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Cover Page, Introduction, Contents, Call for Papers, All papers in one file

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CONTENTS

	Abstract: As about 60% of finished price of goods are allocated to raw material and purchased parts by suppliers in the chain of automotive industry, the importance of supplier management and its performance is an ongoing problem. Moreover the need of monitoring of supplier performance has been emphasized in Quality Management System of automotive industry ISO/TS16949. To meet standard requirement and also continuous improvement in business, companies need to monitor their supplier performance. Delivery and quality are two of the most important indicators of supplier evaluation. This paper introduces a statistical approach to monitor supplier performance over time by using control charts. To monitor supplier delivery performance, a statistical control chart is developed based on conceptual model of how to implement in industry. Normality test is done on data and upper and lower control limits are calculated. Data gathered from supplier of a tier 1 company and out of control signals are recognized on chart. All out of control signals are removed from control chart and updated "In control" is obtained with improved mean and standard deviation. It can be employed in the industry and should result in improvement in supplier performance over time. [Journal of American Science 2010;6(4):5-11]. (ISSN: 1545-1003). **Key words**: Delivery Performance, Statistical Monitoring, Quality Management System (QMS)	
3	**A New Species of Cuttlefish , Sepia vecchioni (Cephalopoda,Sepiidae) from Colachal Coast, South India** Neethiselvan[1] N, Venkataramani[2] VK [1.] Fisheries Training and Research Center,VUTRC,Tamil Nadu Veterinary and Animal Sciences University,Aavin campus, Nanjikottai Road, Thanjavur-613 006, Tamil Nadu, India [2.] Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Thoothukkudi - 628 008, Tamil Nadu, India [email:drneethi@yahoo.co.in] **Abstract:** A new species of cuttlefish Sepia vecchioni sp. nov. hitherto wrongly treated as S. prashadi Winckworth, 1936, collected from the Colachal fish landing centre ($8^{o}10'$ N, $77^{o}15'$E) of South India is described. The dorsal mantle of S. vecchioni sp. nov. has distinct white stripes facing upwards in contrast to transverse zebra type stripes with the ends facing downward in the closely related species, S. prashadi. Unlike S.prashadi, a well-defined sexual dimorphism with respect to shape and length of arm is also seen in S. vecchioni sp.nov. In matured males of this species, the first and fourth pair of arms are greatly extended and the first pair is whip like. Males of this species also differ from that of S.prashadi with regard to sucker arrangement on hectocotylized arm. Though both the species can be classified under Acanthosepion species complex of Rochebrune (1984) based on cuttle bone characteristics, Sepia vecchioni sp.nov. also shares the characters of the Rhombosepion species complex of Rochebrune (1984) by having weakly and incompletely calcified cuttlebone. The comparison of morphological characters, meristic characters and cuttlebone characteristics of S.vecchioni sp.nov. with that of other five closely related species of the genus Sepia of world waters are also discussed. [Journal of American Science 2010;6(4):12-21] (ISSN:1545-1003). **Keywords:** Sepia vecchioni sp.nov.; Sepia prashadi; Acanthosepion species complex; Rhombosepion species complex; Hectocotylization	Full Text
4	**Water quality monitoring in Nigeria; Case Study of Nigeria's industrial cities** Ekiye, Ebiare * and Luo Zejiao China University of Geosciences, Department of Environmental science. 388 Lumo road, Wuhan, 430074, Hubei, P.R. China. luozejiao@hotmail.com **Abstract:** The aim of this study was to analyze the state of water quality management in	Full Text

	Nigeria's industrial cities. In a developing country such as Nigeria, there is immense demand for advancement in various facets of living and economic development is a priority of the government. This has led to increase in industries resulting in an increased quantity of discharge and a wide range of pollutants reaching water bodies. This study indicates that both urbanization and industrialization have contributed to the large scale of pollution currently observed in most Nigerian cities notably those swarming with industries namely; Lagos, Rivers, Kano and Kaduna states. There are no incentives for implementing pollution reduction measures. Wastes are disposed indiscriminately especially for small and medium scale industries. Data for this paper were obtained by observation, investigation and from related studies on the subject matter. Finally, this paper proposes constant river water monitoring as a step towards pollution abatement. [Journal of American Science 2010;6(4):22-28]. (ISSN: 1545-1003). **Key words:** Water, Quality, Monitoring, Nigeria, Industrial Cities	
5	**An investigation on the Strength and workability of cement based concrete performance by using ZrO_2 nanoparticles** Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran. ***** Corresponding Author: Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir **Abstract:** The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase ZrO_2 particles. ZrO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-ZrO_2 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of ZrO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase ZrO_2 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):29-33]. (ISSN: 1545-1003). **Key words:** Nanophase ZrO_2 particles; concrete; compressive strength; workability.	Full Text
6	**Embedded System Using Ultrasonic Waves and Voice Biometric to Build an E-Glass for the Blinds** Kenza Meridji, Khalid T. Al-Sarayreh. School of Higher Technology (ÉTS), University of Québec, Montréal, Québec H3C 1K3, Canada. kenza.meridji.1@ens.etsmtl.ca, khalid.al-sarayreh.1@ens.etsmtl.ca **Abstract:** Currently, embedded and real time systems are used in wide range of a related human applications to improve the quality of our lives such as embedded systems for communication (Mobile, satellite, and avionics systems), and control systems such as (microwaves, refrigerators and embedded system in vehicles). Nevertheless, embedded and real time systems are still immature. The application of these systems is used for various devices. However, these systems are never used for a human body to complete human missing-part functionality; which means the embedded system can be used as part of natural neural networks in a human system nerve. This paper proposes a technical view to build an electronic glass (E-Glass) for the blind people. Moreover, this paper provides the complete E-	Full Text

	Glass electronic circuit in which the electronic scanning system to tackle the objects and time signals are included. This E-Glass could be used by the blind to assist them in their ways without any human assistance. Moreover, it will be used by the blind to make them self confidence, to let them walk independently and to increase their morality. It is important to note that the hardware and software components of the E-Glass are not expensive. This work could be provided to the practitioner's people in the industry or to the students of the department of electrical or biomedical engineering. [Journal of American Science 2010;6(4):34-42] (ISSN: 1545-1003). **Keywords:** Embedded System, Neural Networks, Ultra Sonic Wave, Artificial Intelligence, Voice Biometric.	
7	**Assessment of the effects of the cement paste composite in presence TiO_2 nanoparticles** Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran. * Corresponding Author: Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir **Abstract:** The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase TiO_2 particles. TiO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-TiO_2 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of TiO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase TiO_2 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):43-46]. (ISSN: 1545-1003). **Key words:** Nanophase TiO_2 particles; concrete; compressive strength; workability.	Full Text
8	**Treatment of Light Contaminated Surface Water Using Slow Sand Filtration in China** Gracious Grace Lwesya*, Yilian Li China University of Geosciences, School of Environmental Sciences, 388 Lumo Road, Hongshan District, Wuhan City, Hubei province, 430074, P.R.China. graciouslwesya@yahoo.co.uk; yl.li988@yahoo.com.cn **Abstract:** This was a comparative study to investigate the effectiveness of slow sand filtration with the best type of sand in filtering water from the domestic lake at China University of Geosciences (CUG) in Wuhan. It was a laboratory scale experiment which had four columns with all having a length of 100 cm in height, 3cm in diameter, and the sand was filled to a depth of 80cm with sand sizes of 0.075-2mm, 0.075-0.5 mm, 0.5-2mm and a control of 0.075-2mm with no pre-growth of bio-film. The rate of trickling water was set at 2 rounds per meter (rpm) and the filter run period was 15 days with 7 days wet and 3 days dry cycle to prevent clogging. COD, TN, TP, DO and OC were analyzed. Overall, fine sand column had the best results but specifically, COD efficiency rate was best in column of fine sand with 83%, TP in mixed sand with 81%, TN in fine sand column with 67% and DO in the control column with 8.15mg/L and OC was best in fine sand column with 22.59g. The best type of sand would be considered as 0.075-0.5 mm because it dominated in most results. With all the conditions in place, slow sand filtration was very effective as it removes most of the organic matter and suspended materials hence the water can easily be re-used not only due to its efficiency but also its simplicity in operation, cost effectiveness as well as being environmentally sound. [Journal of American Science 2010;6(4):47-57]. (ISSN: 1545-1003). **Keywords:** Sand, filtration, bio-film, sand effectiveness.	Full Text

9	**Mineralization of organic compounds in wastewater contaminated with petroleum hydrocarbon using Fenton's reagent: a kinetic study** Gbehou Nounagnon Achille [1,*], Li Yilian [2] 1. School of Environmental Studies, China University of Geosciences, Hubei province, 388. Lumo Road, 430074 Wuhan, P.R China, achillegbehou@yahoo.fr, 008615927014830 2. China University of Geosciences, Department of Environmental Sciences, 388 Lumo Road, Hongshan District, Wuhan City, Hubei province, 430074, P.R.China, yl.li988@yahoo.com.cn **Abstract:** In the present work, the possibility of using chemical oxidation through Fenton's reaction for the pre-treatment of wastewater contaminated with petroleum hydrocarbon was investigated as laboratory-scale experiments. The effect of different operational conditions, namely, hydrogen peroxide, ferrous ion concentrations, temperature, and initial pH were evaluated. Operating at initial pH of 3, with a temperature of 28°C and a molar ratio H_2O_2/Fe^{2+}=9:1, it gave us 70.58% removal of COD. A kinetic study was carried out using a modified pseudo-first-order model. The experiment was performed at different temperatures hence allowed the calculation of Arrhenuis equation parameters and the global activation energy for the first-order reaction. [Journal of American Science 2010;6(4):58-66]. (ISSN:1545-1003). **Key words:** Chemical oxidation, wastewater, COD, activation energy	Full Text
10	**Similarity Identification and Measurement between Ontologies** Amjad Farooq and Abad Shah Computer Science and Engineering Department University of Engineering and Technology, Lahore – Pakistan **amjadfarooquet@gmail.com** **Abstract:** The retrieval of relevant and precise information from web has always been remained a serious problem. To address this problem, the idea of ontologies-based web, so-called semantic web, was proposed in 2001. But the problem is not completely solved due to the semantic heterogeneity suffered by ontologies. In this paper we propose a semi-automatic technique to measure the explicit semantic heterogeneity. The proposed technique identifies all candidate pairs of similar concepts without omitting any similar pair. The proposed criteria for similarity measurement are based on theme semantic web. The proposed technique can be used in different types of operations on ontologies such as merging, mapping and aligning. By analyzing its results a reasonable improvement in terms of completeness, correctness and overall quality of the results has been found. [Journal of American Science 2010; 6(4):67-85]. (ISSN: 1545-1003). **Keywords:** Semantic Web, Heterogeneity, Ontology Matching, Similarity Identification	Full Text
11	**Embedded ZrO_2 nanoparticles mechanical properties monitoring in cementitious composites** Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran. *** Corresponding Author:** Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir **Abstract:** In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase ZrO_2 particles has been studied. ZrO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-ZrO_2	Full Text

	particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of ZrO_2 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of ZrO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase ZrO_2 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):86-89]. (ISSN: 1545-1003). **Key words:** Nanophase ZrO_2 particles; concrete; split tensile strength; flexural strength.	
12	**The effects of incorporation Fe_2O_3 nanoparticles on tensile and flexural strength of concrete** Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran. *** Corresponding Author:** Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir **Abstract:** In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase Fe_2O_3 particles has been studied. Fe_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano- Fe_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of Fe_2O_3 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of Fe_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Fe_2O_3 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):90-93]. (ISSN: 1545-1003). **Keywords:** Nanophase Fe_2O_3 particles; concrete; split tensile strength; flexural strength.	Full Text
13	**Mechanical properties of cement mortar with Al_2O_3 nanoparticles** Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran. *** Corresponding Author:** Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir **Abstract:** In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase Al_2O_3 particles has been studied. Al_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano- Al_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of Al_2O_3 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of Al_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Al_2O_3 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):94-97]. (ISSN: 1545-1003). **Keywords:** Nanophase Al_2O_3 particles; concrete; split tensile strength; flexural strength.	Full Text
14	**Improvement the mechanical properties of the cementitious composite by using TiO_2 nanoparticles**	Full Text

Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno
Department of Technical and Engineering Sciences, Islamic Azad University (Saveh Branch), Felestin Sq., Saveh, Islamic Republic of Iran.
*** Corresponding Author:** Ali Nazari, Assistant professor, Tel: + 98 255 2241511, E-mail: alinazari84@aut.ac.ir

Abstract: In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase TiO_2 particles has been studied. TiO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-TiO_2 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of TiO_2 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of TiO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase TiO_2 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):98-101]. (ISSN: 1545-1003).
Key words: Nanophase TiO_2 particles; concrete; split tensile strength; flexural strength.

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Benefits of Fe_2O_3 nanoparticles in concrete mixing matrix

Full Text

Ali Nazari*, Shadi Riahi, Shirin Riahi, Seyedeh Fatemeh Shamekhi and A. Khademno
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Abstract: The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase Fe_2O_3 particles. Fe_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-Fe_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of Fe_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Fe_2O_3 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):102-106]. (ISSN: 1545-1003).
Keywords: Nanophase Fe_2O_3 particles; concrete; compressive strength; workability.

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Biodegradation of Produce Water Hydrocarbons by Pure Cultures of *Alcaligenes* sp.

Full Text

Chuma C Okoro [1], Olukayode O Amund [2]
[1] Department of Biological Sciences and Biotechnology, Caleb University, Lagos
[2] Department of Botany and Microbiology, University of Lagos, Nigeria
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Abstract: Biodegradation studies of hydrocarbons in untreated produce water from an oil production facility in Nigeria was undertaken over a period of time using pure cultures of *Alkaligenes* sp. Isolated from Escravos River where produce water was being discharged as at the time the studies were carried out. Gas chromatography and mass spectrometry were used to monitor the rate of reduction in some petroleum hydrocarbon fractions while the index used to evaluate biodegradation was the decreasing trend in the ratios of nC17/Pristane and nC18/Phytane. Gas chromatographic analysis showed that untreated produced water used for the study had an oil and grease content of 1407mg/L, this includes n-alkanes (608mg/L), Aromatics (13.88mg/L), NSO compounds (12.68mg/L) PAHs(0.833mg/L) and some unidentified greasy components. Upon mechanical treatment, the oil and grease component of

	produce water was reduced to 44mg/L comprising of n-alkanes (38.40mg/L), Aromatics (2.65mg/L), NSO compounds (1.78mg/L), PAHs (0.0655mg/L) and some unidentified greasy component. A pure culture of *Alcaligenes* sp. after 40 days of exposure to untreated produced water reduced the oil and grease content to 19.58mg/l comprising of n-Alkanes (16.87mg/l), Total aromatics (1.25mg/l), NSO compounds (0.98mg/l) and PAH (0.0096mg/l). This result indicate that produce water is readily biodegradable and pure cultures of *Alcaligenes* sp. used for the study were very efficient in the degradation of produced water hydrocarbons especially the recalcitrant PAH component when compared with the conventional mechanical treatment process. [Journal of American Science 2010;6(4):107-113]. (ISSN: 1545-1003). **Keywords**: Pure culture, Produce water, Biodegradation, Hydrocarbon, *Alcaligenes* sp.	
17	**Recognition of Nonrandom Patterns on Supply Performance by Employing Statistical Monitoring** Soroush Avakh Darestani [1], Professor Dr. Md. Yusof Ismail [2], Associate professor Dr. Napsiah bt. Ismail [3] [1.] Department of Mechanical and manufacturing engineering, University Putra Malaysia (UPM), 43400, Malaysia, soroushavakh@yahoo.com [2.] Department of Manufacturing Engineering, University Malaysia Pahang (UMP), 26300, Malaysia [3.] Department of Mechanical and manufacturing engineering, University Putra Malaysia (UPM), 43400 Malaysia **Abstract:** This paper introduces a practical methodology of assignable signals and Run chart tests for identification of nonrandom patterns of supplier performance by statistical monitoring. The assumption of normal distribution is one of the important factors to implement a control chart in industry and service. It is supposed that natural data shows lack of any nonrandom pattern signals or out of control points on control chart. The data of supplier's on-time delivery for automotive industry has been gathered and illustrated on control chart by employing appropriate transformation and assignable signals and run chart were tested on the control chart accordingly. The results show that tests were able to identify nonrandom patterns of supplier performance data. Out of control signals were removed from the control chart and show that on-time delivery performance was increased accordingly. The control chart with natural pattern can be used as pilot for monitoring supplier delivery over time and improve supplier delivery performance. [Journal of American Science 2010;6(4):114-122]. (ISSN: 1545-1003). **Key words:** Run Chart Pattern Recognition (RCPR); Johnson Transformation; Supply Performance; Statistical Process Control (SPC); Anderson-Darling test (AD); On-Time Delivery (OTD)	Full Text
18	**Estimation of Shelf Life for Water-based Paints Using Regression Methods** Obidi Olayide F [1], Nwachukwu Simon C[1], Aboaba, Olusimbo O [1], Nwalor, John U[2] [1]Department of Botany and Microbiology, University of Lagos, 11001, Nigeria [2]Department of Chemical Engineering, University of Lagos, 11001, Nigeria laideob@yahoo.com **Abstract:** The shelf lives of water-based paints made in Nigeria were investigated. The mean changes in the microbial population count of six freshly made paint samples (PS1 – PS6) were monitored fortnightly for a period of 10 months. The growth data of isolated organisms from the fresh and spoilt paint samples were fitted into a multiple linear regression model to predict shelf life for the fresh paint samples. The microbial population ranged from $1.0 \times 10^1 - 4.7 \times 10^5$ cfu/ml and from $1.0 \times 10^1 - 5.5 \times 10^3$ cfu/ml for bacteria and fungi over the study period. Physico-chemical parameters such as specific gravity (SG), optical density (OD),	Full Text

	transmittance (TR), pH and viscosity (VIS) were also determined every two weeks for the fresh paint samples over the ten-month study period. The measurements of the physico-chemical parameters suggested deterioration related to microbial population count of the paint samples. Consequently, the model developed comprised of two equations with particular attention to microbial population count and physico-chemical parameters of the paint samples. The microbial population counts of the spoilt paint samples were 3.4 x 10^{10} cfu/ml and 3.2 x 10^{5} cfu/ml for bacteria and fungi respectively. The changes in the physico-chemical parameters ranged from 2.8658 – 1.0853, 1.49 – 3.91, 6.9 – 2.3, 8.5 – 5.6, 11.7cst –10.8cst for SG, OD, TR, pH and VIS in fresh paint samples. The percentage residual error between the shelf life predicted and the shelf life experimental ranged between 0.001 and 0.500. The shelf lives obtained for the fresh paint samples were 19, 21, 23, 22, 37, and 22 months respectively. [Journal of American Science 2010;6(4):123-127]. (ISSN: 1545-1003). K**ey words:** Shelf life; Paints; Regression; Physico-chemical parameters	
19	**SOME STUDIES ON LEAD TOXICITY IN MARINO SHEEP** Mona S. Zaki[(1)]; Susan Mostafa[(2)]; and Isis Awad[(2)] [(1)] Dept. of Hydrobiology, National Research Center,Cairo, Egypt [(2)] Dept. of Biochemistry, National Research Center, Cairo, Egypt dr_mona_zaki@yahoo.co.uk **Abstract:** The problem of lead toxicity originated in a private farm in *El- Katta* "Giza governorate", due to ingestion of plant polluted with lead. About 8 out of 50 Marino sheep animals showed lead toxicity. The animal's age was 6 months. The animals suffered from depression, pressing head against objects, dilatation of eye pupils, total blindness (in 2 cases) with normal light reflex in both eyes, edema in briskets, enteritis with bloody diarrhea and pupil dilation. Also there were lacrimation, pale dirty mucous membrane and sunken eyes. Serum analysis from these animals revealed high lead concentration. In addition too, significant decrease in the levels of testosterone, LH, FSH. PCV, haemoglobin, R.B.C.s and total proteins were also decreased. Highly degeneration of kidney, and liver accompanied with elevation of AST, ALT, Urea, creatinine, cortisol, sodium, and potassium. Moreover, *S.epidermidis* and *S. Aeruginosa* were isolated. We conclude that the cause of animals morbidity and mortality in this farm was not due to bacterial infections but due to lead toxicity and we can say that polluted environment, especially with lead, can cause severe harm to animal health, in addition to serious danger on human health, by eating food polluted with lead. [Journal of American Science 2010;6(4):128-131]. (ISSN: 1545-1003). **Keywords:** Lead toxicity in Marino sheep, environmental pollution, biochemical and microbial changes	Full Text
20	**Perception of Women towards Family Values and Their Marital Satisfaction** Ali Edalati & Ma'rof Redzuan*. *Faculty of Human Ecology,University Putra Malaysia* *43400Serdang, Selangor, malaysia* **Corresponding Author*: E-mail: marof@putra.upm.edu.my; Tel: +60123484810 **Abstract:** The purpose of the current study is to examine the relationship between perceptions of women toward Iranian traditional values and their marital satisfaction. The 337 of women were selected as the population of the study. Stratified random sampling was used to select samples. The Kansas Marital Satisfaction (KMS) is used to measure marital satisfaction. It shows a positive relationship between perception of women toward traditional values (inequality in family affairs and inequality in regulation) and marital satisfaction. It has also shown that there is no relationship between perception of women toward traditional values of inequality in access job and inequality in political in relation to marital satisfaction. The multivariate regression analysis is utilized to answer the last objective. The analysis showed that among the two predictor variables, the variable of inequality in affairs was found to be	Full Text

	significant in explaining the observed variation in marital satisfaction (DV). Generally, the final model has explained 4.6% of total observed variance in marital satisfaction. The findings show one reflection of the inequality and discrimination that there is in family affairs and inequality in regulation between men and women in the Iranian society. The findings highlight the need for screening and identification of other factors and unequal situations in the family and society in which women are growing. [Journal of American Science 2010;6(4):132-137]. (ISSN: 1545-1003). **Keywords:** Family Values, Marital Satisfaction, Women perception.	
21	**The effect of *Diphenyl Dimethyl Bicarboxylate* and *Dexamethasone* on Immunological and parasitological parameters in murine *Schistosomiasis mansoni*** Ibrahim RB Aly[*], Mohamed A Hendawy[*], Eman Ali[*], Mohammed S. Hedaya[**] Mona MF Nosseir[***]. Departments of Parasitology[*], Surgery[**] and Pathology[***] Theodor Bilharz Research Institute, Giza, Egypt dr_mona_zaki@yahoo.co.uk **Abstract:** This work aimed to evaluate the effect of Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone either alone or combined with praziquantel (PZQ) on different parasitological, immunological, and pathological parameters that reflect disease severity and morbidity in murine schistosomiasis. Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone had no effect on worm burden but altered tissue egg distribution. This indicates that under the schedule used, both drugs did not interfere with the development of adult worms or oviposition but it can modulate liver pathology. Meanwhile, dexamethsone showed a marked reduction of granuloma size more than DDB. Dexamethasone-treated mice, also, showed lower levels of serum gamma interferon (IFN-γ), interleukin-12 (IL-12), and IL-4 together with higher IL-10 level compared to infected untreated control animals. These data suggested that dexamethasone is a convenient and promising co adjuvant agent causing decreased morbidity in murine schistosomiasis. [Journal of American Science 2010;6(4):138-145]. (ISSN: 1545-1003). **Keywords:** Schistosomiasis – Morbidity – Cytokines – Treatment.	Full Text
22	**The role of catechin against doxorubicin – induced cardiotoxicity in Ehrlich Ascites Carcinoma Cells (EAC) bearing mice** Samiha Abd El Dayem, Fatma Foda , Mona Helal, Asmaa Zaazaa**.** Zoology Department-Women`s College for Arts, Science and Education. Ain Shams University, 1 Asmaa Fahmy Street Heliopolis, Cairo, Egypt samdayem1153@yahoo.com **Abstract :** Doxorubicin (Dox) is a chemotherapy drug used for treatment of wide variety of cancers. It known that, Dox may cause cardiotoxicity by producing free radicals and oxidative stress along the period of treatment. Catechin is considered one of the flavonoids which has powerful antioxidant properties and free radicals scavenger. The present work was designed to investigate the protective role of catechin on doxorubicin – induced cardiotoxicity in Ehrlich Ascites Carcinoma (EAC) bearing- mice and to test whether catechin has an effect on the antitumor properties of the Dox. Mice were divided into five groups as follows: (G1): Control group, (G2) Mice were injected with Ehrlich Ascites Carcinoma (EAC) cells (2.5 x 10^6 EAC/ml) to form a solid tumor , (G3) Mice were inoculated with (2.5 x 10^6 EAC/ml) and injected (i.p.) with Doxorubicin (15 mg/kg), (G4) Mice were inoculated with (EAC) at the same dose and were injected (i.p.) with (200mg/kg) Catechin , Group5 (G5) Mice were injected (i.p.) with Doxorubicin 15mg/kg of and 200mg/kg of Catechin in addition to the inoculation with EAC (2.5 x 10^6 EAC/ml). Dox (15mg/kg) and /or Catechin (200mg/kg) were administrated after 10 days in EAC bearing- mice through a period of 2 weeks in six equal	Full Text

	injections. Results showed that, EAC -bearing mice treated with Dox plus Catechin recorded decrease in the mean tumor weight and significant increase in the cumulative mean survival time as compared to the other treated groups. Biochemical studies of EAC inoculation showed decline in serum total protein and lactate dehydrogenase activities, while serum total lipid has significantly increased. The treatment of tumor-bearing mice with Dox plus Catechin (G5) improved these levels. Significant increase in cardiac lipid peroxidation and glutathione contents for both tumor-bearing mice (G2) and doxorubicin groups (G3) were recorded. Combined treatment of Dox and Catechin (G5) caused amelioration in these contents. Glutathione peroxidase and superoxide dismutase activities showed highly significant increase in all treated groups. Administration of Dox plus Catechin (G5) modulate these activities. In conclusion, the present study suggested that Catechin treatment may significantly reduce cardiotoxicity induced by doxorubicin in Ehrlich Carcinoma - bearing mice by the induction of the cardiac antioxidant enzymes and blocking lipid peroxidation. Also, Catechin enhances the antitumor properties of doxorubicin by increasing its inhibitory effect on tumor growth. [Journal of American Science 2010;6(4):146-152]. (ISSN: 1545-1003). **Keywords**: Ehrlich Ascites Carcinoma (EAC); Doxorubicin; Catechin; Antioxidant enzymes; Lipid peroxidation; Heart; Mice.	
23	**Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.** Amal I Hassan* and Abeer H. Abd El-Rahim** *Radioisotope Department, Nuclear Research Centre, Atomic Energy Authority, Egypt. ** Department of Cell Biology, Genetic Engineering and Biotechnology Research Division, National Research Center, Egypt. dr_mona_zaki@yahoo.co.uk **Abstract:** The present investigate the various biological changes induced by hyperthermia (at 42°C) in female rats and the mode of recovery at 1, 6 , 24 & 72 hrs at different ages (2, 6 , 12 & 24 months) . Biological parameters studied were red blood cells (RBCs), white blood cells (WBC's), hemoglobin (Hb) , B% & T% lymphocytes. Immunoglobulin G &A (IgG & IgA) and serum activities of triiodothyronin and thyroxin (T_3 & T_4), the heat shock protein 70(HSP70). Besides, the chromosomal aberrations test and micronucleus formation were investigated in female rats. In attempt to find out the interaction between age and hyperthermia in such parameters in normal female rats. The results revealed that Highly significant increases of WBC's, B%, IgG and HSP70 at 1 till 72hr post WBH in aged 2 and 6 months. On the other hand, WBH caused a significant decrease in each RBC's, T_3 & T_4 at 6 till 72 hr post WBH. As well as, the count of Hb decreased in age 2 month at 1 till 72 hr post the heat exposure but increased at 1 & 6 hr in 6 month aged post WBH then decrease at 24 hr & 72 hr post WBH. T% lymphocyte count significantly decreased at 1 hr post WBH and increased at 6 hr & 24 hr then decrease again at 72 hr post WBH in ages 2 & 6 month. IgA level significantly increased in 6 aged rats at 1, 6 & 24 hr post WBH then decreased at 72 hr below the control value post WBH. The results revealed that WBH caused a significant increase of B% lymphocyte, Hb and IgA at 1 & 72 hr post WBH in age 12 & 24 months, except Hb in 12 month decreased at 72 hr post heat exposure. On the other hand, T% lymphocyte, RBC's , IgG and serum T_3 &T_4 decreased at 1 & 72 hr post heat exposure except IgG level increased at 72 hr post WBH . The level of HSP70 increased significantly at 1 till 24 hr post WBH in 12 month and reached to the control value at 72 hr post WBH. On the contrary, HSP70 decreased significantly at 1hr in aged rats (24 month), then increased significantly at 6 and 24 hr post heat exposure then decreased below the control value at 72 hr post WBH. In the chromosomal aberrations test, we observed positive responses at all ages but in different frequencies and recover may occur at 72 hr for the all except young age (2 month) which needed more time to completely recover., in the micronucleus test, we observed positive responses in all ages at 24hr only, while at 72hr post heat exposure the mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) were within the vehicle control group at all ages except 2 month which increased significantly than control group. The	Full Text

	results suggest that hyperthermia can induce both chromosomal aberrations and micronucleus formation. [Journal of American Science 2010;6(4):153-166]. (ISSN: 1545-1003). **Key words:** Hyperthermia –HSP70, chromosomal aberration –micronucleus.	
24	**Some Promising Wild Edible Plants of Srinagar and its Adjacent Area in Alaknanda Valley of Garhwal Himalaya, India** J. K. Tiwari[1], R. Ballabha[1] and P. Tiwari[1] [1]Department of Botany, HNB Garhwal University, Srinagar Garhwal, Uttarakhand- 246 174, India jktiwari31@rediffmail.com, radhekuniyal.2007@rediffmail.com, ptiwari29@rediffmail.com **Abstract:** The present communication deals with the ethnobotanical exploration, identification, concerns and future potentialities of the wild edible plant species consumed by the local people inhabiting in the hilly areas of Alaknanda valley that fall in the Uttarakhand state of India. A total of 55 plant species belonging to 35 families were recorded from the study area. Amaranthaceae, Lamiaceae and Moraceae were the dominant families with 4 species each, while Anacardiaceae, Fabaceae, Rosaceae and Rutaceae followed with 3 species and rest were represented by one species from each family. The four major life forms were herbs, shrubs, climbers and trees. Herbs made the highest proportion of the edible species (18) followed by trees (17), shrubs (13) and climbers (7). The plant species were divided into two classes - prepared in to vegetables and consumed as raw. 23 species belonged to the former category while the later was represented by 32 plants. [Journal of American Science 2010;6(4):167-174]. (ISSN: 1545-1003). **Keywords:** Wild edible plants, Garhwal Himalaya, indigenous knowledge, local inhabitants.	Full Text
25	**Genetic alterations induced by toxic effect of thermally oxidized oil and protective role of tomatoes and carrots in mice** Mariam G. Eshak[1], Inas S. Ghaly[1]*, Wagdy K. B. Khalil[1]*, Ibrahim M. Farag[1], Kadry Z. Ghanem[2] [1]Cell Biology Department, [2]Food Science and Nutrition Department, National Research Centre. 12622 Dokki, Giza, Egypt wagdykh@yahoo.com ; Inas.ghali@yahoo.com **Abstract:** The present study was designed to investigate the genetic alterations and sperm abnormalities in male mice fed diet containing thermally oxidized sunflower oil repeatedly used for frying processes (FO). Also, tomatoes and carrots were added to FO diet to test their protecting ability against potential hazards caused by oxidized oil rich foods. The genetic alterations including DNA fragmentation and chromosome aberrations as well as changes of mRNA expression of some lipid metabolism related-proteins were determined. The results revealed that rate of DNA fragmentation was significantly higher in animals fed FO diet than those of animals fed normal oil diet (NO). On the other hand, the rates of DNA fragmentation decreased in animals fed FO diets plus low (5%) or high (10%) concentration of tomatoes (FOT1 or FOT2) or carrots (FOC1 or FOC2) compared with those fed FO diet. Chromosome examination showed that total structural aberrations increased significantly in animals fed FO diet than those fed NO diet. On the other hand, animals fed diets containing FO plus low or high concentration of tomatoes or carrots had significantly lower frequencies of total structural aberrations than those fed FO diet. Sperm studies showed a significant increase in the number of morphologically abnormal sperms and a significant decrease in the sperm count in animals fed FO diet compared with those fed NO diet. However, the animals fed diets containing low or high level of tomatoes or carrots showed significant decreases of sperm abnormalities. The mRNA expressions of the lipid metabolism related-genes, RBP, H-FABP and C-FABP were significantly higher in liver tissues of mice fed FO diet than those found in mice fed NO diet. However, the expression of all tested genes was down-regulated in FOT1 and FOT2 or FOC1	Full Text

	or FOC2 groups compared with those detected in the FO group. In conclusion, the present study adds evidence for a link between prolonged feeding intake of FO diet and induced mutagenic effects in animal cells. However, tomatoes and carrots proved to be good protective agents against hazards of such mutagenic foods. [Journal of American Science 2010;6(4):175-188]. (ISSN: 1545-1003). **Keywords:** Thermally oxidized oil, mice, tomato and carrot, genetic alterations, RT-PCR, sperm morphology.	
26	**Preparation and Characterization of Amine-Imine Derivatives Used in Organic Thin Film Transistor** [1]Chien-Chih Lin, [2]Hsien-Chiao Teng, [3]Shen Cherng, [1]An Chi Yeh [1]Department of Chemical and Material Engineering, Chengshiu University, Niaosong, Taiwan, RO China [2]Department of Electrical Engineering, ROC Military Academy, Fengshan, Taiwan, RO China [3]Department of Computer Science and Information Engineering, Chengshiu University, Niaosong, Taiwan, RO China cherngs@csu.edu.tw **ABSTRACT:** In this report, synthesis and characterization of Amine-imine derivatives of BIP and NIP are presented. Amine-imine derivatives have more delocalization molecular orbits having excitation spectra with red shift. Additionally, the different distribution of molecular energy levels for BIP and NIP causes the emission and absorption of different wavelengths. In this study, both BIP and the NIP were used as the organic thin film transistor active layer deposited on a silicon wafer substrate and the surface morphology, structure of permutation as well as carrier mobility rate were discussed. [Journal of American Science 2010;6(4):189-192]. (ISSN: 1545-1003). **Keywords:** molecular orbits, carrier mobility, surface morphology	Full Text
27	**Cyanobacteria of a Tropical Lagoon, Nigeria.** Adesalu, Taofikat Abosede [1], Nwankwo, Dike Ikegwu.[2] [1]Department of Botany and Microbiology, University of Lagos, Nigeria. [2]Department of Marine sciences, University of Lagos, Nigeria. boseadesalu@yahoo.com. **Abstract**: Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa, Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanosarcina, Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa, M. flos-aquae, M. wesenbergii* and *Anabaena flos-aquae.* In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanosarcina hueberliorum* is new record for Nigeria. [Journal of American Science 2010;6(4):193-199]. (ISSN: 1545-1003). **Keywords:** Cyanophytes, tropical, bloom, Lagos lagoon complex	Full Text

Relationship between Body Fat Percent and Maximal Oxygen Uptake among Young Adults

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Abstract: The present study was conducting to examine the relationship between maximum oxygen uptake (VO_2max) and body fat percent among international students in Universiti Putra Malaysia. VO_2max and body fat percent are importance factors at health and sport research. Twenty six male student (26 +/- 5 years old in age and 168 +/- 5 cm in high and 73 +/- 5 kg in weight) at Universiti Putra Malaysia. Maximal were participated at this investigation. Oxygen Uptake and body fat percent have been measured by the routine protocols. At the end of this research have been shown signification and negative correlation (-0.042) between the VO_2max and body fat percent. Results of this investigation show that there is negative correlation between maximum oxygen uptake and body fat percent. Increase the VO_2max have been shown with decrease and improve on body composition. [Journal of American Science 2010;6(4):1-4]. (ISSN: 1545-1003).

Key Words: Maximum Oxygen Uptake, Body Fat Percent, Aerobic Capacity, Physical Activity

1. Introduction

Living in recent century and specifically after Second World War is difference with the all of previous centuries. Non-activity and changing over in life style is the result of machining method in every work. Advancements in using the technology may help the human to make daily work easier and also in most jobs technology has helped to alleviate the high risk of injury for many type of physically demanding jobs. However while the technology has increased the safety of the daily work and also was the main cause to reduce the physical activity level among the people (Brooks, 2002). There are several factors which are importance in health and fitness level. Cardiovascular, muscular, cardio respiratory fitness with normal and standard level of the body composition are the main factors that researcher have been focused. Have been reported several investigation to examine effect of the exercise and training methods to theses factors (LaMonte et al., 2000; Sergi et al., 2009; Suzuki et al., 1998). Have been shown that functional capacity and cardiovascular fitness improved by the regular exercise (Collins et al., 2004). Among the fitness factors, aerobic fitness is the main factors that enhance human to doing daily jobs and also improve the ability to long time duration exercise. VO_2max is maximum oxygen that human cans consume during exercise in one minute (D. W. Hill & Rowell, 1996). There are several methods to improve aerobic capacity. Regular aerobic exercise is the most importance factors to improve the human aerobic power and also maximum oxygen uptake (Tan & Yang, 2007). Have been shown other fitness factor such as body composition is closely related with aerobic fitness and VO_2max. Daily diet are using to doing daily work. Food before using by the body to provide the energy was converted to ATP. Adenosine three phosphates is the main source of the energy. Extra foods in body will be stored as the fat in some area of the bodies such as the abdominal (Gause-Nilsson & Dey, 2005; White et al., 2008). Regular exercise and physical activity is the main cause to improve body composition and cardiovascular fitness. Body fat percent, body mass index, water cells, muscle mass are factors which are influenced by exercise. Recent investigations have been shown that there are the closely relationship between the body composition factors and aerobic and cardiovascular and aerobic fitness (Bandyopadhyay et al., 2006). Have been reported improve at both of the cardiovascular fitness and aerobic capacity with improve at body composition. And also improve at some body composition aspect such as body fat percent, body mass index and body muscle mass at result by the exercise and improve on maximum oxygen uptake (A. M. Hill et al., 2007; Tomassoni et al., 1985). There are several aims to do exercise. One of the main reason to doing exercise is improve the body composition factors. This research is going to examine relationship between the body fat percent and maximum oxygen uptake among health student. Actually the health and fitness are the importance issue among student and specifically among international student which have sedentary manner their daily works.

2. Methods and Procedure of Research

Twenty six volunteer's male students (26 +/- 5 years old in age and 168 +/- 5 cm in high and 73 +/- 5 kg in weight) were participated in this investigation. The students were recruited among postgraduate international student from the University Putra Malaysia. In physical activate level, all participate were considered untrained and had not participated in regular exercise for at least six months period to the start of this investigation. All subjects were attended on physiology libratory to measurement or estimating the variables in this investigation. All tests were conducted at the laboratory condition with 22-25C temperature. And also all measurement and VO_2max test was at the morning and before the lunch time. In the initial visit to the laboratory, subjects were asked to fill up the question paper which contained name, age and physical activity level. At the first time weight was measured by the calibrated electronic balance scale with division of 100grams. High has been measured by the standard height meter with division of the 1cm. three skin fold methods have been used to estimating the body fat percents. All measurement was in left side of the subjects (Deurenberg et al., 2007). All the measurements were made by the researcher for reducing the experimental error. Skin fold thickness measurements were done from standard anthropometrical reference points as well as the Chest, Abdomen and Thigh. Body fat Percent value was estimated by applying the standard equation. To measurement the maximum oxygen uptake researcher has been used Bruce protocol on the treadmill and gas analyzer. The Bruce treadmill protocol has now become a standardized procedure used to evaluate the aerobic capacity and cardiovascular fitness of athletes or non athletes health people. Nowadays there is the other protocol to estimate the VO_2max for patients and for clinical VO_2max or maximum oxygen uptake is simply the most amount of oxygen that a subject can consume from the air and utilize and is measured by the volume of oxygen per minute per kg body weight per time (mL/kg/min). Some other unite is for VO_2max volume as well as oxygen per whole body weight per minutes (lit/m). To doing the Bruce protocol, subject after warm up (3-5 minutes) was stand on the treadmill and out the mask on their face hardware and software was calibrated before start the test. Maske was put on their face at star the test. Treadmill was turn on run and with click on the start menu at monitor test was started. The following steps were pointed on the default program on the software and Subject could stop their test every time that the received to exhaustion or they can not continue for every reason such as hearth disease. Treadmill had change in the shape automatically in per step. And also in the per step was increasing the speed in treadmill.

Walk
Stage 1 = 1.7 mph at 10% Grade
Stage 2 = 2.5 mph at 12% Grade

Jog/walk
Stage 3 = 3.4 mph at 14% Grade
Stage 4 = 4.2 mph at 16% Grade

Run:
Stage 5 = 5.0 mph at 18% Grade
Stage 6 = 5.5 mph at 20% Grade
Stage 7 = 6.0 mph at 22% Grade

Recovery:
00-02 min 2.5 Speed slope 12

In the recovery phase the subject should continue their walking on the treadmill for tow minute and after that device was stop automatically.

3. Statistical Analysis and Results

Correlation Body fat percent and maximum oxygen uptake was conducted by Paired Samples Statistics. A p-value 0.05 was considered statistically significant. Analysis proces were performed using SPSS 17 on the Windows XP (SPSS Inc, Chicago, IL, USA).

Correlation between body fat percent (BFP) and maximum oxygen uptake (VO_2max) is shown at figure1. Data are expressed as the mean and standard deviation in body fat percent and maximum oxygen uptake at table 1.

After the Paired Samples Statistics has been shown that, there were relationships between the BFP and VO2 max among these two groups (correlation was equal -0.402). Actually with increasing BFP, maximum oxygen reduces and by decreasing BFP, maximum oxygen increases (table 2).

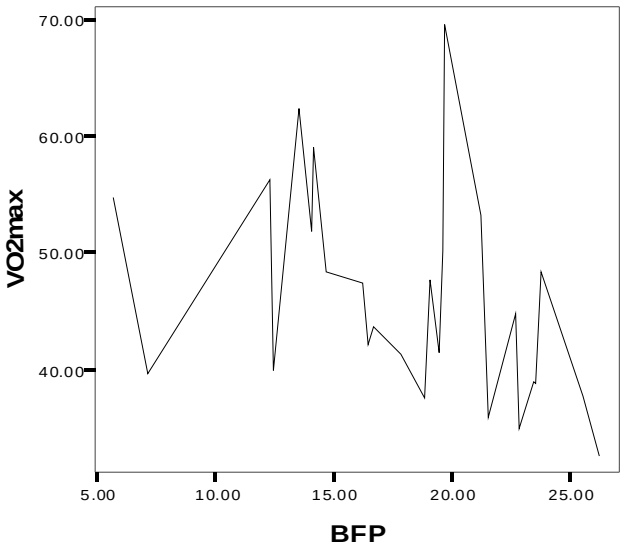

Figure 1Figure 1: Correlation between maximum oxygen uptake (VO_2max) and body fat percent (BFP) among students.

Table 1: Discreptive Data for VO_2max and BFP

	N	Mean	Std. Deviation	Std. Error Mean
Body Fat Percent	26	18.0449 (%)	5.290	1.037
Maximum Oxygen Uptake	26	46.1035 (ml/kl/min)	9.116	1.7

Table 2: Correlation between BFP and VO_2max

	N	Correlation	Signification
BFP-VO_2max	26	-0.402	0.042

4. Discussion

Result of this investigation has been shown that there are a strong relationships between the Body Fat Percent and Maximum Oxygen Uptake. Body composition is the main factors that may influence on human ability to doing works. And also have been shown that any improve on aerobic ability may enhance body composition factors. Previous studies have been clarified that, there are strong relationships between healthy folks and their Body Fat Percent, also was proved that there are relationships between maximum oxygen uptake and endurance performance. Improving endurance performance can increase cardiovascular ability for working. Struggling to improve the cardiovascular performance can reduce cardiovascular diseases. In this study, shows that, increasing in body fat percent can cause reducing VO_2max. Wrong methods in livening and non-activity can bring obesity among young men. Tennis, running, swimming and some other aerobic sports, need high performance in cardiovascular system. Some factors can affect this ability. Body fat percent increases with unsuitable diet in food eating and unbalancing between the out put and input of food which is eaten. With endurance training and diet suitable, people can reduce body fat percent and reduce cardiovascular diseases. One of the most importance issue among student and international student is non activity that may increase risk factors such as body fat percent and at this result reduce ability to doing daily works. Researcher purpose at the final of this research,

giving the information to people and students may improve the physical activity level among them.

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[1] Bandyopadhyay, A., Chatterjee, S., Chatterjee, P., Papadopoulou, S. K., & Hassapidou, M. (2006). VO2max of boys according to obesity status. Journal of Human Movement Studies, 51(3), 167-180.

Brooks, L. L. (2002). THE EFFECTS OF AFTER-SCHOOL PHYSICAL ACTIVITY AND ADULT ENCOURAGEMENT ON ADOLESCENTS. University of Wisconsin.

Collins, E., Langbein, W. E., Dilan-Koetje, J., Bammert, C., Hanson, K., Reda, D., et al. (2004). Effects of exercise training on aerobic capacity and quality of life in individuals with heart failure. Heart & Lung: The Journal of Acute and Critical Care, 33(3), 154-161.

Deurenberg, P., Pieters, J. J. L., & Hautvast, J. (2007). The assessment of the body fat percentage by skinfold thickness measurements in childhood and young adolescence. British Journal of Nutrition, 63(02), 293-303.

Gause-Nilsson, I., & Dey, D. K. (2005). Percent body fat estimation from skinfold thickness in the elderly. Development of a population-based prediction equation and comparison with published equations in-75 year-olds. Journal of Nutrition, Health and Aging, 9(1), 19-24.

Hill, A. M., Buckley, J. D., Murphy, K. J., & Howe, P. R. C. (2007). Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. American Journal of Clinical Nutrition, 85(5), 1267-1274.

Hill, D. W., & Rowell, A. L. (1996). Running velocity at VO2max. Medicine and Science in Sports and Exercise, 28(1), 114-118.

LaMonte, M. J., Eisenman, P. A., Adams, T. D., Shultz, B. B ,.Ainsworth, B. E., & Yanowitz, F. G. (2000). Cardiorespiratory fitness and coronary heart disease risk factors: The LDS Hospital Fitness Institute Cohort. Circulation, 102(14), 1623-1628.

Sergi, G., Coin, A., Sarti, S., Perissinotto, E., Peloso, M., Mulone ,S., et al. (2009). Resting VO2, maximal VO2 and metabolic equivalents in free-living healthy elderly women. Clinical Nutrition.

Suzuki, I., Yamada, H., Sugiura, T., Kawakami, N., & Shimizu, H. (1998). Cardiovascular fitness, physical activity and selected coronary heart disease risk factors in adults. Journal of Sports Medicine and Physical Fitness, 38(2), 149-157.

Tan, S., & Yang, F. (2007). The effects of regular resistance training on the elders' aerobic work capacity. Chinese Journal of Rehabilitation Medicine, 22(9), 776-778.

Tomassoni, T. L., Blanchard, M. S., & Goldfarb, A. H. (1985). Effects of a rebound exercise on aerobic capacity and body composition. Physician and Sportsmedicine, 13(11), 110-115.

White, M., Davies, P., & Murphy, A. (2008). Validation of percent body fat indicators in pediatric oncology nutrition assessment. Journal of Pediatric Hematology/Oncology, 30(2), 124-129.

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An Investigation on Supplier Delivery Performance by using SPC Techniques for Automotive Industry

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Abstract: As about 60% of finished price of goods are allocated to raw material and purchased parts by suppliers in the chain of automotive industry, the importance of supplier management and its performance is an ongoing problem. Moreover the need of monitoring of supplier performance has been emphasized in Quality Management System of automotive industry ISO/TS16949. To meet standard requirement and also continuous improvement in business, companies need to monitor their supplier performance. Delivery and quality are two of the most important indicators of supplier evaluation. This paper introduces a statistical approach to monitor supplier performance over time by using control charts. To monitor supplier delivery performance, a statistical control chart is developed based on conceptual model of how to implement in industry. Normality test is done on data and upper and lower control limits are calculated. Data gathered from supplier of a tier 1 company and out of control signals are recognized on chart. All out of control signals are removed from control chart and updated "In control" is obtained with improved mean and standard deviation. It can be employed in the industry and should result in improvement in supplier performance over time. [Journal of American Science 2010;6(4):5-11]. (ISSN: 1545-1003).

Key words: Delivery Performance, Statistical Monitoring, Quality Management System (QMS)

1-Introduction:

SPC has its origins in the 1920s. Dr Walter A. Shewhart of the bell Telephone laboratories was one of the early pioneers of the field. SPC is a set of problem-solving tools may be applied to any process. The control chart is the most powerful of the SPC tools (Montgomery et al., 2007). SCM performance, supplier selection, SCM quality, Customer satisfaction and so on are the most researched area in the scope of supply chain management since 1980. Many studies have been done in the scope of supplier selection and evaluation. Nowadays, automotive industries are one of the industries which have implemented On time delivery system for their suppliers. Just in Time is a production system in which the movement of goods during production and deliveries from suppliers are carefully timed so that the right parts, in the right quantities are provided at the right time. The success of JIT approach depends on the capability of the company's suppliers to meet several criteria on quality quantity and delivery performance (Alwan, 2000).

From QMS approach in automotive industry, the ISO/TS 16949 was jointly developed by the IATF members and coupled with customer-specific requirements defines quality system requirements for use in the automotive supply chain(IATF, 2002). Based on QMS, the organization shall evaluate and select the suppliers based on their ability to supply products in accordance with the organization's requirements.

2- Literature review:

Supply Chain Management can be seen as the process of strategically managing the procurement, movement and storage of materials, parts and finished inventory and also related information flows through the organization and its marketing channels in such a way that current and future profitability are maximized through the cost effective fulfillment of orders (Rahul andAltekar, 2005). As stated in BMW supplier Quality Management System (BMW, 2008), they are looking for suppliers who are best in "Product Quality", "Life Time Cost" and "Delivery Process Capability and On-time Delivery".

PSA PEUGEOT CITROËN stated in their supplier Quality Principles in 2006 that the quality of their

vehicles depends directly on the quality of the external supplies that represent over 70% of the manufacturing cost of a vehicle(PSA PEUGEOT CTROEN, 2006).

A study also has been conducted in PROTON on determining what supplier development programs are being undertaken by PROTON as Malaysian car-manufacturing firm. PROTON establishes supplier rating schemes which track supplier performances in terms of management, financial, technical capability, quality, delivery, services, price, etc(Abdullah et al., 2008).

Supplier linkage and internal departmental linkage have significant effect on overall SCM performance where the supplier linkage is the most important significant on SCM overall performance(lee et al., 2007). On time deliveries, reducing response time, determining customer future needs were also highly ranked on SCM performance (Tan et al., 2002). Process management has been about improving the linkages between internal processes and supply chain management has been about improving the linkages between firms(McAdam and McCormac, 2001). Supply chain performance is significantly affected by the strategic nature of the purchasing function (Paulraj et al., 2006).

Traditionally, purchasing was considered as a clerical function, where the relationships between suppliers and buyers tended to be adversarial. However, many organizations are now moving towards a more collaborative approach (Humphreys et al., 2001). Many manufacturers embraced the concept of supply chain management to improve product development, quality and delivery goals, and to eliminate waste.

An insufficient level of communication between internal customer and supplier could be among the reasons conceivable for the situation detected. Issues that give rise to criticism or dissatisfaction are either not articulated clearly enough to be understood or are not perceived, realized or accepted as a source of improvement(Large andKonig, 2009).

There is always a need for a tool to select potential suppliers and continually monitoring and assessing the performance of the suppliers. What manufacturers are doing is to establish dynamic trading relationships. However, it needs a tool to select potential suppliers with the capability of continually monitoring and assessing the performance of their suppliers (Liu et al., 2000). The prioritization of suppliers for development depends upon, for example, the supplier's quality performance and the importance of the product supplied. Supplier performance shall be monitored through delivered product quality and delivery schedule performance. Moreover, the organization shall promote supplier monitoring of the performance of their manufacturing processes. The organization shall determine, collect and analyze appropriate data generated as a result of monitoring and managing from other relevant sources such as suppliers (ISO, 2002). Moreover, Performance measurement describes the feedback or information on activities with respect to meeting customer expectations and strategic objectives (Lehtonen, 2001).

The aim of any type of data analysis is to gain understanding from data. When we collect process performance data we see that it varies. The information in this variation is important to the understanding of how the process is performing and statistical process control (SPC) is primarily the tool for understanding variation(Stapenhurst, 2005). A large number of statistical tools and methods are applied in manufacturing and service firms. Statistical process control (SPC) includes a number of them. Shewhart introduced control charts (CCs) in the beginning of the 1930s and currently it is one of the most widely discussed statistical techniques(Xie andGoh, 1999).

SPC is not really about statistics or control, it is about competitiveness. Organizations, whatever their nature, compete on three issues: quality, delivery and price if the quality is right, the chances are the delivery and price performance will be competitive too(Oakland, 1999). It was found that control charts could be adapted to monitor the supplier/retailer interface and that the results could potentially be used to monitor and manage the buyer/supplier relationship effectively. Moreover, SPC is one of the techniques used in quality assurance programs and/or Total Quality Management (TQM), for controlling, monitoring and managing a process either manufacturing or service through the use of statistical methods(Anthony et al., 2000).

SPC is a technique of investigating of the dynamics of failing supplier's performance. A good perspective can be obtained by considering three basic market perspectives(Crichton et al., 2003). A well established quality principle is that strategies that lead to improving supplier performance should be based by the first instance on improving supplier capability; and, in the second instance on moving the average performance towards the overall target performance required by the customer(Besterfield, 1994). To achieve this, the use of SPC control charts seems to offer a simple solution to establishing problem-solving dialogues between buyers and suppliers. An exploratory investigation done in food multiple retailers on their suppliers performance

monitored using SPC techniques has demonstrated the value of SPC in consistently monitoring performance; of presenting this information in an understandable format; of using this information to stimulate buyer/supplier problem solving activities; of focusing on process consistency; and, of linking measurement protocols throughout the supply network. Both SPC and conventional statistical analysis can contribute to this paradigm. It was found that these control charts could be adapted to monitor the supplier/retailer interface and that the results could potentially be used to monitor and manage the buyer/supplier relationship effectively(Morgan and Dewhurst, 2008).

Most investigation and research have been done on method of supplier selection and performance evaluation and few researches have been done on supplier monitoring and tools of monitoring of suppliers. The performance monitoring is one of the important clauses in the standard.

This paper employed SPC Control Charts for monitoring of supplier performance in the automotive industry in a Quality Management System environment. Based on the literature, Figure 1 is depicted to employ SPC as monitoring tool to cover the need of supplier monitoring in both ISO/TS and SCM environments.

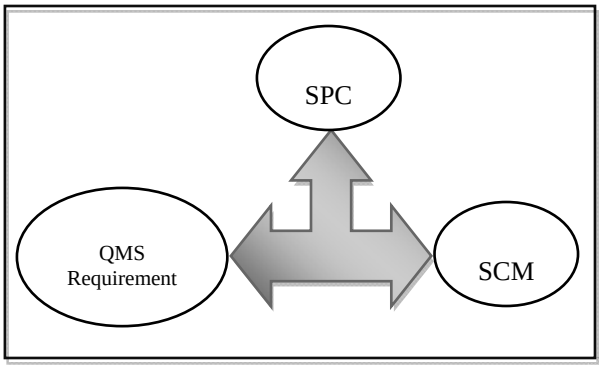

Figure 1, Relation between three main scope of thesis

3- Methodology

From the literature, delivery is performance is selected through investigation by statistically controlling of supplier's performance.

Hereby, a conceptual model has been proposed (Figure 2) and it shows the consecutive steps to develop the statistical analysis.

To develop the control chart, the first step is to post preliminary data to the chart along with the control limits and central lines. The next step is to adopt standard values with the available data. If an analysis of the preliminary data shows good control, then central lines can be considered as representative of the process and these become the standard values. Good control can be briefly described as that which has no out-of-control points, no long runs on either side of the control line, and no unusual patterns of variation (Besterfield, 2009).

The delivery measures by On Time Delivery (OTD) indicator on a monthly basis has been defined by automotive supply chain according to below:

$$OTD = \frac{\text{The number of on time delivered parts}}{\text{The number of ordered parts}} \times 100$$

For employing mean and range chart, the OTD indicator should be defined according to the literature of statistical process control philosophy. Suppose the supplier supplies m lots in specific time (weekly, monthly, and quarterly). If the gathered data approximates normal distribution, individual mean OTD and moving range can be employed otherwise, to deal with normality assumption on data, Central limit theorem should be applied with $n \geq 4$ and the proposed control chart will be substitute with OTD mean and range according to:

$i = 1, 2, \ldots\ldots, n$ and $j = 1, 2, \ldots\ldots, m$

n = sample size of supplier's OTD

m = the number of OTD samples for establishing control chart

OTD = the percentage of On – Time Delivery

R_i = range of the ith OTD's subgroup

$\bar{R}$ = average of the OTD's subgroup ranges

A_2, D_3 and D_4 factors for computing central lines of X bar and R chart(Besterfield, 2009)

Where:

$$\overline{OTD_j} = \frac{\sum_{j=1}^{m} \overline{OTD_j}}{m} \quad ,$$

$$\overline{\overline{OTD}} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{m} OTD_{ij}}{n \times m} \quad ,$$

$$UCL_{\overline{OTD}} = \overline{\overline{OTD}} + A_2\bar{R} \, , LCL_{\overline{OTD}} = \overline{\overline{OTD}} - A_2\bar{R}$$

$$UCL_R = D_4\bar{R} \quad , \quad UCL_R = D_3\bar{R}$$

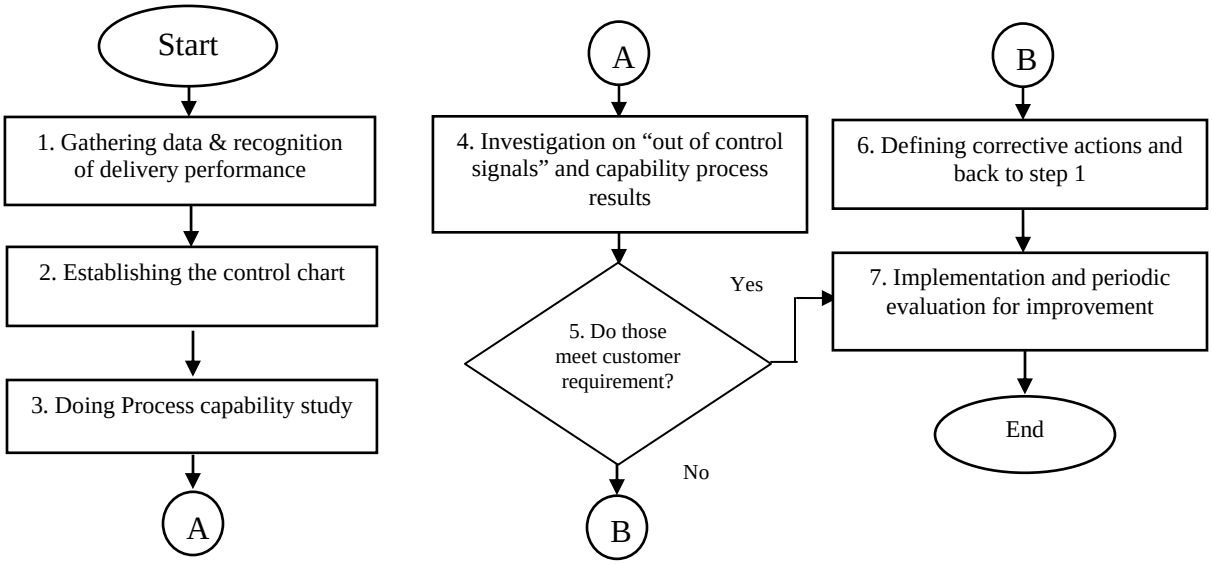

Figure 2, Conceptual model for monitoring of delivery Performance

4- Case study:

Based on the equations and according to the proposed conceptual model, an automotive vendor was selected as case study. This company is a Tier 1 for automotive industry. The OTD was measured for 88 arrival lots according to Table 1.

According to the propose methodology, the distribution of data approximated to small extreme value in 1% significant level according to figure 3. To establish the control chart, the data should show normal distribution. In this situation, the research used central theorem limit to meet this assumption where $n \geq 4$. All calculations and charts have been produced by using Minitab software version 15.1.0.0.

Table 1, Basic statistics of supplier delivery performance

Variable	count	mean	St. Dev	variance	minimum	maximum	skewness	kurtosis
Supplier OTD	88	74.31	17.81	317.11	9	97	-1.18	1.45

Delivery mean and range chart is depicted in Figure 4. According to gathered data 22 points were drawn on OTD mean and range chart and 13 points from mean chart and 5 points from range chart were out of control. It shows caused signal on data. The test on data also demonstrates that data have clustering caused signal as well. The upper control limit of OTD chart is obtained as 91.23 where it may be 100 in a perfect situation.

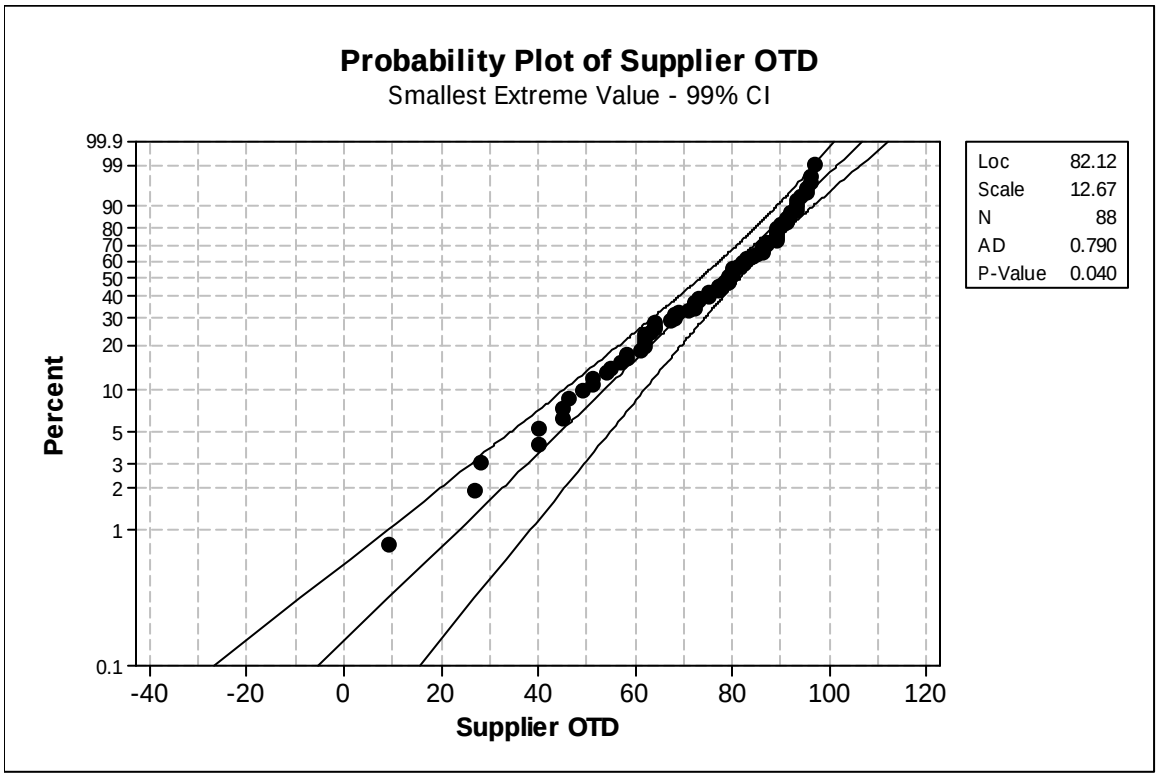

Figure 3, Probability Plot for Delivery performance data

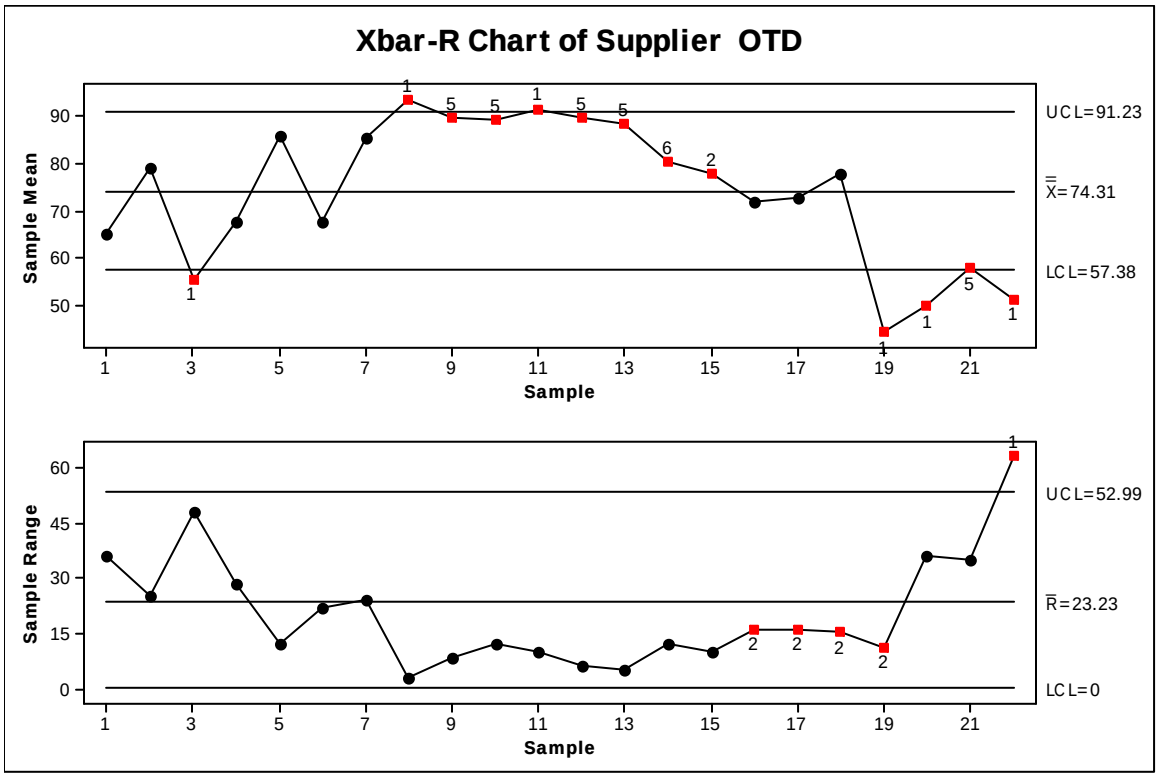

Figure 4, Deliver mean and range chart for n=4

To see whether the observed delivery performance meet customer expectation, the gathered data should be examined with upper and lower specification limit (USL and LSL of delivery by customer). It is required by customer that the lower limit is 75 and the upper limit should be 100. Capability study has been done based on approximated data to smallest extreme value distribution in 95% confidence interval.

For OTD's lower that 75, it can be stated that it does not meet customer expectation. Based on the capability study equation according to:

$$P_p = \frac{Min\left\{(USL - \bar{X}), (\bar{X} - LSL)\right\}}{3S}$$

$$PPU = \frac{USL - \bar{X}}{3S}$$

Hereby, a delivery capability study has been done and result depicted in figure 5. The Target defined 90 out of 100. As suppliers try to deliver the lots on time with same ordered quantity, it is obvious that the histogram has right skewness and majority of data has kurtosis to upper delivery limit. The minimum delivery capability is defined by customer 1 where the result shows that both $P_p = 0.23, PPU = 0.79$ have not met minimum customer expectation and corrective actions are required to increase delivery performance.

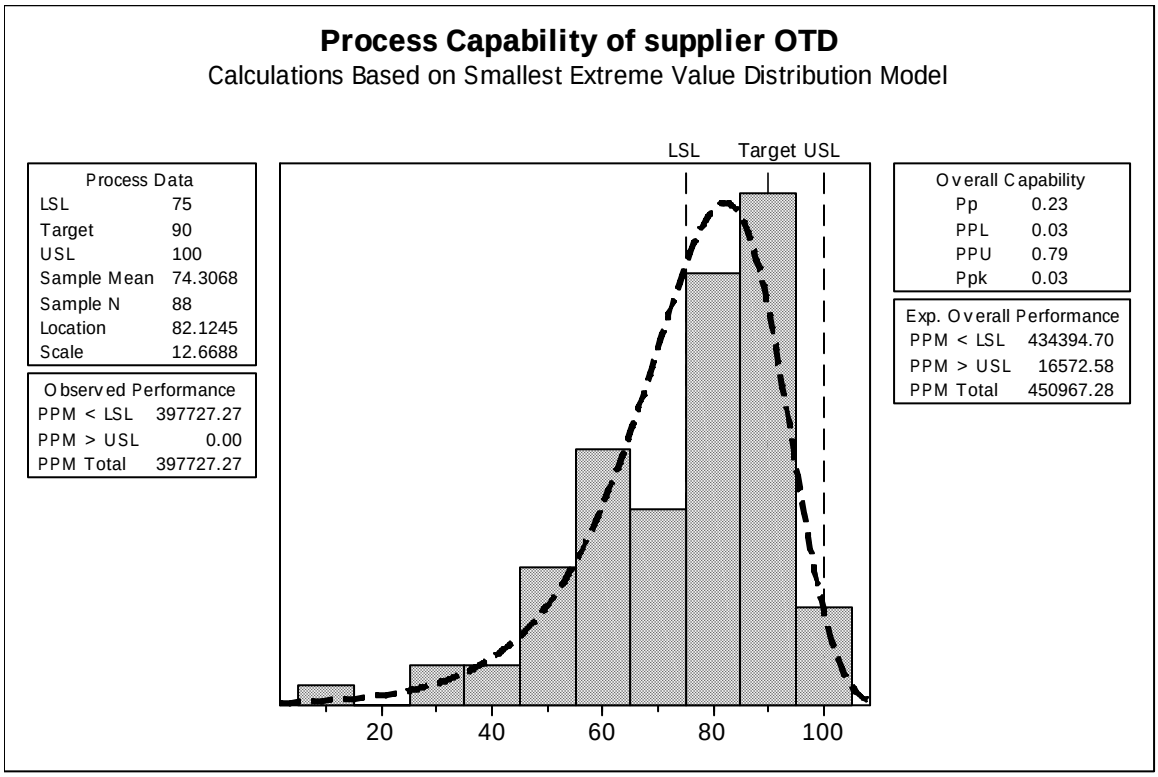

Figure 5, Delivery capability study

5- Result:

Investigation on delivery performance shows that it can be modeled for monitoring by control chart. Out of control and caused signals pattern may be recognized on supplier on time delivery data. It is important to identify the distribution of data before any action. It can work nicely when the numbers of arrival lots are in continuous supply.

For the low rate of arrival, short run chart may be used in this respect. The result shows that OTD data approximates to smallest extreme value distribution. It is because the data tend toward upper specification limit and supplier trying to deliver right quality with right ordered quantity "on time' to customer. The low delivery capability was because of two major issues.

Firstly, the target was appointed 90 out of 100 by customer where the mean of the OTD was 74.31 and it makes a bias from target and results in lower capability. Secondly, due to extensive standard deviation, as the overall process capability is based

on overall standard deviation, a centralized standard deviation will achieve a higher delivery capability.

The corrective action by supplier can lead to improvement on two phases. First, by reducing the deviation and range of deliveries and second by adjusting the mean with target and improve mean of delivery. According to SCM literature, for a long term partnership both supplier and customer effort is needed in this respect to obtain good result.

6- Conclusion

From the literature, delivery performance is one of the most important indicators of supplier performance. This paper intended a model "on time delivery" using control chat. It is shown that control charts can be used to monitor delivery performance. It also may help organizations to enhance their supplier performance monitoring via corrective actions and improvement on caused signals patterns. When the rate of lot arrival is low, standardized or individual chart also recommended.

References

1. Abdullah, R., Lall, K. L., & Tatsuo, K. (2008). Supplier development framework in themalaysian automotive industry. International journal of economicsand management, 2(1), 29--58.
2. Alwan, L. C. (2000). Statistical Process Analysis (first ed.): McGraw-Hill.
3. Anthony, J., Balbontil, A., & Taner, T. (2000). Key ingredients for the effective implementation of statistical process control. Integrated manufacturing system, 49, 242-247.
4. Besterfield, D. H. (1994). Quality controll (4 ed.): Prentice Hall, Englewood.
5. Besterfield, D. H. (2009). Quality Control (8 ed.): Prentic Hall.
6. BMW. (2008). Supplied Part Quality management.
7. Crichton, G., Zammit, V., Hughes, J., & Vammen, S. (2003). Connecting purchasing and supplier strategies to shareholder value: emerging trends for executive action, (Executive report of the future purchasing alliance): Charted inistitute of purchasing andsupply
8. Humphreys, P. K., Shiu, W. K., & Chan, F. T. (2001). Collabrotive buyer-supplier relationship Hong Kong manufacturing firms. Supply chain management journal, 6(4), 152.
9. IATF. (2002). ISO/TS16949:2002. 2009, from http://www.iatfglobaloversight.org/
10. ISO. (2002). Quality management system-Particular requirement for the application of ISO9001:2000 for automotive production and relevant service part organizations (2 ed.): ISO copyright office.
11. Large, R. O., & Konig, T. (2009). A gap model of purchasing internal service quality: Concept Case and internal survey. Journal of purchasing and supply management, 24-32.
12. lee, C. W., Kwon, I.-w. G., & Severance, D. (2007). Relationship between supply chain performance and degree of linkage among supplier internal integration. Supply chain management: An international journal, 12(6), 444-452.
13. Lehtonen, T. W. (2001). Performance measurement in construction logistics. International journal of production economies, 69, 107-116.
14. Liu, J., Ding, F. Y., & Lall, V. (2000). Using data envolement analysis to compare suppliers for supplier selection and performance improvement. Supply chain management: An international journal, 5(3), 143-150.
15. McAdam, R., & McCormac, D. (2001). International business processes for global and supply chain management. Business process management journal, 7(2), 114.
16. Montgomery, D. C., Runger, G. C., & Hubele, N. F. (2007). Engineering Statistics (4 ed.): John Wiley & Sons, Inc.
17. Morgan, C., & Dewhurst, A. (2008). Multiple retailer supplier performance: An exploratory into using SPC techniques. International journal of production economies, 111, 13-26.
18. Oakland, J. (1999). Statistical process control (4 ed.): Butterworth Heinemann Oxford.
19. Paulraj, A., Chen, I. J., & Flynn, J. (2006). Level of strategic purchasing: Impact on supply chain integration and performance. Journal of purchasing and supply management, 12, 107-122.
20. PSA PEUGEOT CTROEN. (2006). Supplier Quality Management Principles. Retrieved Dec, 2006
21. Rahul, V., & Altekar. (2005). Supply chain management: Printice Hall.
22. Stapenhurst, T. (2005). Mastering Statistical Process Control (1 ed.): ELSEVIER.
23. Tan, k. C., Lyman, S. B., & Wisner, J. D. (2002). Supply chain management a strategic perspective. International journal of operation and production management, 22(6), 614-631.
24. Xie, M., & Goh, T. N. (1999). Statistical techniques for quality. The TQM magazine, 11, 238-242.

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A New Species of Cuttlefish , *Sepia vecchioni* (Cephalopoda,Sepiidae) from Colachal Coast, South India

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Abstract: A new species of cuttlefish *Sepia vecchioni* sp. nov. hitherto wrongly treated as *S. prashadi* Winckworth, 1936, collected from the Colachal fish landing centre (8°10' N, 77°15'E) of South India is described. The dorsal mantle of *S. vecchioni* sp. nov. has distinct white stripes facing upwards in contrast to transverse zebra type stripes with the ends facing downward in the closely related species, *S. prashadi*. Unlike *S.prashadi*, a well-defined sexual dimorphism with respect to shape and length of arm is also seen in *S. vecchioni* sp.nov. In matured males of this species, the first and fourth pair of arms are greatly extended and the first pair is whip like. Males of this species also differ from that of *S.prashadi* with regard to sucker arrangement on hectocotylized arm . Though both the species can be classified under *Acanthosepion* species complex of Rochebrune (1984) based on cuttle bone characteristics, *Sepia vecchioni* sp.nov. also shares the characters of the *Rhombosepion* species complex of Rochebrune (1984) by having weakly and incompletely calcified cuttlebone. The comparison of morphological characters, meristic characters and cuttlebone characteristics of *S.vecchioni* sp.nov. with that of other five closely related species of the genus *Sepia* of world waters are also discussed.[Journal of American Science 2010;6(4):12-21] (ISSN:1545-1003)

Keywords: *Sepia vecchioni* sp.nov.; *Sepia prashadi*; A*canthosepion* species complex; *Rhombosepion* species complex; Hectocotylization

1.Introduction

Out of the 14 species of cuttlefishes so far recorded from Indian waters, 13 species belong to the genus *Sepia*, Linnaeus, 1758 and one species belongs to the genus *Sepiella*, Grey, 1849 namely *Sepiella inermis*. Based on the shape of cuttlebone, Khromov *et al.* (1998) have classified the cuttlefishes of the genus *Sepia* into 6 species complexes. According to their classification, the 13 species of cuttlefishes of the genus *Sepia* of Indian waters can be classified under three different species complexes namely, *Acanthosepion* (Rocheburne, 1884), *Sepia senu stricto* (Linnaeus, 1758) and *Doratosepion* (Rocheburne, 1884). Seven species *viz*. *Sepia aculeata* Orbigny, 1848, *S. brevimana* Steestrup,1875, *S. thurstoni* Adam & Rees,1966, *S. prashadi* Winckworth,1936, *S. stellifea*, Homenko and Khromov,1984, *S. elliptica* Hoyle,1885 and *S. prabahari* Neethiselvan and Venkataramani, 2002(Neethiselvan and Venkataramani, 2002) can be classified under the species complex *Acanthosepion*. Three species such as *Sepia pharaonis* Ehrenberg, 1831, *S. latimanus* Quoy and Gaimard, 1832, and *Sepia ramani* Neethiselvan, 2001(Neethiselvan, 2001) belong to *Sepia sensu stricto* species complex. The three species *viz Sepia arabica* Massy, 1916, *S. trygonia* Rochebrune, 1884, and *Sepia kobiensis*, Hoyle1885 which are found distributed in Indian coast have been classified under *Doratosepion* species complex of Rochebrune, 1884 (Khromov *et.al.* 1998). The present study deals with the taxonomic description of a new species of cuttle fish belonging to the genus *Sepia* with the comparison of its closely related species *S. prashadi* Winckworth, 1936 and five other closely related species of world waters

Etymology

Dr.Michael Vecchione, is a renowned cephalopod taxonomist working as Director in National Marine Fisheries Service, National Systematic Laboratory of National Museum of Natural History, Smithsonian Institution, Washington DC, USA. In honor of his excellent contribution to the field of Cephalopod taxonomy, the newly described species is named after him.

2.Materials and methods

The present work is based on 50 live specimens collected from the catches of trawls operated at a depth range of 70-100m off Colachal (8°10' N, 77°15'E)coast on 15th December 2006. Three specimens were also collected from Pazhayar(11 ^{0} 23.5 ' N, 79 ^{o} 41.5 ' E) coastal waters at depth of about 10m on 5th January 2007.

The fifty live specimens of *Sepia vecchioni* sp.nov collected from Colachal fish landing centre included 25 males and 25 females representing a wide length group . Fifty live specimens of its closely related species, *S. prashadi*, Winckworth, 1936 were collected on 25 th December 2006 covering 25 males and 25 females from the catches of trawls operated off Thoothukudi (8°48'N, 78°9'E) coast at a depth of range of 70-100 m.

Twenty morphometric measurements, two meristic characters and six cuttlebone measurements were recorded for each specimen of both the species. The morphometric and cuttlebone measurements were taken nearest to the millimeter with the help of a divider and a scale. As clear-cut sexual dimorphism was observed in *S.vecchioni* sp.nov, with respect to length of arms and cuttlebone characteristics, the sexes were separately treated with regard to length of arms and cuttle bone characteristics. However for other characters, the data were pooled irrespective of sexes. In the case of *S.prashadi*, care was taken to include both juveniles and matured specimens. Since Silas *et al* (1985) have reported that the length at first maturity of this species vary from 6.7 to 7.2 cm, specimens with the Dorsal Mantle Length (DML) ranging from 6.0 to 8.9 cm were used for the study for reasonable comparison. Though no notable sexual dimorphism with respect to any of the body proportions has been observed for *S.prashadi* in the studies carried out along Chennai coast(13°5.6' N, 80°18.1'E) of India which is type locality of *S.prashadi* (Jothinayagam, 1987), the morphometric measurements with respect to length of arms and cuttlebone were separately recorded for comparison with that of *S.vecchioni* sp.nov. The morphometric measurements were expressed as percentage of DML and those of cuttlebone were expressed as the percentage of Cuttle Bone Length (CBL). The percentage overlapping of body proportions of *S. vecchioni* sp.nov. with that of *S. prashadi* were worked out as per the method of Hubs and Hubs (1953).

3. Results

Type locality Colachal, (8°10' N, 77°15'E) South India

Distribution

Sepia vecchioni sp.nov. is found to have distribution mainly in the extreme coast of India in Arabian sea, mainly in offshore fishing grounds with the depth ranging from 70 to 100m. Its sporadic occurrences was recorded in Bay of Bengal along Northern Tamil Nadu coast (South east coast of India) in coastal waters with the depth as low as 10m .

Materials.Holotype

LA 5 and LA 6 (DML 126mm male; DML108 mm female) in Fisheries College and Research Institute Reference Museum (FCRIRM), Thoothukudi, Tamil Nadu, India, 15th December 2006 coll. by N.Neethiselvan.

Paratypes

(i) 50 specimens with DML 65 to 140 mm with same details as above. (ii) 2 specimens deposited in Fisheries college and Research Institute Reference Museum (LA 7 and LA 8: DML 116 mm male and 104 mm female) (iii) 2 specimens deposited in Marine Biological Station Reference Museum (MBSRM) at Parangipettai (Porto-Novo), Annamalai University, Tamil Nadu, India. (DML 116 mm male and 105 mm female)

Other materials

Two male (110 & 140 mm DML) and one female (115 mm DML) in coastal waters of Pazhayar (11°23.5' N, 79°41.5'E) at a depth of about 10m coll. by N. Neethiselvan, 5th January 2007.

Diagnosis

Sepia vecchioni sp. nov. can be well identified with the following diagnostic characters: (i)distinct white stripes with ends facing upwards on dorsal mantle, white patches and dots on lateral sides of dorsal mantle head and arms, white patches and dots on lateral sides of dorsal mantle arranged vertically (Figure 1a Figure 1b);

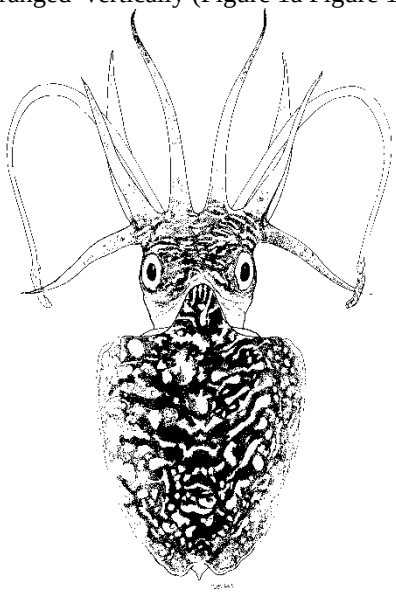

Figure1a. Dorsal view of *Sepia vecchioni* sp. nov. (Male) (Illustration by D'Antony Manuela, FAO copy right, 2007)

(ii) well defined sexual dimorphism noticeable with respect to length of arms, matured males with first and fourth arms much extended, first pair whip like, arm formula being 1.4.3.2(Figure 1a), arms in female not notably extended although with the same formula (Figure 1b);

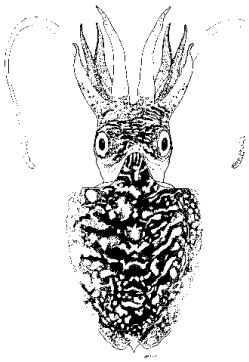

Figure 1b. Dorsal view of *Sepia vecchioni* sp. nov. (Female)(Illustration by D'Antony Manuela, FAO copy right,2007)

(iii) left ventral arm in male hectocotylized, two middle series of suckers in hectocotylized arm completely absent even from basal portion, basal region with 2-3 rows of bi-serially arranged normal suckers proximally followed by a ventral and dorsal series, each series with 18-20 reduced minute suckers in hectocotylized portion , hectocotylized portion follows 8 to 10 normal quadriserial minute suckers, there after suckers very minute up to arm tip (Figure 2);

Figure 2.Hectocotylized arm of *Sepia vecchioni* sp. nov. (Male) (Illustration by D'Antony Manuela, FAO copy right,2007)

(iv) tentacle slender; tentacular club elongate with four extremely enlarged median suckers, the middle two being very much enlarged (Figure 3)

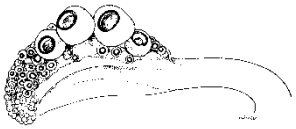

Figure 3.Tentacular club of *Sepia vecchioni* sp. nov. (Illustration by D'Antony Manuela, FAO copy right, 2007)

Common names

Sepia vecchioni sp. nov. is popularly called by fishermen as '*Paper cuttle fish'* because of its translucent and thin mantle tissue and is also called as *'Kuttan'* by vernacular name. It is also called as 'dotted cuttle fish' as the dorsal mantle has white patches and dots.

Fishery

Sepia vecchioni sp. nov. forms commercial fishery off Kanyamumari caost from August to November in trawlers operated at a depth of ranging from 70 – 100 m. It constitutes mono species fishery during peak fishing season and form sporadic fishery in the motorized traditional fishing in hook and lines along with other cuttlefish species .

Description

The morphometric measurements of *S. vecchioni* sp.nov. and *S. prashadi* are given in Table 1 and the percentage overlapping of body proportions of *S. vecchioni* sp.nov. with that of *S. prashadi* are given in Table 2.The description of various body parts of *S. vecchioni* sp.nov. are given below.

Mantle

Mantle elongate , ovate, broadest at anterior end, mid dorsal part in anterior end produced into a triangular lobe, mantle so soft that the ridges of cuttlebone visible through dorsal mantle and can be felt by hand, dorsal mantle pinkish brown with distinct white stripes and spots in fresh condition and persist even after death, stripes with undulations on mid dorsal mantle, stripes discontinuous, stripes on anterior triangular lobe very much curved and facing upwards, white patches and spots on lateral sides of dorsal mantle arranged almost vertically, (Figure1a, Figure 1b); ventral mantle with numerous minute pinkish brown spots and12 distinct luminescent streaks, 6 on each side, adjacent and parallel to fin base, maximum width 63.34% (55.81-84.53%) of DML

Fins

Fins very soft, narrow, fin breadth not uniform, wider near posterior end , a narrow white line runs along fin base on dorsal mantle demarcating mantle and fin ;fin starts slightly below the anterior margin of mantle with the distance of 5.84 %(2.29-20%) of DML, fin width 7.65% (2.94- 13.05) of DML.

Head

Head prominent, short and as long as inter orbital width, dorsal side with numerous white spots, patches, inter orbital width30.46 (20.94 –43.06) of DML; eye prominent and its diameter 16.30% (10.78—30.00) of DML.

Funnel

Funnel large, thin walled, reaches almost base of fourth ventral arm, funnel length 36.09 % (30.00 -- 46.14) of DML

Arms

Arms generally elongate, dorsal side of arm with white patches and dots, arms soft with pointed end, I pair slender, II and III pair slightly flattened and ventral arms (IV pair) long with well developed keels and tapering ends, arms in matured male I and IV pair notably elongated , I pair very elongate and whip like , arm formula being 1.4.3.2 (Figure 1a), arms in female not remarkably extended though general arm formula being the same (Figure 1b), lateral sides of arms with numerous minute pinkish brown spots, arms with quadriserial suckers arranged in oblique rows throughout; suckers with horny rings with vertical sides, basal rows of suckers large and get gradually reduced towards distal end, those at tips very minute; left dorsal arm in males (I)69.92 (51.20-83.33 %) of DML, left dorsal lateral arm (II)48.99% (40.00-61.52%) of DML; left ventral lateral arm (III)49.76% (33.32-65.25%) of DML and left ventral arm

(IV)62.35 (45.21-80.04%) of DML, in females left dorsal arm (I) 61.26%(49.31-81.51 %) of DML, left dorsal lateral arm (II) 47.24% (33.32-68.62%)of DML, left ventral lateral arm (III) 47.85% (36.86-61.00%) of DML and left ventral arm IV 59.47% (46.66-76.16%) of DML

Hectocotylus

Left ventral arm in male hectocotylized, two middle series of suckers completely absent even from basal portion, basal portion with 3 rows of biserial normal suckers proximally followed by a ventral and a dorsal series of minute suckers each consisting of 18-20 suckers in hectocotylized portion, two series of suckers of the hectocotylised portion widely separated from each other with distinct gap, hectocotylized portion follows 7 to 8 rows of minute quadri serial suckers and there after suckers very minute up to arm tip (Figure 2).

Tentacles

Tentacles long, slender, longer than body 178.04%(150.78-256.85%) of DML, tentacular stem rounded in cross section; tentacular club slender and elongate 17.21%(13.05-27.68%) of DML, club with four enlarged median suckers , two middle suckers being extremely enlarged (Figure 3), distal end of club with about 20 minute suckers, swimming membrane at anterior end broad which gradually tapers towards posterior end, protective membranes on ventral side narrow with 1 or 2 suckers , on contrary dorsal protective membrane broad with 4 to 6 minute suckers on its boarder, protective membranes do not extend beyond corpus and united at base.

Buccal membrane

Buccal membrane thick, rostrum of horny beak dark in colour, embedded in buccal mass seen in middle.

Colour

Body pinkish brown with white dots and conspicuous white stripes on dorsal mantle; 12 luminescent vertical streaks 6 on each side of the ventral mantle persist even after death and preservation in ice.

Cuttlebone

Cuttlebone fragile, elongate, elliptical, acuminate on both anterior and posterior ends, color pale pink, dorsal surface mostly flat, slightly convex and highly granulose, cuttlebone of female slightly broader than that of male, mid dorsal side of the cuttlebone weakly calcified, covered with a peelable smooth chitinous membrane, $3/4^{th}$ of anterior lateral edges calcareous, polished and yellowish in color, dorsal surface with one median and two longitudinal lateral ribs which can be felt through dorsal mantle of animal, lateral ribs diverge slightly towards distal end; ventral surface with one medial longitudinal groove and two lateral grooves one on each side, running along entire length of cuttlebone , lateral grooves fades towards the distal end and become less distinct, medial groove also becomes shallow and get widened towards distal end of loculus; loculus slightly convex, upper margin of striated zone inverted 'V' shaped, inner cone short with pocket like cavity, limbs of inner cone narrow; sulcus deep; outer cone translucent with poorly developed wings; spine short, prominent and curved upward.

