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Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture

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Abstract

Soil contaminated with heavy metals cadmium (Cd) and lead (Pb) is hard to be remediated. Phytoremediation may be a feasible method to remove toxic metals from soil, but there are few suitable plants which can hyperaccumulate metals. In this study, Cd and Pb accumulation by four plants including sunflower (*Helianthus annuus* L.), mustard (*Brassica juncea* L.), alfalfa (*Medicago sativa* L.), ricinus (*Ricinus communis* L.) in hydroponic cultures was compared. Results showed that these plants could phytoextract heavy metals, the ability of accumulation differed with species, concentrations and categories of heavy metals. Values of BCF (bioconcentration factor) and TF (translocation factor) indicated that four species had dissimilar abilities of phytoextraction and transportation of heavy metals. Changes on the biomass of plants, pH and Eh at different treatments revealed that these four plants had distinct responses to Cd and Pb in cultures. Measurements should be taken to improve the phytoremediation of sites contaminated with heavy metals, such as pH and Eh regulations, and so forth.

Key words: phytoextraction; heavy metals; plants; cadmium; lead

Introduction

Heavy metal level of the biosphere has accelerated rapidly since the onset of the industrial revolution which poses major environmental problems, including the damaging land surface and cultivated land pollution (Gisbert *et al*., 2003). Unlike organic compounds, heavy metals can not be degraded, and the cleanup usually requires their removal. Thus remediation of sites contaminated with toxic metals is particularly challenging (Lasat, 2002). Current practice for remediating heavy metal-contaminated soils relies heavily on "dig-and-dump" or encapsulation, neither of which addresses the issue of decontamination of the soil (Mai *et al*., 2003). Some reported that activated carbon could be widely used for heavy metal removal, however, this could be expensive and there had been considerable interest in the use of other adsorbent materials, particularly biosorbents (Wase and Forster, 1997; Ajmal *et al*., 2000). Some other methods, such as soil washing, had an adverse effect on biological activity, soil structure and fertility (Pulford and Watson, 2003). Furthermore conventional cleanup technology is generally too costly, and often harmful to desirable soil properties (i.e., texture, organic matter) for the restoration of contaminated sites. More recently, increasing attention has been given to the development of a plant-based technology (phytoremediation) to remediate heavy metal contaminated soils (McGrath *et al*., 1993; Raskin *et al*., 1994; Chaney *et al*., 1997).

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless (Salt *et al*., 1998). The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a "green", sustainable process (Pulford and Watson, 2003). In general, the ideal plant species to remediate a heavy metal-contaminated soil should be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest (Ebbs and Kochian, 1997). Some evidences were provided that sunflower could phytoremediate soil polluted by Cd^{2+} in association with *Pseudomonas putida* (Cindy *et al*., 2006) and Pb-contaminated soil (Begonia, 1997); ricinus could accumulate Cd in pot experiment at the concentrations from 10 to 400 mg/kg during 60 d (Lu and He, 2005); Miller *et al*. (1995) reported that afalfa had ability of accumulating Cd in soils receiving high rates of sewage sludge (equivalent to 4.6 kg Cd/hm^2); and in the study of Begonia (1997), mustard was considered as a hyperaccumulator which had the most tolerant to lead. But few reports compared their abilities of phytoextracting heavy metals.

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Hyperaccumulators can be selected by growing in hydroponic culture contaminated with metals. The advantages of this method are short-period and maneuverable though there are some distinct characteristics between soil and liquid, and it is easy to observe changes in rhizosphere of plants. In addition, the success of phytoextraction mainly depends on the interaction between medium, metals, and plant (Lasat, 2002), characteristics in rhizosphere of plants are completely different and they can affect efficiency of phytoextration consequentially, thus, it is necessary to understand changes in rhizosphere of species when exposed to heavy metals.

The purpose of this paper was to evaluate the ability of bioaccumulation of Cd and Pb by four plants: sunflower (*Helianthus annuus* L.), mustard (*Brassica juncea* L.), alfalfa (*Medicago sativa* L.), ricinus (*Ricinus communis* L.) in hydroponic culture. Relationships of changes of pH, Eh and biomass of plants between phytoextraction were also monitored to supply some available information for thorough phytoremdiation of toxic metal polluted soil.

