The role of *Cladophora sp.* and *Spirulina platensis* in the removal of microbial flora in Nile water

[1]Osman, G. A., [2]Ali, M. S., [1]Kamel, M. M. and [3]Amber, S. Gad

1Bacteriology Lab., Water Pollution Research Dept.,2Agriculture Microbiology Department, 3 Chemistry of Natural and Microbial products Dep., National Research Center, Dokki, Cairo, Egypt.
gamalosmanali2005@yahoo.com

Abstract: The main aim of this study is to evaluate the role of *Cladophora sp* or *Spirulina platensis* (as biofilters) for the reduction of microbial load in Nile water as well as reduce the organic matter content (which produce carcinogenic compound when exposed to the chlorine) before chlorine treatment in Water Treatment Plants for potable water. The results showed that *Cladophora sp* succeeded in removing tested microbial spp., with, a ratio of 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,and 22 ^{o}C) total coliform, fecal coliform, fecal streptococci, salmonellae group, *Pseudomonas spp.*, total staphylococci, yeasts and fungi, respectively. On the other hand, the total organic carbons (TOC) were 9 before filtration, while after filtration was 7.5 ppm, but *Spirulina platensis* reduced TOC from 9 to 6.25 ppm. In addition, the tested microorganisms in Nile water passed through biofilters were absent by treatment with 2ppm chlorine dose for 30 min. While in case of unfiltered Nile water samples some microbial groups were present even with chlorine dose 6 ppm. *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) for 10 minutes, the tested microbial groups were absent. In the unfiltrated water sample for some microbial spp. were present for 60 minutes after treatment with chlorine dose 6 ppm. These results confirmed the reduction of the already applied chlorine concentration and decrease the presence of carcinogenic compound in drinking water to improve water quality.
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1. Introduction

River Nile is the main source of water in Egypt and divided into seven segments. Cairo segment is considered the most important one because it represents the major cluster of drinking water treatment plants. Changes in water quality are expected to be more pronounced as the river penetrates densely populated urban areas and various industrial regions. Therefore, a wide variety of pathogens may be transmitted by fecally polluted water.

Conventional water purification is a process of removing undesirable chemicals, materials, and biological contaminants from contaminated water. Generally, the methods used were physical processes such as , filtration and sedimentation; Rushton *et al* (1996), biological processes such as slow sand; filters or activated sludge, chemical processes such as flocculation and chlorination and the use of electromagnetic radiation. Rose *et al* (2006) used solar radiation for disinfection. The purification process of water may reduce the concentration of particulate matter including suspended particles, parasites, bacteria, algae, viruses, fungi range of dissolved and particulate material derived from the surfaces that water may have made contact with after falling. Bansal *et al* (1988) used active carbon for clarification. Some metals were removed by algae; Horikoshi *et al (* 1979); Darnell *et al* (1988);. Tsezos and Volesky (1988). Biofiltration in a media populated with microorganisms could be applied using sludge. Chlorine is one of the most common oxidants used to disinfect drinking water and to limit bacterial re-growth in water distribution systems. It reacts with various cellular compounds and affects metabolic and physiological processes (McKenna and Davies, 1988). It can damage bacterial membranes modifying their permeability (Sips and Hamers, 1981), inhibit ATP production (Barrette *et al,* 1989), fragment proteins (Thomas, 1979), cause nucleic acid damage (Phe *et al,* 2004).

Chlorination is one of the main procedures for disinfecting raw water for potable purposes, but this process results in the formation of mutagenic/carcinogenic disinfection by-products (DBPs) deriving from the reaction of the chlorine with organic compounds (humic and fulvic acids) naturally or by pollution present in water (Boorman *et al,* 1999). Chlorinated water is mutagenic in bacteria (Kargalioglu *et al,* 2002) and induces a genotoxic effect on mammalian cells (Lu *et al,* 2002 and Plewa *et al,* 2002). Traditionally, elevated counts

of *Escherichia coli* are presumed to indicate the presence of sewage, mostly derived from nearby point sources. Donovan *et al*. (2008) discussed the gastrointestinal disease associated with exposure to pathogens. The relationship between fecal indicator bacteria and *Cladophora sp.* remains under investigation. The local and regional density of *Escherichia coli* and *Enterococci* in Cladophora mats strongly correlated in both *E. coli* and *Enterococci* survived for over 6 months in sun-dried *Cladophora sp.* mats stored at 4°C; the residual bacteria in the dried alga readily grew upon re-hydration.However, The oxygenation caused by photosynthesis reduces the total coliform count Mezzomo *et al* (2010). Also, Doke, *et al*. (2004) used *Spirulina ps.* to remove COD, BOD, heavy metals and bacteria in wastewater.

