The role of *Cladophora spp* and *Spirulina platensis* in the removal of microbial Nile water

*Osman, G. A., **Ali, M. S., *Kamel, M. M. and***Amber, A. Gad

*Bacteriology Lab., Water Pollution Research Department,**Agriculture Microbiology Department, ***Natural and Microbial products Department, National Research Center, Dokki, Cairo, Egypt.
Corresponding author, Osman, G. A., E-mail: gamalosmanali2005@yahoo.com

Abstract: The main aim of this study was to evaluate the removal of some microbial in Nile water passed through some algae (*Cladophora sp* and *Spirulina platensis*) as filters before the chlorine treatment. The other target from this filtration were to reduce the organic matter which product carcinogenic compound when exposed to the chlorine as well as reduction the chlorine doses used in Water Treatment Plants for potable water. The results show that *Cladophora spp* succeeded in removing microbial tested, however, the ratio removals were 12.7, 21.1, 33.3, 11.1, 32.1, 27.2, 27.6 30.8 34.4 and 33.3% for total viable bacterial count at 37 ^{o}C, 22 ^{o}C, total coliform, fecal coliform, fecal streptococci, salmonellae group, *Pseudomonas spp.*, total staphylococci, yeasts and fungus, respectively. On the other hand, the total organic carbons (TOC) were 9 before filtration, while after filtration was 7.5 ppm, but *Spirulina platensis* made reduction from 9 to 6.25 ppm. In addition, the microbial tested in Nile water samples were absent after filtrated with chlorine dose 2ppm for 30 minutes while in case Nile water samples without filtrated some microbial were present with dose 5ppm. *Spirulina platensis* was more efficiency where the results show that the ratio removals were 42.3, 51.5, 77.1, 80.6, 75, 45.5, 62.1, 92.3, 56.3 and 50% for total viable bacterial count at 37^{o}C, 22^{o}C, total coliform, fecal coliform, fecal streptococci, salmonellae group, *Pseudomonas spp.*, total staphylococci, yeasts and fungus, respectively. After filtrated the Nile water through *Spirulina platensis* and treated with chlorine (dose 2ppm) for10 minutes, the microbial tested were absent while the water sample (without filtrated), some microbial were present for 60 minutes with dose 5ppm. These treatments concluded that reduction of chlorine used and carcinogenic compound in drinking water treatments as will as water quality.
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Key words: Nile water, Classical bacterial indicators, Salmonellae group, Total staphylococci, *Pseudomonas spp.* yeasts, fungus, and chlorine.

Introduction

Material and methods

● Sample collection

Nile water samples were collected at inlet of El-Giza Water Treatment plant. It collected in 10 liters sterile glass bottles (10 bottles) and then transferred from the sites to the lab in ice box. The water was putting in sterile container (150 litters) and transfer by motor with flow rate 2 liters / minute to the system figure (1). Water sampling was taken at 0, 10, 30, 60 and 120 minutes in 1 liter sterile glass bottles. Sodium thiosulphate crystals (18 mg/L) were added only to the bottles samples of chlorinated drinking water (**APHA, 2005**).

● Chlorine water

Chlorine water was prepared from the lab of El-Giza Water Treatment plant. It added to the Nile water and filtrated water with doses 2 ppm and 5ppm, respectively according to **APHA, 2005**.

● Estimated the total organic carbons (TOC) and carcinogenic materials

The total organic carbons (TOC) and carcinogenic materials were estimated in water samples according to **APHA, 2005**.

● Microbiological examination

Enumeration of Classical bacterial indicators: Total bacterial counts (at 22ºC and 37ºC), total coliform, facal coliform and facal streptococci were carried out using poured plate and MPN methods according to **APHA (2005)**, for raw water and drinking water.

Detection and enumeration of *Pseudomonas aeruginosa* was using asparagine broth media as a presumptive test (MPN methods for raw water and drinking water according to **APHA, 2005**). The positive tubes produced a greenish fluorescent color after exposing to long-wave ultraviolet light. These tubes were used to streak the surface of acetamide agar slants as confirmation test. Positive confirmed tubes with the purple color indicated to high pH value after incubated at 37ºC for 24 hours **(APHA, 2005)**.

