH is one of the most important factors controlling the adsorption of dye onto adsorbent particles, which affects the surface charge of the adsorbents as well as the degree of ionization of different pollutants. The hydrogen ion and hydroxyl ions are adsorbed quite strongly and therefore the adsorption of other ions is affected by the pH of the solution. Change of pH affects the adsorptive process through dissociation of functional groups on the adsorbent surface active sites. This subsequently leads to a shift in reaction kinetics and equilibrium characteristics of adsorption process. As the pH increases, it is usually expected that the cationic dye adsorption also increases due to increasing of the negative surface charge of adsorbents [63]. The effect of initial pH of the MB dye solution on the amount of dye adsorbed was studied by varying pHs under constant process parameters (Fig.7). The amount of MB adsorbed by HOSDC increased with pH change of dye solution from 1 to 12 at 22°C. With increasing pH values the adsorption of MB on HOSDC tends to increase, which can be explained by the electrostatic interaction of cationic MB species with the negatively charged hydrolyzed oak sawdust composite surface. The electrostatic attraction force of the dye compound with the HOSDC surface is likely to be raised when the pH increases. A similar behavior was observed for methylene blue adsorption on wheat shells [64] and on oak sawdust [36].

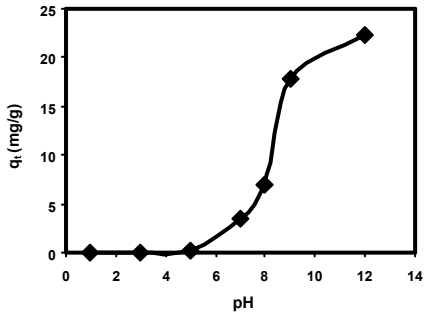


Figure 7. Effect of the different pH on the adsorption of MB dye onto HOSDC (initial dye concentration = 200 mg/L, adsorbent dose = 5 g L^{-1}, contact time = 120 min, solution temp. =22±2°C and agitation speed= 250 rpm).

3.2.2. Effect of adsorbent doses

Adsorbent dosage is representing an important parameter due to its strong effect on the capacity of an adsorbent at given initial concentration

of the adsorbate. Fig. 8 shows that the increase in adsorbent dosage from 0.5 to 10 g/L resulted in a decrease of uptake capacity from 53.51 to 11.37 mg/g.

The adsorption capacity was found to be high at low dosages. Many factors can contribute to this adsorbent concentration effect. The most important factor is that adsorption sites remain unsaturated during the adsorption reaction. This decrease in adsorption capacity with the increase in the adsorbent dosage is mainly attributed to the non-saturation of the adsorption sites during the adsorption process [65, 66].This result confirms the previously studies concerning the removal of unwanted materials from aqueous solution by sawdust [35].

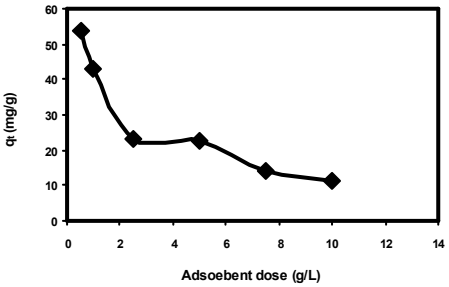


Figure.8. Effect of adsorbent dose on the adsorption of MB dye onto HOSDC (initial dye concentration =200 mg/L, contact time = 120 min, pH= 12, solution temp. = 22 ±2^{O}C and agitation speed = 250 rpm)

3.2.3. Agitation speed

Agitation is an important parameter in sorption phenomena, which has a serious action on the distribution of the solute in the bulk solution and the formation of the external boundary film. The effect of agitation speed (in rpm) on the up take capacity of the original dye concentration was investigated in Fig. 9. The up take capacity seemed to be affected by the agitation speed for values between 0 and 250 rpm, thus confirming that the influence of external diffusion on the sorption kinetic control plays a significant role. Also it is clear that while increasing mixing rate from 250 to 400 rpm, uptake capacity (q_t mg/g) decreased from 22.4 to 11.92 mg/g. This decrease in uptake capacity (q_t mg/g) may be attributed to an increase desorption tendency of dye molecules and/or having similar speed of adsorbent particles and adsorbate ions (i.e. the formation of a more stable film around the adsorbent particles). This desorption tendency may be attributed to high mixing speed which means more energy input and higher shear force causing break of bounds between MB and the adsorbent . This also indicates that a 250 rpm shaking rate is sufficient to assure that all the surface binding sites are made readily available for dye uptake. Then the effect of external film diffusion on adsorption rate can be assumed to be not significant. The results were in agreement with Batzias F.A., and D.K. Sidiras [24].

