Bull. Environ. Contam. Toxicol. (2004) 73:182–189

DOI: 10.1007/s00128-004-0411-3

Bulletin of Environmental Contamination and Toxicology

Lead and Cadmium Accumulation and Toxicity in the Presence of EDTA in *Lemna minor* L. and *Ceratophyllum demersum* L.

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Received: 4 June 2003/Accepted: 21 April 2004

In contaminated aquatic environments several toxic metals occur, often creating undesirable living conditions for many plants and animals (Palmer and Wittbrot 1991). Vascular aquatic macrophytes may accumulate considerable amounts of heavy metals in their tissues (Kovacks et al. 1984). In the recent past, several of the submerged, emergent and free-floating aquatic macrophytes are reported to bioconcentrate heavy metals in natural waters as well as after exposure to wastewaters (Greger 1999). Salt et al. (1995) reported that aquatic or semiaquatic vascular plants such as *Eichhornia crassipes*, *Hydrocotyle umbellata*, *Lemna minor* and *Azolla pinnata*, can take up Pb, Cu, Cd, Fe and Hg from contaminated solutions that have been around for a long time. Influence of heavy metals on plant metabolism has been studied for many years, mainly as effect of plant exposure to a single heavy metal (Siedlecka and Krupa 1999). Several investigators have shown that Pb and Cd in sediments and the water column can have an adverse effect on plant growth (Miller et al. 1977). The concentrations of lead in polluted sources waters lie typically in the range 1-100 μg mL^{-1} . These concentrations are, however, frequently reduced during treatment, prior to discharge to the receiving waters (Harrison and Laxen, 1981). Cadmium in wastewaters may range from less than 0.01 μg Cd mL^{-1} in relatively clean water to greater than 10 μg Cd mL^{-1} in contaminated water (Chen et al., 1977).

In recent years, the remobilization of metals by synthetic anthropogenic chelating agents has received much attention. The use of EDTA (ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid) has especially been questioned because of their potential for increasing the solubilization and remobilization of heavy metals from aquatic sediments (Müller and Förstner 1976). The chelator EDTA forms a soluble complex with many metals, such as Pb (Kroschwitz 1995). The literature to date reports a number of chelators that have been used for chelate-induced hyperaccumulation. (Huang et al. 1997). EDTA is the most commonly used chelate because of its strong chelating ability for different heavy metals (Norvell 1991). EDTA has two advantages with respect to other chelates – its relatively low biodegradability in groundwater systems (Nowack 1996) and its strong complexing capacity with heavy metals (Kedziorek and Bourg 2000). Potentially, chelates are known to render insoluble cations soluble and thus, they become available to plants (Lindsay 1974). Dirilgen (1998) reported that the effects of

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EDTA and other chelators on the uptake and toxicity of metals in aquatic biota have been investigated by numerous researchers. This paper deals with the effects of EDTA on Pb^{2+} and Cd^{2+} accumulation and their toxicity in terms of changes in total chlorophyll in *Lemna minor*, a free-floating aquatic macrophyte and *Ceratophyllum demersum*, a rootless submerged aquatic macrophyte.

MATERIALS AND METHODS

Lemna minor L. (duckweed) and *Ceratophyllum demersum* L. (coontail) are commonly available aquatic macrophytes in Adana, Turkey. We chose *L. minor* based on the research of Mohan and Hosetti (1997) and *C. demersum* Ornes and Sajwan (1993). The macrophytes were collected from the local water bodies. These were acclimatized in 5 % Arnon and Hoagland (1940) nutrient solution under 25-27 °C , 14 h light (6000 lux) and 10 h dark periods. The macrophytes were treated with 0.5 mM EDTA as $C_{10}H_{14}N_2Na_2O_8.2H_2O$ (MERCK), 50 μg Pb mL^{-1} as $Pb(CH_3COO)_2 . 3H_2O$ (MERCK), 0.5 μg Cd mL^{-1} as $CdCl_2 . H_2O$ (MERCK), 50 μg Pb mL^{-1} plus 0.5 mM EDTA, 0.5 μg Cd mL^{-1} plus 0.5 mM EDTA, 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} plus 0.5 mM EDTA. For *C. demersum* 10.1-11.2 g and *L. minor* 5.2-5.5 g initial fresh weights were used in 3000 mL plastic pods. Macrophytes were placed in each concentration and this process repeated three times. A sample of 5 % nutrient medium was used as control. The pH's of solutions were adjusted to 7.0 using N/10 H_2SO_4 or N/10 NaOH. Both *C. demersum* and *L. minor* were harvested after 7-days of exposure and analyzed for metals and chlorophyll content. Chlorophyll content was determined following Arnon (1949). The extraction of pigment was done in acetone (80 % v/v, MERCK). For extraction 100 mg of *Lemna* fronds or *Ceratophyllum* leaves were used. Then, the extraction solution was filtered and absorbance of the filtrate was recorded at 652 nm spectrophometrically (SHIMADZU UV mini 1240). Extraction solution was used as blank.

