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Atmospheric Deposition-Carried Pb, Zn, and Cd from a Zinc Smelter and Their Effect on Soil Microorganisms*[1]

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ABSTRACT

Dust emissions from smelters, as a major contributor to heavy metal contamination in soils, could severely influence soil quality. Downwind surface soils within 1.5 km of a zinc smelter, which was active for 10 years but ceased in 2000, in Magu Town, Guizhou Province, China were selected to examine Pb, Zn, and Cd concentrations and their fractionation along a distance gradient from a zinc smelter, and to study the possible effects of Pb, Zn, and Cd accumulation on soil microorganisms by comparing with a reference soil located at a downwind distance of 10 km from the zinc smelter. Soils within 1.5 km of the zinc smelter accumulated high levels of heavy metals Zn (508 mg kg^{-1}), Pb (95.6 mg kg^{-1}), and Cd (5.98 mg kg^{-1}) with low ratios of Zn/Cd (59.1–115) and Pb/Cd (12.4–23.4). Composite pollution indices (CPIs) of surface soils (2.52–15.2) were 3 to 13 times higher than the reference soils. In metal accumulated soils, exchangeable plus carbonate-bound fractions accounted for more than 10% of the total Zn, Pb, and Cd. The saturation degree of metals (SDM) in soils within 1.5 km of the smelter (averaging 1.25) was six times higher than that of the reference soils (0.209). A smaller soil microbial biomass was found more frequently in metal accumulated soils (85.1–438 μg C g^{-1}) than in reference soils (497 μg C g^{-1}), and a negative correlation ($P < 0.01$) of soil microbial biomass carbon to organic carbon ratio (C_{mic}/C_{org}) with SDM was observed. Microbial consumption of carbon sources was more rapid in contaminated soils than in reference soils, and a shift in the substrate utilization pattern was apparent and was negatively correlated with SDM ($R = -0.773$, $P < 0.01$). Consequently, dust deposited Pb, Zn, and Cd in soils from zinc smelting were readily mobilized, and were detrimental to soil quality mainly in respect of microbial biomass.

Key Words: composite pollution index, heavy metals, microbial biomass, saturation degree of metals, sequential extraction

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Eco-environmental pollution caused by mining and smelting activities is a worldwide problem and has aroused increasing concerns among scientists. In addition to vegetation destruction and erosion of cultivated land caused directly by mining and smelting activities, scientists have placed more focus on peculiar environmental problems caused by the release of harmful substances, particularly heavy metals. Severe soil heavy metal accumulations from metal smelters have been well reported (Rieuwerts and Farago, 1996; Ullrich *et al.*, 1999; Basta and McGowen, 2004). Fugitive dust emission from smelters is regarded as one of the most severe causes attributing to heavy metal accumulations in soils (Rolfe and Jennett, 1975; Rieuwerts and Farago, 1996).

However, heavy metal toxicity to a soil eco-environmental system depends largely on the bioavailability of heavy metals. At present, difficulty still remains how to directly assess heavy metal bioavailability. Substitution methods, for example, sequential extraction techniques (Tessier *et al.*, 1979; Li *et al.*, 1995), are frequently applied and performed effectively. Ivask *et al.* (2003) have applied a sequential extraction procedure to predict the mobile fraction of heavy metals near two metal smelters, and have concluded

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that chemically mobile fractions must be regarded as bioavailable fractions. Others have widely applied this technique to evaluate heavy metal bioavailability in soils near metal smelters (Verner *et al.*, 1996). Therefore, sequential extraction techniques are still regarded as one of the most effective methods to evaluate heavy metal bioavailability in soils.

Soil microorganisms can be good indicators of soil quality, and the adverse influence of heavy metals on soil microorganisms has been included as one aspect of soil quality (Hinojosa *et al.*, 2004). Microbial activities have been noted as one important aspect influenced by heavy metals (Anand *et al.*, 2003). These include decreasing soil microbial biomass (Fritze *et al.*, 1996), weak enzyme activity (Kandeler *et al.*, 1996), and increasing microbial respiration rate (Bogomolov *et al.*, 1996). Microbial metabolic quotient qCO_2 (Fliessbach *et al.*, 1994), and the efficiency of microbial carbon utilization (Bardgett and Saggar, 1994) are both parameters of microbial activities and can be correlated with soil heavy metal accumulation.

Microbial community structure and functional diversity, characterized by Biolog, were regarded as another important aspect that was affected by heavy metals (Zak *et al.*, 1994). The Biolog method, which was developed by Garland and Mills (1991), was commonly applied to determine the response of soil microbial communities to heavy metal stress (Campbell *et al.*, 1995; Yang *et al.*, 2006b). However, understanding the impact of heavy metal accumulation on soil microorganisms during and after zinc smelting activities requires a great deal of further study.

