Copper and Cadmium Tolerance, Uptake and Effect on Chloroplast Ultrastructure. Studies on *Salix purpurea* and *Phragmites australis*

Abdelmalek Hakmaoui[a], Mohammed Ater[a], Károly Bóka[b], and Matilde Barón[c,*]

[a] Department of Biology, Abdelmalek Essaâdi University, BP 2121-Tetouan, Morocco
[b] Department of Plant Anatomy, Eötvös Lorand University, Pázmány Peter, Stny 1/C, Budapest, H-1117 Budapest, Hungary
[c] Department of Biochemistry and Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC Profesor Albareda, 1, 18008 Granada, Spain. Fax: +34 958 12 96 00. E-mail: mbaron@eez.scic.es

* Author for correspondence and reprint requests

Z. Naturforsch. **62 c**, 417–426 (2007); received December 1, 2006/January 22, 2007

We have compared the effect of toxic Cu and Cd concentrations on growth, metal accumulation, and chloroplast ultrastructure of willow (*Salix purpurea* L.) and reed [*Phragmites australis* (Cav.) Trin. ex Steud.]. After a 10-day treatment, both species have tolerated to some extent the lowest concentration of both metals; however, plant growth was strongly reduced at the highest Cu and Cd concentrations. These plants could be described as Cu-tolerant at the lowest concentration tested, showing a higher tolerance index in reed than in willow; in contrast, willow exhibited higher tolerance against Cd. Both plants appeared to be moderate root accumulators of Cu and Cd. Ultrastructural studies revealed special features that can provide some protection against heavy metals stress, such as ferritin aggregates in the stroma. In addition, Cu and Cd induced distortion of thylakoids, reduction of grana stacks, as well as an increased number and size of plastoglobuli and peripheral vesicles.

Key words: Heavy Metal Toxicity, *Phragmites australis*, *Salix purpurea*

Introduction

Heavy metals (HMs) are natural elements that can contaminate the soil by different human activities such as mining, industries, atmospheric deposition, excessive use of agrochemicals and waste disposal. The toxicity of HMs such as Cd, Ni, Cu and Pb for animals and plants is well known (Kabata-Pendias and Pendias, 2000).

Copper is an essential trace element for all plants and it is required for different enzyme systems, *e.g.* plastocyanin, superoxide dismutase and amine oxidase (Yruela, 2005). However, exposure to excess Cu has a detrimental effect on plant growth, triggering oxidative stress in plant cells, inhibiting the photosynthetic electron transport (Drążkiewicz *et al.*, 2004) and diminishing the content of photosynthetic pigments (Barón *et al.,* 1995).

Cadmium is a toxic element with no known physiological function in plant metabolism. Cd generally inhibits plant growth and influences nutrient distribution (Lidon and Henriques, 1991). The phytotoxic effect of Cd in photosynthesis has been studied in various species (Krupa *et al.,* 1993; Ouzounidou *et al.,* 1997). This metal is also involved in the formation of active oxygen species and membrane damage (Iannelli *et al.,* 2002; Pietrini *et al.*, 2003).

At toxic concentrations in leaves, HMs can damage leaf organelles, particularly the chloroplast (Peng *et al.,* 2005). Furthermore, the impact of HMs on the chloroplast ultrastructure is the key in understanding the physiological alterations induced, because of the relationship between chloroplast structure, photosynthetic ability, and plant growth.

Willow (*Salix purpurea* L.) is a fast-growing tree used as a biological filter for wastewater as well as in the remediation of sludge and industrially polluted lands (Landberg and Greger, 1994; Robinson *et al.*, 2000). In addition, its fast growth and high biomass productivity makes it an attractive crop for bio-fuels. However, the large number of species and hybrids of *Salix* spp. (Landberg and Greger, 1994) with a wide genetic variability pre-

Abbreviations: HMs, heavy metals; PV, peripheral vesicles; TI, tolerance index.

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sents different tolerance levels to specific HMs. Numerous studies on metal tolerance and accumulation have been made on willow species but few studies have focused on *S. purpurea.*

Reed [*Phragmites australis* (Cav.) Trin. ex Steud.] is a rhizomatous plant of the Poaceae family with a broad geographical distribution in the world (Haslam, 1973). It can withstand extreme environmental conditions, including toxic concentrations of heavy metals such as Zn, Pb, Cu and Cd (Stoltz and Greger, 2002; Ait Ali *et al.,* 2002, 2004; Batty and Younger, 2004; Deng *et al.,* 2004). Reed has been widely used in constructed wetlands for treating wastewater (Rai *et al*., 1995; Batty and Younger, 2004; Samecka-Cymerman *et al*., 2004) because of its multiple positive effects on aquatic ecosystems resulting from its high water-purifying capacity. In fact, reed and willow have been used together in the same wetlands for urban wastewater treatment (Ezzahri *et al*., 2001).