Harvested macrophytes were washed three times with distilled water, kept on filter paper for a few seconds to remove excess water and weighed. Then, they were dried at 80 °C to constant weight. Metal concentrations were determined according to Fargasova (1998). For mineralization dried samples (0.02 g) were dissolved in 2 mL of HNO_3 (65 % p.a. MERCK) with two drops H_2SO_4 (95-97 % p.a. MERCK). After mineralization, metals were determined by Atomic Absorption Spectrophotometer (Perkin Elmer 3100). Control samples were also treated the same way because of determination of their lead and cadmium content. Values of uptake were given after deducting metal contents of control macrophytes. Concentration factor (CF) was calculated following Foster (1976).

$$CF = \frac{\text{Metal concentration in macrophyte (μg } g^{-1} \text{ dry weight)}}{\text{Initial metal concentration in media (μg } mL^{-1})}$$

Metal concentrations and chlorophyll amounts in both aquatic macropyhtes are the means of the three replicates. For statistical analyses we chose the analysis of

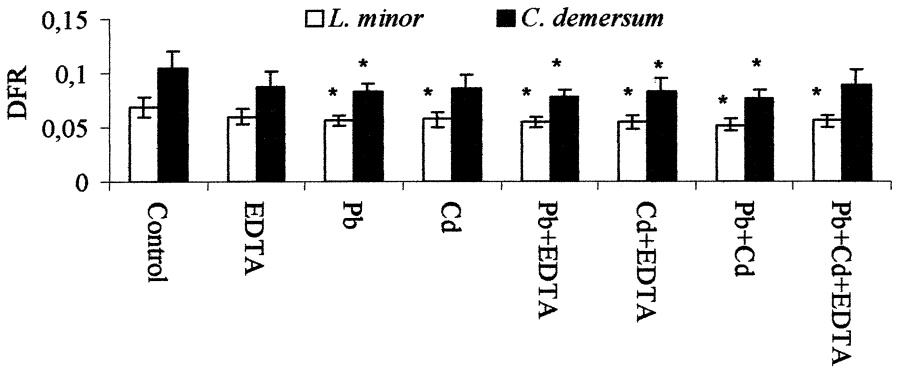

Figure 1. Effects of Pb and Cd supplement with and without EDTA on DFR of *L. minor* and *C. demersum.* Bars are represent mean±standard deviation. *P<0.05.

variance (ANOVA) in SPSS package programme (11.0 for windows). The significance of differences between mean values a multiple range test (LSD; Least Significant Difference) was used. For this reason, alpha (α) was preferred to be 0.05, which corresponds to a confidence level of 95 %.

RESULTS AND DISCUSSION

Single and combined Cd and Pb with and without EDTA concentrations affected growth in both macrophytes after seven days exposure. As a rule, Cu^{2+} inhibited growth more than other metals, and the effect of Pb^{2+} exceeded that of Cd^{2+} (Arambasic et al. 1995). According to our observation, both macrophytes were adversely affected in 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} combination more than other tested concentrations. *C. demersum* was affected adversely in 50 μg Pb mL^{-1} more than *L. minor*. However, *L. minor* was affected adversely in 0.5 μg Cd mL^{-1} more than *C. demersum.*

