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Potential of *Azolla caroliniana* for the removal of Pb and Cd from wastewaters

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A b s t r a c t. Heavy metals constitute a serious health risk because they accumulate in soils, water and organisms. One of the methods of removing these pollutants from water and soil is the use of plants (phytoremediation). There are many plants (hyperaccumulators) which have the ability to accumulate large amounts of heavy metals. One of them is the aquatic fern *Azolla* sp., which can bind some substances. The aim of this study was to verify the ability of *Azolla caroliniana* Willd. (*Azollaceae*) to fix Pb and Cd from polluted waters. During the experiment, *A. caroliniana* was grown in water solution enriched in Pb(II) and Cd(II), each at concentrations of 0.1, 0.5 and 1 mg dm^{-3}. The presence of lead and cadmium ions caused an inhibition of *A. caroliniana* growth by about 30-37 and 24-47%, respectively. After the end of the experiment, the content of the metals tested was determined in the medium and in the biomass (after mineralization). In the water medium, the decrease of Pb(II) amounted to 90% and that of Cd(II) to 22%. In the *A. caroliniana* tissues, the content of lead was up to 416 mg Pb per kg d.m., and that of cadmium – up to 259 mg Cd per kg d.m.

K e y w o r d s: *Azolla*, bioaccumulation, heavy metals: lead, cadmium

INTRODUCTION

Heavy metals are common in human activity and constitute a serious health risk because they easily accumulate in soils, water and organisms. Lead (Pb) and cadmium (Cd) are among such metals. They are quite widespread (Evanko and Dzombak, 1997) and expose people to high toxicity. Cadmium has a mutagenic effect on live organisms (Watanabe and Endo, 1997). Also lead is a very dangerous metal which easily accumulates in tissues, especially in bones and liver (Krieger, 1990).

One of the methods of lowering the content of heavy metals in the environment is to use plants to remove them from the substratum, this is called phytoremediation. There are many plants, which can absorb extremely high amounts of heavy metals. They are called hyperaccumulators and they are exploited to clean up the environment (Evanko and Dzombak, 1997).

Alfalfa (*Medicago sativa* L., *Trifolieae*) is highly adept at binding lead and cadmium (Gardea-Torresdey *et al.*, 1996). Also *Thlaspi caerulescens* Presl. (*Brassicaceae*) can bind large amounts of cadmium (Nedelkoska and Doran, 2000). Hop (*Humulus lupulus* L., *Cannabinaceae*) can be used to remove lead ions from contaminated water (Gardea-Torresdey *et al.*, 2002) and the willow (*Salix* sp. L., *Salicaceae*) can bind cadmium (Klang-Westin and Perttub, 2002). *Taraxacum officinale* Weber (*Asteraceae*) and *Ambrosia artemisiifolia* L. (*Asteraceae*) have the ability to remove Pb and Cd from soil (Pichtel *et al.*, 2000). *Phaseolus vulgaris* L. (*Fabaceae*) could be used to absorb pollutants from water contaminated with lead (Piechalak *et al*., 2002) and Indian mustard (*Brassica juncea* L., *Brassicaceae*) is a high-biomass Pb accumulator (Gleba *et al.*, 1999). Also duckweed (*Lemna minor* L., *Lemnaceae*) can effectively remove lead from water (Rahmani and Sternberg, 1999).

The aquatic fern *Azolla caroliniana* Willd. (*Azollaceae*) is a small plant, common in many parts of the world, especially in tropical environments (Watanbe *et al.*, 1992). A specific feature of this fern is its symbiosis with the cyanobacterium *Anabaena azollae* Strasb. (*Nostoceae*) which can bind atmospheric nitrogen. Due to this, *Azolla* sp. is used as a green manure, especially in rice fields in Asia (Carrapiço, 2001). The fern has other applications (Bennicelli *et al.*, 2004), and one of them is the bioaccumulation of heavy metals. Some experiments were conducted

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which, prove that *Azolla* sp. can bind metals like Zn, Pb, Cu, Cd, Au, Ni, Sr, Cr and Hg (Gaur and Noraho, 1995; Sanyahumbi *et al.*, 1998; Antunes *et al*., 2001; Cohen-Shoel *et al*., 2002; Bennicelli *et al*., 2004). This fern can also remove nutrients (Forni *et al*., 2001) and organic substances like sulphonamides (Forni *et al*., 2002).

