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RESEARCH ARTICLE

Metal contamination status of the soil-plant system and effects on the soil microbial community near a rare metal recycling smelter

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Abstract Four heavy metals (Cd, Cu, Pb and Zn), two metalloids (As and Sb) and two rare metals (In and Tl) were selected as target elements to ascertain their concentrations and accumulation in the soil-plant system and their effects on the structure of the soil microbial community in a typical area of rare metal smelting in south China. Twenty-seven soil samples 100, 500, 1000, 1500 and 3000 m from the smelter and 42 vegetable samples were collected to determine the concentrations of the target elements. Changes in soil microorganisms were investigated using the Biolog test and 454 pyrosequencing. The concentrations of the eight target elements (especially As and Cd) were especially high in the topsoil 100 m from the smelter and decreased markedly with increasing distance from the smelter and with increasing soil depth. Cadmium bio-concentration factors in the vegetables were the highest followed by Tl, Cu, Zn, In, Sb, Pb, and then As. The concentrations of As, Cd and Pb in vegetables were 86.7, 100 and 80.0 %, respectively, over the permissible limits and possible contamination by Tl may also be of concern. Changes in soil microbial counts and average well colour development were also significantly different at different sampling distances from the smelter. The degree of tolerance to heavy metals appears to be fungi > bacteria > actinomycetes. The 454 pyrosequencing indicates that long-term metal contamination from the smelting activities has resulted in shifts in the composition of the soil bacterial community.

Keywords Bacterial community · BCF · Rare metals · Soil contamination · South China

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Introduction

Contamination of agricultural land and groundwater by heavy metals is usually linked to human activities. A major problem with heavy metals is that they cannot be biologically degraded and they therefore remain in the environment for long periods of time if they are not removed. Soil metal pollution with potentially toxic elements is receiving increasing attention worldwide. Metal smelting activities are major pollution sources leading to high levels of soil metal pollution near the smelters and threatening adverse effects on plant growth and soil microorganisms (Cui et al. 2004; Kachenko and Singh 2006; Wang et al. 2007a; Jia et al. 2013). Most previous studies have focused on smelters of commonly investigated metals such as Cu (Cui et al. 2004). However, in addition to the large quantities of heavy metals that can be released by human activities, increasing amounts of rare metals such as gallium (Ga), indium (In), thallium (Tl), and germanium (Ge) can be released simultaneously into the environment, leading to contamination of the

surrounding atmosphere, surface waters, groundwaters and soils (Álvarez-Ayuso et al. 2013; Baceva et al. 2014; Li et al. 2014). Rare metals have high monetory values and their recycling from waste materials is a very profitable activity. The effects of rare metals such as In and Tl on environmental and human health have recently raised concerns (Madejón 2013; Jia et al. 2013).

Indium and Tl are potentially toxic elements that are widely distributed in the natural environment at very low concentrations but they may be mobilized by the combustion of fuels and other industrial processes and their tendency to persist in soils depends on soil type (Madejón 2013). These elements are highly toxic to living organisms and are relatively easily taken up by food plants to enter the food chain and thus may represent a chronic risk to human health. Indium, in particular, can impair many organs such as the lungs, testicles, and spleen, and thus induce dysmorphosis and embryotoxis (Ayadi et al. 2014). Moreover, soil metal pollution is a serious concern for animal and human health and also has deleterious effects on bacteria. It has been demonstrated repeatedly that heavy metals affect microorganisms in soils in various ways. They shift the structure of microbial populations, reduce their diversity, and affect the species composition, reproduction and activity of indigenous microorganisms (Wyszkowska et al. 2013; Mikryukov et al. 2015; Chen et al. 2015). Some studies have focused on soil metal contamination and the effects on plants and microbes around mineral mining and smelting areas where high levels of metal pollution occur in soils and plants (Cui et al. 2004; Kachenko et al. 2006; Wang et al. 2007a). However, contamination of both soils and vegetables in specific areas such as near rare metal recycling smelters has been little investigated and the effects of contamination by metals including rare metals on soil microbial communities require investigation.

Rare metal recycling from industrial wastes has been practised in Yongxing city, Hunan province, south China for many years but there is little information on the environmental effects of the recycling processes. We hypothesized that rare metal smelters have polluting effects on the local environment and thus represent a risk to human health via the food chain or to the local environment in terms of soil quality. The objectives of the present study were therefore to investigate the levels of contamination of the metal(loid)s Cu, Zn, Pb, Cd, As and Sb and also the rare metals In and Tl near a rare metal recycling smelter to reveal the influence of metal contamination on the soil microbial community, to compare the changes in concentrations of polluting elements in both soil and vegetable samples with increasing distance from the smelter, and to characterize the effects of the pollutants on soil microorganisms and the risk to human health and thus provide useful information for controlling the metal pollution of agricultural soils.

Materials and methods

Sample collection and pre-treatment

All the soil (Typic Fe-accumuli-Stagnic Anthrosols) and vegetable samples were collected from the area affected by a typical rare metal recycling smelter (26° 05′ 28″ N, 113° 05′ 59″ E) at an altitude of 350 m above sea level within the mid-subtropical humid monsoon climatic zone. This smelter has recycled rare metals including Au, Ag, Se and In from industrial wastes for 26 years. Local farmers grow vegetables in the vicinity of the smelter. Topographic features were carefully explored before sampling on 21 March 2013 in Hunan province, south China. Samples of soil and vegetables were collected on 22 March 2013 from a transect along the prevailing wind direction (NW to SE in winter, SE to NW in summer) at locations that were different distances (100, 500, 1000, 1500 and 3000 m) from the smelter as shown schematically in Fig. 1. At each distance, a soil sample was removed from the line and two samples were removed from each side of the line at locations 20 m from the line. Within a circle of 5 m at each location, a separate core was collected from each of three sampling points using a soil corer and these were mixed thoroughly to give a composite soil sample of about 1 kg. A GPS positioning system was employed for the accurate determination of the sampling distances from the smelter. Thus, the composite soil samples, each 1 kg fresh weight, were collected in triplicate from each distance from the smelter from the top 20 cm of the soil profile. In addition, at 3000 m distance from the smelter, additional soil samples were taken from the same sampling locations at depths 20–40, 40–60, 60–80 and 80–100 cm in the soil profile, but only from one sampling point so that these deeper samples were not composite samples and comprised about 330 g fresh soil. Soil subsamples were obtained by the standard quartering method and were then numbered and stored in sample bags before transfer to the laboratory.

At each soil sampling location, the edible parts of any vegetables growing within a distance of 1 m were collected to give three replicate plant samples for every species at each distance from the smelter. The same agricultural production system was in use throughout the area, and factors such as agricultural management were therefore not considered. A total of 27 soil samples and 42 vegetable samples comprising 10 vegetable species or varieties were collected.

