Journal of Hazardous Materials 186 (2011) 683–689

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Lead accumulation and tolerance characteristics of *Athyrium wardii* (Hook.) as a potential phytostabilizer

Tongjing Zou [a], Tingxuan Li [a,*], Xizhou Zhang [a], Haiying Yu [a], Hongbing Luo [b]

[a] College of Resources and Environmental Science, Sichuan Agricultural University, 46 Xinkang Road, Ya'an, Sichuan 625014, China
[b] College of Urban and Rural Construction, Sichuan Agricultural University, 288 Jianshe Road, DuJiangyan, Sichuan 611830, China

ARTICLE INFO

Article history:
Received 4 June 2010
Received in revised form
12 November 2010
Accepted 12 November 2010
Available online 20 November 2010

Keywords:
Lead phytostabilization
Athyrium wardii
Mining ecotypes
Tolerance
Biochemical response

ABSTRACT

Lead (Pb) pollution poses great threats to human health and can trigger serious environmental consequences. *Athyrium wardii* (Hook.), a new plant with the potential for phytostabilization of Pb, was identified in a field survey of plant species in a lead–zinc mine tailing located in Sichuan Province, China. The growth, Pb concentration and some physiological and biochemical characteristics of mining ecotypes (ME) and non-mining ecotypes (NME) were analyzed by pot experiment employing different concentrations of $Pb(NO_3)_2$ in tested soil during four weeks period. The results showed that the *A. wardii* has a higher tolerance to excessive levels of Pb soil contamination. The concentrations of Pb in the shoots and roots of the ME were 3.5 and 3.0 times higher, respectively, than those of the NME when plants were supplied with Pb at 800 mg Pb kg^{-1}. *A. wardii* of ME showed higher bio-activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and lower concentrations of malondialdehyde (MDA) and membrane permeability under higher Pb levels, while the opposite tendency was observed in NME. These findings demonstrated that the mining ecotype of *A. wardii* has the potential for phytostabilization of Pb contaminated soils.

1. Introduction

Heavy metal pollution of soils has become a widespread problem in recent years. Such pollution originates from continuous exploitation of mineral resources, electronic waste, sewage sludge, and widespread use of fertilizers, herbicides and pesticides [1,2]. According to the US Environmental Protection Agency (EPA), lead (Pb) is the most common heavy metal contaminant in the environment [3]. Lead pollution is a problem that cannot be neglected because it exists in many forms in natural sources and remains over long periods in soil with high toxicity levels, which adversely affects crop production and has the potential to cause human health problems [4].

Pb is known to cause cellular damage by inducing oxidative stress via the over-production of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (–OH) and hydrogen peroxide (H_2O_2), which react very rapidly with DNA, lipids and proteins, thereby causing cellular damage [5–7]. However, a number of autochthonous plant species in mining areas have the potential to survive and accumulate excessive amounts of Pb in their biomass without incurring damage to basic metabolic functions [8]. Therefore, phytoremediation has emerged as a viable biotechnology to decontaminate heavily polluted sites. The two main plant-based strategies of soil pollution remediation are phytoextraction and phytostabilization [9,10]. Previous studies have primarily focused on phytoextraction, which uses hyperaccumulator plants to absorb Pb into shoot tissue. Cropping and harvesting these shoots reduce the level of contaminants. These plant species have special mechanisms to cope with higher levels of Pb stress in their growth environment. To combat oxidative damage, these plants have a defense system composed of a variety of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which can neutralize, convert and scavenge ROS [5,11,12]. Free radicals and hydrogen peroxide have been reported to cause damage to membrane permeability, which is often related to lipid peroxidation. Malondialdehyde (MDA) is one of the decomposition products of polyunsaturated fatty acids of biomembranes. Cell membrane stability has been widely applied to study the effects of Pb stress on plants.

However, although heavy metals can be absorbed by hyperaccumulators, these plants are not suitable for every type of mining site. Mine tailings at unreclaimed mining sites generally remain unable to sustain plant life for tens to hundreds of years, and exposed tailings can spread over tens of hectares via aeolian dispersion and water erosion, which can pose particular threats to the health of people and plants [13,14]. Therefore, phytoextraction is not suit-

* Corresponding author. Tel.: +86 835 2882216; fax: +86 835 2882216.
E-mail address: litinx@263.net (T. Li).

0304-3894/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2010.11.053

able for such sites. Conversely, phytostabilization primarily focuses on sequestration of metals within the roots and rhizosphere [15]. This remediation strategy creates a vegetative cap for the long-term stabilization and containment of tailings. Plant canopies serve to reduce aeolian dispersion, while plant roots prevent water erosion, immobilize heavy metals by adsorption or accumulation, and provide a rhizosphere wherein metals precipitate and stabilize [16,17]. Consequently, phytostabilization has great practical significance and flexibility in the ecological restoration of mining tailings and remediation of soil polluted by heavy metals. However, there is little information available regarding the physiological and biochemical mechanisms of potential plants for Pb stabilization and tolerance.

Through a survey of plant species during 2007 and 2008, we identified a new ecotype, *A. wardii*, growing in lead–zinc mine tailings in the Sichuan Province of China. The Pb concentrations of shoot and root of other herbaceous plants were 41–285 mg kg^{-1} and 62–883 mg kg^{-1}, however, *A. wardii* was able to accumulate as much as 15,542 mg kg^{-1} in roots with a fast growth rate and relatively large amount of biomass. The objectives of this study were (1) to investigate the effects of Pb on the growth and uptake of *A. wardii* within two ecotypes and (2) to evaluate the effects of Pb on the physiological and biochemical characteristics of the ecotypes to enable a better understanding of the stabilization ability and tolerance of Pb.

2. Materials and methods

2.1. Field survey and sample collection

The surveyed area was located in the Sanhe Pb/Zn mine in Yingjing County, Ya'an (102°31′E, 29°47′N) City in Sichuan Province of China, which is located at an elevation of 1358–1445 m. The area has a subtropical moist monsoon climate with an average temperature of 15.3 °C and annual rainfall of over 1500 mm. This mine was in operation from the 1950s to 2003. The average content of lead in the mine tailings is about 7.62%. Field surveys were conducted from May to August at 2008 (during the entire growth period) to determine the potential for stabilizing lead. A contrasting ecotype in the normal agricultural soils from the Yucheng District of Ya'an (102°51′–103°12′E, 29°40′–30°14′N) City in Sichuan Province that had similar climatic and topographic conditions to the mining area was also identified.

