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Effect of cations, including heavy metals, on cadmium uptake by *Lemna polyrhiza* L.

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Cations, including calcium, magnesium, potassium, sodium, copper, iron, nickel and zinc, inhibited (up to 40%) extracellular binding and intracellular uptake of cadmium by *Lemna polyrhiza* in solution culture. Test plants showed a high capacity of extracellular cadmium binding which was competitively inhibited by copper, nickel and zinc; however, calcium, magnesium and potassium caused non-competitive inhibition. Iron and sodium increased K_m and decreased V_{max}, thereby causing mixed inhibition of extracellular binding. Intracellular cadmium uptake displayed Michaelis–Menten kinetics. It was competitively inhibited by calcium, magnesium, iron, nickel and zinc. Monovalent cations (sodium and potassium) caused non-competitive and copper caused mixed inhibition of intracellular cadmium uptake. Thus, high levels of cations and metals in the external environment should be expected to lower the cadmium accumulation efficiency of *L. polyrhiza*.

Keywords: cations, cadmium, extracellular binding, heavy metals, inhibition kinetics, intracellular uptake, *Lemna polyrhiza*

Introduction

While a great deal of information has accumulated on the toxicity and uptake of single species of heavy metal ions in aquatic plants and algae, relatively little is known about the combined effects of two or more metals (Braek *et al.* 1980, Wong *et al.* 1980). This ignorance is concerning as natural waters and effluents always contain a mixture of metal ions. There is a pressing need to understand how uptake and toxicity of a metal is influenced by the presence of other metal ions. This exigency gets support from Wong *et al.* (1978) who carried out algal bioassays with a mixture of metals whose individual concentrations were within the recommended discharge levels. They found metals to be extremely toxic when present in combinations.

In general, a mixture of heavy metals can produce three possible types of interactions: synergistic, antagonistic and non-interaction (Ting *et al.* 1991). Most studies on combined effects show simple cases of either synergism or antagonism. Several explanations have been proposed for these synergistic and antagonistic actions. For instance, when the combined effect of copper and nickel on the cell population of *Chlorella vulgaris* was found to be synergistic, it was supposed that this resulted from an increase in membrane permeability (Hutchinson & Stokes 1975). Passow *et al.* (1961) showed that the cell membrane is the target site of activity of cupric and mercuric ions. In contrast, antagonistic interaction was reported in the case of cadmium and selenium for the same alga, and it was suggested that screening or competition for the binding sites on the cellular surfaces had resulted in the metal ions mutually alleviating their individual toxic effects.

An extensive literature survey by Ting *et al.* (1991) reveals that a few researchers have employed metal uptake as the test criterion in algae–metal interaction. Stokes (1975) found that copper and nickel enhanced each others uptake in *Scenedesmus acutiformis*, and thus acted synergistically. Mierle (1982) observed inhibition of copper uptake by a number of metals. However, Braek *et al.* (1980) reported both antagonistic and synergistic action for zinc and cadmium uptake by different algae. Similar investigations are fewer in the case of lichens (Beckett & Brown 1984), bryophytes (Brown & Beckett 1985, Wells & Brown 1990) and higher plants, including aquatic macrophytes (Huebert & Shay 1991).

Cadmium is regarded as one of the most toxic heavy metals. The principal uses of cadmium are in metal electroplating, in alloys, as stabilizing material for plastics

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and in batteries (Wong *et al.* 1980). Mine waters may contain up to 42 mg Cd l^{-1} (Fleischer *et al.* 1974). It may be possible to use aquatic plants for stripping cadmium from wastewaters since they are known to accumulate high concentrations of this metal (Hutchinson & Czyrska 1972, Moore & Ramamoorthy 1984, Sela *et al.* 1989, Huebert & Shay 1992). The present study deals with the influence of certain metals (copper, nickel, iron and zinc) and cations (calcium, magnesium, potassium and sodium) on the extracellular binding and intracellular uptake of cadmium in *Lemna polyrhiza*. Attempts were also made to see if these metals and cations could change the kinetic parameters for cadmium uptake in test plants.