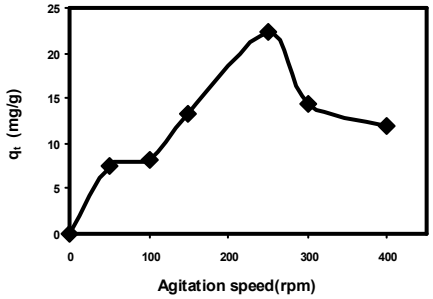


Figure 9. Effect of agitation speed on the adsorption of MB dye onto HOSDC (initial dye concentration =200 mg/L, contact time = 120 min, pH= 12, solution temp. = 22 ±2^{O}C and adsorbent dose=5g/L)

3.2.4. Binding polymer

The effect of binding polymer on the dye removal has been studied as show in Fig. 10. The alginate matrix has low efficiency toward MB removal. On the other hand, the presence of the polymer matrix decrease the percentage dye removal than the free HOSD which may be due to some of the composite area is now not available. The percentage removal decreased from 89.7% to 72.75% after 270 min, upon using composite beads instead of free HOSD. However the adsorption rate decreased through the first adsorption hour may be due to the diffusion limitation of dyes through the composite beads to reach the active adsorption sites (hydrolyzed oak sawdust). As the mean reason for such research is to overcome handling problems of classic adsorbents such as sawdust by converting them into composites. Although the polymeric composite sawdust has lower removal efficiency than sawdust but the advantage of using the composite make this difference disregarded.

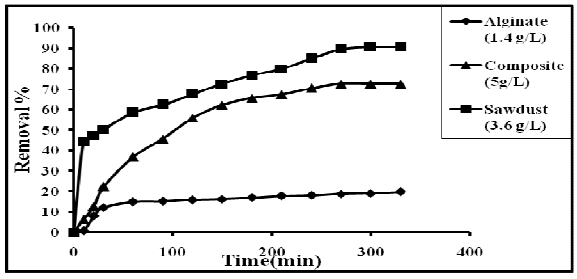


Figure 10. Effect of binding polymer on the percentage dye removal (initial dye concentration =200 mg/L contact time up to 330 min, pH= 12, solution temp. = 22 ±2^{O}C and agitation speed = 250 rpm).

3.2.5. Initial dye concentration and contact time

The effect of contact time on the adsorption of MB dye was investigated at different initial dye concentration (25-200 mg/L) onto HOSDC adsorbents. It can be noticed from Figs.11-12, that the removal of dye by adsorption on HOSDC was found to be rapid at the initial period of contact time and then to slowdown with time. The reason for this from my opinion is the attractive forces between the dye molecule and the adsorbent such as Vander Waals forces and electrostatic attractions; fast diffusion onto the external surface was followed by fast pore diffusion into the intraparticle matrix to attain equilibrium at 270 min.

Also it can be seen from Fig.12 that the amount of dye adsorbed (mg/g) increased with increased initial dye concentration and remained constant after equilibrium time (270 min). The concentration provides an important driving force to overcome all mass transfer resistance of the dye between the aqueous and solid phases [67, 68]. Hence a higher initial concentration of dye will enhance the adsorption process. The equilibrium sorption capacity of the HOSDC increased with the increase of the initial dye concentration, while the % removal of dye showed the opposite trend (Fig.11). When the initial dye concentration increased from 25 to 200 mg/L, the actual amount of dye adsorbed per unit mass of HOSDC increased from 4.66 to 29.15 mg/g and the percentage removal decreased from 93.2 to 72.75 %. A similar trend was also observed for MB adsorption onto Parthenium hysterophorus [69] and MB onto bamboo-based activated carbon [70].

