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Bioremediation of different types of polluted water using microalgae

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Abstract The present investigation was attempted to reduce the toxic pollutants from the different mixtures of water samples (sewage, sea and well) using the freshwater alga, *Chlorella vulgaris* and the marine alga *Chlorella salina.* The results revealed that both algae species were highly efficient and having a potential to reduce pH, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, ammonia, phosphate, sulphate, calcium, magnesium, sodium, potassium, heavy metals (Zn, Cu, Mn, Ni, Co, Fe and Cr) and the number of total Coli-form bacteria after 10 days of treatment compared to the untreated water samples. The removal efficiency of heavy metals was 13.61–100 %. In general, *C. vulgaris* shows higher removal efficiency in most of parameters than *C. salina.*

Keywords Bioremediation · Wastewater · Seawater · Well water · Chlorella · Physico-chemical parameters

1 Introduction

Bioremediation is a friendly approach for remediation of contaminated water and soil. Biological treatment can be accomplished by different ways, through use of mixed microbial culture such as bacteria, fungi and algae for the bioremediation of pollutants (Azab 2008).

Phycoremediation is the use of micro or macro algae for the removal of pollutants including xenobiotics and nutrients from wastewater and CO_2 from emitted waste air (Olguín 2003). Bioremediation with microalgae is particularly effective because of their capabilities converting solar energy into useful biomasses and assimilate nutrients such as phosphorus and nitrogen which cause eutrophication in the process of photosynthesis (De la Noüe and De Pauw 1988). In tertiary wastewater treatment, microalgae offer effective low cost approach to remove contaminants and excess nutrients and produce potentially valuable biomass, because of its high ability for inorganic nutrient uptake (Bolan et al. 2004; Munoz and Guieyssea 2006). Zhang et al. (2008) found that *Scendesmus* sp. showed high ability to remove inorganic nutrients from artificial domestic secondary effluents.

Microalgae assimilate a significant amount of nutrients because they require high amounts of phosphorus and nitrogen for the synthesis of 45–60 % proteins of microalgal dry weight, nucleic acids and phospholipids. Nutrient removal can also be increased by NH_3 stripping or phosphorus precipitation due to the rise in the pH associated with photosynthesis (Oswald 2012).

The removal of biochemical oxygen demand (BOD) in the wastewater treatment system, suspended solids, nutrients (NO_3^-–N; NO_2^-–N; NH_4^+–N and PO_4^{-3}–P), coliform bacteria, and toxicity are the main goal for getting purified wastewater. Biological oxygen demand (BOD) exploits the ability of microorganisms to oxidize organic material to CO_2 and water using molecular oxygen as an oxidizing agent. Therefore, BOD can deplete the dissolved oxygen of receiving water leading to fish kills and anaerobiosis, hence its removal is a primary aim of waste water

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treatment. Suspended solids are removed principally by physical sedimentation (Choi and Lee 2012; Abdel-Raouf et al. 2012).

Algae take up metals through adsorption over the cell surface very quickly in a few seconds or minutes; this process is called physical adsorption. Then, these ions are transported slowly into the cytoplasm in a process called chemisorptions (Dwivedi 2012). Microalgae are efficient absorbers of heavy metals (Chaisuksant 2003; Akhtar et al. 2004). Therefore, this property of heavy metal absorption has application in wastewater and different mixtures of water samples (sewage, sea and well) treatment. *Chlorella pyrenoidosa* had a high protein content when grown on sewage sludge and the aqueous extract retained a comparatively low level of various heavy metals, i.e. Cu^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+} (Wong and Tam 1984). Cyanobacteria and algae were evaluated as biosorbents for removing nickel (Ni) at concentration of <20 ppm from a chemically complex wastewater effluent. Cyanobacteria and algae were chosen because they were easy to grow and could withstand processing into biosorbent materials (Corder and Reeves 1994).

This work aimed to study the ability of two green microalgae *Chlorella vulgaris* and *Chlorella salina* for removal of pollutants from sewage and different mixtures of water samples (sewage, sea and well water) for eventual goal for recycling water.

2 Materials and methods

2.1 Water sampling

Waste water samples were collected from Al-Salhya sewage station, Qena, Egypt. Sea water samples were collected from the Red Sea at Hurghada region. Well water samples were collected from the wells of South Valley University.

2.2 Biological methods

2.2.1 Isolation and culturing of algae

Chlorella vulgaris (unicellular, green fresh microalga) was isolated from sewage water of El-Salhya sewage station, Qena, Egypt and *Chlorella salina* (unicellular, green marine microalga) was isolated from lake Marriott, Alexandria, Egypt. For cultivation and isolation of *C. vulgaris*, solid and liquid Beijernick's nutritive medium (Stein 1966) were used. *C. salina* was cultivated and isolated by using solid and liquid F/2 nutritive medium (Guillard and Ryther 1962). The growth of different algal species was examined by means of a compound microscope (Leica DM500) and identified according to Prescott (1982).

