Vol.53, n. 1: pp. 235-240, January-February 2010
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Bioaccumulation and Chemical Forms of Cadmium, Copper and Lead in Aquatic Plants

JinZhao Hu [1*], AiZhen Zheng[1], DongLi Pei[1] and GuoXin Shi[2]
[1]Key Laboratory of Plant and Microorganism interaction; Department of Life Science; Shangqiu Normal University; Shangqiu 476000; China. [2]Institute of Plant Cell and Molecular Biology; College of Life Science; Nanjing Normal University; Nanjing 210097; China

ABSTRACT

The cadmium(Cd), copper(Cu) and lead(Pb) accumulation, as well as their relative content of different chemical forms in Sagittaria sagittifolia *L. and* Potamogeton crispus *L. were determined. The results showed that both the plants had the ability to accumulate large amounts of Cd, Cu and Pb, and they absorbed metals in dose-dependent manners. The roots of* S. sagittifolia *appeared more sensitive to Cd and Pb than the leaves of* P. crispus*. The potential of Cu uptake by these two plant tissues was similar. Under the same concentration, the uptake of Cu for both the plants was higher than Pb and Cd, while that of Pb was lowest. The Cd, Cu and Pb existed with various forms in the plants. Cd and Pb were mainly in the NaCl extractable form in* S. sagittifolia *and* P. crispus*. The HAc and ethanol extractable Cu were the main forms in the root, whereas the ethanol extractable form was the dominant chemical form in the caulis and bulb of the* S. sagittifolia *L.*

Key words: heavy metals, phytoextraction, macrophytes, *Sagittaria sagittifolia* L., *Potamogeton crispus* L.

INTRODUCTION

Water contamination by heavy metals in some area is practically inevitable due to natural process (weathering of rocks) and anthropogenic activities (industrial, agricultural and domestic effluents). Waste water from the industries of mining, electroplating, paint or chemical laboratories often contains high concentrations of heavy metals, including cadmium (Cd), copper (Cu) and lead (Pb). These elements, at concentrations exceeding the physiological demand of the plants, not only could administer toxic effect in them, but also could enter food chains, get biomagnified and pose a potential threat to human health (Sugiyama, 1994). Therefore, the determination of these toxic metals in wastewater is of substantial importance for environmental monitoring. In recent years, it has been reported that some plant species, known as hyperaccumulators, which are usually present in heavy metal-contaminated areas, have the ability to accumulate unusually high concentration of heavy metals without dramatically being physiologically impacted (Reeves and Brooks, 1983; Baker and Brooks, 1989). Other studies (Dirilgen and Inel, 1994; Zaman and Zereen, 1998) postulated the possibility of using aquatic plants in biological treatment of water. The use of biological organisms as indicators of metal toxicity was considered a relatively inexpensive, simple, and reliable alternative to chemical analyses (Hernandez et al., 1987). Among aquatic

[*]Author for correspondence: hujinzhaobio@hotmail.com

macrophytes, the role of submerged plants in relation with the accumulation of metals and toxicity has been well documented (Gupta et al., 1996; Sinha et al., 2002; Hu et al., 2007). However, there are few reports on Cd, Cu and Pb accumulation in *Sagittaria sagittifolia* L. and *Potamogeton crispus* L., which are widely distributed in China. *S. sagittifolia* is an important economic hygrophyte because of the quality of its products and its great medicinal functions, while *P. crispus* is a submerged aquatic plant, and can accumulate toxic metals, such as Fe, Pb, Ni, Cd and Cu (Ali et al., 1999; Rai et al., 2003). The major objective of this investigation was to assess the possible accumulation of Cd, Cu and Pb in these plants. The study could be helpful to understand the mechanisms adopted by *S. sagittifolia* and *P. crispus* under Cd, Cu and Pb stress.