In this study, we compare the behaviour of these two plants under metal toxicity while being used in phytoremediation. Cu and Cd were used to compare the toxic effect of a metal essential for plant growth with that effect induced by an element having no metabolic function in the plant. The Cu and Cd concentrations used here are based on preliminary assays using multiple concentrations (Kohl and Lösch, 1999). We assessed the Cu and Cd sensitivity of willow and reed plants, analyzing the metal uptake, accumulation levels in different tissues, as well as metal-induced changes in growth and chloroplast ultrastructure. Perturbations affecting the chloroplast and consequently photosynthesis would be an indicator of the impact of metal toxicity on the aerial parts of the plant. This combined analysis of metal uptake and stress on willow and reed may contribute to the optimization of phytoremediation processes.

Materials and Methods

Plant culture

Plants were cultivated in a growth chamber at 120 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), generated by a combination of Sylvania VHO cool-white fluorescent and incandescent lamps (Danvers, MA, USA), with a 16 h/8 h photoperiod, a temperature regime of 25 °C/20 °C (day/night) and a relative humidity of 60–70%.

Reed rhizomes were taken from adult plants growing on the shores of Martil river (Tetouan, Morocco), placed in plastic trays with vermiculite and watered for 20 d until root and shoot development. Willow cuttings (approx. 18 cm long) were obtained from one willow clone growing in the same area. Shoot cuttings of uniform size were rooted in water.

About 20-day-old reed sprouts (12 cm) and willow cuttings with three branches and six roots were transferred to hydroponic culture in polyethylene pots with 1.5 L of a modified Hoagland nutrient solution (Hoagland and Arnon, 1941). The solution was aerated continuously and buffered to pH (5.6 ± 0.1) with 0.5 mM MES [2-(*N*-morpholino)ethanesulphonic acid]. When plants were placed in the hydroponic culture, copper was added (in the form of $CuSO_4 \cdot 5H_2O$) at increasing concentrations of 15.7, 47.2 and 78.6 μM, and cadmium ($CdCl_2$) at concentrations of 44.5, 89 and 133.5 μM. All solutions were changed twice weekly to prevent depletion of metals and nutrients. The metal treatment was continued for 10 d. Control plants were grown in the absence of HMs.

Three replicates were used per treatment and 6 plants per container.

Determination of growth parameters and tolerance index

Total fresh weight, shoot length, total root length (the sum length of all roots) and the number of roots per plant were determined at the beginning of the treatment and after 10 d of growth. Changes in these parameters were used to evaluate metal toxicity.

The tolerance index (TI) was calculated at different Cu and Cd concentrations by dividing the root length of the plant exposed to different metal concentrations by that measured during growth in the control solution. The following equation was used: TI (%) = 100 × (root length under metal treatment)/(root length in the control solution).

Metal content

After 10 d of either Cu or Cd treatment, roots and shoots were separated. Samples were washed with deionized water, dried for 48 h at 80 °C and then ground to a fine powder. Dry plant material was wet digested in cylinders filled with a mixture of $HNO_3/HClO_4$. After cooling, the metal concentrations were determined by a Perkin-Elmer 5000 Atomic absorption spectrophotometer.

Electron microscopy

All samples were harvested after 10 d of HM treatment. Willow samples were taken from old (4^{th} and 5^{th} leaves from the lower part of the stem) and young leaves (2^{nd} and 3^{rd} leaves close to the apex), affected by the metal toxicity in their mature or developing stage, respectively. Reed samples were taken only from the 4^{th} leaf of the plant, which emerged after the treatment.

Leaf samples were fixed in a mixture of 4% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 d. Samples were rinsed several times in phosphate buffer to remove aldehyde fixatives and post-fixed with 1% OsO_4 for 3 h. Next, the tissue samples were rinsed in phosphate buffer, dehydrated in ethanol series and embedded in Durcupan resin. Sections were cut with an ultramicrotome Reichert-Jung Ultracut E instrument, stained with uranyl acetate (2% in methanol) and lead citrate and examined in a Hitachi 7100 TEM instrument at 75 kV accelerating voltage.

