Accumulation and localization of cadmium in *Echinochloa polystachya* grown within a hydroponic system

F.A. Solís-Domínguez[a], M.C. González-Chávez[b],
R. Carrillo-González[b], R. Rodríguez-Vázquez[a,*]

[a] *CINVESTAV-IPN, Dpto. de Biotecnología y Bioingeniería, Av. Instituto Politécnico Nacional No. 2508, 07360 México, D.F., México*
[b] *Colegio de Postgraduados en Ciencias Agrícolas, IRENAT, Carr. Mex-Texcoco km 36.5, Montecillo, Estado de México 56230, México*

Abstract

Phytoremediation is a technology for extracting or inactivating pollutants. *Echinochloa polystachya* [(H.B.K.) Hitchcock] (Poaceae) is a fast-growing perennial grass that is common in tropical areas and is often found in oil-polluted soils that contain high concentrations of heavy metals. However, its tolerance to heavy metals, and its ability to accumulate them, has yet to be investigated. Here we test the hypothesis that *E. polystachya* is able to accumulate high concentrations of cadmium (Cd). Plants were grown hydroponically with different levels of Cd^{2+} (0, 0.25, 1, 2, 10, 50, and 100 mg L^{-1}), and were found to be tolerant to Cd^{2+} at all levels. No metal-toxicity symptoms were observed at any Cd^{2+} level. Root and leaves Cd concentrations were 299 ± 13.93 and 233 ± 8.77 mg kg^{-1} (on a dry weight basis), respectively. Scanning electron microscopy showed the inclusion of Cd within the xylem; this result was confirmed by energy dispersive X-ray spectrometry. Leaf tissues also accumulated Cd, especially within the bulliform cells of the epidermis. We conclude that *E. polystachya* is a hyperaccumulator of Cd. While data for other metals are not yet available, *E. polystachya* shows promise in the phytoextraction of Cd from polluted tropical sites.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Cadmium; *Echinochloa polystachya*; Hyperaccumulator plants; Heavy-metal tolerance; Phytoremediation; Hydroponic

1. Introduction

Cadmium (Cd) is not essential to plant growth, but is very toxic to many organisms [1]. In humans, Cd causes health problems such as damage to kidneys and lung tissues, emphysema, and carcinogenesis [2].

The accumulation of Cd within soil, sediments, and the aquatic environment is of concern because following uptake by plants or other life forms Cd can be passed on to all members of the food chain. It is therefore important to develop methods of rehabilitating Cd-polluted soils.

Phytoremediation is a technology used to restore polluted sites. It is cost-effective, environmentally friendly, and technically applicable *in situ*, making it preferable to other chemical or mechanical techniques [3–5]. Phytoremediation using plant species with an exceptional capacity to accumulate metals is a potentially important technique [6–9], however, only a few plant species are known to hyperaccumulate Cd, including *Thlaspi caerulescens* [10–13].

Plants show Cd toxicity like, chlorosis, reddish veins and petioles, curled leaves, severe reduction in growth of roots, tops, and number of tillers [1]. The range of approximate concentration of Cd in leaf tissue (dry weight) of some species is 0.05–0.2 mg kg^{-1} considered normal, and 5–10 up to 30 mg kg^{-1} considered excessive or toxic [1]. However, Cd hyperaccumulator plants accumulate above 100 mg kg^{-1} dry weight [1,11].

Grasses such as *Festuca ovina*, *Festuca rubra*, *Agrostis capillaris*, *Agrostis delicatula*, and *Agrostis stolonifera* are tolerant to different metals [14] but they do not hyperaccumulate metals. In Mexico, *Echinochloa polystachya* is commonly found at oil-polluted soils (gleysol) in tropical areas, and has therefore been suggested for use in the phytoremediation of soils [15]. This plant grows in either humid or flooded areas, as it can be terrestrial or aquatic [16]. Its leaves can reach up to 60 cm of length and its roots up to 2.5 m

Table 1
Shoot number and leaf, root and total dry biomass and length of 90-day-old *E. polystachya* plants grown in hydroponics and exposed for 58 days to different Cd^{2+} concentrations