Detection and enumeration of salmonellae group were carried out using membrane filter technique. A 100 ml drinking water samples were separately filtrated through the membrane filter (0.45 µm pore size and 47 mm diameter). The membrane was transferred onto bismuth sulphate agar as a confirmed test. **(APHA, 2005)**. On the other hand, for Nile water samples (ml samples), salmonellae groups was counted from inoculated 5ml autoclaving buffer peptone water **(British Standard Institute, 2002)** tubes (incubated at 37ºC for 24 hours) which used as MPN technique**.** 0.2 ml from these tubes was streaked (by rod glass) on the plates of bismuth sulphate agar as a confirmed test. After incubation at 37ºC for 48 hours, typical black colonies with or without metallic sheen and blackening extended beyond the colonies considered as confirmed positive results for the presence of salmonellae **(APHA, 2005)**.

Detection and enumeration of *Staphylococcus sp*. were carried out using membrane filter technique. A 100 ml drinking water samples were separately filtrated through the membrane filter (0.45 µm pore size and 47 mm diameter). The membrane was transferred onto Baird-Parker agar Base ((Himedia, India) for the selective isolation of coagulase-positive *Staphylococcus aureus* in water **(APHA, 2005).** 1ml of Nile water samples was inoculated into 5ml autoclaving buffer peptone water **(British Standard Institute, 2002)** tubes (incubated at 37ºC for 24 hours) which used as MPN technique**.** 0.2 ml from these tubes was streaked (by rod glass) on the plates of Baird-Parker agar medium**.** After incubation (37ºC for 24 hours)**,** typical black colonies with or without metallic sheen and blackening extended beyond the colonies considered as confirmed positive results for the presence of *Staphylococcus aureus* **(APHA, 2005)**.

Detection and enumeration of fungi and yeasts were using Saparoud agar medium and molt yeast extract medium respectively, for raw water and drinking water according to **APHA, 2005**.

● Algae preparing

From Nile water *Spirulina platensis* was isolated and used in this study. The preparation and maintenance of the inoculums was accomplished using Zarrouk 's medium according to standard for the cultivation of this micro-alga. **(Aly and Amber 2010).**

Cladophora spp were collected in ice boxes (put into glass jars) from the beaches of River Nile in Port Said, area every week during May and November, 2010 and stored as mats at 10^{o}C.

In this experiment were made two times every week (during May and November, 2010), Kg of both *Cladophora sp.* and *Spirulina platensis* (wet weight) was added to the lagoon of glass and passed the raw Nile water with flow rate 2 liters / minute (figure 1).

Concentrations A,B ; algal biomass ,and combined treatment effect of algal biomass and Cl_2 concentrations .A=6 ppm,B=2 ppm/

Results and Discussion :

Effect of Chorination on Nile water putification: Disinfection by chlorination can be problematic, in some circumstances. Chlorine can react with naturally occurring organic compounds found in the water supply to produce compounds known as disinfection byproducts (DBPs). The most common DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs). Due to the potential carcinogenicity of these compounds, drinking water regulations across the developed world require regular monitoring of the concentration of these compounds in the distribution systems of municipal water systems. The World Health Organization has stated that the "Risks to health from DBPs are extremely small in comparison with inadequate disinfection."There are also other concerns regarding chlorine, including its volatile nature which causes it to disappear too quickly from the water system, and aesthetic concerns such as taste and odor. Chlorination is the process of adding the element chlorine to water as a method of water purification to make it fit for human consumption as drinking water. Water which has been treated with chlorine is effective in preventing the spread of waterborne disease. The chlorination of public drinking supplies was originally met with resistance, as people were concerned about the health effects of the practice. The use of chlorine has greatly reduced the prevalence of waterborne disease as it is effective against almost all bacteria and viruses, as well as amoeba.

Table 1. The log average counts for classical bacterial indicators in Nile wate sitesr with Cl_2 sample .(the control).

