

Full Length Research Paper

Lead tolerance and detoxification mechanism of *Chlorophytum comosum*

Youbao Wang*, Jiemin Tao and Jie Dai

College of Life Sciences, Anhui Normal University, No. 1 Beijing East Road, Wuhu, 241000, Anhui Province, People's Republic of China

Accepted 26 August, 2011

Chlorophytum comosum seedlings were grown in soils containing different lead (Pb) concentrations (CK, 200, 400, 600, 800, 1000, 1250, 1500 and 2000 mg/kg-dry soil) for three months and the effects on the growth, physiological indexes and the accumulation of *C. comosum* were examined. The results show that the tolerance index (TI) of *C. comosum* were all above 100 in soils containing 500 mg/kg or lower Pb. Malondialdehyde (MDA) level and electrical conductivity (EC) of *C. comosum* differed insignificantly with rising Pb concentration. The activities of catalase (CAT) and peroxidase (POD) increased significantly in lower Pb concentration and reached their maximum at 1000 and 250 mg/kg, respectively. In addition, *C. comosum* plant could maintain a lower Pb level in vivo in soils containing higher Pb concentration. These data indicate that *C. comosum* is surprisingly tolerant to Pb stress.

Key words: *Chlorophytum comosum*, lead, accumulation, tolerance, physiological index.

INTRODUCTION

With the rapid development of industries, soil heavy metal pollution becomes an increasingly important issue worldwide. Among various toxic metals, lead (Pb) which interferes with many physiological processes was considered as the most phytotoxic agent to plants and human beings (Poskuta et al., 1996; Bradshaw, 1997; Xia, 2004). Pb pollution had drawn international attention and it is urgent to develop methods to clean up Pb-contaminated soils. As compared to physical and chemical remediation, phytoremediation is preferred because of safety and lower cost (Raskin et al., 1994; Wong, 2003; Singer et al., 2007). Phytoremediation of heavy metal-contaminated soils basically includes phytoextraction and phytostabilization (Vangronsveld et al., 1995; Salt and Krämer, 2000; Dahmani-Müller et al., 2000). The normal phytoremediation practice is to choose metal-tolerant and fast-growing plants with high biomass that can survive in metal-contaminated and nutrient deficient soils. Plant species suitable for revegetation of mine tailings should have evolved biological mechanisms

substrates (Tordoff et al., 2000; Whiting et al., 2004).

Nowadays, ornamental plants have become a new source of phytoremediation because they are not only used for landscaping but also have practical applications in air pollution monitoring and control (Hemdez et al., 2005). Consideration of ornamental plant in the reclamation of heavy metal polluted soil has important practical significance (Zhou, 2006). *Chlorophytum comosum* (Thunb.) Baker is an evergreen horticultural plant native to southern Africa, which is characterized by high biomass, easy cultivation, intense competitive ability, high tolerance to heavy metal stress and wide geographic distribution (Bai et al., 2010; Wang et al., 2011). As a popular horticultural plant, *C. comosum* has high economic benefits and when they are removed, the roots, a main part containing much heavy metal, are also moved from the soil, thereby avoiding secondary pollution. The heavy metal accumulated will not be transferred to the food chain and consequently reduce the risk to human health. The objectives of this current research were to investigate the effects of Pb on the growth and Pb accumulation, and to explore the tolerance mechanisms of *C. comosum* to Pb, and to provide scientific basis for the reclamation of Pb polluted soil.

*Corresponding author. E-mail: wyzbl@tom.com. Tel: +86-553-3869193.

Table 1. Effect of Pb on *C. comosum* growth matters.