A. wardii is a perennial herb. We found underground roots and tubers of *A. wardii* can survive many years, however, shoots changed in growth period of one year. Therefore, according to the changes of morphology and biomass of shoots, we distinguished the three growth stages at early growth stage (May), vigorous stage (August) and late growth stage (October). Two ecotypes of *A. wardii* were collected at the three growth stages. The shoots and roots were then washed and oven-dried at 70 °C for 48 h, after which the oven-dried samples were ground with a stainless steel grinder (FW-100, China) to be able to pass through a 100 mesh sieve. Next, plant samples were digested with HNO_3:$HClO_4$ (5:1, v/v) in closed Teflon vessels until the liquid was clear. The digested material was then washed into a 50 ml flask and diluted to volume using de-ionized water [18]. The Pb concentration in the solution was determined by flame atomic absorption spectrophotometry (Mk M6, Thermo elemental, USA). EPA publication SW-846 7420 is taken for standardizing the AAS for lead. The instrument working conditions were wavelength 283.3 nm, slit 0.7 nm, atomization 2000 °C, read time 3 s, sample volume 10 μL [19].

2.2. Pot experiment

2.2.1. Plant materials and lead treatments

Seedlings from the mining ecotype (ME) and non-mining ecotype (NME) were obtained from the Sanhe Pb/Zn mine tailings and uncontaminated agricultural soils in Ya'an, respectively, in June at 2009. The fern was separated into similar size plant segments composed of 8–9 fronds. Healthy plants of similar size were selected and cultured for two weeks in quartz sand with 1/10 Hoagland's solution, which was continuously watered every three days.

The pot experiment of *A. wardii* was conducted in plastic boxes (400 × 300 × 140 mm) filled with 7.5 kg of sterilized soil. A soil sample of Ochric Aquic Cambosol (1–10 cm) was collected in Daxing, Ya'an and then sieved (2 mm). The Pb treatments consisted of the addition of 0, 200, 400, 600, 800 and 1000 mg Pb kg^{-1} to soil supplied as $Pb(NO_3)_2$, with six replicates in each treatment. The control was the treatment without any Pb. To simulate the Pb impacted soils, pot soils were homogenized for four weeks with the additive Pb prior to the transplantation of seedlings. The basic characteristics of the soil after homogenization are presented in Table 1.

After growing for 14 days in quartz sand, the plants were transferred to the pots, which each received eight fronds. The pot experiment was conducted in a net house with transparent polythene sheets on the roof. The water-holding capacity of filled soil in plastic boxes was maintained at 70–80% by applying de-ionized water every three days during the plant growth.

2.2.2. Determination of Pb in soil and plants

After four weeks of growth, the vegetation of *A. wardii* was harvested and rinsed thoroughly with de-ionized water. The samples were then divided into above-ground (shoots) and below-ground (roots plus rhizomes) parts. The non-rhizosphere soil was collected from near the roots, and the tightly adhering rhizosphere soil was collected by brushing with a paintbrush [20].

The method of determining plant Pb concentrations was described in detail in Section 2.1. To determine the total Pb content, soil samples were digested in the same way with HNO_3:$HClO_4$:HF (5:1:1, v/v/v) [21]. The content of exchangeable Pb was measured by 1 g of soil with 1 M $MgCl_2$, the samples were shaken for 2 h then centrifugated at 3000 r min^{-1} for 30 min, extract supernatant fluid after filtrated [22]. Next, the concentrations of lead in the plants and soil were measured using a flame atomic absorption spectrophotometer (Mk M6, Thermo Elemental, USA). Available phosphorus concentration, available potassium concentration and available nitrogen were determined by molybdenum blue method,

Table 1
Basic physico-chemical characteristics of the tested soil after 4 weeks of homogenization.

Applied (mg Pb kg^{-1} soil)	Treatment	EPb (mg kg^{-1})	TPb (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	AN (mg kg^{-1})	OM (g kg^{-1})	pH
0	Ck	ND	35.34 ± 2.72f	13.96 ± 2.23	63.53	22.74	2.3	7.29
200	Pb200	2.34 ± 0.09e	196.49 ± 0.68e					
400	Pb400	7.39 ± 0.26d	393.78 ± 1.60d					
600	Pb600	13.33 ± 0.41c	601.29 ± 8.35c					
800	Pb800	18.01 ± 0.56b	827.59 ± 7.75b					
1000	Pb1000	35.31 ± 0.21a	1091.34 ± 16.78a					

Note: EPb: exchangeable Pb, TPb: total Pb, AP: available phosphorus, AK: available potassium, AN: available nitrogen and OM: organic mater. ND: Not detected. Different letters in each column indicate a significant difference ($p < 0.05$).

flame spectrophotometer and alkaline hydrolysis diffusion method, respectively. Organic matter of the soil was determined by potassium bichromate titrimetric method. The soil pH (1:2.5 soil to water, w/w) was measured with a conventional pH meter [21].

2.2.3. Other physical–chemical analyses

The fresh samples were quickly frozen in liquid nitrogen and stored at −80 °C (Thermo Freezer 700, USA). For the enzyme assays, 0.4–0.5 g of leaves were ground in 8 ml of ice-cold 50 mM phosphate buffer solution (pH 7.8) containing 0.2 mM Na_2-EDTA. The homogenate was then centrifuged at 4 °C for 20 min at 10,000 × *g* (model ALLEGRA-64R, Beckman Coulter. Inc., USA), and the resulting supernatants were used for determination of the enzyme activity.