MATERIALS AND METHODS

Bulbs of *S. sagittifolia* were collected from Shuang Zhazhen Plantation Farm (Nanjing). For acclimation, healthy and equal-sized bulbs were pre-cultured in 5% Hoagland solution. After a first true leaf outspread entirely, the seedlings were separated into three groups randomly, transplanted into beakers, and received 1, 3, 5, 10 and 20 mg L^{-1} of Cd^{2+}, Cu^{2+} and Pb^{2+} in 5% Hoagland solution. The *P. crispus* plants were collected from Gaoyou Lake (a freshwater lake in Jiangsu Province) and acclimatized for more than six months in large hydroponic tubs under natural conditions. Thereafter, the same weight (approximately 5 g fresh weight) of plants were detached, separated into three groups randomly, followed by growth for two weeks in 5% Hoagland solution (pH 6.5). Subsequently, the plants were transplanted into beakers and also received 1, 3, 5, 10 and 20 mg L^{-1} of Cd^{2+}, Cu^{2+} and Pb^{2+}. The plants grown in 5% Hoagland solution without additional Cd^{2+}, Cu^{2+} and Pb^{2+} ions served as control. All the beakers were placed into a Forma Scientific-3744 enclosed incubator (made in USA) under the laboratory conditions (Light: Dark, 14:10 h, 114 μmolphotons m^{-2} s^{-1} illumination provided by fluorescent tube light at 25°C ± 2°C). Nutrient solutions were changed every 48 h. On the fourth day of the treatment, the leaves between the third and the tenth leaf from the top of *P. crispus* (for both control and treated) and the root, caulis and bulb of the *S. sagittifolia* (control and treated) were harvested and analyzed separately.

In two independent experiments, the isolated plant samples were harvested, washed with distilled water and blotted between paper towels prior to weighing. Approximately 0.5 g of each control or treated sample was digested in supra-pure concentrated HNO_3: $HClO_4$ (3:1, v/v) at 60 °C in a water bath until about 2 mL acid digest was left. After cooling, the acid digest was diluted with 1 M HCl and made up to a final volume of 25 mL for the determination of metal contents on an Avanta Atomic Absorption Spectrophotometer (AAAS) (GBC, Australia). On the basis of the currently accepted protocol for metal uptake testing, the accumulation rates (R) of Cd, Cu and Pb were evaluated by the following formula,

$$R = C_1*25/C_2/G$$

where C_1: the metal concentration of the plant issue (mg g^{-1} FW)

C_2: the metal concentration of the solutions (mg L^{-1})

G: the fresh weight of plant (g)

For the evaluation of Cd, Cu and Pb chemical forms, a five-step sequential extraction procedure based on Xu et al. (1991) was used immediately after the samples were harvested and washed. The procedure was illustrated as follows: Total 37.5 mL 80% ethanol was added to 2 g fresh plant tissues in a 50 mL centrifuge tube. The tissues were submerged in the ethanol for 17-18 h at 30°C. Subsequently, the extract was reclaimed, and a second 37.5 mL aliquot of 80% ethanol was added to the centrifuge tube. The process was repeated every two hours for four times within 24 h, and the final volume of 150 mL extract was obtained. The following four steps were conducted similarly to the first step. The extractants were deionized water, 1M NaCl, 2% acetic acid and 0.6M HCl in sequence. At the end of each extraction step, total 150 mL extract was evaporated until almost dry. The residue was digested in supra-pure concentrated HNO_3: $HClO_4$ (3:1, v/v), and the concentrations of Cd, Cu and Pb in different chemical forms were analyzed by an AAAS (GBC, Australia).

Three replicates were conducted to assess the precision of the analytical techniques. The result was presented as average with standard deviation of three replicates. Statistical analysis was made by SPSS for Windows, *r* represents the correlation coefficient. All weights of the plant materials (g) were fresh weights.

RESULTS AND DISCUSSION

Plant metal absorption capacity can be represented by accumulation rate (R). In general, the higher the R value, the greater uptake capacity the plant has. In the present study, Cd, Cu and Pb contents in *P. crispus* leaves and *S. sagittifolia* roots increased significantly with increase in metal concentrations (Fig. 1A and 2A). For *S. sagittifolia* roots, *r* values of Cd, Cu, Pb were 0.991, 0.993 and 0.975. Corresponding correlation coefficient values for *P. crispus* leaves were *r* Cd =0.994, *r* Cu =0.966, and *r* Pb =0.995. Although both the plants absorbed metals in a dose-dependent manner, the R values of the three metals were different. The accumulation rates of Cd and Pb for *S. sagittifolia* roots increased continuously with increasing Cd and Pb concentrations (R_{Cd} ranged from 26 to 78 and R_{Pb} ranged from 14 to 104, Fig. 1B), while the same rates for *P. crispus* leaves changed slightly (R_{Cd}: 204~258 and R_{Pb}: 130~192, Fig. 2B). It seemed that *S. sagittifolia* possessed the maximum potential in accumulating Cd and Pb, especially at the highest concentrations, and its roots appeared more sensitive to Cd and Pb than the leaves of *P. crispus*. In contrast, the maximum accumulation rate value of Cu for both the plants occurred at 3 mg L^{-1} Cu. R_{Cu} ranged from 122 to 197 for *S. sagittifolia* roots and 246 to 524 for *P. crispus* leaves, (Fig. 1B and 2B), indicating that the maximum potential of Cu uptake by these two plant tissues was similar. Under the same concentration, the uptake of Cu for both the plants was higher than Pb and Cd, while that of Pb was lowest (Fig. 1B and 2B). This was probable because Cu was essential for the plants (Prasad et al., 2001) while Pb was a relatively immobile element (Dushenkov et al., 1995), and a mechanism of precipitation of Pb as sulphate at the root and leaf systems (Reeves and Brooks, 1983).