Zinc smelting in the Hezhang County, Guizhou Province in southwestern China, was developed 300 years ago and profited from the regionally abundant coal resources and Pb and Zn mine ores—two essential conditions for traditional smelting activities. Zinc smelting in Magu Town of the Hezhang County started in the late 1980s, and even though it was halted in 2000, there remain large quantities of waste slag heaps piled up around the abandoned smelting furnaces, and huge tracts of formerly cultivated land remain barren. Yang *et al.* (2006a) reported severe heavy metal pollution in soils adjacent to the zinc smelters in the Hezhang County. High Zn concentrations could be found in mosses near the zinc smelters in the Hezhang County, indicating that atmospheric deposition could transport large amounts of heavy metals (Bi *et al.*, 2006).

Therefore, the authors hypothesized that atmospheric deposition from smelting activities could accumulate Pb, Zn, and Cd in soils downwind and would consequently be responsible for the abnormal activity of soil microorganisms. The objectives of this investigation were to examine Pb, Zn, and Cd concentrations and their fractionation along a distance gradient from a zinc smelter, and to study the possible effects of Pb, Zn, and Cd accumulation on soil microorganisms by comparing the area adjacent to the zinc smelters with a reference area located 10 km downwind from the zinc smelter.

MATERIALS AND METHODS

Magu Town in the Hezhang County (26° 57′ N, 104° 32′ E), Guizhou Province, China, with a history of 10-year zinc smelting, was selected in this study. The elevation was around 1 900 to 2 100 m above sea level, with relatively low topography. The annual mean temperature was about 12 °C, and the average yearly rainfall was 900 to 1 200 mm. Smelting activities were performed in a valley surrounded by steep hills (about 10 km^2 area was affected by these smelting activities), and slag was piled concentrically around the smelting furnace. No Pb or Zn mines were found in this valley, and smelting ores were conveyed from a Zn mine 10 km away. To avoid smelting slag and to ensure that dust deposition inputs during smelting was the sole heavy metal source in soils, the soils were collected downwind at distances of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0, and 1.5 km, respectively, from the smelting furnaces. At each site, four replicate top soil samples (0–20 cm) were randomly collected along a transect, using a stainless steel spade, in January 2001, except for the site at 1.5 km, where duplicate samples were collected. Each sample was a mixture of ten random small spades in a one square meter section. An additional downwind location behind a hill, approximately 10 km away from the smelting furnaces, was selected as the reference site, to compare the influence of dust deposition on soil heavy

metal accumulation. Thirty surface soils at this reference site were randomly collected using the same method.

Both macrograph and binocular microscopic-based observations confirmed no slag residues in the soil samples. Therefore, smelting slag as a contributor of heavy metal could be excluded. However, atmospheric dust deposition contributing as Pb, Zn, and Cd source in soils was confirmed when considering the geological setting of the Zn smelting valley.

Fresh soils were passed through a 6-mm stainless steel sieve, and then a 2-mm sieve to eliminate all plant debris. The fresh soils were stored in a refrigerator at 4 °C before microbial biomass measurement and Biolog tests were performed. Then the soils were sub-sampled, and air-dried for measurement of basic soil properties and determination of Pb, Zn, and Cd fractions.

Fresh soils were used to determine the soil moisture content and loss on ignition (LOI) by using the oven-dry method at 107 and 900 °C for 4 and 12 h, respectively. Soil organic carbon content was then calculated using LOI (Yang *et al.*, 2006b). Air-dried soils were used for measurement of pH in the slurry with deionized water to a sample ratio of 2.5:1 following the method of ISSCAS (1978). Portions of air-dried soils were ground to less than 0.125 mm and digested by HF-$HC1O_4$-HNO_3 mixture, and then the contents of Al and Fe were determined with the help of atomic absorption spectrometry (AAS) and by graphite atomic absorption spectrometry (GFAAS) (model PE5100PC, Manufactured by PERKIN ELMER, USA), respectively, as described by Tessier *et al.* (1979) and Li *et al.* (1995). International reference material MAG 1 (U.S. Geological Survey) was used as the reference for quality control. Good recovery rates of MAG 1 (95%–106% for Fe and 80%–90% for Al, respectively) were obtained (Yang *et al.*, 2006a).

Air-dried soils were ground to finer than 0.125 mm prior to the determination of the chemical fractionation of heavy metals. Tessier's sequential extraction method (Tessier *et al.*, 1979) was used to distinguish the chemical fractions of heavy metals in the soils. The fractions of heavy metals determined using this method included exchangeable, carbonate-bound, Fe-Mn oxide-bound, organic (sulfide) matter associated, and the residue forms. The first two fractions were usually considered as the readily available portions of metals (Zalidis *et al.*, 1999), and were of great eco-environmental significance, whereas, the others became available to microorganisms in the long term.

The concentrations of Pb, Zn, and Cd in the extracts were determined by using AAS (Yang *et al.*, 2006a). The total metal contents of the soils were then calculated from the cumulative concentrations of different extraction steps. Recovery rates of MAG 1 were 90%–112% for Pb, 98%–103% for Zn, and 105%–116 % for Cd, respectively (Yang *et al.*, 2006a).

