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Accumulation of heavy metals by the marine microalga *Chlorella marina* Butcher (Chlorophyceae) in culture.

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Abstract

The green alga *Chlorella marina* Butcher was investigated in the laboratory for accumulation of six heavy metals, such as chromium, cadmium, cobalt, copper, nickel and lead. The cultures were grown in estuarine water medium mixed with different concentrations of the individual metals. The baseline metal concentrations in *C. marina* were shown to be in an order of Cu> Pb> Co> Cd> Ni> Cr under normal culture conditions. In the experimental groups, the metal uptake capacity of the alga was Ni> Pb> Co> Cu> Cd> Cr at equilibrium. When assessed using the bioconcentration factors, metal accumulation by *C. marina* was demonstrated to be the most efficient at a concentration of 0.001 mg L^{-1} for Cr, Cd and Co and at 0.01 mg $L^{§1}$ for Cu and Pb. This study suggests that *C. marina* can be a source of mineral supplements in mariculture.

Keywords: bioaccumulation, bioconcentration factor, green alga, heavy metal concentration, mariculture, mineral supplement

INTRODUCTION

Microalgae are sensitive indicators of environmental change and are widely used in the assessment of risk and development of environmental regulations for metals (Levy *et al*., 2007). Microalgae are able to accumulate metal ions from aquatic solutions in a short time by biosorption in uncomplicated systems, without any problems of toxicity (Fathi and Falkner, 1997; Fathi *et al*., 2000; Giusti, 2001; Afkar *et al*., 2010).

Among biosorbents, green algae are attractive as they are ubiquitous in natural environment, have large surface area to volume ratio and high binding affinity to pollutants (Chong *et al*., 2000). *Chlorella marina* is a green microalgal species, with a spherical to ellipsoidal shape having diameter of 2–3µm with a surface area to volume ratio of 1.1. Species of microalgae belonging to the genus *Chlorella* have been widely utilized in marine fish hatcheries in view of transferring the essential fatty acids, EPA (Eicosapentaenoic acid) and DHA (Decosa hexaenoic acid), and other dietary component from the algae *via* the rotifers to fish larvae (James *et al*., 1987; James and Abu- rezeq, 1988; Langton and Waldock, 1981; Devresse *et al*., 1990; Hana *et al*., 2007).

Little is known, however, about mineral concentrations and metal uptake capacities in marine *Chlorella* species while such information is increasingly demanded in the assessment of algal food values (Aksu *et al*., 1997; Aksu *et al*., 1999; Wong *et al*., 2000; Mehta and Gaur, 2001; Tam *et al*., 2001; Han *et al*., 2006; Hana *et al*., 2007). As bioaccumulation of heavy metals occurs mainly through a food chain with phytoplankton at the base, it is also important to understand the patterns of heavy metal accumulation by microalgal species such as *C. marina* which have been used as food in mariculture.

The present study was to investigate the accumulation of the heavy metals, chromium, cadmium, cobalt, copper, nickel and lead, by *Chlorella marina*, against a wide range of concentrations. Baseline metal concentrations in *C. marina* were assumed to be constituted in cultures from the control group. Secondly, it is also aimed to estimate the potential ability of the alga for use in wastewater treatment based on a comparison of bioconcentration factors between *C. marina* and other commercially used marine microalgae. It is anticipated that this work will provide information for the further utilization of *C. marina* in either mariculture or wastewater treatment or the microalgal food industry.

MATERIALS AND METHODS

Cultures of *Chlorella marina* Butcher were provided by the Central Marine Fisheries and Research Institute, Vizhinjam, Tamil Nadu, South India. The algal cultures were grown in 500 mL Ehrlenmeyer flasks containing 200 mL estuarine water medium as previously described (Walne, 1946) and exposed constantly to fluorescent tubes with a light intensity of 1000 μmol m^{-2} s^{-1}. The containers were held in a shaker working at a

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speed of 90 oscillations min^{-1} and at a temperature of 25ºC.

Aliquots with cultures in mid-log growth phase were inoculated into new culture medium at concentrations between 3×10^6 and 4×10^6 cells mL^{-1} (20 mL aliquots: 200 mL medium) for subsequent tests and grown under the conditions described above. The culture medium for the experimental groups was mixed with individual metal salts, including $K_2Cr_2O_7$, $CdCl_2/5H_2O$, $CoCl_2/6H_2O$, $CuSO_4/5H_2O$, $Ni(NO_3)_2/6H_2O$ and $Pb(NO_3)_2$, to designated nominal concentrations (Table 1).