The aim of the research presented in this paper was to study the effect of Pb and Cd concentration in nutrient solution on biomass growth of *A. carolininana* and the accumulation of these metals.

METHODS

The fern was put into aquariums containing a 3 dm^3 liquid nutrient medium prepared according to the International Rice Research Institute (IRRI) recommendation (Watanabe *et al.*, 1992). The metals examined were introduced into each aquarium in their salt form ($PbCl_2$ and $CdCl_2\ 2\frac{1}{2}H_2O$) in 3 concentrations: 0.1, 0.5 and 1 mg dm^{-3}. These did not contain nitrates, so that the *A. caroliniana* could use the nitrogen provided by the *A. azollae*. In this way 9 treatments (3 per metal) were obtained. A 10th aquarium was used as a control, which contained only a nutrient medium. The initial *A. caroliniana* biomass was 20 g.

The experiment was carried out at a photoperiod of 18/6, air temperature of 25°C, and air humidity of about 70%. The *A. caroliniana* was cultured for 2 weeks with 6 control points during this period. The following actions were performed: monitoring of air temperature and humidity, observations of the fern and of the concentration of Pb(II) and Cd(II) in the solution. At the end of the experiment, the biomass of *A. caroliniana* was collected, washed and weighed to determine its fresh mass. After that the dry mass of the plants was determined after drying at 80°C and used to determine the metals content in the biomass. These measurements, after mineralizing by acid digestion (HNO_3) in a microwave closed system, were conducted by the FAAS (Flame Atomic Absorption Spectrometry) method in liquid samples and the ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) method.

Statistical analysis was performed to describe the significance of the influence of heavy metals on *A. caroliniana*. One-way analysis of variance (ANOVA) was done to examine differences in metal contents in the solution. Results were presented as mean values with Lowest Significance Differences (LSD) and a 95% confidence level. Also, correlation coefficients (R^2) and regression equations were calculated to show interactions between the values analyzed.

RESULTS AND DISCUSSION

The decrease of Pb(II) concentration in the nutrient medium is shown in Fig. 1. Generally, a rapid reduction of Pb(II) concentration was noted in the treatments with 0.1 and 0.5 mg dm^{-3} of lead ions. Already on the 2nd day of the experiment in the 0.1, 0.5 and 1 mg Pb(II) dm^{-3} treatments there remained 0.029, 0.272 and 0.619 mg dm^{-3} of lead ions, respectively. In the following days, the concentration of lead ions was further reduced. Only in the 0.5 mg Pb(II) dm^{-3} treatment it decreased quickly to 0.028 mg dm^{-3} on the 4th day of the study. Finally, the decline constituted 82 and 90% of the initial Pb(II) concentrations of 0.5 and 0.1 mg dm^{-3}, respectively. In the course of the 1 mg dm^{-3} Pb(II) treatment, this decrease was lower and amounted to 47.6%. This may mean that *A. caroliniana* can remove lead ions from solutions after 2 days. All the values were highly correlated (R^2 = 0.85-0.97) and the Pb(II) decrease was statistically significant (P<0.00005).

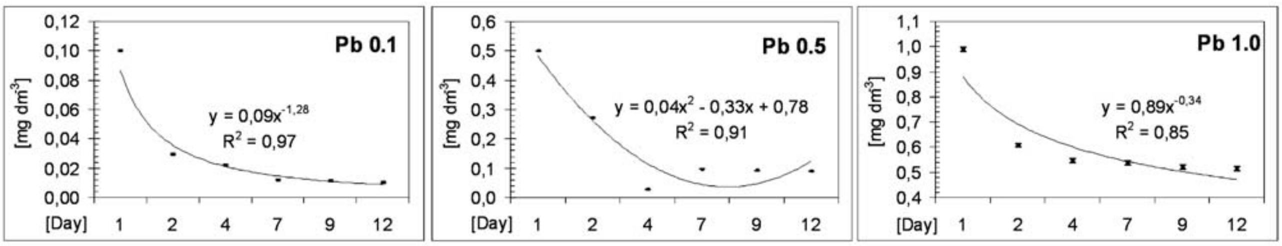

Fig. 1. Changes in Pb(II) concentration in the nutrient medium during the experiment.