Statistical analysis

A one-way ANOVA was run on the SPSS computer program, and data from the different treatments and control were compared by Duncan's multiple-range test at $p < 0.05$.

Results

Effect of copper and cadmium on plant growth and tolerance index

The data for plant growth parameters after 10 d of either Cu or Cd treatment are summarized in Table I and Table II, respectively.

Under Cu treatment (Table I), the reduction observed in all the growth parameters measured proved highly significant ($p < 0.001$) in both plants, except for the fresh weight in reed ($p < 0.05$). All growth parameters in willow and shoot and root lengths in reed underwent significant reductions ($p < 0.05$) at 47.2 μM Cu. At this concentration, the percentage decrease in fresh weight, total stems, root length, and number of roots per plant in Cu-treated willow compared with the control values were 57, 37, 57 and 58, respectively. In Cu-treated reed the decrease in shoot and root length was less pronounced, with percentages of 26 and 31, respectively, compared with the control. At the highest concentration (78.6 μM Cu), growth

Table I. Growth parameters for *Salix purpurea* and *Phragmites australis* after 10 days growth under Cu treatments. Values are means ± s.e. ($n = 18$).

Treatment	Total fresh weight [g/plant]		Total stems/shoot length [cm/plant]		Root length [cm/plant]		Number of roots/plant	
	Willow	Reed	Willow	Reed	Willow	Reed	Willow	Reed
Control	3.62 ± 1.07^{a}	1.75 ± 0.66^{a}	31.01 ± 5.88^{a}	7.46 ± 1.50^{a}	114.04 ± 29.88^{a}	31.87 ± 9.23^{a}	8.91 ± 2.96^{a}	4.50 ± 1.44^{a}
15.7 μM Cu	3.56 ± 0.86^{a}	1.59 ± 0.61^{a}	28.62 ± 9.57^{a}	6.00 ± 1.22^{ab}	100.62 ± 29.31^{a}	33.42 ± 9.52^{a}	7.75 ± 1.28^{a}	4.08 ± 1.50^{a}
47.2 μM Cu	1.57 ± 0.73^{b}	1.50 ± 0.31^{a}	19.42 ± 7.65^{b}	5.50 ± 1.22^{b}	49.37 ± 14.05^{b}	21.98 ± 5.63^{b}	3.83 ± 1.52^{b}	3.75 ± 1.28^{a}
78.6 μM Cu	0^{c}	1.04 ± 0.54^{b}	1.98 ± 0.77^{c}	4.16 ± 1.32^{c}	0^{c}	4.16 ± 1.60^{c}	0^{c}	0.83 ± 0.38^{b}
ANOVA	***	*	***	***	***	***	***	***

Values followed by the same letter are not significantly different according to Duncan's test ($p < 0.05$); the one-way ANOVA shows significant difference at: *** $p < 0.001$ and * $p < 0.05$.

Table II. Growth parameters for *Salix purpurea* and *Phragmites australis* after 10 days growth under Cd treatments. Values are means ± s.e. (n = 18).

Treatment	Total fresh weight [g/plant]		Total stems/shoot length [cm/plant]		Root length [cm/plant]		Number of roots/plant	
	Willow	Reed	Willow	Reed	Willow	Reed	Willow	Reed
Control	3.62 ± 1.07^{a}	1.75 ± 0.66^{a}	31.01 ± 5.88^{a}	7.46 ± 1.50^{a}	114.04 ± 29.88^{a}	31.87 ± 9.23^{a}	8.91 ± 2.96^{a}	4.50 ± 1.44^{a}
44.5 μM Cd	3.33 ± 0.97^{a}	1.12 ± 0.40^{b}	25.21 ± 6.49^{b}	5.17 ± 1.84^{b}	104.21 ± 19.54^{a}	10.04 ± 3.73^{b}	8.16 ± 2.65^{a}	2.75 ± 0.96^{b}
89 μM Cd	2.33 ± 0.81^{b}	0.72 ± 0.41^{c}	15.83 ± 5.24^{c}	3.46 ± 1.32^{c}	37.33 ± 13.81^{b}	3.83 ± 1.11^{c}	7.75 ± 2.70^{a}	1.58 ± 0.51^{c}
133.5 μM Cd	0.86 ± 0.41^{c}	0.33 ± 0.15^{d}	8.62 ± 3.74^{d}	2.79 ± 1.05^{c}	16.54 ± 5.57^{c}	3.04 ± 0.75^{c}	5.25 ± 1.76^{b}	1.16 ± 0.57^{c}
ANOVA	***	***	***	***	***	***	***	***

Values followed by the same letter are not significantly different according to Duncan's test ($p < 0.05$); the one-way ANOVA shows significant difference at: *** $p < 0.001$.

of the aerial parts and roots was totally inhibited in willow but not in reed, in which the decrease in fresh weight and shoot length was about 50% and the decrease in root growth parameters (length and number) was approx. 85%.