Soil microbial biomass was measured using the chloroform fumigation-0.5 mol L^{-1} K_2SO_4 extraction procedure (Wu *et al.*, 1990), and the concentrations of organic carbon in the extracts before and after fumigation were determined by using a TOC analyzer (Shimazu, TOC-500, Japan). Measured organic C was converted to microbial biomass C (C_{mic}) using a transformation coefficient of 2.22.

Sole-carbon-source utilization of soil microbes was determined by the routine Biolog method (Garland and Mills, 1991; Campbell *et al.*, 1997). A total of 95 carbon sources (excluding the blank) were measured on GN plates. Ten grams of fresh soil were added to a 250 mL flask containing 100 mL of sterile distilled water and shaken on a wrist shaker at full speed for 10 min. The average well color development (AWCD) of all C sources was calculated to reflect the total activity of the microorganisms, Principal component analyses (PCA) of the Biolog data was applied to discriminate microbial community shifts according to Campbell *et al.* (1997). In this study, one Biolog GN plate per sample was used.

All the data processing and statistical analyses were performed on Genstat Rel 5.3 software (NAG Ltd., Oxford, UK).

RESULTS AND DISCUSSION

Accumulation of Pb, Zn, and Cd in soils

Concentrations of heavy metals Zn (508 mg kg^{-1}), Pb (95.6 mg kg^{-1}), and Cd (5.98 mg kg^{-1}) in

the surface soil of the smelting region were apparently higher than those in the reference area, however, soils in the reference area had a similar concentration trend of Zn (74.7 mg kg^{-1}), Pb (30.6 mg kg^{-1}), and Cd (0.24 mg kg^{-1}) to soil background levels in Guizhou Province (Fig. 1), except that the Pb, Zn, and Cd concentrations were above their soil limits in China (MEPPRC, 1995), being 250, 200, and 0.30 mg kg^{-1} for Pb, Zn, and Cd, respectively, as measured in the smelting region. The ratios of Zn/Cd (59.1–115) and Pb/Cd (12.4–23.4) were low, compared to 360 and 141 of Zn/Cd and Pb/Cd, respectively, in the reference soils (Fig. 1).

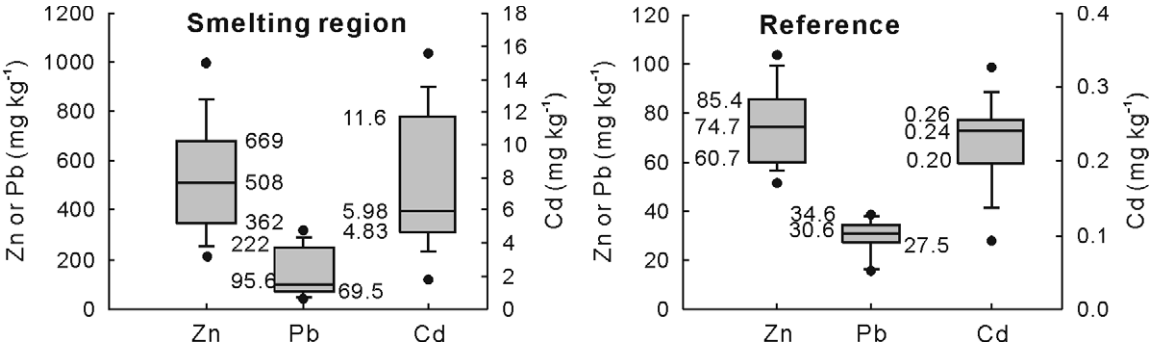

Fig. 1 Concentrations of Pb, Zn, and Cd in the soils of the two regions studied. The circles represent 5 and 95 percentile of the data and the error bars represent 10 and 90 percentile of the data. The middle line is the median value of the data range. The upper value of the box represents the 75 percentile and the lower value of the box represents the 25 percentile.

Rapid decreases of heavy metal concentrations in soils downwind from the smelting furnaces were observed within a distance of 200 m, and the highest concentrations of Zn (917 mg kg^{-1}), Pb (303 mg kg^{-1}), and Cd (14.5 mg kg^{-1}) were observed at 20 m from a zinc smelter (Fig. 2). Zinc, Pb, and Cd concentrations had a tendency to gradually decrease with increasing distance from the smelting location (Fig. 2).

Statistically, Zn, Pb, and Cd concentrations in soils within 1.5 km of the smelting activities were significantly higher than those in soils of the reference area located 10 km from the smelting activity ($P < 0.001$).

Composite pollution index (CPI) of soils is defined as

$$\mathrm{CPI} = \Sigma[(C_i \times P_i)/C_\mathrm{o}]$$

where C_i is the concentration of Pb, Zn or Cd in soils, C_o is the soil background concentration of Pb, Zn or Cd in Guizhou Province (31.3, 86.9, and 0.21 mg kg^{-1} for Pb, Zn, and Cd, respectively, CEMS, 1990), and P_i is the molar percentage of Pb, Zn or Cd and can be calculated from the equation $P_i = (C_i/\mathrm{AW}_i)/\Sigma(C_i/\mathrm{AW}_i)$, where AW_i is the atomic weight of Pb, Zn or Cd, and can be applied to evaluate the levels of soil contamination by heavy metals (Yang *et al.*, 2004). The CPI (2.52–12.2) of soils in the smelting region was 3–13 times higher ($P < 0.05$) than that (0.923) in the reference area (Table I). Contamination of heavy metals in soils within 1.5 km of the smelting activity was apparent compared to soils in the reference area 10 km away.

