The physiological response and sub-cellular localization of lead and cadmium in *Iris pseudacorus* L.

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Abstract  The seedling development and physiological responses of *Iris pseudacorus* L. to Pb and Cd and their combination were studied for 28 days liquid culture and sub-cellular localization of Pd and Cd in the root tip cells treated with 2,070 mg L\(^{-1}\) Pb and 1,000 mg L\(^{-1}\) Cd for 16 days sand culture was evaluated. Results showed that the dry weights (DWs) of shoots and roots of *I. pseudacorus* were significantly decreased at 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\) Cd treatments and the root DWs under all treatments were significantly decreased in comparison with that of control. The concentrations of Chl\(a\) in the leaves were decreased at all treatments, while, the concentrations of Chl\(b\) and total carotenoids were not significantly decreased under 25 mg L\(^{-1}\) Cd treatment. The MDA and proline concentrations and POD activities in the shoots and roots were increased under treatments of 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\) Cd + 500 mg L\(^{-1}\) Pb treatments and the root MDA concentrations in the shoots were significantly decreased at 25 mg L\(^{-1}\) Cd treatment. The results of sub-cellular localization of Pb and Cd showed that numerous Pb deposits were found on the inner surface of died cell walls in the cortex treated with 2,070 mg L\(^{-1}\) Pb and Cd deposits were found in the cell wall treated with 1,000 mg L\(^{-1}\) Cd. Pb and Cd deposits were not found in the cytoplasm. The results indicated that POD and proline showed strong beneficial properties against Pb and Cd stress and there were some mechanisms keeping most cells with normal activities in the plant from Pb toxicity by sacrificing a few cells that accumulated a large amount Pb. Sub-cellular localizations of Pb and Cd in the root tip cells of *I. pseudacorus* were little difference with the localizations in other species of *Iris* in the previous studies.

Keywords  *Iris pseudacorus* L. · Cadmium (Cd) · Lead (Pb) · Physiological response · Tolerance · Sub-cellular distribution

Introduction

Heavy metals produced by the modern industrial activity in the past have reached the toxic levels in the land and water of many parts of the world (Roy et al. 2005). Aquatic and terrestrial habitats are becoming progressively polluted due to indiscriminate discharge of heavy metals (Issa et al. 1995). Lead (Pb) and cadmium (Cd) are significant risk to environment. This becomes an urgent problem for cleaning up by environmental approaches. Several approaches have been suggested for remediation of soils and water contaminated with heavy metals. One of them is phytoextraction which means using plants to remove heavy metals from polluted environments (Salt et al. 1998). The value of metal accumulating plants for environmental remediation has been fully realized to be attractive, economic and non-invasive alternatives. However, the utilizations of such
plants could not fully meet the needs of bioremediation due to the limitation of the physical and chemical conditions of the habitats, biomasses and propagations of these plants (Bhattacharya et al. 2006). Therefore, there is a need for effective use of these plants and searching more plants which could accumulate lead in the above-ground parts and adapt different environments (Demirezen and Aksoy 2004; Han et al. 2008).

The present biochemical and physiological knowledge of the mechanisms controlling stress resistance of plants suggests that membranes are amongst the main cellular targets common to different stresses (Sayed 1999; Yu and Gu 2007). Pb and Cd oxidation may induce oxidative stress that damage plant cell membrane and the increase of malondialdehyde (MDA) concentration, thus MDA is an indicator of oxidative damage resulting in the loss of cell membrane lipid caused by free radicals and hydroperoxides (Ali et al. 2003). Chloro plast membrane was also damaged by heavy metals and indirectly effected chlorophyll contents in the leaves (Burzyski and Kobus 2004). Stress-induced antioxidant enzymes and penetrative materials are dependent of the severity of the treatment and also the species and age of the plant (Qureshi et al. 2007; Yu and Gu 2007). Many antioxidant enzymes such as peroxidase (POD) and penetrative materials such as proline enable the cell to quench the oxidative stress in some species, therefore, antioxidant enzymes and penetrative materials are used as physiological parameters in the study of Pb stress (Guo et al. 2004).