3.2.6. Thermal effect

The dependence of the adsorption capacity of MB on temperature has been investigated at 22, 40, 50 and 60°C. The temperature has two major effects on the adsorption process. The adsorption capacity increases with temperature due to the increase of the rate of diffusion of the adsorbate molecules across the external boundary layer and the internal pores of the adsorbent particle, whose decreases in case viscosity of the solution for highly concentrated suspensions. In addition, changing the temperature will change the equilibrium capacity of the adsorbent for a particular adsorbate [71, 36]. Fig. 13 presents the temperature versus adsorbed amount. It is clear that with the increase in temperature the amount of adsorbed dye increases, indicating the process to be endothermic. This kind of temperature dependence of the adsorbed amount of MB may reflect the increase in the case with which the dye penetrates into HOSDC because of its larger diffusion coefficient. In fact, a possible mechanism of interaction is the reaction between the chromophore

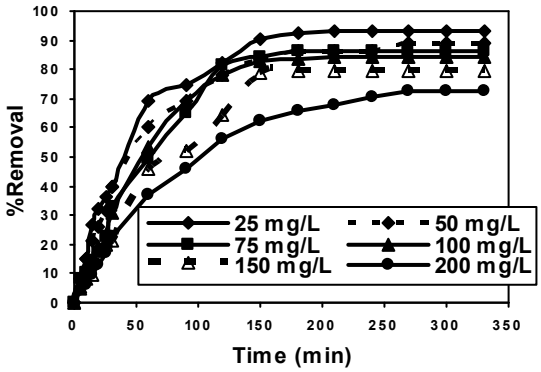


Figure 11. Effect of contact time and initial dye concentration on the removal of MB dye onto HOSDC (adsorbent dose=5g/L, pH= 12, solution temp. = 22 ±2°C and agitation speed = 250 rpm).

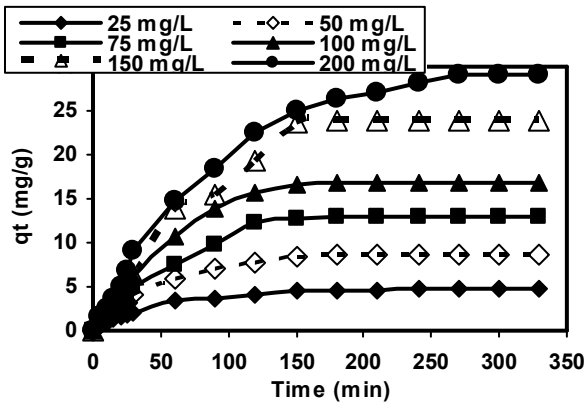


Figure 12. Effect of contact time and initial dye concentration on the adsorption of MB dye onto HOSDC (adsorbent dose=5g/L, pH= 12, solution temp. = 22 ±2°C and agitation speed = 250 rpm).

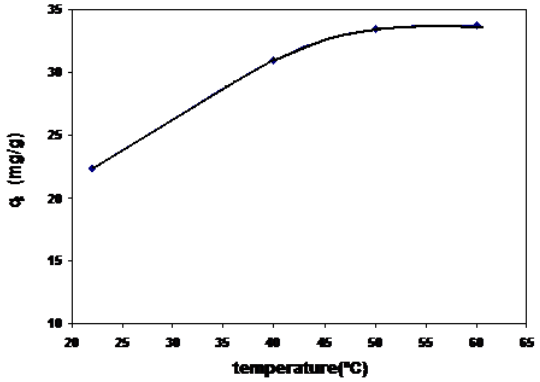


Figure 13. Effect of solution temperature on the adsorption of MB dye onto HOSDC (initial dye concentration =200 mg/L, contact time = 120 min, pH= 12, adsorbent dose = 5g/L and agitation speed = 250 rpm).

groups such as alcoholic, carbonylic and phenolic of the HOSDC and the cationic group in the dye molecule; such a reaction could be favored at higher temperatures. Hydrogen bond can occur between OH groups of HOSDC and nitrogen atom of dye; electrostatic attractive forces between cationic dye ions and the surface of HOSDC as depending on pH [72].

3.3. Isotherm analysis

Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems [73]. In this work, the equilibrium data for MB on hydrolyzed oak sawdust composite were modeled with the Langmuir, Freundlich and Temkin models. The linear plot of specific adsorption (C_e / q_e) against the equilibrium concentration (C_e), (Fig. 14) shows that the adsorption obeys the Langmuir model. The Langmuir constants q_m and K_a were determined from the slope and intercept of the plot and are presented in Table 1. The value of the correlation coefficient (R^2 = 0.97 obtained from Langmuir expression indicates that Langmuir expression provided a good linearity.

