The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter

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Abstract

The environmental risk of heavy metal pollution is pronounced in soils adjacent to large industrial complexes. It is important to investigate the functioning of soil microorganisms in ecosystems exposed to long-term contamination by heavy metals. We studied the potential effects of heavy metals on microbial biomass, activity, and community composition in soil near a copper smelter in China. The results showed that microbial biomass C was negatively affected by the elevated metal levels and was closely correlated with heavy metal stress. Enzyme activity was greatly depressed by conditions in the heavy metal-contaminated sites. Good correlation was observed between enzyme activity and the distance from the smelter. Elevated metal loadings resulted in changes in the activity of the soil microbe, as indicated by changes in their metabolic profiles from correlation analysis. Significant decrease of soil phosphatase activities was found in the soils 200 m away from the smelter. Polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) analysis demonstrated that heavy metals pollution had a significant impact on bacterial and actinomycetic community structure. There were negative correlations between soil microbial biomass, phosphatase activity, and NH₄NO₃ extractable heavy metals. The soil microorganism activity and community composition could be predicted significantly using the availability of Cu and Zn. By combining different monitoring approaches from different viewpoints, the set of methods applied in this study were sensitive to site differences and contributed to a better understanding of heavy metals effects on the structure, size and activity of microbial communities in soils. The data presented demonstrate the role of heavy metals pollution in understanding the heavy metal toxicity to soil microorganism near a copper smelter in China.

Keywords: Availability; Community composition; Heavy metals; Soil microorganism

1. Introduction

Mining, processing, and smelting activities have contaminated soil and water resources with heavy metals throughout the world (Valery and Eugene, 1998). Geochemical weathering processes acting upon metallurgical wastes and by-products initiate the process of transporting heavy metals from contaminated areas and redistributing them to surrounding soils, streams, and groundwater. Thus, heavy metals can adversely affect soil and water resources and endanger the health of surrounding ecosystems and human populations (McGrath et al., 2001; Shi et al., 2002).

Many reports have shown that short-term or long-term exposure to toxic metals results in the reduction of microbial diversity and activities in soil (Lasat, 2002; McGrath et al., 2001). Diversity and activity of microbial communities are important indices of soil quality. Soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and the control of plant pests and plant growth (Elsgaard et al., 2001; Filip, 2002). Alterations in the composition of microbial communities have often been

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proposed to be an easily and sensitive indicator of anthropogenic effects on soil ecology (Renella et al., 2005).

With the increasing emphasis on sustainable fertility and environmental benefits, the restoration of soil microbial activity has become more important. This is especially so since the negative effects of heavy metals on plant growth and their possible entry into the food chain have been well documented (Brun et al., 2001; Cornelia and Franz, 2004). Despite the demonstrated importance of soil microorganisms, the environmental state and the future of the soil ecosystem are still largely unknown. In addition, only a few studies including this component have been conducted on heavy metals smelters in China (Liu, 2003).

In this study, a typical heavy metal smelter (copper smelter) was selected. The landscape presents itself as an important case study not just to ecologists interested in the complex effects of man-made disturbance on ecosystems, but also to those involved with the determination of public policy related to environmental pollution. Soil microbiological and biochemical properties (biomass C, phosphatase activities) were measured to determine the effects of heavy metals on microbial activities. Water and NH4NO3 test are used as a method for multielement extraction of micronutrients and has been used for assessing the bioavailability of nonessential trace metals (Krishnamurti and Naidu, 2002; Song et al., 2004). Soil bacteria and actinomycetes community analysis were carried out using activation-independent methods. Partial 16S rDNA genes were amplified from soil bacterial community DNA by polymerase chain reaction (PCR), using primers that bind to evolutionarily conserved regions within these genes in the bacteria and actinomycete. The diversity of PCR-amplified products was transformed to genetic fingerprints using denaturing gradient gel electrophoresis (DGGE) (Heuer et al., 1997; Muyzer et al., 1993).

2. Materials and methods

2.1. Study site

The study area selected is near to and downwind of a copper–zinc smelter started in 1985 in Zhujiawu county, Zhejiang province, China. The soil was contaminated by heavy metals, mainly Cu and Zn. According to USDA soil taxonomy (United States Department of Agriculture (USDA), 1988), the alluvial sandy loam, paddy soil that developed on the river alluvium is a fluvatic. This area was formerly used to cultivate cereal grass. After 1990s the site was not longer cultivated due to the reduction of crop output and contamination of the crop by heavy metals. A sward consisting of plants tolerant to heavy metals covered the ground at the time of sampling.

