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Heavy metal and sulfur concentrations and mycorrhizal colonizing status of plants from abandoned lead/zinc mine land in Gejiu, Southwest China

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A field survey of heavy metals and sulfur concentrations and mycorrhizal status of plants grown in abandoned lead/zinc mine land was conducted in Gejiu City, Yunnan Province, Southwest China. Low or no root arbuscular mycorrhizal fungi (AMF) infection was detected in most of the plants for 21 species of 13 families collected from the abandoned Pb/Zn mine land. The maximum concentrations of Pb, Zn, Cd and As was up to 11255.3 ± 1887.2, 5884.2 ± 531.9, 70.5 ± 0.8 and 498.9±38.1 mg/kg in soils, respectively. The maximum concentration of Pb, Zn, Cd and As in plants shoot was 2323.4 ± 125.0, 1273.7 ± 140.0, 238.5 ± 19.5 and 326.7 ± 53.2 mg/kg, and which in plants root was 3480.0 ± 642.9, 1421.6 ± 251.3, 291.8 ± 11.2 and 390.6 ± 30.1 mg/kg, respectively. The average S concentrations were 0.29 and 0.19% DW in plants shoots and roots, respectively. Significant correlation between S and Pb, Zn, As concentrations in plants shoots, and between S and Pb, Zn, Cd, As concentrations in plants roots was observed.

Key words: Lead/zinc mine, heavy metal, sulfur, arbuscular mycorrhizal fungi, relation, Gejiu.

INTRODUCTION

Southwest China is rich in metallic mineral resources including tin, copper, lead and zinc (Cheng et al., 2011). This region is an important metallic material foundation supporting economic development of China. Great achievements have been obtained in the survey and development of China's mineral resources in the past five decades since the founding of New China. The mining activities have brought not only economic prosperity, but also environmental problems. Abandoned mine land can result in severe pollution and have aesthetic impacts on the local environment. Mining have already become major sources of heavy metal contamination for soils and water bodies (Cheng et al., 2010). Cultivation of crops for human consumption on contaminated soils and waters can potentially lead to the uptake and accumulation of heavy metals in the edible plant parts with a resulting risk to human and animal health (Zhuang et al., 2009a, b). Increasing evidence shows that heavy metal pollution of mined areas are considered potential carcinogens and are associated with etiology of a number of diseases, especially cardiovascular, kidney, nervous system, blood as well as bone diseases to the local inhabitants (Jarup,

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2003).

Abandoned mine land usually provide an unfavorable substrate for plant growth because of their high concentrations of toxic metals and low nutrient content. Only a restricted number of plants from the local flora are able to grow in metalliferous soils. The high concentrations of heavy metals in the soils and the native plants grown in the abandoned mine area of Les Malines (Southern France), NW Madrid (Spain), Northern Vietnam, Guangxi (South China) and so on were found (Moreno-Jiménez et al., 2009; Escarre et al., 2011; Ha et al., 2011; Li et al., 2007). So, the heavy metal contamination of abandoned mine soils and plants in the mining areas has been regarded as a great environmental problem.

It is well known that arbuscular mycorrhiza fungi (AMF) is widely distributed on metal contaminated sites (Sonjak et al., 2009; Zarei et al., 2010). Many studies have indicated that mycorrhizal colonization might increase plant tolerance to heavy metal contamination and appear to protect plants partially against the toxicity of heavy metals. Some recent studies have focused on the status of AMF associated with the plants on metal polluted ecosystems (Chen et al., 2005a; Sonjak et al., 2009).

Sulfur (S) is an essential nutrient for plants. It is fourth in importance after N, P and K, and is considered vital for proper plant growth and development. Recently, it has been demonstrated that S plays a pivotal role in plants tolerance to heavy metals. The mechanisms proposed that S-containing compounds like glutathione (GSH), phytochelatins (PCs) and metallothioneins (MTs) can improve the tolerance of plants to several metals and metalloids through complexation and/or further sequestration of toxic forms inside cellular vacuoles (Cobbett and Goldsbrough, 2002; Gill and Tuteja, 2011; Na and Salt, 2011). In recent years, more studies have been focused on heavy metals uptake, accumulation and tolerance in relation to sulfur availability to plants (Feng et al., 2009; Srivastava and D'Souza, 2009; Fan et al.; 2010). However, there are only a few reports on the S contents of wild plants in heavy metals contaminated environment (Huseyinova et al., 2009).

Plants take up S primarily as the sulfate anion, which was often found in low concentrations in the soil (Rausch and Wachter, 2005; Rennenberg et al., 2007). Most of the sulfur in soil environments (>95% of total sulfur) is bound to organic molecules, and is therefore not directly plant-available (Kertesz and Mirleau, 2004). On the other hand, under conditions of low S availability, AMF symbiosis can increase the percentage of S in pot-grown onions and maize (Banerjee et al., 2003; Guo et al., 2007). The ability of mycorrhizal fungi to transfer S suggests that mycorrhizal plants might obtain S from organic sources (Allen and Shachar-Hill, 2009), and also indicates the possibility that AMF might enhance tolerance of heavy metals by mycorrhizal plants because of improvement of the S nutrition of plants.