So, the aim of this investigation is to use some algae (*Cladophora sp* or *Spirulina platensis*) as filters before the chlorine treatment as stage in water treatment processes for potable water.

2. 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 transferred to the lab in ice box. The water was putting in sterile container (150 litters) and transfer by motor with flow rate 3 liters / minute to the system (fig. 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 obtained from the lab of El-Giza Water Treatment plant and added to raw water and filtrated Nile water with doses 2 ppm and 6ppm, respectively in the presence of aluminum sulfate (25-40 ppm) as flocculation and neutralization according to APHA, (2005).

● The Estimated total organic carbons (TOC) and trihalomethanes

The total organic carbons (TOC) and trihalomethanes 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 sp* was using asparagine broth media as used a presumptive test (MPN methods for raw water and drinking water according to APHA (2005). The e tubes produced a greenish fluorescent color after exposing to long-wave ultraviolet light was considered as positive. 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. 100 ml drinking water aliquots were separately filtrated through the membrane filter (0.45 µm pore size and 47 mm diameter). The membranes wrere transferred onto bismuth sulphate agar as a confirmed test, (APHA, 2005). On the other hand, for Nile water samples, salmonellae groups were 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 aliquotes were separately filtrated through the membrane filter (0.45 µm pore size and 47 mm diameter). The membranes were 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 were 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, (according to Carmichael, 1986) and was used in this study. Algal identification has been done according to Komárek & Fott (1983), and Komárek, & Anagnostidis (1989). 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 sp 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.

Filtration experiment were made twice every week (during May to November, 2010), Kg of both *Cladophora sp.* or *Spirulina platensis* (wet weight) were placed in glass basin and the raw Nile water was passed through it with flow rate 3 liters / minute (figure 1).

● **Estimation of pH and Turbidity pH** was estimated in the water sample by pH meter (ENWAY, 3505 pH meter, France). Turbidity test was carried out using turbidity meter (HACH, 2100 AN, USA).

3. Results and Discussion:

Data given in Table (1) show that the average values (48 values) of total organic carbons (TOC), pH, turbidity (NTU) and trihalomethanes (chlorine 2 ppm), were 7.5 ppm, 7.3, 4, and 0.02 ppm for *Cladophora sp*, respectively after filtration the Nile water. On the other hand, *Spirulina platensis* recorded 6.25 ppm, 7.1, 3 and 0.01 ppm for total organic carbons (TOC), pH, turbidity (NTU) and trihalomethanes(chlorine 2 ppm). These data were accepted the safety of drinking water according to the Egyptian Standard (2007) for drinking water declared that potable water must be 6.5 to 8.5 for pH and 0.1 ppm for trihalomethanes.

Table (1): Physo-chemical tested of Nile water passed through some algae filters (*Cladophora sp*and *Spirulina platensis*) after treatment with different doses of Chlorine (2 ppm and 6 ppm)

Algae / Parameters	*Cladophora sp*		*Spirulina platensis*	
	before	after	before	after
Total organic carbons / ppm	9	7.5	9	6.25
Trihalomethanes / ppm				
Chlorine (2 ppm)	0.3	0.02	0.2	0.01
Chlorine (6 ppm)	0.4	0.2	0.4	0.04
Turbidity (NTU)	5	4	5	3
pH	7.5	7.3	7.5	7.1

Note: - these figures were an average for 48 values.

Chlorine is added to Nile water to reduce or eliminate microorganisms, which can be present in water supplies. The effect of different doses of chlorine (2 and 6 ppm) for removal of classical bacterial indicators counts (total bacterial counts at 37ºC and 22ºC, total and fecal coliform as well as fecal streptococci) were expressed as a log average numbers in Table (2).

Data given in Table (2) show that the initial average log numbers cfu / 100 ml of total viable bacterial count of Nile water at 37 ºC and 22 ºC were 7.1 and 6.6 cfu / 100, respectively but when Nile water was with exposed them to different chlorine doses they were absent after 120 minutes at dose 6 ppm but some bacterial were present at 2 ppm. Also, samples of non-treated Nile water in the present investigation supported higher log average counts of total coliform, fecal coliform and fecal streptococci being in the order of 4.8, 3.6 and 2.8 MPN-index / 100ml, respectively. These bacteria were killing after 120 minutes except fecal streptococci in different chlorine doses tested.