About 100 g of each soil sample were air-dried at room temperature and passed through 0.15-mm and 0.85-mm sieves for analysis of total metals and other soil properties, respectively. The texture of the local soil was (dry weight basis) 43.5 % clay, 40.7 % silt, 15.8 % sand, and 2.98 % organic matter. Because of the small area of uniform soil type sampled, the soil physicochemical properties 3000 m from the smelter were considered to be typical of the whole area. The

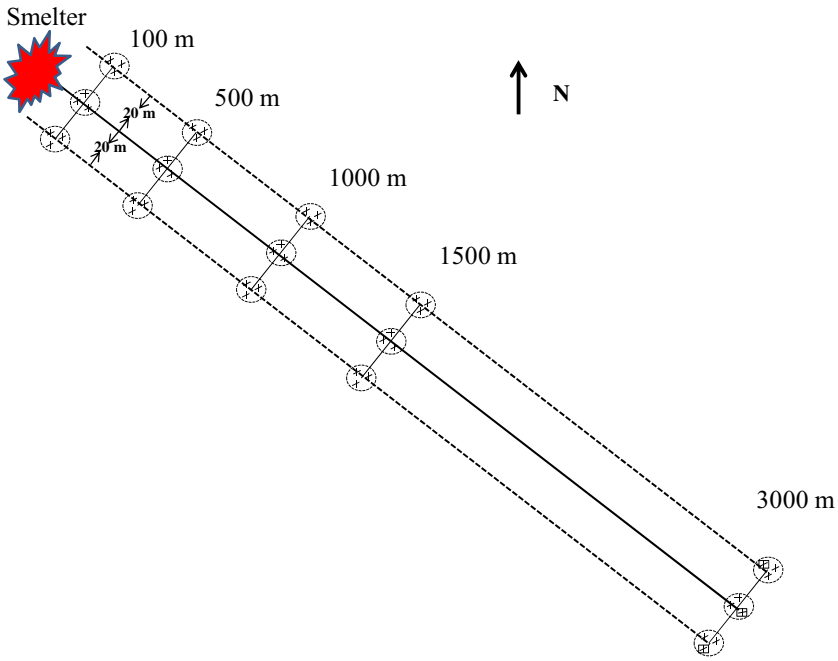

Fig. 1 Schematic representation of the sampling strategy. Sampling locations were at specific distances along the prevailing wind direction and perpendicular on both sides at a distance of 20 m. Within each sampling location, three separate soil cores were removed from randomly selected sampling points. These were mixed to give three 1-kg composite replicate samples per location (i.e. at each distance from the smelter). Additional samples were collected from the three replicate locations 3000 m from the smelter from deeper in the soil profile. These consisted of one soil core at each location

soil pH (1:2.5 soil:water) was 5.20 and the CEC, exchangeable Ca and exchangeable Mg were 11.4, 3.10 and 0.25 cmol(+)kg^{-1}, respectively (Chinese Society of Soil Agricultural Chemical Professional Committee). After transport to the laboratory, all the fresh samples of edible parts of the vegetables were immediately washed with tap water, rinsed with distilled water and wiped dry with paper tissue, then dried at 105 °C in an oven for 30 min to destroy enzymes and then oven-dried to constant weight at 85 °C to minimize the volatilization of organic constituents. The plant samples were then homogenized with a stainless steel mill for the determination of the target metal concentrations. The bioconcentration factors (BCF) of the different elements in the edible parts of vegetables were calculated by dividing the plant metal concentrations on dry weight basis (determined) by the soil metal concentrations.

Determination of the eight target elements

Concentrations of Cu, Zn, Pb, Cd, As, Sb, In, and Tl in soil samples were determined after digestion with 4:1 *v*/*v* HCl/HNO_3 and concentrations in the plant samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X7, Thermo Scientific, Waltham, MA) after digestion with 3:2 *v*/*v* HNO_3/$HClO_4$. Certified reference materials GBW07404 (GSS-4) and GBW07603 (GSV-2) (provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei province, China) were used as part of the quality control process in soil and plant analysis, respectively. The results for the standard reference materials were all within the published confidence intervals.

All reagents were guaranteed laboratory reagents and all equipment was soaked in 10 % HNO_3 for > 24 h before washing with deionized water and drying. Concentrations of different elements in all plant samples were calculated by fresh weight (FW). Blank controls and national standard reference materials were included in each batch of samples analysed and the number of parallel samples was ≥ 10 %.

Microbial counts and community level physiological profiling (CLPP) analysis

Bacteria, actinomycetes and fungi were enumerated on LB, modified Gause's No. 1 and modified Martin agar plates, respectively, following Shen et al. (1989). The community level physiological profiling (CLPP) of the microbial communities was examined using Eco microplates (BIOLOG Inc.) according to the method of Garland and Mills (1991). The average well colour development (AWCD) values of the BIOLOG data were calculated according to Zak et al. (1994).

DNA extraction, PCR and pyrosequencing

Community DNA was extracted from six samples of soil collected to a depth of 0–20 cm at 100 and 3000 m (three replicates from each site) distance from the smelter using the FastDNA SPIN for Soil Kit (MP Biomedicals, Illkirch, France), according to the manufacturer's instructions. Extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). The integrity of the DNA extracted from the soil was confirmed by electrophoresis in a 0.8 % agarose gel with 0.5

Tris-boric acid (TBE) buffer (45 mM Tris-borate; 1 mM EDTA, pH 8.0).

PCR amplification of the hypervariable V4-V5 region of the 16S rRNA gene was performed using the 8 bp key-tagged eubacterial primers 515F and 907R (http://wildpigeon.cme.msu.edu/pyro/help.jsp). PCR mixtures contained 1 mM each primer (Integrated DNA Technologies, Coralville, IA), 1.8 mM $MgCl_2$, 0.2 M dNTPs, 1.5× bovine serum albumin (New England Biolabs, Ipswich, MA), 1 U of FastStart high-fidelity PCR system enzyme blend (Roche Applied Science, Indianapolis, IN) and 10 ng of the DNA template. The PCR program consisted of a 3-min initial denaturation at 95 °C; 30 cycles of 95 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min, and a 4-min final extension at 72 °C. For each sample, amplicons of three replicate PCRs were recovered using a QIA quick gel extraction kit followed by a QIA quick PCR purification kit (Qiagen, Germantown, MD). Equimolar amplicons were combined and submitted to pyrosequencing using a Genome Sequencer FLX system (454; Life Sciences, Branford, CT) at the Majorbio Medicine Science and Technology Co Ltd in Shanghai, China.