Added Cd^{2+} concentration in the nutritive solution (mg L^{-1})	Accumulated Cd^{2-} concentration per experimental unit (mg)	Number of shoots	Leaf length (cm)	Root length (cm)	Leaf biomass dry weight (g)	Root biomass dry weight (g)	Total biomass dry weight (leaf + root) (g)
0 (control)	0	11.5 ± 2.64 a	33.1 ± 2.29 a	23.1 ± 1.38 a	4.89 ± 0.42 a	3.43 ± 0.6 a	8.32 ± 0.47 a
0.25	0.4	13.5 ± 1.91 a	31.2 ± 5.1 a	24.8 ± 1.65 a	4.72 ± 0.77 a	3.62 ± 0.7 a	8.34 ± 0.59 a
1	1.7	13.0 ± 2.58 a	33.4 ± 2.52 a	23.9 ± 2.75 a	4.82 ± 0.60 a	3.33 ± 0.6 a	8.15 ± 0.85 a
2	3.5	12.7 ± 2.62 a	33.1 ± 5.36 a	22.2 ± 1.26 a	5.48 ± 1.02 a	3.65 ± 0.8 a	9.13 ± 1.75 a
10	17.4	12.7 ± 2.98 a	33.6 ± 4.03 a	22.3 ± 1.9 a	4.65 ± 0.62 a	3.33 ± 1.3 a	7.98 ± 0.84 a
50	87	14.2 ± 1.5 a	29.2 ± 2.92 a	22.3 ± 2.81 a	4.64 ± 0.51 a	3.15 ± 0.6 a	7.79 ± 0.87 a
100	174	12.2 ± 2.06 a	30.6 ± 5.36 a	22.4 ± 2.7 a	4.67 ± 0.42 a	2.91 ± 0.5 a	7.58 ± 0.69 a

The letter "a" indicate no significant difference ($p > 0.05$), Tukey's test. Values are means ± S.D. ($n = 7$).

of depth [17]. The oil-polluted sites contain heavy metals, the concentration of some heavy metals found in sites with oil spills are: Pb (1.95–10 mg kg^{-1}), Cd (0.4–3.9 mg kg^{-1}), V (0.4–17.3 mg kg^{-1}), Cu (3.39–11.4 mg kg^{-1}), Ni (11.2–30.8 mg kg^{-1}), and Cr (5.4–55.5 mg kg^{-1}) [18–21]. The speciation of Cd in natural freshwater and rivers is mainly in the form of the free ion Cd^{2+} [22–24], which also occurs in soil solution [1,25]. The aim of the present study is to determine Cd accumulation within *E. polystachya* grown within a hydroponic system, including Cd localization within roots and leaves.

2. Materials and methods

2.1. Cadmium accumulation in plants of E. polystachya

Tillers of *E. polystachya* with two nodes were disinfected with 96% ethanol for 20 s and 5% NaClO for 15 min, and rinsed five times with distilled water. This was done to eliminate microorganisms that could potentially interfere with the production of biosurfactants by the plant (data not shown). The experimental unit was a polyvinyl-chloride cylinder (7 cm diameter, 20 cm length) filled with 1 kg of acid-washed (2% HCl, 12 h) and autoclaved (121 °C, three cycles, 1 h each) river sand, and planted with one tiller of the grass (3 days old) per cylinder.

Tillers were watered every other day with Long-Ashton nutrient solution [26] buffered at pH 5.8 with 0.5 mM MES (2-(*N*-morpholino)ethanosulphonic acid) and containing Cd as

$Cd(NO_3)_2 \cdot 4H_2O$ at one of eight different concentrations as Cd^{2+} (0, 0.25, 1, 2, 10, 50, and 100 mg L^{-1}). To develop the radical system, the plants received 50 mL of nutrient solution without Cd every other day for the first 28 days (14 waterings). After this, it was added the nutrient solution with Cd^{2+} for the next 43 days (22 waterings), and 80 mL nutrient solution with Cd during 15 days more (eight waterings). The total volume of nutrient solution with Cd^{2+} was 1740 mL, corresponding to 0, 0.4, 1.7, 3.5, 17.4, 87, and 174 mg of cumulative Cd^{2+} added per unit to the respective experiments (Tables 1 and 2). The experimental design was completely randomized, conducted in a greenhouse from May to July (latitude 19°39′41″N, longitude 99°07′49″W, 2261 m above sea level, minimum average temperature 10 °C and maximum 40.5 °C, 13 h light period). Each treatment (Cd^{2+} level) was replicated seven times. Watering with the Cd solution began at day 28, and plants were harvested 2 days after the last watering (90 days old).