One litre aliquots of stationary phase cells (13 d after inoculation) collected from five replicate cultures were centrifuged at 600g for 20 minutes and the pelleted cells were washed with estuarine water. The samples were subsequently dried under vacuum at 60ºC for 6 h before weighing. Metal concentrations in dried materials were measured using an inductively coupled plasma optical emission spectrophotometer (Perkin Elmer, Optima 2100). Data were organised based on triplicate experiments. One way ANOVA was used to interpret the results.

The bioconcentration factors were calculated using the following formula (Sadiq, 1992)

$$BF = \frac{\text{metal concentration in algal cultures}}{\text{metal concentration in culture medium}}$$

RESULTS

Table 1 gives the average concentrations of the metals Cr, Cd, Co, Cu, Ni and Pb in cultures of *Chlorella marina* from control and experimental groups. In the control group, which was free from heavy metal contamination, metal concentrations were shown to be in the order of Cu> Pb> Co> Cd> Ni>Cr (Fig. 1).

In the experimental groups, the equilibrium concentration of different metals were found to be varied (Figure. 2a &b). The six heavy metals displayed concentrations in an order of Ni> Pb> Co> Cu> Cd >Cr in the cultures when the respective equilibrium was reached. This did not, however, constantly reflect the metal uptake capacity of the alga. When exposed to an equal concentration of 1.0 mg L^{-1}, for example, Pb level in the algal cultures was not significantly different from Cd (P>0.10) and was conspicuously lower than Cu (Table 1). Accumulation of Ni was lower than half that of Co at a concentration of 0.50 mg L^{-1} metal.

The metal uptake capacity of *Chlorella marina* was also estimated based on bioconcentration factors, which indicate a constant proportion between the internal and external metal concentrations. Except for Cd, most metals were shown to have two peaks along the BF curves (Figure 3a, b,d,e,f and g), but the BFs of Co (Fig. 3d) were not significantly different at a concentration range between 0.005 and 0.10 mg L^{-1} (P> 0.10). The two peak values of BFs were similar in Cr (Fig. 3a&b) and Pb (Fig. 3g) (P>0.10), respectively. They posed 60%

Table 1. Concentrations of heavy metals Cr, Cd, Co, Cu, Ni and Pb in cultures of *Chlorella marina* from both control and experimental groups 13 days after inoculation (sample size = 3).

Metals in medium (mg L^{-1})	Concentration of heavy metals tested (mg L^{-1}) in cultures of *C. marina*					
	Cr	Cd	Co	Cu	Ni	Pb
0.000	0.12± 0.22	2.25 ± 1.20	3.02±1.02	18.6±0.12	80.76±0.14	3.78±0.05
0.001	1.10± 0.41	2.95 ±2.14	7.20±1.25	------	------	------
0.005	3.90± 0.23	5.33 ±1.23	28.6±1.04	-----	-----	------
0.01	8.50± 0.14	6.90 ±2.56	53.8±2.25	24.8±1.24	4.12±1.42	8.60±1.23
0.05	47.1 ±0.47	23.4 ±0.12	252 ±0.44	43.9±2.14	20.0±1.56	23.9±2.16
0.10	105± 1.42	43.6 ±0.23	559 ±2.12	-----	-----	-------
0.20	150± 3.25	85.8 ±1.24	990 ±1.52	125±3.28	29.6±1.02	81.2±0.32
0.50	160± 2.45	209 ±2.01	1043 ±1.24	283±1.23	598±2.05	204±1.28
1.00	----	299 ±2.10	----	439±0.12	1030±2.36	321±2.48
1.50	-----	386 ±0.12	----	-----	-----	-----
2.00	---	----	----	447.14	1680±1.05	1450±0.26
3.00	----	-----	----	------	1860±0.08	1220±1.24
5.00	----	-----	-----	------	------	1560±0.41

——— * Not tested for the related concentration

difference for Cu (Fig. 3e) and 65% for Ni (Fig. 3f). With regard to the bioconcentration factors, metal uptake by *C. marina* was most efficient at a concentration of 0.001 mg L^{-1} for Cr, Cd and Co, and at 0.01 mg L^{-1} for Cu and