Compared to the reference soils 10 km from the zinc smelter, soils within 1.5 km of the zinc smelter showed a high accumulation of the heavy metals Pb, Zn, and Cd (Fig. 1), which suggested severe soil pollution by heavy metals near the zinc smelter. This result was supported by high composite pollution index (CPI) scores (Table I). Sharp decreases in Pb, Zn, and Cd concentrations in soils at a greater distance from the zinc smelter (Fig. 2) offered important evidence of dust deposition carrying pollutants (Rolfe and Jennett, 1975; Rieuwerts and Farago, 1996). Bi *et al.* (2006) found that dust deposition-derived heavy metals in soil have the features of low ratios of Zn/Cd (< 200) and Pb/Cd (< 60), which are favored by similar low Zn/Cd and Pb/Cd ratios in the moss growing near the zinc smelters. Low Zn/Cd and Pb/Cd ratios in the smelting affected soils in this study confirmed dust deposition carrying

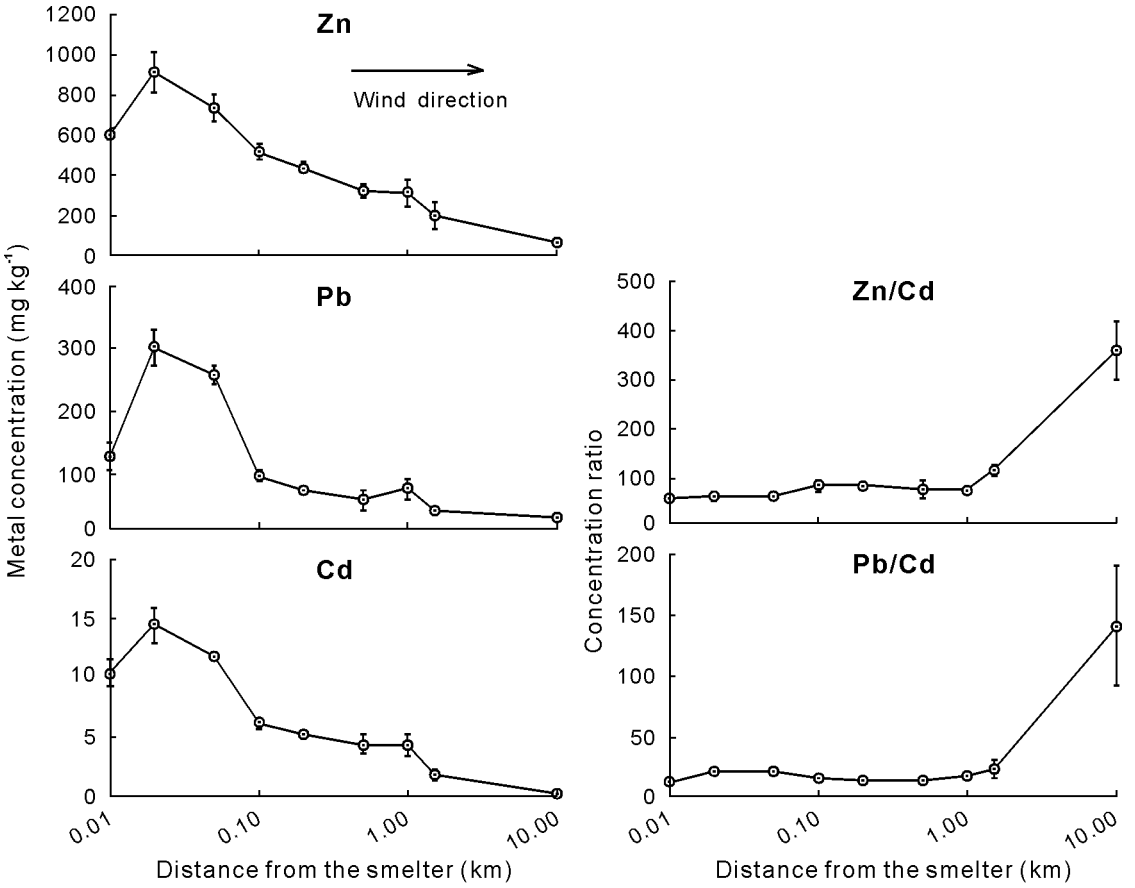

Fig. 2 Concentrations of Pb, Zn, and Cd in the soils studied, relative to distances from a zinc smelter expressed in the common logarithmic scale.