The shoot number per experimental unit was determined and the lengths of roots and leaves were measured. Roots and leaves were analyzed separately. Plant parts were dried for 72 h at 70 °C, and dry biomass was determined. Root and leaves samples were ground within an "IKA A11 basic" mill with a stainless rotating blade and analyzed for Cd content. Half a gram of each sample was digested in a digester DigiPREP with H_2O_2 (1 mL)/HNO_3 (3.5 mL)/HCl (7 mL) following the EPA Method 7130 [27]. Samples were analyzed using an atomic spectrometer AVANTA-GBC. To determine plant translocation

Table 2
Accumulation of Cd in root, leaf and total biomass of 90-day-old *E. polystachya* plants grown in hydroponics and exposed for 58 days to different Cd^{2+} concentrations

Added Cd^{2+} concentration in the nutritive solution (mg L^{-1})	Accumulated Cd^{2+} concentration per experimental unit (mg)	Accumulated Cd in leaf per experimental unit (mg)	Accumulated Cd in root per experimental unit (mg)	Cd in the total biomass (leaf + root) (mg)	Transport index (T_i)
0 (control)	0	0	0	0	
0.25	0.4	0.008 ± 0.0002 a	0.044 ± 0.019 a	0.05 ± 0.019 a	13.4 ± 1.45
1	1.7	0.046 ± 0.009 a	0.044 ± 0.006 a	0.09 ± 0.013 ab	71.1 ± 13.21
2	3.5	0.057 ± 0.012 a	0.053 ± 0.035 a	0.11 ± 0.045 ab	72 ± 16.47
10	17.4	0.14 ± 0.021 a	0.17 ± 0.068 a	0.31 ± 0.07 b	52.5 ± 5.22
50	87	0.67 ± 0.098 b	0.51 ± 0.128 b	1.18 ± 0.19 c	89 ± 7.34
100	174	1.09 ± 0.062 c	0.87 ± 0.075 c	1.96 ± 0.03 d	77.8 ± 3.65

Different letters are significantly different ($p < 0.05$), Tukey's test. Values are means ± S.D. ($n = 7$).

ability (from roots to leaves) at different Cd^{2+} concentrations, the transport index was calculated as: $T_i = [$shoot Cd concentration $(mg\,kg^{-1})$/root Cd concentration $(mg\,kg^{-1})] \times 100$, as proposed by Ghosh and Singh [9].

2.2. Localization of cadmium accumulation in E. polystachya determined by scanning electron microscopy and energy dispersive X-rays

Root and leaf segments were analyzed to determine the sites of Cd accumulation. Tissue segments were washed with deionized water and fixed in the presence of 1% Na_2S with 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7). Post-fixation in 1% OsO_4 was performed before dehydratation with ethanol series (50, 60, 70, 80, 90, and 100%). Samples were dried at critical point and coated with a thin layer of Au [28]. The scanning electron microscope (SEM) analysis was carried out using a JEOL JSM-6300 microscope adapted with a NORAN energy dispersive X-ray spectrometer with VOYAGER II 1100/1110 software.

2.3. Localization of cadmium accumulation in E. polystachya determined by transmission electron microscopy

Selected root and leaf segments were treated as described above for SEM analysis. After dehydration with ethanol series, samples were treated with propylene oxide and infiltrated in Spurr resin. Thin slides (70–90 nm) were prepared using a Reichert-Jung Ultracut-E l ultramicrotome. Samples were analyzed without contrasting in a JEOL JEM-2010 F TEM microscope adapted with a NORAN energy dispersive X-ray spectrometer.

2.4. Statistical analysis

Differences between treatments were tested by ANOVA followed by Tukey's test ($p < 0.05$) using the software SAS (statistical analysis system) v6.12.

3. Results and discussion

3.1. Cadmium accumulation by E. polystachya

Heavy metals such as Cd^{2+} cause cell damage in general because they act as enzyme inhibitors or precipitate essential elements or metabolites [9]. Heavy metals bind to protein sulphydryl groups involved in catalytic function or in the structural identity of the protein [29,30].

These injures are commonly observed in plant tissues exposed to high metal concentrations. Growth is also reduced as a result of Cd toxicity, however, we did not observe tissue damage or growth retardation in *E. polystachya* even at the highest Cd^{2+} concentrations used in the present experiments. There were no significant differences in root or leaves dry weight, root and leaf length, or the number of shoots among the experiments with different Cd^{2+} concentrations ($p > 0.05$; Table 1).
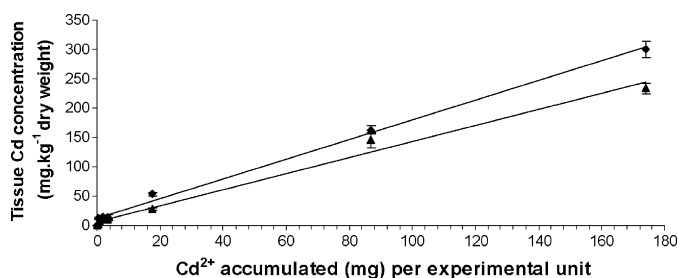

Fig. 1. Cadmium accumulation in the roots (♦) and leaves (▲) of plants of *E. polystachya* grown under hydroponic conditions with varying Cd^{2+} concentrations. Error bars indicate standard deviation of each point ($n = 7$).