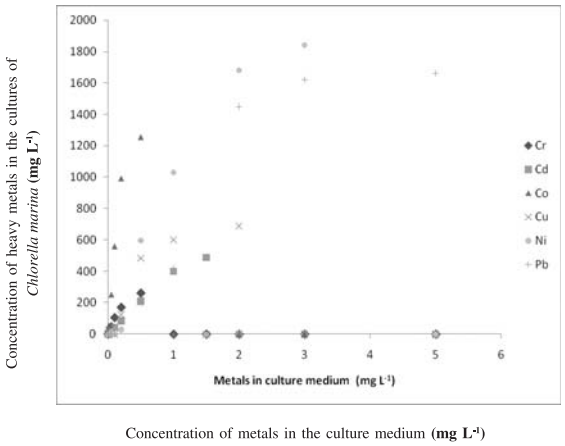
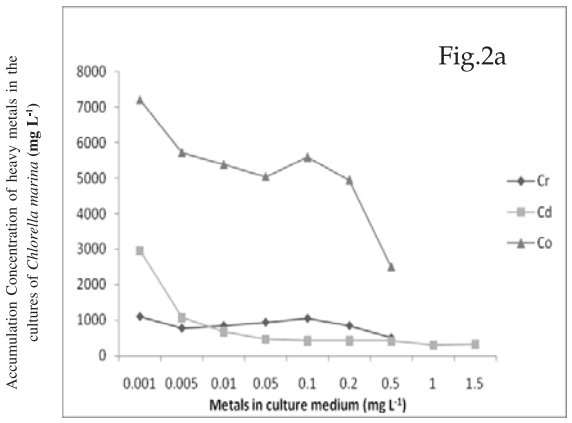


Figure 1. Scatterplot diagram showing concentration difference between metals contained in the cultures of *Chlorella marina* from both control and experimental groups, 13 days after inoculation (sample size = 3).



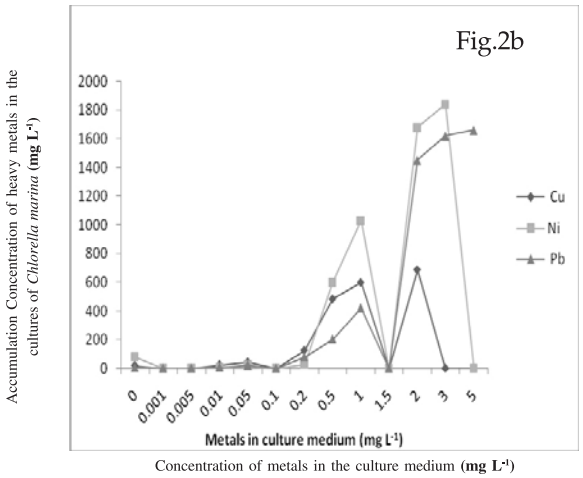
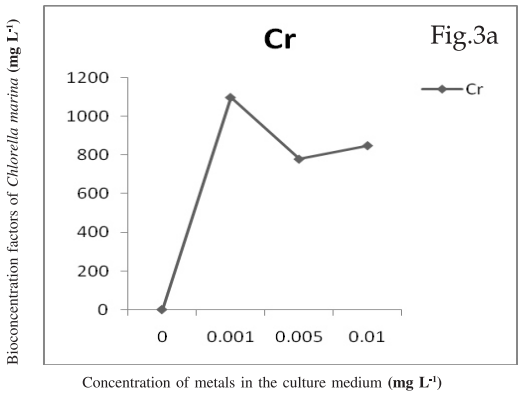
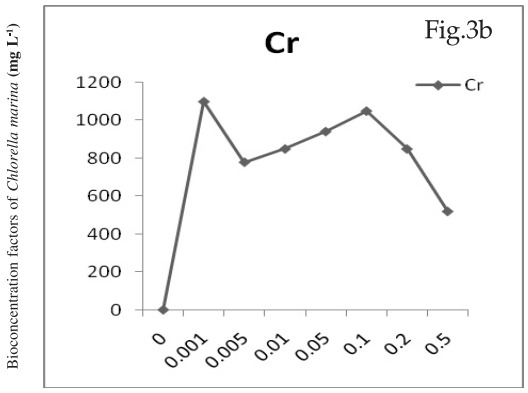
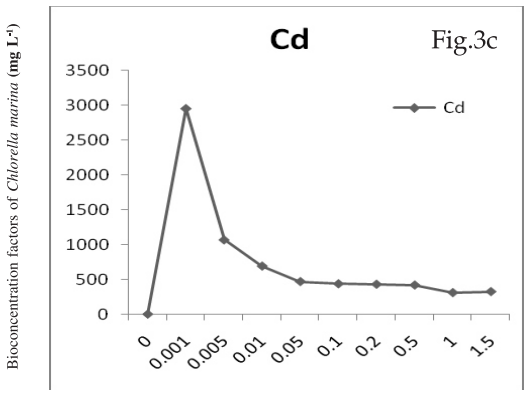
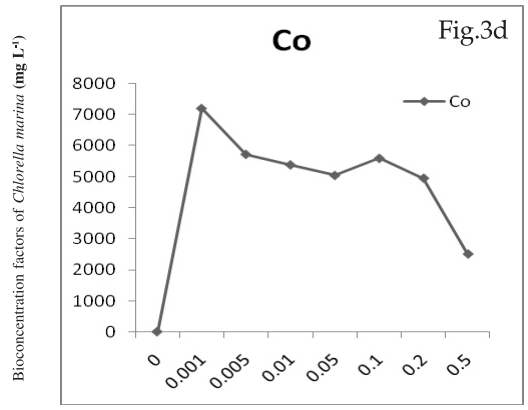