Pb stress (mg/kg)	Root length (cm)	Length of aboveground part (cm)	Root volume (cm ³)	Fresh weight (g)	TI ^a (%)
CK	13.21±0.68ab ^b	21.53±1.10a	2.71±0.49b	5.37±0.52ab	100ab
250	13.31±0.58ab	22.39±1.92a	2.95±0.37b	5.37±0.49ab	100.75ab
500	15.49±0.33a	22.51±2.10a	3.01±0.17b	5.93±0.14a	109.69a
750	11.29±4.09b	23.89±1.30a	4.87±1.00a	5.72±1.67a	85.46b
1000	10.23±1.54bc	24.43±2.24a	5.15±0.58a	4.87±1.11b	74.18bc
1250	9.86±2.46bc	17.20±1.64b	2.92±0.91b	3.92±1.26c	77.44bc
1500	9.64±2.08bc	12.87±1.90c	2.43±1.10b	4.80±0.90b	72.98bc
2000	6.46±1.65c	11.33±1.68c	1.57±0.54c	3.53±0.77c	48.90c

a TI: Tolerance index: the average root length of experimental group to that of control × 100. ^b: Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test.

MATERIALS AND METHODS

Experimental setup

C. comosum seedlings with aerial root were collected from the same matrix plant and were used in the experiment after one-week acclimation at room temperature in the laboratory. The test soil is yellow brown soil collected from Zheshan located in Wuhu, Anhui province, China. The pH value, electrical conductivity (EC), total amount of N, P, K and organic matter (OM) are 5.33, 101 $\mu\text{S}/\text{cm}$, 1.55, 2.06, 9.69 and 25.55 mg/kg, respectively. The soil was homogenized, screened with a 3 mm-sieve and air dried. About 250 g test soil was filled in each plastic pot ($\Phi = 12.5$ cm). Pb was applied as $\text{Pb}(\text{NO}_3)_2$ to each pot and the eight treatment concentrations including control were CK (a soil sample without exogenous Pb), 250, 500, 750, 1000, 1250, 1500 and 2000 mg/kg of dried soil. After equilibration for two weeks at room temperature, two seedlings were transplanted to each pot. The soil moisture was maintained at 80% of WHC. Each treatment had three replicates.

Sampling and analysis

About 90 days later, *C. comosum* was carefully uprooted and washed with distilled water. Roots and aboveground parts were cut with scissors, and their length and fresh weight were determined. The tolerance index was calculated. The samples were dried at 70°C for 48 h until constant weight. The physiological indicators of fresh leaves were measured, and the contents of photosynthetic pigment were determined spectrophotometrically. EC was measured with conductivity meter (DDS-11A, Shanghai) and MDA content was determined according to Yan et al. (1997). POD were determined by guaiacol colorimetric method and CAT were measured by the decrease of absorbance at 240 nm (Wu and Tiedemann, 2002). All enzyme activities were calculated on the base of fresh weight (FW).

The physical and chemical properties of the soil samples were determined according to the Environmental Monitoring Center of China (1992). The plant samples were dried with oven for half an hour at 105°C and then stood over night at 75°C until constant weight. The dried samples were digested in mixed acids ($\text{HNO}_3 : \text{HClO}_4 : \text{H}_2\text{SO}_4 = 8:1:1$). Pb content was analyzed with an atomic absorption spectrophotometer (AA6800, Shimadzu, Japan) (Environmental Monitoring of China 1992). To avoid cross-

contamination, all containers were washed in 2% HNO_3 for more than 24 h before used.

Data treatment

Data were analyzed by the SPSS 17.0 software package and Microsoft Excel. All measurements were replicated three times. Average values and standard deviations (S.D.) were calculated by the Microsoft Office Excel 2003.

TI (Tolerance index) (%) = the average root length of experimental group × 100 / the average root length of control group (Rout et al., 1999).

BC (Bioaccumulation coefficient) = the Pb concentration in plant / the Pb concentration in soil (Tanhan et al. 2007).

TF (Translocation factor) = the Pb concentration in aboveground parts / the Pb concentration in roots (Baker et al., 1994).