2.2.2 Algal growth and culture conditions

Axenic algal samples of *C. vulgaris* and *C. salina* were grown separately in 500 mL culture flasks containing nutritive medium. The cultures were incubated at 25 ± 1 °C and illuminated with cool white fluorescent lamps at an intensity 100 μmol photons m^{-2} s^{-1}. The algal cultures were supplied with dry air (Lorenzen 1964) to provide CO_2 necessary for photosynthesis, to prevent the settling of the cells at the bottom of the containers and to maintain the algae in suspension without mechanical stress (Persoone et al. 1980). The algae were kept under optimum conditions and subcultured routinely.

2.3 Treatment of water samples by algae

2.3.1 Experimental layout

The cells of *C. vulgaris* and *C. salina* were inoculated in glass tanks with 10 liter capacity containing different mixtures of water samples; sewage (100 % sewage waste water), mixture 1 (70 % sewage:30 % sea water), mixture 2 (70 % sewage:30 % well water) and mixture 3 (70 % sewage:15 % sea:15 % well water). Preliminary experiments were carried out to determine the optimum concentration of water samples mixtures. Water samples were inoculated with algae by adding algae cell cultures to give a concentration of 5 %. Treatment of water samples with algae was carried out under illumination and aeration for 10 days. The treated water samples were centrifuged to precipitate the algal mats. The supernatant was used to determine the physico-chemical characteristics of water samples.

2.3.2 Physico-chemical characteristics of water samples

The initial physico-chemical analysis of water sample was made before inoculation of algae and at final stage, the total content in each flask was filtered to remove algae and then used for the analysis of various parameters (pH value, total dissolved salts (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, ammonia and phosphate) (APHA 2005).

Calcium, magnesium, sodium, potassium and heavy metals were determined by using atomic absorption (Spectrometer: MESLO). Total coli-forms bacteria were determined by multiple tube fermentation method "Most Portable Number of bacteria (MPN method)" according to the standard methods for the examination of water and wastewater (APHA 1971).

2.4 Statistical analysis

The data were analyzed statistically by one way analysis of variance test using statistical computer program SPSS (version20).

3 Results and discussion

3.1 pH value

A marked reduction in pH values was noticed in water samples after the treatment with algae, where the algal treatment retained the pH value around neutral value (Fig. 1). *C. vulgaris* reduced the pH values in sewage, mixtures$_{1,2\&3}$ from 7.70, 7.87, 7.68 and 7.77 to 6.90, 7.01, 7.04 and 6.82, respectively, while *C. salina* reduced the pH values from 7.7 to 7.1 in sewage, 7.87 to 7.2 in mixture$_1$, 7.68 to 7.36 in mixture$_2$ and 7.77 to 7.37 in mixture$_3$ water samples. Similar observation recorded by Aarti et al. (2008).

3.2 Total dissolved salts (TDS)

The TDS of water samples were significantly decreased with algal treatment. The removal percentages of TDS were 68.42, 38.52, 43.37 and 33.47 % in sewage, mixtures$_{1,2\&3}$, respectively, by *C. vulgaris* and 37.59, 34.40, 42.17 and 24.86 % in sewage, mixtures$_{1,2\&3}$, respectively, by *C. salina* compared to untreated water samples (Fig. 2). This reduction in TDS may be due to the utilization of various nutrients by algae (Rao et al. 2011; Ahmad et al. 2013). The unique mechanism of bioabsorption/adsorption of different types of dissolved solids in wastewater is responsible to reduce TDS to lowest level (Nanda et al. 2010; Azarpira et al. 2014).

3.3 Biological oxygen demand (BOD) and chemical oxygen demand (COD)

The algal treatment of water samples induced a progressive reduction in both BOD and COD values. *C. salina* was found to be more efficient than *C. vulgaris* in BOD and COD reduction. Where BOD removal efficiency was ranged from 83.17 to 90.63 % and from 87.01 to 90.75 % by *C. vulgaris* and *C. salina*, respectively, (Fig. 3). According to Ganapathy et al. (2011) the value of BOD indicates level of toxicity of wastewater and they further reported the reduction in BOD of distillery effluent by 53 % using *Nostoc* species. According to Abdel-Raouf et al. (2012) BOD indicates the respiratory demand of bacteria and algae metabolizing the organic matter present in