2.2. Soil samples

Soil samples were taken in May 2004 at seven points along a gradient of pollution of Cu and Zn (Table 1). Each point contained five replicated samples, each composed of three soil cores 5 cm in diameter and 15 cm deep (each soil core about 500 g) were taken randomly from different areas at each site. Each replicate sample was mixed thoroughly. Field moist soils were sieved (<2 mm) by nylon sieve and large pieces of plant material, stones, and soil animals were removed. Part of the samples was kept moist in the dark at 4 °C to determine the concentration of heavy metals in the soil solution and to assess soil enzyme activity and microbial biomass. The remaining soil was stored at −20 °C to extract soil DNA. Subsamples was air dried at ambient temperature, crushed, and sieved through a 0.2-mm nylon sieve to analyze pH, organic matter, and total metal content.

2.3. Soil analysis

Soil pH was measured with a glass electrode using a 1:2.5 soil-to-water ratio. Organic C was determined by dichromate digestion. Total contents of Cu/Zn in the soil were analyzed with graphite and flame atomic absorption spectrophotometry (GFAAS) (Thermo Element MKII-M6) by digesting 100 mg of soil in a mixture of HF–HClO4–HNO3 (Lu, 1999). Soil bioavailability of heavy metals was estimated by extracting with 1 M NH4NO3 (1:2.5 w/v) in triplicate. The soil suspensions were centrifuged at 4000 rpm for 10 min and filtered. Contents of Cu and Zn in each filtrate were determined by FAAS (Lu, 1999). The certified standard samples were included at every stage of soil heavy metals analysis.

2.4. Saturation water extract

Sufficient amounts of deionized, distilled water were added to 50 g of the fresh soils, in duplicate, to bring the soil to saturation (100% of water holding capacity). The soil was mixed thoroughly to form a slurry and equilibrated for 24 h in a 250-mL plastic container. The soil solutions were recovered from the slurry by filtration under vacuum using filter paper. The solutions were centrifuged at 12,000g for 30 min and passed through 0.25-μm millipore filters (Vulkan et al., 2000). The concentration of the metals in the supernatants was determined by GFAAS.

Table 1

Selected properties of soil samples collected along a heavy metals pollution gradient

<table>
<thead>
<tr>
<th>Soil no.</th>
<th>Distance (m)</th>
<th>pH</th>
<th>Total Zn (mg kg⁻¹)</th>
<th>Total Cu (mg kg⁻¹)</th>
<th>Organic matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>7.75 ± 0.13a</td>
<td>3194 ± 562a</td>
<td>658 ± 123a</td>
<td>3.59 ± 0.20a</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>7.89 ± 0.13b</td>
<td>2386 ± 311ac</td>
<td>438 ± 41b</td>
<td>3.65 ± 0.05a</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>7.86 ± 0.10a</td>
<td>3219 ± 1357a</td>
<td>319 ± 74cd</td>
<td>3.69 ± 0.21a</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>7.97 ± 0.14bd</td>
<td>2795 ± 674a</td>
<td>245 ± 15cd</td>
<td>3.52 ± 0.20a</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>8.12 ± 0.13c</td>
<td>1650 ± 824bc</td>
<td>185 ± 17cf</td>
<td>3.75 ± 0.12a</td>
</tr>
<tr>
<td>6</td>
<td>400</td>
<td>8.08 ± 0.06ed</td>
<td>1281 ± 148b</td>
<td>170 ± 10ef</td>
<td>3.77 ± 0.10a</td>
</tr>
<tr>
<td>7</td>
<td>600</td>
<td>8.12 ± 0.07c</td>
<td>1140 ± 141b</td>
<td>144 ± 17ef</td>
<td>3.61 ± 0.44a</td>
</tr>
</tbody>
</table>

Note: The arithmetic mean of five replicates is shown with its arithmetic standard deviation (Mean ± SD). Different letters in the same column indicate significant differences (P < 0.05).

aDistance from the smelter.

bPH (H2O:soil) = 2.5:1.
2.5. Microbial biomass carbon

Microbial biomass carbon (biomass C) was determined by the fumigation-extraction (FE) method (Vance et al., 1987). Three subsamples of moist soil (see soil samples) were extracted with 20 mL 0.5 M K2SO4. The samples were shaken for 30 min, filtered, and frozen at -20 °C. Simultaneously, three other subsamples of soil (also equivalent to 5.0 g dry soil) were fumigated with ethanol-free chloroform for 24 h at 25 °C and then extracted and frozen similarly. Biomass C (BC) was calculated from 

\[ \text{BC} = 2.22 \times \frac{E_C}{E_X} \]

where \( E_C \) (C extracted from fumigated soil) and \( E_X \) (C extracted from nonfumigated soil). Carbon in the extracts was determined by an automated TOC Analyzer (Shimazu, TOC-500, Japan).

