# CHARACTERIZATION OF SOIL PHYSICO-CHEMICAL AND MICROBIAL PARAMETERS AFTER REVEGETATION NEAR SHAOGUAN Pb/Zn SMELTER, GUANGDONG, P.R. CHINA

CHONG-BANG ZHANG[1,4], LI-NAN HUANG[1], MING-HONG WONG[2], JIN-TAO ZHANG[3], CHENG-JIE ZHAI[3] and CHONG-YU LAN[1]

[1]*School of Life Sciences and State Key Laboratory of Biocontrol, Sun Yet-Sen (Zhongshan) University, Guangzhou 510275, P.R. China;* [2]*Croucher Institute for Natural Resources and Environmental Sciences, and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong;* [3]*Shaoguan Pb/Zn Smelter, Shaoguan 512024, P.R. China;* [4]*School of Life Sciences, Taizhou University, Linhai 317000, P.R. China*

*(*author for correspondence, e-mail: ls04@zsu.edu.cn; Tel.: (8620) 84036296, Fax: (8620) 84113652)*

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**Abstract.** The criterion for judging the successful revegetation largely focuses on the aboveground indicators, whereas the information for soil ecosystem during the revegetation is often ignored. To better understand the effects of the revegetation on the development of the soil ecosystem near Shaoguan Pb/Zn Smelter, Guangdong Province of Southern China, we compared the difference of the microbial and physico-chemical parameters between the four revegetated sites and two control sites (bare ground and native forest area). The soil organic C, total N, total P, $NH_4$-N, $NO_3$-N, available P, WHC and porosity significantly increased and bulk density decreased in the four revegetated sites compared with those in bare ground, indicating the processive effects of the revegetation on the reestablishment of the soil nutrient pools. The heavy metal contents were higher in the four revegetated sites than in the bare ground, thus the revegetation resulted in the accumulation of heavy metals released from smelter in surface soil. The soil microbial composition and activities, except that the oligotrophic bacterial number decreased over revegetation time, significantly increased in the revegetated sites compared with those in the bare ground, and predominantly correlated with soil organic C, total N, $NH_4$-N, $NO_3$-N and WHC. The soil oligotrophic bacteria was negatively related to all individual heavy metal contents, thus was the most sensitive indicator in reflecting heavy metal stress, while other microbial parameters, despite not showing negative relationships to the individual heavy metal contents, were sensitive to the potential availability of Pb and Cu (ratio of available to total heavy metal contents), but less sensitive to those of Zn and Cd. Both the principal component analysis (PCA) and the discriminant analysis (DA) resulted from microbial and physico-chemical datasets not only revealed the shifts of the soil physico-chemical and microbial patterns from the unrevegetated to non-polluted conditions, but also implied the possible loss of effects of revegetation on soil remediation in the sites revegetated for four (RIV) and five (RV) years, respectively.

**Keywords:** heavy metal contents, industrial barrens, microbial composition and activity, physico-chemical properties, revegetation, soil development

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## 1. Introduction

Metal smelting activities generate a large amount of dust containing heavy metals depositing on surface soil. The direct effect of dust deposition might result in the loss of the aboveground vegetation, and create large areas of industrial barrens (Helmisaari *et al*., 1995). The indirect effects not only include the potential health risks of heavy metals to humans through contaminating food chains and water (Tordo *et al*., 2000; Wong, 2003), but also the loss of structure and function of microbial communities which mediate soil processes such as organic matter decomposition, nutrient transforming and aggregate forming (Preston *et al*., 2001). Therefore, revegetation is necessary for limiting dispersion of heavy metals by fixing and stabilizing the top layer of soil (Vangronsveld *et al*., 1996). Meanwhile, a successful vegetation cover might restore the ecological integrity such as biodiversity, ecological processes, and structure relationships of the disturbed ecosystem of these industrial barrens (Montalvo *et al*., 1997).