The ratio of dry to fresh weight (DFR) was not significant with respect to their control for both macrophytes in 0.5 mM EDTA concentration (P>0.05) (Figure 1). In *C. demersum* treated with 0.5 μg Cd mL^{-1} and Pb+Cd+EDTA medium, DFRs did not differ significantly with respect to the control (P>0.05). In other tested concentrations DFRs decreased significantly with respect to their control for both macrophytes (P<0.05). In *L. minor* and *C. demersum* the lowest DRFs, 24.0 and 26.5, was observed at 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} combination, respectively. Ornes and Sajwan (1993) observed that dry weights of *C. demersum* exposed to 0.01 and 0.04 μg Cd mL^{-1}, were higher than controls. This suggests a simulatory effect of Cd^{2+} at these levels. However, dry weights of the macrophytes exposed to 1.03 μg Cd mL^{-1} were decreased after 1 week. In the present study, similar findings were found for *C. demersum* after treating with 0.5 μg Cd mL^{-1}.

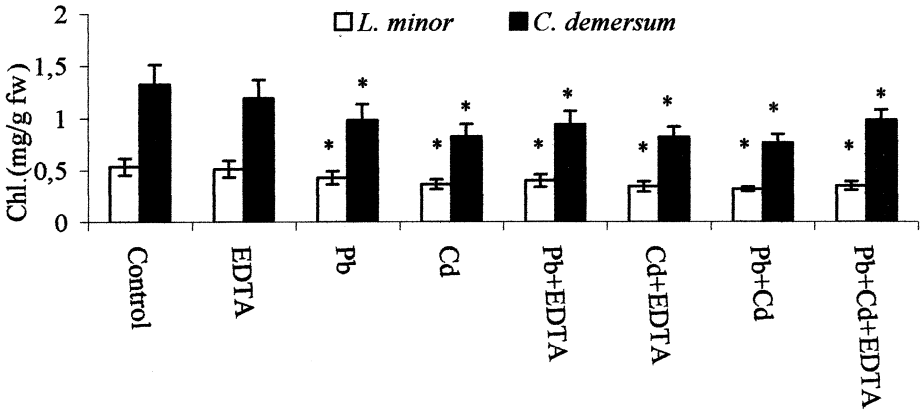

Figure 2. Effects of Pb and Cd supplement with and without EDTA on total chlorophyll content of *L. minor* and *C. demersum.* Bars are represent mean±standard deviation. *P<0.05.

Reductions of total chlorophyll contents were not be significant with respect to their control in 0.5 mM EDTA concentrations (P>0.05) (Figure 2). In other tested concentrations, reductions of total chlorophyll contents in *L. minor* and *C. demersum* were significant with respect to their control (P<0.05). In 50 μg Pb mL^{-1}, 50 μg Pb mL^{-1} plus 0.5mM EDTA, 0.5 μg Cd mL^{-1}, 0.5 μg Cd mL^{-1} plus 0.5 mM EDTA, 50 μg mL^{-1} Pb plus 0.5 μg Cd mL^{-1}, 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} plus 0.5 mM EDTA medium, total chlorophyll contents decreased by 20.4, 25.3, 31.7, 36.0, 40.5 and 34.7 %, respectively, in *L. minor* and they decreased 25.9, 29.1, 37.8, 38.0, 42.6 and 25.2 %, respectively, in *C. demersum.*

Chlorophyll content is a parameter that is sensitive to heavy metal toxicity (Gupta and Chandra,1996). The effects of Cd^{2+} and Pb^{2+} on photosynthesis and chlorophyll synthesis are more thoroughly studied (Seregin and Ivanov, 2001). By comparison, cadmium induced toxicity was more than lead toxicity. Cd decreased chlorophyll content in *Lemna* plants at 0.5, 1.0, 5.0 and 10.0 μM Cd concentrations after four and eight days treatment. In a similar way lead also exerted its adverse effects on the *Lemna* plants by decreasing the chlorophyll pigment at 0.25, 0.5, 0.75 and 1.0 mM Pb concentrations after four and eight days treatment. (Mohan and Hosetti, 1997). At 1.0 μg Cd mL^{-1} level chlorophyll content of *C. demersum* reduced to almost half of the control values in 168 h (Gupta and Chandra,1996). Because cadmium is more toxic than lead, Cd^{2+} adversely affected chlorophyll content more than Pb^{2+} in both macrophytes (Figure 2). Nasu et. al. (1983), reported that EDTA mitigated the toxic effect of copper but not of cadmium. According to the our findings, EDTA didn't mitigate toxic effects of $Pb^{2+}$and Cd^{2+}on chlorophyll content, when tested separately. On the other hand, EDTA decreased toxic effects of $Pb^{2+}$and Cd^{2+}on chlorophyll content in macrophytes treated with Pb+Cd+EDTA combination. The highest decrease was 42.6% in *C. demersum* in Pb+Cd combination compared to control. In *L. minor* in the same medium, the decrease in total chlorophyll was 40.5%. Cd^{2+} and Pb^{2+} disrupt photosynthesis in different