RESULTS AND DISCUSSION

Effects of Pb on growth of *C. comosum*

During the 90-day treatment, lead affected markedly the growth of *C. comosum* seedlings (Table 1). The morphological features were quantitatively confirmed by measuring the length of roots and aboveground parts, root volumes and fresh weights (FW). These physiological indexes all increased significantly at lower Pb concentration. At 250 and 500 mg/kg Pb concentrations, the length of roots and fresh weights increased as compared to the controls, and both reached their maximum at 500 mg/kg Pb concentration, which were 1.17 and 1.10 times higher than the control, respectively. The length of leaves and root volumes reached their peak values in 1000 mg/kg, which were 1.13 and 1.90 times higher than the control, respectively. In addition, at 2000 mg/kg Pb concentration, the parameters mentioned

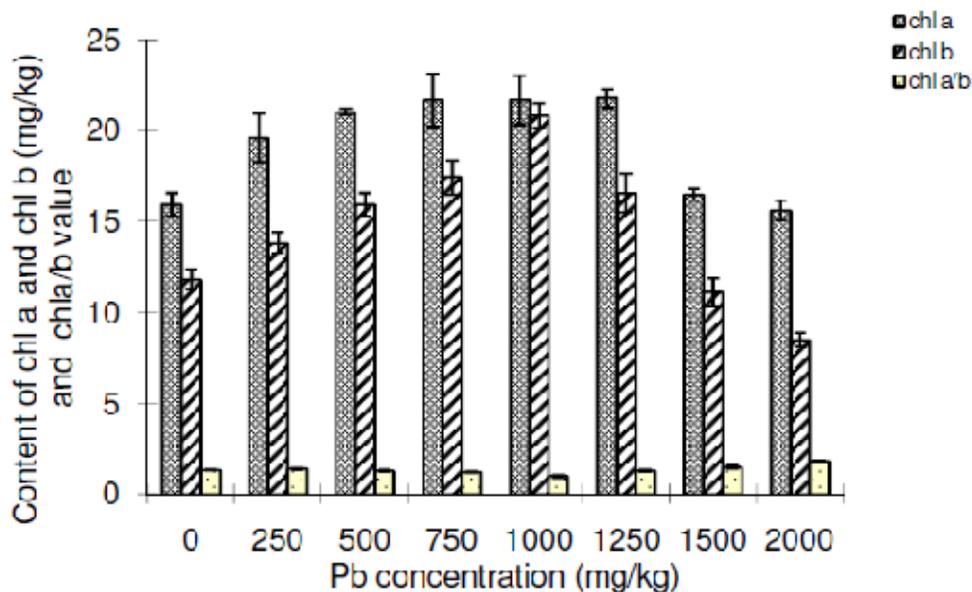


Figure 1. Effects of Pb on the photosynthetic pigments.

earlier were 48.90, 52.62, 57.93 and 65.73% of the control, respectively.

Effects of Pb on tolerance index

The primary effect of lead toxicity on plants is the rapid inhibition of root growth, probably due to the inhibition of cell division in root tips (Eun et al., 2000). Root system is the main part that absorbs and excludes heavy metal. The root length of sensitive plant is obviously inhibited by heavy metal, while that of tolerant plant is only slightly affected. TI is an important indicator that reflects the heavy metal tolerance of plants (Yan et al., 1997). During 90 days of Pb exposure, the TI values of *C. comosum* were not significantly different from the control, except for that at the 2000 nmg/kg Pb treatment. The TI of *C. comosum* were all above 100 in soil Pb concentration less than 500 mg/kg and the peak value was 109.69 at 500 mg/kg (Table 1). When compared with *Acacia farnesiana*, a lead bioaccumulator, when Pb^{2+} concentration in the medium was increased to 1000 mg/L, the TI showed an important decrease, as low as 8 to 10% after 60 days exposure (Amalia et al., 2011). Our findings indicate that *C. comosum* could maintain normal growth under high Pb stress and the plant is tolerant to Pb pollution.