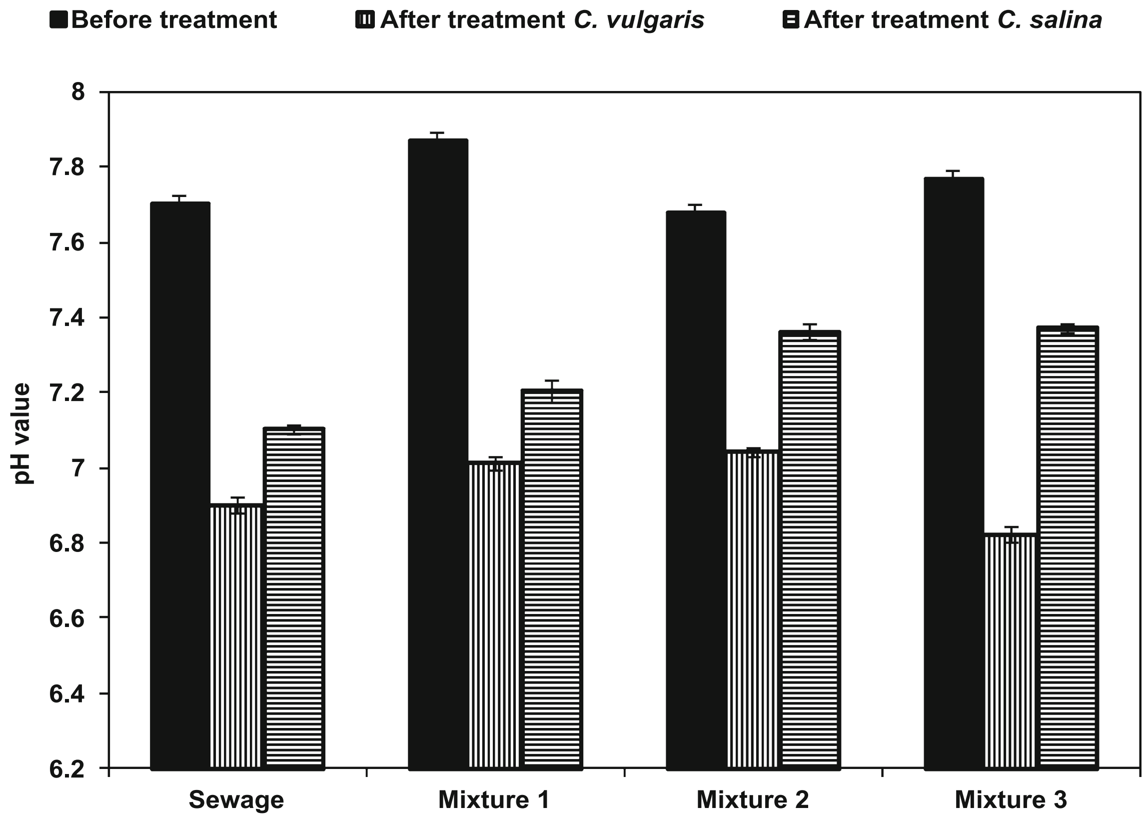

Fig. 1 Changes in pH values of water samples before and after treatment with algae. The values are the mean of 3 replicates ± SD

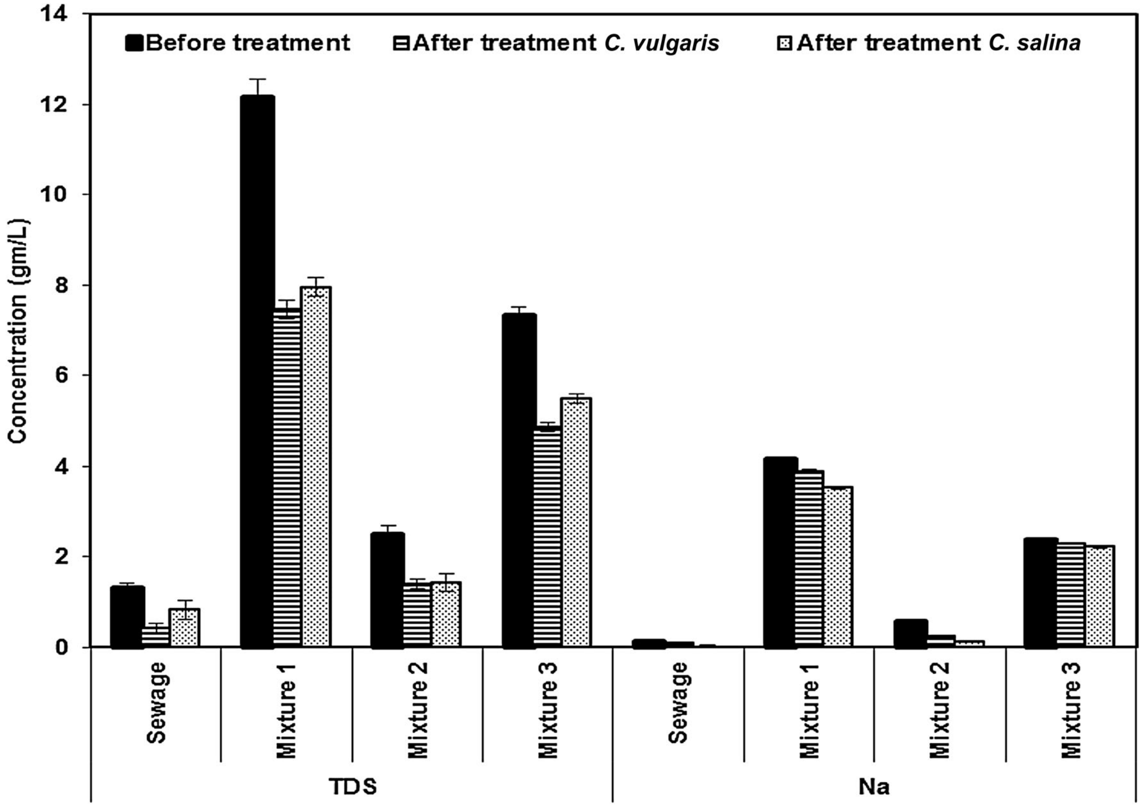

Fig. 2 Changes in TDS and Na^+ contents of water samples before and after treatment with algae. The values are the mean of 3 replicates ± SD

