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Removal of heavy metals (Cr, Cd, Ni and Pb) using fresh water algae (*Utricularia tenuissima, Utricularia tenuis* & *Zygogonium* ericetorum) from contaminated water

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Abstract

A study was conducted to check the efficiency of different fresh water algae for removing heavy metals (Cr, Cd, Ni and Pb) from contaminated water. The three most abundant indigenous algal species namely *Ulothrix tenuissima, Oscillatoria tenuis and Zygogonium* ericetorum were collected from fresh water channels of Parachinar, Pakistan and brought to the laboratory of Soil and Environmental Sciences Department at the University of Agriculture, Peshawar Pakistan for proper identification. To check the efficiency for removing heavy metals artificial contaminated water was prepared and was inoculated with mix culture of above mentioned algae and incubated for 10 days. After incubation algal species were removed from water through centrifugation and was dried, digested and analyzed for heavy metals. The results showed that the concentration of all heavy metals was substantially reduced in the algal inoculated contaminated water. The analysis of algal biomass showed that considerable amount of metals and other elements were recovered in algae. Among the tested algal species, *Zygogonium ericetorum* showed maximum removal Ni(99.40ug) and Cr(66.84ug) from contaminated water followed by *Oscillatoria tenuis* with 84ug(Ni) and 64.83ug(Cr) respectively. However *Oscillatoria tenuis* showed maximum removal of Cd(41.00ug) than the other algal species. Similarly *Zygogonium ericetorum* showed maximum removal of Pb (451ug) followed by *Ulothrix tenuissima* where 441ug was recorded. Highest amount Cd, and Ni were recovered in *Zygogonium ericetorum biomass while highest amount of Cr and Pb were recorded in the biomass of Oscillatoria tenuis.* Finally it could be concluded that algae have efficiently removed heavy metals from contaminated water. Further research is needed to test other algal species for removal of heavy metal and other elements from the contaminated water.

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Introduction

The surface water quality is of great importance due to its effects on health of human as well as plants. Earth atmosphere and natural waters are polluted by municipal, industrial and agricultural wastes (McGrath *et al.,* 2001). The presence of heavy metals in these wastes is of major concern because of their toxicity, bioaccumulating tendency, and threat to human life and the environment (Igwe & Abia, 2006). Heavy metals finally reach to water where they impair its use for drinking, industrial, agricultural, recreation or other purposes (Nriagu, 1988, Donmez, 2002, Sheng *et al.,* 2004). This polluted water causes health hazards to different consumers such as animals, human and crops (Correia *et al*., 2000; Dixie *et al.,* 1998; Derek, 1999; Dias *et al.,* 2002). Moreover farm productivity decrease in toxic heavy metals polluted areas (Gosavi *et al.,* 2004). Among the heavy metals, Cadmium, Chromium, Lead and Nickel are the big four heavy metals posing the greatest hazard to human health and cause serious damage to ecosystem (Shanab *et al.,* 2012). In Pakistan, due to lack of proper facilities of waste disposal only 1 % of wastewater is treated before being discharged directly into water bodies (Lewis *et al*., 2007). Removal of heavy metals from water system is the initial step in an ecological restoration process. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis and evaporation are not cost effective and have many disadvantages like incomplete metal removal and toxic sludge generation (Brauckmann, 1990; Volesky, 2001). Hence biological approach has been considered as an alternative strategy to remove heavy metals from contaminated water. The ability of algae, bacteria, fungi and yeast has been extensively studied in the last two decades. Of the microorganism studied, algae are gaining popularity, due to the fact that algae, are the most efficient and effective organism to remove heavy metals from the contaminated water (Gekeler *et al.,* 1998). A number of algal species are known to remove nitrogen, phosphorous and heavy metals from contaminated water (Laliberte *et al*., 1994; Oswald, 1988; Pavasant *et al*., 2006; Yoshida *et al.,* 2006). Unfortunately little attention is paid to the biological treatment of contaminated water in Pakistan. This contaminated water loaded with heavy metals generated in industries is discharged directly into rivers and irrigation channels. These heavy metals accumulate in agriculture fields and crops primarily because of use of contaminated waters for irrigation (Khan *et al*., 2003; Murtaza *et al*., 2007; Mussarat *et al*., 2007). The crops *raised* on such contaminated soils contained a number of heavy metals (in leaves and fruits) in amount beyond the permissible limits for human consumption. In such case, it is important that less expensive and environmentally friendly biological approach will have to be explored for removal of toxic heavy metals from contaminated water. Therefore the present study was focused on removal of heavy metals using some fresh water algal species.