Figure 2a &b. Accumulation of heavy metals Cr, Cd, Co, Cu, Ni and Pb in the cultures of *Chlorella marina* exposed to various metal concentrations









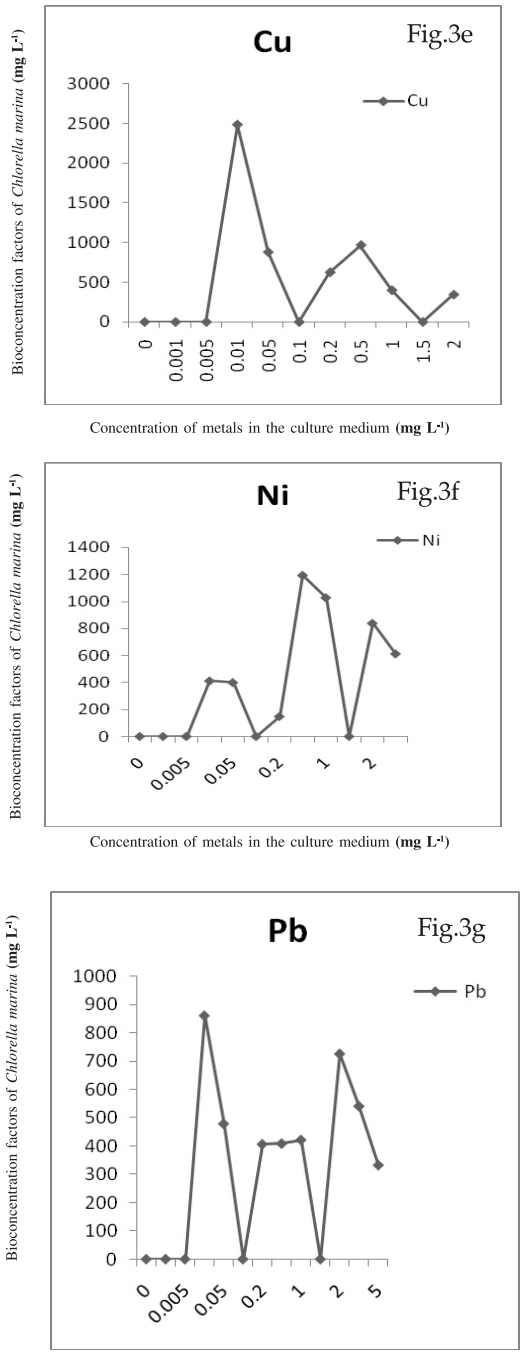


Figure 3a-g. Bioconcentration factors (BF) of *Chlorella marina* versus metal concentrations in the culture medium.

Pb. Similar effects were also found for Cr at 0.10 mg L^{-1} and Pb at 2.0 mg L^{-1}. The highest value of BF for Ni occurred against an external concentration of 0.50 mg L^{-1}, but a higher value could be expected at a concentration of < 1 mg L^{-1} based on an examination of the BF patterns as seen in Fig. 3f.

DISCUSSION

Bioremoval, the use of biological systems for the removal of metal ions from polluted waters, has the potential to achieve greater performance at lower cost than conventional wastewater treatment technologies for metal removal (Wilde and Benemann, 1993). In this context, selection of specific microalgae strains for cultivation and processing are needed for definite bioremoval applications, in order to provide significant improvements in dealing with the world-wide problems of metal pollution.

