Bioaccumulation of metals by *Scenedesmus, Selenastrum* and *Chlorella* algae

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Abstract

Three species of algae were investigated for their ability to accumulate metal ions. *Scenedesmus*, *Selenastrum* and *Chlorella* species were found to be capable of accumulating metals such as Cu^{2+}, Pb^{2+}, and Cr^{3+} with 67 to 98% efficiency. Although *Chlorella* was less capable of accumulating these cations than the other two organisms, it possessed a greater capacity for the $Cr_2O_7^{2-}$ anion. A suspension of *Selenastrum* was used to accumulate Cr^{3+} from a sample of post-anaerobic digester tannery effluent. The algae removed 39% of the chromium from solution. The rate of metal (Cu^{2+}, Pb^{2+}, Cr^{3+}) accumulation by *Scenedesmus* was rapid, occurring in the first 4 min. Of the 4 metals investigated, Cu^{2+}, Cr^{3+}, Pb^{2+} and $Cr_2O_7^{2-}$, the former 2 were more toxic to the algae than the latter two.

Introduction

Bioaccumulation of metals by algae may present a feasible method for remediating waste waters contaminated with metals (Darnall et al., 1986; Jackson, 1978; Nakajima et al., 1981). One of the other advantages of algae is that they may be grown in ponds with little nutritional input or maintenance. Moreover algae can be considered to be non-pathogenic, which gives these organisms an advantage over many other forms of microbial biomass. Algal ponds are a final stage of sewage treatment in many sewage treatment plants, and the use of algal ponds for the bioremediation of tannery effluents has also been investigated recently (Laubscher et al., 1990).

Field experiments reported by Gale (1986) indicate that live photosynthetic micro-organisms can be effective in metal detoxification of mine waste waters. By using cyanobacteria in a system of artificial pools and meanders, 99% of dissolved and particulate metals could be removed. McHardy and George (1990), like Vymazal (1984), studied *Cladophora glomerata* in artificial freshwater channels and found the alga to be an excellent accumulator of zinc, which was concentrated 2 to 5 thousand times.

There have also been reports of accumulation of Cu^{2+}, Pb^{2+} and Cr^{3+} as well as Ni^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+} and Mn^{2+} by algae (Sicko-Goad and Stoermer, 1979; Vyzamal, 1984). Algae in experimental rice paddies were found to accumulate and concentrate Cd^{2+} by a factor of about 10 000 times when compared to the ambient water (Reiniger, 1977). Reports of algal species present in algal ponds are inevitably mixed and may include both algae (eukaryotes) and blue-green algae (prokaryotes) and data from such experiments should be interpreted with this in mind.

The mechanism of metal accumulation by algae is primarily via binding of metals to the cell wall surface, although intracellular uptake also contributes to the total accumulation (Vyzamal, 1984). The binding of metal to algae could be either ionic or by complex formation with ligands on the cell wall. The polymers which constitute the cell wall are rich in phosphoryl, carboxyl, aromatic and hydroxyl groups (Ehrlich, 1986) which bind cationic metals (Crist et al., 1981). Investigations of zinc accumulation by algal cultures under various lighting conditions proved that light was not necessary for the common freshwater alga *C. glomerata* to accumulate zinc, as accumulation levels were independent of the photosynthetic period. This in turn implied that energy-dependent mechanisms were not necessary for metal accumulation (Vymazal, 1987) and it was concluded that the dominant Zn cation accumulation process was therefore an adsorptive mechanism. It has been found that pH, outside the 5 to 7 unit range, decreases bioaccumulation of metals by algae (Schenck et al., 1988).

In this study, the accumulation of Cu^{2+}, Pb^{2+} and Cr^{3+} from solution by 3 freshwater algal species, namely *Scenedesmus* sp., *Selenastrum* sp. and *Chlorella* sp. (which will henceforth be referred to by genus), was studied. The objective was to use these algae for accumulation of metals from solutions, including a preliminary study of Cr^{3+} accumulation from tannery effluent.