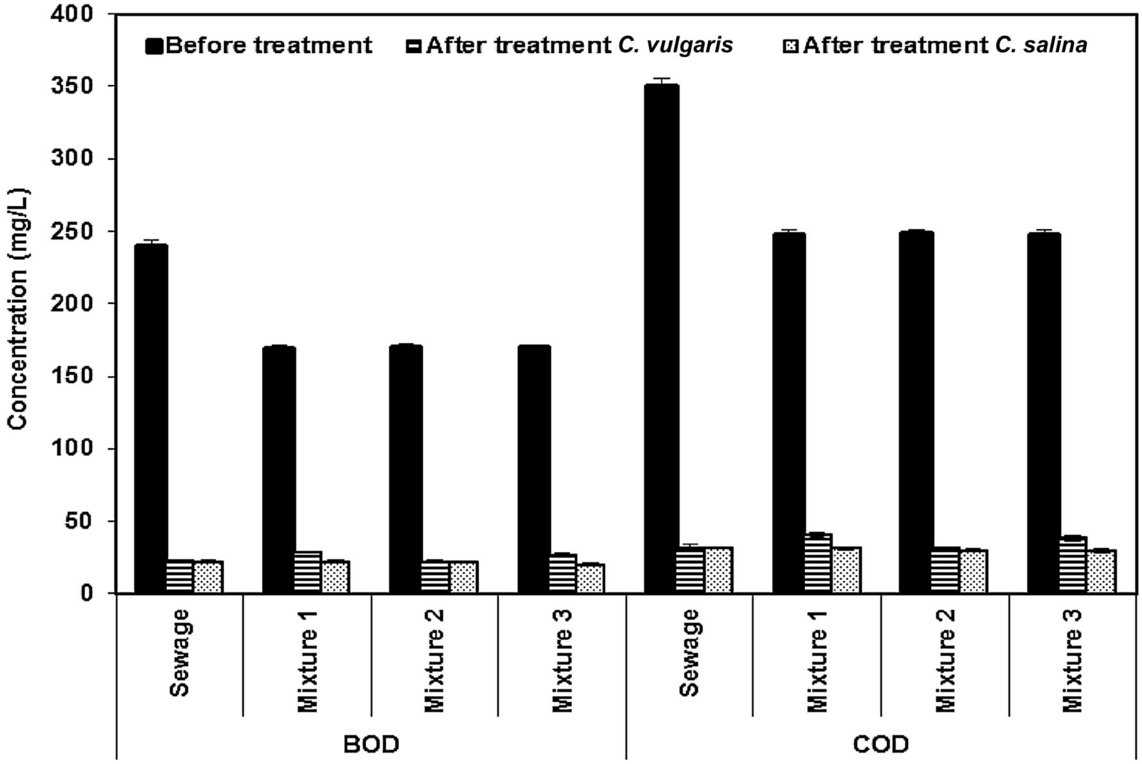

Fig. 3 The removal efficiency of BOD and COD from water samples by *C. vulgaris* and *C. salina.* The values are the mean of 3 replicates ± SD

wastewater and excess BOD usually depletes the dissolved oxygen. Sengar et al. (2011), Kshirsagar (2013), Azarpira et al. (2014) have reported very high reduction in BOD using different algal species and confirmed that microalgae are the best candidates for purification of wastewater and improvement in its physico-chemical parameters.

In addition, the reduction in COD values was ranged from 83.56 to 90.83 % by the treatment with *C. vulgaris* and ranged from 87.32 to 90.97 % in case of *C. salina* compared to untreated water samples (Fig. 3). Similar observation recorded by Sharma and Khan (2013), Elumalai et al. (2013), Azarpira et al. (2014). The chemical oxidations of carbon present in organic pollutants releasing carbon dioxide is responsible for reduction of COD value, similarly faster biodegradation and bioconversion of organic matter due to algae might be the additional reason Abdel-Raouf et al. (2012).

3.4 Removal of some nutrients from water samples

3.4.1 Nitrate

It is apparent from the data in Fig. 4 that algal treatment of water samples was accompanied by remarkable reductions in the nitrate content in water samples. Nitrate removal by *C. vulgaris* was 70.00, 60.00, 93.43 and 89.84 % in sewage and mixtures$_{1,2\&3}$, respectively, corresponding to 40.00, 53.33, 93.38 and 86.53 % by *C. salina* respectively, compared to untreated water samples. Similar observation recorded by Kshirsagar (2014).

