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Sorption of mercury, cadmium and lead by microalgae

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ABSTRACT Forty-six strains of microalgae from BIOTECH Culture Collection (NSTDA), Microbiological Resources Center (TISTR) and Institute of Research and Food Development, 3 strains collected from Thai natural and industrial areas and 3 strains from the Gottingen University culture collection were tested for Mercury (Hg), Cadmium (Cd) and Lead (Pb) removal in aqueous solutions. In green algae, the highest Hg removal was by *Scenedesmus sp.*, *Chlorococcum sp.*, *Chlorella vulgaris var. vulgaris* and *Fischerella sp.*, (97%, 96%, 94% and 92%, respectively). In blue green algae, highest Hg removal was by *Lyngbya spiralis*, *Tolypothirx tenuis*, *Stigonema sp.*, *Phormidium molle* (96%, 94%, 94% and 93%, respectively). For Cd removal in green algae, the highest was by *Chlorococcum sp.*, T5, *Fischerella sp.*, *Chlorella vulgaris var. vulgaris* and *Scenedesmus acutus* (94%, 94%, 91%, 89% and 88%, respectively). In blue green algae, highest Cd removal was by *Lyngbya heironymusii*, *Gloeocapsa sp.*, *Phormidium molle*, *Oscillatoria jasorvensis* and *Nostoc sp.* (97%, 96%, 95%, 94% and 94%, respectively). In green algae, highest Pb removal was by *Scenedesmus acutus*, *Chlorella vulgaris var. vulgaris*, *Chlorella vulgaris*, *Scenedesmus vacuolatus* and *Chlorella vulgaris*, (89%, 88%, 85%, 85% and 84%, respectively). In blue green algae, highest Pb removal was by *Nostoc punciforme*, *Oscillatoria agardhii*, *Gloeocapsa sp.*, *Nostoc piscinale*, *Nostoc commune* and *Nostoc paludosum* (98%, 96%, 96%, 94%, 94% and 92%, respectively). *Scenedesmus acutus* had the highest concentration factor (CF) at 3,412, 4,591 and 4,078 for Hg, Cd and Pb, respectively. *Tolypothrix tenuis* had the highest maximum adsorption capacity of 27 mg Hg/g dry wt. at a minimum concentration of 1.04 mg/l, *Scenedesmus acutus* had the highest maximum adsorption capacity of 110 mg Cd/g dry wt. at a minimum concentration of 48 mg/l and *Chlorella vulgaris* had the highest maximum adsorption capacity of 127 mg Pb/g dry wt. at a minimum concentration of 130 mg/l.

KEYWORDS: microalgae, concentration factor, heavy metal removal, mercury, cadmium and lead.

INTRODUCTION

The release of heavy metals from industries into the environment has resulted in many problems for both human health and aquatic ecosystems.[1, 2] One strategy to reduce heavy metal solution is to use microorganisms. Microalgae, due to their ubiquitous occurrence in nature, have been studied extensively in this regard. They can sequester heavy metal ions by adsorption and absorption, as do by other microorganisms. This ability may be induced in response to stress by toxic heavy metal exposure.[3] The use of microalgae for metal removal has the potential to achieve greater performance at a lower cost than conventional wastewater treatment technologies. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metals from dilute aqueous waste. New biosorbent materials are already being used.[4]

In this study, a number of microalgal strains potentially suitable for Cd, Pb and Hg removal in aqueous solution were selected and the ranking table as removal index and concentration factor index were also calculated. Efficiency of removal, maximum adsorption capacity (q_{max}) and binding constants (K_b) were determined.

MATERIALS AND METHODS

Microorganisms and culture conditions

The strains of algae used and their sources are shown in Table 1. Altogether, 52 strains were tested. Of these, 18 strains were green algae and 34 blue green algae. The experiments were performed as described previously.[5]

Collection of microalgae

Surface water from canals and rivers was sampled in 500 ml sampling bottles. In addition, microalgae were harvested using nylon screens (30 μm) drawn across the surface of the same water body for addition to the same sampling bottle at the same sampling place and time. Some filamentous strains were picked up from rocks or soil at the sampling sites. Temperature and pH were measured at the water surface. Samples were transported to the laboratory and further proceeded within a day and a 2 ml aliquot from each was added to individual test tubes containing 8 ml of modified Medium 18 and incubated at 28°C with illumination (white fluorescent light 4,000 lux) on a 12 hour light and 12 hour dark cycle for 2 weeks.

The samples were then streaked on agar modified Medium 18 plates with 1.5 % agar and incubated at 28°C under the same illumination program for 2 weeks or until the appearance of algal colonies. Single algal colonies were selected and re-streaked on agar medium plates several times to obtain unialgal cultures. Single colonies were maintained in 2 ml of modified Medium 18 under the same conditions, and with weekly transfer for stock cultures.

