Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 147 (2007) 806–816
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Effect of Pb toxicity on root morphology, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*

Ejazul Islam, Xiaoe Yang, Tingqiang Li *, Dan Liu, Xiaofen Jin, Fanhua Meng

MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Natural Resources and Environmental Science, Zhejiang University, Huajiachi Campus, Hangzhou 310029, China

Received 19 October 2006; received in revised form 21 January 2007; accepted 22 January 2007
Available online 2 February 2007

Abstract

Seed germination and hydroponics experiments were conducted to underpin the effects of Pb on mined ecotype (ME) and non-mined ecotype (NME) of *Elsholtzia argyi* from Pb/Cu mining areas and the non-contaminated agricultural areas, respectively. In both experiments, ME exhibited higher tolerance to excessive levels of Pb in the growth medium. Various Pb treatments caused a stimulatory effect on seed germination of both the ecotypes. Concentrations of Pb in the leaves and the stem of the ME were 2.6 and 4.5 times respectively higher than those of the NME when plants were supplied with Pb level of 200 μM. Pb posed adverse effects on root morphological organization and root activity of both the ecotypes but decrease was not sharp and root activity was recovered in ME plants. Root ultrastructural studies revealed that in ME, Pb was detected as fine particles dispersed throughout the cell membrane and cell wall fraction, whereas most of the Pb was found as large aggregates deposited in the cell walls of NME plants. Comparatively better growth, higher tolerance and accumulation of Pb expressed by ME plants is mainly attributed to the maintenance of its root growth and activity as well as integrity of cell organelles.

Keywords: *Elsholtzia argyi*; Pb toxicity; Root morphology; Seed germination; Transmission electron microscopy

1. Introduction

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems on world scale. According to the environmental protection agency (EPA), Pb is the most common heavy metal contaminant in the environment [1]. It is a nonessential element in metabolic processes and may be toxic or lethal to organisms even when absorbed in small amounts [2]. Pb contamination in the plant environment is known to cause highly toxic effects on processes such as depression on seed germination [3], the disturbance in mitosis [4,5], induction of leaf chlorosis [6], toxicity of nucleoli [4], inhibition of root and shoot growth [4,7], reduction in photosynthesis [8,9] transpiration [10], DNA synthesis [11] and inhibition and activation of enzymatic activities [12]. Pb not only affects plant growth and productivity but also enters into the food chain causing health hazards to man and animals [13].

Due to potential hazards associated with Pb pollution and its widespread contamination, increased attention has been paid to develop methods for cleaning up Pb at minimal costs with the fewest environmental side effects [14]. Phytoremediation, the use of vascular plants to remove pollutants from the environment or to render them harmless [15] has emerged as an alternative technique for removing toxic metals from soil and offers the benefits of being in situ, cost-effective and environmentally sustainable. In the recent years, great interest has been developed for the identification of autochthonous plant species, which can accumulate elevated amounts of heavy metals in their tissues, with the aim of employing them for phytoremediation of contaminated soils [16–20]. These plant species have special mechanisms to cope with higher levels of metals in growth medium.

Pb accumulation in the soils affects plants primarily through their root systems. It has been reported that roots can take

* Corresponding author. Tel.: +86 571 86971907; fax: +86 571 86971907.
E-mail addresses: ejazulislam75@yahoo.com (E. Islam), xyang581@yahoo.com (X. Yang), litq76@yahoo.com.cn (T. Li).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.01.117

Table 1
Heavy metal concentrations in the soil and different plant parts of the mined and non-mined sites

Site	Metal concentration (mg kg^{-1})	Soil		Plant			
		Total	Extract.	Flower	Leaf	Stem	Root
Mined site	Pb	10167.0	193.8	25.7	99.1	184.1	299.6
	Cu	886.6	7.1	13.7	40.7	90.2	189.7
	Zn	8955.2	155.1	101.4	411.7	315.9	508.2
Non-mined site	Pb	187.2	1.4	0.38	12.9	59.4	101.9
	Cu	20.7	1.5	6.5	11.2	43.7	86.6
	Zn	100.4	7.7	20.3	75.4	192.2	680.8

up 3–50 times more Pb than leaves [21]. Plant root rapidly responds to absorbed Pb by changes in its growth rate and branching pattern while the aboveground organs respond later and not so evidently to the concentrations that are sublethal for the roots [22,23]. Although several workers have reported the inhibition of root growth under Pb toxicity, however most of the studies were mainly focused on the root elongation and fresh and dry weights and little information is available concerning Pb toxicity and root morphology.

A number of plants belonging to genus *Elsholtzia* have been recognized as Cu tolerant plants. *E. splendens* is first reported to be an endemic Cu tolerant and accumulating plant species based on field survey in an old Cu-mining area of Zhejiang Province of China as well as nutrient solution culture [24,25]. *E. argyi*, another species belonging to same taxonomic family as that of *E. splendens* exists widely in the ancient Pb/Cu-mining areas of Southeast of China, can grow up to 160 cm high and has a greater biomass than that of *E. splendens*. This plant species is ideal for the phytoremediation of Cu and Pb contaminated soils and the volatile constituents in its flowers give a possible utilization of plant resources after they are being used for phytoremediation purposes [26]. In the old Pb/Cu mined area, healthy growth of *E. argyi* was observed in the soil with extractable Pb, Cu and Zn of 193.8, 7.1 and 155.1 mg kg^{-1}, respectively (Table 1). Shoot concentrations of Pb, Cu and Zn were 102.9, 48.1, and 276.3 mg kg^{-1}, respectively. We also found a contrasting ecotype of *E. argyi* growing in the normal agricultural soils of Hangzhou suburbs Zhejiang Province of China that has much lower metal concentrations as compared to the Pb/Cu mining sites (Table 1). The ecotype of *E. argyi* growing in mining areas was designated as mined ecotype (ME), whereas one growing in the non-contaminated agricultural soils was designated as non-mined ecotype (NME). Although previous studies by Jiang et al. [27] reported the differences in tolerance and uptake of Cu by the two contrasting ecotypes of *E. argyi*, no study has been conducted to explore the Pb tolerance and uptake by two different edaphic ecotypes of this species.

The aim of the present study was to investigate the effects of Pb on the seed germination, and root growth, morphology, physiology and ultrastructural localization of Pb in root tips of the two edaphically isolated ecotypes of *E. argyi* and to find out the mechanism involved in the uptake of Pb by these plants.

2. Materials and methods

2.1. Plant materials and seed germination experiment

Seeds of the two ecotypes of *E. argyi* were collected from mature plants growing near the old abandoned Pb/Cu mines in San Men area of Thai Zhou, Zhejiang Province of China, and from the non-contaminated agricultural fields of Xiao Zhang Cun area of Hangzhou, Zhejiang Province, China. Soil and plant samples of both areas were also collected for the heavy metal analysis.

For seed germination experiment, healthy seeds of both the ecotypes were surface sterilized by 1% sodium hypochlorite (NaOCl) for 20 min. After sterilization seeds were washed thoroughly with distilled water and divided into six portions for each ecotype and placed in 100 ml beakers (approximately 200 seeds in each beaker). Around 35 ml of de-ionized water or test solution having 25, 50, 100, 200 and 400 μM Pb was added to these beakers and 10 ml of aliquots containing about 60–65 seeds were added to 7 cm Petri dishes having a layer of three filter papers. Source of Pb was $Pb(NO_3)_2$. The experiment was randomly arranged with each treatment replicated for three times. The seeds were incubated under dark conditions in the growth chambers having day/night temperature of 30/25 °C and day/night humidity of 70/90%. After 96 h of incubation in dark, germination percentage was recorded. For the determination of radical and hypocotyl lengths, each Petri dish was divided into four portions with the help of a marker and then eight plants were randomly selected from each portion and radical and hypocotyl lengths were measured.