Statistical analysis

Sequencing analysis was conducted using the microbial ecology community software program Mothur 1.35.0 (Schloss et al. 2011). The reads were processed by removing barcode and primer, only accepting reads with an average quality score > 25 and read lengths > 250 bp. A total of about 73680 partial 16S rRNA sequences were obtained from the six soil samples. Unique sequences were aligned to the reference SILVA database by default settings and chimeric sequences were removed. Sequences passing these screens were classified using a ribosomal database project naïve Bayesian rRNA classifier with a confidence of 80 % (Wang et al. 2007b). At each taxonomic level, the proportion of sequence identities was calculated as a percentage of all sequences classified in that sample. OTUs (operational taxonomic units) were classified at similarities of 97 % after normalization to 5353 sequences per sample. The α-diversity indices, namely observed OTUs (Sobs), Chao, Ace, InvSimpson and Shannon, were calculated using MOTHUR. The data were plotted using Microsoft Excel 2007 and the statistical analysis was performed using the SPSS v.16.0 software package.

Results

Concentrations of the eight target elements in soil

Concentrations of the eight target elements in the top 20 cm arable layer at distances of 100, 500, 1000, 1500 and 3000 m from the smelter are listed in Table 1. The concentrations of the different pollutants declined markedly with increasing distance from the pollution source. Most severe contamination occurred at the nearest sampling location (100 m) where the concentrations of As, Cd, Cu, In, Pb, Sb, Tl and Zn were, respectively, 5.7, 12.3, 5.2, 11.3, 7.8, 25, 19, and 3.1 times those at 500 m. All of the metal(loid) concentrations (Cu, Zn, Pb, Cd, As) at 100 m far exceeded the Chinese second grade standard soil quality values (GB15618-1995) while only As (56.9 mg kg^{-1}) and Cd (4.18 mg kg^{-1}) exceeded the standard values (As 30 mg kg^{-1}, Cd 0.30 mg kg^{-1}) at a distance of 3000 m from the smelter. In addition, as shown in Table 1, soil Sb concentrations were 223 and 7.43 mg kg^{-1} at 100 and 3000 m from the smelter. Soil In and Tl concentrations were 1.38 and 1.08 mg kg^{-1}, respectively, 100 m from the smelter. These two values are higher than or near to the maximum levels of the natural concentrations of In and Tl in topsoils of China, with ranges of 0.022 to 0.167 and 0.292 to 1.172 mg kg^{-1}, respectively, according to Qi et al. (1992). The concentrations of the different elements in the soil profile 3000 m from the smelter are shown in Table 1. Elemental concentrations always appeared to be highest down to 20 cm depth. This indicates again that the sources of the pollutants in soil are anthropogenic.

Eight target elements in the edible parts of vegetables

The concentrations of the eight target elements in 42 samples (including 10 vegetable types) collected within 3000 m of the smelter and the calculated bio-concentration factors (BCF) are listed in Table 2. According to the Chinese contamination value limits for food in terms of fresh weight (FW) (GB2762-2005), the maximum limit of As for vegetables is 0.05 mg kg^{-1}, Cd limits for corm vegetables, leafy vegetables and other vegetables are 0.1, 0.2 and 0.05 mg kg^{-1}, and Pb limits for corm, leafy and other vegetables are 0.3, 0.3 and 0.1 mg kg^{-1}. As shown in Table 2, the percentages of plants with excessive concentrations of As, Cd and Pb (data with lines) above the standards in the edible parts of sampled vegetables were 86.7, 100 and 80 %, respectively. Concentrations of Sb in the sampled vegetables over the standard value were found only in the garlic samples collected 500 m from the smelter. The highest concentration of Tl was found in cabbage with about 0.17 mg kg^{-1} (FW) in cabbage 1000 m from the smelter.

The values in parentheses in Table 2 are the element BCF of some vegetables and the values with italics and bold fonts indicate that the BCF exceeded 1.0, for example Tl in cabbage, eggplant and water spinach, Cd in baby Chinese cabbage, cabbage sprout, lettuce, and water spinach, In in eggplant, and In and Zn in water spinach, indicating that these vegetables have a relatively high capacity to accumulate metals. In general, the order of the BCF values of the polluting elements in different vegetables was Cd > Tl > Cu > Zn > In > Sb > Pb > As. The

Table 1 Concentrations of the eight target elements in the top 20 cm of the soil profile at different distances from the smelter and the soil profile at 3000 m (mg kg^{-1})

Distance from smelter (m)	Soil depth (cm)	As	Cd	Cu	In	Pb	Sb	Tl	Zn
100	0–20	564 ± 29a	28.7 ± 11.3a	146 ± 58a	1.38 ± 0.08a	751 ± 62a	223 ± 18a	1.08 ± 0.06a	379 ± 42a
500	0–20	99.0 ± 9.3b	2.34 ± 0.50b	28.0 ± 1.7b	0.12 ± 0.02b	96.4 ± 12.3b	8.91 ± 0.24b	0.57 ± 0.01b	121 ± 12b
1000	0–20	84.6 ± 12.3b	7.24 ± 4.06b	24.4 ± 4.2bc	0.14 ± 0.12b	85.6 ± 29.5b	8.21 ± 6.70b	0.33 ± 0.02c	149 ± 26b
1500	0–20	65.8 ± 6.7bc	5.46 ± 0.40b	23.0 ± 0.7c	0.18 ± 0.01b	101 ± 2b	9.99 ± 0.08b	0.30 ± 0.01cd	77.8 ± 6.6c
3000	0–20	56.9 ± 9.8c*	4.18 ± 0.42b*	21.7 ± 0.9c*	0.10 ± 0.01b*	69.4 ± 15.9b*	7.43 ± 0.68b*	0.29 ± 0.01d*	88.8 ± 8.8c*
3000	20–40	36.4 ± 2.2	0.33 ± 0.00	17.0 ± 0.6	0.05 ± 0.01	24.6 ± 6.5	5.16 ± 0.22	0.26 ± 0.02	69.9 ± 5.0
3000	40–60	33.9 ± 3.3	0.22 ± 0.00	17.3 ± 0.5	0.05 ± 0.00	24.7 ± 5.7	5.55 ± 0.35	0.26 ± 0.02	70.9 ± 5.6
3000	60–80	36.0 ± 2.0	0.62 ± 0.01	18.8 ± 0.6	0.06 ± 0.00	38.5 ± 5.9	7.19 ± 0.42	0.28 ± 0.01	77.1 ± 4.7
3000	80–100	29.6 ± 2.5	0.12 ± 0.00	11.8 ± 0.4	0.03 ± 0.00	17.0 ± 2.3	3.30 ± 0.58	0.25 ± 0.01	49.4 ± 2.5

NB: Each value is the mean of three replicates ± standard error of the mean (SEM). Different letters denote significant difference at $p < 0.05$ level for each element at 0–20 cm soil depth compared with sample from 100 m. "*" denotes significant difference at $p < 0.05$ level for deeper soils at 3000 m compared with the topsoil (0–20 cm)

higher BCF of Cd than of the other elements indicates the strong migration ability of Cd and its ready uptake by the vegetables. The BCF of Tl differed in different plant species but was relatively high in cabbage in line with the results of other investigations (Xiao et al. 2004) and may be related to crop variety and the mode of occurrence of Tl in the soil in addition to other factors.