Fundamentals to assessment of ecological integrity in a revegetated ecosystem are the relationships of biotic and abiotic components (Mummey *et al*., 2002). We here select microbial parameters (number, biomass and activities) as biotic indicators for evaluating quality of the revegetated soils. On one hand, visually distinguishable vegetation indicators often pose a transitory artefact of successful revegetation to environmental managers, whereas soil microbial indicators are not able to be faked (Harris, 2003). On the other hand, some studies indicated that the culturable microorganisms are the most sensitive and active components in the environment (Ellis *et al*., 2003; Söderberg *et al*., 2004), whereas the numerically dominant components of microbial communities, as revealed by DNA-based techniques (Kell *et al*., 1998), can exist in dormant forms. Therefore, these culturable microorganisms are the most important contributors to their ecosystem (Ellis *et al*., 2003). In addition, microbial biomass, accounting for 1–4% of soil total C, and 2–6% of total organic N (Anderson and Domsch, 1989; Jenkinson, 1988), and the microbial activities such as basal respiration, substrate induced respiration and cellulose decomposition, are strongly related to soil physico-chemical parameters such as aggregates, pH and nutrient availability (Pascual *et al*., 2000; Margesin *et al*., 2000; Chew *et al*., 2001). Therefore, these microbial measurements have been suggested as possible indicators of soil environmental quality (Hargreaves *et al*., 2003). In this paper, four revegetated sites, resulting from a full-scale revegetation work near Shaoguan Pb/Zn Smelter in Guangdong Province of Southern China, and two control sites including the bare ground and unpolluted native forest area were selected for comparing the differences in microbial and physico-chemical properties. Our objectives were: (1) to test if the microbial and physico-chemical parameters are positively developing over revegetation ages, (2) to test responses of individual microbial parameters to heavy metal fluctuations in long-term metal contamination soils over revegetation ages, and (3) to determine whether soil microbial and physico-chemical

patterns distinguish between revegetated soils and controls or within revegetated soils.

## 2. Material and Methods

The revegetation sites are located at the northern slope of the Southern mountain opposite the Shaoguan Pb/Zn Smelter located in the vicinity of Shaoguan city in the north of Guangdong Province, Southern China (24°47′N, 113°36′E), and their topsoil were deposited with heavy metal dust from the smelting activities from 1976–1999 at which dust emission began to be purified. It once was a subtropical forest area in which the climax vegetation, which has almost disappeared due to smelting activities, dominated by *Castanopsis jucunda, Schima crenata, Cinnamomum camphora* and *Pinus massoniana*. The vegetation cover resulted from a revegetation program in 1999 is currently dominated by *Paulownia fortunei* and accompanied by *Cynodon dactylon, Leucaena glauca, Ligustrum lucidum, Nerium indicum, Fices altissima, Ailanthus altissima* and *Sophora japonica*. The soil in this area is classified as red-yellow podzolic sandy loam. This area has a subtropical monsoonal climate with a mean annual precipitation of 1619.6 mm mainly occurring from March to September. The average annual temperature is 19.6 °C, with the average minimum temperature occurring in January (11 °C), and average maximum temperature in July (28 °C). The prevalent wind direction is from NE to SW.

Four revegetated sites and two control sites (one bare ground and another unpolluted forest site) were included in the study. The four revegetated sites were chosen based on the revegetation ages (revegetated for two (RII), three (RIII), four (RIV) and five years (RV), respectively). The location of the bare ground (BG) was located at the same slope as the revegetated sites, but was not revegetated. The native forest site (FP), regarded as undisturbed site, was located at the upwind of the smelter, and its northeastern distance from the smelter was 10 km (Figure 1).

Soil samples were collected in April 2004. In each of the six sampling sites, three bulked soil samples were collected with cutting rings (64 mm in diameter and 100 mm in length). Each bulked sample consisted of five subsamples, randomly collected at 0–10 cm depth outside a distance of 50 cm from the rhizosphere of individual plants. The field-moist soil samples were sieved ($< 2$ mm), and immediately divided into two subsamples according to different experiment analyses. In laboratory, one soil subsample was stored at 4 °C for the determination of the microbial parameters (microbial number, biomass, respiration and cellulose mineralization). Another subsample was allowed to dry at room temperature for the analyses of physico-chemical properties (including heavy metal contents).