1 Materials and methods

1.1 Seed sources and preparation

Seeds of tested plants (sunflower, mustard, alfalfa, ricinus) were obtained from Shenyang University Key Laboratory of Environmental Engineering. Seeds were surface sterilized by immersion in 20% (v/v) commercial bleach and shaken at 144 r/min on an orbital shaker in sterile distilled water for 6 h. Then they were sown onto stainless place with aseptic gauze in incubator, the temperature and moisture were kept at 28°C and 60%, respectively. Seedlings grew at a length of 2 cm and then transplanted to hydroponic culture under sterile conditions.

1.2 Establishment of hydroponic cultures and growth condition

Plants were grown hydroponically to study their ability to accumulate and tolerate different concentrations of cadmium and lead. Seedlings of plants were placed through a perforation in a plastic platform in a 450-ml plastic jar containing 400 ml of Hoagland's solution (Hoagland and Arnon, 1938), so that the root was immersed in liquid medium and the shoot was above the platform. Sterility checks were conducted in preparation cultures simultaneously.

The heavy metal salts (reagent grade) used in this study included $CdCl_2 \cdot 2.5H_2O$, $Pb(NO_3)_2 \cdot H_2O$. The salts were separately diluted in deionized water and added into hydroponic plant culture respectively. Treatment were prepared at the concentrations of (1) control; (2) Cd 5, 10, 20 mg/L (following as Cd5, Cd10, Cd20, respectively); (3) Pb 50, 100, 200 mg/L (following as Pb50, Pb100, Pb200, respectively) and (4) Cd 20 mg/L + Pb 200 mg/L (following as Cd20+Pb200). All solutions were adjusted to pH 7.0–7.2.

These four plants were grown in half-strength modified Hoagland's solution in the first 1 week, and then cultures were changed to full Hoagland's solution. Plants were maintained at 25°C with a 16-h photoperiod in a green house and arranged in a completely randomized design. During 5 weeks, cultures were replaced every 4 d and supplied deionized water to maintain 400 ml in all treatments. Controls and treatments were in triplicates for analysis.

1.3 Analysis of biomass, pH, Eh, and contents of cadmium and lead

At the end of the experiment (5 weeks), each plant was harvested by clipping the shoot at the culture level. The roots and aerials were washed in dilute detergent solution, followed by several rinses in distilled water. All plant parts were dried in an oven at 70°C for 72 h, and the dry weights were recorded by electronic balance (the limit is 0.1 mg). Values of pH and Eh in hydroponic cultures of treatments were determined using a glass electrode and platinum electrode. Parts of plants including roots and aerials were digested, and the digestion was accomplished using an electric hot plate (Beijing) at 105°C for 15 min with 10 ml of concentrated HNO_3 (trace pure). Subsequently, the sample volume was adjusted to 20 ml with double deionized water and all sample extracts were analyzed using a flame atomic absorption spectroscopy (Spectra AA220, Varian).

Two bioconcentration factors, BCF (bioconcentration factor) and TF (translocation factor), as defined in Eqs.(1) and (2) which was computed from the treatments concentrations, will be used to discuss the results from this study.

$$\mathrm{BCF} = \frac{C_{\mathrm{plants}}}{C_{\mathrm{culture}}} \tag{1}$$

$$\mathrm{TF} = \frac{C_{\mathrm{aerial}}}{C_{\mathrm{root}}} \tag{2}$$

All the data obtained from this study were subjected to statistical analysis of variance (ANOVA). Differences at the $P < 0.05$ level were considered significant.

2 Results and discussion

2.1 Bioaccumulation of Cd and Pb by four plants

After 5 weeks, all these plants could uptake Cd and Pb dissolved in hydroponic culture (Fig.1). But every plant showed different ability of accumulating Cd or Pb.

