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Bioaccumulation of Aluminium in *Dunaliella tertiolecta* in Natural Seawater: Aluminium–Metal (Cu, Pb, Se) Interactions and Influence of pH

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Aluminium (Al) is the third most abundant crustal element and is ubiquitous in the environment. The chemical form, and hence solubility, of Al is influenced by environmental factors, of which pH is the most important. Its solubility in receiving waters increases 1000 times for each one unit drop in pH (Havens and Decosta 1987). Elangovan et al. (1997) have recorded Al levels of up to 700 µg. L^{-1} in neutral waters. Under acidic conditions, Al is highly soluble while at neutral pH, mostly exists as the insoluble $Al(OH)_3$ (Martin 1986). Although the entry of Al into the food chain at neutral pH is thought to be limited owing to its low solubility (Driscoll and Schecher 1989), the Al concentration (200 µg. g^{-1} dry weight) in marine algae and plants is higher than that of many heavy metals such as copper (1-16 µg. g^{-1} dry weight) lead (<5 µg. g^{-1} dry weight) and etc. (Depledge et al. 1995). The chemical speciation of Al changes upon acidification from an insoluble, $Al(OH)_3$, to negatively charged, $Al(OH)_4^-$, and positively charged hydroxo complexes, $Al(OH)_2^+$, $AlOH^{+2}$ and free Al^{+3} ions (Folsom 1986). Not only the free Al^{+3} species but also polynuclear Al-species which occur at neutral pH (Hörnstorm et al. 1995) and the highly reactive hydroxo complexes are considered to be toxic to algae (Lindemann 1990). Rueter et al. (1987) reported that the addition of Al to acidified waters, however, not only increases the total amount of this metal but can also change the concentration of organic and inorganic complexes with other metals and, consequently, the activities of the other metals. Therefore, metal pair interaction is a potent factor to be considered.

For a complete description of the effects of increased Al in ecosystem it is necessary to centre on green algae as important primary producers in aquatic systems. In the present study, *Dunaliella tertiolecta* was selected for the bioaccumulation experiments since it fulfils most of the criteria for a bioassay organism. Under the microscope, it appears to be unicellular ellipsoid with diameter ranges of 8.25±1.25 and 6.41±1.5 µm.

In this study, the bioaccumulation of Al in 24 hours by the marine phytoplankton *D. tertiolecta* was investigated both at pH 6.2 and 8.2 and over a range of exposure concentrations. The bioaccumulation was then compared to that of two essential and one nonessential elements, namely copper and selenium, and lead, respectively. Subsequently, metal ion combinations were used for the examination of synergism and antagonism in Al-Cu, Al-Pb, and Al-Se pairs at pH 8.2.

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MATERIALS AND METHODS

This study used the unicellular green alga *D. tertiolecta* supplied by Dr. Okay from Marmara Scientific Research Center (Turkey). The cells were cultured in 500 ml batches maintained in a growth chamber (LEEC) continuously illuminated with two warm light fluorescent lamps (2x40 W) at 21±2°C. The algae were inoculated in natural seawater enriched by modified f/2 medium (Okay and Gaines 1996). Natural seawater ($S=24.7^{0}/_{00}$) used in the preparation of the cultures and for all experiments was collected from the Samatya coast of Istanbul in September 1997. Before the inoculation, seawater was filtered through a 0.47 μm GF/C glass fibre filter (Whatman) and sterilized in an autoclave.

The time required for the equilibration of metals between seawater and algae were analysed (Ercan, 1998). To provide the same experimental conditions for the four metals, algal cells were exposed to the metals for 24-hr. Batch bioaccumulation experiments were conducted by exposing 100 ml of exponentially growing cell cultures to metal ion solutions of known concentrations. $Cu(NO_3)_2.5H_2O$ (Merck), $Pb(NO_3)_2$ (Merck), $Na_2SeO_3.5H_20$ (Riedel-de Haën) and $Al(NO_3)_3.9H_2O$ (Merck) were used for the preparation of 1000 mg. L^{-1} stock solutions of cations in distilled water. After 24-hr, triplicate 10 ml samples were taken and filtered through prewashed and preweighed cellulose nitrate filter (Millipore HA 0.45 μm) and the filtrate was analysed for its concentration of metal ions. The pH of the culture medium in seawater was 8.2±0.2. 0.1 M HNO_3 was used to adjust the pH to 6.2±0.2. Synergistic and antagonistic effects of metal pairs were investigated only at the pH value of 8.2±0.2. The bioaccumulation of metals in *D. tertiolecta* was calculated on the basis of dry weight.

