Effects of sulfur application on sulfur and arsenic absorption by rapeseed in arsenic-contaminated soil

L. Zhong, C. Hu, Q. Tan, J. Liu, X. Sun

Microelement Research Center of Huazhong Agricultural University, Wuhan, P.R. China

ABSTRACT

A pot experiment was conducted to study the effects of arsenic (As) and sulfur (S) interaction on yield and their accumulation and distribution in rapeseed (*Brassica napus* L.). The results showed that (1) at the same level of S treatment, application of As significantly decreased rapeseed grain and biomass yield; (2) Application of S significantly increased the grain and biomass yield of rapeseed when As was applied; (3) When As application rate increased, As content significantly increased in different parts of rapeseed, and reached their highest level at 120 mg/kg As. Arsenic content from seed were all below 1 mg/kg AS. Addition of S significantly reduced As contents in root and grain of rapeseed.

Keywords: accumulation; distribution; seeds; thiols

Arsenic (As) is a toxic metalloid element in ground and surface waters (Tripathi et al. 2008). As-contaminated soil in China is widespread covering an area of 14 million ha, in 11 provinces (Gu and Zhou 2002). Chen and Zhou (2002) reported that there were large areas of farmland and groundwater that were polluted by As around mining areas. The concentration of arsenic in soils around local mining areas was as high as 237.2 mg/kg, and the average As concentration in vegetables reached 0.74 mg/kg (Liao et al. 2005). Arsenic cannot only affect crop growth, but also bioaccumulate through the food chain and affect the human health. Arsenic can cause skin cancer, affect bladder, liver, kidney, lung and prostate, cause black-foot disease, coronary artery disease and other chronic arsenic poisoning. Rapeseed (*Brassica napus* L.) was planted in arsenic-contaminated fields and it was reported that rapeseed has a high tolerance and absorptive capacity for heavy metals (Ebbs 1997). However, our knowledge on rapeseed growth and yield and absorption of As by rapeseed in arsenic contaminated soils is still limited.

Sulfur (S) is an essential element for plants. Some chemical compounds which contain S, such as glutathione (GSH), phytochelatins (PCs; the polymers of GSH) etc., are some of the main components in plant that can eliminate heavy metal stress and play an important part in non hyperaccumulator plants' resistance to heavy metal (Steffens 1990, Salt et al. 1998, Cobbett 2000). In China, some soils are low in S, because of the application of fertilizers that are low in S (Liu 1995). Rapeseed is one of the plants that need significant S (Hrivna et al. 2001). Application of S to rapeseed can increase its yield (Blake-Kalff et al. 1998). Sulfur has some functions of notable mitigative effect to both plants' environmental stress and heavy metal pollution (Fitzgerald et al. 2001). However, our understanding on the role that S plays in rapeseed's ability to tolerate As is limited. This knowledge is important in our use of rapeseed to decontaminate As-polluted soils.

MATERIAL AND METHODS

Soil used. The top soil layer (0–20 cm) of Alfisols (yellow brown), sampled from the Huazhong Agricultural University, Wuhan, China, was used for the pot experiment. Its characteristics were: pH 4.93 (soil: water ratio of 1:2.5), organic matter 15.17 g/kg soil, alkaline hydrolysis N 100.28 mg/kg soil, Olsen-P 19.18 mg/kg soil, available K 80.67 mg/kg,

Supported by the Program for New Century Excellent Talents of the Education Ministry of China, Project No. NCET-04-0731.

available S 15.80 mg/kg, available As 0.04 mg/kg soil. The soil was mixed, air-dried, and ground to a particle size of < 2 mm.

Experimental design. Four levels of S (0, 50, 100, and 150 mg/kg soil) and As (0, 60, and 120 mg/kg soil) were used in this experiment, resulting in a total of 12 treatments (As_0S_0; As_0S_{50}; As_0S_{100}; As_0S_{150}; $As_{60}S_0$; $As_{60}S_{50}$; $As_{60}S_{100}$; $As_{60}S_{150}$; $As_{120}S_0$; $As_{120}S_{50}$; $As_{120}S_{100}$; $As_{120}S_{150}$). Each treatment had 4 replicates. Plastic pots with 20 cm in height and 20 cm in diameter were used, and each pot was filled with 5 kg soil.