2.2. Hydroponics culture

Seeds were surface sterilized, rinsed, and sowed in substrate containing perlite and vermiculite 3:1 (v/v) and moistened with distilled water. After emergence of seedlings, 1/2 strength basal nutrient solution was supplied until seedlings with 2-leaf pairs were established. Then these young seedlings were transplanted in plastic trays containing 1/2 strength basal nutrient solutions continuously aerated with a pump.

After 2 weeks seedlings with uniform size of both ecotypes were selected and transferred to plastic pots containing about 3.2 l of modified nutrient solution, in which KH_2PO_4 concentration was adjusted to 0.01 mM in order to prevent precipitation of Pb. After 4 days different Pb treatments were

given. The composition of the nutrient solution used for Pb treatment was as follows (in μM): 2000 KNO_3, 50 KCl, 500 $Ca(NO_3)_2{\cdot}4H_2O$, 200 $MgSO_4{\cdot}7H_2O$, 100 NH_4NO_3, 10 KH_2PO_4, 12 H_3BO_3, 2.0 $MnSO_4{\cdot}H_2O$, 0.5 $ZnSO_4{\cdot}7H_2O$, 0.2 $CuSO_4{\cdot}5H_2O$, 0.1 Na_2MoO_4, 0.1 $NiSO_4$, 20 Fe-EDTA. There were four treatments: (1) control (CK), (2) 50 μM, (3) 100 μM and (4) 200 μM Pb, respectively. Pb was supplied as $Pb(NO_3)_2$. The experiment was randomly arranged with each treatment in triplicate. Plants were grown under glasshouse conditions with natural light, day/night temperature of 30/25 °C and day/night humidity of 70/90%. Nutrient solution pH was adjusted to 5.5 with 0.1 M NaOH or 0.1 M HCl and was continuously aerated and renewed at every fourth day during the experiment.

2.3. Plant growth observations

The Pb-treated plants were harvested on 12th day after the treatment. At harvest, roots of intact plants were washed with distilled water, and then immersed in 20 mM Na_2EDTA for 15–20 min to remove Pb adhering to the root surfaces. After that, plants were washed thrice with distilled water and finally with de-ionized water. Root–shoot lengths were recorded, different plant parts were separated and their fresh weights were noted. These plant parts were oven-dried at 70 °C for approximately 72 h and dry weights were recorded.

2.4. Measurement of root morphological parameters and activity

Root morphological parameters were determined after plants were grown at different Pb levels for 12 days. In each replicate, four plants were selected randomly and root length, surface area, volume, diameter and number of root tips of each plant were determined using root automatism scan apparatus (MIN MAC, STD1600$^+$), equipped with WinRHIZO software offered by Regent Instruments Co. Average values of these four plants were considered as one replicate.

For determination of root activity, three treatments CK, 50 and 100 μM Pb were selected with three replicates. Each replicate was having 12 pots and in each pot three plants were grown in about 1.3 l of modified nutrient solution, in which KH_2PO_4 concentration was adjusted to 0.01 mM in order to prevent precipitation of Pb. Root activity was determined after plants were treated with different Pb levels for 0, 2, 4, and 8 days, respectively. At each sampling time, three pots were harvested randomly and each pot was considered as one replicate. Roots were washed thoroughly with distilled water and finally with de-ionized water and cut into small pieces of 3–4 mm. A 0.5 g portion of these roots sample was placed into scaled tube, 5 ml 0.4% TTC (triphenyl tetrazolium chloride) and 5 ml 0.1 mM phosphatic buffer solution (pH 7.0) were added to the tube and reacted for 2 h at 37 °C. Then 2 ml of 1 M H_2SO_4 was added to the tube to stop the reaction. The root activity was expressed by the amount of TPF (triphenyl formazan) deoxidized by TTC [28].

2.5. Transmission electron microscopy

Plants of both the ecotypes treated with two levels of Pb, i.e. CK and 200 μM for 12 days were selected for the TEM studies. Small sections of root tips, 1–3 mm in length, were fixed in 4% glutaraldehyde (v/v) in 0.2 M PBS (sodium phosphate buffer, pH 7.2) for 6–8 h and post-fixed in 1% OsO_4 (osmium(VIII) oxide) for 1 h, then in 0.2 M PBS (pH 7.2) for 1–2 h. Dehydration was performed in a graded ethanol series (50, 60, 70, 80, 90, 95, and 100%) followed by acetone, then samples were infiltrated and embedded in Spurr's resin. Ultra-thin sections (80 nm) were prepared and mounted on copper grids for viewing in the transmission electron microscope (JEOL TEM-1200EX) at an accelerating voltage of 60.0 kV.

2.6. Analytical methods

Total and available Pb, Cu and Zn in the soils were estimated by digesting with mix acid and extracting with 5 mM DTPA (pH 7.3), respectively. For plant samples, portion of 0.200 g was ashed in a muffle furnace at 550 °C for 12 h. The ash was dissolved in 1:1 (v/v) HCl and then washed into 50 ml flasks and made volume using de-ionized water. Metal concentrations in both soil and plant samples were determined using Integrated Couple Plasma Mass Spectrophotometer (Agilent 7500a).

2.7. Statistical analyses

All data were processed by statistical package SAS (Version 9.0). Values reported here are means of three replicates. Data were tested at significant levels of $P < 0.05$ using one way ANOVA. Graphical work was carried out using Sigma Plot software v.10.

3. Results

3.1. Effects of Pb on seed germination, hypocotyl and radical lengths

Application of different concentrations of Pb posed variable effects on seed germination, radical and hypocotyl lengths in both the ecotypes (Table 2). The results showed a stimulatory effect of Pb on the seed germination of both ecotypes of *E. argyi*. Also there was earliness in the seed germination of non-mined ecotype (NME) at highest Pb concentrations. However, radical and hypocotyl lengths in both ecotypes were adversely affected as compared to the corresponding controls, except for the 25 μM Pb treatment in which there was an increase in the radical and hypocotyl lengths of the mined ecotype (ME). Inhibition of radical lengths started at 100 μM for ME, while it started at 25 μM Pb in NME. Similarly inhibition of hypocotyl lengths started at 200 μM for ME whereas in NME it started at 25 μM Pb treatment, respectively.

3.2. Effects of Pb on plant growth

In hydroponics experiment, the ME of *E. argyi* grew well at all supplied Pb levels, and no toxic symptoms were observed

Table 2
Seed germination percentages, and the radical and hypocotyl lengths of the two ecotypes of *E. argyi* incubated under different Pb levels for 96 h

E. argyi ecotypes	Pb treatment (μM)	Germination percentage (%)	Radical length (mm)	Hypocotyl length (mm)
Mined ecotype (ME)	0 (control)	61.9 d	9.9 ab	8.6 b
	25	63.2 d	10.4 a	10.8 a
	50	71.8 c	9.4 b	8.5 b
	100	77.9 b	7.5 c	8.3 b
	200	83.5 a	4.7 d	7.0 c
	400	83.4 a	3.3 e	5.1 d
Non-mined ecotype (NME)	0 (control)	74.2 c	12.0 a	15.0 a
	25	81.5 b	9.2 b	11.2 b
	50	83.1 b	5.6 c	6.9 c
	100	92.9 a	4.2 d	5.1 d
	200	93.8 a	2.6 e	3.0 e
	400	93.0 a	1.5 e	1.9 f

Different letters in the same column indicate significant differences ($P < 0.05$) among the treatments and control.

(Fig. 1A). However, plant growth in NME was inhibited significantly (Fig. 1B). Plants of ME displayed better growth with fully expanded green leaves, while after 3 days of treatment with Pb, NME showed toxicity symptoms and leaves started lynching, their color turned to dark green and having purplish lower epidermis. After 1 week of treatment there were wilting symptoms on leaves and putrescence on the root tips in NME. It can be noted from Fig. 1B that at the time of harvest, plants of NME were showing obvious symptoms of growth inhibition and plants growing under higher Pb levels were having few and brittle leaves with dark purplish lower epidermis especially at 200 μM Pb treatment.