As per the classification of the genus *Sepia* by Khromov *et al* (1998) into six different species complexes, *Sepia vecchioni* sp.nov can be classified under *Acanthosepion* species complex of Rochebrune (1984) due to distinct pocket like cavity of the inner cone (Figure 4a,Figure 4b). However, it also shares some of the characters of *Rhombosepion* species complex of Rochebrune (1984) by having weakly calcified cuttlebone and partially calcified dorsal surface at the distal end. Cuttlebone in female relatively broader than that of males, in males, width of cuttlebone 27.06% (24.60-29.88) of CBL, length of loculus 28.33%(24.61-31.98%) of CBL, indicating that the length of loculus is almost equal or slightly higher than width ; length of striated zone 64.49% (60.44-71.45) of CBL, inner cone length about 2.06% (1.30-3.28%) of CBL.

In females, width of cuttlebone 28.91% (25.97-31.82) of CBL, length of loculus 28.06%(23.91-31.26%)of CBL, indicating that the length of loculus is almost equal or little less than width, length of striated zone 63.37% (55.86-70.90) of CBL, inner cone length about 1.89%(1.09-2.22%) of CBL

Affinity

Sepia vecchioni sp. nov. is closely related to *S. prashadi* by having 4 extremely enlarged median suckers on the tentacular club (Figure 3 , Figure 5a).

The ventral mantle of both the species has 12 distinct luminescent vertical lines 6 on each side, adjacent and parallel to fin base, Both the species are found matured in the size range of 7.0 to 7.5 cm. They also resemble each other in body proportions as evidenced from the high percentage of overlapping (Table.2). The cuttle bone of both the species is also pale pink in color. Apart from *S prashadi*, other five species of *Sepia* such as *S. omani* Adam and Rees, 1966, *Sepia vossi*, Khromov, 1996, *Sepia papillata* Quoy and Gaimard, 1832, *Sepia chirotrema* Berry, 1918 and *Sepia opipara* Iredale, 1926 also show resemblances with *Sepia vecchioni* sp. nov. by having 3 to 5 extremely enlarged suckers on tentacular club occupying majority of the club surface (Khromo *et al*.,1998; Lu, 1998 & Jereb and Roper,2005).

Table1. Morphometric measurements of *Sepia vecchioni* sp.nov. and *Sepia prashadi* (in cm)

Characters	*Sepia vecchioni* sp.nov.			*Sepia prashadi*		
	Mean	SD	Range	Mean	SD	Range
Maximum width of mantle	6.6429	0.6711	5.5 – 8.4	4.8352	0.3312	4.0 – 5.8
Fin width	0.8175	0.3364	0.3 - 1.5	0.3130	0.1139	0.2 – 0.7
Fin length	9.1048	1.4601	6.0 – 13.0	5.6944	0.6172	4.5 - 7.4
Maximum width including fin	7.6429	1.0899	5.8 – 10.7	5.2815	0.3781	4.2 – 6.5
Eye diameter	1.7175	0.3701	1.0 – 3.0	1.211	0.1560	1.0 – 1.5
Inter orbital width	3.1921	0.4480	2.2 – 4.0	2.6185	0.3086	2.0 – 3.5
Free height	0.6143	0.2878	0.3 – 1.8	0.6630	0.1725	0.3 – 1.0
Length of funnel	3.7937	0.4886	2.6 – 5.0	2.7444	0.2910	2.2 – 3.5
Tentacle length	18.6460	1.8892	15.0– 23.5	16.3389	1.6308	12.0-21.0
Tentacular club length	1.8095	0.2964	1.4 – 2.5	1.3944	0.1446	1.0 – 2.0
Male						
Left first arm length	8.2593	1.7041	5.2 – 11.5	3.6487	0.5926	2.5 – 5.5
Left second arm length	5.5852	1.1323	3.6 – 8.0	3.4000	0.5588	2.4 – 4.5
Left third arm length	5.6519	1.1777	3.3 – 7.9	3.3538	0.4997	2.5 – 4.9
Left fourth arm length	7.0741	1.3424	4.7 – 10.1	3.6590	0.5895	2.6 – 5.9
Female						
Left first arm length	6.0833	0.942	4.2 – 8.0	3.2133	0.7455	2.5 – 5.3
Left second arm length	4.6389	0.7088	3.0 – 6.2	3.0267	0.6361	1.9 – 4.6
Left third arm length	4.7639	0.7739	3.4 - 6.0	3.0867	0.6054	2.2 – 4.5
Left fourth arm length	5.9250	1.1302	4.0 – 8.0	3.6000	0.4980	2.6 – 4.8
Male						
Cuttle bone length	10.5000	1.4958	7.7 – 12.6	7.2308	0.5630	6.0 – 8.9
Cuttle bone width	2.8750	0.2861	2.3 – 3.2	2.3897	0.1410	2.1 – 2.6
Length of loculus	3.0250	0.4521	2.3 – 3.9	2.8359	0.3000	2.2 – 3.3
Striated zone length	6.7125	0.7574	5.5 – 8.0	3.9744	0.4419	3.3 – 5.3
Inner cone length	0.2250	0.0829	0.1 – 0.3	0.2641	0.0480	0.2 – 0.3
Spine length	0.2875	0.0331	0.2 - 0.3	0.3605	0.0630	0.3 - 0.5
Female						
Cuttle bone length	10.2263	0.8252	9.0 – 12.5	6.9933	0.7672	6.0 – 8.8
Cuttle bone width	3.0158	0.2661	2.6 – 3.5	2.4600	0.2154	2.1 – 2.9
Length of loculus	2.9263	0.2988	2.2 – 3.5	2.9467	0.5352	2.2 – 3.7
Striated zone length	6.6105	0.7040	5.7 – 8.3	3.6800	0.5062	3.1 – 4.6
Inner cone length	0.1947	0.0223	0.1 – 0.2	0.2333	0.0471	0.2 – 0.3
Spine length	0.2684	0.0567	0.2 - 0.4	0.3400	0.0611	0.2 - 0.4

Table 2. Percentage overlapping of body proportions of *Sepia vecchioni* sp.nov. with that of *Sepia prashadi*

Characters	Overlapping range	Overlapping ratio	Extreme range	Extreme ratio	Percentage of overlapping
Maximum width of mantle	59.81 - 76.68	16.87	55.81 – 84.59	28.78	58.62
Fin width	2.94 - 9.23	6.29	2.67 - 13.05	10.38	60.60
Fin length	72.42 - 85.74	13.32	71.45 - 96.00	24.55	54.26
Maximum width including fin	67.95 - 88.35	20.40	58.31-92.28	33.97	60.05
Eye diameter	14.08 - 20.23	6.15	10.78 - 30.00	19.22	32.00
Inter orbital width	25.00 - 43.06	18.06	20.94 - 44.60	23.66	76.33
Free height	4.29 - 12.67	8.38	2.29 - 20.00	17.71	47.32
Length of funnel	31.25 - 46.14	14.89	30.00 - 49.22	19.22	77.47
Tentacle length	192.92 -256.85	63.93	150.78 - 284.53	133.75	47.80
Tentacular club length	16.18 - 27.68	11.50	13.05 - 28.98	15.93	72.19
Male					
Left first arm length	51.20 – 61.82	10.62	34.25 – 95.80	61.55	17.25
Left second arm length	40.00 – 59.14	19.14	36.36 – 62.48	26.12	73.28
Left third arm length	37.88 – 56.32	18.44	33.32 – 65.25	31.93	57.75
Left fourth arm length	45.21 – 68.99	23.78	40.91 – 80.04	39.13	60.77
Female					
Left first arm length	46.66 – 64.66	18.00	37.67 – 76.16	38.49	46.77
Left second arm length	36.86 – 56.12	19.26	30.15 – 61.00	30.85	62.43
Left third arm length	35.49 – 54.90	19.41	33.32 – 68.62	35.30	54.99
Left fourth arm length	49.31 – 63.06	13.75	41.94 – 81.51	39.57	34.75
Male					
Cuttle bone width	29.22 – 29.88	0.66	24.60 – 36.36	11.76	5.61
Length of loculus	28.16 – 31.98	3.82	24.61 – 46.37	21.76	17.56
Striated zone length	60.44 – 65.15	4.71	47.23 – 71.45	24.22	19.45
Inner cone length	2.56 – 3.28	0.72	1.30 – 5.48	4.18	17.22
Spine length	0	0	2.38 – 7.69	5.31	0
Female					
Cuttle bone width	30.50 – 31.82	1.32	25.97 – 38.16	12.19	10.83
Length of loculus	0	0	23.91 – 49.22	25.31	0
Striated zone length	55.86 – 62.71	6.85	45.93 – 70.90	24.97	27.43
Inner cone length	0	0	1.09 – 4.61	3.52	0
Spine length	3.41 – 3.60	0.19	1.79 – 6.67	4.88	3.89

Figure 4a.Ventral view of Cuttle bone of *Sepia vecchioni* sp. nov. (Male)
(Illustration by D'Antony Manuela, FAO copy right,2007)

Figure 4b.Ventral view of Cuttle bone of *Sepia vecchioni* sp. nov. (Female)
Illustration by D'Antony Manuela, FAO copy right,2007)

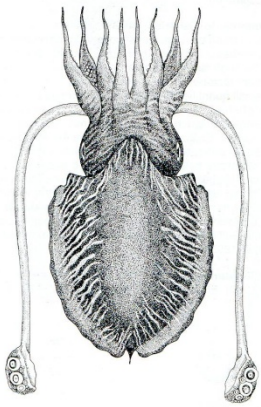

Figure 5a. Dorsal view of *Sepia prashadi*
after Jereb and Ropper (2005)

Figure 5b.Ventral view of Cuttle bone of *Sepia prashadi*

4. Discussions

Despite the similarities in body proportions as revealed by the high percentage of overlapping (Table 2), *S.vecchioni* sp.nov. can easily be distinguished from *S.prashadi* based on the stripe pattern. While the dorsal mantle of *S. Prashadi* has 'transverse zebra type stripe pattern' with the ends facing downward(Figure 5a), the stripes of *S.vecchioni* sp.nov are not of 'zebra type ' and with the ends generally facing upward (Figure 1a, Figure 1b). Another important diagnostic character of *S.vecchioni* sp.nov is the existence of sexual dimorphism with respect to length of arms in matured animals (Figure 1a). The first pair of arms in male are notably long and whip like in contrast to sub equal in both the sexes of *S. prashadi* (Jereb and Roper,2005). This is also evidenced from the relatively low percentage of overlapping (17.25%) with respect to first arm length between the males of *S.vecchioni* sp.nov and *S.prashadi* (Table.2).

The hectocotylization pattern is yet another important character which can be used to distinguish males of *S. vecchioni* from that of *S. prashadi*. In *S.vecchioni* sp.nov, the suckers are biserial both on the basal as well as on the hectocotylized portion .They are quadriserial only beyond the hectocotylized portion near the arm tip. However in the case of *S. prashadi*, the suckers are quadri-serial through out the hectocotylised arm, despite their reduction in size in the hectocotylized portion. Further, there exists difference with respect to number of reduced suckers on the hectocotylized portion also . The hectocotylized portion of *S.vecchioni* sp.nov has a ventral and a dorsal series of suckers each with 18-20 reduced minute suckers, however in the case of *S.prashadi* , there are 12 to 14 rows of reduced suckers. (Jereb and Roper, 2005). The distinctly wide gap between the two series of suckers in the hectocotylized portion in the case of *S.vecchioni* sp.nov is due to the complete absence of two middle series of suckers.

In the case of tentacular club, though there are four enlarged median suckers with the two middle suckers being extremely large in both the species, the club is short in *S.prashadi* (Jereb and Roper, 2005) and is elongate in *S.vecchioni* sp.nov.(Figure 3). Further, the protective membranes are not united at base in *S.prashadi*. However, in the case of *S.vecchioni* sp.nov., the protective

membranes are united at base. Regarding cuttle bone characteristics of *S. vecchioni* sp.nov., cuttle bone is fragile with weakly calcified portion on dorsal side especially at the distal end. However, the cuttlebone of *S. prashadi* is rigid with complete calcification. Further, the anterior striations in the striated zone of *S. vecchioni* sp.nov. are inverted 'V' shaped (Figure 4a&4b) while they are shallow 'M'shaped in *S. prashadi* (Figure 5b).

The analysis of percentage overlapping of body proportion of *S. vecchioni* sp.nov. with that of *S. prashadi* clearly indicates that both the species resemble each other in many of their body proportion. However, four valid diagnostic characters can be derived based on cuttle bone characteristics which showed nil / low percentage of overlapping *viz* (i) cuttlebone width, (ii) length of loculus, (iii) inner cone length and (iv) spine length .These morphometric measurements as the percentage of CBL are lower for *S.vecchioni* sp.nov. than that of *S.prashadi* .This may be attributed to relatively elongated nature of cuttle bone of *S. vecchioni* sp.nov.

Since the cuttle bone of *S.vecchioni* sp.nov. shares the characters of both *Acanthosepion* species complex and *Rhombosepion* species complex , it appears that this kind of mixed character has some evolutionary significance. Khromov (1998) viewed the emergence of *Rhombosepion* species complex followed by *Acanthosepion* species complex during the course of evolution. This species might be probably a link in the evolutionary process of *Rhombosepion* species complex followed by the *Acanthosepion* species complex as viewed by Khromov (1998).

Apart from *S.prashadi* , *S. vecchioni* sp.nov. can easily be distinguished from the other closely related species such as *S. omani*, *S. vossi*, *S. papillata*, *S. chirotrema* and *S. opipara* based on the differences in characteristics of tentacular club, hectocotylization pattern and cuttlebone characteristics(Table 4). Though *Sepia vecchioni* sp.nov resembles *S. chirotrema*, by having tentacular club with protective membranes united at the base, it can be differentiated based on the cuttle bone characteristics, as *S. chirotrema* belongs to *Sepia sensu stricto* species complex (Khromov *et al*.1998). *Sepia vecchioni* sp nov. has some resemblances with *S.omani* and *S. vossi* regarding hectocotylization in males . However, it distinctly differs from these two species with respect to cuttle bone characteristics (Table.4).Further, *S. vecchioni* sp.nov notably deviates also from *S.papillata* and *S.opipara* as each of them belongs to different species complex based on the cuttle bone characteristics.

Table 3. Distinguishing morphological and meristic characters of closely related species of *Sepia vecchioni* sp.nov.

Species	Characters		
	Tentacular club	Hectocotylization	Cuttle bone
Sepia vecchioni sp.nov.	4 median suckers greatly enlarged, of which two middle sucker extremely enlarged; protective membranes are united at base	3 rows of biserial normal suckers proximally followed by 18-20 rows of reduced suckers in a ventral series and a dorsal series separated by a wide gap, then 7 to 8 rows of minute quadriserial suckers	Fragile, weekly calcified, elliptically oval shaped; median and lateral ribs distinct, anterior striae inverted 'V' shaped; inner cone
Sepia prashadi[2,4]	4 median suckers greatly enlarged, of which two middle suckers extremely enlarged; protective membranes not united at base	4 rows of quadri serial normal suckers proximally, followed by 12-14 rows of reduced suckers medially, then normal suckers up to arm tip	Thick, fully calcified,oblong in outline; median and lateral ribs distinct, anterior striae shallow 'M' shaped, inner cone forms distinct cup like cavity;spine long pointed and straight; belongs to *Acanthosepion* species complex
Sepia omani[2,4]	5 median suckers greatly enlarged, of which three middle suckers extremely enlarged; protective membranes not united at base	2 or 3 rows of quadri serial normal size suckers proximally, followed by reduced suckers medially up to 40% of arm with 2 dorsal and 2 ventral series of suckers displaced laterally	Thick fully calcified acuminate; dorsal median and lateral ribs distinct; anterior striae shallow 'M'shaped; innercone 'U' shaped; spine long and straight; belongs to *Rhombosepion* species

		with a gap	complex
Sepia vossi[2]	5 median suckers greatly enlarged, of which three middle suckers extremely enlarged; protective membranes not united at base	3 rows of normal quadri serial suckers proximally, followed by reduced suckers in 2 dorsal and 2 ventral series of suckers displaced laterally with gap followed by normal suckers up to arm tip.	Blunt-pointed anteriorly ; median rib distinct ,lateral ribs indistinct; anterior striae inverted 'U' shaped; inner cone forms 'V'shaped posterior ledge ; spine long pointed straight; spine with dorsal and ventral keel
Sepia papillata[4]	4 median suckers greatly enlarged, of which 2 middle suckers extremely enlarged; protective membranes not united at base	----------	Broadly oval; ventral surface with median furrow; anterior striae 'L' shaped innercone without spine; belongs to *Sepia sensu stricto* species complex
Sepia chirotrema[4,6]	3median suckers greatly enlarged, of which middle sucker extremely enlarged; protective membranes united at base	Distal end attenuated, narrow, and compressed laterally, bearing microscopic suckers.	Anterior end acuminate, posterior end broadly rounded; median rib distinct, lateral ribs less distinct; anterior striae inverted 'V' shaped; Inner cone lateral limbs fused with outer cone; spine strong, not pointed and curved with out keel; belongs to *Sepia sensu stricto* species complex
Sepia opipara[2,4]	4 or 5 median greatly enlarged suckers, of which 2nd proximal sucker extremely enlarged; protective membranes not united at base,	5 or 6rows of normal quadri serial suckers proximally followed by 6 or 7 rows of reduced suckers medially, ventral suckers smaller than dorsal suckers, then normal suckers up to arm tip	Thick, irregularly calcified; elongate oval; median and lateral ribs distinct; anterior striae inverted 'U' shaped; innercone narrow 'U' shaped; spine short; belongs to *Rhomposepion* species complex

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References

1. Hubbs CL, Hubbs. An improved graphical and comparison of series of samples. Syst.Zool, 1953; 2 (2):49-56.

2. Jereb P, Ropper CFE.(eds.) Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Chambered nautiluses and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae).FAO Species Catalogue for Fishery purposes.2005;1(4)262.
3. Jothinayagam JT. Cephalopoda of Madras Coast. Zoological Survey of India,Technical Monograph, 1987;15 pp.26-9.
4. Khromov DN, Lu C C, Guerra A, Dong Zh, Boletzky SV. A Synopsis of Sepiidae outside Australian waters (Cephalopoda: Sepioidea). In "Systematics and biogeography of cephalopods" Voss N A, Vecchione M, Toll RB, Sweeny M J. (eds.) Smithsonian Contribution to Zoology, 1998;1(586) pp.77-157.
5. Khromov DN. Distribution patterns of Sepiidae.In: Systematics and biogeography of cephalopods, Voss N A, Vecchione M, Toll R B, Sweeny M J. (eds.), Smithsonian Contribution to Zoology, 1998;1(586) pp.191-206.
6. Lu CC. A synopsis of Sepiidae in Australian waters (Cephalopoda: Sepioidea).In "Systematics and biogeography of cephalopods" Voss NA, Vecchione M, Toll R B, Sweeny M J. (eds.) Smithsonian Contribution to Zoology, 1998;1(586). pp.159-190.
7. Neethiselvan N. A new species of cuttle fish *Sepia ramani* sp.nov.(Class:Cephalopoda) from Tuticorin Bay,Southeast coast of India.Indian Journal of Marine Sciences,2001;30:81-86.
8. Neethiselvan N,Venkataramani VK. *Sepia prabahari* sp.nov.(Mollusca/Cephalopoda),a new species of Acanthosepion species complex from Tuticorin Bay, Southeast coast of India. Indian Journal of Marine Sciences,2002;31(1):45-51.
9. Silas EG, Sarvesan R, Nair KP, Sastri YA, Sreenivasan, PV, Meiyappan, MM., Vidyasagar K, Rao KS, Rao BN. Some aspects of the biology of cuttlefishes. Cephalopod bionomics fisheries and resources of the exclusive economic zone on India: Some aspects of biology of cuttle fishes. In "Cephalopods bionomics, Fisheries and Resources of the exclusive economic zone of India" Vol.37 Silas E. (edt.), Bulletin of Central Marine Fisheries Research Institute, Cochin, 1985; pp. 49-70.

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Water quality monitoring in Nigeria; Case Study of Nigeria's industrial cities

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Abstract: The aim of this study was to analyze the state of water quality management in Nigeria's industrial cities. In a developing country such as Nigeria, there is immense demand for advancement in various facets of living and economic development is a priority of the government. This has led to increase in industries resulting in an increased quantity of discharge and a wide range of pollutants reaching water bodies. This study indicates that both urbanization and industrialization have contributed to the large scale of pollution currently observed in most Nigerian cities notably those swarming with industries namely; Lagos, Rivers, Kano and Kaduna states. There are no incentives for implementing pollution reduction measures. Wastes are disposed indiscriminately especially for small and medium scale industries. Data for this paper were obtained by observation, investigation and from related studies on the subject matter. Finally, this paper proposes constant river water monitoring as a step towards pollution abatement. [Journal of American Science 2010;6(4):22-28]. (ISSN: 1545-1003).

Key words: Water, Quality, Monitoring, Nigeria, Industrial Cities

1. Introduction

Nigeria is the most populous country in Africa. With a population of over 140 million, the country is endowed with generous resources of water bodies. The span of water bodies within the country is estimated at 900 km^2. This water provides resources for fishery, transportation, irrigation, recreation and domestic uses. Different regulations put in place to protect the marine environment in Nigeria have not been effective in controlling the indiscriminate dumping of effluent into open water bodies. These effluents range from chlorides, phosphates, oil and grease, nitrates, heavy metals to name a few. The heavy metals present in most Nigerian rivers and found in concentrations well above acceptable and permissible levels are lead, copper, zinc, nickel, chromium, Cadmium and Iron (Olayinka and Alo 2004; Esoka and Umaru 2006, Eniola et al, 2010). Degradation of water quality is most severe in the four states that contain 80 percent of the nations industries; Lagos, Rivers, Kano and Kaduna States. This has continued to pose a threat on health and economic development in Nigeria (Ajibade 2004; Olayinka and Alo 2004; Adekunle et al., 2007; Adeyemi et al., 2008; Mustapha, 2008; Adewolu et al., 2009).

Enforcement of emission standards have not been done strictly and the Federal Ministry of Environment still faces some challenges in helping industries adopt cleaner technologies (Adelegan, 2004). Although findings related to industrial pollution of water resources have been disturbing, the category of pollution that has received much attention in Nigeria is sewage pollution of portable drinking water. This has been managed with the weight of such bodies as UNICEF established in Nigeria in 1952 and WaterAid which began work in Nigeria in 1995 to assist with the vast water and sanitation needs found and has since been assisting the water and sanitation units (WASU)

of local government councils to deliver water and sanitation services to the poor. However, about 60 percent of the Nigerian populace both rural and some urban dwellers still source for domestic water and sometimes drinking water from ponds, streams and shallow wells justifying the concern for increases in the level of pollutants in surface and groundwater thus making water monitoring even more vital. (Adelegan 2004; WaterAid 2007; Morenikeji 2010).

Presently, very little if any has been done on an integrated level concerning industrial pollution abatement in Nigerian waters (Egbu, 2004; Olayinka and Alo; 2004, Essoka and Umaru, 2006). The Federal Government of Nigeria only gave attention to environmental abuse after the discovery of an Italian ship dumping toxic wastes in Nigeria in May 1998, giving rise to the establishment of the "Federal Environmental Protection Agency" FEPA later that year. The establishment of FEPA was also followed by the publication of "National Guidelines and Standards for Environmental Pollution" which focused mainly on industrial pollution. This body was renamed in September 1999 and is presently the 'ministry of environment'.

2. Materials and Methods:

Study Area:

Figure 1 shows the map of Nigeria, illustrating the four key industrial cities and the Federal capital Territory Abuja.

Data collection:

Data for this paper was obtained by observation, investigation and by review of several literatures on industrial pollution especially in the specified areas.

The information needed for this study was collected between August and December 2009. Information on water quality and supply within Nigerian cities was obtained at the Federal ministry of Agriculture and water resources, Abuja. A review of the water quality status for the major industrial cities in Nigeria and physio-chemical characteristics of effluent discharge in these cities is given below:

Lagos State:

Lagos is the largest city in Nigeria and the second largest in Africa. It contains over 40% of Nigeria's manufacturing activities with the highest level of emission of 8000 tons of hazardous waste per year, most of which is discharged directly into the Lagos lagoon.

Lagos Lagoon lies between longitudes 3° 22' E and 3° 40' E and Latitude 6° 17'N and 6° 28' N. The lagoon is generally shallow with a depth of between 0.3 and 3.2 m in most parts with the exception of some dredged parts, notably in the Lagos Harbor, where depth is greater than 10 m. The tidal range is 0.3-1.3 m.

Waters in the lagoon and ocean surrounding

Lagos do not meet required standards and thus are not fit for human consumption. Experts have reported that pollution of the lagoon is responsible for the fast depleting of its coastal terrain. Studies have also shown that urban and industrial wastes discharged into the Lagos Lagoon have had a significant impact on the ecosystem (Okoye, 1991; Sangodoyin 1995; Adebayo et al, 2007).

Hence, public authorities in Lagos have had to source for water from neighboring states. In 1910, Lagos installed its first water treatment plant in Iju village, Ogun state with subsequent expansions almost every decade due to consequent rise in population. In a 1985 study, it was discovered that only 47% of the people living in the metropolis were served with portable water at reduced level of service. This figure has since reduced.

Lagos is the only state that charges pollution levies and although the measure is expected to serve as some disincentive to pollution generation and also for the alleviation of pollution problems in the state, it is better seen as a revenue generation effort on the part of the government.

Kaduna State:

Kaduna city is the capital of Kaduna State in north-central Nigeria. The Kaduna River is a tributary of the Niger River which flows for 550 km through Nigeria. It runs along the town from the north to the south of the city covering extensive areas and with low current during the dry season.

The river has a storage reservoir (Kangimi Dam) in the upper region. It is diverted at two points, the north and south water serves as a source of drinking water with a capacity of 240 and 35 million liters per day respectively. The major drains carrying effluents are sited at points beyond the south water works.

The river has a strong seasonal pattern with a flow of 2,000 m^3/s in the rainy season and 1 m^3/s in the dry season and is the major sink of most industrial effluents in the city (Essoka and Umaru, 2006). The concern for pollution is within the six months of the dry season (November to May) and particularly in march, which is the peak of the dry season. The river achieves a 200:1 dilution in the rainy season. However, during the dry season, the river becomes an open sewer for waste pollutants.

The only extensive study on this river was the 'Kaduna River Pollution Study' carried out in 1987 (KRPS, 1987).

Kano State:

The main water body in Kano state is the Kano River basin. It serves as the major source of water supply to the city and is also the major sink for wastes from industrial estates within the state. The river has numerous tributaries but the main rivers that make up the Kano river basin are the Salanta, Challawa and Bompai rivers.

In a study of the effects of industrial effluent in this river basin, the river was found to be contaminated. It was found to contain the following pollutants in significant concentration; high Chemical Oxygen Demand, total solids, hardness, Calcium Carbonate, and ammonia nitrogen. These rivers are used extensively for domestic water supply, irrigation, fishing and recreation but the quality of the water has been found to be unsuitable for these purposes. (Bichi and Anyata, 1999; Mashi and Alhassan 2007).

Rivers State:

Rivers state, as the name suggests is bound by many rivers, notably, the Atlantic Ocean to the North of the state. The state has twenty four administrative divisions with the city of Port Harcourt as its capital.

The major source of pollution in the state is the Petroleum industry. The city of Port Harcourt serves as the nerve center to most transnational petroleum processing companies in the region. Other major contributors to this pollution include iron and steel, fertilizer and petrochemical plants. Another issue

of concern is the mixed disposal of municipal and industrial solid wastes in water bodies (Solomon, 2009) and poor sewerage systems. Effluents from households are discharged into open drains, gutters and water bodies. (Ajao and Anurigwo, 2002).

3. Results and discussion:

Lagos State:

The study through Table 1 shows composition of waste water discharge from two manufacturing industries; a textile and an alcoholic beverage company within Lagos state.

It shows effluents characteristics from both companies (Afprint [textile] and Nigeria Breweries Plc) that discharge into the lagoon.

Table 1. Physio-chemical parameters of effluents from two Lagos industries

Industries	Afprint	NBPlc	**Total (Rounded Figure)**
Temp (^oC)	27.6	30.3	**50.0**
pH	7.6	4.8	**-**
Conductivity	761.0	1156.6	**1,900.0**
Turbidity	11.6	573.6	**580.0**
Salinity	0.1	0.5	**-**
Alkalinity	766.6	445.0	**1,200.0**
Total Hardness	1233.3	4083.3	**5,300.0**
TSS	320.0	833.3	**1,100.0**
Oil/Grease	20.0	0.0	**20.0**
BOD_5	534.0	1352.3	**1,800.0**
COD	850.6	2253.3	**3,100.0**
H_2S	17.2	130.0	**140.0**

Source: Adebayo et al., 2007

All values in mg/ L [except those indicated]

Kaduna State:

The concentration of select pollutants (Cl^-, No_3, NH_3, Po_4, S^{2-}) and other physico-chemical indexes associated with textile processing are shown in Table 2. It shows average values of effluent from five different textile companies that discharge into the Kaduna River.

Table 2. Physio-chemical parameters of effluents from Kaduna industries

Industries	**Total (Rounded Figure)**
Temp. (^oC)	**30.0**
Color	**2,400.0**
TDS	**1,100.0**
Free Chlorine	**0.5**
Nitrate	**4.0**
Ammonia	**1.0**
Phosphate	**1.0**
TSS	**400.0**
Oil/Grease	**7.0**
BOD_5	**300**
COD	**1,800.0**
Sulphide	**0.6**

Source: Yusuf and Sonibare, 2004

All values in mg/ L [except temperature]

Kano State:

Dominant pollutants discovered in Kano river basin from an extensive study of the basin are listed in Table 3. This table shows significantly high concentrations of COD, TSS, hardness, $CaCo_3$, NH_3-N in the three rivers that make up the Kano river basin.

Table 3. Concentration of pollutants in Kano river basin

Rivers	Salanta	Bompai	Challawa	**(Total Rounded Figure)**
COD	8557.4	1166.9	598.7	**2,600.0**
TSS	16934.6	1458.0	1609.0	**20,000.0**
Hardness	1349.6	2506.8	1332.0	**5,100.0**
$CaCo_3$	5150.0	530.0	400.0	**6,000.0**
NH_3-N	5150.0	530.0	400.0	**6,000.0**

Source: Bichi and Anyata, 1999.

[All values in mg/ L]

Rivers state:

Table 4 shows Total effluents from industry and households (in tones/ yr) in Port Harcourt. Pollutants of concern as listed in the table include Oil and grease, Nitrogen, Phosphorus and Biological oxygen demand.

Table 4. Total effluents from industry and households (in tones/ yr) in Port Harcourt.

	Industries	Septic/Oil	**Total (Rounded Figure)**
BOD_5	4374.0	8808.0	**13,000.0**
TSS	3533.0	6178.0	**19,500.0**
OIL	2343.0	2343.0	**4,700.0**
N	362.0	2966.0	**3,300.0**
P	836.0	360.0	**1,200.0**

Source: Ajao and Anuriguo, 2002.
[All values in mg/ L]

Recent efforts, such as rehabilitation of water pumping stations and replacement of outdated pipe networks are in place to improve water quality and supply within Rivers state and although these efforts are commendable, the issue of industrial pollution is still ignored.

Water quality monitoring in Nigeria

The basis of water quality monitoring is to obtain information which will be useful in management of water resources in the country. It would prove useful in management, control and investigation of pollution cases, Classification of water resources, Collection of baseline data, Water quality surveillance and Forecasting water quality.

In Nigeria most of the portable water used for both domestic and industrial purposes is channeled from rivers and groundwater. The present water quality monitoring status in Nigeria involves monitoring only groundwater once every year by each states' water board using FEPA standards. There is no integrated river water quality monitoring scheme in Nigeria.

FEPA and EPA:

The United States Environmental Protection Agency (EPA) sets standards that, when combined with protecting ground water and surface water, are critical to ensuring safe drinking water. The EPA also regulates about 90 contaminants and so does FEPA but the EPA works with its regional offices, states, tribes and its many partners to protect public health through implementing the Safe Drinking Water Act.

Amongst unfavorable legislative, technical and operational constraints, the existing water quality monitoring status in Nigeria is affected principally by institutional barriers: which are constraints or difficulties encountered by the various institutions as a result of the defective organizational framework, these include; inadequate and untimely funding, shortage of requisite personnel, lack of central coordination body of the agencies activities, poor maintenance culture of infrastructures and obsoleteness of the legislations that established some of the institutions with the resultant effect of mild or no penalties for culprits (Egbu, 2004).

Conclusion and Recommendations:

The issue of water in Nigeria may marvel a lot of people considering its abundance in the area. However, the question is whether what is available is good enough for consumption. The desire and determination to provide good quality water that is affordable to the citizenry therefore should influence constant monitoring. The lack of information on pollution is a serious hindrance to pollution management directly or remotely. Thus, in addition to treatment of waste water before disposal, appraisal of water resources would offer proficient information able to indicate areas of main concern. This would prove useful in detection of threats to human and environmental health. Data from monitoring would also fine-tune the control strategies and approaches

already identified thereby helping to develop practical guidelines for pollution reduction. In Nigeria where environmental regulatory measures are relatively frail, and most of the populace still obtain water from rivers and shallow wells, constant water quality monitoring would serve as a means of checking and averting pollution and also upgrading outdated standards. This would be most practical in cities with high incidence of industries resulting in discharge of a great deal of industrial waste into water bodies. It should be noted that the establishment of FEPA signifies a landmark in environmental management efforts in Nigeria but the laws and guidelines published by FEPA need a strict follow up to achieve a pollution free environment.

Faced with this condition, it is suggested that water quality monitoring be contracted to private environmental monitoring companies who will liaise with the government for a healthier environment, this is to ensure that regularity of monitoring activities is not affected by change in government. There is also, little or no institutional memory in Nigeria on the influence of industrial waste on human health, thus, a detailed campaign should be put in place, elucidating the mechanism of water pollution especially with regard to toxic substances. Also, because of the seasonal nature of most Nigerian rivers, for river water monitoring to be very effective, it is proposed that monitoring frequency be upgraded from once to twice a year. This would help ensure that water decline and reduced dilution point due to seasonal changes do not affect the consistency of monitoring results. Better sewerage systems should also be constructed for wastewater from households.

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References

1. Adebayo OT, Balogun AM and Olubiyi OA. Chemical Analysis of Some Industrial Effluents That Discharge into Lagos Lagoon, Nigeria. Research Journal of Environmental Sciences. 2007. 1 (4): 196-199
2. Adekunle IM, Adetunji MT, Gbadebo AM and Banjoko OB. Assessment of Groundwater Quality in a Typical Rural Settlement in Southwest Nigeria. International Journal of Environmental Research and Public Health. 2007. 4 (4): 307-318
3. Adelegan JA. The history of environmental policy and pollution of water sources in Nigeria (1960-2004): The way forward.
4. Adewolu MA, Akintola SL, Jimoh AA, Owodehinde FG, Whenu, OO and Fakoya KA. Environmental Threats to the Development of Aquaculture in Lagos State, Nigeria, European Journal of Scientific Research. 2009. 34 (3): 337-347
5. Adeyemi D, Ukpo G, Anyakora C, and Unyimadu JP. Organochlorine Pesticide Residues in Fish Samples from Lagos Lagoon, Nigeria. American Journal of Environmental Sciences. 2008. 4 (6): 649-653
6. Ajao EA. and Anurigwo S. Land – based sources of pollution in the Niger Delta, Nigeria. Ambio

Journal of the human environment. 2002. 31 (5): 442 - 445.

7. Ajibade LT. Assesment of water quality near river Asa, Ilorin, Nigeria. The environmentalist, Springer. 2004. 24 (1): 11-18
8. Alamu O. Sustainability in the Nigerian water sector: Danger of heavy metal poisoning. American society of Agricultural and Biological engineers, Asabe technical Library. 2005
9. Bichi MH and Anyata BU. Industrial waste pollution in the Kano river basin. Journal of environmental management and health. 1999. 10 (2): 112 – 116
10. Egbu AU. Constraints to effective pollution control and management in Nigeria. The environmentalist Journal, Springer link. 2004. 20 (1): 13-17
11. Eniola EB, Chukwu LO and Olaide BS. Hydro-Chemistry, Macro-Invertebrate Fauna and Fish Production of Acdja Fishing Sites in A Tropical Lagoonal Ecosystem. Journal of American Science. 2010:6(1)
12. Essoka PA and Umaru JM. Industrial effluent and water pollution in Kakuri area, Kaduna South, Nigeria. Journal of Industrial pollution and control. 2006. 22 (1).
13. Mashi SA and Alhassan MM. Effects of Wastewater Discharge on Heavy Metals Pollution in Fadama Soils in Kano City, Nigeria. Journal of biomedical and Environmental sciences. 2007. 20 (1).
14. Morenikeji OA. The Final Hurdle to Be Crossed In the Eradication of Dracunculiasis in Nigeria. Journal of American Science, 2010: 6(2)
15. Mustapha MK. Assessment of the water Quality of Oyun Reservoir, Offa, Nigeria, Using Selected Physico-Chemical Parameters. Turkish Journal of Fisheries and Aquatic Sciences. 2008. 8: 309-319
16. Ogbuagu JO and Ajiwe VI. Industrial Pollution Survey in Anambra State, Nigeria. Journal of environmental contamination and toxicology Springer link. 1998. 61 (1): 269-275
17. Okoye BC. Heavy metals and organisms in the Lagos Lagoon. International Journal of Environmental studies. 1991. 37 (4): 285 – 292
18. Olayinka KO and Alo BI. Studies on industrial pollution in Nigeria: the effects of textile effluents on the quality of groundwater in some parts of Lagos. Nigeria Journal of Health and Biomedical Sciences. 2004. 3(1): 44-50
19. Solomon UU. The state of solid waste management in Nigeria. Waste Management Journal, Elsevier. 2009. 29 (2009): 2787 - 2790
20. Sangodoyin AY. Characteristics and control of industrial effluent generated pollution. Environmental management and Health. 1995. 6 (4): 15 - 18
21. Yusuff RO and Sonibare JA. Characterization of textile industries' effluents in Kaduna, Nigeria and pollution implications. 2005. 6 (3): 212-221
22. Christine Jaulmes - UNICEF - At a glance: Nigeria - New EU donation will help provide safe water for 2.1 million people <http://www.unicef.org/infobycountry/nigeria_28236.html>
23. Federal Republic of Nigeria-Federal Ministry of Environment National Guidelines and Standards for water Quality in Nigeria.
24. United States Environmental Protection Agency. 1997. Monitoring water Quality.
25. Water Aid Nigeria – WaterAid.org/International/Nigeria

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An investigation on the Strength and workability of cement based concrete performance by using ZrO_2 nanoparticles

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Abstract: The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase ZrO_2 particles. ZrO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-ZrO_2 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of ZrO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase ZrO_2 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):29-33]. (ISSN: 1545-1003).

Key words: Nanophase ZrO_2 particles; concrete; compressive strength; workability.

1. Introduction

Concrete is a newer construction material compared to steel and stone. Use of concrete in constructions and buildings may have begun less than a century ago. But in recent century, very wide and effective research has seen on improving the properties of concrete with incorporating wide range of supplementary cementing materials such as pozzolans and nanoparticles due to increasing the use of concrete from decade to decade. Recently, nanotechnology has attracted great scientific attention because of the new potential uses of particles in nanometer (10^{-9} m) scale. This may be due to the nanoscale size of particles being able to result in significantly improved properties from predictable grain-size materials of the same chemical composition. As a consequence, industries can be able to design new and novel products and to re-engineer many existing products that function at unprecedented levels.

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the compressive strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as compressive strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In view of these advances, the aim of this study is to investigate the influences of nano-ZrO_2 on workability and compressive strength of binary blended concrete. ZrO_2

reacts with calcium hydroxide produced from the hydration of calcium zirconias. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano-ZrO_2 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

HPC and HSC are very useful in constructions and multistory buildings because they can decrease the cross-sectional area of the structural fundamentals.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano-ZrO_2 particles

Nano–ZrO_2 with average particle size of 15 nm was used as received. The properties of nano-ZrO_2 particles are shown in Table 2.

Table 2. The properties of nano-ZrO_2

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	160 ± 12	< 0.14	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm^3 was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano-ZrO_2 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano-ZrO_2 particles blended concretes

Sample designation	nano-ZrO_2 particles	Quantities (kg/m^3)	
		Cement	nano-ZrO_2 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano-ZrO_2] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano-ZrO_2 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the workability following the mixing procedure. Cubes of 100 mm edge were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The compressive strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Compressive strength of nano-ZrO_2 particles blended concrete

Compressive strength of nano-ZrO_2 particles blended cement concrete cubes was determined as per ASTM C 39 after 7, 28 and 90 days of moisture curing.

2.4. Workability

Standard slump tests conforming to ASTM C143 were used to determine the workability of the concrete.

3. Experimental results and discussion

The compressive strength results obtained from the experimental investigations are showed in tables and the comparison between the results of workability test is presented in form of bar chart. All the values are the average of the three trails in each case in the testing

program of this study. The results are discussed as follows.

3.1. Compressive strength

The compressive strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the compressive strength increases with nano-ZrO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano-ZrO_2 particles decreases the compressive strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano-ZrO_2 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Compressive strength of nano-ZrO_2 particle blended cement mortars

		Compressive strength (MPa)		
Sample designation	nano-ZrO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	27.3	36.8	42.3
N1	0.5	31.6	42.7	46.5
N2	1.0	33.1	43.6	48.1
N3	1.5	32.2	42.9	47.7
N4	2.0	28.5	39.7	44.3

Water to binder [cement + nano-ZrO_2] ratio of 0.40

The high enhancement of compressive strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-ZrO_2 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano-ZrO_2 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste.

3.2. Workability

A high-quality concrete is one which has acceptable workability (around 6.5 cm slump height) in the fresh condition and develops sufficient strength. Basically, the bigger the measured height of slump, the better the workability will be, indicating that the concrete flows easily but at the same time is free from segregation [12, 13]. Maximum strength of concrete is related to the workability and can only be obtained if the concrete has adequate degree of workability because of self compacting ability. Self-compacting repair mortars, as new technology products, are especially preferred for the rehabilitation and repair of reinforced concrete structures [14]. The water/powder (cement, fly ash, limestone filler, silica fume, nano-particles, etc.) ratio of mortar and the type of chemical admixtures should be determined, in order to place the fresh mortar without any external compaction and at the same time without causing any segregation [15]. In other words, the rheology of paste phase of self-repairing mortar should have suitable properties from flowability and segregation point of view [16–19].

The workability of C0 and N series concrete are presented in Figure 1. The figure shows the influence of nano-ZrO_2 particles content on the workability of mixtures at constant water to binder ratio of 0.40. The results show that unlike the C0 series, all investigated nano-ZrO_2 particles blended mixtures had low slump values and non-acceptable workability. This may be due to the increasing in the surface area of powder after adding nanoparticles that needs more water to wetting the cement particles.

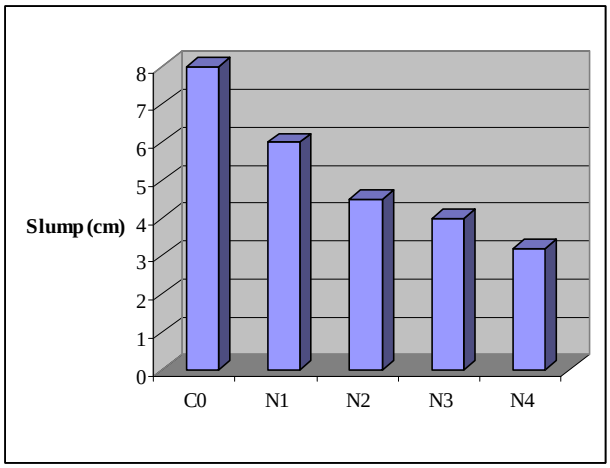

Figure 1. Particle size effects of nano-ZrO_2 on workability of concrete. N1,N2,N3 and N4 are the series N blended concrete with 0.5, 1.0, 1.5 and 2.0 percent of nano-ZrO_2 particles, respectively.

With the improvement of novel plasticizers, to obtain high filling rates is possible even for compound molding systems. The fresh characteristics of concrete, strength and durability of mortars can be improved by the addition of inert or pozzolanic [20]. The selection of the amount and the type of cementitious or inert powders depends on the physical and physico-chemical properties of these powders which are affecting the performance of fresh paste such as particle shape, surface texture, surface porosity and rate of superplasticizer adsorption, surface energy (zeta potential), finest fraction content, Blaine fineness and particle size distribution.

There is no universally accepted agreement on the effect of these factors due to the complex influence of the

combination of these factors [21].
Usually, increasing the fine particles content in cements changes the rheological properties of pastes and consequently influences the workability of mortars and fresh concrete mixtures. The observed changes can be advantageous or not as a result of many factors influencing the rheology of cement pastes [22]. It is usually expected that, if the volume concentration of a solid is held constant, for a specific workability, the replacement of cement with a fine powder will increase the water demand due to the increase in surface area. This is more observed for nanoparticles blended concrete. However, in some cases, the above-mentioned conclusion is not appropriate. [23] obtained same results with fly ash blended concrete. But In this study, the addition of nano-ZrO_2 particles decreased the fluidity and increased the water demand for normal consistency

Conclusions

The results show that the nano-ZrO_2 particles blended concrete had significantly higher compressive strength compare to that of the concrete without nano-ZrO_2 particles. It is found that the cement could be advantageously replaced with nano-ZrO_2 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano-ZrO_2 particles content was achieved with 1.0% replacement. Partial replacement of cement by nano-ZrO_2 particles decreased workability of fresh concrete; therefore use of super plasticizer is substantial.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).
[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.
[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.
[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa compressive strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.
[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO_2 particles. Const Build Mater 2007; 21(6): 1351-1355.
[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.
[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.
[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.
[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.
[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.
[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.
[12] Lin YH, Tyan YY, Chang TP, Chang CY. An assessment of optimal mixture for concrete made with recycled concrete aggregates. Cem Concr Res 2004; 34(8): 1373-1380.
[13] Tattersall GH, Baker PH, An instigation of the effect of vibration on the workability of fresh concrete using a vertical pipe apparatus, Magazine of Concrete Research 1989; 14 (146): 3–9.
[14] L. Courard, A. Darimont, X. Willem, C. Geers, R. Degeimbre, Repairing concretes with self-compacting concrete: testing methodology assessment, Proceedings of the First North American Conference on the Design and Use of Self-Consolidating Concrete, 2002, pp. 267–274.
[15] Felekoglu B, Tosun K, Baradan B, Altun A, Uyulgan B. The effect of fly ash and limestone fillers on the viscosity and compressive strength of self-compacting repair mortars. Cem Concr Res 2008; 36: 1719–1726.
[16] P.F.G. Banfill, The Rheology of Fresh Cement and Concrete—A Review, 11th International Cement Chemistry Congress, Durban, 2003. 13 pp.
[17] C. Lemmer, J. Hartman, T. Wolf, Rheological properties of mortars for self-compacting concrete, http://www.massivbau.tu-darmstadt.de/user/ lemmer/web/scc/LeDaCon199.htm, 13 pp.
[18] C.F. Ferraris, J.M. Gaidis, Connection between the rheology of concrete and rheology of cement paste, ACI Mater. J. (July–August 1992) 388–393.
[19] C.F. Ferraris,Measurement of the rheological properties of high performance concrete: state of the art report, J. Natl. Inst. Stand. Technol. 104 (1999) 461–478.
[20] M. Cyr, C. Legrand, M. Mouret, Study of the shear thickening effect of superplasticizers on the rheological behavior of cement paste containing or not mineral admixtures, Cem. Concr. Res. 30 (2000) 1477–1483.
[21] C.F. Ferraris, K.H. Obla, R. Hill, The influence of mineral admixtures on the rheology of cement paste and concrete, Cem. Concr. Res. 31 (2001) 245–255.
[22] S. Grzeszczyk, G. Lipowski, Effect of content and particle size distribution of high-calcium fly ash on the rheological properties of cement pastes, Cem. Concr. Res. 27 (1997) 907–916.
[23] F. Lange, N. Mortel, V. Rudert, Dense packing of cement pastes and resulting consequences on mortar properties, Cem. Concr. Res. 27 (1997) 1481–1488.

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Embedded System Using Ultrasonic Waves and Voice Biometric to Build an E-Glass for the Blinds

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Abstract:

Currently, embedded and real time systems are used in wide range of a related human applications to improve the quality of our lives such as embedded systems for communication (Mobile, satellite, and avionics systems), and control systems such as (microwaves, refrigerators and embedded system in vehicles). Nevertheless, embedded and real time systems are still immature. The application of these systems is used for various devices. However, these systems are never used for a human body to complete human missing-part functionality; which means the embedded system can be used as part of natural neural networks in a human system nerve. This paper proposes a technical view to build an electronic glass (E-Glass) for the blind people. Moreover, this paper provides the complete E-Glass electronic circuit in which the electronic scanning system to tackle the objects and time signals are included. This E-Glass could be used by the blind to assist them in their ways without any human assistance. Moreover, it will be used by the blind to make them self confidence, to let them walk independently and to increase their morality. It is important to note that the hardware and software components of the E-Glass are not expensive. This work could be provided to the practitioner's people in the industry or to the students of the department of electrical or biomedical engineering. [Journal of American Science 2010;6(4):34-42] (ISSN: 1545-1003).

Keywords: *Embedded System, Neural Networks, Ultra Sonic Wave, Artificial Intelligence, Voice Biometric.*

1. Introduction

Currently, there are an estimated of 45 million blind people and 135 million visually impaired people worldwide [World Health Organization 2001]. Unfortunately, 90% of people who are blind live in developing countries this represents around 33.3 million [World Health Organization 2001]. Moreover, in developing countries, it is believed that 60-80% of children who become blind die within 1-2 years [Lewallen, S et al, 2001].

Furthermore, more than half of the world's blind live in India (9 million), Africa (7 million) China (6 million) and Arab region (7 million). If trends continue 75 million people will be blind by 2020 worldwide.

The idea of this research is to build a first generation of an electronic glass (E-Glass) for the blind. This could open the avenues for the researchers in the electrical, biomedical and computer engineering departments to improve this kind of research based on some foundations provided in this paper.

This work will eventually be the first step to use the technology as external functionality to help the blind persons to indemnify the missing part.

This paper is organized as follows: section 2 presents an overview of the related work. Section 3 presents the research methodology. Section 4 presents the theoretical part. Section 5 presents the practical part. Conclusions and future work are presented in section 6.

2. An overview of the related work

To date, there are no similar research studies related to this topic. However, there are few references that may be used to build the proposed E-Glass such as engineering and information technology field.

Based on the literature [DuBose TJ, Baker AL 2009] and [Doppler Ultrasound History 2008] the

Ultrasound signals are a Real Time Locating System (RTLS) or Indoor Positioning System (IPS) technology used to automatically track and identify the location of objects in real time using simple, inexpensive nodes (badges/tags) attached to or embedded in objects and devices, which then transmit an ultrasound signal to communicate their location to microphone sensors.

2.1 Ultrasonic wave and echo

A sound wave is typically produced by a piezoelectric transducer encased in a probe [*disambiguation needed*] [Ang Jr., ES; Gluncic V, Duque A et al. 2006]. Strong, short electrical pulses from the ultrasound machine make the transducer ring at the desired frequency. The frequencies can be anywhere between 2 and 18 MHz The sound is focused either by the shape of the transducer, a lens in front of the transducer, or a complex set of control pulses from the ultrasound scanner machine (Beam forming) [Bricker L, Garcia J, Henderson J, et al. 2000]. This focusing produces an arc-shaped sound wave from the face of the transducer. The wave travels into the body and comes into focus at a desired depth.

Older technology transducers focus their beam with physical lenses. Newer technology transducers use phased array techniques to enable the sonographic machine to change the direction and depth of focus. Almost all piezoelectric transducers are made of ceramic [Bricker L, Garcia J, Henderson J, et al. 2000].

Materials on the face of the transducer enable the sound to be transmitted efficiently into the body (usually seeming to be a rubbery coating, a form of impedance matching). In addition, a water-based gel is placed between the patient's skin and the probe.

The sound wave is partially reflected from the layers between different tissues. Specifically, sound is reflected anywhere there are density changes in the body: e.g. blood cells in blood plasma, small structures in organs, etc. Some of the reflections return to the transducer [Edler I, Hertz CH. 2004].

The return of the sound wave to the transducer results in the same process that it took to send the sound wave, except in reverse. The return sound wave vibrates the transducer; the transducer turns the vibrations into electrical pulses that travel to the ultrasonic scanner where they are processed and transformed into a digital image [Donald I, Mac Vicar J, Brown TG, 1995].

2.2 Forming the image

The sonographic scanner must determine three things from each received echo [The History of Ultrasound 2006]:

- How long took the echo to be received when the sound was transmitted.
- The focal length stored in the phased array is deduced enabling a sharp image of that echo at that depth (this is not possible while producing a sound wave).
- How strong the echo was. It could be noted that sound wave is not a click, but a pulse with a specific carrier frequency. Moving objects change this frequency on reflection, so that it is only a matter of electronics to have simultaneous Doppler sonography.

2.3 Displaying the image

Images from the sonographic scanner can be displayed, captured, and broadcast through a computer using a frame grabber to capture and digitize the analog video signal. The captured signal can then be post-processed on the computer itself [Woo, Joseph 2002].

2.4 Studies on the safety of ultrasound

A study at the Yale School of Medicine found a correlation between prolonged and frequent use of ultrasound and abnormal neuronal migration in mice. A meta-analysis of several ultrasonography studies found no statistically significant harmful effects from ultrasonography, but mentioned that there was a lack of data on long-term substantive outcomes such as neurodevelopment [Merritt, CR 1989].

2.5 Voice biometric

Voice biometric consists of two major tasks, that is, Feature Extraction and Pattern Recognition. Feature extraction attempts to discover characteristics of the sound signal, while pattern recognition refers to the matching of features in such a way as to determine, within probabilistic limits, whether two sets of features are located from the same or different domain [Rabiner and Juang, 1993].

In general, voice biometric can be subdivided into voice identification, and voice verification. Voice identification will be used in this paper to send the sound to blind person who used this system.

2.6 Digital Signal Processing (DSP)

The Digital Signal Processing (DSP) is the study of signals in a digital representation and the processing methods of these signals [Kuo and Gan, 2004].

The DSP and analogue signal processing are subfields of signal processing. Furthermore, the DSP includes subfields such as audio signal processing, control engineering, digital image processing, and speech processing. RADAR Signal processing, and communications signal processing are two other important subfields of DSP [Lyons, 1996].

3. Research Methodology

In this paper, first we have build two electronic circuits integrated with each other, see figure 4 the first circuit generating ultra sonic signals that send/receive signals to get and analyse the information about the objects from the environment like a radar system.

The second circuit received the processed ultra sonic signals into voice biometric chip to send different types of alarming voice to help the blind person to avoid any obstacles in his/her way.

4. Theoretical Part

This section provides the procedure for producing the proposed and the future view for this E-Glass. By forming and displaying the image.

4.1 E-Glass system parameters and constraint

The first circuit generating ultra sonic signals that send/receive signals to get and analyse the information about the objects from the environment like a radar system. This part could be extended to form and display the objects-image by using the following steps:

- In E-Glass application the source of ultrasound is the transducer.
- A typical disc transducer produces a field roughly in the form of a beam, as you see in figure-1 [Valma J Robertson, Kerry G Baker 2001].

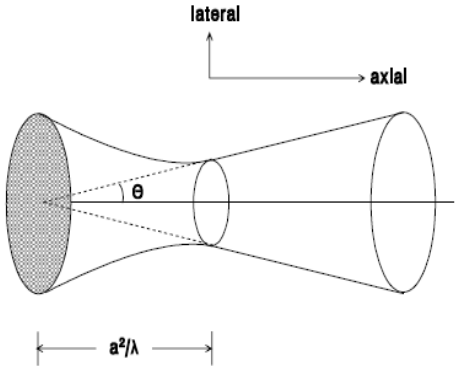

Figure1: Beam Behaviour.

- The analysis is simplified for operation at a single frequency, and this will be assumed. For distances less than a2/λ, where a is the transducer radius, and λ the wave length, the beam contracts to a radius of about a/2. This region is referred to as the near field.
- The transducer is characterized by its total power output, corresponding to the integral of the time-averaged intensity over the face of the transducer. Formally this can be written as follow:

$$W=\frac{1}{T}\int_{t=0}^{t=T}\int_{S} i(\mathbf{r},t)\,d^2\mathbf{r}dt \qquad (1)$$

Where T is the period and S is the face of the transducer. The quantity I, where A is the effective area of T 'W/A the transducer is the time-averaged intensity, spatially averaged over the face of the transducer. The boundary of the transducer face is determined by intensity drop off and hence is not exactly equal to the geometrical intensity.

- The boundary is taken where the intensity has dropped to 25% of the maximum transmitting ultra sonic waves. The quantity *ISATA* (*z*) is similarly defined as the spatial and temporal average over the effective beam area at distance z from the face of the transducer.

- Beyond this point the intensity quickly approaches an inverse dependence on axial distance. *ISPPA* is the spatial peak pulse average in

$$I_{SPPA}=\frac{1}{T}\int i(a^2/\lambda,t)dt \qquad (2)$$

For a pulsed system, the quantity T is the repetition period. In this case, as mentioned previously, the variation is less extreme, since it is the superposition of curves for each spectral component and the positions of the extreme are frequency dependent.

4. 2 E-Glass and dimensional sonic wave

The ultra sonic wave used in this paper based on one dimensional sonic wave, to the future use, can improve the electronic circuit in figure 5, to work with two or three or fourth dimensional waves [Gale Encyclopedia of Medicine 2001]. For one dimensional wave, one can fellow these steps:

- Sonic disturbance [Valma J Robertson, Kerry G Baker 2001] is propagated by the motion of the particles of the propagating medium vibrating around their equilibrium positions. The motion may be described by Newtonian mechanics. For mathematical simplicity consider a fictitious medium in which motion is restricted to one dimension. If $u(x)$ is the particle displacement from equilibrium at x then the force on a volume element about y is given by:

$$dF=(\rho dxdydz)\cdot\frac{\partial^2 u}{\partial t^2} \qquad (3)$$

Where: ρ is the density of the medium. The net force in the x direction is the difference between the forces at $x+dx$ and x operating on the area $dydz$.

- If the stress σ is defined as the force per unit area then

$$dF=[\sigma(x+dx)-\sigma(x)]dydz=\frac{\partial\sigma}{\partial x}\cdot dxdydz \qquad (4)$$

- This leads to the continuum equation:

$$\frac{\partial\sigma}{\partial x}=\rho\frac{\partial^2 u}{\partial t^2} \qquad (5)$$

- Since the displacement depends upon position the system becomes distorted. An element originally of length dx extending from x to $x+dx$ will now extend from $x+u(x)$ to $x+dx+u(x+dx)$ resulting in an increase in length of the element of $u(x+dx)-u(x)$. The dimensionless quantity strain is defined as the fractional distortion and hence is given by:

$$\varepsilon(x)=\frac{u(x+dx)-u(x)}{dx}=\frac{\partial u}{\partial x} \qquad (6)$$

- After steps in sections 4.1 and 4.2, one can build matrix of signal through the reflected sonic wave signals or (echo) and then forming displaying the image for the entire object, this part are not described in this paper, which is considered as a future view for the upcoming E-Glass research.
- Based on sections 4.1 and 4.2 in this paper, the E-Glass behaviour waves are described and the constraints defined the waves dimensional then the output of the first circuit will be used as input to the second circuit or (voice circuit).

4.3 Forming and displaying image in E-Glass

This part is still under investigation, this section will be discussed in the next generation of this E-Glass.

4.4 Neuron node connection for E-Glass

Neuron node connection is a mediator part between the embedded E-Glass system and the human optical nerves, the idea is to capture the sending human optical nerve into E-Glass system and through the artificial neuron node, there will be testing for sending and receiving a sonic wave's data between the embedded system and the human nerve.

This part is still under investigation, this section will be discussed in the next generation of this E-Glass.

5. Practical Part

This section covers what we achieved from E-Glass project, this section includes some preliminary results that may improve the second E-Glass generation.

5.1 The proposed E-Glass system

The proposed E-Glass works through an electronic circuit to scan and to collect information about the entire objects using ultrasonic signals (echo system) such as the one used for a radar system. This electronic circuit could be developed to be used by the blind.

The E-glass system for the blind could be built utilizing the communication passive signal theory

between the environment and the blind persons see (Gonzalez et al 2002). The passive signal sends and receives data to and from the objects (Guo and Liddell, 2002). The signals will analyse these objects based on the length of the collected signals per a time using a quantization sampling signal (G. Fant 1970).

The analyzed data can then be used by the voice circuit by comparing this collected data with the predefined conditions on the voice circuit or system threshold (B. Gold and N. Morgan 2000). This will allow taking decisions by the proposed system to give different types of alarms.

5.2 General View of the E-Glass

The E-Glass research is to build a discipline view between the engineering and medical fields and utilizes this discipline to help blind persons.

This E-Glass system is composed of software based on the artificial intelligence programming capabilities and hardware components to install these programs.

The software use VHDL (verlog hardware description language) programming to define the functionality of the re-programmable microcontroller; this is considered as the brain of the E-Glass system.

The hardware is composed of two electronic circuits systems. The first electronic circuit is composed of two radars build on a PCP card see figure 2 and the second electronic circuit is composed of a wire or a wireless voice chip.

5.3 How the E-Glass Works?

This E-Glass receives the information from the two radars systems that are embedded into the E-Glass. This information will be analyzed in the artificial intelligence software and will produce a warning about any obstacle objects through a voice system.

This E-Glass works through the ultra sonic waves by sending radar signals for a distance from 2 meters to 50 meters and 120 degrees as a vertical and horizontal cover angle along with 60 degrees to cover the right and left, as a result, in total the cover angle will be 270 degrees. See figure 2. One of the characteristics of this E-Glass is that it can identify any small object with a 2 cm^2 area or more from 2 meters distance.

This E-Glass will work through the following two phases:

5.3.1 First phase

The first radar will be put on the left part of the E-Glass.

This radar will send signals to the left part and will receive signals from the left part. Then, this radar will analyse all the data which are collected, to cover the left part. The second radar will be put on the right part of the E-Glass. It will send signals to the right parts and analyse all the data which are collected from the radar, to cover the right part see-figure 1.

Therefore, after receiving the information from the two radars, it will be analyzed in the artificial intelligence software. This analysis could be done through the first electronic circuit programs that are connected to the two radars.

5.3.2 Second phase:

The second electronic circuit program will analyze the signals that will be received from the electronic warning and alarming device (voice system).

This second electronic signal contains the predefined data (these data is considered as a system specifications) to give a scaled warning to the blind persons. This will be based on the dangerous degree of the situation facing the blind persons using the analysis constraints for timing diagram system.

5.4 The proposed E-Glass Components

The proposed E-Glass system is composed of the following components micro controller, memory, electrical circuits, timing system and a voice chip.

The first generation of this embedded E-Glass system prototype was built in Jordan see-figure 1.

This prototype model was build on the bases of the electronic circuit in figure 4, which actually includes two integrated circuits; the first circuit is in the above part of figure 4; it will collect information about objects or obstacles from the real environment, While the second circuit is in the bottom part of figure 4; it will analyse the collected information to take decision and to give a scaled warning voice to blind persons based on the system condition.

5.4.1 First Generation of the E-Glass Prototyping

Figure 1 presents the first generation of the E-Glass embedded system, this prototype could be built in engineering laboratories based on the electronic circuit listed in figure 4.

5.4.2 The Electronic Scanning System to Tackle the Objects

Figure 2 describes the scanning system embedded into the E-Glass through the electronic circuit on figure 4.

The scanning system sends signal to tackle the objects vertically and horizontally.

Assuming that the E-glass system is fixed on the middle area of the human body; the vertical signals covers from 0 degree to 180 degree range; while the horizontal signals, covers from 90 to 270 degree range as seen in figure 2.

Figure 2: The E-Glass Embedded System.

5.4.3 Sending and Receiving Signals Timing System.

The E-Glass system will send signal to capture the environmental object each 100 ms and receive signals each 10 ms. The timing system diagram in figure 3 works as follows:

The embedded timing module generates ultra sonic signals based on 8 bits cycle for each pulse.

The embedded timing system for the E-Glass receives input signals from the environmental objects each 10 ms.

The embedded timing system for the E-Glass sends input signals from the system to the environment each 100 ms see-figure4.

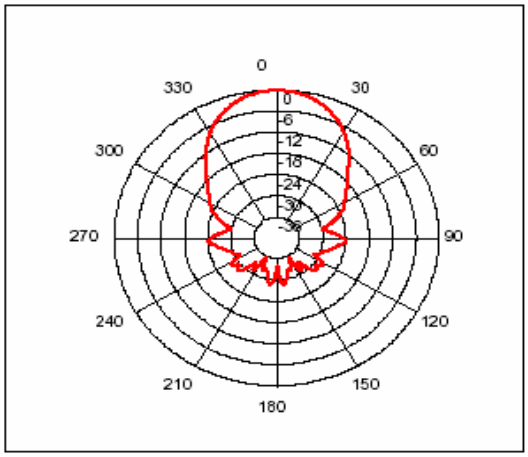

Figure 3: The Electronic Scanning System to Tackle the Objects

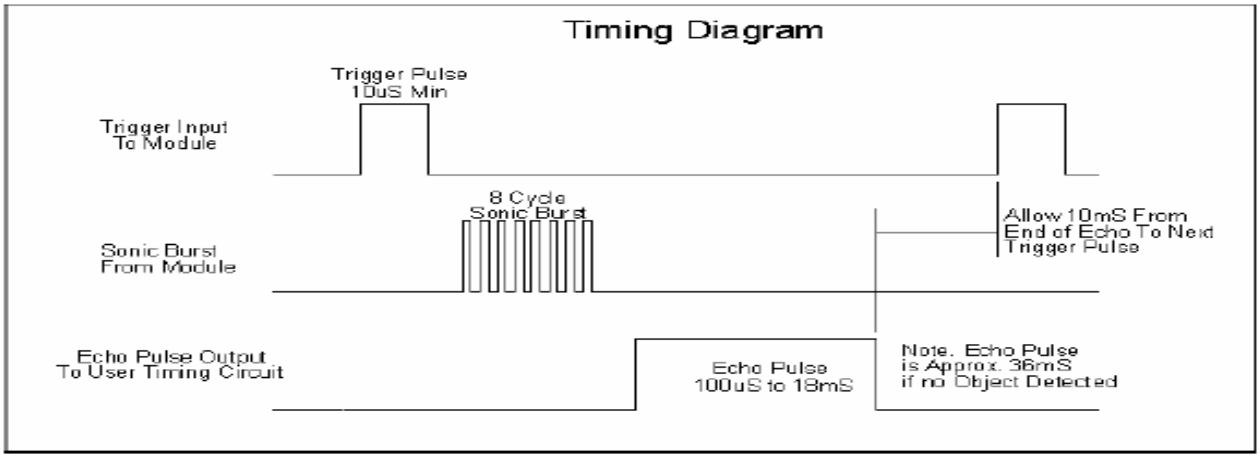

Figure 4: Sending and Receiving Signals Timing System

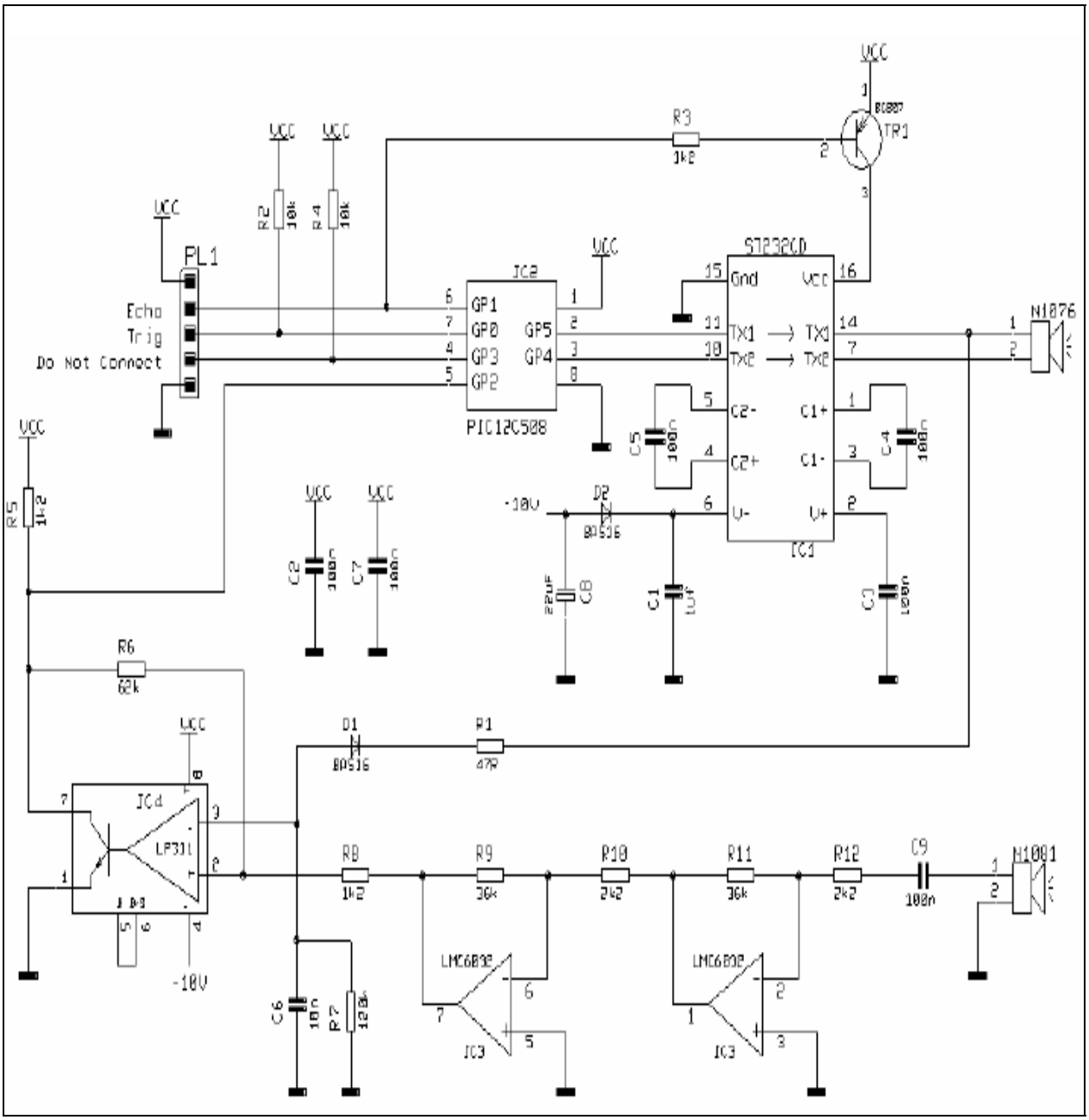

Figure 5: The Completed E-Glass Electronic Circuit for the first generation.

6. Conclusion and Future work

The research in the field of using information technology IT to be part of a human functionality to assist them in their daily life is not developed yet.

The work presented in this paper is innovative from the social view. As one note that there is no similar work undertaken to date. Therefore, this research opens avenues to a new research field to fulfil special needs people.

As a future work, this kind of research should be explored through the medical engineering fields in order to improve it. For instance, the future view of this E-Glass system is to build it as a replaceable part of the damaged human neural system. This will be used as an internal part of a human body utilizing the

ultra sonic capabilities especially the one used in medical and pattern recognition. This system will directly replace the human optical nerves instead of the damaged eyes.

References

(World Health Organization 200)1, blind statistics http://www.uniteforsight.org/eye_stats.php.

(Lewallen, S and Court right, P. 2001), Blindness in Africa: present situation and future needs.

(DuBose TJ, Baker AL; 2009) "Confusion and Direction in Diagnostic Doppler Sonography http://jdm.sagepub.com/cgi/content/abstract/25/3/173/ref=sib_dp_ptu#reader-link.

(Doppler Ultrasound History 2008). http://www.obgyn.net/ultrasound/ultrasound.asp?page=feature/doppler_history/history_ultrasound.

(Ang Jr., ES; Gluncic V, Duque A et al. 2006). "Prenatal exposure to ultrasound waves impacts neuronal migration in mice". Proc Natl Acad Sci USA 103 (34): 12903–10. doi:10.1073/pnas.0605294103.

(Bricker L, Garcia J, Henderson J, et al. 2000). "Ultrasound screening in pregnancy: a systematic review of the clinical effectiveness, cost-effectiveness and women's views". Health technology assessment (Winchester, England) 4 (16): i-vi, 1–193.

(Edler I, Hertz CH. 2004). The use of ultrasonic reflects scope for the continuous recording of movements of heart walls.

(Donald I, MacVicar J, Brown TG, 1995).Investigation of abdominal masses by pulsed ultrasound. Lancet 1958;1(7032):1188-95.

(The History of Ultrasound 2006): A collection of recollections, articles, interviews and images". www.obgyn.net.

(Woo, Joseph 2002). "A short History of the development of Ultrasound in Obstetrics and Gynecology". http://www.ob-ultrasound.net/history1.html. Retrieved 2007-08-26.

(Merritt, CR (1 November 1989). "Ultrasound safety: what are the issues?". Radiology 173 (2): 304–306. PMID 2678243. http://radiology.rsnajnls.org/cgi/reprint/173/2/304. Retrieved 2008-01-22.(Rabiner, L. R. and Juang, H., 1993), Fundamentals of Speech Recognition, Prentice Hall, Englewood Cliffs, New Jersey, USA.

(Kuo, S. M., Gan, W.-S., 2004), Digital Signal Processors: Architectures, Implementations, and Applications, Prentice Hall, Englewood Cliffs, New Jersey, USA.

(Lyons, R. G., 1996), Understanding Digital Signal Processing, Pearson Education, London, UK.

(Valma J Robertson, Kerry G Baker 2001), A Review of Therapeutic Ultrasound: Effectiveness Studies, Physical Therapy. Volume 81. Number 7.

(Gale Encyclopedia of Medicine 2001). Capture and Store Gynecological Ultrasounds. The, 2nd Edition Volume 1 A-B. Page no.4.

(G. Fant 1970), Acoustic Theory of Speech Production, Mouton & Co., The Hague, 1970 And Some of its Implications", Journal of Speech and Hearing Research, 4:303-320, 1961

(Gonzalez, Rafael. and Woods, Richard. 2002). Digital image processing, second edition, Prentice-hall.

(Guo and Liddell, 2002).. Support Vector Regression and Classification Based Multi-view Detection and Recognition IEEE International Conference on Automatic Face& Gesture Recognition, pp.300-305.

(B. Gold and N. Morgan 2000), Speech and Audio Signal Processing: Processing And Perception of Speech and Music, John Wiley & Sons, Inc., New York, 2000

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Assessment of the effects of the cement paste composite in presence TiO_2 nanoparticles

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Abstract: The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase TiO_2 particles. TiO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano- TiO_2 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of TiO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase TiO_2 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):43-46]. (ISSN: 1545-1003).

Key words: Nanophase TiO_2 particles; concrete; compressive strength; workability.

1. Introduction

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the compressive strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as compressive strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano- TiO_2 on compressive strength and workability of binary blended concrete has been studied. Nanoparticles react with calcium hydroxide produced from the hydration of calcium silicates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano- TiO_2 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

HPC and HSC are very useful in constructions and multistory buildings because they can decrease the cross-sectional area of the structural fundamentals.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The

chemical and physical properties of the cement are shown in Table 1.

2.1.2. Nano- TiO_2 particles

Nano– TiO_2 with average particle size of 15 nm was used as received. The properties of nano- TiO_2 particles are shown in Table 2.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

Table 2. The properties of nano- TiO_2

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	155 ± 12	< 0.13	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm³ was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano- TiO_2 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano- TiO_2 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the workability following the mixing procedure. Cubes of 100 mm edge were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The compressive strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

Table 3. Mixture proportion of nano- TiO_2 particles blended concretes

Sample designation	nano- TiO_2 particles	Quantities (kg/m^3)	
		Cement	nano- TiO_2 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano- TiO_2] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.3. Compressive strength of nano- TiO_2 particles blended concrete

Compressive strength of nano- TiO_2 particles blended cement concrete cubes was determined as per ASTM C 39 after 7, 28 and 90 days of moisture curing.

2.4. Workability

Standard slump tests conforming to ASTM C143 were used to determine the workability of the concrete.

3. Experimental results and discussion

The compressive strength results obtained from the experimental investigations are showed in tables and the comparison between the results of workability test is presented in form of bar chart. All the values are the average of the three trails in each case in the testing program of this study. The results are discussed as follows.

3.1. Compressive strength

The compressive strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the compressive strength increases with nano- TiO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano- TiO_2 particles decreases the compressive strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano- TiO_2 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength

[11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

The high enhancement of compressive strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano- TiO_2 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano- TiO_2 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste.

Table 4. Compressive strength of nano- TiO_2 particle blended cement mortars

		Compressive strength (MPa)		
Sample designation	nano- TiO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	27.3	36.8	42.3
N1	0.5	30.8	41.9	45.5
N2	1.0	31.9	43.4	46.9
N3	1.5	31.5	42.5	45.9
N4	2.0	28.7	39.3	44.8

Water to binder [cement + nano- TiO_2] ratio of 0.40

3.2. Workability

A high-quality concrete is one which has acceptable workability (around 6.5 cm slump height) in the fresh condition and develops sufficient strength. Basically, the bigger the measured height of slump, the better the workability will be, indicating that the concrete flows easily but at the same time is free from segregation [12, 13]. Maximum strength of concrete is related to the workability and can only be obtained if the concrete has adequate degree of workability because of self compacting ability. Self-compacting repair mortars, as new technology products, are especially preferred for the rehabilitation and repair of reinforced concrete structures [14]. The water/powder (cement, fly ash, limestone filler, silica fume, nano-particles, etc.) ratio of mortar and the type of chemical admixtures should be determined, in order to place the fresh mortar without any external compaction and at the same time without causing any segregation [15]. In other words, the rheology of paste phase of self-repairing mortar should have suitable properties from flowability and segregation point of view [16–19].

The workability of C0 and N series concrete are presented in Figure 1. The figure shows the influence of nano- TiO_2 particles content on the workability of mixtures at constant water to binder ratio of 0.40. The results show that unlike the C0 series, all investigated nano- TiO_2 particles blended mixtures had low slump values and non-acceptable workability. This may be due to the increasing in the surface area of powder after adding nanoparticles that needs more water to wetting the cement particles.

With the improvement of novel plasticizers, to obtain high filling rates is possible even for compound molding systems. The fresh characteristics of concrete, strength and durability of mortars can be improved by the addition of inert or pozzolanic [20]. The selection of the amount and the type of cementitious or inert powders depends on the physical and physico-chemical properties of these powders which are affecting the performance of fresh paste such as particle shape, surface texture, surface porosity and rate of superplasticizer adsorption, surface energy (zeta potential), finest fraction content, Blaine fineness and particle size distribution.

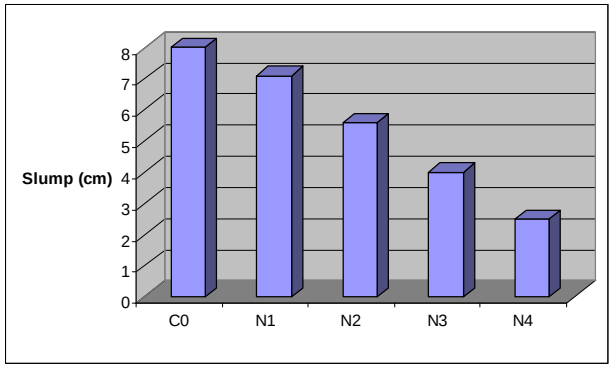

Figure 1. Particle size effects of nano- TiO_2 on workability of concrete. N1,N2,N3 and N4 are the series N blended concrete with 0.5, 1.0, 1.5 and 2.0 percent of nano- TiO_2 particles, respectively.

There is no universally accepted agreement on the effect of these factors due to the complex influence of the combination of these factors [21].

Usually, increasing the fine particles content in cements changes the rheological properties of pastes and consequently influences the workability of mortars and fresh concrete mixtures. The observed changes can be advantageous or not as a result of many factors influencing the rheology of cement pastes [22]. It is usually expected that, if the volume concentration of a solid is held constant, for a specific workability, the replacement of cement with a fine powder will increase the water demand due to the increase in surface area. This is more observed for nanoparticles blended concrete. However, in some cases, the above-mentioned conclusion is not appropriate. Lange et al. [23] obtained same results with fly ash blended concrete. But In this study, the addition of nano- TiO_2 particles decreased the fluidity and increased the water demand for normal consistency

Conclusions

The results show that the nano- TiO_2 particles blended concrete had significantly higher compressive strength compare to that of the concrete without nano- TiO_2

particles. It is found that the cement could be advantageously replaced with nano- TiO_2 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano- TiO_2 particles content was achieved with 1.0% replacement. Partial replacement of cement by nano- TiO_2 particles decreased workability of fresh concrete; therefore use of super plasticizer is substantial.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).
[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.
[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.
[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa compressive strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.
[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO2 particles. Const Build Mater 2007; 21(6): 1351-1355.
[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.
[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.
[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.
[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.
[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.
[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.
[12] Lin YH, Tyan YY, Chang TP, Chang CY. An assessment of optimal mixture for concrete made with recycled concrete aggregates. Cem Concr Res 2004; 34(8): 1373-1380.
[13] Tattersall GH, Baker PH, An instigation of the effect of vibration on the workability of fresh concrete using a vertical pipe apparatus, Magazine of Concrete Research 1989; 14 (146): 3–9.
[14] L. Courard, A. Darimont, X. Willem, C. Geers, R. Degeimbre, Repairing concretes with self-compacting concrete: testing methodology assessment, Proceedings of the First North American Conference on the Design and Use of Self-Consolidating Concrete, 2002, pp. 267–274.
[15] Felekoglu B, Tosun K, Baradan B, Altun A, Uyulgan B. The effect of fly ash and limestone fillers on the viscosity and compressive strength of self-compacting repair mortars. Cem Concr Res 2008; 36: 1719–1726.
[16] P.F.G. Banfill, The Rheology of Fresh Cement and Concrete—A Review, 11th International Cement Chemistry Congress, Durban, 2003. 13 pp.
[17] C. Lemmer, J. Hartman, T. Wolf, Rheological properties of mortars for self-compacting concrete, http://www.massivbau.tu-darmstadt.de/user/ lemmer/web/scc/LeDaCon199.htm, 13 pp.
[18] C.F. Ferraris, J.M. Gaidis, Connection between the rheology of concrete and rheology of cement paste, ACI Mater. J. (July–August 1992) 388–393.
[19] C.F. Ferraris,Measurement of the rheological properties of high performance concrete: state of the art report, J. Natl. Inst. Stand. Technol. 104 (1999) 461–478.
[20] M. Cyr, C. Legrand, M. Mouret, Study of the shear thickening effect of superplasticizers on the rheological behavior of cement paste containing or not mineral admixtures, Cem. Concr. Res. 30 (2000) 1477–1483.
[21] C.F. Ferraris, K.H. Obla, R. Hill, The influence of mineral admixtures on the rheology of cement paste and concrete, Cem. Concr. Res. 31 (2001) 245–255.
[22] S. Grzeszczyk, G. Lipowski, Effect of content and particle size distribution of high-calcium fly ash on the rheological properties of cement pastes, Cem. Concr. Res. 27 (1997) 907–916.
[23] F. Lange, N. Mortel, V. Rudert, Dense packing of cement pastes and resulting consequences on mortar properties, Cem. Concr. Res. 27 (1997) 1481–1488.

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Treatment of Light Contaminated Surface Water Using Slow Sand Filtration in China

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Abstract: This was a comparative study to investigate the effectiveness of slow sand filtration with the best type of sand in filtering water from the domestic lake at China University of Geosciences (CUG) in Wuhan. It was a laboratory scale experiment which had four columns with all having a length of 100 cm in height, 3cm in diameter, and the sand was filled to a depth of 80cm with sand sizes of 0.075-2mm, 0.075-0.5 mm, 0.5-2mm and a control of 0.075-2mm with no pre-growth of bio-film. The rate of trickling water was set at 2 rounds per meter (rpm) and the filter run period was 15 days with 7 days wet and 3 days dry cycle to prevent clogging. COD, TN, TP, DO and OC were analyzed. Overall, fine sand column had the best results but specifically, COD efficiency rate was best in column of fine sand with 83%, TP in mixed sand with 81%, TN in fine sand column with 67% and DO in the control column with 8.15mg/L and OC was best in fine sand column with 22.59g. The best type of sand would be considered as 0.075-0.5 mm because it dominated in most results. With all the conditions in place, slow sand filtration was very effective as it removes most of the organic matter and suspended materials hence the water can easily be re-used not only due to its efficiency but also its simplicity in operation, cost effectiveness as well as being environmentally sound. [Journal of American Science 2010;6(4):47-57]. (ISSN: 1545-1003).

Keywords: Sand, filtration, bio-film, sand effectiveness.

1. Introduction

Treatment of water has for more than decades been a problem in both developed, developing and third world countries. Industrialization, globalization, population growth and some other factors continue to pose threats on both surface waters and underground water hence this has aroused the need to devise new methods and enforce the ones already existing to manage the water resources. In developed countries, treatment of contaminated water is not really a critical issue however it is still not economically viable since most activities of life and non-life revolve around water. If treatment of wastewater is done appropriately and recycled, wastewater can become a vital option in water resources at the same time, meeting the definition of sustainable development as in “Our Common Future”, 1987. However, the greatest challenge therefore comes in where monitory funds are a constraint and this is usually the case in most developing countries in Africa, Asian, and South America. Not only lower cost alternative methods have to be adopted in treating contaminated water, but also technologies which would have optimal use without causing damage on the environment and one of it, is Sand Filtration method. Though the method is archaic, its efficiency has been proven to be about 85% - 90% and it has been accepted and widely recognized as well as being adopted by World Health Organization (WHO), Oxfam, United States Environmental Protection Agency (US-EPA), and other UN organization. There are various types of sand filters namely; Rapid Sand Filtration (RSF), Up-flow Sand filtration and Slow Sand Filtration. This research focussed on treatment of surface water which is light contaminated using Slow Sand Filtration (SSF). The method was first established by John Gibb from Scotland around 1804 and in 1829, it was first adopted in London, WHO (1989).

As the name itself suggests, Slow Sand Filtration is a water treatment process that uses sand in treating water and in the process, it naturally uses biological activity. Slow sand filters have been in use for centuries, and are time-tested systems for cleaning drinking water. It is used to eliminate chemical oxygen demand (COD), organic content like phosphorous, suspended materials through decomposition, adsorption, absorption, electrostatic force and van der waals force, Shenkut Mesfin, (1996). Therefore, the objectives of this study were to investigate the quality of water after trickling through sand filtration using different types of sand and find the best sand size hence in the process, promoting the use of sand filtration. In this research, a

comparative study was conducted using different types of sand and the parameters which were analyzed after treatment of the effluent were; Chemical Oxygen Demand (COD), Total Phosphorous (TP- PO_4^{3-}), total nitrogen (TN) which comprises Nitrate $(NO_3 - N)$ and Nitrite $(NO_2 - N)$, Organic Nitrogen and Ammonium $(NH_4 - N)$, pH, Dissolved oxygen and Organic Content (OC) in soil after the experiment.

According to the definition given by WHO, safe water is the one which cannot harm the consumer when utilized. It may be colored, hard, with unpleasant odor, bitter, salty but as long as the values are within the threshold limits, it is considered "safe" or "portable" hence SSF can make the water safe if it is properly or effectively treated. SSF has been widely adopted since within a single unit, it incorporates settlement, filtration, organic removal and inactivation, and partly chemical and physical change.

From to Huisman I and Wood (1974) and Logsdon S.G et al, (2002), the principle of SSF is simple in such a way that a layer known as schmutzdecke which is a bio-film develops with microbes such as fungi, bacteria, protozoa, rotifers and other aquatic animals such as bryozoa, snails, annelid worms, insect larvae which break down the organic compound in the waste water. In addition, McMeen and Benjamin, (1997); Ellis (1985) states that the sand grains of the filter bed provide additional biological and physical mechanisms that contribute to removal efficiency. Therefore in the experiment, four PVC columns were used containing fine, course and mixed types of sand sizes; of which all of them had schmutzdecke grown for 7 days before commencing treatment except the forth column which was used as a control.

2. Materials and Methods

2.1. Wastewater and sand origin

The waste water used was obtained from the Eastern side of China University of Geosciences' Lake, of which it is the domestic influent from the staff as well as students apartments and from literature, concentration values (mg/L) of COD, TP, TN, DO, normally ranges from 100 to 400, 2 to 12, 20 to 75, 0.1 to 10, respectively and pH of 5 to 8, Temperature of 15 to 25 ℃, Chen H.Q et al (2008). The sand used was quartz type which is from Yangtze river in Wuhan hence comprise minerals such as K^+, Na^+ Ca^{2+}, iron, oxides, others, Achak M et al, (2009).

2.2. Sand and column characterization

Column 1; 0.075-2mm (mixture of fine and medium sand hence termed as mixed sand), Column 2; 0.075-0.5 mm (fine sand), Column 3; 0.5-2mm (course sand), Column 4; 0.075-2mm mixture of fine and medium sand- mixed sand which begun to treat water without pre-growth of schmutzdecke and this was just used as a control to show the importance of micro-organisms.