The content of Cd in the S (sunflower) + Cd20 treatment was the highest (327.34 mg/kg) ($P < 0.05$); and the content of Cd was the lowest (42.56 mg/kg) in the R (ricinus) + Cd5 treatment ($P < 0.05$). There were no obvious differences between the accumulation of mustard and alfalfa. Meanwhile, quantities of Cd accumulated by plants increased with the increment of Cd concentrations. In the treatments of Pb, the contents of Pb in sunflower were 589.50 mg/kg, 703.21 mg/kg and 917.82 mg/kg in the treatments of Pb50, Pb100 and Pb200, respectively. Pb accumulation by mustard was the highest (835.54 mg/kg) at the concentration of 200 mg/L ($P < 0.05$). The enrichment of Pb uptaken by ricinus was the lowest ($P < 0.05$). The same as Cd treatments, accumulating of Pb by plants

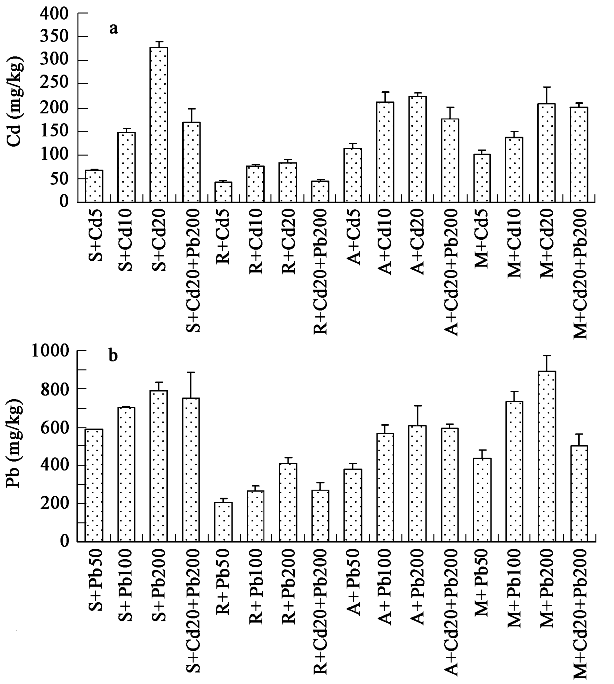

Fig. 1 Bioaccumulation of Cd and Pb by four plants in hydroponic culture. S: sunflower, R: ricinus; A: alfalfa, M: mustard.

increased with the increasing of contents of Pb. In addition, the present of Cd or Pb in cultures treated by Cd20+Pb200 showed inhibition effects to the accumulation of Pb or Cd when different species were used.

Over this experiment the abilities of enrichment by plants were different among species. Some reasons might contribute for the differences. First of all, the level of Cd in plants might be affected by several physiological factors of plants, including Cd uptake from the solution, xylem translocation from root to shoot, sequestration of Cd (in subcellar compartments or as organic complexes) (Hart *et al*., 1998). Bioaccumulation depends not only on the characteristics of the organism itself, but also on the characteristics of the substance and the environment factors.

In this experiment, heavy metals used had particular toxicity to plants, as a result that plants showed dissimilar response to Cd or Pb. Mohan and Hosetti (1997) considered that there were many enzymes involved in RNA, DNA and protein metabolism. Therefore, cadmium or lead posed deleterious effects on much of the biochemical machinery required for cell survival. Cadmium or lead had numerous sites of action within plants. It is more likely that accumulation will be associated with a mechanism that sequestering it in a less toxic form, the mechanism of heavy metals accumulation and the response of plants to these toxic metals are quite complex and can not be explained without throughout investigation.

In general, Cd has more toxic to plants than Pb, the damage to plants made by Cd may affect the growth of plants and tolerance to heavy metals much more than Pb. Thus, these four plants in this experiment accumulated more Pb than Cd.