Taking garlic as an example, samples 500, 1500 and 3000 m from the smelter have been listed and compared in Table 2. The sharpest decline with increasing distance was that of Zn, followed by As and Sb. The Cu concentration showed little variation with increasing distance from the smelter. The rates of decline of As, Cd, Cu, In, Pb, Sb, Tl and Zn were 84, 63, 20, 77, 69, 78, 84 and 92 %, respectively. Water spinach exhibited higher accumulation than almost every other vegetable and this also indicates the risk of long-term consumption of water spinach.

Changes in soil microbial index

Figure 2 shows that the average well colour development (AWCD) values of the soil 3000 m away calculated from the BIOLOG data were always higher than that at the other distances. In contrast, the lowest AWCD was always in the soil 100 m from the smelter. Overall, the AWCD values increased with increasing distance from the smelter. However, the AWCD values of soil samples at 500 m were close to that at 3000 m (Fig. 2). The number of carbon sources used by the microbial community was significantly lower in the soil at 500 m than at the other distances and the Shannon H' index also had the lowest value in the soil at 500 m (Table 3).

Bacteria, actinomycetes and fungi are the most widely studied groups of soil microorganisms (Vig et al. 2003). The numbers of different microbial groups increased with decreasing concentrations of the target metals in the soil (Table 3). Moreover, the numbers of bacteria, actinomycetes and fungi in soil at 100 m were significantly lower than at other distances ($p < 0.05$). Fungal abundance in soil > 100 m from the smelter did not change markedly; however, bacteria and actinomycetes in soil at 500 m were significantly higher than at 100 m. It is apparent that soil bacteria and actinomycetes were strongly affected by the heavy metals.

Bacterial community structure at the two sites

The rarefaction analyses show that the libraries provided an adequate sampling of bacterial diversity in samples from different distances (Fig. S1). A total of 19 bacterial phyla were found in the two soil samples. 454 pyrosequencing reveals that the soil at 3000 m was affiliated with six major bacterial lineages (> 1 %), namely *Proteobacteria* (30.7 %), *Firmicutes* (28.9 %), *Bacteriodetes* (15.3 %), *Actinobacteria* (10.8 %) *Acidobacteria* (8.0 %) and *Planctomycetes* (3.0 %). The phylogeny of the bacterial community of site 3000 m is shown in Fig. 3b. Seven major bacterial phylogroups (> 1 %) recovered from the site at 100 m were affiliated with *Firmicutes* (40.6 %), *Proteobacteri*a (35.4 %), *Bacteriodetes* (7.5 %), *Actinobacteria* (7.0 %) *Acidobacteri*a (3.3 %), *Chloroflexi* (3.9 %) and *Planctomycetes* (2.0 %). Phylogenetic relationships of bacteria recovered from the site at 100 m are shown in Fig. 3a. Overall, there was significant enhancement of phyla *Firmicutes*, *Proteobacteria*, and *Chloroflexi* at the highly metal-contaminated site (100 m) and, conversely, the others all decreased (Fig. 3). This suggests that the adaptabilities of *Firmicutes*, *Proteobacteria*, and *Chloroflexi* to contamination were stronger than those of the other phyla. The results of richness and diversity of 16S rRNA gene sequences in soil at 100 and 3000 m are listed in Table 4. All four indices (Chao, Ace, InvSimpson and Shannon) suggest a decrease in the

Table 2 Concentrations and bio-concentration factors (BCF) values of the eight target elements in the edible parts of different vegetable samples (mg kg^{-1} FW)