The application of different Pb concentrations caused pronounced effect on the plant height (Fig. 2A) and root length

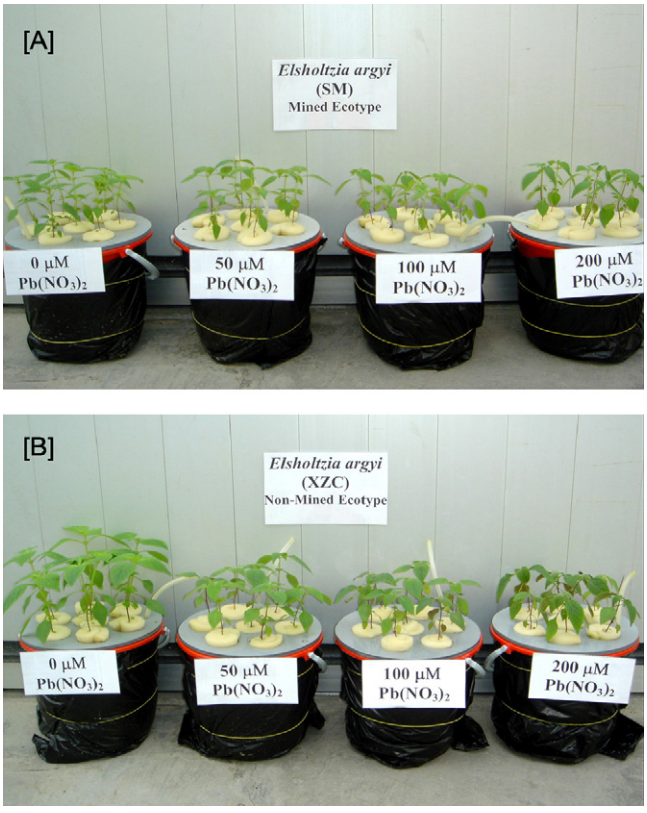

Fig. 1. Effect of Pb on the plant growth of mined ecotype (A) and non-mined ecotype (B) of *E. argyi* at the time of harvest. Plants were exposed to different levels of Pb for 12 days.

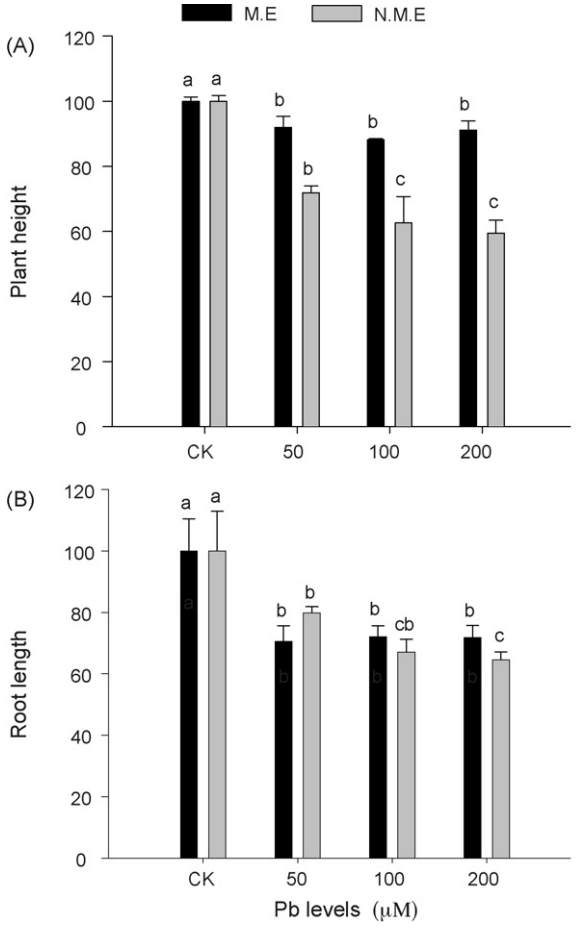

Fig. 2. Effects of Pb on plant height (A) and root length (B) of the two ecotypes of *E. argyi*. Plants were exposed to different levels of Pb for 12 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatment and control (CK). In CK, plants heights were 13.8 and 19.2 cm plant^{-1} and root lengths were 20.3 and 25.7 cm plant^{-1} for ME and NME, respectively.

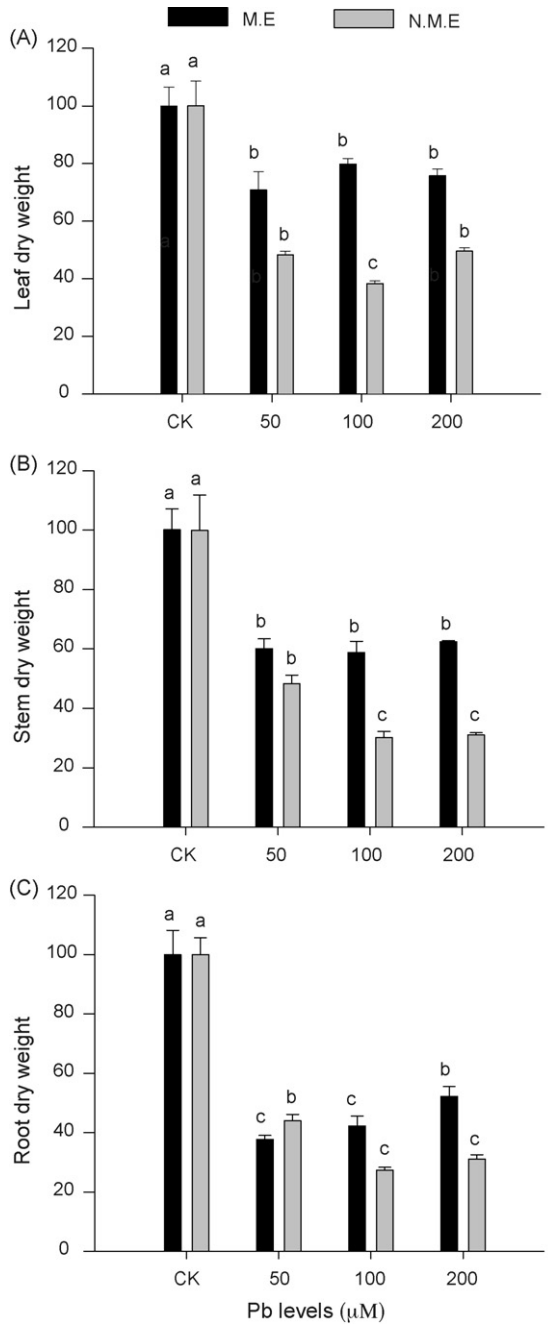

Fig. 3. Dry weights of the leaf (A), stem (B) and roots (C) of the two ecotypes of *E. argyi* grown under different levels of Pb for 12 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatment and control (CK). In CK, leaf dry weights were 0.055 and 0.125 g plant^{-1}, stem dry weights were 0.013 and 0.038 g plant^{-1} and root dry weights were 0.021 and 0.051 g plant^{-1} for ME and NME, respectively.