The ability of microalgae to accumulate metals from aqueous solution is well documented (Fathi and Falkner, 1997; Fathi *et al.*, 2000; Giusti, 2001; Fathi, 2008) as well as the possibility of using microbial biomass to remove metals from effluents (Macaskie, 1991; Hamdy, 2000). Algae take metals up both passively and actively. As passive biosorption mainly depends on binding to functional surface ligands, the cell wall structure is most important for rapid metal ion uptake (Afkar *et al.*, 2010). Some, such as Pb and Sr, may be passively adsorbed by charged polysaccharides in cell wall and intracellular matrix (Fathi *et al.*, 2000; El-Sheekh *et al.*, 2003; Osman *et al.*, 2004; Fathi *et al.*, 2005). Other metals (e.g., Zn, Cd) are taken up actively against large intracellular concentration gradients. On the other hand, some authors have reported that the phenomenon of metal accumulation by microbial cells is quite complex, two principal mechanisms are reported *viz.*, adsorption on to the surface of the cell and a slower, active uptake into the cytoplasm (Barbara and Michael, 1994). It is observed that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water (Fathi *et al.*, 2005). There also reports (Hamdy, 2000) on the metal uptake which is dependent on the type of biosorbent, with different accumulation affinities towards the tested elements and the amount of metal uptake increases steeply with increase in the biomass.

During the present study, the pattern of accumulation of all the six heavy metals (Cr, Cd, Co, Cu, Ni and Pb) in the tested microalga *Chlorella marina* demonstrated a slow increment that gradually approached an apparent upper asymptote which is in accordance with the previous reports (Sharif *et al.*, 2007). This pattern of metal accumulation is in contrast with metal sorption reported by several authors in microalgae which is often characterized by an initial phase of rapid sorption followed by a second phase of slower uptake (Khummongkol *et al.*, 1982; Genter *et al.*, 1988; Reinfelder and Chang, 1999). In such a biphasic model of metal uptake, metal ions are believed to bind to and saturate negatively charged sites (e.g. carboxylic groups) on the exterior of cells in the first phase and are then

transported to the interior of the cell *via* biologically active processes in the second phase (Xue *et al.*, 1988).

A model for metal uptake based on surface adsorption for the uptake of cadmium by *Chlorella vulgaris* has been developed (Khummongkol *et al*., 1982) and established a linear equilibrium relationship between metal in the solution and that adsorbed on the cell surface at low cadmium concentrations. The metal binding characteristics of *C. vulgaris* to assess the role of the surface charge site for the metal binding have been investigated (Cho *et al*., 1994). In this study, the carboxyl-modified algae show major decreases in the adsorption capacity of Cd (II) and Zn (II) binding and the amine-modified algae also display some decreases in metal adsorptions.

The mineral fraction in microalgal cells constitutes a major proportion of the dry weight, ranging from 6 to 39% (Brown *et al.*, 1989). Metal concentrations in cells vary considerably both with species and environment (Eisler, 1981). Cu concentrations, for example, are shown to be 65 and 652 $\mu g\ g^{-1}$, respectively, in the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecic*a (Fabregas and Herrero, 1986). Maximum intracellular accumulation under low cadmium concentration in *Chlorella* sp. was estimated to be 2.4 mg Cd/g dry cells (Matsunaga *et al*., 1999). There are previous studies regarding the cadmium concentration in several species of *Chlorella* at the range of 5 to 7.9 mg g^{-1} (Sakaguchi *et al*., 1979; Geisweid and Urbach, 1983). In the present study, the maximum Cd metal concentration accumulated by *Chlorella marina* was found to be 0.386 mg g^{-1}.

In the present work, data from the control group represented baseline metal concentrations. Such information is useful in the evaluation of algal food values in mariculture (Riley and Roth, 1971) and in the assessment of algae as effective agents of heavy metal removal in environmental treatments.

Microalgae have long been recommended as a source of minerals in mariculture (Stanley and Jones, 1976); Ogino and Yang, 1980; Fabregas and Herrero, 1986). They are easy to grow and their mineral contents can be enhanced by increasing metal concentrations in the culture medium (Chen *et al*., 1998). This is found to be in agreement with the present study also. The capacity of *C. marina* for metal accumulation has been demonstrated to be flexible in response to external metal concentrations during the present investigation (Fathi *et al*., 2005). Compared with the control group in this investigation, 'Co' concentration in the algal cultures, for example, was doubled when the medium contained 0.001 mg L^{-1} Co, and the amount of Cu increased about 400 times as the dosage was raised to 0.5 mg L^{-1} Cu. Of the six heavy metals tested, Co, Cu and Ni are essential to many living organisms. They were all shown to be accumulated at relatively high rates when the alga was exposed to the metals in unpolluting levels. It is, however, necessary to remember that data presented in Table 1 can only be consulted, as indicated by the BFs, when the culture medium is enriched with individual metals. The metal accumulation patterns may also be changed significantly when the culture medium is mixed with different metal components (Skjak and Jensen, 1991; Wong and Chang, 1991).