After the digestion of algae retained on filters with HNO_3, the analyses for the cellular Al concentration were carried out using the erichrome cyanine R method (APHA 1989). The bioaccumulations of copper and lead were determined by atomic absorption spectrophotometry (Shimadzu AA-680) and of selenium by spectroflourimetry (APHA 1989). To evaluate the possible precipitation of the metals in the culture medium, non-inoculated enriched seawater spiked with the studied concentrations of metals was also filtered. The background metal content of seawater was measured by extracting the preconcentrated seawater sample together with a chelating agent into the organic solvent (Lajunen 1992). The background Cu, Pb, Se and Al levels in seawater were 6.63±0.15 μg. L^{-1}, 0.90±0.24 μg. L^{-1}, 0.14±0.09 μg. L^{-1}, and 0.64±0.20 μg. L^{-1}, respectively.

All assays were carried out in duplicate and on two separate occasions. Data points are averages and error bars represent standard deviations. Al bioaccumulation was analysed by single factor ANOVA (Excel 5.0). Significant differences between metal pair analyses ($p<0.05$) were sought by *Student's t-test* (Statistica, Release 4.5).

RESULTS AND DISCUSSION

Fig. 1 illustrates the bioaccumulation data, for Al over the studied concentration range, at pH 6.2 and 8.2. Perusal of Fig. 1 reveals that for the same equilibrium time, bioaccumulation of Al is higher for greater values of initial Al concentration at both pH values. Statistical analysis showed that the bioaccumulation of Al in *D. tertiolecta* was dependent on pH. A two-fold increase in the bioaccumulation of Al was observed for a pH decrease of two units. At pH 8 Al is found as uncharged and negatively charged hydroxide complexes (Schnour 1985). At this pH, precipitation and adsorption on the cell surface can dictate the behaviour of Al. These processes decrease the permeability of the cell membrane and hence the uptake of Al inside the cell wall may be low. The pH dependence of metal uptake can be largely related to the net ionic charge on the cellular surface and also metal solution chemistry. At pH 6, the algal surface is not totally protonated compared to the lower pH values. At pH 6.5, the most important species of Al are reported as $Al(OH)_2^+$ and $Al(OH)_4^-$ (Lindemann 1990). Adsorption on the cell surface affects the dissolution of these hydroxides. Metabolic uptake of Al by green alga *S. obliquus* at pH 6.8 and 5.2 has been reported (Kong et al. 1999). Since bioaccumulation is largely related to binding of metals to cell surface components and intracellular accumulation, the increase in bioaccumulation of Al at pH 6.2 may be due to both adsorption and intracellular uptake. Additionally, the surface of algae can behave as a colloidal surface with double layer. However, more works such as X-ray fluorescence in association with scanning electron microscopy are necessary to characterise the mechanisms that govern the bioaccumulation of metals in the alga.

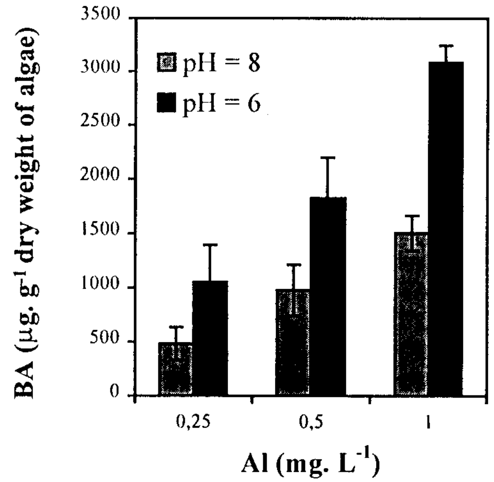
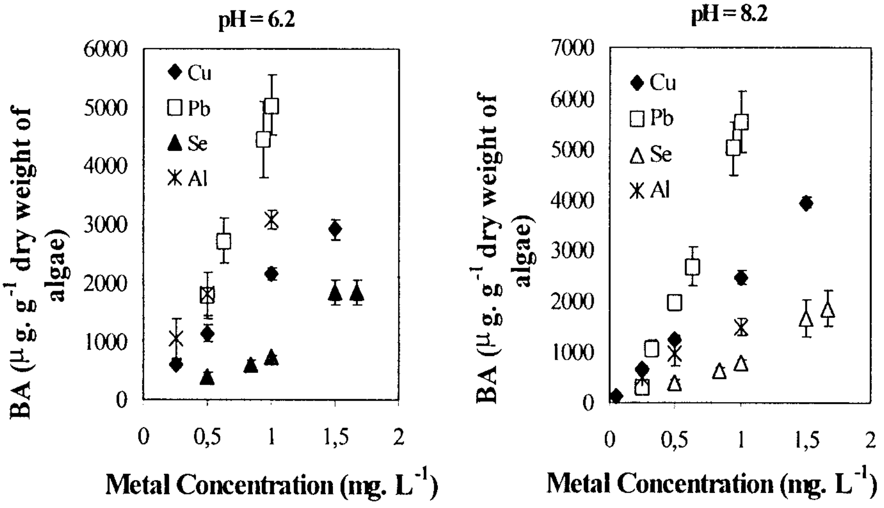


Figure 1. The relationship between the bioaccumulation (BA) of Al in *D. tertiolecta* and the total Al concentration in seawater.