The sub-cellular localization of Pb and Cd reflects their chemical properties and/or the role in plant’s physiology and ecology. Pb and Cd uptake and accumulation through roots of higher plants have been expounded in some species of plants (Salt et al. 1998). Accumulation and location of Pb and Cd occurred as electron dense granules in the roots of some plants (Han et al. 2007, 2008; Sridhar et al. 2005). However, the mechanism of uptake, translocation and accumulation of Pb and Cd in plants should be better understood.

Heavy metals in contaminated water are collected with wetland plants, such as Typha spp. (Demirezen and Aksoy 2004), Scirpus spp. (Bhattacharya et al. 2006) and Lemna spp. can uptake metals, concentrating them in both aboveground and belowground biomass. Yellow flag (Iris pseudacorus L.) is a perennial with nice leaves and yellow flowers and widely planted in the gardens, streets or planted in shallow water as ornamental plants in the world. I. pseudacorus was also used as phytoremediating plant in wastewater or wetlands for the removal of organic matter and nutrients (Ansolà et al. 1995). I. pseudacorus could accumulate high quantity of Pb (Han 2007) and Cd (Huang et al. 2008). The aim of this study was to research the physiological response of I. pseudacorus to the Pb, Cd and their combination and to investigate the difference of Pb and Cd localization in the root cells between aquicolous species (I. pseudacorus) and terraneous species of Iris (I. tectorum and I. japonica) in the previous studies.

Materials and methods

Cultivation and experimental design

Seedlings culture of I. pseudacorus was according to the method of Han et al. (2007). When the seedlings reached 10 cm height and they were removed from the pots. The roots were carefully and gently washed and the seedlings were transferred into 500 mL pots (6 seedlings/pot) and supplied with the nutrient medium of 1/2 Hoagland nutrient solution. After 2 weeks, the plants were treated with nutrient solution free of Pb (CK), and addition of 500 mg L⁻¹ Pb supplied as Pb (NO₃)₂, 25 mg L⁻¹ Cd supplied as CdCl₂ and 500 mg L⁻¹ Pb + 25 mg L⁻¹ Cd for the biomass and physiological parameters determination for 28 days. Each experiment was consisted of three replicates. The seedlings were cultivated in clean sand and treated with nutrient solution without Cd and Pb as control, and addition of 1,000 mg L⁻¹ Cd and 2,070 mg L⁻¹ Pb, respectively, for the determination of sub-cellular localization of Pb and Cd in root cells for 16 days. The solutions were supplemented with nutrient solution containing the different levels of Pb and Cd weekly. The experiment was conducted in a greenhouse at ambient temperature (15–25°C) under the natural light.

Measurement of biomass

The seedlings were harvested and washed thoroughly with running tap water after being treated for 28 days and divided into two parts, shoots (leaves and small rhizomes) and roots. The dry weights (DW) were measured after the shoots and roots were dried at 80°C to constant weight.

Measurement of photosynthetic pigments

Leaves and roots were taken from plants at 28 days after Pb treatments and used for determinations. After extraction with 80% acetone, Chlorophyll a (Chla), chlorophyll b (Chlb) and total carotenoids were measured spectrophotometrically in the extracts at 470, 647 and 664.5 nm, respectively, according to Lichtenthaler (1987).

Detection of the activity of POD and the concentrations of MDA and proline

The malondialdehyde (MDA) concentrations and activity of superoxide dismutases (POD) were assayed according to
the method of Guo et al. (2004). The level of lipid peroxidation was expressed as MDA concentration and was determined as 2-thiobarbituric acid (TBA) reactive metabolites. POD activity was measured with guaiacol as the substrate in a total volume of 3 mL. The reaction mixture consisted of 50 mmol L\(^{-1}\) potassium phosphate buffer (pH 6.1), 1% (w/v) guaiacol, 0.4% (v/v) H\(_2\)O\(_2\) and enzyme extract. Increase in the absorbance due to oxidation of guaiacol was measured at 470 nm. A unit of POD activity was expressed as the change in absorbance per minute and specific activity as enzyme units per g fresh weight (FW). Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf segments were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined. Amounts of proline were expressed as \(\mu g\) g\(^{-1}\) FW.