Columns used were of 100 cm in height, 3cm in diameter, and the sand was filled to a depth of 80cm. Using Darcy's law of which

$$Q = \frac{KA(H_1 - H_2)}{L}$$

Where by; Q= Volumetric flow rate (m^3/s or ft^3/s), K= hydraulic conductivity (m/s or ft/s),
A= surface flow area perpendicular to L or direction of flow (m^2 or ft^2)
H= average depth of water above the filter (m or ft), L= length of the medium (m or ft)

The permeability (K) and porosity (n) of mixed sand is: L =30cm, A=$7.065 \times 10^{-4}m^2$ H_1 =75cm, H_2 =47.5cm, ΔH=27.5cm=0.275m

Q= $KA\frac{\Delta H}{L} \Leftrightarrow K = \frac{QL}{A(\Delta H)}$ = 0.242

For (n); Volume of medium= 50 mℓ, Initial volume of water = 20 mℓ, absolute volume of water = 17.15; $\frac{V of\ water}{V of\ sand} = \frac{17.15}{50}$ = 0.343

For Fine sand:

L = 0.3m, A= 7.065×10^{-4}, H_1 = 78.5cm, H_2 = 47.3cm, ΔH=31.2cm=0.312m

$$K = \frac{QL}{A(\Delta H)} = 0.207$$

(n); V of water = 21.5, V of sand = 50 then n =0.43

For course sand:

L = 0.3m; A= $7.065 * 10^{-4}$, H_1 = 85cm, H_2 = 80cm, ΔH=5cm=0.05m

K = 5.6619

n ; V of water = 17.5, V of sand = 50 then n = 0.35

2.3. Experimental set-up

Three columns were filled with mixed sand, fine sand and course sand as shown in fig.1 and trickled with water for 7 days to allow the micro-organisms to grow and form the bio-film layer. All the columns were filled with 74 cm of pure sand and 3 cm of gravel at the top and bottom. An average of 3.5L of influent was trickled through the columns using a pump at a rate of 2 rounds per meter (rpm). After the elapse of this first phase, water was being filled following 7days wet/ 3 days dry cycle. The water was made to pass through the column by gravitational flow. The forth column was used as a control hence started treating wastewater influent without the first phase of growing the microbes and it used the mixed sand.

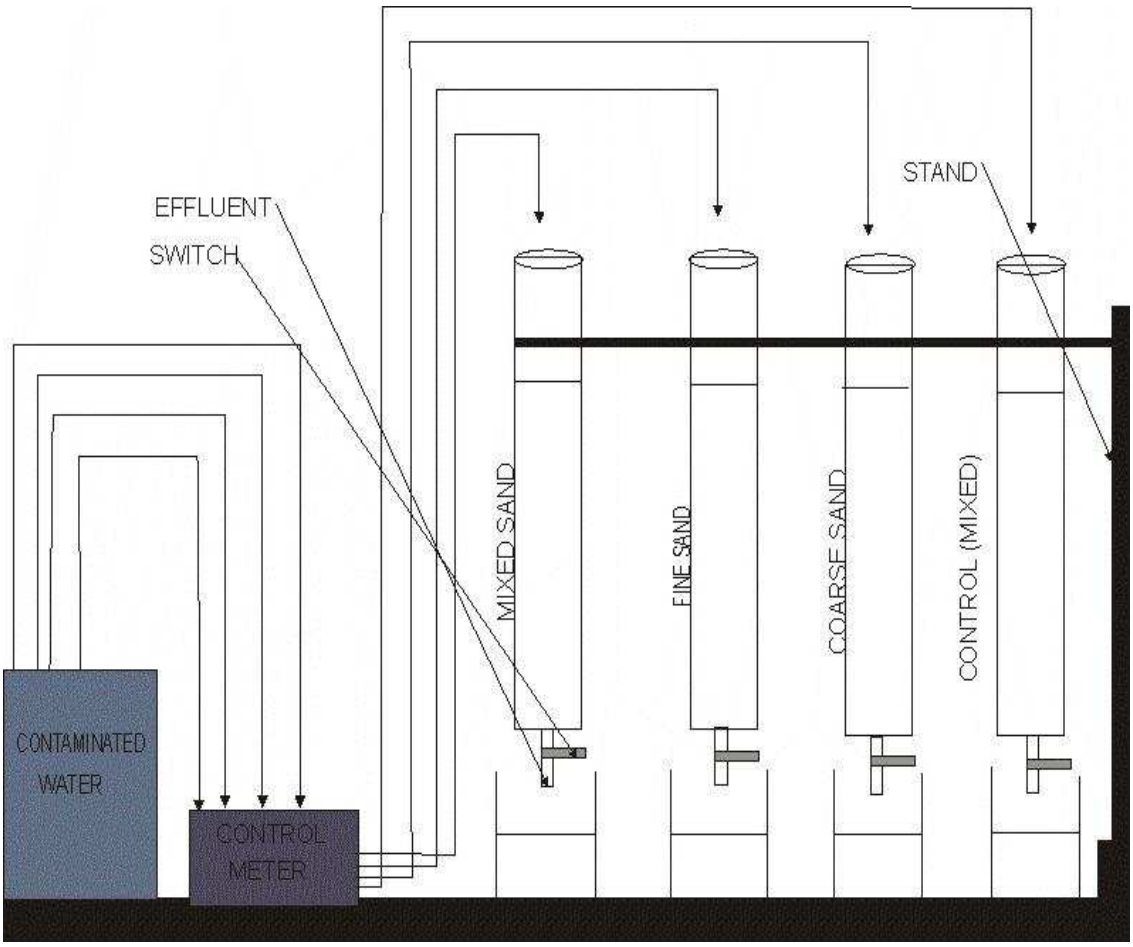

Fig. 1. Schematic diagram of the experiment with 4 columns.

2.4. Physical-chemical analysis of wastewater samples

All the samples were being analyzed on daily basis using the US-EPA standard methods. COD was measured using titration method with potassium dichromate, sulphuric acid, ammonium ferrous sulphate and phenanthroline indicator. Total phosphorous (TP) and total nitrogen (TN) was analyzed using DRB200 reactor machine through Test 'N Tube procedure for PhosVer with Acid persulfate Digestion and TNT Persulfate Digestion Method respectively. For TP, Total and Acid Hydrolyzable Test Vial, Potassium persulfate Powder Pillow, 1.54 N sodium hydroxide, Phosver 3 Phosphate Reagent Powder Pillow, were used. For TN, Total Nitrogen Persulfate Reagent Powder Pillow, Total Nitrogen Hydroxide Reagent Vials, TN Reagent A, B and C powder Pillow chemicals were used. pH was determined using a pH meter and DO also used a DO machine. All the chemicals were supplied by Chinese company known as Wuhan Heng Ling Technology and Tianjin City Fuchen Chemical Reagents Factory.

2.5. Physical analysis of sand

Sand samples were weighed before and after heating for organic content. The crucibles for drying were pre-heated for 4 hours at 400℃ and after putting soil, they were then put in first furnace for 4 hours at 105 ℃ and second furnace at 650℃ for 4 hours, as in ohlinger, (1995). For all columns, three points were taken at the top (0-7cm), middle (35-42cm) and bottom (70-77cm).

3. Results and Discussion

3.1. COD Removal

World Health organization, US-EPA and other UN organizations have proved that sand filtration can treat water from 80-90%. COD was measured every day for 20 days in 7days wet/ 3days cycle. On day 15, the columns were left to recover for one week. Table 1 shows the results for the analysis.

Table 1
Variations of COD in different columns (Mg/L)

	SAMPLE NAME				
DAYS	CONTAMINATED WATER	COLUMN 1	COLUMN 2	COLUMN 3	COLUMN 4
day 1	133.90	23.34	22.25	30.64	35.32
day 2	140.08	20.69	19.94	26.69	32.91
day 3	191.86	20.98	20.34	25.42	40.03
day 4	162.29	11.84	8.19	13.48	37.12
day 5	196.00	19.2	16.00	22.40	33.60
day 6	128.39	16.46	14.23	29.63	19.75
day 7	123.20	18.8	15.20	32.00	28.80
day 8	139.68	12.90	11.68	17.74	15.65
day 9	115.66	20.88	17.43	25.70	22.49
day 10	124.80	19.20	12.80	27.20	20.80
day 11	177.24	27.27	26.02	33.55	27.77
day 12	199.19	31.16	30.52	33.19	32.59
day 13	222.16	32.74	33.31	26.06	31.94
day 14	209.32	33.26	36.23	21.48	24.19
day 15	214.61	39.61	40.13	29.36	36.45
day 16	179.20	45.6	46.20	27.60	40.60
day 17	199.18	49.64	49.89	36.02	42.76
day 18	150.00	48.2	49.40	40.00	43.40
day 19	127.42	39.39	44.42	38.41	39.01
day 20	151.97	47.14	50.02	44.88	59.89

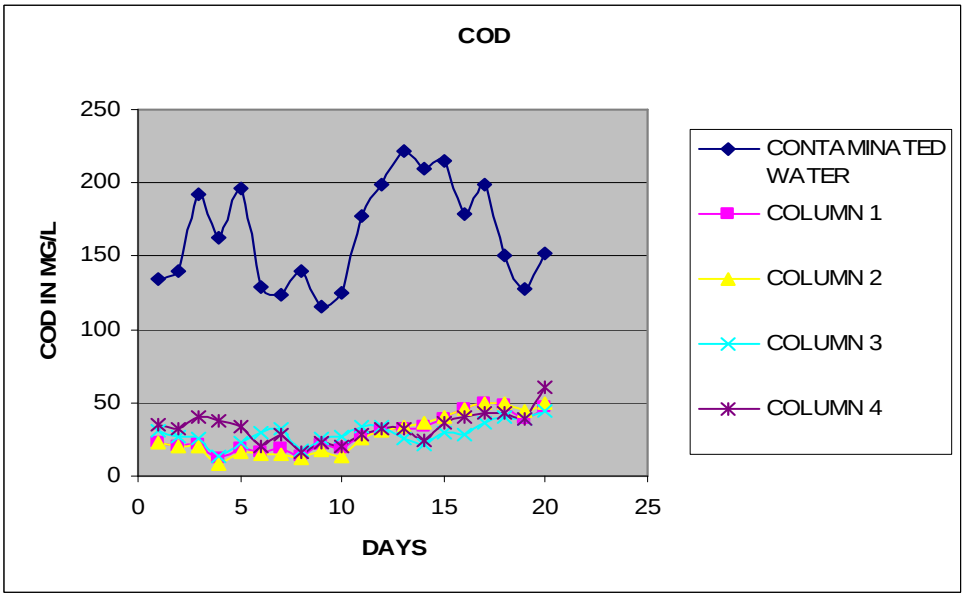

Fig.2. quantity of COD removal in different columns (mg/L)

The best results were obtained from the column with fine sand of 0.075-0.5 mm that is column 2. This is so because the there is little space in between the sand grain particles hence the organic compounds are easily trapped. Most of the COD is removed at the top of the column and the efficiency is reduced towards the bottom because of absence of oxygen. From fig. 2, fine sand column started with the efficiency of 83%. Unlike column 4 which was only 73% efficient for four days and its efficiency still remained below 79% due to insufficient micro-organisms since there was no pre-growth of the bio-film but on day 5, its efficiency increased to 82 % due to growth of microbes. In column 2, maximum COD removal was attained on day 4 at 94% and it was maintained between 80% and 90% until day 15 and this was found to be the best column with the best type of sand. After being left to recover again, the efficiency dropped to 67% hence it can be deduced that the life span of the column was 15 days. The overall COD concentration in filter effluent was measured as 28.21025 mg/L with min: 8.197mg/L; Max: 50.023mg/L. However, the efficiency of column 1 was not really significant from column 2. Column 3 was the third in its efficiency and column 4 was the forth for 5 days but the values interchanged with column 4 being the third since the microbes had grown by then and the values were close to the ones for column 1 since the same type of sand was used but the difference was in the amount of microbes which was lower in the column 4. Column 3 was most of the times the least efficient until day 12 since it had the highest sand size and this has an effect on filtration rate in such a way that it was high hence causing reduction in the organic loading and which reduced the removal rate of COD. Generally columns 1,2,3,4 were efficient in COD removal with ranges from 68 to 92%, 65 to 94%, 70 to 91%, 60 to 88% and average efficiency rates of 82%, 83%, 81% and 79% respectively. The efficiency was found by the formula $\frac{C_{in} - C_{out}}{C_{in}} \times 100$ whereby C represents concentration value. COD values from all columns were below 50mg/L which is the standard treated effluent in China. Achak M. et al, (2009) also found an efficiency range of 69 to 89%. Satoshi, (1998) in Achak M. et al, (2009) also reported as average of removal efficiency of 81.2% and similar results were experienced by Oladoja et al, (2006) who reported 80% COD removal. The removal efficiency for various days has been shown in fig.3.

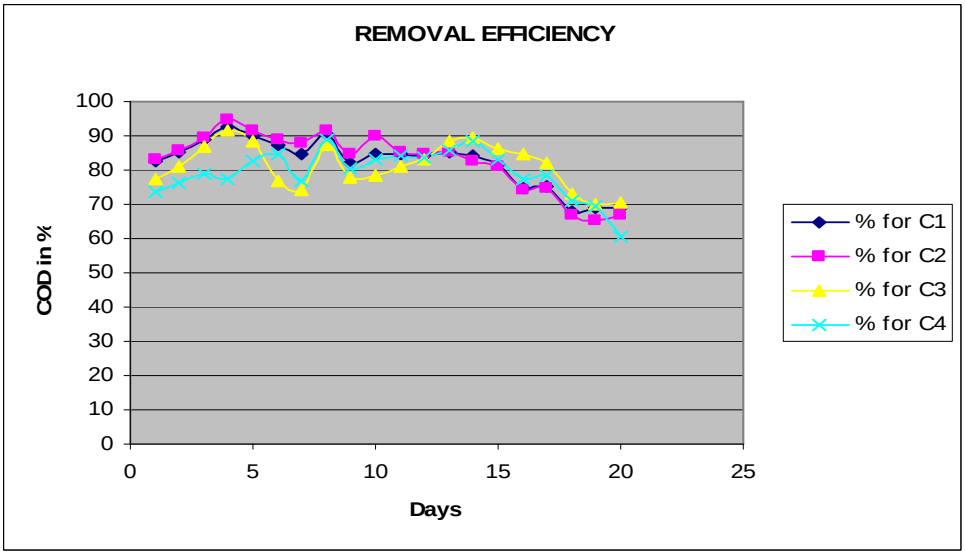

Fig. 3. COD removal in %

Achak M. et al, (2009) explains the removal of COD is eliminated through physical and biological phenomena. The particulate matter is filtered and sedimentation takes place and adheres itself to the sand grain particles. Biological degradation then takes place under oxygenated conditions. Jianmin Hua, et al (2003) reports that about 75 to 80 % of COD is removed in the first 25 to 30cm of sand in the column. The efficiency reduces due to clogging which reduces the filtration rate and the availability of the oxygen content.

3.2. Total Phosphorous (TP) removal

In this study, TP was measured every 2days and the removal capacity was the best and recorded data ranges with 0- 5mg/L as recommended by China Standards of surface water of which column 1- min: 0.04; max: 0.69, column 2- min: 0.08; max: 0.63, column 3- min: 0.25; max0.736, column 4- min: 0.1; max: 0.695 as shown in table 2 and the trend as shown in fig. 4.

Table 2
Variations of TP in different columns

DAYS	RAW WATER	COLUMN 1	COLUMN 2	COLUMN 3	COLUMN 4
day 1	2.18	0.11	0.16	0.25	0.20
day 2	3.36	0.04	0.08	0.39	0.1
day 3	2.42	0.06	0.19	0.3	0.21
day 4	2.8	0.12	0.34	0.39	0.35
day 5	2.54	0.25	0.33	0.45	0.38
day 6	3.58	0.36	0.41	0.46	0.39
day 7	4.02	0.45	0.46	0.51	0.48
day 8	4.28	0.52	0.58	0.67	0.59
day 9	3.89	0.57	0.55	0.68	0.58
day 10	4.7	0.69	0.63	0.74	0.70

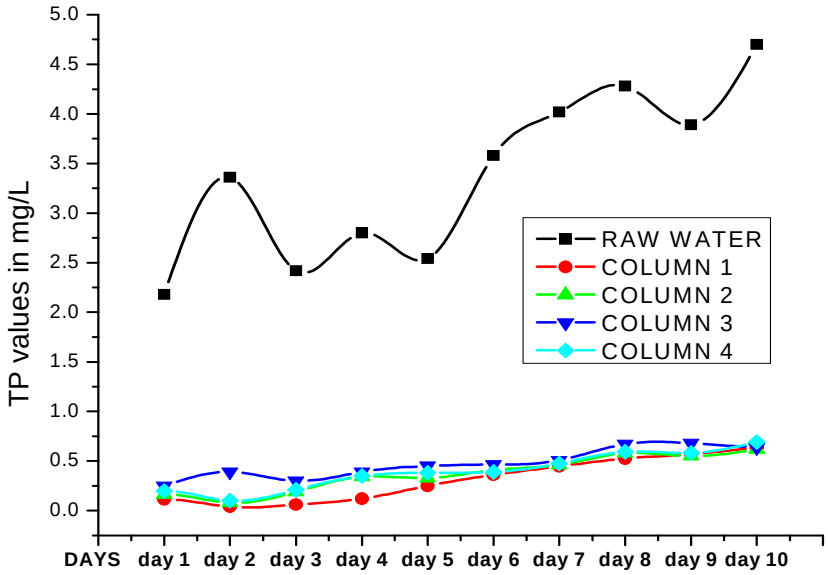

Fig.4. Quantity of TP removed (mg/L)

The average efficiency rates were 91%, 89%, 86%, 89% respectively. Removal of phosphorous is complex and sand grain size is a component that has to be considered. Rehan Sadiq et al, (2002); Jenkins et al, (1971) in Andrew J Erickson et al, (2007) illustrate that to remove dissolved phosphorous, it has to be converted to a solid phase and be removed as a particulate matter as well as being removed by sedimentation and physical sieving for solids materials. Billore et al, 1999 in Achak M. et al, (2009) explains that phosphorous is removed by adsorption and precipitation, ionic exchange. However, in long term it can also be removed as substrate as a main sink. Andrew J Erickson et al, (2007); Stumm and Morgan, (1981); Reddy and D'Angelo, (1994) explain the actual process of adsorption and precipitation that in acidic soils, it is dominated by iron oxides and aluminum. Phosphorous gets adsorbed and precipitates, hence becomes immobilized by ferric oxyhydroxide and forms ferric and aluminum phosphates. In alkaline soils, phosphorous retention or precipitation is dominated by calcium and magnesium, Weber-Shirk, Monroe L. (1997b). It has to be noted that the adsorption and precipitation process is highest in acidic conditions which are close to neutral. However, some phosphorous can still be adsorbed with iron in alkaline conditions like pH 10. Since this requires the use of oxygen, most of the processes occur on top and the capacity to retain it, decreases with depth.

In this study, column 1 which had mixed sand had the best results as shown in fig.4 since there is need of pores to provide space for iron oxides for phosphorous adsorption and precipitation. This should go hand in hand with amount of microbes which are responsible for the uptake. Column 3 had the worst because the pores are large and lacks microbes hence the precipitates would easily fall back in the water. In other words, the positive retention suggests that the retention capacity still exists and after a couple of days, the retention capacity decreases as the precipitates clogs the filter and brings complication of adsorption sites. That suggests the need of backwashing in the field to be cleaning the soil after a specific period because if this is overlooked, the precipitates may fall back in the effluent when they have saturated.

3.3. Total Nitrogen (TN) Removal

TN was also measured every 2 days. According to China standards, recommended surface water to be disposed off is 15mg/L. The ranges were; column 1 – min: 6.3; max: 16.5, column 2- min: 6.1; max 15.9, column 3- min: 6.5; max 17.3, column 4- min: 6.8; max: 16.8 as shown in table 3. The average removal efficiency rates were 65%, 67%, 62%, 64% respectively.

Table 3
Variations of TN in different columns

DAYS	CONTAMINATED WATER	COLUMN 1	COLUMN 2	COLUMN 3	COLUMN 4
day 1	28.4	6.8	6.6	6.9	7.2
day 2	30.8	6.4	6.1	6.5	6.9
day 3	27.5	6.3	5.9	6.6	6.8
day 4	26.3	7.2	6.6	7.4	7.9
day 5	29.4	7.9	7.5	8.8	8.5
day 6	28.8	8.5	8.3	9.2	8.6
day 7	25.2	10.8	9.6	11.5	10.2
day 8	27.3	11.4	11.5	13.9	12.7
day 9	28.1	15	14.8	16.8	15.3
day 10	28.9	16.5	15.9	17.3	16.8

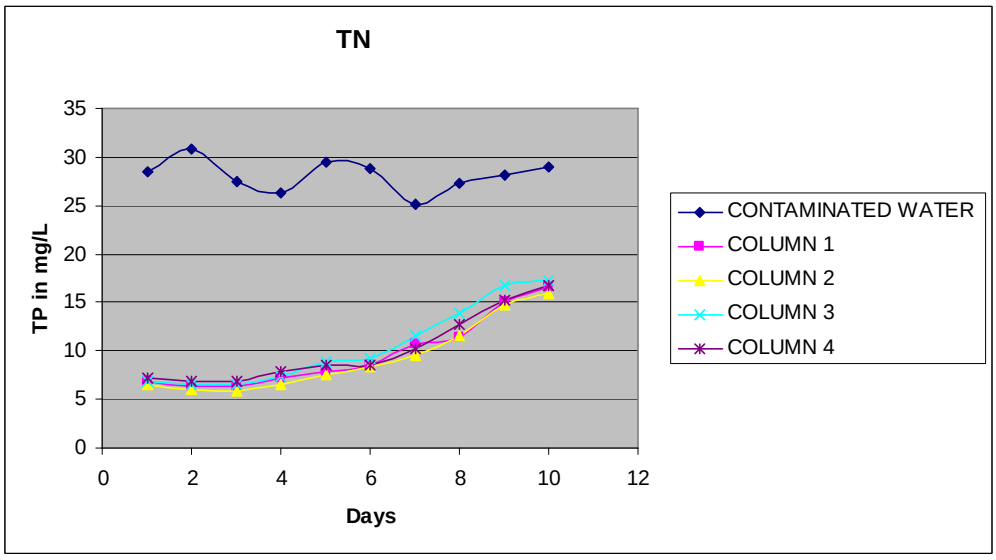

Fig. 5. Quantity of removed TN (mg/L)

The study of removal of nitrogen is complex and through literature, one of the removal process is through nitrification and de-nitrification though Gumes K and Tuncsiper B, (2009) states that the major removal mechanism is basically through de-nitrification of N-NO_3^+ by anaerobic bacteria. However, since the mentioned processes are achieved by both aerobic and anaerobic, the removal might have taken place from the top of the column to the bottom. The best type of sand was found to be column 2 with fine sand since it has a lot of microbes and the soil is compact which entails that the oxygen content would be lower than column 1 hence providing adequate growing and living conditions for anaerobic bacteria. In Achak M et al, (2009), it is explained that apart de-nitrification, microbes largely utilize nitrogen in form of NH_3 for the manufacturing of cellular components hence physical and chemical adsorption of NH_4^+ on organic matter. In nitrification, $N-NH_4$ is transformed into NO_2^- and to NO_3^- and in de-nitrification which occurs in anoxic zones, NO_3^- is changed to NO_2^- then to N_2O and to N_2. Hammer and Knight, (1994); Gumes K and Tuncsiper B, (2009), also explains that ammonium can be volatilized when NH4 is transformed to volatile NH3 especially when the pH is between 7.5 and 8.4 and in this study, this could be also the case as the pH had similar range. The fact that the

efficiency of TP was better than TN, it indicates that the sand used was rich in iron and calcium. The overall procedure of nitrification, Davis L.M. and Masten J.S, (2008);

$$NH_4^+ + 2O_2 \Leftrightarrow NO_3^- + H_2O + 2H^+$$

De-nitrification;

$$2NO_3^- + organic\ matter \Leftrightarrow N_2 + CO_2 + H_2O$$

From fig.5, column 1 was the second, column 4 third because they might had relatively fewer organisms than column 2 and column 3 which had the least as well due to its large sand size hence bigger spaces in between the sand making the medium have less nutrients for the growth of bio-film.

3.4. Dissolved Oxygen (DO)

The DO of influent had arrange of 0.1 to 1.84 mg/L and after running through the sand filter, the effluent in column 1, 2, 3, 4 had a range of 5.42 to 9.23; 5.78 to 9.28; 6.6 to 9.3; 6.75 to 9.32; and an average of 7.00, 7.35, 7.91, 8.15 mg/L as illustrated in the fig.6. Column 4 had the best results due to few micro-organisms that use oxygen hence the bio-film took time to grow and column 1 did not have relatively good amount of DO as there was competition in the utilization of DO. The filter traps all the organic matter hence the DO is replenished in the process.

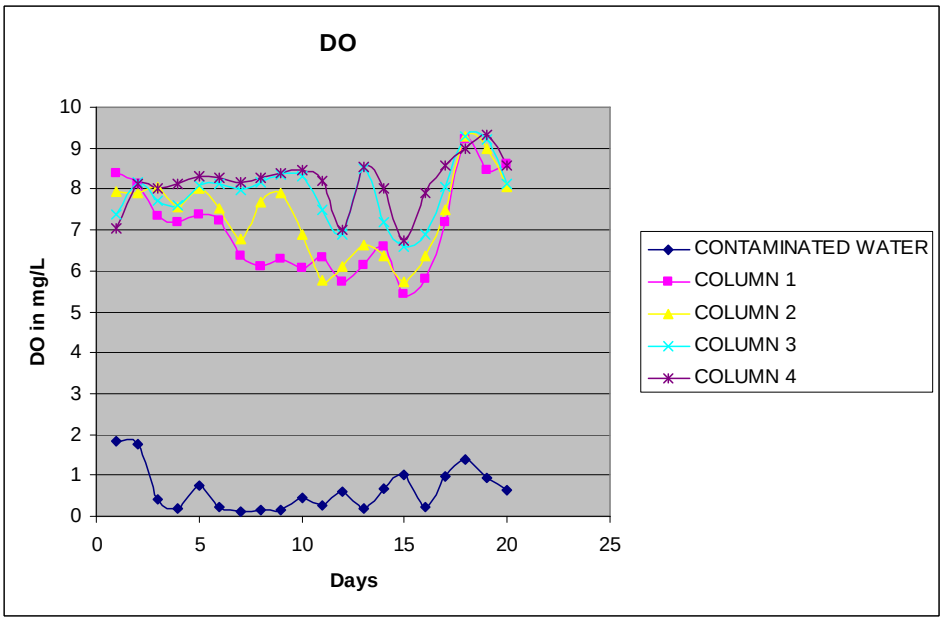

Fig.6. DO removal (mg/L)

3.5. Organic Content (OC)

OC in soil was weighed at the end of the experiment with column 2 having the highest mass and column 1 was the second as shown in table 4. This also entails why most parameters had good results in column 2.

Table 4
Organic compound from columns

Sample point (in cm)	Sand from columns (g)				
	Column 1	Column 2	Column 3	Column 4	Blank Sand
Top	4.14	4.87	2.49	3.29	0.26 (mixed)
Middle	4.81	5.61	3.45	3.32	0.34(fine)
Bottom	10.34	12.11	5.18	8.59	0.17(course)
Total	19.29	22.59	11.12	15.2	

4. Conclusion

Based on results, SSF has proved to be effective in treating light contaminated water in terms of its effluent quality with COD removal being the best in fine sand which was column 2 of 0.075-0.5 mm. TP removal was more effective in mixed sand column which was column 1 of 0.075-2mm, TN was best in column 2 just like COD. DO was effective in all columns though column 4 (0.075-2mm) outweighed with a marginal difference which comprised of mixed sand without pre-growth of micro-organisms and seconded by column 3 with course sand (0.5-2mm). In overall, fine sand should be regarded as the best because two parameters (COD and TN) studied in detail, proved more effective than in other types of sand, and for DO, the values were all in the accepted range and most of the results in fine sand were of good quality for many days as compared to other types of sand. The high amount of OC in column 2 also suggests the reason of it being more effective than other columns as it has been researched that OC is required in the treatment process of the water and growth of bio-film layer hence the higher the content, the better the efficiency but care should be taken, as too much of it would result to clogging of the column. Apart from its effectiveness, sand filtration should be promoted especially in developing areas due to its simplicity in its operation, cost effectiveness as well as being environmentally sound. Water from this system can be re-used for gardening, building, home use, and safety measures, chlorine ($C\ell$) should be added when drinking. The best efficiency rates were obtained within a period of 15 days hence it can be concluded that it was the maximum time to operate.

List of symbols

COD = Chemical Oxygen Demand
CUG = China University of Geosciences
DO = Dissolved Oxygen
$NO_3 - N$ = Nitrate
$NH_4 - N$ = Ammonium
NTU = Nephelometric Turbidity Unit
OC = Organic Content
PO_4^{3-} = Phosphate
RPM = Round per Meter
SSF = Slow Sand Filtration
TN = Total Nitrogen
TP = Total Phosphorous
UN = United Nations
WHO = World Health Organization

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References;

1) Achak M, L. Mandi, N. Ouazzani. Removal of organic pollutants and nutrients from olive mill wastewater by a sand filter, Morocco, Journal of Environmental Management, Elsevier. 2009; (90): 2771-2779.
2) Andrew J. Erickson, John s, Gulliver, Peter T. Weiss. Enhanced Sand Filtration for Storm Water Phosphorous Removal, Journal of Environmental Engineering, ASCE,2007: 485-497.
3) Chen H.Q, Hua Qing Chen, Yi Lian Li, Yu Ning, Feng Lan Lv. Experimental study on blockage law of constructed rapid infiltration system, School of Environmental Studies, China University of Geosciences, Wuhan 430074, P. R. China, 2008
4) Davis L Mackenzie, Masten J. Susan. Principles of Environmental Engineering and Science, 2nd Edition, McGraw Hill, Tsinghua Press, 2008.
5) Ellis, K.V. Slow Sand Filtration. CRC Critical Reviews in Environmental control, 1985.
6) Gary S. Logsdon, Roger Kohne, Solomon Abel and Shawn LaBonde. Slow sand filtration for small water systems, Journal of Environmental Engineering Science 2002; (1): 339-348, Canada.
7) Hammer D.A Knight R.L. Designing constructed wetlands for nitrogen removal, Water Science Technology 1994; 29 (4): 15-27.

8) Huisman I and Wood, W.E, F.I.C.E. Slow Sand Filtration, World Health Organization, Geneva, 1974:20-43.
9) Jenkins, D., Ferguson J.F., and Menar, A.B. Chemical processes for phosphate removal, Water Resources, 1971;(5): 369-89.
10) Jianmin Hua, Pinglin An, Josef Winter, Claudia Gallert. Elimination of COD, Microorganisms and Pharmaceuticals from sewage by trickling through sandy soil below leaking sewers, Water Research, Elsevier. 2003;(37):4395-404.
11) Kemal Gunes and Bilal Tuncsiper. A serially connected sand filtration and constructed wetland system for small community wastewater treatment, Ecological Engineering, Elsevier, 2009; (35): 1208-1215.
12) McMeen, C.R. and Benjamin, M. NOM. Removal by slow sand filtration through iron oxide-coated olivine, Journal of the American Waterworks Association, 1997.
13) Ohlinger, R, Soil respiration by titration. In Schinner, F Ohlinger R, Kandeler E. Margesen R.(EDs.) Methods in Soil Biology, Springer, Berlin 1995: 95-98.
14) Oladoja, N.A., Ademoroti, C.M.A., Asia, I.O. Treatment of industrial effluents using fortified soil-clay. Desalination 2006;(179): 247–261.
15) Reddy K. R. and D'Angelo E.M. Soil processes regulating water quality in wetlands, Global Wetlands: Old World and New, Elsevier, New York, (1994):309-324.
16) Rehan Sadiq, Tahi Husan, A.M. Al-Zahrani, Anwar Khalil Sheikh and Shaukat Farooq, Secondary Effluent Treatment by Slow Sand Filters: Performance and Risk Analysis, Institute for Research in Construction, National Research Council, Canada, 2002.
17) Satoshi, M., 1998. Natural process, Water Quality International March/April, in Achak M, L. Mandi, N. Ouazzani, Removal of organic pollutants and nutrients from olive mill wastewater by a sand filter, Morocco, 2009:2771-779.
18) Shenkut Mesfin. Experience in Slow Sand Filtration in Ethiopia, Advances in Slow Sand and Alternative Biological Filtration, edited by Niglel Graham and Robin Collins, John Wiley and Sons, USA, 1996.
19) Stumm W. and Morgan J.J. Aquatic chemistry: An Introduction Emphasizing chemical equilibria in natural water, Wiley, New York, 1981.
20) Weber-Shirk, Monroe L. Biological Mechanisms in Slow Sand Filters. Journal of the American Water Works Association, 1997b.
21) WHO Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture, Report of Scientific Group, Geneva, 1989.
22) World Commission on Environment and Development, Our Common Future, 1987.

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Mineralization of organic compounds in wastewater contaminated with petroleum hydrocarbon using Fenton's reagent: a kinetic study

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Abstract: In the present work, the possibility of using chemical oxidation through Fenton's reaction for the pre-treatment of wastewater contaminated with petroleum hydrocarbon was investigated as laboratory-scale experiments. The effect of different operational conditions, namely, hydrogen peroxide, ferrous ion concentrations, temperature, and initial pH were evaluated. Operating at initial pH of 3, with a temperature of 28 ^{o}C and a molar ratio H_2O_2/Fe^{2+} = 9:1, it gave us 70.58% removal of COD. A kinetic study was carried out using a modified pseudo-first-order model. The experiment was performed at different temperatures hence allowed the calculation of Arrhenuis equation parameters and the global activation energy for the first-order reaction. [Journal of American Science 2010;6(4):58-66]. (ISSN: 1545-1003).

Key words: Chemical oxidation, wastewater, COD, activation energy

1-Introduction

The world is facing the challenge of purification of surface and groundwater contaminated with petroleum hydrocarbon. In spite of its many uses, petroleum hydrocarbons are toxic in nature and environmental contamination by these toxic products is emerging as a serious global problem. Hydrocarbons are quantitatively most important constituents of petroleum, and arise from natural as well as anthropogenic sources. Their accidental releases in the natural environment have resulted into the gross contamination of soil, surface and groundwater. In this case, they constitute a major environmental problem due to the high organic compounds load and the great volume of contaminated water generated. In the last few years there has been a great effort to develop new solutions for the remediation of hydrocarbon from water. The best and economical way is to control the release of petroleum in the natural environment.

Biological treatments have been suggested by many authors and institutions. One of those institutions is RIMAX BIO which suggested the use of BIO PETRO TREET – Bioremediation system for removing petroleum hydrocarbon from soil and water. The Bio Petro Treet system consist of a highly concentrated microbial formulation containing a proprietary blend aerobic bacteria having petroleum molecule metabolizing capacities for hydrocarbon removal from soil and aqueous environments. Although, the high concentration of organic compounds load make the petroleum hydrocarbon recalcitrant to aerobic degradation and inhibit the efficiency of anaerobic digestion. Physico-chemical processes have been also applied for petroleum hydrocarbon treatment. The reduction of hydrocarbon pollution can be increased if biological treatments are combined with chemical or physical processes. Advanced oxidation processes (AOPs) are known for their capacity to mineralize a wide range of organic compounds. The concept has been defined by Glaze et al. in 1987 as "near ambient temperature and pressure water treatment processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification".

AOPs employ reactive oxidizing agents such as hydrogen peroxide or ozone, with or without addition of catalysts or photolysis. These oxidation systems have been used to treat both individual organic and inorganic substances in water under laboratory conditions as well as real effluents from the different sources. Among AOPs, the Fenton's reagent (Fe^{2+}/H_2O_2) becomes more and more common for the treatment of

contaminated water. Many researches had demonstrated that the oxidation mechanism by Fenton's reagent was due to the reactive hydroxyl radicals generated in an acidic solution by the catalytic decomposition of hydrogen peroxide (Lopes De Morais and Zamora,2005; Kang and H. Wang 2000). In Fenton's reagent, iron and hydrogen peroxide are two major chemicals determining operation cost as well efficacy (Zhang, 2005). The equation of hydroxyl radicals generation is below:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO\bullet$ (a)

In addition to the main reaction, various additional competitive reactions are also possible involving Fe^{2+}, Fe^{3+}, H_2O_2, superoxide and hydroxyl radicals (E. Neyens, J. Baeyens, 2003).

$Fe^{2+} + HO\bullet \rightarrow Fe^{3+} + OH^-$ (b)

$H_2O_2 + HO\bullet \rightarrow H_2O + HO_2\bullet$ (c)

$Fe^{2+} + HO_2\bullet \rightarrow Fe^{3+} + HO_2^-$ (d)

$Fe^{3+} + HO_2\bullet \rightarrow Fe^{2+} + O_2 + H^+$ (e)

In the presence of organic substrates (RH) the highly reactive hydroxyl radical, a species with a relatively short life-span (rate constants in the range 107 to $1010 M^{-1} s^{-1}$), undergoes oxidation generating a new radical (C.Walling, 1974).

$RH + HO\bullet \rightarrow R\bullet + H_2O$ (f)

Possible organic compounds present in reaction mixture can suffer an abstraction of a hydrogen atom or addition of radical HO•, in the case of olefins, creating a new radical (R•), which can subsequently be oxidized by Fe^{3+}:

$R\bullet + Fe^{3+} \rightarrow Fe^{2+} +$ product (g)

The equation above regenerates Fe^{2+} which ensures the continuity of the chain reaction that can lead ultimately to the decomposition of organic substrate in carbon dioxide and water.

The purpose of this work is the application of Fenton's reagent to partially degrade the organic load in water contaminated with petroleum hydrocarbon, expressed in terms of COD. The aim is to experimentally determine the optimal conditions for Fenton's reagent application, studying the effect of different operational variables such as pH, temperature and H_2O_2 and Fe^{2+} concentrations. Also, a kinetic study using a modified pseudo-first-order model was made in order to determine the kinetic constants.

2- Materials and methods

2-1- Contaminated water

The oxidization reaction was conducted as laboratory-scale experiments for lowering the oil-products content dissolved in water solution. To evaluate the effectiveness of petroleum hydrocarbon removal from water by oxidization method, a model wastewater was prepared. It consisted of petroleum hydrocarbon (**5ml of crude oil**) which was left in water (20L) over 3 days to let it completely dissolve in water medium. Before performing the Fenton's oxidation, the initial COD of the sample was tested. The characterization of the wastewater is presented in the table below.

Table1: Characterization of wastewater

COD (mg/l)	pH
98	8.6

2-2 Experiments

Fenton's reagent experiments were carried out at room temperature in a 1 liter reactor using varying hydrogen peroxide and $FeSO_4$ dosages at varying pH values in order to determine the optimum conditions for best COD removal. Optimum pH determination was carried out for chemical dosages of H_2O_2 and, in order to find optimum chemical dosages at a predetermined optimum pH, H_2O_2 and $FeSO_4$ dosages varied. The pH adjustment was carried out with diluted sulphuric acid (H_2SO_4) and sodium hydroxide (NaOH) solutions. Typical experiments were carried out with 500mL wastewater to which an amount of $FeSO_4 \cdot 7H_2O$ was added and mixed by stirring. The Fenton oxidation began with the addition of hydrogen peroxide solution. To avoid the coagulation of chemical, samples were taken to the mixing machine. The mixing rate was set at 120r/min. Temperature was also regulated. Samples were withdrawn from the reactor at pre-determined times. Control experiments were performed in the absence of either Fe^{2+} or H_2O_2.

2-3 Reagents

During the experiments, the following reagents were used:

- H_2O_2 (1%, 2% and 3%)
- $FeSO_4 \cdot 7H_2O$, 0.1mol/L
- NaOH
- H_2SO_4
- $K_2Cr_2O_7$
- $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$
- Phenanthroline

2-4 Analytical methods

The COD analyses were performed by titration method using ammonium sulfate $(NH_4)_2Fe(SO_4)_2$, potassium dichromate ($K_2Cr_2O_7$) and phenanthnoline. The residual hydrogen peroxide present in the reaction media was checked by peroxides test. Dissolved oxygen was measured with DO machine. Others wastewater parameters were analyzed according to standard methods. All of oxidation experiments were performed, at least, three times and the observed margin of error was always less than 4% of the reported value.

3- Results and discussions

Table 2: Experimental conditions, COD values, COD removal (%) obtained after oxidation of petroleum hydrocarbon with Fenton's reagent

Experiments	concentration H_2O_2 (%)	T (^oC)	pH	Molar ratio (H_2O_2/Fe^{2+})	COD Initial (mg/l)	COD_{Final} (mg/l)	Removal COD (%)
Exp 1	1%	28	3	9:1	98.34	87.33	11.20
Exp 2	2%	28	3	9:1	94.56	57.13	39.25
Exp 3	3%	28	3	9:1	97.25	28.61	70.58
Exp 4	3%	28	2	9:1	97.15	40.53	58.28
Exp 5	3%	28	5	9:1	95.48	32.96	65.48
Exp 6	3%	28	7	9:1	93.83	48.57	48.23
Exp 7	3%	28	9	9:1	95.35	73.84	22.55
Exp 8	3%	10	3	9:1	94.56	50.83	46.24
Exp 9	3%	20	3	9:1	95.74	32.60	65.94
Exp 10	3%	40	3	9:1	93.45	28.43	69.57
Exp 11	3%	50	3	9:1	95.43	31.45	67.04
Exp 12	3%	28	3	4.5:1	94.28	26.88	71.48
Exp 13	3%	28	3	18:1	96.50	38.48	60.12

Control experiments

Experiments	Fe^{2+} (M)	H_2O_2 (%)	T (^oC)	pH	COD Initial (mg/l)	COD_{Final} (mg/l	Removal COD (%)
C1	0	3	28	3	96.50	89.35	7.4
C2	0.1	0	28	3	97.15	95.60	1.6

Fenton's oxidation was carried out at a laboratory scale in order to minimize the impact of petroleum hydrocarbon on natural water. The Fenton's oxidation pathway system to the reduction of COD is always summarized as follow:

- **Step 1**:
  $COD + H_2O_2 + Fe^{2+} \rightarrow$ partially oxidized species (h)

- **Step 2**:
  Partially oxidized species + H_2O_2 + Fe^{2+} $\rightarrow$ CO_2 + H_2O + inorganic salts (i)

The extent of oxidation determines the extent of reduction of COD and the costs of application of Fenton's reagent depend on the amount of hydrogen peroxide used. Many cases have been faced whereby the complete mineralization of the organic compounds which produces carbon dioxide and water is not necessary achieved. The intermediate compounds minimize the consumption of chemical reagent and results in the great reduction of COD. Table 2 gives the initial and the final values of COD for each experiment and the removal COD obtained has been defined as:

$$COD(\%) = \frac{COD_0 - COD_f}{COD_0} \times 100 \qquad (k)$$

The table also summarizes also the experiments performed where-by the hydrogen peroxide concentration, temperature, pH and the initial concentration of ferrous ions were modified. As it can be observed, the removal COD for Fenton experiment after 2h of reaction, lay between 11.20% and 71.48% depending on the operating condition. These COD removal is attributed by the high reactivity of organic compounds with hydroxyl radicals. In Fenton's reagent, the hydrogen peroxide dosage could also be referred to as the stoichiometric weight ratio between the hydrogen peroxide and COD, which are calculated assuming the complete oxidation of COD.

3.1 Effect different dosage of hydrogen peroxide.

In the first set of experiments, the pH of the medium and the molar ratio (hydrogen peroxide and ferrous ions) were kept constant at 3.0 and 9:1 respectively. The values were chosen based on many researches and recommendations. (Stuart M. Peters, et al, 2001). The temperature was kept at 28 ^{0}C. The hydrogen peroxide concentration was changed between 1 and 3%. Figure 1 shows the rate of COD reduction over time. It can be observed that COD reduction occurs principally at an initial period (20- 40 min) and as the reaction time increased the COD concentration profile was approaching a plateau. It is possible to observe that COD is significantly reduced within two hours of reaction. The kinetic attention will be focused on two hour of reaction in this study. The figure shows also that increasing the concentration of hydrogen peroxide (and proportionally ferrous ions concentration) has a positive effect on the COD removal rate. For the maximum hydrogen peroxide concentration studied (3%), the COD removal reaches 70.58%. This shows the Fenton's reagent ability to degrade a great amount of oxidizable organic matter in our water sample. The increase in COD conservation with the raise of hydrogen peroxide concretion results from the generation of hydroxyl radicals and, therefore, a greater extent of oxidation reaction. (P. Bautista, et al, 2007)

3.2 Effect of initial pH

The pH effect was studied through experiments Exp 3, Exp 4, Exp 5, Exp 6 and Exp 7. Figure 2 shows the COD reduction of petroleum wastewater for different initial values of pH. It reveals that Fenton's reagent is a highly pH sensitive process. The COD removal performance declined for pH values greater than 5.0. This occurs due to the formation of the ferric hydroxo-complexes, namely the precipitation of Fe^{3+} as $Fe(OH)_3$, hindering the reaction between $Fe^{3+}$and H_2O_2, and therefore the regeneration of Fe^{2+}. Besides, $Fe(OH)_3$ catalyzes the self-decomposition of H_2O_2 (particularly unstable in basic media) to oxygen molecular and water, resulting in a decline of its oxidative capacity (P. Bautista, et al, 2007). On the other hand, for pH values lower than 2.0 the reaction of hydrogen peroxide with Fe^{2+} is seriously affected due to the formation of complex species $[Fe(H_2O)_6]^{2+}$, which reacts slower with peroxide when compared to that of $[Fe(OH)(H_2O)_5]^{2+}$. In addition, the peroxide gets solvated in the presence of high concentration of H^+ ion to form stable peroxone ion $[H_3O_2]^+$. The peroxone ion it leads to an electrolytic behavior on the part of hydrogen peroxide improving its stability and substantially reducing the reactivity with ferrous ion.

Therefore, the initial pH value has to be in the acidic range (3–4) to generate the maximum amount of HO • and oxidize the organic compounds. In particular, the optimal value of pH is 3, which is in agreement with previous results obtained in other studies using Fenton's reagent.

3.3 Effect of temperature

A temperature range from 10 to 50^{o}C was studied in order to observe the effect of temperature on Fenton's reagent (Exp 3, Exp 8, Exp 9, Exp 10 and Exp 11). The obtained COD removal efficiencies after oxidation with Fenton's reagent are presented in figure 3. It can be deduced from this figure that the optimal range for the temperature is in the range of 28 to 40 ^{o}C. For lower temperatures studies, the COD reduction indicates that the extent of the reaction was not complete within 2h of reaction. For 10 ^{o}C the removal COD 46.24% and for 20 ^{o}C was 65.94%. This hypothesis is confirmed by the presence in the reaction medium of residual levels of hydrogen peroxide. The removal COD rate went down above the optimal range of temperature. The hydrogen peroxide self-decomposition into molecular oxygen and water is being significant at 50 ^{o}C, partially reducing the effectiveness of the oxidation process and the generation of hydroxyl radicals. The Fenton's reaction is an exothermic process. The moderation of the temperature in the reactor is important for safety reasons and to avoid runway situations.

3.4 Effect of ferrous ions concentration

To determine the effect of the concentration of ferrous ions they were carried out as experiments 3, 12 and 13. As a control experiment, it is possible to confirm that using only hydrogen peroxide, the obtained COD reduction is very small (7%). The results indicated that by increasing the Fe^{2+} ion concentration in the solution, the COD removal increased. Oxidation rate increases with Fe2+ ion concentration as the results of higher production of hydroxyl radicals. In spite of the improvement in reaction kinetics achieved with higher Fe^{2+} concentration, it is important to reduce costs in chemicals and minimize the production of Fe^{3+} that will probably precipitate as Fe (OH) $_3$. Consequently, it appears that in the case of Exp 12 where H_2O_2:Fe^{2+} = 4.5:1, the final conversion of COD (71.48%) is only slightly above the Exp 3 (70.58%), where the initial concentration of iron was half (H_2O_2:Fe^{2+}= 9:1). Probably a greater concentration of Fe^{2+} can cause the recombination of radical HO•. In this case, the Fe^{2+} reacts with the hydroxyl radical functioning as a scavenger (C. Walling, 1974). It is therefore desirable that the amount of iron used must be a compromise that can minimize the production of sludge from iron complex. The value of H_2O_2:Fe^{2+}= 9:1 was selected and used as a reference for other tests. Finally, to confirm the symbiotic importance of hydrogen peroxide in Fenton's reagent using only ferrous ions (C2), there is practically no decrease on COD (only about 1.6%).

3. 5 Kinetic study

It must be remarked that since we have a very complex reaction mixture, the overall kinetics will be analyzed by taking into the total organic compound present within the reactor. Due to the complexity of chemical compounds formed as intermediates in the Fenton reagent oxidation, make virtually impossible carry out a detailed kinetic study with the different individual reactions that take place during chemical oxidation. However, it is possible to conduct an approximated kinetic study according to some parameter that represents the overall organic matter, in this case, the COD. Figure 4 shows the effect of temperature in COD reduction during Fenton's reagent application to the wastewater contaminated with petroleum hydrocarbon. Through this study it will be possible to assess and obtain a series of apparent kinetic constants that reflect the greater or lesser speed with which follows the process of oxidation in the different conditions set operative.

The progressive disappearance of organic load, measured as COD, by Fenton reagent may be represented by a simple irreversible reaction of pseudo-first-order with respect to COD:

Organic matter (COD) + Fenton → P + CO_2 +H_2O (l)

P symbolizes the intermediate products of degradation.

By assuming that these reactions follow pseudo-first-order kinetics with respect to COD, we may write:

$$-\frac{dCOD}{dt} = kCOD \qquad \text{(m)}$$

which can be integrated between t = 0 and t = t, yielding:

$$\ln\left(\frac{COD_O}{COD}\right) = kt \qquad \text{(n)}$$

According to this expression, a linear representation of the first term versus time, should lead to a straight line for each experiment whose slope is k

Figure 5 shows this kinetic of pseudo-first-order for the Fenton system in the case of experiments in which we varied the temperature. Least squares regression analysis gave the k values listed below:

K=**0.00635 min^{-1}** at 10 °C, **0.01205 min^{-1}** at 20 °C, **0.02165min^{-1}** at 28 °C, **0.02465min^{-1}** at 40 °C and **0.03 min^{-1}** at 50 °C. The kinetic reaction for the Fenton system is generally limited by the speed of HO• radical generation but also dependent on specific wastewater to be treated.

A multiple regression analysis permits to calculate the value of k for experiments with Fenton's reagent at pH 3. To analyze the experimental data, the integral method was used for the first step. The results are shown in figure 5, which clearly gives evidence and is in agreement with the equation (n). From the slopes of lines, the apparent second-order kinetic constants were obtained and from the corresponding Arrhenuis-type plot (figure 6) k values can be correlated by an Arrhenius-type expression. The graphical representation of ln k on the basis of 1/T (Figure 6) allows the calculation of the parameters of Arrhenius equation:

$$\ln k = \ln A_o - \frac{E_a}{RT} \qquad \text{(o)}$$

Where E_a is the energy of activation (kJ mol^{-1}) and A_0 the pre-exponential factor (min^{-1})

The regression analysis with expression of constants against temperature led the following values of: $E_a = 31.877 kjmol^{-1}$ and $A_0 = 4.29\times10^{-2}\ \text{min}^{-1}$

For the zero-order reaction rate data, no correlation could be established. This is probably due to the decomposition of hydrogen peroxide, which strongly increases with the temperature - approximately 2.2 times for each 10°C rise in the range 20 – 100 °C (US peroxide, 2001). This work shows that the use of the Fenton's reagent requires a very fine control of the operating conditions to achieve high performances, otherwise, limitations will unavoidably arise from hydrogen peroxide decomposition via non-producing HO• reactions, reaction of the HO• species with non-target chemicals or even other well-know reactions reported in the literature that reduce the amount of hydrogen peroxide available for Fenton's reaction (Hunling SG, et al. 2000).

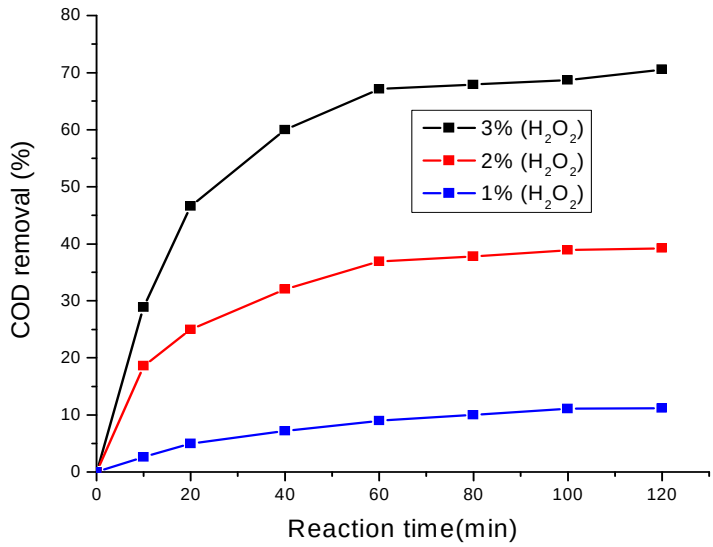

Figure1: Effect of hydrogen peroxide concentration on the COD removal

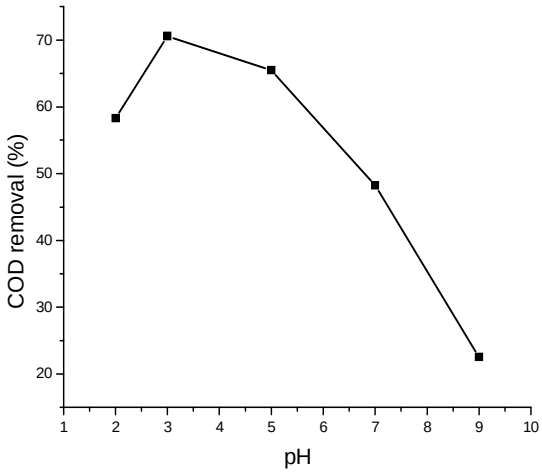

Figure2: influence of pH on the COD removal

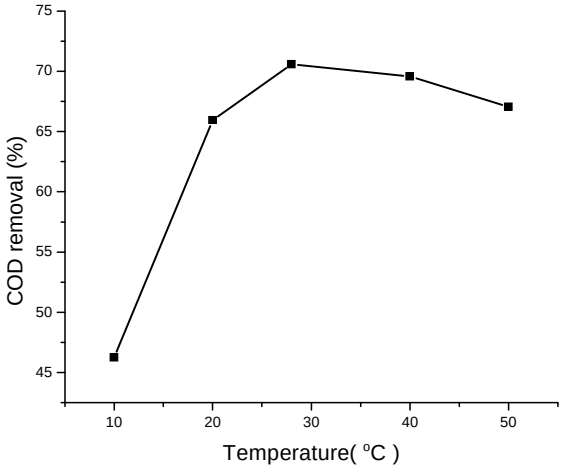

Figure3: initial temperature influence on the COD removal

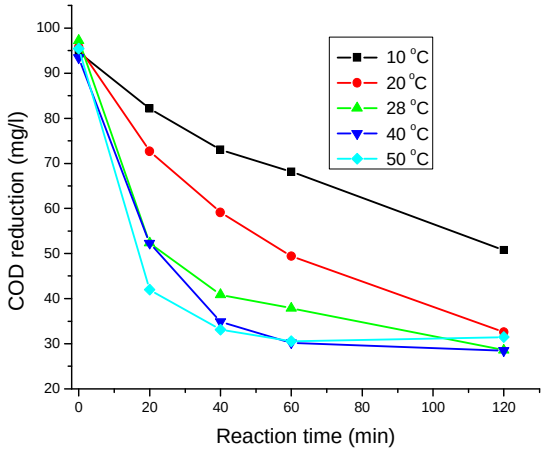

Figure4: Effect of temperature on the COD reduction

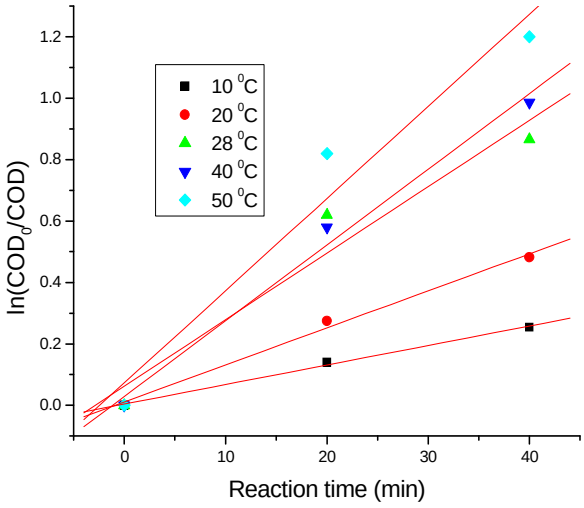

Figure5: Determination of the first-order rate constants k for different temperatures

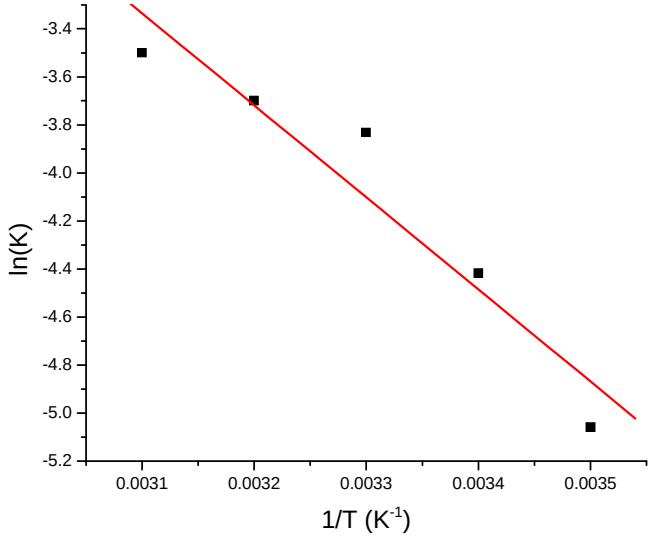

Figure6: Arrhenuis plot of the apparent first-order kinetic constants for the reaction

4- Conclusion

The overall results of this study indicate that the application of Fenton's reagent is a feasible method to partially treat water contaminated with petroleum hydrocarbon achieving a significant decrease of COD. Fenton's reagent at initial pH 3, temperature of 28 ^{o}C, molar ratio H_2O_2:Fe^{2+}= 9:1 and H_2O_2 3%, leads to a COD reduction of 70.58%. A simple kinetic analysis based on COD was carried out. A modified pseudo-first-order equation allowed to describe the process well and led to the determination of kinetic parameters useful for the design of industrial reactors. A value of $31.877 kjmol^{-1}$ was obtained for the apparent activation energy (Ea) in Arrhenius equation.

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References:

BOWERS A. R., CHO S. H., SINGH A. Chemical oxidation of aromatic compounds: comparison of H_2O_2, $KMnO_4$ and O_3 for toxicity reduction and improvements in biodegradability. In "Chemical Oxidation Technologies for the Nineties", Eckenfelder W. W., Bowers A. R., Roth J. A., ads. Technomic Publishing Company, Lancaster, PA. 1991:11

Bryant, J.D. and Wilson, J.T. Fenton's in-situ reagent chemical oxidation of hydrocarbon contamination in soil and groundwater. *Remediation*. Autumn, 1999: 13-25.

E. Neyens, J. Baeyens. A review of classic Fenton's peroxidation as an advanced oxidation technique, Journal of Hazardous Materials 2003; (98): 33 -50.

Flaherty KA, Huang CP. Continuous flow applications of Fenton's reagent for the treatment of refractory wastewaters. In: Eckenfelder WW, Bowers AR, Roth JA, editor. Proceedings of the second International Symposium on Chemical Oxidation— Technologies for the Nineties, Vanderbilt University, Nashville, Tennessee, USA, 19–21 February 1992, vol. 2, Switzerland: Technomic Publishing AG Basel, 1994: 58–77.

Huling, S.G., Arnold, R.G., Jones, P.K., and Sierka, R.A. Predicting Fenton-driven degradation using contaminant analog. *Journal of Environmental Engineering*. April. 2000:348-353.

Kang YW, Hwang K -Y. Effects of reaction conditions on the oxidation efficiency in the Fenton process. Water Res 2000; (34):2786–90.

Liang, L., Hofmann, A., and Gu, B. Ligand-induced dissolution and release of ferrihydrite colloids. Geochimica et Cosmochimica Acta, 2000; 64(12):2027-37.

LIN S. H., LIN C. M., LEU H. G. Operating characteristics and kinetic studies of surfactant wastewater treatment by Fenton oxidation. Wat. Res. 1999; (33):1735

Lopez de Morais, J. and P.P Zamora,Use of advanced oxidation processes to improve the biodegradability of mature landfill leachates, J. Hazardous Materials, 2005 ;(B123): 181-6

Lou, J.C., and Lee, S.S., Chemical oxidation of BTX using Fenton's reagent. *Hazardous Waste and Hazardous Materials*, 1995;12(2): 185-193.

P. Bautista, A.F.Mohedano, M.A. Gilarranz, J.A. Casas, J.J. Rodriguez, Application of Fenton oxidation to cosmetic wastewaters treatment, Journal of Hazardous Materials. 2007;(143): 128–134.

Siegrist, R. L., K. S. Lowe, D. R. Smuin, O. R. West, J. S. Gunderson, N. E. Korte, D. A. Pickering, and T. C. Houck. Permeation Dispersal of Reactive Fluids for In Situ Remediation: Field Studies. ORNL/TM-13596. Oak Ridge, Tenn.: Oak Ridge National Laboratory. 1998c

Siegrist, R. L., K. S. Lowe, L. C. Murdoch, T. L. Case, and D. A. Pickering. "In Situ Oxidation by Fracture-Emplaced Reactive Solids," Journal of Environmental Engineering 1999;125(5):429–40.

Siegrist, R. L., K. S. Lowe, L. D. Murdoch, W. W. Slack, and T. C. Houck.X-231A Demonstration of In Situ Remediation of DNAPL Compounds in Low-Permeability Media by Soil Fracturing with Thermally Enhanced Mass Recovery or Reactive Barrier Destruction. ORNL/TM-13534. Oak Ridge, Tenn.: Oak Ridge National Laboratory.1998b

US Peroxide. Reference Library Peroxide Applications.URL:http://www.h2o2.com/intro/overview.html. Last updated: 99/10/06. Last visited: April, 2001.

Watts, R.J., and Dilly, S.E. Evaluation of iron catalysts for the Fenton-like remediation of diesel contaminated soils. Journal of Hazardous Materials. 1996;(51): 209-24.

Zhang, H., H.J. Choi and C.P. Huang. Optimization of Fenton process for the treatment of landfill leachate, J. Harzardous Materiels, 2005;(B125): 166-74

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Similarity Identification and Measurement between Ontologies

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Abstract: The retrieval of relevant and precise information from web has always been remained a serious problem. To address this problem, the idea of ontologies-based web, so-called semantic web, was proposed in 2001. But the problem is not completely solved due to the semantic heterogeneity suffered by ontologies. In this paper we propose a semi-automatic technique to measure the explicit semantic heterogeneity. The proposed technique identifies all candidate pairs of similar concepts without omitting any similar pair. The proposed criteria for similarity measurement are based on theme semantic web. The proposed technique can be used in different types of operations on ontologies such as merging, mapping and aligning. By analyzing its results a reasonable improvement in terms of completeness, correctness and overall quality of the results has been found. . [Journal of American Science 2010; 6(4):67-85]. (ISSN: 1545-1003).

Keywords: Semantic Web, Heterogeneity, Ontology Matching, Similarity Identification

1. Introduction

The World Wide Web (or the Web) is a global source of information, which includes information about almost every topic that a person can think. But it is difficult to retrieve relevant, specific and precise information due to semantic heterogeneity and the lack of machine understandability of contents. It has been estimated that only 37 percent to 52 percent relevant results are retrieved and other retrieved results are irrelevant (Lewandowski, 2008). The idea of semantic web was envisioned by Lee (Lee et al., 2001), which provides a promising solution to overcome the retrieval performance problem of the web. According to the theme of the semantic web, the web-contents need to be structured, formalized, stored and retrieved through ontologies.

When multiple ontologies are simultaneously used in the integrating operations such as merging, mapping and aligning then they may suffer from different types of heterogeneities such as semantic heterogeneity and non-semantic or syntactic heterogeneity (Shvaiko & Euzenat, 2008; Hauswirth & Maynard, 2007). The syntactic heterogeneity occurs due to the use of different languages. The semantic heterogeneity includes terminological, conceptual and contextual heterogeneities. The terminological heterogeneity arises when different terms are used to represent the same concept or the same term is used to represent different concepts. The conceptual heterogeneity between two concepts may occur due to their different level of granularities i.e., when a concept is sub-concept or super-concept of the other, or both are overlapped. Similarly, two concepts are explicit-semantically heterogeneous if they are terminologically and taxonomically similar but they have different roles or functionalities in their respective ontologies.

To handle the problem of ontological semantic heterogeneity, it is required to identify the similarity between ontologies. For this purpose different techniques have been proposed and reported in the literature (Shvaiko & Euzenat, 2009; Maedche & Staab, 2002; Hariri et al., 2006; Aleksovski et al., 2006; Trojahn et al., 2008; Jeong et al., 2008;Noy & Musen, 2001; Melnik et al., 2002). However, some issues are still unsolved. Explicit semantic similarity needs to be measured in order to carry the vision of semantic web (González, 2005; Uschold, 2003; Uschold, 2002). The measurement of degree of similarity (*DoS*) based on Edit-distance formula, is unreliable because it measures the *DoS* based on the criteria of finding terms-similarity rather than finding similarity between concepts represented by the terms. The criteria as reported in (Shvaiko & Euzenat, 2005; Erhard & Philip, 2001; Lambrix &Tan, 2006), used for the identifying taxonomic similarity between concepts of two ontologies declare certain pairs of similar concepts as dissimilar due to the biasness of these criteria towards those concepts whose siblings-concepts, sub-concepts or direct super-concepts are not similar. Most of the existing similarity measurement techniques only compute the *DoS* between concepts of two ontologies (Buccella et al., 2005; Giunchiglia et al., 2007), which is inadequate to determine that which concept is more generic or more specific than the other, and this issue is considered as an open research issue (Janowicz et al., 2008). Similarly, some existing techniques compute only the Semantic Relation (*SR*) between two

concepts (Giunchiglia et al., 2007). Although, *SR* shows that one concept is more generic, or more specific than the other concept, yet it does not give the level of generality. Furthermore, the measurement of semantic similarity is a complex and is inefficient in execution-wise (Janowicz et al., 2008).

The above mentioned shortcomings in the existing similarity measurement techniques motivate us, to propose an integrated technique based on innovative vision of semantic web to achieve the following objectives: (i) identifying all pair of similar concepts without omitting any candidate pair of similar concepts. (ii) Identifying and measuring the explicit semantic similarity between intellectual concepts of ontologies.

The remainder of the paper is organized as follow. In Section 2, the background and related work is presented. The proposed technique is given in Section 3, and it is validated via case studies in Section 4. Results are analyzed and discussed in Section 5 and finally the paper is concluded in Section 6.

2. Background and Related Work

For aligning ontologies, several techniques have been proposed (Duchateau et al., 2007; Alasoud et al., 2008; Sherman & Price, 2001; Shvaiko & Euzenat, 2005; Erhard & Philip, 2001; Lambrix &Tan, 2006). On the basis of similarity-measuring criteria, these techniques are categorized into schema-based and instance-based techniques. In schema-based techniques, similarity between concepts is measured at structure-level while ignoring their actual data, whereas in instance-level techniques, similarity is measured by taking actual data into consideration. In structural aligning, the taxonomic characteristics of concepts are mostly considered. The two concepts are rendered taxonomically similar (Shvaiko & Euzenat, 2005; Erhard & Philip, 2001; Lambrix &Tan, 2006) if (i) their direct super-concepts are similar; (ii) their sibling-concepts are similar; (iii) their direct sub-concepts are similar; (iv) their descendant-concepts are similar; (v) their leaf-concepts are similar and vi) concepts, in the paths from the root to those concepts, are similar. Irrespective of the structural aligning technique used, it has been observed that certain pairs of similar concepts are categorized dissimilar because of bias of above mentioned criteria towards those concepts whose siblings-concepts, sub-concepts or direct super-concepts are not similar.

In (Aleksovski et al., 2006), the background knowledge of domain has been used via ontology to determine similarity between concepts of two ontologies, especially for those concepts which are not lexically and structurally similar. It has been evaluated by matching a medical ontology to another while using comprehensive medical domain ontology as background knowledge. This technique is well suited for those ontologies having very poor taxonomic and non-taxonomic relations between concepts. There are some other approaches for measuring semantic similarities between concepts of XML schemas, database schemas and some graph-like structures (Giunchiglia et al., 2007, Janowicz et al., 2008; Jeong et al., 2008; Noy & Musen, 2001; Melnik et al., 2002; Duchateau et al., 2007). In these schemas, the explicit meanings of concepts are determined either from their respective attributes or from their hierarchical positions. The meanings of concepts in terms of their interactions with other concepts are not explicitly defined in these schemas. Therefore these approaches seem to be inappropriate for measuring the similarities between concepts of ontologies schemas.

Ontology matching technique, proposed in (Alasoud et al., 2008) has three phases. It uses Levenshtein Distance (Cohen et al., 2003) and WordNet (Pedersen et al., 2004) techniques in first phase. A matrix with binary values is the output of first phase. For a_i there may be multiple corresponding b_js that are similar and only one among them is short listed on the bases of high score computed in the third phase from the neighbors of those concepts search in the second phase. This algorithm does not properly differentiate between concepts and its data properties and object properties. Data types and constraints are ignored while measuring similarity between data properties. Due to use of Levenshtein Distance formula of degree of similarity, the completeness and the correctness of the result is comparative low. Secondly, the pairs of similar concepts are not accompanied by their semantic relations. Whole-part relationships are only considered whereas the role-based and taxonomic characteristics are overlooked.

In order to detect and retrieve relevant ontologies Alexander Maedche, and Steffen Staab (Maedche & Staab, 2002) proposed a set of similarity measures for ontologies. The lexical and conceptual aspects of concepts of ontologies are considered. In lexical level measures, the terms used to name concepts are compared and their similarity is computed using well known method known as edit distance (Cohen et al., 2003) and they proposed a lexical metric for similarity computing which is equal to $MAX\ (0,\ MIN\ (|Li|,\ |Lj|) - ed\ (Li,\ Lj)\ /\ MIN\ (|Li|,\ |Lj|))$, where Li and Lj are two lexical entities whose similarity is being computed. The metric value varies in between 0 and 1. The 0 means both are dissimilar whereas the 1 indicates the similarity exactness of terms. The *ed* is a function that returns an integer which is equal to number of insertions, deletions or

substitutions to transform one lexical term into other. At conceptual level, the similarity is computed from the similarities of their respective super-entities. Two entities are similar if their direct super-entities in their respective taxonomies are similar or all super-entities of first entity are similar to super-entities of second entity used in comparison.

In (Trojahn et al., 2008), composite ontology mapping technique has been proposed. Different existing matchers have been collectively used in this technique. The technique has been automated through agent-based scenarios. For lexical similarity measuring, they use the string-based measures and to examine the linguistic semantics of terms, the WordNet has been used. The structural similarity between two terms has been computed based on the similarities of their respective super and sub concepts. The overall degree of similarity has been computed from the lexical, linguistic and structure similarities of terms.

In (Buccella et al., 2005), syntactic and semantic matchers are used to compute similarity, and final decision is made by the user. The syntactic matcher uses string-based techniques, known as edit distance and n-gram to measure the degree of similarity between two input terms. For semantic comparison, a thesaurus is searched for synonyms of input terms and then comparison is made using synonyms. During semantic matching, the depth of concepts from their common super-concepts in their respective taxonomies, are also considered. The overall degree of similarity is computed from the results of syntactic matcher and semantic matcher.

In HCONE (Kotis & Vouros, 2004), the ontology is defined as a set of terms used to represent concepts, their relationships and data-properties alongwith the axioms for interpretation of terms. Using WordNet and semantic index method, the highly ranked sense of each term is located and identified. For each term, all generic and specific terms are also retrieved from wordNet and then semantic relation between two terms, based on this information is identified. Finally, the merging decision based on the semantic relation, is made.

There are some others ontology merging, mapping and alignment tools (McGuinness et al., 2000; Maedche & Staab, 2002; Bouquet et al., 2003; Hariri et al., 2006; Lambrix &Tan, 2006). Each of them uses almost the same matching techniques to measure the similarity between concepts of ontologies. These toots use string-based techniques such as edit distance and n-gram to measure the degree of similarity between terms used for representing concepts. Some of them use WordNet to get linguistic information such as synonyms and hyponyms while measuring similarity and then the structural information of terms are further used to compute the overall degree of similarity.

Most of the existing works as summarized in Table 1 are about the measurement of similarity between two concepts based on their names, linguistic semantics, and the similarities of their taxonomic characteristics such as super-concepts, sub-concepts and sibling-concepts. However, no attention has been given on the explicit semantics based similarity measurement between concepts of ontologies. Secondly, the existing techniques compute only the *DoS* between concepts of two ontologies (Buccella et al., 2005; Giunchiglia et al., 2007). The value of *DoS* remains between 0 and 1 which is inadequate to determine as to which concept is more generic or more specific than the other one. It has been considered as open research issue (Janowicz et al., 2008). Similarly, some existing techniques compute only the Semantic Relation (*SR*) between two concepts (Giunchiglia et al., 2007). Although, *SR* shows that one concept is more generic, or more specific than the other concept, yet it does not give the level of generality. Therefore, each pair of similar concepts should be accompanied by their both *DoS* and *SR* in order to take a better decision while performing the aligning, merging and mapping operations of ontologies.

The measurement of degree of similarity (*DoS*) based on Edit-distance formula may produce incorrect results because the *DoS* is measured based on terms rather than concepts represented by those terms. That is, some pairs of similar concepts are declared dissimilar because of the heterogeneous terms used for the names those concepts. Similarly, some pairs of dissimilar concepts are declared similar because of the similarity of terms used for those concepts. Some approaches consider the synonyms provided by the WordNet while measurement of similarity. Their main considerations are the terms or the synonyms of terms rather than concepts represented by those terms and secondly, most of the tools consider the taxonomic characteristics of concept i.e., their relations with parents and children. The taxonomic similarity measurement criteria (Shvaiko & Euzenat, 2005; Erhard & Philip, 2001; Lambrix &Tan, 2006), as discussed before, declare certain pairs of similar concepts as dissimilar because of the biasness of these criteria towards those concepts whose siblings-concepts, sub-concepts or direct super-concepts are not similar.

3. Proposed Similarity Identification and Measurement Technique

First we give and list the basis of our proposed technique:

Table 1. A comparison of some techniques for similarity measurement between ontologies

Techniques	**Name-based similarity**	**Linguistic-based similarity**	**Taxonomic-based similarity**	**NonTax. based similarity**	**DOS**	**SR**
TAOM (Buccella et al., 2005)	Edit-distance , n-gram	Thesaurus	Parents	N	Y	N
MSBO (Maedche & Staab, 2002)	Edit-distance	N	Parents	N	Y	N
SEMC (Bouquet et al., 2003)	Edit-distance	WordNet	Parents Children	N	Y	N
HCONE (Kotis & Vouros, 2004)	String-based Techniques.	N	Parents, Children	N	N	Y
Chimaera (McGuinness et al., 2000)	Edit-distance	N	Parents, Children	N	Y	N
SSMO (Hariri et al., 2006)	String-based Techniques.	N	Parents, Children	N	N	Y
SAMBO (Lambrix &Tan, 2006)	Edit-distance	N	Parents, Children	N	N	Y
EOMT (Alasoud et al., 2008)	Edit-distance	WordNet	Parents	N	Y	N
CACOM(Trojahn et al., 2008)	Edit-distance	WordNet	Parents , Children	N	Y	N

i) Concepts are compared instead of terms used to represent concepts.
ii) Domain-specific semantics (i.e., explicit semantics of concepts) are being used in similarity measurement process, rather than their linguistic semantics.
iii) The super-concepts based contextual similarity measurement is computed and relaxing the similarities between their respective sub-concepts (or sibling concepts).
iv) The layered matching strategy is adopted to make the measurement process more efficient.

The proposed technique works in three phases as shown in Figure 1. The three phases are: i) IPS - Identifying Primary Similarity, ii) ICS - Identifying Contextual Similarity, iii) IRS - Identifying Role-based Similarity. There are some preprocessing tasks before the technique starts its actual working. These tasks are: (a) acquisition of concepts, (b) acquisition of super-concepts of primarily similar concepts, and (c) acquisition of roles of contextually similar concepts, are performed by the three phases, respectively. The structure diagram of proposed technique is shown in Figure 1. In the figure, *M* and *N* are two RDF models of the two input ontologies *A* and *B*, respectively. The *CA*, *PA* and *RA* represent concepts-acquisition, parent-acquisition and roles-acquisition processes, respectively. The *IPS*, *ICS* and *IRS* represent processes of identifying primary similarity, identifying contextual similarity and identifying role-based similarity, respectively. The label *1* represents two separate lists of concepts acquired from the models *M* and *N*, respectively. The label *2* represents a list of pairs of primarily similar concepts. The label *3* represents two separate lists of parents of primarily similar concepts, and the label *4* represents a list pairs of concepts possessing contextual similarity. Label *5* represents two separate lists of roles of contextually similar concepts, and the label *6* represents a list of pairs of concepts possessing role-based similarity. In the figure, O_1, O_2 and O_3 are the three (3) vectors containing pairs of primarily, contextually and explicit semantically similar concepts, respectively.

3.1 Definitions

(a) In an ontology we define a *concept* as a class of objects sharing common elementary, taxonomic and non-taxonomic characteristics. We define a concept as a 5-tuple i.e. <*T, P, C, S, R*>; where *T, P, C, S* and *R* are sets of terms, parents, children, siblings and roles respectively, that a concept may have. These sets are formally defined as:

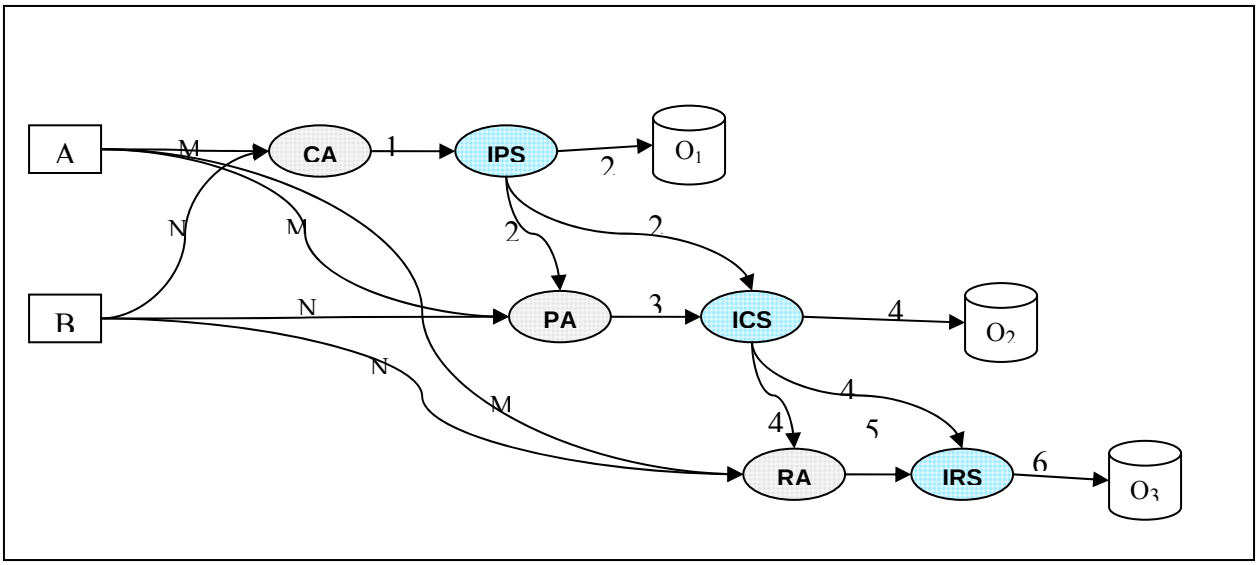

Figure 1: Structure diagram of the proposed technique

$T = \{term_i \mid 1 \le i \le N_1\}$ (1)
$P = \{parent_i \mid 1 \le i \le N_2\}$ (2)
$C = \{child_i \mid 1 \le i \le N_3\}$ (3)
$S = \{sibling_i \mid 1 \le i \le N_4\}$ (4)
$R = \{role_i \mid 1 \le i \le N_5\}$ (5)

A concept has linguistic and explicit semantics. The synonyms of a concept represent its linguistic or implicit semantics whereas the explicit semantics of a concept are defined in terms of its roles (or responsibilities), which it plays in a certain domain. In other words, the explicit semantic of a concept is domain dependent. If a concept *C* plays the roles r_1, $r_2 \ldots r_n$ in a domain *D*, then the explicit semantic of the concept *C* is formally defined as follows:

$${}_{ES}C^D = \{r_1, r_2 \ldots r_n\} \quad (6)$$

(b) We refer to the 1st level similarity as the *Primary Similarity*. Two concepts are primarily similar if and only if either their names belong to *T* (see Equation (1)) of the concept C_i where $1 \le i \le N$; or the first-name belongs to *T* and second-name belongs to *P* (see Equation (2)) or versa where *T* and *P* both belong to the same concept C_i. The primary similarity is denoted as $\approx^1$, and can formally be written as follows:

$$a \approx^1 b \text{ Iff } ((a \wedge b) \in T(c_i)) \vee ((a \in T(c_i) \wedge b \in P(c_i)) \vee (b \in T(c_i \wedge a \in P(c_i))) \quad (7)$$

In Equation (7), $T(c_i)$ and $P(c_i)$ are the two sets of terms and parents of the concept c_i of the target domain.

(c) The 2nd level similarity is referred it to as *contextual similarity*. Two concepts are contextually similar if and only if they possess the primary similarity and have one or more common concepts in their respective list of super-concepts. It can formally be written as follows:

$$a \approx^2 b \text{ Iff ((condition given in Eq. (7) is true)} \wedge ((P_a \cap P_b) \neq \phi)) \quad (8)$$

In Equation (8), P_a and P_b are the two respective sets of parents of the concepts *a* and *b* as we have already defined in Equation (2).

(d) We refer to the 3rd level of similarity (or explicit-semantics based similarity) as r*ole-based similarity*, and it is especially used for identifying similarity between two intellectual concepts. Two concepts possess the role-based similarity if and only if they possess contextual similarity and they have one or more common roles in their respective list of roles.

$$a \approx^3 b \text{ Iff ((condition given in Eq. (8) is true)} \wedge (R_a \cap R_b \neq \phi)) \quad (9)$$

In Equation (9), R_a and R_b are the respective sets of roles (as we have defined in Equation (5)) of concepts *a* and *b*.

(e) Since there may be multiple roles of same concept, therefore, while identifying the similarity, we consider common roles of two concepts *a* and *b*, then the *role-based DoS* of the concept *a* with respect to the concept *b* is computed by dividing total number of common roles by total number of roles in their union. Assume *M* is a set of roles of a concept *a* and *N* is a set of roles of another concept *b*. Bother sets belong to two different ontologies *A* and *B* respectively, then *DoS* between the concepts *a* and *b* is computed by using the following empirical formula.

$$DoS = \frac{|M \cap N|}{|M \cup N|} \quad (10)$$

Role-Based SR between pairs of similar concepts *a* and *b* may is denoted as follows.

(a, b) = *SR* (11)

The criteria for computing *SR* are listed as follows:

i. SR = '=' ; a is equivalent to b;
   a = b iff ((| M ∩ N | = | M|) & (|M| - |N| = 0))
ii. SR= '≥ '; a is more generic than b;
   a ≥ b iff (| M ∩ N | = | N|)
iii. SR='≤'; a is less generic than b;
   a ≤ b iff (| M ∩ N | = | M|)
iv. SR= 'x'; otherwise semantic relation is undefined; (x), take manual decision

There may be no b_j that is exactly similar to a_i, there may be multiple b_js that are more specific than a_i, or multiple b_js that are more generic than b_js. In these cases, we have adopted two strategies, i.e., up-ward and down-ward strategies. In the up-word strategy, we choose a pair of concepts (a_i, b_j) with *SR* such that b_j is least granular in all b_js. Similarly, in the down-ward strategy we choose a pair with b_j having the maximum granularity.

(f) Granularity of a concept (*Gc*) is proportional to its level of generality. The generality of a concept may vary from the most generic to least generic or vice versa. Let *g* be the generality of the concept c and *k* is the constant of proportionality, then we define *Granularity-Based Degree of Similarity Gc* as follows:

$Gc = k * g$ (12)

In Equation 12, *g* can vary between *1* and *n*, where *n* is an integer value. If *g* is equal to 1, then the concept is considered to be the most generic concept, and if *g* is equal to *n*, then the concept is considered to be the least generic. We compute the *DoS*, between two concepts particularly the non-intellectual concepts from their granularities. Let *Ga* and *Gb* be the granularities of two primarily similar concepts *a* and *b* respectively, then their *DoS* is computed by using the empirical formula given in Equation (13).

$$DoS = \frac{|Ga - Gb|}{Max(Ga, Gb)} \quad (13)$$

If *Ga* and *Gb* are the granularities of primarily two similar concepts *a* and *b* respectively, then *SR* between them can be computed by Equation (14).

(a, b) = *SR* (14)

The criteria for computing SR are given below:

(i) SR = '=' ; a is equivalent to b;
   a = b iff (Ga = Gb)
(ii) SR= '≥ '; a is more generic than b;
   a ≥ b iff (Ga < Gb)
(iii) SR= '≤'; a is less generic than b;
   a ≤ b iff (Ga > Gb)

3.2 IPS - Identifying Primary Similarity Phase

The primary similarity (defined earlier) is not the same as terminological similarity because we mainly focus on logical meaning of concepts instead of terms used to represent the concepts. The identifying process of the primary similarity is given in algorithmic form in Figure 2. The terms used to represent concepts in both source ontologies *A* and *B*, as obtained in the vectors CS_A and CS_B (defined in Equation (15)-(16)) are the input of this phase. The vector Sim_{PS} (defined in Equation (17)) containing pairs of primarily similar concepts is the output of this phase.

$CS_A = \{a_i \mid \forall\ a_i \in A; 1 \le i \le M\}$ (15)

$CS_B = \{b_j \mid \forall\ b_j \in B; 1 \le j \le N\}$ (16)

$Sim_{PS} = \{(a, b, DoS, SR) \mid \forall\ ((a \in CS_A \wedge b \in CS_B) \wedge (a \approx^1 b)\ )$ (17)

In Equation (17), the symbol $\approx^1$ represent the primary or the first level similarity (defined in Equation (7)) whereas *DoS* and *SR* (defined in Equation (10) – (11) and Equation (13) – (14)) based on the roles and granularities of concepts, respectively.

3.3 ICS - Identifying Contextual Similarity Phase

Since the conceptual similarity between two concepts (defined earlier), is based on the similarity of their respective parent concepts, therefore, we need the parent-concepts of all those concepts which are declared primarily similar concepts in the previous phase. Hence, for all concepts in the resultant vector, Sim_{PS}, obtained from Phase-1, their respective parent concepts from the ontologies *A* and *B* are separately extracted in the two vectors, i.e., C^PS_A and C^PS_B, which are formally defined as follows:

```
Input:  CS_A and CS_B  Vectors
(DV-Domain Vocabulary, an implicit input)
Output: Sim_PS -
          a vector containing pairs of primarily
        similar concepts
Begin
For each a in CS_A
For each b in CS_B
aId= DV.getId(a);Ga = DV. getGranularity(a)
bId= DV.getId(b);Gb = DV. getGranularity(b)
S1 = aId.size();    S2 = bId.size
If (S1 = S2) then
If aId.equal(bId) then Temp.SR = '='
Else       Temp.SR = 'x'
Else if (S1 < S2) Then  T = bId.substr(1,s1)
If T.equal(aId) Then Temp.SR = ' ⊇ '
Else Temp.SR = 'x'
Else if (S1 > S2) Then T = aId.substr(1,s2)
If T.equal(bId) Then Temp.SR = ' ⊆ '
Else       Temp.SR = 'x'    End if
Temp.DOS = absolute (Ga – Gb) / Maximum
(Ga, Gb); Sim_PS.add(temp)
   Next
  Next
End
```

Figure 2: A slice of pseudo code for identifying primary similarity

$$C^{P}S_A = \{(a_i, (p_i, p_{i+1}, \ldots, p_k)) \mid \forall\ a_i, p_i \in A \wedge p_i \text{ isParentOf } (a_i)\} \quad (18)$$

$$C^{P}S_B = \{(b_j, (p_j, p_{j+1}, \ldots, p_k)) \mid \forall\ b_j, p_j \in B \wedge p_j \text{ isParentOf } (b_j)\} \quad (19)$$

This phase takes $C^{P}S_A$, $C^{P}S_B$ (see Equation (18) – (19)) vectors, populated in the acquisition process and Sim_{PS} (see Equation (17)) populated in the previous phase, as the input and returns a set Sim_{CS}, (defined in Equation (20)), containing pairs of taxonomically similar concepts as the output.

$$Sim_{CS} = \{(a, b, DoS, SR) \mid \forall\ ((a, b) \in Sim_{PS} \wedge (a \approx^2 b))\} \quad (20)$$

```
Algorithm: Identifying contextual similarity
Input :(i) C^pS_A and C^pS_B vectors
       (ii) Sim_PS vector
Output: Sim_CS (as defined in Eq.20); a vector containing
pairs of taxonomically similar concepts
Begin
       For each p in Sim_PS
       parentC_a = C^pS_A.getParents(p.C_a)
       parentC_b = C^pS_B.getParents(p.C_b)
       same = isSameParent(parentC_a, parentC_b)
              If same Then Sim_CS.add(p)
       Next
Function isSameParent(Vector V_a, Vector V_b): Boolean
       {match=False
        For each p_a in V_a
         For each p_b in V_b
              If p_a = p_b Then
                     {match= True;
                     Break ;}
    Next
        Return match
       }
End
```

Figure 3: A slice of pseudo code for identifying contextual similarity

In Equation (20), the symbol $\approx^2$ represents contextual of the 2nd level similarity as defined in Equation (8). The contextual similarity is based on taxonomic positions of a_i and b_j. To measure this similarity, it is necessary to measure the similarity between their respective parents. A segment of algorithm of the identifying process of contextual similarity is given in Figure 3.