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) [23]. The absorbance was recorded at 560 nm. One unit of SOD was defined as the amount in a volume of extract that caused inhibition of the photo-reduction of NBT by 50%. CAT activity was measured based on the decline in absorbance at 240 nm due to the decrease in the extinction of hydrogen peroxide. The reaction mixture contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml enzyme extracts. The reaction was started by adding H_2O_2. Analysis of the POD capacity was based on the oxidation of guaiacol using H_2O_2. The increase in absorbance was read at 420 nm [24] (model UN-2600A, UNICO).

Approximately 0.2 g of leaves were ground with a mortar and pestle and extracted in 80% acetone. The total chlorophyll a and chlorophyll b were estimated according to the procedure described by Lichtenthaler [25].

Lipid peroxidation products were measured using the method described by El-Moshaty et al. [26], and the lipid peroxides were expressed as nmol Malondialdehyde (MDA) (g FW)$^{-1}$. Membrane permeability was measured with the method described by Lutts et al. [27].

2.3. Statistical analyses

The Pb concentration and accumulation of the samples are presented for five individual replicates. Statistical analysis of all data was conducted by one-way ANOVA with LSD using the SPSS statistical software package (Version 11.0).

3. Results

3.1. A field survey of ecotypes of A. wardii in accumulating Pb

In the field survey, the soil from the mining area had a high Pb content. The total content of lead in this area was 268–9978 mg kg^{-1}, and the available content of lead was 56–274 mg kg^{-1}. Due to the high Pb content in the soils, the shoot biomass of ME was lower than that of NME (Table 2). Conversely, the root biomass of ME was 2.5 times higher than that of NME.

The concentrations of lead in roots and shoots of ME were 15,542.1 and 656.8 mg kg^{-1} during the early growth stage, respectively. Although the Pb concentrations in the shoots and roots of *A. wardii* decreased significantly ($p < 0.05$) with prolonged growth time, the concentrations of ME were 20–30 times and 235–445 times higher than those of the NME in the shoots and roots, respectively (Table 3). The average translocation factor was 0.04 at three growth stages.

Table 2
Biomass of two ecotypes of *A. wardii* during the three growth stages evaluated in the field survey (g).

Growth stage	Shoots		Roots	
	ME	NME	ME	NME
ES	1.1 ± 0.3bB	2.8 ± 0.3cB	2.2 ± 0.2cC	0.9 ± 0.2cB
VS	2.3 ± 0.3aA	5.5 ± 0.2aA	3.9 ± 0.3bB	2.1 ± 0.1aA
LS	2.6 ± 0.2aA	3.4 ± 0.3bB	5.2 ± 0.2aA	1.6 ± 0.1bA

Note: ES: early growth stage; VS: vigorous growth stage; LS: late growth stage. Different lowercase letters in each column indicate a significant difference at $p < 0.05$, different capital letters in each column indicate a significant difference at $p < 0.01$.

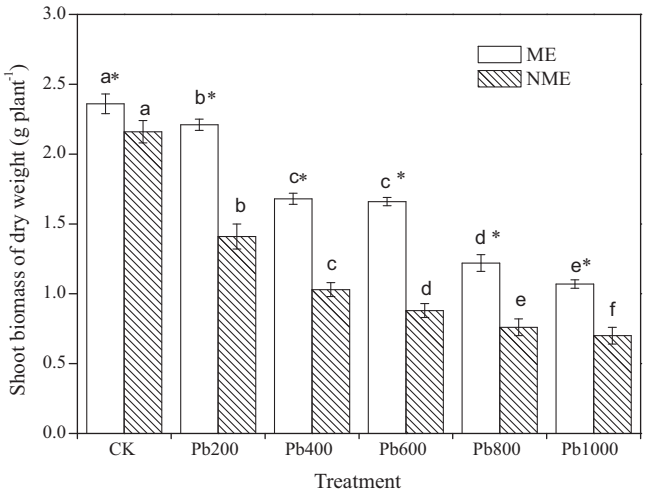

Fig. 1. Shoot biomass of the two ecotypes of *A. wardii* grown under different levels of Pb for 4 weeks. Data are mean ± SD of three individual replicates. Mean values labeled with different letters in each series are significantly different ($p < 0.05$) at different Pb concentrations of soil. *Significantly different ($p < 0.05$) at different ecotypes.

3.2. Effects of Pb treatment on growth and accumulation of A. wardii

In the pot experiment, a significant decrease ($p < 0.05$) in shoot biomass was observed in *A. wardii* with increasing Pb concentration in soil (Fig. 1). The biomass of NME declined by 35% when compared with the control, which was more greatly affected than that of ME when the Pb concentration was at 200 mg Pb kg^{-1} of soil. The shoot biomass of the NME and ME decreased by 55% and 68% at 1000 mg Pb kg^{-1}, respectively. The average biomass in ME was up to 1.5 times higher than that of NME.

Table 3
Pb concentration of two ecotypes of *A. wardii* during the three growth stages evaluated in the field survey (mg kg^{-1}).

Growth stage	Shoot		Root	
	ME	NME	ME	NME
ES	656.8 ± 6.0aA	24.9 ± 3.0aA	15542.1 ± 50.8aA	36.5 ± 2.4aA
VS	434.4 ± 6.9bB	14.5 ± 0.1bB	10720.1 ± 23.0bB	24.1 ± 0.4bB
LS	188.0 ± 2.8cC	9.5 ± 1.1cB	4132.2 ± 23.2cC	17.6 ± 0.4cB

Note: ES: early growth stage; VS: vigorous growth stage; LS: late growth stage. Different lowercase letters in each column indicate a significant difference at $p < 0.05$, different capital letters in each column indicate a significant difference at $p < 0.01$.