These results are in the line with those obtained by El-Taweel & Shaban (2003) who noticed that the range for river Nile samples at the different sites of Greater Cairo were 10^5 to 10^7 cfu / 100 ml for bacterial counts at 22 ºC and 37 ºC or 10^3 to 10^5, 10^3 to10^5 and 10 to10^4 MPN-index / 100 ml for total coliform, faecal coliform and faecal streptococci, respectively. The same results when Ali, et al (2008) who found that the bacterial counts at 22 ºC and 37 ºC in river Nile samples at El-Giza site were 8.3 x 10^5 and 5.0 x 10^5 cfu / 100 ml, respectively. These results are in line with those obtained by Shash *et al*. (2010) who enumerated the total coliform and fecal coliform in 100% tested samples of Nile water at Greater Cairo with the mean value 10^4 and 10^3 cfu / 100 ml respectively, and compatible with Egyptian Standard (2007).

Table (2): Effect of different doses of chlorine (2 and 6 ppm) for removal classical bacterial indicators in Nile water

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_2 A 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_2 B 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

Note:- these log numbers were a average for 48 values.
Cl_2 A :- chlorine dose 6 ppm Cl_2 B:- chlorine dose 2 ppm TC:- Total coliform FC:- Fecal coliform FS:- Fecal streptococci

With respect to some pathogenic microorganisms of Nile water, data recorded in Table (3) show that the log average numbers of salmonellae group, *Pseudomonas sp* and total staphylococci in all Nile water samples were 2.2, 2.9 and 2.6 cfu / 100 ml respectively. Chlorine treatments in this investigation effect on the survival of these counts were decrease as to be absent at 120 minutes. The results demonstrated that total staphylococci more resistant than other bacteria tested. These results corresponding with Saleh, (2009) who demonstrated that the mean log counts of *Staphylococcus sp. and Salmonella sp.* were 3.0 and 4.0 cfu / 100ml respectively, in Nile water samples from different sites at Greater Cairo, Egypt.

Table (3): Effect of different doses of chlorine (2 and 6 ppm) for removal of some pathogenic microbial in Nile water

Treated samples	Log number of cell forming unit (cfu) / 100 ml				
	Salmonellae group	*Pseud. sp.*	Total Staph.	Total molds	
				yeasts	Fungi
Nile water	2.2	2.9	2.6	3.2	3.6
With Cl_2 A 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_2 B 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

Note:- e log numbers were a average for 48 values.
Cl_2 A :- chlorine dose 6 ppm Cl_2 B:- chlorine dose 2 ppm *Pseud. sp:- Pseudomonas sp* Total staph.:- total staphylococci Cl_2 A :- chlorine dose 6 ppm Cl_2 B:- chlorine dose 2 ppm *Pseud. sp:- Pseudomonas sp* Total staph.:- total staphylococci

On other hand, the average log number of total yeasts and fungi were determined during the period of tested Nile water samples were being 3.2 and 3.6 cfu /100 ml, respectively. The results show that the mold especially fungi more resistant than bacteria for different chlorine doses tested. These results are in agreement with those of El-Taweel and Shaban (2003) who found that the log count range of yeast in Greater Cairo Nile water was from 1.3 to 4.1 cfu / 100 ml.

While the high results were obtained by El-Taweel (1998) and Ali *et al* (2000) for the average log number of yeast being 4.0 and 4.61 cfu / 100 ml, respectively. These results are in agreement with those obtained by Shaban and El-Taweel (2002) who found that the count of yeast ranged from 0.0 to 23 cfu / 100 ml in drinking water. Also, Kelley *et al.* (2003) reported that the count of yeast was 28 cfu / L in tap water. The average filamentous fungi counts of 3.7×10^2 were reported by Gonçalves *et al.* (2006) in chlorinated water.

Data presented in Table (4) revealed that classical bacterial indicators in Nile water were significantly affected by filtration through *Cladophora sp* than control as will as affected with chlorination. The results show that the ratios removal were 12.7, 21.1, 33.3, 11.1, and 32.1% for total bacterial counts at 37ºC, 22ºC, total coliform, fecal coliform, and fecal streptococci, respectively. In addition, all bacteria tested were absent at 60 minutes in different doses of chlorine. On the other hand, *Spirulina platensis* was the better than *Cladophora sp.* where the ratios removal were 42.3, 51.5, 77.1, 80.6 and 75% for total bacterial counts at 37ºC, 22ºC, total coliform, fecal coliform, and fecal streptococci, respectively. Also, the same results were obtained in this investigation where all bacteria tested were absent after 30 minutes in the presence of chlorine.