So the authors conducted this preliminary study to investigate lead (Pb), zinc (Zn), cadmium (Cd) and arsenic (As) concentrations in soils and plants, S concentrations in plants and the mycorrhizal status of plants colonizing abandoned Pb/Zn mine land in Gejiu City, Yunnan Province, Southwest China, in order to investigate the relations among the heavy metal and S contents and AMF colonization of plants grown in heavy metals contaminated environment. The results would help us understand the mechanisms of plant adaptation to the adverse environments of abandoned Pb/Zn mine land.

MATERIALS AND METHODS

Study site

Gejiu is the largest polymetallic tin ore field in the world (~300 Mt of Sn, and ~700 Mt of Cu, Pb, Zn, Sb, Ag, Mo, Au and Bi) (Chen et al., 1992; Cheng, 2007; Cheng and Mao, 2010). This study has been carried out at abandoned Laochang Pb-Zn mine land, located in Gejiu City, Yunnan Province, Southwest China, between latitudes 23°18′09 N and longitudes 03°10′42 E and at an elevation above sea level of 2353 to 2378 m. The positions of the sampling sites were recorded using a Global Position System (GPS). Sampling site was abandoned at least 30 years before the study.

Plant and soil sampling

In total, 21 species of 13 families were collected from abandoned Pb/Zn mine land in October 2010 (dry season). That included 7 species of Compositae, 2 species of Poaceae and Polygonaceae, and 1 species each of Dryopteridaceae, Leguminosae, Labiatae, Ranunculaceae, Equisetaceae, Moraceae, Rosaceae, Gentianaceae and Euphorbiaceae. Plant species of Compositae were dominant in abandoned Pb/Zn mine land.

Plant samples included shoots and roots for herbaceous. At least 3 individual plants of the plant species with high biomass were randomly collected within the sampling area. 9~15 individual plants of the plant species with little biomass were randomly collected and then 3~5 individual plants were mixed together to give at least 3 composite plants samples. The plants were carefully dug from the substrate and the majority of bulk soil samples were manually removed from the roots. Plant samples were placed loosely in a labeled cloth bag, and were transported to the laboratory as quickly as possible.

The soils samples were collected from the roots horizon of each plant. Maximum sampling depth was 5–20 cm. Forty two (42) soil samples were collected corresponding to the plants samples. Only the substrate closely attached to the root system was analyzed.

Soil analysis

The soil samples were air-dried at room temperature for 2 weeks, and then stored in sealed plastic bags at room temperature for up to 2 months until samples could be treated.

100.0 g dried soil samples were detached for determining AMF spores density. The spores were extracted by wet sieving of the soil samples and a sucrose gradient centrifugation method (Daniels and Skipper, 1982) and observed under light microscopes. Some spores were tightly grouped in sporocarps and it was difficult to count the number of spores per sporocarp, so, in these cases, a sporocarp was referred to as one spore.

The remaining soils were ground into fine powder and sieved through 0.25 mm nylon sieve. 5.0 g soil samples were mineralized by wet digestion in 20 ml of ultrapure mixture of concentrated

HNO_3/HCl/$HClO_4$ (1:3:2) on a thermo block at 200 ~ 250°C until the color of the soils was off-white and then were diluted with 50 ml of 0.2% HNO_3. The total concentrations of Pb, Zn and Cd were determined by the flame atomic absorption spectrometry (FAAS) using a TAS-990 atomic absorption spectrometer (Beijing Puxi Instrument Factory, Beijing, P.R. China). The total concentrations of As were determined by silver diethyl dithiocarbamate method. Standard materials were included for assurance control. Standard materials were $Pb(NO_3)_2$, $ZnCl_2$, $CdCl_2$ and $As(NO_3)_2$. Means of Pb, Zn, Cd and As were calculated from three composite samples.

Plant analysis

Prior to the analysis of the plant material, leaves and roots of plants were separated and carefully washed with tap and deionized water in order to remove any surface soil or dust deposits, and then the height of shoots was measured.

A part of roots were collected from sampled plants and fixed in 5 ml formalin, 5 ml acetic acid, and 90 ml of 70% alcohol, diluted twice when used (1/2 FAA), and stored at 4°C. Roots were taken from the 1/2 FAA, washed several times in tap water and cleared in 10% (w/v) KOH by heating to approximate 90°C in a water bath for 2 to 3 h, the time depending on the size/structure of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5 to 1 cm segments and stained with 0.5% acid fuchsin according to Berch and Kendrick (1982) method. Fifty 0.5 to 1 cm root fragments were examined per sample for their arbuscular mycorrhizal status under a compound microscope (160 to 800×).

Plants shoots and the remaining roots were oven-dried at 105°C for 30 min and 60°C for 48 h, then the dry weight (DW) was weighed by analytical balance. Dry plants shoots and roots were ground into fine powder sieved through 1 mm nylon sieve. 1.0 g plant samples were mineralized by wet digestion in 15 ml of ultrapure mixture of concentrated HNO_3/$HClO_4$ (3:1) (v/v) on a thermo block at 200 ~ 250°C until a transparent solution was obtained and then were diluted with 50 ml of 0.2% HNO_3. The total concentrations of Pb, Zn, Cd and As were determined by the methods used for soils. The total concentrations of S were determined by barium sulfate turbidimetry.