TABLE I

Composite pollution index (CPI) and saturation degree of metals (SDM) of soils at different distances downwind from a zinc smelter

Distance from the smelter	CPI		SDM	
	Average	Range	Average	Range
km				
0.01	8.09	7.74–8.60	1.83	1.72–1.90
0.02	12.20	11.00–13.20	2.75	2.43–3.20
0.05	9.83	9.23–10.70	2.31	1.68–3.10
0.10	6.52	6.03–6.84	1.25	1.18–1.37
0.20	5.53	5.33–6.04	1.24	1.22–1.31
0.50	4.16	3.86–4.66	0.78	0.75–0.80
1.00	4.09	3.20–4.98	0.91	0.78–1.18
1.50	2.52	1.99–3.04	0.59	0.48–0.70
10.00	0.92	0.68–1.27	0.23	0.15–0.40
$LSD_{0.05}$	0.65		0.27	

Pb, Zn, and Cd from the zinc smelter (Fig. 2).

Fractionation of Pb, Zn, and Cd in soils

Fractionation by the sequential extraction method indicated that residual and Fe-Mn oxide bound

fractions were the two main chemical forms of Pb and Zn present in soils, regardless of their distances from the zinc smelter (Fig. 3). The residual fraction of Zn (averaging 58%) was larger than that of Pb (averaging 41%), whereas, the Fe-Mn oxide bound fraction of Pb (averaging 36%) was larger than Zn (averaging 25%). This finding was consistent with the findings of former researchers (García Sánchez *et al.*, 1999), who found that anthropogenic Pb usually accumulated in the oxide-bound fraction. In contrast to Zn and Pb, the exchangeable fraction was dominant in Cd, accounting for an average of 42% of the total Cd in soils, and it had a tendency to decrease with increasing distances from the zinc smelter (Fig. 3). This result supported the hypothesis that anthropogenic Cd is considered readily mobile in soils (García Sánchez *et al.*, 1999).

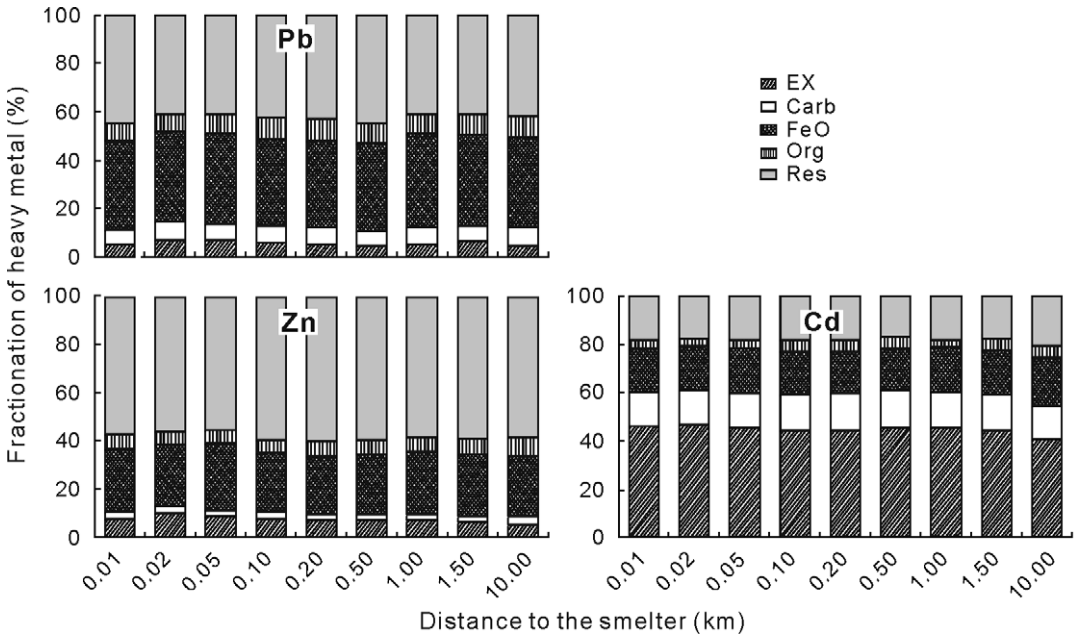

Fig. 3 Fractionation of Pb, Zn, and Cd in soils at different distances from a zinc smelter. EX = exchangeable fraction; Carb = carbonate bound fraction; FeO = iron and Mn bound fraction; Org = organic bound fraction; Res = residual fraction.

The first two fractions of the sequential extraction (exchangeable and carbonate-bound forms) are often regarded as the readily bioavailable fractions (Zalidis *et al.*, 1999). In this study, these two fractions accounted for 12%, 9.5%, and 56% of Pb, Zn, and Cd, respectively.

Toxicity of heavy metals to soil microorganisms is closely related to their bioavailability (Yang *et al.*, 2006b). However, the bioavailability of heavy metals to soil microorganisms remains difficult to be quantitatively determined (Giller *et al.*, 1998). Therefore, indirect ways, such as growth of organism and succedent evaluation of the uptake or toxicity, were frequently applied (Tiensing *et al.*, 2001). Chemical speciation extraction, such as the sequential procedure (Tessier *et al.*, 1979; Rauret *et al.*, 1999), is one of the most frequently applied methods. Hlavay *et al.* (2001) found less than 10% of the mobile fractions of the metals Pb, Zn, and Cd in sediments, concluding no significant release risk of heavy metals under natural environmental conditions. In this study, the mobile fraction was above 10% for Pb and Zn, whereas, over 50% for Cd (Fig. 3), which suggested a higher mobilization risk of heavy metals in the smelting affected soils.