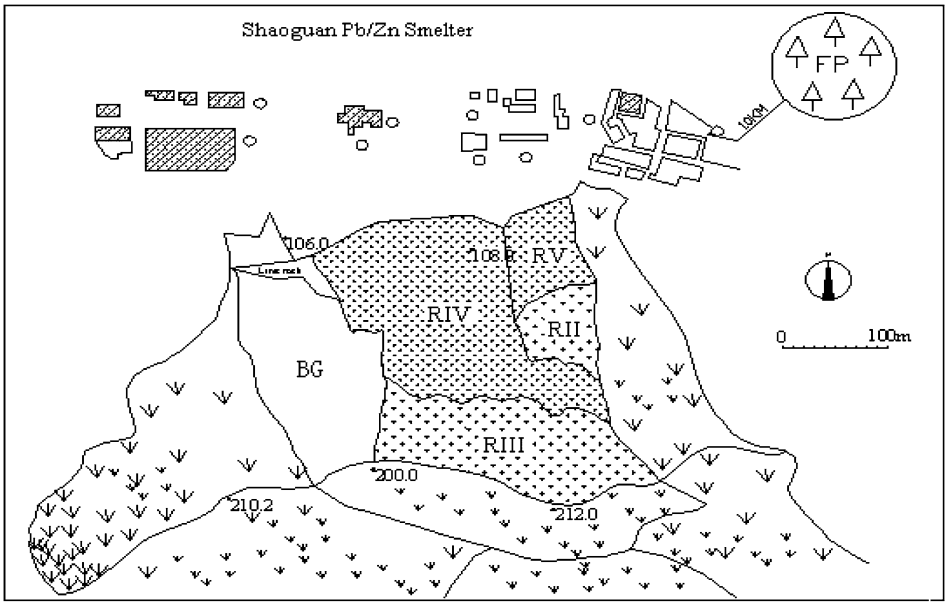


*Figure 1*. Distribution of studied sites on the southern mountain and native forest area which are situated at downwind and upwind of the Shaoguan Pb/Zn Smelter, Guangdong Province of Southern China, respectively. Revegetated sites include four areas revegetated for two, three, four and five years (RII, RIII, RIV and RV), respectively. Control sites include BG (bare ground), where no plant species grow and FP (forest area), where no heavy metal pollution occurs.

## 3. Microbial Analyses

The determinations of the microbial numbers were conducted by using a diluted standard plate method. The copiotrophic bacteria were estimated on full strength nutrient agar. The actinomycete were grown on Gause's No.1 synthetic medium (Bunt and Rovira, 1955), and the fungi were grown on Martin' medium (Martin, 1950). The oligotrophic bacteria were firstly grown on 100 fold diluted nutrient broth agar (DNB), and transplanted to the fresh DNB again (Hattori and Hattori, 1980). Microbial number was computed according to cfu (colony forming unit) in plates on dry weight basis.

The microbial biomass C and N were determined by using the fumigation-extraction method (Sparling and West, 1988; Joergensen and Brookes, 1990), respectively. The biomass C ($C_{mic}$) was calculated from the difference between fumigated and non-fumigated soil using a conversion factor $K_{EC} = 0.35$. The biomass ninhydrin reactive N ($N_{mic}$) was calculated from the difference ninhydrin-N extracted by $K_2SO_4$ from fumigated samples and ninhydrin-N extracted by $K_2SO_4$ from non-fumigated samples using a conversion factor $K_{EN} = 3.1$.

The basal respiration was measured by the NaOH absorption method (Isermayer, 1952). The substrate induced respiration (SIR) was analyzed by using the method described by Anderson and Domsch (1978), plus with Isermayer technique. The cellulose mineralization was analyzed using 100% cotton strips method (Department of Soil Microbiology, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China 1985).