Site	Log number of cell forming unit (CFU) / 100 ml				
	Total viable bacterial count at:-		Bacterial indicators (MPN-index)		
	37 °C	22 °C	TC	FC	FS
Nile water	7.1	6.6	4.8	3.6	2.8
With Cl_2a					
0 time	6.8	6.1	4.2	3.2	2.4
10 minutes	3.8	3.5	3.2	1.1	1.8
30 minutes	1.8	1.1	1.1	0	1.1
60 minutes	1.0	0.8	0	0	0.7
120 minutes	0	0	0	0	0
With Cl_2b					
0 time	6.6	6.4	4.2	3.2	3.6
10 minutes	6.3	4.1	3.6	2.8	3.2
30 minutes	3.9	3.8	2.4	1.7	2.8
60 minutes	2.3	2.1	1.1	0.7	1.7
120 minutes	1.1	0.7	0	0	0.6

Bromine in a concentration of 1·4 mg/l was injected for 9 hr; during eight consecutive nights into the Jordan channel. Chlorine and bromine were injected for the same time during two nights, to yield a total of 0·4 mg/1 residual. After 15 min contact, coliforms and fecal coli counts showed full correlation between disinfection capacity of bromine under these conditions to that achieved in the laboratory.*Cosmarium*, known to be resistant to chlorine, proved to be sensitive to bromine. *Chroococcus* found naturally in the water was shown to be sensitive to bromination. *Chlorella*, placed into the channel water in dialysing tubes, showed to react as in previous laboratory experiments.The combined use of chlorine and bromine in field experiment confirmed laboratory results in which higher algal and bacterial kill was achieved with mixtures of halogens than with any one of them separately.Table 2 indicated the gradual decrease in the pathogens count in all microorganisms concerned with the two concentrations used with Cl_2.

Table(2). The log average counts for pathogen counts in Nile water after Cl_2 treatment

Treated sample	Log number of cell forming unit (CFU) / 100 ml				
	Salmonellae group	*Pseudo.sp.*	*Total Staph*	Total molds	
				yeast	fungi
Nile water	7.1	6.6	4.8	3.6	2.8
With Cl_2a					
0 time	1.8	2.6	2.4	2.9	3.4
10 minutes	0	1.1	1.8	1.1	2.6
30 minutes	0	0	1.4	0	1.8
60 minutes	0	0	0	0	0.9
120 minutes	0	0	0	0	0
With Cl_2b					
0 time	2.4	2.4	2.1	2.7	3.2
10 minutes	1.8	1.8	2.9	2.1	2.8
30 minutes	0.7	0.7	0.7	1.4	1.8
60 minutes	0	0	0.2	0.9	1.1
120 minutes	0	0	0	0	0.3

Effect of Cladophora Algae on water purification

As it could be seen from table 3, either concentrations of the used Cl_2 treatment resulted in decreasing the pathogens count especially with increasing the time of exposure .Cladophora aegagrophila is not really a plant, but a ball of algae, so it is a decorative exception from the rule about avoiding algae at all costs. It is normally found in shallow lakes, where the movement of the waves forms it into a sphere. In an aquarium it must be turned regularly to keep it in shape. Cladophora aegagrophila can be divided into smaller pieces, which become spherical with time, or which form a carpet, if attached to roots and stones. Associations between Cladophora and microbial communities are not well understood, although some research has presented evidence of a relationship between Cladophora and bacilliform bacteria (The cell wall of Cladophora provides a suitable attachment and grazing surface for many other organisms, such as diatoms, protozoa, mollusks, rotifers, and young crayfish, and links between bacteria and algae have been found frequently in aquatic environments). Few number of researches findings the that demonstrate the presence of fecal indicator bacteria, E. coli and enterococci, on Cladophora.