In soils, oxides (hydroxides) of Fe and Al have a strong fixation ability on heavy metals that significantly affect their availability in these soils (Aboulroos *et al.*, 2006; Rose *et al.*, 2006). Therefore, an index, saturation degree of metals (SDM) is introduced to describe the fixation ability of oxides (hydroxides) and the availability of heavy metals in soils. SDM is defined as:

$$\mathrm{SDM} = 1000 \times \Sigma(C_i/\mathrm{AW}_i)/\Sigma(\mathrm{O}_i/\mathrm{OAW}_i)$$

where O_i is the concentration of Fe or Al in soils, and OAW_i is the atomic weight of Fe or Al. Higher SDM represents more availability, whereas, lower SDM represents a stronger fixation of heavy metals in soils, suggesting the possibilities of mobilization or stabilization of heavy metals. In the studied soils, higher SDM (> 2.0) was measured within 50 m from the smelter, and SDM > 1.0 within 200 m, however, SDM was less than 0.4 in the reference area (Table I). The average SDM (1.25) within 1.5 km from the smelter was almost six times as high as that in the reference area (0.209). This result suggested a higher impairment probability from heavy metals in soils within 1.5 km of the smelter than in the reference area 10 km away.

Even though SDMs of the studied soils were generally less than 3 (Table I), smelting-affected soils had six times higher SDM than that in the reference area. This agreed with the results from sequential extraction (Fig. 3), further confirming a severe risk of heavy metal mobilization from smelting affected soils.

Soil microbial characteristics in relation to Pb, Zn, and Cd availability

Soil microbial biomass can effectively reflect the size of the microbial community (Insam *et al.*, 1991). In this study, soil microbial biomass varied greatly, ranging from 95.1 μg C g^{-1} near the Zn smelter to 497 μg C g^{-1}, 10 km away from the smelter (Table II). This suggested that there existed a large size of soil microbial community in the reference area. Due to the influence of Zn smelting practices, soils within 0.1 km of the smelter had low vegetation coverage and low organic matter (Table II). A similar phenomenon has been noticed by Kuperman and Carreiro (1997). Compared to the soils within 50 m of the zinc smelter, soils at the reference area (10 km away from the smelter) had significantly higher organic carbon (Table II).

TABLE II

Some chemical and biological parameters of soils collected at different distances from a zinc smelter in Magu Town, Hezhang County

Distance to zinc smelter	pH	Microbial biomass	Organic carbon	Fe_2O_3	Al_2O_3	MnO
km		μg kg^{-1}	g kg^{-1}			
0.01	5.98±0.33[a)]	106.71±26.04	13.00±4.47	128.51±9.80	131.30±9.15	1.53±032
0.02	5.98±0.28	95.06±11.12	14.10±3.72	150.64±15.60	129.83±16.36	2.09±0.68
0.05	6.05±0.17	177.72±60.77	13.69±4.67	136.80±10.05	133.65±39.72	2.61±0.33
0.10	5.88±0.44	183.25±85.54	22.60±2.26	145.42±9.22	168.83±12.99	1.86±0.22
0.20	5.90±0.24	206.61±50.44	21.88±1.72	140.77±7.83	136.05±7.96	1.82±0.39
0.50	5.95±0.27	267.51±93.65	24.56±4.67	138.83±9.87	173.98±18.04	1.77±0.32
1.00	6.34±0.13	333.22±18.17	21.48±1.56	131.00±17.75	143.87±20.07	1.71±0.48
1.50	6.20±0.02	437.51±53.08	20.37±0.08	110.90±7.21	146.00±6.36	1.52±0.02
10.00	5.92±0.90	497.43±183.67	25.34±25.34	115.09±115.09	141.85±26.34	1.66±0.63
$LSD_{0.05}$	1.03	210.50	5.36	16.93	34.86	0.80

[a)] Mean±standard deviation.

To avoid confounding effects of different soil C content, a ratio of soil microbial biomass carbon to soil organic carbon (C_{mic}/C_{org}) was applied to compare the effects of soil metals. Apparently, a negative relationship between soil C_{mic}/C_{org} and SDM, with a correlation coefficient of -0.664 ($P < 0.01$), could be simulated (Fig. 4). However, correlation between C_{mic}/C_{org} and soil pH was not statistically significant ($R = 0.232$, $n = 60$). This result suggested that heavy metals with higher availability in soils might have more adverse effects on the soil microbial biomass.