Cultivation system

Microalgae were inoculated into modified Medium 18 consisting of (per liter) 1.50 g $NaNO_3$, 380 mg $MgSO_4.7H_2O$, 120 mg K_2HPO_4, 110 mg $CaCl_2.2H_2O$, 70 mg NaCl, 10 mg $Fe_2(SO_4)_3.nH_2O$, 9 mg $Na_2MoO_4.2H_2O$, 3 mg H_3BO_3, 2 mg $MnSO_4.4H_2O$, 0.3 mg $ZnSO_4.7H_2O$, 0.08 mg $CuSO_4.5H_2O$, and 0.04 mg $CoCl_2.6H_2O$. The pH was adjusted to 7.5 and the modified Medium 18 was then autoclaved. Microalgae were cultivated in 200 ml test tubes under the conditions previously described (1) for 7- 10 days before use as inoculum for experiments.

Chemical reagents

$Cd(NO_3)_2$ and $Pb(NO_3)_2$ were from Unilab, Australia. $HgCl_2$, HNO_3 90% and $NaNO_3$ were from Carlo Erba Italy. HNO_3 69%, $ZnSO_4.7H_2O$ and $CoCl_2.6H_2O$ were from BDH Laboratory Supplies, England. H_2O_2, NaOH, $MgSO_4.7H_2O$, K_2HPO_4 and $CaCl_2.2H_2O$ were from Merck, Germany. $Fe_2(SO_4)_3.nH_2O$, $NaMoO_4.2H_2O$, H_3BO_3, $MnSO_4.4H_2O$ and $CuSO_4.5H_2O$ were from May and Baker ltd. Dagenham, England. Agar powder (commercial grade) was from Whongtavee Company, Thailand. Methyl alcohol was from the government pharmaceutical organization, Thailand. NaCl was from Riedel-de HaenAG Seeize-Hannover, Germany.

Preparation of Hg, Cd and Pb solutions

Hg, Cd and Pb were prepared as 100 ml stock solutions of $HgCl_2$, Cd $(NO_3)_2$ and $Pb(NO_3)_2$, in water purified by Milli-Q Jr. (Milli-Q UF Plas, Millipore, France), sterilized using membrane filter (Millipore comp. 0.22µ) and kept in the refrigerator. Heavy metal concentrations were calculated from the equation; $M_1V_1 = M_2V_2$ where M_1 was the stock solution concentration, M_2 the required concentration, V_1 the volume of the stock solution and V_2 the volume of the solution at required concentration.

Hg, Cd and Pb removal

For Hg, Cd and Pb removal in aqueous solution experiments, a cell concentration of 1 g dry wt./l from inoculum was used. Cells (50 mg dry wt.) were centrifuged and washed twice with deionized water before resuspension in 50 ml of each heavy metal solution at 1 mg/l, pH 7, in 200-ml Erlenmeyer flasks. After mixing at 200 rpm, 28°C, for 30 min, the solution was centrifuged and the supernatant solution sampled for its heavy metal concentration by atomic absorption spectrophotometry. The centrifuged cells were oven dried at 100°C until constant weight. Heavy metal removal ability was calculated from the equation $(C_i\text{-}C_f)/\ C_i \times 100$ (%), where C_i was the initial concentration (mg/l) and C_f the equilibrium (final) heavy metal concentration (mg/l).

Selected microalgal strains (50 mg dry wt.) were suspended in 50 ml of various heavy metal solutions. Hg concentrations were 0.5, 1, 2, 5, 10, 20, 40, 80 and 150 mg/l, Cd concentrations were 5, 10, 20, 40, 80, 100, 150 and 200 mg/l and Pb concentrations were 5, 10, 20, 40, 80, 160, 200, 400 and 600 mg/l. Each sample was incubated at 200 rpm, 28°C for 30 min and then sampled for metal concentration using an atomic absorption spectrophotometer. Adsorption of Hg, Cd and Pb, q (mg/g dry wt.), was determined q_{max} and K_b were calculated by using Langmuir equation as :

$$q = \frac{q_{\max}C}{K_b + C}$$

Where q is the heavy metal adsorbed to the solid phase (mg/g dry wt.)

q_{max} is the maximum adsorption capacity (mg/g dry wt.)

K_b is the binding constant (mg/l)

C is the equilibrium concentration of heavy metal in solution (mg/l)

Atomic absorption analysis

For Hg analysis, samples were diluted using milli-Q water before being measured in the range of 25-40 ppb by using the graphite system wavelength at 253.7nm on an atomic absorption spectrophotometer

(Varian, Spectr AA600, Australia). For Cd and Pb analysis, the sample solution was measured by atomic absorption spectrophotometer (Z-8000 Varience, Hitachi, Japan) using the graphite system wavelength at 228.8 nm. Sample solutions were fixed using 0.05 M HNO_3.