The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor R_L that is given by the following equation [74]:

$R_L = 1/ (1+ K_a C_o)$ (14)

where C_0 (mg/L) is the initial concentration of adsorbate, and K_a (L/mg) is Langmuir constant. The value of R_L indicates the shape of the isotherm which is unfavorable ($R_L > 1$), linear ($R_L = 1$), favorable ($0 < R_L < 1$), or irreversible ($R_L = 0$). The R_L values for the adsorption of MB onto HOSDC (Table 2) are observed to be in the range 0–1, indicating that the adsorption was a favorable process. The equilibrium data were further analyzed using the linearized form of Freundlich isotherm, by plotting log q_e versus log C_e (Fig. 15). The calculated Freundlich isotherm constants (KF, n) and the corresponding coefficient of correlation, R^2 are shown in Table 1.

The coefficient of correlation is (R^2 = 0.99) which expressing an agreement with the experimental data of MB on HOSDC. The result shows that the value of n is greater than unity (n = 1.818) indicating that the dye is favorably adsorbed on HOSDC. This is in great agreement with the findings regarding to R_L value. The magnitude of Freundlich constant indicates easy uptake of MB from aqueous solution.

The adsorption data for MB on HOSDC were analyzed by a regression analysis to fit the Temkin isotherm model (Fig. 16). The parameters of Temkin model as well as the correlation coefficient are listed in Table 1. The coefficient of correlation was (R^2 = 0.83) showing the poorest fit to the experimental adsorption equilibrium data. Considering that Langmuir isotherm assumes a monolayer coverage and uniform activity distribution on the adsorbent surface, this is an expected result. But adsorption of methylene blue is quite a complex process, probably forming multi layers and even closing some of the pores. Also, a variation of sorption activity is expected with surface coverage.

So it can be conclude that the Freundlich isotherm model was more suitable for the experimental data than other isotherms because of the high value of correlation coefficient (R^2 = 0.99). A similar result was reported for MB adsorption on granular and powdered activated carbon [75].

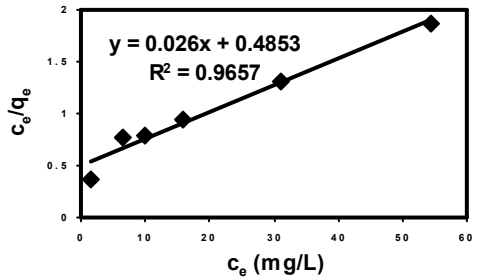


Figure.14. Langmuir isotherm plot for adsorption of MB dye onto HOSDC.

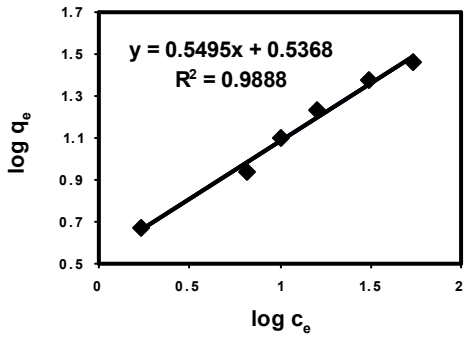


Figure 15. Freundlich isotherm plot for adsorption of MB dye onto HOSDC.

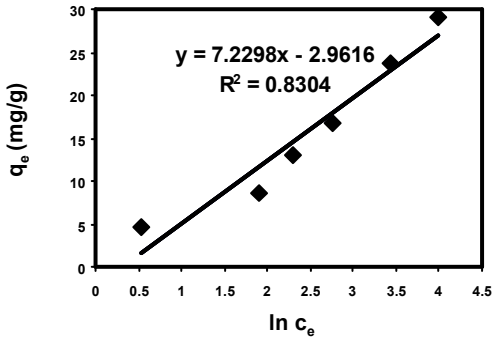


Figure 16. Temkin isotherm plot for adsorption of MB dye onto HOSDC.

Table 1. Isotherms parameters for removal of MB on HOSDC.