## 4. Physico-Chemical Analyse

Total organic carbon (Nelson and Sommers, 1982), Kjeldahl nitrogen (Bremner and Mulvaney, 1982), total P and available P (Bray and Kurtz, 1945) and $pH_{KCl}$ (Smith and Doran, 1996) were determined in dry soil samples, respectively. The $NH_4$-N and $NO_3$-N were determined in fresh soil samples by steam distillation as described by Keeney and Nelson (1982), respectively. The bulk density and porosity were analyzed in the same soil cores following techniques described by Misra (1968). To determine the water-holding capacity (WHC), fresh soil samples were firstly saturated with water and then sucked off the surplus water under sand bath. The remaining water in soils represented the water-holding capacity (Jaggi, 1976).

Other part of soil samples were digested with 16M $HNO_3$ + 12M $HClO_4$ (5:1, v/v) for total Pb, Zn, Cu, Cd (McGrath and Cunliffe, 1985), and extracted with the DTPA -diethylenetriamin epentaacetic acid (soil to DTPA, 1:2, w/v) for extractable Pb, Zn, Cu and Cd by using method proposed by Baker and Amacher (1982). The digests and extracts were stored in polyethylene bottles at 4 °C and analyzed by using an atomic absorption spectrophotometer (Model 3030, Perkin-Elmer, USA). Ratio of available to total heavy metal content was computed as the potential bioavailability of heavy metals in soils according to reports of Singh *et al*. (1998) and Remon *et al*. (2005).

## 5. Statistical Analyse

All data were analyzed by using SAS for Windows version 6.12. Because of the soil heterogeneity in studied sites, all parameters were given on an oven-dry weight basis and were means ($\pm$ the standard deviations) of three replicates unless stated otherwise. The significant differences of all measurements were subjected to one-way ANOVA, followed by LSD test. The coefficients between measurements were computed by simple correlation analysis, followed by the Pearson coefficient. We selected those variables most effective in separating revegetated sites from control sites on the basis of stepwise discriminant analysis (DA) in which partial $F$-statistics and Wilk's Lambda criterion were used, and then the physico-chemical and microbial patterns were evaluated by both principal component analysis (PCA) and stepwise discriminant analysis (DA) methods for determining the separation of

these studied sites. In the present study, all figures were plotted by means of Sigma plot software version 9.0.

## 6. Results

Compared with those in the site BG, the revegetation resulted in significant increases of some soil nutrients (organic C, total N, total P, $NH_4$-N, $NO_3$-N and available P, $P < 0.05$) and physical conditions (WHC and porosity, $P < 0.05$), as well as decrease of bulk density (statistically not significant, $P < 0.05$) in the four revegetated sites (Table I). Likewise, soil heavy metal contents (except for the total Cu content) in the four revegetated sites were significantly higher than in the BG site (Table II, $P < 0.05$). The ratio of available to total Pb contents (heavy metal potential availability) showed a trend of decrease in the revegetated sites (except for the site RII), whereas the ratio for Cd showed a consistent increase trend from BG to RV sites. The peak of the ratio for Zn occurred in the RIII site, whereas the peak of the ratio for Cu was contrary to that of Zn ratio (Figure 2).

Soil microbial properties such as copiotrophic bacteria, actinomycete and fungal numbers, biomass C, basal respiration, SIR and cellulose mineralization differently increased in the four revegetated sites compared with those in the BG site (Table III, $P < 0.05$). The microbial biomass N, although of not significantly ($P < 0.05$), increase in revegetated sites compared with those in the BG site. The oligotrophic bacteria number was significantly lower in the revegetated sites than levels of the BG and FP site ($P < 0.05$). The microbial metabolic quotients ($qCO_2$) were higher in the BG, RIV and RV sites than in the RII, RIII and FP sites ($P < 0.05$). The ratio of copiotrophic bacteria (CB) to oligotrophic bacteria (OB) also differently increased in the four revegetated sites, whereas $C_{mic}/C_{org}$ (biomass C/organic C) and $N_{mic}/ N_{org}$ (biomass N/ organic N) fluctuated similar to the microbial biomass C and N.