The hability of *E. polystachya* to tolerate high concentration of Cd may be related with the production of γ-glutamylcysteine. Cd is an inducer of γ-Glu-Cys peptides, which provide thiols for binding the metals and detoxify the cell [31]. (γ-Glu-Cys)$_n$-Ser has been detected in certain species of the Poaceae [32].

The accumulation of Cd in the plant tissue as $mg\,kg^{-1}$ dry weight is reported in Fig. 1, in addition the accumulated Cd in the plant tissue per experimental unit is reported in Table 2.

It is observed that roots and leaves of *E. polystachya* treated with lower Cd^{2+} concentrations (0.25, 1, and 2 $mg\,L^{-1}$) did not show significant increases in Cd accumulation ($p > 0.05$); however, at higher concentrations (10, 50, and 100 $mg\,L^{-1}$) Cd accumulation increased significantly ($p < 0.05$). The highest Cd accumulation was recorded within the plant subjected to the highest Cd concentrations. Roots and leaves accumulated 299 ± 13.93 and 233 ± 8.77 $mg\,kg^{-1}$ of Cd dry weight, respectively, at the highest level of applied Cd^{2+} (Fig. 1). Root Cd accumulation was higher than that in leaves by a factor of 1.3 ± 0.06, as was also observed for other plants in previous studies [7,9,10,12,33] (Table 3). Cadmium accumulation in roots and leaves is represented by Eqs. (1) and (2) ($R^2 = 0.9954$, S.E.M. = 9.14; $R^2 = 0.9883$, S.E.M. = 11.05), respectively. *F*-Test showed a significant difference between root and leaves Cd accumulation ($p < 0.05$; Fig. 1).

$$y = 1.6791x + 11.239 \qquad (1)$$

$$y = 1.3651x + 5.7453 \qquad (2)$$

The elevated plant Cd contents demonstrate that this grass is able to tolerate and to translocate Cd to leaves. This is the first report of *E. polystachya* as a Cd hyperaccumulator plant (Table 3).

This is an important finding, as the highest levels of Cd^{2+} applied here (50 and 100 $mg\,L^{-1}$), are greater than those found in many polluted soils [34,7,35]. It has been reported that plants are considered to be Cd hyperaccumulators when they are able to accumulate Cd to a level that is more than 100 $mg\,kg^{-1}$ in leaves or 0.01% dry weight [1,4,11]. In the current study, *E. polystachya* accumulated 0.023% and 0.029% of Cd in leaves and roots, respectively. This plant species can therefore be used in phytoremediation as an agent of phytoextraction from tropical soils or wetlands that are polluted with Cd and perhaps even other heavy metals.

Table 3
Comparison of Cd accumulation in some species of hyperaccumulator and accumulator plants

Species and family	Cd concentration in roots (mg kg^{-1})	Cd concentration in leaves (mg kg^{-1})	References
Thlaspi caerulescens[a] (Brassicaceae)	Nr	116–263	[13]
		1020	[10]
Arabidopsis halleri[a] (Brassicaceae)	660[b]	157[b]	[12]
Echinochloa polystachya[a] (Poaceae)	299	233	This study
Brassica junceae (Brassicaceae)	81.9	43.2	[9]
Phragmytes karka (Poaceae)	53	39.5	[9]
Brassica campestris (Brassicaceae)	64.5	34	[9]
Dhatura innoxia (Solanaceae)	37	28.5	[9]
Equisetum ramosisti (Equiselaceae)	Nr	28	[33]
Ipomoea carnea (Convolvulaceae)	51	20.1	[9]
Juncus effusus (Juncaceae)	Nr	20	[33]
A. petraea (Brassicaceae)	336[b]	15[b]	[12]
Poa labillardieri (Poaceae)	3.4[b]	3.0[b]	[7]

Nr, not reported.
[a] Hyperaccumulator plant.
[b] The original units are μg g^{-1}, they were homogenized to mg kg^{-1}.

Many plant species identified as hyperaccumulators have been discovered in temperate climates, but have the disadvantage of producing little foliar biomass [1]. Little work has been done on hyperaccumulators in tropical climates, with the exceptions of Cuba and Brazil [11]. In Mexico, *E. polystachya* grows abundantly in gleysoles [15], and is found throughout central and south America [17], growing year-round with a high rate of primary production (2.8 kg m^{-2} dry weight). Based on our results, this represents a potential extraction of 6.4 kg of Cd per ha (1 ha = 10,000 m^2). In flooded soils, where *E. polystachya* can grow by as much as 4 cm d^{-1} and post-flowering biomass can reach 8 kg m^{-2} dry weight [16], potential Cd extraction may be as high as 18 kg ha^{-1}. These projections indicate the potential of further research on the phytoextraction of other heavy metals by *E. polystachya*.