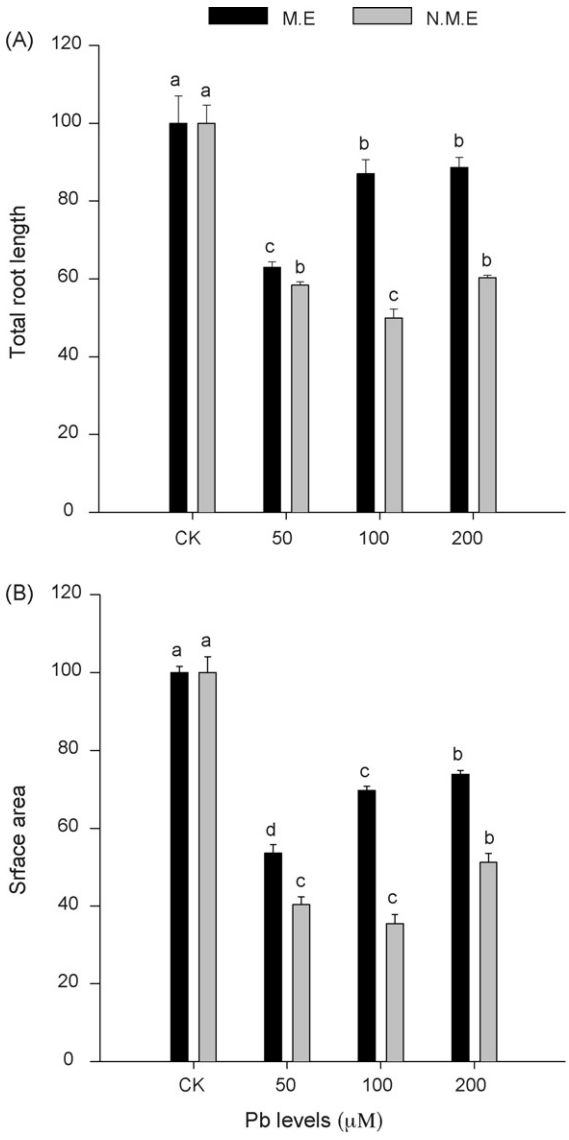

Fig. 4. Total root lengths (A) and surface area (B) of the two contrasting ecotypes of *E. argyi* grown under different levels of Pb for 12 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatment and control (CK). In CK, total root lengths were 240 and 435 cm plant^{-1} and surface areas were 47 and 93 cm^2 plant^{-1} for ME and NME, respectively.

(Fig. 2B) of *E. argyi*. The plant height and root length in both ecotypes decreased significantly ($P < 0.05$) over control, however, the relative decrease was not significant within different Pb treatments for ME. On the other hand in NME, both plant height and root length had an inverse relation with the increasing Pb concentrations. Dry weights of leaf, stem and root of *E. argyi* were also adversely affected by different Pb treatments (Fig. 3). Both leaf and stem dry weight of ME decreased significantly over the control, however, no significant ($P < 0.05$) differences were observed within the different Pb levels. Root dry weights were also decreased, however, a quadratic response was noted as it increased at 200 μM Pb treatment. In NME, dry weights of leaf, stem and root were also decreased and plants showed a definite quadratic response (Fig. 3A–C).

3.3. Effect of Pb on root morphology

The total root lengths and surface area were evidently different for both ecotypes at different Pb supply levels (Fig. 4A and B). Root length and surface area of the NME was significantly reduced by Pb treatments and plants showed a quadratic response as root length and surface area were increased at 200 μM Pb

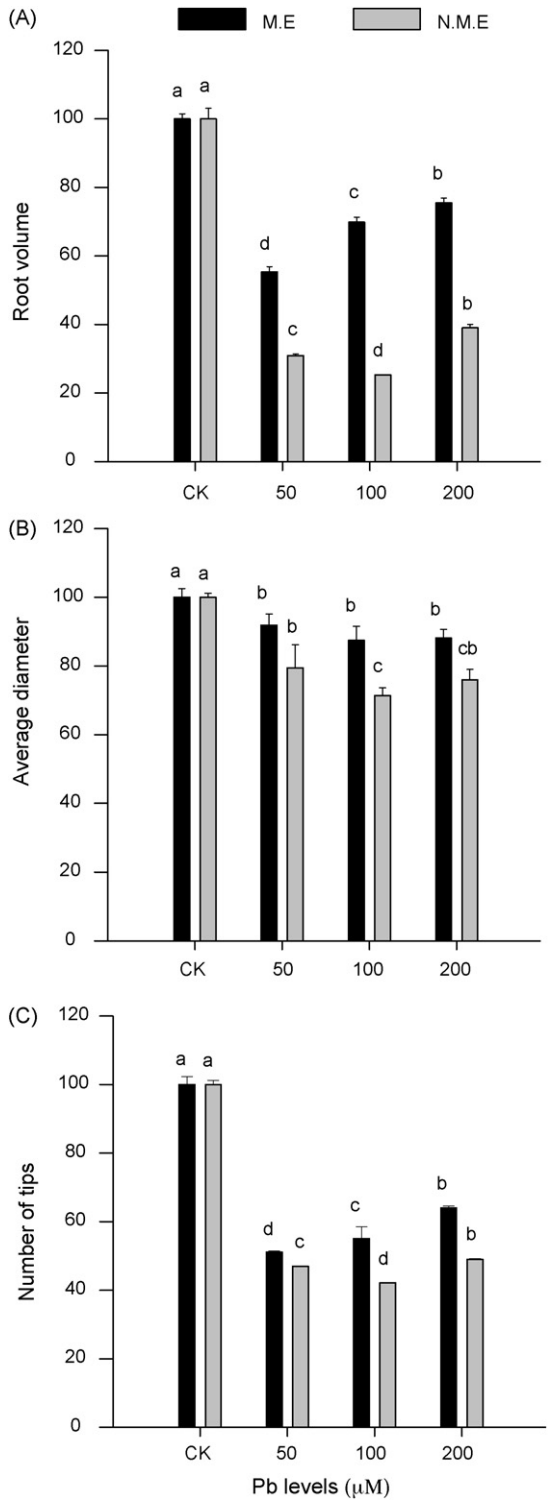

Fig. 5. Root volume (A), average diameter (B) and number of root tips (C) of the two ecotypes of *E. argyi* under different levels of Pb for 12 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatment and control (CK). In CK, root volumes were 0.61 and 1.6 cm^3 $plant^{-1}$, average diameters were 0.6 and 0.7 mm and number of root tips was 733 and 994 $plant^{-1}$ for ME and NME, respectively.

treatment. Although root length and surface area were also reduced in ME, decrease was not sharp except at 50 μM Pb treatment, where both root length and surface area were decreased to 37 and 46% of their corresponding controls.

Different Pb levels had variable but significant ($P < 0.05$) impact on root volume, diameter and number of root tips of both ecotypes (Fig. 5A–C). Data on root volume of both ecotypes (Fig. 5A) showed a trend similar to total root length and surface area, respectively. Root diameters in both ecotypes were slightly reduced by the application of different Pb treatments (Fig. 5B). In ME it decreased significantly ($P < 0.05$) over control but decrease was non-significant within the treatments. However, in NME it decreased in a quadratic manner. Number of root tips in both ecotypes was decreased significantly over the control showing a quadratic response as number of root tips was increased at 100 for ME and 200 for NME, respectively (Fig. 5C).

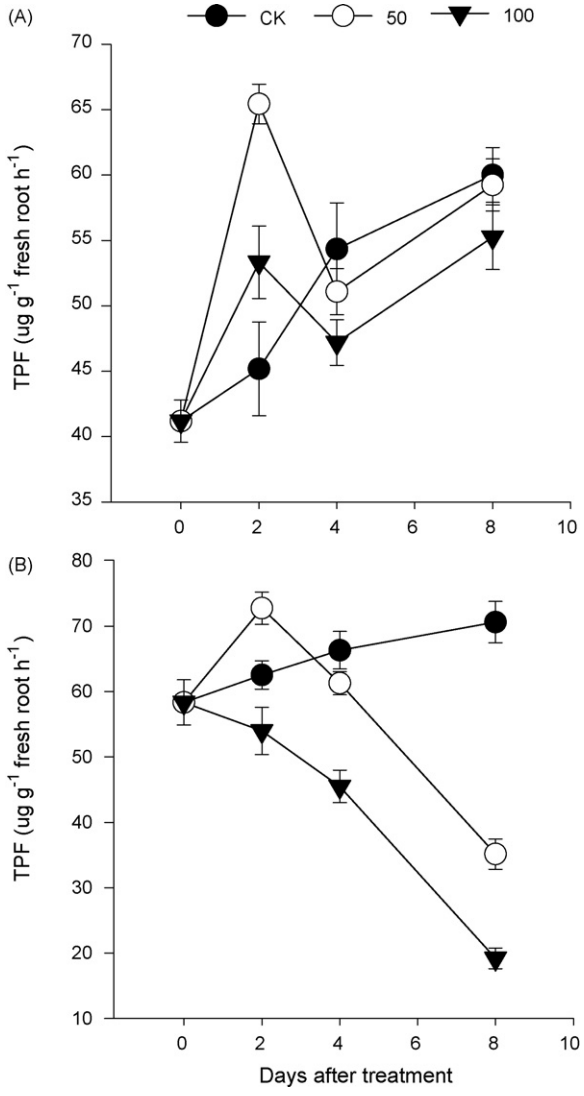

Fig. 6. Effect of Pb on root dehydrogenase activity of the two contrasting ecotypes of *E. argyi*. Plants were exposed to different levels of Pb for 0, 2, 4 and 8 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Means were significantly ($P < 0.05$) different among the treatments and control at days 2 and 4 of treatment for ME and at days 2, 4 and 8 of treatment for NME, respectively.