During the present study, the accumulation of all the heavy metals tested were found to increase with their increasing concentrations in the culture medium. This is in agreement with the recent reports (Afkar *et al*., 2010) that accumulations of Co, Cu and Zn by *Chlorella vulgaris* cells are parallel to increasing the concentrations in the culture medium. The report has concluded that the bioaccumulation factors (the ratio of concentration of an element in dry biomass and in the surrounding medium) of the three tested metals are also increasing with their concentrations in the culture medium. Metal accumulation by *C. marina* was shown to be in an order of Ni> Pb> Co> Cu>Cd>Cr during the present experiment.

Microalgae have been considered to have better advantages over the traditional methods in the elimination of heavy metals from aquatic systems (Wilde and Benemann, 1993; Sandau *et al*., 1996a; Sandu *et al*., 1996b). Although only a few marine microalgae have been investigated for metal accumulation ability, some are already commercially available for heavy metal removal in the treatment of wastewaters (Sandau *et al*., 1996a). According to US Environmental Protection Agency (EPA), the maximum admissible limits of Cd, Cu, Ni and Pb in drinking waters cannot exceed 5.0, 1300, 100, and 15 $\mu g\ L^{-1}$, respectively. According to the Bureau of Indian Standards (BIS), the maximum concentrations of Cd, Cu, Ni and Pb in drinking waters cannot exceed 10, 1500, 20 and 100 $\mu g\ L^{-1}$, respectively. At these limits, the bioconcentration factors of *Chlorella marina* were shown to be >1000 for Cd and Cu; < 1000 for Pb; ~1000 for Cr and Ni; and > 5000 for Co under the current experimental conditions. These are found to be particularly attractive figures compared with those reported (Wilde and Benemann, 1993) for other microalgae though there is probably scope for enhancing accumulation by studies on the influence of growth conditions, such as duration of cultivation, pH and chemical composition of the medium (Shulz – Baldes and Lewin, 1976; Drba *et al*., 1976; Sakaguchi *et al*., 1979). In addition, as shown in Fig. 3, *C. marina* has been shown to have bioconcentration factor values between 1000 and 8000 at other metal concentrations.

In general, the results of this investigation suggest that *Chlorella marina* can be a mariculture mineral supplement in terms of its potential ability to accumulate

essential metals, such as Co, Cu and Ni, in an unpolluted environment. The alga seems potentially more useful as an agent for heavy metal removal in wastewater treatment, though it is worth making further investigations based on the variation of environmental factors. Studies are also in line by various researchers for improving biomass containment or immobilization techniques through developing bioremoval process steps using metabolically active microalgal cultures.

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REFERENCES

Afkar, E., Ababna, H., and Fathi, A. A. 2010. Toxicological Response of the Green Alga *Chlorella vulgaris*, to Some Heavy Metals. *Am. J. Environ Sci.,* 6: 230 - 237.

Aksu, Z., Acikel, U. and Kutsal, T. 1997. Application of multi component adsorption isotherms to simultaneous biosorption of iron (III) and chromium (VI) on *C. vulgaris*. *J. Chem. Tech. Biotechnol.,* 70: 368 – 378.

Aksu, Z., Acikel, U. and Kutsal, T. 1999. Investigation of simultaneous biosorption of copper (II) and chromium (VI) on dried *Chlorella vulgaris* from binary metal mixtures: application of multi component adsorption isotherms. *Separ. Sci. Technol.*, 34: 501 -524.

Brown, M.R., Jeffrey, S.W. and Garland, C.D. 1989. Nutritional Aspects of Microalgae Used in Mariculture: A Literature Review. *In: CSIRO Marine Laboratories Report*, CSIRO, Australia. P. 205.

Barbara, W. and Michael, W. 1994. Comparative studies of the heavy metal uptake of whole cells and different types of cell walls from *Chlorella fusca*. *Biotechnol. Tech.*, 8: 227 -232.

Eisler, R. 1981. *Trace Metal Concentrations in Marine Organisms*. Pergamon Press, New York.

Chong, A.M.Y., Wong, Y.S. and Tam, N. F. Y. 2000. Performance of different microalgal species in removing nickel and zinc from industrial wastewater. *Chemosphere*, 41: 251–257.

Cho, D.Y., Lee, S.T., Park, S.W. and Chung, A.S. 1994. Studies on the biosorption of heavy metals onto *Chlorella vulgaris*. *J.environs. Sci. heal.,* Part A: Toxic/ Hazardous Substances and Environmental Engineering, 29: 389 – 409.

Chen, B. L., Huang, Q., Lin, X. J., Shi, Q. Q. and Wu, S. G. 1998. Accumulation of Ag, Cd, Co, Cu, Hg, Ni and Pb in *Pavlova viridis* Tseng (Haptophyceae). *J. Appl. Phyco.,* 10: 371 -376.