Statistical analysis

Translocation factor (TF) was described as the ratio of heavy metals concentration in plant shoot to that in plant root. Enrichment coefficient (EF) was described as the heavy metals concentration in plant above ground part divided by this heavy metal element concentration in soil (Zu et al., 2005).

Pearson's correlation relationship analysis was undertaken to assess the relationship between concentrations of Pb, Zn, Cd, As, S and AMF colonization rates or spores density, between concentrations of Pb, Zn, Cd, As and S between shoots and roots at $P<0.05$ or $P<0.01$ level according to SPSS.

RESULTS

Dominant plant species on abandoned Pb/Zn mine land and mycorrhizal status of plants

Very little or no AMF colonization was observed in most of the plants examined (Table 1). But extensive mycorrhizal colonization was recorded in the roots of *Cirsium japonicum* Fisch. ex DC which was up to 89.3%. Spore density ranged from 55 to 1500 per 100.0 g soil, with an average of 576, for rhizosphere soils sampled in the present study, and it was not always related to the arbuscular mycorrhizal status of the corresponding plant.

Concentrations of Pb, Zn, Cd and As in soils

Pb, Zn, Cd and As concentrations in plants soils are shown in Table 2. For different plant species, Pb, Zn, Cd and As concentrations in soils were different. The average concentration of Pb, Zn, Cd and As in soils was 4853.0, 2718.6, 32.3 and 237.1 mg/kg, maximum was 11255.3 ± 1887.2, 5884.2 ± 531.9, 70.5 ± 0.8 and 498.9 ± 38.1 mg/kg in soils, minimum was 2491.8 ± 352.7, 919.2 ± 101.5, 18.7 ± 1.5 and 76.3 ± 1.9 mg/kg in soils.

Concentration and enrichment of heavy metals in plants

Pb, Zn, Cd and As concentrations in plants are shown in Table 2. For different plant species, Pb, Zn, Cd and As concentrations in plants were different. The average concentration of Pb, Zn, Cd and As in plants shoot was 452.0, 382.3, 51.8 and 121.1 mg/kg, maximum was 2323.4 ± 125.0, 1273.7 ± 140.0, 238.5 ± 19.5 and 326.7 ± 53.2 mg/kg and minimum was 118.7 ± 8.8, 86.2 ± 6.8, 8.7 ± 0.7 and 12.5 ± 3.8 mg/kg in shoot. The average concentration of Pb, Zn, Cd and As in plants root was 1079.5, 415.7, 88.3 and 116.8 mg/kg, maximum was 3480.0 ± 642.9, 1421.6 ± 251.3, 291.8 ± 11.2 and 390.6 ± 30.1 mg/kg and minimum was 206.5 ± 6.9, 99.1 ± 9.1, 8.7 ± 0.6 and 20.0 ± 1.2 mg/kg in root.

Translocation factor and enrichment coefficient of Pb, Zn, Cd and As are shown in Table 3. Translocation factor of Pb, Zn, Cd and As was 0.08~2.37, 0.28~2.55, 0.30~2.88 and 0.13~5.63 respectively, and those of 4, 7, 10 and 12 plant species were higher than 1. Enrichment coefficient of Pb, Zn, Cd and As was 0.02~0.23, 0.03~0.37, 0.23~5.37 and 0.09~1.97, respectively. Pb and Zn enrichment coefficients of all samples were lower than 1. Cd and As enrichment coefficients of 12 and 4 plants species were higher than 1.

Sulfur concentrations in plants

Sulfur concentrations in plants are shown in Table 4. S concentration was 0.08% (*Erianthus rufipilus* (Steud.) Griseb) to 0.99% (*Equisetum arvense* L) and 0.06% (*Eupatorium adenophorum* Spreng) to 0.71% (*Equisetum arvense* L), and the average S concentrations was 0.29 and 0.19% in plants shoot and root, respectively.

DISCUSSIONS

Relationship between AMF colonization rates or spore density and heavy metals concentrations in plants

Relatively high spore densities were found in rhizosphere soils of some possibly nonmycorrhizal plants (876 AMF spores in 100.0 g soil from the rhizosphere of *Ficus tikoua* Bur.), and they were sometimes higher than those

Table 1. Mycorrhizal infection rates of plants' roots and spore density in abandoned Pb/Zn mine land.