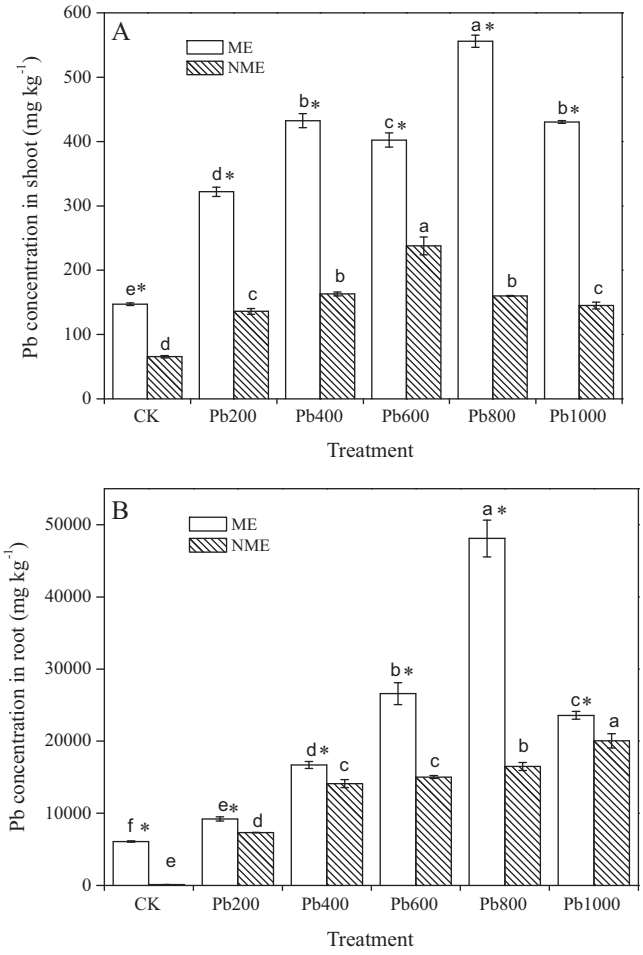

Fig. 2. Pb concentrations in the shoot (A) and root (B) of the two ecotypes of *A. wardii* grown under different levels of Pb for 4 weeks. Data are mean ± SD of three individual replicates. Mean values labeled with different letters in each series are significantly different ($p < 0.05$) at different Pb concentrations of soil. *Significantly different ($p < 0.05$) at different ecotypes.

Fig. 2 presents the Pb concentration of the two ecotypes of *A. wardii* grown under different Pb conditions for four weeks. Increasing Pb accumulation in *A. wardii* was observed with increasing soil Pb levels (Fig. 2). For ME, the Pb concentration in shoots and roots at 800 mg Pb kg^{-1} of soil was higher than that in other Pb treatments. The high Pb concentrations in shoots (556 mg kg^{-1}) and roots (48,110 mg kg^{-1}) increased by 278% and 717%, respectively, when compared with the control. For the NME, the Pb concentration in shoots was highest at 600 mg Pb kg^{-1}, after which it decreased. The concentration in NME roots increased with increasing Pb, with the Pb concentration being 169 times higher than that of the control and close to the concentration of ME at 1000 mg Pb kg^{-1} of soil. The Pb concentration of ME in the shoots and roots was 163% and 66% higher than that of NME on average under Pb stress conditions. At 800 mg Pb kg^{-1} of soil, the Pb concentration between ME and NME varied significantly.

3.3. Effects of Pb treatment on the biochemical characteristics of *A. wardii*

3.3.1. Concentration of chlorophyll a and chlorophyll b

Fig. 3 shows the concentration of chlorphyll a, chlorphyll b and chlorphyll a/b in the leaves of the two ecotypes of *A. wardii* grown under different levels of Pb for four weeks. The concentration of chlorophyll a of ME was significantly higher than that of NME. A

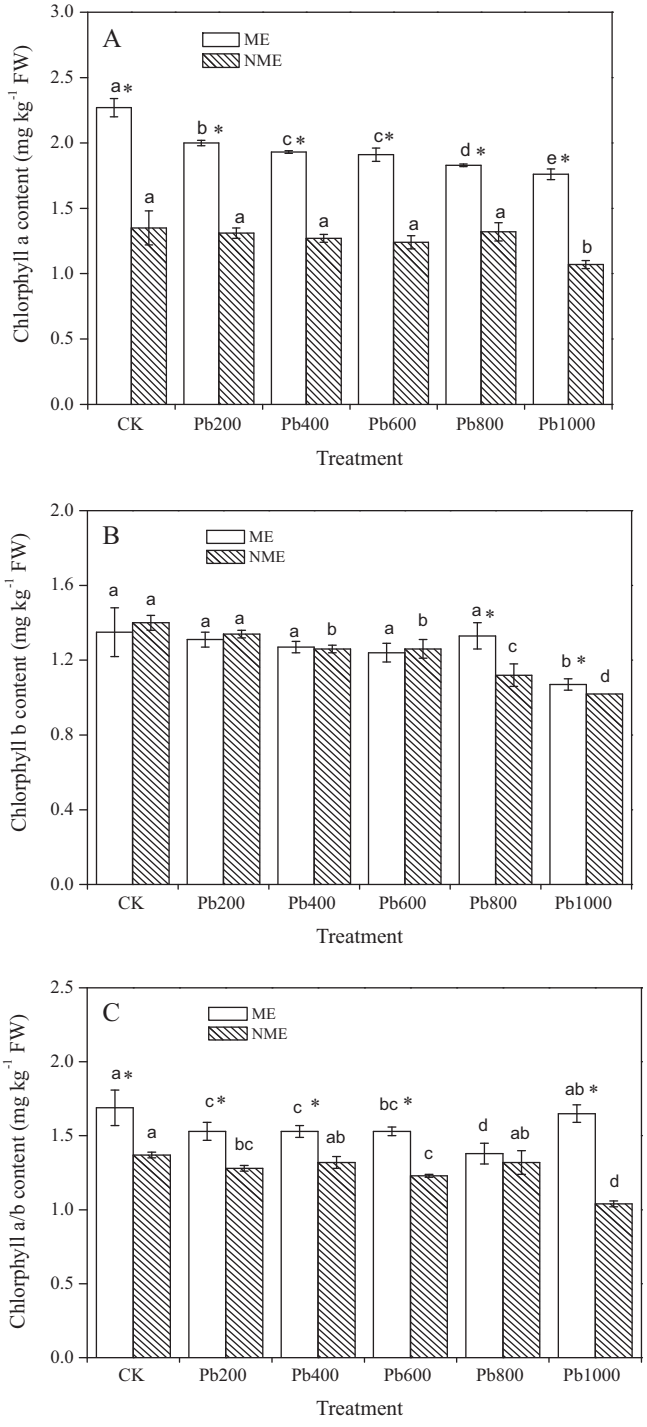

Fig. 3. Concentration of chlorphyll a (A), chlorphyll b (B) and chlorphyll a/b (C) in the leaves of the two ecotypes of *A. wardii* grown under different levels of Pb for 4 weeks. Data are mean ± SD of three individual replicates. Mean values labeled with different letters in each series are significantly different ($p < 0.05$) at different Pb concentrations of soil. *Significantly different ($p < 0.05$) at different ecotypes.