Growth of Cd-treated species (Table II) was progressively inhibited with increasing metal concentration in the nutrient solution. The reduction for all the growth parameters measured was highly significant ($p < 0.001$) in both plants. At the lowest and intermediate Cd concentrations (44.5 and 89 μM), the decline in growth (roots and aerial parts) in reed was more pronounced than in willow. At the highest Cd concentration the differences between the two species were less obvious.

The tolerance index (TI), based on root elongation, for different Cu and Cd treatments is presented in Figs. 1 and 2, respectively. In the case of reed, the TI at all Cu concentrations appeared to be significantly higher than that of willow. At the lowest Cu concentration (15.7 μM), the root growth seemed to be stimulated in reed, compared with the control. In willow, root growth was completely inhibited at the most severe treatment (78.6 μM); however, the TI for reed was approx. 10% at the same concentration (Fig. 1).

The Cd treatments gave rise to the opposite situation (Fig. 2). The TI proved higher for willow at all the concentrations analyzed. With increasing Cd concentrations, the difference in the TI between the two plants became less notable.

Fig. 1. Tolerance index of *Salix purpurea* and *Phragmites australis* at different Cu concentrations, calculated using the total root length (n = 18). The one-way ANOVA shows significant differences between *S. purpurea* and *P. australis* at: *** $p < 0.001$, ** $p < 0.01$; ns, not significant.

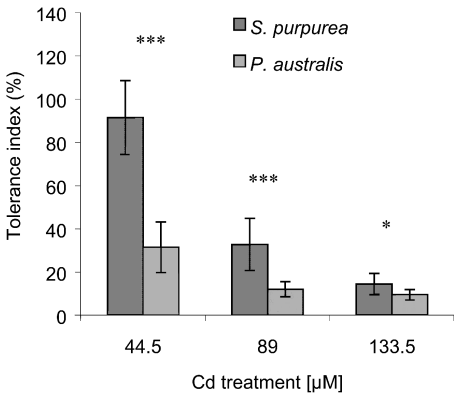

Fig. 2. Tolerance index of *Salix purpurea* and *Phragmites australis* at different Cd concentrations, calculated using the total root length (n = 18). The one-way ANOVA shows significant differences between *S. purpurea* and *P. australis* at: *** $p < 0.001$, * $p < 0.05$.

Metal accumulation

The accumulation of Cu and Cd in roots and shoots of the two species after 10 d of treatment is illustrated in Fig. 3. The Cu concentration in roots and shoots of the two species increased with the external HM concentrations and became constant at the highest concentrations (Figs. 3A, C). However, the Cd content, in roots as well as shoots, increased almost linearly with the Cd concentration available in the growth medium (Figs. 3B, D). The two species differed in their accumulation power, depending on the HM and the part of the plant. Indeed, reed accumulated higher Cu and lower Cd concentrations in their roots than did willow (Figs. 3A, B). The Cu levels in shoots were similar for the two species, whereas in the case of Cd, accumulation in reed was higher than in willow (Figs. 3C, D).