Vegetable	As	Cd	Cu	In	Pb	Sb	Tl	Zn
Baby Chinese cabbage—3000 m	0.27 ± 0.02 (0.047)	0.58 ± 0.02 (***1.388***)	0.42 ± 0.03 (0.194)	0.001 ± 0.000 (0.096)	0.62 ± 0.05 (0.089)	0.06 ± 0.00 (0.081)	0.004 ± 0.000 (0.137)	2.24 ± 0.14 (0.252)
Cabbage—1000 m	0.41 ± 0.05 (0.032)	0.33 ± 0.10 (0.304)	0.31 ± 0.04 (0.085)	0.001 ± 0.000 (0.048)	0.51 ± 0.06 (0.04)	0.08 ± 0.02 (0.065)	0.17 ± 0.03 (***3.46***)	6.39 ± 0.54 (0.29)
Cabbage sprout—1000 m	0.50 ± 0.05 (0.039)	1.46 ± 0.16 (***1.344***)	0.44 ± 0.03 (0.12)	0.002 ± 0.000 (0.096)	1.28 ± 0.17 (0.099)	0.08 ± 0.01 (0.065)	0.02 ± 0.00 (0.407)	4.28 ± 0.19 (0.192)
Cayenne pepper—500 m	0.17 ± 0.06 (0.011)	0.10 ± 0.07 (0.259)	1.73 ± 0.04 (0.385)	0 ± 0 (0)	0.13 ± 0.01 (0.008)	0.019 ± 0.005 (0.013)	0.035 ± 0.002 (0.382)	4.86 ± 0.27 (0.251)
Cayenne pepper—1000 m	0.13 ± 0.02 (0.009)	0.40 ± 0.04 (0.338)	1.44 ± 0.14 (0.369)	0 ± 0 (0)	0.10 ± 0.01 (0.008)	0.021 ± 0.001 (0.016)	0.010 ± 0.001 (0.1914)	2.57 ± 0.23 (0.108)
Cayenne pepper—3000 m	0.14 ± 0.01 (0.015)	0.17 ± 0.00 (0.247)	1.24 ± 0.01 (0.356)	0 ± 0 (0)	0.07 ± 0.01 (0.006)	0.015 ± 0.000 (0.013)	0.004 ± 0.000 (0.085)	2.31 ± 0.06 (0.163)
Corn—500 m	0.03 ± 0.00 (0.001)	0.13 ± 0.03 (0.133)	1.41 ± 0.05 (0.124)	0 ± 0 (0)	0.07 ± 0.00 (0.002)	0.011 ± 0.001 (0.003)	0 ± 0 (0)	14.57 ± 0.98 (0.297)
Eggplant—1500 m	0.04 ± 0.01 (0.007)	0.26 ± 0.04 (0.082)	1.14 ± 0.09 (0.020)	0 ± 0 (0)	0.03 ± 0.00 (0.004)	0.003 ± 0.000 (0.045)	0.003 ± 0.001 (***1.513***)	2.38 ± 0.21 (0.006)
Garlic—500 m	1.27 ± 0.02 (0.051)	0.57 ± 0.08 (0.971)	0.64 ± 0.05 (0.091)	0.005 ± 0.000 (0.157)	2.64 ± 0.45 (0.110)	0.25 ± 0.02 (0.112)	0.05 ± 0.01 (0.349)	19.24 ± 3.88 (0.636)
Garlic—1500 m	0.38 ± 0.01 (0.023)	0.22 ± 0.04 (0.161)	0.60 ± 0.04 (0.104)	0.002 ± 0.000 (0.034)	0.90 ± 0.22 (0.036)	0.08 ± 0.01 (0.032)	0.01 ± 0.00 (0.135)	3.94 ± 0.72 (0.203)
Garlic—3000 m	0.20 ± 0.01 (0.014)	0.20 ± 0.05 (0.191)	0.52 ± 0.02 (0.096)	0.001 ± 0.000 (0.042)	0.64 ± 0.09 (0.037)	0.05 ± 0.01 (0.027)	0 ± 0 (0)	3.17 ± 0.68 (0.143)
Green Chinese onion—3000 m	0.40 ± 0.08 (0.047)	0.22 ± 0.03 (0.351)	0.58 ± 0.06 (0.178)	0.002 ± 0.000 (0.128)	1.24 ± 0.19 (0.119)	0.14 ± 0.01 (0.126)	0.004 ± 0.000 (0.091)	4.77 ± 0.65 (0.358)
Lettuce—3000 m	0.60 ± 0.24 (0.132)	0.91 ± 0.27 (***2.721***)	0.55 ± 0.06 (0.317)	0.003 ± 0.001 (0.361)	1.85 ± 0.60 (0.333)	0.18 ± 0.10 (0.303)	0.01 ± 0.00 (0.427)	4.70 ± 1.34 (0.662)
Water spinach—500 m	1.13 ± 0.02 (0.657)	0.37 ± 0.03 (***9.207***)	2.12 ± 0.09 (***4.353***)	0.003 ± 0.001 (***1.416***)	1.09 ± 0.05 (0.648)	0.12 ± 0.01 (0.795)	0.104 ± 0.008 (***10.46***)	14.60 ± 0.31 (***6.95***)
Water spinach—3000 m	0.95 ± 0.02 (0.962)	0.21 ± 0.00 (***2.94***)	2.890 ± 0.221 (***7.672***)	0.001 ± 0.000 (0.554)	0.551 ± 0.027 (0.457)	0.082 ± 0.002 (0.636)	0.010 ± 0.000 (***1.966***)	4.40 ± 0.25 (***2.86***)

NB: Each value is the mean of three replicates ± SEM. FW denotes fresh weight. Values higher than standards in GB 2762–2005 are underlined. Bio-concentration factors (BCF) are calculated by plant metal concentration on DW basis (determined) divided by soil metal concentration and shown in parenthesis. BCF values >1 are listed in italics and bold fonts. The scientific names of the plants listed in the tables, i.e. baby Chinese cabbage, cabbage, cabbage moss, cayenne pepper, corn, eggplant, garlic, green Chinese onion, lettuce and water spinach, are *Brassica rapa* L. var. *glabra Rege*, *Brassica oleracea* var. *capitata*, *Brassica rapa* var. *chinensis*, *Ipomoea aquatic*, *Zea mays*, *Solanum melongena*, *Allium sativum*, *Allium fistulosum*, *Lactuca sativa* and *Ipomoea aquatic*, respectively

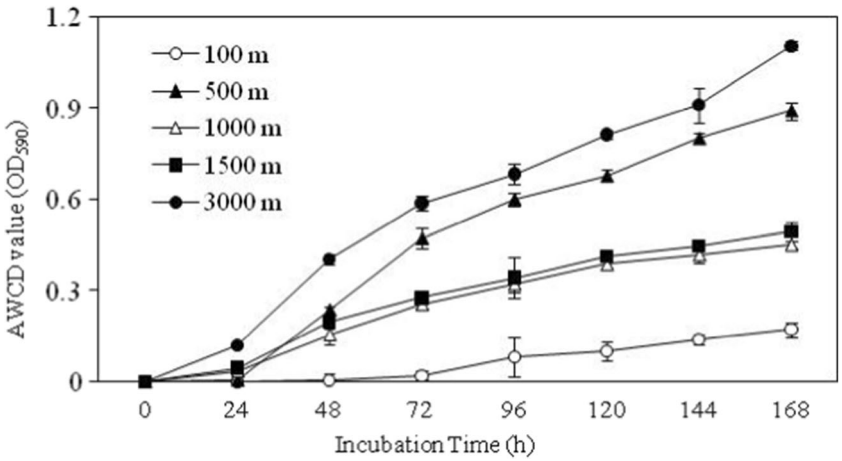

Fig. 2 Analysis of colour development in BIOLOG plates inoculated with soil suspensions of soil samples from different distances from the smelter. Mean values of the average well colour development (*AWCD*) calculated using three replicates. The *bars* indicate the MSD as determined by ANOVA

Table 3 Microbial counts (mg kg^{-1}) and functional diversity indices of the soil community in the top 20 cm of the soil profile at different distances from the smelter (mg kg^{-1})

Distance (m)	Microbial counts			Functional diversity		
	Actinomycetes ($\times10^6$ cfus g^{-1} soil)	Bacteria ($\times10^7$ cfus g^{-1} soil)	Fungi ($\times10^5$ cfus g^{-1} soil)	Shannon H'	Number of carbon sourced used	Evenness
100	1.4 ± 0.5d	0.8 ± 0.2d	2.8 ± 0.1d	3.79 ± 0.07a	70.7 ± 1.3a	0.992 ± 0.001c
500	3.3 ± 0.3c	2.9 ± 0.2bc	3.0 ± 0c	3.44 ± 0.30b	42.0 ± 12.1b	0.993 ± 0.001bc
1000	7.8 ± 0.6b	2.6 ± 0.3c	3.4 ± 0a	3.64 ± 0.16ab	80.5 ± 12.5a	0.992 ± 0.001c
1500	10.8 ± 0.6a	3.3 ± 0.2b	3.5 ± 0.1a	3.73 ± 0.03ab	86.7 ± 2.1a	0.994 ± 0.001ab
3000	11.6 ± 0.7a	4.2 ± 0.3a	3.2 ± 0.1b	3.68 ± 0.07ab	76.0 ± 10.1a	0.995 ± 0.001a

NB: Each value is the mean of three replicates ± SEM. Different letters denote significant difference at $p < 0.05$ level according to Duncan's multiple range test

diversity of highly metal contaminated soils at the site 100 m from the smelter compared to the site at 3000 m (Table 4).