Methods

Culture maintenance

The starting cultures were obtained from the Department of Zoology and Entomology at Rhodes University. Isolates of the 3 species were obtained by the spray plate method in which filtered air was forced into a vessel containing an algal culture, which consequently propelled the medium out of a fine outlet (in this case a Pasteur pipette). The resultant aerosol was directed onto BG 11-agar plates. The plates were incubated at 22°C on a light table of 165.4 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity. Working cultures of each species were grown from a single colony. The cultures were maintained on 2% agar plates (prepared by adding 2 g of normal nutrient agar to 100 mℓ of BG 11 medium, autoclaving for 15 min, and then pouring 15 mℓ into sterile disposable petri dishes).

All cultures were grown in BG 11 medium, pH 7.4, prepared according to Allen (1968) with ultra-pure water (Milli-Q). The medium had low turbidity, allowing for penetration of light required by the photosynthetic algae. The medium was sterilised by autoclaving at 121°C for 15 min. The medium was stored at 4°C until inoculated. The 3 species (one from each of the genus of *Scenedesmus*, *Selenastrum* and *Chlorella*) were harvested in the log phase.

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The cultures were scaled up by adding 200 ml of the inoculum to 2.5 *l* of BG 11 medium. Samples were taken every 48 h using sterile glass pipettes, and these were used for cell counts and contamination checks. When the cultures reached the stationary phase they were harvested and used for metal ion bioaccumulation experiments. Duplicate 5 ml samples were drawn from the stationary phase cultures and filtered with Whatman glass fibre (GF/A) filter disks through a Venturi pump. The filters were dried at 30°C overnight and weighed to determine the cell mass per volume of culture.

Metal bioaccumulation studies

Algal metal bioaccumulation was assayed by suspending algae in aqueous solutions of Cu^{2+}, Pb^{2+}, Cr^{3+} or $Cr_2O_7^{2-}$ as metal salts ($CuSO_4.5H_2O$, $Pb(CH_3COO)_2.3H_2O$, $K_2Cr_2O_7$ and $Cr(SO_4)_3.6H_2O$ respectively). The concentrations of metal ions added were 2.5, 10, 25 or 100 mg·l^{-1} and are representitive of those to be found in industrial wastewaters generally. The assays were performed in duplicate or triplicate. All glassware used in metal bioaccumulation assays was made of borosilicate glass because of its relatively low adsorption of metal cations. All solutions were made with ultra-pure water. All glassware used in metal bioaccumulation studies was washed with 20% HCl, rinsed twice with ultra-pure water, and air-dried before use.

Aliquots of 0.25, 1.0, 2.5 or 10 ml of 1 000 mg·l^{-1} metal stock solutions were added to 90 ml of algal culture in a 250 ml conical flask and diluted with ultra-pure water to yield metal concentrations of 2.5, 1.0, 25, or 100 mg·l^{-1} respectively. The conical flask was stoppered with a cotton wool bung and incubated in an orbital shaker at 80 r·min^{-1} and 22°C overnight. A 5 to 15 ml sample was drawn from the flask and filtered through a GF/A filter disk using the Venturi pump. The remaining culture was retained for pH determination and then allowed to settle overnight, after which the flask was observed for algal flocculation. The filtrate from the Venturi pump was collected in glass vials and analysed by atomic absorption spectroscopy. The filter paper and the retentate were dried in a 30°C incubator overnight and then weighed. The net gain in mass of the filter gave the cell dry mass with bound metal per 10 ml of treated algal culture.

Toxicity determinations

To determine the effect which exposure to metal ions had on the algae, the algae were streaked out on 2% BG 11-agar plates. A sample was streaked out from each of the cultures treated, at each of the metal concentrations tested. The plates were incubated as described above for 96 h and then observed for algal colony growth.

Results

The 3 algal species used in this study were able to accumulate Cu^{2+}, Pb^{2+} and Cr^{3+} cations with approximately 90% efficiency over a range of initial metal concentrations that varied by up to nearly 2 degrees of magnitude (Tables 1, 2 and 3). Unlike cationic trivalent chromium ions, the anionic dichromate ions were only accumulated to a small degree, and accumulation was influenced by the initial concentration of the anion (Table 4). Determination of the pH values during the metal binding reactions showed that the pH was maintained within pH 6.4 to 8.4.