The sub-cellular localization of Cd and Pb

The root segments of approximately 5 mm long were collected after 16 days exposure for electron transmission microscopy (TEM). The sub-cellular localization of Cd in root cells was evaluated according to Sridhar et al. (2005) with modifications. The samples were fixed in 2.5% glutaradehyde in 50 mmol L\(^{-1}\) -potassium phosphate buffer (pH 7.1) for 2 h at room temperature, then washed with deionised water, and treated with 0.1% Na\(_2\)S for 0.5 h. The samples were washed with deionised water thoroughly and fixed in 2.5% glutaradehyde for 4 h again. The sub-cellular localization of Pb in root cells was evaluated according to Sahi et al. (2002) with slight modifications. Pieces of the roots were fixed in 2% glutaradehyde in 50 mmol L\(^{-1}\) PIPES (pH 6.8), and incubated in the fixative for 3 h at room temperature. The samples were washed in 50 mmol L\(^{-1}\) PIPES buffer. All root segments were post-fixed in 2% OsO\(_4\) for 2 h and then dehydrated through an ethanol series and embedded in Spur epoxy resin. Ultrathin sections were obtained using ultramicrotome (POW-TIME-XL), collected on copper-supported grids, and observed with H-7650 Transmission Electron Microscope (TEM) at 80 kV.

Statistical treatment

All data presented are mean values. The measurements were done with three replicates for the biomass and physiological parameters. One-way analysis of variance (ANOVA) in randomized complete block design was performed to check the variability of data and validity of the results. The data were analyzed with SAS software system (SAS Institute Inc. 1994).

Results

The development of seedlings

The effects of different concentrations of Pb and Cd on the seedling biomass of *I. pseudacorus* after 28 days exposure were shown in Fig. 1. The DWs of shoots were decreased significantly treated with 500 mg L\(^{-1}\)Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\)Pb \((P < 0.05)\), while the DW of shoots treated with 25 mg L\(^{-1}\)Cd was no significant differences compared with that of control. However, the root growth under all treatments was significantly inhibited and the DWs of roots were dropped to 78, 86 and 71% of the control, respectively.

The concentrations of photosynthetic pigments

The concentrations of Chl\(_a\), Chl\(_b\) and total carotenoids in the leaves of *I. pseudacorus* for 28 days exposure were showed in Fig. 2. The concentrations of Chl\(_a\) in the leaves treated with Cd and Pb were significantly decreased compared with that of control \((P < 0.05)\). The concentrations of Chl\(_b\) were significantly decreased under the stress of 500 mg L\(^{-1}\)Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\)Pb, while, the decrease of Chl\(_b\) concentrations under the treatment of 25 mg L\(^{-1}\)Cd was no significant difference with that of control. However, total carotenoids concentrations in the leaves under the treatments of 25 mg L\(^{-1}\)Cd and 500 mg L\(^{-1}\)Pb dropped significantly compared with that of control \((P < 0.05)\) (Fig. 2), but no significant difference was found under the treatment of 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\)Pb. The effect of Cd, Pb and their combination on the Chl\(_b\) concentrations was similar with the effects on DW of shoots. The largest decreases of Chl\(_a\), Chl\(_b\) and Car concentrations were found
Different letters showed that the MDA concentrations in the roots of I. pseudacorus increased in the treatments of 25 mg L\(^{-1}\)Cd, 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb were raised about 9, 11 and 25% of the control, respectively. The concentrations of MDA in the shoots treated with 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb was significantly increased compared with that of control, while the concentrations of MDA were not significantly increased (500 mg L\(^{-1}\) Pb) or decreased significantly (25 mg L\(^{-1}\) Cd) compared with that of control \((P < 0.05)\). The results indicated that Pb and Cd toxicity in the roots and shoots of I. pseudacorus was linked to lipid peroxidation.

The proline concentrations in roots of I. pseudacorus increased significantly under the stress of 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb (Table 1) compared with that of control \((P < 0.05)\). The changes of proline concentrations in the shoots showed the same manner as in the roots, while, the increase of the proline concentrations in the treatments of 500 mg L\(^{-1}\)Pb and 25Cd mg L\(^{-1}\) + 500 mg L\(^{-1}\)Pb were raised to 16.9 and 8.2 times of the control respectively.