Soil in rhizoboxes received (per kg) a basal application of 200 mg P as KH_2PO_4; 200 mg K as KH_2PO_4 and KCl; 200 mg N as urea and $(NH_4)_2SO_4$; 10 mg Mn as $MnCl_2$; 1 mg Cu as $CuCl_2{\cdot}H_2O$; 2 mg Zn as $ZnCl_2$; 50 mg Mg as $MgCl_2{\cdot}6H_2O$; 1 mg B as H_3BO_3; 0.1 mg Mo as $Na_2MoO_4{\cdot}2H_2O$ per kg soil, As as $Na_2HAsO_4{\cdot}7H_2O$, and S as $(NH_4)_2SO_4$. Ammonium sulphate ($(NH_4)_2SO_4$) was used as the N fertilizer. Differences in N were adjusted by urea. These nutrients were added to the soil as solution and mixed thoroughly before potting.

Ten rapeseed (*Brassica napus* L.) seeds of cultivar No. 2 Zhong-You-Za were directly sowed in each pot. The plants were thinned to 5 per pot three weeks later, and to one plant per pot one month later. Water content of the soil maintained at 80% of water holding capacity, and N fertilizer was added as urea (200 mg/kg soil) after two months. The plants were harvested and were separated to roots, stems, grain and pod, and dried in the oven at 70°C.

Chemical analysis. The plant samples were digested with a solution of 5:1 concentrated HNO_3:$HClO_4$ (v/v). Total As in the plant was determined by an atomic absorption spectrophotometer (GBC 906AA, Australia, VARIAN) (He et al. 2002). Total S in the plant was determined by inductively coupled plasma-atomic emission spectroscopy (ICPVISTA-MPX) (Huang and Schulte 1985).

Available As was analyzed by an atomic absorption spectrophotometer after Extraction in 0.5 mol/L $NaHCO_3$. Available S was analyzed by inductively coupled plasma-atomic emission spectroscopy after extraction in 2 mol/L Ca $(H_2PO_4)_2$ (Huang and Schulte 1985, He et al. 2002). Available K was analyzed by flame photometer after extraction in 1 mol/L $CH_3COO(NH_4)_2$.

Data analysis. All data were statistically analyzed with a two-way ANOVA procedure using SAS 8.1 software, and the mean values of each treatment underwent multiple comparisons using the Tukey's test at the $P < 0.05$ level. Correlation coefficients between two variables were determined by the CORR procedure of SAS 8.1.

RESULTS AND DISCUSSION

Effect of sulfur application on yield. There was no significant effect of sulfur application on the biomass yield of rapeseed when no As was applied; seed yield significantly decreased in the treatment S_{100} and S_{150} compared with the treatment S_{50} at As_0, though (Table 1). However, when As was applied at 120 mg/kg, sulfur application to the rapeseed significantly increased the biomass and grain yield, especially in the S_{150} treatment (Table 1). There was 15% increase in biomass and 21% increase in grain yield compared with the S_0 treatment. Arsenic has a high affinity with sulfur, it can exert its toxicity to plants after reduction to arsenite ($As^{(III)}$), through interaction

Table 1. Effect of sulfur application on biomass of rapeseed exposed to different As levels