In Table 5 it can be seen that the accumulation of Cr^{3+} from tannery effluent only reached a maximum of 39%, far lower than that seen in the case of artificial chromium solutions. The reason for this could be that most of the chromium in solution has been oxidised to chromate. An alternative explanation is that the chromium cations may be chelated to organic compounds in the waste water, including perhaps the extracellular metal binding agents of bacteria such as *Zoogloea ramigera* that are present. The ligands on the cell surface would then have to compete with the chelating agents in solution.

The biosorption of metals onto the cell surface allows for cross-bridging of anionic ligands on different cells by multivalent metal cations, with resultant flocculation. This is reflected in the results presented in Table 6. *Chlorella* cells, which showed generally poorer accumulation of metals than the other 2 strains, were the least flocculated by Cr^{3+} and *Chlorella* was the only strain that was flocculated by the addition of anionic dichromate ions, implying a higher ratio of anionic to cationic ligands on the cell surface, which explains the lower cation accumulation values of this strain.

The rate of metal cation accumulation by *Scenedesmus* biomass

TABLE 1
ACCUMULATION OF Cu^{2+} BY ALGAL CELLS (MEAN ± SD)

Initial Cu concentration	Culture	Cell dry mass (mg·100 ml^{-1})	% Metal accumulation	Metal accumulation (mg·g^{-1})
2.5 mg·l^{-1}	*Sce*	32.3 ± 2.1	92.0 ± 2.0	7.1
	Sel	29.0 ± 1.7	95.3 ± 1.2	8.2
	Chl	32.0 ± 1.7	91.3 ± 3.8	7.1
25.0 mg·l^{-1}	*Sce*	34.3 ± 1.5	92.3 ± 1.2	67.3
	Sel	29.3 ± 2.5	98.3 ± 0.6	83.9
	Chl	32.3 ± 3.1	87.3 ± 1.5	67.6
100.0 mg·l^{-1}	*Sce*	38.7 ± 2.5	82.7 ± 1.5	213.7
	Sel	40.7 ± 1.2	96.7 ± 2.3	237.5
	Chl	38.3 ± 7.1	67.0 ± 7.0	174.9

TABLE 2
ACCUMULATION OF Pb^{2+} BY ALGAL CELLS (MEAN ± SD)

Initial Pb concentration	Culture	Cell dry mass (mg·100 $m\ell^{-1}$)	% Metal accumulation	Metal accumulation ($mg \cdot g^{-1}$)
10.0 $mg \cdot \ell^{-1}$	Sce	35.0 ± 0	72.0 ± 1.4	20.6
	Sel	27.5 ± 0.7	94.5 ± 0.7	34.3
	Chl	34.0 ± 0	67.0 ± 2.8	19.7
100.0 $mg \cdot \ell^{-1}$	Sce	43.0 ± 2.8	94.5 ± 0.7	219.8
	Sel	39.5 ± 0.7	97.5 ± 0.7	246.8
	Chl	45.5 ± 2.1	81.5 ± 3.5	179.1

TABLE 3
ACCUMULATION OF Cr^{3+} BY ALGAL CELLS (MEAN ± SD)

Initial Cr concentration	Culture	Cell dry mass (mg·100 $m\ell^{-1}$)	% Metal accumulation	Metal accumulation ($mg \cdot g^{-1}$)
10.0 $mg \cdot \ell^{-1}$	*Sce*	35.0 ± 1.4	95.0 ± 1.4	27.1
	Sel	28.5 ± 0.7	97.5 ± 0.7	34.2
	Chl	36.0 ± 0	83.0 ± 0	23.1
100.0 $mg \cdot \ell^{-1}$	*Sce*	54.0 ± 0	96.5 ± 0.7	178.7
	Sel	48.5 ± 0.7	99.0 ± 0	204.1
	Chl	49.0 ± 1.4	86.5 ± 0.7	176.5

TABLE 4
ACCUMULATION OF $Cr_2O_7^{2-}$ BY ALGAL CELLS (MEAN ± SD)