The sub-cellular distribution of Cd and Pb

The transmission electron micrographs of root cells of I. pseudacorus grown in sand with 1/2 Hoagland nutrient solution composed of control (without addition of Pb and Cd), and with addition of 2,070 mg L\(^{-1}\) Pb and 1,000 mg L\(^{-1}\) Cd were showed in Fig. 3. There were no Pb deposits in cell wall (CW) and intercellular space (ICS) of the cells grown in 1/2 Hoagland nutrient solution without addition of Pb and Cd (Fig. 3a). Micrograph B showed a cell of meristem treated with 2,070 mg L\(^{-1}\) Pb, there were

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**Fig. 2** Interactive effects of Pb and Cd stress on photosynthetic pigment contents of Iris pseudacorus L. Values are the means ± SE of three replicate measurements. Different letters in the same color columns indicate significant difference at the different Pb and Cd levels \((P < 0.05)\)

under the stress of 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb, 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb and 500 mg L\(^{-1}\) Pb and the concentrations of them were dropped by 24, 24 and 20%, respectively compared with that of control.

The POD activity and the concentrations of MDA and proline

In general, POD activities in the roots remained the same manner with those in the shoots after 28 days exposure (Table 1). POD activities in the shoots treated with 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb were higher and treated with 25 mg L\(^{-1}\)Cd were significantly lower than that of control \((P < 0.05)\). However, The POD activities in the roots were relatively higher than those in the shoots in same treatment. The POD activities treated with 500 mg L\(^{-1}\)Pb both in roots and in shoots were higher than those treated with 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\)Pb. The results indicated that the POD activity in the roots and shoots could be promoted by 500 mg L\(^{-1}\)Pb stress and this promotion could be restrained by 25 mg L\(^{-1}\)Cd.

The results in Table 1 showed that the MDA concentrations in the roots of I. pseudacorus increased in the treatments of 25 mg L\(^{-1}\)Cd, 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb were raised about 9, 11 and 25% of the control, respectively. The concentrations of MDA in the shoots treated with 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb was significantly increased compared with that of control, while the concentrations of MDA were not significantly increased (500 mg L\(^{-1}\) Pb) or decreased significantly (25 mg L\(^{-1}\) Cd) compared with that of control \((P < 0.05)\). The results indicated that Pb and Cd toxicity in the roots and shoots of I. pseudacorus was linked to lipid peroxidation.

The proline concentrations in roots of I. pseudacorus increased significantly under the stress of 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb (Table 1) compared with that of control \((P < 0.05)\). The changes of proline concentrations in the shoots showed the same manner as in the roots, while, the increase of the proline concentrations in the treatments of 500 mg L\(^{-1}\)Pb and 25Cd mg L\(^{-1}\) + 500 mg L\(^{-1}\)Pb were raised to 16.9 and 8.2 times of the control respectively.

The sub-cellular distribution of Cd and Pb

The transmission electron micrographs of root cells of I. pseudacorus grown in sand with 1/2 Hoagland nutrient solution composed of control (without addition of Pb and Cd), and with addition of 2,070 mg L\(^{-1}\) Pb and 1,000 mg L\(^{-1}\) Cd were showed in Fig. 3. There were no Pb deposits in cell wall (CW) and intercellular space (ICS) of the cells grown in 1/2 Hoagland nutrient solution without addition of Pb and Cd (Fig. 3a). Micrograph B showed a cell of meristem treated with 2,070 mg L\(^{-1}\) Pb, there were

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**Table 1** Interactive effects of Pb and Cd stress on the contents of MDA and proline, the membrane permeability and the POD activity of Iris pseudacorus