2.6. Soil enzyme activity

Soil alkaline phosphatase activity was measured spectrophotometrically by the disodium phenyl phosphate method of Li (Li, 1996). Briefly, 10 g of soil sample were carefully transferred into a 100-mL measuring flask and 2 mL of toluene were added to inhibit the growth of microorganisms. After standing for 15 min, 10 mL of 0.5% (w/v) disodium phenyl phosphate and 10 mL of 0.2 M borate buffer (pH 9.6) were incorporated. The soil sample and the added solutions were mixed evenly and incubated at 37 °C for 24 h. Then, the solution was made up to 100 mL volume with 38 °C distilled water and filtered. One milliliter of the filtrate was transferred into another 100-mL volumetric flask; then 5 mL of 12.5 mM boric acid buffer (pH 9.6), 24 mL of distilled water, and 1 mL of 0.2% (w/v) 2,6-dibromoquinone-chloride (C6H3Br2Cl2NO) were added and carefully mixed. After color development for 20 min, the solution was diluted to 100 mL and the absorbance of the diluted solution was measured at 578 nm. The soil alkaline phosphatase activity was expressed as mg phenol produced (g dry soil 24 h-1).

2.7. DNA extraction

The soils of plots situated at 50, 200, and 600 m were selected in the present study to extract total DNA by placing approximately 500 mg of soil in tubes containing lysing matrix. Isolation of total DNA was accomplished with a FastPrep DNA isolation kit according to the manufacturer's protocol (BIO101).

2.8. PCR–DGGE microbial community analysis

Primers F338: 5′-CTACGGGAGGCAGCAG-3′ and R518: 5′-ATTACCGCGGCTGCTGG3′ were used in this study for the amplification of bacterial 16S rDNA genes and Primers F243: 5′-GATGAGCCCGGC-GGCCTA3′ and R518 for the amplification of soil actinomycetes. The GC clamp (CGCCGGCCGGCCGGCCGGCCGGCCGGCCGGCCGGCCGGCCGGCCCGC-CGGC) described by Muyzer et al. (1993) was added to the forward primers to facilitate the DGGE. PCR reaction was executed in a Mastercycler gradient (Eppendorf, Germany) in 0.2-mL tubes using a reaction volume of 50 μL. The reaction mixture contained 25 pmol of both primers, 20 μmol of each dNTPs, 5 μL of 10 × reaction buffer, and 2.5 units of Taq DNA polymerase. Cycling conditions used to amplify the soil bacterial and actinomycetic 16S rDNA gene fragment were 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min (for bacteria) or 53 °C for 1 min (for actinomycetes), and 72 °C for 2 min. A final extension period of 72 °C for 10 min was used. An aliquot of 5 μL PCR products was checked by electrophoresis in 1.5% (wt/vol) agarose gels stained with ethidium bromide prior to DGGE. For DGGE analysis, PCR products generated from each sample was separated on a 10% acrylamide gel with a linear denaturant gradient range from 35% to 60% using the Bio-Rad D-GENE System. DGGE was performed using 30 μL of the PCR product in 1 × TAE buffer at 60 °C, and 160 V for 300 min. Gels were stained with silver staining (Bassam et al., 1991) and the gels scanned (Bio-Rad, USA).

2.9. Analysis of DGGE patterns

Digitized DGGE images were analyzed with Quantity One image analysis software (Version 4.0, Bio-Rad, USA). This software identifies the bands occupying the same position in the different lanes of the gel. Using PCA analysis, the matrix was used to calculate the similarity of the gel pattern. It took into account the presence or absence of individual bands in all lanes (binary matrix) (Christopher et al., 2003). The Shannon index \( H \) was used to estimate soil bacterial diversity based on the intensity and number of bands using the equation:

\[ \text{Shannon index} (H) = - \sum_{i=1}^{N} \left( \frac{n_i}{N} \right) \ln \left( \frac{n_i}{N} \right) \]

where \( n_i \) is the peak height of the band \( i \), \( i \) the number of bands in each DGGE gel profile on \( N \) is the sum of peak heights in a given DGGE gel profile. Regression analysis was performed by SPSS 11.0 (SPSS Inc., USA) to investigate the correlation between the Shannon index and operational parameters.