Materials and methods

Collection and Processing of Algae

Three algal samples were collected from different fresh water channels of Parachinar, the capital of Kurram Agency of Pakistan. The samples were collected by hand picking and preserved in 5% formalin (Sarim and Farida, 1978). Then were brought to the laboratory of Soil and Environmental Sciences Department, the University of Agriculture, Peshawar Pakistan.

Procedure for Identification of Algae

Algal sample was transferred to the microscope slide and observed under the light microscope (40x magnification) and specimens identified with the help of guidelines as described in Prescott (1951), and Tiffany and Britton (1952). Algal specimens were first transferred to a clean Petri dish and homogenized using a couple of drops of formalin from specimen bottle. A thin smear was transferred to a clean dry microscope slide and covered gently with microscope slide. The slide was observed under the light microscope initially at 10x and finally at 40x magnification. Five slides per sample were prepared in similar manner and 15 random areas per slide were observed for algal species. The specimen was identified taxonomically with the help of guidelines given in the literature (Prescott, 1951; Tiffany and Brittonn, 1952).

Testing of Algae for the Removal of heavy metals from contaminated water

Desired numbers of fresh algal samples were collected from the selected areas and were tested for the removal of heavy metals from contaminated water. Artificial contaminated water was prepared as per detail given in Table 1.

Known amount (50 ml) of contaminated water was transferred to a container in triplicate and was inoculated with desired type of algae. The container was covered with aluminum foil to avoid evaporation. Algae were removed from water through centrifugation after 10 days and was dried, digested and analyzed for heavy metals.

Measurement of Heavy Metals in Algae

Heavy Metals in algal sample were determined by the wet digestion method of Watson *et al.* (1992). In this method, algal sample was digested with 10 ml conc HNO_3 (overnight treatment) and 4 ml perchloric acid at 100 to 350ºC for about 1 ½ hr. After cooling, the digest was filtered through Whatman No. 42 and diluted to 25 ml, and then concentrations of heavy metals such as Chromium, Cadmium, Lead and Nickel were evaluated by atomic absorption spectrophotometer (Shimadzu, Model AA-6300).

Statistical Analysis

Descriptive statistics was used for calculation of means of three replications (Bhatti, 2006).

Results and discussion

Chromium (Cr)

The results obtained on the effect of algal inoculation on Cr removal from contaminated water are presenting in Table 2. The results revealed that algae removed considerable amount of Cr from contaminated water during 10 days of incubation period. On average, the Cr concentration decreased from 81.61ug per 50mL in without algae to 17.55 ug per 50mL with algal inoculation during 10 days. These results indicated that algae removed 64.05 ug per 50mL Cr where as only 32.19 ug of it was recovered in algal biomass. No considerable differences were observed among the three algal samples for their efficiency in removing Cr from contaminated water. But results indicated that the amount of Cr recovered in algal biomass was less than its amount removed by algae.

Table 1. Concentration of heavy metals and other elements in artificial contaminated water.

Element		X	(mg L^{-1})	XX	(mg L^{-1})
Pb	Cd	5.00	0.01	10.0	1.0
Ni	Cr	0.02	0.10	3.0	2.0

X: maximum recommended concentration in irrigation water.

XX: concentration in artificial contaminated water.

Table 2. Effect of algal inoculation on removal of chromium (Cr) from artificial contaminated water during 10 days of incubation.

Type of algae	Cr concentration contaminated water (ug per 50 mL)		Removal of Crby algae from Contaminated water (ug per 50 mL)	Cr (ug) recovered in algal biomass
	Without algal inoculation	With algal Inoculation		
Ulothrix tenuissima	76.50	16.00	60.50	27.58
Oscillatoria tenuis	80.33	15.50	64.83	34.66
Zygogonium ericetorum	88.00	21.16	66.84	34.33
Means	81.61	17.55	64.05	32.19