The results of uptake of Cd, Cu and Pb suggested that the absorption mechanism of these metals could be quite different due to plant species, metal characteristics, and the element density of the coexistence ion in the environment as well as their solubility. This reflected the complexity of plant metal uptake in the environment (Seregin and Ivanov, 2001).

However, total concentrations of metals can not provide sufficient toxic information. Metal mobility, bioavailability and toxicity often depend on not only their total concentration, but also their physicochemical forms (Yuan et al., 2004). The results of the sequential extraction procedure showed that the Cd, Cu and Pb existed in various forms, which correspond to different bioavailability after absorbed by the plant. In the present study, both Cd and Pb were mainly in the NaCl extractable form in the root, caulis and bulb of *S. sagittifolia* (Table 1) and *P. crispus* leaf (Table 2). The HAc and ethanol extractable, Cu was the main form in the root, whereas the ethanol extractable form was the dominant chemical form in the caulis and bulb of the *S. sagittifolia* (Table 1). The HCl extracted form in *P. crispus* leaves contained the most Cu (Table 2). Cd and Pb were mainly in the pectic acid and water-soluble forms in *S. sagittifolia* organs and *P. crispus* leaves, which potentially combined with proteins or presented in an absorbed form (Xu et al., 1991).

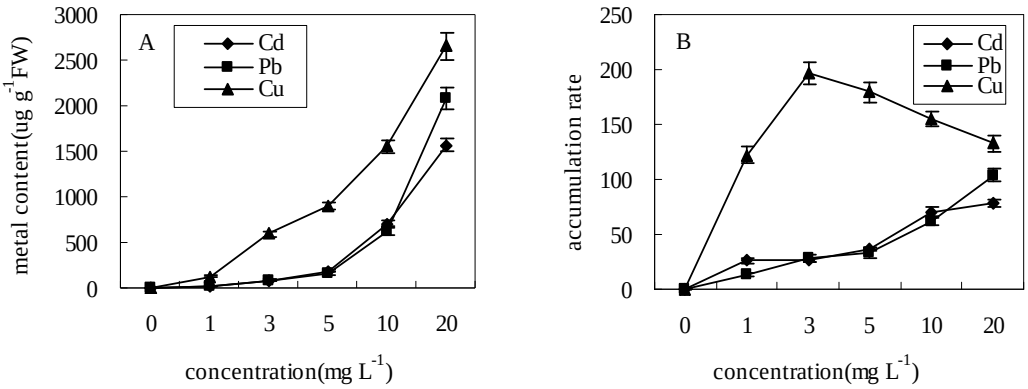

Figure1 - Bioaccumulation of Cd, Cu and Pb in *S. sagittifolia* L. root. Values are mean of triplicates ± SD. Standard deviations are represented by vertical bars. R^2 (Cd) =0.991, **P<0.01; R^2 (Cu) =0.993, **P<0.01; R^2 (Pb) =0.975, **P<0.01. F =0.889, one way ANOVA.

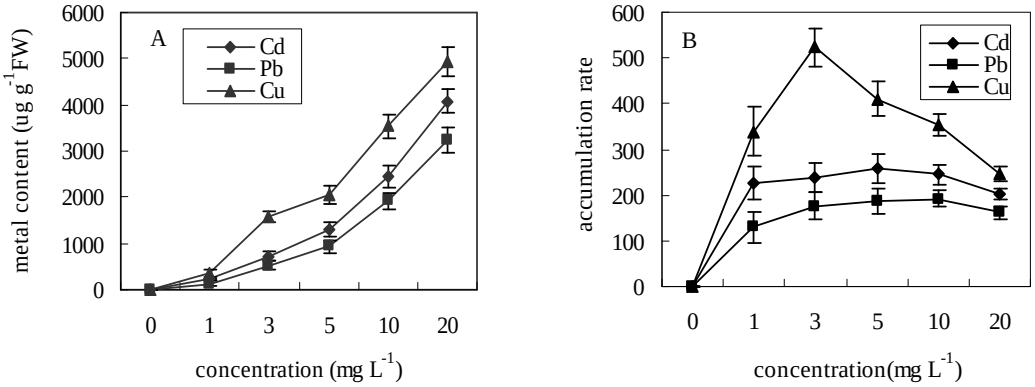

Figure 2 - Bioaccumulation of Cd, Cu and Pb in *P. crispus* L. leaf. Values are mean of triplicates ± SD. Standard deviations are represented by vertical bars. R^2 (Cd) =0.994, **P<0.01; R^2 (Cu) =0.966, **P<0.01; R^2 (Pb) =0.995, **P<0.01. F = 0.701, one way ANOVA.