3.4.2 Ammonia

The obtained results elucidated that remarkable decrease in ammonia concentration was observed in water samples after algal treatment. The removal efficiency ranged between 59.99 and 75.00 % by *C. vulgaris* treatment and 62.00 and 73.03 % by *C. salina* treatment compared to untreated water samples (Fig. 4).

Rao et al. (2011) reported that, *C. vulgaris* was able to reduce all forms of nitrogen substantially, and ammonia and nitrate levels, in particular. They revealed also that the phosphate removal efficiency of *C. vulgaris* was nearly 100 % in the wastewater.

3.4.3 Phosphate

From the data in Fig. 4, it is clear that the application of algal treatment reduced the phosphate contents in water samples after 10 days of treatment. The removal efficiency of *C. vulgaris* was ranged between 87.14 and 90.08 %. On

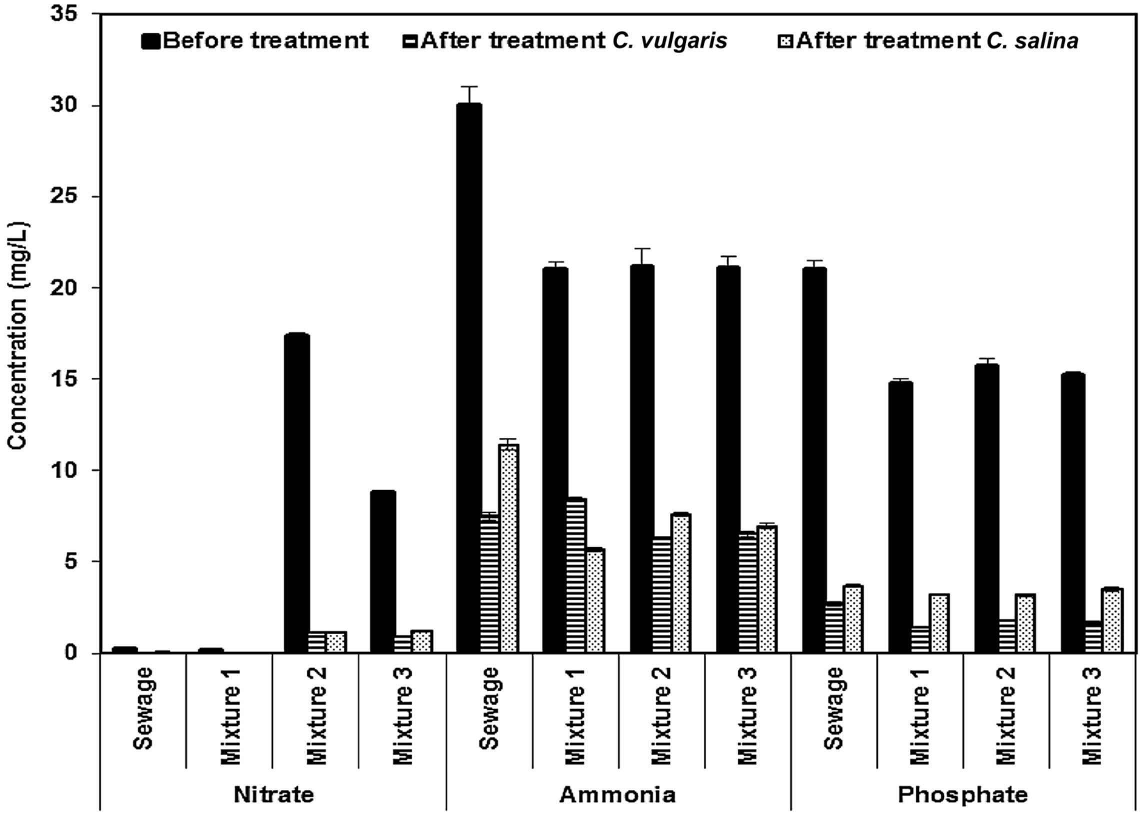

Fig. 4 The removal efficiency of nitrate, ammonia and phosphate from water samples by *C. vulgaris* and *C. salina*. The values are the mean of 3 replicates ± SD

the other hand, the removal efficiency of *C. salina* was ranged between 76.97 and 82.48 %. These results were in agreement with results obtained by Kshirsagar (2014).

The removal of P was mainly according to biological uptake mediated by the metabolic pathways of aerobic organisms (Maris et al. 1983). Phosphate removal by algae during phycoremediation is due to the utilization of phosphorus for growth. The phosphorus, which is used in the algal cells mainly for production of phospholipids, adenosine triphosphates (ATP) and nucleic acids, gets assimilated as inorganic orthophosphate and the uptake process is active, i.e. it requires energy (Becker 1994). Microalgae are able to assimilate phosphorus in excess, which is stored in the cells as polyphosphate granules, and magnesium and potassium are co-transported along with phosphate (Bitton 1990).