Table 3 indicated that Cladophora can be a secondary habitat for indicator bacteria that could potentially influence water quality. The long-term survival of E. coli and enterococci in Cladophora mats also has important ecological and public health implications. Masses of floating Cladophora, as a result of wave action, can release indicator bacteria and elevate their levels in the water. Also, algal mats washed onto beach sand may get buried in the sand by wave action or human activities, where they are protected from sunlight and desiccation. Here, indicator bacteria may multiply due to available nutrients from the decomposing mats; in turn, the beach sand can serve as a source of indicator bacteria for the nearshore water, especially when waves re-suspend buried mats. Previously, studies have shown that pathogenic bacteria (e.g., vibrios) are often associated with cladophora. It is possible that Cladophora provides a niche for pathogenic bacteria.

Combined effect of Cladophora and Cl2 on Nile water purification:

Salmonella count is much more than other pathogens 7.1 compared to log CFU/100ml 6.6 and 4,8 for pseudcocci and staphilococci respectively. application of 6ppm Cl_2 resulted in total disinfection ,however fungi still present with log. CFU/100ml 0.3after 120 min. Cladophora is a branching, green filamentous alga found naturally along the coastline of most of the Great Lakes There has been a recent resurgence of macroalgae, predominantly Cladophoral. These algae blooms lead to unsightly and foul-smelling beaches and have negative economic consequences as a result of the lowered beach use. In addition, Cladophora blooms result in reduced quality of drinking water and decreased property values. The offending plant is primarily Cladophora, a common filamentous green alga.? Growing on submerged rocks, it looks like long green hair waving in the water.? Cladophora is an important component of freshwater ecosystems, providing food and shelter for invertebrates and small fish.? The recent excessive blooms in the Great Lakes, however, signal an ecosystem responding to both natural changes and human impacts.

E. coli and enterococci survived for over 6 months in sun-dried and refrigerated Cladophora, perhaps other factors (competition, predation, and sunlight) were responsible for the gradual disappearance of E. coli and enterococci in naturally occurring Cladophora.While the case for natural multiplication needs further validation, Cladophora can be a reservoir for E. coli and enterococci. To understand the ecological and environmental implications of the present findings, more laboratory studies are necessary. These might include (i) in vitro studies showing the range of tolerance and growth potential of subject bacteria under a variety of environmental conditions (insolation, desiccation, and temperature), (ii) a thorough investigation of the genomic and phenotypic relationships of algae and ambient bacteria to investigate clonality or source-sink relationships further, (iii) noninvasive sterilization and inoculation of algae using wild and lab strains to discover intrinsic growth potential, maximum carrying capacity, and associated limiting factors, (iv) high-resolution microscopic studies of algal thalli and biofilm to further understand the physical association of algae and indicator bacteria, and (v) more investigations of the health implications of these findings. Our findings clearly suggest that Cladophora can be a secondary habitat for indicator bacteria that could potentially influence water quality in affected Great Lakes swimming areas. The long-term survival of E. coli and enterococci in Cladophora mats also has important ecological and public health implications. Masses of floating Cladophora, as a result of wave action, can release indicator bacteria and elevate their levels in the water.). As it could be seen from table 4, Cladophora macroalagae is more efficient in removing the pathogens especially when combined with Cl_2.

Table(3).Classical bacterial count on using *Spirulina platensis* and *Cladophora.*

Site	Log number of cell forming unit (CFU) / 100 ml				
	Total viable bacterial count at:-		Bacterial indicators (MPN-index)		
	37 °C	22 °C	TC	FC	FS
Nile water	7.1	6.6	4.8	3.6	2.8
Algae 1	6.2	5.2	3.2	3.2	1.9
Removal (%)	12.7	21.1	33.3	11.1	32.1
Algae1 Cl_2a					
0 time	5.5	4.9	2.8	2.4	1.4
10 minutes	2.2	1.8	0.7	0	0.7
30 minutes	1.1	0.6	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0
Algae 2	4.1	3.2	1.1	0.7	0.7
Removal (%)	42.3	51.5	77.1	80.6	75
Algae2 Cl_2b					
0 time	3.9	2.9	0.7	0.7	0.7
10 minutes	1.6	1.1	0	0	0
30 minutes	0	0	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0

FS: fecal streptococcus; FC fecal coliform; TC total colifom Aga 1 Cladophora Alga 2 *Spirulina* A Cl_2 6ppm B 2ppm