Initial Cr concentration	Culture	Cell dry mass (mg·100 $m\ell^{-1}$)	% Metal accumulation	Metal accumulation ($mg \cdot g^{-1}$)
10.0 $mg \cdot \ell^{-1}$	*Sce*	30.3 ± 0.6	10.0 ± 2.0	3.3
	Sel	24.0 ± 1.0	8.3 ± 3.5	3.5
	Chl	25.0 ± 3.6	9.7 ± 5.0	3.9
100.0 $mg \cdot \ell^{-1}$	*Sce*	27.7 ± 1.5	19.0 ± 1.0	68.6
	Sel	24.7 ± 1.5	18.0 ± 3.5	72.9
	Chl	21.7 ± 1.5	22.0 ± 1.0	101.4

was relatively rapid for all metals studied (Fig. 1). Moreover the final percentage of the metals accumulated from the artificial solutions was almost 100% in each case.

The toxicity of the metal ions to the cells is important if the culture is to be used as biomass during metal bioaccumulation reactions. In Table 7 the results of culturing algae after metal bioaccumulation show that Cu^{2+} and $Cr_2O_7^{2-}$ dichromate ions were the greatest inhibitors of cell biomass increase.

TABLE 5
ACCUMULATION OF Cr FROM TANNERY EFFLUENT

Sample	Initial Cr $mg \cdot \ell^{-1}$	% Accumulation
Filtered effluent	13	-
Sel. + effluent	8	39

Discussion

The 3 freshwater algae investigated in the present study were each capable of accumulating Cu^{2+}, Pb^{2+}, and Cr^{3+} cations. *Scenedesmus*

TABLE 6
FLOCCULATION OF TREATED ALGAL CULTURES

Treatment		*Scenedesmus*	*Selenastrum*	*Chlorella*
Cu	2.5 mg·t^{-1}	+	+	+
	25.0 mg·t^{-1}	++	++	++
	100.0 mg·t^{-1}	++	++	++
Pb	10.0 mg·t^{-1}	++	++	++
	100.0 mg·t^{-1}	++	++	++
Cr(III)	10.0 mg·t^{-1}	+	+	+
	100.0 mg·t^{-1}	++	++	+
	Effluent	+	+	+
Cr(VI)	10.0 mg·t^{-1}	-	-	-
	100.0 mg·t^{-1}	-	-	-

++ Settling of the algae and clearing of the medium
+ Settling of the algae with no clearing of the medium
- Algae remain mostly in suspension

TABLE 7
METAL TOXICITY TO ALGAL CULTURES, GROWTH OF STREAKS OF METAL TREATED CULTURES ON 2% BG 11 - AGAR PLATES

Treatment		*Scenedesmus*	*Selenastrum*	*Chlorella*
Cu	2.5 mg·t^{-1}	+	+	+
	25.0 mg·t^{-1}	+	+	-
	100.0 mg·t^{-1}	-	-	-
Pb	10.0 mg·t^{-1}	+	+	+
	100.0 mg·t^{-1}	+	+	+
Cr(III)	10.0 mg·t^{-1}	+	+	+
	100.0 mg·t^{-1}	+	+	+
Cr(VI)	10.0 mg·t^{-1}	+	+	+
	100.0 mg·t^{-1}	-	-	+

Growth (+), No growth (-)

and *Selenastrum* were the most efficient accumulators of these cations, while the least efficient, *Chlorella*, accumulated greater quantities of the anion $Cr_2O_7^{2-}$ than the other two strains. Accumulation of Cu^{2+}, Pb^{2+}, and trivalent Cr^{3+} was partially dependent on the initial ambient metal concentration in that a similar percentage of the metal was accumulated over one or more orders of magnitude of initial ambient metal concentration. Similarly Costa and Leite (1991) found that the amount of Cd^{2+} and zinc accumulated by species of *Chlorella* and *Scenedesmus* was dependent on the external metal concentration, with increasing metal accumulation at increased external metal concentrations until a level was reached at which suspected toxic effects resulted in reduced Cd^{2+} accumulation. Vymazal (1987) concluded that metal uptake was linear over a certain range, but as the quantity of biomass in relation to the available metal is increased, the proportional accumulation diminishes hyperbolically as a "weight dilution effect" occurs.