Drba, K., Véber, K. and Zahradnik, J. 1976. Toxicity and accumulation of copper and cadmium in the alga *Scenedesmus obliquus* LH. *Bull. Envir. Contam. Toxicol.*, 34: 904 – 908.

Devresse, B., Romdhane, M.S., Buzzi, M., Rasowa, J., Legar, P., Brown, J. and Sorgeloos, P. 1990. Improved larviculture outputs in giant fresh water prawn *Machrobranchium rosenbergii* fed a diet of *Artemia* enriched with n - 3 HUFA and Phospho lipid, *World Aquaculture*, 21 : 123 -125.

El-Sheekh, M. M., El-Naggar, A. H., Osman, M. E. H. and El-Mazaly, E. 2003. Effect of Cobalt on growth, pigments and the photosynthetic electron transport in *Monoraphidium minutum* and *Nitzchia perminuta*. *Braz. J. Plant. Physiol.*, 15: 159 -166.

Fabregas, J. and Herrero, C. 1986. Marine microalgae as a potential source of minerals in fish diets. *Aquaculture*. 52: 237 – 243.

Fathi, A. A. and Falkner, G. 1997. Adaptation to elevation of the concentration of the trace element copper during growth of *Scenedesmus bijuga* is reflected in the properties of the copper uptake system. *J. Trace. Microprobe. Techn.*, 15: 321-333.

Fathi, A. A., Zaki, F.T. and Fathy, A. A. 2000. Bioaccumulation of some heavy metals and their influence on the metabolism of *Scenedesmus bijuga* and *Anabaena spiroides*. *Egypt. J. Biotechonl.*, 7: 293-307.

Fathi, A. A., El-Shahed, A. M., Shoulkamy, M. A., Ibraheim, H. A. and Abdel Rahman, O. M. 2008. Response of Nile water phytoplankton to the toxicity Cobalt, Copper and Zinc. *Res. J. Environ. Toxicol.,* 2: 67 -76.

Fathi, A. A., Zaki, F. T. and Ibraheim, H. A. 2005. Response of tolerant and wild type strains of *Chlorella vulgaris* to copper with special references to copper uptake system. *Protistology.*, 4: 73 -78.

Genter, R.B., Colwell, F.S., Pratt, J.R., Cherry, D.S. and Cairns J Jr. 1988. Changes in epilithic communities due to individual and combined treatments of zinc and snail grazing in stream mesocosm. *Toxicol. Ind. Health.,* 4:185 -201.

Geisweid, H.J., Urbach, W. 1983. Sorption of Cd by the green microalgae *Chlorella vulgaris, Ankistrodesmus braunii* and *Eremosphaera viridi*. *Zeitschrift für Pflanzenphysiologie,* 109: 127 -141.

Giusti, L. 2001. Heavy metal contamination of brown seaweed and sediments from the UK coastline between the Wear River and the Tees River. *Environ. Int.*, 26: 275 -286.

Han, X., Wong, Y.S.S., Wong, M. H. and Tam, N. F. Y. 2007. Biosorption and bioreduction of Cr (VI) by a microalgal isolate *Chlorella miniata*. *J. Hazard. Mater.,* 146: 65 – 72.

Han, X., Wong, Y. S. and Tam, N. F.Y. 2006. Surface complexation mechanism and modeling in Cr (III) biosorption by a microalgal isolate, *Chlorella miniata*. *J. Colloid. Interface Sci.*, 303: 365 -371.

Hamdy, A. A. 2000. Biosorption of heavy metals by marine algae. *Curr. Microbiol.*, 41: 232 -238.

James, C. M., Dias, P. and Salman, A. E. 1987. The use of marine yeast *(Candida sp.)* and baker's yeast *(Saccharomyces cerevisiae)* in combination with *chlorella sp.* for mass culture of the rotifer *Brachinous plicatilis*. *Hydrobiologia.*, 147: 263 – 268.

James, C.M. and Abu-rezeq, T. S. 1988. Effect of different cell densities of *Chlorella capsulatea* and a marine

chlorella sp. for feeding the rotifer *Brachionous plicatilis*, *Aquaculture*., 69: 43 – 56.

Khummongkol, D., Canterford, G.S. and Fryer, C. 1982. Accumulation of heavy metals in unicellular algae. *Biotechnol. Bioeng*., 24: 2643 -2660.

Levy, J. L., Stauberand, J. L. and Jolley, D.F. 2007. Sensitivity of marine microalgae to copper - The effect of biotic factors on Copper adsorption and toxicity. *Sci Total Environ*, 387: 141 -154.