Plant specie	Root colonization rates[a] (%)	AMF Spore density[b]
Sonchus oleraceus L.	6.3	200
Eupatorium adenophorum Spreng	24.6	1004
Artemisia japonica Thunb.	12.5	1224
Cirsium japonicum Fisch. ex DC.	89.3	1500
Dichrocephala benthamii C. B. Clarke	10.5	240
Sonchus arvensis L.	30.5	216
Picris divaricata Vaniot	21.8	804
Eramopogon delavayi (Hack.) A. Camus	8.8	496
Erianthus rufipilus (Steud.) Griseb.	5.0	424
Pteris multifida Poir	42.5	1104
Dryopteris labordei (Christ) C. Chr.	8.3	404
Desmodium yunnanense Franch.	2.5	528
Origanum vulgare L.	48.3	756
Anemone vitifolia Buch.-Ham.	26.0	204
Fagopyrum dibotrys (D. Don) Hara	ND	204
Polygonum nepalense Meisn	ND	316
Equisetum arvense L.	ND	76
Ficus tikoua Bur.	ND	876
Rubus yunnanicus Ktze.	ND	428
Swertia punicea Hemsl.	ND	316
Phyllanthus urinaria L.	ND	776

a) ND, Not detected; b) number of AMF spores in 100.0 g soil from the corresponding plant rhizosphere.

associated with mycorrhizal plants (200, 204, 216 and 240 AMF spores in 100.0 g soil from the rhizosphere of *Sonchus oleraceus* L., *Anemone vitifolia* Buch.-Ham., *Sonchus arvensis* L. and *Dichrocephala benthamii* C. B. Clarke, respectively). In some cases, this may have been due to the fact that plant roots were interwoven in the same field sample so that mycorrhizal plants may have influenced sporulation in the rhizosphere of a plant that was non-mycorrhizal.

In this study, negative correlation of AMF colonization rates or spore density with heavy metals concentrations in mycorrhizal plants shoots and roots was found. But only the significantly negative correlation of AMF colonization rates or spore density with As concentrations in roots was found (Table 5).

AMF are ubiquitous soil microbes occurring in almost all habitats and climates, including metal contaminated soils (Sonjak et al., 2009). When Compared with stress-free conditions, polluted lands contain reduced population diversity and number of autochthonous AMF strains which are heavy metal tolerant (Del Val et al., 1999; Gonzalez-Guerrero et al., 2008). In general, inoculation of heavy metals tolerant AMF could reduce heavy metals toxicity effects on plant growing and improve plant tolerance to heavy metals contamination (Chen et al., 2005b; Vivas et al., 2006; Arriagada et al., 2010). In fact, there were many research achievements on the mechanisms of plants heavy metals tolerance enhanced by AMF. Studies with beneficial impacts of mycorrhizal colonization on plant growth had focused on their ability to enhance nutrient uptake, especially phosphorus in heavy metals contaminated soils (Vogel-Mikus et al., 2006; Chen et al., 2007). Furthermore, AMF colonisation of plants under heavy metal stress resulted in increase of binding capacity of root cell walls (Zhang et al., 2009), enhancement of antioxidant activities (Azcon et al., 2009) and expression of specific genes responsible for production of proteins (including GSH, PCs and MTs) (Ouziad et al., 2005; Rivera-Becerril et al., 2005; Hildebrandt et al., 2007), that increase the tolerance of plants to heavy metals stress. So, AMF were considered being essential for the survival and growth of some plants growing in heavy metals contaminated soils (Leung et al., 2007). The prospect of fungal symbionts existing in metal contaminated soils has important implications for restoration of abandoned metalliferous mine lands of metal contaminated soils use of local plant species in combination with AMF.

Relationship between sulfur and heavy metals concentrations in plants

In the present study, significantly positive correlation between S and Pb, Zn and As concentrations in plants shoots, and very significantly positive correlation between S and the four heavy metals concentrations in plants

Table 2. Pb, Zn, Cd and As concentrations in soils and plants from abandoned Pb/Zn mine land.