significant decrease ($p < 0.05$) in the concentration of chlorophyll a was observed in ME with increasing Pb concentration in soil, while the concentration of chlorophyll a in NME remained unchanged at 0–800 mg Pb kg^{-1} and then decreased by 21% when compared with the control. Similarly, the concentration of chlorophyll b of both ecotypes decreased in response to the Pb treatments, but the reduction in ME was greater than in NME in Fig. 3B.

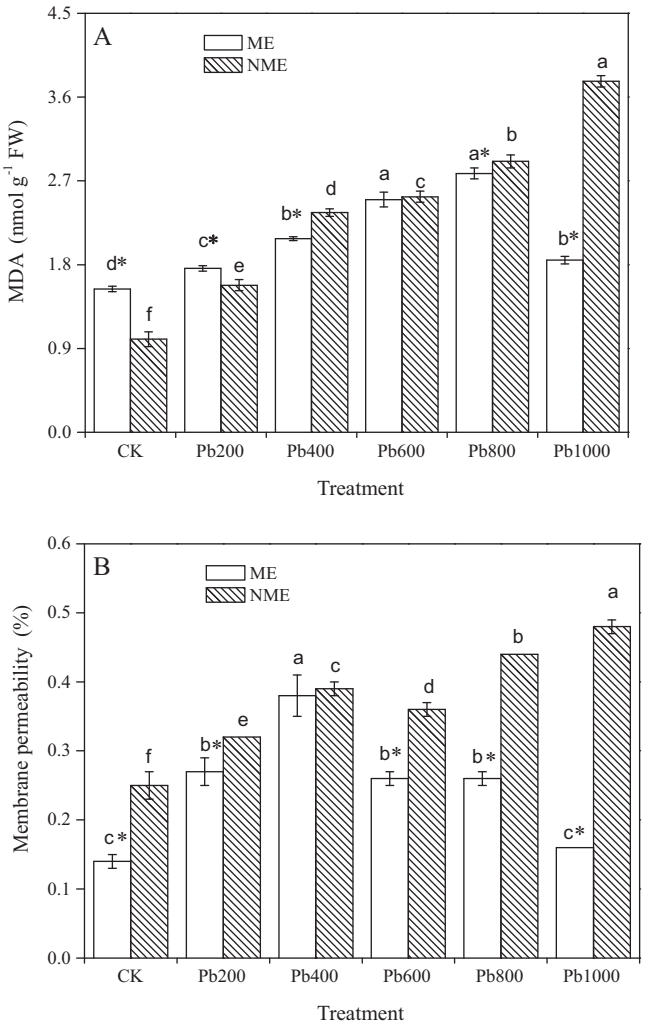

Fig. 4. Effects of Pb on the MDA content (A) and membrane permeability (B) in the leaves of the two ecotypes of *A. wardii* grown under different levels of Pb for 4 weeks. Data are mean ± SD of three individual replicates. Mean values labeled with different letters are significantly different ($p < 0.05$) at different Pb concentrations of soil. *Significantly different ($p < 0.05$) at different ecotypes.

The concentration of chlorophyll a/b of the ME was significantly higher than that of the NME (Fig. 3C). The concentration of chlorophyll a/b of the ME was lowest at 800 mg Pb kg^{-1}, while the differences between other treatments and the control changed little. Conversely, in NME, the concentration of chlorophyll a/b decreased by 24% at 1000 mg Pb kg^{-1}.

3.3.2. Lipid peroxidation products and membrane permeability

Fig. 4A and B shows that increasing concentrations of Pb in the growth medium caused lipid peroxidation and membrane damage in *A. wardii*. Lipid peroxidation in the leaves of both ecotypes was measured based on the MDA content (Fig. 4A). Significant differences in MDA were observed in both ecotypes with increasing concentrations of Pb in soil. The concentration of MDA in the leaves of the ME increased by 81% at 800 mg Pb kg^{-1}, and then decreased to 33%, which was close to the control level, in response to treatment with 1000 mg Pb kg^{-1}. A continuous increase in the concentration of MDA was observed in the leaves of the NME with increasing Pb levels, with an overall increase of 58–277% when compared with the control being observed.

As shown in Fig. 4B, the membrane permeability of both ecotypes showed a similar trend in the concentration of MDA. The membrane permeability of ME peaked at 400 mg Pb kg^{-1} and then decreased with high Pb stress. However, the membrane permeability of NME increased with increasing Pb treatment and was higher than that of the ME.