A high Cd transport index (T_i) [9] was calculated for plants, in the present study, exposed to the highest Cd concentrations (Table 2), indicating that *E. polystachya* translocates Cd efficiently from roots to leaves, although by a mechanism that is poorly understood.

3.2. Localization of cadmium accumulation in E. polystachya determined by scanning electron microscopy and energy dispersive X-rays

3.2.1. Cd localization in roots determined by SEM

Observations of roots treated with different levels of Cd^{2+} revealed small inclusions (Fig. 2a) that were not observed in the roots of control plants. Energy dispersive X-ray (EDX) observations reveal that the inclusions are rich in Cd (Fig. 2b). There were more inclusions in the xylem than in the cortex. The reason to detect easily Cd in the xylem could be due to metal ligands reported in the xylem and phloem [1]. The prevalence of inclusions within the xylem indicates that it is the main pathway of Cd transport from root to leaves [3]. Cd could be chelated to organic compounds, as the EDX spectrum showed oxygen and carbon peaks (Fig. 2b). The role of chelating ligands in Cd translocation should therefore be investigated.

3.3. Detection of Cd in leaves via SEM

Abundant inclusions were observed in leaves of plants treated with Cd. These were more frequently seen in buliform cells (Fig. 3a and b) than in the mesophile region. EDX analyses confirmed that these inclusions contain Cd (Fig. 3c). Buliform cells are larger cells than epidermis cells, and are totally occupied by vacuoles and do not contain chlorophyll [36]. Our results show that these cells accumulate Cd within their vacuoles.

3.4. Localization of cadmium accumulation in E. polystachya determined by transmission electron microscopy

TEM observations confirmed the results obtained by SEM analysis. Thin sections from Cd-treated plants showed abundant clusters inside the vacuoles of root cells (Fig. 4b), while
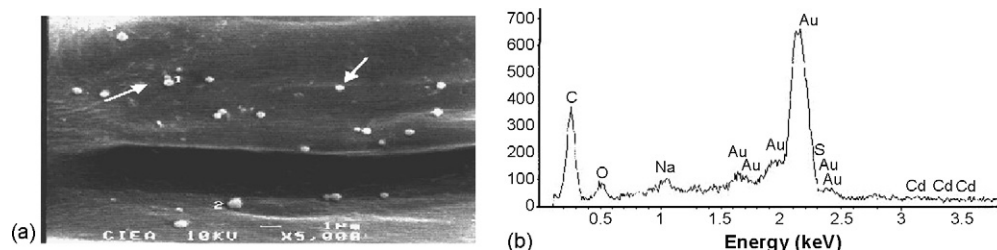