The possible reason could be that some algal biomass might have been lost in the process of collection, drying and weighing. Among the algal species, *Zygogonium* ericetorum was found to effective in removal of 66.84ug per 50mL Cr from contaminated water followed by *Oscillatoria tenuis* (64.83ug per 50 mL). The Minimum amount of Cr was removed by *Ulothrix tenuissim*. In algal biomass highest amount of Cr (ug) was recovered from *Oscillatoria tenuis* (34.66ug) followed by *Zygogonium* ericetorum (34.33ug) and *Ulothrix tenuissim* (27.58ug). Similar results were obtained by the researcher Dwivedi *et al.* (2010) who explored the absorbing ability of metals (Cr, Cu, Fe, Mn, Ni and Zn) and metalloid (As) by green algae and blue green microalgae growing naturally in selected Cr-contaminated sites. The maximum absorbance of Cr was shown by *Phormedium bohneri* (8550 μg g^{-1} dw) followed by *Oscillatoria tenuis* (7354 μg g^{-1} dw), *Chlamydomonas angulosa* (5325 μg g^{-1} dw), *Ulothrix tenuissima* (4564 μg g^{-1} dw), and *Oscillatoria nigra* (1862 μg g^{-1} dw).

Table 3. Effect of algal inoculation on removal of cadmium (Cd) from artificial contaminated water during 10 days of incubation.

Type of algae	Cd concentration in contaminated water (ug per 50 mL)		Removal of Cd by algae from Contaminated water (ug per 50 mL)	Cd (ug) recovered in algal biomass
	Without algal Inoculation	With algal inoculation		
Ulothrix tenuissima	40.83	5.16	35.67	21.58
Oscillatoria tenuis	46.00	5.00	41.00	23.08
Zygogonium ericetorum	42.66	5.50	37.44	52.16
Means	43.16	5.22	38.03	32.27

* Values are mean of three replicates.

Table 4. Effect of algal inoculation on removal of Lead (Pb) from artificial contaminated water during 10 days of incubation.

Type Of algae	Pb concentration in contaminated water (ug per 50 mL)		Removal of Pb by algae from Contaminated water (ug per 50 mL)	Pb (ug) recovered in algal biomass
	Withoutalgal inoculation	With algal inoculation		
Ulothrix tenuissima	480	38	441	195
Oscillatoria tenuis	447	33	414	225
Zygogonium ericetorum	480	29	451	221
Means	469	34	436	214

* Values are mean of three replicates.

The results also specify that the phytoplankton diversity was changed by Chromium pollution. Blue green algae represented the dominant community where Cr concentration was higher (11.84 and 2.27 mg l^{-1}) (r = 0.695), whereas green algae showed negative correlation with respect to Cr concentration (r = −0.567). So it was found that different algal species were capable to grow in Cr-contaminated sites and to accumulate considerable amounts of Chromium. Deng *et al.* (2006) investigated the reduction of Cr (VI) in the presence of *Chlorella vulgaris*. Gupta *et al.* (2008b) reported, 1 kg dry weight biomass of filamentous algae *Spirogyra species* removed approximately 14.7 x 10^3 mg Cr (VI) from polluted water. Onyancha *et al.* (2008) studied the Cr removal from tannery waste waters. The algal species Spirogyra *condensate* and *Rhizoclonium hieroglyphicum* have been used for the absorbance of Cr from tannery waste water. Results revealed that *Spirogyra condensate* exhibited maximum absorbance of about 14 mg Cr (III) g^{-1} of algae whereas Rhizoclonium *hieroglyphicum* had 11.81 mg of Cr (III) g^{-1} of algae.

Table 5. Effect of algal inoculation on removal of nickel (Ni) from artificial contaminated water during 10 days of incubation.

Type of algae	Ni concentration in contaminated water (ug per 50 mL)		Removal of Ni by algae from Contaminated water (ug per 50 mL)	Ni (ug) recovered in algal biomass
	Without algal inoculation	With algal inoculation		
Ulothrix tenuissima	74.66	8.66	66.00	24.58
Oscillatoria tenuis	99.50	8.66	90.84	41.50
Zygogonium ericetorum	110.56	11.16	99.40	48.50
Means	94.90	9.49	85.41	38.19

* Values are mean of three replicates.

Cadmium (Cd)