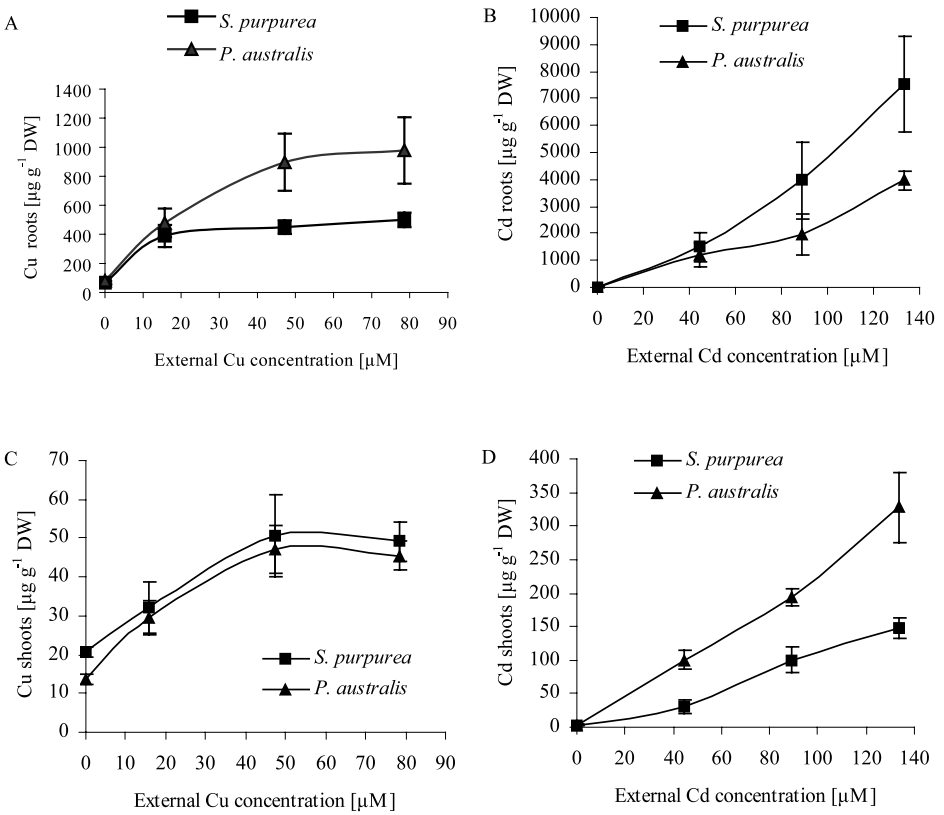

Fig. 3. Copper (A, C) and cadmium (B, D) accumulation in roots (A, B) and in shoots (C, D) of *Salix purpurea* and *Phragmites australis* after 10 days of treatment. Values are means ± s.e. (n = 4).

Changes in chloroplast ultrastructure induced by HM toxicity

The HM-induced changes were examined in the chloroplast ultrastructure in terms of chloroplast size, shape, thylakoid system and starch content. In addition, the presence of storage inclusions such as plastoglobuli and ferritin deposits was analyzed.

Plastids from control willow plants are shown in Figs. 4A (old leaves) and B (young leaves). Fig. 4A displays chloroplasts with a lenticular shape, dense stroma showing low contrast with the thylakoid membrane, as well as high and wide grana. Ferritin aggregates were present at the end of the organelle (Fig. 4A and inset). Chloroplasts from young leaves (Fig. 4B) exhibit the usual appearance: stroma with lower density and low grana. Starch was absent in both samples. Peripheral vesicles, the small invaginations originating from the inner envelope membrane, were rarely visible.

The highest Cd concentration used (133.5 μM) induced numerous alterations in the cells of old leaves (Fig. 4C). Chloroplasts showed swollen but organized thylakoids with thylakoid-free stroma areas. Compact and electrodense tannin precipitates were often visible in the cells. In the mesophyllar cells with low tannin content, chloroplasts showed slight alterations in their shape but not in the inner structure. In the young leaves of plants treated with the same Cd concentration (Fig. 4D), the tannin-containing cells had extremely aggregated and dense cytoplasm; because of the low contrast, chloroplast structure was hardly visible in these cells. The number of peripheral vesicles was slightly higher in both kinds of samples, mainly in plastids of tannin-containing cells. The number of plastoglobuli was not influenced by the Cd treatment.

The Cu-treated *Salix* plants showed some disturbances in chloroplast ultrastructure at the lowest concentration (15.7 μM): appearance of enlarged plastoglobuli in chloroplast from old leaves (Fig. 4E) and thylakoid swelling in amoeboid-shaped plastids from young leaves (Fig. 4F). Peripheral vesicles (Fig. 4E and inset) were more frequent than in Cd treatments. At the highest Cu concentration analyzed (78.6 μM), numerous plastoglobuli were observed in old and young leaf samples (Figs. 4G, H), also increasing their size in the old leaves. In addition, the thylakoid system had a wavy appearance. Thylakoid-free stroma areas were expanded in both samples, as in young