2.10. Statistical analysis

All data were analyzed using Microsoft Excel and SPSS 11.0. A probability level of 0.05 was considered to be statistically significant.

3. Results

3.1. Soil solid-phase properties

For the past 20 yr, Cu and Zn have been the dominant sources of pollution in this area. The soils varied greatly in heavy metal concentrations with their distances from the smelter (Table 1). Soil pH varied from 7.75 to 8.12 and increased away from the smelter. Soil pH of the plots situated 50 and 100 m from the smelter was significantly lower than for the soils situated at 250, 400, and 600 m. The range of soil organic C was from 3.52% to 3.77%. There was a large variation in the concentration of total Cu (144–658 mg kg-1) and Zn (1140–3218 mg kg-1). The highest concentration of Cu was found in the soil nearest to the smelter but of Zn in 150 m away from the smelter (Table 1). No significant correlation was found between soil total heavy metals and soil organic C.

3.2. Concentrations of extractable heavy metals

The concentrations of soluble Cu and Zn in the soil solution samples varied significantly (Fig. 2). Concentration of Cu and Zn in the soil solution decreased with increasing distance from the smelter. The concentrations of water-extractable Cu and NH4NO3-extractable heavy metals in the plots located within 50 m from the smelter were significantly higher than those of other soils. A significant decrease was observed between the concentrations of NH4NO3-extractable heavy metals in the soils situated within 50 to 150 m (Table 2), while there was no significant decrease in soils lying between 250 and 600 m. A significant relationship between extractable Cu (NH4NO3-extractable and water-soluble) and total Cu was observed and there was no correlation between extractable Zn and total Zn in the soil (Table 2).
Table 2
Distribution of NH4NO3-extractable and soil solution heavy metals, microbial biomass, and phosphatase activity of the tested soils away from the copper smelter.

<table>
<thead>
<tr>
<th>Soil no.</th>
<th>Water-extractable Cu, Zn (mg kg⁻¹)</th>
<th>NH4NO3-extractable Cu, Zn (mg kg⁻¹)</th>
<th>Microbial biomass C(mg kg⁻¹)</th>
<th>Phosphatase activity(mg phenol g⁻¹ dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn</td>
<td>Cu</td>
<td>Zn</td>
<td>Cu</td>
</tr>
<tr>
<td>1</td>
<td>1.53±0.99a</td>
<td>3.61±0.90a</td>
<td>15.68±3.72a</td>
<td>9.22±2.18a</td>
</tr>
<tr>
<td>2</td>
<td>1.06±0.40ac</td>
<td>2.08±1.42b</td>
<td>8.35±3.21b</td>
<td>7.05±2.25b</td>
</tr>
<tr>
<td>3</td>
<td>0.53±0.29bcd</td>
<td>0.86±0.51c</td>
<td>5.01±1.89c</td>
<td>3.51±1.61c</td>
</tr>
<tr>
<td>4</td>
<td>1.05±0.42ade</td>
<td>0.60±0.26c</td>
<td>4.38±1.47ce</td>
<td>3.57±0.44c</td>
</tr>
<tr>
<td>5</td>
<td>0.71±0.46bece</td>
<td>0.42±0.19e</td>
<td>2.56±0.77ce</td>
<td>2.14±0.30ce</td>
</tr>
<tr>
<td>6</td>
<td>0.21±0.15b</td>
<td>0.44±0.34c</td>
<td>2.32±0.82ce</td>
<td>1.95±0.22ce</td>
</tr>
<tr>
<td>7</td>
<td>0.33±0.13b</td>
<td>0.78±0.33c</td>
<td>1.97±0.56de</td>
<td>1.63±0.19de</td>
</tr>
</tbody>
</table>

*Note:* The arithmetic mean of five replicates is shown with their arithmetic standard deviations (Mean ± SD). Different letters in the same column indicate significant differences (P<0.05).

Table 3
Shannon diversity index for the different heavy-metals-contaminated soil samples

<table>
<thead>
<tr>
<th>Microorganism distance</th>
<th>Shannon index (Mean β±SD γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil bacteria</td>
</tr>
<tr>
<td>50 m</td>
<td>2.20±0.14a</td>
</tr>
<tr>
<td>200 m</td>
<td>2.68±0.22b</td>
</tr>
<tr>
<td>600 m</td>
<td>2.81±0.16b</td>
</tr>
</tbody>
</table>

The arithmetic mean of five replicates is shown with their arithmetic standard deviations. Different letters in the same column indicate significant differences (P<0.05).