Plant specie	Pb (mg/kg)			Zn (mg/kg)		
	Shoot	Root	Soil	Shoot	Root	Soil
Sonchus oleraceus L.	2323.4±125.0^{A}	3480.0±642.9^{A}	11255.3±1887.2^{A}	1229.4±145.3^{A}	1421.6±251.3^{A}	5884.2±531.9^{A}
Eupatorium adenophorum Spreng	118.7±8.8^{G}	215.3±9.9^{HI}	2652.1±141.1^{FG}	92.1±6.7^{G}	195.1±17.4^{EFG}	919.2±101.5^{J}
Artemisia japonica Thunb.	174.9±9.9^{FG}	259.4±22.6^{GHI}	3520.1±379.5^{DEFG}	158.6±17.8^{EFG}	192.7±23.8^{EFG}	1654.2±102.8^{GHIJ}
Cirsium japonicum Fisch. ex DC.	581.2±40.9^{CD}	245.1±3.9^{GHI}	2491.8±352.7^{G}	316.3±58.7^{DEF}	132.1±9.6^{EFG}	1236.7±116.7^{IJ}
Dichrocephala benthamii C. B. Clarke	584.6±48.3^{CD}	988.1±33.9^{EFGH}	6927.2±304.7^{BC}	875.5±123.2^{B}	561.9±19.9^{BC}	2345.0±208.8^{FGH}
Sonchus arvensis L.	678.0±34.5^{C}	2165.1±336.1^{BC}	6103.1±519.7^{CD}	1273.7±140.0^{A}	498.6±60.9^{BCD}	3728.3±299.8^{DE}
Picris divaricata Vaniot	307.4±29.2^{EF}	1121.6±54.1^{DE}	4723.3±833.5^{CDEFG}	208.6±2.1^{EFG}	367.6±24.9B^{CDEF}	3880.8±453.5^{CDE}
Eramopogon delavayi (Hack.) A. Camus	161.4±21.7^{FG}	312.9±51.4^{FGHI}	5683.9±332.3^{CDE}	95.8±9.4^{FG}	347.5±34.5^{BCDEFG}	3102.5±118.4^{EF}
Erianthus rufipilus (Steud.) Griseb.	158.4±13.0^{FG}	281.9±19.3^{GHI}	2799.3±400.7^{FG}	174.2±13.6^{EFG}	274.5±13.7^{DEFG}	4772.5±445.9^{BC}
Pteris multifida Poir	284.4±11.6^{FG}	2253.2±230.3^{BC}	3331.8±253.8^{EFG}	185.6±6.9^{EFG}	514.1±44.6^{BCD}	1422.5±79.4^{HIJ}
Dryopteris labordei (Christ) C. Chr.	201.7±15.1^{FG}	724.9±82.9^{EFGHI}	5220.5±428.3^{CDEF}	86.2±6.8^{G}	129.9±33.5^{FG}	1700.8±138.2^{GHIJ}
Desmodium yunnanense Franch.	1141.4±125.9^{B}	1018.6±46.6^{EFG}	9082.6±937.2^{AB}	567.4±44.6^{C}	565.7±54.3^{B}	2314.2±56.1^{FGH}
Origanum vulgare L.	213.5±3.1^{FG}	1087.1±100.8^{DEF}	2650.5±432.9^{FG}	184.4±3.3^{EFG}	396.1±16.4^{BCDE}	1723.3±297.6^{GHIJ}
Anemone vitifolia Buch.-Ham.	193.4±12.0^{FG}	206.5±6.9^{I}	2836.6±130.3^{FG}	173.9±20.0^{EFG}	166.3±11.3^{EFG}	2387.5±176.8^{FG}
Fagopyrum dibotrys (D. Don) Hara	134.1±5.9^{FG}	241.8±22.5^{HI}	3361.8±274.5^{EFG}	132.9±12.3^{FG}	156.9±17.5^{EFG}	1920.8±335.2^{GHI}
Polygonum nepalense Meisn	153.9±6.9^{FG}	1840.1±139.3^{CD}	3855.0±274.3^{DEFG}	189.9±53.6^{EFG}	313.9±33.1^{BCDEFG}	1361.7±224.2^{IJ}
Equisetum arvense L.	231.0±53.8^{FG}	2920.7±382.7^{AB}	9475.1±1323.9^{AB}	364.5±60.3^{CDE}	1217.9±128.7^{A}	4335.8±43.5^{BCD}
Ficus tikoua Bur.	150.5±6.5^{FG}	1200.2±117.3^{DE}	3989.1±624.1^{DEFG}	127.5±13.3^{FG}	298.2±22.2^{CDEFG}	1585.8±77.2^{GHIJ}
Rubus yunnanicus Ktze.	664.4±49.8^{C}	590.9±35.7^{EFGHI}	4407.6±807.1^{CDEFG}	850.2±5.7^{B}	333.5±34.9^{BCDEFG}	5070.8±119.0^{AB}
Swertia punicea Hemsl.	561.4±33.2^{CD}	1302.7±276.2^{DE}	4469.6±396.1^{CDEFG}	510.3±45.0^{CD}	545.8±64.5^{BC}	3639.2±178.3^{DE}
Phyllanthus urinaria L.	475.1±43.3^{DE}	213.8±31.6^{HI}	3077.3±457.8^{EFG}	231.6±7.8^{EFG}	99.1±9.1^{G}	2104.2±118.6^{GHI}

Capital letters indicate very significant difference between different plants at $p < 0.01$ by LSD's t-test.

roots was observed (Table 6). This indicated the important role of S nutrition in plant tolerance and accumulation of heavy metals.

Sulfur-containing compounds like GSH, PCs and MTs could bind toxic heavy metals in thiolate complexes (Cobbett and Goldsbrough, 2002). Many studies confirmed enhancement of heavy metal accumulation through over expression of enzymes and genes involved in S-assimilation and GSH/PCs biosynthesis (Dominguez-Solis et al., 2004; Wawrzynski et al., 2006; Guo et al., 2008). Increasing sulfur supply could enhance tolerance to As and its accumulation in *Hydrilla verticillata* (Lf) Royle (Srivastava and D'Souza, 2009), significantly increase Cd concentrations in shoot of *Tagetes erecta* L. with increase of sulfur levels in the culture solution (Feng et al., 2009). However, excessive sulfur supply reduces cadmium accumulation in brown rice (Fan et al., 2010).

These results indicated that S nutrition play an important role in heavy metals tolerance and accumulation of plants.

Relationship between S concentrations and AMF status of plants grown in heavy metals contaminated soils

There were no significant correlations between S concentrations and AMF status in this study (Table 6). In consideration of the importance of S

Table 2. Contd.