The bioaccumulation of Al was compared with our previous study (*Türker Saçan et al. in press*) in which we measured the bioaccumulation of Cu, Pb and Se at the same pH values. Relationships between the bioaccumulation of four metals and metal concentrations at pH 6.2 and 8.2 are shown in Fig. 2. (a) and Fig. 2. (b), respectively.



(a) (b)

Figure 2. The relationships between the bioaccumulation of four metals and metal concentrations a) at pH 6.2 and b) at pH 8.2.

The order of bioaccumulation was found as Pb> Al> Cu> Se at pH 6.2, whereas the order of bioaccumulation was found as Pb> Cu> Al> Se at pH 8.2. Decreasing the pH increases the bioaccumulation of Al, but decreases the bioaccumulation of Cu. The pH dependence of heavy metals uptake can be related to the distribution of functional groups on the algal surface and to changes in the free aquo metal ion activities (Xue and Sigg 1990). The dissolved Cu pool is dominated by free hydrated metal ions at low pH and uncharged inorganic complexes at high pH (Peterson and Nyholm 1993). The decrease in the bioaccumulation of Cu can be explained simply competition between the free hydrated Cu ions and the monovalent hydrogen ions for binding to the cell surfaces at low pH. Protons have no effect on metal adsorbtion to the algae near pH 8. In the normal pH range, the phosphate groups are the ionogenic sites which bind metals such as Cu (II) without releasing any protons (Davilla et al. 1995a). On the other hand, as proteins which are part of the membranes possess hydroxyl and carboxyl groups for coordinate binding of metal ions. This binding often involves exchange of protons. Al shows high affinity to bind organic acids. Hydroxyl and carboxyl groups are able to mobilize Al^{+3} from Al-hydroxo complexes (Schnitzer and Kenndorf 1981). In other words, this change in the order of bioaccumulation may be attributed to the differences between Cu-surface and Al-surface binding sites and the aqueous form of metal ions at pH values of 6.2 and 8.2.

In another study, it was reported that the uptake rate of Al and Cu by duckweed was lower than the uptake rate of Pb over the pH values 4.0-5.0 (Mo et al. 1988).

It was also found that Al was more toxic than Cu for duckweed at lower pH and higher ambient concentrations but the relationships were reversed at higher pH values. Although toxicity does not always parallel bioaccumulation and may depend on the nature and amount of aquatic plankton and its stage of development, the toxicity studies on duckweed are consistent with our bioaccumulation data. The bioaccumulation of Se was the lowest of the four metals even at the highest concentration tested, but looking through the bioaccumulation values of the four metals, it can be concluded that the alga has a great capability of accumulating metals.

The bioaccumulation results of Al-Cu, Al-Pb and Al-Se in algae are tabulated in Table 1 together with the bioaccumulation values of the four metals when they were added singly. No significant difference ($p>0.05$) was observed in the bioaccumulation of Al in algae in the presence of Cu for different Al-Cu concentrations. An indirect toxicity of Al to the green alga *Scenedesmus* via liberation of Cu from organic complexes has been reported at pH 7.3 and for Al and Cu concentrations of 80μmol. L^{-1} and 5μmol. L^{-1}, respectively (Rueter et al. 1987). Our bioaccumulation results are not parallel to these toxicity studies because the algal-metal sequestring process occurs by different mechanisms, depending on the algae, pH and the solution conditions. Futhermore, the concentrations of metal were much lower than those studied in this work. Davilla et al. (1995b) suggested that the adsorption of metal ions can be correlated with a equilibrium surface complex formation considering the existence of the two kinds of sites, high affinity and low affinity. These metals may not compete for the binding sites on the surface of the algal cell wall and the binding and/or adsorption sites of the two metals seem to be different. Although electron micrographs indicated a shrinking of the cell wall of *Chlorella saccarophila* in the presence of both Cu and Al (Folsom et al. 1986), no quantitative interaction was observed between the bioaccumulation of these two metals in *D. tertiolecta*.