Dry weight determination

To separate cells from aqueous solutions, blue green algae were filtered using a nylon screen size 30 µm while green algae were collected by centrifugation at 4000 rpm for 10 min. Cells were washed 3 times with deionized water and dried in an oven at 100°C for 24 hours or until constant weight. Samples were cooled in a dessicator for 30 min before dry weight was measured.

Calculation of concentration factors

The concentration factor (CF) was used to compare heavy metal removal ability among different strains of microalgae. It was calculated as follows:

$$CF = \frac{\mu g \text{ metal removed/g dry wt.}}{\mu g \text{ metal in solution/ml solution}}$$

Digestion of microalgal cells

Dried algal samples were digested with 1 ml of conc. HNO_3 in a dry thermo bath (Boekel series 02344, USA) until the solution was dry. After cooling, 1 ml of 30% H_2O_2 was added. The sample was then further digested for 1 hour or until the solution had evaporated to dryness. This step was repeated 2 times until a white ash was obtained. Five ml of 0.5 N HNO_3 was added to the digested sample and heavy metal concentration was measured using an atomic absorption spectrophotometer as described above. All the experiments were performed in three replicates.

RESULTS

Collection of microalgae

Ten strains of microalgae were screened from natural water and industrial areas. Three of them as green algae (T1, T5 and T10) were used in this experiment.

Hg, Cd and Pb removal in aqueous solution

Hg, Cd and Pb removal capacity by some green algae and blue green algae was high (Table 1). Within the green algae, Hg removal efficiency was highest at 97%, 96%, 94% and 92% for *Scenedesmus sp.*, *Chlorococcum sp.*, *Chlorella vulgaris var. vulgaris* and *Fischerella sp.*, respectively and for blue green algae, Hg removal efficiency was highest at 96%, 94%, 94%, 93% and 92% for *Lyngbya spiralis*, *Tolypothrix tenuis* BBC 100, *Stigonema sp.* BCC 92, *Phormidium molle* and *Lyngbya heironymusii.Within the green algae,* Cd removal efficiency was highest at 94%, 94%, 91%, 89% and 88%, for *Chlorococcum sp.*, T5, *Fischerella sp.*, *Chlorella vulgaris var. vulgaris* and *Scenedesmus acutus* and for blue green algae Cd efficiency was highest at 97%, 96%, 95%, 94% and 94% for *Lyngbya heironymusii, Gloeocapsa sp., Phormidium molle, Nostoc sp. and Oscillatoria jasorvensis,* respectively. Within the green algae, Pb removal efficiency was highest at 89%, 88%, 85% , 85% and 84% for *Scenedesmus acutus*, *Chlorella vulgaris var. vulgaris*, *Chlorella vulgaris* BCC 15, *Scenedesmus vacuolatus* and *Chlorella vulgaris* CCAP211/11B and for blue green algae, Pb removal efficiency was highest at 98%, 96%, 96%, 94%, 94% and 92% for *Nostoc punctiforme*, *Oscillatoria agardhii*, *Gloeocapsa sp.*, *Nostoc piscinale*, *Nostoc commune and Nostoc paludosum,* respectively.

In order to simplify the ability of each microalga to remove each or all three heavy metals, we converted % removal into removal index by dividing % removal by the highest % removal and multiply the value obtained by 10. This would facilitate the comparison of each heavy metal removal ability by various microalgae. At the same time the additive total index in the last column of Table 1 was very helpful as to the effectiveness of each microalga in removing the combined Hg, Cd and Pb in the wastewater. The concentration factor index and the total index given in Table 2 were also obtained in a similar manner to that of Table 1.

Concentration factors for Hg, Cd and Pb removal

Concentration factors (CF) for heavy metal removal are shown in Table 2. For green algae *Chlamydomonas sp.*, *Scenedesmus acutus*, *Scenedesmus pertoratus* and *Myxosarcina sp.* had the highest CFs for Hg (3,590, 3,412, 2,711 and 2,210, respectively) while *Scenedesmus acutus*, *Scenedesmus pertoratus*, *Myxosarcina sp.* and *Chlorella vulgaris* BCC 15 had the highest CFs for Cd (4,591, 3,730, 2,742 and 2,601, respectively) and *Scenedesmus acutus*, *Scenedesmus pertoratus*, *Chlorella vulgaris* BCC 15 and T10 had the highest CFs for Pb (4,078, 3,083, 2,293 and 1,442, respectively). For blue green algae, *Nostoc punctiforme, Nostoc sp.*, *Tolypothrix tenuis* BCC 100 and *Phormidium angustissimum* had the highest CFs for Hg (4,218, 2,383, 1,976, 1,967, respectively), while *Tolypothrix tenuis*,

Table 1. Heavy metals removal in aqueous solution by microalgae.