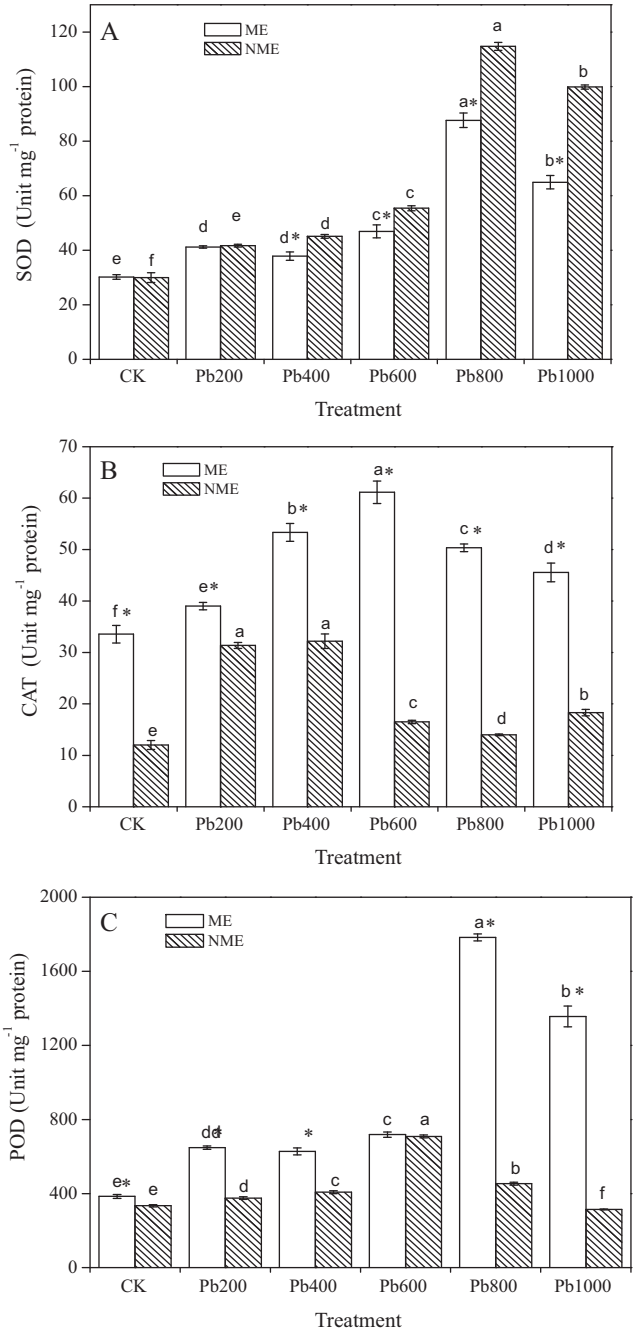

Fig. 5. Activity of antioxidative enzymes SOD (A), CAT (B) and POD (C) in the leaves of the two ecotypes of *A. wardii* grown under different levels of Pb for 4 weeks. Data are mean ± SD of three individual replicates. Mean values labeled with different letters are significantly different ($p < 0.05$) at different Pb concentrations of soil. *Significantly different ($p < 0.05$) at different ecotypes.

3.3.3. Antioxidant enzymes activities

As shown in Fig. 5A–C, Pb induced a strong antioxidative response in the two ecotypes of *A. wardii*. The activity of antioxidant enzymes followed patterns similar to the Pb concentration, with an increase with increasing soil Pb concentration followed by a decrease in response to high soil Pb treatments being observed (Fig. 5A). The SOD activity increased by 190% and 283% in the leaves of ME and NME at 800 mg Pb kg^{-1} in soil, respectively, when com-

pared with the control. The increasing percentage of SOD activity of ME was significantly higher than in NME, which was 1.3–53.8% under Pb stress conditions.

As shown in Fig. 5B, the CAT activity of the ME and NME increased to the maximum value at 400 mg Pb kg^{-1} and 600 mg Pb kg^{-1}, respectively, after which it decreased. The CAT activity of ME was about 24.3–148.6% higher than that of NME under Pb treatment. The trend of POD activity of both ecotypes was similar to the CAT activity (Fig. 5C). A significant increase in the activity of POD was observed in ME at 800 mg Pb kg^{-1}, with a value of 364% compared to the control and a value four times higher than that of NME being observed. The POD activity of the NME peaked at 600 mg Pb kg^{-1}, and significantly decreased to a level close to that of the control in response to the highest Pb treatment.

4. Discussion

A. wardii, which belongs to the Athyriaceae family, is a popular perennial plant and a type of fern that grows in fascicles. This fern has suitable features for phytostabilization, such as a well developed root system, high biomass and the ability to maintain a high content of Pb in its root tissue. Although there has been some research conducted to evaluate the use of plants such as *Lolium perenne* [28], *Atriplex lentiformis* [29], *Atriplex canescens* [30] and *Pistacia terebinthus Bieberstein* [31] for phytostabilization and revegetation of polluted soils, there has been little research on the phytostabilization of Pb polluted soil, which is difficult to reclaim. However, the results of the present study indicate that the ME of *A. wardii* may be suitable for stabilizing Pb.

The increase in lead concentration was greater in ME than in NME under stress conditions. In addition, an increasing uptake of Pb in both ecotypes was observed with increasing Pb concentration (Fig. 2). Specifically, the roots absorbed lead from soil and there was limited translocation to the shoots. It has also been shown that Pb is unevenly distributed in roots, where different root tissues act as barriers to apoplastic and symplastic Pb transport, thereby restricting Pb transport to the shoot [32]. In a survey of the three growth stages, the ME with the highest tolerance took-up the smallest proportion of total soil Pb and had the lowest Pb contents in the shoots. Additionally, both ecotypes of *A. wardii* in the pot experiment accumulated low concentration in the shoots, with shoot/root ratios of 0.01–0.03 and 0.007–0.020 being observed for ME and NME, respectively. Comparison of the Pb concentration of the two ecotypes revealed a decrease in the Pb concentration in the shoots after 800 mg kg^{-1} in ME and 600 mg kg^{-1} in NME. These findings indicated that ME had significantly higher tolerance than NME owing to the high induction of oxidative stress by Pb, which elevated the activity of antioxidative enzymes that may serve as important components of the antioxidative defense mechanism against oxidative injury. Due to the high Pb concentration in the roots of ME, our study indicated that the *A. wardii* of ME had the ability to uptake more Pb in the roots under high Pb stress conditions. Compare with Islam's study [2] that Pb concentration in the roots of ME was as high as 40,000 mg kg^{-1} and in the stem was 1720 mg kg^{-1} at a Pb level of 200 μM, our study showed that *A. wardii* may be suitable for phytostabilization since it had low Pb concentrations in the shoots and could stabilize Pb in the roots to prevent it from entering the food chain via water and wind erosion. Therefore, using tolerant plants that can accumulate metals in their root tissues or aid in their precipitation in the root zone is being encouraged for the phytostabilization of soils contaminated with high levels of metals from mining.