3.4. Effect of Pb on root activity

Effects of different Pb concentrations upon root activity (as TTC reducing capacity) of *E. argyi* varied significantly ($P < 0.05$) among the ecotypes. For ME (Fig. 6A), root activity was increased at 50 and 100 μM Pb on day 2 of the treatments while root activity of NME was only increased by 50 μM Pb and was decreased by Pb level of 100 μM on the same day of treatment (Fig. 6B). On day 4 of the treatments, root activities in both ecotypes were slightly decreased by 50 μM Pb treatment while at Pb level of 100 μM, decrease in the root activity of NME was more sharp as compared with ME plants. However, root activity of ME was recovered, and it was close to the control at day 8 of the metal treatment, whereas for the NME, it decreased at both 50 and 100 μM Pb and did not recover.

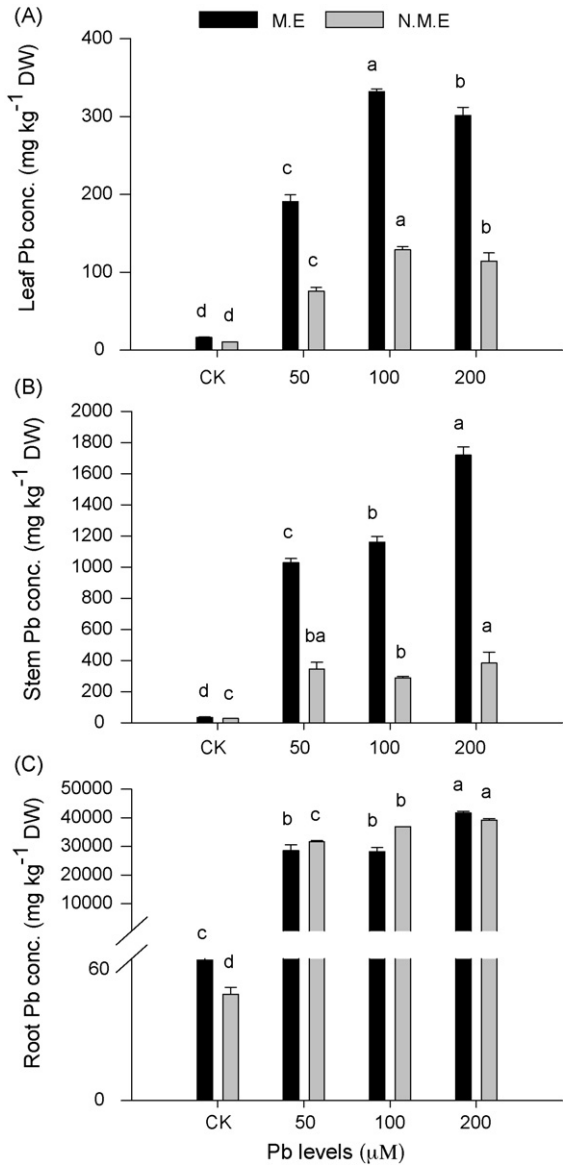

Fig. 7. Pb concentrations in the leaf (A), stem (B), and roots (C) of the two ecotypes of *E. argyi* grown under different levels of Pb for 12 days. Data points and error bars represent means ± S.D. of three replicates ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatment and control (CK).

3.5. Metal concentrations in plant tissues

Varying amounts of Pb concentrations were noted in plant organs of ecotypes under investigation (Fig. 7). The Pb concentrations in leaf and stem of ME were significantly ($P < 0.05$) higher than that of NME. However, much smaller differences regarding Pb concentrations in the roots were noted among ecotypes, and Pb concentration in the roots of ME plants were lower than that of the NME when the plants were grown at 50 and 100 μM Pb, respectively (Fig. 7C). In both the ecotypes, Pb concentrations in the tissue decreased in the order of root > stem > leaf.

3.6. Transmission electron microscopy

Transmission electron micrographs of the root tips cells of mined ecotype (A and B) and non-mined ecotype (C and D) are shown in Figs. 8 and 9. In control, root cells of both, i.e. mined ecotype (Fig. 8A and B) and non-mined ecotype (Fig. 8C and D) of *E. argyi* were having rich cytoplasm and organelles, smaller vacuoles, smooth and continuous cell wall, and bigger nucleus and nucleolus. The cell walls were clean, endoplasmic reticulum and numerous plasmodesmata were visible.

Ultrastructural studies revealed that Pb caused adverse effects on cell organelles in both ecotypes (Fig. 9A–D), but damage was more pronounced in the NME. At 200 μM Pb, the meristematic cells of both ecotypes especially of NME were having sparse cytoplasm and organelles including some mitochondria, which were swollen, bigger or more vacuoles, shrinked nucleus with indistinct nucleolus. Cell wall was not smooth and had many attachments. Heavy Pb depositions were located on the cell wall and cell membranes. An interesting feature noted was that both ecotypes exhibited variable patterns of Pb deposition in the root tip cells. In ME (Fig. 9A and B), Pb taken by roots was found as fine particles dispersed throughout the cell membrane and cell wall fraction, whereas in the roots of NME (Fig. 9C and D) although concentration of Pb was almost equal to ME, most of the Pb was found as large aggregates deposited in the cell wall fractions.

4. Discussion

Pb is an environmental pollutant and is extremely toxic to plants and other living organisms including human. Its concentration in normal plants does not exceed 5 mg kg^{-1} usually [29]. Unfortunately, the mechanisms of Pb toxicity in plants are still poorly understood. *E. argyi*, exists widely in the ancient Pb/Cu-mining areas of Southeast of China and previous studies by Jiang et al. [27] indicated that Sanmen-ecotype (from mining area) of *E. argyi* is a Cu-tolerant ecotype, and its tolerance to high Cu levels was mainly related to its extraordinary capability to restrict Cu uptake, especially Cu translocation from root to the shoot. Peng et al. [30] reported that *E. argyi* has high Pb tolerance and may accumulate large amounts of Pb in its aerial tissues but studies were focused only on one ecotype. In the present study, where we compared two contrasting ecotypes of *E. argyi*, the ME was found more tolerant to Pb than the NME and proved