Discussion

Effects of the smelter on concentrations of elements in soils and plants

Results indicate that the smelter is the main pollution source in the study area. The concentrations of all eight target elements in the top 20 cm arable layer of the soil profile declined markedly with increasing distance from the pollution source. Concentrations of Cu, Zn, Pb, Cd and As in soil 100 m from the smelter were several times higher than the Chinese second grade standard soil quality values (GB15168-1995). In particular, Cd and As concentrations (even in soil 3000 m from the smelter) exceeded the standard values. Moreover, the concentrations of As, Cd and Pb in the edible parts of most of the vegetable samples (≥ 80 % of plants collected) were above the permissible levels. These results indicate that As, Cd and Pb are major pollutants resulting from the activities of the smelter in this area and represent high potential risk to human health from the consumption of the polluted vegetables.

High soil concentrations of Sb can usually be detected only where Sb has been mineralized near the smelting area but it is present at < 1 mg kg^{-1} in most agricultural soils. The maximum allowable concentrations of Sb in soils in Germany and the Netherlands are 3.5 and 5 mg kg^{-1}, respectively (Eikmann and Kloke 1993; Crommentuijn et al. 1997). The background concentration of Sb in China has been suggested to be 0.38–2.98 mg kg^{-1} after an investigation of Sb distribution characteristics in 40 soil types in 34 provinces or cities (Qi and Cao 1991). In the present study, soil Sb concentrations were 223 and 7.43 mg kg^{-1} at 100 and 3000 m from the smelter. This indicates that Sb is one of the main soil pollutants. Dissolved Sb in the soil may be freely taken up by plants but Sb is not normally considered to be an essential element for plant growth (Baroni et al. 2000). Kabata-Pendias and Pendias (2001) believed that toxic effects of Sb would appear when the concentration was between 5 and 10 mg kg^{-1}, but they suggested that the threshold value of Sb should be 5 mg kg^{-1}.

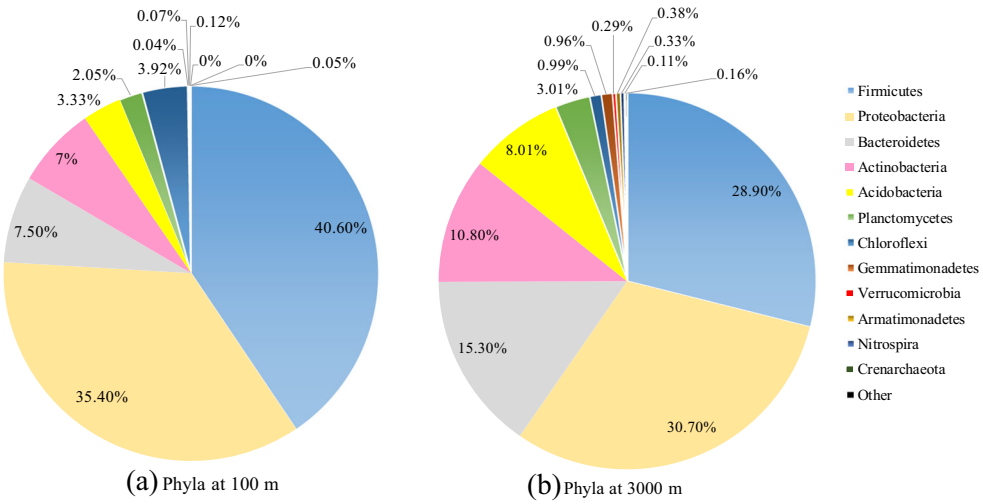

Fig. 3 Distribution of partial sequences of bacterial 16S rRNA genes from the soil samples from **a** 100 m and **b** 3000 m. Proportions were calculated based on sequences classified at the phylum level using RDP classifier

Table 4 Richness and diversity of 16S rRNA gene sequences from different treatments

Treatment	Sobs	Chao	Ace	InvSimpson	Shannon
3000 m	1595 ± 144a	3791 ± 319a	6182 ± 853a	47 ± 16a	5.82 ± 0.31a
100 m	966 ± 326b	2207 ± 562b	3972 ± 855b	25 ± 12a	4.68 ± 0.77b

NB: Each value is the mean of three replicates ± SEM. Averages of replicates with standard error in parenthesis; mean values followed by different letters are significantly different at $p < 0.05$ according to independent sample T test

The World Health Organization (WHO) has stipulated Sb as a carcinogen (DFG 2007) and set the acceptable daily intake (ADI) value and the total daily intake (TDI) value at 0.86 and 6 μg kg^{-1} (by body weight), respectively (WHO 2003). In the present study, the Sb concentration in the garlic samples collected 500 m from the smelter was above the standard value. These results indicate that Sb is a major pollutant in soil near the smelter but Sb might represent a lower potential risk to humans than As, Cd or Pb.

There have been few reported studies on the typical rare metal elements In and Tl in soils. In China, the background concentrations of In and Tl in topsoils are in the ranges 0.022 to 0.167 and 0.292 to 1.172 mg kg^{-1} according to Qi et al. (1992) after analysis of 853 soil samples collected throughout the country. In the present study, soil concentrations of In (1.38 mg kg^{-1}) 100 m from the smelter were much higher than the maximum value of unpolluted soil In in China (Qi et al. 1992). However, In concentrations in none of the plant samples collected were higher than the standard values, indicating that In is not a major pollutant threatening human health under current cropping patterns. Furthermore, Tl has been listed in Canada as a priority detected inorganic pollutant and its safety threshold value in farmland soils is 1 mg kg^{-1} (CCME 1999). Previous studies consider that the soil cannot be available for agricultural production when the soil Tl concentration exceeds 1.05 mg kg^{-1} (Zhang et al. 1998). In the present investigation with long-term emissions of dust and wastewater, the concentration of Tl in soils 100 m from the smelter was 1.08 mg kg^{-1}. Although the Tl concentrations in this study are much lower than that reported by Wierzbicka et al. (2004), Tl in soils can be readily taken up by plants and enter the food chain to exert adverse effects on human health (Xiao et al. 2003). Studies have also shown that the most commonly occurring forms of Tl in soils are water-soluble, silicate-bound, bound state sulfide and organic matter-bound, and the water soluble Tl can be directly taken up by plants or leached to deeper parts of the soil profile or migrate with leaching solutions (Lehn and Schoer 1987; Xiao et al. 2003; Jia et al. 2013).