The oligotrophic bacteria number was negatively correlated to the total and available heavy metal contents (Figure 3, $P < 0.05$ or $P < 0.01$), while other microbial properties did not show any significant correlations with individual heavy metal contents. Nevertheless, all microbial properties were negatively related to Cu and Pb ratios (except for the copiotrophic and oligotrophic bacterial number, Table IV, $P < 0.05$ or $P < 0.01$), but less related to Zn and Cd ratios (except for $N_{mic}$ and OB). On the other hand, most of microbial properties determined in the present study except for the oligotrophic bacteria predominantly and positively correlated with soil organic C, total N, $NH_4$-N, $NO_3$-N and WHC (Table V, $P < 0.05$ or $P < 0.01$), less (except for $N_{mic}$ and CB) correlated with total P (TP), available P (AP) and pH, but no parameters correlated with bulk density and porosity (data not shown).

According to stepwise discriminant analysis (DA), the important factors that contribute the most to the discriminant functions in each dataset studied were

TABLE I

Soil physico-chemical properties (means ± standard deviation, $n = 3$) in the revegetated sites and two control sites

| Physico-chemical properties | BG | RII | RIII | RIV | RV | FP |
|---|---|---|---|---|---|---|
| Organic C (g $kg^{-1}$ dw) | 14.19 ± 3.1d | 21.88 ± 1.8abc | 25.24 ± 0.9ab | 15.34 ± 1.5cd | 17.33 ± 1.5cd | 28.25 ± 1.0a |
| Total N (g $kg^{-1}$ dw) | 0.41 ± 0.07b | 0.51 ± 0.02c | 0.52 ± 0.01c | 0.46 ± 0.02c | 0.71 ± 0.10c | 0.87 ± 0.02a |
| Total P (g $kg^{-1}$ dw) | 0.16 ± 0.007e | 0.36 ± 0.003b | 0.27 ± 0.010cd | 0.25 ± 0.017d | 0.34 ± 0.042bc | 0.44 ± 0.030a |
| $NH_4$-N (mg $kg^{-1}$dw) | 5.43 ± 1.0c | 8.22 ± 0.7abc | 9.96 ± 1.0b | 7.33 ± 0.2bc | 9.71 ± 1.5b | 15.97 ± 1.5a |
| $NO_3$-N (mg $kg^{-1}$ dw) | 2.91 ± 0.5c | 4.09 ± 0.5bc | 4.87 ± 0.5ab | 2.64 ± 1.0c | 4.03 ± 0.7bc | 6.32 ± 0.5a |
| Available P (mg $kg^{-1}$ dw) | 5.62 ± 0.7dc | 21.14 ± 4.8a | 15.46 ± 0.9ab | 10.59 ± 1.4bc | 12.52 ± 1.1b | 20.84 ± 0.3a |
| pH | 3.5 ± 0.03a | 3.7 ± 0.06a | 3.7 ± 0.07a | 3.8 ± 0.66a | 4.6 ± 0.04a | 4.4 ± 0.12a |
| WHC (%) | 51.0 ± 4.7d | 64.1 ± 0.6b | 69.3 ± 1.7b | 60.8 ± 3.9bc | 55.6 ± 1.1cd | 88.9 ± 2.0a |
| Bulk density (g $cm^{-3}$) | 1.4 ± 0.04a | 1.3 ± 0.10a | 1.2 ± 0.27a | 1.3 ± 0.01a | 1.1 ± 0.10a | 1.2 ± 0.01a |
| Porosity (%) | 39.7 ± 1.3b | 46.7 ± 2.9b | 49.5 ± 7.9b | 41.1 ± 4.3b | 61.5 ± 0.6a | 51.0 ± 1.2ab |

*Note*. BG: bare ground; RII, RIII, RIV, RV: sites revegetated for two, three, four and five years, respectively; FP = native forest site. The data with the same letter indicate no significant difference, and the data with different letter indicate significant difference at $P < 0.05$ levels according to LSD test.