Microalgae	% Removal			Removal index*			Total index
	Hg	Cd	Pb	Hg	Cd	Pb	
Green algae							
Chlorella vulgaris var. vulgaris BCC 15	94	89	88	9.7	9.5	9.9	29.0
Scenedesmus acutus TFRPD 1020	85	88	89	8.8	9.4	10.0	28.1
Chlorococcum sp. BCC 16	96	94	71	9.9	10.0	8.0	27.9
Chlorella vulgaris BCC 15	74	86	85	7.6	9.1	9.6	26.3
Chlorella vulgaris CCAP211/11B	75	83	84	7.7	8.8	9.4	26.0
Scenedesmus pertoratus IFRPD 1010	79	83	80	8.1	8.8	9.0	26.0
T5	73	94	75	7.5	10.0	8.4	26.0
Scenedesmus vacuolatus CCAP211	81	73	85	8.4	7.8	9.6	25.7
*Chlorella sp.*BCC 13	71	88	77	7.3	9.4	8.7	25.3
Kirchnerriella sp. TISTR	72	80	81	7.4	8.5	9.1	25.0
Chlorella saccharophilla CCAP211/1A	70	81	79	7.2	8.6	8.9	24.7
T10	70	81	74	7.2	8.6	8.3	24.1
T1	68	84	72	7.0	8.9	8.1	24.0
Fischerella sp. BCC 22	92	91	35	9.5	9.7	3.9	23.1
Scenedesmus sp. BCC 82	97	58	58	10.0	6.2	6.5	22.7
Chamydomonas sp. BCC 11	86	86	30	8.9	9.1	3.4	21.4
Myxosarcina sp. BCC 59	91	53	53	9.4	5.6	6.0	21.0
Chlorella eillipsoidea BCC 12	81	84	23	8.4	8.9	2.6	19.9
Blue green algae							
Phormidium molle BCC 71	93	95	90	9.7	9.8	9.2	28.7
Lyngbya heironymusii BCC 41	92	97	80	9.6	10.0	8.2	27.7
Oscillatoria jasorvensis BCC 56	89	94	85	9.3	9.7	8.7	27.6
Oscillatoria agardhii BCC 52	73	90	96	7.6	9.3	9.8	26.7
Tolypothrix tenuis TISTR 8063	81	88	88	8.4	9.1	9.0	26.5
Rivularia sp. BCC 80	86	88	76	9.0	9.1	7.8	25.8
Lyngbya spiralis BCC 42	96	80	73	10.0	8.2	7.4	25.7
Gloeocapsa sp. BCC 25	50	96	96	5.2	9.9	9.8	24.9
Nostoc sp. BCC 50	86	94	58	9.0	9.7	5.9	24.6
Phormidium angustissimum BCC 68	74	87	77	7.7	9.0	7.9	24.5
Tolypothrix tenuis BCC 100	94	53	90	9.8	5.5	9.2	24.4
Nostoc punctiforme BCC 48	66	73	98	6.9	7.5	10.0	24.4
Stigonema sp. BCC 90	92	89	52	9.6	9.2	5.3	24.1
Stigonema sp. BCC 92	94	80	59	9.8	8.2	6.0	24.1
Calothrix sp. BCC 8	86	88	59	9.0	9.1	6.0	24.1
Calothrix parietina TISTR 8093	50	88	91	5.2	9.1	9.3	23.6
Nostoc paludosum BCC 46	44	92	92	4.6	9.5	9.4	23.5
Calothrix marchica var. intermedia BCC 6	92	87	43	9.6	9.0	4.4	22.9
Calothrix sp. BCC 5	37	84	88	3.9	8.7	9.0	21.5
Calothrix sp. TISTR 8130	40	82	86	4.2	8.5	8.8	21.4
Hapalosiphon welwitschii BCC 34	85	75	47	8.9	7.7	4.8	21.4
Nostoc commune BCC 76	43	69	94	4.5	7.1	9.6	21.2
Cylindrospermum sp. BCC 20	83	65	52	8.6	6.7	5.3	20.7
Nostoc piscinale BCC 47	22	82	94	2.3	8.5	9.6	20.3
Mastogocladus sp. BCC 36	89	78	29	9.3	8.0	3.0	20.3
Calothrix sp. BCC 10	92	89	13	9.6	9.2	1.3	20.1
Hapalosiphon hibernicus BCC 27	84	90	13	8.8	9.3	1.3	19.4
Oscillatoria amoena BCC 53	12	83	89	1.3	8.6	9.1	18.9
Anabaena sp. BCC 2	68	85	29	7.1	8.8	3.0	18.8
Nostoc punctiforme BCC 49	46	84	51	4.8	8.7	5.2	18.7
Stigonema sp. BCC 90	82	90	5	8.5	9.3	0.5	18.3
Nostoc micropicum BCC 77	26	72	80	2.7	7.4	8.2	18.3
Calothrix marchica BCC 4	84	57	20	8.8	5.9	2.0	16.7
Hapalosiphon sp. BCC 30	86	62	11	9.0	6.4	1.1	16.0

* Strains of algae and blue green algae arranged in descending order according to additive removal index. The removal index for each metal is the % removal divided by the highest % removal times 10. Total index represents the sum of the removal index of all three metals.