3.5 Removal of some minerals from water samples

3.5.1 Calcium

Application of *C. vulgaris* and *C. salina* treatment of water samples induced remarkable decrease in calcium contents (Fig. 5). *C. vulgaris* decreased the calcium contents from 105.23 to 2 mg/L in sewage, from 199.66 to 50 mg/L in mixture$_1$, from 137.86 to 6 mg/L in mixture$_2$ and from 168.76 to 26 mg/L in mixture$_3$. *C. salina* decreased the calcium contents from 105.23 to 4 mg/L in sewage, from 199.66 to 70 mg/L in mixture$_1$, from 137.86 to 30 mg/L in mixture$_2$ and from 168.76 to 46 mg/L in mixture$_3$ compared to untreated water samples.

3.5.2 Magnesium

The treatment with *C. vulgaris* induced a remarkable reduction in the magnesium contents amounted to 84.23, 58.46, 76.59 and 67.57 % in sewage and mixtures$_{1,2\&3}$, respectively. While *C. salina* reduced the magnesium contents by 58.75, 50.67, 55.40 and 40.20 %, respectively (Fig. 5).

3.5.3 Sodium

The data in Fig. 2 indicated that sodium content decreased markedly in water samples as a result of algal treatments.

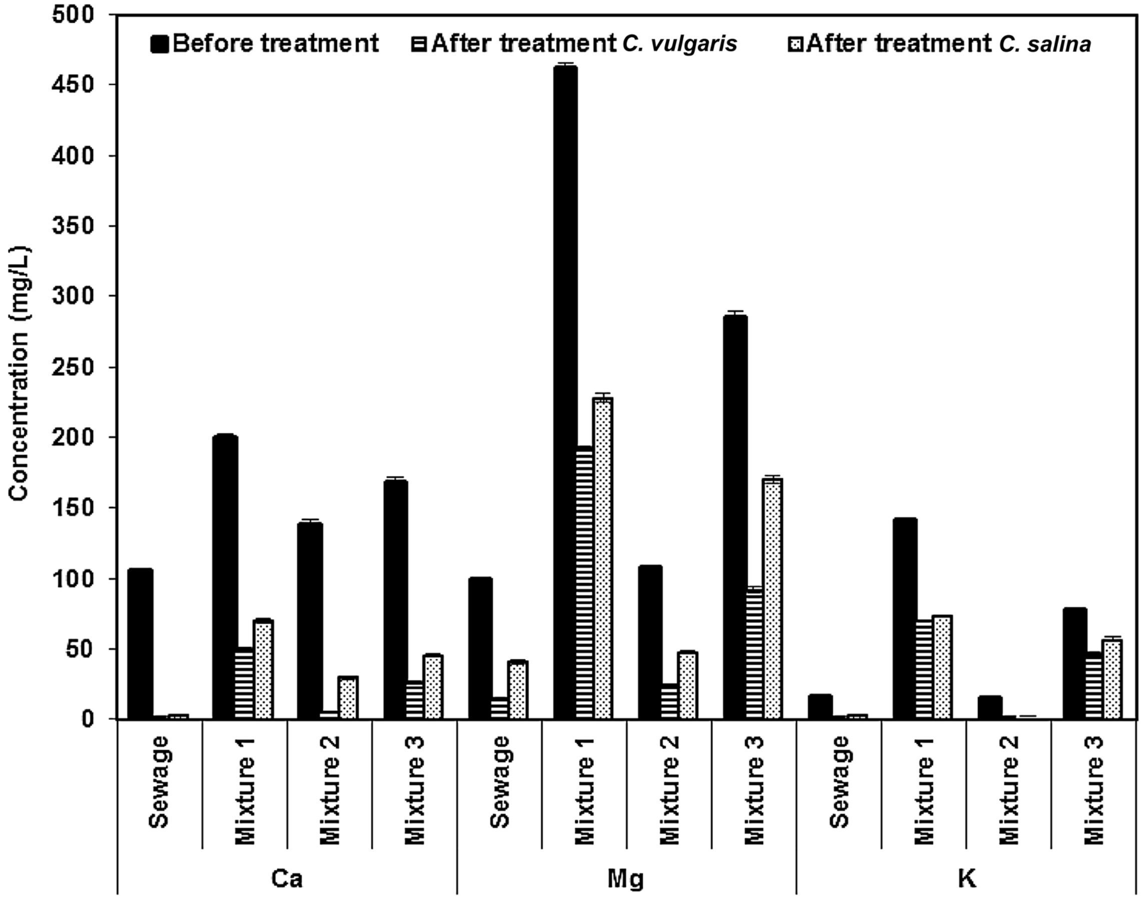

Fig. 5 The removal efficiency of Ca^{2+}, Mg^{2+} and K^{+} from water samples by *C. vulgaris* and *C. salina*. The values are the mean of 3 replicates ±SD

The percentage of reduction fluctuated between 3.38 and 54.39 % by *C. vulgaris* and 5.91 and 75.44 % by *C. salina* compared to untreated water samples.