3.4 IRS - Identifying Role-based Similarity Phase

In this phase, the role based similarity, as defined in Equation (9), is measured between two contextually similar concepts. Figure 4 shows a segment of algorithm of the identifying process of the role-based similarity. Similarly, to measure *SR* we acquire the roles of each concept. The roles of each concept of *A* and *B* ontologies are separately acquired in two vectors i.e. $C^{R}S_A$ and $C^{R}S_B$, formally defined as:

$$C^{R}S_A = \{(a_i, (r_i, r_{i+1}, \ldots, r_n)) \mid \forall\ a_i, r_i \in A \wedge r_i \text{ isRoleOf}(a_i)\} \quad (21)$$

$$C^{R}S_B = \{(b_j, (r_j, r_{j+1}, \ldots, r_n)) \mid \forall\ b_j, r_j \in B \wedge r_j \text{ isRoleOf}(b_j)\} \quad (22)$$

$C^{R}S_A$, and $C^{R}S_B$ (see Equation (21) – (22)) are populated in the role-acquisition process and Sim_{CS}

(see Equation (20)) is populated in the previous phase, both are the input of the process and Sim_{RS} - a set containing pairs of similar concepts based on their roles (defined in Equation (23)), is the output of this phase.

$$Sim_{RS} = \{(a, b, DoS, SR) \mid \forall\ ((a, b) \in Sim_{CS}) \wedge (a \approx^{3} b)\} \quad (23)$$

```
Algorithm: Measuring of role-based similarity
Input: (i) C^RS_A, C^RS_B Vectors
       (ii) Sim_CS Vector
Output: Sim_RS - a vector containing pairs of role-based similar concepts.
Begin
For each p in Sim_cs
rC_a= C^RS_A.getRoles(p.C_a);rC_b= C^RS_B.getRoles(p.C_b)
T = countSame(rC_a, rC_b)
DoS = T / ((rCa.size() + rCb.size()) – T)
SR = computeSR (T, rCa.size(), rCb.size())
temp.Ca = p.Ca;  temp.Cb = p.Cb
temp.SR = SR; temp.DoS = DoS; Sim_RS.add(temp)
Next
End Sub
Function countSame(Vector V_a, Vector V_b): Return Boolean
{same = 0
 For each r_a in V_a
 For each r_b in V_b
 If r_a = r_b Then {same = same +1;  Next
 Next;  Return same}
End Function
Function computeSR(Integer T, integer n, integer m): Return Char {
If (t = n) && (n – m = 0) Then Return '='
Else If (t = m) && (n – m > 0) Then Return '≥'
Else If (t = n) && (n – m < 0) Then Return '≤'
                Else Return 'X' }
End Main
```

Figure 4: A slice of pseudo code for identifying role-based similarity

In Equation (23), the symbol $\approx^{3}$ represents the role-based or the 3rd level similarity as defined in Equation (9). In order to identify the 3rd level similarity of contextual similar concepts short listed in the previous phase, we need to acquire their roles from their respective ontologies.

In Table 2, we give a comparison between the existing techniques and proposed technique; *SM*, *DoS* and *SR* represent Similarity Measurement, Degree of Similarity and Semantic Relation, respectively. The explicit semantic similarity measurement is the key point of the proposed technique. According to theme of Semantic Web, the short comings of the current web can be overcome by formalizing explicit semantics of web-contents using ontologies. However, ontologies may themselves suffer from the explicit semantic heterogeneity problem when their lexically and contextually similar concepts have different or overlapped explicit semantics. In order to resolve such type of heterogeneity, the similarity measurement based on explicit-semantics is essential.

Table 2: Existing techniques vs. proposed technique

	Parameters	**Existing Techniques**	**Proposed Technique**
i	**Explicit-semantics based SM**	Not supported	Supported
ii	**Lexical SM**	- Terms are compared;- *DoS* is computed through string-based techniques(edit-distance, prefix, suffix and n-gram)	- Concepts are compared. - *DoS* is computed from granularities and explicit-semantics of concepts

iii	**Linguistic-semantics based SM**	Supported	Domain specific semantics of concepts
iv	**Contextual SM**	Both the optional and mandatory characteristics are considered	Only mandatory characteristic with different criterion is considered
v	**Output of overall SM**	Pairs of similar concepts with either *DoS* or *SR*	Pairs of similar concepts with both *DoS* and *SR*
vi	**Matching Strategy**	Individual Matching	Integrated and layered matching

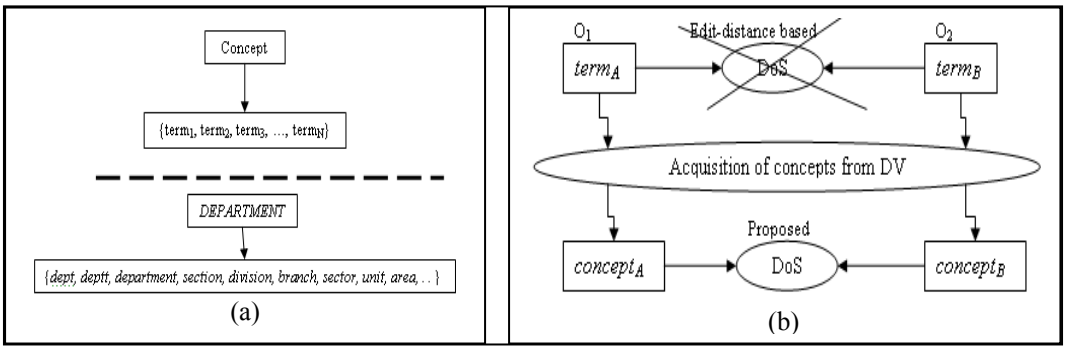

Figure 5. The *DoS* through edit-distance based formula and through proposed formula

A concept is represented by a set of terms, including its synonyms such as shown in Figure 5 (a). The existing techniques, as summarized in Table 1, use edit-distance based formula to compute *DoS* between two concepts.

In some cases, the edit-distance based *DoS* can be incorrect such as the pair (O1:dept, O2:department) shown in Figure 5(a), is declared dissimilar when edit-distance based DoS measurement formula is used Similarly, some pairs of dissimilar concepts are declared as similar pairs such as (*Software Design*, *Software Designer*) and (*System Analyst*, *System Analysis*) because the edit-distance based *DoS* between concepts of these pairs are 0.86 and, 0.85 respectively. In proposed technique, the measurement of *DoS* is performed on concepts themselves represented by the terms given in ontologies. The measurement process of *DoS* is accomplished through domain vocabulary (*DV*), as shown in Figure 5 (b).

In linguistic-semantic based matching, the concepts and their respective synonyms are examined. That is, if one concept is a synonym of other concept or vice versa, then both concepts are considered as equivalent concepts. The current techniques use WordNet to fetch the synonyms of concepts. However a domain may have some abbreviated, acronyms or composite named concepts which are not found in WordNet. In proposed technique we use domain specific vocabulary in place of WordNet to get `better results of linguistic semantic matching.

The context of a concept is usually known by its Super, Sub and Sibling (*3S*) concepts in its respective ontology. Usually, a concept may or may not have sub or sibling concepts but it always has some parents. This means that while identifying contextual similarity between two concepts, the similarity between their respective super concepts should be considered only. We have empirically observed that while measuring contextual similarity between two concepts, if the similarities of *3S* concepts are taken into consideration then some pairs of similar concepts may be declared dissimilar. This is because of dissimilarity of their respective sub concepts or sibling concepts. Furthermore, while measuring contextual similarity between two concepts, the similarity between their respective immediate super-concepts is not mandatory. In proposed technique, we have taken into consideration the similarity of their super-concepts while relaxing the similarities of sub and sibling concepts.

The proposed technique compute both the *DoS* and *SR* between concepts, As mentioned earlier, the value of *DoS* between two concepts remains in the range of 0 and 1which is inadequate to determine which concept is more generic or more specific than the other concept? Similarly, the semantic relations such as $\supseteq$ and $\subseteq$ between two similar concepts

show that one concept is more generic or more specific to the other concept. However, it does not reflect the *DoS* between the two concepts. Therefore, each pair of similar concepts should be accompanied with both *DoS* and *SR* in order to take better decision while aligning, merging and mapping ontologies.

We have empirically observed that within a certain domain, the lexically dissimilar concepts are always contextually dissimilar. Similarly the contextually dissimilar concepts are always explicit semantically dissimilar. That is, there is no need to measure the contextual similarity between lexically dissimilar concepts. And, there is no need to measure the explicit-semantics similarity between contextually dissimilar concepts. Secondly, the direct measurement of contextual similarity without measuring the lexical similarity may produce inaccurate result. This suggests that, if the similarity measurement is performed in some integrated and layered fashion to enable the measurement process more efficient. Most of the existing techniques follow the individual matching. The individual matching strategy reduces the efficiency of overall similarity computing process because of the maximum input for all matchers. For example, there are N numbers of candidate pairs whose similarities are to be measured. In individual matching strategy each matcher gets same and the maximum input i.e. N, whereas, in integrated and layered strategy the input of second and third matchers are N_1 (where $N_1 < N$) and N_2 (where $N_2 < N_1$) number of pairs respectively. That is, the input of 2nd Matcher of proposed technique is less than the input to the second matcher of existing techniques and same is the case with third matchers of proposed and existing techniques. Furthermore, the 1st level matcher used in proposed technique, identifies similarity between input terms, based on the actual concepts represented by those terms whereas the lexical matcher, used in existing techniques, measures similarity through string-based approaches.

4. Case Studies

We evaluate the proposed technique through case studies targeting its objectives that are given earlier. The Education and the Business domains have been taken as sample domains for testing the working of the proposed technique. We take *Software Development Organization (SDO)* from Business domain and *University* from Education domain. From these two domains, different pairs of ontologies are chosen as the input ontologies to the proposed technique. We have implemented the proposed technique in Java language by using an integrated development environment - NetBeans IDE 6.1 (NetBeans, 2009). In order to load and parse ontologies, OWL API (Bechhofer et al., 2003; Horridge et al., 2007) has been used.

The ontologies of SDO, which we have selected, they mainly concentrate on human resources and their roles, i.e., the intellectual concepts and their interactions with non-intellectual concepts. A software organization has different categories of the intellectual concepts such as technical and non-technical human resources. The category of technical human resources is further divided in different teams such as Analysis-team, Design-team, Implementation-team, SQA-team, Supplemental-team and Deployment-team. There are different concepts in each team such as Analyst, Use-Case Engineer, Software Engineer, Programmer, Coder, SQA-Engineer, Technical-Writer, Librarian, and Project Manager. They work on different projects, and each project has many different modules. These intellectual concepts are commonly used in different software development organizations with same, overlapped or different roles. In order to manually trace the proposed technique, we have taken a subset of commonly used roles by the intellectual concepts of these ontologies, which are listed in Figure 6. The list of sample concepts of the first input ontology *dataSoft.owl* is shown in Table 3. For the sake of simplicity, we have chosen only those concepts which are contextually similar. The domain vocabulary includes the concepts of this ontology.

(r1)	Analyze Hardware Requirements	(r2)	Analyze Software Requirements
(r3)	Analyze Functional Requirements	(r4)	Analyze Non Functional Requirements
(r5)	Analyze Cost Benefit	(r6)	Design Database
(r7)	Design Algorithms	(r8)	Design Reports
(r9)	Design Input Screens	(r10)	Design Structure
(r11)	Design Graphics	(r12)	Design Web Pages
(r13)	Implement Database	(r14)	Implement Algorithm
(r15)	Implement Reports	(r16)	Implement GUI
(r17)	Implement Structure	(r18)	Write Requirements Specifications

(r19)	Write Design Documents	(r20)	Write Code Documents
(r21)	Test Functional Requirements	(r22)	Test Non Functional Requirements
(r23)	Test Procedures	(r24)	Tune Database
(r25)	Backup Database	(r26)	Cost Management
(r27)	Resource Management	(r28)	Define standard operating procedures
(r29)	Change Management	(r30)	Write User Manual
(r31)	Software configuration control	(r32)	Storing final released products
(r33)	Developing a test plan for the project	(r34)	Allocating database resources to projects
(r35)	Compiling source code/linking/building	(r36)	Defining user profiles
(r37)	Creating test baselines	(r38)	Ensuring Inter-group coordination
(r39)	Deploying applications in virtual machine	(r40)	Ensuring successful project closure
(r41)	Establishing SCCB and SCRB for projects	(r42)	Ensuring SQA activities
(r43)	Faxing, mailing, shipping	(r44)	Ensuring the security of project databases
(r45)	Handling and maintaining the store	(r46)	Identification of project based SCM tool(s)

Figure 6. A subset of roles in software development organization

4.1 List of Concepts of First Input Ontology

Table 3. A sample slice of intellectual concepts form A ontology

Id	Concept	Roles
(a1)	SoftwareEngineer	r7, r10, r13, r16
(a2)	SeniorSoftwareEngineer	r3, r4, r7, r10
(a3)	Programmer	r12, r13, r14,r15,r16,r17
(a4)	SeniorProgrammer	r6, r7, r8, r9, r10
(a5)	Designer	r11, r12
(a6)	Analyst	r1,r2, r3
(a7)	SeniorAnalyst	r3,r4, r5
(a8)	SQAEngineer	r21, r22, r23
(a9)	DBA	r6, r13, r24, r25
(a10)	TechnicalWriter	r18,r19, r20,r30
(a11)	ProjectManager	r26,r27
(a12)	ProcessManager	r28

4.2 List of Concepts of Second Input Ontology

The ontology *ridos.owl* is chosen as the second input ontology. This ontology is also considered while populating the domain vocabulary. A subset of its concepts is shown in Table 4 .

Table 4. A sample slice of intellectual concepts form B ontology

Id	Concept	Roles
(b1)	ProjectManager	r26,r27, r28
(b2)	SofConfigManager	r29
(b3)	SoftwareEngineer	r4, r7, r10, r13
(b4)	SQAEngineer	r21, r22
(b5)	Programmer	r6, r7, r8, r9,r10,r12-r17
(b6)	Designer	r11, r12
(b7)	Analyst	r1,r2, r3, r4
(b8)	Coder	r12, r13, r14, r15, r16, r17
(b9)	DBA	r13, r24, r25
(b10)	SoftwareArchitect	r8, r9, r11, r12
(b11)	TechnicalWriter	r18, r19, r20

Table 5. A slice of role-based similar concepts with a threshold-value

Pairs	Pair of Concepts	DoS	SR
(a1, b3)	(A:SoftwareEngineer, B:SoftwareEngineer)	0.60	X
(a2, b3)	(A:SenSoftwareEngineer, B:SoftwareEngineer)	0.60	X
(a3, b5)	(A:Programmer,B:Programmer)	0.55	<
(a3, b8)	(A:Programmer, B:Coder)	1.00	=
(a4, b5)	(A:SeniorProgrammer, B:Programmer)	0.45	<
(a5, b6)	(A:Designer, B:Designer)	1.00	=
(a5, b10)	(A:Designer, B:SoftwareArchitect)	0.50	<
(a6, b7)	(A:Analyst, B:Analyst)	0.75	<
(a8, b4)	(A:SQAEngineer, B:SQAEngineer)	0.66	>
(a9, b9)	(A:DBA, B:DBA)	0.75	>
(a10, b11)	(A:TechnicalWriter, B:TechnicalWriter)	0.75	>
(a11, b1)	(A:ProjectManager, B:ProjectManager)	0.66	<

In the second case study, we take *csuet.owl* and *lcwu˙owl* as ontology *A* ontology *B,* respectively. The semantic relation between a pair of concepts has been computed based on their respective granularities.

The sample concepts that are taken from the ontology *A* are*: (a_1) Project, (a_2) ITConsultant, (a_3) Director, (a_4) Manager, (a_5) UnderGradStudent, (a_6) Convener, (a_7) Course, (a_8) Professor, (a_9) Quiz, (a_{10}) Workshop, (a_{11}) NationalConference, (a_{12}) ResearchCentre, (a_{13}) PostGradStudent, (a_{14}) Person, (a_{15}) Deptt.* The sample concepts that are taken from the ontology *B* are taken: *(b_1) TermProject, (b_2) Consultant, (b_3) Director, (b_4) SupportManager, (b_5) ConvenerAdmission, (b_6) Student, (b_7) Professor, (b_8) PostGradCourse, (b_9) Workshop, (b_{10}) Conference, (b_{11}) ResearchCentre, (b_{12}) Department, (b_{13}) SoftwareEngineer, (b_{14}) Person, (b_{15}) Employee, (b_{16}) Faculty*. The sample pairs are *(a_1, b_{12}), (a_2, b_2), (a_3, b_3), (a_4, b_4), (a_5, b_6), (a_6, b_5), (a_7, b_7), (a_8, b_7), (a_9, b_8), (a_9, b_{10}), (a_{10}, b_9)* and *(a_{11}, b_{10})*, respectively.

4.3 Primary Similarity Identification and Measurement: As mentioned earlier, it is the first phase of proposed technique. Here, the pairs of concepts possessing primary similarity are identified.

Input: *A= (a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8, a_9, a_{10}, a_{11}, a_{12}); B = (b_1, b_2, b_3, b_4, b_5, b_6, b_7, b_8, b_9, b_{10}, b_{11}, b_{11}).*

The output: According to the algorithm for primary similarity identification, given in Figure 2 the following pairs are identified as primarily similar pairs:

Sim^{PS} = {(a_2, b_2, 0.80, '≤'), (a_3, b_3,1.00, '='), (a_4, b_4, 0.75,'≥'), (a_5, b_6,0.84, '≤'), (a_6, b_5,0.80,'≥'), (a_8, b_7, 1,'='), (a_9, b_8, 0.66,'≥'), (a_{10}, b_9,1, '='), (a_{11}, b_{10},0.86 '≤')}

4.4 Contextual Similarity Identification and Measurement: it is the second phase of proposed technique. Here, the pairs of concepts obtained in previous phase, possessing contextual similarity are identified.

Input: *Sim_{PS}* and super-concepts of *(a_2, a_3, a_4, a_5, a_6, a_8, a_9, a_{10}, a_{11})* and super-concepts of *(b_2, b_3, b_4, b_5, b_6, b_7, b_8, b_9 and b_{10})*.

Output: According to the algorithm for contextual similarity identification, given in Figure 3 the following pairs are identified as contextually similar pairs:

Sim_{CS} = {(a_2, b_2, 0.80, '≤'), (a_3, b_3, 1, '='), (a_4, b_4, 0.75, '≥'), (a_5, b_6, 0.84, '≤'), (a_8, b_7, 1, '='), (a_9, b_8, 0.66, '≥')}

4.5 Role-based Similarity Identification and Measurement: it is the third and the final phase of proposed technique. Here, the pairs of concepts obtained in previous phase, possessing role-based similarity are identified.

Input: *Sim_{CS}* and roles of concepts as short-listed in the previous phase i.e. roles *of concepts (a_2, a_3, a_4, a_5, a_6, a_8, a_9)* and *(b_2, b_3, b_4, b_6, b_7, b_8) respectively.*

Output: According to the algorithm for role-based similarity identification, given in Figure 4 the following pairs are identified as role-based similar pairs:

Sim_{RS} = {(a_3, b_3, 1, '='), (a_5, b_6, 0.84, '≤'), (a_8, b_7, 1, '='), (a_9, b_8, 0.66, '≥')}

5. Results: Analysis and Discussion

We have the following observations about the results of *IPS*, *ICS* and *IRS* phases as they have been computed in previous section. These observations are listed as follows:

i) If the 1st level similarity for a pair of concepts is *true*, then it may be *true* or *false* for the next levels of similarities.

ii) If the 1st level similarity for a pair of concepts is *false*, then its 2nd level and 3rd level of similarities are always *false*.

iii) The 3rd level similarity is *null* for a pair of concepts of the non-intellectual concepts possessing 2nd level of similarity.

(iv) There is a role-based similarity between pair of concepts (a_{13}, b_7), i.e., *A: PostGradStudent* and *B: Professor*, because both work-on the research-project, and also there is contextual-similarity between these concepts. Same is the case of the pair of concepts (a_8, b_{13}) i.e. *A:Professor* and *B:SoftwareEngineer* both are working on Project. These pairs are not primarily similar because the main motive behind finding the similarity between concepts is merging, aligning or mapping of two ontologies for the knowledge sharing, therefore, the merging, aligning or mapping of the *PostGradStudent* concept with the *Professor* concept, is not recommended. In the proposed technique, a pair of concepts having no primary similarity is simply discarded.

From these above mentioned observations, we conclude the correctness of the layer strategy adopted in our proposed technique. The primary similarity of concepts is the prerequisite of the contextual similarity, and it is prerequisite of the role-based similarity. However, it is not necessary that two primarily similar concepts are also the contextually similar or two contextually similar concepts are the role-based similar.

To realize the achievement of the different objectives, as listed before, we compare the results of proposed technique with the results from some existing techniques. The criteria for comparison include the (i) completeness; (ii) correctness and (iii) overall quality of results.

Completeness: The completeness of a similarity identifying technique is just like the precision measures used in information retrieval (Trojahn et al., 2008; Euzenat, 2007; Ehrig & Euzenat, 2005). It is the ratio of correct number of pairs found divided by the total number of pairs found. Let *totalPairsFound* be the total number of pairs found in which *CorrectPairsFound* number of pairs are correct, such as *totalPairsFound* >= *CorrectPairsFound*, then the completeness can be formally written as:

$$\text{Completeness} = \frac{Correct\_Pairs\_Found}{Total\_Pairs\_Found} \quad (24)$$

Correctness: The correctness of a similarity identifying technique is just like the recall measures used in information retrieval (Trojahn et al., 2008; Euzenat, 2007; Ehrig & Euzenat, 2005). The correctness is the ratio of correct number of pairs found, divided by the expected number of correct pairs. Let $Correct\_Pairs\_Expectea$ be the total number of correct pairs expected and $Correct\_Pairs\_Found$ number is of correct pairs found by a technique such as $Correct\_Pairs\_Expectea >= Correct\_Pairs\_Found$, then the correctness can be formally written as

$$\text{Correctness} = \frac{Correct\_Pairs\_Found}{Correct\_Pairs\_Expected} \quad (25)$$

Overall Quality of Result: The overall quality (OQ) of result is based on correctness and completeness of result. It is computed just as f-measure (Trojahn et al., 2008; Euzenat, 2007; Ehrig & Euzenat, 2005), used in information retrieval.

$$OQ = 2 * \frac{Completeness * Correctness}{Completeness + Correctness} \quad (26)$$

Through layered strategy, the output of first layer is used as input for the second layer and so on, whereas the output of first layer is set of pairs of concepts having primary similarity while all other concepts are discarded in the output. This means that the input to second layer is a short list of concepts instead of all concepts which reduce a reasonable execution-time for 2nd level of similarity identification. Similarly the concepts shorted-listed in second layer are input to third layer. Therefore, the overall execution-time of proposed technique is comparatively short.

Test Cases for Evaluating Performance

We have taken four pairs of ontologies as shown in Table 6 to evaluate the completeness, correctness and overall quality of results of proposed technique up to second level of similarity. And, then it is followed by the evaluation of the role-based similarity i.e. the 3rd level similarity, based on new criterion. Comparisons of results are then made with expected results and with the results of existing matching techniques used in different tools and systems

5.1 Evaluating Performance with 1st Test Case

Sample input pairs: 37; Pairs of similar concepts (expected): 25; Similar pairs (out of 25) with different terms: 10. With respect to test case 1, the results from proposed technique (SIMTO) and from some existing techniques are compared with respect to their completeness, correctness and overall quality. The graphical representation of comparison is also given in the Figures 7, 8 and 9 respectively. A comparative improvement in result of proposed technique, with respect to completeness is realized.

Table 6: Test-cases for evaluating performance of different techniques

TestCase	Ontologies	Input Pairs	Similar Pairs (expected)	Similar Pairs (with different terms)
1	A1, B_1	37	25	10
2	A2, B_2	40	22	5
3	A_3, B_3	28	12	2
4	A4, B_4	25	15	12

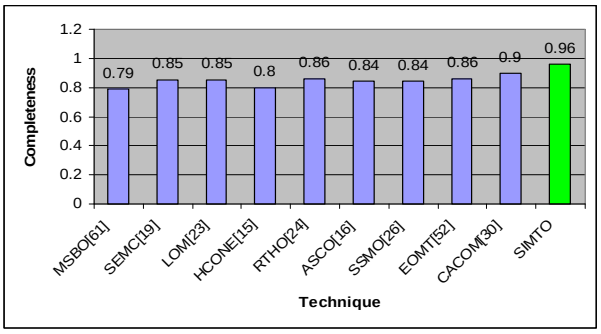

Figure 7. Completeness wise comparison of results with respect to first test case

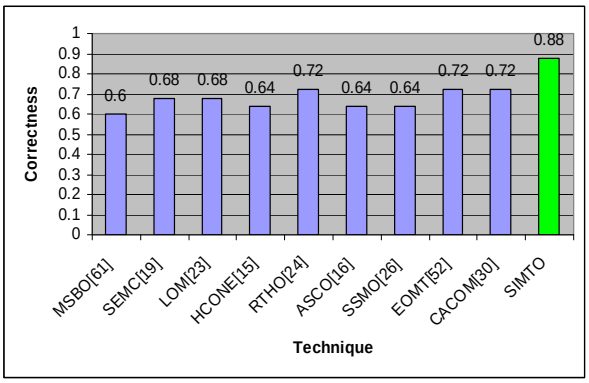

Figure 8. Correctness wise comparison of results with respect to first test case

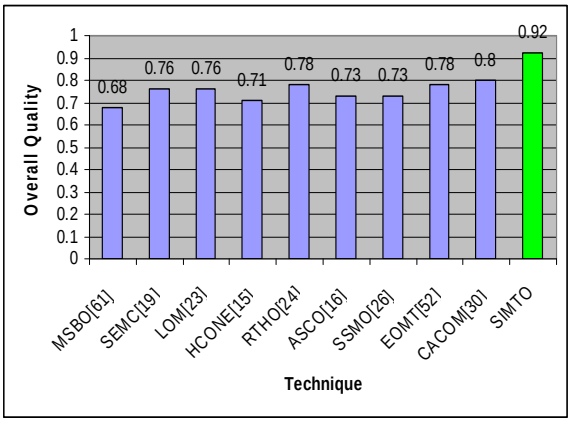

Figure 9. Overall quality wise comparison of results with respect to first test case

5.2 Evaluating Performance with 2nd Test Case

Sample input pairs: 40; Pairs of similar concepts (expected): 22; Similar pairs (out of 22) with different terms: 5. With respect to test case 2, the results from *SIMTO* and from some existing techniques are compared with respect to their completeness, correctness and overall quality. The graphical representation of comparison is also given in the Figures 10, 11 and 12 respectively. It has observed that when the number of similar pairs having different names, decrease, the completeness of results increases. Furthermore, the result of proposed technique, with respect to completeness is better than the results of existing techniques.

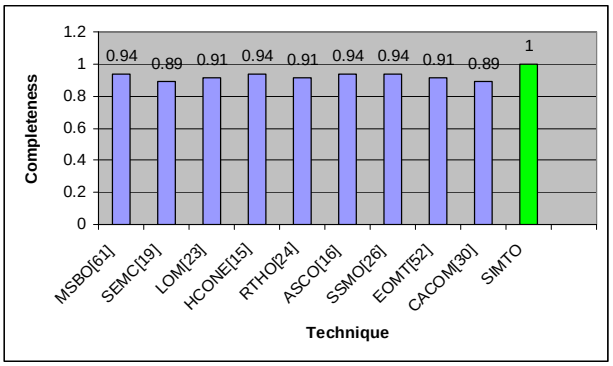

Figure 10. Completeness wise comparison of results with respect to second test case

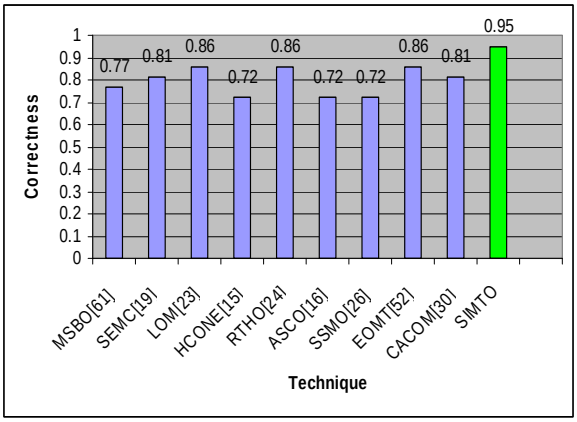

Figure 11. Correctness wise comparison of results with respect to second test case

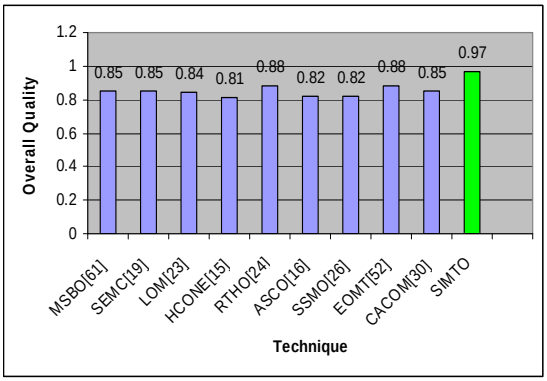

Figure 12. Overall quality wise comparison of results with respect to second test case

5.3 Evaluating Performance with 3rd Test Case

Sample input pairs: 28; Pairs of similar concepts (expected): 12; Similar pairs (out of 12) with different terms: 2. With respect to test case 3, the results from *SIMTO* and from some existing techniques are compared with respect to their completeness, correctness and overall quality. The graphical representation of comparison is given in the Figures 13, 14 and 15 respectively. An improvement in result of proposed technique, with respect to completeness, correctness and overall quality, is realized in comparison. It has also observed that when the number of similar pairs having different names, decreases, the completeness of results increases. Furthermore, the result of proposed technique, with respect to completeness, correctness and overall quality is better than the results of existing techniques.

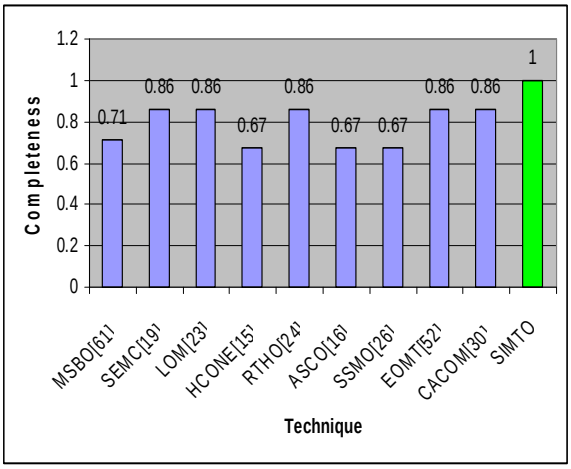

Figure 13. Completeness wise comparison of results with respect to third test case

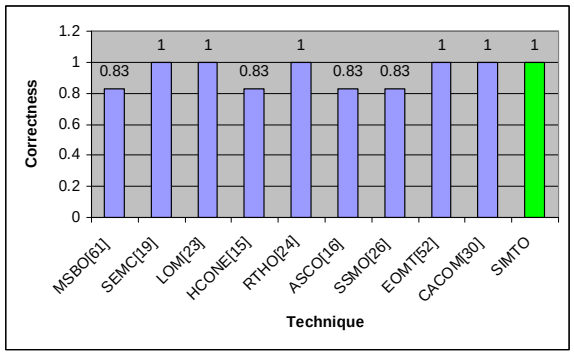

Figure 14. Correctness wise comparison of results with respect to third test case

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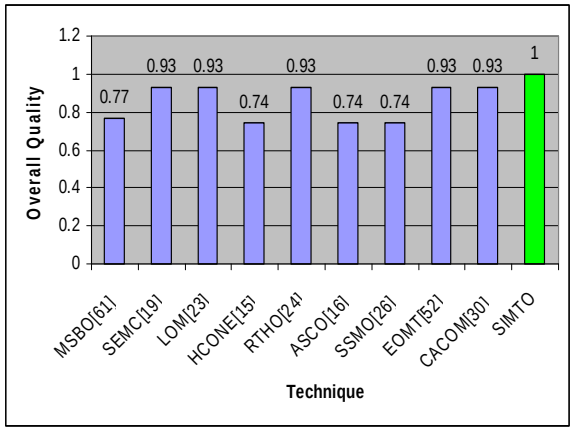

Figure 15. Overall quality wise comparison of results with respect to third test case

5.4 Evaluating Performance with 4th Test Case

Sample input pairs: 25; Pairs of similar concepts (expected): 15. Similar pairs (out of 15) with different terms: 12. With respect to test case 4, the results from *SIMTO* and from some existing techniques are compared with respect to their completeness, correctness and overall quality. The graphical representation of comparison is given in the Figures 16, 17 and 18 respectively. It has observed that when the number of similar pairs having different names increase, the completeness of results decreases. There is a considerable decrease in correctness of results from existing techniques particularly the techniques excluding the linguistic similarity of terms. Furthermore, the overall qualities of results are also badly affected. However, the result of proposed technique, with respect to completeness is better than the results of existing techniques. The comparisons between results from some current techniques and from proposed technique (up to 2nd level of similarity) are shown in Figures 7-18.

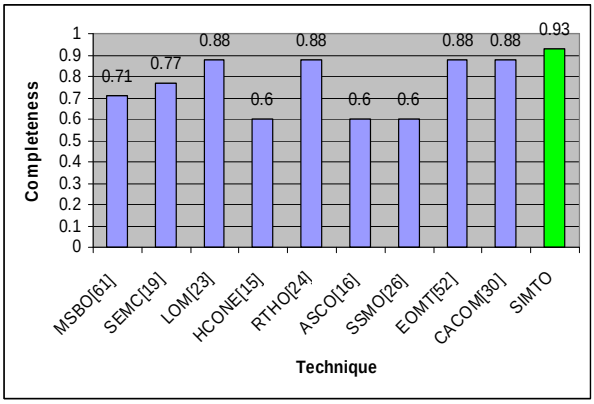

Figure 16. Completeness wise comparison of results with respect to fourth test case

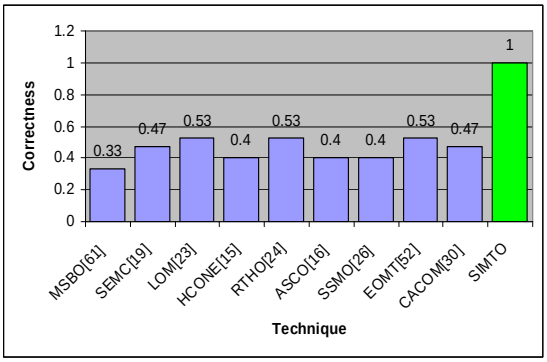

Figure 17. Correctness wise comparison of results with respect to fourth test case

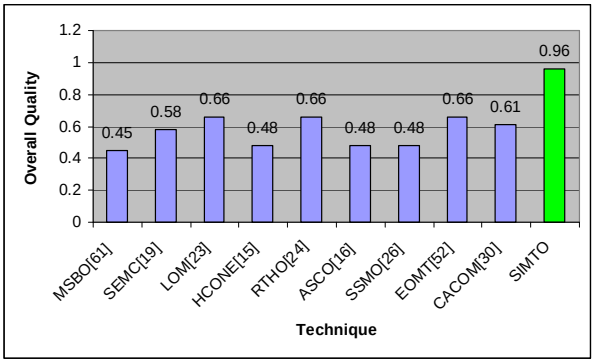

Figure 18. Overall quality wise comparison of results with respect to fourth test case

Furthermore, a comparison between results from proposed technique with the new criterion (i.e. role-based similarity) and expected results has also made in Figure 19. We examined more than ten ontologies of different software houses for evaluating the new criterion of proposed technique. The results are verified by respective domain experts and are declared satisfactory.

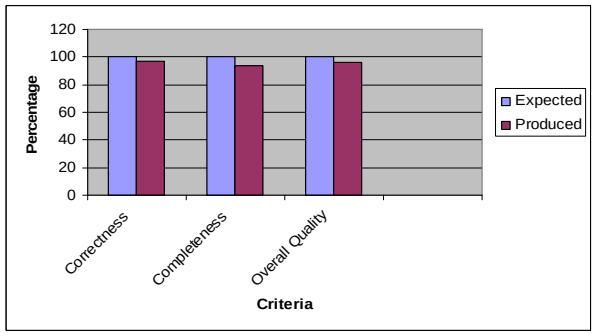

Figure 19. Role-based similarity: expected results vs. produced results

Through analysis of results, we come to some conclusions which are described next.

(i) Through string-based approaches, as used in existing techniques, some dissimilar pairs are declared similar pairs, which decrease the completeness and overall quality of results.

Overall results of existing techniques heavily rely on the heterogeneity of terms as shown in Table 7; higher the number of concepts represented with different terms, lower the completeness and overall quality of results will be.

(ii) Although in existing techniques, the WordNet has good support for matching linguistics semantics of terms, but linguistic semantics of several domain-specific terms particularly the abbreviated terms and composite terms are not supported by WordNet. Also, due to same linguistic semantic of different terms, some unnecessary pairs are identified, which reduce the completeness and overall quality of result.

(iii) Although the proposed technique is also dependent on domain-specific vocabulary, but we empirically observed that domain-specific vocabulary is much better than WordNet.

(iv) The proposed technique may produce 100 percent complete and correct result, but it is not always true, due to absence of some new concepts in domain-specific vocabulary.

(v) We manually populate domain-specific vocabulary and it is some time consuming task. Domain vocabulary is not a static, it is updated dynamically

6. Conclusion and Future Directions

In this paper a semi-automatic, integrated and layered technique has been presented for identification and measurement of similarity between two ontologies. The proposed technique is based on the innovative theme of the semantic web. The proposed technique is not only helpful in different

.Table 7: Comparison of results related to Heterogeneous Pairs of Similar Concepts (HPoSCs)

TestCase	HPoSCs	Identified (Existing Techs.)	Identified (Proposed Tech.)	Correctness (Existing Techs.)	Correctness (Proposed Techs.)
1	10	3	7	**0.30**	**0.70**
2	5	1	4	**0.20**	**0.80**
3	2	2	2	**1.00**	**1.00**
4	12	2	11	**0.17**	**0.92**

ontology integration operations such as merging, mapping, alignment and querying but also in engineering new ontologies

Identification and measurement of similarity between the ontologies is a mandatory pre-requirement of various reuse operations of ontologies such as merging, mapping and alignment. It is also a mandatory requirement for engineering new ontologies by assembling exiting ontologies or components of ontologies. Although the proposed similarity identification technique uses, as core, the innovative ideas of semantic web however essential modifications related to the issues and trends specific to the similarity between concepts of ontologies has been made. The proposed technique upgrades similarity measurement criteria, from terms to concepts, from linguistic semantic to explicit semantic and from all taxonomic characteristics of concepts to mandatory and optional characteristics. In addition we introduced the concepts of similarity levels: primary similarity or 1st level similarity; contextual similarity or 2nd level similarity and the role-based similarity or 3rd level similarity.

We conclude the research result as follows:

- Similarity measurement techniques used for database schemas and XML schemas are not well suited for identifying and measuring of similarity between ontologies schemas.
- The role of domain-specific vocabulary is vital in measurement of similarity between ontologies.
- Primary similarity measurement is the prerequisite for the contextual similarity measurement whereas the contextual similarity measurement is the prerequisite for the role-based similarity identification.
- For a pair of concepts, the degree of similarity and semantic relation are complements to each others.
- It is difficult to get hundred percent correct and complete results due to the lack of standardization in the use of terminologies for concepts and their roles.

As discussed above, the similarity identification is a core and prerequisite task for ontologies integration operations. In addition, this task is also required for ontologies engineering through reuse of ontologies. We plan to work on design and development of methodologies for reuse and integration of ontologies.

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References

1. Alasoud, A., Haarslev,V., & Shiri, N. (2008). An Effective Ontology Matching Technique. *Proceedings of the 17th International Symposium on Methodologies for Intelligent Systems (ISMIS'08), LNAI 4994, 585–590*.
2. Aleksovski, Z., Kate, W., & Harmelen, F. (2006). Exploiting the structure of background knowledge used in ontology matching. *Proceedings of International Workshop on Ontology Matching collocated with the 5th International Semantic Web Conference, pp. 13-24 , USA*.
3. Aleksovski, Z., Klein, M., Kate, W., & Harmelen, F. (2006). Matching Unstructured Vocabularies Using Background Ontology. *Proceedings of 15th International Conference on Knowledge Engineering and Knowledge Management Managing Knowledge in a World of Networks, 182-197, Czech Republic.*
4. Bechhofer, S., Lord, P., & Volz, R. (2003). Cooking the Semantic Web with the OWL API. *2nd International Semantic Web Conference, ISWC, Sanibel Island, Florida*.
5. Bouquet, P., Serafini, L., & Zanobini, S., (2003). Semantic Coordination: A New Approach and an Application. *LNCS 2870, pp.130-145*.
6. Buccella, A., Cechich, A., & Brisaboa, N. (2005). A Three-Level Approach to Ontology Merging. *MICAI , LNAI 3789, 80 – 89*.
7. Cohen, W., Ravikumar, P., Fienberg, S.(2003). A Comparison of String Distance Metrics for Name-Matching Tasks. *IJCAI-03: 3-78*.
8. Duchateau, F., Bellahsene, Z., & Roche, M. (2007). Context-based Measure for Discovering Approximate Semantic Matching between Schema Elements. *Proceedings of The International Conferences on Research*

Challenges in Information Science (RCIS), Morocco.

9. Ehrig, M., & Euzenat, J.(2005). Relaxed precision and recall for ontology matching. P*roceedings of K-Cap 2005 workshop on Integrating ontology, Banff (CA)* 25-32.
10. Erhard, R., & Philip, B.A. (2001). A survey of approaches to automatic schema matching. *The VLDB Journal, 10(4), 334-350, Springer Berlin*.
11. Euzenat, J. (2007). Semantic Precision and Recall for Ontology Alignment Evaluation. *In proceedings of International Joint Conference on Artificial Intelligence, 348-353*.
12. Giunchiglia, F., Yatskevich, M., & Pavel Shvaiko, P. (2007). Semantic Matching: Algorithms and Implementation. *Journal on Data Semantics*. 9 (2007),1-38, Germany.
13. Giunchiglia, F., Yatskevich, M., Shvaiko, P. (2007). Semantic Matching: Algorithms and Implementation. *LNCS Journal on Data Semantics 9 (2007), 1-38*, Germany.
14. González R. G. (2005). A Semantic Web approach to Digital Rights Management. *Ph.D. Thesis, Universitat Pompeu Fabra, Barcelona*.
15. Hariri, B., Abolhassani, H., Khodaei, A.(2006). A new Structural Similarity Measure for Ontology Alignment. *In proceedings of the 2006 International Conference on Semantic Web & Web Services, pp.36-42 , USA*.
16. Hauswirth, M., & Maynard, D. (2007). Knowledge web 2.2: Heterogeneity in the semantic web. *Technical report, NoE Knowledge Web project*.
17. Horridge, M., Bechhofer, S., & Noppens, O. (2007). Igniting the OWL 1.1 Touch Paper: The OWL API. *OWLED 2007, 3rd OWL Experienced and Directions Workshop, Innsbruck, Austria*.
18. Janowicz, K., Raubal, M., Schwering, A., & Kuhn, W. (2008). Semantic Similarity Measurement and Geospatial Applications. *Transactions in GIS, 12(6), 651-659*.
19. Jeong, B., Lee, D., Cho, H., & Lee, J. (2008). A novel method for measuring semantic similarity for XML schema matching. *Expert Systems with Applications, 34(3), 1651–1658, Elsevier*.
20. Kotis, K., & Vouros, G.A. (2004). The HCONE Approach to Ontology *Merging. Proceedings. of the First European Semantic Web Symposium, LNCS 3053, Springer, 137-151*.
21. Lambrix, P., & Tan, H. (2006). SAMBO - A System for Aligning and Merging Biomedical Ontologies. *Journal of Web Semantics, 4(3), 196-206.*
22. Lee, T. B., Hendler, J. and Lassila, O. (2001). The SemanticWeb. Scientific America, 284(5),34-43.
23. Lewandowski, D. (2008). The Retrieval Effectiveness of Web Search Engines: Considering Results Descriptions. *Journal of Documentation, 66(6), 915-937*.
24. Maedche, A., & Staab, S. (2002). Measuring similarity between ontologies. *Proceedings of the International Conference on Knowledge Engineering and Knowledge Management (EKAW), 251–263, Spain*.
25. McGuinness, D., Fikes, R., Rice J. & Wilder, S. (2000). An Environment for Merging and Testing Large Ontologies. *Proceedings of the Seventh International Conference on Principles of Knowledge Representation and Reasoning. 483-493*.
26. Melnik, S., Garcia-Molina, H., & Rahm, E. (2002). A Versatile Graph Matching Algorithm and Its Application to Schema Matching. *Proceedings of International Conference on Data Engineering (ICDE), 117-128, USA*.
27. NetBeans IDE 6.1 (2009). http://www.netbeans.org/community/releases/61/ , Retrieved on October 20, 2009.
28. Noy, N., & Musen, M. (2001). Anchor-prompt: using non-local context for semantic matching. *Proceedings of the workshop on Ontologies and Information Sharing at the International Joint Conference on Artificial Intelligence (IJCAI), 63–70, USA*.
29. Pedersen, T., Patwardhan, S., & Patwardhan, S. (2004). WordNet::Similarity – Measuring the Relatedness of Concepts. *Proceedings. of 19th National Conference on AI, San Jose, CA*.
30. Sherman, C., & Price, G. (2001). The Invisible Web: Uncovering Information Sources Search Engines Can't See. *CyberAge Books*.
31. Shvaiko, P., & Euzenat, J. (2005). A Survey of Schema-based Matching Approaches. *Journal on Data Semantics, 146-171*, *Germany*.
32. Shvaiko, P., & Euzenat, J. (2008). Ten Challenges for Ontology Matching. *In Proceedings of the 7th International Conference on Ontologies, Databases, and Applications of Semantics*.
33. Shvaiko, P., & Euzenat, J. (2009). Ontology Matching web-site;

http://www.ontologymatching.org/. Retrieved on October 4, 2009.

34. Trojahn, C., Moraes, M., Quaresma, P., & Vieira, R.(2008). A Cooperative Approach for Composite Ontology Mapping. *Journal on Data Semantics X, LNCS 4900, 237–263*.
35. Uschold, M. (2002) A semantic continuum on the semantic web. *The Knowledge Engineering Review, 17(1), 87-91*.
36. Uschold, M. (2003) Where Are the Semantics in the Semantic Web?. *AI Magazine 24(3): 25-36*.
37. Visser, P., Jones, D., Bench-Capon, T., Shave, M. An Analysis of Ontology Mismatches; Heterogeneity versus Interoperability. *AAAI Spring Symposium on Ontological Engineering*, 1997.
38. W3C; The World Wide Web Consortium; http://www.w3.org

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Embedded ZrO_2 nanoparticles mechanical properties monitoring in cementitious composites

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Abstract: In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase ZrO_2 particles has been studied. ZrO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-ZrO_2 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of ZrO_2 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of ZrO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase ZrO_2 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):86-89]. (ISSN: 1545-1003).

Key words: Nanophase ZrO_2 particles; concrete; split tensile strength; flexural strength.

1. Introduction

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the tensile strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as tensile strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano-ZrO_2 on flexural and tensile strength together with the setting time of binary blended concrete have been investigated. ZrO_2 reacts with calcium hydroxide produced from the hydration of calcium zirconias. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano-ZrO_2 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano-ZrO_2 particles

Nano–ZrO_2 with average particle size of 15 nm was used as received. The properties of nano-ZrO_2 particles are shown in Table 2.

Table 2. The properties of nano-ZrO_2

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	160 ± 12	< 0.14	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm^3 was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano-ZrO_2 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano-ZrO_2 particles blended concretes

Sample designation	nano-ZrO_2 particles	Quantities (kg/m^3)	
		Cement	nano-ZrO_2 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano-ZrO_2] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano-ZrO_2 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the flexural strength following the mixing procedure. Cylinders with the diameter of 150 mm and the height of 300 mm for split tensile strength and cubes with 200 mm × 50 mm × 50 mm edges for flexural strength tests were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The tensile strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Split tensile strength of nano-ZrO_2 particles blended concrete

Split tensile test was carried out in accordance to the ASTM C 496-90 standard. After the specified curing period was over, the concrete cylinders were subjected to split tensile test by using universal testing machine. Tests were carried out on triplicate specimens and average split tensile strength values were obtained.

2.4. Flexural strength of nano-ZrO_2 particles blended concrete

Flexural test were done in accordance to the ASTM C293 Standard. Similar to the tensile tests, flexural tests were carried out on triplicate specimens and average flexural strength values were obtained.

3.4. Setting time of nano-ZrO_2 particles blended concrete

Setting time of the specimens was regulated according to the ASTM C191 standard.

3. Experimental results and discussion

3.1. Split Tensile strength

The split tensile strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the split tensile strength increases with nano-ZrO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano-ZrO_2 particles decreases the split tensile strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano-ZrO_2 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica

leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Split Tensile strength of nano-ZrO_2 particle blended cement mortars

		Split Tensile strength (MPa)		
Sample designation	nano-ZrO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	1.5	1.8	2.3
N1	0.5	2.5	2.9	3.4
N2	1.0	3.0	3.3	3.6
N3	1.5	2.9	3.0	3.2
N4	2.0	2.0	2.1	2.4

Water to binder [cement + nano-ZrO_2] ratio of 0.40

The higher the split tensile strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-ZrO_2 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano-ZrO_2 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste. However, the value of split tensile strength in the specimens is not high and better reinforcements such as needle-type nanoparticles are requested.

3.2. Flexural strength

The flexural strength results of series C0 and N mixtures are shown in Table 5. Similar to the tensile strength, the flexural strength of the specimens increases with nano-ZrO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). Again, the increasing in the flexural strength is due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-ZrO_2 particles.

3.3. Setting time

The obtained results from the initial and final setting times of the cement mortars with nano-ZrO_2 particles are shown in Figures 1 and 2, respectively. Figures 1 and 2 shows that by increasing the volume fraction of nanoparticles, the setting time is decreased indicating that nano-ZrO_2 has a faster hydration reaction speed than did the cement, because nano-ZrO_2 is characterized by its unique surface effects, smaller particle sizes, and higher surface energy [12]. Smaller particle sizes allow a rapid increase in surface area leading to a fast rise in the number of surface atoms. These surface atoms are highly active and unstable, which results in a faster reaction speed. Hence, a cautious approach should be taken for the setting time of the paste during the utilizing of nano-ZrO_2 [12].

Table 4. Flexural strength of nano-ZrO_2 particle blended cement mortars

		Flexural strength (MPa)		
Sample designation	nano-ZrO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	4.2	4.4	4.7
N1	0.5	5.0	5.3	5.5
N2	1.0	5.5	5.8	6.2
N3	1.5	5.2	5.6	5.9
N4	2.0	4.8	5.0	5.2

Water to binder [cement + nano-ZrO_2] ratio of 0.40

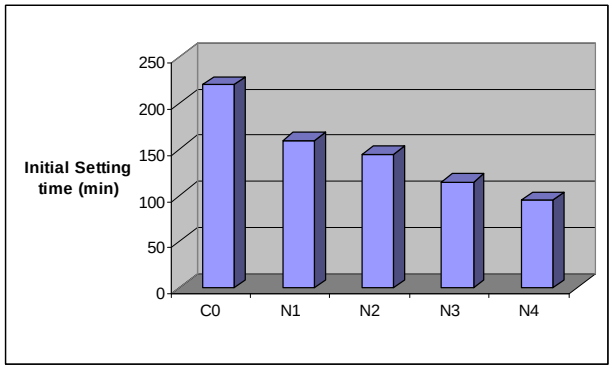

Figure 1. Influence of nano-ZrO_2 particles on the initial setting time of cement paste.

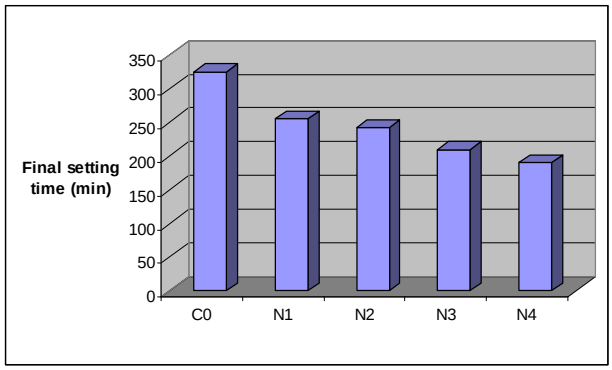

Figure 2. Influence of nano-ZrO_2 particles on the initial setting time of cement paste.

Conclusions

The results show that the nano-ZrO_2 particles blended concrete had higher split tensile and flexural strength compare to that of the concrete without nano-ZrO_2

particles. It is found that the cement could be advantageously replaced with nano-ZrO_2 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano-ZrO_2 particles content was achieved with 1.0% replacement. However, the split tensile strength of the concrete could be improved by using more suitable reinforcements such as needle type nanoparticles.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).
[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.
[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.
[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa tensile strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.
[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO_2 particles. Const Build Mater 2007; 21(6): 1351-1355.
[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.
[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.
[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.
[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.
[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.
[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.
[12] Kuo WT, Lin KL, Chang WC, Luo HL, Effects of Nano-Materials on Properties of Waterworks Sludge Ash Cement Paste. Journal of Indian Engineering Chemistry 200; 12(5): 702-709.

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The effects of incorporation Fe_2O_3 nanoparticles on tensile and flexural strength of concrete

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Abstract: In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase Fe_2O_3 particles has been studied. Fe_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano- Fe_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of Fe_2O_3 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of Fe_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Fe_2O_3 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):90-93]. (ISSN: 1545-1003).

Key words: Nanophase Fe_2O_3 particles; concrete; split tensile strength; flexural strength.

1. Introduction

Concrete is a newer construction material compared to steel and stone. Use of concrete in constructions and buildings may have begun less than a century ago. But in recent century, very wide and effective research has seen on improving the properties of concrete with incorporating wide range of supplementary cementing materials such as pozzolans and nanoparticles due to increasing the use of concrete from decade to decade. Recently, nanotechnology has attracted great scientific attention because of the new potential uses of particles in nanometer (10^{-9} m) scale. This may be due to the nanoscale size of particles being able to result in significantly improved properties from predictable grain-size materials of the same chemical composition. As a consequence, industries can be able to design new and novel products and to re-engineer many existing products that function at unprecedented levels.

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the tensile strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as tensile strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano- Fe_2O_3 on flexural and tensile strength together with the setting time of binary blended concrete have been investigated. Alumina

component reacts with calcium hydroxide produced from the hydration of calcium silicates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano- Fe_2O_3 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

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Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano- Fe_2O_3 particles

Nano– Fe_2O_3 with average particle size of 15 nm was used as received. The properties of nano- Fe_2O_3 particles are shown in Table 2.

Table 2. The properties of nano- Fe_2O_3

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	155 ± 12	< 0.13	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm^3 was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano- Fe_2O_3 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano- Fe_2O_3 particles blended concretes

Sample designation	nano- Fe_2O_3 particles	Quantities (kg/m^3)	
		Cement	nano- Fe_2O_3 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
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N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano- Fe_2O_3] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano- Fe_2O_3 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the flexural strength following the mixing procedure. Cylinders with the diameter of 150 mm and the height of 300 mm for compressive strength and cubes with 200 mm × 50 mm × 50 mm edges for flexural strength tests were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The tensile strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Split tensile strength of nano- Fe_2O_3 particles blended concrete

Split tensile test was carried out in accordance to the ASTM C 496-90 standard. After the specified curing period was over, the concrete cylinders were subjected to split tensile test by using universal testing machine. Tests were carried out on triplicate specimens and average split tensile strength values were obtained.

2.4. Flexural strength of nano- Fe_2O_3 particles blended concrete

Flexural test were done in accordance to the ASTM C293 Standard. Similar to the tensile tests, flexural tests were carried out on triplicate specimens and average flexural strength values were obtained.

3.4. Setting time of nano- Fe_2O_3 particles blended concrete
Setting time of the specimens was regulated according to the ASTM C191 standard.

3. Experimental results and discussion

3.1. Split Tensile strength
The split tensile strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the split tensile strength increases with nano- Fe_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano- Fe_2O_3 particles decreases the split tensile strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano- Fe_2O_3 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Split Tensile strength of nano- Fe_2O_3 particle blended cement mortars

		Split Tensile strength (MPa)		
Sample designation	nano- Fe_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	1.5	1.8	2.3
N1	0.5	2.1	2.4	2.7
N2	1.0	2.5	2.8	3.2
N3	1.5	2.7	2.5	3.0
N4	2.0	1.8	1.9	2.3

Water to binder [cement + nano- Fe_2O_3] ratio of 0.40

The higher the split tensile strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano- Fe_2O_3 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano- Fe_2O_3 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste. However, the value of split tensile strength in the specimens is not high and better reinforcements such as needle-type nanoparticles are requested.

3.2. Flexural strength
The flexural strength results of series C0 and N mixtures are shown in Table 5. Similar to the tensile strength, the flexural strength of the specimens increases with nano- Fe_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). Again, the increasing in the flexural strength is due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano- Fe_2O_3 particles.

Table 4. Flexural strength of nano- Fe_2O_3 particle blended cement mortars

		Flexural strength (MPa)		
Sample designation	nano- Fe_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	4.2	4.4	4.7
N1	0.5	4.9	5.0	5.7
N2	1.0	5.4	5.2	6.0
N3	1.5	5.0	5.0	5.3
N4	2.0	4.3	4.8	5.0

Water to binder [cement + nano- Fe_2O_3] ratio of 0.40

3.3. Setting time
The obtained results from the initial and final setting times of the cement mortars with nano- Fe_2O_3 particles are shown in Figures 1 and 2, respectively. Figures 1 and 2 shows that by increasing the volume fraction of nanoparticles, the setting time is decreased indicating that nano- Fe_2O_3 has a faster hydration reaction speed than did the cement, because nano- Fe_2O_3 is characterized by its unique surface effects, smaller particle sizes, and higher surface energy [12]. Smaller particle sizes allow a rapid increase in surface area leading to a fast rise in the number of surface atoms. These surface atoms are highly active and unstable, which results in a faster reaction speed. Hence, a cautious approach should be taken for the setting time of the paste during the utilizing of nano- Fe_2O_3 [12].

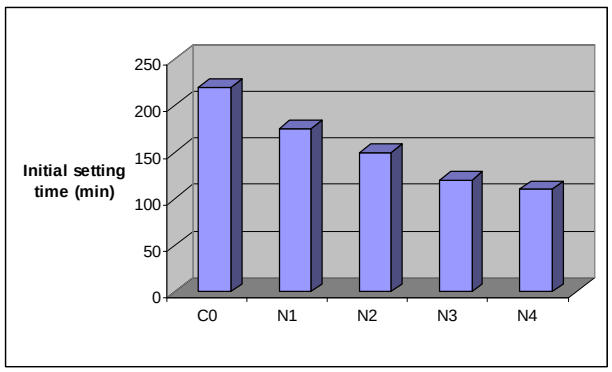

Figure 1. Influence of nano- Fe_2O_3 particles on the initial setting time of cement paste.

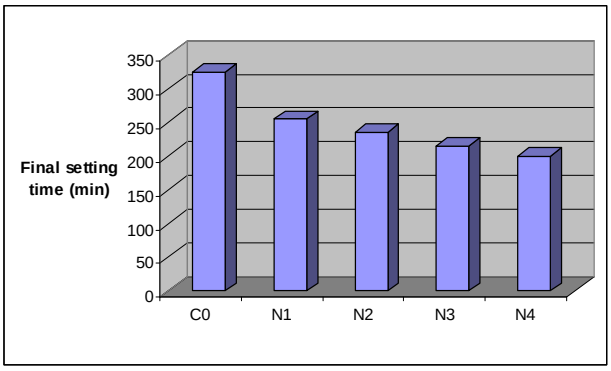

Figure 2. Figure 1. Influence of nano- Fe_2O_3 particles on the initial setting time of cement paste.

Conclusions

The results show that the nano- Fe_2O_3 particles blended concrete had higher split tensile and flexural strength compare to that of the concrete without nano- Fe_2O_3 particles. It is found that the cement could be advantageously replaced with nano- Fe_2O_3 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano- Fe_2O_3 particles content was achieved with 1.0% replacement. However, the split tensile strength of the concrete could be improved by using more suitable reinforcements such as needle type nanoparticles.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).

[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.

[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.

[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa tensile strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.

[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO_2 particles. Const Build Mater 2007; 21(6): 1351-1355.

[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.

[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.

[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.

[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.

[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.

[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.

[12] Kuo WT, Lin KL, Chang WC, Luo HL, Effects of Nano-Materials on Properties of Waterworks Sludge Ash Cement Paste. Journal of Indian Engineering Chemistry 200; 12(5): 702-709.

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Mechanical properties of cement mortar with Al_2O_3 nanoparticles

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Abstract: In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase Al_2O_3 particles has been studied. Al_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano- Al_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of Al_2O_3 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of Al_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Al_2O_3 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):94-97]. (ISSN: 1545-1003).

Key words: Nanophase Al_2O_3 particles; concrete; split tensile strength; flexural strength.

1. Introduction

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the tensile strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as tensile strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano- Al_2O_3 on flexural and tensile strength together with the setting time of binary blended concrete have been investigated. Alumina component reacts with calcium hydroxide produced from the hydration of calcium silicates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano- Al_2O_3 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano- Al_2O_3 particles

Nano– Al_2O_3 with average particle size of 15 nm was used as received. The properties of nano- Al_2O_3 particles are shown in Table 2.

Table 2. The properties of nano- Al_2O_3

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	165 ± 12	< 0.1	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm³ was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano- Al_2O_3 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano-Al_2O_3 particles blended concretes

Sample designation	nano- Al_2O_3 particles	Quantities (kg/m^3)	
		Cement	nano- Al_2O_3 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano-Al_2O_3] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano-Al_2O_3 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the flexural strength following the mixing procedure. Cylinders with the diameter of 150 mm and the height of 300 mm for split tensile strength and cubes with 200 mm × 50 mm × 50 mm edges for flexural strength tests were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The tensile strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Split tensile strength of nano-Al_2O_3 particles blended concrete

Split tensile test was carried out in accordance to the ASTM C 496-90 standard. After the specified curing period was over, the concrete cylinders were subjected to split tensile test by using universal testing machine. Tests were carried out on triplicate specimens and average split tensile strength values were obtained.

2.4. Flexural strength of nano-Al_2O_3 particles blended concrete

Flexural test were done in accordance to the ASTM C293 Standard. Similar to the tensile tests, flexural tests were carried out on triplicate specimens and average flexural strength values were obtained.

3.4. Setting time of nano-Al_2O_3 particles blended concrete

Setting time of the specimens was regulated according to the ASTM C191 standard.

3. Experimental results and discussion

3.1. Split Tensile strength

The split tensile strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the split tensile strength increases with nano-Al_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano-Al_2O_3 particles decreases the split tensile strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano-Al_2O_3 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during

the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Split Tensile strength of nano-Al_2O_3 particle blended cement mortars

		Split Tensile strength (MPa)		
Sample designation	nano-Al_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	1.5	1.8	2.3
N1	0.5	2.2	2.3	2.6
N2	1.0	2.6	2.8	3.1
N3	1.5	2.4	2.5	2.8
N4	2.0	1.8	1.9	2.3

Water to binder [cement + nano-Al_2O_3] ratio of 0.40

The higher the split tensile strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-Al_2O_3 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano-Al_2O_3 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste. However, the value of split tensile strength in the specimens is not high and better reinforcements such as needle-type nanoparticles are requested.

3.2. Flexural strength

The flexural strength results of series C0 and N mixtures are shown in Table 5. Similar to the tensile strength, the flexural strength of the specimens increases with nano-Al_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). Again, the increasing in the flexural strength is due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-Al_2O_3 particles.

3.3. Setting time

The obtained results from the initial and final setting times of the cement mortars with nano-Al_2O_3 particles are shown in Figures 1 and 2, respectively. Figures 1 and 2 shows that by increasing the volume fraction of nanoparticles, the setting time is decreased indicating that nano-Al_2O_3 has a faster hydration reaction speed than did the cement, because nano-Al_2O_3 is characterized by its unique surface effects, smaller particle sizes, and higher surface energy [12]. Smaller particle sizes allow a rapid increase in surface area leading to a fast rise in the number of surface atoms. These surface atoms are highly active and unstable, which results in a faster reaction speed. Hence, a cautious approach should be taken for the setting time of the paste during the utilizing of nano-Al_2O_3 [12].

Table 4. Flexural strength of nano-Al_2O_3 particle blended cement mortars

		Flexural strength (MPa)		
Sample designation	nano-Al_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	4.2	4.4	4.7
N1	0.5	4.6	5.1	5.7
N2	1.0	5.3	5.2	6.0
N3	1.5	5.0	5.0	5.3
N4	2.0	4.4	4.8	4.8

Water to binder [cement + nano-Al_2O_3] ratio of 0.40

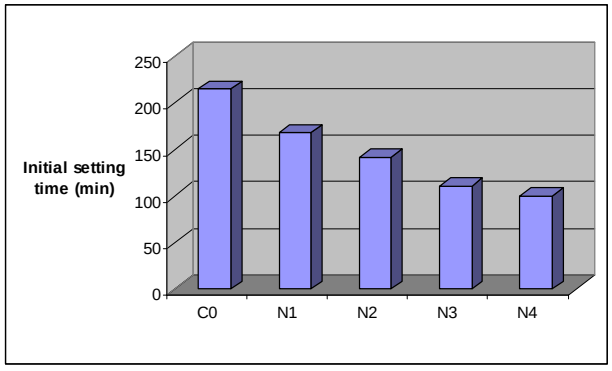

Figure 1. Influence of nano-Al_2O_3 particles on the initial setting time of cement paste.

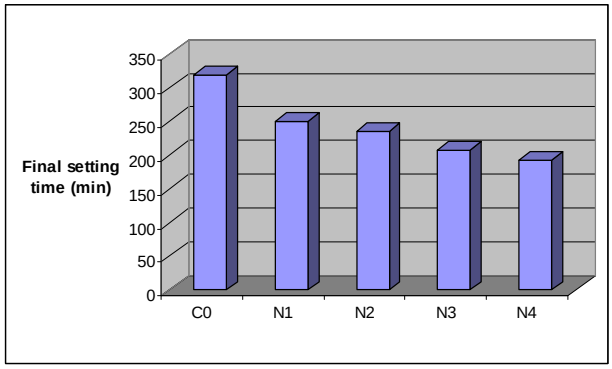

Figure 2. Figure 1. Influence of nano-Al_2O_3 particles on the initial setting time of cement paste.

Conclusions

The results show that the nano-Al_2O_3 particles blended concrete had higher split tensile and flexural strength

compare to that of the concrete without nano-Al_2O_3 particles. It is found that the cement could be advantageously replaced with nano-Al_2O_3 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano-Al_2O_3 particles content was achieved with 1.0% replacement. However, the split tensile strength of the concrete could be improved by using more suitable reinforcements such as needle type nanoparticles.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).
[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.
[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.
[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa tensile strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.
[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO_2 particles. Const Build Mater 2007; 21(6): 1351-1355.
[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.
[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.
[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.
[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.
[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.
[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.
[12] Kuo WT, Lin KL, Chang WC, Luo HL, Effects of Nano-Materials on Properties of Waterworks Sludge Ash Cement Paste. Journal of Indian Engineering Chemistry 200; 12(5): 702-709.

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Improvement the mechanical properties of the cementitious composite by using TiO_2 nanoparticles

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Abstract: In this Paper, the split tensile and flexural strength together with the setting time of concrete by partial replacement of cement with nano-phase TiO_2 particles has been studied. TiO_2 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-TiO_2 particles up to maximum replacement level of 2.0% produces concrete with improved split tensile strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The flexural strength of fresh concrete was increased by increasing the content of TiO_2 nanoparticles. The setting time of fresh concrete was decreased by increasing the content of TiO_2 nanoparticles. It is concluded that partial replacement of cement with nanophase TiO_2 particles improves the split tensile and flexural strength of concrete but decreases its setting time. [Journal of American Science 2010;6(4):98-101]. (ISSN: 1545-1003).

Key words: Nanophase TiO_2 particles; concrete; split tensile strength; flexural strength.

1. Introduction

Concrete is a newer construction material compared to steel and stone. Use of concrete in constructions and buildings may have begun less than a century ago. But in recent century, very wide and effective research has seen on improving the properties of concrete with incorporating wide range of supplementary cementing materials such as pozzolans and nanoparticles due to increasing the use of concrete from decade to decade. Recently, nanotechnology has attracted great scientific attention because of the new potential uses of particles in nanometer (10^{-9} m) scale. This may be due to the nanoscale size of particles being able to result in significantly improved properties from predictable grain-size materials of the same chemical composition. As a consequence, industries can be able to design new and novel products and to re-engineer many existing products that function at unprecedented levels.

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the tensile strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as tensile strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable

fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano-TiO_2 on flexural and tensile strength together with the setting time of binary blended concrete have been investigated. Alumina component reacts with calcium hydroxide produced from the hydration of calcium silicates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano-TiO_2 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano-TiO_2 particles

Nano–TiO_2 with average particle size of 15 nm was used as received. The properties of nano-TiO_2 particles are shown in Table 2.

Table 2. The properties of nano-TiO_2

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	150 ± 12	< 0.12	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm^3 was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano-TiO_2 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano-TiO_2 particles blended concretes

Sample designation	nano-TiO_2 particles	Quantities (kg/m^3)	
		Cement	nano-TiO_2 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano-TiO_2] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano-TiO_2 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the flexural strength following the mixing procedure. Cylinders with the diameter of 150 mm and the height of 300 mm for split tensile strength and cubes with 200 mm × 50 mm × 50 mm edges for flexural strength tests were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The tensile strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Split tensile strength of nano-TiO_2 particles blended concrete

Split tensile test was carried out in accordance to the ASTM C 496-90 standard. After the specified curing period was over, the concrete cylinders were subjected to split tensile test by using universal testing machine. Tests were carried out on triplicate specimens and average split tensile strength values were obtained.

2.4. Flexural strength of nano-TiO_2 particles blended concrete

Flexural test were done in accordance to the ASTM C293 Standard. Similar to the tensile tests, flexural tests were

carried out on triplicate specimens and average flexural strength values were obtained.

3.4. Setting time of nano-TiO_2 particles blended concrete

Setting time of the specimens was regulated according to the ASTM C191 standard.

3. Experimental results and discussion

3.1. Split Tensile strength

The split tensile strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the split tensile strength increases with nano-TiO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano-TiO_2 particles decreases the split tensile strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano-TiO_2 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Split Tensile strength of nano-TiO_2 particle blended cement mortars

		Split Tensile strength (MPa)		
Sample designation	nano-TiO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	1.5	1.8	2.3
N1	0.5	2.3	2.6	2.9
N2	1.0	2.8	3.0	3.3
N3	1.5	2.6	2.7	3.0
N4	2.0	1.9	1.9	2.4

Water to binder [cement + nano-TiO_2] ratio of 0.40

The higher the split tensile strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-TiO_2 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano-TiO_2 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste. However, the value of split tensile strength in the specimens is not high and better reinforcements such as needle-type nanoparticles are requested.

3.2. Flexural strength

The flexural strength results of series C0 and N mixtures are shown in Table 5. Similar to the tensile strength, the flexural strength of the specimens increases with nano-TiO_2 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). Again, the increasing in the flexural strength is due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-TiO_2 particles.

Table 4. Flexural strength of nano-TiO_2 particle blended cement mortars

		Flexural strength (MPa)		
Sample designation	nano-TiO_2 particle (%)	7 days	28 days	90 days
C0 (control)	0	4.2	4.4	4.7
N1	0.5	4.9	5.1	5.6
N2	1.0	5.6	5.5	6.1
N3	1.5	5.1	5.4	5.7
N4	2.0	4.5	5.1	5.0

Water to binder [cement + nano-TiO_2] ratio of 0.40

3.3. Setting time

The obtained results from the initial and final setting times of the cement mortars with nano-TiO_2 particles are shown in Figures 1 and 2, respectively. Figures 1 and 2 shows that by increasing the volume fraction of nanoparticles, the setting time is decreased indicating that nano-TiO2 has a faster hydration reaction speed than did the cement, because nano-TiO2 is characterized by its unique surface effects, smaller particle sizes, and higher surface energy [12]. Smaller particle sizes allow a rapid increase in surface area leading to a fast rise in the number of surface atoms. These surface atoms are highly active and unstable, which results in a faster reaction speed. Hence, a cautious approach should be taken for the setting time of the paste during the utilizing of nano-TiO2 [12].

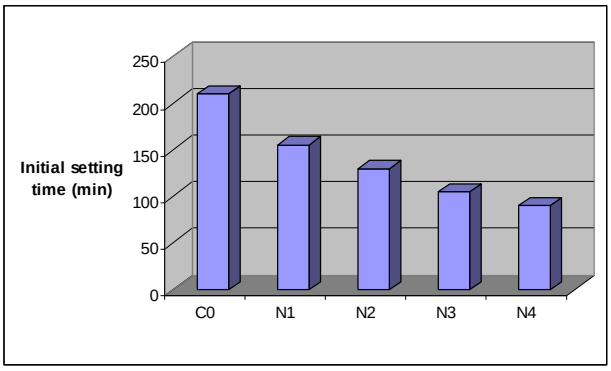

Figure 1. Influence of nano-TiO2 particles on the initial setting time of cement paste.

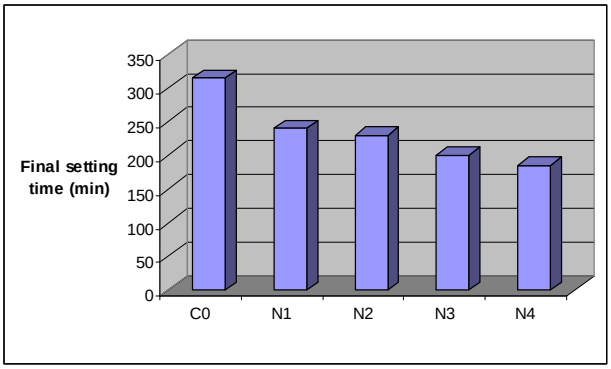

Figure 2. Figure 1. Influence of nano-TiO2 particles on the initial setting time of cement paste.

Conclusions

The results show that the nano-TiO_2 particles blended concrete had higher split tensile and flexural strength compare to that of the concrete without nano-TiO_2 particles. It is found that the cement could be advantageously replaced with nano-TiO_2 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano-TiO_2 particles content was achieved with 1.0% replacement. However, the split tensile strength of the concrete could be improved by using more suitable reinforcements such as needle type nanoparticles.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).

[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.

[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.

[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa tensile strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.

[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO_2 particles. Const Build Mater 2007; 21(6): 1351-1355.

[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.

[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.

[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.

[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.

[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.

[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.

[12] Kuo WT, Lin KL, Chang WC, Luo HL, Effects of Nano-Materials on Properties of Waterworks Sludge Ash Cement Paste. Journal of Indian Engineering Chemistry 200; 12(5): 702-709.

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Benefits of Fe_2O_3 nanoparticles in concrete mixing matrix

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Abstract: The purpose of this study is to investigate the compressive strength and workability of concrete by partial replacement of cement with nano-phase Fe_2O_3 particles. Fe_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-Fe_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0% of cement replacement. The workability of fresh concrete was decreased by increasing the content of Fe_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Fe_2O_3 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(4):102-106]. (ISSN: 1545-1003).

Key words: Nanophase Fe_2O_3 particles; concrete; compressive strength; workability.

1. Introduction

Concrete is a newer construction material compared to steel and stone. Use of concrete in constructions and buildings may have begun less than a century ago. But in recent century, very wide and effective research has seen on improving the properties of concrete with incorporating wide range of supplementary cementing materials such as pozzolans and nanoparticles due to increasing the use of concrete from decade to decade. Recently, nanotechnology has attracted great scientific attention because of the new potential uses of particles in nanometer (10^{-9} m) scale. This may be due to the nanoscale size of particles being able to result in significantly improved properties from predictable grain-size materials of the same chemical composition. As a consequence, industries can be able to design new and novel products and to re-engineer many existing products that function at unprecedented levels.

There are few reports on incorporation of nanoparticles in cement-based concrete. Hui Li et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the compressive strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved 800 MPa strengths on compressed samples, and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) ranging from 200 to 800 MPa and fracture energies up to 40 kJ m^{-2}. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

The definition of high performance concrete (HPC) and high strength concrete (HSC) have been changing from time to time. Until the late 1960s 35 MPa and 42 MPa were considered as HSC while in the mid 1980s 55 MPa concrete was considered as HSC**.** Perhaps by the end of this century, 150 MPa will be branded as HSC [6]**.** Production of HPC and HSC are a challenge and depends upon so many factors. Also In the last 15 years Ultra High Performance Concrete (UHPC) has become a vanguard product in industrial and structural applications gratitude to outstanding properties, such as compressive strength of 150–200 MPa, tensile strength of 8–15 MPa with significant remaining post-cracking bearing capacity, and remarkable fracture energy of 20–30 kJ/m^2 [7,8].

In this work, the influences of nano-Fe_2O_3 on compressive strength and workability of binary blended concrete has been studied. Nanoparticles react with calcium hydroxide produced from the hydration of calcium silicates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano- Fe_2O_3 of a high purity (99.9%) and a high

Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

HPC and HSC are very useful in constructions and multistory buildings because they can decrease the cross-sectional area of the structural fundamentals.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)*

Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano- Fe_2O_3 particles

Nano– Fe_2O_3 with average particle size of 15 nm was used as received. The properties of nano- Fe_2O_3 particles are shown in Table 2.

Table 2. The properties of nano- Fe_2O_3

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	155 ± 12	< 0.13	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96g/cm^3 was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano- Fe_2O_3 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550kg/m^3. The proportions of the mixtures are presented in Table 3.

Table 3. Mixture proportion of nano- Fe_2O_3 particles blended concretes

Sample designation	nano- Fe_2O_3 particles	Quantities (kg/m^3)	
		Cement	nano- Fe_2O_3 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano- Fe_2O_3] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano- Fe_2O_3 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the workability following the mixing procedure. Cubes of 100 mm edge were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The compressive strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

2.3. Compressive strength of nano-Fe_2O_3 particles blended concrete

Compressive strength of nano-Fe_2O_3 particles blended cement concrete cubes was determined as per ASTM C 39 after 7, 28 and 90 days of moisture curing.

2.4. Workability

Standard slump tests conforming to ASTM C143 were used to determine the workability of the concrete.

3. Experimental results and discussion

The compressive strength results obtained from the experimental investigations are showed in tables and the comparison between the results of workability test is presented in form of bar chart. All the values are the average of the three trails in each case in the testing program of this study. The results are discussed as follows.

3.1. Compressive strength

The compressive strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the compressive strength increases with nano- Fe_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano- Fe_2O_3 particles decreases the compressive strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano- Fe_2O_3 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Compressive strength of nano-Fe_2O_3 particle blended cement mortars

		Compressive strength (MPa)		
Sample designation	nano- Fe_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	27.3	36.8	42.3
N1	0.5	30.1	41.0	44.5
N2	1.0	31.2	42.5	46.1
N3	1.5	31.0	41.9	45.2
N4	2.0	28.1	38.9	43.8

Water to binder [cement + nano- Fe_2O_3] ratio of 0.40

The high enhancement of compressive strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano- Fe_2O_3 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano- Fe_2O_3 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste.

3.2. Workability

A high-quality concrete is one which has acceptable workability (around 6.5 cm slump height) in the fresh condition and develops sufficient strength. Basically, the bigger the measured height of slump, the better the workability will be, indicating that the concrete flows easily but at the same time is free from segregation [12, 13]. Maximum strength of concrete is related to the workability and can only be obtained if the concrete has adequate degree of workability because of self compacting ability. Self-compacting repair mortars, as new technology products, are especially preferred for the rehabilitation and repair of reinforced concrete structures [14]. The water/powder (cement, fly ash, limestone filler, silica fume, nano-particles, etc.) ratio of mortar and the type of chemical admixtures should be determined, in order to place the fresh mortar without any external compaction and at the same time without causing any segregation [15]. In other words, the rheology of paste phase of self-repairing mortar should have suitable properties from flowability and segregation point of view [16–19].

The workability of C0 and N series concrete are presented in Figure 1. The figure shows the influence of nano- Fe_2O_3 particles content on the workability of mixtures at constant water to binder ratio of 0.40. The results show that unlike the C0 series, all investigated nano- Fe_2O_3 particles blended mixtures had low slump values and non-acceptable workability. This may be due to the increasing in the surface area of powder after adding nanoparticles that needs more water to wetting the cement particles.

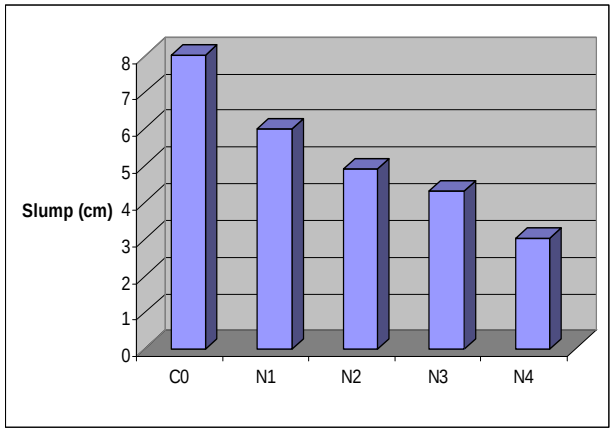

Figure 1. Particle size effects of nano- Fe_2O_3 on workability of concrete. N1,N2,N3 and N4 are the series N blended concrete with 0.5, 1.0, 1.5 and 2.0 percent of nano- Fe_2O_3 particles, respectively.

With the improvement of novel plasticizers, to obtain high filling rates is possible even for compound molding systems. The fresh characteristics of concrete, strength and durability of mortars can be improved by the addition of inert or pozzolanic [20]. The selection of the amount and the type of cementitious or inert powders depends on the physical and physico-chemical properties of these powders which are affecting the performance of fresh paste such as particle shape, surface texture, surface porosity and rate of superplasticizer adsorption, surface energy (zeta potential), finest fraction content, Blaine fineness and particle size distribution.

There is no universally accepted agreement on the effect of these factors due to the complex influence of the combination of these factors [21].

Usually, increasing the fine particles content in cements changes the rheological properties of pastes and consequently influences the workability of mortars and fresh concrete mixtures. The observed changes can be advantageous or not as a result of many factors influencing the rheology of cement pastes [22]. It is usually expected that, if the volume concentration of a solid is held constant, for a specific workability, the replacement of cement with a fine powder will increase the water demand due to the increase in surface area. This is more observed for nanoparticles blended concrete. However, in some cases, the above-mentioned conclusion is not appropriate. Lange et al. [23] obtained same results with fly ash blended concrete. But In this study, the addition of nano- Fe_2O_3 particles decreased the fluidity and increased the water demand for normal consistency

Conclusions

The results show that the nano- Fe_2O_3 particles blended concrete had significantly higher compressive strength compare to that of the concrete without nano- Fe_2O_3 particles. It is found that the cement could be advantageously replaced with nano-Fe_2O_3 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano- Fe_2O_3 particles content was achieved with 1.0% replacement. Partial replacement of cement by nano- Fe_2O_3 particles decreased workability of fresh concrete; therefore use of super plasticizer is substantial.

References

[1] Li H, Xiao HG, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Composites Part B: Engineering 2003; 35(March).

[2] Nazari A., Riahi, Sh, Khademno A., "Effects on nano-SiO_2 on mechanical properties of binary blended concrete", To be published.

[3] Lu P, Young JF. Hot pressed DSP cement paste, Material Research Society Symposium Proceedings, 1992; 245.

[4] Richard P, Cheyrezy M. Reactive powder concretes with high ductility and 200- 800 MPa compressive strength, San Francisco: ACI Spring Convention, SP 144–24, 1994.

[5] Jo BW, Kim CH, Tae G, Park JB. Characteristics of cement mortar with nano-SiO2 particles. Const Build Mater 2007; 21(6): 1351-1355.

[6] Ismail MS, Waliuddint AM. Effect of rice husk ash on high strength concrete. Const Build Mater1996; 10(1): 521-526.

[7] Sun MQ, Liu QP, Li ZQ, Hu YZ. A study of piezoelectric properties of carbon fiber reinforced concrete and plain cement during dynamic loading. Cem Concr Res 2000; 30: 1593– 1595.

[8] Sorelli L, Constantinides G, Ulm FJ Toutlemonde F. The nano-mechanical signature of Ultra High Performance Concrete by statistical nano indentation techniques. Cem Concr Res 2008; 38: 1447–1456.

[9] Zivica V. Effects of the very low water/cement ratio. Const Build Mater 2009; 23(8): 2846-2850.

[10] Bui DD, Hu J, Stroeven P. Particle size effect on the strength of rice husk ash blended gap-graded Portland cement concrete. Cem Concr Compos 2005; 27(3): 357–366.

[11] AI-Khalaf MN, Yousift HA. Use of rice husk ash in concrete. Int J Cem Compos Lightweight Concr 1984; 6(4): 241-248.

[12] Lin YH, Tyan YY, Chang TP, Chang CY. An assessment of optimal mixture for concrete made with recycled concrete aggregates. Cem Concr Res 2004; 34(8): 1373-1380.

[13] Tattersall GH, Baker PH, An instigation of the effect of vibration on the workability of fresh concrete using a vertical pipe apparatus, Magazine of Concrete Research 1989; 14 (146): 3–9.

[14] L. Courard, A. Darimont, X. Willem, C. Geers, R. Degeimbre, Repairing concretes with self-compacting concrete: testing methodology assessment, Proceedings of the First North American Conference on the Design and Use of Self-Consolidating Concrete, 2002, pp. 267–274.

[15] Felekoglu B, Tosun K, Baradan B, Altun A, Uyulgan B. The effect of fly ash and limestone fillers on the viscosity and compressive strength of self-compacting repair mortars. Cem Concr Res 2008; 36: 1719–1726.

[16] P.F.G. Banfill, The Rheology of Fresh Cement and Concrete—A Review, 11th International Cement Chemistry Congress, Durban, 2003. 13 pp.

[17] C. Lemmer, J. Hartman, T. Wolf, Rheological properties of mortars for self-compacting concrete, http://www.massivbau.tu-darmstadt.de/user/ lemmer/web/scc/LeDaCon199.htm, 13 pp.

[18] C.F. Ferraris, J.M. Gaidis, Connection between the rheology of concrete and rheology of cement paste, ACI Mater. J. (July–August 1992) 388–393.

[19] C.F. Ferraris,Measurement of the rheological properties of high performance concrete: state of the art report, J. Natl. Inst. Stand. Technol. 104 (1999) 461–478.

[20] M. Cyr, C. Legrand, M. Mouret, Study of the shear thickening effect of superplasticizers on the rheological behavior of cement paste containing or not mineral admixtures, Cem. Concr. Res. 30 (2000) 1477–1483.

[21] C.F. Ferraris, K.H. Obla, R. Hill, The influence of mineral admixtures on the rheology of cement paste and concrete, Cem. Concr. Res. 31 (2001) 245–255.

[22] S. Grzeszczyk, G. Lipowski, Effect of content and particle size distribution of high-calcium fly ash on the rheological properties of cement pastes, Cem. Concr. Res. 27 (1997) 907–916.

[23] F. Lange, N. Mortel, V. Rudert, Dense packing of cement pastes and resulting consequences on mortar properties, Cem. Concr. Res. 27 (1997) 1481–1488.

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Biodegradation of Produce Water Hydrocarbons by Pure Cultures of *Alcaligenes* sp.

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Abstract: Biodegradation studies of hydrocarbons in untreated produce water from an oil production facility in Nigeria was undertaken over a period of time using pure cultures of *Alkaligenes* sp. Isolated from Escravos River where produce water was being discharged as at the time the studies were carried out. Gas chromatography and mass spectrometry were used to monitor the rate of reduction in some petroleum hydrocarbon fractions while the index used to evaluate biodegradation was the decreasing trend in the ratios of nC17/Pristane and nC18/Phytane. Gas chromatographic analysis showed that untreated produced water used for the study had an oil and grease content of 1407mg/L, this includes n-alkanes (608mg/L), Aromatics (13.88mg/L), NSO compounds (12.68mg/L) PAHs(0.833mg/L) and some unidentified greasy components. Upon mechanical treatment, the oil and grease component of produce water was reduced to 44mg/L comprising of n-alkanes (38.40mg/L), Aromatics (2.65mg/L), NSO compounds (1.78mg/L), PAHs (0.0655mg/L) and some unidentified greasy component. A pure culture of *Alcaligenes* sp. after 40 days of exposure to untreated produced water reduced the oil and grease content to 19.58mg/l comprising of n-Alkanes (16.87mg/l), Total aromatics (1.25mg/l), NSO compounds (0.98mg/l) and PAH (0.0096mg/l). This result indicate that produce water is readily biodegradable and pure cultures of *Alcaligenes* sp. used for the study were very efficient in the degradation of produced water hydrocarbons especially the recalcitrant PAH component when compared with the conventional mechanical treatment process. [Journal of American Science 2010;6(4):107-113]. (ISSN: 1545-1003).

Key words: Pure culture, Produce water, Biodegradation, Hydrocarbon, *Alcaligenes* sp.

1. Introduction

Produce water is a by-product of the production of oil and gas hydrocarbons from underground oil reservoirs. When the hydrocarbons are produced, the water component is separated from the oil and gas at the initial stage of processing. For offshore operations, separated produce water is treated and discharged into the sea. Produced water constitutes a source of chronic pollution to the marine environment since it is continuously discharged into the sea over a very long period of time.

The chemical properties of produce water that could cause harmful effect to marine organisms include its elevated salinity, altered ion ratios, low dissolved oxygen, heavy metals, biocides, petroleum hydrocarbons and other organics (Middleditch, 1984). The fate of these components when discharged into the environment will depend upon the chemical and physiochemical properties of the individual components, the properties of the receiving environment as well as the metabolic versatility of the biota in that environment (Neff, 1988).

Produce water are known to be toxic to fishes and other marine animals (Middleditch, 1984). A major cause for concern in recent times has been the presence of polycyclic aromatic hydrocarbons (PAHs) such as anthracene, phenanthrene, benzo(a)pyrene, and benzo(a) anthracene in produced water (OGP, 2002). Some of these compounds are recalcitrant and potential carcinogens and they have the capability to bio-accumulate in food chains since they are not easily degradable (Neff, 1985).

The issue of bio-accumulation or recalcitrant organic compounds from produce water in the marine sediment is a possibility because the produce water treatment systems currently in use by most oil production companies are primarily designed to remove particulate or dispersed oil and therefore has little effect on the concentrations of the dissolved petroleum hydrocarbons such as aromatics and other organics in produce water (Lysyj, 1982).

Biological treatment (breakdown of hydrocarbons by microorganisms) has been suggested in some circles as the best means of breakdown and removal of dissolved aromatic hydrocarbons in produced water (OGP, 2002). Produce water has been proved to be readily biodegradable and both pure and mixed microbial cultures have been used successfully to degrade petroleum hydrocarbons in produce water (Andrea et al, 2001, Okoro and Amund, 2002, Okoro, 2008).

In the present study, an attempt was made to use an ubiquitous bacterial isolate from Escravos river, an *Alcaligenes* sp. which was isolated around the produce water discharge zone to degrade various

components of produce water hydrocarbons with much emphasis on the recalcitrant polycyclic aromatic hydrocarbons (PAHs). *Alcaligenes* sp. has been known to be excellent hydrocarbon degraders (Krooneman et al, 1996), they are small gram negative rod like bacteria and most species are found in marine environment. Some members of this group like the *A. dinifricans, A. odorans,* and *A. eutrophus* are known to be excellent hydrocarbon degraders including the polycyclic aromatic hydrocarbons (Weissenfeis et al, 1990, Harayama et al, 1999).