Plant specie	Cd (mg/kg)			As (mg/kg)		
	Shoot	Root	Soil	Shoot	Root	Soil
Sonchus oleraceus L.	153.1±34.2^{B}	291.8±11.2^{A}	62.0±7.9^{AB}	275.0±21.5^{ABC}	239.3±29.3^{BC}	370.7±47.4^{BC}
Eupatorium adenophorum Spreng	14.4±3.7^{G}	10.4±1.3^{I}	52.4±1.3^{BC}	35.3±2.9^{G}	39.2±5.1^{G}	135.0±11.0^{FG}
Artemisia japonica Thunb.	14.8±2.0^{G}	10.2±2.1^{I}	22.8±1.5^{DE}	84.5±15.0^{FG}	43.8±11.0^{FG}	374.8±47.4^{BC}
Cirsium japonicum Fisch. ex DC.	12.6±0.9^{G}	11.5±1.5^{I}	20.2±2.2^{E}	56.2±14.5^{FG}	34.9±11.1^{G}	117.5±13.0^{FG}
Dichrocephala benthamii C. B. Clarke	65.9±6.0^{CDEF}	133.5±10.3^{DEF}	21.2±1.3^{DE}	279.7±25.4^{AB}	240.7±45.4^{BC}	206.4±27.7^{EF}
Sonchus arvensis L.	97.8±17.3^{C}	116.8±20.5^{EFG}	22.2±0.6^{DE}	326.7±53.2^{A}	219.1±66.0^{BCD}	166.0±10.3^{FG}
Picris divaricata Vaniot	100.6±2.4^{C}	197.1±25.7^{BC}	18.7±1.5^{E}	130.5±9.9^{EF}	171.9±22.0^{CDE}	100.8±15.3^{G}
Eramopogon delavayi (Hack.) A. Camus	10.8±1.1^{G}	14.5±2.1^{I}	32.6±8.2^{D}	26.1±2.6^{G}	21.5±4.7^{G}	284.8±27.9^{CDE}
Erianthus rufipilus (Steud.) Griseb.	11.1±1.1^{G}	10.5±1.7^{I}	45.5±11.3^{C}	18.3±2.3^{G}	20.0±1.2^{G}	124.1±13.7^{FG}
Pteris multifida Poir	43.8±1.7^{DEFG}	135.5±23.1^{DEF}	32.9±3.1^{D}	28.8±6.6^{G}	22.8±8.7^{G}	281.5±30.9^{CDE}
Dryopteris labordei (Christ) C. Chr.	29.1±1.3^{FG}	72.3±1.5^{GH}	21.5±1.0^{DE}	55.6±14.4^{FG}	37.9±9.1^{G}	330.2±22.5^{CD}
Desmodium yunnanense Franch.	24.3±4.8^{G}	175.5±24.4^{BCD}	22.7±3.2^{DE}	193.3±39.3^{CDE}	272.1±36.8^{B}	202.2±27.1^{EF}
Origanum vulgare L.	8.7±0.7^{G}	8.7±0.6^{I}	18.8±3.7^{E}	36.7±3.4^{G}	45.1±2.9^{FG}	76.3±1.9^{G}
Anemone vitifolia Buch.-Ham.	34.7±0.6^{EFG}	32.6±3.6^{HI}	18.8±1.5^{E}	187.5±26.4^{DE}	135.6±5.5^{DEF}	111.6±17.4^{FG}
Fagopyrum dibotrys (D. Don) Hara	12.1±0.3^{G}	9.8±0.2^{I}	52.3±3.5^{BC}	21.6±4.1^{G}	21.3±4.1^{G}	444.8±35.0^{AB}
Polygonum nepalense Meisn	17.7±1.2^{G}	15.7±0.9^{I}	19.9±2.1^{E}	95.2±30.3^{FG}	28.3±6.1^{G}	310.8±11.3^{CD}
Equisetum arvense L.	70.6±8.4^{CDE}	227.2±35.0^{B}	70.5±0.8^{A}	130.9±9.8^{EF}	252.0±44.2^{BC}	498.9±38.1^{A}
Ficus tikoua Bur.	12.6±1.3^{G}	142.8±20.5^{CDE}	19.2±2.7E	12.5±3.8^{G}	95.1±11.7^{EFG}	112.7±3.4^{FG}
Rubus yunnanicus Ktze.	238.5±19.5^{A}	82.8±2.6^{FGH}	55.7±1.5^{BC}	247.1±41.7^{ABCD}	43.9±4.0^{FG}	328.2±22.5^{CD}
Swertia punicea Hemsl.	36.1±6.5^{EFG}	119.5±13.5^{EFG}	22.6±2.1^{DE}	227.3±24.6^{BCD}	390.6±30.1^{A}	268.6±25.1^{DE}
Phyllanthus urinaria L.	78.8±11.2^{CD}	35.2±1.9^{HI}	25.0±1.0^{DE}	74.6±7.9^{FG}	77.6±9.4^{FG}	132.5±13.6^{FG}

nutrition for plants heavy metals tolerance, effects of AMF on plants S nutrition under heavy metals stress attracted researchers' attention. Galli et al. (1995) reported that there was an increase in the contents of cystein, gamma EC and GSH in the mycorrhizal maize roots under Cu stress. Rivera-Becerril et al. (2005) reported that *hgsh2* (coding for hGSH synthetase) gene expression was enhanced by *Glomus intraradices* colonization of pea roots, particularly in the presence of Cd (+86%), *PsMT*$_A$ (coding for MTs), *gsh2* (coding for GSH synthetase) and *gr* (coding for glutathione reductase) gene expression and thiol groups was not modified by mycorrhizal colonization.