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CK</th>
<th>25Cd</th>
<th>Pb500</th>
<th>Cd25 + Pb500</th>
</tr>
</thead>
<tbody>
<tr>
<td>POD activities (ΔOD(_{470}) g(^{-1}) min(^{-1}))</td>
<td>R: 233.33c</td>
<td>211.11d</td>
<td>377.78a</td>
<td>316.67b</td>
</tr>
<tr>
<td></td>
<td>S: 211.11c</td>
<td>183.33d</td>
<td>255.56a</td>
<td>227.78b</td>
</tr>
<tr>
<td>MDA concentrations (µmol g(^{-1}) FW)</td>
<td>R: 6.56c</td>
<td>7.14b</td>
<td>7.26b</td>
<td>8.19a</td>
</tr>
<tr>
<td></td>
<td>S: 10.52b</td>
<td>9.34c</td>
<td>11.85ab</td>
<td>12.20a</td>
</tr>
<tr>
<td>Pro concentrations (µg g(^{-1}) FW)</td>
<td>R: 30.28c</td>
<td>33.57c</td>
<td>37.38b</td>
<td>70.57a</td>
</tr>
<tr>
<td></td>
<td>S: 9.97c</td>
<td>11.58c</td>
<td>168.48a</td>
<td>82.14b</td>
</tr>
</tbody>
</table>

Values were the means of three replicate measurements. Different letters in the same row indicate significant difference at the different Pb and Cd levels \((P < 0.05)\)

S: Shoots, R: Roots
no Pb deposits in the cell. Numerous Pb deposits were on the inner surface of died cell walls in the cortex treated with 2,070 mg L\(^{-1}\) Pb (Fig. 3c, d, Pb deposits were indicated with white arrowhead), there were not any Pb deposits in the cell walls and cytoplasm of the neighbor cells. However, numerous Cd deposits were found in the cell wall (Fig. 3e, Cd deposits were indicated with black arrowhead) and on the out surface of the cells (Fig. 3f) in a triangular intercellular space bordering with three cortical cells treated with 1,000 mg L\(^{-1}\) Cd. The Cd deposits were not found in the cytoplasm.

**Discussion**

Plant exposition to heavy metals has always resulted in a strong reduction of plant growth as a consequence of significant alterations in many metabolic pathways and photosynthetic activities (Burzyski and Kobus 2004; Yu and Gu 2006). The effects of Pb and Cd on the DWs of shoots and roots of *I. pseudacorus* were much more obvious in our experiment. The maximum decreases of the DWs of leaves and roots were found under the stress of 25 mg L\(^{-1}\)Cd + 500 mg L\(^{-1}\) Pb and the DWs were...
dropped to 55 and 71% of control, respectively (Fig. 1). The DW of leaves treated with 25 mg L\(^{-1}\)Cd was not significantly different with that of control indicating that the leaves of *I. pseudacorus* was more tolerant than roots. Singh et al. (2003) considered that plants were exposed in certain concentrations of heavy metals, the roots of plant are affected by the exposure of heavy metals first and some sensitive plants will show sign and the growth of the roots is restrained.

The excess of Pb and Cd usually decreased the concentrations of chlorophylls, often by damaged the membrane of the organelles, such as the structure of chloroplasts and indirectly affected the concentrations of chlorophylls (Ewais 1997; Han 2008). Burzyski and Kobus (2004) obtained that the chlorophylls concentrations in the leaves of cucumber treated with 1,000 µM Pb decreased up to 80 or 90%. Ewais (1997) found that the chla concentrations in the leaves of *Cyperus difformis, Chenopodium ambrosioides* and *Digitaria sanguinolos* were decreased as treated with 200 mg L\(^{-1}\) Pb and 20 mg L\(^{-1}\) Cd, while the chlb concentrations in the leaves of *C. difformis* and *D. sanguinolos* were not decreased. In our experiment, the concentrations of Chla and Chlb and total carotenoids were decreased under Pb and Cd stress, but Chlb concentration treated with 25 mg L\(^{-1}\) Cd and total carotenoids concentration under 500 mg L\(^{-1}\) Pb stress were not significantly decreased (Fig. 2). Kosobrakhov et al. (2004) obtained similar results that increasing the Pb concentration in soil from 500 to 2,000 mg kg\(^{-1}\) did not result in a further decrease of the pigment concentrations in leaves of *Plantago major*.