Hapalosiphon sp., Nostoc micropicum, Anabaena sp. had the highest CFs for Cd (3,028, 2,416, 2,404 and 2,368, respectively) and *Gloeocapsa sp., Nostoc commune, Nostoc paludosum* and *Tolypothrix tenuis* had the highest CFs for Pb (4,154, 3,269, 3,022 and 2,963, respectively).

Based on results for removal ability, high concentration factor and high tolerance on agar plate cultures (data not shown), 3 strains of green algae (*Chlorella vulgaris* BCC 15, *Chlorella vulgaris* CCAP 211/11B and *Scenedesmus acutus*) were selected for the further experiments. In addition, 2 strains of blue green algae (*Tolypothrix tenuis* TISTR 8063 and *Calothrix parietina*) were selected for the further study because they had high removal capacity and were easily separated from water by filtration because of filamentous morphology. This would be amenable for application in simple outdoor conditions.

Hg, Cd and Pb removal at various heavy metal concentrations

Hg, Cd and Pb removal by the three selected strains of green algae and two selected strains of blue green algae were assessed using the Langmuir equation for maximum adsorption capacities (q_{max}) and binding constants (K_b) as shown in Table 3. This isotherm can be described by the Langmuir adsorption isotherm, which is often reported for biological adsorption by other kinds of biomass[5]. The Langmuir equation is given by

$$q = \frac{q_{\max} C}{K_b + C} \qquad (1)$$

Where q is the metal adsorption to the solid phase (mg/g dry wt.), $q_{\max}$ is the maximum adsorption capacity (mg/g dry wt.), K_b is the binding constant (mg/l), and C is the equilibrium metal concentration (mg/l). The Langmuir equation 1 can be rearranged as

$$\frac{C}{q} = \frac{C}{q_{max}} + \frac{K_b}{q_{max}} \qquad (2)$$

The adsorption of Hg, Cd and Pb fitted well with the Langmuir equation. From equation 2, the maximum adsorption capacities ($q_{\max}$) for Hg by the two blue green algae strains, *Tolypothrix tenuis* TISTR 8063 and *Calothrix parietina* were 27 and 19 mg Hg/g dry wt., respectively. For the green algae, *Chlorella vulgaris* BCC 15, *Chlorella vulgaris* CCAP 211/11B and *Scenedesmus acutus*, they were 18, 16 and 20 mg Hg/g dry wt., respectively. For Cd, the green algae, gave the highest capacity at 110 mg Cd/g dry wt. followed by the two blue green algae, *Tolypothrix tenuis* TISTR 8063 and *Calothrix parietina* at 90 and 79 mg Cd/g dry wt. For Pb adsorption the green alga *Chlorella vulgaris* BCC 15 gave the best capacity at 127 mg Pb/g dry wt. followed by *Scenedesmus acutus* at 90 mg Pb/g dry wt.

With respect to the binding constant value (K_b), lower values indicated higher affinity for the heavy metal. For Hg, *Tolypothrix tenuis* TISTR 8063 had the lowest K_b value (0.01) and thus the highest affinity. For Cd, *Tolypothrix tenuis* and *Calothrix parietina* had the highest affinity ($K_b = 0.62$ and 0.92, respectively) and for Pb, *Chlorella vulgaris* BCC 15 and *Chlorella vulgaris* CCAP 211/11B had highest affinity ($K_b = 3.06$ and 3.06, respectively) (Table 3).

Figure 1 shows the equilibrium isotherm for the adsorption of Hg, Cd and Pb by three microalgae and two blue green algae. *Tolypothrix tenuis* was the best among other algae, adsorbing Hg at 27 mg Hg/g dry wt. at a minimum concentration of 1.04 mg/l (Fig. 1 A). For Cd adsorption, *Scenedesmus acutus* was best at 110 mg Cd/ g dry wt. at a minimum concentration of 48 mg/l (Fig. 1 B). For Pb adsorption, *Chlorella vulgaris* was best at 127 mg Pb/g dry wt. at a minimum concentration of 130 mg/l (Fig. 1 C).