Isotherms	Parameters	Value
Langmuir	q_m (mg/g)	38.46
	k_a (L/mg)	0.0536
	R^2	0.97
Freundlich	KF(mg/g(L/g)$^{1/n}$)	3.442
	1/n	0.55
	R^2	0.99
Temkin	A (L/g)	0.664
	B (J/mol)	7.23
	R^2	0.83

Table 2. R_L values for different concentrations

Dye concentration (mg/L)	Value of R_L
25	0.427
50	0.272
75	0.199
100	0.157
150	0.111
200	0.085

3.4. Adsorption kinetics

Adsorption is a physiochemical process that involves the mass transfer of a solute (adsorbate) from the liquid phase to the adsorbent surface. A study of kinetics of adsorption is desirable as it provides information about the mechanism of adsorption, which is important for efficiency of the process. The applicability of the pseudo-first order and pseudo-second-order was tested for the adsorption of MB onto HOSDC. The best-fit model was selected based on the linear regression correlation coefficient, R^2, values.

The kinetics of MB adsorption on HOSDC was studied at different initial concentrations. Using Eq. (7), ln ($q_e - q_t$) versus t was plotted at different MB concentrations and is shown in Fig. 17.The Lagergren first-order rate constant (k_1) and q_e,cal determined from the model are presented in Table 3 along with the corresponding correlation coefficients. In spite of correlation coefficients $R^2 \geq 0.88$ for all initial MB concentrations studied, the first order kinetic models is not suitable for the data, as is shown in Fig.17. which indicates that ln ($q_e - q_t$) is slightly increased after 180 min. The first order model clearly does not fit in the region when the time is larger than 180 min. Therefore, the experimental kinetic data were further analyzed using the pseudo-second-order model. By plotting t/q_t against t for different initial MB concentrations (Fig. 18), a straight line was obtained in all cases and using Eq. (8) the second order rate constant (k_2) and q_e were determined from the plots. The q_e,cal values along with correlation coefficients for the pseudo-second-order models are shown in Table 3.It can be noticed that k_2 decreases with increasing initial MB concentration.

The decrease in the rate of MB removal with increasing initial MB concentration may be due to decreasing MB diffusivity as a result of the association of dye molecules to form bulky aggregates which becomes more pronounced at high dye concentration [76]. The model fits the kinetic data very well with $R^2 \geq 0.94$, which is better than pseudo-first, order kinetic (Table 3). These results suggest that the adsorption of MB on HOSDC may be best described by the pseudo-second-order kinetic model with high correlation coefficients.A number of authors have reported pseudo-second-order kinetics for adsorption of methylene blue on wheat shells [77], palygorskite [78] and dehydrated wheat bran carbon [5].

The half-adsorption time, $t_{0.5}$, is defined as the time required for the adsorption to take up half as much HOSDC as its equilibrium value. The half-adsorption time is often used as a measure of the adsorption rate and was calculated using the following equation [79]:

$$t_{0.5} = 1/ k_2 q_e \quad (15)$$

The diffusion coefficient largely depends on the surface properties of adsorbents. The diffusion coefficient for the intra particle transport of different initial concentrations of MB were also calculated using the following relationship [80]:

$$t_{0.5} = 0.03 r_o^2 / D \quad (16)$$

where $t_{0.5}$ is the half life in seconds as calculated from Eq. (15), r_o the radius of the adsorbent particle in centimeters and D is the diffusion coefficient value in cm^2/s. In these calculations, it has been assumed that the solid phase consists of spherical particles with an average radius between the radii corresponding to upper- and lower-size fractions. The value of r_o was calculated to be 1.625×10^{-1} cm for HOSDC samples. Calculated values of $t_{0.5}$ and D are given in Tables 3. Pore diffusion coefficient was found to be in the order of 10^{-7} cm^2 s^{-1} as illustrated in table 3, which indicating that the rate-controlling step is mainly pore diffusion [45,81-82] because the overall rate of the removal process is controlled by whichever is the slowest process.