	As treatments (mg/kg)	S treatments (mg/kg)				
		S_0	S_{50}	S_{100}	S_{150}	mean
Biomass yield[1] (g/pot)	As_0	47.75^{abc}	49.42^{ab}	48.42^{abc}	44.74^{abc}	47.58
	As_{60}	42.40^{c}	43.21^{c}	45.32^{abc}	45.27^{abc}	44.05
	As_{120}	43.93^{bc}	43.76^{bc}	46.65^{abc}	50.66^{a}	46.25
	mean	44.69	45.46	46.80	46.89	
Grain yield (g/pot)	As_0	20.23^{ab}	21.39^{a}	18.12^{cde}	19.23^{bcd}	19.74
	As_{60}	17.20^{ef}	17.94^{def}	18.50^{cde}	17.43^{ef}	17.77
	As_{120}	16.54^{f}	17.51^{ef}	19.57^{bc}	19.98^{ab}	18.40
	mean	17.99	18.95	18.73	18.88	

[1]biomass yield = stem weight + pod weight + grain yield; [2]bars represent SEM (n = 4). Mean values for treatments with different lowercase letters indicate significant differences by the Tukey's test (P < 0.05)

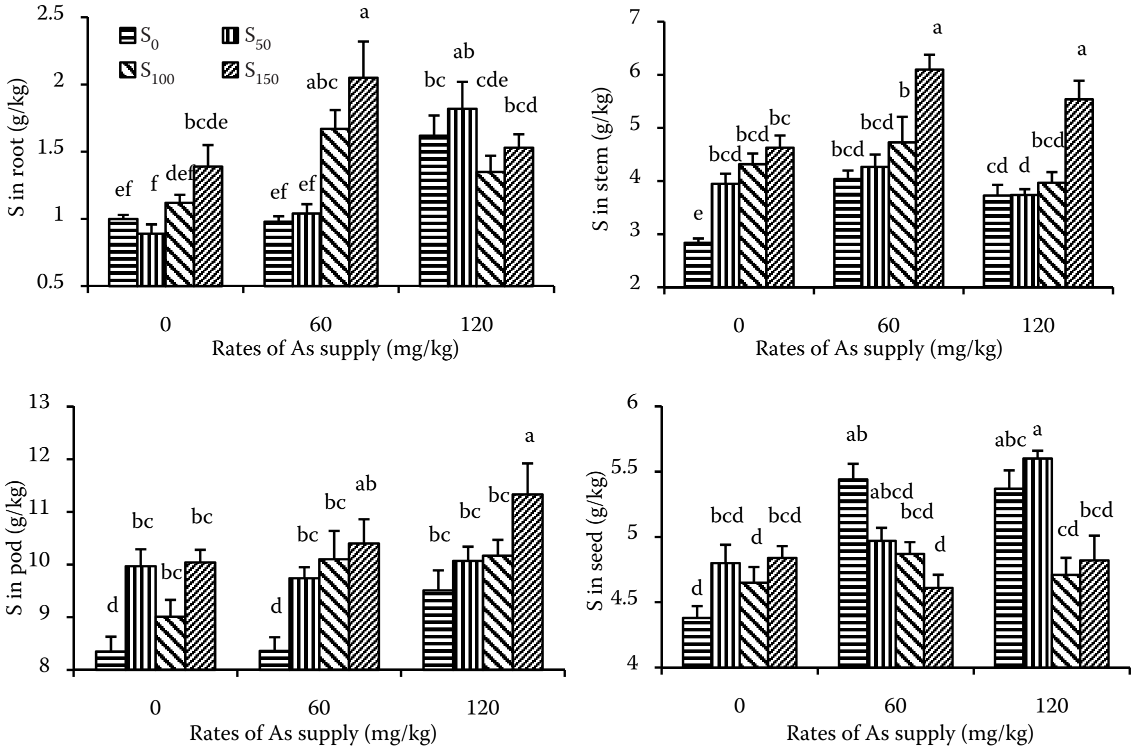

Figure 1. Interactive effects of arsenic and sulfur on S contents of rapeseed in different parts. Bars represent SEM (n = 4). Mean values for treatments with different lowercase letters indicate significant differences by the Tukey's test (P < 0.05). Initial S content was 0 ppm (S_0), 50 ppm (S_{50}), 100 ppm (S_{100}) and 150 ppm (S_{150}), respectively