Pb stress in the present study resulted in a significant decrease in the shoot biomass of both ecotypes that were studied. In the pot experiment, a sharp decrease in the shoot biomass of both ecotypes was observed with increasing Pb treatment. These findings correspond to those of other published reports [2,33]. A possible explanation for the reduction of plant biomass in response to high Pb levels may be related to its high Pb accumulation. Ferns may use energy to cope with the high Pb concentration in the tissues [34]. The shoot biomass of ME was higher than that of NME with increasing soil Pb. This might suggest that the inhibiting effect on the plant growth of NME was more prominent. The ME showed better growth and tolerance to higher Pb concentration in soil.

Since photosynthesis is highly sensitive to Pb toxicity, the physiological characteristics of the leaves of *A. wardii* were investigated under different soil Pb concentrations. In this study, a decrease in chlorophyll a and chlorophyll b was observed as the soil Pb concentration increased, and the decrease was lower in ME than in NME. The strong relationship between Pb application and the decrease in photosynthesis of the entire plant was comparable to scenarios using *Ceratophyllum demersum* L. [35] and *Raphanus sativus* [4]. Reduction of the chlorophyll content may be attributed to reduced chlorophyll synthesis caused by Pb, impaired uptake of essential elements such as Mn and Fe, damage to the photosynthetic apparatus or chlorophyll degradation in response to increased chlorophyllase activity [36].

In many plant species, heavy metals have been reported to cause oxidative damage due to the production of ROS [37]. This study showed that Pb toxicity caused the production of lipid peroxides, and membrane damage and that it induced some key enzymes of the antioxidant defense system in *A. wardii*.

Lipid peroxidation and membrane permeability are important indices for determination of free radical mediated injury [17]. In this study, MDA content and membrane permeability increased significantly ($p < 0.05$) in the leaves of the NME with increasing Pb concentration, while the ME decreased in response to higher Pb levels. The reduction of MDA concentration and membrane permeability were due to increased antioxidative enzyme activities, which reduced the H_2O_2 levels and membrane damage. The MDA and membrane permeability in the NME was higher than that of the ME under Pb stress. These findings imply that the endurance capability of ME of *A. wardii* under high Pb stress was stronger than that of NME, and that it can rapidly regulate the antioxidative system.

Plants have evolved complex antioxidant systems to repair ROS induced damage. Generally, various antioxidant enzymes exhibit different responses, showing an increase in response to lower concentrations followed by a decline in response to higher stress, which was consistent with the results of this study.

As shown in Fig. 5A, increased SOD activity was observed in the leaves of both ecotypes in response to 800 mg Pb kg^{-1}. This was attributed to an increase in the superoxide radical concentration, which then decreased in response to the highest concentration of soil Pb. The reason for the decrease may have been inactivation of the enzyme by H_2O_2 [38] or binding of the metal to the active centers of the enzyme [39], in which case this decrease would indicate that the oxygen scavenging function of SOD was impaired. The SOD activity in the leaves of the ME was lower than that of NME under Pb stress, which was not consistent with the results of the study conducted by Islam et al. [2]. The activity of CAT and POD significantly increased ($p < 0.05$) in both ecotypes in response to low concentrations of Pb and declined in response to the highest concentration. The ME of *A. wardii* was able to maintain high levels of POD and CAT activities at higher concentrations of Pb. There was also a significant difference in the POD and CAT activities between ME and NME. These findings demonstrate the effectiveness with which the scavenging mechanism removed H_2O_2 from oxidative damage. Therefore, ME can be presumed to have a better system of antioxidant enzymes than NME.

5. Conclusion

To date, no plant species for phytostabilization of Pb have been reported in China, although some investigations have been conducted. In our survey of tailings from a Pb/Zn mine in Sichuan Province of China, a mining ecotype of *A. wardii* was found to accumulate 15,542 mg kg^{-1} Pb in its roots. Additionally, pot experiments in this study revealed that the response of ME was quite different from that of the NME. The extent of the decline in biomass of the NME was greater than that of the ME after Pb treatment. *A. wardii* had a higher Pb concentration in the roots of both ecotypes. Owing to the stronger activity of the antioxidant enzymes, high Pb stress caused less damage in the ME than the NME. The results of this study show that *A. wardii* can be a basic material which is suitable for phytostabilization strategy. Future studies will include a small-scale field experiment of *A. wardii* for Pb phytostabilization.

Acknowledgments

This work was financially supported by the Sichuan Youth Science & Technology Foundation (06ZQ026-020), the Youth Foundation of the Sichuan Education Bureau (2006B009), a key project grant from the Sichuan Education Bureau (2006A008), and the Open Research Fund Program of the Ministry of Education Key Laboratory of Polluted Environmental Remediation and Ecological Health, Zhejiang University, Huajiachi Campus, Hangzhou 310029, China. The authors are thankful to Dr. C. Hu (Sichuan Agriculture University) for his help in plant determinations, and to Dr. H.B. Luo, Dr. H. Huang and Dr. P. Huang for their critical comments regarding the language and construction of the manuscript.