The pseudo-first-order and pseudo-second-order kinetic models could not identify the diffusion mechanism. Thus the kinetic results were then analyzed by using the intraparticle diffusion model. Fig. 19 represents the dependence of the amount of the dye adsorbed at time t (q_t) on the square root of time($t^{0.5}$) this dependence had been used before by Weber and Moris [45] to investigate intraparticle

diffusion mechanism. For intraparticle diffusion model, Ho [83] pointed out that it is essential for the q_t versus $t^{0.5}$plots to go though the origin if the intraparticle diffusion is the sole rate-limiting step. The intraparticle diffusion plots are given in Figure 19 for different initial MB concentration. The linearity of the plots demonstrated that intraparticle diffusion played a significant role in the uptake of MB by HOSDC. In the present study, any plot did not pass through the origin. This indicates that although intraparticle diffusion was involved in the adsorption process, it was not the sole rate-controlling step. This also confirms that adsorption of MB on the adsorbent was a multi-step process, involving adsorption on the external surface and diffusion into the interior [84].

From the previous figure, the sorption process tends to be followed by two phases. It was found that an initial linear portion ended with a smooth curve followed by a second linear portion. The two phases in the intraparticle diffusion plot suggest that the sorption process proceeds by surface sorption and the intraparticle diffusion. The initial curved portion of the plot indicates boundary layer effect while the second linear portion is due to intraparticle or pore diffusion. The slope of second linear portion of the plot has been defined as the intraparticle diffusion parameter k_{i2} [85]. Table 4 shows the corresponding model fitting parameters, indicating the adsorption mechanism follows the intraparticle diffusion process. It was found that the values of k_{i2} increased with increasing initial MB concentration. The driving force of diffusion is very important for adsorption processes. Generally, the driving force changes with the adsorbate concentration in the bulk solution. The increase of adsorbate concentration results in an increase of the driving force, which will increase the diffusion rate of MB [86].On the other hand, the intercept of the plot reflects the boundary layer effect. Larger the intercept, greater is the contribution of the surface sorption in the rate-limiting step. The calculated intraparticle diffusion coefficient k_{i2} value at different initial dye concentrations was shown in Table 4.

3.5. Adsorption mechanism

The prediction of the rate-limiting step is an important factor to be considered in sorption process. For solid–liquid sorption process, the solute transfer process was usually characterized by either external mass transfer (boundary layer diffusion) or intraparticle diffusion or both. The mechanism for the removal of MB by adsorption may be assumed to involve the following steps [87]:

1. Migration of dye from the bulk of the solution to the surface of adsorbent.
2. Diffusion of dye through the boundary layer to the surface of adsorbent.
3. Adsorption of dye at an active site on the surface of adsorbent.
4. Intraparticle diffusion of dye into the interior pore structure of adsorbent.

The boundary layer resistance will be affected by the rate of adsorption and increase in contact time, which will reduce the resistance and increase the mobility of dye during adsorption [77].

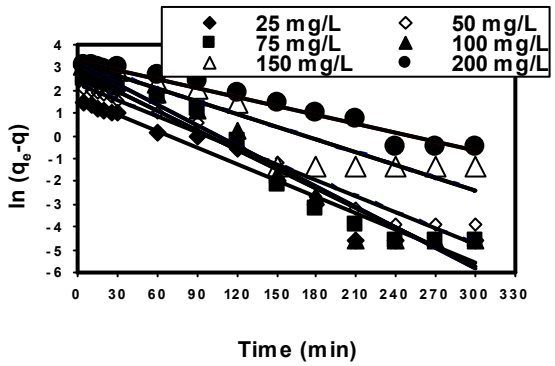


Figure 17. First-order plots of MB dye adsorption onto HOSDC.