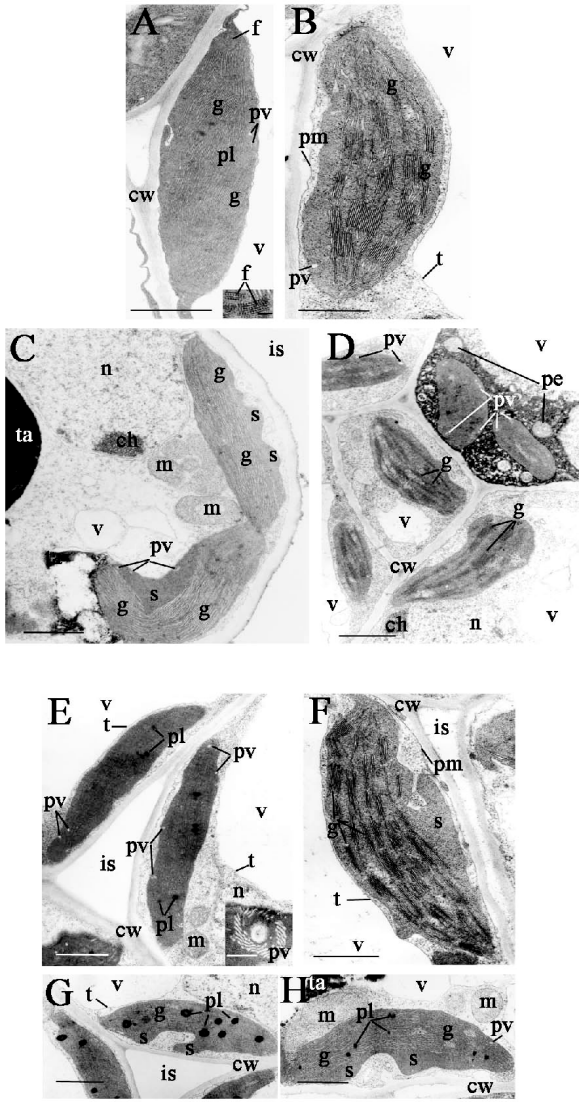

Fig. 4. Electronmicrographs from leaves of control and heavy metal-treated (Cd, Cu) *Salix*. (A, B) Chloroplasts from old and young leaves, respectively, of control plants; inset, crystalline ferritin. (C, D) Cells and chloroplasts from old and young leaves, respectively, of 133.5 μM Cd-treated plant. (E, F) Chloroplasts from young and old leaves of 15.7 μM Cu-treated plant; inset: peripheral vesicles at the end of the plastids (plane of section is nearly parallel to the envelope membrane). (G, H) Chloroplasts from old and young leaves of 78.6 μM Cu-treated plant.
Cb, crystalloid body; ch, chromatin; cw, cell wall; f, ferritin; g, granum; is, intercellular space; l, lipid; v, vacuole; n, nucleus; m, mitochondrion; pe, peroxisome; pl, plastoglobuli; pm, plasma membrane; pv, peripheral vesicles; t, tonoplast; ta, tannin content; s, thylakoid-free region of stroma.