Effect of Pb on the photosynthetic pigments

With the absorption and accumulation of heavy metal, the plant will perform many physiological changes (Liu et al.,

2009). As indicated in Figure 1, with increasing Pb concentration, the photosynthetic pigment contents showed a downward trend after rising at first. The contents of chlorophyll a and b reached their maximum at 1250 and 1000 mg/kg, respectively which were 1.21 and 1.76 times that of the control separately. The chl a and b contents maximized at 2000 mg/kg were 97.81 and 72.36% of CK. Meanwhile, with increasing Pb concentration, the ratio of chlorophyll a content to that of chlorophyll b (chl a/b) increased slightly. These information indicated that the damage degree of photosynthetic pigments was chl a < chl b, but their susceptibility to Pb damage was almost equal.

Chlorophyll content increased with Pb dosage, primarily because exogenous heavy metal dosage enhanced the synthesis of chlorophyll. But with increasing Pb concentration in soils, chlorophyll content was decreased. A possible reason for the decrease of plant chlorophyll was that the heavy metals has replaced the central Mg ion or inhibited chlorophyll synthesis by inhibiting chlorophyll synthesizing enzyme activity (Gupta and Chandra, 1996; Ewais, 1997; Pflugmacher et al., 1999; Manios et al., 2003). Finally, it can be assumed that the decrease in chlorophyll concentration was insignificant and it did not affect the plant growth, as growth was not inhibited throughout the experiment.

Effects of Pb on lipid peroxidation and the activity of antioxidative enzymes

A suitable criterion to select plants for metal phyto-remediation purposes could be the presence of

Table 2. Effects of Pb on EC, MDA content, POD and CAT activities of *C. comosum*.

Pb stress (mg/kg)	EC ($\mu\text{s/cm}$)	MDA content ($\mu\text{mol/g}$)	POD activity (mg/g-min)	CAT activity (mg/g-min)
CK	0.13±0.06bc ^a	0.20±0.13c	75.13±6.45a	2.01±0.53f
250	0.15±0.03a	0.29±0.14ab	82.15±3.73a	2.63±0.14bcd
500	0.13±0.02cd	0.26±0.02b	50.12±2.56b	2.87±0.02abc
750	0.13±0.01c	0.29±0.04a	49.13±5.78b	2.90±0.04ab
1000	0.12±0.01d	0.30±0.07a	49.12±3.20b	3.00±0.07a
1250	0.12±0.01d	0.25±0.03b	48.12±6.20bc	2.53±0.03cd
1500	0.14±0.01b	0.24±0.04b	40.14±4.40c	2.41±0.04de
2000	0.11±0.01d	0.21±0.01bc	47.11±6.35bc	2.12±0.01ef

^a: Values followed by the same letter within a column are not significantly ($P>0.05$) different according to the least significant difference (LSD) test.

Table 3. Pb Concentration and distribution in *C. comosum*.

Pb stress (mg/kg)	Concentration in above ground part (mg/kg)	Concentration in root (mg/kg)	BC ^a		TF ^b
			Aboveground part	Root	
CK	12.21±2.20d ^c	15.34±3.15f	0.48±0.09a	0.60±0.12b	0.80±0.02b
250	68.69±9.10c	180.16±36.67e	0.27±0.05b	0.72±0.18a	0.38±0.02d
500	91.50±4.88c	189.64±15.76e	0.18±0.01c	0.38±0.31c	0.48±0.02d
750	154.03±11.58b	229.19±39.81d	0.21±0.02c	0.31±0.53c	0.67±0.10bc
1000	172.50±17.68b	239.84±30.20c	0.17±0.02cd	0.24±0.30cde	0.72±0.17bc
1250	201.53±25.50a	315.75±15.31a	0.16±0.02cd	0.25±0.01cd	0.64±0.10c
1500	163.75± 22.29b	265.59±39.80b	0.11±0.05de	0.18±0.04de	0.62±0.01c
2000	181.81±10.03ab	191.75±9.66e	0.09±0.01e	0.10±0.05e	0.95±0.01a

^a: BC: Bioaccumulation coefficient; ^b: TF: translocation factors; ^c: Values followed by the same letter within a column are not significantly ($P>0.05$) different according to the least significant difference (LSD) test.