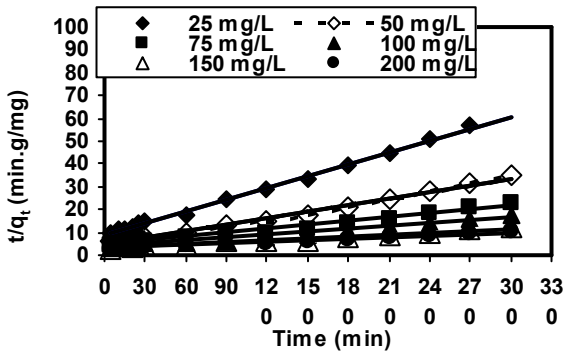


Figure 18. Second-order plots of MB dye adsorption onto HOSDC

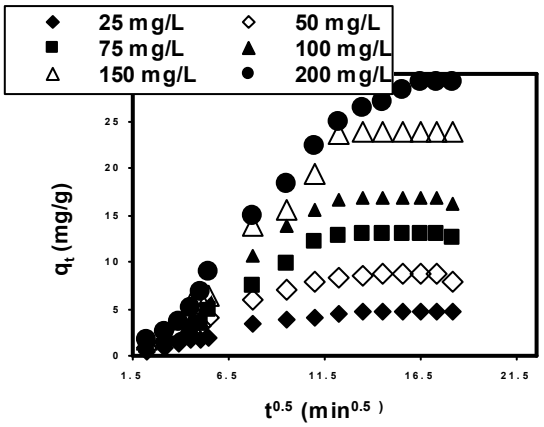


Figure 19. Intra-particle diffusion plots of MB dye adsorption onto HOSDC

3.6. Thermodynamics studies

The thermodynamic parameters for the sorption of MB onto HOSDC at various temperatures were calculated and listed in Table 5. The values of ΔH° and ΔS° have been computed from the slope and the intercept of the plot of ln kc vs. 1/T (Fig. 20) which gives a straight line with acceptable coefficient of determination (R^2), while the Gibbs free energy change ΔG° was calculated using eq. (11). The value of ΔH° was positive, indicated that the adsorption reaction was endothermic. The positive value of ΔS° shows that increasing randomness at the solid/liquid interface during the adsorption of MB on HOSDC. The negative values of ΔG° indicate the spontaneous nature of adsorption with a high preference of methylene blue onto HOSDC. The decrease in the negative value of ΔG° with an increase in temperature indicates that the adsorption process of methylene blue on HOSDC becomes more favorable at higher temperatures [88].

In this study, the activation energy values were higher than 42kJ mol^{-1} as presented in Table5 indicating chemically controlled process; also the high values of the activation energy indicated that diffusion is not a limiting factor controlling the rate of adsorption. Consequently, adsorption of MB dye by HOSDC appears to occur by chemisorption Spontaneous and endothermic adsorption has also been reported for the system of basic dyes on tree fern [89], wheat shell [90], and mansonia wood sawdust [91].

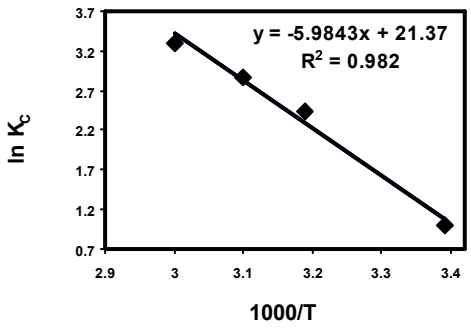


Figure 20. Effect of temperature on MB kinetic sorption for HOSDC (initial dye concentration = 200mg/L, adsorbent dose = 5 g/ L, pH =12, contact time = 270 min and agitation speed = 250 rpm).

3.7. Single-stage batch adsorber

Adsorption isotherm studies can also be used to predict the design of single stage batch adsorption systems [92-94]. The schematic diagram for a single-stage adsorption process is shown in Fig. 21. The solution to be treated contains V (L) of water and an initial MB concentration C_o (200 mg/L), which is to be reduced to C_e in the adsorption process. In the treatment stage, the amount ofadsorbent W (g) added is added to solution and the dye concentration on the solid changes from q_0 =0 to q_e. The mass balance for the dye in the single stage is given by

$V(C_o - C_e) = W(q_e - q_o) = W\, q_e$ (15)

The Freundlich isotherm data may now be applied to Eq. (15) since the Freundlich isotherm gave well fit to experimental data.

$W/V= (C_o - C_e) / KF\, C_e^{1/n}$ (16)

Fig.22 shows a series of plots derived from Eq. (16) for the adsorption of MB on the adsorbent and depicts the amount of effluent which can be treated to reduce the MB content by 90, 80, 70, 60 and 50% using various masses of adsorbent.

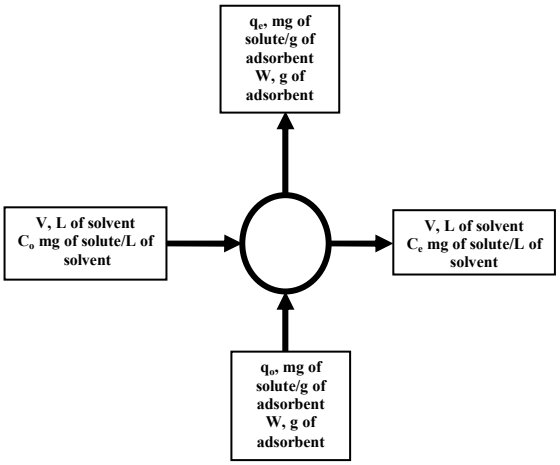


Figure 21. A single-stage batch adsorber.