3.3. Soil microbial biomass

Microbial biomass carbon (Cmic) in the soils, measured by the FE method, ranged from 22.5 to 48.1 mg g⁻¹ (Table 2). Although Cmic increased from the nearest plot (50 m) to the remotest plot (600 m), significant decrease was found in the soils 250 m away from the smelter. A strong relationship was observed between Cmic and the distance from the smelter (r = 0.89). Soil-soluble Zn was significantly correlated with soil Cmic (r = 0.90); however, there was no significant correlation between the microbial biomass C and soil-soluble Cu. Soil Cmic was negatively correlated with NH4NO3-extracted Cu, total Cu, extractable Zn, and total Zn (Table 3). Extracting with NH4NO3 was better than with water in predicting the effect of heavy metals on Cmic. In our study, no significant correlation was observed between the microbial biomass C and soil organic C.

3.4. Enzyme activity

Variations of the alkaline phosphatase activity in the soil samples away from the smelter are shown in Table 2. Enzyme activity was greatly depressed by conditions in the heavy metal-contaminated sites. Good correlation was observed between enzyme activity and distance from the smelter. Phosphatase activities in soil samples were the lowest near the smelter. Significant decrease was found in the soils 200 m away from the smelter. Soil phosphatase activities showed an increased trend with a decrease in heavy metals content. Soil phosphatase activity was negatively correlated with soil solution Cu (r = −0.82), NH4NO3-extractable Cu (r = −0.92), total Cu (r = −0.95), soil solution Zn (r = −0.80), NH4NO3-extractable Zn (r = −0.87), and total Zn (r = −0.84) (Table 3).

3.5. Soil microorganism community composition analyzed by DGGE

The DGGE profiles were highly reproducible, in replicated samples (Figs. 1 and 2). Although lots of bands were detected in all samples, apparent differences in the bacterial community among the soils from 50, 200, and 600 m away from the smelter were readily observed (Fig. 1). As shown in Fig. 1, the numbers of bands in plot situated at 200 and 600 m were significantly increased as compared to that situated at 50 m. In contrast to the nearest plot (50 m), it is apparent that the number of bands in soil was...
enhanced for heavy metals decreasing. For soil actinomy-
cete community composition, we also found that the
number of bands bands in the plot situated of 600 m was
significantly increased as compared to that situated at 50 m
(Fig. 2).

Subsequently, DGGE gels were interpreted using the
Shannon index and principal component analysis (PCA),
in which data were transformed in two ways, the former
taking into account the relative intensity and the latter the
presence of the bands. The Shannon index indicated in the
most polluted soil decreased the bacteria and actinomyce-
diversity in soil as compared to that in soils in the remotest
soil (600 m) (Table 3). PCA proved to be a very powerful
way of analyzing DGGE profiles. The PCA plots showed a
clear separation due to different distance, indicating altered
structure in diversity. Using the presence of the bands data,
the first two principal components (PC1 and PC2) were
sufficient to explain 54.6% and 44.6% of the variance for
soil bacteria and actinomycete, respectively (Fig. 3).

4. Discussion

Recent results showed that it is the concentration of a
metal in its available form that is crucial to understanding
metal toxicity. The availability of heavy metals is related to
their chemical forms in the soils. Several fractions or
compartments of the soil act as reservoirs of available
metals. Water and NH₄NO₃ extraction, which is a widely
used soil testing method (Kot and Namiesniık, 2000;
Krishnamurti and Naidu, 2002; Song et al., 2004), provide
an operationally defined soil compartment that is char-
acterized by its solubility. Water can release the soil
solution heavy metals; NH₄NO₃ mainly extracts soluble
and exchangeable metals. The heavy metal concentrations
in soil solution are generally reduced at neutral or alkaline
pH (Muñoz-Meléndez et al., 2000). The study sites were
alkaline, and the average concentrations of Cu and Zn in
the soil solution were only 0.34% and 0.03% of total Cu
and Zn, respectively (Table 1). These small but active
fractions, however, may provide some indication of the
forms of heavy metals that are most available for soil
microorganisms (Brooke, 1995; Bruce et al., 2003; Huang
and Shindob, 2000).