In the present study it was found that Cu^{2+}, Pb^{2+}, and Cr^{3+} were accumulated rapidly. Vymazal (1984) found that *C. glomerata* was found to accumulate metal cations at two distinct rates, dependent on the type of metal cation being accumulated. Ni^{2+}, Cr^{3+}, Fe^{2+} and Mn^{2+} were found to be accumulated at a steady rate over an extended period, while Cu^{2+}, Pb^{2+}, Cd^{2+} and Co^{2+} were rapidly accumulated after which accumulation was relatively minimal.

Chlorella vulgaris has previously been shown to be capable of accumulating a wide variety of metal cations (Darnall et al., 1986)

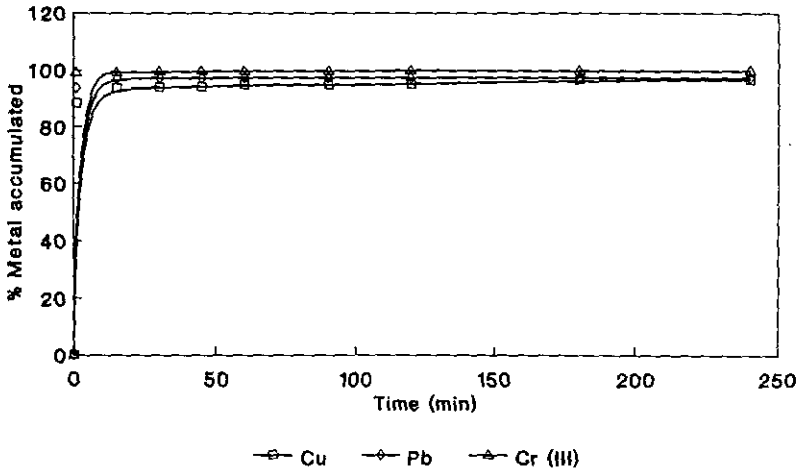

Figure 1
Rate of metal accumulation by Scenedesmus

where the alga simultaneously accumulated a variety of metal cations, and the metals may be selectively desorbed. Most metals, such as Cu^{2+}, were eluted by reducing the pH to 2, while others, such as Hg^{2+}, Au^{2+} and Ag^{+} remained firmly bound. The Au^{2+} and Hg^{2+} could then be selectively eluted by addition of mercaptoethanol (which presumably dissociates the metals which bind more firmly to thiol groups on the biomass). This added selectivity may make the biomass more viable than synthetic ion exchange resins which usually possess a single ligand type.

Cr^{3+} is used in the leather tanning process, and a considerable amount of this metal is found in most tannery waste waters. Studies on post-anaerobic digestion tannery effluent showed that algae were not entirely effective at removing Cr^{3+} from solution. Although 95% of the Cr^{3+} from an artificial solution could be accumulated by *Scenedesmus*, only 39% could be removed from the tannery effluent. A possible explanation is that the tannery waste water contains many organics, for example skin proteins, which bind Cr^{3+}, and thereby compete with the biomass for the metal.

Other investigations have been carried out involving the addition of sewage to metal-rich mine waters to induce algal blooms (which utilise the available nutrients from the sewage), and thereby to facilitate algal accumulation of metals (Jackson, 1978). However, problems with this method may arise because certain types of sewage in themselves may be loaded with toxic concentrations of metals, thereby inhibiting the growth of the algae (Wong and Lay, 1980; Tam and Wong, 1983).