with thiol (–SH) groups of proteins, amino acid and enzymes (Abedin et al. 2002, Meharg and Rahman 2003). However, reduction of $As^{(V)}$ to $As^{(III)}$ and complexation of $As^{(III)}$ with glutathione (GSH) and phytochelatins (PCs) which were S-containing metabolites followed by sequestration of these complexes in vacuoles was considered as a major strategy of As detoxification in plants (Koch et al. 2000, Bleeker et al. 2006). This study showed that biomass yield as well as grain yield of rapeseed were significantly decreased under high As (120 mg/kg) stress. Yet, S application increased the biomass and grain yield under high As (120 mg/kg) stress (Table 1).

Sulfur concentration and distribution in different plant parts. Sulfur was mainly accumulated in the pods and seeds of rapeseed, but was low in roots: the S concentration in pods were 6–9 times higher than that in roots (Figure 1). With the S_{150} treatment, the sulfur concentration in the stem and pod significantly increased and reached a maximum when As was added. There was an obvious interaction between As and S additions on the concentrations of S in the root, stem and pod of rapeseed. Application of arsenic tended to increase S concentrations in the root and pod (except for $As_{60}S_0$ in root and $As_{60}S_{50}$ in pod), and the highest S concentration was observed at the level of 120 mg/kg As soil.

Most of the sulfur, 74–84%, taken up was retained in the pod and seed (Table 2). Furthermore, application of S decreased the distribution of S in seed and increased the distribution of S in stem, as compared with the control, irrespective of As supply. With the As treatments, the distribution of S in pod increased with the S addition.

In this study, application of As increased S concentrations in root, pod (Figure 1) and whole-plant (data not shown). Upon exposure to As stress plants need to harmonize biosynthesis and consumption of thiols to achieve a new state of metabolic equilibrium to combat the stress, and sulfur was the main component of thiols (Friedrich and Schrader 1978, Mishra 2008). Once exposed to As, level of thiols would decline due to their consumption in As detoxification. It would lead to an increase in S demand resulting in derepression of the whole pathway to tackle the stress imposed (McMahon and Anderson 1998, Nikiforova et al. 2005). Therefore, under As stress, the rapeseed would absorb more S, which resulted in increased S concentrations in root and pod.

Arsenic concentration and distribution in different parts of rapeseed. The concentration of arsenic was the highest in the root, and the lowest in the seed. Arsenic concentration in different parts of rapeseed significantly increased

Table 2. Response of S distribution in different parts of rapeseed to arsenic and sulfur

As treatments (mg/kg)	Distribution ratio of S (%)	S treatments (mg/kg)			
		S_0	S_{50}	S_{100}	S_{150}
As_0	root	1.2	0.8	1.3	1.5
	stem	14.9	16.8	20.8	18.7
	seed	35.4	33.5	28.6	32.0
	pod	48.5	48.8	49.3	47.8
As_{60}	root	1.1	1.0	1.4	1.9
	stem	23.1	18.1	18.8	24.5
	seed	31.7	32.5	29.9	24.7
	pod	44.1	48.4	49.9	48.9
As_{120}	root	1.4	1.7	1.3	1.3
	stem	17.8	16.7	17.9	20.7
	seed	33.9	34.2	31.7	26.9
	pod	46.9	47.4	49.0	51.1

with increasing As concentration in the soil, and reached the maximum in the As_{120} treatment (Figure 2). Arsenic concentration of the seed significantly decreased with increasing S application. The concentration of As in the seed decreased 51.9% in $As_{60}S_{150}$ treatment compared with that in the $As_{60}S_0$ treatment.

Arsenic was mainly accumulated in pod and stem. About 60–89% of As absorption by the plant was concentrated in its pod and stem (Table 3).