The general concentration of Tl in the normal human body is minimal as it is not an essential element. According to early reports, the minimum lethal dose (MLD) of Tl to a 70-kg adult is 50–80 mg (Moeschlin 1980) and its maximum non-effect dose for adults is about 0.056 mg per day (Sager 1998). It has been reported that the consumption of vegetables containing Tl at > 0.3 mg kg^{-1} (FW) may trigger chronic disease in the human body (Bowen 1979). In the present investigation, the highest Tl concentration was found in cabbage with about 0.17 mg kg^{-1} (FW) in cabbage 1000 m from the smelter. This result is consistent with previous studies in which cabbage was likely to accumulate Tl to much higher concentrations than other plant species (Xiao et al. 2003; Jia et al. 2013). However, the Tl concentration in plants in the present study is much lower than that in cabbage (818 mg kg^{-1} DW) found by Jia et al. (2013) and > 500 mg kg^{-1} (DW) by Xiao et al. (2004). This may be because the pollution sources in their studies were sulfide mineralization of Tl and mining activities (Xiao et al. 2004; Jia et al. 2013). The contamination of soils by Tl in the neighbourhood of the smelter should therefore be of considerable concern if vegetables that readily accumulate Tl such as cabbage are grown in the area near the smelter. The above results indicate that Tl might be a potentially toxic pollutant especially when plants that easily accumulate Tl (such as cabbage) are grown in the area.

Soil microbial characteristics affected by the smelter

It is generally accepted that test samples with larger changes in AWCD might have a higher microbial carbon source utilization ability which may correspond with higher microbial activity (Classen et al. 2003). In the present study, the AWCD of the soil 3000 m away was always the highest and the lowest AWCD was present in soil 100 m from the smelter. This result is consistent with soil metal concentrations differing between distances. Studies have shown that the soil microbial biomass has a strong relationship with soil distance from metal smelters (Wang et al. 2007a). This indicates that soil metal concentrations can significantly affect soil microorganisms. Although the concentrations of most of the target elements in soil samples at 500 m were higher than those at 1000 and/or 1500 m from the smelter, the AWCD values of soil samples at 500 m were higher than at 1000 or 1500 m in the current study. This may have resulted from the sudden decline in some toxic elements such as Cd in soil at 500 m, which was respectively about 1/3 and 2/5 of that at 1000 and 1500 m. In fact, Cd is inhibitory to the microbial population below a certain level (Dar 1996), and Teng et al. (2005) also found that Cd is the metal with maximum biological toxicity in a situation of combined pollution by the heavy metals Cu, Zn,

Pb, and Cd. The lowest soil Cd concentration at 500 m might be responsible for the high AWCD value. Unexpectedly, although the investigated element concentrations in soil at 500 m were lower than at 100 m, the Shannon H' index and the number of carbon sources used by the microbial community in the soil at 500 m were lower. The unknown contribution of other soil properties not investigated in this study might explain this phenomenon and further study is required to investigate this possibility.

Bacteria, actinomycetes and fungi were investigated in the present study. Numerous previous studies have found that fungi are more resistant than bacteria or actinomycetes to long-term heavy metal contamination (Fliessbach et al. 1994). Moreover, estimates of microbial populations in soil by conventional plate count methods have also indicated that bacteria are more sensitive than fungi to metals such as Cd (Vig et al. 2003). In the present study, the change in biomass followed the order fungi < bacteria < actinomycetes, indicating that the degree of tolerance to heavy metals appears to be fungi > bacteria > actinomycetes, a similar result to that of Hiroki (1992).

Metal stress adversely affects soil quality by both reducing microbial biomass and inhibiting specific functional groups of microbes (Linton et al. 2007). Metal stress severely affects rare and sensitive species and decreases their competitive ability, resulting in an abundance of metal-resistant species capable of adapting to stress (Giller et al. 1998; Wolinsky et al. 2005). This phenomenon was apparent in our study which clearly suggests an increase in *Firmicutes*, *Proteobacteria* and *Chloroflexi* with a loss of the bacterial phyla Bacteriodetes, *Actinobacteria*, *Acidobacteria*, *Planctomycetes*, *Gemmatimonadetes* and the other minor phyla in response to high metal stress in the soil at the site at 100 m. Results obtained in this study corroborate previous reports detecting structural shifts within bacterial communities at long-term metal-contaminated sites (Smit et al. 1997; Muller et al. 2003; Desai et al. 2009). *Firmicutes* have been found to constitute a dominant portion of bacterial populations in the metal-polluted soils (Ellis et al. 2003), and *Firmicutes* and *Chloroflexi* have higher relative abundances in highly metal contaminated samples (Yin et al. 2015). In particular, the phylum *Actinobacteria* decreased from 10.8 % (3000 m) to 7.0 % (100 m). Hiroki (1992) found that *Actinobacteria* can be strongly affected by heavy metals even at relatively low concentrations and decrease significantly with increasing heavy metal content. These results indicate that the bacterial community composition has shifted because of the metal pollution arising from the recycling of rare metals.

Conclusions

The soils around the rare metal smelter were contaminated by the eight target elements, and As, Cd and Pb were three main elements polluting vegetables and soils around the smelter, and the possibility of Tl and In contamination is of some concern. Actinomycetes were the microorganisms most strongly affected by the heavy metals. The degree of tolerance to heavy metals appeared to follow the sequence: fungi > bacteria > actinomycetes. Soil metal pollution significantly shifted the bacterial community composition and *Firmicutes, Proteobacteria* and *Chloroflexi* had higher adaptability to the pollution. The activities of the smelter have had a detrimental effect on the local environment and may represent a potential threat to both human health and soil quality.

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References

Álvarez-Ayuso E, Otones V, Murciego A, García-Sánchez A, Santa Regina I (2013) Zinc, cadmium and thallium distribution in soils and plants of an area impacted by sphalerite-bearing mine wastes. Geoderma 207:25–34

Ayadi A, Maghraoui S, Kammoun S, Tekaya L (2014) Effects of the presence of indium on the mammary gland ultrastructure, body weight, food intake and plasmatic prolactin concentration. Microscopy 63:383–389

Baceva K, Stafilov T, Sajn R, Tanaselia C, Makreski P (2014) Distribution of chemical elements in soils and stream sediments in the area of abandoned Sb-As-Tl Allchar mine, Republic of Macedon. Environ Res 133:77–89

Baroni F, Boscagli A, Protano G, Riccobono F (2000) Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area. Environ Pollut 109: 347–352

Bowen HJM (1979) Environment al chemistry of the elements. Academic, London, pp 1–316

CCME (Canadian Council of Ministers of the Environment) (1999) Canadian environmental quality guidelines. Available from: http: //www.ccme.cayceqg_ rcqeyindex. Html.