If live algae are to be utilised in continuous bioaccumulation of metals then stable algal populations must be maintained. This necessitates control of the toxic levels of the metals being accumulated and a knowledge of their effects. In the present study, growth of all 3 of the strains of algae investigated was most inhibited by high concentrations of Cu^{2+}, although chromate anions also inhibited growth. Capolino et al. (1991) also found Cd^{2+} to be toxic to species of *Chlorella* and *Scenedesmus* - more so than a range of other metals such as Cu^{2+}, Pb^{2+} and Hg^{2+}. Poisoning of *Scenedesmus* and *Chlorella* species by metals (especially Cd^{2+}) can be a major factor in their operational parameters (Capolino et al., 1991; Costa and Leite, 1991). Attempting to grow algae in toxic metal-containing waste waters would therefore be problematic. Reports of metal toxicity to algae are numerous and often contradictory. Albergoni et al., (1980) have reported that Cu^{2+} is more toxic than Cd^{2+}, while others provide evidence to support the opposite effect (Wren and McCarroll, 1990). The variable factor here may be the tolerance of the species involved and more specifically the amount of the toxic metal accumulated in each case. The presence of Cu^{2+} may often lengthen the lag period of algal growth, but not affect the exponential growth rate, which has led certain authors to suggest that perhaps the first cell division after a period of inactivity is more sensitive to Cu^{2+} toxicity, because of unique biochemical conditions, than are later periods of division (Morel et al., 1978). Alternatively this may signify a period of biochemical adaption or selection for tolerant mutants. Meticulous investigations of these mechanisms are imperative for the effective maintenance of the algal bioaccumulation process.

Temperature may enhance metal toxicity to algae. For instance *Scenedesmus acutus* was more sensitive to mercury at higher temperatures (Huisman et al., 1980). This is also true of other algae (Knowles and Zingmark, 1978). However temperature may be positively linked to accumulation of metals within certain temperature ranges (Huisman et al., 1980; Darnall et al., 1986), which means that sacrifice of the cells is necessary for optimal metal bioaccumulation. This may require separation of algal growth pools from pools where the bioaccumulation process occurs and occasionally tapped for fresh biomass when the biomass in the bioaccumulation pools is saturated.

Alternatively metal-tolerant algal cultures may be used. Tolerance to metal toxicity may be achieved by algae in various ways. For instance bioaccumulation may be reduced by a decrease in the permeability, active accumulation and absorption surfaces, while active excretion could also play a role (Albergoni et al., 1980). Algae may express intracellular chelators in the presence of metal ions. A copper-tolerant strain of *Scenedesmus* has been reported to produce a metallothionein type protein, while *Chlorella pyrenoidosa* and *Dunaliella* produced metallothionein type proteins when exposed to high cadmium concentrations (Gadd, 1990). Albergoni et al. (1980) determined that *Euglena gracilis* was capable of producing two glycoproteins; the larger one (of over 100 kDaltons) accumulated Cd^{2+}, while a smaller one (6.5 to 8.0 kDaltons) accumulated Cu^{2+} and differed from the larger glycoprotein in apparently being excreted extracellularly into the medium. Reports of possible exo-chelators in natural freshwater bodies by blue-green algae (*Aphanizomenon* spp.) have been published (De Haan et al., 1981).

In conclusion it was found in this study that the algae *Scenedesmus*, *Selenastrum* and *Chlorella* were capable of accumulating Cu^{2+}, Pb^{2+}, and Cr^{3+} from solutions of 100 mg·t^{-1} metal with 67 to 99% efficiency. The chromate anion was accumulated at only 8 to 22% efficiency, indicating that there are more binding sites for cations than anions. Chromium from tannery effluent was only accumulated to 39% of the total, possibly due to to binding competition with organics or a high level of oxidation of Cr^{3+} to $Cr_2O_7^{2-}$. Algal flocculation tended to follow the same trend as metal accumulation. Metal toxicity, a limiting factor when considering the growing algae in metal containing wastewaters, was seen to occur in the presence of 100 mg·t^{-1} Cu^{2+} and 100 mg·t^{-1} $Cr_2O_7^{2-}$, but not equal concentrations of Pb^{2+} or trivalent Cr^{3+}. The simultaneous growth of algae and metal bioaccumulation would therefore be possible if the ambient metal concentration was below the toxic level for that metal.

Recently higher plants have been shown to accumulate metals from metal-contaminated waters (De Wet et al., 1990). In the present study it has been shown that unicellular freshwater algae may represent an alternative bioaccumulant biomass for treating metal-contaminated waters. Further research into the possibility of utilising algae and yeast for remediating metal-contaminated waste waters is being conducted in this laboratory.

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