The results obtained on the effect of algal inoculation on removal of Cd from contaminated water are presented in Table 3. Algal species removed considerable amount of Cd from contaminated water during 10 days of incubation. On average, the Cd concentration decreased from 43.16 ug per 50mL in without algae to 5.22 ug per 50mL with algal inoculation during 10 days. These results indicated that algae removed 38.03 ug per 50mL Cd where as only 32.27 ug of it was recovered in algal biomass. No considerable differences were observed among the three algal samples for their efficiency in removing Cd from contaminated water. But it is noticed that the amount of Cd recovered in algal biomass was less than its amount removed by algae. For removal of Cd from contaminated water *Oscillatoria tenuis* was remarkable than other algal species with 41.00 ug per 50 ml contaminated water. . Highest amount of Cd was recovered from the biomass of *Zygogonium* ericetorum (52.16 ug) followed by Oscillatoria spp (23.08ug) while minimum Cd was recovered from the *Ulothrix tenuissima* (21.58ug) biomass. Our results support the findings of Castro *et al.* (2004), who conducted an experiment in which microalga *Scenedesmus incrassatulus* was grown as single metal species and as mixtures of two or three metals. He reported that Cr and Cd(II) increased the removal percentages of both these metals. *S. incrassatulus* was able to remove all the tested metals (25–78%).Chromium(VI) was efficiently removed in continuous cultures as compare to batch culture, due to actively growing algae, absorbance of chromate could be increased. Wang *et al.* (2010) also investigated the assessment of the growth of green algae *Chlorella sp.* and found that how well the algal growth removed Cd from the wastewaters. Kaonga *et al.* (2008) studied *Spirogyra aequinoctialis* and reported that it has the ability of absorbing Mn, Cd and Pb from the polluted water.

Lead (Pb)

The results obtained on Pb concentration in contaminated water in the absence or presence of algal inoculation and its subsequent recovery in algal biomass are presented in Table 4. All the three algal samples removed substantial amount of Pb from contaminated water (50mL) during 10 days of incubation. The maximum 451 ug of Pb was removed by *Zygogonium* ericetorum followed by *Ulothrix tenuissima* (414 ug per 50mL) whereas minimum Pb was removed by *Oscillatoria tenuis*. On average, algae removed 436 ug per 50mL from contaminated water during 10 days of incubation. The amount of Pb recovered by different algal samples in their biomass was in accordance to the disappearance of Pb from contaminated water due to some algal species. It was however noticed that the amount of Pb recovered in algal biomass was lower than its disappearance from contaminated water, in all the three samples. This may be due to the loss of some algal biomass during the process of collecting, drying and weighing the algal biomass. Similar experiment was conducted by Vilar *et al.* (2007) who studied the efficiency of *Gelidium spp* to absorb Cu ions and Pb (II) from the industrial wastes. Lodi *et al.* (2003) conducted an experiment on *Spirulina spp* and found that its biomass decrease the concentration of NO_3 and PO_4 from polluted water. Gupta and Rastogi (2008a) studied the adsorption of Pb(II) from aqueous solutions by biomass of *Spirogyra sp*. It was found that one gram biomass of Spirogyra sp. absorb 140 mg Pb(II) metal.

Nickel (Ni)

The results obtained regarding Ni concentration in contaminated water in the presence or absence of algal inoculation and its subsequent recovery in algal biomass are shown in Table 5. On average, the Ni concentration decreased from 94.90 ug per 50mL in without algae to 9.49 ug per 50mL with algal inoculation during 10 days. Overall mean showed that algal species removed 85.41 ug per 50mL Ni from the contaminated water whereas only 38.19 ug Ni was recovered in their biomass. Though there was a remarkable difference for their efficiency in removing Ni from contaminated water. The maximum 99.40 ug per 50mL of Ni was removed by *Zygogonium* ericetorum followed by *Oscillatoria tenuis* where it removed 90.84 Ni from the contaminated water (50mL) while lowest (66.00ug) amount of Ni was removed by *Ulothrix tenuissima*. And it was also noticed that removal of Ni from contaminated water was greater than its amount recovered in algal biomass. The possible reason could be that some algal biomass might have been lost in the process of collection, drying and weighing. These findings that algal species are effectively removing Ni^{2+} from contaminated water are in consistent with the results of Wong *et al.* (2000) who performed an experiment to compare the efficiency of two unicellular green algae, *Chlorella vulgaris* and *Chlorella miniata* in removing Ni^{2+} from nickel solutions. The Ni^{2+} removal performance of *C. vulgaris* was significantly lower than that of *Chlorella miniata*. The maximum Ni^{2+} removal by *C. vulgaris* and *Chlorella miniata* was 641.76 and 1367.62 µg g^{-1}, respectively. Guha *et al.* (2001) studied the algal species (*Shewanella alga)* which was found to effectively reduced amount of Cr from different industrial wastes.

Conclusion

The results of the current findings demonstrated that algal species significantly remove the heavy metals from the contaminated water. It can also be concluded from the results that among algal species, *Zygogonium* ericetorum and *Oscillatoria tenuis* were superior in performance in removing the heavy metals than *Ulothrix tenuissima*.

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