Langdon, C. J. and Waldock, M. J. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassosirea gigas spat*. *J. Mar. Biol. Assoc. UK.*, 61: 431 – 448.

Mehta, S.K. and Gaur, J. P. 2001. Characterization and optimization of Ni and Cu sorption from aqueous solution by *Chlorella vulgaris*. *Ecol. Eng.*, 18: 1 -13.

Macaskie, L. E. 1991. The application of biotechnology of the treatment of acid sites of biosorbents peat moss and *Vaucheria* and model substances alginic and humic acids. *Environ. Sci. Technol.*, 33: 2252 -2256.

Matsunaga, T., Takeyama, H., Nakao, T. and Yamazawa, A. 1999. Screening of marine microalgae for bioremediation of cadmium-polluted seawater. *J. Biotechnol.*, 70: 33-38.

Osman, M. E. H., El-Naggar, A. H., El-Sheekh, M. M. and El-Mazally, E. 2004. Differential effects of Co^{2+} and Ni^{2+} on protein metabolism in *Scenedesmus obliquus* and *Nitzschia perminuta*. *Environ. Toxicol. Pharmacol.*, 16: 169 -178.

Ogino, C. and Yang, G.Y. 1980. Requirements of carp and rainbow trout for dietary manganese and copper. *Bull. Jpn. Soc.Sci.Fish*. 46: 455- 458.

Reinfelder, J.R. and Chang, S.I. (1999). Speciation and microalgal bioavailability of inorganic silver. *Environ. Sci. Technol*., 33: 1860 -1863.

Riley, J. P. and Roth, I. 1971. The distribution of trace elements in species of phytoplankton grown in culture. *J. mar. Biol. Ass. UK.*, 51: 63 - 72.

Sandau, E., Sandau, P. and Pulz, O. 1996b. Heavy metal sorption by microalgae. *Acta Biotechnol*. 16: 227 – 235.

Sandau, E., Sandau, P., Pulz, O. and Zimmermann, M. 1996a. Heavy metal sorption by marine algae and algal by-products. *Acta Biotechnol*. 16: 103 – 119.

Schulz-Baldes, M. and Lewin, R.A. 1976. Lead uptake in two marine phytoplankton organisms. *Biol. Bull*., 150: 118 – 127.

Sharif, I. M., Nezhad, M.E. and Zereshki, S. 2007. Different patterns of uptake and depuration of cadmium by periphyton community and a grazer species (*physa* sp.): a mesocosm evaluation, *Freshwater. Forum.*, 27: 15-26.

Sakaguchi, T., Tsuji, T., Nakajima, A. and Horikoshi, T. 1979. Accumulation of Cd by green microalgae. *Eur. J. Appl. Microbiol*. 8: 207 -215.

Skjak, G. and Jensen, A. 1976. Heavy metal tolerance of marine phytoplankton. III. Combined effects of copper and zinc ions on cultures of four common species. *J. exp. Mar. Biol. Ecol.*, 225: 37- 50.

Sadiq, M. 1992. Toxic Metal Chemistry in Marine Environments. In: Dekker, M. (Ed.), New York. P.15 – 19.

Stanley, J. G. and Jones, J. B. 1976. Feeding algae to fish. *Aquaculture*., 7: 219- 223

Tam, N. F.Y., Wong, J. P. K. and Wong, Y. S. 2001. Repeated use of two *Chlorella* species, *C. vulgaris* WW1for cyclic nickel biosorption. *Environ. Pollut.*, 114: 85 -92.

Wong, J. P. K., Wong, Y. S. and Tam, N. F. Y. 2000. Nickel biosorption by two *Chlorella* species, *C. vulgaris* (a commercial species) and *C. miniata* (a local isolate), *Bioresour. Technol.*, 73: 133 -137.

Walne, P. R. 1974. Culture of bivalve molluscs: 50 years experience at Conway. *Fishing News* (Books) *Ltd.* P. 1-173.

Wilde, E. W. and Benemann, J. 1993. Bioremoval of heavy metals by the use of microalgae. *Biotech. Adv.*, 11: 781 -812.

Wong, P. K. and Chang, L. 1999. Effects of copper, chromium and nickel on growth, photosynthesis and chlorophyll *a* synthesis of *Chlorella pyrenoidosa* 251. *Envir. Pollut*., 72: 127 -139.

Xue, H.B., Stumm, W. and Sigg, L. (1988). The binding of heavy metals to algal surfaces, *Water. Res*., *22*: 917 -926.