biochemical mechanisms related to heavy metal tolerance (Amalia et al., 2011). Membrane lipids are the major target of free radical attack, since the protonation of superoxide radical ($\bullet\text{O}_2^-$) can produce hydroperoxyl radical ($\bullet\text{HO}_2$), which can convert fatty acids to toxic lipid peroxides. Therefore, measurement of the products of lipid peroxidation, such as MDA, has been commonly used to assess oxidative stress/injury (Diwan et al., 2010). According to our research, both EC and MDA production changed insignificantly with increasing Pb concentration. As the most widely accepted indicator of oxidative damage, the maximum of the MDA content was 0.24 in 1000 mg/kg, which was 1.5 times of the control, then it decreased with increasing Pb stress.

We further investigated the activities of POD and CAT, which play an important role in the protective enzyme system. As a defensive mechanism, SOD, POD and CAT could protect plant from damage induced by Pb toxicity. POD and CAT are key enzymes in the detoxification of H_2O_2 , so protective enzymes can prevent the accumulation of $\bullet\text{O}_2^-$ and H_2O_2 effectively and limit membrane lipid peroxidation caused by free radicals (Diwan et al., 2010). With the raise of Pb concentration,

the activities of POD and CAT in leaves showed a downward trend after rising initially (Table 2). The activities of POD and CAT reached their peaks in 250 and 1000 mg/kg, which were 1.09 and 1.49 times, respectively of the control. In addition, when Pb concentration was up to 2000 mg/kg, activities of POD and CAT were 62.70 and 105.47% of the control, respectively. Higher Pb concentration would impair the structure and synthesis of enzyme and protective enzyme system would be damaged. In the evolution and elimination processes of free radicals, only the active oxygen species scavengers can maintain the biological free radicals at a low level in order to maintain the normal physiological activity.

Pb accumulation

The accumulation and compartmentation of heavy metals in plant were important indexes in choosing tolerant plant species. As seen in Table 3, the Pb concentrations in roots and aboveground parts of *C. comosum* reached their peaks at 1250 mg/kg Pb, that is, 315.75 and 201.53 mg/kg, respectively. The Pb concentration in the roots

and aboveground parts of *C. comosum* was 191.75 and 181.81 mg/kg in soils treated with 2000 mg/kg Pb. The translocation factor was decreased at low Pb concentration, but increased significantly when Pb concentration in soil reached 750 mg/kg. The translocation factor was 0.95 at 2000 mg/kg Pb concentration.

Plant tolerance to a particular metal is governed by an inter-related network of physiological and molecular mechanisms. The apparent tolerance to increasing levels of toxic elements can result from the exclusion of toxic elements or the metabolic tolerance of plants to specific elements (Sun and Zhou, 2005). Table 3 shows that *C. comosum* was extremely tolerant to lead stress and with increasing Pb concentration, *C. comosum* could maintain a lower Pb level in plant tissues in soil with higher Pb concentration. Metal uptake depends primarily on metal bioavailability (Jabeen et al., 2009). Root secretion which can absorb heavy metals could inhibit the bioavailability of soil pollutants. Glue-like substance secreted by roots, though competitive binding with Pb^{2+} , Cu^{2+} , Cd^{2+} , could keep those heavy metals outside the roots (Sun and Zhou, 2005; Li et al., 2002).

Some researches showed, under Pb stress, plants could secrete citric acid, malic acid and acetic acid which could form soluble complexes with lead. Those soluble complexes could inhibit the transmembrane transport of lead and reduce the bioavailability of lead ions in the environment (Shen et al., 1997). Besides, Li et al. (2002) demonstrated that NtCBP4 and AtCNGC1 are the two components of a transport pathway which are responsible for the entry of Pb ion into roots. Kim et al. (2006) found that AtPDR12 which is located in the cell membrane has the efflux mechanism of Pb^{2+} and AtPDR12-overexpression enhanced the resistance in plants to Cd and Pb toxicity.