DISCUSSION

Hg, Cd and Pb removal in aqueous solutions

Our results indicated that both green algae and blue green algae had very high Hg, Cd and Pb removal capacities and that adsorption occurred within the relatively short time of 10-20 min.[5] The green alga *Chlorella vulgaris* is often used to study adsorption of heavy metals[6-9] but in this study, the green alga *Scenedesmus acutus* had a higher removal capacity. In general heavy metals are taken in by blue green algal cells by adsorption followed in sequence by metabolism-dependent intracellular cation intake as applicable to Zn[10], Cu, Cd and Zn[11], Cd [12], Al[13], Ni[14] and Hg.[15] In other studies, blue green algae had lower capacities than those found herein. For example, Kitjaharn[16, 17] showed that the blue green algae *Aphanothece halophytica* and *Spirulina platensis* could remove only 22% and 35% Pb, respectively, from battery factory wastewater. In spite of this, we selected blue green algae to test for heavy metal adsorption because they have high growth rates and are easy to separate from solution by simple filtration.

Table 2. Heavy metals removal in aqueous solution by microalgae calculated as concentration facctor (CF).

Microalgae	Concentration Factor (CF)			Concentration index*			Total index
	Hg	Cd	Pb	Hg	Cd	Pb	
Green algae							
Scenedesmus acutus IFRPD 1020	3412	4591	4078	9.5	10	10	29.5
Scenedesmus pertoratus IFRPD1010	2711	3730	3083	7.6	8.1	7.6	23.3
*Chamydomonas sp.*BCC 11	3590	2217	796	10	4.8	2.0	16.3
Chlorella vulgaris BCC 15	1785	2601	2293	5.0	5.7	5.6	16.3
Myxosarcina sp. BCC 59	2210	2742	422	6.2	6.0	1.0	13.2
T10	1460	1878	1442	4.1	4.1	3.5	11.7
Scenedesmus sp. BCC 82	1262	2164	749	3.5	4.7	1.8	10.0
Scenedesmus vacuolatus CCAP211	1274	1351	1358	3.5	2.9	3.3	9.7
Kirchnerriella sp. TISTR	1066	1372	1066	3.0	3.0	2.6	8.6
Fischerella sp. BCC 22	1656	1354	405	4.6	2.9	1.0	8.5
T1	1060	1537	793	3.0	3.3	1.9	8.2
Chlorella sp. BCC 13	857	1184	1167	2.4	2.6	2.9	7.9
Chlorella eillipsoidea BCC 12	1778	982	228	5.0	2.1	0.6	7.7
Chlorella saccharophilla CCAP211/1A	869	1196	895	2.4	2.6	2.2	7.2
Chlorella vulgaris var. vulgaris BCC15	1150	805	914	3.2	1.8	2.2	7.2
Chlorococcum sp. BCC 16	1131	1086	660	3.2	2.4	1.6	7.2
Chlorella vulgaris CCAP211/11B	488	980	863	1.4	2.1	2.1	5.6
T5	327	774	586	0.9	1.7	1.4	4.0
Blue green algae							
Nostoc punctiforme BCC 4	4218	1110	2735	10.0	3.7	6.6	20.2
Tolypothrix tenuis TISTR 8063	919	3028	2963	2.2	10.0	7.1	19.3
Nostoc paludosum BCC 46	1656	1980	3022	3.9	6.5	7.3	17.7
Nostoc sp. BCC 50	2383	2210	1331	5.6	7.3	3.2	16.2
Lyngbya spiralis BCC 42	1552	2329	1788	3.7	7.7	4.3	15.7
Phormidium angustissimum BCC 68	1967	1847	1919	4.7	6.1	4.6	15.4
Anabaena sp. BCC 2	1937	2368	780	4.6	7.8	1.9	14.3
Nostoc commune BCC 76	819	1355	3269	1.9	4.5	7.9	14.3
Gloeocapsa sp. BCC 25	1424	238	4154	3.4	0.8	10.0	14.2
Nostoc micropicum BCC 77	740	2404	1767	1.8	7.9	4.3	13.9
Cylindrospermum sp. BCC 20	1656	1537	1866	3.9	5.1	4.5	13.5
Nostoc punctiforme BCC 49	1283	2181	1301	3.0	7.2	3.1	13.4
Hapalosiphon sp. BCC 30	1608	2461	422	3.8	8.1	1.0	13.0
Calothrix sp. BCC 8	451	1688	2489	1.1	5.6	6.0	12.6
Tolypothrix tenuis BCC 100	1976	1135	1675	4.7	3.7	4.0	12.5
Oscillatoria agardhii BCC 52	1264	1117	1998	3.0	3.7	4.8	11.5
Rivularia sp. BCC 80	1380	1493	1356	3.3	4.9	3.3	11.5
Lyngbya heironymusii BCC 41	1625	1341	1317	3.9	4.4	3.2	11.5
Calothrix sp. TISTR 8130	825	1722	1478	2.0	5.7	3.6	11.2
Calothrix parietina TISTR 8093	1096	1292	1784	2.6	4.3	4.3	11.2
Oscillatoria jasorvensis BCC 56	1923	1758	313	4.6	5.8	0.8	11.1
Calothrix sp. BCC 5	725	1749	1404	1.7	5.8	3.4	10.9
Hapalosiphon welwitschii BCC 34	1636	1263	954	3.9	4.2	2.3	10.3
Nostoc piscinale BCC 47	533	1454	1764	1.3	4.8	4.2	10.3
Calothrix marchica var. intermedia BCC 6	1626	1391	774	3.9	4.6	1.9	10.3
Oscillatoria amoena BCC 53	269	1942	945	0.6	6.4	2.3	9.3
Stigonema sp. BCC 91	1155	15.6	489	2.7	5.0	1.2	8.9
Calothrix sp. BCC 10	776	1906	283	1.8	6.3	0.7	8.8
Stigonema sp. BCC 90	1396	1493	86	3.3	4.9	0.2	8.4
Stigonema sp. BCC 92	995	1035	1083	2.4	3.4	2.6	8.4
Mastogocladus sp. BCC 36	1135	1141	427	2.7	3.8	1.0	7.5
Calothrix marchica BCC 4	1263	1025	338	3.0	3.4	0.8	7.2
Hapalosiphon hibernicus BCC 27	718	1422	194	1.7	4.7	0.5	6.9
Phormidium molle TISTR 8246	953	807	799	2.3	2.7	1.9	6.8