3.5.4 Potassium

The treatment of water samples with algae was accompanied by remarkable decreases in the potassium content. The treatment with *C. vulgaris* reduced the potassium contents by 86.67, 50.00, 85.98 and 39.77 % in sewage and mixtures $_{1,\ 2\&\ 3}$, respectively. On the other hand, the treatment with *C. salina* reduced potassium contents by 77.50, 48.05, 84.28 and 27.25 %, respectively (Fig. 5). This observation is in agreement with Azab (2002) who reported that the application of algae for wastewater treatment exhibited variable percentages of reduction in minerals. In this context Rao et al. (2011) reported a drastic reduction in magnesium levels and moderate decrease in potassium levels by using *C. vulgaris* in phycoremediation, although they observed that reduction in sodium levels and a significant reduction in calcium.

3.6 Removal of heavy metals from water samples

The ability of *C. vulgaris* and *C. salina* to remove toxic heavy metals from water samples after 10 days incubation was presented in Table 1. The data elucidated that the removal efficiency differed according to the types of heavy metal and microorganism used. It was obvious that the chlorophyte algae used here exhibited high ability to remove heavy metals from water samples.

3.6.1 Zinc

The water samples that treated with algae exhibited remarkable reductions in zinc concentrations. The highest removal efficiency of *C. vulgaris* (64.96 %) was recorded in mixture$_2$. On the other hand, the removal efficiency of *C. salina* was ranged between 15.16 and 28.52 %.

3.6.2 Copper

The results showed that *C. vulgaris* was able to remove copper from sewage and mixtures$_{1,2\&3}$ by 98.64, 100,100 and 55.95 %, respectively, while the removal efficiency was 90.74, 100, 89.95 and 90.31 % by *C. salina* respectively.

3.6.3 Manganese

It is apparent from the results in Table 1 that, both *C. vulgaris* and *C. salina* showed a great ability to absorb manganese from the water samples. *C. vulgaris* absorbed all manganese contents in the water samples. In addition, the absorption of manganese by *C. salina* ranged between 89.94 and 93.71 %.

3.6.4 Nikel

From the data in Table 1, it can be noticed that there was a remarkable reduction in nickel contents after the application of biotreatment. The highest removal efficiency of *C. vulgaris* (100 %) was obtained in mixtures $_{1\&2}$ and the absorption reached to 90.95 and 51.11 % in sewage and mixture$_3$, respectively. while *C. salina* absorb 100 % of nickel contents in mixtures$_{1\&3}$ and 81.90 % in sewage. The lowest absorption value (13.61 %) of *C. salina* was recorded in mixture$_2$ compared with control (initial concentration).

3.6.5 Cobalt

The removal of cobalt from the water samples ranged between 32.29 and 59.28 % by *C. vulgaris* and ranged between 47.92 and 100 % by *C. salina* compared to untreated water samples.

3.6.6 Iron

The data in Table 1 revealed that high removal efficiency of iron was recorded by both algal species. *C. vulgaris* and *C. salina* absorb all iron contents in the water samples, except *C. salina* absorbs the iron contents by 97.24 % in mixture$_3$ after 10 days of incubation.

3.6.7 Chromium

The results indicated that *C. vulgaris* and *C. salina* show variable efficiencies in chromium removal. *C. vulgaris* absorb 21.74, 66.46 and 41.85 % of chromium content from mixtures$_{1,2\&3}$, respectively. While *C. salina* absorbs 5.13, 19.72 and 30.59 % of chromium contents from mixtures$_{1,2\&3}$, respectively. Chromium was not detected in the sewage water.

3.6.8 Cadmium and lead: cadmium and lead were not detected in the water samples

The results of our investigation are in agreement with Hamdy (2000), El-Sheekh et al. (2005) who found that the different metals uptake depended upon the type of biosorbent, which has different accumulation affinities towards the tested elements. In addition, Kaplan et al. (1986) found that the efficiency of absorption of metals depends on the nature and charge of the cell wall polysaccharides of *C. stigmaphora* which contain high

Table 1 The removal efficiency of Heavy metals (ug/ml) and total coli-form bacteria from water samples using *Chlorella vulgaris* and *Chlorella salina* after 10 days of incubation