Also, concentrations of heavy metals in cultures could influence the abilities of phytoextraction. Sunflower, ricinus, alfalfa, and mustard used in this experiment showed distinct accumulation at different concentrations, higher concentration maybe increase the content of heavy metals in tissue compartments of plants, at the same time; it could make damage to the growth of plants, and decreased accumulation of metals conversely, this result agreed with Lu and He (2005) who reported that *R. communis* could bioaccumulate Cd in soil at concentrations ranged from 10 to 400 mg/kg. Growth of ricinus began to be slow and inhibited at concentrations beyond 40 mg/kg. Bioaccumulation reached the maximum (4460.3 mg/kg) at the concentration of 360 mg/kg but 400 mg/kg, it meant that higher concentration affected the metabolism of ricinus greatly, which weaken the accumulation ability. However, Han *et al*. (2005) considered that accumulation of Cd, Cr and Pb by *Vetiveria zizanioides* was negative correlated with concentrations. That may be a result of different response or tolerance of species to metals kinds and concentrations.

The present of one ion could decrease extraction of the other ion in the hydroponic cultures in this experiment. This result agreed with Mohan and Hosetti (1997), who observed that toxic ion (Cd^{2+}) inhibited uptake of other metal ions (Cu^{2+} and Pb^{2+}) by roots in pot experiment. Whereas, Lin *et al*. (2000) reported that 5 mg/kg Cd could improve the accumulation of Pb; 10 mg/kg Cd could active Pb in soil when grown with wheat. In addition, Chakravarty and Srivastava (1997) noted that the interaction of Zn and Cd at equimolar concentration could overcome the toxicity of cadmium to linseed (*Linum usitatissimum*). It can be seen that accumulation of complexes of heavy metals by plants depends on plant species, kinds or concentrations of metals.

2.2 BCF and TF of plants in Cd and Pb treatments

BCF values were studied in each treatment (Fig.2). In Cd treatments, BCF values of alfalfa and mustard were the highest in Cd5 treatments (22.67, 20.38, respectively) ($P < 0.05$), which meant that they had better ability of bioaccumulation of Cd than sunflower and ricinus. BCF values of all plants decreased with the increasing of the concentration of Cd except sunflower. The values of TF (1.27) showed that there was much more Cd moved into aerial of ricinus in the treatment of Cd5 than others. And TF of plants tended to decrease with the increasing of Cd. In treatments of Pb, values of BCF decreased with increasing of the concentrations. Among these plants tested, sunflower had the highest BCF (11.79) in the Pb50 treatment ($P < 0.05$). It was apparent that sunflower had better ability of bioaccumulating Pb in Hydroponic culture than other three plants when the concentration was relatively lower. The BCFs of these plants in Cd20 + Pb200 were the lowest among all treatments. The TF value in M (mustard) + Pb200 treatment was 1.32, higher than the others. In treatments by ricinus, TF values showed that ricinus tended to transfer more Pb to leaves and stems than others when Pb concentrations increased.

Values of BCF can be an index to estimate a plant's

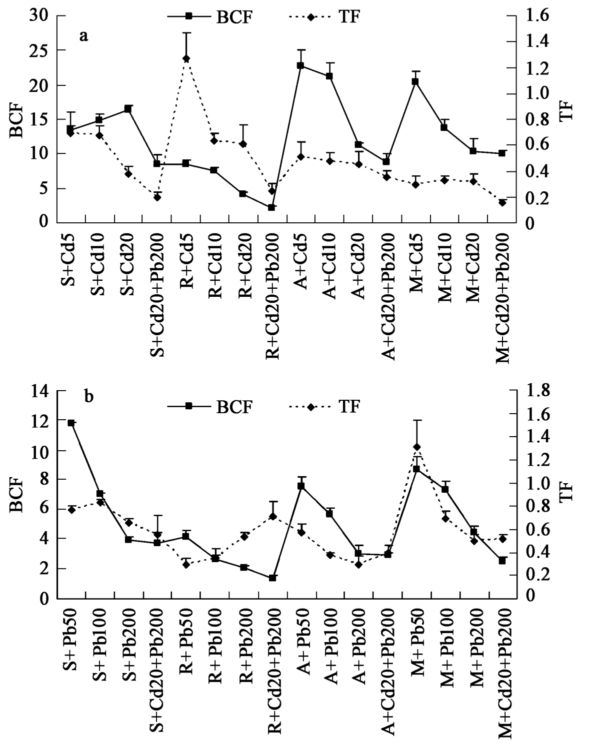

Fig. 2 Values of BCF and TF of plants in Cd and Pb treatments. S: sunflower; R: ricinus; A: alfalfa; M: mustard.