* Strains of algae and blue green algae arranged in descending order according to additive Concentration Factor index. The CF index for each metal is the CF divided by the highest CF times 10. Total index represents the sum of the CF index of all three metals.

Table 3. Maximum adsorption capacities (q max) and binding constants (K b) for various microalgae with Hg, Cd and Pb.

Microalgae	Heavy Metal					
	Hg		Cd		Pb	
	Kb	q max (mg/g dry wt.)	Kb	q max (mg/g dry wt.)	Kb	q max (mg/g dry wt.)
Blue green algae						
Tolypothrix tenuis TISRT 8063	0.01	27	0.62	90	7.83	31
Calothrix parietina TISTR 8093	0.03	19	0.92	79	6.87	45
Green algae						
Chlorella vulgaris BCC 15	0.15	18	3.83	76	3.06	127
Chlorella vulgaris CCAP211/11B	0.03	16	1.60	62	3.06	39
Scenedesmus acutus IFRPD 1020	0.11	20	1.57	110	9.45	90

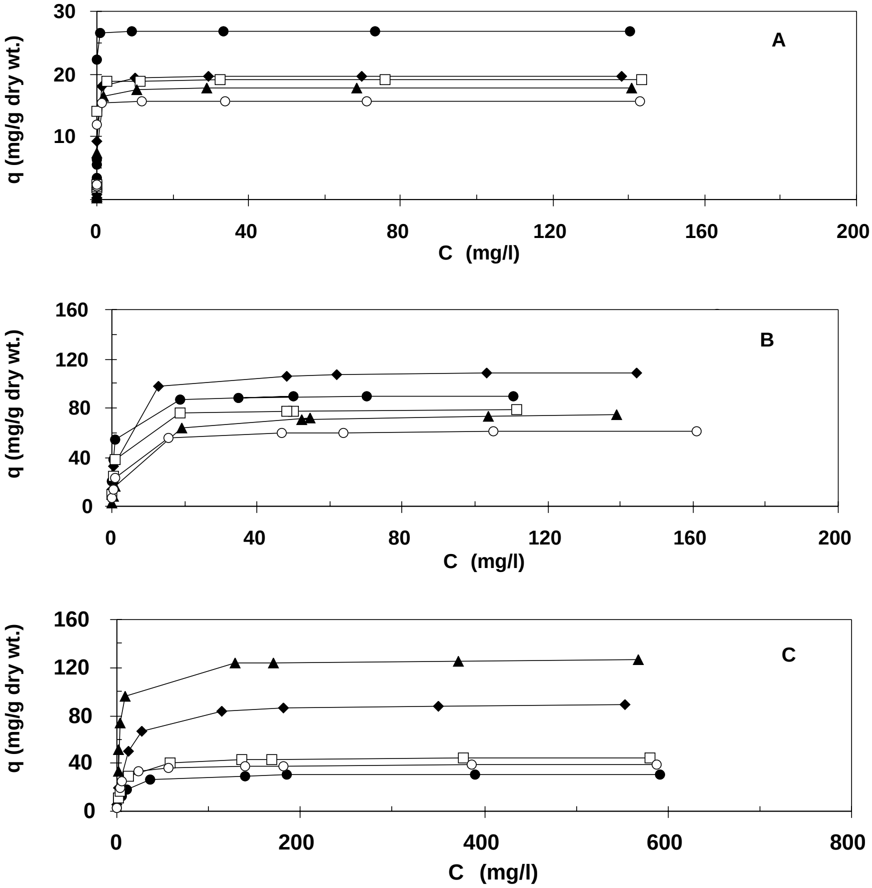

Fig 1. Adsorption of Hg (A), Cd (B) and Pb (C) by five microalgae at various heavy metal concentrations. Microalgae cells (0.5 g dry wt.) were suspended in 50 ml solution containing various concentrations of heavy metals. Symbols: ●, *Tolypothrix tenuis*; □, *Calothrix parietina*; ▲, *Chlorella vulgaris*: ○, *Chlorella vulgaris* (CCAP211/11B); ◆, *Scenedesmus acutus*.

Concentration factors for Hg, Cd and Pb

The concentration factor (CF) is the ratio of the metal concentration in dry biomass to that in aqueous solution and it is defined as (μg metal removed/g dry wt.)/(μg metal in solution/ml solution). It is used to compare heavy metal removal ability among different microbial strains. In our study *Chlamydomonas sp.* gave a CF of 3,590 for Hg and this was far lower than 9,060 reported for *Chlamydomonas sp.* by Hassett.[18] By contrast, our

CF of 4,218 for Hg for the blue green alga *Nostoc punctiforme* was much higher than 1,950 reported by Hassett for *Nostoc sp.*.[18] So also was our CF of 2,404 for Cd for *Nostoc micro-picum* when compared to reports of 1,140-1,470 µg/g for *Nostoc sp.*.[18] Cd removal values reported for other cyanobacteria included 3,250 µg/g for *Chro-ococcus paris*[11] and 1,160-1,310 µg/g for *Oscillatoria sp.*.[18] Our value for *Oscillatoria sp.* was nearly the same at 1,117-1,942 µg/g. We found that *Nostoc commune* gave the highest CF of 3,269 for Pb and this compared to 1,920 in *Nostoc sp.* by Hassett.[18] He used a microplate technique to determine CF for various algal species, under various conditions (eg, metal concentration and type, culture age, pH, etc) and found that green algae and blue green algae accumulated the metals Hg, Cd, Pb and Zn better than green algae, while green algae accumulated Cd better than blue green algae. In other studies, concentration factors for heavy metals in the green alga *Scenedesmus