To screen the dominant factors of soil chemical parameters affecting soil microbial community size, a stepwise regression analysis was accomplished among soil C_{mic}/C_{org} ratio, availability of heavy metals

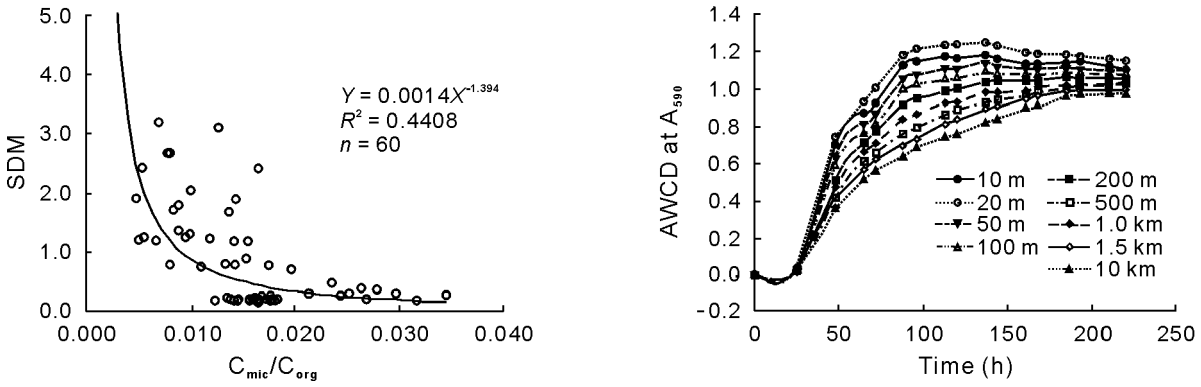

Fig. 4 Relations between saturation degree of metals (SDM) in soils and the ratio of soil microbial biomass carbon to soil organic carbon (C_{mic}/C_{org}).

Fig. 5 Average well color development (AWCD) of microorganisms in soils collected at different distances from a zinc smelter. A_{590} = absorbent wavelength of 590 nm.

(SDM), soil pH, Organic carbon, Fe_2O_3, and Al_2O_3 contents. Therefore, a stepwise regression equation could be obtained according to the entry criteria of variables when the probability of $F \leq 0.050$:

$$C_{mic}/C_{org} = 0.0412 - 0.0097C_{org} - 0.0051SDM$$

The R-value of this equation was 0.587 for a total of 60 samples. Apparently, soil C_{mic}/C_{org} was initially, negatively affected by soil organic carbon content, and then by the available portions of soil Pb, Zn, and Cd. This result further proved that Pb, Zn, and Cd accumulation negatively affected soil microbial community size.

Soil microbial community

Average well color development (AWCD) patterns of microorganisms in soils within gradient distances from the smelter varied similarly with the incubation time. However, after 48 h of incubation, microorganisms of soils 10 km away from the zinc smelter had relatively slower carbon source utilization than those of soils within 1.5 km of the smelter at the same incubation time (Fig. 5). Given an AWCD of 1.0, it took < 100 h for microorganisms in soils 100 m inside the zinc smelter, whereas, > 100 h for soils 200 m outside the smelter (Fig. 5), indicating a quicker consumption of carbon sources by microorganisms in soils closer to the smelter. However, AWCD was not apparently correlated negatively or positively with soil microbial biomass (Fig. 5). This observation verified that AWCD could provide information on differences in community structure other than microbial biomass (Yao *et al.*, 2003).

Soil microbial community structure and functional diversity, as revealed by factor loadings of principal component analysis of Biolog data, can reflect the physiological changes in soil microbial communities under heavy metal stress (Zabinski and Gannon, 1997). Apparently, plots of the principal component scores can be significantly distinguished on the X-axis (Fig. 6). This suggested there existed different patterns of substrate utilization and different microbial communities of soil microorganisms at varying smelting-affected sites. Furthermore, the most influential C sources, deduced from PC1, demonstrated that soil microbial communities at sites close to the zinc smelter would have improved utilization of carbon sources, such as, α-ketoglutaric acid, methyl pyruvate, and L-asparagine, however, decreased utilization of glucose-1-phosphate, α-cyclodextrin, and *N*-acetyl-D-galactosamine.

In this study, the principal components PC1 and PC2 of substrate utilization contributed to about 52% and 25% of the variance. PC1 scores were found to correlate negatively with SDM ($r = -0.773$, $P < 0.01$), although positively with soil microbial biomass ($r = 0.584$, $P < 0.01$), soil organic carbon ($r = 0.497$, $P < 0.01$), and C_{mic}/C_{org} ratios ($r = 0.573$, $P < 0.01$). However, PC1 scores had no

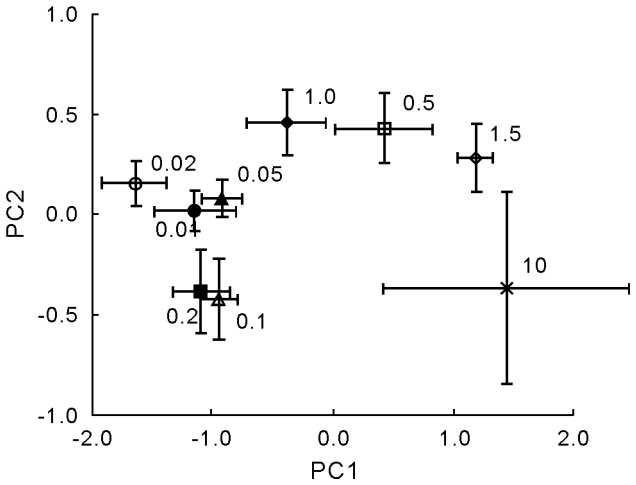

Fig. 6 Principal component (PC) score plots of Biolog data showing microorganism community structures of soils at different distances from a zinc smelter. Plots are present as mean±standard deviation; Data marks represent the distances of soils to the zinc smelter.