This data demonstrated that it could be possible to reduce the chlorine dose which used in water treatment for drinking water and lowest the amounts of trihalomethanes compounds.

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 (Richard, 2003). Few number of researches demonstrated the presence of fecal indicator bacteria, *E. coli* and *enterococci*, on *Cladophora*. Cladophora can be a secondary habitat for indicator bacteria that could potentially influence water quality (Matsuo, 2003; Richard, 2003; Ishii et al 2006)

Similarly, the data in this study were in the same line with Doke, *et al.* (2004) who found that the ratio removal of total bacterial counts were 75% in wastewater by *Spirulina SP.* Lodi *et al.* (2003) studied the removal of some pollutant materials 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.

With respect to the occurrence of some pathogenic bacteria and total mold in Nile water, data recorded in Table (5) which filtrated through *Cladophora sp.* and *Spirulina platensis* in the present chlorine with different doses (2 and 6 ppm). The results show that *Cladophora sp.* could removed these pathogenic microbes with ratios 2.2, 2.9, 2.6, 3.2and 3.6% while *Spirulina platensis* can remove their with ratios 45.5, 62.1, 93.3, 56.3 and 50% for salmonellae group, *Pseudomonas sp*, total staphylococci, salmonellae group, yeasts and fungi, respectively. On the other hand, after filtration through algae and the chlorine treatment Nile water, these microbial were not detected after 10 and 30 minutes for 6 and 2 ppm of chlorine doses, respectively. Also, the results shows that the pathogenic bacteria tested were more sensitive than the mold as will as Staphylococci more resistant than *Pseudomonas sp* and salmonellae group.

These results are in the line with those obtained *by Lodi et al. (2003) who reported that Cladophora and/or Spirulina platensis* can be used an alternative to assist in the Nile water treatment, reducing the environmental impact caused by their pollutants. Also, these results are in agreement with those obtained by Ishii *et al* (2006) suggest that *Cladophora* is a likely secondary habitat for pathogenic bacteria (*E. coli*, *Shigella*, *Salmonella* and *Campylobacter*) in lake Michigan and that association of these bacteria with *Cladophora warrants* additional to assess the potential health impact on beach users.

From these results microbically are in the line with those reported by Egyptian standard for potable water. So, the aim of this study reduce the microbial by natural product (algae) as a filter with low cost and to reduce the carcinogenic material during water treatment processes for produce drinking water.

Table (4): Effect of *Cladophora sp.*, *Spirulina platensis* and different doses (2 and 6 ppm) of chlorine on removal classical bacterial indicators in Nile water.