obliguus* (11 days old, 3 hours, 22 °C in the dark) were 5,040 for Hg and 3,600 for Cd. These differed somewhat from the values reported herein for *Scenedesmus acutus* at 3,412 for Hg and 4,591 for Cd. For the blue green alga, *Nostoc* reported concentration factors were 1,950 for Hg and 1,920 for Cd[19] but the CF values herein were only 234 and 343, respectively. Differences probably depended on strain and on experimental conditions such as strain source, culture age, pH and time of exposure.

Hg, Cd and Pb removal at various heavy metal concentrations

Srikrajib[20] reported maximum adsorption capacities (q_{max}) of 103 and 95 mg Cd /g dry wt from the isotherm of Cd for *Sargassum polycystum* dried at 80 °C and 100 °C, respectively. Herein, the highest maximum adsorption capacity (q_{max}) for Cd was found with *Scenedesmus acutus*, at 110 mg Cd/g dry wt. Values for other strains ranged from 62 to 90 mg Cd/g dry wt. *Sargassum polycystum* and *Scenedesmus acutus* had similar maximum adsorption capacities (q_{max}) although the former comprised dead cells and the latter living cells. Aksu et al[21] studied adsorption of lead from waste water using the green alga *Chlorella vulgaris* in a single stage batch reactor and reported a value for maximum adsorption capacity (q_{max}) of 83 mg Pb /g dry wt. They also found that adsorption increased with increasing pH and temperature. Herein, the highest maximum adsorption capacity (q_{max}) for lead was found with *Chlorella vulgaris*, at 127 mg Pb/g dry wt, while values for other strains ranged from 31 to 90 mg Pb/g dry wt. Value differences for *Chlorella vulgaris* were probably due to differences in experi-mental conditions.

The ranking table in Table 1 and Table 2 were done as % removal index and concentration factor index in order to rank the ability of those microalgae. Some algae had % removal indexes of 9-10 for all three metals but none did so for CF index. Based on these results, we suggested that the mixture of algae should be used to obtain the best result in real wastewater condition which usually contains more than one metal type. For mixed cultures the organisms to be combined should have similar growth requirements and should be physiologically and biochemically compatible under hard-water field conditions or real wastewater system.

Strains that will be useful as biosorbents must have the ability to function in hard water. Thus, tests for Cd removal, for example should also be carried out in solutions containing Ca^{2+} or Mg^{2+} at concentrations usually present in hard water. Only strains that pass such tests will be suitable for application under outdoor conditions.[1]

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