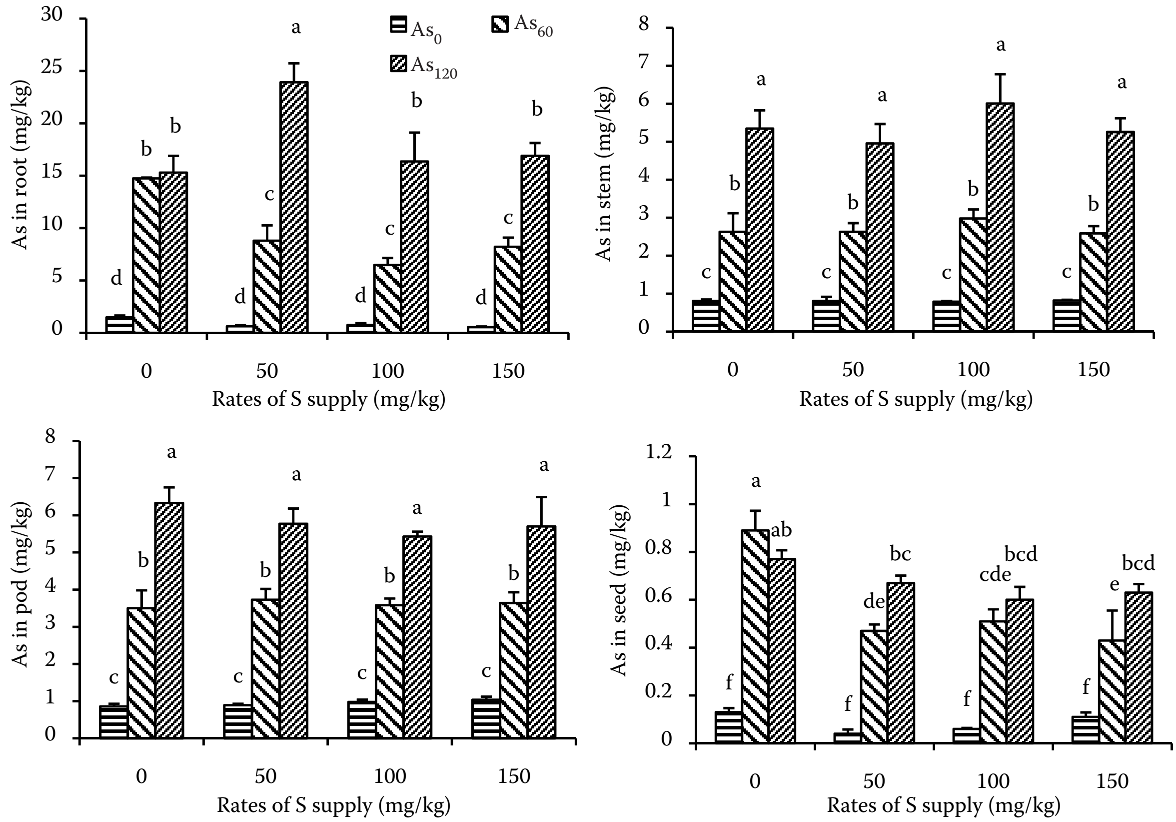

Figure 2. Interactive effects of arsenic and sulfur on As contents of rapeseed in different parts. Bars represent SEM ($n = 4$). Mean values for treatments with different lowercase letters indicate significant differences by the Tukey's test ($P < 0.05$). Initial As content was 0 ppm (As_0), 60 ppm (As_{60}) and 120 ppm (As_{120})

Table 3. Response of As distribution in different parts of rapeseed to arsenic and sulfur

As treatments (mg/kg)	Distribution ratio of As (%)	S treatments (mg/kg)			
		S_0	S_{50}	S_{100}	S_{150}
As_0	root	14.4	7.3	8.5	6.2
	stem	35.5	39.0	36.6	34.8
	seed	8.4	3.5	3.3	7.4
	pod	41.7	50.1	51.6	51.7
As_{60}	root	30.6	20.8	14.5	20.2
	stem	26.4	26.9	31.0	27.9
	seed	9.6	7.5	8.2	6.2
	pod	33.4	44.8	46.2	45.7
As_{120}	root	17.9	29.8	21.4	23.1
	stem	33.2	29.0	36.8	31.5
	seed	6.6	5.5	5.6	5.7
	pod	42.3	35.7	36.3	39.7