Changes in the concentration of cadmium ions in all the treatments are shown in Fig. 2. Concentrations of Cd(II) in all the treatments tested underwent small changes during the experiment. Decrease of the metal concentration constituted 10, 18 and 22% for the 0.1, 0.5 and 1 mg Cd(II) dm^{-3} treatments, respectively. Negative correlation of Cd(II) concentration with time increased with metal dosage (R^2 = 0.75-0.95) and the decrease of the concentration with time was not significant only in the Cd 0.1 treatment (P=0.0588). Reduction of Cd(II) ions was considerable in the remaining treatments (P<0.00005).

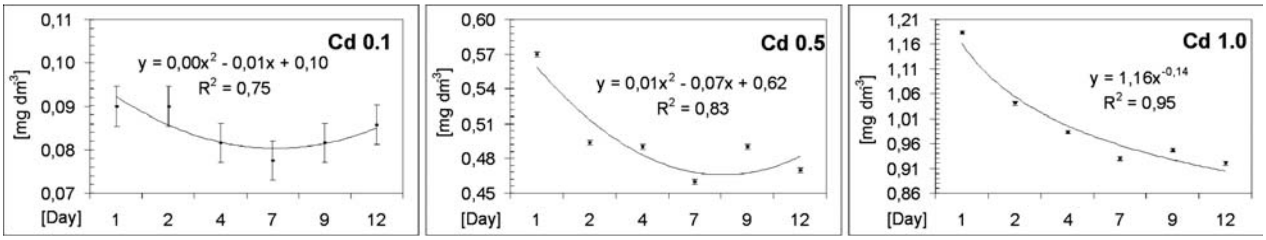

Fig. 2. Changes in Cd(II) concentration in the nutrient medium during the experiment.

The growth of *A. caroliniana* in all the treatments is shown in Fig. 3. In the control treatment there occurred about a 4.5-fold increase of biomass up to 89.9 g. In the remaining treatments the growth rate was lower; in the case of Pb, an addition 0.5 mg dm^{-3} of lead ions caused the strongest inhibition of *A. caroliniana* growth (36.8%). For the 0.1 and 1 mg dm^{-3} Pb(II) treatments, this inhibition amounted to 29.5 and 31.6%, respectively. The lowest dose of cadmium (0.1 mg dm^{-3}) caused the lowest decrease in biomass equal to 23.6%. Higher doses of this metal limited the increase of biomass by more than 40% (40.8% for 0.5 Cd(II) treatment and 46.5% for 1 mg dm^{-3} Cd(II) treatment). This suggests, that cadmium is a more toxic metal to *A. caroliniana* than lead in higher doses, and that low concentrations of cadmium are only marginally harmful to this plant, when the same dose of Pb(II) was more harmful (about 6% lower biomass) for the fern.

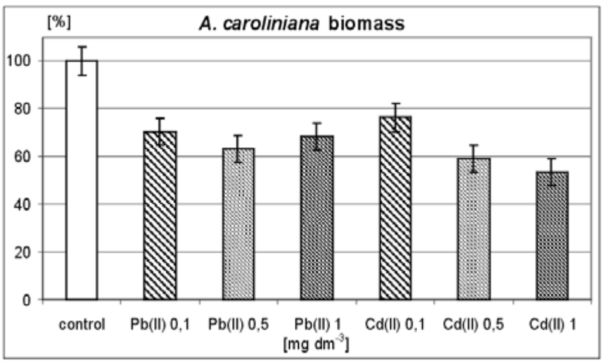

Fig. 3. The growth of *A. caroliniana* in each combination after 12 days of culturing.

The amount of the metals absorbed by *A. caroliniana* is shown in Figs 4A-B. The left part of the figure shows the concentration of lead taken from all the treatments. The amounts of accumulated metals depended on the initial doses – the bigger the initial dose the higher the accumulation. This relationship was described by the regression equation and showed a high correlation (R^2 equal to about 0.99).

A comparison of these two graphs shows that *A. caroliniana* has taken up more lead than cadmium. Lead amounts to 53-416 mg kg^{-1} d.m. and cadmium to 23-259 mg kg^{-1} d.m. This data suggests, that *A. caroliniana* is more tolerant to lead than to cadmium, and this confirms the earlier results about the higher toxicity of Cd.