This experiment showed long-term effects of nearly 20 yr
of heavy metal contamination near a copper smelter. It was
found that heavy metals could affect the activity and
community composition of soil microorganisms (Table 2,
Figs. 1 and 2). The activity and community composition of
soil microorganism are closely related to soil fertility and
environmental quality. Brookes has provided evidence that
heavy metals decrease the proportion of microbial biomass
C in total soil organic matter, and soil microbial C has been
proposed as a useful measure of soil pollution by heavy
metals (Brooke, 1995).

The above-mentioned hypothesis might also explain the
reduction of enzyme activities observed in the Cu/Zn-
contaminated soils. The results obtained for soil phospha-
tase indicated that the heavy metal-contaminated soil, with
increasing distance from the smelter, had significant

Fig. 2. DGGE profiles of actinomycete communities inhabiting each
heavy metal-contaminated soil sample. PCR products were synthesized
with the primer pair F243–R518. Labels above each lane indicate which
site the pattern represents.

Fig. 3. Score plots from the principal component analysis (PCA) based on data from soil plots situated of 50, 200, and 500 m (A, soil bacteria; B, soil
actinomycete).
The variability in the metabolic profiles indicated the possibility that the metal contamination may result in a community that is more variable and less stable. In our study, genetic structure of indigenous soil microorganism communities was assessed by molecular methods, based on DGGE fingerprinting of PCR-amplified 16S rDNA sequences from soil-extracted DNA. The PCR–DGGE technique has become a widespread molecular technique for monitoring changes in soil microflora due to pollution and changes in agricultural practices (Heuer et al., 1997; Muyzer et al., 1993). We detected Cu and Zn effects, namely an increased relative abundance of some populations with increasing distance from the smelter. Molecular fingerprinting of soil bacterial communities provided clear evidence that heavy metals were the main factor influencing microbial diversity by promoting changes in species composition (position of bands) and in species richness (number of occurring bands) (Table 2, Figs. 1–3).

Soil microbial activity and diversity are difficult to elucidate, and no single approach provides a complete depiction of the soil microbial situation. However, by combining different monitoring approaches, better insight can be gained into the microbial environment. The set of methods applied in this study were sensitive to site differences and contributed to a better understanding of heavy metal effects on the structure, size, and activity of microbial communities in soils. Previous research, essentially based on culturing and physiological assays, has shown that soil heavy metals were toxic to microorganisms and might reduce the soil microbial community composition (Kunito et al., 1999). The availability of heavy metals is an important factor affecting the soil microorganism community composition.

According to our results, there is a negative correlation between soil bacteria activity, community diversity, and NH₄NO₃-extractable heavy metal.

Although we did not determine the speciation of metals in the soil solution in present studies (Lofts et al., 2004; Sauvé et al., 1998), the results showed similar correlations between total and extractable contents of the heavy metals in soils and soil microbial activity and community composition; this suggests that NH₄NO₃-extracted heavy metals and total heavy metals can probably be used as predictors of the effect of heavy metals on soil microorganisms. Although the duration of our experiment does not allow prediction of microbial genetic diversity and potential functional abilities (Prus-Głowacki et al., 1999), this will provide an explanation of the influence of combined pollution of Cu and Zn on soil microorganism activity and community composition near a copper smelter in China.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble¹ NH₄NO₃,² Total³</td>
<td>Soluble NH₄NO₃ Total</td>
</tr>
<tr>
<td>Total Cu</td>
<td>0.96**</td>
<td>0.98**</td>
</tr>
<tr>
<td>Total Zn</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cmic⁴</td>
<td>−0.73</td>
<td>−0.88**</td>
</tr>
<tr>
<td>P</td>
<td>−0.82*</td>
<td>−0.92**</td>
</tr>
</tbody>
</table>

*P < 0.05.
**P < 0.01.
¹Soluble, soil solution heavy metal.
²NH₄NO₃, NH₄NO₃-extractable heavy metal.
³Total, total heavy metal.
⁴Cmic, soil microbial biomass.
⁵P, soil phosphatase activity.

5. Conclusions

Our study demonstrated a highly significant correlation between the total contents of Cu and their fractions extracted by water and NH₄NO₃ in the metal-contaminated soils under study. Subsequently, by combining different monitoring approaches from different viewpoints, better insight can be gained into the microbial environment. The set of methods applied in this study were sensitive to site differences and contributed to a better understanding of heavy metals effects on the structure, size, and activity of microbial communities in soils. Finally, the results showed similar correlations between total and extractable contents of the heavy metals and soil microbial activity and community composition. This suggests that NH₄NO₃-extracted heavy metals and total heavy metals can probably be used as a predictor of bioavailability of heavy metals in soil.

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### References