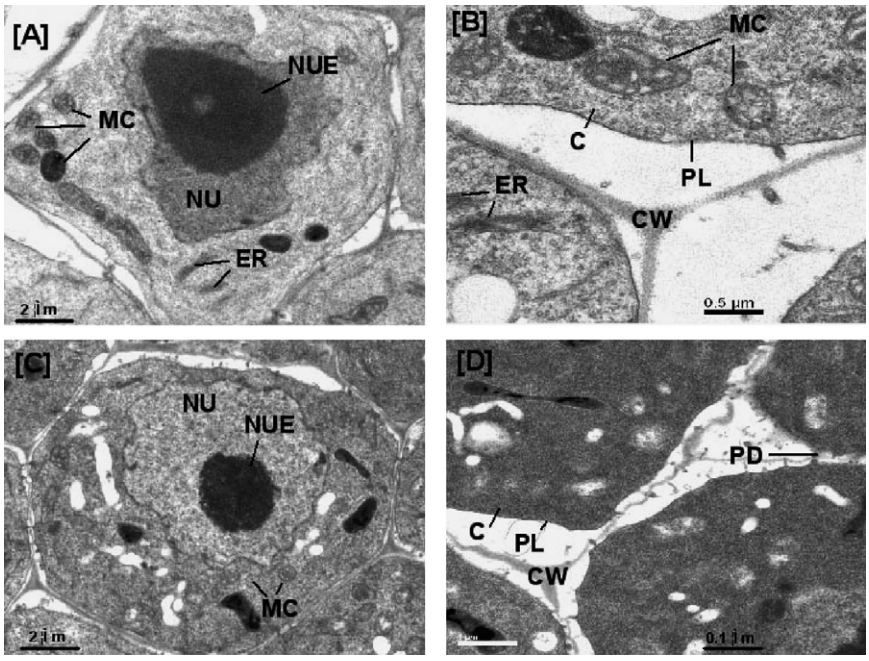

Fig. 8. Transmission electron micrographs of the root tips cells of mined ecotype (A and B) and non-mined ecotype (C and D) exposed to 0 μM Pb (CK) for 12 days. Bars: A = 2 μm, B = 0.5 μm, C = 2 μm, D = 0.1 μm. Labels: NU, nucleus; NUE, nucleolus; C, cytoplasm; CW, cell wall; MC, mitochondria; PD, plasmodesmata; ER, endoplasmic reticulum; PL, plasmalemma.

to be a shoot hyperaccumulator of Pb. McGrath et al. [31] also found significant differences among the two ecotypes of *Thlaspi caerulescens* in Cd uptake. Coughtrey and Martin [32] showed that the translocation of metals to the shoot of *Holcus lanatus* is prevented in ecotypes from metal-contaminated sites, and Landberg and Greger [33] obtained same results for different *Salix* species.

4.1. Effect of Pb on plant growth

Various authors have reported inhibition of seed germination by Pb but results of the present study showed a stimulatory effect of Pb on the seed germination of both ecotypes. This might be due to the reason that seeds were freshly harvested and were still under physiological dormant conditions, which

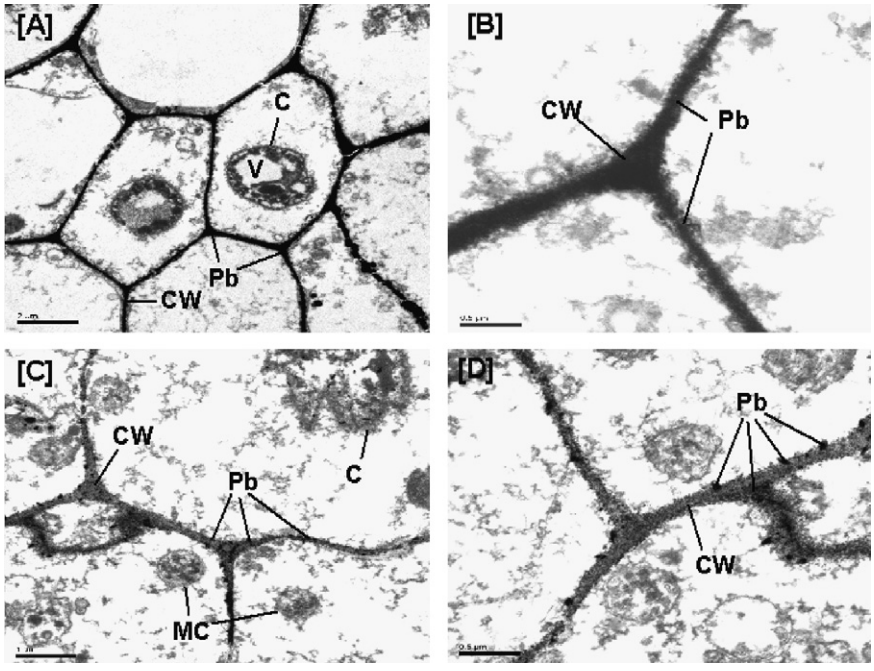

Fig. 9. Transmission electron micrographs of the root tips cells of mined ecotype (A and B) and non-mined ecotype (C and D) exposed to 200 μM Pb for 12 days. Bars: A = 2 μm, B = 0.5 μm, C = 1 μm, D = 0.5 μm. Labels: C, cytoplasm; CW, cell wall; MC, mitochondria; V, vacuole; Pb, Pb deposits.

is normally overcome by post-harvest ageing. Such dormancy is also reported to be broken by several stress conditions, for example, low pH solutions [34], heavy metals [35] and high temperatures [36]. In the present study application of different Pb treatments caused breakage of seed dormancy and stimulated the seed germination. Our results are in agreement with the results of Jeliazkova and Craker [37] who also found an increase in the germination percentage of *Carum carvi* L. (caraway) seeds exposed to Pb and Cd toxicity at 100 and 6 mg l^{-1}, respectively. The radical and hypocotyl lengths of both ecotypes were adversely affected as compared with the corresponding controls, but decrease was sharper in the NME plants. The results showed that ME have higher tolerance to the excessive levels of Pb in the growth medium during seed germination stage.

The effect of Pb on fresh and dry biomass accumulation of the plant parts seems to be differential with regards to plant species, plant cultivars, plant organs and the metabolic processes [38–41]. In hydroponics experiment when plants were treated with varying concentration of Pb, growth of ME plants was not severely inhibited in comparison with the control while for NME, plant growth was severely inhibited which may be due to the strong inhibition of root growth under varying concentrations of Pb. In most of the studies a decrease in fresh and dry weights of plants was reported under Pb treatment [42,43]. However in certain cases, as in corn seedlings, an apparent increase in dry weight of plant organs was reported which was due to an increase in the synthesis of cell wall polysaccharides resulting from Pb exposure [44]. In the present study an increase in the dry weights of NME was noted at 200 μM Pb treatment may also be due to the increased synthesis of cell wall polysaccharides in response to higher Pb concentrations in the nutrient solution.

4.2. Effect of Pb on root activity and morphology

Root activity (as TTC reducing capacity) is used as an important physiological parameter for evaluation of ion uptake, but few reports are available concerning the effect of Pb on root activity. In plant tissue, TTC is mainly reduced by the activity of dehydrogenase enzymes, which is related positively to respiration capacity, and has been widely used in the study of the viability of different tissues, for example, seeds, leaves, and roots [46–48]. In present study, root activity of ME was recovered and close to the control at day 8 of the treatment but for the NME, it decreased 50 and 72% of control at 50 and 100 μM Pb treatments, respectively. The recovery of root activity in the mined ecotype of *E. argyi* might be partly attributed to the stimulated synthesis of compounds with sulphydryl group [49] and/or due to the root exudation of some organic acids, which can chelate Pb and consequently reduce its toxicity. Li et al. [29] also reported that root activity of the hyperaccumulator ecotype (HE) of *Sedium alfredii* Hance treated with Pb was recovered close to the control at day 10 of the treatment whereas that of non-hyperaccumulator ecotype (NHE) was not recovered with the advance of treatment time but in contrast to results of present study, no increase was detected in the root activity of both the ecotypes on days 2 and 4 of treatment with Pb. Increase in the root activity of control plants might be due to positive response of plants to frequent changes in nutrient solution as just 4 days before the application of Pb treatments, plants were growing in large trays having 1/2 strength nutrient solution. The results of the present study showed that better root growth and increased Pb uptake by ME are attributed to the maintenance of its root activity under Pb application.