The aims and objectives of the present study therefore is to evaluate the biodegradation potentials of the *Alcaligene*s sp. isolated around the discharge zone of produce water discharge into the marine environment, especially as it relates to the polycyclic aromatic hydrocarbon components of the produce water hydrocarbon.

2. Materials and Methods:

Sample Collection

Untreated produce water samples were collected with sterile 1000 L Wheaton glass bottles at a point before the final process stream of the Wemco treatment plant at Chevron's Escravos tank farm while the treated produce water samples were collected at a point after the final process stream where it is being discharged to the receiving water.

Enumeration of hydrocarbon utilizing bacteria:

Hydrocarbon utilizing bacterial counts in produced water samples were obtained by using the mineral salts medium of Mills *et al* (1978). The composition of the medium is as follows in (g/L): NaCl (10), $MgSO_4.7H_20$ (0.42), KCl (0.29), KH_2PO_4 (0.83), $Na_2HP\ O_4$ (1.25), $NaNO_3$ (0.42), Agar bacteriological (15), and pH adjusted to 7.2. The medium was autoclaved at 1.1 kg/cm^2 for 15 min. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light) and held in the lid of the Petri dishes. The dishes were wrapped round with a masking tape so as to increase the vapor pressure within the Petri dishes while the plates were incubated at 29 ^{0}C for 6 days after which the growth of hydrocarbon degrading bacteria were observed and counted.

Analytical methods:

Separation of Aliphatic and Aromatic components of hydrocarbons in produced water using High Performance Liquid Chromatography (HPLC).

A measured quantity of the oil sample (10ml) was introduced into the bond elute filter to separate the hydrocarbons from the Nitrogen, Sulfur and Oxygen (NSO) containing components of the petroleum mixture. The filtrate (2 ml) containing both the aliphatic and the aromatic components of the petroleum mixture was injected into the HPLC (WATERS 486). The aliphatic component eluted after 18mins while the aromatic component after 45mins. Each fraction (0.2 µL) was subsequently analyzed by means of Gas chromatography attached to a mass selective detector.

Analysis of Total hydrocarbons, n-Alkanes and Polyaromatic hydrocarbons.

The method used in the analysis was described by Neff et al, (1989). The hydrocarbon extract was concentrated in a Kuderna-Danish flask on a 70^{0}C water bath to approximately 1.0 ml. The concentrated extract was transferred to a 1-dram vial with a disposable pipette and the flask rinsed twice with 1 ml methylene chloride. The rinses were added to the vial and the volume of the extract was reduced to about 1ml with a gentle stream of purified nitrogen gas.

Total n-alkanes and aromatic concentrations were determined by GC-MS analysis of the F1 and F2 fractions respectively. Both resolved and unresolved hydrocarbons were quantified. The resolved concentrations were determined by summing the total resolved area with valley integration and then using an average n-alkane or PAH response factors to calculate an amount relative to the internal standard. The unresolved concentrations were calculated by integrating the total area of the chromatogram (both resolved and unresolved complex mixture (ucm area), subtracting the resolved area and determining the amount relative to the internal standard.

Gas Chromatography of Oils

Fresh and degraded oil were analyzed by Gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1micron film thickness was used. A temperature program of 50-305^{0}C increasing at 3.5^{0}C per minute for 27.15min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was 325^{0}C while the injection port temperature was 305^{0}C. The oil extracts of culture supernatants were dissolved in methylene chloride while a sample volume of 0.2µl was injected. The nC17/Pristane and nC18/Phytane ratios were subsequently calculated from the height of various chromatograms.

Biodegradation and Growth studies

Growth and degradation studies over a time course were carried out using untreated produce water from Escravos tank farm as the sole carbon and energy source. The untreated produce water

used for the study had an initial oil and grease content of 1407 mg/l. Starter cultures were originally prepared using the minimal salts formulations of Mills *et al*, 1988 and the produce water as the sole carbon and energy source. 5 ml. of the pure bacterial culture broth *(Alcaligenes sp.*) was introduced into 500mL of produce water in a 1000 mL capacity wheaton glass bottle. The bottle was covered with a non-absorbent cotton wool and placed in a slanted position to allow air passage through the pores of the cotton wool. Appropriate controls were set up with sterile produce water broth without the test organism. The bottles were shaken manually at regular intervals to allow adequate mixing and homogeneity of the contents. The experimental setup was monitored for a period of 40 days and at every 10 day interval, cultural samples were collected and analysed for microbial load while the residual hydrocarbon was extracted with methylene chloride and analysed by Gas chromatography.

3. Results

The Untreated and Mechanically Treated Produce Water

The untreated produce water used for the experiment had an oil and grease content of 1407mg/L comprising of n-Alkanes (608mg/l), aromatics (13.88mg/l), NSO compounds (12.68 mg/l) and PAHs (0.833mg/l) and an initial nC17/Pristane and nC18/Phytane ratios of 1.41 and 2.93 respectively while the mechanically treated produced water used as a reference had an initial oil and grease content of 44mg/l comprising of n–alkanes (38.4), aromatics (2.65), NSO compounds (1.78) and PAHs (0.0655) and an initial nC17/Pristane and nC18/Phytane ratios of 1.24 and 3.0 respectively. The GC chromatograms of both the untreated and the mechanically treated produced water are shown in Figs. 1 and 2 respectively.

Biodegradation of Produced water hydrocarbons with pure cultures of *Alcaligenes* sp.

The GC Chromatograms of untreated produce water after 40 days of exposure to pure cultures of Alkagenes sp. are shown in Figure 3, while the corresponding nC17/pr. And nC18/ph. Ratios are shown in Figure 4. It was observed that after 40 days of exposure, the pure culture of *Alcaligenes* sp. reduced the oil and grease content of produced water from 1407mg/l to 19.58mg/l comprising of n-Alkanes (16.87mg/l), Total aromatics (1.25mg/l), NSO compounds (0.98mg/l) and PAH (0.0096mg/l). On the corresponding levels of nC17/pr. and nC18/ph. Ratios, it was observed that both ratios dropped below 1 at day 40 and this signifies significant biodegradation.

4. Discussion

Alkagenes sp is a predominant microbial flora of Escravos river, found mostly within the discharge zone of produce water effluents (Okoro, 1999). The evaluation of its biodegradation potential is therefore necessary because of the persistence of significant soluble hydrocarbon fractions from produce water around the discharge zone.

Most *Alkaligenes sp*. are known to be good hydrocarbon degraders (Krooneman et al, 1996), some species like *Alkaligenes odorans, A. dinifricans, and A.eutropheus* have been implicated in the degradation of Polyclorinated biphenyls in mixed culture (Clark, 1979) while others have been implicated in the degradation of Polycyclic aromatic hydrocarbons (Weissenfel et al, 1990) but produce water hydrocarbons have not been implicated in any of these studies.

The *Alkaligenes sp*. used in the present study degraded total petroleum hydrocarbons in produce water from its original concentration of 1407 mg/l to 19.58mg/l after 40 days of exposure. The PAHs were equally degraded from its original concentration of 0.833mg/l to 0.0096mg/l. These results were better when compared with the total TPH and PAH levels of mechanically treated produce water which were 44 and 0.0655 mg/l respectively. The degradation profile and pattern of *Alkaligenes sp.* used in the study was similar to the results obtained with pure cultures of *Achromobacter sp*. (Okoro and Amund, 2002), the only difference was that *Alkaligenes* species were more predominant than *Achromobacter* in mixed cultures.

The index used to monitor the progress of biodegradation is the rate of decrease in the ratios of nC17/Pristane and nC18/Phytane. Pritchard and Coaster (1991) used the same index to monitor the progress of biodegradation during the EPA Alaska oil spill biodegradation project. The application of this concept is based on the principle that during biodegradation, decreases of total oil residues could occur because of other non biological processes, thus changes in hydrocarbon composition that are indicative of biodegradation must be measured accurately. This is done historically by examining the weight ratios between hydrocarbons known to be readily biodegradable such as the C17 and C18 alkanes and those that biodegrade slowly such as the branched alkanes (Pristane and Phytane) but with very close chromatographic behaviour. A weight ratio less than 1 signifies considerable biodegradation Pritchard and Coaster (1991).

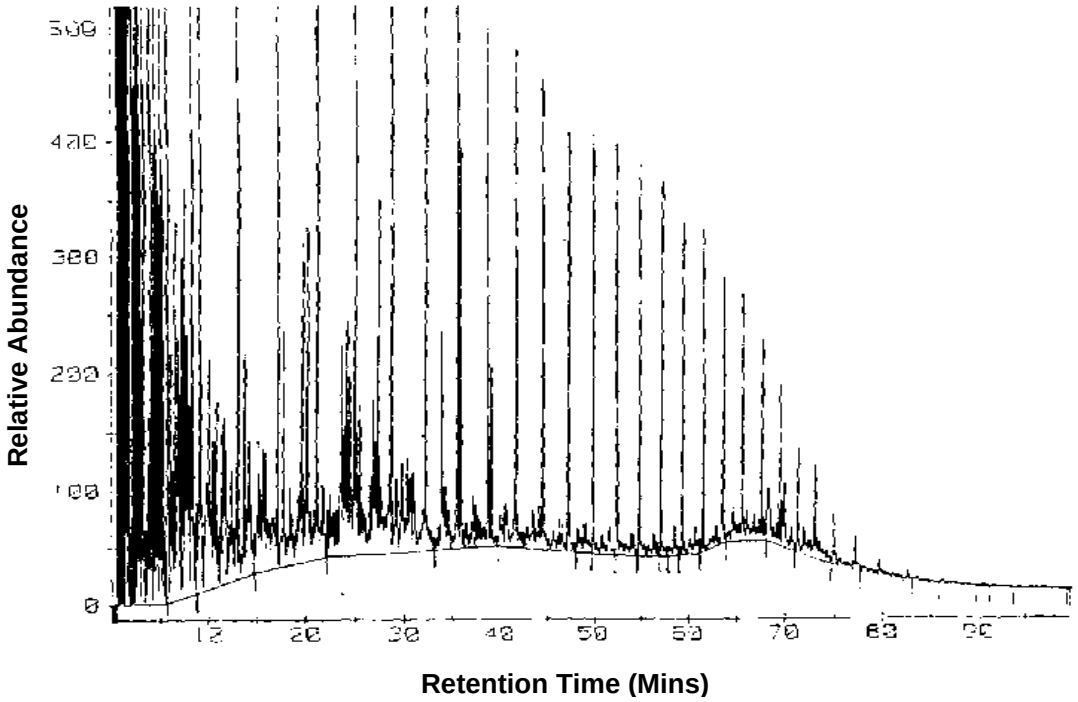

Figure 1: GC Chromatogram of untreated produced water from Escravos tank farm (nC17/pr. Ratio = 1.41, nC18/ph. Ratio = 2.93)

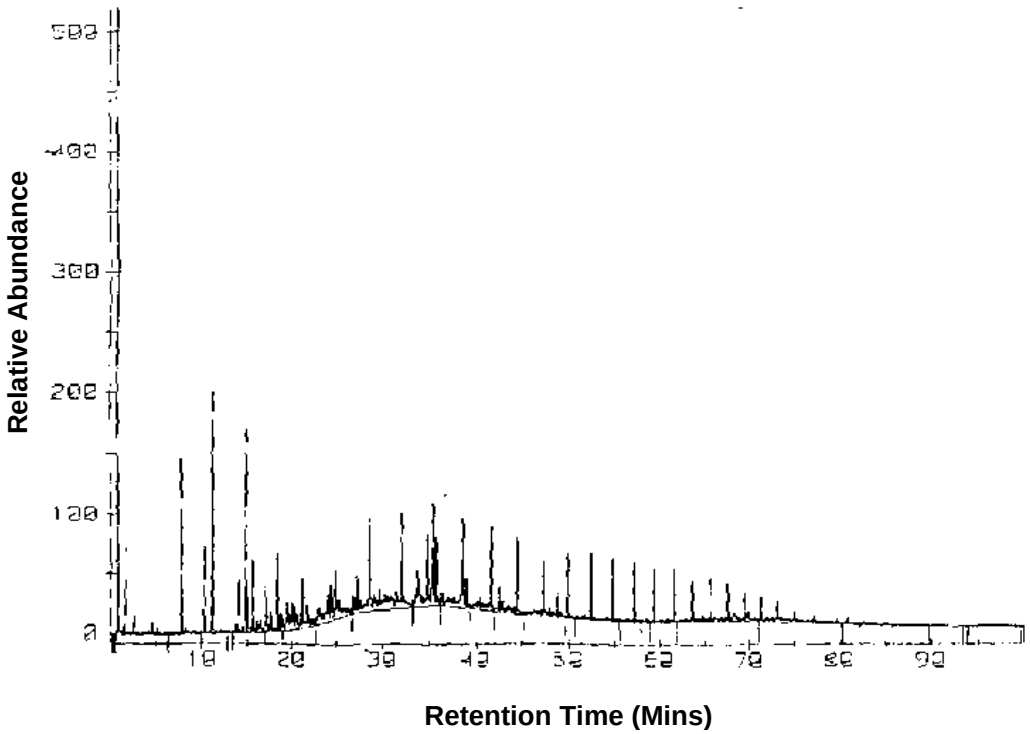

Figure 2: GC Chromatogram of mechanically treated produced water from Escravos tank farm (nC17/pr. Ratio = 1.24, nC18/ph. Ratio = 3.00)

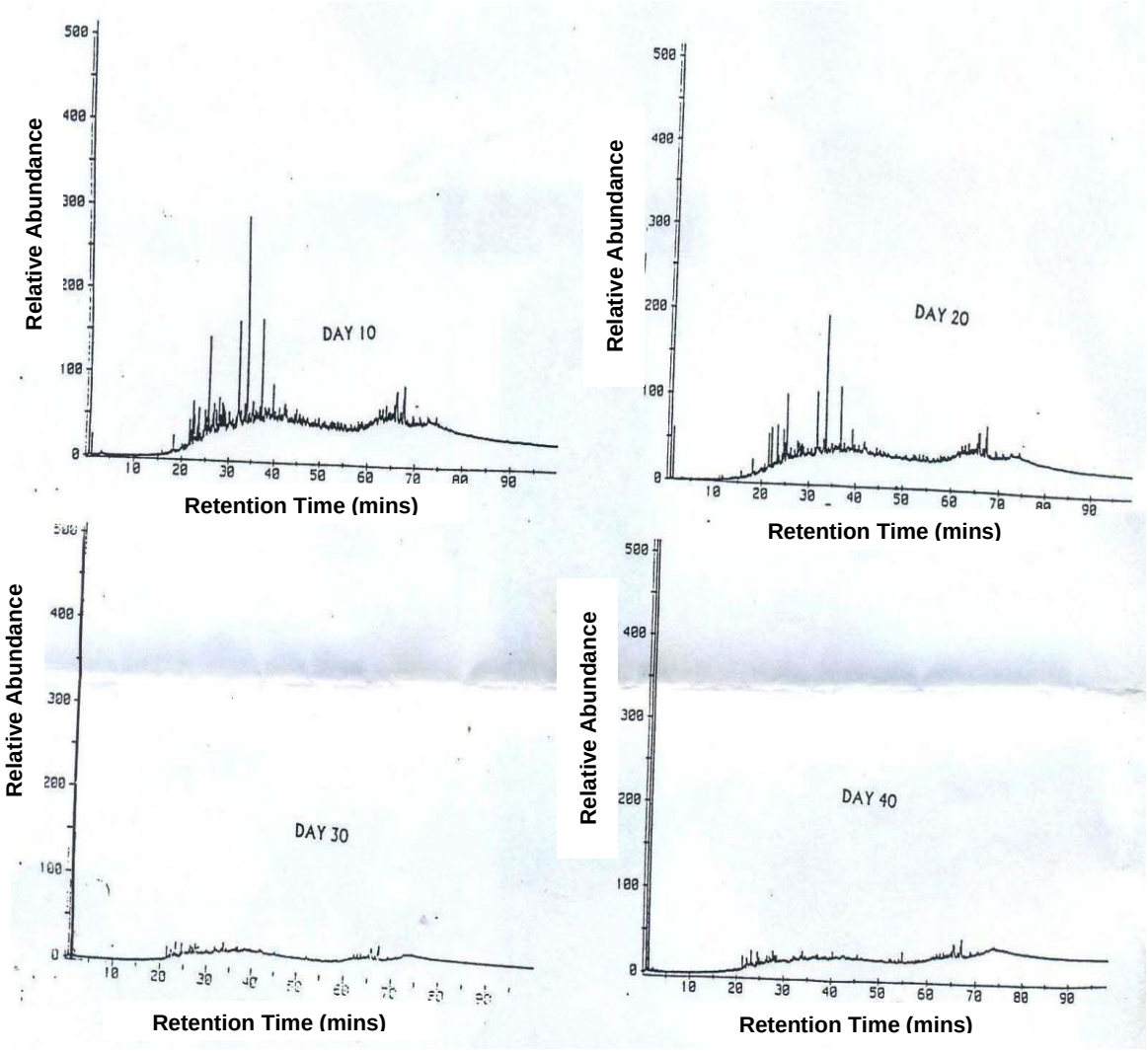

Fig.3. GC Chromatograms of untreated produce water after a 40 day exposure to a pure culture of Alcaligenes sp.

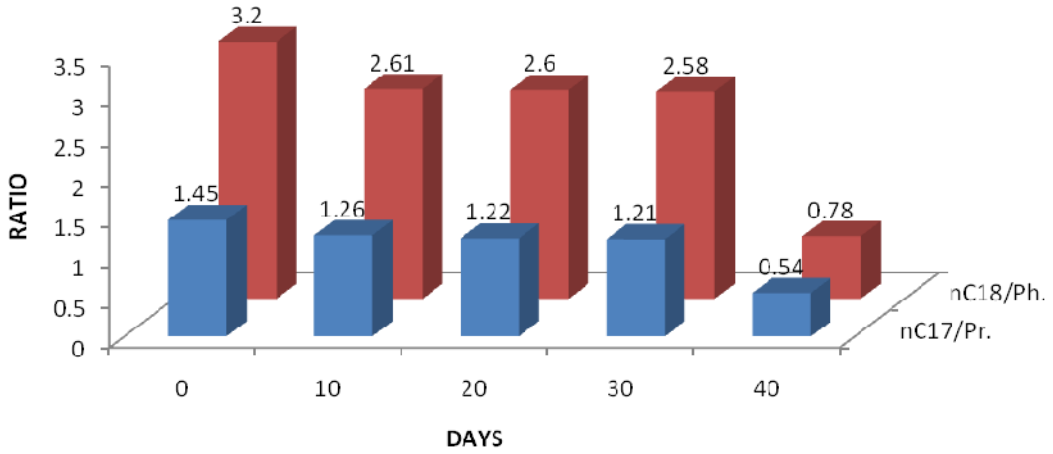

Fig. 4: Biodegradation of untreated produce water with pure cultures of *Alcaligenes sp*. The result is evaluated as decrease in nC17/Pristane and nC18/Phytane ratios

After 40 days of exposure of produce water with pure cultures of *Alkaligenes sp*., the weight ratios of nC17/Pristane and nC18/Phytane was less than 1 (0.54 and 0.78 respectively) and this according to Pritchard and Coaster (1991) signifies significant biodegradation.

In conclusion therefore it can be advanced that Produce water effluents from Chevrons Escravos tank farm used in the study is readily biodegradable and the *Alkaligenes sp*. found within the vicinity of produce water discharge zone are capable of degrading produce water hydrocarbons and most especially the sparingly soluble components such as PAHs that are very difficult to remove with the conventional mechanical treatment presently in use. This is a sure indication that the *Alkaligenes* sp. that dominate microbial activities at the discharge zone of produce water effluents can be beneficial to the environment because of its excellent hydrocarbon degradation potential.

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References:

1. Andrea RC, Tania AA, Lucia RD Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Braz. J. Microbiol. 2001; 32(4):124-129.
2. Ayers RC, Parker, M. Offshore Produce water management-Technical report to CAPP. 2003. No:29125.
3. Clark RR. Biodegradation of Polychlorinated biphenyls with mixed cultures of Alkaligenes sp. Appl. Environ. Microbiol. 1979; 37(4):680-685.
4. Harayama S, Keshira H, Kasai Y, Shutsubo K. Petroleum Biodegradation in Marine Environments. J. Molec. Microbiol. Biotechnol. 1999;1(1):63-70
5. Krooneman, J, Wieringa EB, Moore ER, Gerritse J, Prius RA, Gottschal JC. Isolation of Alkaligenes sp. strain L6 at low oxygen concentration and degradation of 3-chlorobenzoate via a pathway not involving Chlorocathecols. Appli. Environ. Microbiol. 1996;62(7):2427-2434.
6. Lysyj L. Chemical composition of produced water in some off- shore oil platforms. Report to USEPA Municipal Environmental Research Lab. Cincinnati, OH. 1982 ;600/ 282-034.
7. Middledich BS. Ecological effects of produced water discharges from offshore oil and gas production platforms. Final report on API project. American Petroleum Institute, Washington, D.C. 1984 NO. 248.
8. Mills AL, Breuil C, Colwell RR. Enumeration of petroleum-degrading marine and estaurine microorganisms by the most probable number method. Can. J. Microbiol. 1978. 24: 552-557
9. Neff JM. The potential impacts of drilling fluids and other effluents from exploratory drilling in the living resources.. R.H.Bachus (ed.) Cambridge Massachusetts. 1985;224
10. Neff JM, Sauer TC, Maciolec N.. Fate and Effects of Produce water discharges in near shore marine waters. American Petroleum Institute, Washington D.C. API Publication No. 4472. 1989.
11. OGP. Aromatics in Produce water: Occurence, Fate, Effects and Treatment. OGP Publications. No. 1.20/324, 2002. International Oil and Gas Producers England.
12. Okoro CC. Microbial degradation of hydrocarbons in produced water from crude oil production operations in Escravos tank farm. Ph.D. Thesis, University of Lagos. 1999;269 .
13. Okoro CC, Amund OO. Biodegradation of hydrocarbons in untreated produce water using pure and mixed microbial cultures.;J. Sci. Tech. and Environ. 2002;2(2): 14-23
14. Okoro CC. Biodegradation of hydrocarbons in untreated produce water using pure fungal cultures. Afr. J. Microbiol. Res. 2008 2:217-223.
15. Pritchard, P. H. and Coaster, C.F. EPA'S Alaska oil spill bioremediation project. Environ. Sci. Technol. 1991;25 (3): 372-373
16. Read AD. Treatment of oily water in North Sea Oil installations. In: Oil Water Discharges; Regulatory, Technical and Scientific Considerations. Johnson C.S.and Morris, R.J. (ed). Applied Science Publishers, Barking Essex, England. 1978;127-136.
17. Weissenfeis WD, Beyer M, Klein J, Rehm HJ. Microbial metabolism of Fluoranthene by pure bacterial cultures. Appl. Microbiol. Biotechnol. 1990;32:479-484.

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Recognition of Nonrandom Patterns on Supply Performance by Employing Statistical Monitoring

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Abstract:

This paper introduces a practical methodology of assignable signals and Run chart tests for identification of nonrandom patterns of supplier performance by statistical monitoring. The assumption of normal distribution is one of the important factors to implement a control chart in industry and service. It is supposed that natural data shows lack of any nonrandom pattern signals or out of control points on control chart. The data of supplier's on-time delivery for automotive industry has been gathered and illustrated on control chart by employing appropriate transformation and assignable signals and run chart were tested on the control chart accordingly. The results show that tests were able to identify nonrandom patterns of supplier performance data. Out of control signals were removed from the control chart and show that on-time delivery performance was increased accordingly. The control chart with natural pattern can be used as pilot for monitoring supplier delivery over time and improve supplier delivery performance. [Journal of American Science 2010;6(4):114-122]. (ISSN: 1545-1003).

Key words: Run Chart Pattern Recognition (RCPR); Johnson Transformation; Supply Performance; Statistical Process Control (SPC); Anderson-Darling test (AD); On-Time Delivery (OTD)

1. Introduction:

In the 70's supply chain concentrated mainly on integration of warehousing and transportation within the company. In the 80's the focus of supply chain management moved to re-engineering of cost structured. At the end of 80's the focus again shifted from cost reduction to enhancing customer service. Today, successful SCM needs the recognition that the firm is simply one of the players in the long chain that begins with suppliers and includes transportation, manufacturers and customers (Rahul & Altekar, 2005). Firms cannot effectively compete in isolation of their suppliers and other parties in the supply chain. Interest in the approach of supply chain management has steadily increased since the 1980s when companies faced the benefits of cooperative relationships within and beyond their own company (Lummus & Vokurka, 1999).

Araz and Ozkarahan (2007) asserted that collaborating with the appropriate suppliers and managing them is taking more important now with the strategic cooperation being implemented with suppliers to obtain a competitive advantage and the involvement of suppliers in product development phases. Therefore, effective methods that have the capability of evaluating and continually monitoring suppliers' performance are still needed. Modern markets are competitive business environments where customers need their suppliers to be dependable in delivering on-time lots. One of the important goals of supply chain management is to improve delivery performance. In this context, the investment is needed to reduce delivery variance to a targeted goal as a part of an overall continuous improvement plan to improve supply chain performance (Guiffrida and Jaber, 2008). Moreover, delivery capabilities are highly important followed by production abilities, while value-adding capabilities such as process, managerial, financial, as well as communication/networking capabilities are also concerned as important when selecting a supplier (Pressey et al., 2009).

Ittner et al. (1999) examined whether supplier selection and monitoring practices affect the integration between supplier strategies and organizational performance. Automakers make significantly greater employ of suppliers for new product and process ideas and for accelerating the development process, and attend sessions at the

supplier's firm more frequently. It is concluded that higher use of advanced selection and monitoring practices tends to increase profitability, product quality, and supplier performance in companies following supplier collaborative strategies, but has little effect on the performance of firms utilizing arms-length transactions. According to the conducted researches and cited by authors (Shin et al., 2000; Toni and Tonchia, 2001; Paulraj et al., 2006; Robb et al., 2008), it has been concluded that delivery performance, quality and cost are as most important indicators of supplier performances and monitoring tools are needed to control and improve supply performance indicators accordingly.

On the other hand, Statistical Process Control (SPC) is an effective tool for monitoring and evaluation of indicators over time. SPC is one of the techniques employed in quality assurance programs, for evaluating, monitoring and managing a process either manufacturing or service through the use of statistical methods (Anthony et al., 2000). The aim of any type of data analysis is to obtain understanding from data. When process performance data are monitored, it represents that it varies. The information in this variation is important to the understanding of how the process is performing and SPC is primarily the tool for understanding variation (Stapenhurst, 2005). It is because that the importance of quality has been long concerned as vital for both competition and survival in the business world. As such, more firms have adopted the use of SPC as a tool for achieving higher product quality (Duffuaa and Ben-Daya, 1995). This paper is aimed to represent the employing of control chart to monitor supply performance and how it can help companies to track their supplier performance by identification of caused signals on control charts. Moreover, using run chart test will be discussed accordingly. In this context, authors review on literature of assignable signals and common signals and patterns recognition. Montgomery (1997) cited that most processes do not perform in a state of statistical control. Consequently, the routine and attentive use of control charts will recognize assignable causes. If these causes can be removed from the process, variability will be reduced and the process will be improved. Figure 1 shows the process improvement tasks using the control chart. However, the process is out of control if any one or more of special caused rules for Shewhart's control chart is happened. A control chart may represent out-of-control signals either when one or more points fall beyond the control limits or the plotted points show some nonrandom pattern of behavior. The problem is pattern recognition, which is a systematic or nonrandom pattern on the control chart and identifying the reason for this behavior. Levine et al. (2001) asserted that a stable process is in a state of statistical control and has just chance or common signals of variability performing it.

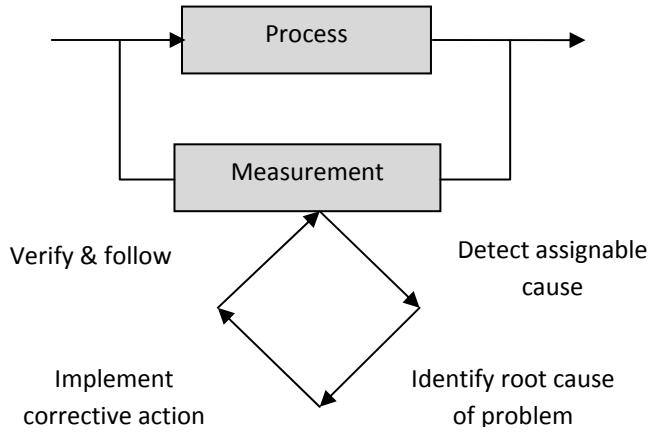

Figure 1. Process improvement using control chart (Montgomery, 1997)

Likewise, special causes of variability have relatively large impacts on the process and are not inherent to it. The circumstances or factors that cause this kind of variability may be recognized. The simplest rule for detecting the presence of a special cause is one or more points that fall beyond $\pm 3\sigma$ limits of the control charts. Monitoring of a process with a control chart can also support in process improvement. Pyzdek (2003) mentioned that a phenomenon will be cited to be controlled when, through the use of past history, it can be forecasted at least between control limits, how the phenomenon may be expected to vary in the future. A process control system is essentially a feedback system that integrates process outcomes with process inputs as depicted in Figure 2.

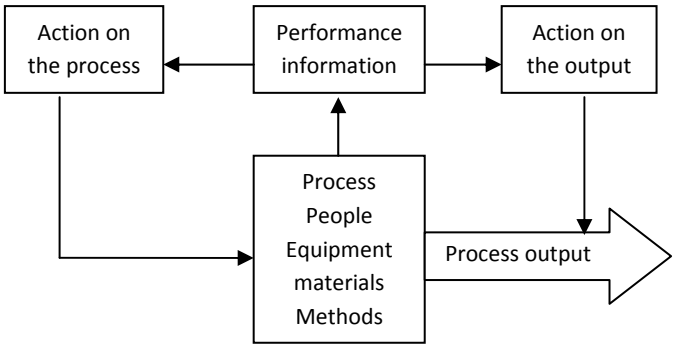

Figure 2. A process control system (Pyzdek, 2003)

Moreover, control chart interpretation is an art that can only be developed by looking at many

control charts and probing patterns to identify the underlying system of cause at work. Eight sensitive run tests were indicated for analyzing the patterns variation in the various zones of control chart.

Besterfield (2009) asserted that when a process is in state of control, there occurs a normal pattern of variation, which is represented by control chart. Control limits (UCL and LCL) are usually structured at 3 standard deviations from the central line (CL). They are used as a basis to judge whether there is evidence of lack of control. When a process is out-of-control, the cause signals responsible for the condition must be identified. As such, Evan and Lindasay (2008) mentioned that when a process is in statistical control, the points on a control chart fluctuate randomly between the control limits with no recognizable pattern. Table 1 represents the recommended nonrandom patterns by several authors. It can be concluded that trend pattern, cyclic pattern, mixture and shift in mean are most highlighted patterns which should be recognized and corrective actions should be taken in account.

Table 1. Recommended nonrandom patter

Authors	Non random recommended patterns
Montgomery, (1997)	Cyclic pattern; Mixture pattern; Shift in process level pattern; Trend pattern; Stratification pattern
Levine et al., (2001)	The cyclic pattern; The mixture pattern; The trend pattern; The stratification pattern; Shift in process level pattern
Pyzdek, (2003)	Freaks; Drift; Cycles; Repeating patterns; Discrete data; Planned changed; Suspected differences; Mixture
Evan and Lindasay (2008)	On point out of control limits; Sudden shift in process average; Cycles; Trends; Hugging the centre line; Hugging the control limits pattern instability
Besterfield, (2009)	Change or jump in level; Trend or steady change in level; Recurring cycles; Two populations; Mistakes

Alwan (2000) discussed that how assignable patterns can be recognized by run chart tests and hypothesis testing evaluate them. In this context; a simple numerical check of randomness of a series is named a runs test. The run test classifies observations as being above (+) or below (-) some central line, usually the sample mean, which is the default value in Minitab software. To place the problem in a statistical hypothesis-testing framework, the following proposed hypothesis can be considered:

- *Null hypothesis* H_0:
  Process is random
- *Alternative hypothesis* H_1:
  Process is nonrandom

The number of runs observed from a process is one possible test statistic for deciding whether a process is random or not. A p *value* of hypothesis is defined as the probability of obtaining as observed sample value that deviate as far, or farther, from the expected value of the test statistic when the null hypothesis is true. In general, for a specified value of significant level α :

- If p *value* $\geq \alpha$, *accept* H_0
- If p *value* $< \alpha$, *reject* H_0

This paper aims to create connection between supply chain performance measurement by statistical monitoring and then test whether nonrandom of suppliers' delivery indicator can be recognized based on the run chart test or not. As nowadays, application of SPC has deployed to service and measuring performance indicator of systems. Although control charts were first developed and used in a manufacturing context, they are easily applied to service organizations such as hospital, bank, insurance company, post office, ambulance, police department, hotel transportation and auto service (Evans &Lindsay, 2008).

2. Methodology

Minitab identifies special cause signals as presented in Table 2. Out-of-control points appeared on the control charts labeled with rule 1 and caused signal patterns appeared and labeled from rule 2 until 8. Out of control signals are removed from variable and attribute control charts to make them "In control".

Many analyses require an assumption of normality. In SPC methodology, quantitative standard control charts are often based on the assumption that the observations are normally distributed. In practice, normality can fail and consequently the determination of assignable causes may result in error (Fournier et al., 2006). The Anderson-Darling statistic is a measure of how far the plot points fall from the fitted line in a probability plot. A smaller Anderson-Darling statistic indicates that the distribution fits the data better. If the $p-value$

(when available) for the Anderson-Darling test is lower than the chosen significance level (0.05 in this research), conclude that the data do not follow the specified distribution. Moreover, the Johnson transformation function is selected from three types of functions in the Johnson system. Consequently, the run chart shows if special causes are influencing your process. Run Chart performs two tests for randomness that provide information on the non-random variation due to trends, oscillation, mixtures, and clustering (Minitab, 2006).

Table 2 Assignable caused signals (Minitab, 2006)

Rules	Assignable caused signals
1	1 point > 3 standard deviation from centre line
2	9 points in a row on same side of centre line
3	6 points in a row, all increasing or all decreasing
4	14 points in a row, alternating up and down
5	2 out of 3 points > 2 standard deviation from centre line
6	4 out of 5 points > 1 standard deviation from centre line
7	15 points in a row within 1 standard deviation of centre line
8	8 points in a row > 1 standard deviation from centre line

Also, Castagliola and Castellanos (2008) investigated on process capability indices for bi-variate non normal distributions and cited that one possible solution for solving the problem of non normality is the use of Johnson system of distributions. According to reviewed literature, proposed methodology has been depicted in Figure 3.

The proposed methodology model is titled Run Chart Pattern Recognition algorithm (RCPR). To synchronization with process improvement in SPC as last authors have mentioned before, RCPR is homogenous built based on PDCA philosophy. It begins with identification of performance indicator of supply chain and goes through gathering data. Then gathering data, normality test and if required, doing transformation on data to meet data normality distributed. According to Minitab, eight special caused signals will be tested and moreover, run test is employed to identify nonrandom patterns on data. Root cause analysis is planned either to define corrective action or aiming with improvement plans.

To test the proposed methodology, data were gathered from automotive industry in Iran. The data are about on-time delivery of an OEM's vendor for 88 deliveries over time. The on-time delivery formulation was defined by the automaker as equation 1:

Equation 1:

$$OTD = \frac{\text{The number of on time delivered parts}}{\text{The number of ordered parts}} \times 100$$

3. Result and discussion

According to RCPR, on-time delivery (OTD) was selected as an important indicator of supply performance measurement. Data were depicted in Figure 4.

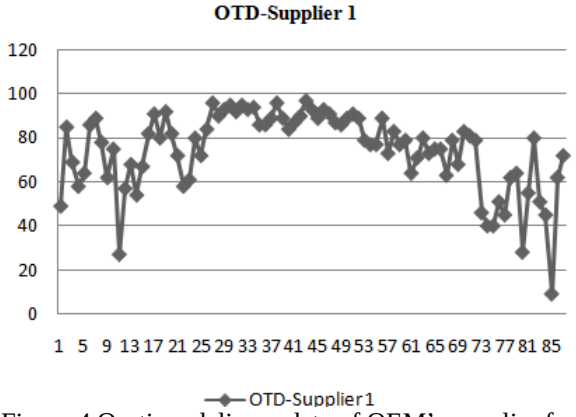

Figure 4 On-time delivery data of OEM's supplier for 88 deliveries to automaker

Data gathered of OEM's supplier was tested by Anderson-Darling (AD) normality test by significant level $\alpha = 0.05$ and results show that supplier OTD data are not normality distributed. Based on the proposed methodology, AD normality test shows that P-value is lower than 0.005 and the transformation should be employed. Johnson transformation was done on OTD-Supplier 1 data and results demonstrate that data are normally distributed in significant $\alpha = 0.05$. The optimal transformation function was recognized as equation 2:

Equation 2:

$$OTD\ Transformation\ function = -1.48174 + 0.946084 \times Ln\left(\frac{X +}{98.}\right.$$

$$Where\ X: on-time\ delivery\ \%$$

Figure 5 illustrates that outcome of transformation function on data before and after conversion.

As shown in Figure 5, $P-value$ for best fit after transformation is gained as 0.986781 which is higher than significant level $\alpha = 0.05$.

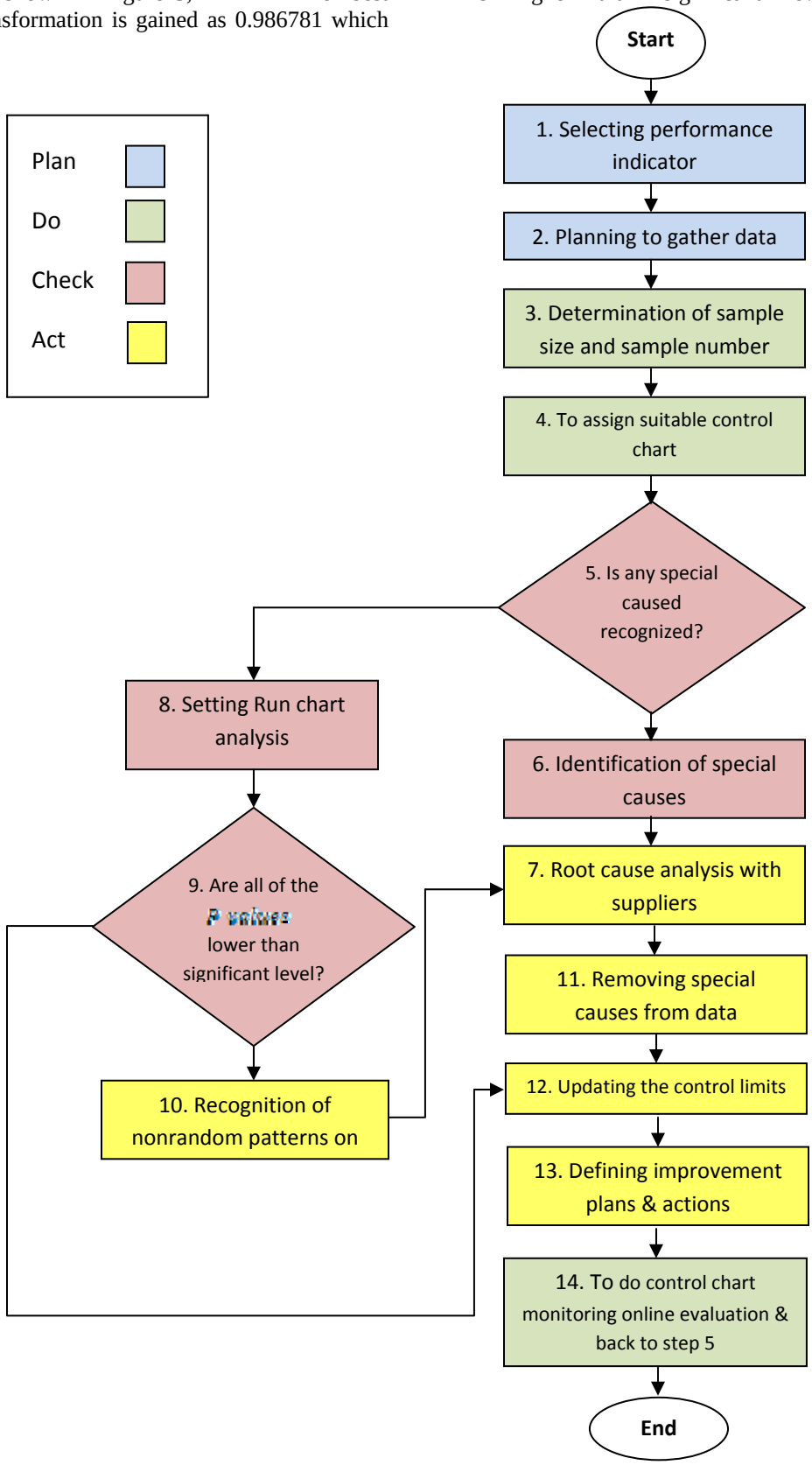

Figure 3. Process improvement by employing Run Chart Pattern Recognition algorithm (RCPR)

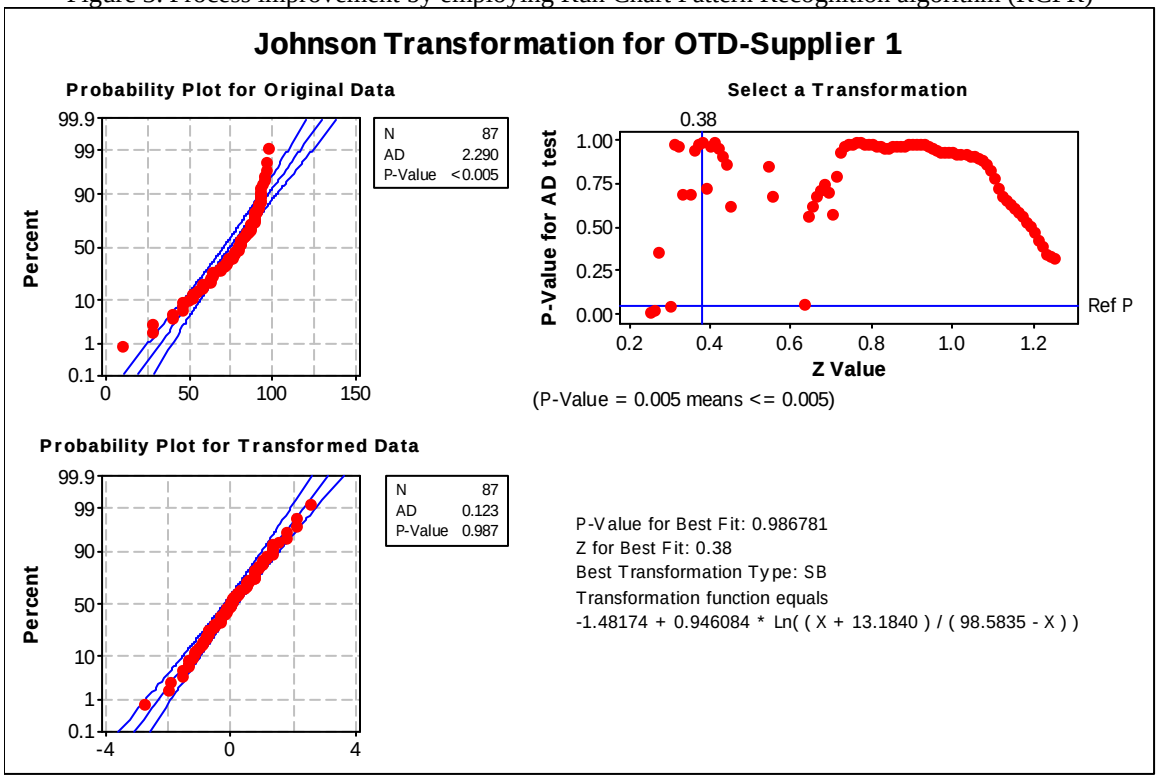

Figure 5. Johnson's transformation on "on-time delivery" data

Now the transformed data can be used for running control charts. An important point should not be forgotten that when data are not normally distributed, Central Limit Theorem (CLT) also may be employed. According to Hayter (2007), if $X_1, \cdots, X_n$ is a sequence of independent identically distributed random variables with a mean μ and a variance σ^2, then the distribution of their average $\bar{X}$ can be approximated by a $N\left(\mu, \frac{\sigma^2}{n}\right)$ distribution. Similarly, the distribution of the sum $X_1 + \cdots + X_n$ can be approximated by a $N(n\mu, n\sigma^2)$. When data are not normally distributed and we are interested to use $\bar{X}, R$; $\bar{X}, S$ control charts, it can be concluded that CLT can be employed. In this research, individual delivery monitoring was purposed and due to that the sample size or each delivery amount was targeted. In this context, individual X and moving range control chart (Figure 6) was illustrated and out-of-control signals were identified with red points accordingly. Moreover, Figure 6 represents that special cause signals as labeled by 1, 2, 5 and 6 were recognized on control charts according to mentioned rules in Table 2. Those signals should be analyzed and root causes to be identified by supplier cooperation.

Assignable caused signals are represented by red points on the control chart (Figure 6). It shows that delivery performance is affected with nonrandom condition and those should be identified and to be removed from control chart. It should be mentioned here that additive trend on delivery performance shows a desirable situation for both suppliers and their client even it is an out of control signals or assignable pattern. However, it should be investigated for further improvement in delivery process. In practice, when customers are monitoring their suppliers, while assignable cause signals alarmed (red points such as 11, 15, 74, 75, 80 and so on) root cause analysis should be taken in account to prevent delivery downturn.

Based on the RCPR algorithm, two consecutive steps were designed to identify out-of-control signals on data, out-of -control tests on control chart (Table 2's rules) and run chart tests (pattern recognition tests) included clustering, trends, mixtures and oscillation tests.

Figure 7 depicts the run chart test on OTD-Supplier 1 data and either the clustering test's $p-value$ is less than the significant level

($\alpha = 0.05$) or trend test and indicates a tendency for clustering and trend on on-time delivery data. The p value of mixtures and oscillation tests is higher than significant level. It can be concluded the data are included nonrandom patterns and according to RCPR algorithm, root cause analysis should be placed in account.

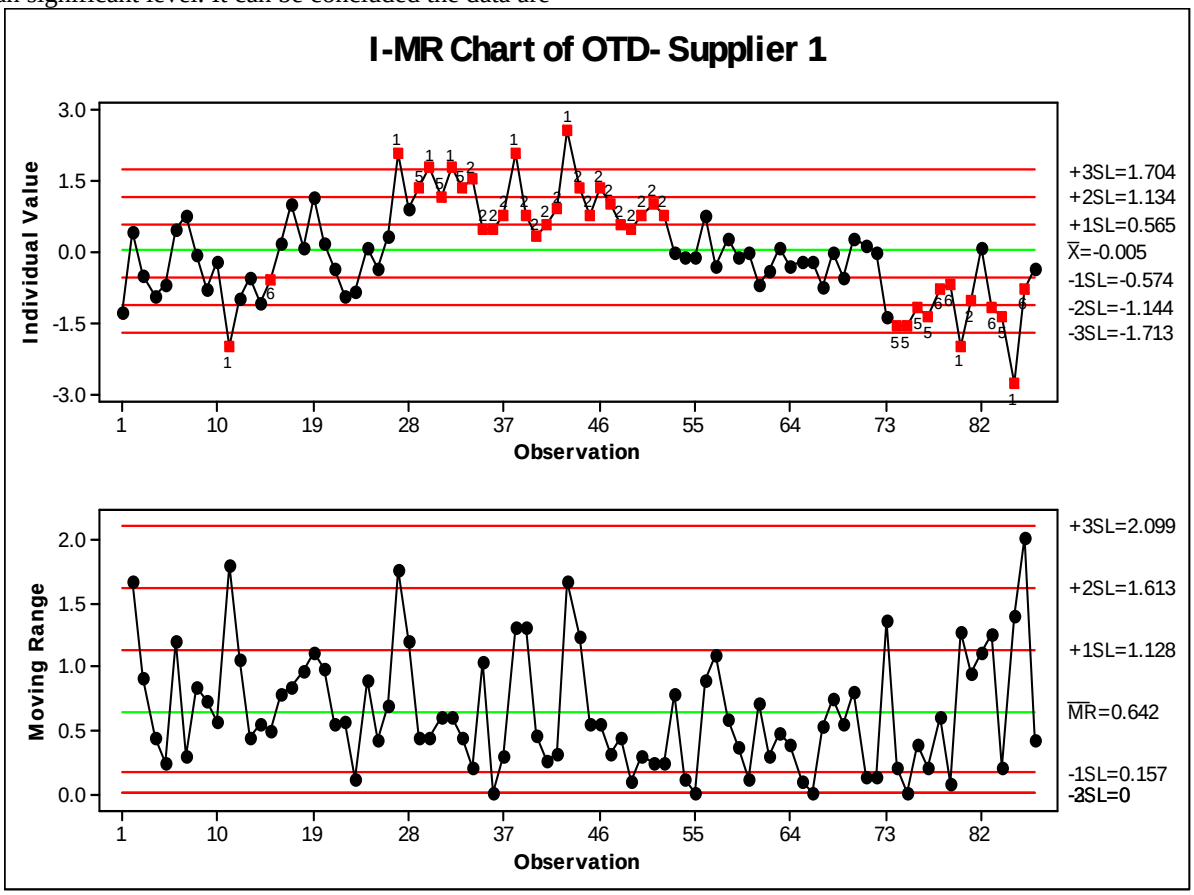

Figure 6. Individual X and moving range control chart for OTD transformed data

According to RCPR, the hypothesis in this section test whether OTD data has any nonrandom pattern signals. To test:

H_0:
$OTD - Supplier\ 1\ data\ has\ a\ random\ sequence$
H_1:
$OTD - supplier\ 1\ data\ has\ not\ a\ random\ sequence$

At Significant level $\alpha - 0.05$

$P - Value\ for\ clustering = 0.000$,
$P - Value\ for\ Mixtures = 1.000$
$P - Value\ for\ Trends = 0.043$,
$P - Value\ for\ Oscillation = 0.957$

A $p - Value\ for\ clustering = 0.000 < 0.05$, the null hypothesis is rejected and can be concluded that data has nonrandom sequence as recognized as clustering and likewise, as $p - Value\ for\ Trends = 0.043 < 0.05$, the null trend hypothesis test is rejected and can be concluded that data has nonrandom sequence as recognized by trend. According to RCPR, the next step is to identify the source of out of control signals and removing out of control points from primary control chart. In the first attempt to remove out of control signals from Individual X control chart (Figure 6), it was resulted that the mean of OTD was increased from -0.005 to 0.029. It can be interpreted that eliminating of out of control signals can lead to increase the delivery performance over time.

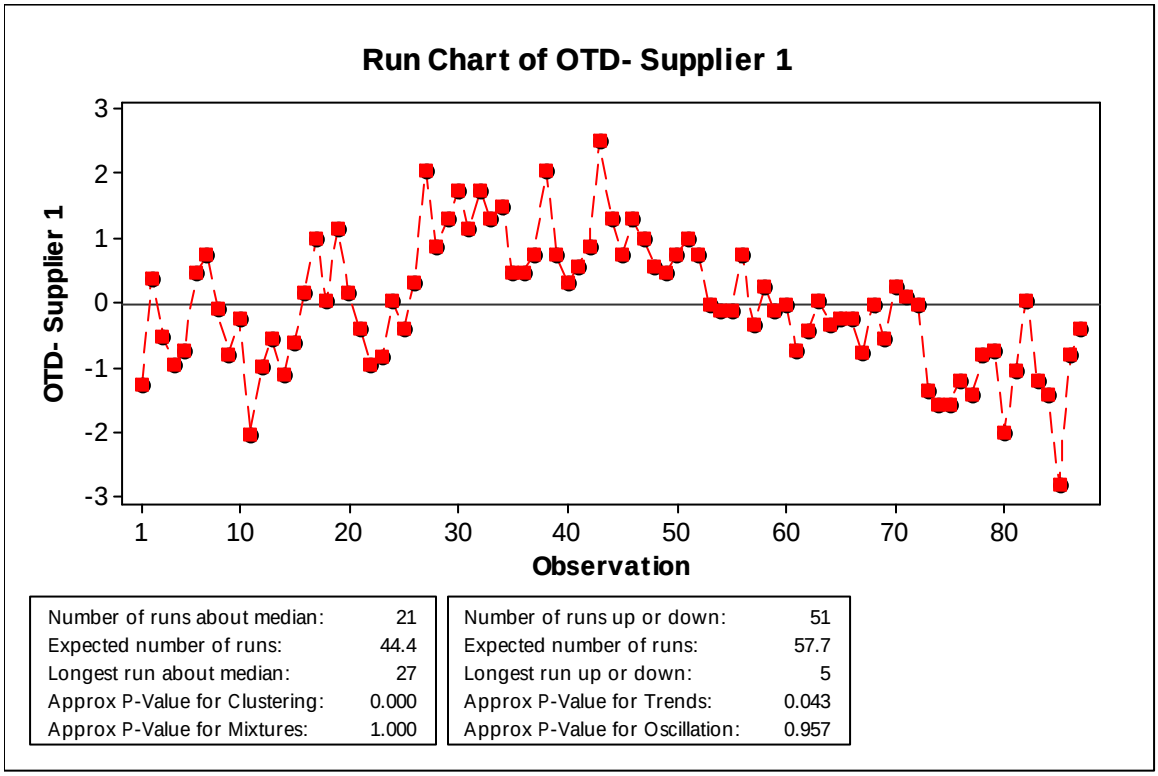

Figure 7 Run chart analysis on OTD-Supplier 1 data

4. Conclusion

Based on the mentioned methodology, run chart pattern recognition algorithm (RCPR) algorithm was proposed to identify the nonrandom supply performance patterns. The results show that caused signals can be identified by two consecutive steps. First, users can use Showhart's rules to identify the caused out of control signals and likewise run chart test which included trend, mixtures, oscillation and clustering tests. According to the gathered data from OEM for on-time delivery indicator, individual delivery and moving range chart was employed to monitor the supply trend and recognition of out of control signals. Afterwards, the run chart was provided with sample size $n = 1$ to recognition of caused signals. The results support that runs chart tests can recognize the nonrandom patterns on data in significant $\alpha = 0.05$. According to the results, it can be concluded the supplier delivery performance was affected by assignable trend and clustering pattern which does not let the process smoothly perform. Eliminating assignable signals (both run tests and Table 1's rules) assist to enhance delivery performance accordingly. In practice, central theorem limit (CLT) also can be used to establish the control chart. One of the advantages is that scale of data will not be changed and the essence of data will be kept accordingly. It was resulted that the mean value of transformed OTD was enhanced from -0.005 to 0.029. It can be interpreted that eliminating of out of control signals can lead to increase the delivery performance over time. In practice, when customers are monitoring their suppliers; while assignable cause signals root cause analysis should be taken in account to prevent delivery downturn. It make a feed back to supplier and let them go through their firm's processes and do problem solving activities to improve the performance and finally increasing OTD control chart central limit accordingly.

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References

1. Alwan, L. C. (2000). *Statistical Process Analysis* (first ed.): McGraw-Hill.
2. Anthony, J., et al. (2000). Key ingredients for the effective implementation of statistical process control. *Integrated manufacturing system, 49*, 242-247.

3. Araz, C., & Ozkarahan, I. (2007). supplier evaluation and management system for strategic sourcing based on a new multicriteria sorting procedure. *International journal of production economies, 106*, 585-606.
4. Besterfield, D. H. (2009). *Quality Control* (8 ed.): Prentic Hall.
5. Castagliola, P., & Castellanos, J.-V. G. (2008). Process capability indices dedicated to bivariate non normal distributions. *Journal of Quality Maintenance engineering, 14*(1), 87-101.
6. Duffuaa, S. O., & Daya, M. B. (1995). Improving maintenance quality using SPC tools. *Journal of quality in maintenance engineering, 1*(2), 25-33.
7. Evans, J. R., & Lindsay, W. M. (2008). *Managing for Quality and Performance Excellence* (7 ed.): THOMSON.
8. Fournier, B., et al. (2006). Application of the generalized lambda distributions in a statistical process control methodology. *Journal of Process Control, 16*(10), 1087-1098
9. Guiffrida, A. L., & Jaber, M. (2008). Managerial and economic impacts ofreducing delivery variance in the supply chain. *Applied Mathematical Modeling, 32*, 2149-2161.
10. Hayter, A. (2007). *Probability and Statistics for engineers and scientifics* (3 ed.): THOMSON
11. Ittner, C. D., et al. (1999). Supplier selection, monitoring practices, and form performance. *Journal ofAccounting and Public Policy, 18*, 253-281
12. Levine, D. M., et al. (2001). *Applied Statistics for engineers and scientists*: Prentice Hall.
13. Lummus, R. R., & Vokurka, R. (1999). Definig supply chain management: a historical perpective and practical guidelines. *Industrial management and data systems, 99*(1), 11-17.
14. Minitab. (2006). Minitab (Version 15.1.0.0): Minitab Inc.
15. Montgomery, D. C. (1997). *Introduction to Statistical Quality Control* (3 ed.): John Wiley & Sons, Inc.
16. Paulraj, A., et al. (2006). Level of strategic purchasing: Impact on supply chain integration and performance. *Journal of purchasing and supply management, 12*, 107-122.
17. Pressey, A. D., et al. (2009). Purchasing practices in small-to medium- sized enterprices: An examination of sterategic purchasing adoption, supplier evaluation and supplier capabilities. *Journal of purchasing and supply management, 15*, 214-226.
18. Pyzdek, T. (2003). *Quality Engineering Handbook* (2 ed.). New York: Marcel Dekker Inc.
19. Rahul, V., & Altekar. (2005). *Supply chain management*: Printice Hall.
20. Robb, D. J., et al. (2008). Supply chain and operations practice and performance in Chinese furniture manufacturing. *International journal of production economies, 112*, 683-699.
21. Stapenhurst, T. (2005). *Mastering Statistical Process Control* (1 ed.): ELSEVIER.
22. Shin, H., et al. (2000). Supply management orientation and supplier/buyer performance. *Journal of operations management, 18*, 317-333.
23. Toni, A. D., & Tonchia, S. (2001). Performance measurement systems; Models characteristics. *International journal of operations and production management, 21*(1/2), 46-70.

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Estimation of Shelf Life for Water-based Paints Using Regression Methods

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Abstract: The shelf lives of water-based paints made in Nigeria were investigated. The mean changes in the microbial population count of six freshly made paint samples (PS1 – PS6) were monitored fortnightly for a period of 10 months. The growth data of isolated organisms from the fresh and spoilt paint samples were fitted into a multiple linear regression model to predict shelf life for the fresh paint samples. The microbial population ranged from $1.0 \times 10^1 - 4.7 \times 10^5$ cfu/ml and from $1.0 \times 10^1 - 5.5 \times 10^3$ cfu/ml for bacteria and fungi over the study period. Physico-chemical parameters such as specific gravity (SG), optical density (OD), transmittance (TR), pH and viscosity (VIS) were also determined every two weeks for the fresh paint samples over the ten-month study period. The measurements of the physico-chemical parameters suggested deterioration related to microbial population count of the paint samples. Consequently, the model developed comprised of two equations with particular attention to microbial population count and physico-chemical parameters of the paint samples. The microbial population counts of the spoilt paint samples were 3.4×10^{10} cfu/ml and 3.2×10^5 cfu/ml for bacteria and fungi respectively. The changes in the physico-chemical parameters ranged from 2.8658 – 1.0853, 1.49 – 3.91, 6.9 – 2.3, 8.5 – 5.6, 11.7cst –10.8cst for SG, OD, TR, pH and VIS in fresh paint samples. The percentage residual error between the shelf life predicted and the shelf life experimental ranged between 0.001 and 0.500. The shelf lives obtained for the fresh paint samples were 19, 21, 23, 22, 37, and 22 months respectively. [Journal of American Science 2010;6(4):123-127]. (ISSN: 1545-1003).

K**ey words:** Shelf life; Paints; Regression; Physico-chemical parameters

1. Introduction

Paint is the general term for a family of products applied to various surfaces such as wood, metal or stone to protect the surface from corrosion, oxidation and environmental weathering. It also provides decorative finish (Adeleye and Adeleye, 1999). Water-based paints are prone to biodegradation because of their aqueous nature and presence of microbial nutrients. Therefore, the monitoring of paint quality is one of the main goals of the paint industry. The shelf life of water-based paints is influenced by a number of factors such as initial microbiological quality of raw materials, packaging materials and the manufacturing plant itself (Gillatt, 1999, Obidi *et al.*, 2009). The paucity of information on paint shelf life is a major problem bewildering the paint industry. This is the reason for the extensive use of lead to improve paint durability and hence, shelf life (Rabin, 1989). However, the use of lead in paints has posed significant health hazard since the mid 1920's (Rabin, 1989, Mathee *et al.*, 2007). The aims of the present study were to determine the shelf lives of paints using a multiple linear regression model and to evaluate the validity of the developed model in predicting shelf life by comparing the observed result with the model prediction using the residual error factor (Neter *et al.*, 1983).

2. Materials and Methods

Microbiological Enumeration and Identification

Six paint samples (PS1-PS6) from a major paint industry in Lagos, Nigeria were collected randomly shortly after completion of production. They were monitored for microbial growth using standard plate count technique. Aliquots (0.1 ml) from both low (10^{-2}, 10^{-4}) and high (10^{-6}, 10^{-8}) ten -fold serial dilutions of paint samples were plated out on Nutrient agar (NA), Mac Conkey agar (MCA) and Potato dextrose agar (PDA) plates in three replicates. NA and MCA plates were incubated aerobically at 37^0C for 1-2 days and PDA plates at room temperature (30 ± 2^0C) for 3-5 days. Precautionary measures such as aseptic techniques were adhered to and found effective. In addition, spoilt paint samples were analyzed to know the level of microbial contaminants that could cause spoilage of water-based paint samples. The colonies that developed in the plates were enumerated. Pure cultures of isolates

were obtained by sub-culturing and identified by reference to API (Analytical Profile Index) test systems (bioMerieux Vitek, Inc. Hazelwood, MO. USA.

Determination of Physico-chemical Parameters
Specific gravity

Specific gravity was determined by pycnometry as described by Ohwoavworhua and Adelakun (2005). A sterile pycnometer of 50 ml capacity was weighed and the weight recorded as M1. Paint sample (50 ml) was transferred into the pycnometer. The pycnometer and its content were weighed and the weight recorded as M2. The pycnometer containing the paint sample was filled with distilled water and shaken many times to allow all trapped air within the pycnometer to be expelled. The weight of the pycnometer was recorded as M3. The pycnometer was emptied, washed and refilled with distilled water and the weight recorded as M4. Specific gravity was then calculated using the formula:

$$SG = \frac{(M2-M1)}{(M4-M1)(M3-M2)}$$

Optical Density and Transmittance

This was determined colorimetrically (Rieck *et al.,* 1993) with a photoelectric colorimeter (Model: AE-11C Tokyo Erma Optical works Ltd, Japan). The colorimeter was standardized by adjusting it to read 100% light transmittance with 5 ml distilled water at a wavelength of 660 nm. The colorimeter had two scales. The bottom scale displayed the absorbance while the top scale, % transmittance. Five (5 ml) of serially diluted paint samples were poured in the cuvette and subsequently placed in the colorimeter to determine the optical density and the transmittance.

Data Analysis
Mean pH

The pH of the paint samples was determined with a pH meter (Model: Jenway M50/Rev CE350EU) in 1: 200 solution of the paint samples in distilled water. The pH meter was calibrated using phthalate buffer (pH, 4.0) and phosphate buffer solutions (pH, 7.0).

Viscosity

Viscosity was determined using a glass capillary tubular viscometer (Model: Capirograph Toyoseikl Seisaku-Sho Ltd) as described by Rammohan and Yassen (2003). The paint sample was allowed to flow through an outlet tube (measuring tube which is narrowed into a capillary tube above the outlet). Two annular reference marks on the measuring tubes were used. The time it took the sample meniscus to drop from the upper to the lower reference mark was measured manually with a stop-watch. The viscosity was then calculated by multiplying the measured time by the viscometer calibration factor at room temperature (30 ± 2^0C).

The microbial growth data for the fresh and spoilt paint samples were fitted into the first equation of a multiple linear regression model (Neter *et al*., 1983). The regression parameter estimates ($ß_0$ – $ß_3$) were generated with data from the initial to the final microbial population count of fresh paint samples during the study period using the JMPIN software.

$$Y_i = ß_0 + ß_1 (\log X_1) + ß_2 (\log X_2) + ß_3 (\log X_3) + e \quad (1)$$

Where $ß_0$ is the regression intercept, $ß_1$- $ß_3$ are regression parameter estimates (at 0 – 10 months), X_1– X_3 (represented by the highest value of total bacterial count, total coliform count and total fungal count respectively) are the regressor coefficients at the spoilt state, Y_i is the time (in months) and e is the error factor. The data for the physico-chemical parameters during the study period were fitted into the second equation of the model:

$$Y_i = ß_0 + ß_1 (X_1) + ß_2 (X_2) + ß_3 (X_3) + ß_4 \ln (X_4) + ß_5 (X_5) + e \quad (2)$$

Where $ß_0$ is the regression intercept, $ß_1$- $ß_5$ are regression parameter estimates (at 0 – 10 months), X_1– X_3 (represented by SG, OD, TR, pH and VIS) are the regressor coefficients at the spoilt state, Y_i is the time (in months).

Comparison Between Observed and Predicted Shelf Life

This was based on the residual error factor ($e= Y_i - \hat{Y}$.) The observed microbial population count and physico-chemical parameters were compared with the predicted values.

$$e = Y_i - \hat{Y}$$

Where Y_i is observed result, $\hat{Y}$ is predicted result and e is the error factor

3. Results and Discussion

Ten morphologically different microorganisms were isolated following initial ten-fold serial dilution and standard plate count from fresh paint samples (PS1– PS6) that have been processed to meet company's regulations and specifications. The bacterial isolates which were identified by the identification profiles generated using the database code obtained from the API identification software (APIWEB) included the following: *Bacillus polymyxa*, (OB-1) *Bacillus brevis*, (OB-2) *Bacillus laterosporus*,(OB-3) *Proteus mirabilis*, (OB-4) *Escherichia coli*, (OB-5) *Lactobacillus gasseri* (OB-7) and *Lactobacillus brevis*

(OB-8) The fungal isolates were identified as *Aspergillus niger*, (OB-9) *A. flavus* (OB-10) and *Penicillium citrinum* (OB-11) respectively based on macroscopic and microscopic characteristics. The microbial population count data from the six paint samples (PS1– PS6) during the ten-month study period showed an increase from 1.0 x 10^1 to 4.7 x 10^5 cfu/ml. The mean changes in microbial population density of the paint samples are shown in Figure 1. There was a protracted lag period of 4 – 5 months in the paint samples. Subsequently, there was steady exponential growth till the 10^{th} month. The high counts of bacteria observed in the fresh paints (Figure1) suggests that the shelf life of the paints would be rather short. The results obtained in this study demonstrate that microorganisms utilized the paints as a source of nutrients and that the constituents of paints were conducive to increased cell multiplication and population buildups in the paint. These were similar to the observations made by Gillatt (1992) that a can of water-based paint is highly susceptible to deterioration. Similar observation by Da Silva (2003) also proved that the various organic constituents of paints such as pigments, additives, binders etc. act as nutrients for microorganisms and help to stimulate microbial growth. In addition to these organisms, the spoilt samples were observed to contain *Pseudomonas aeruginosa* (OB-6). The microbial population densities of the spoilt paint samples are presented in Table 1. Previous investigators have shown that *Pseudomonas aeruginosa* is a commonly encountered organism in spoilt paints (Ogbulie, 2004) and constitutes at least 75% of isolates from spoilt emulsion paints (Dey *et al*., 2004). Spoilt emulsion paints have become a source of concern to marketers and consumers and now constitute a major problem bewildering the paint industry in Nigeria. The mean changes in physico-chemical parameters in the six paint samples are illustrated in Figures 2 & 3. There were increases in optical density over the period for all the samples ranging from 1.49– 3.91. In contrast, there was a decreasing trend in other parameters ranging from 2.8658 – 1.0853, 5.2 – 2.3, 8.4 – 5.6 and 11.7cst – 10.8cst for SG, TR, pH and VIS respectively during the period. The physico-chemical parameters of the spoilt paint samples were 0.1058, 7.51, 0.15, 4.12 and 3.0cst for SG, OD, TR, pH and viscosity respectively. The changes observed for these parameters emphasized the increase in microbial growth and hence increase in acidic metabolites and lower viscosity. The physico-chemical parameters measured in the study appear to provide a sensitive and reliable measure of paint deterioration. Furthermore, the changes in the physical appearance of the paint samples over the study period showed a steady and gradual loss of the original colour, texture and viscosity. In view of the consistent increase in microbial population counts and the corresponding steady decline in physico-chemical parameters, the model used in this study was based on changes in microbial population count and physico-chemical parameters. Shelf life determination must therefore, depend on such criteria which include the rate of deterioration of quality parameters. Due to the fact that the stability of these physico-chemical parameters infer quality, their stability was regarded as a key factor or determinant of shelf life of paints. The data from the microbial population count and the physico-chemical parameters of fresh paints monitored during the period were combined with the corresponding data of the spoilt paint and fitted into the developed model to extrapolate the shelf life of paints. The shelf lives of the paints were therefore estimated to be 19, 21, 23, 22, 37, and 22 months respectively. The percentage residual error calculated by deducting the predicted results from the observed results was found to be marginal, ranging from 0.001 to 0.5 %. The regression (ANOVA) was highly significant at a P value of 0.0001 indicating a good fit, which implies that the regression is very useful. Therefore, changes in physico-chemical parameters and microbial population counts can be used as indices to predict shelf life of products such as paints.

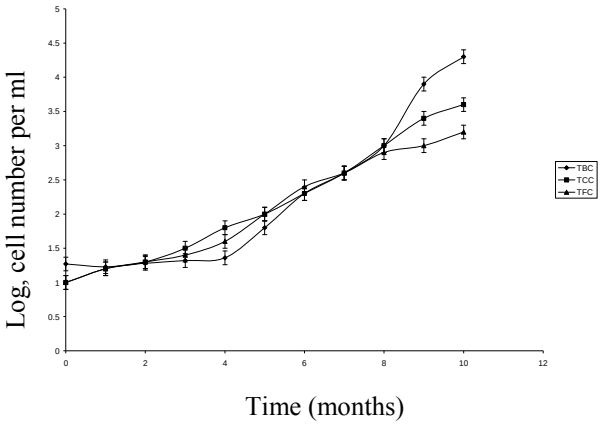

Figure 1. Mean changes in microbial population density in fresh paint samples PS1-PS6. TBC, total bacterial count; T sCC, total coliform count; TFC, total fungal count. Data represent the averages of triplicate determinations.

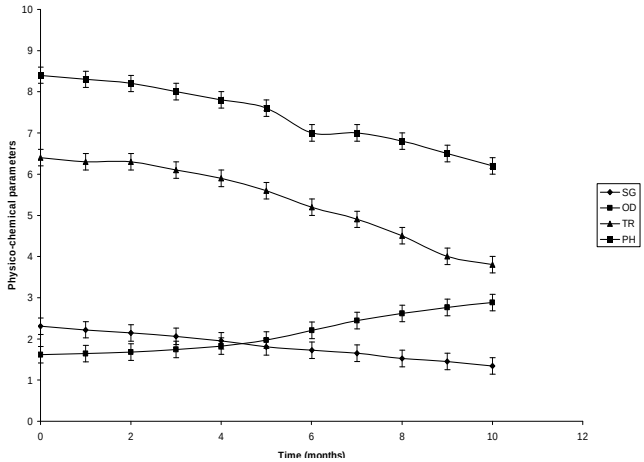

Figure 2. Mean changes in the physico-chemical parameters in fresh paint samples PS1 - PS6. SG, specific gravity; OD, $_{600\ nm}$; TR, transmittance. Data represent the averages of triplicate determinations.

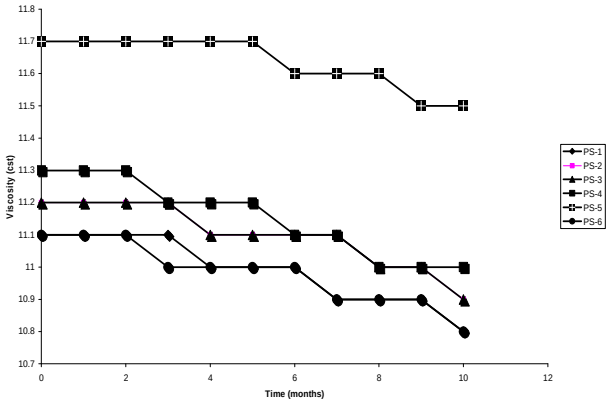

Figure 3. Mean changes in viscosity of fresh paint samples PS1-PS6 monitored for ten months after production

Table 1. Microbial Population Densities in Spoilt Paint Samples

Paint sample	Total bacterial counts (x 10^{10} cfu/ml)	Total coliform counts (x 10^{7} cfu/ml)	Total fungal counts (x 10^{5} cfu/ml)	Fungal isolates	Bacterial isolates
PSA	2.9	1.1	2.5	OB-9	OB-2, OB-3, OB-4, OB-6, OB-7
PSB	3.4	1.1	3.2	OB-9, OB-11	OB-1 OB-6, OB-7, OB-8
PSC	3.0	1.0	2.8	OB-10, OB-11	OB-3, OB-4, OB-6, OB-7
PSD	2.5	2.9	2.5	OB-10	OB-2, OB-4, OB-6
PSE	3.1	1.1	2.2	OB-11	OB-1, OB-5, OB-6

Values presented are means of triplicate samples.

Conclusion

The microbial population count appeared to correlate with the degree of acceptability as indicated by changes in physico-chemical parameters of the paints. The model developed in this study was based on the microbial growth data and the physico-chemical parameters. On the basis of the results above, the microbial population count equation of the regression model predicted the paints shelf life to have an average nominal shelf life of 21 months, while the physico-chemical parameter equation predicted an average nominal shelf life of 24 months. The developed model can be adopted by the paint industry to make a reliable prediction of paint shelf life. The adequacy and accuracy of the developed model determined by the correlation coefficient which was greater than 0.90 indicate a good fit.

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References

1. Adeleye IA, Adeleye OA. Isolation and identification of microbes associated with paints and weathered painted walls. J. Sci. Res. Devt. 1999; 4: 71-76.
2. Da Silva VQ. Microbial deterioration of paints. Microbiologist 2003; 4 (1): 43.
3. Dawes EA. Quantitative problems in biochemistry. 4th Edn. E. and S Livingstone Ltd., Edinburg and London., 2003. pp. 220-239.
4. Dey BK., Hashim MA., Hassan S, Gupta BS. Microfilteration of water-based paint effluents. Adv. Env. Res. 2004; 8 (3): 455-466.
5. Gillatt AC. Bacterial and fungal spoilage of water borne formulations. Additives. 1992; 10: 387-393.
6. Mathee A, Rollin H, Levin J, Naik I. Lead in paints: Three decades later and still a hazard for African children. Environmental Health Perspectives 2007; 1151 (31): 321- 322.
7. Obidi OF, Nwachukwu SCU, Aboaba OO. The use of predictive modeling in shelf life determination of paints. Academia Arena. 2009; 1 (4): 58-63.
8. Ogbulie JN. Microbial deterioration of surface paint coatings. Global J. of Pure and Appl. Sci. 2004; 10 (4): 485-490.
9. Ohwoavworhua FO, Adelakun TA. Phosphoric acid-mediated depolymerization and decrystallization of cellulose obtained from corn cob: preparation of low crystallinity cellulose and some physico-chemical properties. Trop. J. Pharm. Res. 2005; 4 (2): 509-516.
10. Rabin R. Warnings unheeded: A history of child lead poisoning. American J. Pub. Health. 1989; 79 (12): 1668-1674.
11. Rammohan RMV, Yassen M. Determination of intrinsic viscosity by single specific viscosity measurements. J. Appl.Polymer Sci. 2003; 31 (8): 2501-2508.
12. Rieck P, Peters D, Hartmann C, Coutois Y. A new rapid colorimetric assay for quantitative determination of cellular proliferation, growth inhibition and viability. Methods in Soil Science. 1993; 15 (1): 37-41.
13. Neter J, Wasserman W, Kutner MH. General multiple regressions. In: Applied Linear Regression Models. Richard, D. (ed.). Homewood, Illinois: Irwin, Inc., 1983; pp. 226-294.

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SOME STUDIES ON LEAD TOXICITY IN MARINO SHEEP

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Abstract: The problem of lead toxicity originated in a private farm in *El- Katta* "Giza governorate", due to ingestion of plant polluted with lead. About 8 out of 50 Marino sheep animals showed lead toxicity. The animal's age was 6 months. The animals suffered from depression, pressing head against objects, dilatation of eye pupils, total blindness (in 2 cases) with normal light reflex in both eyes, edema in briskets, enteritis with bloody diarrhea and pupil dilation. Also there were lacrimation, pale dirty mucous membrane and sunken eyes. Serum analysis from these animals revealed high lead concentration. In addition too, significant decrease in the levels of testosterone, LH, FSH. PCV, haemoglobin, R.B.C.s and total proteins were also decreased. Highly degeneration of kidney, and liver accompanied with elevation of AST, ALT, Urea, creatinine, cortisol, sodium, and potassium. Moreover, *S.epidermidis* and *S. Aeruginosa* were isolated. We conclude that the cause of animals morbidity and mortality in this farm was not due to bacterial infections but due to lead toxicity and we can say that polluted environment, especially with lead, can cause severe harm to animal health, in addition to serious danger on human health, by eating food polluted with lead. [Journal of American Science 2010;6(4):128-131]. (ISSN: 1545-1003).

Keywords: Lead toxicity in Marino sheep, environmental pollution, biochemical and microbial changes

1. Introduction

Lead is a major environmental pollutant and its toxicity continues to be a major public health problems, and there is a growing consensus that lead cause toxic injury to human at a level of exposure that was considered to be safe only a decade ago. As the chronic exposure to lead, even at low levels, can result in slow progressive, in most of time, irreversible damage to haematopoeitic, nervous and renal systems [1]. Recent researches predicted that high levels of toxic metals in scalp hair, due to environmental pollution, in addition to deficiency in trace metals, play a role in the development of heart diseases [2].

In recent years, research efforts are directed towards quantification of the impact of lead exposure on human health, particularly from environment. The diagnosis based on blood lead levels doesn't always give an accurate estimate of total body burden of lead, so it's important to detect subcellular damage using reliable sensitive biomarkers [3]. Animals are very good indicator of the environmental pollution, as they inhabit the same space as humans and are exposed to the action of the same pollutants, for that reason, it's appropriate and advantageous to evaluate the negative impact of the polluted environment by heavy metals, and their influences load on human health by parallel evaluation of their load on animals [4, 5], so measuring of lead in blood animals [6], in cows and especially in lactating one, proved to be a good indicator of environmental contamination and also for food contamination from polluted animal [7].

2. Material and Methods

Animals:

8 young Marino sheep of 6 months old were used in the present study. They were obtained from a private farm in *El-Katta* "Giza governorate". The animals suffered from depression, pressing head against objects, dilatation of eye pupils, total blindness (in 2 cases) with normal papillary light reflex in both eyes, edema in briskets, enteritis with bloody diarrhea and papillary dilation. Also there were lacrimation, pale dirty mucous membrane and sunken eyes.

Haematological studies:

Blood samples were collected from the jugular vein on EDTA as anticoagulant for determination of Hb, PVC, ESR, RBC's count, and WBC's count, according to [8].

Biochemical and Hormonal studies:

The activities of aspartic aminotransferase (AST) and alanine aminotransferase (ALT) as well as cholesterol, urea and creatinine levels were determined according to the method of Varley *et al*.,[9] by using commercial kits (Bio Merieus, France).

Total serum protein was estimated according to Drupt [10]. Serum cortisol was analyzed by a Gamma counter using 125 I cortisol radioimmunassay kit (Baxter Health Care Corporation USA) according to the method described by Pickering and Pottinger [11]. Potassium, Sodium and lead concentrations were determined by atomic absorption spectrophotometry.

Bacteriological studies:

Swabs from internal organs (liver, kidney, intestine and lungs) were collected under aseptic condition. The inoculated plates were incubated at 37°c for 24-48 h. The suspected colonies were picked and purified by further subculturing after which they were stained with Gram stain, for further biochemical identifications, the subjected isolates were classified according to Buchman et al., [12] and Wilson & Miles [13].

Soil Forage:

The lead content of the soil and forage was measured by atomic absorption according to the method of Rodrigues and Castellon [14].

Statistical analysis:

The obtained data were subjected to the student t-test according to Gad and Well [15].

3. Results

In the present study, haematological examination showed significant decrease in haemoglobin, PCV, and RBC's count and significant increase was found in ESR and W.B.C's count in all animals (Table 1).

Biochemical results detected significant increase in AST, ALT, urea, creatinine, cortisol, sodium, potassium and lead (Table 2). While, there was significant decrease in total protein level (Table 2). Serum LH, F.S.H, and testosterone hormones were significantly decreased (Table 3).

The lead content was found to be 293.72 p.p.m in the polluted soil and 164.3 p.p.m in forage (Table 4).

Bacteriological results revealed that the most predominant isolated micro-organisms was *S. epidermis and S. Aeuroginosa* (Table 5).

Table (1): Effect of lead toxicity on some hematological parameters (Mean values ± SE) in Marino sheep.

Parameters	Control (8)	Marino sheep (8)
P.C.V	32±1.2	32±1.4
E.S.R	2.1±0.01	2.8±1.3*
R.B.C's count 10^6 /ml	7.33±0.28	5.4±1.24*
W.B.C's count 10^3 /ml	9.35±0.23	10.03±0.49*
HB g/dl	9. 1±0.01	8.13±0.14*

*P <0.01

Table (2): Effect of lead toxicity on some biochemical parameters (Mean values ± SE) in Marino sheep.

Parameters	Control (8)	Marino sheep (8)
AST U/l	132±1.23	173±2.45*
ALT U/l	32±1.46	63±1.62*
Total Protein g/ dl	11.4±1.68	9±0.27*
Urea mg/dl	2.80±0.62	3.01±0.82*
Creatinine mg/dl	0.97±0.03	1.8±0.02*
Cortisol ng/dl	0.8±0.01	1.77±0.23*
Na^+ M.E.Q	98±2.2	124±3.3*
K^+ M.E.Q	4.8±1.3	6.3±1.9*
Lead p.p.m	0.88±0.12	1.78±0.10*

*P <0.01

Table (3): Effect of lead toxicity on L.H, F.S.H, and testosterone hormones (Mean values ± SE)

Parameters	L.H (mu/ml)	F.S.H (mu/ml)	Testosterone (ug/dl)
Control	3.24 ± 0.32	5.5 ± 0.32	5.2 ± 0.042
Affected Animal	2.2 ± 0.14*	4.2 ± 0.42*	3.9 ± 0.23*

*P <0.01

Table (4): Mean level of lead in soil and vegetables in the studied area

Samples	Polluted area concentration of lead in p.p.m	Control Area
Soil	294.72 ± 32.24 *	75.23 ± 9.12
Forage	165.3 ± 6.32 *	65.01 ± 6.23

*P <0.01

Table (5): Bacterial determined in internal organs liver and kidney in Marino sheep

Bacterial Isolated	Degree of presence
Streptococcus sp.	30 %
S. epidermidis	70 %
S. aeurogenosa	25 %

4. Discussions

From our results, we noticed a decrease in the level of hemoglobin, PVC, and R.B.C's count, owing to the fact that lead intoxication causes a documented defect in haem synthesis. The results obtained agreed with several authors [16, 8] because lead pollution has an inhibitory effect on globin synthesis, inhibits iron to form haem and inhibits delta amino levulinic acid dehydratase in red cells.

Moreover, we can conclude that lead toxicity has dangerous effect on animals in the studied areas where the lead content of soil measured was about 293.73 p.p.m, and in contaminated pasture was 164.3 p.p.m, the lead poisoning in the studied Marino sheep may be due to grazing this contaminated pasture which may be due to their contamination by industrial wastes [17]. Hob & Kirn [18] reported that, lead concentration in plants was 80-160 p.p.m and in soil was 100-300 p.p.m which was considered as toxic level. Referring to the FAO-WHO[19] recommendations, the acceptable daily lead intake is 0.05 p.p.m. and our finding agreed with that of Bryant and Rose [20]; Fayed and Abdallah [21]; Zaki et al., [22]

Bacterial microorganism e.g. Streptococcus Sp, S. Epidermis and were isolated from internal organs, similar finding were reported by Fingold & Martin [23] and Ducan & Prasse [24] who stated that bacterial microorganism are present in animals and birds suffering from high pollution with lead due to immunological suppression.

Both clinical signs and ocular changes, which were observed in the present work, might be attributed to the toxic effect of lead on the C.N.S as mentioned by Krameller-Froetcher, [25] and Schlerka [26], this toxic effect was characterized by severe cerebral disturbances leading to blindness in some cases which may be due to cerebrocoritcal oedema, or may be also due to associated optic neuritis and optic atrophy. We can concluded, that all the clinical symptoms reported in the studied Marino sheep might be due to acute lead intoxication, as mentioned by Ozmen and Mor [27].

The biochemical results detected in Table 2, showed significant increase in AST, ALT, while there was significant decrease in total protein level (Table 2). These findings agreed with those found by Swarap et, al [28], as the as the elevation in transaminases activities and the decrease in total protein level may be attributed to the liver injury, so the exposure to lead in polluted environments alters serum biochemical parameters indicative of liver functions. The biochemical results, detected in table 2, showed significant increase in urea and creatinine, which are indicative of abnormal kidney functions, agreed with those of Goswami and Gachhui [29]. The disturbed liver and kidney functions have been seen on the last stage of lead toxicity, this agred with Zaki et al., [22]. The hormonal results, detected in Table 3, showed significant decrease in L.H., F.S.H. and testosterone, which are affected in the early stages of lead poisoning [30 and 31].

In conclusion lead toxicity cause atrophy of liver, kidney, gonads blindness, in addition to the locomotor disturbances. And the cause of animal morbidity and mortality in this farm was not due to bacterial infections but due to lead toxicity. We can also say that polluted environment, especially with lead, can cause severe harm to animal health, in addition to serious danger on human health, by eating food polluted with lead.

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References

1. Silbergeld E.K. 1997: Preventing lead poisoinning in children, Ann Rev. Public Health; 18:801-810.
2. Afridi H.I., T.G. Kazi, G.H. Kazi, M.K Jamali., G.O. Shar, (2006): Essential trace & toxic element distribution in the scalp hair of Pakistani myocardial infarction patients, Biol.Trace elem. Res. 113(1):11-34.
3. Endo G., S. Honiglechi, I. Kiyota, (1990): Urinary NaG in lead exposed workers, J Appl. Toxicol, 10:235-38.
4. Korak M., E. Kralova, P. Sviatko, J. Bilek, A. Bugarsky, (2002): Study of the content of heavy metals related to environmental load in urban areas in Slovakia. Bratsil Lek. Listy; 103 (7-8): 231-7.
5. Rashed M.N. and M.E. Soltan, (2005): Animal hair as indicator for heavy metal pollution in urban & rural areas, Env. Monit Assess; 110 (1-3) : 41-53.

6. Zadnik T., 2004: Lead in top soil, hay, silage& blood of cows from farm near a former lead mine & current smelting plant before and after installation of filters. Vet. Hum. Toxicol.; 46(5): 287-90.
7. Swarp D. , R.C. Patral, R. Naresh, P. Kumar, P. Shekhar, (2005): Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Sci Total Environ.; 347 (1-3): 106-110.
8. Jain S.D. (1986): Evaluation of Haemogram in healthy and diseased sheep. Res. Vet. Sci. 33,21.
9. Varley H., A.H. Gwenbek and M. Bell, (1980): Practical clinical chemistry vol. I Genera] I top-'scomnoner test 5s1 ed. London, William medical books Ltd
10. Drupt, F., (1974): Estimation of total protein, Pharm Biol, 9, 77,
11. Pickering, A.D. and J. Pottinger, (1983): Analysis of Hormone, Gen Comp Ender. 49,232.
12. Buchman R.E, W. E Gibbuns and R.Y Stajner (1975): Bergeyes Manual of determinative Bacteriology, Ed. Millions and Wilkins co Baltimore.
13. Wilson G.S. and A.A. Miles, (1975): Topley and Wilsonies principles of Bacteriology and immunity 6th Ed, Edwards and Annald, publishers LTD, London.
14. Rodrigues MF and Castellon (1982): Lead and cadmium levels in soil and plants near highways and their correlation with traffic density. Environ. Pollute. (B) 4:281-290.
15. Gad W. and G. Well (1976): Statistical methods 6th Ed the lowa state Univ, Press Iowa U.S.A.
16. Soliman, M.N. (1983): Toxicity resulting from lead compounds in Veterinary practice. Ph. D. Thesis, Cairo University Egypt.
17. Lemos R.A., D. Driemeier, E.B. Guimacaes, I.S. Dutra, A.E. Mori and C.S. Barros, (2004): Lead Poisoning in cattle grazing pasture contaminated by industrial Waste, Vet Hum Toxicol, 46 (6) 326-8.
18. Hob and Kirn (1988): Elevated levels of a lead and other metals in roadside soil and grass and their use to monitor heavy metal depositions in Hong Kong. Environ. Pollut.,49: 34-51.
19. FAO/WHO 1983: 6th joint expert committee on food additives, evaluation of mercury, lead, cadmium and the food additives diethyl pyrocarbonate and acetyl gallate, WHO food additivitie Ser. No.4.
20. Bryant SL and RW Rose, (1985): Effect of cadmium on the reproductive organs of the male Ram "Australian Journal of Biological -sciences, 28(3); 305-311-15
21. Fayed A.H and E.B. Abdallah, (1997): "Technique for studying the morphology of mammalian spermatozoa which are eosinophilic in a different live/dead stain. J. Report. Fertile; 29; 443.
22. Zaki M.S., A.M Haman and F.S Bayaumi and M.N Shalaby, (2001): Some clinicopathological and microbial studies in lead toxicity on cows, Egypt comp path & clinic pathology vol 14 29-35.
23. Fingold S.M. 1982: Diagnostic microbiology, 6th ed., Mosby co. st. Lowis, Toronto, London.
24. Ducan J. and K.W. Prasse (1986): Vet laboratory medicine-clinical pathology, 2nd ed. Iowastate Univ., press. Ames; Iowa U.S.A.
25. Krametter-Frooestscher R., F. Tataruch, S. Hauser, M. Leschnick, A. Url and W. Baumgartner, (2007): Toxic effects in herd of beef cattle, following exposure to ash residue contaminated by lead and mercury. Vet. J.174 (1): 99-105.
26. Scherleka G., F. Tataruch, R. Krametter-Frooestscher, A. Url, D. Kossler, S. Hogler and P. Schmidt, (2004): Acute lead poisoning in cows due to feeding of lead contaminated ash residue, Berl Mumch Tierarztl Wochenschr, 117 (1-2):52-6.
27. Ozmen O. and F. Mor (2004): Acute lead intoxication in an old Battery Factory, Vet Hum.Toxicol. 46 (5):255-6.
28. Swarp D. , R. Naresh, V. P. Varshney, M. Balgangatharathilagar, P. Kumar, D. Nandi and R.C. Patral, (2007): Changes in plasma hormone profile and liver functions in cows naturally exposed to lead and cadmium around diffirent industrial areas, Res. Vet. Sci. 82 (1):16-21.
29. Goswami K., R. Gachhui and A. Bandopadhyay, (2005): Hepatorenal dysfunction in lead pollution. J. Environ. Sci. Eng.: 47 (1)75-80.
30. Gorbel F., M. Boujelbene, F. Makni Ayadi, F. Gucimazi, F. Croute J.P Soleilhcvoup and A.l. El-Feki, (2002): Cytotoxic effects of lead on the endocrine & exocrine sexual function of pubescent male & female rats. Demonstration of apoptotic activity, C R Biol; 325 (9): 927-40.
31. Srivastava V., R.K. Dearth, K.J. Hiney, L.M. Ramisez, G. Bratton and W.L. Dees, (2004): The effects of low level Pb. on steroidogenic acute regulatory protein in the pubertal ratovary, Toxicol Sci 77(1): 35-40.