Heavy metal toxicity can cause excessive ROS production, including superoxide radical (\(O_2^{-}\)), hydroxyl radical (\(\cdot OH\)) and hydroxyl peroxide (\(H_2O_2\)), which can cause oxidative damage to biomolecules such as lipid, protein and nucleic acids, and disrupt cellular metabolism (Ali et al. 2003; Yu and Gu 2007). When exposed to environmental stress, plants will be induced to develop a defense system. The antioxidant enzymes are considered to be an important defense system of plants against oxidative stress caused by heavy metals and the activities of antioxidant enzymes represent the power of plant against oxidative stress (Weckx and Clijsters 1996). Under heavy metals stress, POD acts as an enzymatic protector (antioxidant) against peroxidation (Ali et al. 2003; Guo et al. 2004; Lagriffoul et al. 1998; Scebbas et al. 2006; Qureshi et al. 2007). The seedlings of *I. pseudacorus* under Pb and Cd stress showed that POD activities were significantly stimulated when plants were exposed to 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\) Cd + 500 mg L\(^{-1}\) Pb stress both in the leaves and roots (Table 1). Hu et al. (2007) obtain the similar result. Scebbas et al. (2006) found that the lower Cd concentration (2.2 µmol L\(^{-1}\)) increased the enzymes activities after 3 months in leaves and only after 1 month in roots of *Miscanthus sinensis*, while a decrease in POD activity was observed at higher Cd concentrations (6.6 µmol L\(^{-1}\)). However, Guo et al. (2004) observed that a stimulation of POD activity was recorded in the plants subjected to 1.0 µmol L\(^{-1}\) Cd treatment, and the extent of the increase varied greatly depending on concentration and time of exposure. Lagriffoul et al. (1998) found that POD activity was increased in the leaves of young maize plants (*Zea mays* L.) after 3 and 4 days Cd treatment, but not in roots. In our experiment, POD activities in the roots and leaves of *I. pseudacorus* exposed to 25 mg L\(^{-1}\) Cd was significantly decreased (*P* < 0.05) and the interactive effect of Pb and Cd could reduce the increase of POD caused by Pb (Table 1). The result showed that the increase of POD activity prevent plant cell membrane damage from excessive ROS to a great extent and defense plant against oxidative stress caused by Pb.

Oxidative damage is often exclusively associated with peroxidation reactions in membrane lipids. Thus, membrane lipid peroxidation is an indicator of oxidative damage resulting in the loss of cell membrane lipids caused by free radicals and hydroperoxides (Smirnoff 1993). The plant membranes are considered as primary sites of metal injury and membrane destabilization is directly correlated with MDA production, which is one of the decomposition products of polyunsaturated fatty acids of membranes (Ali et al. 2003). In the present study, the MDA concentrations in roots of *I. pseudacorus* were significantly increased under the Pb and Cd treatments compared with that of control (*P* < 0.05) (Table 1). The result was similar with the experiment of Ali et al. (2003) in which the contents of MDA in *Salix acmophylla* increased significantly at 60 days Pb treatment. The MDA contents in leaves of *I. pseudacorus* were not significantly increased under the 25 mg L\(^{-1}\) Cd treatment (Table 1). However, Balestrasse et al. (2005) found that proline concentration increased significantly in nodules and roots under Cd treatments, more markedly under 200 µmol L\(^{-1}\) Cd.

Proline accumulation in plants is a response to heavy metals to maintain the osmotic balance in the cells of plant, which can be used as a physiological parameter for the tolerance response of plant. Singh et al. (2003) suggested that the accumulation of proline in plants protect the cell membrane and proton pump against damage. The results of our study showed that the concentrations of proline in the leaves and roots of *I. pseudacorus* were increased under 500 mg L\(^{-1}\) Pb and 25 mg L\(^{-1}\) + 500 mg L\(^{-1}\) stress (*P* < 0.05) (Table 1), indicating that *I. pseudacorus* was able to respond against Pb stress by proline osmotic regulation. Han (2008) obtained similar result in the research of effect of Pb and NaCl on the growth and physiological response of *I. halophila*. However, Qureshi et al. (2007) in the experiment of Pb-induced oxidative stress and metabolic alterations in *Cassia angustifolia* found that proline
concentrations increased at 60 days after sowing but declined thereafter. The proline concentrations in the leaves and roots were not increased at the treatment of 25 mg L\(^{-1}\) Cd (Table 1). However, Balestrasse et al. (2005) found that proline concentration increased significantly in nodules and roots of soybean plants under Cd treatments.