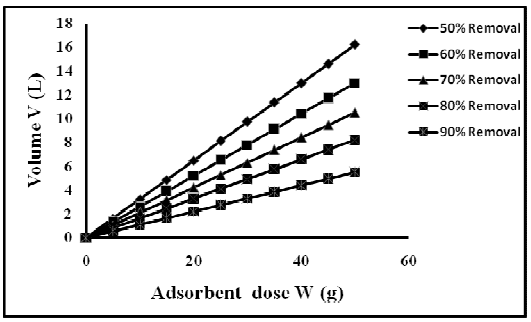


Figure 22. Volume of effluent treated against adsorbent dose for different percentages of MB removal.

Table 3. Kinetic parameters for the removal of MB by HOSDC at 295K

Dye concentration (mg/L)	1st order			2nd order			$t_{0.5}$ (min)	$D*10^{-7}$ (cm^2/s)
	K_1 min^{-1}	R^2	$qe_{calc.}$ (mg/g)	K_2 (g mg^{-1} min)	R^2	qe_{cal} (mg/g)		
25	0.0241	0.955	5.12	0.266	0.996	5.76	0.65	203
50	0.0233	0.974	9.46	0.045	0.993	10.65	2.09	63.2
75	0.0287	0.961	17.56	0.015	0.965	17.92	3.72	35.5
100	0.0298	0.96	23.5	$6.95*10^{-3}$	0.961	23.47	6.13	21.5
150	0.0188	0.88	24.63	$2.57*10^{-3}$	0.942	36.1	10.78	12.2
200	0.0134	0.972	27.59	$1.61*10^{-3}$	0.968	43.48	14.29	9.2

Table 4. Intraparticle diffusion rate parameter at different initial dye concentration

Dye concentration (mg/L)	K_{1d}	C_1	R^2_1	K_{2d}	C_2	R^2_2
25	0.4772	0.5781	0.991	0.1486	2.437	0.881
50	0.6859	0.9355	0.915	0.3026	3.3713	0.8506
75	1.0617	2.059	0.855	0.595	3.8778	0.756
100	1.4117	2.3085	0.928	0.6404	7.4521	0.7558
150	1.6305	2.4385	0.908	1.2652	5.0692	0.8305
200	2.2145	4.149	0.927	1.6442	3.2734	0.9564

Table 5. Thermodynamic parameters and activation energy for dye sorption onto HOSDC

Temperature K	ΔG° (kJ mol^{-1})	Ea (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (kJ mol^{-1})
295	-2.43	52.2	49.75	177.67
313	-6.35	52.35		
323	-7.73	52.44		
333	-9.12	52.52		

4. Conclusions

The present study confirmed that the prepared hydrolyzed oak sawdust calcium alginate composite has an effective adsorbent for removal of methylene blue dye from aqueous solution. Removal of methylene blue dye is pH dependent and the maximum removal was attained at pH 12. The equilibrium adsorption is practically achieved through a time of 270 min. It was also a function of initial adsorbent dose, dye concentration, agitation speed and temperature of the solution. Also adsorption equilibrium data follows; Freundlich isotherm models. The kinetic study of methylene blue dye on HOSDC was performed based on pseudo-first order, pseudo-second-order and intraparticle diffusion models.

The data indicate that the adsorption kinetics follow the pseudo-second-order model with intra-particle diffusion as one of the rate determining steps. The determination of the thermodynamic parameters (ΔG°, ΔH° and ΔS°) indicates the spontaneous and endothermic nature of the adsorption process. The positive value of ΔS° indicates that increasing randomness at the solid/liquid interface during the adsorption of MB on HOSDC. The activation energy of adsorption of methylene blue dye was found to be higher than 42 kJ mol^{-1} indicating chemically controlled process.

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3/3/2010

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The Journal of American Science

ISSN 1545-1003

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ISSN 1545-1003