(Paul et. al., 1995). Van Assche and Clijters (1990) reported that the reduction in chlorophyll content in the presence of the Pb^{2+} may be due to an inhibition of chlorophyll biosynthesis. One of the important enzymes of chlorophyll biosynthesis is aminolevulinic acid (ALAD) which catalyses the formation of porphobilinogen. Lead inhibits ALAD activity by binding with –SH group of the enzyme and overall chlorophyll biosynthesis through Mg^{2+} (Singh 1995).

Pb^{2+} amounts in control *L. minor* and *C. demersum* were determined as 7±3 and 19±8 μg g^{-1}, respectively. In Tablo 1, values are shown after deducting lead contents of control macrophytes. In *L. minor* and *C. demersum* exposed to 50 μg Pb mL^{-1}, metal concentrations were 1116 and 3858 μg g^{-1} , respectively (Table 1). These results show that Pb^{2+} accumulation was higher in *C. demersum* compared to *L. minor* ($P<0.05$). Although in *L. minor* in 50 μg Pb mL^{-1} plus 0.5 mM EDTA combination, Pb^{2+} concentration did not significantly increase ($P>0.05$), however, in *C. demersum* in the combination, Pb^{2+} concentration increased significantly ($P<0.05$). Our findings demonstrated that tested combinations affected Pb^{2+} accumulation in both macrophytes. In spite of statistically significant enhancement in Pb^{2+} content in *L. minor* exposed to 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} combination ($P<0.05$), in *C. demersum*, Pb^{2+} amount decreased indifferently ($P>0.05$). In Pb+Cd+EDTA combination, Pb^{2+} accumulation decreased in *C. demersum* but increased in *L. minor*. These show significant differences in metal accumulation in comparison to 50 μg Pb mL^{-1} ($P<0.05$).

Cd^{2+} concentrations in control *L. minor* and *C. demersum* were determined as 1.2±0.6 and 3.7±0.9 μg g^{-1}, respectively. In Tablo 2, values are shown after deducting cadmium contents of control macrophytes. In *L. minor* and *C. demersum* treated with 0.5 μg Cd mL^{-1} the metal amounts were estimated to be 1136 and 420 μg g^{-1}, respectively (Table 2). These results show that Cd^{2+} accumulation was higher in *L. minor* compared to *C. demersum* ($P<0.05$). In 0.5 μg Cd mL^{-1} plus 0.5 mM EDTA combination, EDTA significantly decreased Cd^{2+} accumulation in both macrophytes ($P<0.05$). In 50 μg Pb mL^{-1} plus 0.5 μg Cd mL^{-1} combination, Cd^{2+} accumulation of both macrophytes were adversely affected by Pb^{2+} and these values were found to be significant when compared to 0.5 μg Cd mL^{-1} ($P<0.05$). In Pb+Cd+EDTA combination, the lowest Cd^{2+} amount was determined as 71 in *C. demersum*, while it was 290 μg g^{-1} in *L. minor* ($P<0.05$).

Concentration factor (CF) was estimated from ratio of metal concentrations in macrophyte (μg g^{-1} dw) to metal in test medium (μg mL^{-1}). According to this, the highest CF for Pb^{2+}, 117, was estimated in *C. demersum* treated with 50 μg Pb mL^{-1} plus 0.5 mM EDTA combination. The lowest CF, 22, was observed in *L. minor* exposed to 50 μg Pb mL^{-1} (Table 1). In *L. minor*, the highest CF for Cd^{2+}, 2272, was observed with 0.5 μg Cd mL^{-1} and the lowest factor, 142, was determined in *C. demersum* in Pb+Cd+EDTA combination (Table 2).