TABLE II

Heavy metal contents (mg $kg^{-1}$ dw, means ± standard deviation, $n = 3$) in the revegetated sites and two control sites

| Metal contents | BG | RII | RIII | RIV | RV | FP |
|---|---|---|---|---|---|---|
| Total Pb | 468.4 ± 58.0bc | 1066.6 ± 200.9ab | 867.8 ± 53.1ab | 939.2 ± 106.2ab | 1170.0 ± 219.9a | 67.3 ± 7.4c |
| Total Zn | 253.0 ± 38.1bc | 751.0 ± 142.8b | 493.0 ± 40.5bc | 753.0 ± 9.7b | 1315.0 ± 215.8a | 75.0 ± 11.4a |
| Total Cu | 16.7 ± 0.9a | 42.7 ± 4.0a | 29.9 ± 0.4a | 29.7 ± 2.4a | 50.4 ± 6.1 a | 16.3 ± 0.8a |
| Total Cd | 11.2 ± 1.4cd | 28.0 ± 6.2b | 22.4 ± 1.3bc | 27.1 ± 3.6b | 46.7 ± 6.4a | 1.5 ± 0.2d |
| Available Pb | 209.9 ± 33.3c | 493 ± 88.6a | 330.6 ± 41.1bc | 381.4 ± 39.3ab | 454.6 ± 56.2ab | 15.3 ± 2.3d |
| Available Zn | 64.1 ± 8.9c | 166.7 ± 25.4b | 153.0 ± 23.7b | 183.6 ± 29.4b | 286.8 ± 29.5a | 7.6 ± 1.4c |
| Available Cu | 3.6 ± 0.7bc | 9.2 ± 1.4a | 5.1 ± 0.5b | 5.9 ± 0.5b | 10.8 ± 1.3 a | 1.1 ± 0.1c |
| Available Cd | 2.3 ± 0.5cd | 8.5 ± 3.1bc | 8.0 ± 0.9bc | 11.7 ± 2.1b | 21.1 ± 4.0a | 0.3 ± 0.1d |

*Note*. Abbreviations of the sites studied referes to Table I.

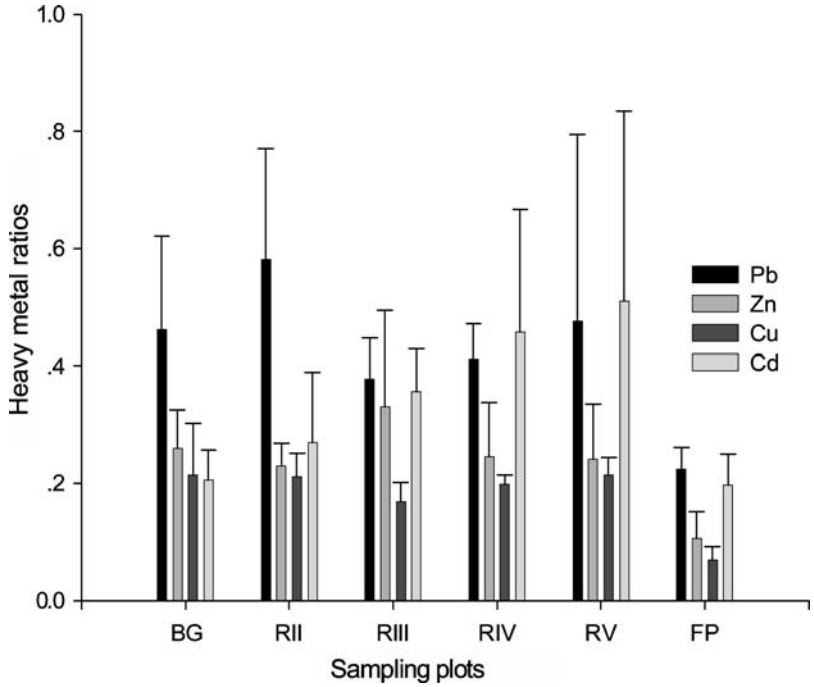


*Figure 2*. Variation for ratios of available to total heavy metal content (means ± standard deviation, $n = 3$).