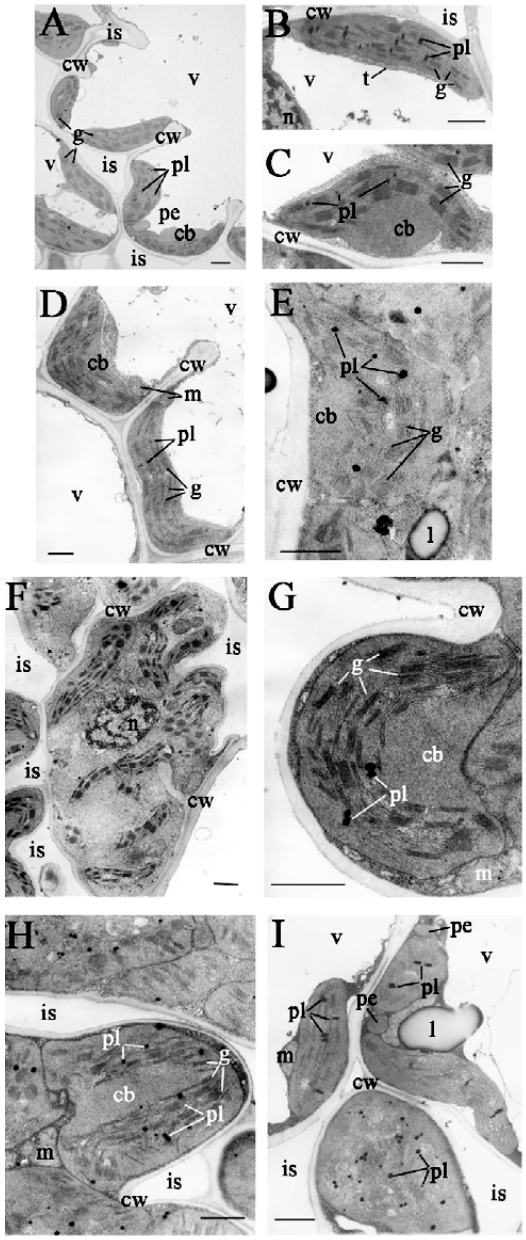

Fig. 5. Electronmicrographs from leaves of control and heavy metal-treated (Cd, Cu) *Phragmites*. (A) Chloroplasts in pockets of a mesophyllar cell. (B, C) Single plastids from control leaf. (D) Chloroplasts in pockets from 44.5 μM Cd-treated plant. (E) Chloroplast from a leaf of 133.5 μM Cd-treated plant. (F, G) Mesophyll cell and chloroplast from 15.7 μM Cu-treated plant. (H) Chloroplast from 47.2 μM Cu-treated plant. (I) Cells from a leaf of 78.6 μM Cu-treated plant.
For abbreviations see Fig. 4.

leaves treated at the lowest Cu concentration. Some peripheral vesicles are visible and the starch content did not increase.

In control reed plants (Figs. 5A–C), plastids were located in well developed lobes of mesophyll cells (intracellular pockets) formed by cell wall invaginations. Chloroplasts showed various elongated shapes, in some cases displaying thylakoid-free areas, due to the presence of crystal-like bodies (Fig. 5C), present mostly in stroma dilations. They were probably built up from ferritin-like material, usually visible in ferritin pockets of chloroplasts (Harrison and Arosio, 1996). The thylakoid system was well developed; grana were composed of 10–20 thylakoids. Small electrodense plastoglobuli were present in low number (5–10) and starch was absent in all sections.

In Cd-treated reed plants, the lowest Cd concentration (44.5 μM) appeared to exert a weak influence on the cell mesophyll and chloroplast ultrastructure (Fig. 5D). At the highest Cd concentration applied (133.5 μM), cells showed a less developed vacuolar system (not shown in this picture) and some membrane injuries appeared. Chloroplasts with crystalloid bodies displayed a disturbed shape, wavy appearance of grana and stroma thylakoids and swollen intrathylakoidal space (Fig. 5E). In addition, electron-dense plastoglobuli and light cytoplasmic lipid droplets were visible.

Figs. 5F–I show the ultrastructural changes induced by the Cu treatment in reed plants. At the lowest concentration used (15.7 μM) chloroplasts showed a well organized thylakoid system (grana and stroma membranes; Fig. 5F). Some chloroplasts presented dark plastoglobuli and crystalline deposits disturbing their shape (Fig. 5G). Similar almost rounded plastids with inclusions also appeared in plants exposed to 47.2 μM Cu. Thylakoids were swollen with increasing lumen space and plastoglobuli were numerous (Fig. 5H). At the highest Cu concentration (78.6 μM) (Fig. 5I) chloroplasts showed various shapes; their thylakoid system was dramatically affected, displaying membrane swelling and fewer grana. Numerous plastoglobuli in chloroplasts, and light lipid droplets in the cytoplasm, could also be seen. Crystalloid bodies were also visible inside the chloroplast and in other sections (not shown) of these samples. Peroxisomes and mitochondria near the chloroplasts were light and swollen.

Discussion

Growth parameters, such as biomass as well as shoot and root growth, have been used to evaluate metal toxicity in plants (Lee *et al.*, 1981). Root growth was particularly sensitive to metal toxicity (Baker and Walker, 1989). The TI, based on root growth, provides an estimate of the short-term effect of HM toxicity. Our experiments showed that both reed and willow could be described as Cu-tolerant plants at the lowest concentration used (15.7 μM), the TI for reed being higher than for willow. In the standard tolerance test, 7.85 μM Cu has often been used to discern tolerant and non-tolerant plants (Ait Ali *et al.,* 2002). In the case of Cd, willow appeared more tolerant than reed. At the highest toxic concentrations of both metals, root extension was negligible.

The different uptake patterns observed between Cu and Cd (Fig. 3) could be a consequence of the different role of these elements in the plants (Hardiman *et al.,* 1984). The essential element Cu was transported under metabolic control, reaching a constant concentration in roots and shoots despite the variations in the growth medium. By contrast, Cd, a non-essential element, was taken up passively to an extent proportional to the concentration in the solution.