Materials and methods

L. polyrhiza was collected from paddy fields at Jakhama (Kohima district, Nagaland, India) and cultivated in AAP medium (Wang 1986) at 25 ± 1 °C under a 14 h light (PAR 45 μmol m^{-2} s^{-1}) and 10 h dark cycle, and at pH around 7.

Healthy plants of *L. polyrhiza* were selected for experiments. Prior to incubation in test solutions, selected plants were gently washed in double-distilled water for 10 min. For each experiment, freshly-prepared test solutions were used in the following forms: cadmium as cadmium acetate, calcium as calcium chloride, magnesium as magnesium chloride, potassium as potassium carbonate, sodium as sodium chloride, copper as cupric chloride, iron as ferric chloride, nickel as nickel chloride and zinc as zinc sulfate.

The purpose of washing test plants before setting up the experiments was to remove nutrient solution generally present on the plant surfaces and to avoid interference of nutrient cations in the kinetic studies. However, washing test plants with double-distilled water will not completely remove cations adhering to the walls of root cells and other parts of test plant, but this provided uniformity in the experiments.

All experiments were performed at 20 °C and at pH 7. All the test solutions were unbuffered. Experiments were carried out in double-distilled water by adding the desired doses of cadmium or the cations. For the control experiment, five concentrations of cadmium (i.e. 10, 20, 40, 80 and 100 μM) were taken. In each of these solutions, about five fronds of *L. polyrhiza* were floated and incubated for 2 h. Each treatment had a minimum of five replicates. In order to determine the kinetics of inhibition of cadmium uptake in test plants by different competing cations and metals (cadmium, magnesium, potassium, sodium, copper, iron, nickel and zinc), *L. polyrhiza* was incubated in test solutions containing 100 μM of interacting cation and 10, 20, 40, 80 and 100 μM of cadmium, in a manner similar to that described by Brown & Beckett (1985). Reports are available showing such high concentrations of cations and metals in certain unpolluted and polluted waters (Taylor & Demayo 1980, Demayo & Taylor 1981, Taylor *et al.* 1981, Wehr & Whitton 1983, Pringle *et al.* 1986). Test plants were harvested after a 2 h treatment.

Several chemicals, such as EDTA and $NiCl_2$, have been recommended to displace heavy metals adsorbed onto plant surfaces (Bates *et al.* 1982, Beckett & Brown 1984). In the present work, the desorption of extracellular cadmium was carried out by shaking and washing the plant biomass with 20 ml of 10 mM EDTA for 10 min followed by rinsing with chilled double-distilled water. The analysis of this washing gave the extracellular metal level and the analysis of EDTA-washed plant material gave the intracellular metal level. Plant samples were dried at 60 °C until constant weights were obtained.

Dried plant materials were weighed and digested following the method used by Bates *et al.* (1982). Weighed plant samples were taken in borosilicate test tubes along with 5 ml of digestion mixture consisting of concentrated nitric acid, hydrogen peroxide 30% (v/v) and double distilled water in 1:1:3 ratio (v/v/v). Each sample was then gently digested on a hot plate until a clear solution of about 0.5 ml was left in the test tube. The volume of this solution was made to 5 ml with 2% (v/v) nitric acid. The digested samples were analyzed for cadmium content at the Regional Sophisticated Instrumentation Centre, NEHU, Shillong, using a Perkin-Elmer 2380 atomic absorption spectrophotometer.

Kinetic parameters were calculated for intracellular uptake and extracellular binding of cadmium by *L. polyrhiza* using procedures outlined by Wells & Brown (1987). Since intracellular cadmium uptake followed typical Michaelis–Menten kinetics, K_m (Michaelis–Menten constant; the ion concentration required for half maximal uptake) and V_{max} (maximal uptake rate) were calculated. These kinetic constants could not be calculated for extracellular cadmium binding, which did not occur at a constant rate throughout the experiment. The kinetic constants for extracellular cadmium binding calculated for the present work are K_s (dissociation constant; the ion concentration required for half maximal uptake) and U_{max} (maximal uptake capacity) (see Wells & Brown 1990).