Metal bioaccumulation depends upon plant species, its organs, and numerous abiotic factors such as temperature, pH, transport of metal-contaminated particles and dissolved ions in water (Lewis 1995; Lewander et al. 1996). According to our

results, the submerged macrophyte, *C. demersum* accumulated more Pb^{2+} than the free-floating plant *L. minor* in 50 μg Pb mL^{-1} medium. *L. minor*, however, accumulated more Cd^{2+} than *C. demersum* in 0.5 μg Cd mL^{-1} medium. When combined, Cd^{2+} and Pb^{2+} usually produced synergistic effects and infrequently were antagonistic (Lepp 1977). Pb^{2+} content in the roots of beech seedlings treated with low concentrations of Cd^{2+} and Pb^{2+} was lower under the combined treatment than when treated only with Pb^{2+} (Breckle 1991). In Pb+Cd interaction, Cd^{2+} enhanced Pb^{2+} accumulation in *L. minor*, but insignificantly decreased in *C. demersum* in comparison to individual Pb^{2+}. In same interaction, Cd^{2+} accumulation was decreased by Pb^{2+} in both *C. demersum* and *L. minor*.

Chelating agents such as EDTA have the potential to increase the bioavailability of metal leading to increased metal accumulated in plant tissue (Kroschwitz 1995). The results in the present investigation indicate that EDTA enhanced Pb^{2+} accumulation in both macrophytes, in particular *C. demersum*, and decreased Cd^{2+} accumulation particularly in *L. minor*. Nevertheless, EDTA decreased Cd^{2+} accumulation in the combination Pb+Cd+EDTA. In Pb+Cd+EDTA combination CF, which indicates the efficiency of the macrophytes in accumulating the metals, is found to increase Pb^{2+} accumulation compared to individual Pb^{2+}treatment.

Table 1. Content and concentration factor (CF) of Pb in *L. minor* and *C. demersum* with and without EDTA treatment.

	Pb (μg g^{-1} dry weight)		CF	
Concentration	*L. minor*	*C. demersum*	*L. minor*	*C. demersum*
Pb	1116±262 ax	3858±335 ay	22±7 ax	77±15 ay
Pb+EDTA	1265±192 ax	5833±490 by	25±9 ax	117±32 by
Pb+Cd	2208±187 bx	3823±290 ay	44±12 ax	77±16 ay
Pb+Cd+EDTA	2215±196 bx	2240±211 cx	44±11 ax	45±9 cx

Values are mean± standard deviation. Means with different letters are significantly different from one another at 0.05 level. a, b and c indicate difference among exposure concentrations, and x and y indicate difference between macrophytes.

Table 2. Content and concentration factor (CF) of Cd in *L. minor* and *C. demersum* with and without EDTA treatment.

	Cd (μg g^{-1} dry weight)		CF	
Concentration	*L. minor*	*C. demersum*	*L. minor*	*C. demersum*
Cd	1136±154 ax	420±68 ay	2272 ±325 ax	840±189 ay
Cd+EDTA	115±15 bx	335±32 ay	230±56 bx	670±165 ay
Pb+Cd	770±151 cx	150±22 by	1540±256 cx	300±59 by
Pb+Cd+EDTA	290±41 dx	71±13 by	580 ±145 dx	142±29 by

Values are mean± standard deviation. Means with different letters are significantly different from one another at 0.05 level. a, b, c and d indicate difference among exposure concentrations, and x and y indicate difference between macrophytes.

As treatment tools, because metal contamination in the aquatic systems is still an important environmental problem, the use of macrophytes, which have high metal

accumulation ability such as *L. minor* and *C. demersum*, can reduce this problem to some extent. Results obtained in the present study can be a source for further investigation dealing with phytoremediation of metal contaminated waters.

Acknowledgements: This study was supported partially by Çukurova University Research Fund.

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