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Perception of Women towards Family Values and Their Marital Satisfaction

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Abstract: The purpose of the current study is to examine the relationship between perceptions of women toward Iranian traditional values and their marital satisfaction. The 337 of women were selected as the population of the study. Stratified random sampling was used to select samples. The Kansas Marital Satisfaction (KMS) is used to measure marital satisfaction. It shows a positive relationship between perception of women toward traditional values (inequality in family affairs and inequality in regulation) and marital satisfaction. It has also shown that there is no relationship between perception of women toward traditional values of inequality in access job and inequality in political in relation to marital satisfaction. The multivariate regression analysis is utilized to answer the last objective. The analysis showed that among the two predictor variables, the variable of inequality in affairs was found to be significant in explaining the observed variation in marital satisfaction (DV). Generally, the final model has explained 4.6% of total observed variance in marital satisfaction. The findings show one reflection of the inequality and discrimination that there is in family affairs and inequality in regulation between men and women in the Iranian society. The findings highlight the need for screening and identification of other factors and unequal situations in the family and society in which women are growing. [Journal of American Science 2010;6(4):132-137]. (ISSN: 1545-1003).

Keywords: Family Values, Marital Satisfaction, Women perception.

1. Introduction

This article examines the relationship between perceptions of women toward Iranian traditional values and marital satisfaction. Accumulating evidence indicates that new women generation in Iran challenges traditional values. Research showed that the degree of modernity and liberalism are variables that have a significant effect on marital satisfaction. Research has consistently demonstrated that women are rejecting the old values in society, and consider the women status is lower than men. Since the 1960s, there has been a sudden and sharp decline in marriage rates, an increase in the age at marriage, and a significant increase in divorce rates in all over the world. The roots of these changes are in the demographic transition arising out of the social and economic developments of the last century. This is also true in Iran. In this country, the structure of family is changing and is under the processes of structuring and restructuring continuously. Therefore, any attitude towards family should benefit from the new generation's attitude toward the concept of family as well as the society values. This will ensure a permanent and sustainable development of value-system with respect to individuals' rights regardless of their gender. In the context of the current research, female understands and even experiences the gender differences may separate her from the opposite gender within family, and even cause certain behaviors and reflections from other fellows toward her (Ehzazi, 2002). Therefore, female always select and develop family values and cultural models from among these male-oriented views. This perception is, however, observed to differ in degree and intensity across human society and must be separately investigated prior to any generalizations. Studies focusing on people's understanding and perception of social and psychological structures require drawing a comprehensive and illustrative sketch of the context of the study.

Marriage, as the first issue to discuss in the context of this study, has obtained the most controversial debates in Iran for many years. While monogamy was commonly practiced by the majority of people, polygamy has been in Iran from many years ago, and Islam has emphasized on this custom and state's civil law originated from Islamic jurisprudence permits it. Although, currently in Iran polygamy is committed rarely, yet it remains as an addressed issue of certain criticism for the Iranian society. The history of Iran is known as masculine dominant history, i.e. Iranian culture has been masculine-oriented (Sarookani, 2005). In other words, families have been under the influence of the fathers. For instance, children would have the father's

surname in as their surname and the father in the family would decide on future marriages of the family members. Undoubtedly, the Islamic values and the Islamic teachings, which have shaped the way of life in the Iranian society for many centuries, will continue to support the patriarchal family framework for family decision-making, individual behavior and action. Sexual behavior will continue to be controlled within this framework. Family and the state will discourage sexual activities out of marriage by various methods and heavy punishments. Hence, marriage will continue to be a marked transitional stage in life of an individual and family will have primacy in mate selection.

The unit of family is the second issue of concern in this discussion. When more social cooperation and organizations have more interrelations, the family duties decrease, i.e. when social organizations such as insurances, hospitals and education departments are more developed, the family duties become less significant, as some of these functions are performed by these social organizations. The changes in the functions of the families become so vast and important that it is not possible to consider family as a productive unit. Nowadays, family has delivered the production to industry and is considered as a consumer unit. In Iran, like other countries, the functions of the family have changed and will keep changing in line with economic advancements which are rapidly affecting the family forms regarding their values.

The other issue of a prime concern is the observed changes of attitudes and values across generations in the Iranian society. Since two decades ago, women have some independence and are able to benefit from social and economic advantages. Apparently, because of the economic and social changes and the urbanization, the influence of Western attitudes through mass media and internet, the Iranian young generations seem to be changing with respect to religious affairs. Nowadays, the youths continue to study and think that it is necessary to be educated. This may reveal the reason why the marriage age has increased (Sadeghi & Kalhor, 2007). In the past, boys didn't have freedom to marry any girl they liked. Family-arranged marriages were so common that they had to marry the girl recommended by their parents. Contrary to this tradition, today the situation is different. Kinship network has been replaced with social system of values. Therefore, today marriage factors are mostly social ones and are directly related to the youths, and not their parents. All the things aforesaid are the factors justifying why marriage age is increasing, and people marry at older ages; that is why there are more unmarried people in the villages and cities, and the family is a device for the youths to find their ways to socialize and enter into the bigger community. As women become more aware of their right and try to employ it, this would lead to higher number of divorces for women, where they are in abusive marriage relationships. Interestingly, it was found out that higher education and formal employment of women are both correlated with divorce rate (Riyahee, Aliverdynia, & Bahrami Kakavand, 2006).

The Iranian society is passing from a traditional society to a modern status; therefore, the Iranian families, especially the women in these families, have always been traditionally facing more problems compared to other family members. In other words, the modern society values on the one hand and the current views on the other hand, put the woman in appalling conditions. Yet, the adoption of Western ideas in all aspects of life by younger generations will be a continued source of stress on family. This will also reinforce the existing male and female conflict and unsatisfaction in their relationship, where young generation of women is exposed to the gender egalitarian values of western culture. They look for new values that consider them equal as men. This article studies the relationship between perception of women toward Iranian traditional family values and marital satisfaction. The general objective of this study is to determine the relationships between perception of women toward family values and marital satisfaction among married women. Specifically, the objectives were to describe the perception of women toward Iranian traditional family values and marital satisfaction; to examine the relationships between Iranian family values (inequality in getting job, regular, family affairs and politic) and female marital satisfaction; and to determine the factor which predicts marital satisfaction.

2. Literature Review

Marital satisfaction is related to general happiness and pleasure with the relationships between couples. Marital satisfaction means a good feeling of marriage. Kaplan & Maddux (2002) stated that marital satisfaction is an individual experience in marriage which can only be evaluated by each person in response to the degree of marital pleasure. They believed that, it depends upon the individual's expectations, needs, and desires in their marriage. Marital satisfaction refers to the degree of satisfaction between couples. This would mean the degree of satisfaction they feel with their relationship. This satisfaction could be addressed both from the perspective of wife toward the husband, or the husband toward the wife. Nowadays, because of new values and changes in the societies, cultures, women

situations, and women educations, the women's expectation toward marital relationship has also been changed. As mentioned by Chafez, (1992):

"The average, once acceptable marriage increasingly becomes re-conceptualized by many women as short on intimacy and equality and therefore as unacceptable. The feminist message functions to raise the ideal standards or expectations against which marriages are increasingly measured, and therefore raises the frequency with which they will be found wanting" (p. 18)

Women's greater involvement in higher education has brought about a change in attitudes towards traditional marital roles, family values, and difficulties in reconciling changing roles within their marriage (Chafetz, 1992; Beck, 1995). According to Bureau of the Census (1992):

"Younger people in the U.S. who are marrying for the first time face roughly a 40-50% chance of divorcing in their lifetime under current trends" (p. 5).

With respect to the context of the current Iranian condition, Shadi Talab (2001) believes that at present times there is more agreement with women's employment outside the house, girls' marriage with non relatives, acquiring knowledge, equal cooperation of both partners in housework and opposition to polygamy. Among the educated women, it was found that the higher the educational level, the greater is the marital satisfaction. They have more hope and faith in life and there is more connection between their ideals and realities. Meanwhile, Shachar (1991) studied marital satisfaction with a sample of 206 couples who were married up to four years for the first time. The result revealed a degree of modernity and liberalism and the husband's desire to marry were variables that had a significant effect on marital satisfaction. On the other hand, Danesh & Heydarian (2006) investigated the relationship between mutual interest and respect among couples and their marital satisfaction. Result showed that couples, and also men and women, who were more respectful and loved their spouses, were more satisfied in their married life. There was a positive correlation between the amount of respect and love among couples. The couples who were respectful to their spouses were more satisfied of their married life. Also, couples who loved their spouses will be more satisfied of their married life as well.

3. Research Methodology

A study was carried out in Shiraz City, Fars Province, Iran. Iran is an Islamic country, with its population of approximately 70,000,000 people (Iran Statistical Centre, 2006). The majority of Iranians practice the Shiite sect of Islam. In the study, a number of 337 women were selected as the respondents. Stratified random sampling was used to select samples. The Kansas Marital Satisfaction (KMS) is used to measure the marital satisfaction (Schumm, Bugaighis, Paff-Bergen, Hatch, Obiorah, Meens, & Copeland, 1986). The satisfaction of the respondents towards their marital relationship was evaluated through this scale. It includes three questions in which the responses range from 1 (extremely dissatisfied) to 7 (extremely satisfied). The sum of these three responses was the total score. This scale is reported to have high internal consistency with alphas ranging from 0.89 to 0.98 and high inter-correlations among items ranging from 0.93 to 0.95 (Schumm, Nichols, Schectman, & Grisby, 1983). In this study, the level of marital satisfaction reported by women was used. In this particular study, the alpha reliability of women marital satisfaction was observed as 0.95.

Meanwhile, to assess the perceptions of women toward family values, a family value scale was used. The scale was a combination of four sub-scales that contains of inequality in family affairs, inequality in employment, inequality in regulation implementation and inequality in political issues. This scale comprises 49 items. There are 25 items about negative aspects and 24 items about positive features of gender inequality and values exhibited in Iranian society. Women's opinions related to the family values have been categorized under the five groups of strongly disagree, disagree, neutral, agree and strongly agree. For example, inequality in family affairs relates to free selection of the husband (without the permission and satisfaction of father and elder brother as an example), similar rights of man and woman for getting divorce (divorce right, etc.), family directorship (man's right), marriage proposal (man's right), man's ability to prevent his wife from choosing a job that is against the benefits and interests of the family, the guardianship of children (to be undertaken by father and grandfather), men's polygamy, training and taking care of children, housework and duties and providing family expenses (to be undertaken by men). Women's opinions related to the traditional values of Iran have been categorized under the five groups of strongly disagree, disagree, neutral, agree and strongly agree. Pearson product moment correlation coefficient and multiple regressions were employed in this research in order to achieve the stated objectives..

4. Results and Discussions

In this section, the descriptive data is presented in Table 1 which includes the perception of women toward Iranian family values and marital satisfaction.

Table 1: Perception of Women toward Iranian Traditional Family Values and Their Marital Satisfaction

Variables	n	%	Mean	SD.
Perception towards family values			50.23	7.03
Inequality in Family Affairs				
Agreed With Tradition Values (<43)	59	17.5		
Somewhat agreed (43-59)	253	75.1		
Not Agreed with Tradition Values (>59)	25	7.4		
Inequality in Regulations			36.83	8.45
Agreed (<32)	103	30.6		
Somewhat agreed (32-46)	198	58.8		
Not Agree (>46)	36	10.7		
Inequality in Access to Job			22.07	5.66
Agreed (<20)	130	38.6		
Somewhat agreed (20-31)	191	56.7		
Not Agreed (>31)	16	4.7		
Inequality in Politics			14.53	4.74
Agreed (<14)	179	53.1		
Somewhat agreed (14-22)	140	41.5		
Not Agree (>22)	18	5.3		
Marital Satisfaction (n=337)			15.40	5.82
Low (<9)	72	21.4		
Medium (9-15)	65	19.3		
High (>15)	200	59.3		

The participants for this study were 337 women (wives). For "the mean for family affairs" it was observed to be M=50.23, SD=7.04. Approximately, 59 (17.5%) of the respondents have agreed with traditional family values, while 253 (75.1%) were located as mediocre and 25 (7.4%) were found to disagree with the traditional values. It can be interpreted that majority of the respondents were either be unsupportive to the traditional values or were examining their position to adopt the new family values. For the values of inequality in politics, it was observed that M=14.53, SD=4.74; for values inequality in access to job M=22.07, SD=5.67; for inequality in family affairs M=50.23, SD=7.03, while for inequality in regulations M=36.83, SD=8.45. Generally, majority of the respondents were in the medium level (somewhat agreed) of the four domains of perceptions of family values. However, when high level (agreed) and the low level (not agreed) of the perception towards family values is compared, more respondents were found to be in the high level in all four domains of perceptions. It reflects that more respondents perceived the occurrence of inequalities in family affairs, regulations, access to job and politics compared to those who disagreed. In Iranian culture, there are new values emerged in relations to family values.

The other variable in this tabulated data represents the "level of marital satisfaction". As shown in Table 1, the level of their marital satisfaction is high, where the calculated mean was M=15.40 and the standard deviation was SD=5.82. From this total figure, 72 (21.4%) of respondents had low satisfaction, and 65 (19.3%) had medium satisfaction, whereas, 200 (59.3%) had a high level of marital satisfaction.

The Pearson's product-moment correlation test was utilized to determine the relationship between perception of women toward four types of Iranian traditional values (inequality in affairs, inequality in regulation, inequality in access job and inequality in political) and marital satisfaction. The results are shown in Table 2.

Table 2: Correlation Coefficient (r) between Inequality in Affairs, Inequality in Regulation, Inequality in access Job, and Inequality in Political

Variables	Marital Satisfaction
Inequality in affairs	.212**
Inequality in regulation	.143**
Inequality in access job	-.035
Inequality in political	-.003

**P≤.01; *p≤.05

The results show that there are positive and significant relationships between marital satisfaction and perception of women toward inequality in family affairs, r = .212 and p<0.01; and towards inequality in regulation, r = .143 and p<0.01. However, there are no significant relationships between marital satisfaction and perception towards inequality in access job, r = -.035 and p<0.05.; and towards inequality in politics, r = -.003 and p<0.05.

The Multivariate Regression Analysis was used to answer the last objective. The analysis shows that from the four predictor variables, only inequality in family affairs was found to be significant in explaining the observed variation in marital satisfaction (the dependent variable). Generally, the final model has explained only 4.6% of total observed variance of marital satisfaction.

Table 3: Summary of Regression Analysis of the Main Variables Contributing to Marital Satisfaction

Predictors of marital satisfaction	Unstandardized coefficients	Std. Error	Beta Unstandardized coefficients	t	p-value
constant	6.450	2.250		2.860	.004
*Sum famil	.156	.052	.188	3.005	.003
**Sum ineq	.031	.043	.044	710	.487

R^2 =4.6
*Sum famil=Sum family affairs
** Sum ineq= Sum inequality in family regulation

The regression model shows that inequality in family affairs is one of the most important factors that play a great role in women marital satisfaction in Iranian society. Marital satisfaction means good feeling of marriage.

The findings of this study imply that women gradually understand the traditional family values in their society as against their rights, and thus some of them reject these values. After their marriage, women are more in contact with all sections of the society and turn out to be more familiar with the values and norms found in their society. Today, women with higher education have more awareness about the rights and outlook for equality in their lives; thus, they reject traditional values and norms which may consider them as second class members of their families. The findings of the study show that there was an increase in the number of modern women turned out to be more satisfied with their lives. As the women become more knowledgeable, the level of marital satisfaction might also increase. It is believed, modern women can think well than traditional women because they have more relation and connection with other people, where they can spend more times with their friends. In terms of hobby and entertainments, modern women looked more satisfied compare to the traditional women. Modern women understand that if there is any inequality in the society, it belongs to the structure of their society that must be change. In the society of Iran nowadays, women are more educated compared from the past. They are more knowledgeable in spite of the existence of so many difficulties in their relationship, and new generation struggle to discover, learn and adopt new values. Being more knowledgeable of the new values make them more aware about their rights and lives in the society.

5. Conclusion

As we can see, finding showed that women had low level of perception towards family values (inequality in family affairs and inequality in regulation), and also towards government rules. However, these inequalities belong to the past and old generation, and must be changed in all section of the society, especially in the governmental sections. Today, women learned new values through their formal educations, and through the mass media in their society. Moreover, Bandura (1977) stated that most learning takes place indirectly by modeling, which entails receiving information by observing others. It means that observer extracts underlying rules inside the behavior. This theory clearly recognizes the fact that people can learn behavior without directly experiencing and in absence of any rewards (new values and norms). The presence of modernization, new ideas and values in our society, influence the women to go against women discriminations and want to effect change in all sections of the society, especially in the families. They emphasize that these changes must be accepted by society and government.

As a conclusion, our society is passing from a traditional to a modern society. In this situation, compared to men women are more facing difficulty and problem. However, with the increased level of their education, the percentage of their employment in all the sections of the society is also increased. They will become more participative in social activity, such that, it also increases the activities where boys and girls will have more contacts and relations with each other. Through these contacts

they can be more familiar with new norms and values, and therefore, they will know each other better than before. Such contacts will allow them to choose their suitable partners, and this could lead them living happily and feeling satisfy after marriage. In order to have a better society and more satisfied women, the unequal rules (norms), especially those that cause female unsatisfied in their marital relation, the inequalities between male and female that are found in all sections of society must be changed..

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References

Armstrong, T., Wernke, J. Y., Lisdahl Medina, K., & Schafer, J. (2002). Do partner agreee about the occurence of intimate partner violence: A review of the current literature. *Trauma, violence, & Abuse, 3*(3), 181-193.

Bandura, A. (1977). *Social learning theory*. Englewood Cliffs: Prentice-Hall.

Beck, U. (1992). *Risk society: towards a new modernity*. London: Sage.

Chafetz Saltzman, J. (1992). *Chicken or egg? A theory of the relationship between feminist movements and family change in industrial societies.* Paper presented at the Seminar on Gender and Family Change in Industrialized countries, Rome, Italy, January 1992.

Danesh, E., & Heydarian, M., M (2006). The relationship between mutual interest and respect among couples and their marital satisfaction in Qom city. *Journal of Counseling research and development, 5*(18), 59-76.

Ehzazi, s. (2002). *Sociology of family*. Tehran: Roshangaran publishing co.

Iran Statistical Centre, R. N. (2006). Retrieved 20 November 2006, from http://www.sci.org.ir

Kaplan, M., & Maddux, J. E. (2002). Goals and marital satisfaction: Perceived support for personal goals and collective efficacy for collective goals. *Journal of Social and Clinical Psychology, 21*, 157-164.

Riyahee, M. S., Aliverdynia, A., & Bahrami Kakavand, S. (2006). Sociological analysis of divorce (Case study Kermansha). *Women Research, 5*(3), 109-130.

Sadeghi, F. S., & Kalhor, S. (2007). Gap between genders in Iranian 3th development strategy. *Journal of Women studies, 2*, 5-28.

Sarookani, B. (2005). Women power and family. *Women Research, 3*(2), 29-50.

Shachar, R. (1991). His and her marital satisfaction: The double standard. *Sex Roles, 25*, 451-467.

Shadi Talab, J. (2001). Iranian women and their expectations. *Namey-E-Oloome Ejtemae, 18*, 31-62.

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The effect of *Diphenyl Dimethyl Bicarboxylate* and *Dexamethasone* on Immunological and parasitological parameters in murine *Schistosomiasis mansoni*

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Abstract: This work aimed to evaluate the effect of Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone either alone or combined with praziquantel (PZQ) on different parasitological, immunological, and pathological parameters that reflect disease severity and morbidity in murine schistosomiasis. Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone had no effect on worm burden but altered tissue egg distribution. This indicates that under the schedule used, both drugs did not interfere with the development of adult worms or oviposition but it can modulate liver pathology. Meanwhile, dexamethsone showed a marked reduction of granuloma size more than DDB. Dexamethasone-treated mice, also, showed lower levels of serum gamma interferon (IFN-γ), interleukin-12 (IL-12), and IL-4 together with higher IL-10 level compared to infected untreated control animals. These data suggested that dexamethasone is a convenient and promising co adjuvant agent causing decreased morbidity in murine schistosomiasis. [Journal of American Science 2010;6(4):138-145]. (ISSN: 1545-1003).

Keywords: Schistosomiasis – Morbidity – Cytokines – Treatment.

1. Introduction

Schistosomiasis is a chronic and debilitating disease that remains one of the most prevalent parasitic infections in the humid tropics, with an estimated 650 million people at risk of infection and 200 million actually infected in 74 countries (WHO, 2002). It is encouraging that significant progress in the control of schistosomiasis has been achieved over the last several years in Brazil, China and Egypt. However, because of environmental changes linked to water resources development and the rapidly increasing sizes and movements of population, the disease has spread to previously non-endemic or low endemic areas (Engels *et al.* 2002).

The main cause of morbidity and mortality in human schistosomiasis is hepatic fibrosis, which essentially involves portal spaces, without severe lesions in the hepatic parenchyma. Management of schistosomiasis has focused primarily on treating and preventing the complications of portal hypertension. Unfortunately, no therapy has been proved to prevent progressive hepatic fibrosis which is associated with granulomatous hypersensitivity to parasite eggs. A proportion of patients with chronic schistosomiasis retain the hepatic fibrous scarring of the liver, following antihelminthic treatment. This problem leads to the suggestion that addition of anti-fibrotic agents as an adjuvant to anti-schistosomal chemotheraby may be useful in the treatment of *Schistosoma mansoni (S. mansoni)* infection (Mohamed *et al.* 1991).

Praziquantel (PZQ) remains the only antibilharzial drug effective against the four main schistosomes pathogenic to man (Gönnert & Andrews, 1977; WHO, 2002). Although it has been reported that PZQ has minimal side effects, control of schistosomiasis using PZQ at a population level faces some problems. Resistance to PZQ has been recently induced in schistosomes by laboratory selection (Fallon & Doenhoff, 1994). Reduced cure rates and failure of treatment after PZQ have been reported in Senegalese, Kenyan and Egyptian patients (Ismail *et al.* 1999; Fallon *et al.* 2000, Gryseels *et al.* 2001).

DDB (dimethyl – 4 , 4 '- dimethoxy – 5 , 6 , 5 ', 6' -dimethylene dioxybiphenyl- 2,2'-dicarboxylate), a component derived from *Shizandrae,* is a curative agent for the treatment of hepatitis used clinically in East Asia (e.g. China & Korea). It protects liver tissue against carbon tetrachloride-, galactosamine-, thioacetamide- or prednisolone-induced injuries, and enhances antibody production (Liu, 1987; Salama *et al*. 2004). A long term randomized controlled human study has shown DDB to substantially improve the liver function of patients with the hepatitis B virus (Liu, 1987). We have reported that the pharmacological effect of DDB was associated with the inhibition of NF-KB activation and TNFβ (Salama *et al*. 2004).Previous studies on the effect of corticoids in murine schistosomiasis showed variations according to the dose, the type and the schedule of treatment used (Harrison & Doenhoff , 1983, Morrison *et al. 1986,* Hermeto *et al* . 1990). It was proposed that the decrease in worm burden was due to impairment in the initial phase of parasite penetration into host tissues (Hermeto *et al . 1994*). Dexamethasone also decreased the level of collagen synthesis and the level of post-translational enzymes associated with

collagen synthesis (Newman & Cutroneo, 1978; James *et al*. 1983).

This study aimed to explore the effects of DDB or dexamethasone either alone or in combination with PZQ on several parasitological, immunological, and pathological parameters in murine schistosomiasis.

2. Material and Methods

Materials:

Animals:

Male, Swiss albino Laboratory-bred mice, each weighing 18-20 grams were used in this study. They were maintained, in conditioned rooms at 21ºC, on sterile water adlibitum and balanced dry food containing 24% protein. The animal experiment was carried out according to the internationally valid guidelines (Nessim *et al.* 2000) at Schistosome Biological Supply Program Unit of Theodor Bilharz Research Institute (SBSP/TBRI, Giza, Egypt).

Cercariae:

Schistosoma mansoni cercariae suspension (0.2 ml) was obtained from SBSP/TBRI and placed drop by drop on a glass plate. The cercariae on the plate were killed by the addition of one drop of 1% iodine. With the aid of a dissecting microscope, the number of cercariae was determined. Generally five counts were made to calculate the number of cercariae per ml of the suspension and the average number per 0.1 ml was used. Infection was performed by the subcutaneous injection of 100 *S. mansoni* cercariae to each mouse (Stirewalt & Dorsey, 1974).

Drug regimen:

Praziquantel

Tablets (600mg) were grinded as white powder and suspended in 13 ml of 2% cremophore-EL, as it is insoluble in water. The drug was freshly prepared before oral administeration to mice using a stainless steel oral canula. The dose given was 500 mg/kg body weight for two consecutive days.

Diphenyl Dimethyl Bicarboxylate (DDB)

Bimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene-dioxybiphenyl-2,2′ dicarboxylate was supplied by Dongkwang Pharmaceutical Co. DDB was administered orally to mice using a stainless steel oral canula. The dose given was 25mg/kg body weight, three times a week until the end of the experiment.

Dexamethasone disodium phosphate

Decadron (Prodome, Brazil) was injected by the intramuscular route at 1 mg/kg body weight three times a week until the end of the experiment.

Experimental design:

Mice (70) were divided into seven groups (each composed of ten mice) as follows:

Group 1: Normal control group.

Group 2: Infected untreated control group, in which mice were infected with 100 *S. mansoni* cercariae.

Group 3: Infected-treated group, with 25 mg/kg DDB orally, three times/ week from the first day of infection to the end of the experiment.

Group 4: Infected-treated group, with 25 mg/kg DDB orally, three times/ week from the first day of infection to the end of the experiment and 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Group 5: Infected-treated group, with intramuscular injection of 1 mg/kg dexamethasone, three times/week from the first day of infection to the end of the experiment.

Group 6: Infected-treated group, with intramuscular injection of 1 mg/kg dexamethasone, three times/week from the first day of infection to the end of the experiment and 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Group 7: Infected with *S. mansoni* cercariae and treated with 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Animals of all groups were killed under anesthesia, 8 weeks post infection.

Methods:

Parasitological Parameters

Worm burden: Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting (Duvall & DeWitt, 1967).

Tissue egg load: The number of ova/gm intestinal or hepatic tissue was counted after digestion overnight in 5% KOH (Cheever, 1968; Kamel *et al.* 1977).

Percentage egg developmental stages "Oogram pattern": The percentage of eggs at different developmental stages were examined in three samples/mouse and the mean of each stage/animal was obtained (Pellegrino *et al.* 1962).

Immunological study

Serum enzyme assessment

Animals of all groups were weighed, then killed and blood was collected. The serum was separated by centrifugation at 3000×*g* for 10 minutes and stored at −20°C for the assay of ALT [EC 2.6.1.2] (Reitman & Frankel, 1957), GGT [EC 2.3.2.2] using Boehringer reagent kit (Mannheim, Germany), AP [EC 3.1.3.1] (Kind & King, 1954), total protein (Weichselbaum, 1946) and albumin (Doumas et al. 1971).

Cytokine assay

Serum IFN-γ, IL-12, IL-4, and IL-10 levels were measured 8 weeks post-infection by a sandwich enzyme-linked immunosorbent assay technique with capture and detection antibodies according to the instructions of the manufacturer (PharMingen, San Diego, Calif.). Recombinant cytokines were used as standards. Briefly, plates (Nunc, Roskilde, Denmark) were coated with capture antibodies with 100 ul of serum sample or recombinant cytokine. Following addition of the biotinylated detection antibody and streptavidin-alkaline phosphatase conjugate, the reaction was developed with *para*-nitrophenyl phosphate (Sigma). Absorbance at 405 nm was measured with a Benchmark reader (Bio-Rad Laboratories Inc., Hercules, Calif.). Assays were performed in duplicate. The cytokine concentration was obtained from a regression curve prepared with the help of Microplate Manager software (Bio-Rad).

Histopathological study

Liver specimens were fixed in 10% buffered formalin and embedded in paraffin blocks. The prepared 4μm thick sections were examined by light microscopy using Hematoxylin and eosin and Masson trichrome stains.

Measurement of mean granuloma diameter per group was calculated at a microscopic magnification of X100 using an occular micrometer. Only lobular granulomas containing eggs in their centers and non-confluent ones were measured (Lichtenberg, 1962).

Statistical analysis

The data were presented as mean ± standard error of the mean (X±SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05. different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

3. Results

The Worm burden and tissue egg load in the intestine and liver of each studied group were calculated as the mean ± SE. In the infected control group, the total number of worms counted was 29.6±0.26, divided between liver (43%) and portomesenteric vein (57%). Oral administration of DDB to infected mice with *S. mansoni* reduced the total number of worm burden to 23.3±0.29 (21.37% reduction); treatment of mice with dexamethasone alone reduced the total number of worm burden to 25.6 ± 0.20 (13.5 % reduction) especially those in the liver (Table 1). On the other hand, PZQ caused a marked reduction in worm burden reaching 95.6%, with 60% of the worms shifted to the liver; this inhibition was slightly improved when PZQ was given in combination with DDB (95.9%). The oogram pattern after PZQ treatment showed a complete disappearance of all immature ova from the wall of the intestine, a reduction in the number of mature ova and a four fold increase in dead ova. dexamethsone alone affected the number of dead ova significantly, reduced the number of mature ova (19–20%) while hardly affecting the immature ova. Combination of PZQ with DDB or dexamethasone augmented its effect on the mature ova to reach 85% 95% respectively (Table 2). In principle, the same observation was noted in egg load, where PZQ reduced it in both intestine (95.7%) and liver (96.4%), slightly expanded upon combination with DDB. DDB or dexamethsone alone showed a decrease also in the egg load mounted to 76.8%, 72.7% in the intestine, 76.1% and 66.5% in the liver.

Hepatic granuloma in each studied group was measured as the mean of granuloma diameter± SE and it was 318.8 μm ± 26.3 for the infected control group. Oral administration of DDB to infected mice with *S. mansoni* decreased the granuloma diameter to 194.1 μm ± 21.2 (39.1 % reduction) while administration of DDB in combination with PZQ decreased the mean of granuloma diameter to 168.1μm ± 32.11 (47.3% reduction). Intramuscular administration of dexamethsone to infected mice with *S. mansoni* decreased the granuloma diameter to 142.2 μm ± 25.1 (55.4 % reduction) that reached 119.2 μm ± 29.5 (62.6 % reduction) (Table 3). Accordingly, a significant reduction ($p<0.001$) was observed in granuloma diameter in the two treated groups either alone or combined with PZQ relative to infected control.

Table 1: **Effect of oral administration of DDB and intramuscular administration of Dexamethasone on Worm burden and tissue egg load in different studied groups**

Animal group	Mean No. of worms ± SE	% Reduction	Mean no. of ova count ± SE			
			Intestine	% reduction	Liver	% reduction
Infected Control	29.6 ± 0.26	-	14199±1342	-	2877±411	-
Dexamethson			***		***	
Dexamethson +PZQ	25.6 ± 0.31	13.5 %	3877± 211	72.7%	965±255	66.5 %
DDB	*** 1.6 ± 0.35	94.6 %	*** 715 ± 121	95 %	*** 178± 40	93.8 %
DDB + PZQ	*** 23.3 ± 0.29	21.3 %	*** 3292 ± 233	76.8 %	*** 689 ± 98	76.1 %
PZQ	*** 1.2 ± 0.29	95.9 %	*** 545 ± 133	96.2 %	*** 94±13	96.7 %
	*** 1.3 ± 0.35	95.6 %	*** 612 ± 156	95.7 %	*** 101±13	96.4 %

***** Statistically significant difference at p< 0.001 compared to infected control group.**
***** Statistically significant difference at p< 0.001 compared to infected control group.**

Table 2: Effect of oral administration of DDB and intramuscular administration of Dexamethasone oogram pattern of mice infected with 80 *S. mansoni* cercariae and sacrificed 8 weeks postinfection.

Group Name	**Oogram pattern (% ova)**		
	Immature	**Mature**	**Dead**
Infected Control	65.3 ± 5.4	31.1 ± 2.6	3.6 ± 0.7 *
Dexamethson	50.2± 4.2	30.7 ± 3.4	19.1 ± 1.5
Dexamethson +PZQ	*** 5.3 ± 5.1	*** 4.3 ± 1.4	*** 90.4 ± 8.3
DDB	** 20.2± 5.4	43.2± 6.1	** 36.6± 2.4
DDB + PZQ	*** 10.3 ± 1.9	*** 6.5 ± 1.4	*** 83.2 ± 5.4
PZQ	*** 2.3 ± 0.4	*** 1.9 ± 0.2	*** 95.8 ± 4.9

* Statistically significant difference at p< 0.05 compared to infected control group
** Statistically significant difference at p< 0.01 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Table 3:- Effect of oral administration of DDB and intramuscular administration of Dexamethasone on hepatic granuloma diameter of mice infected with *S. mansoni*.

Group Name	Hepatic granuloma diameter X GD ± SE	% Reduction
Infected Control	318.8 ± 26.3	- ***
Dexamethson	142.2 ± 25.1	55.4 % ***
Dexamethson +PZQ	119.2 ± 29.5	62.6% **
DDB	194.1 ± 21.2	39.1% **
DDB + PZQ	168.1 ± 32.11	47.3 % **
PZQ	201.1 ± 25.3	24.1 %

GD :- Granuloma diameter
SE:- Standard Error.
** Statistically significant difference at p< 0.01 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Table 4:- Effect of oral administration of DDB or intramuscular injection of dexamethasone either alone or combined with PZQ on cytokine production of mice infected with 100 *S. mansoni* cercariae and sacrificed 8 weeks post-infection

Group Name	IL-4 pg/ml X ± SE	IL-10 pg/ml X ± SE	IL-12 pg/ml X ± SE	IFN-γ pg/ml X ± SE
Infected Control	770±160.1	512±19.1	150±10.1	672±74.0
DDB	687±144.1	612±46.5***	131±9.6	607±63.0
DDB + PZQ	712±155.2	668±50.3	138±12.5	613±72.0
Dexamethasone	272±57.1***	711±23.1***	52±16.1***	127±54.0***
Dexamethasone+PZQ	288±100.3	698±33.0	45±14.4	192±94.0
PZQ	711±133.0	588±45.5	163±11.7	633±53.0

X = Mean SE= Standard Error.
**** Significant difference at p< 0.01 compared to infected control group.**
*** significant difference at p< 0.001 compared to infected control group.

Cytokines assy

Cytokines are believed to modulate the amount of fibrosis and granuloma size and play a fundamental role in the pathology of schistosomal infection. In order to investigate if the modulatory effects of both DDB and dexamethasone on granulomas were mediated through alteration of cytokine production, the levels of these mediators in serum were measured. Intramuscular administration of dexamethasone to infected mice induced significant decrease in the levels of IL-4, IFN-γ or IL-12 when compared their levels on infected untreated control group, while treatment of mice with DDB induced insignificant decreases in the levels of IL-4, IFN-γ, and IL-12 (Table 4). On the other hand, significant increases in serum IL-10 levels were detected in groups treated with DDB or dexamethasone comparing to the infected untreated control group.

Liver Enzymes Assay.

Treatment of mice with DDB or dexamethasone was found to reduce serum enzyme levels characteristic of hepatic damage induced by infection, as indicated by a lowering in the raised levels of serum ALT (78%, 85% respectively), GGT (73%, 86% respectively) and AP (76%, 93% respectively). Treatment of mice with DDB or dexamethasone, also tended to normalize the lowered levels of serum albumin. Untreated infected mice showed a two fold elevation of liver enzymes as compared with normal control animals. Treatment with PZQ alone reduced liver enzymes insignificantly compared to untreated infected mice. The highest significant reduction (p<0.100) in liver enzymes was observed in combination of PZQ with DDB or dexamethasone.

4. Discussions

Schistosomiasis is a major public health problem in tropics, with tens of millions infected and many more at risk (Boros, 1999). It has been estimated that greater than 250,000 deaths per year are directly attributable to this disease (Botros et al. 2000), and the subtle morbidities associated with chronic infection have a more serious impact.

Treatment relies on a single drug, praziquantel, to eliminate the adult worms but this has no prophylactic properties and is ineffective against resistant strains (Botros et al. 2000).

Previous studies have been reported using non-steroidal anti-inflammatory drugs (NSAIDs) e.g tiaprofenic acid and piroxicam either alone or as adjuvant to praziquantel in treating hepatic granuloma in *S.mansoni*-infected mice (Hegazy et al. 1997). The possibility of using another NSAIDs namely, ibuprofen (CAS 15687-27-1) and naproxen (CAS 22204-53-1), either alone or in combination with praziquantel (CAS 55268-74-1) has been studied to induce regression of hepatic morbidity or to ameliorate the biochemical and histopathological consequences and intensity of infection (Mahmoud et al. 2002). However, in the current study, we aimed to investigate the possible role of DDB or dexamethasone alone or as co adjuvant therapy in the treatment of murine schistosomiasis.

Oral administration of DDB to *S. mansoni*-infected mice showed insignificant decrease in the worm burden, and alteration of egg load in intestinal and liver tissue (76.8% and 76.1% reduction, respectively). However, combination with PZQ decreased the egg load in the intestinal and hepatic tissue giving 96.2% and 96.7% reduction, respectively. Moreover, reduction in granuloma diameter and cytokine production, indicated that administration of DDB to *S. mansoni*-infected mice may improve disease morbidity.

The protective effects of DDB on chemically induced damage of isolated suspended rat hepatocytes were studied by Fu and Liu (1992) and its reversing effect on the phenotyes of human hepatocarcinoma cell line has been evaluated (Liu et al. 1996). Recently, administration of DDB on tamoxifen-induced liver injury in rats showed that prolonged treatment revealed a potent anti-fibrogenic role (El-Beshbishy, 2005). Moreover, a pharmaceutical composition of garlic oil and DDB, as active ingredients for enzyme induction and liver protection has been used as a curative preparation for patients with acute or chronic viral hepatitis (Park et al. 2005).

On the other hand, dexamethasone was previously investigated as a co adjuvant immunomodulator in treatment of chronic schistosomiasis (Pyrrho *et al.* 2002).

Our results showed insignificant effect of dexamethasone on parasite number which agreed with Lambertucci et al. (1989). However, investigators who used hydrocortisone or a dose of dexamethasone 50 times higher than ours reported decrease in the parasite burden (Coker, 1957, Hermeto *et al. 1993*). Following oviposition, the eggs are carried mainly to intestinal and hepatic veins then to the lungs and other tissues. Although a lack of *S. mansoni* fecundity using dexamethasone in vitro has been described (Morrison, 1986), another decrease in the amount of oviposition was reported after oral administration of dexamethasone in vivo (Lambertucci et al. 1989). Our results demonstrated that with the therapeutic schedule used, neither worm development nor oviposition was significantly modified, but the treatment altered egg distribution in tissue, favoring a more intense deposition in the intestine. The mechanism by which dexamethasone alters the egg distribution in tissue is unknown. Also, little is known about the effect of this glucocorticoid on the migration of female parasites and on the intravascular sites of oviposition. A reduction in the rate of egg excretion following treatment of infected mice with corticosteroids or hydrocortisone acetate was observed (Newsome, 1963; Doenhoff et al. 1978) with consequent changes in the places where the eggs are trapped in the tissues. Since granulomas are composed of several cell types and extracellular matrix components, the action of dexamethasone on these elements is pleiotropic and difficult to evaluate in vivo. However, granuloma size in animals treated with dexamethasone showed significant decrease, probably due to the high levels of IL-10 induced by treatment. This observation is in accordance with Franchimont et al. (1999) who showed that administration of exogenous IL-10 resulted in reduction of granuloma size. Furthermore, an opposite effect was seen in IL-10-deficient mice (Wynn et al. 1998). Rezende et al. (1997) suggested that immunocomplexes from patients with chronic intestinal schistosomiasis are able to modulate granulomatous hypersensitivity to *S. mansoni* eggs by inducing prostaglandin E production that augments IL-10 level.

Cytokines are believed to modulate the amount of fibrosis and granuloma size and play a fundamental role in the pathology of schistosoma infection. In order to investigate if the modulatory effects of both DDB and dexamethasone on granulomas were mediated through alteration of cytokine production, the levels of these mediators in serum were measured. Administration of exogenous IL-4 increases the amount of fibrosis (Yamashita & Boros. 1992), while administration of anti-IL-4 or exogenous IFN-γ decreases the level of collagen deposition (Cheever et al. 1994, Czaja et al. 1989). In murine models, IL-12 was also involved in reduction of the amount of fibrosis and granuloma size (Wynn et al. 1995, Hoffmann et al. 1998). However, compared to wild-type mice, mice lacking IL-4 (IL-4 knockout mice) showed diminution in catalase levels, increased hepatotoxicity that resulted in early

mortality (Fallon et al. 2000, La Flamme et al. 2001). In our study, administration of DDB or dexamethasone to *S. mansoni*-infected mice decreased serum IL-12 and IFN-γ levels and induced a pronounced reduction of IL-4 levels, but it is possible that despite the decrease in the level of IL-4 production, the circulating levels of this cytokine are enough to exert a protective effect and has been suggested that it plays an important role in the severity of *S. mansoni* infection and may influence the course of disease (Brunet et al. 1998, Fallon et al. 2000). Since dexamethasone also increased serum IL-10 levels, our data are in agreement with those from previous reports (Hoffmann et al. 1999; 2000; Wynn et al. 1997), indicating that production of IL-10 is the key factor in preventing the polarization toward a Th1 or Th2 profile and therefore avoiding an increase in rates of disease morbidity.

In conclusion, the use of DDB or dexamethasone as a coadjuvant treatment with praziquantel in murine schistosomiasis, in addition to minimizing the morbidity of infection, may give an insight into the mechanisms involved in its pathogenesis.

References

1. Boros DL 1999. T helper cell populations, cytokine dynamics, and pathology of the schistosome egg granuloma. Microbes Infect. 1:511–516.
2. Boros DL, Pelley RP, Warren KS 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. J. Immunol. 114:1437–1441.
3. Botros SS, Makary EA, Ahmed KM, Ibrahim AM, Nashed NN, El-Nahal HM, Doughty BL, Hassanein HI 2000. Effect of combined praziquantel and recombinant glutathione S-transferase on resistance to reinfection in murine schistosomiasis mansoni. Int. J. Immunopharmacol. 22:979–988.
4. Brunet LR, Dunne DW, Pearce EJ 1998. Cytokine interaction and immune responses during *Schistosoma mansoni* infection. Parasitol. Today 14:422–427.
5. Cheever AW, Williams ME, MET A, Wynn TA, Finkelman FD, Seder RA, Cox TM, Hieny SP, Caspar P, Sher A 1994. Anti-IL-4 treatment of *Schistosoma mansoni*-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. J. Immunol. 153:753–759.
6. Chiaramonte MG, Cheever AW, Malley JD, Donaldson DD, Wynn TA 2001. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. Hepatology 34:273–282.
7. Coker C 1957. Effect of cortisone on natural immunity to *Schistosoma mansoni* in mice. Proc. Soc. Exp. Biol. Med. 96:1–3.
8. Czaja MJ, Weiner FR, Takahashi S, Giambrone MA, van der Meide PH, Schellekens H, Biempica L, Zern MA 1989. Gamma-interferon treatment inhibits collagen deposition in murine schistosomiasis. Hepatology 10:795–800.
9. Doenhoff M, Musallam R, Bain J, McGregor A. 1978. Studies on the host-parasite relationship in *Schistosoma mansoni*-infected mice: the immunological dependence of parasite egg excretion. Immunology 35:771–778.
10. Duvall RH, Dewitt WB 1967. An improved perfusion technique for recovering adult Schistosoma from laboratory animals. Am J Trop. Med Hyg. 16: 483- 486.
11. Gönnert, R, Andrews, P 1977. Praziquantel a new board spectrum antischistosomal agent. *Z. Parsitenkd*. 52: 120 – 129
12. Gryseels B, Mbaye A, De Vlas SJ, Stelma F, Guisse F, Van Lieshout L, Faye D, Diop M, Ly A, Tchuem-Tchuente L, Engels D, Polman K 2001. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Trop Med Int Health*, 6: 864-873.
13. Engels D, Chisulo L, Montresor A, Savioli L 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta Trop., 82: 139-146.
14. Ismail MM, Botros S, Metwally A, William S, Farghally A 1999. Resistance to praziquantel: Direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am J Trop Med Hyg. 60:932–935.
15. Lichtenberg EV 1962. Host response to eggs of *Schistosoma mansoni* .Granuloma fromation in the unsensitized laboratory mouse. *Am J Pathol*, 41:711.
16. Fallon P, Doenhoff M 1994. Drug-resistant schistosomiasis: Resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific.*Am J Trop Med Hyg*, 51:83-88.
17. Fallon P G, Richardson EJ, McKenzie GJ, McKenzie AN 2000. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J. Immunol. 164:2585–2591.
18. Flores Villanueva PO, Zheng XX, Strom TB, Stadecker MJ 1996. Recombinant IL-10 and IL-10/Fc treatment down-regulate egg antigen- specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. J. Immunol. 156:3315–3320.

19. Harrison RA, Doenhoff MJ 1983. Retarded development of *Schistosoma mansoni* in immunosuppressed mice. Parasitology 86:429–438.
20. Hermeto MV, Bicalho RS Melo AL, Pereira LH 1990. Kinetics of the pulmonary phase of *Schistosoma mansoni* in mice treated with dexamethasone. Rev. Inst. Med. Trop. Sa˜o Paulo 32:168–171.
21. Hermeto MV, Melo AL, Bicalho RS, Vargas AP, Favaretto FJ, Pereira FH 1993. Dexamethasone does not reduce the worm burden in mice infected with in vivo obtained schistosomules of *Schistosoma mansoni*. Rev Inst Med Trop Sa˜o Paulo 35:389–390.
22. Hermeto MV, Bicalho RS, da Silva RE, de Melo AL, Pereira LH 1994. Oogram studies in mice infected with *Schistosoma mansoni* and treated with dexamethasone. Rev. Inst. Med. Trop. Sa˜o Paulo 36:99–104.
23. Hoffmann KF, Caspar P, Cheever AW, Wynn TA 1998. IFNgamma, IL-12, and TNF-alpha are required to maintain reduced liver pathology in mice vaccinated with *Schistosoma mansoni* eggs and IL-12. J. Immunol. 161:4201–4210.
24. Hoffmann KF, Caspar P, Cheever AW, Wynn TA. 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. J. Immunol. 164:6406–6416.
25. Hoffmann K F, James SL, Cheever AW, Wynn TA 1999. Studies with double cytokine-deficient mice reveal that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccineinduced immunity to *Schistosoma mansoni*. J. Immunol. 163:927–938.
26. Salama HM, Amer AR, Hammad OM, El-Sayed WF 2004. Effect Of Ddb Monotherapy And In Combination With Amantadine Hydrochloride And Ribavirin In Patients With Chronic Hepatitis C Virus Infection. Sci. Med. J. ESCME, Vol.16,No. 1
27. James SP, Hoofnagle JH, Strober W, Jones EA 1983. NIH conference: primary biliary cirrhosis: a model autoimmune disease. Ann. Intern. Med. 99:500–512.
28. La Flamme AC, Patton EA, Bauman B, Pearce EJ 2001. IL-4 plays a crucial role in regulating oxidative damage in the liver during schistosomiasis. J. Immunol. 166:1903–1911.
29. Lambertucci JR, Modha J, Curtis R, Doenhoff M 1989. The association of steroids and schistosomicides in the treatment of experimental schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. 83:354–357.
30. Liu GT 1987: Clinical uses and pharmacology of DDB. A new drug for chronic viral hepatitis B. Proc. CAMS and PUMC.; 2, 228-233.
31. Morrison DD, Waa EAV, Bennett. JL 1986. Effects of steroids and steroid synthesis inhibitors on fecundity of *Schistosoma mansoni* in vitro. J. Chem. Ecol. 12:1901–1908.
32. Newsome J 1963. Observations on corticosteroid treatment of schistosomiasis in hamsters and baboons. Trans. R. Soc. Trop. Med. Hyg. 57:425–432.
33. Nessim NG, Hassan SI, William S, El-Baz H 2000. Effect of the broad spectrum anthelmintic drug flubendazole upon *Schistosoma mansoni* experimentally infected mice. *Arzneimitted-Forrschung/Drug Research, 50: 1129-1133.*
34. Pellegrino J, Oliveira CA, Faria J, Cumha AS 1962. New approach to the screening of drugs in experimental *Schistosoma mansoni* in mice. Am. J. Trop. Med. Hyg., 11: 201-215.
35. Rezende SA, Silva Teixeira DN, Drummond SC, Goes AM 1997. IL-10 plays a role in the modulation of human granulomatous hypersensitivity against *Schistosoma mansoni* eggs induced by immune complexes. Scand J. Immunol. 46:96–102.
36. Stirewalt M, Dorsery CH 1974. *Schistosoma mansoni* penetration stimuli for schosome cercariae. *Exp Parasitol, 35: 10.*
37. World Health Organization 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. World Health Organ Tech Rep Ser, 912.
38. Wynn TA, Cheever AW, Jankovic D, Poindexter RW, Caspar P, Lewis FA, Sher A 1995. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. Nature 376:594–596.
39. Wynn TA, Cheever AW, Williams ME, Hieny S, Caspar P, Kuhn R, Muller W, Sher A 1998. IL-10 regulates liver pathology in acute murine schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. J. Immunol. 160:4473–4480.
40. Wynn TA, Morawetz R, Scharton Kersten T, Hieny S, Morse HC, Ku¨hn R, Mu¨ller W, Cheever AW, Sher A 1997. Analysis of granuloma formation in double cytokine-deficient mice reveals a central role for IL-10 in polarizing both T helper cell 1- and T helper cell 2-type cytokine responses in vivo. J. Immunol. 159:5014–5023.
41. Yamashita T, Boros DL 1992. IL-4 influences IL-2 production and granulomatous inflammation in murine schistosomiasis mansoni. J. Immunol. 149:3659–3664.

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The role of catechin against doxorubicin – induced cardiotoxicity in Ehrlich Ascites Carcinoma Cells (EAC) bearing mice

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Abstract : Doxorubicin (Dox) is a chemotherapy drug used for treatment of wide variety of cancers. It known that, Dox may cause cardiotoxicity by producing free radicals and oxidative stress along the period of treatment. Catechin is considered one of the flavonoids which has powerful antioxidant properties and free radicals scavenger. The present work was designed to investigate the protective role of catechin on doxorubicin – induced cardiotoxicity in Ehrlich Ascites Carcinoma (EAC) bearing- mice and to test whether catechin has an effect on the antitumor properties of the Dox. Mice were divided into five groups as follows: (G1): Control group, (G2) Mice were injected with Ehrlich Ascites Carcinoma (EAC) cells (2.5 x 10^6 EAC/ml) to form a solid tumor , (G3) Mice were inoculated with (2.5 x 10^6 EAC/ml) and injected (i.p.) with Doxorubicin (15 mg/kg), (G4) Mice were inoculated with (EAC) at the same dose and were injected (i.p.) with (200mg/kg) Catechin , Group5 (G5) Mice were injected (i.p.) with Doxorubicin 15mg/kg of and 200mg/kg of Catechin in addition to the inoculation with EAC (2.5 x 10^6 EAC/ml). Dox (15mg/kg) and /or Catechin (200mg/kg) were administrated after 10 days in EAC bearing- mice through a period of 2 weeks in six equal injections. Results showed that, EAC -bearing mice treated with Dox plus Catechin recorded decrease in the mean tumor weight and significant increase in the cumulative mean survival time as compared to the other treated groups. Biochemical studies of EAC inoculation showed decline in serum total protein and lactate dehydrogenase activities, while serum total lipid has significantly increased. The treatment of tumor-bearing mice with Dox plus Catechin (G5) improved these levels. Significant increase in cardiac lipid peroxidation and glutathione contents for both tumor-bearing mice (G2) and doxorubicin groups (G3) were recorded. Combined treatment of Dox and Catechin (G5) caused amelioration in these contents. Glutathione peroxidase and superoxide dismutase activities showed highly significant increase in all treated groups. Administration of Dox plus Catechin (G5) modulate these activities. In conclusion, the present study suggested that Catechin treatment may significantly reduce cardiotoxicity induced by doxorubicin in Ehrlich Carcinoma - bearing mice by the induction of the cardiac antioxidant enzymes and blocking lipid peroxidation. Also, Catechin enhances the antitumor properties of doxorubicin by increasing its inhibitory effect on tumor growth. [Journal of American Science 2010;6(4):146-152]. (ISSN: 1545-1003).

Keywords : Ehrlich Ascites Carcinoma (EAC) ; Doxorubicin ; Catechin ; Antioxidant enzymes ; Lipid peroxidation ; Heart ; Mice.

1. Introduction

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev et al., 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans (Gupta et al., 2004). Anthracyclines constitute a major class of cytotoxic agents for the treatment of cancer. Among these, doxorubicin possesses a broad spectrum of antitumor activity, exhibits a wide activity against leukemia's breast, lung, thyroid and ovarian carcinomas (Lonnie 2002 and Aleisa et al., 2007).

Doxorubicin caused cardiotoxic effect in animals (Kalender et al., 2002) and in patients (Bast et al., 2007). It is widely accepted that oxidative stress and the production of free radicals are involved in doxorubicin both in terms of antitumor effects and cardiotoxicity (Singal et al., 2000). Olson et al. (1988) suggested that this toxicity could involve doxorubicinol, the primary circulating metabolite of doxorubicin. Doxorubicinol could accumulate in the heart and contribute significantly to the chronic cumulative cardiotoxicity of doxorubicin therapy. Hence, different treatment schedules were used to decrease drug levels in plasma and myocardium and it has also been proposed free radical scavengers and flavonoids might be effective in lessening the pathological changes observed after anthracycline treatment (Kalender et al., 2002). Antioxidants protect cells and tissues against free radical which caused oxidative damage and injury (Nakagawa and Yokozawa et al., 2002). Green tea's antioxidant effects seem to be dependent upon the polyphenol (Catechin) fraction (Kumamoto, 1998). Several epidemiological and in vitro studies suggest that Catechins have beneficial effects on human health, serving to protect against congestive heart failure, cancer, myoglobinuric acute renal failure, reduce the incidence of myocardial ischemia and the risk of ischemic heart disease

mortality, due to their antioxidant activities (Du and Lou 2008 ; Korish and Arafah 2008).

So, the present work was designed to investigate the protective role of Catechin against doxorubicin – induced cardiotoxicity in Ehrlich carcinoma cells (EAC) bearing- female mice and also to test whether Catechin has an effect on the antitumor properties of doxorubicin.

2. Materials and Methods

2.1 Tumor cell line:

Because Ehrlich Ascites Carcinoma cells were reported to show greater initial growth and total cell count in female mice than male mice (**Vincent and Nicholls, 1967**), the present study used female mice as experimental animals. The initial inoculation of Ehrlich Ascites Carcinoma (EAC) cells was kindly provided by the National Cancer Institute (Cairo University, Egypt). The tumor line was maintained in female mice by weekly intra-peritoneal injection of $2.5x10^6$ cells/mouse according to the method recommended by the Egyptian National Cancer Institute, Cairo University. Such developed tumor is characterized by its moderate rapid growth which could not kill the animal due to the accumulation of ascites before about 14 days after transplantation. Cells were counted before injection using the bright line haemocytometer and dilutions made by physiological saline. The desired number of cells was injected in a volume of 0.5 ml/mouse. Solid Ehrlich carcinoma was induced by inoculation of $2.5x10^6$ cells in the back between the thighs of each animal (Fahim et al., 1997).

2.2 Animals:

Adult female Swiss albino mice, weighing 22-25 gm were obtained from the animal house of Theodore Bilharis Institute (Cairo, Egypt).The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). The animals were maintained on a standard pellet diet and tap water *ad libitum*..

2.3 Chemical compounds:

Doxorubicin used in the present work was manufactured by Pharmacia Italia S.P.A., with molecular weight 543.5262 and chemical formula: C_{27}-H_{29}-NO_{11}. CAS Number is 29042-30-6. Doxorubicin was administered intraperitoneally in six equal injections (each containing 2.5 mg/kg) to animals over a period of 2weeks for accumulative dose of 15 mg/kg body wt. according to (Timao and Singal, 2000). All mice were treated for two weeks with doxorubicin after bearing 9-day –old solid Ehrlich carcinoma.

(+)-Catechin was purchased from Fluka Chemicals (Ronkonkama, NY). Catechin chemical formula is C_{15} H_{14} O_6 with molecular weight 290.272 and CAS Number 7295-85-4. Catechin is soluble in water and was administered at dose (200 mg/kg body wt) in mice in six intraperitoneal (i.p.) injections alone or 30 min after doxorubicin (2.5 mg/kg body wt) administration according to **(**Kalender et al., 2005).

2.4 Groups of Experimental Animals:

A total number of 125 female Swiss albino mice were used in the experimental groups. Female mice used in the present work according to many previous studies (Ali, 2005 and Fouda, 2005). Animals were divided into five groups, each containing 25 mice as follows: **Group1**: Control group (G1): Mice of this group maintained on a standard pellet diet and water applied *ad libitum* were used as a normal or negative control group. **Group2**: Ehrlich tumor group (G2): Mice of this group were inoculated in the back between the thighs of each animal with Ehrlich Ascites Carcinoma (EAC) cells. Each mouse was injected subcutaneously with 2.5×10^6 EAC/ml to form a solid tumor (Fahim et al., 1997) this group known as tumor or positive control group. **Group3**: Tumor and Doxorubicin treated group (G3): Mice of this group were inoculated with 2.5×10^6 of (EAC) in the back between the thighs of each animal and injected (i.p.) with Doxorubicin (15 mg/kg)**. Group4**: Tumor and catechin treated group (G4): Mice of this group were inoculated with Ehrlich Ascites Carcinoma (EAC) in the back between the thighs of each animal with the above dose. Also these mice were injected intraperitoneally (i.p.) with (200mg/kg) catechin according to (Kalender et al., 2005).**Group5**: Tumor, doxorubicin and catechin treated group (G5): Mice of this group were injected (i.p.) with doxorubicin 15mg/kg of and 200mg/kg of catechin additional to the inoculation of EAC in the back between the thighs of each animal.

2.5 Anti-tumor studies

The anti-tumor activities of doxorubicin and/or catechin were assessed on 168 mice bearing solid Ehrlich Carcinoma that were classified into 4 equally sized groups (Gs.2-5) each group containing 42 mice. Ten days after inoculating the EAC cells (when the tumor became papale), the animals in Gs (3-5) were i.p. injected with the Doxorubicin (15 mg/kg) in six equal injections (each containing 2.5 mg/kg) over a period of 2 weeks and/or Catechin (200 mg/kg) was administered in mice in six injections (i.p.) 30 min after Doxorubicin administration, then left untreated until the end of the experiment. The animals in G (2) received no treatment and served as a control group. The weight of the solid tumor was determined by killed 3 animals every week and the mean weight of the tumors, the cumulative mean survival time (CMST) of the animals and the tumor growth inhibition ratio (T/C %) were recorded according to Fahim et al. (2003). Where: The cumulative mean survival time (CMST) is the days which animals were leaved, Increase of life span (ILS %) = Mean survival time of test / Mean survival time of control **x**100 and the tumor growth inhibition ratio (T/C %)= Mean

tumor weight of control - Mean tumor weight of test / Mean tumor weight of control **x**100.

2.6 Blood sampling:

At the end of the experimental period, mice were over night fasted, anaesthetized under diethyl ether (Sigma Co. USA) and blood was collected by cardiac puncture where blood was collected in dry clean tubes. The blood percolates along the wall of the centrifuge tube to prevent risk of hemolysis. The blood tubes were left 30 minutes then centrifuged at 3000 rpm for 30 minutes. After centrifugation the serum was separated at once, divided into aliquots and stored at -70^{o}C until used in the biochemical analysis.

2.7. Biochemical measurement:

2.7.1. Serum analysis:

Total protein in serum was determined by the method of (Doumas, 19**75***)*. Using a commercial kit purchased from Stanbio chemicals (USA) through Gamma Trade Company. Total lipids in serum were determined by the method of Zollner *(*1962*)* using a commercial kit purchased from Bio-diagnostic Company (Egypt). LDH kit for the quantitative determination of Lactate Dehydrogenase in serum was determined by the method of Kachmar (1976*)*. This kit was purchased from Stanbio chemicals (USA) through Gamma Trade Company.

2.7.2. Tissue analysis:

Heart were excised immediately from animals (mice) after dissection and carefully washed with cold saline, dried and weighed. Fresh heart usually 0.1gm was used for tissues analysis. Estimation of lipid peroxidation (MDA) carries out according to the method of Ohkawa et al. (1979). This method is based on the measurement of malondialdehyde (MDA) as one of the main end products of lipid peroxidation by the thiobarbituric acid test. Determination of glutathione (GSH) content carries out according to the method of Beutler et al. (1963). The method upon determination of a yellow color that developed 5,5dithiol-bis (2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. Glutathione peroxidase (GSH-PX) was measured colorimetrically according to the method of Gross et al. (1967). Estimation of super oxide dismutase (SOD) carries out according to the method of Minami and Yoshikawa (1979). This method is based on the generation of superoxide anions by pyrogallol autoxidation.

2.8 Statistical analysis:

Data were analyzed using Students "t" test according to the method of Hin and Wetherill (1975). Analysis of variance (ANOVA) and Fisher's significant difference test (Winer, 1971) were also used. P values of 0.05 or less were considered statistically significant.

3. Results

3.1 Anti-tumor studies:

Treatment of tumor-bearing animals with the doxorubicin (G3) and doxorubicin plus catechin (G5) recorded an increase in the life span (ILS %), which reached to 112.5% and 131.25%, respectively, compared to tumor-bearing controls (G2). On day 60, at which all animals of both (G 2) and (G 4) were dead, no marked difference in the mean tumor weight were recorded (8.21±0.57g) and (8.91±0.68g) respectively , (Table 1). These results were markedly reduced to (5.12±0.29g) in G (3) and (5.28±0.38g) in G (5). Also, the most elevation of the tumor growth inhibition ratio (T/C %) was recorded in the doxorubicin treated group (G3) as compared to G (4) and G (5).

3.2 Biochemical studies:

The sera levels of total protein in the groups of (2), (3) and (4) were significantly decreased ($P<0.001$) compared to the normal control group (Table 2). On contrast, G 5 showed no difference ($P>0.05$) compared to G1. Tumor bearing mice (G2) and catechin treated (G4) exhibited a very highly significant increase of serum total lipid concentration, the mean total lipid were recorded (595.38 ± 15.37 mg/l) and (591.32 ± 10.06 mg/l), respectively as compared to G1 (159.73 ± 4.55 mg/l). While G3 and G5 showed a highly significant improvement in serum total lipid level as compared to G2 recording (366.11 ± 10.35 mg/l) and (170.11 ± 12.28 mg/l) , respectively. A very highly significant decrease of serum lactate dehydrogenase was observed in G2 (460.47± 12.99 U/L) in relation to G1 (770.61 ± 10.45 U/L). The treatment of tumor bearing mice with doxorubicin (G3) or catechin (G4) recorded a very highly significant elevation of serum LDH as compared to G1 and G2. The cardiac MDA levels of all groups except G5 showed marked elevation. A very highly significant increase in the GSH level was observed in G2 (28.42 ± 2.01 mg/g tissue) compared to G1 (21.73 ± 1.26 mg/g tissue). On the other hand, the rest of other groups showed no statistical difference when compared to G1. Cardiac GPx activity was significantly increased in G2, G3 and G4 where recorded (1072.64 ± 27.62 U/mg), (923.98 ± 23.79 U/mg) and (945.74 ± 16.50 U/mg) in compared to G1 (583.92 ± 16.00), while the GPx level in G5 showed no statistical difference (617.59 ± 20.43 U/mg). The SOD activity of all groups recoded a highly significant increased. This elevation was strongly observed in G3, where it recorded (1.96 ± 0.13 U/g) when compared to G1.

Table (1): Anti-tumor activities of Doxorubicin and/or catechin on Ehrlich carcinoma compared to tumor-bearing controls.

PTI (days)	G2		G3		G4		G5	
	M	MTW±SE	M	MTW±SE	M	MTW±SE	M	MTW±SE
10	0/8	1.10±0.11	0/8	1.12±0.21	0/8	1.20±0.13	0/8	1.11±0.12
17	0/8	2.60±0.26	0/8	1.49±0.15	0/8	2.52±0.28	0/8	1.44±0.16
24	0/8	4.23±0.35	0/8	1.41±0.42	0/8	4.32±0.18	0/8	1.38±0.18
30	0/8	4.54±0.28	0/8	1.71±0.26	0/8	4.61±0.34	0/8	1.72±0.26
36	0/8	5.21±0.45	0/8	2.97±0.17	0/8	5.42±0.31	0/8	2.88±0.43
42	1/8	5.98±0.51	0/8	3.30±0.25	0/8	6.13±0.62	0/8	3.48±0.68
48	2/8	6.52±0.38	1/8	3.84±0.36	3/8	6.82±0.32	0/8	3.93±0.34
54	4/8	7.82±0.44	2/8	4.53±0.18	4/8	7.93±0.71	0/8	4.89±0.29
60	8/8	8.21±0.57	6/8	5.12±0.29	8/8	8.91±0.68	2/8	5.28±0.38
66			8/8	5.68±0.81			5/8	6.24±0.52
72							8/8	6.53±o.67
MTW	5.13		3.12		5.32		3.53	
MST days	48		54		46.50		63	
ILS%			112.50		96.88		131.25	
T/C%			39.4		3.58		31.19	

PTI = Post tumor inoculation. M = Mortality.
MTW = Mean tumor weight (g) MST (days) = mean survival time.
ILS= Increase of life span % T/C= Tumor inhibition ratio

Table 2: The effect of doxorubicin and/or Catechin on heart tissue parameters in Ehrlich carcinoma bearing mice

Groups	Total protein g/dl	Total lipid mg/L	LDH U/L	MDA n mol/gm	GSH mg/g	GPx U/mg	SOD U/g
GI	7.30±0.08	159.73±4.55	770.61±10.45	3.25±0.17	21.73±1.26	583.92±16.00	1.07±0.05
G2	5.68±0.21 -22.19% a ***	595.38±15.3 272.73% a ***	460.47±12.99 -40.18% a ***	5.48±0.22 68.63% a ***	28.42±2.01 30.80% a **	1072.64±27.62 83.69% a ***	1.50±0.08 40.17% a ***
G3	6.63±0.13 -9.18% a ***	366.11±10.35 129.20% a ***	975.71±31.04 26.61% a ***	8.05±0.25 147.52% a ***	24.87±1.60 14.45% N.S.	923.98±23.79 58.24% a ***	1.96±0.13 83.33% a ***
G4	5.55±0.13 -23.97% a ***	591.32±10.06 270.19% a ***	1219.60±16.31 58.26% a ***	5.28±0.15 62.44% a ***	26.01±2.01 19.72% N.S.	945.74±16.50 61.97% a ***	1.47±0.07 38.02% a ***
G5	7.23±0.07 -0.96% N.S.	170.11±12.28 6.49% N.S.	891.69±21.43 15.71% a ***	3.00±0.18 -7.60% N.S.	24.25±1.60 11.59% N.S.	617.59±20.43 5.77% a ***	1.56±0.15 45.79% a ***
ANOVA F(4.45)	P<0.001 39.15	P<0.001 372.72	P<0.001 197.53	P<0.001 106.10	P<0.001 2.03	P<0.001 103.69	P<0.001 9.80

Values are presented as: mean±S.E.
%=Percentage of change from normal control (G1)
a*** = highly significant (P<0.001) from control (G1)
a** = highly significant (P<0.01) from control (G1)
N.S. Non significant

4. Discussion

4.1. Anti-tumor studies:

In the present study, treatment of tumor-bearing mice with doxorubicin only and with both doxorubicin plus catechin exerted a marked effect in the retardation of tumor growth. This is demonstrated by marked increase in the percentage of survivors, the cumulative mean survival time on day 40 and the marked decrease in the mean tumor weigh as compared to tumor-bearing mice group. In addition, the present data indicate that the combined treatment of doxorubicin and catechin caused a remarkable increase in mean survival time (63days) as compared to single doxorubicin treatment (54day). It merits note that a more pronounced effect was observed in the combined treatment represented by higher increases in the life span of animals (131.25%) and the tumor inhibition ratio (31.19%), compared to tumor-bearing animals treated with doxorubicin which recorded (112.50% and 39.4%), respectively. These observations are consistent with the previous findings by Balasubashini et al.(2006) and Majumder et al. (2006). It has been shown by Gewirtz (1999) that the capacity of doxorubicin to inhibit DNA synthesis has been proposed as a mechanism of action of doxorubicin on cancer cells and may be also due to the effect of Catechin which increased concentration of doxorubicin in cancer cells and may have anticarcinogenic and antiproliferatic properties (Ahmed and Mukhtar,1999; Crespy and Williamson,2004). Moreover, Sadzuka et al. (1998) found that green tea components, such as caffeine, theanine, (-)-epigallocatechin gallate (EGCG) and flavonoids have inhibitory effects on the doxorubicin efflux from Ehrlich ascites carcinoma cells. Thus, it is suggested that EGCG and flavonoids may enhance DOX induced antitumor activity and increase the DOX concentrations in tumors through the inhibition of DOX efflux. However, the present findings are in line with the main concept of cancer research that evaluation of any tested substance depends on extension of the survival time of cancer patients and that an increase in the life span of drug-treated tumor bearing mice $\geq$ 125% is considered indicative of presumptive drug activity (Fahim et al., 2003).

4.2 Biochemical studies:

The inhibitory effect of tumor inoculation on protein content may be explained based on that the tumor cells tend to concentrate amino acids for its protein synthesis and growth while normal host suffered from this inhibition (Wiseman and Ghadially, 1958). The inhibition in total protein content in doxorubicin treated group may be due to the oxidative stress and reduction of protein synthesis in the tissue (Swift et al., 2002). In doxorubicin and Catechin treated group, protein level back to normal as a result of the antioxidant properties of Catechin and its ability to scavenge ROS induced by doxorubicin administration (Kalender, 2005). The increments in total lipid level of tumor group and doxorubicin treated group may be due to the deamination of protein in tissue, reduction in ATP production due to swelling and vacuolization of mitochondria and disturbance in glutathione synthesis using NADPH as a cofactor (Naidu et al., 2002). In doxorubicin plus Catechin treatment total lipid level back to normal because that Catechin increase the glutathione-S-transferase and decrease glucose-6-phosphate enzyme which consider to be important coenzyme in bio-synthesis of fatty acids. Also, Catechin inhibits the absorption of cholesterol and promotes cholesterol execration (Miura et al., 1994). LDH activity was inhibited in tumor group due to increase in inflammatory cells which caused reduction in protein level (Saad-Hossne et al., 2003). The increase in cardiac enzyme in doxorubicin treated group could to an increase in its release following doxorubicin-induced lipid peroxidation in cardiac membranes (Deepa and Varalakshmi, 2003). Treatment with doxorubicin plus Catechin caused improvement in LDH activity which related to the free radical scavenging activity, so Catechin protect cardiac membrane from free radicals and decrease lipid peroxidation level, as a result of that LDH prevented from releasing outside the cell (Schmidt *et al.*,2005). The significant increase in cardiac MDA in these groups may be due to that cancer cells induced excessive production of free radicals leading to damage lipids and can induced lipid peroxidation (Fouda ,2005) This elevation in MDA level in doxorubicin treated group related to the metabolism of doxorubicin which lead to the release of iron ions that react with cellular components specially the poly unsaturated fatty acids in cell membrane and increase the MDA level (Kalender *et al.*,2002 and 2005). The significant decrease in GSH level in tumor group may be due to lack of amino acids which used in making of GSH (Deepa and Varalakshmi, 2003). The inhibition of cardiac GSH level in the doxorubicin group compared to tumor bearing group explicable by an increased rate of transformation of GSH to oxidized glutathione (GSSG) as a result of GSH consumption to get ride of H2O2 (Fouda, 2005). The significant elevation in cardiac GSH-PX and SOD in tumor group may be correlated to the oxidative stress on the host in response to the continual generation of free radicals by the increasing tumor load (Fahim *et al.*2003). The level of GSH and GSH-Px and SOD activities back to normal in doxorubicin plus Catechin group due to normalization of lipid peroxidation and Catechin scavenges hydroxyl radicals which may initiate lipid peroxidation (Zhao et al., 2001 and Mai and Altucci 2009).

5. Conclusions

From the results of the present work can conclude that: Catechin supplementation might be significantly reducing doxorubicin- induced cardotoxicity in Ehrlich ascites carcinoma cells- bearing mice. This protection

of Catechin was demonstrated by the induction of the antioxidant enzymes systems to increase the disposal of overproduction reactive free oxygen radicals after doxorubicin treatment and by blocking the lipid peroxidation in heart tissue. In addition to Catechin enhances the antitumor properties of doxorubicin by increase the inhibitory effect of doxorubicin on tumor growth.

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6. References

1. Abullaev, FI, Luna, RR , Roitenburd, BV , Espinosa, AJ . Pattern of childhood cancer mortility in Mexico. Arch Med Res. 2000; 31: 526-531.
2. Ahmed, HN. Biological and histochemical studies on the effect of irradiation on Ehrlich tumor. Ph.D. Thesis, Cairo University, 1999; pp.57-64.
3. Aleisa, AM, Al-Rejaie, SS, Bakheet, SA, Al-Bekari, AM, Al-Shabanah, OA, Al-Majed, A, Al-Yahya, AA , Qureshi, S . Effect of metformin on clastogenic and biochemical changes induced by adriamycin in Swiss albino mice. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**.** 2007; 634 (1-2): 93-100**.**
4. Ali RA. Electrophorasis of serum proteins as a tool for early detection of biomarkers of some diseases. Ph.D. thesis Zoology Department-Faculty of science for girls, Al-Azhar University; 2005.
5. Balasubashini MS, Karthigayan S, Somasundaram ST, Balasubramanian T, Viswanathan V, Raveendran P, Menon V P. Fish venom (*Pterios volitans*) peptide reduces tumor burden and ameliorates oxidative stress in Ehrlich's ascites carcinoma xenografted mice. Bioorganic & Medicinal Chemistry Let. 2006; 16(24): 6219- 6225.
6. Bast A , Kaiserová H, Hartog G, Haenen G, Vijgh1 W. Protectors against doxorubicin-induced cardiotoxicity: Flavonoids. Cell Biol. Toxicol. 2007; 23(1):39-47.
7. Beutler E, Duron O, Kelly BM. Improved method of the determination of blood glutathione. J. Lab.& Clin. Med. 1963; 61(5): 882-888.
8. Crespy V, Williamson G. A review of the health effects of green tea catechins in vivo animal model.J. Nutr. 2004; 134: 3431-3440.
9. Deepa PR, Varalakshmi P. Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem. Biol. Interact. 2003;146: 201-210.
10. Doumas BT. Colorimetric determination of total protein based on the Biuret method. Clin. Chem. 1975; 21: 1159-1166.
11. Du Y, Lou H. Catechin and proanthocyanidin B4 from grape seeds prevent doxorubicin-induced toxicity in cardiomyocytes. Eur J Pharmacol. 2008; 591(1-3): 96-101
12. Fahim F A, Esmat A Y, Mady E. A, Amin M A. Serum LDH and ALP isozyme activities in mice bearing solid Ehrlich carcinoma and/or treated with maximum tolerated dose (MTD) of aloin. Disease Markers, 1997; 13: 183-193.
13. Fahim FA, Esmat AY, Mady EA, Ibrahim EK. Antitumor activities of Iodo acetate and Di methylsulphoxide against solid Ehrlich carcinoma growth in mice. Biol. Res. 2003;36: 253-262.
14. Foud FM. Anti-tumor activity of tetrodotoxin extracted from the Masked Puffer fish *Arothron diadematus.* Egyptian Journal of Biology 2005**;** **7**:10-22.
15. Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem. Pharmacol.1999; 57: 727-747.
16. Gross RI, Bracci R, Rudolph N, Schroeder E, Kochen J. Hydrogen peroxide toxicity and detoxification in the erythrocytes of new born infants. Blood 1967; 29(4): 481-493.
17. Gupta A, Mazumder UK, Kumar RS, Kumar TS. Anti-tumor activity and anti-oxidant role of *Bauhinia racemosa* against Ehrlich ascites carcinoma in swiss albino mice. Act. Pharm. Sin. 2004**;** 25(8): 1070-1076.
18. Hin Z, Wetherill G B. The T-test and X^2 goodness of Litchapman and Hall. 1975; London.
19. Kachmar JF, Moss DW. In fundamentals of clinical chemistry. 2nd ed., N. W. Tietz, Editor. W. B. Saunders, Philadelphia, 1976; pp. 652.
20. Kalender S, Kalender Y, Ates A, Yel M, Olcay E, Candan S. Protective role of antioxidant vitamin E and catechin on idarubicin-induced cardiotoxicity in rats. Braz. J. Med. Biol. Res. 2002**;** 35: 1379-1387.
21. Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of

vitamin E and catechin. Toxicology 2005**;** 209: 39-45.
22. Korish AA, Arafah M M. Catechin combined with vitamins C and E ameliorates insulin resistance (IR) and atherosclerotic changes in aged rats with chronic renal failure (CRF). Archives of Gerontology and Geriatrics 2008; 46 (1): 25-39.
23. Kumamoto M. Evaluation of the Antioxidative activity of tea by an oxygen electrode method. Biosci. Biotechnol. Biochem 1998. 62(1): 175-177.
24. Lonnie P, Swift M, Cutts A R, Abraham N, Don R P. Activation of adriamycin by the pH-dependent formaldehyde-releasing prodrug hexamethylenetetramine. Molecular Cancer Therapeutics 2002**;** **2**: 189-198.
25. Mai A, Altucci L. Epi-drugs to fight cancer: From chemistry to cancer treatment, the road ahead. Intern. J. Bioch.& Cell Biol. 2009 ; 41:199-213.
26. Majumder S, Dutta P, Mookerjee A, Choudhuri SK. The role of a novel copper complex in overcoming doxorubicin resistance in Ehrlich ascites carcinoma cells in vivo. Chemico-Biological Interactions 2006;159 (2): 90-103.
27. Minami M, Yoshikawa H. A simplified assay method of super oxide dismutase. Clinica Chimica Acta. 1979**;** **29**: 337-342.
28. Miura S, Watanabe J, Tomita T, Sano M, Tomita I. The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low density lipoprotein. Biol. Pharm. Bull. 1994; 17: 1567-1572.
29. Naidu MU, Kumar KV, Mohan IK, Sundaram C, Singh S. Protective effect of gingko biloba extract against doxorubicin-induced cardiotoxicity in mice. Indian J. Exp. Biol. 2002; 40(8): 894-900.
30. Nakagawa T, Yokozawa T. Direct scavenging of nitric oxide and superoxide by green tea. Food Chem. Toxicol. 2002; 40: 1745-1750.
31. Ohkawa H, Ohish N, Yagi K. Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979; 95:351-358.
32. Olson RD, Mushlin PS, Brenner MD, Fleischer S, Cusack BJ, Chang BK, Boucek RJ. Doxorubicin Cardiotoxicity May Be Caused by Its Metabolite, Doxorubicinol . PNAS. 1988; 85 (10): 3585-3589.
33. Saad-Hossne R, Saad-Hossne W, Prado G R. Neoplasic ascite: effects of phenol, glycerin and acetic in Ehrlich ascites tumor. Acta. Cir. Bras. 2003;18(6): 11-13.
34. Sadzuka Y, Sugiyama T, Hirota S. Modulation of cancer chemotherapy by green tea. Clin. Cancer Res. 1998; 4(1): 153-156.
35. Schmidt M, Schmitz H J, Baumgart A, Guedon D, Netsch M I, Kreuter M H, Schmidlin CB, Schrenk D. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food and Chemical Toxicol. 2005; 43:307-314.
36. Singal P K, Li T, Kumar D, Danelisen I, Iliskovic N. Adriamycin-induced heart-failure: mechanism and modulation. Mol. Cell. Biochem. 2000; 207**:** **77**-85**.**
37. Swift P L, Cutts SM, Phillips DR. Activation of adriamycin by the pH-dependent formaldehyde-releasing prodrug hexamethylenetetramine. Mol. Cancer Therap. 2002; 2: 189-198.
38. Timao L I, Singal P K. Adriamycin-induced early changes in myocardial antioxidant enzymes and their modulation by probucol. Circulation 2000; 102: 2105-2110.
39. Vincent PC, Nicholls A . Comparison of the growth of the Ehrlich Ascites Tumor in male and female mice. Cancer Res. 1967; 27(1): 1058-1065.
40. Winer B J. Statistical principles in Experimental Design. Mc. Graw-Hill. New York, N Y. 1971.
41. Wiseman G, Ghadially F N. A biochemical concept of tumor growth infiltration and cachexia. Br. Med.J. 1958; 11: 18-21.
42. Zhao B, Guo Q, Xin W. Free radical scavenging by green tea polyphenols. Methods Enzymolol. 2001; 335: 217-231.
43. Zollner N, Kirsch K. Colorimetric method for determinationm of total lipids Z. Ges. Exp. Med. 1962; 135: 545.

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Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.

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Abstract: The present investigate the various biological changes induced by hyperthermia (at 42°C) in female rats and the mode of recovery at 1, 6 , 24 & 72 hrs at different ages (2, 6 , 12 & 24 months) . Biological parameters studied were red blood cells (RBCs), white blood cells (WBC's), hemoglobin (Hb) , B% & T% lymphocytes. Immunoglobulin G &A (IgG & IgA) and serum activities of triiodothyronin and thyroxin (T_3 & $T_{4)}$, the heat shock protein 70(HSP70). Besides, the chromosomal aberrations test and micronucleus formation were investigated in female rats. In attempt to find out the interaction between age and hyperthermia in such parameters in normal female rats. The results revealed that Highly significant increases of WBC's, B%, IgG and HSP70 at 1 till 72hr post WBH in aged 2 and 6 months. On the other hand, WBH caused a significant decrease in each RBC's, T_3 & T_4 at 6 till 72 hr post WBH. As well as, the count of Hb decreased in age 2 month at 1 till 72 hr post the heat exposure but increased at 1 & 6 hr in 6 month aged post WBH then decrease at 24 hr & 72 hr post WBH. T% lymphocyte count significantly decreased at 1 hr post WBH and increased at 6 hr & 24 hr then decrease again at 72 hr post WBH in ages 2 & 6 month. IgA level significantly increased in 6 aged rats at 1, 6 & 24 hr post WBH then decreased at 72 hr below the control value post WBH. The results revealed that WBH caused a significant increase of B% lymphocyte, Hb and IgA at 1 & 72 hr post WBH in age 12 & 24 months, except Hb in 12 month decreased at 72 hr post heat exposure. On the other hand, T% lymphocyte, RBC's , IgG and serum T_3 &T_4 decreased at 1 & 72 hr post heat exposure except IgG level increased at 72 hr post WBH . The level of HSP70 increased significantly at 1 till 24 hr post WBH in 12 month and reached to the control value at 72 hr post WBH. On the contrary, HSP70 decreased significantly at 1hr in aged rats (24 month), then increased significantly at 6 and 24 hr post heat exposure then decreased below the control value at 72 hr post WBH. In the chromosomal aberrations test, we observed positive responses at all ages but in different frequencies and recover may occur at 72 hr for the all except young age (2 month) which needed more time to completely recover., in the micronucleus test, we observed positive responses in all ages at 24hr only, while at 72hr post heat exposure the mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) were within the vehicle control group at all ages except 2 month which increased significantly than control group. The results suggest that hyperthermia can induce both chromosomal aberrations and micronucleus formation. [Journal of American Science 2010;6(4):153-166]. (ISSN: 1545-1003).

Key words: Hyperthermia –HSP70, chromosomal aberration –micronucleus.

1. Introduction

Whole body hyperthermia (WBH), as a treatment modality, shows promising results in the management of advanced and refractory cancer in combination with chemotherapy (Ismail- Zade et al. 2002). Stress is a sudden environmental change that induces damage at the molecular, cellular and organismal level (Soti and Peter, 2007). Macromolecules such as proteins are continuously exposed to potential damaging agents that can cause loss of molecular function and depletion of cell populations over the lifetime of essential organs. One of the key homeostatic responses involved in maintaining longevity is the induction of heat shock proteins (HSPs), a conserved reaction to damaged intracellular proteins(Calderwood et al. 2009) Damage to macromolecules is characteristic of aging and degenerative diseases. Beyond DNA damage, protein damage may not only a consequence, but also a causal factor in cellular malfunction. Damage may induce misfolding, and the aggregating oligomeric species may gain a novel toxic property, severely compromising cellular function (Dobson, 2003) .The relationship between thermal resistance and expression of inducible heat shock proteins, especially Hsp70, depends on the species and temperature treatments (Bahrndorff et al. 2009). The major stress of life is oxidative stress. Thus, it is not surprising that oxidized protein level increases with aging of all animal species (Stadtman and Stadman, 2004).

The heat shock response is a highly conserved "stress response" mechanism used by cells to protect themselves from potentially damaging insults. It often involves the upregulated expression of chaperone and Hsps to prevent damage and aggregation at the proteome level (Michael et al. 2007). Induction of Hsp

expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans. This family of proteins can also serve as indicators of thermal stress (Rodwell et al. 2008). Area of focus has been the family of highly conserved stress proteins known as heat shock proteins (HSPs). Some of the well-studied Hsps in mammals are those with molecular masses of 70 kDa, and these ubiquitous proteins include the highly heat inducible 72 kDa protein (Hsp70). There is substantial evidence showing that Hsp70 plays a critical role in providing cellular protection against the adverse effects of a wide array of stress and toxic conditions (Kregel, 2002).. Interestingly, investigators have also shown that the ability to induce Hsp70 under a variety of physiologically-relevant conditions, including hyperthermic challenge, is reduced with aging (Bonelli et al.1999; Jin et al. 2004, Kregel et al. 1995 and Vasilaki et al. 2002). Hsp70 appears to be regulated at both transcriptional and translational levels (Chandolia et al. 1999 and Vivinus et al. 2001). Zhang et al. 2003 demonstrate that the reduction in stress tolerance that accompanies aging is associated with a complex set of integrated alterations in hepatic steady-state levels of reactive oxygen species (ROS), macromolecular damage, redox buffering, and transcription factor regulation. Heat stress proteins (HSPs) are induced by a variety of stimuli. They contribute to maintaining the metabolic and structural integrity of the cell, as a protective response to external stresses. A raised body temperature raises the metabolic rate and makes the immune response more efficient (Broom, 2007).
Levels of protein Hsp70 were not detectable in the control treatment and 2 h after hardening treatment. However, levels of Hsp70 gradually increased thereafter and at 4 and 6 hr after the hardening treatment expression was elevated but with large variation (Bahrndorff, 2009). Several authors reported either a decrease or no change in the number of RBC, s in animals subjected to high environmental temperature (Terajima et al. 2000).As well as these authors reported the destruction of red blood cells at 42°C. Lee and Korean (1987) observed a slight decrease in the Hb content of rabbits exposed to heat stress (37 – 80% RH) for 2- 4 hrs daily for two weeks. They also found a decrease in total leukocytes count and a significant decrease in lymphocytes without a statistically significant change in monocyte count. Whereas, Kamal et al. (1992) reported a significant increase in WBC' s count in heat stressed mature rats. Mostafa et al. (2005) reported that WBH caused significantly decrease in T3 and T4 hormones in rabbits after immediately heat exposure and after 24 hrs post WBH. Furthermore, they observed that WBH caused a significant decrease below the control values in each of RBC' s, Hb and total WBC's counts. Following after the 24 hrs recovery period. The WBC's was significantly higher while T_3, T_4, RBC's , Hb and lymphocytes values were significantly lower than in the acute WBH group.

Hyperthermia exerts numerous effects on mammalian cells including division delay, cell killing and chromosomal aberrations when cells are heated in the S-phase (Dewey et al. 1990 and 1978). Also, Takahashi et al. (2004) suggested that heat-induced DNA double strand breaks contribute to heat-induced cell killing because heat treatment induces histone γ H2AX-containing foci. Such foci have been associated with double strand breaks induced by ionizing radiation, other agents, and other stresses. (Capetillo et al. 2004). DNA synthesis is inhibited by heat, dependent on the time and temperature of heating (Warters et al. 1984).

2. Material and Methods

Two hundred and forty female albino rats (*Rattus rattus*) at 2, 6, 12 and 24 months of age were purchased from the animal house of the National Research Center, Dokki, Egypt. Food and water were provided *ad libitum* under standard condition of light, humidity and temperature. After a period of acclimation the room environmental temperature and relative humidity (RH) will be recorded twice daily using Thermohygrometer.