Conclusion

Instead of inhibition, lower Pb concentration stimulated the growth of *C. comosum*. The TI of *C. comosum* were all above 100 at Pb concentrations below 500 mg/kg and the growth traits increased initially and then declined at higher Pb concentration. The same results also can be observed in physiological characteristics of *C. comosum*. The activities of POD and CAT raised obviously when exposed to lower Pb concentration, as a result, the contents of MDA and chl, which were important indicators of damages caused by adversity, did not differ significantly from controls in Pb concentration below 1000 mg/kg. Furthermore, *C. comosum* could maintain lower Pb level in plant tissues in soils treated with higher Pb concentration.

Our findings suggest that *C. comosum* is highly tolerant to Pb stress, and has the advantages of high biomass, easy cultivation and wide geographic distribution, which most heavy-metal-tolerant plants lack and will supply a gap of most Pb-tolerant plant discovery.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the National Natural Science Foundation of China (No. 31070401), the Key Foundation of Education Department of Anhui Province (No. KJ 2009 A 104, KJ 2010 A 152), the Foundation of the Provincial Key Laboratory of the Conservation and Exploitation of Biological Resources in Anhui and the Key Laboratory of Biotic Environment and Ecological Safety in Anhui Province.

REFERENCES

- Amalia MM, Ernesto FT, Fernando RC, Tania L (2011). Volke-Sepulveda Lead bioaccumulation in *Acacia farnesiana* and its effect on lipid peroxidation and glutathione production. *Plant Soil*. 339: 377-389.
- Bai XY, Liu HH, Han BP (2010). Experimental study on ornamental plants remediation of heavy metals in Sewage Sludge. *Environ. Sci. Technol.*, 10(9):39-44,105.
- Baker AJM, Reeves RD, Hajar ASM (1994). Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl Brassicaceae. *New Phytol.*, 127: 61-68.
- Bradshaw AD (1997). Restoration of mined lands-using natural process. *Ecol. Eng.*, 8: 255-269.
- Dahmani-Müller H, Oort F, Gélie B, Balabane M (2000). Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environ. Pollut.*, 109: 231-238.
- Diwan H, Khan I, Ahmad A, Iqbal M (2010). Induction of phytochelatin and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. *Plant Growth Regul.*, 61: doi:10.1007/s10725-010-9454.
- Environmental Monitoring of China (1992). Modern analytical method of soil. China Environmental Science Press, Beijing, China
- Eun S, Youn HS, Lee Y (2000). Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiol. Plant*. 110: 357-365.
- Ewais EA (1997) Effects of cadmium nickel and lead on growth, chlorophyll content and proteins of weeds. *Biol. Plantarum*, 39: 403-410.
- Gupta M, Chandra P (1996). Bioaccumulation and physiological changes in *Hydrilla verticillata* (L. f.) Royle in response to mercury. *B. Environ. Contam. Toxicol.*, 56: 319-326.
- Hemndez AL, Gasco AM, Gasco JM, Guerrero F (2005). Reuse of waste materials as growing media for ornamental plants. *Bioresour. Technol.*, 96: 125-131.
- Jabeen R, Ahmad A, Iqbal M (2009). Phytoremediation of heavy metals: physiological and molecular mechanisms. *Bot Rev.*, 75:339-364.
- Kim DY, Bovet L, Kushnir S, Noh EW, Martinoia E, Lee Y (2006). AtATM3 is involved in heavy metal resistance in Arabidopsis. *Plant Physiol.*, 140: 922-932.
- Li LY, Zong LG, Huang Y, Zhao YH (2002). Effect of different organic acids on lead uptake by rice. *J. Nanjing Agric. U.*, 25 (3): 45-48.
- Liu ZL, He XY, Chen W, Yuan FH, Yan K, Tao D (2009). Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator-*Lonicera japonica* Thunb. *J. Hazard. Mater.* 169: 170-175.
- Manios T, Stentifor EI, Millner PA (2003). The effect of heavy metals accumulation on the chlorophyll concentration of *Typha latifolia* plants, growing in a substrate containing sewage sludge compost and watered with metaliferous water. *Ecol. Eng.*, 20: 65-74.
- Pflugmacher S, Geissler K, Steinberg C (1999). Activity of phase I phase II detoxification enzymes in different cormus parts of *Phragmites australis*. *Ecotoxicol. Environ. Saf.* 42: 62-66.
- Poskuta JW, Parys E, Romanowska E (1996). Toxicity of lead to photosynthesis, accumulation of chlorophyll, respiration and growth of *Chlorella pyrenoidosa* Protective role of dark respiration. *Acta Physiol. Plant.* 18: 165-171.
- Raskin I, Kumar PBAN, Dushenkov S, Salt SD (1994). Bioconcentration