Results and discussion

The incubation of test plants in 100 μM Cd solution led to cadmium adsorption (extracellular binding) as well as transport (intracellular uptake) (see Table 1). The intracellular uptake occurred at almost a constant rate up to

Table 1. Extracellular binding and intracellular uptake of cadmium by *L. polyrhiza* from a solution containing 100 μM Cd

Time (Min)	Extracellular binding (μmol Cd g^{-1})	Intracellular uptake (μmol Cd g^{-1})
10	6.5 ± 0.7	0.34 ± 0.03
20	12.0 ± 1.1	0.69 ± 0.15
30	15.8 ± 0.7	1.12 ± 0.16
60	22.1 ± 0.8	2.11 ± 0.14
90	24.3 ± 1.0	2.84 ± 0.26
120	26.6 ± 1.4	3.82 ± 0.22
180	26.8 ± 1.2	4.53 ± 0.26

Mean ± SD.

2 h. The extracellular binding occurred at a rapid rate during the initial stage of incubation, but slowed down with the passage of time. It became saturated in 2 h. When test plants were incubated at different concentrations of cadmium (10, 20, 40, 80 and 100 μM) for 2 h at 20 °C and the pH adjusted to 7, both extracellular binding and intracellular uptake showed saturation kinetics. The kinetic constants for intracellular uptake (K_m and V_{max}) and extracellular binding (K_s and U_{max}) are given in Table 2. All of the tested cations and metals, i.e. calcium, magnesium, potassium, sodium, copper, iron, nickel and zinc, inhibited extracellular binding of cadmium. Changes in kinetic parameters for extracellular cadmium binding, i.e. K_s and U_{max}, in the presence of inhibiting cations can be seen in Table 2. It is clear that calcium, magnesium and potassium did not change K_s, whereas U_{max} for extracellular cadmium binding was significantly decreased. Hence, these cations cause non-competitive inhibition. The present findings are contrary to the observations of Wells & Brown (1990) who found calcium and magnesium to be competitive inhibitors for cadmium adsorption. According to Brown & Beckett (1985), the inhibition of extracellular cadmium binding by calcium and magnesium was not wholly competitive in a moss studied by them. The non-competitive inhibition of extracellular binding of cadmium by potassium has been observed by Wells & Brown (1990) as well. A suggestion has been made that the surface binding capacity for divalent cations could be increased by increasing the concentration of a monovalent cation (Demarty *et al.* 1978, Wolterbeek 1987). It is believed that the stimulation of divalent cation binding is due to an increase of electrostatic attraction (rather than chemical binding) by anionic cell wall groups depleted of protons by the monovalent cations. This was definitely not the case in the present study as potassium and sodium inhibited cadmium adsorption.

Copper, nickel and zinc caused competitive inhibition of cadmium binding, but the nature of inhibition of extracellular binding by iron was mixed, as an increase in K_m and a decrease in V_{max} occurred concomitantly (Table 2). Sodium also caused mixed inhibition.

The cations causing competitive inhibition of cadmium binding are obviously competing for the same binding sites. The ability of certain cations (e.g. calcium, magnesium and potassium) to non-competitively inhibit extracellular cadmium binding implies that binding of cations affected the surface charge density of anionic sites available for cadmium binding (see Wells & Brown 1990). It has been suggested that Cd^{2-} and K^+ bind to different sites and K^+ binding reduces the number of Cd^{2+} binding sites (Wells & Brown 1990). The ionic radius of K^+ is 40% greater than that of Cd^{2+} (Weast & Astle 1983). It seems probable that K^+ binding to one site reduces the space available in the extracellular matrix, thereby excluding Cd^{2+} binding.