Most of the previous studies reporting deleterious effects of Pb on the root growth were based merely on parameters like root elongation and fresh and dry weights, whereas in response to Pb toxicity, roots can also respond via changes in surface area, volume and diameter, production or inhibition of lateral roots and tips, and variation in the other morphological parameters. Some studies were also conducted on root morphology but these were mainly focused on the relationship between root morphology and yield [50] and there is little information available on the relationships between root morphology and hyperaccumulation. Results from present study showed that roots of the ME plants have great potential to tolerate and absorb Pb from the growth medium. As compared with control plants toxic symptoms were observed and root growth was dramatically inhibited in the NME of *E. argyi* with Pb treatment while inhibition of root morphological parameters of the ME was not sharp. However, an improvement in the root morphological parameters of NME was noted at 200 μM Pb, which might be due to the stimulated increased synthesis of cell wall polysaccharides to detoxify the higher Pb concentrations via aggregating it mainly into the cell walls. An et al. [51] reported that *Cucumis sativus* could retain greater amount of metals in the roots due to its root morphology. The greater surface area of numerous and thin roots contributed more to the absorption of heavy metals by roots. In the present study, growth cessation of NME may also be due to the reduced root activity and destruction of root structure in response to various Pb concentrations.

4.3. Effect on Pb concentrations in plant tissue and root ultrastructure

Plants differ widely in their capacity to take up and accumulate heavy metals in various organs. In present experiment, Pb concentrations in the stem and leaf of the ME were 1720 and 301 mg kg^{-1} at Pb level of 200 μM. Concentrations in the stem were even higher than that in the representative Pb-hyperaccumulators *Thlaspi rotundifoliun* which was reported to be able to accumulate shoot Pb concentrations of 130–8200 mg kg^{-1} with a mean of 1100 mg kg^{-1} [45]. The results from present study showed that although roots of ME and NME had almost same concentration of Pb at highest metal concentration (200 μM) in the nutrient solution, the Pb concentrations in leaf and stem of ME were 2.6 and 4.5 times higher than those of NME showing greater ability of ME plants to transfer Pb from roots to stem and leaves. Our results are in agreement with Li et al. [29] who also showed that concentration of Pb in the leaf and stem of hyperaccumulating ecotype of *S. alfiedii* H. (from the mined area) were 1.9 and 2.4 times higher respectively than the non-hyperaccumulating ecotype (from the agricultural areas). In

present study higher Pb concentrations in the stem and leaves of ME plants of *E. argyi* might be attributed to a specialized metal transport system that needs to be characterized further.

Toxicity of heavy metals including Pb to large extent depends upon their absorption, transport and cellular localization within the plant tissue [52]. Metals in the tissue can be trapped by the negative charges of the cell walls, i.e. accumulated in the apoplast [53], or may also be taken up into the cell cytoplasm [54]. Such partitioning between compartments may differ between species [54,55] and plant parts [54]. In present study TEM studies indicated that in the root of ME plants, Pb was found as fine particles dispersed throughout the cell membrane and cell wall fraction, whereas in the roots of NME although concentration of Pb was almost equal to ME, most of the Pb was found as large aggregates deposited in the cell wall fractions. These differences in deposition pattern partly explain why roots of the NME were not able to transfer Pb to above ground parts, i.e. stem and leaves as most of the Pb was strongly bound to the carboxyl groups of carbohydrates in cell walls resulting in a diminished transport via apoplast [56]. Movement of Pb in the root is primarily via the apoplast is also supported by the report that a large proportion of Pb is readily extractable in water [57]. The difference observed in the Pb deposition pattern of the ME and NME roots in the present study might be due to the different Pb sensitivity thresholds of the two *E. argyi* ecotypes and remains to be confirmed.

5. Conclusions

- Variable root responses to Pb toxicity were observed in both ecotypes of *E. argyi*.
- Poor growth and susceptibility of NME plants to higher concentrations of Pb in the growth medium may be due to the strong inhibition of root growth associated with reduced root activity and uptake of minerals.
- Comparatively better growth, absence of toxicity symptoms and higher tolerance to Pb expressed by ME are mainly attributed to the maintenance of its root growth and activity, morphological organization and integrity of cell organelles especially membranous organelles in response to Pb toxicity.
- It is suggested that both ecotypes of *E. argyi* have different mechanisms at the cellular level and more than one mechanism might be working simultaneously in the Pb tolerance and hyperaccumulation of these plants and need to be further investigated.

Acknowledgements

The study was financially supported by a grant from the National Natural Science Foundation of China (No. 20477039), Program for Changjiang Schlors and Innovative Research Team in University (No. IRT0536), and “973” project from Ministry of Science and Technology China (No. 2002CB410804). The authors are greatly thankful to Professor Jun Ying Li from the Center of Analysis & Measurement, Zhejiang University, Hangzhou, China for her kind help in the Transmission Electron Microscope analyses of the plant samples.

References

[1] M.A. Watanabe, Phytoremediation on the brink of commercialization, Environ. Sci. Technol. 31 (1997) 182A–186A.
[2] C.H. Walker, S.P. Hopkin, R.M. Sibly, D.B. Peakall, Principles of Ecotoxicology, Taylor and Francis, Bristol, PA, 1996.
[3] M. Wierzbicka, J. Obidzinska, The effect of lead on seed imbibition and germination in different plant species, Plant. Sci. 137 (1998) 155–171.
[4] D. Liu, W. Jiang, W. Wang, F. Zhao, C. Lu, Effects of lead on root growth, cell division, and nucleolus of *Allium cepa*, Environ. Pollut. 86 (1994) 1–4.
[5] M. Wierzbicka, Resumption of mitotic activity in *Allium cepa* root tips during treatment with lead salts, Environ. Exp. Bot. 34 (1994) 173–180.
[6] W.R. Johnson, J.A. Proctor, Comparative study of metal levels in plants from two contrasting lead mine sites, Plant Soil 46 (1977) 251–257.
[7] A. Fargasova, Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapis alba* seeds, Bull. Environ. Contam. Toxicol. 52 (1994) 452–456.
[8] J.W. Poskuta, E. Parys, E. Romanowska, H. Gajdzis-Gujdan, B. Wroblewska, The effects of lead on photosynthesis, 14C distribution among photoassimilates and transpiration of maize seedlings, Acta Soc. Bot. Pol. 57 (1988) 149–155.
[9] J.W. Poskuta, E. Waclawczyk-Lach, In vivo responses of primary photochemistry of Photosystem II and CO_2 exchange in light and in darkness of tall fescue genotypes to lead toxicity, Acta Physiol. Plant. 17 (1995) 233–240.
[10] G.L. Rolfe, F.A. Bazzaz, Effect of lead contamination on transpiration and photosynthesis of loblolly pine and autumn olive, For. Sci. 21 (1975) 33–35.
[11] B. Gabara, B. Wojtyła-Kuchta, M. Tarczynska, The effect of calcium on DNA synthesis in pea (*Pisus sativus* L.) roots after treatment with heavy metals, Folia Histochem. Cytobiol. 30 (1992) 69–73.
[12] F. Van Assche, H. Cliisters, Effects of metals on enzyme activity in plants, Plant Cell Environ. 13 (1990) 195–206.
[13] M.R.D. Seaward, D.H.S. Richardson, Atmospheric sources of metal pollution and effects on vegetation, in: A.T. Shaw (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects, CRC Press Inc., Boca Raton, FL, 1990, pp. 75–92.
[14] M.A. Brennan, M.L. Shelley, A model of the uptake, translocation, and accumulation of Pb (Pb) by maize for the purpose of phytoextraction, Ecol. Eng. 12 (1999) 271–297.
[15] S.D. Cunningham, W.R. Berti, Remediation of contaminated soils with green plants: an overview, In vitro Cell Dev. Biol. (1993) 207–212.
[16] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 643–668.
[17] A.J.M. Baker, R.R. Brooks, Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry, Biorecovery 1 (1989) 81–126.
[18] A.J.M. Baker, S.P. McGrath, C.M.D. Sidoli, R.D. Reeves, The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants, Resour. Conserv. Recyl. 11 (1994) 41–49.
[19] G.M. Tordoff, A.J.M. Baker, A.J. Willis, Current approaches to the revegetation and reclamation of metalliferous mine wastes, Chemosphere 41 (2000) 219–228.
[20] G. García, A. Faz, H.M. Conesa, Selection of autochthonous plant species from SE Spain for soil Pb phytoremediation purposes, Water Air Soil Pollut.: Focus 3 (2003) 243–250.
[21] A. Wozny, M. Krzeslowska, B. Tomaszewska, Pb in Plant Cells, Sorus, Poznan, Poland, 1995.
[22] S.W. Breckle, Growth under stress: heavy metals, in: Y. Waisel, A. Eshel, U. Kafkafi (Eds.), Plant Roots, The Hidden Half, Marcel Dekker, New York, 1991, pp. 351–373.
[23] M.S. Davies, Effect of toxic concentrations of metals on root growth and development, in: D. Atkinson (Ed.), Plant Root Growth: An Ecological Perspective, Blackwell Sci. Publ., Oxford, 1991, pp. 211–227.
[24] X.E. Yang, W.Y. Shi, C.X. Fu, M.J. Yang, F. He, Copper-hyperaccumulators of Chinese native plants: characteristics and possible use for phytoremediation, in: N.E. Bassam (Ed.), Sustainable Agriculture for Food, Energy and Industry I, James & James Science Publishers Ltd., London, 1998, pp. 484–489.