Compartmentalization of heavy metals was another mechanism for detoxification in plants (Han et al. 2007, 2008; Sahi et al. 2002; Samardakiewicz and Woz’ny 2000). Heavy metals accumulated in the cell walls and vacuoles are well known mechanism for plants protecting the plasmalemma and keeping lower concentration of toxic ions in the cytoplasm (Han et al. 2007; Samardakiewicz and Woz’ny 2000). In our research, there were no Pb deposits in the cell of meristem treated with 2,070 mg L\(^{-1}\) Pb, which indicated that the meristematic section of the root tip was not the main heavy metal absorption part of *I. pseudacorus* (Fig. 3b). The Pb deposits were found on the inner surface of a few root tip cells of *I. pseudacorus*. The result indicated that Pb was transported passively by apoplast (cell walls or intercellular space) and actively in the cytoplasm (cytoplasm). Sahi et al. (2002) obtained similar results in their research on *Sesbania drummondii*, but they found that large Pb deposits along the surface of plasma membrane extended deeper into the root cell wall of *S. drummond*. Numerous Pb deposits aggregated in the inner surface of some cells and make them died (Fig. 3c, d), indicating that there were some mechanisms keeping most cells with normal activities in the plant from Pb toxicity by sacrificing a few cells that accumulated a large amount Pb in these cells (Han et al. 2008). Cd accumulated in vacuoles and apoplasts play a significant role in scavenging of free radicals produced in plant cells (Sridhar et al. 2005). Ultrastructure showed that Cd deposits in some cell walls were not well-distributed and not found in the plasma and vacuoles (Fig 3e, f), showing that Cd was mainly transported by the way of apoplasts. However, Han et al. (2007) found that some Cd deposits were located not only in the cell walls, but in the vicinity of the plasma membranes and membrane-bound organelles in the root cells of *I. lactea* var. *chinensis*. Cd deposits within the cell wall in plants exposed to Cd might also suggest the apoplastic transport of Cd, but Cd deposits accumulated in the cell walls might also negatively affect the enzymes contained in this compartment. Moreover, it was not known whether this damage was the result of Cd accumulation, or Cd penetrated the cells that had already been damaged.

Plants have the potential to change redox stratification and metal distribution through at least three distinct processes: (1) release of O\(_2\) through roots into the rhizosphere, (2) primary production, increasing the quantity of labile organic carbon and (3) direct uptake metals into roots, rhizomes, stems and leaves (Koretzky et al. 2007). However, there are two limitations for the phytoextraction of heavy metals. One is that heavy metals have extremely low solubility in soils (Morse and Luther 1999). Another limitation for heavy metals phytoextraction is the lack of plants which are able to hyperaccumulate heavy metals in the shoots (Shibata et al. 2007). Some species including *Typha* (Demirezen and Aksoy 2004) and *Scirpus* (Bhattacharya et al. 2006) have been demonstrated to translocate varying amounts of Pb from wetland soils into plant tissue. *I. pseudacorus* is normally used as ornamental plants having the advantages: perennials with very nice leaves and yellow flowers, good adaptation, easy propagation (Ansola et al. 1995; Huang et al. 2003), relatively higher shoots biomass and easy to harvest. Moreover, *I. pseudacorus* was tolerance and accumulation of Pb (Han 2007) and Cd (Huang et al. 2008). According to the study, we found that chlorophylls and MDA could be the indicators of the plant damage and proline and POD could be the indicators of the plant tolerance in the heavy metals contaminated soils phytoremediation. Authors are studying on the phytoremediation of lead mine tailing and heavy metal contaminated soils of smeltery in Jiangxi Province, China using the species of *Iris* L. and other plants and have obtained some valuable data. From the data we found that *I. pseudacorus* could accumulate more Pb in the roots and shoots and will be a promising plant in the phytoremediation of Pb and Cd contaminated soils, no matter wetland or land.

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