Fig. 2. (a) SEM image of longitudinal sections of *E. polystachya* roots treated with Cd (100 mg L^{-1}). Arrows show inclusions observed in the roots strand vascular. (b) The EDX spectrum confirms the presence of Cd within the inclusions.
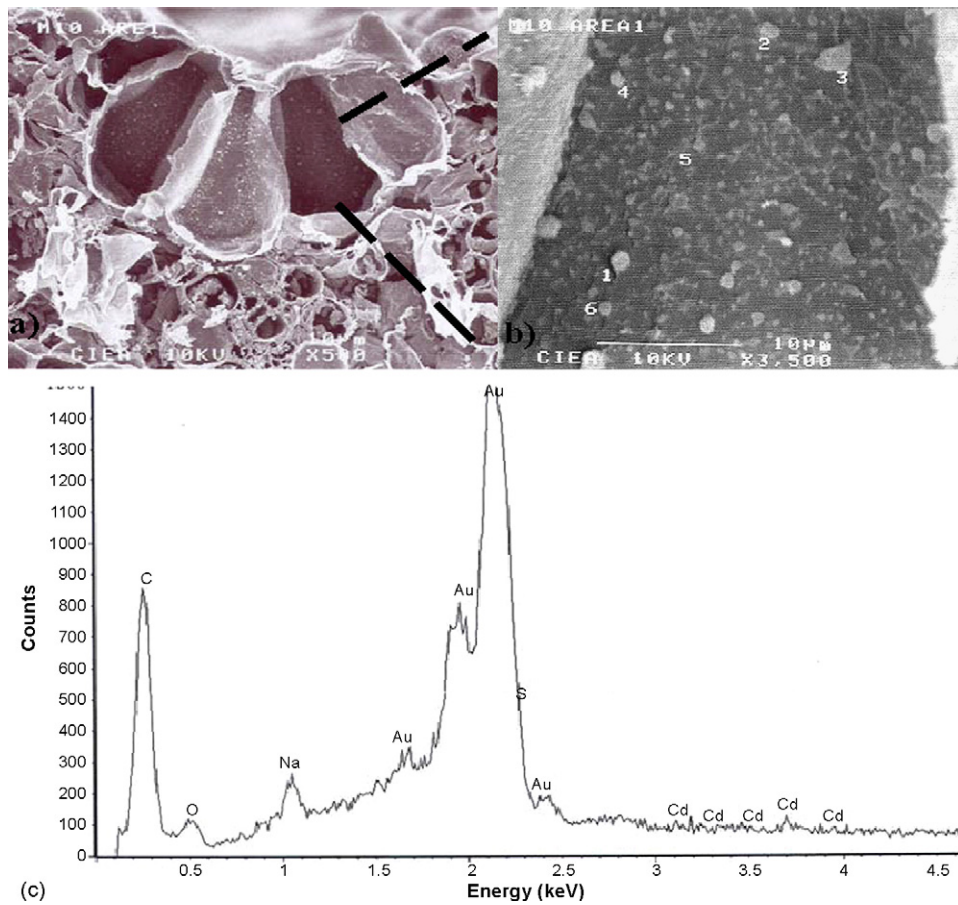
Fig. 3. (a) SEM image of buliform cells in *E. polystachya* leaves after 58 days of growth with Cd ($100\,\mathrm{mg\,L^{-1}}$). (b) SEM image highlighting numerous inclusions within buliform cells. (c) The EDX spectrum demonstrates that the inclusions contain Cd.
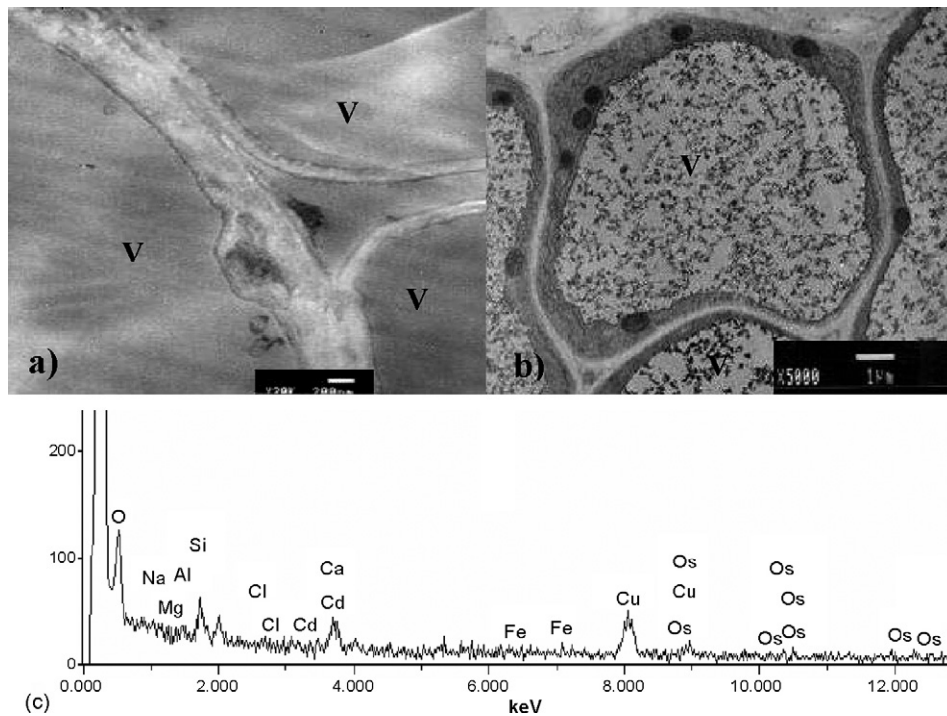

Fig. 4. (a) TEM image of *E. polystachya* roots from a control plant shows vacuoles (V) from Cd-untreated plants. (b) TEM image of *E. polystachya* roots from a plant treated with $100\,\mathrm{mg\,L^{-1}}$ Cd shows abundant clusters into the vacuoles. (c) The EDX spectrum confirms the presence of Cd within the clusters. The peaks labeled copper (Cu) and osmium (Os) are due to the copper grid and the osmium tetroxide fixative, respectively.

clusters were not observed in the controls (Fig. 4a). The clusters observed by TEM within the vacuoles contain Cd (Fig. 4c). Wang et al. [37] reported similar clusters detected by EDX in *Pseudomonas aureoginosa* grown at high concentrations of Cd. Other mechanisms of Cd detoxification in plants are thought to involve chelation through phytochelatins [38] or vacuole compartmentation [39,6].