Also, improvement of S concentrations by AMF had been reported and demonstrated (Banerjee et al., 2003; Guo et al., 2007; Allen and Shachar-Hill, 2009). However, under heavy metals stress, the mechanisms about effects of AMF on plants S nutrition and metabolism were still unknown. The relationship between S nutrition and AMF under

Table 3. Translocation factor and enrichment coefficient of heavy metals from abandoned Pb/Zn mine land to plants.

Plant specie	Translocation factor				Enrichment coefficient			
	Pb	Zn	Cd	As	Pb	Zn	Cd	As
Sonchus oleraceus L.	0.67	0.86	0.52	1.15	0.21	0.21	2.47	0.74
Eupatorium adenophorum Spreng	0.55	0.47	1.38	0.90	0.04	0.10	0.27	0.26
Artemisia japonica Thunb.	0.67	0.82	1.45	1.93	0.05	0.10	0.65	0.23
Cirsium japonicum Fisch. ex DC.	2.37	2.39	1.09	1.61	0.23	0.26	0.62	0.48
Dichrocephala benthamii C. B. Clarke	0.59	1.56	0.49	1.16	0.08	0.37	3.11	1.36
Sonchus arvensis L.	0.31	2.55	0.84	1.49	0.11	0.34	4.41	1.97
Picris divaricata Vaniot	0.27	0.57	0.51	0.76	0.07	0.05	5.37	1.30
Eramopogon delavayi (Hack.) A. Camus	0.52	0.28	0.74	1.21	0.03	0.03	0.33	0.09
Erianthus rufipilus (Steud.) Griseb.	0.56	0.63	1.05	0.92	0.06	0.04	0.24	0.15
Pteris multifida Poir	0.13	0.36	0.32	1.26	0.09	0.13	1.33	0.10
Dryopteris labordei (Christ) C. Chr.	0.28	0.66	0.40	1.47	0.04	0.05	1.35	0.17
Desmodium yunnanense Franch.	1.12	1.00	0.14	0.71	0.13	0.25	1.07	0.96
Origanum vulgare L.	0.20	0.47	1.00	0.82	0.08	0.11	0.46	0.48
Anemone vitifolia Buch.-Ham.	0.94	1.05	1.07	1.38	0.07	0.07	1.85	1.68
Fagopyrum dibotrys (D. Don) Hara	0.55	0.85	1.24	1.01	0.04	0.07	0.23	0.05
Polygonum nepalense Meisn	0.08	0.61	1.12	3.36	0.04	0.14	0.89	0.31
Equisetum arvense L.	0.08	0.30	0.31	0.52	0.02	0.08	1.00	0.26
Ficus tikoua Bur.	0.13	0.43	0.09	0.13	0.04	0.08	0.65	0.11
Rubus yunnanicus Ktze.	1.12	2.55	2.88	5.63	0.15	0.17	4.28	0.75
Swertia punicea Hemsl.	0.43	0.93	0.30	0.58	0.13	0.14	1.60	0.85
Phyllanthus urinaria L.	2.22	2.34	2.24	0.96	0.15	0.11	3.15	0.56

heavy metals stress would be complicated and need further study. Research on effects of AMF on the significant enzymes and gene involved in sulfur uptake, activation and assimilation, GSH and PCs metabolism, such as sulfate transporters (HAST and LAST), ATP sulfurylase, O-acetylserine sulfhydrylase, serine acetyl transferase, γ-glutamylcysteine synthetase, phytochelatin synthase (Mendoza-Cozatl et al., 2005), would contribute to clarifying the way to produce effects on plants S nutrition by AMF under heavy metals stress, and then to expound the mechanisms of plants heavy metals tolerance enhanced by AMF.

Heavy metals concentrations in plants grown in heavy metals contaminated soils

Twenty one plants were collected and analyzed (Table 2). Many of these species showed heavy metals concentrations much higher than the normal levels for plants, but none of the plants analyzed could be classified as hyperaccumulators. Similar results were obtained for high heavy metals concentrations in plants grown in soils surrounding an abandoned mine in NW Madrid (Spain) (Moreno-Jiménez et al., 2009). This plants accumulation and transport of heavy metals from heavy metals contaminated soils could have consequential impact on the environment and the health of the local people.