relation with soil pH ($r = -0.019$). A systematic shift of soil microbial community structure could be observed in soils with gradient distances from the zinc smelter (Fig. 6). Elevated metal concentrations and reduced pH values were considered as two apparent factors significantly influencing the substrate utilization patterns of soil microbial communities (Knight *et al.*, 1997). However, in this study, soil pH was not significantly differentiated (Table II), suggesting that elevated metal concentration, rather than soil pH, could be responsible for the shift in soil microbial community structure in the studied soils.

Negative correlation between soil microbial biomass and heavy metal concentration suggest that heavy metal pollutants negatively impact soil microorganism activities (Fritze *et al.*, 1996). Measurements of substrate utilization (Biolog) in this study provide evidence of shifts in both structure and diversity of soil microbial communities at the smelting affected sites. This finding is consistent with Smit *et al.* (1997), who reported an apparent shift in soil microbial community structure due to heavy metal accumulation. Heavy metal-induced soil microbial biomass decrease has also been reported by Kuperman and Carreiro (1997) and Kandeler *et al.* (1996), even though they found that microbial activities still existed after the soils were contaminated by high level metals for many years. A large reduction in microbial activity may occur on account of a short-term response of microorganisms to toxic metals (Roane and Kellogg, 1996). Metal contamination is considered as an extreme environment produced by humans for microorganisms. This can be exemplified from Barajas Aceves *et al.* (1999), whose study found a significantly negative exponential relationship of soil microbial biomass to heavy metal Zn concentration up to 6 500 mg kg^{-1}. Their result can be fortified by a negative relationship between soil C_{mic}/C_{org} and SDM in this study (Fig. 4). Scarce vegetation coverage in the studied region may be another reason why heavy metals reduce soil microbial populations (Kuperman and Carreiro, 1997).

Community level substrate utilization of soil microorganisms was impaired at heavy metal-accumulated sites (Figs. 5 and 6). This finding agreed with Knight *et al.* (1997) who demonstrated a significant reduction in substrate utilization at the Zn amendment levels of 300 to 350 mg kg^{-1} at low pH (5.1). However, Bååth *et al.* (1998) have found no effects of high levels of Cu, Ni, and Zn on the substrate utilization in the Lee Valley soil. This controversy remains complicated due to the variability of composition and activity of soil microbial communities under heavy metal stress.

It is often difficult to compare results of heavy metal toxicity to soil microorganisms, from previous studies. Giller *et al.* (1998) attributed this to the differences in study methodology: laboratory ecotoxicology studies, "field" ecotoxicology studies, and environmental monitoring. Therefore, two cases

of heavy metal toxicity: "chronic toxicity" and "acute toxicity" could be defined. The former is often related to a constant stress of heavy metals to microorganisms, and the latter to a drastic, sudden disturbance (Grime, 1997). This might explain the difference between this study and the previous documents on heavy metal effects on soil microorganism community structure and diversity. Decreases in soil microbial biomass and differences in substrate utilization patterns in this study did suggest that some changes might have occurred in soil microorganisms, as a result of dust deposited-heavy metal accumulation, within 1.5 km of the zinc smelter, as compared to the reference site.

CONCLUSIONS

Soils within 1.5 km of the zinc smelter accumulated high levels of heavy metals Zn (508 mg kg^{-1}), Pb (95.6 mg kg^{-1}), and Cd (5.98 mg kg^{-1}) with low ratios of Zn/Cd (59.1–115) and Pb/Cd (12.4–23.4), and their CPIs, varying in the range of 2.52–15.2, were 3–13 times higher than those in the reference soils. Heavy metal contamination from smelting atmospheric deposition was apparent in the Magu Town region.

In heavy metal contaminated soils, Zn and Pb were dominated by residual and Fe-Mn oxide bound fractions, whereas, Cd by the exchangeable fraction, and their exchangeable plus carbonate bound fractions accounted for $> 10\%$ of the total soil Zn, Pb, and Cd. The SDM of contaminated soils (1.25) was six times higher than that of the reference soils (0.209), confirming a high risk of heavy metal mobilization.

Both the smaller soil microbial biomass in contaminated soils (85.1–438 μg g^{-1}) than in reference soils (497 μg g^{-1}) and the negative correlation of soil C_{mic}/C_{org} with SDM suggested that accumulation of Pb, Zn, and Cd might impair soil microbial community size. Furthermore, a more rapid consumption of carbon sources by microorganisms in the contaminated soils, together with an apparent shift in substrate utilization and its negative correlation with SDM, confirmed a shift in soil microbial community structure. Apparently, accumulation of Pb, Zn, and Cd from atmospheric deposition during zinc smelting was detrimental to soil quality.

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