4. Conclusions

E. polystachya is a fast-growing plant with high biomass production that grows on soils or flooded areas and has the ability to tolerate ($174\,\mathrm{mg\,L^{-1}}$ Cd^{2+} accumulated per experimental unit during 58 days) and hyperaccumulate high Cd concentrations.

No metal-toxicity symptoms were observed at any of the Cd concentrations analyzed in the present study. Higher Cd concentrations were found in roots than in leaves (299 ± 13.93 and $233 \pm 8.77\,\mathrm{mg\,kg^{-1}}$ dry weight, respectively). The localization of Cd was mainly observed in the root xylem and in leaf tissues, especially in the bulliform cells of the epidermis.

Acknowledgements

References

[1] A. Kabata-Pendias, H. Pendias, Trace Elements in Soils and Plants, 3rd ed., CRC Press, Boca Raton, Florida, 2001.

[2] J.A. Ryan, H.B. Pahren, J.B. Lucas, Controlling cadmium in the human food chain: a review and rationale based on health effects, Environ. Res. 28 (1982) 251–302.

[3] D.E. Salt, M. Blaylock, N.P.B.A. Kumar, V. Dushenkov, B.D. Ensley, I. Chet, I. Raskin, Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants, Biotechnology 13 (1995) 468–474.

[4] T.M. Chaudhry, W.J. Hayes, A.G. Khan, C.S. Khoo, Phytoremediation-focusing on accumulator plants that remediate metal-contaminated soils, Aust. J. Ecotoxicol. 4 (1998) 37–51.

[5] I. Alkorta, C. Garbisu, Phytoremediation of organic contaminants in soils, Biores. Technol. 79 (2001) 273–276.

[6] D.E. Salt, I.J. Pickering, R.C. Prince, D. Gleba, S. Dushenkov, R.D. Smith, I. Raskin, Metal accumulation by aquacultured seedlings of indian mustard, Environ. Sci. Technol. 31 (1997) 1636–1644.

[7] W.J. Hayes, T.M. Chaudhry, R.T. Buckney, A.G. Khan, Phytoaccumulation of trace metals at the sunny corner mine, New South Wales, whit suggestions for a possible remediation strategy, Aust. J. Ecotoxicol. 9 (2003) 69–82.

[8] K. Vogel-Mikus, D. Drobne, M. Regvar, Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia, Environ. Pollut. 133 (2005) 233–242.

[9] M. Ghosh, S.P. Singh, A comparative study of cadmium phytoextraction by accumulator and weed species, Environ. Pollut. 133 (2005) 365–371.

[10] S.L. Brown, R.L. Chaney, J.S. Angle, J.M. Baker, Phytoremediation potential of *Thlaspi caerulescens* and Blader campion for zinc and cadmium contaminated soil, J. Environ. Qual. 23 (1994) 1151–1157.

[11] R.R. Brooks, Plants that Hyperaccumulate Heavy Metals, CAB International, Wallingford, UK, 1998.

[12] V. Bert, P. Meerts, P. Saumitou-Laprade, P. Salis, W. Gruber, N. Verbruggen, Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*, Plant Soil 249 (2003) 9–18.

[13] E. Lombi, F.J. Zhao, S.J. Dunham, S.P. McGrath, Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction, J. Environ. Qual. 30 (2001) 1919–1926.

[14] W.H.O. Ernst, Bioavailability of heavy metals and descontamination of soils by plants, Appl. Geochem. 11 (1996) 163–167.

[15] M.C. Rivera-Cruz, R. Ferrera-Cerrato, P. Sánchez-García, V. Volke-Haller, L. Fernández-Linares, R. Rodríguez-Vázquez, Descontaminación de suelos con petróleo crudo mediante microorganismos autóctonos y pasto alemán [*Echinochloa polystachya* (H.B.K.) Hitch.], Agrociencia 38 (2004) 1–12.

[16] M.L.M. Pompeo, R. Henry, V. Moschini-Carlos, The water level influence on biomass of *Echinochloa polystachya* (Poaceae) in the Jurumirim reservoir (Sao Paulo, Brazil), Rev. Bras. Biol. 61 (2001) 19–26.

[17] A.A. Beetle, F.E. Manrique, S.J.A. Miranda, L.V. Jaramillo, H.A. Chimal, R.A.M. Rodríguez, Las gramíneas de México. Tomo III, Secretaría de Agricultura y Recursos Hidráulicos, Mexico, 1991.