ability of accumulating heavy metals according to the bioconcentration. BCF values differed with concentrations and kinds of heavy metals, the accumulation ability and physiological factors of plants, and environmental conditions. Mattina *et al*. (2003) studied BCF values of lettuce, pump TT, zucchini, cucumber, tomato, thistle, lupin and spinach in soil contaminated with chlordane, Pb, Zn, Cd and As respectively. The results showed that these plants expressed different BCF values when exposed to different heavy metals; In this experiment, the plant, such as sunflower, could uptake much heavy metals, but BCF values were not the highest, this may be due to the physiological and morphology characteristics of sunflower, concentrations of Cd or Pb and conditions of cultures.

TF values can describe movement and distribution of heavy metals in plants. Transport across root cellular membrane is an important process which initiates metal absorption into plant tissues. TF values of plants depend on many factors. Hart *et al*., (1998) studied translocation of Cd from root to shoot in several species, including ryegrass, tomato, bean, maize and durum wheat. Movement of Cd from roots to shoots was likely to occur via the xylem and to be driven by transpiration from the leaves. An explanation for translocation of heavy metals in plants was given by Salt *et al*., (1995), who said that ABA-induced stormatal closure dramatically reduced Cd or Pb accumulation in shoots of Indian mustard. Cellular sequestration of Cd or Pb can have a large effect on the levels of free Cd or Pb in the symplast and, thus, can potentially influence movement of Cd or Pb throughout the plant. One recent study of heavy metals translocation into peanut fruits provided evidence that accumulation occurred predominantly via the phloem (Popelka *et al*., 1996).

2.3 Biomass and accumulation

The results obtained from this experiment showed that sunflower had the highest biomass (6.32 g) in Pb50 treatment ($P < 0.05$), while the mustard biomass was the smallest in Cd20 + Pb200 ($P < 0.05$). The biomass of these plants showed different correlations with concentrations of Cd or Pb (Table 1). In the treatments of Cd20 + Pb200, biomass of plants was less than other treatments ($P < 0.05$), which showed that combination of Cd and Pb was more harmful to growth of plants than Cd or Pb alone (Fig.3).

Biomass can express the tolerance of plants to toxic metals indirectly. But most of metal hyperaccumulators were small and slow growing (Lasat, 2002). High biomass plant species were better suited for phytoremediation of metal-contaminated soils (Papoyan and Kochian, 2004). In this test, biomass of sunflower was higher than other plants after 5 weeks growth, which resulted in that total Cd or Pb in sunflower was more than others. So, it can be applied to phytoextract Cd or Pb from heavy metals contaminated soil *in situ*. Generally, biomass of plant decreases with increment of toxic metals in this experiment. High concentration of phytotoxic metals may be more harmful to tissues of plant than low. Toxic metals ions interfere

Table 1 Correlation coefficients between biomass and Cd or Pb concentrations

Biomass	Cd concentration		Pb concentration	
	r	*P*	*r*	*P*
Sunflower	–0.385	0.157	–0.768**	<0.01
Ricinus	–0.571*	0.026	–0.878**	<0.01
Alfalfa	–0.711**	<0.01	–0.888**	<0.01
Mustard	–0.461	0.084	–0.721**	<0.01

Note: *and **represent significant level at $P < 0.05$ and $P < 0.01$, respectively.