[25] M.J. Yang, X.E. Yang, V. Roemheld, Growth and nutrient composition of *Elsholtzia splendens* nakai under copper toxicity, J. Plant Nutr. 25 (2002) 1359–1375.

[26] H.Y. Peng, X.E. Yang, Volatile constituents in the flowers of *Elsholtzia argyi* and their variation: a possible utilization of plant resources after phytoremediation, J. Zhejiang Univ. Sci. 6B (2005) 91–95.

[27] L.Y. Jiang, X.E. Yang, W.Y. Shi, Z.Q. Ye, Z.L. He, Copper uptake and tolerance in two contrasting ecotypes of *Elsholtzia argyi*, J. Plant Nutr. 27 (2004) 2067–2083.

[28] D.A. Jiang, Experimental Guide for Plant Physiology, Chendu Science and Technological University Publisher, Chendu, China, 1999, pp. 39–42 (in Chinese).

[29] T.Q. Li, X.E. Yang, X.F. Jin, Z.L. He, P.J. Stoffella, Q.H. Hu, Root responses and metal accumulation in two contrasting ecotypes of *Sedum alfredii* Hance under lead and zinc toxic stress, J. Environ. Sci. Heal, Part A 40 (2005) 1081–1096.

[30] H.Y. Peng, S.K. Tian, X.E. Yang, Changes of root morphology and Pb uptake by two species of *Elsholtzia* under Pb toxicity, J. Zhejiang Univ. Sci. 6B (2005) 546–552.

[31] S.P. McGrath, S.J. Dunham, R.L. Correll, Potential for phytoextraction of zinc and cadmium from soils using hyperaccumulator plants, in: N. Terry, G. Banuelos (Eds.), Phytoremediation of Contaminated Soil and Water, Lewis Publishers, 2000, pp. 109–128.

[32] P.J. Coughtrey, M.H. Martin, Cadmium uptake and distribution in tolerant and nontolerant populations of *Holcus lanatus* grown in solution culture, Oikos 30 (1978) 555–560.

[33] T. Landberg, M. Greger, Differences in uptake and tolerance to heavy metal in *Salix* from unpolluted and polluted areas, Appl. Geochem. 11 (1996) 175–180.

[34] C.R. Pelacani, R.S. Barros, D.M. Ribeiro, R.B.C. Frigeri, Breaking dormancy of *Stylosanthes humilis* seeds with low pH solutions, Acta Physiol. Plant. 27 (2005) 383–393.

[35] C.A. Delatorre, R.S. Barros, Germination of dormant seeds of *Stylosanthes humilis* as related to heavy metal ions, Biol. Plant. 38 (1996) 269–274.

[36] R.J. Argel, L.R. Humphreys, Environment effects on seed development and hardseededness in *Stylosanthes hamata* cv. Verano. I. Temperature, Aust. J. Agric. Res. 34 (1983) (1983) 261–270.

[37] E.A. Jeliazkova, L.E. Craker, Seed germination of some medicinal and aromatic plants in a heavy metal environment, J. Herbs Spices Med. Plants 10 (2003) 105–112.

[38] G. Kumar, R.P. Singh, Sushila, Nitrate assimilation and biomass production in *Sesamum indicum* L. seedlings in a lead enriched environment, Water Air Soil Pollut. 66 (1993) 163–171.

[39] N. Bharti, R.P. Singh, Growth and nitrate reduction by *Sesamum indicum* cv. PB-I respond differentially to lead, Phytochemistry 33 (1993) 531–534.

[40] R.P. Singh, R. Maheshwari, S.K. Sinha, Recovery of lead caused decreased in biomass accumulation of mung bean (*Vigna radiata* L.) seedlings of K_2HPO_4 and $CaCl_2$, Indian J. Exp. Biol. 32 (1994) 507–510.

[41] R.P. Singh, N. Bharti, G. Kumar, Differential toxicity of heavy metals to growth and nitrate reductase activity of *Sesamum indicum* seedlings, Phytochemistry 35 (1994) 1153–1156.

[42] B. Gabara, E. Goaszewska, Calcium effect on mitotic activity and frequency of mitotic disturbances in the cortex cells of *Pisum sativum* L. roots treated with heavy metals, Bull. Pol. Acad. Sci. Biol. Sci. 40 (1992) 97–103.

[43] A. Kosobrukhov, I. Knyazeva, V. Mudrik, *Plantago major* plants responses to increase content of lead in soil: growth and photosynthesis, Plant Growth Regul. 42 (2004) 145–151.

[44] M. Wierzbicka, Lead in the apoplast of *Allium cepa* L. root tips: ultra structural studies, Plant Sci. 133 (1998) 105–119.

[45] R.D. Reeves, R.R. Brooks, Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe, Environ. Pollut. 31 (1983) (1983) 277–285.

[46] J.D. Joslin, G.S. Henderson, The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride, For. Sci. 30 (1984) 965–970.

[47] D.P. Knievel, Procedure for estimating ratio of live to dead root dry matter in root core samples, Crop Sci. 13 (1973) 124–126.

[48] B. Huang, R.R. Duncan, R.N. Carrow, Drought-resistance mechanisms of seven warm-season turf grasses under surface soil drying. II. Root aspects, Crop Sci. 37 (1997) 1863–1869.

[49] M.M. Lasat, A.J.M. Baker, M. Kochian, Physiological characterisation of root Zn^{2+} absorption and translocation to shoots in hyperaccumulator and non-hyperaccumulator species of *Thlaspi*, Plant. Physiol. 112 (1996) 1715–1722.

[50] D. Schwarz, R. Grosch, Influence of nutrient solution concentration and a root pathogen on tomato root growth and morphology, Sci. Hortic. 97 (2003) 109–120.

[51] Y.J. An, Y.M. Kim, T.I. Kwon, S.W. Jeong, Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation, Sci. Total Environ. 326 (2004) 85–93.

[52] R.P. Singh, R.D. Tripathi, S.K. Sinha, R. Maheshwari, H.S. Srivastava, Response of higher plants to lead contaminated environment, Chemosphere 34 (1997) 2467–2493.

[53] P. Carrier, A. Baryla, M. Havaux, Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil, Planta 216 (2003) 939–950.

[54] D.E. Salt, R.C. Prince, A.JM. Baker, I. Raskin, I.J. Pickering, Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy, Environ. Sci. Technol. 33 (1999) 713–717.

[55] I. Raskin, R.D. Smith, D.E. Salt, Phytoremediation of metals: using plants to remove pollutants from the environment, Curr. Opin. Biotechnol. 8 (1997) 221–226.

[56] E.V. Rudakova, K.D. Karakis, E.T. Sidorshina, The role of plant cell walls in the uptake and accumulation of metal ions, Fiziol. Biochim. Kult. Rast. 20 (1988) 3–12.

[57] R. Broyer, C.M. Johnson, R.E. Paull, Some aspects of lead in plant nutrition, Plant Soil 36 (1972) 301–313.