Table 1 - Chemical forms of Cd, Cu and Pb in different organs of *S. sagittifolia* L. treated for 4 d with 10 mg L^{-1} Cd, Cu and Pb.

contaminant	organs	Content of different forms (ug g^{-1}FW) Percentage (%)					
		$F_{ethanol}$	F_{water}	F_{NaCl}	F_{HAc}	F_{HCl}	$F_{residue}$
Cd	root	16.3±0.29	96.1±2.52	510.2±4.22	67.7 ±2.37	6.7 ±0.26	0.0 ±0.0
		2.30%	13.80%	73.20%	9.80%	0.90%	0%
	caulis	3.3±0.10	5.3 ±0.14	48.8 ±0.36	1.94±0.08	2.8±0.04	0.55± 0.01
		5.30%	8.41%	77.88%	3.10%	4.42%	0.89%
	bulb	4.13±0.05	11.8±0.09	26.44±0.43	7.16±0.08	0.55±0.01	0.28±0.0
		8.20%	23.50%	52.45%	14.21%	1.09%	0.55%
Cu	root	500.44±19.83	197.9±10.8	113.8±12.5	548.2±50.1	189.4±15.8	1.6±0.1
		32.25%	12.76%	7.33%	35.34%	12.21%	0.10%
	caulis	71.1±9.2	9.1±1.7	7.9±0.7	5.9±0.9	12.4±1.4	1.5±0.3
		65.89%	8.38%	7.41%	5.46%	11.50%	1.36%
	bulb	56.8±10.4	7.6±2.0	4.7±0.7	3.7±0.5	4.1±0.4	0.6±0.2
		73.35%	9.76%	6.07%	4.75%	5.28%	0.79%
Pb	root	14.2±0.9	23.5±0.5	241.7±19.7	135.2±1.4	125.0±7.7	80.7±6.5
		2.27%	4.37%	38.73%	21.67%	20.04%	12.93%
	caulis	21.2±0.8	9.0±0.4	200.1±14.1	81.8±9.9	77.1±3.5	51.6±7.0
		4.80%	2.05%	45.39%	18.56%	17.49%	11.71%
	bulb	7.7±0.3	6.1±0.8	171.0±9.6	77.9±3.0	56.8±5.2	51.8±6.2
		2.07%	1.65%	46.04%	20.98%	15.31%	13.96%

Values are mean ± SD. F: abbreviation of form.

Table 2 - Chemical forms of Cd, Cu and Pb in leaves of *P. crispus* L. treated for 4 d with 10 mg L^{-1} Cd, Cu and Pb.

contaminant	Content of different forms (ug g^{-1}FW) / Percentage (%)					
	$F_{ethanol}$	F_{water}	F_{NaCl}	F_{HAc}	F_{HCl}	$F_{residue}$
Cd	23.5±1.31	53.6±6.93	1624.8±38.61	585.5±19.03	155.0±12.40	0
	1.0%	2.2%	66.5%	24.0%	6.4%	0.0%
Cu	549.9±27.75	185.4±5.01	64.1±2.91	605.2±14.76	2049.5±24.45	79.2±6.68
	15.6%	5.2%	1.8%	17.1%	58.0%	2.2%
Pb	112.2±8.44	113.7±5.60	814.8±23.70	327.3±20.36	505.3±23.97	41.4±6.12
	5.8%	5.9%	42.6%	17.1%	26.4%	2.2%

Values are mean ± SD. F: abbreviation of form.

This was in agreement with their strong affinity to proteins or sulfdryl groups of other organic compounds. The dominant chemical form of Cd showed that most of it might be tied up with pectic acid or Hystidyl sites of the cell wall (Leita et al., 1996). These forms could reduce the concentrations of free Cd and Pb, decrease their availability as well as mobility, and avoid their toxic effect on the plants. On the other hand, these forms could also interfere with the metabolic processes and influence the growth and development of the plants due to the possible combination with enzymes (the functional proteins). The existence of Cu was mainly in difficult soluble phosphate that included divalent phosphate and orthophosphate in the roots of *S. sagittifolia*, of which the mobility was small. Therefore, they mostly existed in the roots, and the only small portion was transferred to the caulis and bulb.