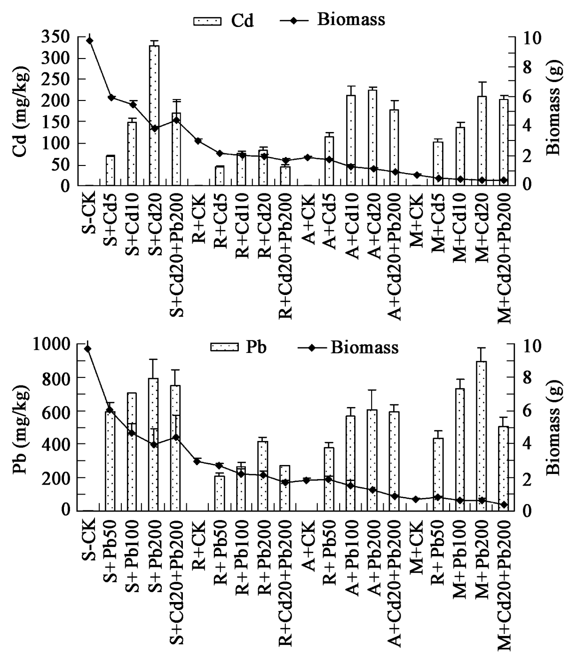

Fig. 3 Relationships between biomass and accumulating of plants. S: sunflower; R: ricinus; A: alfalfa; M: mustard.

with respiratory carbohydrate metabolism in plant cells, probably by substituting irreversibly for another micronutrient in critical enzymes. It also inhibits the formation of chlorophyll by interfering with protochlorophyllide reduction and the synthesis of aminoevulinic acid (Stobart *et al*., 1985). Habash *et al*. (1995) showed that heavy metals might interfere with different steps of the Calvin cycle, resulting in the inhibition of photosynthetic CO_2 fixation. While the study of Lu and He (2005) showed that low concentration of Cd appeared to improve the growth of *R. communis.* Liu and Wang (2002) considered that low concentration of Cu ($\leqslant$ 80 mg/L) could improve the growth of wheat, and high Cu concentration (> 80 mg/L) inhibited germination and seedling growth of wheat. This may be decided by characteristics of plants and heavy metals.

2.4 pH and Eh in hydroponic cultures

After 5 weeks, values of pH and Eh were tested (Fig.4). pH values in treatments of Cd fluctuated from 6.94 to 8.65; the highest pH appeared in Cd5 treated by mustard. But those in treatments of Pb were almost below 7.00, the lowest pH occurred in the treatment Cd20+Pb200 treated by mustard. Data of Eh showed an opposite trend to pH and differed with species. The values of Eh ranged from 141.33 mV to 222.67 mV with the highest values found in M (mustard)+Pb50 treatment and the lowest values in the R (ricinus)+Cd10, respectively.

The distribution of heavy metal is greatly affected by rhizosphere properties such as pH, Eh, organic matter and so on. pH has been regarded as a master variable regulating the mobility of metals (Lim *et al*., 2002). Ervio (1991) found that the most important factor for the uptake of cationic heavy metals by plants is the pH of the soil in which they are growing. In this experiment, pH values increased in Cd treatments; Wang (1991) indicated that the increment of pH could increase the content of exchangeable Cd in soil, that would make Cd more bioavailable, and easy to be phytoextracted by plants. While another study showed that when high levels of sulfur were added the pH dropped significantly, at pH 5 to 5.5 optimum plant growth was achieved, and was accompanied by significantly increased levels of cadmium uptake (Tichy *et al*., 1997). Generally speaking, the soluble plus exchangeable metals increase with the decrease in soil pH (Chen *et al*., 2000). In Pb treatments pH values decreased to make plant uptake metals effectively. Acidification of the rhizosphere could activate some heavy metals (Cao *et al*., 2003). The difference of pH values might be due to the strategies of plants to respond to heavy metals, when exposed to toxic metals, root of plants secreted a diverse array of compound (Prasad, 1995), which could make some complexes with metals, the complexes and exudates changed pH in cultures, and made toxic metals more bioavailable to plants. A study showed that low molecular weight organic acids released from plants might change pH in soil and impact the mechanism of pollutant uptake and transfer (Yang *et al*., 2003).