The distribution of As in seed was decreased by S addition. In addition, when the concentration of As in soil were 0 and 60 mg/kg, the distribution of As in root was decreased by S addition, but the distribution of As in pod increased. However, the distribution of As in pod and root had the opposite effects by S addition in the level of As 120 mg/kg. Therefore, when the concentration of As in the soil is within 0–60 mg/kg, As was promoted to migrate to the aboveground parts with the application of S. However, when the concentration of As in soil reached 120 mg/kg, the distribution ratio of As in aboveground parts decreased with S addition.

In the arsenic treatments, the As concentration and distribution of As in seed decreased with increasing S application in the soil. At the level of 120 mg/kg As, the As concentration in seed had reached 0.7 mg/kg and exceeded Chinese food hygiene and safety standard of 0.7 mg/kg with no S addition (SAC 1994). The content of As in seed was lower than 0.7 mg/kg when S was applied (Figure 2), and it would be safe to eat. This was mainly because of the combination of As with thiol in root, which inhibited As transfer to the shoots. Similarly, in the process of nutrient transport from leaf to seed, it also had the role of retention of As (Kabata-Pendias and Pendias 1984, Liu et al. 2008). This suggested that sulfur application can promote the absorption of arsenic from the soil in rapeseed. Therefore, higher As accumulation in rapeseed with S application may be attributed to the availability of higher sulfate in the soil solution, and to increases of S-containing detoxifying metabolites inside the cell (Nieboer et al. 1984, Hunaiti et al. 2007).

In conclusion, an increase in S supply to rapeseed was found to be effective in increasing the accumulative capability and grain yield of rapeseed in As-contaminated soil. Furthermore, application of S reduced the content of As in seed. Therefore, the phytoremediation capacity and grain yield of rapeseed may be enhanced through exogenous S application.

Acknowledgements

The authors wish to thank Dr. Hong-Jie Di (Professor of Lincoln University, New Zealand) for critical review of the manuscript.

REFERENCES

Abedin M.J., Feldmann J., Meharg A.A. (2002): Uptake kinetics of arsenic species in rice plants. Plant Physiology, *128*: 1120–1128.

Blake-Kalff M.M.A., Harrison K.R., Hawkesford M.J., Zhao F.J., McGrath S.P. (1998): Distribution of sulfur within oilseed rape leaves in response to sulfur deficiency during vegetative growth. Physiologia Plantarum, *118*: 1337–1344.

Bleeker P.M., Hakvoort H.W.J., Bliek M., Souer E., Schat H. (2006): Enhanced arsenate reduction by a CDC25-like tyrosine phosphatase explains increased phytochelatin accumulation in arsenate-tolerant *Holcus lanatus*. The Plant Journal, *45*: 917–929.

Chen T.B., Zhou J.L. (2002): Situation and prospect of research on heavy metal pollution in vegetables and soils for vegetable cultivation in urban areas of China. Journal of Hubei Agricultural College, *22*: 476–479. (In Chinese)

Cobbett C.S. (2000): Phytochelatins and their roles in heavy metal detoxification. Plant Physiology, *123*: 825–832.

Ebbs S.D., Kochian L.V. (1997): Toxicity of zinc and copper to Brassica species: Implications for phytoremediation. Journal of Environmental Quality, *26*: 776–781.

Fitzerald M.A., Ugalde T.D., Anderson J.W. (2001): Sulphur nutrition affects delivery and metabolism of S in developing endosperms of wheat. Journal of Experimental Botany, *52*: 1519–1526.

Friedrich J.W., Schrader L.E. (1978): Sulfur deprivation and nitrogen metabolism in maize seedlings. Plant Physiology, *61*: 900–903.

Gu J.G., Zhou Q.X. (2002): Cleaning up through phytoremediation: a review of Cd contaminated soils. Ecologic Science, *21*: 352–356.