And in other abandoned mine area, several hyperaccumulators such as *Anthyllis vulneraria*, *Thlaspi caerulescens*, *Iberis intermedia* and *Silene latifolia* in the Les Malines Mining District (Southern France) (Escarre et al., 2011); Pteris vittata in Guangxi (South China) (Wang et al., 2012); *Houttuynia cordata* Thunb., *Pteris vittata* L., *Ageratum houstonianum* Mill. and *Potamogeton oxyphyllus* Miq. in Northern Vietnam were found (Ha et al., 2011). These plants could be chosen as pioneer of phytoremediations to restore abandoned mine land. Moreover, in order to utilize native plants for future restoration schemes, more detailed investigations should be conducted to improve our understanding of plant adaptation to abandoned mine land, and then identify the potential role of AMF in phytostabilizing metals in contaminated environments, in enhancing plants tolerance to heavy metals and in stimulating plant growth, so that both plant and fungal symbionts could be used for successful restoration.

Conclusions

There was significant correlation between S and Pb, Zn, Cd and As concentrations in the plants growing on abandoned Pb/Zn mine land. This indicated the importance of S in the heavy metals accumulation by plants in abandoned Pb/Zn mine land.

Table 4. S concentrations of plants grown in abandoned Pb/Zn mine land.

Plant specie	Shoot (%)	Root (%)
Sonchus oleraceus L.	0.621 ± 0.093^{B}	0.422 ± 0.081^{B}
Eupatorium adenophorum Spreng	0.102 ± 0.006^{IJ}	0.063 ± 0.005^{D}
Artemisia japonica Thunb.	0.251 ± 0.016^{DEFGHIJ}	0.087 ± 0.011^{D}
Cirsium japonicum Fisch. ex DC.	0.310 ± 0.013^{CDEFG}	0.119 ± 0.020^{CD}
Dichrocephala benthamii C. B. Clarke	0.294 ± 0.051^{CDEFGH}	0.184 ± 0.012^{CD}
Sonchus arvensis L.	0.418 ± 0.011^{CD}	0.134 ± 0.013^{CD}
Picris divaricata Vaniot	0.377 ± 0.036^{CDE}	0.133 ± 0.028^{CD}
Eramopogon delavayi (Hack.) A. Camus	0.131 ± 0.011^{HIJ}	0.120 ± 0.008^{CD}
Erianthus rufipilus (Steud.) Griseb.	0.084 ± 0.004^{J}	0.078 ± 0.003^{D}
Pteris multifida Poir	0.114 ± 0.010^{IJ}	0.284 ± 0.092^{BC}
Dryopteris labordei (Christ) C. Chr.	0.117 ± 0.008^{HIJ}	0.078 ± 0.014^{D}
Desmodium yunnanense Franch.	0.455 ± 0.108^{BC}	0.147 ± 0.007^{CD}
Origanum vulgare L.	0.279 ± 0.020^{CDEFGHI}	0.195 ± 0.029^{CD}
Anemone vitifolia Buch.-Ham.	0.280 ± 0.108^{CDEFGHI}	0.100 ± 0.011^{D}
Fagopyrum dibotrys (D. Don) Hara	0.185 ± 0.034^{FGHIJ}	0.090 ± 0.007^{D}
Polygonum nepalense Meisn	0.315 ± 0.035^{CDEFG}	0.135 ± 0.031^{CD}
Equisetum arvense L.	0.989 ± 0.061^{A}	0.706 ± 0.140^{A}
Ficus tikoua Bur.	0.138 ± 0.007^{GHIJ}	0.078 ± 0.010^{D}
Rubus yunnanicus Ktze.	0.331 ± 0.045^{CDEF}	0.095 ± 0.003^{D}
Swertia punicea Hemsl.	0.233 ± 0.027^{EFGHIJ}	0.621 ± 0.068^{A}
Phyllanthus urinaria L.	0.138 ± 0.007^{GHIJ}	0.078 ± 0.004^{D}

Capital letters indicate very significant difference between different plants at $p < 0.01$ by LSD's t-test.

Table 5. Relationship among Pb, Zn, Cd, As, S and AMF status of plants.

AMF status	Element	Shoot		Root	
		r	P	r	P
AMF colonization rates	Pb	-0.176	0.547	-0.087	0.768
	Zn	-0.135	0.646	-0.291	0.313
	Cd	-0.210	0.472	-0.314	0.275
	As	-0.246	0.396	-0.338	0.237
	S	-0.035	0.907	-0.007	0.981
AMF spores density	Pb	-0.330	0.249	-0.311	0.280
	Zn	-0.503	0.067	-0.415	0.140
	Cd	-0.444	0.112	-0.317	0.192
	As	-0.610	0.021*	-0.562	0.036*
	S	-0.286	0.322	-0.185	0.527

N =14. "*" significant at $P < 0.05$ level according to SPSS.

Table 6. Relationship among Pb, Zn, Cd, As and S of plants.

Plant part	Heavy metal	r	P
Shoot	Pb	0.440	0.046*
	Zn	0.475	0.029*
	Cd	0.391	0.080
	As	0.495	0.022*
Root	Pb	0.684	0.001**
	Zn	0.776	0.000**
	Cd	0.598	0.004**
	As	0.677	0.001**

N = 21. "*" significant at $P<0.05$, "**" significant at $P<0.01$ level according to SPSS.

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