Eh could change the forms of heavy metal or immobilize heavy metals reducing their toxicity (Shuman and Wang, 1997). Redox potential was mainly influenced by organic matters in cultures. To avoid toxicity, plants had been documented to catalyze redox reactions and alter the chemistry of metal ions (Lasat, 2002). For example, Lytle *et al*. (1998) showed that from a solution supplemented with toxic Cr^{6+}, water hyacinth (*Eichornia crassipes*) accumulated nontoxic Cr^{3+} in root and shoots by changing redox potential in rhizosphere; Chuan *et al*. (1996) said that when solubilities were compared under the same pH values, it was observed that metal solubilities increased as redox potential decreased. Plants may have mechanism to decrease the toxicity of metals to plants by changing factors of their growing condition such as pH and Eh. In addition, values obtained from this experiment showed negative correlation with pH (r = –0.861, P < 0.01), which

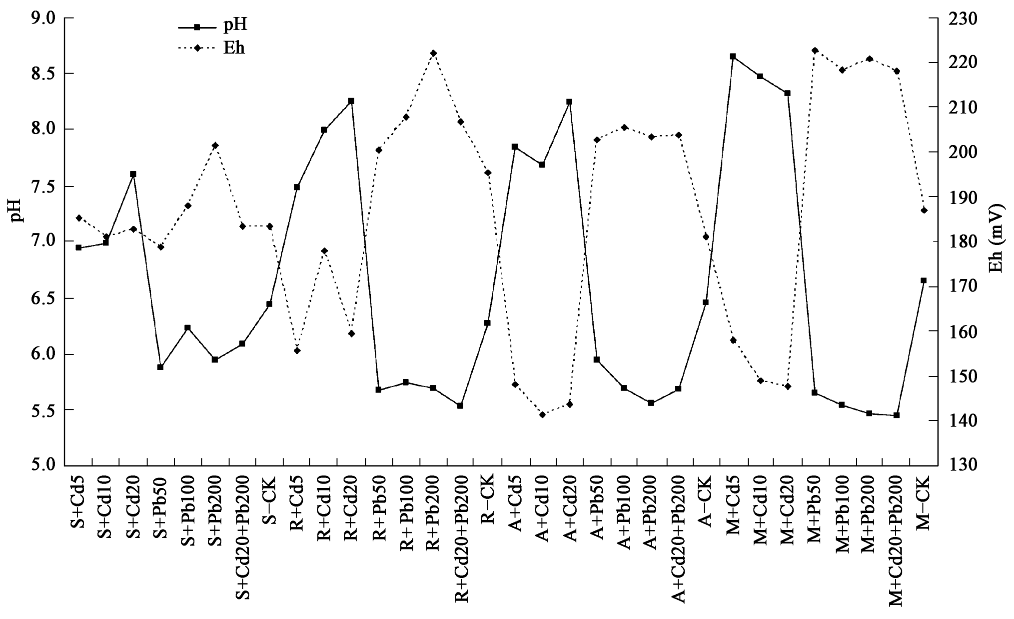

Fig. 4 Comparison of pH and Eh in hydroponic cultures after 5 weeks treatment. S: sunflower; R: ricinus; A: alfalfa; M: mustard.

was a result of root exudates of plant. Organic acids and other compounds excreted by roots could improve the condition of redox and pH, for example, organic ligands could induce changes in soil pH and Eh, solubilization of solid bound Cd may take place as pH and Eh decreases and increases respectively (Collins *et al*., 2003). Moreover, these compounds maybe change the forms of metals, Andrade *et al*. (2004) reported that the lowering of Eh would have contributed to these heavy metals being found almost exclusively in insoluble forms, through the study of Cr, Cu, Ni, Pb, and V in Galician coastal sediments, and to dissolve heavy metals was the key step to the bioavailability of heavy metals to plants.

3 Conclusions

These four plants could phytoextract Cd and Pb in hydroponic cultures, and sunflower showed better ability of accumulation than the others. The ability of accumulation differed with species, concentrations and categories of heavy metals. Therefore, it is necessary to find ideal plants with high biomass which can accumulate toxic metals efficiently according to characteristics of pollutants, plants, and environment conditions. Changes in rhizosphere of plants can affect efficiency of bioaccumulation greatly, thus measurements, including pH and Eh regulations, should be taken to improve the phytoremediation of sites contaminated with heavy metals.

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