- of heavy metals by plants. *Curr. Opin. Biotechnol.*, 5: 285-290.
- Rout GR, Samantaray S, Das P (1999). Differential cadmium tolerance of mung bean and rice Genotypes in hydroponic culture. *Acta Agr. Scand. B-S P.* 49: 234-241.
- Salt DE, Krämer U (2000). Mechanisms of metal hyperaccumulation in plants. In I. Raskin, Ensle (Eds.), *Phytoremediation of toxic metals* New York; John Wiley Sons. Inc., 231-246.
- Shen ZG, Zhao FJ, Mc Grath SP (1997). Uptake and transport of zinc in the hyperaccumulator *thlaspi caerulescens* and non-hyperaccumulator *Thlaspi ochroleucum* plant. *Cell Environ.*, 20:898-906.
- Singer AC, Bell T, Heywood CA, Smith JAC, Thompson IP (2007). Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant *Alyssum lesbiacum*: evidence of histidine as a measure of phytoextractable nickel. *Environ Pollut.*, 147: 74-82.
- Sun RL, Zhou QX (2005). Heavy metal tolerance and hyperaccumulation of higher plants and their molecular mechanisms: a review. *Acta Phytoecol. Sin.* 29(3): 497-504.
- Tanhan MKP, Pokethitiyook P, Chaiyarat R (2007). Uptake and accumulation of cadmium lead and zinc by Siam weed [*Chromolaena odorata* (L.) King & Robinson]. *Chemosphere*, 68: 323-329.
- Tordoff GM, Baker, AJM, Willis AJ (2000). Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*, 41: 219-228.
- Türkan I, Bor M, Özdemir F, Koca H (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci.*, 168: 223-323.
- Vangronsveld J, Assche F, Clijsters H (1995). Reclamation of a bare industrial area contaminated by non-ferrous metals: in situ metal immobilization and revegetation. *Environ. Pollut.*, 87: 51-59.
- Wang YB, Yan AL, Dai J, Wang NN, Wu D (2011). Accumulation and tolerance characteristics of cadmium in *Chlorophytum comosum*: a popular ornamental plant and potential Cd hyperaccumulator. *Environ. Monit. Assess.*, DOI:10.1007/s10661-011-2010-1.
- Whiting SN, Reeves RD, Richard D, Johnson MS, Cook JA, Malaisse F (2004). Research remediation. *Restor. Ecol.*, 12: 106-116.
- Wong MH (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50: 775-780.
- Wu YX, Tiedemann A (2002). Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. *Environ. Pollut.*, 116: 37-47.
- Xia HP (2004). Ecological rehabilitation and phytoremediation with four grasses in oil shale mined land. *Chemosphere*, 54: 345-353.
- Yan CL, Hong YT, Fu SZ, Fang CH, Lei JX, Shen Q (1997). Effect of Cd, Pb stress on scavenging system of activated oxygen in leaves of tobacco. *Acta. Ecol. Sin.*, 17: 488-492.
- Zhou QX (2006). New researching progress in pollution chemistry of soil environment and chemical remediation. *Environ. Chem.*, 25: 257-265.