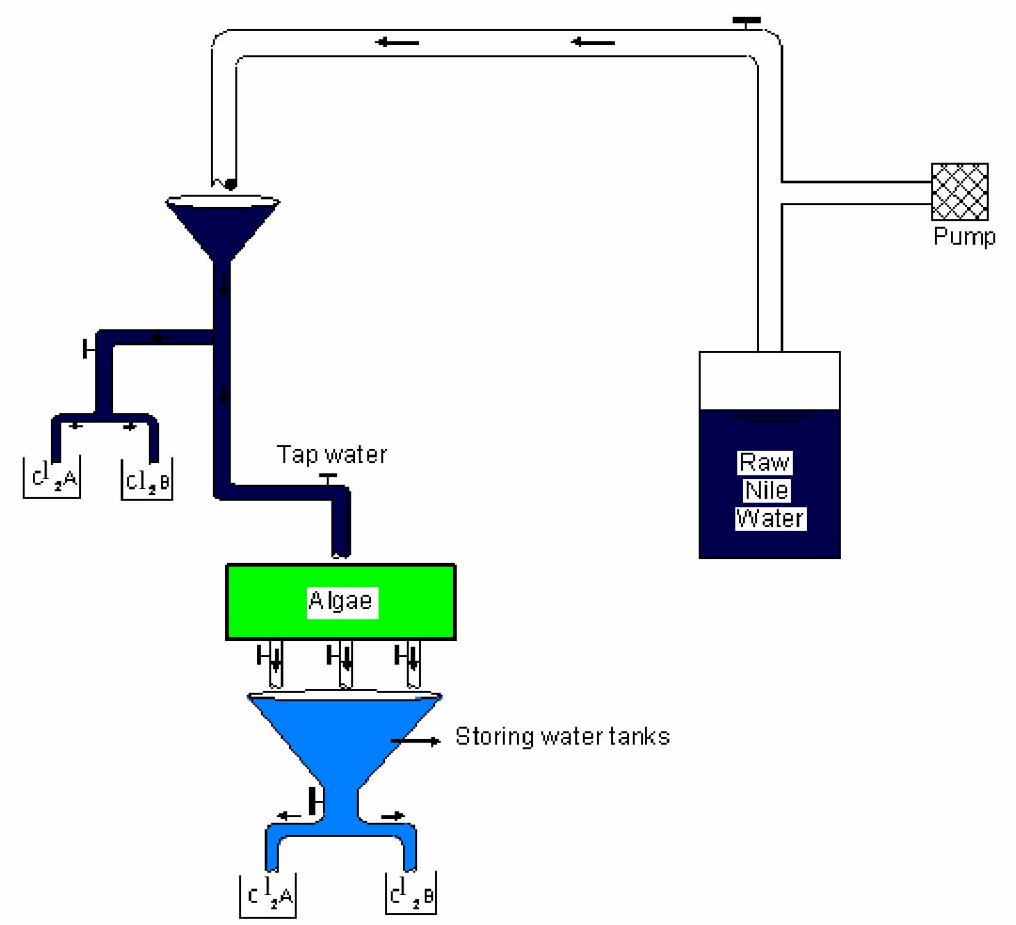
Site	Log number of cell forming unit (cfu) / 100 ml				
	Total viable bacterial count at:-		Bacterial indicators (MPN-index)		
	37 °C	22 °C	TC	FC	FS
Nile water	7.1	6.6	4.8	3.6	2.8
Algae 1	6.2	5.2	3.2	3.2	1.9
Removal (%)	12.7	21.1	33.3	11.1	32.1
Algae1+Cl_2A 0 time	5.5	4.9	2.8	2.4	1.4
10 minutes	2.2	1.8	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
Algae1+ Cl_2B 0 time	5.9	5.3	2.9	2.8	1.8
10 minutes	2.5	2.1	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-$_2$A 0 time	2.4	1.8	0.7	0.7	0.7
10 minutes	1.1	0.2	0	0	0
30 minutes	0	0	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0
Algae2 + Cl-$_2$B 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

Note:- log numbers were a average for 48 values.
Algea1:counts after filter by *Cladophora sp.* Algea 2:-counts after filter by *Spirulina platensis*
Cl_2 A:- chlorine dose 6 ppm Cl_2 B:- chlorine dose 2 ppm TC:- Total coliform FC:- Fecal coliform
FS:- Fecal streptococci

Table (5): Effect of *Cladophora sp.*, *Spirulina platensis* and different doses (2 and 6 ppm) of chlorine on removal of some pathogenic microbial groups in Nile water.

Treated samples	Log number of cell forming unit (cfu) / 100 ml				
	Salmonellae group	*Pseud. sp.*	Total Staph.	Total molds	
				yeasts	Fungi
Nile water	2.2	2.9	2.6	3.2	3.6
Algae 1	1.6	2.1	1.8	2.1	2.4
Removal (%)	27.2	27.6	30.8	34.4	33.3
Algae1+Cl_2A 0 time	1.0	0.8	1.1	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
Algae1+Cl_2B 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
Algae 2	1.2	1.1	0.2	1.4	1.8
Removal (%)	45.5	62.1	92.3	56.3	50
Algae2+Cl_2A 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
Algae2+Cl_2B 0 time	0.2	0.4	1.1	1.5	2.1
10 minutes	0	0	0.1	0.3	0.7
30 minutes	0	0	0	0	0
60 minutes	0	0	0	0	0
120 minutes	0	0	0	0	0

Note:- log numbers were a average for 48 values.
Algea1:counts after filter by *Cladophora sp.* Algea 2:-counts after filter by *Spirulina platensis*
Pseud. sp:- Pseudomonas sp Total staph.:- total staphylococci Cl_2 A:- chlorine dose 6 ppm
Cl_2 B:- chlorine dose 2 ppm



Fig(1). Design used in treating Nile water with Cl_2 concentrations A, B; algal biomass and combined treatment effect of algal biomass and Cl_2 concentrations A=6 ppm, B=2 ppm/

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