In many experiments conducted on live and dried plants at different pH values, *M. sativa* bound 7.1 mg Cd, 7.7 mg Cr(III), 43 mg Pb(II), 4.9 mg Zn(II), but no Cr(VI). The recovery of bound metals from biomass amounted to 90% for Pb, Zn, Cr and 70% for Cd (Gardea-Torresdey *et al.*, 1996). Another hyperaccumulator, *T. caerulescens*, bound 62800 mg Cd(II) in 1 kg of d.m. (Nedelkoska and Doran, 2000). However, in the *H. lupulus* the leaves contained 72400 mg Pb(II) in 1 kg of biomass (Gardea-Torresdey *et al.*, 2002). Willows accumulated 40-80% of Cd introduced into

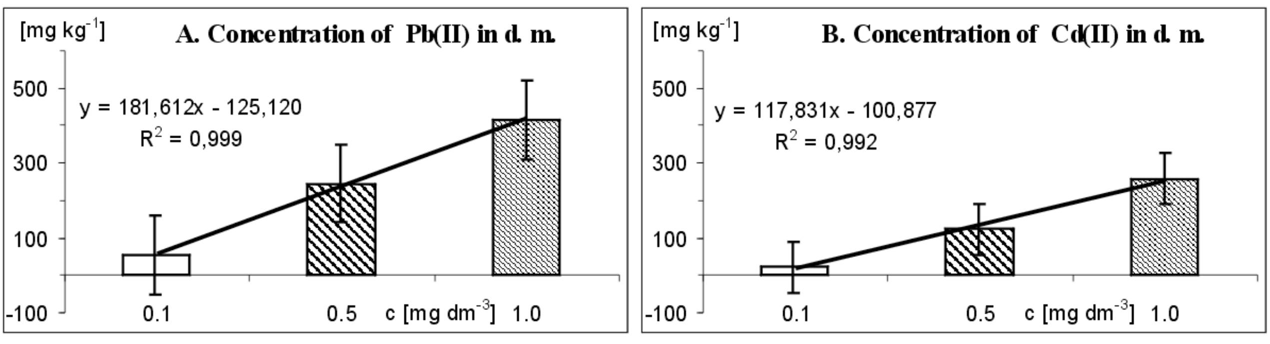

Fig. 4. Concentration of lead (A) and cadmium (B) in biomass taken from all the treatments.

the soil (Klang-Westin and Perttub, 2002). *Agrostemma githago* L. (*Caryophyllaceae*) roots contained 1800 mg Pb per kg and in *T. officinale* there was 15.4 mg Cd per kg (Pichtel *et al.*, 2000). Among the legumes, *P. vulgaris* had in its roots the largest amount of accumulated Pb equal 75 mg per g d.m. (Piechalak *et al.*, 2002). The common aquatic plant *L. minor* can remove up to 90% of soluble Pb from water (Rahmani and Sternberg, 1999).

Sorption of lead ions by dried *Azolla filiculoides* L. depended on pH values. When it was higher (3.5 and 4.5), the sorption was the highest – 95%. At lower pH values (1.5), the accumulation decreased to 30%. This plant removed up to 93000 mg Pb per kg of d.m. (Sanyahumbi *et al.*, 1998). However, *Azolla pinnata* Brown takes up to 90% of Cd from the medium (Gaur and Noraho, 1995). Experiments using *Azolla* sp. until now were performed mostly on dried plant which was packed into columns.

This suggests, that the fern can be used for cleaning waters polluted by heavy metals (phytoremediation). It is made possible by the plants defense mechanisms like the synthesis of detoxifying peptides (phytochelatins) or immobilization in the cell walls (Singh *et al.*, 1997; Jemal *et al.*, 1998; di Toppi and Gabbrielli, 1999; Kubota *et al.*, 2000) as well as in the dried form as has been suggested by many experiments (Sanyahumbi *et al.*, 1998; Fogarty *et al.*, 1999; Antunes *et al.*, 2001; Cohen-Shoel *et al.*, 2002).

CONCLUSIONS

1. The decrease of metal content in the nutrient medium and metal presence in plant tissue prove that *A. caroliniana* can absorb lead more effectively than cadmium.

2. The metals examined showed their toxic effects already in low doses (in comparison to other experiments), which is dangerous to living organisms; so, there is a need to neutralize them. The toxicity of the metals tested caused an inhibition of *A. caroliniana* growth by about 30-46%.

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