Similar to the extracellular cadmium binding, calcium, magnesium, potassium, sodium, copper, iron, nickel and zinc caused a reduction in intracellular cadmium uptake (Table 2). Calcium and magnesium significantly increased the apparent K_m for cadmium uptake, whereas V_{max} remained unchanged. Thus, calcium and magnesium competitively inhibited intracellular cadmium uptake. John (1976) and Wells & Brown (1990) also found competitive inhibition of cadmium uptake by calcium. Magnesium has, however, been found to be a non-competitive inhibitor of cadmium uptake in a moss (Wells & Brown 1990). Beckett & Brown (1984) found competitive inhibition of cadmium uptake by magnesium and postulated that intracellular cadmium uptake occurs by a system normally transporting magnesium. A possible explanation for competitive inhibition of cadmium uptake by calcium could be their ionic similarities, the ionic radius is 99 pm for Ca^{2+} and 97 pm for Cd^{2+}. Iron also caused competitive inhibition of cadmium uptake (Table 2). Gipps & Coller (1982) also reported antagonistic action of iron on cadmium toxicity which obviously resulted through decreased intracellular transport of cadmium.

Potassium and sodium did not bring about any change in apparent K_m for intracellular cadmium uptake; however, V_{max} was significantly decreased. This suggests non competitive inhibition of cadmium uptake. Both of these inhibitory cations are monovalent, that is why they showed a similar effect. Potassium has been found to be a competitive inhibitor of cadmium uptake in oat and lettuce

Table 2. Kinetic constants for the inhibition of extracellular binding and intracellular uptake of cadmium by calcium, magnesium, potassium, sodium, copper, iron, nickel and zinc in *L. polyrhiza*

Inhibiting cation	Extracellular binding		Intracellular uptake	
	K_s (μM)	U_{max} (μmol g^{-1})	K_m (μM)	V_{max} (μmol g^{-1} h^{-1})
None (control)	30.5 ± 4.3	36.0 ± 7.0	56.6 ± 6.2	2.9 ± 0.2
Ca	30.4 ± 0.9	24.5 ± 6.1****	99.0 ± 11.0****	2.8 ± 0.0
Mg	32.2 ± 6.0	28.0 ± 3.3****	90.0 ± 14.0****	2.8 ± 0.6
K	33.4 ± 8.1	29.7 ± 5.2**	56.0 ± 6.6	1.8 ± 0.3**
Na	35.5 ± 4.2**	24.0 ± 5.9****	58.0 ± 8.0	2.0 ± 0.5*
Cu	40.2 ± 6.0****	30.7 ± 4.8	62.0 ± 7.0	1.1 ± 0.5***
Fe	37.7 ± 6.4***	27.5 ± 3.4****	77.0 ± 13.0****	2.8 ± 0.3
Ni	51.0 ± 11.2****	34.3 ± 5.7	78.0 ± 4.0****	2.7 ± 0.2
Zn	45.4 ± 8.3****	32.7 ± 5.2	111.0 ± 18.0****	3.1 ± 0.3

Mean ± SD. Data marked with asterisks are significantly different from control according to Student's *t*-test: **** $P < 0.001$, *** $P < 0.005$, ** $P < 0.01$, * $P < 0.05$.

seedlings (John 1976). Wells & Brown (1990) found that concentration of KNO_3 greater than 1 mM caused both stimulation in transport site activity (increased V_{max}) and reduced affinity for cadmium (increased K_m). The mode of inhibition of intracellular cadmium uptake in *L. polyrhiza* by nickel and zinc was found to be competitive (Table 2). Ting *et al.* (1991) could not find any effect of zinc on intracellular transport of cadmium in a green alga. Others, however, report competitive interaction between zinc and cadmium (Gipps & Coller 1982, Huebert & Shay 1992).

In the presence of copper, the apparent K_m for intracellular uptake of cadmium was increased and V_{max} was decreased concomitantly (Table 2). Therefore, copper caused mixed inhibition of cadmium uptake in test plant. Others have observed the competitive action of copper on cadmium uptake (Schmid *et al.* 1965, Bowen 1969, Brown & Beckett 1985). Beckett & Brown (1984) interestingly found stimulation of cadmium uptake by copper in the lichen *Peltigera*. They suggested that copper perhaps temporarily damaged the plant's plasma membrane making it more permeable to cadmium.

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