The phytotoxicity of the heavy metals contributed to many factors such as the uptake, transportation and chemical forms of the metals inside the plants. In general, the higher the mobility, the more significant the toxic effect. However, the relationship between the toxicity of the metals and their chemical forms was not conclusive. Further experiments are required to address these issues. It is misconception in believing that metal toxicity is high when plant absorbs more metals, or vice versa.

ACKNOWLEDGMENTS

This work was jointly supported by the National Natural Science Foundation of China (30670121) and the Specialized Research Fund for the Doctoral Program of Higher Education (20050319005).

REFERENCES

Ali, M.B., Tripathi, R.D., Rai, U.N., Pal, A., and Singh, S.P. (1999), Physico-chemical characteristics and pollution level of lake Nainital (U.P., India): Role of macrophytes and phytoplankton in biomonitoring and phytoremediation of toxic metal ions. *Chemosphere*, **39**, 2171-2182.

Baker, A.J.M., and Brooks, R.R. (1989), Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and photochemistry. *Biorecovery* **1**, 81-126.

Dirilgen, N., and Inel, Y. (1994) Effects of zinc and copper on growth and *metal accumulation in Duck weed, Lemna minor. Bulletin of environmental contamination and toxicology*, **53**, 442-449.

Dushenkov, V., Kumar, P.B.A.N., Motto, H., and Raskin, I. (1995), Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental science and technology*, **29**, 1239-1245.

Gupta, M., Sinha, S., and Chandra, P. (1996), Copper-induced toxicity in aquatic macrophyte, *Hydrilla verticillata*: effect of pH. *Ecotoxicolgy* **5**, 23-33.

Hernandez, L.M., Rico, M.C., Gonzalez, M.J., and Hernan, M.A. (1987) Environmental contamination by lead and cadmium in plants from urban area of Madrid, Spain. *Bulletin of environmental contamination and toxicology*, **38**, 203-208.

Hu, J.Z., Shi, G.X., Xu, Q.S., Wang, X., Yuan, Q.H., and Du, K.H. (2007), Effects of Pb^{2+} on the active oxygen-scavenging enzyme activities and ultrastructure in *Potamogeton crispus* leaves. Russian *Journal of Plant Physiology*, **54**, 414-419.

Leita, L., Nobili, M.De., Cesco, S., and Mondini, C. (1996) Analysis of intracellular cadmium forms in roots and leaves of bush bean. *Journal of Plant Nutrition*, **19**, 527-533.

Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M., and Strzalka, K. (2001), Physiological responses of *Lemna trisulca* L. (duckweed) to cadmium and copper bioaccumulation. *Plant Science*, **161**, 881-889.

Rai, U.N., Tripathi, R.D., Vajpayee, P., Pandey, N., Ali, M.B., and Gupta, D.K. (2003) Cadmium accunulation and its phytotoxicity in *Potamogeton pectinatus* L.(Potamogetonaceae). *Bulletin of environmental contamination and toxicology*, **70**, 566-575.

Reeves, R.D., and Brooks, R.R. (1983), Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. *Environment Pollution Ser A*, **31**: 277-285.

Seregin, I.V. and Ivanov, V.B. (2001), Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal of Plant Physiology*, **48**, 523-544.

Sinha, S., Saxena, R., and Singh, S. (2002), Comparative studies on accumulation of Cr from metal solution and tannery effluent under repeated metal exposure by aquatic plants: Its toxic effects. *Environmental Monitoring and Assessment*, **80**, 17-31.

Sugiyama, M. (1994) Role of cellular antioxidants in metal-induced damage. *Cell biology and toxicology*, **10**, 1-22.

Xu, J.L., Bao, Z.P., Yang, J.R., Liu, H., and Song, W.C. (1991) Chemical forms of Pb, Cd and Cu in crops. Chinese, *Journal of Applied Ecology*, **2**(3), 244-248.

Yuan, C., Jiang, G., Liang, L., Jin, X., and Shi, J. (2004) Sequential extraction of some heavy metals in Haihe river sediments, People's republic of China. *Bulletin of environmental contamination and toxicology*, **73**, 59-66.

Zaman, M.S., and Zereen, F. (1998), Growth responses of Radish plants to soil cadmium and lead contamination. *Bulletin of environmental contamination and toxicology*, **61**, 44-50.

Received: March 18, 2008;
Revised: September 16, 2008;
Accepted: October 08, 2009.