No significant difference was observed in the bioaccumulation of Al in the presence of Pb but the bioaccumulation of Pb decreased significantly ($p<0.05$) in the presence of Al compared to the bioaccumulation when only lead was added to seawater. Thus, the presence of Al and Pb in a two metal system showed antagonistic behaviour in the bioaccumulation of Pb due to the competition of ions for adsorption sites on the cell wall. This implies that *D. tertiolecta* cells concentrate non metabolic metals such as Al and Pb on the same surface components of the cell wall and Al has a higher affinity to bind the algal surface, and/or adsorption of Al reduces surface charge density thereby making the remaining sites less effective for adsorption of lead.

Although the interference of Al with the uptake, transport and utilisation of several nutrient elements including Ca, Mg, P and K (Foy et al. 1976), and the interactions of Se with Hg, As, Co, Cd, and Ag in organisms living in the aquatic environment (Pelletier 1985; Ting et al. 1991) are known, the combination of Al and Se resulted in no significant difference in bioaccumulation compared with the treatments where metals were added singly at the same concentration.

Table 1. Mean bioaccumulation (BA) of copper, lead, selenium and aluminium in *D. tertiolecta* exposed to metals for 24-hr at pH=8.2±0.2

Cu (mg.L^{-1})	Pb (mg.L^{-1})	Se (mg.L^{-1})	Al (mg.L^{-1})	BA_{Cu}[2] (µg.g^{-1})	BA_{Pb}[3] (µg.g^{-1})	BA_{Se}[4] (µg.g^{-1})	BA_{Al}[5] (µg.g^{-1})
0.5				1262(±150)[1]			
1.0				2480(±143)			
	0.5				1985(±135)		
	1.0				5538(±601)		
		0.5				404(±50)	
		1.0				799(±67)	
			0.5				979(±236)
			1.0				1505(±162)
0.5	-	-	0.5	1245(±131)	-	-	1142(±260)
1.0	-	-	0.5	2814(±809)	-	-	1259(±216)
0.5	-	-	1.0	1172(±131)	-	-	1682(±469)
-	0.5	-	0.5	-	811(±161)	-	945(±121)
-	1.0	-	0.5	-	2934(±924)	-	1207(±280)
-	0.5	-	1.0	-	982 (±260)	-	787(±276)
-	-	0.5	0.5	-	-	335 (±118)	1152(±414)
-	-	1.0	0.5	-	-	599 (±107)	1226(±253)
-	-	0.5	1.0	-	-	294 (±57)	1690(±167)

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[1] standard deviations
[2] µg Cu.g^{-1} dry weight of algae
[3] µg Pb.g^{-1} dry weight of algae
[4] µg Se.g^{-1} dry weight of algae
[5] µg Al.g^{-1} dry weight of algae

Se has chemical similarities with sulphur, can behave as a sulphur analogue in algae and becomes toxic for marine organisms at seawater concentrations higher than required to provide the essential dose. The toxic dose varies also with the nature of the selenium compounds. Selenite and selenate are the most common inorganic forms of selenium. Selenite is more toxic than selenate to some algal species such as *Cricosphaera elongata* (Boisson et al. 1995). However, we observed microscopically that Se administered as selenite prolonged the exponential growth phase of *D. tertiolecta* and suppressed the effect of lead in joint exposure (*Türker Saçan M. et al. in press*). The lack of selenite toxicity was attributed to a capacity of the algae to regulate the influx or efflux of selenite or to detoxify it intracellularly (Boisson et al.1995). However, the bioaccumulation data did not parallel the microscopic observations in joint exposure of Pb-Se. In the present study, a similar trend in bioaccumulation was shown in microscopic observations of joint exposure to Al-Se. Al is not a nutrient for microorganisms and Se is regarded as an essential trace element beneficial to marine algae. The lack of interaction between Al and Se is probably due to the essential and nonessential nature of Al and Se, respectively, and the absence of competition between the two metals for membrane binding sites.

However, these data should be applied to natural water systems with caution since adsorption/desorption equilibrium occur in the natural systems. Other environmental factors such as salinity, temperature and organic carbon content etc. highly effect the speciation of metal ions and greatly interfere with their binding to an algal surface. Therefore, further studies are needed to better understand processes affecting the bioaccumulation of metal ions in natural systems. On the other hand, a practical use and another aspect of the interactions discussed in this study may be the extraction of toxic and/or valuable metals such as cobalt, zinc, copper, silver and gold etc. from waste water by complexation with algae. Such metals could be recovered by metal exchange or acidification, which decreases the number of binding sites thereby releasing the metals.

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