Whole body hyperthermia Treatment: (WBH):

A plastic cage (45 x 25 x 20 cm), conscious and unrestrained. Rectal temperature (Tr) was continuously monitored on a digital display) was established over a 30-min control period for each rat, followed by a heating protocol. An infrared lamp was positioned 40 cm above each rat and either raised or lowered to obtain an ambient temperature of 42°C. Movement of the lamp permitted a constant heating rate (0.06 °C/min) to be attained. Heating was terminated when rectal temperature (Tr) reached 42°C, but was then commenced at an appropriate time to maintain Tr at 42°C for one hour. At the end of this period, the thermistor probe was removed and rats were allowed to passively cool in a cage at room temperature. The (Tr) of the rats will be recorded using a thermocouple (Cole Parmer type T. thermocouple thermometer) connected with a rectal probe, which will be inserted 8 cm beyond the anal sphincter. When Tr reaches (42◦C), directly blood samples will be collected from orbital venous plexus of experimental animals into fresh heparinized tubes. The animals will be allowed recovering at room temperature and blood samples will be taken after 1,6,24 and 72 hr post WBH. Some biochemical parameters, RBC' s and WBC' s counts were determined using a haemocytometer. Hb

concentration was measured by the Cyanmaethmoglobin method, B% & T% lymphocytes. IgG & IgA by ELISA kit from Life Diagnostics and plasma activities of total T_3 & T_4 were measured with radioimmunoassay kits (MP Biomedicals, Eschwege, Germany). Besides, the HSP70 by ELISA kit from Stress Biotechnologies were investigated in female rats.

Bone marrow chromosomal aberrations assay:

After heat exposure, the animals were injected intraperitoneally (i.p) with 0.5 ml colchicines (0.5% mg/kg body weight) one hour and 30 minutes before killing to block the cells in metaphase. Animals were sacrificed and chromosomes of bone marrow were prepared by using methodology of **Yosida and Amano (1965)** with slight modifications. The cells in both femurs were collected by pushing 2-3 times of 0.075M KCL into the marrow cavity, left in hypotonic KCL for 35 min. and then centrifuged at 1000 rpm for 8 min. The supernatant was removed and the pellet was resuspended and fixed in methyl: acetic acid (3:1). Centrifugation and fixation were repeated five times at 20 min. intervals. The pellet was resuspended in a small volume of fixative. Finally, the cells were dropped on a clean wet slide and stained with 5% Giemsa stain. 50 metaphases examined from each animal under 1000X magnification to determine the frequencies of chromosome damage cells. The classification of aberrations was carried out as described by **Venitt and Parry, 1984** and in the international system for Cytogenetic Nomenclature (ISCN) **(Cohen et al. 1993).**

Bone marrow micronucleus assay:

The micronucleus test was performed according to **Schmid (1975) and Heddle (1973).** After heat exposure, animals were killed by cervical dislocation. Both femurs were dissected and bone marrow was flushed from the femoral cavity with fetal calf serum and the cells were centrifuged at 1000rpm for 5 min. The pellets were suspended and smeared on a clean dry slide. The slides were fixed by methanol and stained with Giemsa stain. The micronuclei (MN) were analyzed under a microscope with 1000X magnification using oil immersion. About 1000 polychromatic erythrocytes (PCEs.) were scored for each treated animal and control. To count the number of micronucluated polychromatic erythrocytes (MNPCEs).

Statistical analysis:

All results are expressed as mean ± SEM. The statistical analysis was carried out with Duncan's multiple range test. A $P < 0.05$ was considered the level of statistical significance.

3. Results

Results of the present study are presented in tables (1-4) and (fig. 1-11), demonstrated the effect of WBH 42°C in young, middle and aged rats (2, 6, 12 and 24 months) on hematological indices which manifested a significant decrease of WBCs in the 6 months, rats before heat value of WBC' s increased significantly after 1 hr post WBH in all groups. The highest value was showed in 2 month old rats. The count of WBC' s reached to the control values at 24 &72 hr post WBH in 12 month old rats. On the contrary, reached below the control values in 24 month old rats as shown as in fig (1). The data demonstrated in fig (2) shows the count of RBC' s decreased significantly in all different ages' rats at 1 hr till 72 hr post WBH except in 2 and 24 months' rats. The count of RBC' s in that both groups increased significantly at 24hr and 6hr respectively post WBH. Fig (3) shows the count of T% lymphocytes decreased significantly in all groups after 1 hr post WBH. This decrease was continues till the end of experiment 72 hr in 24 months group but in 2 & 6 month old rats , the count of T% lymphocytes increased significantly at 6 & 24 hr post WBH, then decrease at 72 hr post heat exposure (Table 1a). On the other hand, WBH caused significantly increased of B% in all experimental groups at 1hr till 72 hr post WBH. The maximum increase was recorded in 12 month old rats at 6 &72 hr post heat exposure Fig (4).

Fig 5 & table (1a) illustrated that WBH revealed significant increase of IgG in 2 & 6 month old rats but decreased significantly in 6 & 24 month old rats after 1 hr post WBH. Then IgG values showed increase at 6 &24 hr after heat exposure in aged rats (24 month). This parameter showed fluctuates down and up in 12 months old rats (Fig 5). WBH significantly increased of IgA in rats 6, 12 & 24 months at 1 till 72 hr post heat exposure, the only decrease showed at 72 hr post WBH in 6 month old rats. Fig 6 & table 1b shows the value of IgA in the group 2 month old rats was significantly higher than that in 6, 12 & 24 month old rats by 30.08%, 13.69% and 29.12% respectively (Table 2). The results revealed that WBH increased significantly of Hb count as compared to control values in 6, 12& 24 months at 1 hr post heat exposure (13.76 ± 0.61,12.23 ± 1.55 and 14.34 ± 0.40 respectively). While Hb content showed a significant decrease in 2 month old rats at 1 till 72 hr post WBH. However, the increase of Hb level in aged rats (24 months) continued at 1 till 72 hr post WBH, decreased at 72 hr in 6 & 12 months (Fig. 7). In the present study, WBH caused a significant increase of HSP70 at 1hr post WBH in 2 month old rats and this increase was higher than that of 6,12 &24 months by

49.71%, 20% & 78% respectively (Table 1b) . Then HSP70 decreased at 6, 24 & 72 hr post WBH in 2 month old rats group,1.26 ± 0.43, 2.46 ± 0.99 and 1.40 ± 0.35 respectively Table(1).This decrease was lower than that in 12 month by 139.68%, 30% & 87.14% respectively, but still higher than that control value(Fig 8).The highest decrease of HSP70 was recorded at 6 hr post WBH in aged group (24 month), this decrease was 188.89% lower than that 2 month old rats. Moreover, (Fig 9& 10) shows WBH caused significant decreases of T_3 &T_4 in all groups at 1 hr till 72 hr post heat exposure except in 2 & 6 months showed that T3 approached to the control values at 72 hr post WBH(Table 1b).

- Effect of WBH on chromosomal aberrations of bone marrow cells:

Our experiment was demonstrated the effect of hyperthermia on female rats at different ages (2, 6, 12 and 24 months) and different times (1, 6, 24 and 72 hrs after heat stress).These data are presented in table (3). It can be seen that heat induced various types of structural chromosomal aberrations which consisted of gaps, breaks, deletions, fragments, centromeric attenuations and endomitosis. Numerical aberrations resulted in hypoploidy and hyperploidy only. When heat was applied on rats at 2 month of age, mean values of total chromosomal aberrations were statistically increase ($P<0.05$) in animals sacrificed 1, 6, 24 and 72 hrs after heat stress (19.0±0.41, 29.50±0.29, 27.50±0.29 and 19.75±0.48 respectively) than those of control (11±0.41). While 72 h group statistically decreased than 6 and 24 hrs groups means that at 72 hr the aberrations begin to recover. Also, the number of abnormal metaphases significantly increased in all groups than control.

In female rats at 6 month of age, there were significant increases in the total chromosomal aberrations of animals sacrificed 1, 6, 24 and 72 hr (18.50±0.50, 30.0±0.0, 21.75±0.25 and 10.75±0.25 respectively) than control (9.75±0.63). The value observed 72 hr after heat exposure was within the vehicle control group and thus not considered of biological significance. Abnormal metaphases significant increased than control in all groups except 72 hr post WBH in which the increasing had no significant.

The data obtained from female rats at 12 month old rats recorded that there were significant increase in the total chromosomal aberrations of rats sacrificed 1, 6 and 24 hours after heat shock (24.50±0.50, 34.0±0.41 and 28.75±0.48 respectively) when compared with control (13.50±0.29). While, there was non-significant difference between the frequencies of the total aberrations induced by heat at 72 hr (12.0±0.41) and that observed in the control. The abnormal metaphases decreased at 72 hour group and returned to the control.

In addition, heat caused significant increase in the mean values of total chromosomal aberrations of 1, 6 and 24 hrs (31.0±0.41, 32.50±0.29 and 30.50±0.29 respectively) than those of control (12.0±0.41) at 24 month of age. While between these groups there was no significance. In contrary, 72 hr (14.50±0.50) showed significant decrease in the frequencies of total aberrations than those of other groups and this value was within the vehicle control group and thus not considered of biological significance. Abnormal metaphases reached the control value at 72 hr after treatment.

At 1 hr after heat shock, there were no significance differences between 2 and 6 month of age in the total chromosomal aberrations, while between these and other two groups there were statistically significant difference. 6 h after heat stress show no significant differences between all ages. Also 24 hr after heat had no significant between all groups except 6 month of age this significantly decreased than other groups, so it is demonstrated that young age (2 month) more affected than adult (6 month) which have less frequencies than old age (12 and 24 months). 72 hr showed that there were no significant differences between all ages except 2 month old rats which increased statistically than other groups. Means that young age need more time than other groups to recover. The number of abnormal metaphases increases with increasing the age and begin to recover at 72 hr after heat stress in all ages except 2 month old rats which need more time to complete recover.

Effect of WBH on micronucleus formation of bone marrow cells:

Hyperthermia was tested for induction of micronucleus in bone marrow of female rats (table 4) and (Fig. 11). The number of MNPCEs unaffected with hyperthermia at 1 and 6 hours after heat stress and thus none statistically significant than control and this in all ages (2, 6, 12 and 24 month of age). At 24 hr after heat stress, the number of micronucleus was statistically significant increased ($P<0.05$) for 2, 6, 12 and 24 months of age (6.75±0. 48, 4.0±0.41, 9.0±0.41 and 12.0±1.23 respectively) than those of control (2.0±0.41). Also, there were significantly decrease in the number of MNPCE's at 6 month of age than the all. At 72 hr post treatment, the mean frequencies of MNPCE's at 6, 12 and 24 months of age (2.0±0.41, 2.25±0.25 and 2.75±0.48 respectively) were within the vehicle control group (2.0±0.41). On the other hand, there was a significant increase in 2 month old rats (4.50±0.65) than control group (2.0±0.41).

Table 1a: Effect of WBH at 42 °C and its late effects on various biological parameters in 2,6,12 and 24 months old rats.

Age	Time	*Parameters*				
		WBC's ($X10^3$/cmm)	**RBC's ($X10^6$/cmm)**	**T lymphocytes (%)**	**B lymphocytes (%)**	**IgG (ng/ml)**
2 month	Control	5.36±0.50^{efg}	3.46±0.18^{bc}	23.62±1.01^{fg}	16.59±0.51^{i}	826.60±21.49^{h}
	1 hr	14.51±0.50^{a}	2.01±0.15^{g}	23.15±1.06^{fgh}	21.31±0.92^{ghi}	1436.40±69.68^{b}
	6 hr	6.41±0.51^{defg}	2.56±0.09^{ef}	25.50±0.57^{ef}	32.54±1.20^{b}	1078.50±16.81^{fg}
	24 hr	8.88±0.58 ^{c}	3.94±0.24^{a}	26.91±0.50^{de}	33.48±2.81^{b}	1233.40±48.86^{cdef}
	72 hr	6.67±0.45^{defg}	2.42±0.12^{efg}	20.31±0.41^{h}	26.73±1.43^{defg}	1315.60±69.81^{bcde}
6 months	Control	4.99±0.07^{g}	3.88±0.16^{ab}	28.08±1.9^{bcde}	17.10±0.65^{i}	813.10±11.64^{h}
	1 hr	7.54±0.71^{cd}	2.02±0.05^{g}	20.30±0.76^{h}	27.33±2.41^{bcdef}	942.60±19.58^{gh}
	6 hr	6.78±0.46^{def}	3.13±0.11^{cd}	31.08±0.69^{ab}	26.27±1.59^{efg}	957.70±32.80^{gh}
	24 hr	10.94±0.77^{b}	2.50±0.08^{ef}	30.14±1.38^{abc}	28.44±1.73^{bcdef}	1382.50±53.70^{bc}
	72 hr	11.61±0.70^{b}	2.31±0.14^{fg}	27.19±1.41^{cde}	31.89±1.72^{bcde}	1717.80±49.70^{a}
12 months	Control	7.87±0.63^{cd}	3.85±0.16^{ab}	23.95±1.14^{fg}	23.82±1.69^{fgh}	1226.20±35.95^{cdef}
	1 hr	8.85±0.48^{c}	2.53±0.12^{ef}	21.44±0.80^{gh}	29.32±1.53^{bcdef}	1153.40±79.47^{ef}
	6 hr	13.99±0.47^{a}	3.17±0.19$_{cd}$	22.85±0.53^{fgh}	42.69±4.33^{a}	1304.10±80.79^{bcde}
	24 hr	7.78±0.39^{cd}	3.14±0.17^{cd}	30.56±1.40^{ab}	19.78±1.09^{hi}	1203.50±80.74^{def}
	72 hr	7.58±0.51^{cd}	3.19±0.22^{cd}	27.35±0.92^{cde}	43.19±1.56^{a}	1308.00±47.03bcde
24 months	Control	11.33±0.38^{b}	3.73±0.09^{ab}	31.67±0.39^{a}	27.12±1.55^{cdefg}	1179.00±44.73^{def}
	1hr	5.05±0.24^{fg}	2.06±0.05^{g}	22.55±0.68^{fgh}	33.29±1.86^{bc}	941.50±28.37^{gh}
	6 hr	13.55±0.98^{a}	4.16±0.09^{a}	22.64±0.43^{fgh}	27.63±2.60^{bcdef}	1350.00±70.73^{bcd}
	24 hr	11.35±0.79^{b}	3.49±0.14^{bc}	28.74±0.56 ^{abcd}	39.19±1.15^{a}	1309.00±48.79^{bcde}
	72 hr	6.83±0.38^{de}	2.78±0.11^{de}	28.57±1.39^{abcde}	30.91±1.67^{bcde}	978.20±41.58^{gh}

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05)

Table 2: Effect of WBH at 42 °C and its late effects on various biological parameters in 2, 6, 12 and 24 months old rats (% change).

Age	Time	*Parameters*									
		WBC's	**RBC's**	**T (%)**	**B (%)**	**IgG (mg/dl)**	**IgA (pg/ml)**	**Hb (g/dl)**	**HSP70 (ng/ml)**	**T3 (ng/dl)**	**T4 (µg/dl)**
6 months	Control	6.90	-12.14	-18.88	-3.07	1.63	3.33	5.31	-54.46	6.45	7.65
	1 hr	48.04	-0.49	12.31	-28.25	34.38	-40.87	-78.0	49.71	6.92	18.06
	6 hr	-5.77	-22.27	-21.88	19.26	11.20	6.62	-24.41	30.95	-0.22	9.13
	24 hr	-23.20	36.55	-12.33	15.05	-12.09	-25.39	3.60	-13.41	1.13	-25.04
	72 hr	-74.10	4.55	-33.87	-19.30	-30.57	26.64	12.73	-65.00	4.07	-37.99
12 months	Control	-46.83	-11.27	-1.39	-43.58	-48.34	-6.09	14.47	-157.43	14.29	12.19
	1 hr	39.01	-25.87	7.39	-37.59	19.70	-5.53	-85.21	20.86	25.58	13.13
	6 hr	118.25	-23.83	11.18	-31.19	-20.92	-8.66	15.56	-139.68	19.77	24.87
	24 hr	12.39	20.30	-13.56	40.92	2.42	42.32	-8.00	-30.89	45.20	22.89
	72 hr	-13.64	-31.82	-34.66	-61.58	0.58	-16.77	-6.32	-87.14	30.56	9.22
24 months	Control	-111.38	-7.80	-34.08	-63.47	-42.63	8.89	33.96	-76.24	4.18	8.95
	1hr	65.15	-2.94	2.59	-56.22	34.45	5.78	-85.51	78.00	7.50	8.57
	6 hr	-111.39	-62.50	11.22	15.09	-25.17	30.08	13.29	-188.89	-6.00	9.75
	24 hr	-27.82	11.42	-6.80	-17.05	-6.13	13.96	5.13	3.66	19.02	-0.82
	72 hr	-2.40	-14.88	-40.67	-15.64	25.65	29.12	-33.40	25.71	15.74	16.67

Table (3): Mean values of chromosomal aberrations after heat exposure in female rat bone marrow cells at different ages.

Age	Time	Abnormal metaphases	Types and no. of chromosomal aberrations						Total chromosomal aberrations	Total aberrations excluding gaps
			gap	break	Del & frag	CA	End	Numerical aberrations		
2m	control	11±0.41^{hi}	5	7	6	13	5	8	11.0±0.41^{j}	9.75±0.63^{ij}
	1h	16.25±0.85^{f}	6	3	16	27	0	24	19.0±0.41^{gh}	17.5±0.50^{fg}
	6h	26.50±1.04^{b}	16	3	19	32	11	37	29.5±0.29^{abcd}	25.5±0.29^{c}
	24h	23.75±1.93^{c}	21	5	21	24	5	34	27.5±0.29^{bcd}	22.25±0.63^{d}
	72h	19.50±0.65^{de}	16	0	0	15	18	29	19.75±0.48^{fg}	15.75±0.25^{g}
6m	control	9.50±0.87^{i}	5	5	7	12	4	6	9.75±0.63^{j}	8.5±0.87^{j}
	1h	18.0±0.41^{ef}	6	0	6	27	23	12	18.5±0.50^{ghi}	17.0±0.41^{fg}
	6h	27.50±1.26^{b}	9	5	20	40	11	35	30.0±0.0^{abc}	27.75±0.25^{bc}
	24h	21.0±0.58^{d}	3	0	8	45	11	20	21.75±0.25^{efg}	21.0±0.41^{d}
	72h	10.75±0.25^{hi}	4	0	7	17	7	8	10.75±0.25^{j}	9.75±1.63^{ij}
12m	control	13.50±0.29^{g}	8	4	2	12	5	19	13.50±0.29^{ij}	11.50±0.29^{hi}
	1h	24.0±0.41^{c}	21	4	15	28	11	19	24.50±0.50^{ef}	19.25±0.48^{ef}
	6h	32.50±0.29^{a}	8	8	22	59	13	26	34.0±0.41^{a}	32.0±0.41^{a}
	24h	28.0±0.71^{b}	11	3	24	53	8	12	28.75±0.48^{abcd}	26.0±0.41^{bc}
	72h	12.0±0.41^{gh}	11	0	0	19	5	13	12.0±0.41^{j}	9.25±0.63^{ij}
24m	control	11.75±0.48^{ghi}	3	3	3	15	13	11	12.0±0.41^{j}	11.25±0.48^{hi}
	1h	28.50±0.65^{b}	13	5	29	42	16	19	31.0±0.41^{ab}	27.75±0.75^{bc}
	6h	31.0±0.41^{a}	16	0	24	58	11	21	32.50±0.29^{a}	31.0±2.68^{a}
	24h	28.50±0.29^{b}	9	4	32	41	8	18	30.50±0.29^{ab}	28.25±0.25^{b}
	72h	14.0±0.41^{g}	7	0	7	14	14	12	14.50±0.50^{hij}	12.75±0.48^{h}

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05)

Del: deletion, Frag: fragment, CA: centromeric attenuation, End: endomitosis

Table (4): Mean values of micronucleated polychromatic erythrocytes after heat exposure in female rat bone marrow cells at different ages

times / ages	Mean values of MNPCEs based on 1000 PCEs assessed per animal				
	Control	1h	6h	24h	72h
2 m	2.0±0.41^{f}	1.75±0.48^{f}	2.25±0.48^{ef}	6.75±0. 48^{c}	4.50±0.65^{d}
6m	2.0±0. 41^{f}	2.0±0. 41^{f}	2.0±0. 41^{f}	4.0±0.0.41^{de}	2.0±0.41^{f}
12m	2.0±0. 41^{f}	2.25±0.75^{ef}	2.0±0. 41^{f}	9.0±0. 41^{b}	2.25±0.25^{ef}
24m	2.0±0. 41^{f}	2.25±0.75^{ef}	2.25±0.63^{ef}	12.0±1.23^{a}	2.75±0.48^{ef}

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05).

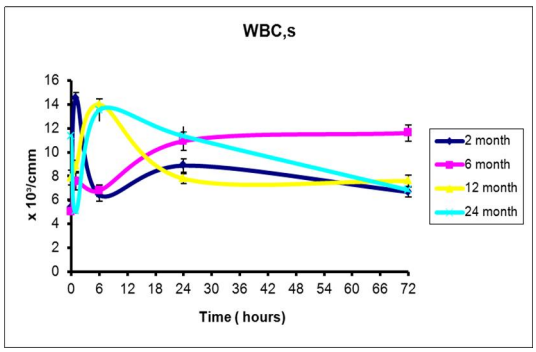

Fig.(1): The effect of WBH on WBC's in rats(2,6,12 and 24 months).

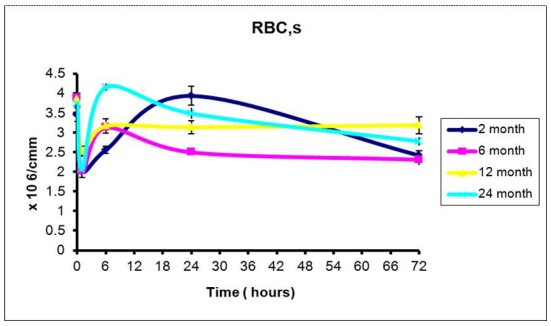

Fig.(2): The effect of WBH on RBC's in rats(2,6,12 and 24 months).

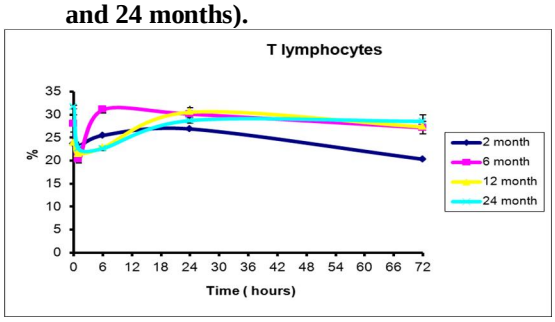

Fig.(3): The effect of WBH on T lymphocytes in rats(2,6,12 and 24 months).

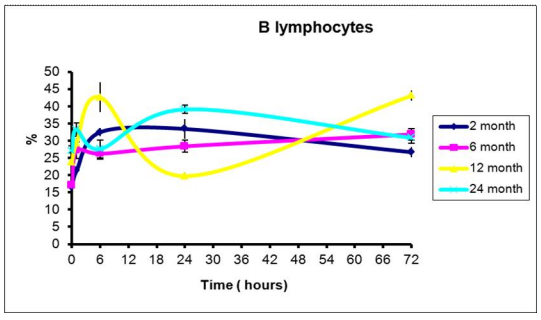

Fig.(4): The effect of WBH on B lymphocytes in rats(2,6,12 and 24 months).

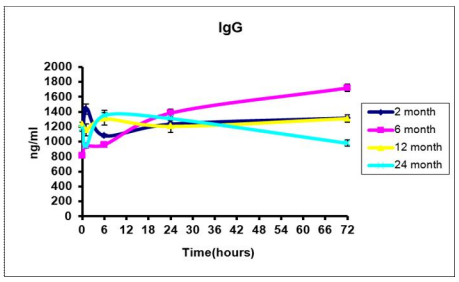

Fig.(5): The effect of WBH on IgG in rats(2,6,12 and 24 months).

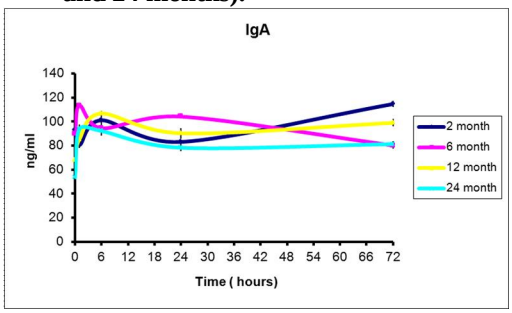

Fig.(6): The effect of WBH on IgA in rats(2,6,12 and 24 months).

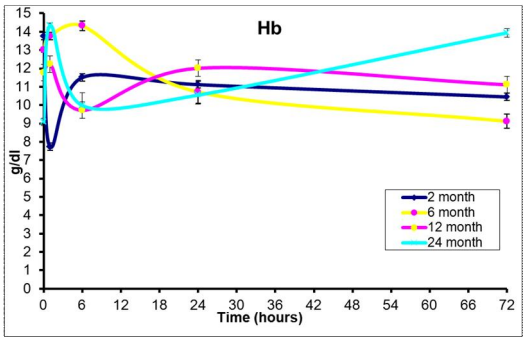

Fig.(7): The effect of WBH on Hb in rats(2,6,12 and 24 months).

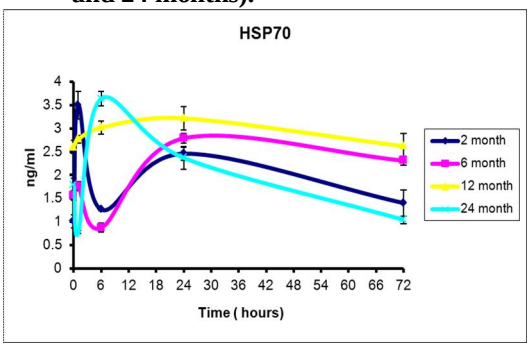

Fig.(8): The effect of WBH on HSP70 in rats(2,6,12 and 24 months).

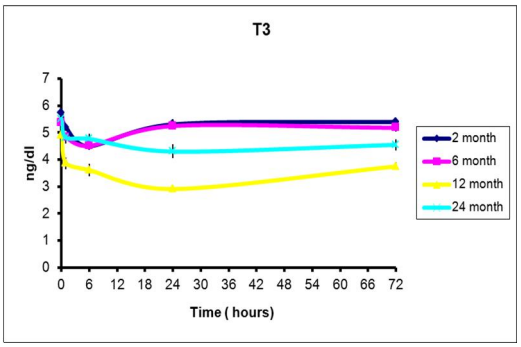

Fig.(9): The effect of WBH on T_3 in rats(2,6,12 and 24 months).

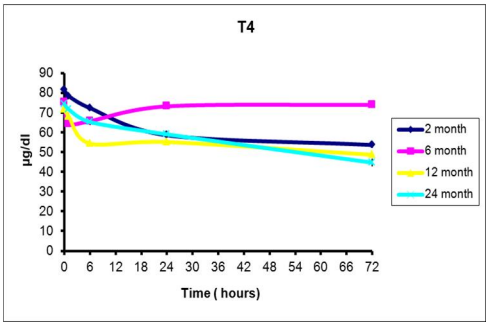

Fig.(10): The effect of WBH on T_4 in rats(2,6,12 and 24 months).

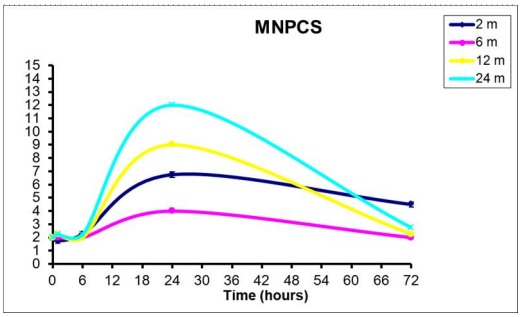

Fig.(11): The effect of WBH on MNPCs in rats(2,6,12 and 24 months).

4. Discussions

In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against heat stress and to permit a rapid recovery from moderate hyperthermic damage (Burdon, 1986 and Hales et al. 1996). However, each tissue and organ has a different sensitivity for sustaining thermal injury (Freeman et al. 1985 and Ando et al. 1994). The biochemical impacts of heat stress on different ages to know the ability of tissues to repair them need to be evaluated. In the present study, the whole body hyperthermia induced increase of WBC's in all different ages after WBH at 1hr till 72 hr except in the aged group (24 month) WBC decreased significantly at 1hr post WBH then increased at 6 hr and approached to control value at 24 hr post heat stress. This decrease may be due to aging as a physiological state affect to decrease of WBCs count in aged rats. Also in most mesenchymal tissues a subcompartment of multipotent progenitor cells is responsible for the maintenance and repair of the tissue following stress with increasing age, the ability of tissues to repair themselves is diminished, which may be due to reduced functional capacity of the progenitor cells Stolzing and Scutt (2006). While WBH caused significantly decreased of RBC in all groups after I hr post the treatment. The significant decrease in the numbers of RBC count is in agreement with the results of Wondergem et al. (1995); Terajima et al.(2000) and Mostafa et al. (2005). The decreased numbers of RBC's may be due to accumulation of blood in different organs as a result of internal hemorrhage as was confirmed by the histopathological alterations observed in liver, kidney and heart in previous studies (Hassan 2006; Abd El- Samea et al. 2007 and Mostafa et al. 2009). These effects might have been also attributed to thrombocytopenia or to decreased blood platelets combined with direct leading to extravasation and escape of red blood cells into the tissue spaces (Terajima et al. 2000 and Mostafa et al. 2005). Another possible reason for the observed decrease of RBCs count could have been the direct hemolytic effect of hyperthermia on the cell membrane resulting in increased membrane fluidity and consequently cell fragility. However, Olayemi and Nottidge (2007) reported the values of RBC, Hb and WBC were similar in the young and adult New Zealand rabbit. The data in the present study revealed a significant increase of Hb level in all groups after 1 hr post WBH except in 2 month's group Hb content decrease in this group till the end of the experiment 72 hr. The present study showed a significant decrease of T lymphocytes percentage in all groups of the experiment after 1hr post WBH which may be due to the stimulating effect of heat on lymphoid organs (Menger ,1983 and Mostafa, 2005).This decreased continued till the end of

experiment in added group 24 months .O' Ahlers et al.(1998) The number of *lymphocytes* decreased significantly from 37°C to 42°C . This effect was mainly caused by a significant decrease of the absolute T4-Cell count and a slight decrease of the T8-Cell count with a resulting significant decrease of **T**-Cells. In addition, IL2-Receptor expression on ***T***-Cells, as a marker for activation, decreased significantly .It seems remarkable, that these effects were reversible in a very short time-period after decrease of temperature. On the other hand, WBH induced a higher significant increase of B lymphocytes percentage in all groups of the experiment.

Induction of Hsp expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans. As well as, the current study was to investigate the impact of both hyperthermia and aging on *in vivo* Hsp70 regulation in response to heat stress. The current results demonstrate that both young animals (2 months) are capable of inducing Hsp70 protein in the early phases of recovery1 hr after a heat challenge. However, at 1 hr and later time points of recovery, old animals (24 months) failed to maintain the high Hsp70 protein levels that were noted in their young counterparts. These results are in accordance with those of Singh et al. (2006) and Soti and Csermely (2006). Our results indicate that both 6 & 12 month groups are capable of up-regulating Hsp70 level at 24 hr & 72 hr following heat stress, while levels of HSP70 in old rats (24 month) decreased significantly at 1 & 72 hours after heat stress. The decrease in HSP70 levels could potentially result from either lowered transcriptional abilities or accelerated mRNA degradation in old rats Zhang et al. (2006). Nevertheless, Singh et al. (2006) speculated that age dependent decline in the ability of peripheral blood mononuclear cells to respond to heat stress in terms of HSP70 induction. Hall et al. (2000) reported that the inducible HSP70 response in the cytoplasm and nucleus was markedly reduced with age at several time points over a 48-h recovery period, although senescent rats were able to strongly express HSP70 early in recovery. Older animals had extensive zone-specific liver injury, which corresponded to the diminished HSP70 response observed in these regions, and a significant reduction in thermotolerance compared with their young counterparts. The process of aging has been associated with increased oxidative damage to macromolecules such as lipids, proteins and DNA in a wide array of tissue types in many eukaryotic species (Dobson, 2003). Therefore, an alteration in the ability of cells to express heat shock proteins could be physiologically important in aging because all living organisms show a reduced ability to respond to stress with increasing age (Bonelli et al.1999; Jin et al. 2004; Kregel et al. 1995 and Vasilaki et al. 2002)). We have shown that the induction of HSP70 expression by heat shock is reduced approximately 188.89% at 6 hr. of recovery in an aged group (24 month old rats) as compared to that in 2 month old group. Other investigators have also shown that the induction of HSP70 expression by heat shock as well as other stresses declines significantly with age in a variety of tissues from rats as well as mononuclear cells from human subjects. Therefore, it appears that a reduced ability to express HSP70 in response to stress may be a common phenomenon underlying the aging process (Heydari et al. 2000). Induction of heat shock proteins including HSP70 that gives a cytoprotective effect against further stress. However, HSP70 induction is attenuated in aged cells. The lower HSP70-levels may contribute to the impaired stress response seen in the aged, and to the higher rates of chronic wounds in aged, which arise from repeated ischemia-reperfusion injury (Andrea et al. 2006).

The prevalence of anabolic activity can trigger the increased basal production of HSP70 in young animals (Maiello et al. 1998). Aging is accompanied by a decay of self-defensive mechanisms and by an accumulation of damages at the molecular, cellular, and organic level as a result of a constant exposure to adverse environmental stresses (28, 30). The impaired response to heat stress observed in old rats could be caused by a failure to induce HSP70 (Fargnoli et al. 1990; Nitta et al. 1994; Heydari et al. 2000; Zhang et al. 2003; Tandara et al. 2006). Because HSP70 is believed to promote the correct refolding of denatured or unfolded proteins damaged by stress and can also act as a chaperone in the ubiquitin–proteasome degradation pathway (Riezman, 2004), the failure of senescent organisms to properly induce the expression of HSP70 and other HSPs would lead to the accumulation of damaged proteins that may be toxic to the cell (Kregel, 2002; Riezman, 2004).

The data in the present study revealed a significant increase of IgG in both groups 2 & 6 months after 1 hr till 72 hr the end of the experiment. On the contrary IgG level in remainder two groups 12 & 24 months showed a fluctuated decrease and increase. On the other hand, WBH induced a significant increase of IgA in 6, 12 and 24 months groups after 1hr post heat exposure. This increase still even 72 hr post WBH in both groups 12 & 24 months. On the contrary, Hietala et al. (2006) reported that hyperthermia has no changes occurred in immunoglobulins or cell-mediated immunity. As well as, Koga et al. (2006) reported that total-body hyperthermia (TBHT) therapy may lead to a reduction in the immune response of cancer patients because of the immediate effect of heat on lymphocytes, the authors studied the immunity of

advanced cancer patients receiving combined total body hyperthermia (TBHT) and anticancer chemotherapy. A decrease was found in their lymphocyte blastogenesis and lymphocyte rosette formation, IgG. These parameters returned to their pretreatment levels at 1 week after completion of TBHT therapy. This result indicates that there is no necessity for giving special consideration to a reduction of cell-mediated immunity in TBHT therapy. Further, Salauze et al. (2004) indicated that both IgM and IgG levels increase gradually with age. By contrast Watt et al. (1986) were observed infants under 6 months failed to respond by the production of IgG antibodies, although increases in IgA and IgM levels.

The endocrine system reacts to the change in the environmental temperature, resulting in change in delivery of thyroid hormones and glucocorticoids (Hardy, 1974) .Thyroid hormones are known to play an important role in adaptive thermogenesis(Arieli and Chint 1986). In the present study there was a dramatic reciprocal change in T3 and T4 during the heat stress of WBH in all groups of the experiment. Although T3 approached to the control value at 72 hr post heat stress in 2 months group and at 24hr in 6 months group. The decrease of T_3 and T_4 hormones is in agreement with other studies (Boiti et al. 1992; Habeeb et al. 1993; Ashour et al. 1995 ; Mostafa et al. 2005). The reduction of T3 and T4 may be due to the direct effect of high temperature on the thermal receptors, which stimulate the hypothalamus to depress its secretion of thyroid stimulating hormone(TSH) releasing hormone(TRH) and consequently the pituitary TSH and thyroid T_3 and T_4 secretion (Kamal, 1975). This decrease conversion of T4 to T3 and faster utilization of T_3 by the respiratory muscles to speed up ventilation in order to enhance energy production. The influence of recovery for 72 in 12 & 24 months groups. So, there is fairly good agreement among authors that the thyroid gland activity in different species increases at moderate cold environment and decreases at high temperature.

There are few reports on the relationship between chromosome aberrations and high body temperature, although there are many in vitro reports of the induction of chromosome aberration by heat treatment (Coss et al. 1982 and Yamada et al. 1989). Here, we demonstrated the effect of hyperthermia on the induction of chromosomal aberrations and micronuclei of bone marrow cells in female albino rats. The results accomplished in this work demonstrated that exposure to hyperthermic treatment (42°C) for 1 hr caused chromosomal aberrations and micronucleus of bone marrow cells at all times but in different frequencies. Our results in agreement with Yamada et al. (1989) and Waissenbourn and Obe, (1992) who reported that structural chromosome aberrations (breaks, stickiness, fragmentation) were observed at 41.5°C and 43 °C respectively . On the other hand, exposure to heat stress in Muscovy ducks leads to negative effects on some physiological, immunological responses and chromosomal aberrations. (El-Badry et al. 2009). Similar findings were obtained in broiler chickens during high temperature exposure (Zhou et al, 1998). These aberrations may because hyperthermia produces little DNA damage (Waters and Axtell, 1992; Jorritsma and Konings, 1993). Heat may induce DNA base damage indirectly via protein damage (Takahashi, 2004) and changes in enzyme complexes for DNA synthesis and repair (Streffer, 1995). Our results demonstrated that at 24 hr, young age (2 month) have more frequencies of chromosomal aberrations than adult (6 months) which have less frequencies than old age (12 and 24 month). 72 hr group show that there were no significant differences between all ages except 2 month which increased statistically than other groups. Mean that young age more affected and need more time than others to recover. This may due to the potential thermotolerance cannot be incorporated into the developing mechanisms of thermoregulation (El-Badry et al. 2009). Laszlo, 1988 suggested that there is a relationship between thermotolerrance and heat shock proteins (hsp) synthesis rate. Furthermore, it has been explained that heat shock proteins bind to chromosomes following heat stress and they take part in chromosome condensation and on recovery induce damages like chromatid stickiness. Mamon and Kutskova, 1993 added that high temperature has a role in inducing damages of mitotic chromosomes in Drosophila melanogaster. From the present results, it was observed that adults were more resistance to heat stress than young and old ages due to potential thermotolerance can incorporat into the developing mechanisms of thermoregulation (Rotwell, 1992). On the other hand, some reports indicate no clear correlation between the frequency of chromosome aberrations and age in the occupationally exposed individuals (Jha and Sharma 1991, Monfared et al. 2003).

Our results also finding that heat shock led to the formation of micronucleus at 24 hour after heat treatment in all ages but more frequencies at 24 month of age. These mean values not significant than control in 1 and 6 hour and recovered at 72 hour after heat at all ages except at 2 month of age. Our findings are in good agreement with other investigations conducted on mammals such as Asanami and Shimono, 1997 and Asanami et al. 2001 they observed positive responses of micronucleus at 31, 33 and 40°C for 24h and 42°C for 2hr. Results suggested that in Chinese hamster cells line, hyperthermic conditions can induce both chromosome aberration and micronuclei. The micronucleus is based on observations that

chromosome fragments and/or entire chromosomes separated (lagging) from the main group at anaphase of mitosis tend to be excluded from a daughter cell nucleus at the telophase stage of mitosis. These chromosomes or fragments are often transformed into micronuclei in the cytoplasm of the daughter cell. As this micronucleus formation may occur spontaneously (not under mutagen influence) at a low rate of incidence, a specific cell type known to have just undergone mitosis under mutagen influence is needed. The mammalian erythrocyte offers this opportunity and has the added advantage of not having a main nucleus so micronuclei can be easily observed. After the last mitotic cycle in the bone marrow, mammalian erythroblasts expel their nuclei but retain any micronuclei and take on a different staining ability. These erythroblasts are polychromatic and stain blue for a period of about 24 hours, then become normochromatic or red staining (Chrisman and Baumgartner 1980 and Schmid, 1975). This may give interpretation for our results that micronucleus significantly increased only at 24 hours after heat stress.

In conclusion, aging and hyperthermia have a synergistic effect on hematological indices as well as, HSP70 that may be essential for surviving and recovering from thermal injury in aged animals. Also, exposure to hyperthermic treatment of 42°C for 1 hr induced chromosomal aberrations and micronucleus formation in female rat bone marrow cells, and recovered at 72 hr. after heat treatment in adults and old ages but in young, it take more time to recover.

5. References

1. Abd El- Samea, I.Z., Mostafa, S.I., Abo-Sinna, G. and Amal.I.H.(2007): Induction of heart injury by hole body hyperthermia in rabbits. J.Egypt. Acad. Soc. Environ. Develop. 8(2):17-29.
2. Ando, M., Katagiri, K., Yamamoto, S., Asanuma, S., Usuda, M., Kawahara, I. and Wakamatsu, K.(1994): Effect of hyperthermia on glutathione peroxidase and lipid peroxidative damage in liver. J Therm Biol 19:177-185.
3. Andrea, T. A.; Oliver, K.; Injoong, K.; Jon, M. E. and Thomas, M. A.(2006): Age effect on HSP70: decreased resistance to ischemic and oxidative stress in HDF. J. Surg. Res., 132(1):32-39.
4. Arieli, A. and Chint, A.(1986): Thyroid status and noradrenaline induce regulatory thermogenesis in heat acclimated rats. Horm. Metab. Res., 7: 222-227.
5. Asanami,S. and Shimono,K. and Kaneda, S.(2001): Effect of temperature on the frequency of chromosome aberrations and micronuclei in cultured Chines hamster cells. The Journal of Toxicological Sciences, 26 (5): 323-326.
6. Asanami,S. and Shimono,K.(1997): High body temperature induces micronuclei in mouse bone marrow, Mutation research/genetic toxicology and environmental mutagenesis. Vplume 390.issues1-2, 24 April 1997, Pages 79-83.
7. Ashour, G., Hassanein, A.M., Gad, H.M., Saeed, A.M. and Shafie, M.M.(1995): Adaptive and reproductive performance of rabbits. Responcse to continuous heat stress. Egypt. J.Anim. Prod., 32(1): 77-90.
8. Bahrndorff, S., Mariën, J., Loeschcke, V. and Ellers, J.(2009): The relationship between thermal resistance and expression of inducible heat shock proteins, especially Hsp70, depends on the species and temperature treatments .Functional Ecology 23, 233–239.
9. Boiti, C.; Chiericato, G.M.; Filotto, U. and Canali, C. (1992): Effects of high environmental temperature on plasma testosterone, cortisol, T_3 and T_4 levels in the growing rabbit. In: Proc. V^{th} world Rabbit. Congress, Oregon statue. Univer. Corvallis, 1: 447-455.
10. Bonelli MA, Alfieri RR, Petronini Pg, Brigotti M, Campanini C, and Borghetti AF. Attenuated expression of 70-kDa heat shock protein in WI-38 human fibroblasts during aging in vitro. Exp Cell Res 252: 20-32, 1999.
11. Broom, M. (2007): Physiology of fever. J. Paediatr Nurs., 19 (6): 40-44.
12. Burdon, R.H. (1986): Heat shock and the heat shock proteins. Biochem J 240:313-324.
13. Calderwood, S.K., Murshid, A. and Prince, T. (2009): The shock of aging: molecular chaperones and the heat shock response in longevity and aging--a mini-review. Gerontology 55(5):550-558.
14. Capetillo, F. O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. DNA Repair (Amst) 2004;3:959–67.
15. Chandolia, R.K., Peltier, M.R., Tian, W., and Hansen, P.J. (1999): Transcriptional control of development, protein synthesis, and heat-induced heat shock protein 70 synthesis in 2-cell bovine embryos. Biol. Reprod., 61: 1644–1648.
16. Chrisman,C. L. and Baumgartner, A. P. (1980): Micronuclei in bone marrow cells of mice subjected to hyperthermia. Mutation Res. 77:95-97.
17. Cohen, M., Kargacin, B. and Klein, M., (1993): Costa M. Mechanism of chromium carcinogenicity. Crit Rev Toxicol. , 23: 255-281.

18. Coss, R.A., Dewey W.C. and Bamburg, J.R. (1982):Effects of hyperthermia on dividing Chinese hamster ovary cells and on microtubules in vitro, Cancer Res., 42, 1059–1071.

19. Dewey, W.C. Li X.L.and Wong, R.S.L. (1990): Cell killing, chromosomal aberrations and division delay as thermal sensitivity is modified during the cell cycle. Radiat.Res. 122 268-274.

20. Dewey, W.C. Sapareto S.A.and Betten, D.A. (1978): hyperthermic radiosesitization of synchronous Chinese hamster cells: relationship between lethality and chromosomal aberrations. Radiat. Res. 76 48-59.

21. Dobson, C. M. (2003): Protein folding and misfolding; Nature 426 884–890.

22. El-Badry, A.S.O., Hassanane, M.M., Ahmed, E.S. and El-Kholy, K.H(2009): Effect of Early-Age Acclimation on Some Physiological, Immunological Responses and Chromosomal Aberrations in Muscovy Ducks During Exposure to Heat Stress, Global Journal of Biotechnology & Biochemistry 4 (2): 152-159.

23. Fargnoli, J. Kunisada, T. Fornace, A.J., Schneider, E.L. and Holbrook, N.J. (1990): Decreased expression of heat shock protein 70 mRNA and protein after heat treatment in cells of aged rats. Proc Natl Acad Sci., 87:846–850.

24. Freeman, M.L., Malcom, A.W. and Meredith, J.W.(1985): Role of glutathione, thermal sensitivity and thermotolerance in Chinese hamster fibroblasts and their heat resistant variants. Cancer Res., 46:1984-1987.

25. Habeeb, A.A.; Abou El Naga, A.I. and Yousef, M.M. (1993): Influence of exposure to high temperature on daily gain, feed efficiency and blood components of growing male Californian rabbits. Egypt. J. Rabbit Sic., 3: 73-80.

26. Hales, J.R.S., Hubbard, R.W. and Gaffin, S.L.(1996): Limitation of heat tolerance. In: Handbook of Physiology (Fregly MJ, Blatteis CM, eds). New York:Oxford University Press, pp.279-355.

27. Hall , D. M., Xu , L. , Drake, V.J. , Oberley, L.W. , Oberley, T.D., Moseley, P.L. and Kregel , K.C. (2000): Aging reduces adaptive capacity and stress protein expression in the liver after heat stress. Appl .Physiol .,89: 749-759.

28. Hassan, A.I.(2006): The relation between hyperthermia and heat shock proteins in carcinoma cell lines with consideration of hyperthermia late effects on rabbits. Ph.D. Thesis. Faculty of Science Ain Shams University.

29. Hardy, R.N.(1974): Temperature and animal life. Edwaed Arnold- London(24-25).

30. Heddle, J.A. (1973): A rapid in vivo test for chromosomal damage. Mutation Res., 18, 187–190.

31. Heydari, A. R., Takahashi, A. Gutsmann, S. Y. and Richardson,A. (2000): Hsp70 and aging.Cellular and Molecular Life Sciences. Exp. Cell Res., 256:83–93.

32. Hietala, J., Nurmi, T., Uhari, M., Pakarinen, A. and K. Kouvalainen , K.(2006): Acute phase proteins, humoral and cell mediated immunity in environmentally-induced hyperthermia in man. Eur. J.Appl.Physio.Occup.Physio.,

33. Ismail-zade, R.S., Buglova, S.E., Potapnev, M.P., Sachivko,N.V., Semenov, A.S., Rudko, V.S., Shman, T.V., and Zhavrid, E.A.(2002): Salvage thermochemo-therapy in managment of advanced refractory malignant tumors in children. Voprosy Oncologii ., 48: 351–355.

34. Jha, A.N. and Sharma, T. (1991): Enhanced frequency of chromosome aberrations in workers occupationally exposed to diagnostic Xrays. Mutat. Res., 260: 343-348.

35. Jin, X.F., Wang, R.B., Xao, C.F., Chang, L.X., Wang,F., Yang, L., Feng, T.Y., Chen, M., Chen, S., Fu, X.Y., Deng, J., Wang, R., Tang, F.F., Wei, Q.Y., Tanguay, R.M., and Wu, T.C.(2004): Serum and lymphocyte levels of heat shock protein 70 in aging: a study in the normal Chinese population. Cell Stress & Chaperones 9: 69-75.

36. Jorritsma, J.B.M. and Konings, A.W.T. (1993): The occurrence of DNA strand breaks after hyperthermic treatments of mammalian cells with and without radiation. Radiation Res. 285: 35-44.

37. Kamal, T.H., Ibrahim, M.S., Mostafa, S.I. and Mostafa A.I.(1992): Effect of controlled high ambient temperature on thyroid activity in men using Radio Immuno Assay. Isotope, Rad, Res., 24(1):31-37.

38. Kamal, T.H.(1975): Heat stress concept and new tracer methods for heat tolerance in domestic animals. In Peacful uses of Atomic Energy for scientific and economic development, Proce. 1st Conf. of Iraqi Atomic Energy Comm. Bagdad, Iraqi, April 1-12, 1975. Atomic Energy Commission (230-235).

39. Kregel K.C. (2002): Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol., 92: 2177-2186,

40. Kregel, K.C., Moseley, P.L., Skidmore, R., Gutierrez, J.A., and Guerriero, V. (1995):HSP70 accumulation in tissues of heat-stressed rats is blunted with advancing age. J Appl. Physiol., 79: 1673-1678.

41. Koga,S., Izumi, A. , Maeta, M., Shimizu, N.,Osaki,Y. and Kanayama,H. (2006): The effects of total-body hyperthermia combined with anticancer drugs on immunity in advanced cancer patients. Cancer 52(7): 7, Pages 1173 – 1177.

42. Laszlo, A., 1988. The relationship of heat-shock proteins thermotolerance and protein synthesis. Exp.Cell Res., 178: 401-414.

43. Lee, B.O. and Korean , S.(1987): Studies on the effect of heat and cold stress on blood picture. Values of serum protein and blood glucose in rabbits. J. Anim. Sci., 29(5):199-207.

44. Maiello ,M., Boeri, D., Sampietro, L., Pronzato, M., Odetti, P. and Marinari, U.M. (1998) : Basal synthesis of heat shock protein 70 increases with age in rat kidneys. Gerontology 44:15-20.

45. Mamon, L.A. and Y.A. Kutskova, (1993): The role of heat shock proteins in high temperature induced damages of mitotic chromosome in Drosophila Melanogaster. Genetika, 29: 604-612.

46. Michael, W. Graner, R.Cumming, I.and Bigner, D. (2007): The Heat Shock Response and Chaperones/Heat Shock Proteins in Brain Tumors: Surface Expression, Release, and Possible Immune Consequences . J.NeuroSci., 27(42):11214-11227.

47. Monfared, A.S., Mozdarani, H., Amiri, M. (2003): Natural background radiation induces cytogenetic radioadaptive response more effectively than occupational exposure in human peripheral blood lymphocytes. Czech. J. Phys., 53 (Suppl A): 791-795.

48. Mostafa,S.I., Bayomy,M. F.F. and Zahran, N.A.R.M.(2005): Hormonal and haematological response to acute whole body hyperthermia (43 C)in nrabbits.J.Egypt.Ger.Soc.Zool.,48A:85-103.

49. Mostafa, S.I., Bayomy, M. F.F., Amal, I.H. and Zahran, N.A.R.M.(2009): Effect of experimental mild and severe whole body hyperthermia. Egypt. J. Med. Lab. Sci., 18(1):1-17.

50. Menger, D.M. (1983): The combination toxicity of hyperthermia and irradiation: studies on bone marrow and peripheral blood of rats. 13Dec. 148 P. Available from the library of Freiburg Univ.(Germany, F.R.).

51. Nitta, Y. Abe, K. Aoki, M. Ohno, I. and Isoyama, S. (1994): Diminished heat shock protein 70 mRNA induction in aged rat hearts after ischemia. Am. J.Physiol.,267:H1795–H1803.

52. O' Ahlers, T., Boehnke, T., Kerner, M. D., Keh, D., Löffel,J. Hildebrandt,B. , Riess, H., Wust, P., Pappert' D.and Gerlach , H.(1998): Induced hyperthermia causes significant changes in lymphocytes. Critical Care, 2(Suppl 1):P002.

53. Olayemi,F.H. and Nottidge, H.O.(2007): Effect of age on the blood profiles of the New Zealand rabbit in Nigeria. African J. Biomed. Res., 10:73-76.

54. Riezman, H. (2004): Why do cells require heat shock proteins to survive heat stress? Cell Cycle 3:61–63.

55. Rodwell, C.E.O., Mackanos,M.A. , Simanovskii, D., Cao, Y., Bachmann, M. H., Schwettman, A. and Contag , C.H.(2008): In vivo analysis of heat-shock-protein-70 induction following pulsed laser irradiation in a transgenic reporter mouse J. Biomed. Opt., Vol. 13, PP. 030501.

56. Rotwell, N.J.,(1992): Hypothalamus and thermogenesis. In: Energy Metabolism: Tissue Determinants and Cellular Corollaries. Raren Press, New York, U.S.A, pp: 229-245

57. Salauze' D., Serre' V. and C. Perrin, C.(2004): Quantification of total IgM and IgG levels in rat sera by a sandwich ELISA technique. Comp. Haem. Int., 4: 30-33.

58. Schmid, W. (1975):The micronucleus test, Mutation Res., 31, 9–15.

59. Singh,R.,Kolvraa,S., Bross, P. , Christensen, K., Gregesen, N., Tan, Q., Jensen, U.B., Eiberg, H. and Rattan, S.I.(2006):Heat shock protein 70 genes and human longevity: a view from Denmark. Ann. N. Y. Acad Sci., 1067:301-308.

60. Soti, C. and Csermely, P.(2006): Aging cellular networks: chaperones as major participants. Ex. Gernotol. 42, 113-119.

61. Soti, C. and Peter, C. (2007): Protein stress and stress proteins: implications in aging and disease. J. Biosci. 32(3): 511–515.

62. Stadtman, C. and Stadtman, E. R.(2004): Role of oxidant species in aging Post-translational modifications of proteins: implications for aging, antigen recognition, and autoimmunity. Curr. Med. Chem., 11: 1105–1112.

63. Stolzing, A. and Scutt, A.(2006): Age-related impairment of mesenchymal progenitor cell function. Aging Cell. 5(3):213-24.

64. Streffer, C. (1995): Molecular and cellular mechanisms of hyperthermia. In Seegenschmiedt MH, Fessenden P, Vernon CC (eds):

Thermoradiotherapy and Thermochemotherapy Volume 1. Berlin: Springer Verlag.

65. Takahashi A, Matsumoto H, Nagayama K., Kitano ,M. ,Hirose , S.,Tanaka' H.,Mori , E., Yamakawa , N.,Yasumoto , J., Yuki , K., Ohnishi , K.and Ohnishi , T.(2004): Evidence for the involvement of double-strand breaks in heat-induced cell killing. Cancer Res.64:8839– 8845.
66. Tandara, A.A.; Kloeters, O.; Kim, I.; Mogford, J.E. and Mustoe, T.A. (2006): Age effect on HSP70: decreased resistance to ischemic and oxidative stress in HDF. J . Surg. Res .,132:32–39.
67. Terajima, H.,Enders, G., Thiaener, A.,Hammerc, K.,Thiery, J. and Yamanoto, Y.,Yamanoko, Y., and Messmer, K.(2000): Impact of hyperthermic preconditioning on postischemic microcirculatory disturbanaces in an isolated perfusion model of rat liver.Hepatol.,31(2):407-415.
68. Vasilaki, A., Jackson, M.J., and McArdle ,A.(2002): Attenuated HSP70 response in skeletal muscle of aged rats following contractile activity. Muscle & Nerve 25: 902-905.
69. Venitt, S., Parry, J.M.,(1984): Mutagenecity Testing: Apractical Approch, IRL Press, Oxford, Washington DC. Pp. 353.
70. Vivinus, S., Baulande, S., Zanten, M.V., Campbell, F., Topley, P., Ellis, J.H., Dessen, P., and Coste, H.(2001): An element within the 5' untranslated region of human Hsp70 mRNA which acts as a general enhancer of mRNA translation. Eur. J .Biochem., 268: 1908–1917.
71. Waissenbourn, U. and Obe, G. (1992). Modification of bleomycin induced chromosome aberration by hyperthermia and under energy depleting conditions in human peripheral lymphocytes. Int. J. Radiat. Biol. 62: 289-296.
72. Warter, R.L.,Arters, R.L. and Stone, O.L. (1984): Macromolecule synthesis in HeLa cells after thermal shock. Radiat. Res. 96:646-652.
73. Waters, R.L. and Axtell, J. (1992): Repair of DNA strand breaks at hyperthermic temperatures in chinese hamster ovary cells. Int. J. Radiat. Biol. 61:43-48.
74. Watt, P.J., Zardis, M. and Lambden, P.R. (1986): Age related IgG subclass response to respiratory syncytial virus fusion protein in infected infants. Clin Exp Immunol. 64(3): 503–509.
75. Wondergem, J., Strebel, F.R., Stephens, L.C.,Siddik, Z.H. and Bull, J.M.C.(1995): Chronic effect of whole body hyperthermia combined simultaneously with cis-diaminedichloroplatinum (II) on normal tissue in rat. Int.J.Hyperther., 11(1):37-47.
76. Yamada, K., Ichikawa, Y., Okumura, H. (1989): Effects of high temperatures on chromosomes of normal and transformed human cell. Human Cell, 2, 80–85.
77. Yosida T.H. and Amano, K.(1965): Autosomal polymorphism in laboratory bred and wild Norway rats, Rattus norvegius, found in Misima, Chromosoma 16 pp. 658.
78. Zhang, H.; Xu, L.; Drake, V.J.; Xie, L. Oberley, L.W. and Kregel, K.C. (2003): Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation. FASEB J 17:2293–2295
79. Zhang, H.J., Doctrow, S.R., Oberley,L. W. and Kregel1,K. C. (2006): Chronic antioxidant enzyme mimetic treatment differentially modulates hyperthermia-induced liver HSP70 expression with aging. Appl .Physiol. 100: 1385-1391.
80. Zhou, W.T., M. Fujita, S. Yamamoto, K. Iwasaki, R. Ikawa, H. Oyama and H. Horikawa, (1998): Effects of glucose in drinking water on the changes in whole blood viscosity and plasma osmosisty of broiler chickens during high temperature exposure. Poultry Sci., 77: 644-647).

2/1/2010

Some Promising Wild Edible Plants of Srinagar and its Adjacent Area in Alaknanda Valley of Garhwal Himalaya, India

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Abstract: The present communication deals with the ethnobotanical exploration, identification, concerns and future potentialities of the wild edible plant species consumed by the local people inhabiting in the hilly areas of Alaknanda valley that fall in the Uttarakhand state of India. A total of 55 plant species belonging to 35 families were recorded from the study area. Amaranthaceae, Lamiaceae and Moraceae were the dominant families with 4 species each, while Anacardiaceae, Fabaceae, Rosaceae and Rutaceae followed with 3 species and rest were represented by one species from each family. The four major life forms were herbs, shrubs, climbers and trees. Herbs made the highest proportion of the edible species (18) followed by trees (17), shrubs (13) and climbers (7). The plant species were divided into two classes - consumed as raw and prepared in to vegetables. 32 species belonged to the former category while the later was represented by 23 plants. [Journal of American Science 2010;6(4):167-174]. (ISSN: 1545-1003).

Keywords: Wild edible plants, Garhwal Himalaya, indigenous knowledge, local inhabitants.

1. Introduction

Millions of people in many developing countries do not have enough food to meet their daily requirements and a further more are deficient in one or more nutrients (FAO, 2004) and the same is true about India, the country with second largest human population on the earth.

Wild edible plants have played an important role in human life since time immemorial. In India most rural inhabitants depend on the wild edible plants to meet their additional food requirements. The diversity in wild plant species offers variety in family diet and contributes to household food security. Today, most human plant food is based on rather limited number of crops, but it is clear that in many parts of the world the use of wild plants is not negligible (Prescott-Allen and Prescott-Allen, 1990; Scherrer *et al*., 2005; Bussmann *et al*., 2006; Bussmann and Sharon, 2006; Kunwar *et al*., 2006; Cavender, 2006; Pieroni *et al*. 2007). Sometimes the nutritional value of traditional wild plants is higher than several known common vegetables and fruits (Nordeide *et al*., 1996; Sundriyal and Sundriyal, 2001; Orech *et al*., 2007).

The Garhwal Himalaya region is the land of many beautiful holy places, valleys and hills. Most of the people of the Garhwal live in the villages. The area forms the middle and outer part of the Himalaya, which is rich in natural resources of which plant resources are prevalent. The forest resource plays an important role in the livelihood of the local communities. The rich plant diversity of the area is utilized by the local inhabitants in various forms as medicine, food, fodder, fuel, timber, agricultural implements, etc. Among these, wild edible plants play an important role in food supplement during scarcity for local inhabitants. Because of small land holdings and subsistence agriculture, the local people collect many wild edible plants for food.

Many works have emphasized on the diversity and traditional uses of wild plants from this part of country (Gaur, 1977; Gaur and Semwal, 1983; Negi, 1988; Negi and Gaur, 1991, 1994; Samant and Dhar 1997; Maikhuri *et al*., 2000; Kala, 2007; Dhyani *et al*., 2007). Although much has been documented on ethnomedicinal and floristic aspects of plants of this area, little has been reported about the wild edible plant resources of Srinagar and its adjacent area. Keeping this in view, the present study was conducted as an attempt from the region to explore and identify the wild edible plant resources, and indigenous traditional knowledge about their utilization.

2. Materials and methods

To undertake the present study the area of Srinagar Garhwal was selected which falls in the subtropical zone of Garhwal Himalaya. Physiographically the area consists of hill slopes and valleys. The study was conducted between 30° 13' 09" - 30° 14' 22" N latitudes and 78° 45' 47" - 78° 51' 58" E longitudes (Figure 1) at the elevation of 535 -1500 m asl. The temperature in summer goes to 35° - 40° C and in winter less than 6° - 7° C due to

heavy frost. Rainfall is less than 200 mm. Regular field surveys were made in the Alaknanda valley during the years 2008 and 2009 in different seasons i.e., rainy, winter and summer, to collect the wild edible plants. Identification of the specimens was done with the help of Garhwal University Herbarium (GUH) and works of Duthie 1906; Osmaston, 1927; Naithani, 1984-85 and Gaur, 1999. Ethnobotanical information on wild plants was collected by interviewing local inhabitants based on a structured questionnaire to collect data on local plant names, uses, parts used and mode of utilization. To determine the authenticity of the information collected during field work, repeated verification of data from different informants was done. Thus, only the specific and reliable information cross-checked with informants has been incorporated in the present study.

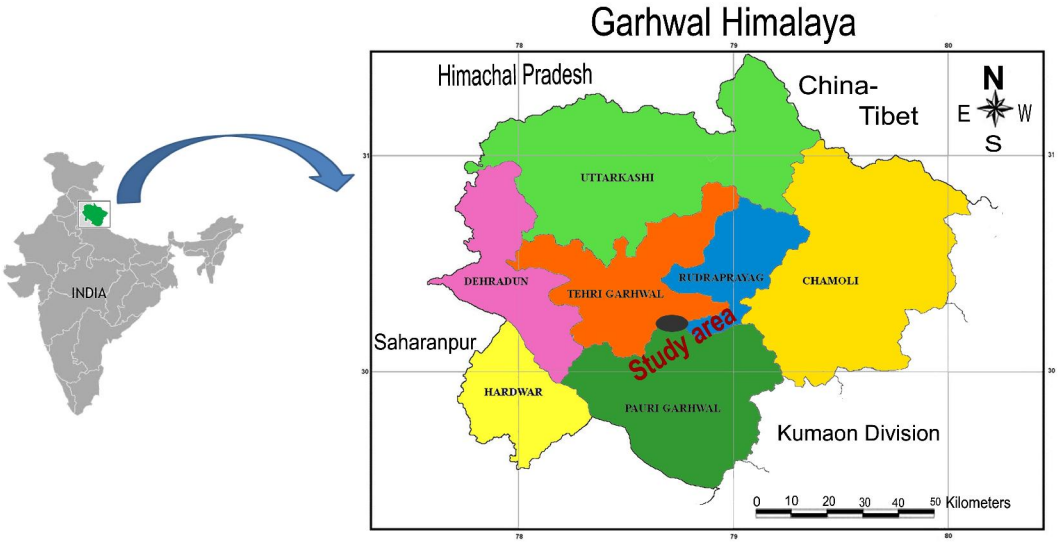

Figure1. Location map of the study area

3. Results and Discussion

The present investigation comprised of 55 species of plants belonging to 35 families. The botanical name, family, local name, parts used and mode of utilization for each species have been enumerated in Table 1. A total of 5 habitats (i.e. shady moist places, exposed slopes, road sides, agricultural fields and forest edges) have been observed. The representation of species is maximum on road sides (37 species) followed by 32 species each in shady moist places and agricultural fields (Figure 2). Among all the species 34 are represented in 3 or >3 habitats, and the remaining restricted to 1 or 2 habitats only.

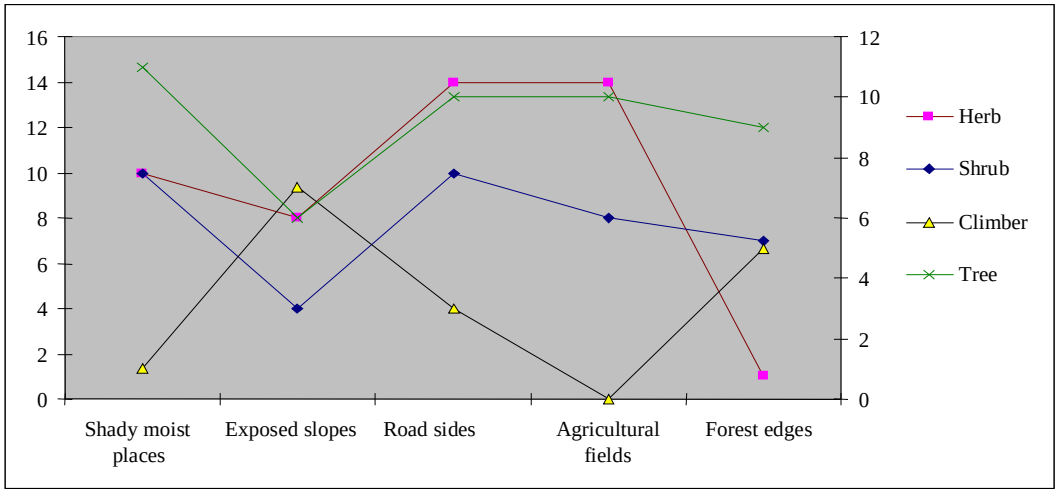

Figure 2. Life forms recorded in different habitats

The plant parts used were leaves, fruits, tubers, flowers and whole plants for food supplements. Herbs made the highest proportion of the edible species followed by trees, shrubs and climbers in descending order. The time and frequency of collecting various plants and plant parts varied from plant to plant depending upon their availability. Method of preparation and uses fall into categories like cooked and eaten as raw.

During the survey, it was observed that the villagers of the area have much faith in using the wild plants as food. The inhabitants of the area are dependent on forests for food up to much extent. They frequently visit forests to collect necessary foods and food supplements (Plate 1 Figure 3-8). Thus, these people have grouped the wild food plants into two categories- made in to vegetables and consumed as raw.

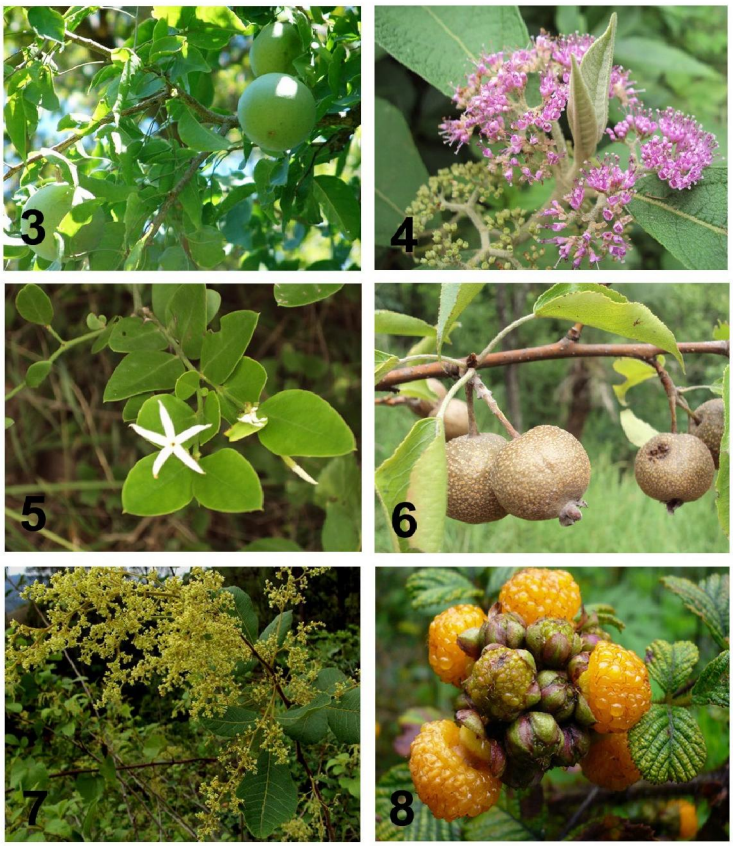

Plate.1 Some important wild edibles used in the study area. Figure 3. *Aegle marmelos* (L.) Correa. 4. *Callicarpa macrophylla* Vahl 5. *Carissa opaca* Stapf ex Haines 6. *Pyrus pashia* Buch.-Ham.ex D.Don 7. *Rhus parviflora* Roxb. 8. *Rubus ellipticus* Smith

The leaves of 18 species are used as vegetables (Figure 9). Species like *Cirsium wallichii*, *Dioscorea bulbifera*, *Gonatanthus pumilus*, *Pueraria tuberosa*, *Vigna vexillata*, etc. bear tuberous roots which provide sufficient minerals. The fruits of 32 species are eaten as raw and sometimes made in to salads or pickles.

The discussions with local inhabitants revealed that the wild food plants are used as common household foods and make a substantial contribution to food security of the people of the area. Therefore, steps are needed to undertake extensive education about their importance and assess their nutritional value to serve as a direct or indirect source of food to the local inhabitants. This may bring to light one or other new food plants from wild resources for ever increasing population of our country. Many of the wild food may not be freely available in future due to overexploitation, habitat destruction, regular forests fires and invasion of exotic plant species. Therefore efforts can be made to bring some of them under cultivation in order to maintain a continuous supply and help in their conservation.

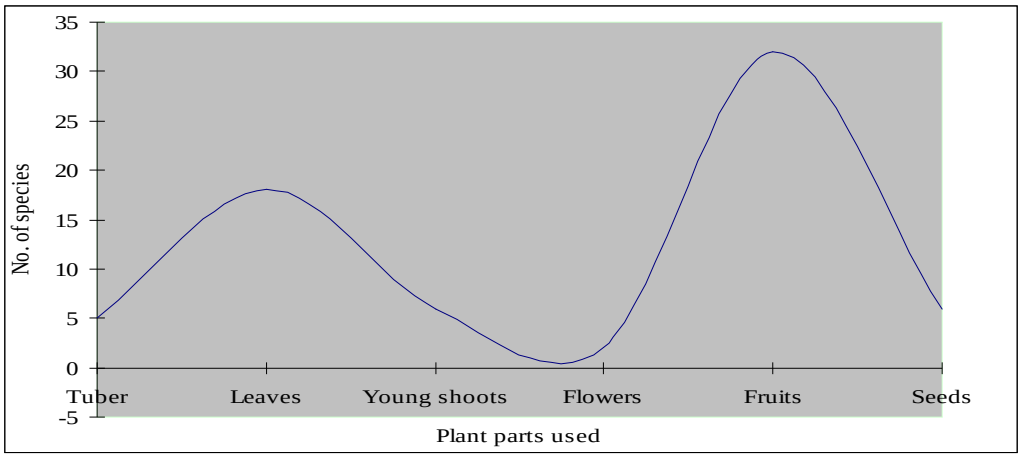

Figure 9. Number of species and plant parts used as wild edibles.

Table 1. List of some promising wild edible plants of Srinagar and its adjacent area in Alaknanda Valley of Garhwal Himalaya, India.

S. No.	Botanical names	Local Name	Family	Life Forms	Habitat (s)*	Plant parts and methods of use
1	*Adhatoda zeylanica* Medikus	Baisingu	Acanthaceae	Shrub	1,3	Young twigs and leaves made into vegetable.
2	*Aegle marmelos* (L.) Correa	Bel	Rutaceae	Tree	1 ,3,5	Fruit pulp is edible, made into juice.
3	*Alternanthera sessilis* (L.) DC.	Ghandugli	Amaranthaceae	Herb	1,3,4	Leaves used as vegetable.
4	*Amaranthus creuntus* L.	Chaulai	Amaranthaceae	Herb	1,3,4	Young shoots and leaves made into vegetable.
5	*A. viridis* L.	Jangli-chaulai	Amaranthaceae	Herb	3,4	Young seeds and leaves used as vegetable.
6	*Ampelocissus latifolia* (Roxb.) Planchon	Bhinura	Vitaceae	Climber	2,5	Fruits are edible.
7	*Berberis asiatica* Roxb. ex DC.	Kingore	Berberidaceae	Shrub	1,3,4,5	Fruits are edible.
8	*B. lycium* Royle	Kingor	Berberidaceae	Shrub	3,5	Fruits are edible and made into sauce.
9	*Bauhinia vahlii* Wight & Arn.	Malu	Caesalpiniaceae	Climber	2,3,5	Roasted seeds are edible.
10	*B. variegata* L.	Kurial,	Caesalpiniaceae	Tree	2,4,5	Young flowers cooked as vegetable.
11	*Bombax ceiba* L.	Semal	Bombaceae	Tree	1,3	Flower buds cooked as vegetable.
12	*Callicarpa*	Daiya	Verbenaceae	Shrub	2,3	Fruits are edible.

	macrophylla Vahl					
13	*Capsella bursa-pastoris* (L.) Medikus	Tuntkya	Brassicaceae	Herb	2,3,4	Young plants used as pot herb.
14	*Carissa opaca* Stapf ex Haines	Karaunda	Apocynaceae	Shrub	1,3,4,5	Fruits are edible
15	*Catunaregam spinosa* (Thunb.) Tirvengadum	Maindul	Rubiaceae	Tree	1,5	Ripe fruits are eaten after roasting or cooked, leaves are cooked as vegetable.
16	*Celastrus paniculatus* Willd.	Malkauni	Celastraceae	Climber	2,3,5	Unripe fruits are boiled and cooked as vegetable.
17	*Celosia argentea* L.	Gadria	Amaranthaceae	Herb	1,3,4	Leaves are cooked as vegetable.
18	*Celtis australis* L.	Khareek	Ulmaceae	Tree	1,3,4	Fruits are edible.
19	*Chenopodium album* L.	Bathua	Chenopodiaceae	Herb	1,3,4	Leaves are used as pot-vegetable.
20	*Cirsium wallichii* DC.	Kandara	Asteraceae	Herb	2,3,4	Tuberous roots are edible after peeling off outer coat and cooked as vegetable.
21	*Cleome viscosa* L.	Jakhya	Cleomaceae	Herb	1,4	Seeds are used as condiments.
22	*Coccinia grandis* (L.) Voigt	Kaduri	Cucurbitaceae	Climber	2,3	Unripe fruits and young shoots are cooked as vegetable and made into pickles.
23	*Cordia dichotoma* Forst.	Lisora	Rutaceae	Tree	2,5	Fruits are edible and made into pickles; young leaves are cooked as vegetable.
24	*Dioscorea bulbifera* L.	Genthi	Dioscoreaceae	Climber	2,5	Tubers are cooked as vegetable.
25	*Duchesnea indica* (Andrews) Focke	Bhium-kaphal	Rosaceae	Herb	2,3,4	Fruits are edible.
26	*Ficus auriculata* Lour.	Timla	Moraceae	Tree	1,3,4	Fruits are eaten raw and cooked as vegetable.
27	*F. palmata* Forsk.	Bedu	Moraceae	Tree	1,3,4	Fruits are edible.
28	*F. semicordata* Buch.-Ham. ex J.E. Smith	Khaina	Moraceae	Tree	2,4,5	Ripened fruits are eaten raw and unripe fruits are made into vegetables.
29	*Gonatanthus pumilus* (D.Don) Engler & Krause	Ban-pindalu	Araceae	Herb	2,3	Tuberous roots and leaves are cooked as vegetable.

30	*Grewia optiva* J. R. Drummond ex Burret	Bheemal	Tiliaceae	Tree	1, 3,4	Fruits are edible.
31	*Mentha arvensis* L.	Pudina	Lamiaceae	Herb	2	Leaves are made into sauce and dried leaves are used as condiments.
32	*M. longifolia* (L.) Hudson	Pudina	Lamiaceae	Herb	2	Leaves used for flavoring and also made into sauces.
33	*Moringa oleifera* Lam.	Sunara	Moringaceae	Tree	1,3,4	Leaves, flowers and fruits are cooked as vegetables.
34	*Morus serrata* Roxb.	Sahtoot	Moraceae	Tree	1, 3	Fruits are edible.
35	*Murraya koenigii* (L.) Sprengel	Kari-patta	Rutaceae	Shrub	1,3,4,5	Ripened fruits are edible and leaves are used to flavor the dishes (*curries*).
36	*Ocimum americanum* L.	Tulsi	Lamiaceae	Herb	1,3,4	Leaves made into sauce.
37	*Opuntia elatior* Miller	Nagfani	Cactaceae	Shrub	1,3,4	Fruits are edible.
38	*Ougeinia oojeinensis* (Roxb.) Hochreutiner	Sandar	Fabaceae	Tree	1,4,5	Flowers are boiled and much sought after mixed with cooked rice and millets.
39	*Oxalis corniculata* L.	Chilmori	Oxalidaceae	Herb	1,2,3,4	Leaves taken as salad and cooked as vegetable
40	*Perilla frutescens* (L.) Britton	Bhangjeera	Lamiaceae	Herb	1,4	Leaves are cooked as vegetable. Seeds are used as spices and condiments.
41	*Phoenix humilis* Royle	Khajoor	Arecaceae	Tree	2,5	Young shoots and ripened fruits are edible.
42	*Phyllanthus emblica* L.	Aunmla	Euphorbiaceae	Tree	1,3,5	Ripened fruits are edible and made into sauce.
43	*Physalis divaricata* D.Don	Damphu	Solanaceae	Herb	1,3,5	Fruits are edible.
44	*Pueraria tuberosa* (Roxb. ex Willd.) DC.	Siralu	Fabaceae	Climber	2,5	The tuberous roots are eaten raw and the older once are boiled and cooked as vegetable.
45	*Pyrus pashia* Buch.-Ham.ex D.Don	Melu	Rosaceae	Tree	2,4,5	Fruits are edible.

46	*Rhus javanica* L.	Damphela	Anacardiaceae	Shrub	2,5	Fruits are edible and made into sauce.
47	*R. parviflora* Roxb.	Tungla	Anacardiaceae	Shrub	1,3,4,5	Ripened fruits are edible.
48	*Rubus ellipticus* Smith	Hinsalu	Rosaceae	Shrub	1,3,4	Fruits are edible.
49	*Rumex hastatus* D.Don	Almora	Polygonaceae	Herb	1,3,4	Leaves are eaten raw as salad and also used as condiments.
50	*Solanum nigrum* L.	Makoi	Solanaceae	Herb	2,3,4	Young shoots and leaves are cooked as vegetable. Ripened fruits are edible.
51	*Spondias pinnata* (L.f) Kurz	Amara	Anacardiaceae	Tree	2,5	Fruits are made into sauce and pickles. Seeds are edible.
52	*Syzygium cumini* (L.) Skeels	Jamun	Myrtaceae	Tree	1,3,4	Fruits are eaten raw or with salt and mustard oil.
53	*Urtica dioica* L.	Kandali	Urticaceae	Shrub	1,2,3,4	Young branches and leaves used as delicious pot herb
54	*Vigna vexillata* (L.) A. Richard	Machali	Fabaceae	Climber	1,2	Tubers are eaten raw after peeling off the outer coat, and also cooked as vegetable. Seeds are edible.
55	*Ziziphus mauritiana* Lam.	Ber	Rhamnaceae	Shrub	1,2,4	Fruits are edible.

***** 1= Shady moist places, 2= Exposed slopes, 3= Road sides, 4= Agricultural fields, 5= Forest edges.

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References

1. Food and Agricultural Organization of the United Nations FAO. The state of food insecurity in the world. Monitoring the progress towards the world food summit 2nd millennium development goals. Annual Report Rome. 2004.
2. Prescott-Allen OC, Prescott-Allen R. How many plants feed the world? Conservation Biology.1990;4:365-374.
3. Scherrer AM, Motti R, Weckerle CS. Traditional plant use in the areas of Monte Vesole and Ascea, Cilento National Park (Campania, Southern Italy). J. Ethnopharmacol. 2005;97:129-143.
4. Bussmann RW, Gilbreath GG, Solio J, Lutura M, Lutuluo R, Kunguru K, Wood N, Mathenge SG. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. J. Ethn. & Ethnomed. 2006;2:22.
5. Bussmann RW, Sharon D. Traditional medicinal plant use in Northern Peru: tracking two thousand years of healing culture. J. of Ethno. & Ethnomed. 2006;2:47.

6. Kunwar RM, Nepal BK, Kshhetri HB, Rai SK and Bussmann RW. Ethnomedicine in Himalaya: a case study from Dolpa, Humla, Jumla and Mustang districts of Nepal. J. Ethno. & Ethnomed. 2006;2:27.
7. Cavender A. Folk medicinal uses of plant foods in southern Appalchia, United States. J. Ethnopharmacol. 2006;108:74-84.
8. Pieroni A, Houlihan L, Ansari N, Hussain B, Aslam S. Medicinal perceptions of vegetables traditionally consumed by south-Asian migrants living in Bradford, Northern England. J. Ethnopharmacol. 2007; 113:100-110.
9. Nordeide MB, Hatloy A, Folling M, Lied E, Oshoug A. Nutrient composition and nutritional importance of green leaves and wild foods in an agricultural district, Koutiala, in Southern Mali. Int J Food Sci Nutr. 1996;47(6):455-468.
10. Sundriyal M, Sundriyal RC. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. Economic Botany.2001;55:377-390.
11. Orech FO, Aagaard-Hansen J and Friis H. Ethnoecology of traditional leafy vegetables of the Luo people of Bondo district, western Kenya. Int. J. Food Sci. Nutr. 2007;58(7):522-530.
12. Gaur RD. Wild edible fruits of Garhwal Hills. JOHSARD. 1977;1:66-70.
13. Gaur RD, Semwal JK. Some little known wild edibles of Garhwal Himalaya. Man & Environment. 1983;7:161-165.
14. Negi KS. Some little known wild edible plants of U.P. hills. J. Econ. Tax. Bot. 1988;12:345-360.
15. Negi KS, Gaur RD. Little endemic wild edibles Allium spp. of U.P. Hills. Mountain Research & Development. 1991;11:162-164.
16. Negi KS, Gaur RD. Principal wild food plants of western Himalaya. U.P. India. In BK Gupta (ed.) Higher Plants of Indian Subcontinent, Vol III. Pp. 1-78. Bishen Singh Mahendra Pal Singh Dehradun, U.P., India. 1994.
17. Samant SS, Dhar U. Diversity, endemism and economic potential of wild edible plants of Indian Himalaya. -International Journal of Sustainable Development and World Ecology.1997;4:179-191.
18. Maikhuri RK, Nautiyal S, Rao KS, Semwal RL. Indigenous knowledge of medicinal plants and wild edibles among three tribal sub communities of the central Himalayas, India. Indigenous Knowledge and Development Monitor. 2000;8:7-13.
19. Kala CP. Prioritization of cultivated and wild edibles by local people in the Uttaranchal hills of Indian Himalaya. Indian Journal of Traditional Knowledge. 2007;6:239-243.
20. Dhyani D, Maikhuri RK, Rao KS, Kumar L, Purohit VK, Sundriyal M, Saxena KG. Basic nutritional attributes of *Hippophae rhamnoides* (sea buckthorn) populations from Uttarakhand Himalaya, India. Current Science. 2007;92:1148-1152.
21. Duthie JF. Catalogue of plants of Kumaon and of the adjacent portions of Garhwal and Tibet based on the collections made by Strachey and Winterbottom during the years 1846-1849. London. Reprint 1994, Bishan Singh Mahendrapal Singh, Dehradun.1906.
22. Osmaston AE. A Forest Flora for Kumaun. Government Press, Allahabad. Reprint 1990, Bishen Singh Mahendra Pal Singh, Dehradun. 1927.
23. Naithani BD. Flora of Chamoli. 2 Vols. Botanical Survey of India, Howrah.1984-85.
24. Gaur RD. Flora of the District Garhwal, N. W. Himalaya (with Ethnobotanical Notes). Transmedia: Srinagar Garhwal.1999.

05/03/2010

Genetic alterations induced by toxic effect of thermally oxidized oil and protective role of tomatoes and carrots in mice

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Abstract: The present study was designed to investigate the genetic alterations and sperm abnormalities in male mice fed diet containing thermally oxidized sunflower oil repeatedly used for frying processes (FO). Also, tomatoes and carrots were added to FO diet to test their protecting ability against potential hazards caused by oxidized oil rich foods. The genetic alterations including DNA fragmentation and chromosome aberrations as well as changes of mRNA expression of some lipid metabolism related-proteins were determined. The results revealed that rate of DNA fragmentation was significantly higher in animals fed FO diet than those of animals fed normal oil diet (NO). On the other hand, the rates of DNA fragmentation decreased in animals fed FO diets plus low (5%) or high (10%) concentration of tomatoes (FOT1 or FOT2) or carrots (FOC1 or FOC2) compared with those fed FO diet. Chromosome examination showed that total structural aberrations increased significantly in animals fed FO diet than those fed NO diet. On the other hand, animals fed diets containing FO plus low or high concentration of tomatoes or carrots had significantly lower frequencies of total structural aberrations than those fed FO diet. Sperm studies showed a significant increase in the number of morphologically abnormal sperms and a significant decrease in the sperm count in animals fed FO diet compared with those fed NO diet. However, the animals fed diets containing low or high level of tomatoes or carrots showed significant decreases of sperm abnormalities. The mRNA expressions of the lipid metabolism related-genes, RBP, H-FABP and C-FABP were significantly higher in liver tissues of mice fed FO diet than those found in mice fed NO diet. However, the expression of all tested genes was down-regulated in FOT1 and FOT2 or FOC1 or FOC2 groups compared with those detected in the FO group. In conclusion, the present study adds evidence for a link between prolonged feeding intake of FO diet and induced mutagenic effects in animal cells. However, tomatoes and carrots proved to be good protective agents against hazards of such mutagenic foods. [Journal of American Science 2010;6(4):175-188]. (ISSN: 1545-1003).

Keywords: Thermally oxidized oil, mice, tomato and carrot, genetic alterations, RT-PCR, sperm morphology.

1. Introduction

Several studies reported that using vegetable oils in prolonged frying processes may be a principle or strong reason for inducing or forming mutagenic or carcinogenic diets (Hamilton et al., 1997; Coultate, 2002; Gouveia De Souza et al., 2004; Pavia & Gordon, 2005; Khalil et al., 2009). The mutagenicity/ carcinogenicity of such diets may be due to the fact that the repeatedly used vegetable oils in frying processes undergo degradation by oxidative reactions that lead to form lipid peroxidation (Nagao et al., 1997; Lampe, 1999; Raloff, 1999).

High intakes of diets containing lipid peroxidation component may be associated with the generation of free radicals (Schut et al., 1997; Lampe, 1999; Coultate, 2002; Guardiola et al., 2008). If not quenched by antioxidants, these highly reactive compounds will react with and potentially alter the structure and function of several cellular components, such as lipid, lipoproteins, proteins, carbohydrates, RNA and DNA. These alterations have been considered as important factors which can affect the gene expression for a lot of genes causing variety of diseases, especially hyperlipidemia, atherosclerosis and cardiovascular diseases (Suganuma & Inakuma, 1998; Lampe, 1999; Nicolle et al., 2003).

Vegetables have been identified to be major dietary sources of antioxidants. Tomatoes and carrots are the main vegetables that have been found to contain a remarkable combination of antioxidant micro-constituents, such vitamins A, C, D and E, polyphenols and various carotenoids, including alpha – and beta – carotene and lycopene (Duthie et al., 1996; Lampe, 1999; Cramer et al., 2001; Nicolle et al., 2003; Gitenay et al., 2007; Devaraj et al., 2008). The activity of antioxidants has been shown to improve immune status, scavenge of free radicals, reduce the production of DNA adducts and could be effective means of preventing variety of diseases (Allard et al., 1994; Brown et al., 1994; Lampe, 1999; Van Breda et al., 2005; Devaraj et al., 2008). A significant decrease in endogenous oxidative base damage in the lymphocyte DNA was detected by Duthie et al. (1996) in both smokers and non-smokers with 20–wk daily supplement of micro-constituents of tomatoes such as vitamin C (100 mg), α–tocopherol (280 mg), and β–

carotene (25 mg). Also, Devaraj et al. (2008) tested the effects of different doses of purified lycopene supplementation on biomarkers of oxidative stress in healthy volunteers. Their results revealed a significant decrease in DNA damage by the comet assay and a significant decrease in urinary 8–hydroxyl deoxaguanosine (8–OhdG) at 8 weeks versus baseline, with 30 mg lycopene/ day. In an additional study, tomatoes were found to have a preventive effect on atherosclerosis by protecting plasma lipids from oxidation. This effect was observed by Suganuma & Inakuma (1998) who found that mice fed the atherogenic diet without tomato for 4 months had a significant increase in plasma lipid peroxide level than those fed the atherogenic diet containing 20% (w/w) lyophilized tomato powder.

On the other hand, Nicolle et al. (2003) investigated the effects of 3-week supplementation of the diet with carrots on lipid metabolism and antioxidant status in rats. They observed significant decreases of cholesterol levels together with reduction of the triglycerides levels in the liver. Also in this experiment, the results revealed that carrot consumption improved the antioxidant status by decreasing the urinary excretion of thiobarbituric acid reactive substances (TBARS), reducing TBARS levels in heart and increasing the vitamin E plastimatic level as compared to the control. The authors concluded that these effects could be interesting for the protection of cardiovascular disease.