He B., Fang Y., Jiang G., Ni Z. (2002): Optimization of the extraction for the determination of arsenic species in plant materials by high-performance liquid chromatography coupled with hydride generation atomic fluorescence spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy, *57*: 1705–1711.

Hrivna L., Richter R., Losak T. (2001): The effect of the content of water-soluble sulphur in the soil on the utilisation of nitrogen and on the yields and quality of winter rape. Rostlinná Výroba, *47*: 18–22.

Huang C.L., Schulte E.E. (1985): Digestion of plant tissue for analysis by ICP-AES. Communications in Soil Science and Plant Analysis, *16*: 943–958.

Hunaiti A.A., Al-Oqlah A., Shannag N.M., Abukhalaf I.K., Silvestrov N.A., Von Deutsch D.A., Bayorh M.A. (2007): Towards understanding the influence of soil metals and sulfate cone tent on plant thiols. Journal of Toxicology and Environmental Health, Part A, *70*: 559–567.

Kabata-Pendias A., Pendias H. (1984): Trace Elements in Soil and Plants. CRC Press, Florida, 171.

Koh I., Want L.X., Olsson C.A., Cullen W.R., Reimer K.J. (2000): The predominance of inorganic arsenic species in plants from Yellowknife, Northwest Territories, Canada. Environmental Science and Technology, *34*: 22–26.

Liao X.Y., Chen T.B., Xie H., Liu Y.R. (2005): Soil As contamination and its risk assessment in areas near the industrial districts of Chenzhou City, Southern China. Environment International, *31*: 791–798.

Liu C.Q. (1995): The importance of sulfur fertilizer and the demand trends of sulfur fertilizer in China. Sulfuric Acid Industry, *5*: 20–23. (In Chinese)

Liu Z.Y., Chen G.Z., Tian Y.W. (2008): Arsenic tolerance, uptake and translocation by seedlings of three rice cultivars. Acta Ecologica Sinica, *28*: 3228–3235. (In Chinese)

McMahon P.J., Anderson J.W. (1998): Preferential allocation of sulphur into γ-glutamylcysteinyl peptides in wheat plants grown at low sulphur nutrition in the presence of cadmium. Physiologia Plantarum, *104*: 440–448.

Meharg A.A., Rahman M.Md. (2003): Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environmental Science and Technology, *37*: 229–234.

Mishra S., Srivastava S., Tripathi R.D., Trivedi P.K. (2008): Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. Aquatic Toxicology, *86*: 205–215.

Nieboer E., Padovan D., Lavoie P. (1984): Anion accumulation by lichens. II. Competition and toxicity studies involving arsenate, phosphate, sulphate and sulphite. New Phytologist, *96*: 83–93.

Nikiforova V.J., Kopka J., Tolstikov V., Fiehn O., Hopkins L., Hawkesford M.J., Hesse H., Hoefgen R. (2005): Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of Arabidopsis plants. Plant Physiology, *138*: 304–318.

Salt D.E., Smith R.D., Raskin I. (1998): Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology, *49*: 643–668.

Standardization Administration of the People's Republic of China (1994): Tolerance Limit of Arsenic in Foods. GB 4811-84. Chinese Standard Press, Beijing.

Steffens J.C. (1990): The heavy metal-binding peptides of plants. Annual Review of Plant Physiology and Plant Molecular Biology, *41*: 553–575.

Tripathi R.D., Srivastava S., Mishra S., Singh N., Tuli R., Gupta D.K., Maathuis F.J.M. (2008): Arsenic hazards: Strategies for tolerance and remediation by plants. Trends in Biotechnology, *25*: 158–165.

Received on April, 2011

Corresponding author:

Dr. Chengxiao Hu, Microelement Research Center of Huazhong Agricultural University, Wuhan 430070, P.R. China
phone: + 86 27 8728 2043, fax + 86 27 8739 5111, e-mail: hucx@mail.hzau.edu.cn