Effect of *Spirulina* on Nile water purification:

Provide specifications for *Spirulina*, which include specifications for protein, lead, minerals, moisture, beta-carotene, total carotenoids, c-phycocyanin, arsenic, cadmium, mercury, pesticides, rodent hairs, and insect fragments. There also established the absence of *E. coli*, *Salmonella*, and *Staphylococcus aure*us, total aerobic bacteria of less than 200,000 colony forming units per gram (cfu/g), and total coliforms of less than 10 cfu/g. the composition of spirulina. As an example of *Spirulina* powder used is Pacifica™ which is is a free-flowing green to bluish-green powder. It has a mild seaweed odor and is not soluble; it forms a suspension. The Particle size is <125 microns and Bulk Density is >0.48 (g/ml). Total aerobic bacteria: <105 cfu/gram Total coliforms: <10 cfu/gram*E. coli*: negativePesticides: negative Arsenic: <0.5 ppm Cadmium: <0.2 ppm each Lead: <0.2ppm Mercury: <0.025 ppm *Salmonella*: negative

Combined effect of *Spirulina* and Cl_2 on Nile water purification:

The initial *Spirulina* inoculum concentrations used ($5x10^3$ cells.mL) correspond to concentrations of around 0.1 g/L Lodi et al. (2003) studied the removal of nitrate and phosphorous from wastewater with *S. platensis* cultivation and concluded that biomass concentrations between 0.25 and 0.86 g/L result in larger removals of these pollutants. Therefore this microalgae can be an alternative to assist in the Nile water treatment, reducing the environmental impact caused by their pollutants and also to use the biomass produced in animal feed and fertilizers, in the production of pigments, vitamins, polysaccharides, including others treating with live *Spirulina platensis* decrease the pathogens to a removal % of 27.2 for samonella ,27.6 for *Pseudococcus* and 30.8 % for *Staphellococci*, 43.4% for yeasts and 33.3 % for fungi.On applying live *Cladophora* the ratio increased to 45.5, 62.1 and 92.3% for bathogen bacteria and 56.3 and 50%for fungi .

Table (4). the log average counts for pathogen counts in nile water after cll2 treatment and algale

Treated sample	Log number of cell forming unit (cfu) / 100 ml				
	Salmonella e group	*Pseudo. sp*	*. Total staph.*	Total molds	
				yeasts	fungi
Nile water	2.2	2.9	2.6	3.2	3.6
Algae1	1.6	2.1	1.8	2.1	2.4
Removal%	27.2	27.6	30.8	43.4	33.3
Algae1+Cl_2 a					
0 time	1.1	1.1	1.5	1.4	1.8
10 minutes	0.2	0.4	0.6	0.7	1.1
30 minutes	0	0	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0
Algae2	1.2	1.1	0.2	1.4	1.8
Removal %	45.5	62.1	92.3	56.3	50
Algae2+Cl_2 b					
0 time	0	0	0	0.4	0.6
10 minutes	0	0	0	0	0
30 minutes	0	0	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0

Krebs & Sisler's method combines photosynthesis with photocatalysis to treat impure water and turn it into safe water, the company explaine the method involves water purification through a rapid growth in biomass, which also can be harvested and used for human or animal consumption, the treatment method is expected to produce potable water for half the cost of reverse osmosis most commonly known to purify seawater as drinking water in large continuous-flow volumes while the biomass is produced and separated for consumption. the storehouse for atmospheric oxygen, carbon dioxide is the resource for recycling both oxygen and carbon. With the new process it can be separated through photosynthesis at a high rate. As the CO2 is separated the carbon grows biomass and the oxygen is released to enrich the air. The process is good for salt water, sewage and industrial wastewater, the company said. The biomass is produced by the concurrent use of photosynthesis and photocatalysis. Light emitting diode lighting and CO2 and balanced nutrients unite, growing a biomass from species of algae such as Spirulina.The biomass growth rate in deep well-lighted enclosed cells is expected to exceed 100 times the natural rate because all factors related to culturing the algae can be optimized in the continuous hydroponic process.

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