Tomatoes and carrots (or its micro-constituents) have also been considered as anti-carcinogenic foods (Cramer et al., 2001). This study estimated the women consumption of carrots and tomatoes of 549 ovarian cancer and 516 control cases. They found that the consumption of such foods that have higher level of carotene and lycopene had reduced the risk of ovarian cancer. Gitenay et al. (2007) used epidemiologic studies and found that high consumption of tomatoes had a protective effect against prostate cancer. Furthermore, few studies have been investigated the effect of vegetables, especially carrots, on gene expression changes (Van Breda et al., 2005). They studied the effect of vegetables on the expression of genes involved in carcinogenic and anti-carcinogenic processes in the lungs of female mice using cDNA microarray technology. During their study, mice fed different diets: a control diet containing no vegetables, a diet containing mixture of vegetables and a diet containing carrots. They investigated the expression of 602 genes involved in pathways of anti–carcinogenesis. The results indicated that carrots were able to modulate most gene expressions and most of these effects occurred in processes that favored lung cancer risk prevention.

Although, humans usually use the vegetable oils in prolonged frying processes, there were no data regarding the possible effects of consuming diets containing repeatedly used frying oils (FO) on genetic alterations and sperm changes in male mice. Also, there were deficient data on the effect of FO diets on the expression of lipid metabolism–related protein genes. Therefore, the present study was aimed to investigate the DNA fragmentation, chromosome aberrations, sperm abnormalities and changes of mRNA expression of some lipid metabolism related–protein genes in mice fed diet containing thermally oxidized sunflower oil repeatedly used for frying process (FO). Also, some vegetables such as tomatoes and carrots were evaluated for their protecting ability against potential hazards caused by the thermally oxidized oil rich foods.

2. Materials and Methods

2.1 Animals: Forty eight adult male Swiss mice, weighting about 25 gm, were obtained from the Animal House Lab., National Research Centre, Giza, Egypt. Apparently healthy animals were randomly assigned into six groups (8 mice each) and housed in stainless steel wire mesh cages on a bedding of wood chips. They were kept in an ambient temperature of 25±3°C on a light/ dark cycle of 12/12 h and supplied with mice chew and fresh water *ad libitum*.

2.2 Diets: Six different diets were used in this study. The ingredients used were according to Phillip et al. (1993) to formulate these diets as shown in Table 1.

2.3 Preparation of frying oil: Sunflower oil was used for frying potatoes. The frying processes were repeated for about 15 times (20 min each). The frying oil was then placed in a glass bottle and mixed with the basal diets, as shown in Table 1.

2.4 Preparation of tomatoes and carrots: The Egyptian types of tomatoes and carrots were purchased from local market. These vegetables were sliced and then dehydrated, separately, by drying at 60°C for three days. Dried tomatoes and dried carrots were also ground, separately, using an electric mill. Five or ten percent of each protector (tomatoes or carrots) were added to the basal diet according to Phillip et al. (1993).

2.5 Experimental design: Mice were divided into six groups as follows: First group, as a negative control was fed basal diet containing the natural sunflower oil (NO) that was not used in frying process. The second group was fed the basal diet containing thermally oxidized sunflower oil repeatedly used for frying process (FO). The third and fourth groups were fed the basal diet containing FO plus dried ground tomatoes at levels of 5% (FOT1) or 10% (FOT2) of the diet. The fifth and sixth groups were fed the basal diet containing FO plus dried ground of carrots at levels of 5% (FOC1) or 10% (FOC2) of the diet. All mice groups were fed the corresponding previously mentioned diets for one month. Afterward, mice were sacrificed for genetic alteration and sperm abnormality studies.

Table 1: The composition of the different diets used.

Ingredient %	Types of diets					
	B+NO	B+FO	B+FOT1	B+FOT2	B+FOC1	B+FOC2
Casein	14	14	14	14	14	14
Sucrose	10	10	10	10	10	10
Salt mixture	5	5	5	5	5	5
Vitamin mix	1	1	1	1	1	1
Sunflower oil	4	-	-	-	-	-
Cellulose	5	5	5	5	5	5
FO	-	4	4	4	4	4
Tomato (5 %)	-	-	5	-	-	-
Tomato (10%)	-	-		10	-	-
Carrot (5 %)	-	-	-	-	5	-
Carrot (10 %)	-	-	-	-	-	10
Starch	61	61	61	61	61	61

B= Basal diet; NO= Normal sunflower oil (not used in frying processes); FO= Thermally oxidized sunflower oil (repeatedly used in frying processes); FOT1= FO + dried ground tomatoes at level 5%; FOT2= FO + dried ground tomatoes at level 10 %; FOC1= FO + dried ground carrots at level 5%; FOC2= FO + dried ground carrots at level 10%

2.6 DNA fragmentation:

Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 1.5 ml of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 min. The samples were centrifuged for 20 min at 10 000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 750 µl of 5% TCA, followed by incubation at 100°C for 20 min. Subsequently, to each sample 2 ml of DPA solution [200 mg DPA in 10 ml glacial acetic acid, 150 µl of sulfuric acid and 60 µl acetaldehyde was added and incubated at room temperature for 24 h (Gibb et al., 1997). The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA Fragmentation} = \frac{\text{OD of fragmented DNA (S)}}{\text{OD of fragmented DNA (S)} + \text{OD of intact DNA (P)}} \times 100$$

2.7 Cytogenetic analysis: For chromosomal analysis, both treated and control animals were sacrificed by cervical dislocation. Two hours before sacrifice, mice were injected with 5 mg colchicine /kg. b.w. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of **Preston et al. (1987)**. Fifty metaphase spreads per animals were analyzed, for scoring the different types of chromosomal aberrations.

2.8 Sperm analysis: For sperm-shape analysis, the epididimus excised and minced in about 8 ml of physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (aqueous), according to the methods of Wyrobek et al. (1983), Farag et al. (2002) and Hana et al. (2008). At least 2500 sperms per group were assessed for morphological abnormalities. Epididymal sperm count was also determined by hemocytometer.

2.9 Semi-quantitative RT-PCR:

The RT-PCR assay was conducted to verify the expression of fried oil-diet on the mRNA expression of the lipid metabolism related-protein genes: retinoid binding protein (RBP), heart fatty acid binding protein (H-FABP), and cutaneous fatty acid binding protein (C-FABP), in the liver tissues. The effect of tomatoes and carrots on the expression of these genes was also examined using the following steps:

2.9.1 RNA extraction: Immediately after animal sacrifice, liver tissues were frozen in liquid nitrogen and stored at −80 °C prior to extraction. Total RNA was extracted from 50 to 100 µg of each tissue by the standard TRIzol extraction method (Invitrogen, Paisley, UK). The solution of extracted RNA was recovered in 100 µl molecular biology grade water. Then, the total RNA samples were pretreated using DNA-free™ DNase treatment to remove any possible genomic DNA contamination. These steps were performed according to manufacturer's protocol (Ambion, Austin, TX, USA).

2.9.2 Reverse transcription: The complete Poly(A)$^+$ RNA isolated from the mice samples was reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo (dT) primer. The composition of the reaction mixture, termed as master mix (MM), consisted of 50 mM $MgCl_2$, 200 U/ µl reverse transcriptase (RNase H free), 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, and 50 µM of oligo (dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards, the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through polymerase chain reaction (PCR) (Brun et al., 2006; Khalil et al., 2009).

2.9.3 Polymerase chain reaction (PCR): The first strand cDNA from different mice samples was used as templates

for RT-PCR with a pair of specific primers. The sequences of specific primers and product sizes are listed in Table (2). The reaction mixture for RT-PCR in a total volume of 20 µl was consisted of 10 mM dNTP's, 50 mM MgCl2, 1 U/ µl taq polymerase,10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3), and autoclaved water. The PCR cycling parameters were one cycle at 95 °C for 4 min, 50 cycles at 94 °C for 30 s, 55 to 60 °C for 30 s, 72 °C for 60 s, and a final cycle at 72 °C for 7 min. The PCR products were then loaded onto 2.0 % agarose gel, with PCR products derived from β-actin of the different mice samples (Kronmiller et al., 1995, Khalil et al., 2009).

2.10 Statistical Analysis: Data for cytogenetic analysis were statistically analyzed by ANOVA. Wilcoxon′ s Signed – rank sum test was used for the statistical analysis of the sperm data. Furthermore, gene expression data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System (SAS, 1982) followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significant were based on probability of $P < 0.05$.

Table 2: Primers and reaction parameters in RT-PCR

Target cDNA	Primer name	Primer sequence (5'–3')	Annealing temperature (°C)	PCR product size (bp)
β-Actin	Act-F	CCC CAT CGA GCA CGG TAT TG	57	189
	Act-R	ATG GCG GGG GTG TTG AAG GTC		
RBP	RBP -F	GC AAG GCT CGT TTC TCT GG	55	178
	RBP -R	GAC TCG TCC CTT GGC TGT AG		
H-FABP	H-FABP -F	CTA GCA TGA GGG AAG CAA GG	55	138
	H-FABP -R	TGC TTC ATC CAG ACA AGT GG		
C-FABP	C-FABP-F	GGG CTG GCT CTT AGG AAG AT	60	100
	C-FABP-R	AAA ACA CGG TCG TCT TCA CC		

3. Results

3.1 DNA fragmentation

As shown in Table 3, the rates of DNA fragmentations were significantly higher (P< 0.001) in animals fed diet containing FO than those in control group. On the other hand, the rates of DNA fragmentations decreased significantly (P< 0.05 or P< 0.01) in animals fed diets containing tomatoes (FOT1 or FOT2) compared with those observed in FO group, in which FOT2 group had the lowest rate of DNA fragmentations. The rates of DNA fragmentations also decreased in animals fed diets containing carrots (FOC1 or FOC2) than those of FO group, however, these decreases were not statistically significant.

Table 3: Rates of DNA fragmentation in male mice fed different diets.

Treatment	DNA fragmentation	
	Rang	Mean± S.E
NO	15.2 - 20.1	17.6 ± 2.4^{c}
FO	37.9- 38.2	38.3 ± 0.1^{a}
FOT1	24.1-30.3	27.2 ± 3.1^{b}
FOT2	18.6- 29.6	23.4 ± 3.2^{bc}
FOC1	33.4 - 37.5	35.5 ± 2.0^{ab}
FOC2	32.7 - 36.0	34.4 ± 1.6^{ab}

[a,b,c,]: Means followed by different superscripts, within columns, differ significantly ($P< 0.05$), NO= Normal oil, FO= Thermally oxidized oil (repeatedly used in frying processes), FOT_1 = FO + dried tomato at level 5%, FOT_2 = FO + dried tomato at level 10%, FOC_1 = FO + dried carrot at level 5%, FOC_2 = FO + dried carrot at level 10%.

3.2 Chromosome examination

Examination the mice chromosomes showed structural aberrations (Table 4), however, numerical aberrations were absent. The structural aberrations of the chromosomes included chromatid gaps, deletions, chromatid breaks, centromeric fusions (C.F), end to end association (E.E) and rings. The mice fed FO diet had higher frequencies of chromosomal aberrations than the control group. Deletions were more frequent than other types of chromosome aberrations. Statistical analysis showed that there were highly significant (p< 0.01) differences between FO and NO groups in the frequencies of total structural aberrations, especially deletions.

The animal fed FO diet plus low level (5%) of tomato (FOT1) had decreased in the frequencies of gaps, deletions, C.F, E.E and total structural aberrations. These decreases were highly significant (p< 0.01) for the frequencies of the deletions and significant (P< 0.05) for the frequencies of the total structural aberrations (Table 4). The frequencies of chromatid breaks were similar in the two mentioned groups. However, the frequencies of rings were non-significantly decreased in the FOT1 group compared to those found in the FO group. Regarding to high concentration of tomato, FOT2 group had the lowest percentages of all structural aberrations. The only exception to this percentage was in the frequency of chromatid breaks which was similar in the FO group. Statistical analysis showed that there were significant

(P< 0.05) differences of each of deletions and total structural aberrations between FO and FOT2 groups (Table 4).

On the other hand, the frequencies of chromosome aberrations were lower (except for chromatid gaps and rings) in the animals fed diet with low level of carrots (FOC1) compared with those of the FO group (Table 4). Statistical analysis showed significant differences (P< 0.05) for the frequencies of deletions and total structural aberrations between FOC1 and FO groups. However, the frequencies of the chromatid gaps and the rings were raised, non-significantly, in the FOC1 group compared with the FO group. Also the frequencies of chromosome aberrations decreased (except for the rings) in animals fed diet with high level of carrots (FOC2) than those of the FO group. Statistical analysis showed that there were significant (P< 0.05) differences in the frequencies of deletions and total structural aberrations between these two groups. However, the ring aberrations were raised, non-significantly, in the FOC2 group compared to those of the FO group (Table 4).

3.3 Sperm examination

3.3.1 Sperm-shape analysis: Sperm examination showed that the abnormalities of sperms (head and tail) were more frequent in mice fed FO diet than those of control group (Table 5). Statistical analysis showed that the differences of the frequencies of head abnormalities (such as amorphous, without look, banana shape and big shape), tail abnormalities and total sperm abnormalities (head + tail) were significant (P< 0.05 or P< 0.01) between FO and control groups. However, the sperm abnormalities in head and tail decreased in animals fed FOT1 and FOT2 diets compared to those of the FO group. These decreases were significant (P< 0.01 or P< 0.05) in the frequencies of banana shape, big shape, tail and total abnormalities (Table 5). The frequencies of sperm abnormalities (banana shape, big shape and total abnormalities) were significantly lower in animals fed FOC1 diet compared to those fed FO diet. Also, the animals fed FOC2 diet had significantly decreases in all sperm abnormalities (amorphous, banana shape, two head and total head abnormalities) compared with that fed FO diet (Table 5).

3.3.2 Sperm counts: Sperm counts decreased significantly (P < 0.01) in animals fed FO diet than those of the control group. In contrast, sperm counts significantly increased (P< 0.05) in animals fed FOT1 diet compared to those of the FO group. The animals fed FOT2 diet had increases in sperm counts than those fed FO diet. However, these increases were not statistically significant. Sperm counts were similar in the FOC1 and FO groups. While, the animal fed FOC2 diet had significant increases (P< 0.05) in sperm counts compared to those fed FO diet.

3.4 Gene expression Patterns

The gene transcripts (mRNAs) of the three genes RBP, H-FABP and C-FABP were successfully detected in all liver tissues within all treated groups (Fig. 1, 3 and 5). The gene expression was normalized with the expression values of the β-Actin gene. The results revealed that RBP, H-FABP, and C-FABP mRNA expression in the liver tissues of the group fed the FO diet was significantly higher (P < 0.0001) than the control group (Figures 2, 4 and 6). On the other hand, tomato treatment was able to inhibit the up-regulation of the gene expression occurred by FO exposure. Where, the mRNA expression of RBP, H-FABP and C-FABP genes in the liver of the FO group was significantly higher (P < 0.001 or P < 0.0001) than those occurred in the FO plus tomato groups (FOT1 or FOT2), respectively (Figures 2, 4 and 6).

Regarding to carrots treatment, the results revealed that the expression of the RBP gene was significantly higher (P < 0.05) in the liver tissues of the FO group compared to those observed in the FO plus carrot groups (FO-C1 or FO-C2) (Figures 1 and 2). The expression levels of the H-FABP and the C-FABP genes were significantly higher (P < 0.05) in the liver of the FO group compared to those found in the FO-C2 group. However, the expression levels of the H-FABP and the C-FABP genes were somewhat higher (P = 0.061) in the liver of the FO group than those detected in the FO-C1 group.

4. Discussion

This study indicated that FO food has a mutagenic effect on the genomic materials of male mice. Mice fed FO diet had more frequent of DNA fragmentation and structural chromosome aberrations than those fed NO diet. This mutagenic effect may be due to the formation of oxidative compounds of lipidperoxid or heterocyclic aromatic amines (HAAs), that have been found to be mutagenic or carcinogenic (Hamilton et al., 1997; Starvic et al., 1997; Raloff, 1999; Coultate, 2002; Guardiola et al., 2008). Lipidperoxid compounds may be associated with generation of free radicals causing DNA fragmentation or DNA mutation (Lampe, 1999; Coultate, 2002; Nicolle et al., 2003; Guardiola, et al., 2008). Also the mutagenic and possibly carcinogenic products of HAAs are metabolized and activated by enzymes of cytochrome system–mediated N-hydroxylation to a number of hydroxylated metabolites which react with cellular DNA to induce fragmentations or mutations (Davis & Snydermine, 1995; Schut et al., 1997; Lampe, 1999; Nicolle et al., 2003; Jackson & Pereira-Smith, 2006; Devaraj et al., 2008). Hydroxyl free radicals are generated due to the cardiotoxic effect of HAAs and

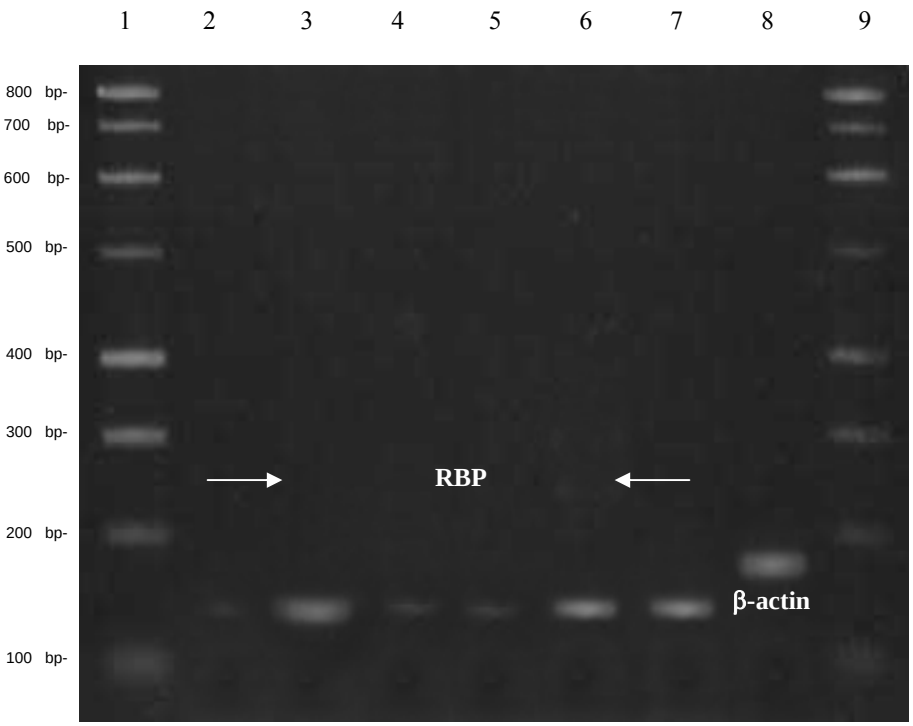

Fig. 1: Semi-quantitative RT-PCR analysis of RBP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10% of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity RBP gene and β-actin gene.

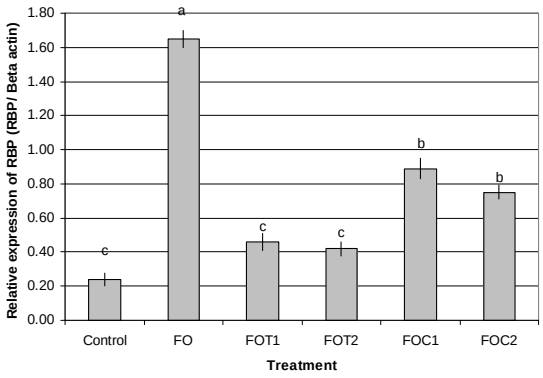

Fig. 2: RBP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

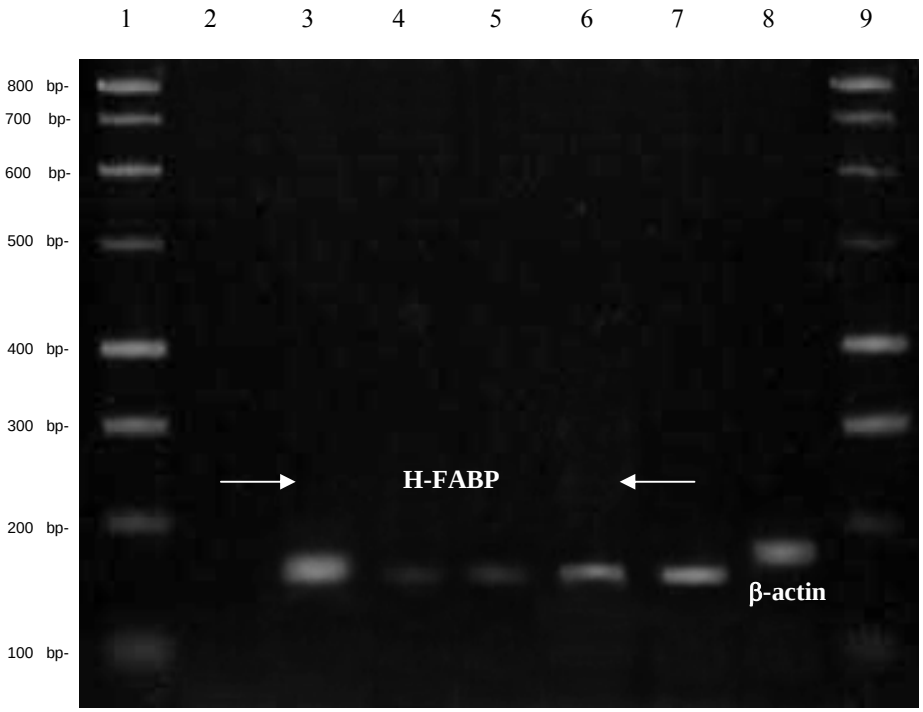

Fig. 3: Semi-quantitative RT-PCR analysis of H-FABP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10% of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity H-FABP gene and β-actin gene.

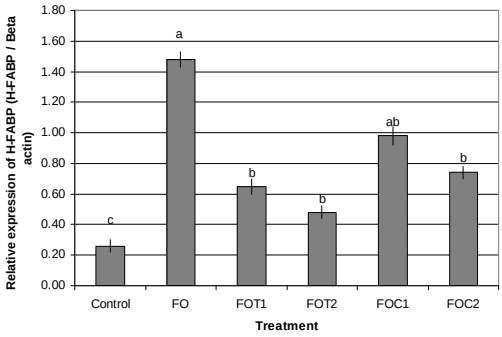

Fig. 4: H-FABP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

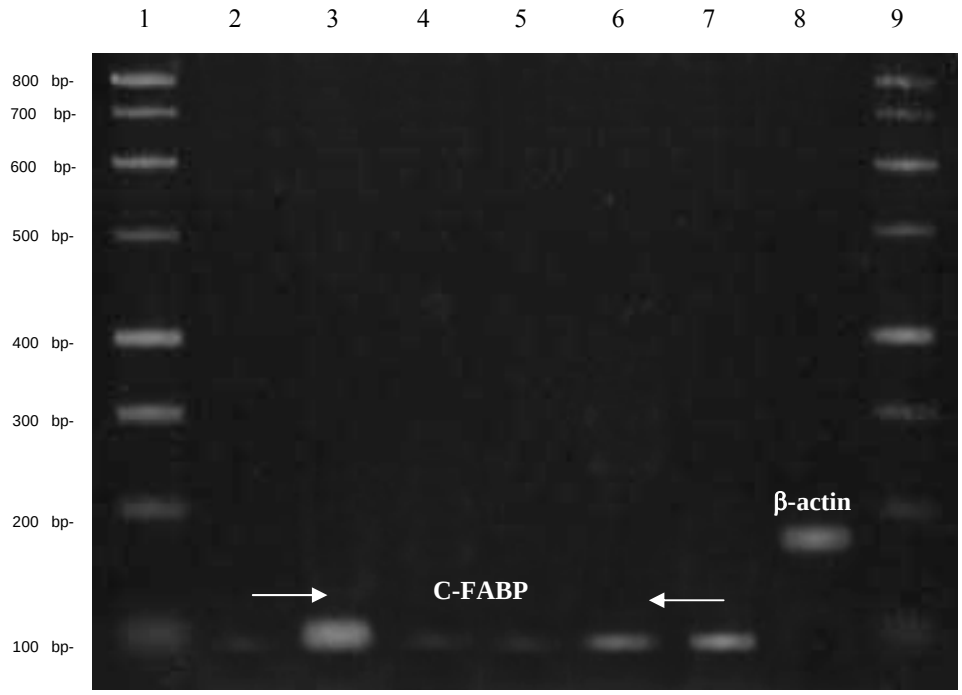

Fig. 5: Semi-quantitative RT-PCR analysis of C-FABP- and β-actin-mRNAs in liver tissues collected from female albino mice fed on standard diet combined with FO and T or C. Lanes 1 and 9 represent DNA ladder. Lane 2 represents liver samples exposed to standard diet mixed with NO. Lane 3 represents liver samples exposed to standard diet mixed with FO. Lane 4 represents liver samples exposed to standard diet mixed with FO and 5% of T (FOT1). Lane 5 represents liver samples exposed to standard diet mixed with FO and 10%of (FOT2). Lane 6 represents liver samples exposed to standard diet mixed with FO and 5% of C (FOC1). Lane 7 represents liver samples exposed to standard diet mixed with FO and 10% of C (FOC2). Lanes 8 represents mRNA of β-actin gene. The RNA recovery rate was estimated as the ratio between the intensity C-FABP gene and β-actin gene.

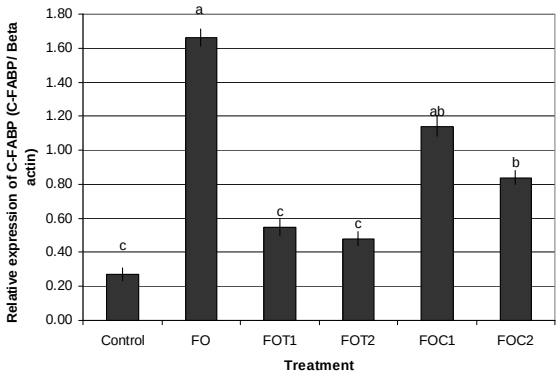

Fig. 6: C-FABP-mRNA expression in the mice liver determined by semi-quantitative RT-PCR. Within each column means superscripts with different letters are significantly different ($P \leq 0.05$).

Table 4: Chromosome aberrations (Mean percentages± SEM) in male mice fed different diets.

Treatment	Chromosome abnormalities						Total structural chromosome aberrations
	Gaps	Deletions	Breaks	Centric Fusions	End to End association	Rings	
NO	1.7±0.3^{A}	2.0±0.0^{A}	0.3±0.3^{A}	0.7±0.3^{A}	0.1±0.0^{AB}	0.0±0.0^{A}	5.6±0.3^{A}
FO	2.7±0.5^{A}	5.5±0.3^{C}	1.0±0.0^{A}	1.5±0.3^{A}	1.8±0.3^{B}	0.1±0.4^{AB}	13.5±0.6^{C}
FOT1	2.7±0.4^{A}	2. 3±0.3^{A}	1.0±1.0^{A}	0.7±0.3^{A}	1.7±0.3^{B}	1.6±0.3^{B}	10.0±0.6 ^{B}
FOT2	2.0±0.0^{A}	3.0±0.6^{AB}	1.0±0.6^{A}	0.3±0.3^{A}	1.3±0.3^{AB}	0.0±0.0^{A}	7.6±1.3^{AB}
FOC1	3.0±1.0^{A}	3.5±0.5^{B}	0.5 ±0.5^{A}	0.5±0.5^{A}	0.5±0.5^{A}	1.0±0.0^{AB}	9.0±0.0^{B}
FOC2	2.0±0. 6^{A}	3. 0±0.0^{AB}	0.0±0.0^{A}	0.3±0.3^{A}	1.6±0.3^{B}	1.0±0.6^{AB}	8.0±1.0^{AB}

^{A,B,C}: Means followed by different superscripts, within columns, differ significantly (P< 0.05), NO= Normal oil (not used in frying processes), FO= Thermally oxidized oil (repeatedly used in frying processes), FOT_1 = FO + dried tomato at level 5%, FOT_2 = FO + dried tomato at level 10%, FOC_1 = FO + dried carrot at level 5%, FOC_2 = FO + dried carrot at level 10%.

Table 5: Sperm abnormalities in male mice fed different diets.

Groups	Types of sperm head abnormalities						Total tail	Total abnormal sperms (head and tail)	(Sperm count x 100)
	Amorphous	Without hook	Banana shape	Two head	Big shape	Total head			
NO	12.0±1.0^{A}	4.5±0.5^{A}	0.0±0.0^{A}	0.0±0.0^{A}	2.0±1.0^{A}	18.5±0.5^{A}	11.5±1.5^{A}	30.0±1.0^{A}	23.6±0.4^{A}
FO	18.8±0.9^{B}	7.5±0.9^{B}	3.5±0.3^{C}	0.3±0.3^{A}	8.5±0.6^{C}	38.5±1. 9^{C}	19.3±0.8^{C}	57.8±1.7^{C}	16.5±0.8^{C}
FOT1	17.5±1.0^{B}	6.3±0.5^{AB}	1.0±0.4^{A}	0.3±0.3^{A}	1.5±0.3^{A}	26.5±1.4^{B}	15.3±1.4^{B}	41.8±1.8^{B}	19.2±1.2A^{B}
FOT2	11.0±1.1^{A}	6.3±0.8^{AB}	0.5±0.3^{A}	0.3±0.3^{A}	6.0±0.8^{B}	24.0±1.1^{B}	14.8±0.6^{AB}	38.8±1.5^{B}	18.4±0.8^{BC}
FOC1	17.0±0.9^{B}	7.0±0.6^{B}	0.3±0.3^{A}	0.0±0.0^{A}	0.3±0.3^{A}	24. 5±1.4^{B}	14.8±0.3^{AB}	39.3±1.3^{B}	16.5±0.6^{C}
FOC2	13.5±1. 0^{A}	6.5±0.3^{AB}	2 .3±0.3^{B}	1.0±0.0^{B}	0.3± 0.3^{A}	23.5±1. 3^{B}	14.0±1.3^{AB}	37.5±0.5^{B}	21.0±0.6^{B}

$^{A,B,C:}$ Means followed by different superscripts, within columns, differ significantly (p< 0.05), number of sperm examined per each animal was 2500, NO= Normal oil (not used in frying processes), FO= Thermally oxidized oil (repeatedly used in frying processes), FOT1=FO+dried tomato at level 5%, FOT2= FO+dried tomato at level 10%, FOC1= FO+dried carrot at level 5%, FOC2=FO+dried carrot at level 10%.

subsequent single and double strand scissor of DNA are produced (Davis et al., 1994; Davis & Syndrmine, 1995). In addition, N-guanine adduct was found in various organs of mice (Tada et al., 1994) and rats (Turesky et al., 1996) fed HAAs containing diets.

The induction of DNA fragmentation or DNA mutation leads to anomalies in the chromosome as a result of disturbance of DNA replication (Sinha & Prasad, 1990; Director et al., 1996; Breneman et al., 1996). To our knowledge, there were no data available regarding the cytogenetic effects of feeding fried oil (FO) to animals. However, in similar studies, Farag et al. (2002) found that prolonged feeding intake of meat (fish or beef) previously exposed to high heat treatment greater than cooking temperature caused induction of chromosome aberrations in rats. On the other hand, Khalil et al. (2009) reported that feeding FO diet induced abnormalities in the reproductive materials and liver enzyme activities of female mice. In a previous study, Breneman et al. (1996) showed a significant response of sister chromatid exchange (SCE) and micronuclei in mice fed MeIQX (2-amino-3, 4-dimethylimidazo (4, 5- f) quinoline) a kind of HAAS diet and suggested that the increase of micronuclei and SCE confirm that MeIQx and/or its metabolites reached peripheral lymphocytes of quantities sufficient to induce chromosome aberrations. Also, Director et al. (1996) reported a significant increase in whole-animal toxicity and the induction of SCE in mice fed PhIP [a kind of HAAs (2- amino-1methyl-6-Phenylimidazo (4, 5-b) Pyridine). Another study by Ohgaki et al. (1987) suggested that the induction of tumor in liver, lung and intestine, in female mice, after receiving MeIQX, may be an indication for observable chromosome damages. In the present study, it was noted that the increase of deletions, in mice bone marrow cells, was more than any other type of structural aberrations. This means that FO induced the chromatid deletion in mice bone marrow cells. Similarly, in another study, high proportion of chromatid deletion, than other structural ones, was observed in mice (Klimova et al., 1990; Khlusova et al., 1992) and in rat (Balabel, 2006) treated with adriamycin.

In the present study a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count occurred in animals fed FO diet. The consistently high incidence of DNA fragmentation and chromosome aberrations, as a result of potential formation of oxidative compounds of lipidperoxid or HAAs (or its metabolites), may be indicative of a general susceptibility of these animals, in the present study, for inducing DNA fragmentation and consequently chromosome derangements of gonadal cells causing abnormalities in sperm shape and reduction in sperm count (Nordenson et al., 1984; Devi and Reddy, 1985; Sailer, et al., 1995; Sakkas et al., 1999; Aitken & Krausz, 2000). Evidence that sperm shape abnormalities, induced by selected mutagens and carcinogens, have been reported by Bruce & Heddle (1979); Wyrobek et al., (1983) and Sinha & Prasad (1990). DNA fragmentation of human genomic of gonadal cells was due to an excessive production of reactive oxygen species (ROS) by oxidative stress and lead to damage in sperm morphology (Twigg et al., 1998; De Lamirande & Gagnon, 1999; Aitken & Krausz, 2000; Muratori et al., 2003). Our results were similar to those reported by Farag et al. (2002) who found a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count in rats fed diets containing meat (fish or beef) previously exposed to high heat treatment, greater than cooking temperature.

Some vegetables such as tomatoes and carrots were tested, in the present study, for their protective action against the mutagenic effects of FO diet. The current results showed that the animals fed diet containing FO plus low or high levels of tomatoes or carrots (FOT1, FOC1 or FOT2, FOC2) had lower DNA fragmentation, chromosome aberrations and morphological sperm abnormalities with an increase in sperm count. These results indicated that tomatoes and carrots have a protective role, in body cells, against the observed mutagenic effect of FO diet. There is an evidence indicated that some components of tomatoes (such as vitamin C, α–tocopherol and β–carotene) have anti-mutagenic effects against smoke induced-lymphocyte DNA damage of smokers (Duthie et al., 1996). Also, many components of tomatoes and carrots (such, polyphenols, various carotenoids and lycopene) were found to improve the immune system, scavenging of free radicals and reduce the production of DNA mutations in different mammalian cells that were previously exposed to variety of oxidative conditions (Nicolle et al., 2003; Van Breda et al., 2005; Srinivasan et al., 2007; Devaraj et al., 2008). Moreover, tomatoes and carrots (or its micro-constituents) were considered to be anti-carcinogenesis. They were able to reduce the risk of ovarian cancer, lung cancer and prostate cancer in human and mice (Cramer, 2001; Van Breda et al., 2005; Gitenay et al., 2007). To our knowledge, there are no published data regarding the effects of tomatoes and carrots on the cytogenetic level in animal. However, the reduction of DNA mutations induced by anti-mutagenic materials such tomatoes and carrots (or its micro-constituents) lead to a reduction in chromosome abnormalities as a result of a decrease in disturbance of DNA replication (Sugimura & Wakabayashi, 1990; Sinha & Prasad,

1990; Breneman et al., 1996; Starvic et al., 1997). Consequently this lead to a reduction in sperm abnormalities (Sailer et al., 1995; Sakkas et al., 1999; Aitken & Krausz, 2000; Farag et al., 2002). The mode of action of these vegetables (tomatoes or carrots) or their constituents against the mutagenic foods (FO) may be due to binding with the mutagens or inhibition of activation of cytochrome systems-mediated N-hydroxylation enzymes with a consequent reduction of genetic material (DNA or chromosomes) and sperm abnormalities (Aitken & Krausz, 2000; Farag et al., 2002; Wang et al., 2004; Devaraj et al., 2008).

Regarding the gene expression alterations our results indicated that RBP, H-FABP, and C-FABP mRNA expression in liver tissues of the group fed the basal diet mixed with FO was significantly higher than the controls. The up-regulation of the RBP mRNA in the present study was compatible with a previous study by Kushiro et al. (2007). Also, Afolabi et al. (2007) reported that a low fat diet reduces plasma RBP in men. Another study showed that there were positive correlations between plasma RBP and total cholesterol in both men and women (Qi et al., 2007). So, our findings of up-regulation of RBP mRNA expression by the FO-diet in male mice is similar to those changes in RBP seen in the above clinical nutrition trials.

In the current study, we also found that mRNA expression of H- and C- FABP was increased in liver tissue of mice fed the FO-diet. These results are similar to those reported by Margareto et al. (2001). They found that FABP mRNA expression increased in the tissues of rats fed a high-fat diet for 30 days. Moreover, Kushiro et al. (2007) found that C-FABP mRNA expression in peri-renal white adipose tissue is high in obese rats.

On the other hand, we found that expression of all genes tested was down-regulated in the liver tissues collected from mice fed the basal diet mixed with frying oil with low or high concentration of tomatoes (FOT1 or FOT2). The mRNA expression of RBP, H-FABP and C-FABP genes in the liver of FO was significantly higher than the FO-T1 and FO-T2 groups. No study was performed yet to investigate the effect of tomatoes or carrots on the expression of the lipid metabolism related-genes, RBP, H-FABP, and C-FABP. However, Bub et al. (2005) reported that tomato juice intake reduced lipid peroxidation in healthy volunteers carrying the R-allele of the PON1-192 genotype.

Concerning carrot treatments, the results revealed that the expression of RBP, H-FABP and C-FABP genes were also significantly down-regulated in the liver tissues of carrot fed groups (FOC1 and FOC2) compared to the FO group. In agreement with our results, Parveen et al. (2000) reported that carrot residue fibers significantly decreased the serum cholesterol, triglycerides, LDL-cholesterol and carcass fat in albino rats. They concluded that carrot residue fibers may be used for the dietary management and control of hyperlipidaemia. We can also suggest that the action mechanism of carrots on lipid metabolism may be attributed to the alteration in the expression of the lipid metabolism related- genes tested in the present study.

The effect of tomatoes or carrots on the expression of the lipid metabolism related-genes, RBP, H-FABP, and C-FABP, require further studies to understand how tomatoes and carrots affect gene expression of the lipid metabolism related-genes in liver and other organs.

Conclusion: the present study adds evidence for a link between prolonged feeding intake of FO diet and induced mutagenic effects in the genomic material and increase the sperm abnormalities in male mice. However, tomatoes and carrots proved to be good protective agents against hazards of such mutagenic foods.

5. References

Afolabi, P. R., Jahoor, F., Jackson, A. A., Stubbs, J., Johnstone, A. M., Faber, P., Lobley, G., Gibney, E., Elia, M. 2007. The effect of total starvation and very low energy diet in lean men on kinetics of whole body protein and five hepatic secretory proteins. *American Journal of Physiolog. Endocrinology and Metabolism* 293: 1580-1589.

Aitken, R. J., Krausz, C. 2000. Oxidative stress, DNA damage and Y chromosome. *Reproduction*, 122: 497-506.

Allard, J. P., Royall, D., Kurian, R., Muggli, R., Jeejeebhoy, K. N. 1994. Effects of β – carotene supplementation on lipid peroxidation in humans. *American Journal of Clinical Nutrition* 59: 884-890.

Balabel, E. A. M. A. 2006. The protective role of royal jelly against chromosomal and DNA changes induced in rats after treatment with adriamycin and cobalt gamma radiation. *Master Thesis. Department of Zoology, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt.*

Breneman, J. W., Briner, J. F., Ramsey, M. J., Director, A., Tucker, J. D. 1996. Cytogenetic results from a chronic feeding study of Me IQx in mice. *Food and Chemical Toxicology* 34: 717-724.

Brown, K. M., Morrice, P. C., Duthie, G. G. 1994. Vitamine E supplementation suppresses indexes of lipid peroxidation and platelet counts in blood of smokers and nonsmokers but plasma lipoprotein concentrations remain unchanged. *American Journal of Clinical Nutrition* 60: 383-387.

Bruce, W. R., Heddle, J. A. 1979. The mutagenic activity of 61 agents as determined by the micronucleus, salmonella and sperm abnormality assay. *Can J Genet Cytol* 23: 319- 333.

Brun, M. E., Gasca, S., Girard, C., Bouton, K., De Massy, B., De Sario, A. 2006. Characterization and expression analysis during embryo development of the mouse ortholog of MLL3. *Gene* 371: 25–33.

Bub, A., Barth, S. W., Watzl, B., Briviba, K., Rechkemmer, G. 2005. Paraoxonase 1 Q192R (PON1-192) polymorphism is associated with reduced lipid peroxidation in healthy young man on a low- carotenoid diet supplemented with tomato juice. *Br J Nutr* 93: 291-297.

Coultate, T. P. 2002. Food: The chemistry of its components. The Royal Society of Chemistry. 4th edn., *Cambridge*, PP: 73-125.

Cramer, D. W., Kuper, H., Harlow, B. L., Titus-Ernstoff, L. 2001. Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenoposal women. *Int J Cancer* 94: 128-134.

Davis, C. D., Farb, A., Thorgeirsson, S. S., Virmani, R., Synderwine, E. G. 1994. Cardiotoxicity of heterocyclic amine food mutagens in cultured myocytes and in rats. *Toxicology and Applied Pharmacology* 124: 201- 211.

Davis, C. D., Snydermine, E. G. 1995. Protective effect of N-acetylcysteine against heterocyclic amine- induced cardiotoxicity in cultured myocytes and in rats. *Fd Chem Toxic* 33: 641-651.

De Lamirande, E., Gagnon, C. 1999. The dark and bright sides of reactive oxygen species on sperm function. In: Gagnon, C. (ed.). The male gamete: From Basic Science to Clinical Applications. Vienna, IL: *Cache River Press*; P.455-467.

Devaraj, S., Mathur, S., Basu, A., Aung, H. H., Vasu, V. T., Meyers, S., Jialal, I. 2008. A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. *Journal of the American College of Nutrition* 27: 267-273.

Devi, K. R., Reddy P. P. 1985. Bisazir induced cytogenetic damage and sperm head abnormalities in mice. *IRCS Med Sci* 13: 1245 -1246.

Director, A., Nath, J., Ramsey, M. J., Swiger, R. R., Tucker, J. D. 1996. Cytogenetic analysis of mice chronically fed the food mutagen 2- amino-1-methyl- 6- phenylimidazo (4, 5b) pyridine (PhIP). *Mutat Res* 359: 53-61.

Duthie, S. j., Ma, A., Collins, A. R. 1996. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res* 56: 1291-1295.

Farag, I. M., Abdou, H. S. A., Ayesh, A. M., Osfr, M. M. H. 2002. Chromosomal and sperm studies on the mutagenic effect of over heated meat and the protective role of green tea and ginseng on rats. *Al-Azhar Bull Sci* 13: 105-120.

Gibb, R. K., Taylor, D. D., Wan, T., Oconnor, D. M., Doering, D. L., Gercel-Taylor, C. 1997. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. *Gynecologic Oncology* 65: 13-22.

Gitenay, D., Lyan, B., Rambeau, M., Mazur, A., Rock, E. 2007. Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamine E deficient rats. *Eur J Nutr* 46: 468-475.

Gouveia De Souza, A., Oliveira Santos, J. C., Conceicao, M. M., Dantas Silva, M. C., Prasad, S. 2004. A thermo-analytic and kinetic study of sunflower oil. *Brazilian Journal of Chemical Engineering* 21(2): 265-273.

Guardiola, F., Ricard, B., Rafael, C., Alba, T., Tric, A. 2008. Determination of hydroperoxides in foods and biological samples by the ferrous oxidation- xylenol orange method: A review of the factors that influence the method's performance. *Analytical Biochemistry* 377: 1-15.

Hamilton, R. J., Kalu, C., Prisk, E., Padley, F. B., Pierce, H. 1997. Chemistry of free radicals in lipids. *Food Chemistry* 60: 193-199.

Hana, H. Y., Khalil, W. K. B., Elmakawy, A. I., Elmeeged, G. A., 2008. Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. *Journal of Steroid Biochemistry & Molecular Biology* 110: 284–294.

Jackson, J. G., Pereira- Smith, O. M. 2006. Primary compensatory role for RB family members at cell cycle gene promoters that are deacetylated and downregulated in doxorubicin- induced senescenceof breast cancer cells. *Molecular Cell Biology* 26: 2501- 2510.

Khalil, W. K. B., Abd El-Kader, H. A. M., Eshak, M. G., Farag, I. M., Ghanem, K. Z. 2009. Biological studies on the protective role of artichoke and green pepper against potential toxic effect of thermally oxidized oil in mice. *Arab J Biotech* 12: 27- 40.

Khlusova, M. I., Novitskii, V. V., Goldberg, E. D. 1992. The cytogenetic effects of antitumor preparations with different mechanisms of action. *Eksperimental'nain Klinicheskaia Farmakologiia* 55: 49-52.

Klimova, M. I., Bogdashin, I. V., Novitskii, V. V., Goldberg, E. D. 1990. Sensitivity of bone marrow cells damaged by antitumor drugs to cytotoxic action of effectors of a normal spleen. *Biulleten Eksperimental'noi Biologii I Meditsiny* 109: 60-62.

Kronmiller, J. E., Nguyen, T., Berndt, W., Wickson, A. 1995. Spatial and temporal distribution of Sonic

hedgehog mRNA in the embryonic mouse mandible by reverse transcription/polymerase chain reaction and in situ hybridization analysis. *Arch Oral Biol* 40: 831–838.

Kushiro, M., Takahashi, Y., Ide, T. 2007. Modulation of cutaneous fatty acid- binding protein mRNA expression in rat adipose tissues by hereditary obesity and dietary fats. *Journal of Oleo Science* 56: 533-541.

Lampe, J. W. 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *American Journal of Clinical Nutrition* 70: 475S-490S.

Margareto, J., Gomez- Ambrosi, J., Marti, A., Martinez, A. 2001. Time- dependent effects of a high- energy- yielding diet on the regulation of specific white adipose tissue genes. *Biochemical and Biophysical Research Communications* 283: 6-11.

Muratori, M., Maggi, M., Spinell, S., Filimberti, E., Forti, G., Baldi, E. 2003. Spontaneous DNA fragmentation in Swin- up selected human spermatozoa during long term incubation. *J Androl* 24: 253-262.

Nagao, M., Ushumia, T., Toyota, M., Inoue, R., Sugimura, T. 1997. Genetic changes induced by heterocyclic amines. *Mutat Res* 376: 161-167.

Nicolle, C., Cardinault, N., Aprikian, O., Busserolles, J., Grolier, P., Rock, E., Demigne, C., Mazur, A., Scalbert, A., Amouroux, P., Remesy, C. 2003. Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol - fed rat. *Eur J. Nutr* 24: 254-261.

Nordenson, L., Abramsson, I., Ducbek, M. 1984. Somatic chromosomal aberrations and male infertility. *Hum Hered*, 34: 240-245.

Ohgaki, H., Hasegawa, H., Suenaga, M., Takayama, S., Sugimura, T. 1987. Carcinogenecity in mice of a mutagenic compound (MeIQx) from cooked foods. *Carcinogenesis* 8:665-668.

Parveen, N., Akhtar, M. S., Abbas, N., Abid, A. R. 2000. Effects of carrot residue fibre on body weight gain and serum lipid fractions. *Int J Agri Biol* 2: 125-128.

Pavia, M. F., Gordon, M. H. 2005. Interaction of ferric ions with olive oil phenolic compounds. *Journal of Agricultural and Food Chemistry* 53: 2704-2709.

Phillip, G. R., Forrest, H., Nielsen, G. C., Fahey, J. R. 1993. AIN-93 purified diet for laboratory rodents: Final Report of American Institute of Nutrition Ad Hoc Writing Committee on The Reformation of The AIn- 76 A Rodent Diet. *J Nutr* 123: 1935-1951.

Preston, R. J., Dean, B. D., Galloway, S., Holden, H., Mcfee, A. F., Shelly, M. 1987. Mammalian in vivo cytogenic assay: Analysis of chromosome aberrations in bone marrow cells. *Mutat Res* 189: 157-165.

Qi, Q., Yu, Z.,Zhao, F., Huang, P., Hu, F. B., Franco, O. H., Wang, J., Li, H., Liu, Y., Lin. X. 2007. Elevated retinol- binding protein 4 levels are associated with metabolic syndrome in Chinese people. *The Journal of Clinical Endocrinology and Metabolism* 92: 4827-4834.

Raloff, J. 1999. New recipes for making serfously browned meats less of a cancer risk. *The Weekly Newsmagazine of Science* 155:17-24.

Sailer, B. L., Jost, L. K., Evenson, D. P. 1995. Mammalian sperm DNA susceptibility to in situ denaturation associated with the prescence of DNA strand breaks as measured by the terminal deoxynucleotidyl transferase assay. *J Androl* 16: 80-87.

Sakkas, D.; Mariethoz, E.; Manicardi, G.; Bizzaro, D.; Bianchi, P. G.; and Bianchi, U. (1999). Origin of DNA damage in ejaculated human spermatozoa. Rev. *Reprod.*, 4: 31-37.

Schut, H. A. J., Wang, C. L., Twining, L. M., Earle, K. M. 1997. Formation and persistence of DNA adducts of 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) in CDFI mice fed a high w-3 fatty acid diet. *Mutat Res* 378: 23-30.

Sinha, S. P., Prasad, V. 1990. Effect of dietary concentration of crude aflatoxin on meiotic chromosomes, sperm morphology and sperm count in mice, Mus musculus. *Proc Indian Natn Sci Acad* 56: 269-276.

Srinivasan, M., Sudheer, A., Menon, V. P. 2007. Ferulic acid: Therapeutic potential through its antioxidant property. *J Clin Biochem Nutr* 40: 92-100.

Starvic, B., Lau, B. P. Y., Matula, T. I., Klassen, R., Lewis, D., Downie, R. H. 1997. Mutagenic heterocyclic aromatic amines (HAAs) in processed food flavour samples. *Food and Chemical Toxicology* 35: 185-197.

Suganuma, H., Inakuma, T. 1998. Protective effect of dietary tomato against endothelial dysfunction in hypercholesterolemic mice. *Bioscience, Biotechnology, and Biochemistry* 63: 78-82.

Sugimura, T., Wakabayashi, K. 1990. Mutagens and carcinogens in the diet. In: Mutagens and carcinogens in the diet. Pariza, M. W.; Felton, J. S.; Aeschbacher, H. U. and Sato, S. (eds). *Wiley-liss, New York,* pp.1-18.

Tada, A. M., Ochiai, K., Wakabayashi, H., Sugimura, T. N., Nagao, M. 1994. Identification of N–(deoxyguanosin-8-yl)-2-amino-3,4-dimethyl imidazo (4,5-f) quinoline (dG-C8-MeIQ) as amajor adduct formed by MeIQ with nucleotides

in vitro and with DNA in vivo. *Carcinogenesis* 15: 1275- 1278.

Turesky, R. J., Markovic, J., Aeschlimann, J. M. 1996. Formation and differential removal of C-8 and N2- guanine adducts of the food carcinogen 2-amino-3-methylimidazo (4,5-f) quinochem. *Res Toxicol* 9: 397- 402.

Twigg, J. P., Fulton, N., Gomez E. 1998. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: Lipid peroxidation, DNA fragmentation and effectiveness of antioxidant. *Hum Reprod* 13: 1429-1436.

Van Breda, S.G., Van Agen, E., Van Sanden, S., Burzykowski, T., Kleinjans, J. C., Van Delft, J. H., 2005. Vegetables affect the expression of genes involved in carcinogenic and anticarcinogenic processes in the lungs of female C57BI/6 mice. *J Nutr* 135: 2546-2552.

Wang, S., Konorev, E. A., kotamraju, S., Joseph, J., Kalivendi, S., Kalyanaraman, B. 2004. Doxorubicin induces apotosis in normal and tumor cells via distinctly different mechanisms. Intermediacy of H(2)O(2)- and p53- dependent pathways. *Journal of Biological Chemistry* 279: 25535- 25543.

Wyrobek, A. J., Gordon, L. A., Burhhart, J. G., Francis, M. W., Kapp, R. W.; Letz, G. Malling, H. V., Topham, J. C., Whorton, M. D. 1983. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. *Mutat Res* 115: 73-148.

Preparation and Characterization of Amine-Imine Derivatives Used in Organic Thin Film Transistor

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ABSTRACT: In this report, synthesis and characterization of Amine-imine derivatives of BIP and NIP are presented. Amine-imine derivatives have more delocalization molecular orbits having excitation spectra with red shift. Additionally, the different distribution of molecular energy levels for BIP and NIP causes the emission and absorption of different wavelengths. In this study, both BIP and the NIP were used as the organic thin film transistor active layer deposited on a silicon wafer substrate and the surface morphology, structure of permutation as well as carrier mobility rate were discussed. [Journal of American Science 2010;6(4):189-192]. (ISSN: 1545-1003).

Keywords: molecular orbits, carrier mobility, surface morphology

Introduction

Recently, the synthesis and characterization of organic complex used for thin film transistor have been developed impressively [1, 2]. In order to improve the surface morphology and structural permutation, synthesis modification producing a new structure can drive a new feature. However, a meaningful modification of organic semiconductor material must have strong supports by valuable mechanism and the application. We develop a couple of new functional OTFTs [3] by using of Amine-imine derivatives BIP and NIP.

Materials and Methods

Active layer as well as the electrode is deposited to substrate by using different mask for defining the pattern where the channel width (W) is 10mm and the channel length (L) is 50μm. The design is depicted in Fig. 1. However, in the evaporation process, mask and substrate may cause the shift of the size of the channel width and length. The accurate channel size can be confirmed by using the optical microscope (Optical Microscope, OM). In Fig. 2., NIP and BIP structures are depicted.

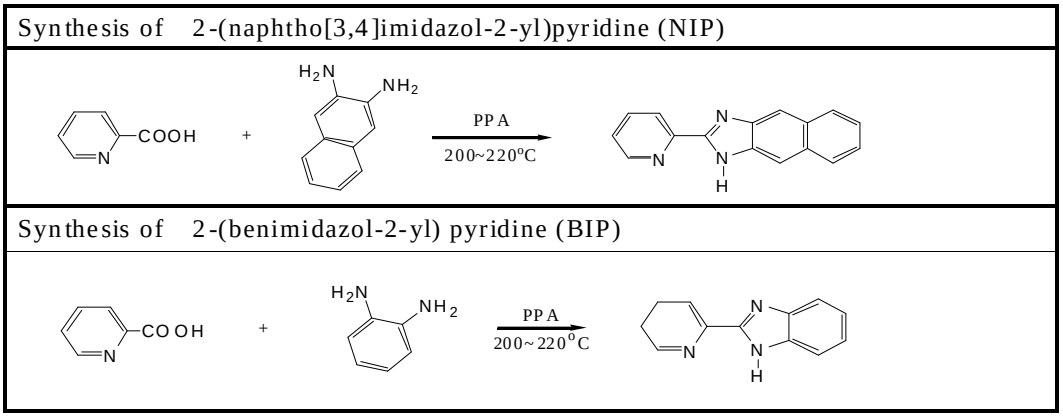


Fig. 2. Structures of NIP and BIP

Results and Discussion

The carrier displacement rate of organic semiconductor materials usually depends on the material purity and grain size. Since the surface is rough, each crystal growth will be interrupted. If the evaporation rate limited to 0.5 Å per second for deposition of film thickness of 100 nm, we can clearly observe the size of the grains and the surface roughness. Fig. 3 shows the SEM photos of NIP and BIP which was used as reference to pick up the optimized evaporation rate.

In this study, Amine-imine derivatives function as active material, the organic thin film transistor output characteristic curve (ID-VD) and the transduction curve (ID-VG) are depicted in Fig. 4. Amine-imine derivatives of BIP and NIP are basically p-type organic semiconductors. Due to intra-molecular Amine-imine hydrogen bonds tend to build their plane structure and associate with inter-molecular hydrogen bonds to turn into three-dimensional planar stack structure, inter-molecular π electrons and π orbital overlap caused by the effective intermolecular charge transfer therefore strengthen the carrier mobility. Since NIP carries one more benzene ring than BIP, it makes the carrier mobility about twice higher. The measured values of SS (Subthreshold Swing) are all in 10V/decade below and which means gate control for the channel current is significant.

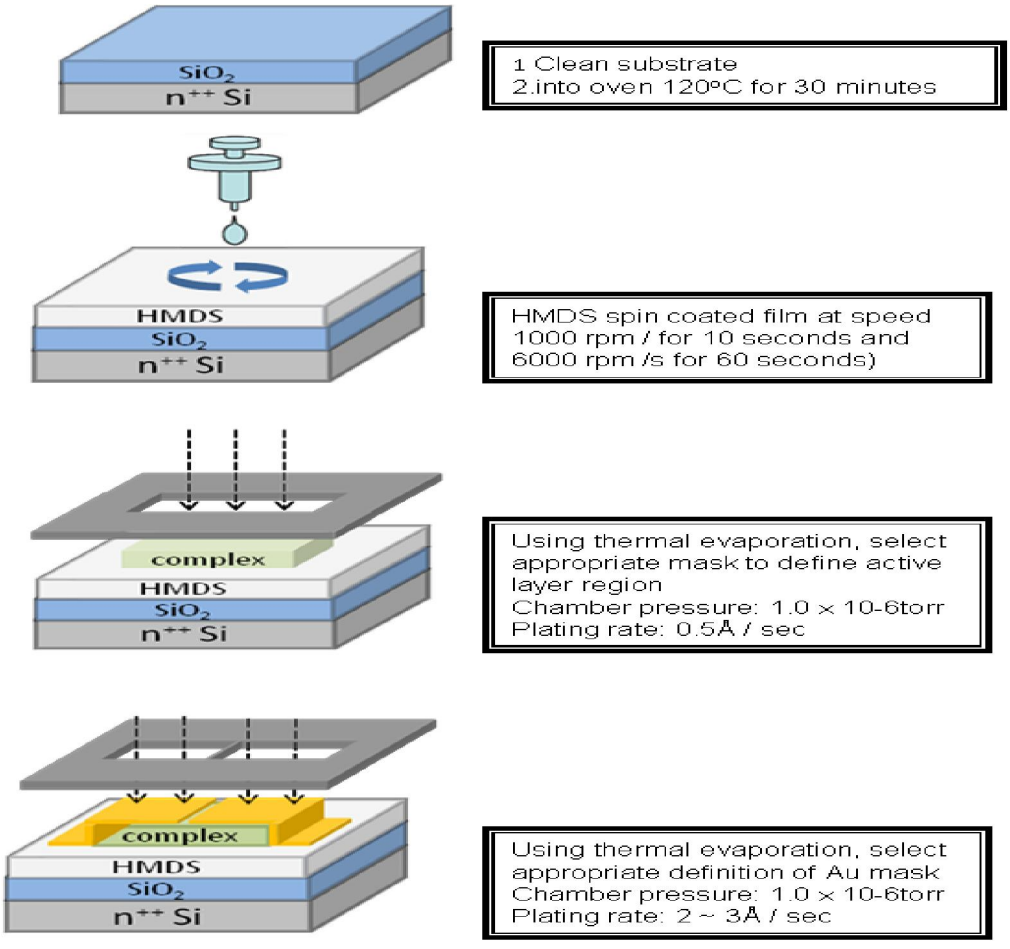
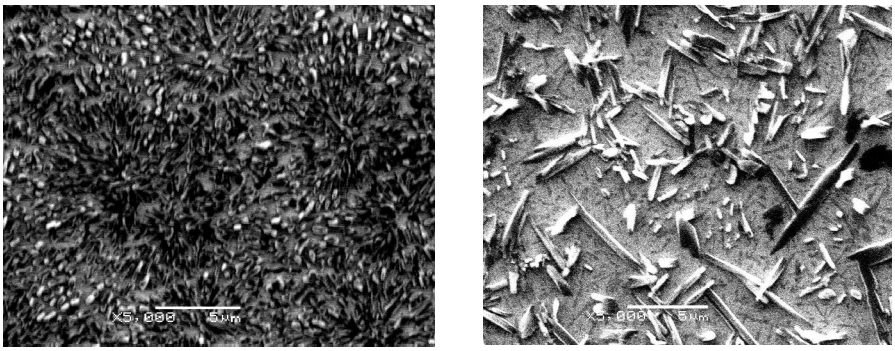


Fig. 2. Schematic drawing of the design of OTFT



(a) (b)

Fig. 3. SEM photos of (a) BIP and (NIP)

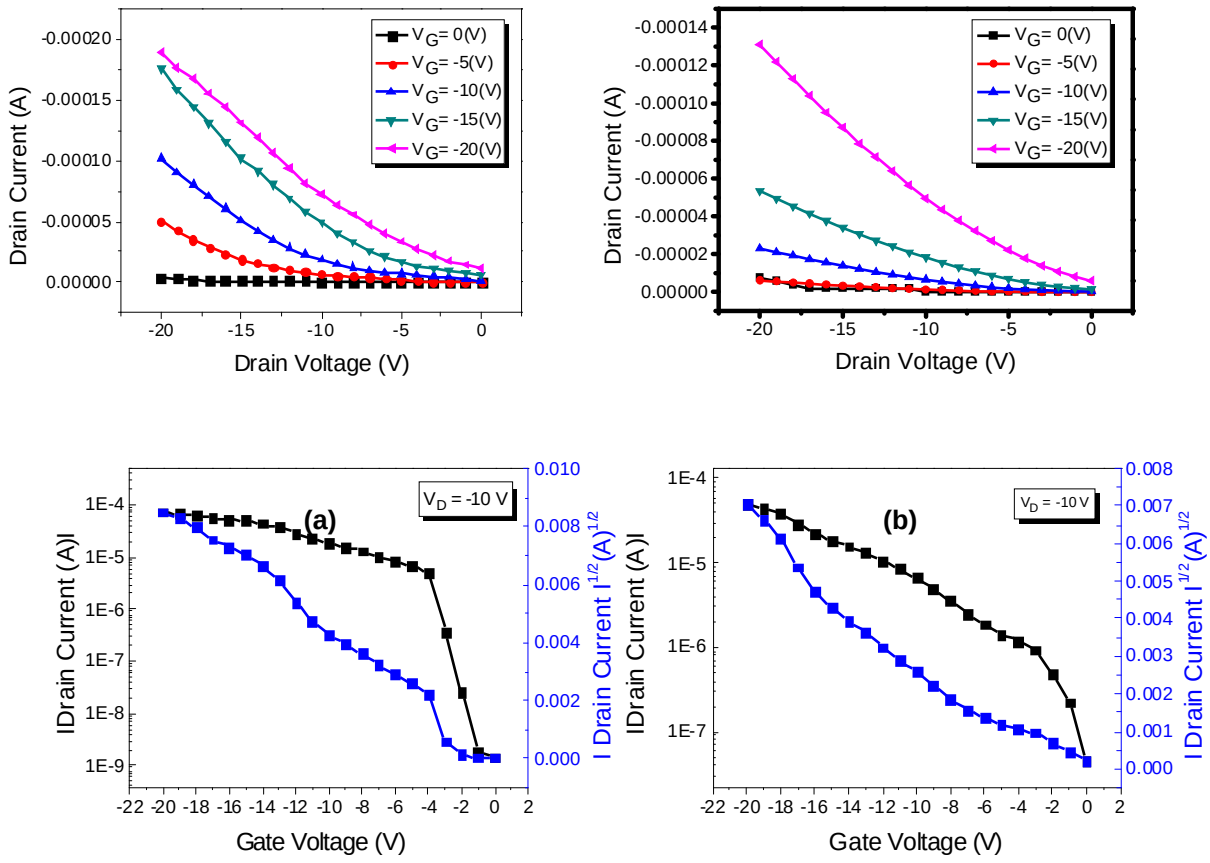


Fig. 4. (a) The OTFT ID-VD curve for BIP thin film as active layer (b) The OTFT ID-VD curve for NIP thin film as active layer (c) The OTFT ID-VG curve for BIP thin film as active layer (d) The OTFT ID-VG curve for NIP thin film as active layer

Table 1. Electrical characteristics under the atmosphere of the OTFT made by BIP and the NIP

Materials	μ_{FET} (cm^2/V_S)	V_{th} (V)	I_{ON}/I_{OFF} ratio	S.S (V/decade)
BIP	0.1173	-12	1.1×10^3	7.6525
NIP	0.2356	-10	2.3×10^4	5.0308

Conclusion

The hydrogen bonds in Amine-imine derivatives build three dimensional planar stack structures which therefore cause intermolecular π electronics and π orbital overlap to produce effective intermolecular charge transfer and strengthen the mobility rate followed enhancing the carriers' mobility. Comparatively, NIP has one more benzene ring than BIP so that to make the carrier mobility promotion from 0.1173 (cm^2/VS) to 0.2356 (cm^2/VS). Therefore, we can conclude that the structural variance of Amine-imine derivatives is the key to be responsible to the rate of carrier mobility in our designed OTFT and Electrical characteristics under the atmosphere of the OTFT made by BIP and the NIP is listed in Table 1.

2/2/2010

References

[1] Horowitz, G., Organic thin film transistors: From theory to real devices. MATERIALS RESEARCH SOCIETY, 2004. 19.

[2] J. Cornil, D.B., J.-P. Calbert , J.-L. Brédas Interchain Interactions in Organic -Conjugated Materials: Impact on Electronic Structure, Optical Response, and Charge Transport. Advanced Materials, 2001. 13(14): p. 1053-1067.

[3] Ingo Salzmann, S.D., Georg Heimel, Martin Oehzelt, Rolf Kniprath, Robert L. Johnson, Ju¨rgen P. Rabe, and Norbert Koch, Tuning the Ionization Energy of Organic Semiconductor Films: The Role ofIntramolecular Polar Bonds. Journal of the American Chemical Society, 2008. 130(39): p. 12870-12871.

Cyanobacteria of a Tropical Lagoon, Nigeria.

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Abstract: Investigations for the first time into the blue green algae of Lekki lagoon were carried out for 24 months (June 2003- May 2005) at monthly intervals using standard plankton net of mesh size 55µm. One hundred and seventy nine species belonging to thirty genera were observed. The filamentous blue green algae *Oscillatoria* formed the most abundant genus making up twenty three species followed by *Phormidium* eighteen species. *Anabaena* and *Chroococcus* recorded thirteen species each while the genera, *Gleocapsa, Merismopedia* and *Microcystis* recorded ten, eight and twelve species respectively. Only one genus each of *Cyanosarcina, Calothrix* and *Scytonema* were encountered. Bloom forming species identified were *Microcystis aeruginosa, M. flos-aquae, M. wesenbergii* and *Anabaena flos-aquae.* In this study, thirty-nine new species were recorded for Lagos lagoon complex in which Lekki lagoon is one of it while *Cyanosarcina hueberliorum* is new record for Nigeria. [Journal of American Science 2010;6(4):193-199]. (ISSN: 1545-1003).

Keywords: Cyanophytes, tropical, bloom, Lagos lagoon complex

Introduction

The coastline of South Western Nigeria is a meandering network of lagoons and creeks of which Lagos lagoon with an area of 208sqkm is the largest (Nwankwo 1989).The geography and hydrology of various parts of Lagos lagoon complex in which Lekki lagoon is one it have been described by several workers. These include Lekki lagoon (Ikusemiju 1973); Lagos lagoon (Hill and Webb 1958) and harbour (Olaniyan 1957). Checklists of planktonic algae in some parts of Nigeria have been documented by different workers. For instance in the North, Holden and Green (1960) studied the phytoplankton of River Sokoto while Khan and Agugo (1990) studied Kongiri dam, Jos mine lakes was studied by Anadu et al. (1990).

In Southern region, studies include Opute (1990,1991,1992) who studied Warri Forcados estuary phytoplankton, New Calabar river by Nwadiaro and Ezefili (1986). Biswas (1984, 1992) had report for eastern region while western region reports include that of Imevbore (1968) on Eleiyele reservoir, Egborge and Sagey (1979) on Ibadan freshwater ecosystem . Nwankwo (1988) studied the planktonic algae of Lagos lagoon, Nwankwo (1993) reported eight cyanobacteria bloom species of coastal waters in South Western Nigeria excluding Lekki lagoon, Nwankwo (1997) reported dinoflagellates list of Lagos lagoon. Adesalu and Nwankwo (2005, 2009) reported the diatoms of Olero creek and Lekki lagoon respectively, Wujek et al. (2003) studied the chrysophytes of Lekki lagoon while Kadiri (1989, 1993, 1999, 2000) reported the rich flora of *Micrasterias*, desmids, algae composition and euglenoids of Ikpoba reservoir respectively.

Of the entire aforementioned checklist, none specifically reported the cyanobacteria checklist in Nigeria coastal waters. The present study was undertaken to investigate the composition of cyanobacteria species of Lekki lagoon for possible biological monitoring since the lagoon is a source of fish supply for people of South Western states and beyond.

Description of study area

Lekki lagoon (Fig.1) a large expanse of shallow freshwater extends between Lagos and Ogun states. It covers an area of about 247km^2. A greater part of the lagoon is shallow (<3.0 m), while some areas are up to 6.4m deep. It lies between longitudes $4^0 00'$ E and $4^0 12'$ E and latitude $6^0 25'$ N and $6^0 37'$ N. The lagoon is fed by river Oni in the north eastern part, while rivers Osun and Saga flow into the north western part. Two peaks of rainfall are associated with this lagoon, a major peak in July and a lesser peak in September. There are two peaks of sunshine hours which approximately correspond to the equinoxes. The mainstay of communities that live around this environment is artisanal fishing.

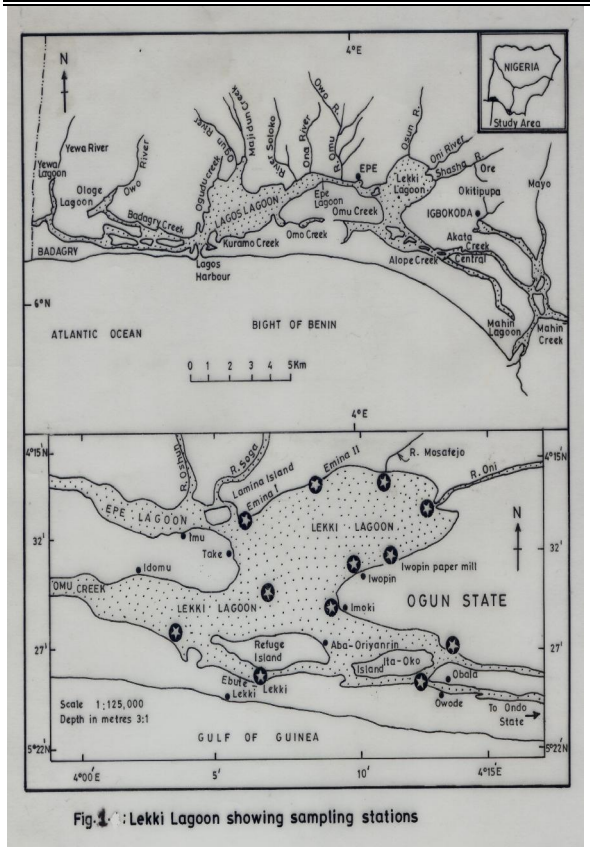

Fig. 1 : Lekki Lagoon showing sampling stations

TABLE 1 : AVERAGE DEPTH (M) AND GEOGRAPHICAL POSITION OF SAMPLING STATIONS

STATIONS	Average depth	Longitudes	Latitudes
Emina 1	2.77	$4^0$5.080E	$6^0$32.754N
Emina II	1.61	$4^0$7.511E	$6^0$34.07N
Entrance of River Mosafejo	1.51	$4^0$10.239E	$6^0$35.344N
Entrance of River Oni	1.80	$4^0$12.153E	$6^0$35.090N
Iwopin 1	1.80	$4^0$13.153E	$6^0$32.309N
Iwopin 11	2.69	$4^0$9.651E	$6^0$32.137N
Imoki	2.17	$4^0$10.048E	$6^0$31.253N
Ise 1	1.81	$4^0$13.413E	$6^0$26.833N
Ise 11	2.41	$4^0$9.788E	$6^0$26.181N
Ebute lekki	1.29	$4^0$5.353E	$6^0$26.685N
Entrance of Omu creek	1.88	$4^0$7.604E	$6^0$28.867N
Lagoon centre	2.23	$4^0$3.348E	$6^0$28.577N

Materials and methods

Collection of sample

Biological samples were collected monthly from twelve stations (Table 1) using Hydrobios plankton net of 55µm mesh size. For quantitative analysis 5litres of the water was concentrated. Biological samples were preserved in 4% unbuffered formalin. Identification was done using Olympus BX51 photomicroscope. Water samples were collected into clean plastic containers for chemical analysis while *in situ* measurements of temperature, transparency, pH and depth were made.

Physical and chemical analysis of water sample

The methods described by America Public Health Association (APHA 1998) were used for physical and chemical analysis. The air and surface water temperature were measured in situ with a simple mercury thermometer while the transparency was measured using a 20cm diameter Secchi disc. The depth was measured with a calibrated pole and the water pH determined using a Phillips pH meter (Model PW950). The chemical factors determined include Salinity, conductivity, dissolved oxygen (DO) and biological oxygen demand (BOD_5). Salinity was determined using the Silver Nitrate Chromate titration method as described by Barnes (1980) while Dissolved oxygen content was determined using a Griffin oxygen meter. Oxygen saturation was recorded in percentage. Biological Oxygen Demand is the measure of the amount of dissolved oxygen that could be depleted from the water body during natural biological assimilation or degradation of organic compounds by the organisms present especially bacteria. This was done after the dissolved oxygen had been measured using the standard method of biochemical consumption of oxygen in 5 days at 20^0C while conductivity was determined using the HANNA instrument (H18733), a wide range conductivity meter that has salinometer range in µS. Conductivity values were recorded as $mScm^{-1}$ at 25^oC (APHA 1998). The department The Federal Meteorological Department, Oshodi, Lagos kindly provided rainfall and sunshine hours data for the period of investigation (Table 2)

Nutrient determination

For nitrate determination, Hach Cadmium reduction method was used (APHA 1998). Phosphate-phosphorus is known to be important in a number of ways, one being that it facilitates the uptake of

nitrogen. It was determined by ascorbic acid method. The values obtained were recorded in milligrams per litre (mgl^{-1}) (APHA 1998) (Table 2)

Water chemistry

The physical and chemical characteristics of the study area are presented in Table 2.The mean pH of the water with a range of 7.41-7.46 indicated that the system is highly buffered. Conductivity which is the numerical expression of the ability of a solution to carry an electric current represents the total ions of water ranged between 0.47-0.56 μScm^{-1} the lowest value for phosphate-phosphorus, nitrate-nitrogen and sulphate were 2.42, 2.70 and 0.002 mgL^{-1}. Salinity recorded the least value of 0.40‰ while chloride ion had the highest value of 10.00 mgL^{-1}.

Table 2: Mean physico-chemical values for Lekki lagoon (concentrations in mg L^{-1})(Stations A-L).

STATIONS	A	B	C	D	E	F
Surface Water tempemperature (^{0}C)	30.54	30.73	30.55	30.61	30.40	30.31
Air tempemperature (^{0}C)	29.68	29.60	29.60	29.71	29.51	29.44
Transparency (cm)	5.74	7.38	11.33	9.25	11.67	10.29
Total suspended solids	9.07	8.56	9.16	8.45	9.28	9.78
pH	7.42	7.43	7.41	7.43	7.44	7.46
Salinity ‰	0.47	0.47	0.45	0.45	0.40	0.55
Phosphate-phosphorus	2.53	2.70	2.42	2.50	2.56	2.61
Nitrate-nitrogen	2.73	2.93	3.42	2.98	2.54	3.85
Chloride	9.23	9.67	9.59	10.00	9.88	9.52
Conductivity (μScm^{-1})	0.56	0.52	0.56	0.47	0.47	0.48
Dissolved Oxygen	4.15	3.46	4.21	4.10	4.16	4.25
Biological Oxygen demand	0.23	0.22	0.26	0.23	0.22	0.25
Chemical oxygen demand	0.36	0.30	0.26	0.25	0.24	0.29
Oil and grease	0.03	0.02	0.02	0.02	0.00	0.01
Turbidity (FTU)	8.59	9.25	10.00	7.46	9.57	8.87
Total dissolved solids	9.48	10.29	10.65	8.51	11.29	11.50
Sulphate	0.03	0.02	0.03	0.04	0.03	0.03
Ca	10.46	11.11	11.92	12.07	11.57	10.71
Fe	0.30	0.23	0.24	0.26	0.22	0.25
Pb	0.01	0.08	0.25	0.08	0.08	0.01
Hg	0.01	0.00	0.00	0.00	0.00	0.00
Cu	0.05	0.02	0.02	0.02	0.03	0.06
Ni	0.02	0.01	0.02	0.01	0.01	0.01
Zn	0.02	0.01	0.01	0.01	0.01	0.02

STATIONS	G	H	I	J	K	L
Surface Water tempemperature (^{0}C)	30.35	30.23	30.38	30.63	30.70	30.55
Air tempemperature (^{0}C)	29.55	29.08	29.29	28.90	29.27	29.21
Transparency (cm)	11.08	10.42	14.42	7.30	10.04	12.33
Total suspended solids	11.76	12.71	8.39	11.50	8.87	8.87
pH	7.44	7.46	7.44	7.42	7.37	7.38
Salinity ‰	0.47	0.44	0.45	0.44	0.50	0.47
Phosphate-phosphorus	2.47	2.49	2.96	2.53	2.54	2.44
Nitrate-nitrogen	3.44	3.25	2.75	3.31	2.70	3.97
Chloride	9.40	9.17	9.31	9.31	9.54	9.99
Conductivity (μScm^{-1})	0.49	0.52	0.46	0.53	0.62	0.56
Dissolved Oxygen	4.25	4.19	4.17	4.16	4.18	4.17
Biological Oxygen demand	0.29	0.30	0.31	0.28	0.26	0.24
Chemical oxygen demand	0.34	0.32	0.30	0.31	0.31	0.25
Oil and grease	0.01	0.01	0.01	0.01	0.01	0.01

Turbidity (FTU)	8.37	7.99	8.42	10.29	9.15	8.69
Total dissolved solids	9.87	9.95	10.31	10.58	10.81	10.29
Sulphate	0.02	0.02	0.05	0.04	0.06	0.03
Ca	11.04	10.96	10.70	10.21	11.40	11.52
Fe	0.21	0.23	0.25	0.28	0.30	0.55
Pb	0.02	0.01	0.00	0.00	0.04	0.02
Hg	0.00	0.00	0.00	0.00	0.02	0.01
Cu	0.01	0.01	0.01	0.00	0.02	0.04
Ni	0.01	0.01	0.01	0.01	0.01	0.01
Zn	0.01	0.01	0.01	0.01	0.01	0.01

Results

Cyanobacteria analysis

In this study, cyanobacteria genera are arranged alphabetically within families and the species in alphabetical order within genera (Table 3).

Table 3: Cyanobacteria checklist at Lekki lagoon, Nigeria.

Division : Cyanophyta

Class : Cyanophyceae

Order: Chroococcales

Family 1: Chroococcaceae

Chroococcus decorticans
C. dispersus (V.Keiss) Lemm.
C. limnecticus Lemm.
**C. limnecticus var. subsalsus* Lemm.
C. minor
*C. minutus (*Kutz.) Rabenh.
C. palidus Nageli
C. prescottii Dr. & Daily
C. turgidus (Kutz.) Lemm.
C. turicensis (Nag.) Hangirg
C. varius A. Braun
Chroococcus sp 1
Chroococcus sp 11
**Cyanosarcina huebeliorum* Komarek & Anagnostids
Dactylococcopsis raphidiodes Hansg.
D. smithii Chodat & Chodat
Dactylococcopsis sp.

Family 2: Merismopediaceae

Agmenellum quadriplicatum
Agmenellum sp.
Aphanocapsa delicatissima West & West
A. elaschista West & West
A. elachista var conferta West & West
**A. nubilum* Nygaard
A.pulverea
A. rivularis
Aphanocapsa sp.
**Aphanothece bullosa var major* Geitler
Aphanothece sp.
Merismopedia angularis Geitler
M. convoluta Breb.
M. elegans A.Br.
M. glauca (Ehr.) Nag.
M. major G.M.Smith
M. marsonii Lemm.
M. punctata Meyen.
M. tennuisima Lemm.

Family 3: Chaemasiphonaceae

Clastidium setigerum
Clastidium sp.

Family 4: Microscystaceae

**Gleocapsa alpicola* (Lyng.) Bornet
**G. arenaria* (Hass.) Rabenh.
G. biformis Novacek
G. compacta Kutz.
G. conglomerata Kutz.
**G. decorticans* (A.Br.) P.Richter
G. delicatissima
G. magma (Breb.) Kutz.
G. quarternata (Breb.) Kutz
Gleocapsa sp.
Gleothece heufleri
Gleothece linearis Nag
Gleothece sp.
Microcystis aeruginosa Kutz.
M. aeruginosa var elongata Rao,C.B
**M. aeruginosa var major* (Wittr.) Smith
M. elongata sp.nov.
**M. firma* (Kutz.) Dr. & Daily
M. flos-aquae (Wittr.) Kirchner
M. paludosus
**M. pulverea* (Wood) Forti
**M. ramosa* Bharadwaja
**M. robusta* (Clack) Nygaard
M. viridis (A. Br.) Lemm.
M wesenbergii Kosinskaja

Order 2: Nostocales

Family: Nostocaceae

Anabaena azollae Strasburger
*A. circinalis (*Kutz.) Rabh.
**A. confervoides* Reinsch
A. constricta Lauter b.
A. cycadeae J.Reinsch
A. cylindrica Lemmermann
A. fircinalis
A. flos-aquae (Lyng.) Breb.
A. limnectica G.M.Smith

A. spiroides Lemm.
**A. torulosa* (Carm.) Lagerh.
Anabaena sp.1
Anabaena sp 11
Aphanabaena sp
Calothrix sp
Cylindrospermum catenatum Ralfs
Cylindrospermum majus Kutz.
Cylindrospermum sp 1
Nostoc carneum
Nostoc linkia
N. muscorum Agardh
N. peltigerae Letellier
N. sphaericum Vauch.
Nostoc sp.1
Nostoc sp 11

Order 3: Oscillatoriales

Family 1: Oscillatoriaceae

Lyngbya birgei G.M.Smith
L. contorta Lemm.
**L lagerheimia* (Mobius) Gom.
L limnectica Lemmermann
**L. martensiana* Menegh.
L versicolor (Wattman)Gomont
Lyngbya sp
Oscillatoria acuminata Gomont
O. acutissima Kufferath
O. agardii Gomont.
O. angustissima West & West
O. articulata
O. brevis Kutz.
O curviceps Agardh
O. formosa Bory.
**O formosa f. edaghica* Novickova
O. germinata Meneghini
O lacustris
**O. lemmermanni* Wolosz
O. limnectica Lemm.
O. limosa (Roth) Ag.
O. margaritifera Kutzing (Gomont)
O. minima
O. planctonica Wolosz
**O. rubescens* DC ex Gomont
O. sancta (Kutz.)Gom
**O simplissima* Gomont.
O. subrevis Schmidle
O. tenuis Ag.
Oscillatoria sp.

Family 2:Phormidiaceae

Arthrospira fusiformis Fott & Karim
Arthrospira sp.
Microcoleus codii Fremy
M. subtorulosus
M. willeana
Microcoleus sp.1
Microcoleus sp 11
Phormidium angustissimum West & West
**P. caerulescens* Geitler
**P. chlorinum* Komarek
P. cortianum
P. crouanii Gomont
P. foveolarum (Mont.) Gomont
**P. insigne* Skuja
**P. laetevirens* Skuja
P. luridum (Kutz.) Gomont
**P luteum* Kosinskaja
**P. molle* Palik
P nigro-viride Gomont
**P. papyraceum* (Ag.) Gom.
P. retzii (Ag.) Gomont
*P. tenue (*Menegh.)Gom.
P. tinctorium Kutz.
Phormidium sp 1
Phormidium sp 11
**Plantothrix clavarata* Skuja
**P. cryptovaginata* Skacelova & Komarek
**P. isothrix* Komarek
P. minor
P. planctonica
Plantothrix sp1
Plantothrix sp 11
Trichodesmium laucustre Klebahn
Trichodesmium sp.

Family 3:Pseudanabaenaceae

**Limnothrix planctonica* Geitler
Limnothrix sp.
**Pseudoanabaena curta* Hollerbach
**P. moniliformis* Komarek & Kling
**P thermalis* Anagnostidis
Pseudoanabaena sp
Spirulina filiformis
S. princeps W.et G.S.West
S. major Geitler
S. meneghiniana Anagnostidis
S. tennerima
Spirulina sp 1
Spirulina sp 11
**Leptolyngbya hypolimnectica*
**Leptolyngbya ocridana* Cardo
L. tenuis

Leptolyngbya sp.
**Plantolyngbya brevicellularis* Cronberg &Komarek
**P. minor* Komarek&Cronberg
**P. tallingii* Komarek & Kling
**P. minor*
Planktolyngbya sp.
Family:Schizotrichaceae
Schizothrix pulvinata
S. friesii (Ag.) Gomont
Schizothrix sp.
Scytonema sp.

Discussion

The cyanobacteria checklist reflects the influence of hydrological conditions of this area. The dominance of *Oscillatoria* throughout the season could be a pointer that the hydrology and salinity of the studied area favours its growth. The particular high diversity of blue-green observed in the lagoon could also be that the water chemistry favours growth of cyanophytes. Five bloom forming cyanophytes identified in this study include *Microcystis aeruginos, M. wesenbergii, , Anabaena flos-aquae, A. spiroides* and *Oscillatoria formosa.*

The variation in physical and chemical parameters observed during the study period may be as a result of the influence of weather conditions. For instance, the rainy season occurring between June and October, characterized by low transparency and pH; increased total suspended solids, higher turbidity and increased flood water condition which might have initiated stressful environmental condition and these conform with Dart and Stretton (1980) who stated that variations in water temperature could cause alterations in the pH due to changes in ionization and increased solubility or precipitation of bottom deposits. Nwankwo and Onitiri (1992) also pointed out that it is possible that rainfall triggers off flood situations which usually increases total solids, reduces transparency and consequently light penetration and also dislodges attached algal forms.The phytoplankton community and the physio-chemical parameters exhibited seasonal changes closely related to the pattern of rainfall. The presence or absence of any blue-green species may be due to the changing physical environment other than pollution (Nwankwo 1994).

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References

1. Adesalu,T.A., Nwankwo, D.I. Studies on the phytoplankton of Olero creek and parts of Benin river, Nigeria. The Ekologia. 2005; 3(2):21-30.
2. Adesalu,T.A., Nwankwo, D. I. A checklist of Lekki lagoon diatoms. International Journal of Botany. 2009; (5) 2: 126-34.
3. American Public Health Association. Standard methods for the examination of water and waste water, 20th ed. American Public Health Association Incorporated, New York. 1998: 1270pp.
4. Anadu, D.I.,Obioma, A., Ejike, C. Water chemistry and plankton periodicity in two contrasting mine lakes inJos,Nigeria. Hydrobiologia, 1990; 206:17-25
5. Biswas, S. Phytoplankton periodicity of Ogelube Lakes, Anambra State, Nigeria. During 1979-1980.Hydrobiologia, 1992; 246: 169-172.
6. Biswas, S. Phytoplankton of Opi Lakes, Anambra State, Nigeria.Verht.Int.Verein. Limnology, 1984; 22:1584-1589.
7. Dart, R. K., Stretton, R. J. Microbiological aspects of pollution control and environmental science. 6 Elsevier Scientific Publishers Amsterdam 1980.
8. Egborge, A. B. M., Sagay, E. G.. The distribution of phytoplankton and zooplankton in some Ibadan freshwater cosystems. Polskie Archiwum Hydrobiologii, 1979; 26: 23-335.
9. Hill, M. B., Webb, J. EThe ecology of Lagos lagoon II. The topography and physical features of Lagos harbour and Lagos lagoon. Phil. Trans. Royal Society London, 1958; 241**:** 319–417.
10. Holden, M. J., Green, J. The hydrology and plankton of the River Sokoto. Journal of Animal Ecology, 1960; 29: 65–84.
11. Ikusemiju, K. A study of catfishes of Lekki lagoon with particular reference to species of *Chrysichthys walkeri* (Bagridae) Ph.D Thesis University of Lagos, Nigeria, 1973.

12. Imevbore, A. M. A. Planktonic algae of Eleiyele reservoir, Nigeria. Journal of Science, 1968; 2: 85–90.
13. Kadiri, M.O. A rich flora of Micrasterias from Ikpoba reservoir, Nigeria. Archiwum Hydrobiologii, 1989; 116(3): 391-399.
14. Kadiri, M.O. Further desmids from Ikpoba reservoir (Nigeria) compared with other records from Africa. Algological studies, 1993; 71:23-35.
15. Kadiri, M. O. A comprehensive preliminary checklist of the algae of the Ikpoba reservoir Edo state,Nigeria. Global journal of pure and applied sciences, 1999; 5 (4): 485-491.
16. Kadiri, M.O. Seasonal trend in the chemical limnology of a shallow Nigerian man-made lake. Acta Hydrobio. 2000; l (42): 29-40.
17. Nwankwo, D. I., Onitiri, A.O. Periphyton (Aufwuchs) community on submerged aquatic macrophytes in Epe lagoon, Nigeria. Journal of Agriculture Science and Technology. 1992; 2 (2): 135- 41.
18. Nwankwo, D. I. A checklist of Nigerian marine algae. (Tarkwa Bay). Nigerian Journal of Botany, 1988; 1: 47–50.
19. Nwankwo, D. I. Cyanobacteria bloom species in coastal waters of South-western Nigeria. Archiwum Hydrobiologie Supplement, 1993*;* 90: 553–542.
20. Nwankwo D. I. Hydrochemical properties and bottom-dwelling of aLagos lagoon sewage disposal site. Polskie Archiwum Hydrobiologia., 1994; 41(1): 35-47.
21. Nwankwo, D. I. A first list of dinoflagellates (Pyrrophyta) from Nigerian coastal waters (creeks, estuaries,lagoons).*Polskie Archiwum Hydrobiologia*, 1997; 44(3): 313–321.
22. Olaniyan, C. I. O. The seasonal variation in plankton in Lagos harbour, Nigeria. Ph.D Thesis, University of London, London, 1957; 173pp.
23. Opute, F. I. Phytoplankton flora of the Warri/Forcados estuaries of Southern Nigeria.Hydrobiologia. 1990; 208:101-109.
24. Opute, F.I. A checklist of the freshwater, brackish and marine phytoplankton of the Warri/Forcados estuaries of southwestern Nigeria. Nigerian Journal of Botany.1991; 4: 227-254.
25. Opute, F.I. Contribution to the knowledge of algae of Nigeria 1: Desmids from the Warri/Forcados estuaries, Part 2. The genera Euastrum and Micrasterias. Archiwum Hydrobiologii. Supplement, 1992; 93: 73-92.
26. Wujek, D. E., Adesalu, T. A., Nwankwo D. I. Silica-scaled chrysophyceae and synurophyceae (chrysophyta) from Nigeria II Lake Lekki. Tropical Freshwater Biology. 2003/2004; 12/13:99-103.

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