References

[1] A.T. Ruley, N.C. Sharma, S.V. Sahi, S.R. Singh, K.S. Sajwan, Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in Sesbania drummondii grown in soil, Environ. Pollut. 144 (2005) 11–18.
[2] E. Islam, D. Liu, T. Li, X. Yang, X. Jin, Q. Mahmood, S. Tian, J. Li, Effect of toxicity of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*, J. Hazard. Mater. 154 (2007) 914–926.
[3] M.A. Watanabe, Phytoremediation on the brink of commercialization, Environ. Sci. Technol. 31 (1997) 182A–186A.
[4] R. Gopal, A.H. Rizvi, Excess lead alters growth, metabolism and translocation of certain nutrients in radish, Chemosphere 70 (2008) 1539–1544.
[5] F. Navari-Lazzo, M.F. Quartacci, Phytoremediation of metals. Tolerance mechanisms against oxidative stress, Miner. Biotechnol. 13 (2001) 73–83.
[6] K. Shaw, R.G. Kumar, S. Verma, R.S. Dubey, Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings, Plant Sci. 161 (2001) 1135–1144.
[7] A.M. Reddy, S.G. Kumar, G. Jyonthsnakumari, S. Thimmanaik, C. Sudhakar, Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.), Chemosphere 60 (2005) 97–104.
[8] S.D. Cunningham, W.R. Berti, J.W. Huang, Phytoremediation of contaminated soils, Trends Biotechnol. 13 (1995) 393–397.
[9] Q. Lin, I.A. Mendelssohn, The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands, Ecol. Eng. l0 (1998) 263–274.
[10] I. Alkorta, C. Garbisu, Phytoremediation of organic contaminants in soils, Bioresour. Technol. 79 (2001) 273–276.
[11] S. Srivastava, R.D. Tripathi, U.N. Dwivedi, Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in *Cuscuta reflexa* – an angiospermic parasite, J. Plant Physiol. 161 (2004) 665–674.
[12] F. Zhang, Y. Wang, Z. Lou, J. Dong, Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*), Chemosphere 67 (2007) 44–50.
[13] G.P. Cobb, K. Sands, M. Waters, B.G. Wixson, E. Dorward-King, Accumulation of metals by vegetables grown in mine wastes, Environ. Toxicol. Chem. 19 (2000) 600–607.
[14] R.C. González, M.C.A. González-Chávez, Metal accumulation in wild plants surrounding mining wastes, Environ. Pollut. 144 (2006) 84–92.
[15] M.O. Mendez, R.M. Maier, Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology, Environ. Health Perspect. 116 (2008) 278–283.
[16] A. Boularbah, C. Schwartz, G. Bitton, W. Aboudrar, A. Ouhammon, J.L. Morel, Heavy metal contamination from mining sites in South Morocco: assessment of metal accumulation and toxicity in plants, Chemosphere 63 (2006) 811–817.
[17] S. Claudia, V. Cesar, G. Rosanna, Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*, Sci. Total Environ. 395 (2008) 1–10.
[18] B. He, X. Yang, W. Ni, Y. Wei, X. Long, Z. Ye, Sedum alfredii: a new lead-accumulating ecotype, Acta Bot. Sin. 44 (11) (2002) 1365–1370.
[19] J. Szkoda, J. Żmudzki, Determination of lead and cadmium in biological material by graphite furnace atomic absorption spectrometry method, Bull. Vet. Inst. Pulawy 49 (2005) 89–92.
[20] L.X. Zhu, J.E. Zhang, W.G. Liu, Review of studies on interactions between root exudates and rhizopheric microorganisms, Ecol. Environ. 12 (2003) 102–105 (in Chinese).
[21] R.K. Lu, Analysis of Soil Agrochemistry, Chinese Agricultural Science and Technology Press, Beijing, 1999 (in Chinese).
[22] X. Xian, Effect of chemical forms of cadmium, zinc, and lead in polluted soils on their uptake by cabbage plants, Plant Soil 113 (1989) 257–264.
[23] K.V.M Rao, T.V.S. Sresty, Antioxidant parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) *Millspaugh*) in response to Zn and Ni stresses, Plant Sci. 157 (2000) 113–128.
[24] I. Cakmak, H. Marschner, Magnesium deficiency and high light intensity enhance activities of superoxide dismutase ascorbate peroxidase, and glutathione reductase in bean leaves, Plant Physiol. 98 (1992) 1222–1227.
[25] H.K. Lichtenthaler, Chlorophylls and Carotenoidspigments of Photosynthesis Biomembranes, Academic Press, New York, 1987.
[26] F.I.B. El-Moshaty, S.M. Pike, A.J. Novacky, O.P. Sehgal, Lipid peroxidation and superoxide production in cowpea (Vigna unguiculata) leaves infected with tobacco ringspot virus or southern bean mosaic virus, Physiol. Mol. Plant Pathol. 43 (1993) 109–119.
[27] S. Lutts, J.M. Kinet, J. Bouharmont, NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance, Ann. Bot. 78 (1996) 389–398.
[28] C. Santibáñez, C. Verdugo, R. Ginocchio, Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*, Sci. Total Environ. 395 (2008) 1–10.
[29] M.O. Mendez, E.P. Glenn, R.M. Maier, Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes, J. Environ. Qual. 36 (2007) 245–253.
[30] K. Rosario, S.L. Iverson, D.A. Henderson, S. Chartrand, C. McKeon, E.P. Glenn, Bacterial community changes during plant establishment at the San Pedro River mine tailings site, J. Environ. Qual. 36 (2007) 1249–1259.
[31] L. Johansson, C. Xydas, N. Messios, E. Stoltz, M. Greger, Growth and Cu accumulation by plants grown on Cu containing mine tailings in Cyprus, Appl. Geochem. 20 (2005) 101–107.
[32] P.M. Kopittke, C.J. Asher, R.A. Kopittke, N.W. Menzies, Toxic effects of Pb^{2+} on growth of cowpea (*Vigna unguiculata*), Environ. Pollut. 150 (2007) 280–287.
[33] X. Cao, L.Q. Ma, C. Tu, Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.), Environ. Pollut. 128 (2004) 317–325.
[34] S. Trivedi, L. Erdei, Effects of cadmium and lead on the accumulation of Ca^{2+} and K^+ and on the influx and translocation of K^+ in wheat of low and high K^+ status, Physiol. Plantarum 84 (1992) 94–100.
[35] S. Mishra, S. Srivastava, R.D. Tripathi, R. Kumar, C.S. Seth, D.K. Gupta, Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation, Chemosphere 65 (2006) 1027–1039.
[36] P. Sharma, R.S. Dubey, Lead toxicity in plants, Br. J. Plant Physiol. 17 (2005) 35–52.
[37] V. Dixit, V. Pandey, R. Shyam, Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum*), J. Exp. Bot. 52 (2001) 1101–1109.
[38] L.M. Sandalio, H.C. Dalurzo, M. Gómez, M.C. Romero-Puertas, L.A. del Río, Cadmium induces changes in the growth and oxidative metabolism of pea plants, J. Exp. Bot. 52 (2001) 2115–2126.
[39] R.A. Fatima, M. Ahmad, Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater, Sci. Total Environ. 346 (2004) 256–273.