Both species appeared to be moderate accumulators of Cu and Cd, more in root tissues than in shoots. This limited translocation to the aerial parts was also shown in previous experiments with different wetland species, suggesting a widespread exclusion strategy for metal tolerance in such plants (Batty and Younger, 2004; Deng *et al.,* 2004). The poor translocation to the aerial parts may be part of a defense strategy (Arduini *et al.*, 1996) to avoid serious shoot damage. Low quantities of both metals in leaves or chloroplasts may block photosynthetic processes (Lidon and Henriques, 1991; Krupa *et al.*, 1993).

At the highest concentration of either Cu or Cd used in our experiments, the amount of metal in shoots of both reed and willow appeared to be sufficient to damage the cell ultrastructure. Direct interaction of these metals with chloroplast components cannot be excluded; however, for some authors (Barceló and Poschenrieder, 1999) chloroplast ultrastructural alterations are indirect effects of metal toxicity.

In our study, chloroplasts from untreated plants of willow and reed exhibited some peculiar features that could help overcome metal toxicity such as ferritin aggregates in the stroma and peripheral vesicles, originating from the inner envelope membrane. Ferritin participates in iron storage and buffering of the mineral availability, avoiding oxidative stress (Kumar and Prasad, 1999). Peripheral vesicles, which increased in number due to the metal treatment, could form a peripheral reticulum, associated with an intense metabolite transport across the envelope between the stroma and cytosol (Mosejev *et al.*, 1987). Increases in the peripheral reticulum have been reported in plants under HM treatments (Ciscato *et al.*, 1997).

Despite the strategies of willow and reed to counteract the effect of metal toxicity, Cu and Cd, in the present experiment, damaged the cell and organelle ultrastructure. The chloroplast disturbances observed were similar to those reported by other authors. For Ouzounid *et al.* (1997) HM-induced changes in chloroplast ultrastructure resemble those induced by senescence. Changes in chloroplast ultrastructure and lipid composition of the thylakoid membranes alter the operability of the photosynthetic electron-transport chain (Lidon and Henriques, 1991; Malik *et al.,* 1992; Barón *et al.,* 1995; Ciscato *et al.*, 1997; Ouzounidou *et al.,* 1997). The increased size and number of plastoglobuli detected in our HM-treated plants might also be an indication of lipid peroxidation in chloroplasts, being a result of thylakoid lipid breakdown accumulated in them (Panou-Filotheou *et al.,* 2001). This correlates with the progressive disorganization of chloroplasts and the poor development of thylakoid membranes and grana, indicating also a HM-induced increase of chloroplast senescence (Vassilev *et al.,* 2003). No starch accumulation in leaves of either Cu- or Cd-treated plants occurred in our experiments. These results agree with results for Cd-treated wheat and bean (Barceló *et al.,* 1988; Ouzounidou *et al.,* 1997). By contrast, some authors have found an increase of starch content in Cu-treated plants (Ciscato *et al.,* 1997). In this case, the discrepancy with our data could be attributed to different growth conditions, HM concentrations, and time of treatment.

In summary, our results show that HM tolerance differs between the two species studied, reed being more tolerant to Cu and more sensible to Cd than willow. Both species restricted the translocation of either Cu or Cd to the aerial parts; however, the uptake pattern differed for each metal. In terms of chloroplast sensitivity against metal toxicity, Cu appeared more toxic than Cd in both plant species,

this being confirmed by preliminary assays *in vitro* with isolated chloroplasts (data not shown). In any case, reed proved to be more tolerant to Cu than to Cd, the alteration in chloroplast architecture not being determinant for metal tolerance. This agrees with the conclusion of some authors studying wetland plants (Deng *et al.,* 2004) which maintain metal concentrations at low levels in shoots, metal tolerance depending mainly on their metal exclusion ability.

It should be taken into account that HM tolerance mechanisms and plant growth inhibition by HMs are complex phenomena with contribution to different processes (efficiency in uptake, transport or metal avoidance, sensitivity of photosynthesis and other pathways of the energy metabolism, production of stress-protecting substances).

Concerning the potential value of *Salix* and *Phragmites* in phytoremediation, we conclude that, like other wetlands species, they could play a role in metal removal from polluted water by metal immobilization in the roots and rhizosphere. In addition, their ability to reduce metal translocation from roots to shoots make them suitable as phytostabilizers.

Acknowledgements

The authors thank AECI (Agencia Española de Cooperación Internacional) for providing the grants in the frame of the Spanish-Moroccan Cooperation Programme and acknowledge the financial support of the Collaborative Linkage Grant-977480 from the NATO Cooperative Science & Technology Sub-Programme.

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