[18] L.C. Osuji, C.M. Onojake, Field reconnaissance and estimation of petroleum hydrocarbon and heavy metal contents of soils affected by the Ebocha-8 oil spillage in Niger Delta, Nigeria, J. Environ. Manage. 79 (2006) 133–139.

[19] R. Prego, A. Cobelo-García, Cadmium, copper and lead contamination of the seawater column on the Prestige shipwreck (NE Atlantic ocean), Anal. Chim. Acta 524 (2004) 23–26.

[20] S. Al-Muzaini, P.G. Jacob, An assessment of toxic metals content in the marine sediments of the shuaiba industril area, Kuwait, after the oil spill during the gulf war, Water Sci. Technol. 34 (1996) 203–210.

[21] J. Sneddon, C. Hardaway, K.K. Bobbadi, J.N. Beck, A study of a crude oil spill site for selected metal concentrations remediated by a controlled burning in Southwest Louisiana, Microchem. J. 82 (2006) 8–16.

[22] S.I. Korfali, B.E. Davies, Speciation of metals in sediment and water in a river underlain by limestone; role of carbonate species for purification capacity of rivers, Adv. Environ. Res. 8 (2004) 599–612.

[23] E. Tipping, S. Lofts, A.J. Lawlor, Modelling the chemical speciation of trace metals in the surface waters of the Humber system, Sci. Total Environ. 210/211 (1998) 63–77.

[24] D.R. Turner, M. Whitfeld, A.G. Dickson, The equilibrium speciation of dissolved components in freshwater and sea water at $25\,^\circ\mathrm{C}$ and 1 atm pressure, Geochim. Cosmochim. Acta 45 (1981) 855–881.

[25] F.T. Bingham, G. Sposito, J.E. Strong, The effect of sulphate on the availability of cadmium, Soil Sci. 141 (1986) 172–177.

[26] E. Hewitt, Sand and Water Culture Methods used in the Study of Plant Nutrition. Comonwealth Bureau Technical Communication 22, Commonwealth Bureau, Farnham Royal, UK, 1966.

[27] EPA, Method 7130, Cadmium: atomic absorption, direct aspiration, 1986, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/7130.pdf.

[28] J.J. Bozzola, L.D. Russel, Electron Microscopy; Principles and Techniques for Biologists, Jones and Bartlett Publishers, USA, 1992.

[29] A.A. Meharg, Integrated tolerance mechanisms: constitutive and adaptive plant responses to elevated metal concentrations in the environment, Plant Cell Environ. 17 (1994) 989–993.

[30] F. Van Assche, H. Clijsters, Effect of metals on enzyme activity in plants, Plant Cell Environ. 13 (1990) 195–206.

[31] W.E. Rauser, Phytochelatins and related peptides: structure, biosynthesis, and function, Plant Physiol. 109 (1995) 1141–1149.

[32] S. Klapheck, W. Fliegner, I. Zimmer, Hydroxymethyl-phytochelatins [(γ-glutamylcysteine)$_n$-serine] are metal-induced peptides of the Poaceae, Plant Physiol. 104 (1994) 1325–1332.

[33] H. Deng, Z.H. Ye, M.H. Wong, Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China, Environ. Pollut. 132 (2004) 29–40.

[34] G.R. Carrillo, L.J. Cajuste, Heavy metals in soils and alfalfa (*Medicago sativa* L.) irrigated with three sources of wastewater, J. Environ. Sci. Health 27 (1992) 1771–1783.

[35] A.G. Khan, T.M. Chaudry, W.J. Hayes, C.S. Khoo, L. Hill, R. Fernandez, P. Gallardo, Physycal, chemical and biological characterisation of a steel-works waste site at port Kembla, NSW, Australia, Water Air Soil Pollut. 104 (1998) 389–402.

[36] W.C. Dickison, Integrative Plant Anatomy, Academic Press, USA, 2000.

[37] C.L. Wang, P.C. Michels, S.C. Dawson, S. Kitisakkul, J.A. Baross, J.D. Keasling, D. Clark, Cadmium removal by a new strain of *Pseudomonas aeuruginosa* in aerobic culture, Appl. Environ. Microbiol. 63 (1997) 4075–4078.

[38] K. Taulavuori, M.N.V. Prasad, E. Taulavuori, K. Laine, Metal stress consequenses on frost hardiness of plants at northern high latitudes: a review and hypothesis, Environ. Pollut. 135 (2005) 209–220.

[39] D.E. Salt, G.J. Wagner, Cadmium transport across tonoplast of vesicles from oat roots, J. Biol. Chem. 268 (1993) 12297–12302.