Water samples	Treatment	Parameter	Zn	Cu	Mn	Ni	Co	Fe	Cr	Total coli-form bacteria
Sewage	Before		36.80 ± 0.60	81.00 ± 1.50	108.70 ± 2.20	21.00 ± 0.50	0	110.50 ± 1.50	0	>2400
	After biotreatment	*C. vulgaris*	23.90* ± 0.20	1.10* ± 0.05	0.00	1.90* ± 0.20	–	0.00	–	46
		Removal efficiency %	35.05	98.64	100.00	90.95	–	100.00	–	–
		C. salina	29.80* ± 0.30	7.50* ± 0.10	9.20* ± 0.10	3.80* ± 0.20	–	0.00	–	13
		Removal efficiency %	19.02	90.74	91.54	81.90	–	100.00	–	–
Mixture 1	Before		28.85 ± 0.50	56.79 ± 1.20	76.69 ± 1.50	15.15 ± 0.10	0.57 ± 0.02	92.35 ± 2.35	27.09 ± 0.50	>2400
	After biotreatment	*C. vulgaris*	12.60* ± 0.20	0.00	0.00	0.00	0.30* ± 0.01	0.00	21.20* ± 0.20	33
		Removal efficiency %	56.33	100.00	100.00	100.00	47.37	100.00	21.74	–
		C. salina	24.40* ± 0.40	0.00	5.60* ± 0.10	0.00	0.00	0.00	25.70* ± 0.20	<2
		Removal efficiency %	15.42	100.00	92.70	100.00	100.00	100.00	5.13	–
Mixture 2	Before		27.11 ± 0.50	56.73 ± 1.23	76.36 ± 1.06	14.70 ± 0.10	3.84 ± 0.04	88.75 ± 0.05	9.84 ± 0.04	>2400
	After biotreatment	*C. vulgaris*	9.50* ± 0.10	0.00	0.00	0.00	2.60* ± 0.10	0.00	3.30* ± 0.03	4
		Removal efficiency %	64.96	100.00	100.00	100.00	32.29	100.00	66.46	–
		C. salina	23.00* ± 0.50	5.70* ± 0.10	4.80* ± 0.10	12.70* ± 0.20	2.00* ± 0.05	0.00	7.90* ± 0.20	49
		Removal efficiency %	15.16	89.95	93.71	13.61	47.92	100.00	19.72	–
Mixture 3	Before		27.98 ± 0.40	56.76 ± 0.10	76.53 ± 2.50	14.93 ± 0.50	2.21 ± 0.10	90.55 ± 1.50	18.47 ± 0.37	>2400
	After biotreatment	*C. vulgaris*	23.80* ± 0.30	25.00* ± 0.20	0.00	7.30* ± 0.30	0.90* ± 0.02	0.00	10.74* ± 0.04	23
		Removal efficiency %	14.94	55.95	100.00	51.11	59.28	100.00	41.85	–
		C. salina	20.00* ± 1.00	5.50* ± 0.10	7.70* ± 0.20	0.00	0.70* ± 0.01	2.50* ± 0.20	12.82* ± 0.32	<2
		Removal efficiency %	28.52	90.31	89.94	100.00	68.33	97.24	30.59	–

The values are the mean of 3 replicates ± SD

* The mean difference is significant at the 0.05 level

amount of uronic acids and consequently, a high copper-complexing capacity compared to the less charged *C. salina* polysaccharides. Similar finding was concluded by Karamushka et al. (1994) who mentioned that the rate and magnitude of accumulation of heavy metals like gold substantially depended on the physiological state of the cells of *Spirulina platensis*. Moreover, the removal of heavy metal attributed to mechanisms other than the tendency of cells for bioaccumulation (Pena-Castro et al. 2004).

In our study, it was observed that green algal cells cultivated in water samples with high metal contents also accumulated higher metal contents. This observation has been emphasized by Priyadarshani et al. (2011) who mentioned that algal cells cultivated in the media with very high metal contents also accumulated higher metal contents. However, in few cases, the metal uptake was independent on the external metal concentration.

3.7 Removal of coli-form bacteria

The number to total Coli-form bacteria was >2400 cell/100 mL water samples before biotreatment in all water samples mixtures. The treatment with *C. vulgaris* and *C. salina* exerted considerable decrease in the total Coli-form bacteria. The maximum decrease was recorded in mixture$_2$ by *C. vulgaris* treatment (4 cell/100 mL water samples). On the other hand, the highest effect of treatment with *C. salina* on total Coli-form bacteria was observed in mixture$_1$ and mixture$_3$ where the number of total Coli-form bacteria decreased to <2 cell/100 mL water samples. This ensured that the main removal mechanisms of pathogen and coli-forms are ruled by algal activity (Curtis 1994). Moawad (1968) observed that the environmental factors which were favorable for algal growth were unfavorable for the survival of coli-forms. A similar observation on the percent reduction of coli-forms and *Salmonella* was also made by Colak and Kaya (1988). Mezrioui et al. (1994) found that green algae reduce *Vibrio cholerae* abundance more than *E. coli* (Fecal contamination bacteria) and that the die off of *E. coli* appears to be more reduced in presence of cyanobacteria.

4 Conclusion

From the results, it is concluded that recycling and reusing of different mixtures of water samples is possible by Phycoremediation using different algal species like *C. vulgaris* and *C. salina*. The present investigation showed that both the algal species had very good potential to reduce the toxic level of all physico-chemical parameters. These experiments confirm that *C. vulgaris* and *C. salina* may be considered efficient nutrient removers.

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