Chen QL, Yang BS, Wang H, He F, Gao YC, Scheel RA (2015) Soil microbial community toxic response to atrazine and its residues under atrazine and lead contamination. Environ Sci Pollu Res 22: 996–1007

Classen AT, Boyl SI, Haskins KE, Overby ST, Hart SC (2003) Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. FEMS Microbiol Ecol 44:319–328

Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qiu Y, Liang JZ (2004) Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environ Int 30:785–791

Crommentuijn T, Polder MD, Plassche EJ (1997) Maximum permissible concentrations and negligible concentrations of metals, taking background concentrations into account. National Institute of Public Health and the Environment, The Netherlands, Bilthoven, pp 1–260

Dar GH (1996) Effects of cadmium and sewage-sludge on soil microbial biomass and enzyme activities. Bioresour Technol 56:141–145

Desai C, Parikh RY, Vaishnav T, Shouche YS, Madamwar D (2009) Tracking the influence of long-term chromium pollution on soil bacterial community structures by comparative analyses of 16S rRNA gene phylotypes. Res Microbiol 160:1–9

DFG (Deutsche Forschungsgemeinschaft) (2007) Antimony and its inorganic compounds (inhalable fraction). In: Greim H (ed) The MAK collection for occupational health and safety, Part I: MAK value documentations. Weinheim, Wiley-VCH, pp 1–73

Eikmann T, Kloke A (1993) Nutzungsund schutzgutbezogene orientierun gswerte fur (Schad-) stoffe in Boden. Erich Schmidt, Berlin, pp 1–527

Ellis RJ, Morgan P, Weightman AJ, Fry JC (2003) Cultivation-dependent and -independent approaches for determining bacterial diversity in heavy-metal-contaminated soil. Appl Environ Microbiol 69:3223–3230

Fliessbach A, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol Biochem 26:1201–1205

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57:2351–2359

GB15618-1995 (1995) Environmental quality standards for soils, State Bureau of Environmental Protection, State Bureau of Technical Supervision, July 13

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Hiroki M (1992) Effects of heavy metal contamination on soil microbial population. Soil Sci Plant Nutr 38:141–147

Jia YL, Xiao TF, Zhou GZ, Ning ZP (2013) Thallium at the interface of soil and green cabbage (*Brassica oleracea* L. var. *capitata*): soil-plant transfer and influencing factors. Sci Total Environ 450:140–147

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton

Kachenko A, Singh B (2006) Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. Water Air Soil Pollut 169:101–123

Lehn H, Schoer J (1987) Thallium transfer from soils to plants: correlation between chemical form and plants uptake. Plant Soil 97:253–265

Li ZG, Feng XB, Bi XY, Li GH, Lin Y, Sun GY (2014) Probing the distribution and contamination levels of 10 trace metal/metalloids in soils near a Pb/Zn smelter in Middle China. Environ Sci Pollut Res 21:4149–4162

Linton PE, Shotbolt L, Thomas AD (2007) Microbial communities in long-term heavy metal contaminated ombrotrophic peats. Water Air Soil Pollut 186:97–113

Madejón P (2013) Thallium. Environ Pollut 22:543–549

Mikryukov VS, Dulya OV, Vorobeichik EL (2015) Diversity and spatial structure of soil fungi and arbuscular mycorrhizal fungi in forest litter contaminated with copper smelter emissions. Water Air Soil Pollut 226:114

Moeschlin S (1980) Thallium poisoning. Clin Toxicol 17:133–146

Muller D, Lievremont D, Simeonova DD, Hubert JC, Lett MC (2003) Arsenite oxidase aox genes from a metal-resistant β-proteobacterium. J Bacteriol 185:135–141

Qi WQ, Cao JS (1991) Study on the soil environmental background values of antimony. Chinese J Soil Sci 22:209–211

Qi WQ, Cao JS, Chen YL (1992) Study on the soil environmental background values of In and Tl. Chinese J Soil Sci 23:31–33

Sager M (1998) Thallium in agricultural practice. In: Nriagu JO (ed) Thallium in the environment. Wiley, New York

Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. Plos One 6, e27310

Shen P, Fan XR, Li GW (1989) Experiment of microbiology (In Chinese). Beijing: High Education Press pp 92.

Smit E, Leeflang P, Wernars K (1997) Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. Fems Microbiol Ecol 23:249–261

Teng Y, Huang CY, Luo YM, Li ZG (2005) Changes in microbial activities and its community structure of red earths polluted with mixed heavy metals. Acta Pedol Sin 42:819–828 (in Chinese)

Vig K, Megharaj M, Sethunathan N, Naidu R (2003) Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Adv Environ Res 8:121–135

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007a) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Wang YP, Shi JY, Wang H, Lin Q, Chen XC, Chen YX (2007b) The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicol Environ Safe 67:75–81

Wierzbicka M, Szarek-Lukaszewska G, Grodzinska K (2004) Highly toxic thallium in plants from the vicinity of Olkusz (Poland). Ecotoxicol Environ Safe 59:84–88

WHO (2003) Antimony in drinking water, Background document for development of WHO guidelines for drinking water quality (S)

Wolinsky M, Cans J, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309:1387–1390

Wyszkowska J, Borowik A, Kucharski M, Kucharski J (2013) Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. J Elementol 18:769–796

Xiao TF, Chen JA, Hong B (2003) Thallium contamination in soils and its environmental impact. Bull Mineral Petrol Geochem 22:140–143 (in Chinese)

Xiao TF, Guha J, Boyle D, Liu CQ, Chen JG (2004) Environmental concerns related to high thallium levels in soils and thallium uptake by plants in southwest Guizhou, China. Sci Total Environ 318:223–244

Yin HQ, Niu JJ, Ren YH, Cong J, Zhang XX, Fan FL, Xiao YH, Zhang X, Deng J, Xie M, He ZL, Zhou JZ, Liang YL, Liu XD (2015) An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. Sci Rep 5:14266

Zak JC, Willig MR, Moorhead DL, Wildman HG (1994) Functional diversity of microbial communities: a quantitative approach. Soil Biol Biochem 26:1101–1108

Zhang SX, Dong SP, Yan W (1998) The geochemical behaviours of thallium in soils and sediments of the grass and river region. J Agro-Environ Sci 17:113–117 (in Chinese)