selected as the copiotrophic bacteria, oligotrophic bacteria, actinomycete, biomass C, biomass N, SIR and CM for the microbial properties; the organic C, total N, total P, $NH_4$-N, pH and WHC for the soil physico-chemical properties, and the total Pb, Cu, available Pb, Zn and Cu for the soil heavy metals. The PC and DA scores based on the microbial and physico-chemical data selected from DA clearly separated the revegetated sites from control sites (Figure 4), whereas the PCA and DA scores based on heavy metal data did not (data not shown). The first two axes of PCA explained 82.69% of the variation in physico-chemical data. The BG site, which overly degraded owing to the earlier heavy metal dust deposition, was located at the left, and the FP site, which did not pollute by heavy metals, was located at the right on the graph. Others were found in the middle on the graph, where the RII and RIII sites were classified into one group, and RIV and RV sites into other groups and closed to BG site. The separation of the physico-chemical patterns along PC1 (explained 67.79% of the data variation) appeared to reveal a shift of soil nutrient pools from the polluted toward the non-polluted conditions. PCA based on the microbial dataset also clearly separated the four revegetated sites from two control sites (Figure 4). The first two axes of PCA explained 82.02% of the data variation. Differing from the soil physico-chemical pattern, the PCA of the microbial data

TABLE III

Microbial properties (means ± standard deviation, $n = 3$) in revegetated sites and two control sites

| Microbial properties | BG | RII | RIII | RIV | RV | FP |
|---|---|---|---|---|---|---|
| CB | 10.1 ± 0.8e | 20.5 ± 0.8c | 25.1 ± 0.5b | 13.2 ± 0.48d | 14.7 ± 0.32d | 31.9 ± 1.4a |
| OB | 17.7 ± 0.4b | 13.6 ± 0.7c | 14.1 ± 0.7c | 11.3 ± 0.5d | 10.0 ± 0.7d | 26.5 ± 0.4a |
| Actinomycete | 8.5 ± 1.59d | 13.1 ± 0.32c | 18.3 ± 0.88b | 12.9 ± 0.35c | 10.8 ± 1.79cd | 25.2 ± 1.34a |
| Fungi | 0.8 ± 0.07d | 1.4 ± 0.1cd | 2.2 ± 0.27ab | 1.5 ± 0.06c | 1.7 ± 0.03bc | 2.7 ± 0.35a |
| $C_{mic}$ | 44.8 ± 10.5b | 106.2 ± 36.4ab | 187.1 ± 35.5a | 71.9 ± 32.3b | 81.6 ± 32.5b | 129.5 ± 41.0ab |
| $N_{mic}$ | 0.59 ± 0.12b | 0.89 ± 0.01b | 1.08 ± 0.27b | 0.87 ± 0.07b | 1.55 ± 0.20ab | 2.52 ± 1.47a |
| BR | 0.017 ± 0.003c | 0.023 ± 0.006bc | 0.038 ± 0.017a | 0.025 ± 0.002abc | 0.026 ± 0.003abc | 0.035 ± 0.004ab |
| SIR | 4.27 ± 0.9c | 5.57 ± 1.8bc | 12.21 ± 1.9b | 11.14 ± 0.9b | 10.27 ± 1.8b | 25.27 ± 1.7b |
| CM | 1.51 ± 0.3c | 2.34 ± 0.2c | 5.47 ± 0.4a | 1.54 ± 0.2c | 3.83 ± 0.0b | 5.46 ± 0.5a |
| $qCO_2$ | 0.00049 ± 0.0001abc | 0.00017 ± 0.0001c | 0.00017 ± 0.0001c | 0.00074 ± 0.0004a | 0.00057 ± 0.0002ab | 0.00021 ± 0.0001bc |
| CB/OB | 0.604 ± 0.06c | 1.553 ± 0.25bc | 2.616 ± 0.35b | 1.014 ± 0.07c | 1.238 ± 0.09c | 4.424 ± 1.83a |
| $C_{mic}/C_{org}$ | 2.67 ± 1.04c | 6.45 ± 0.32b | 8.79 ± 1.07a | 2.64 ± 0.96c | 3.23 ± 1.57c | 5.97 ± 1.32b |
| $N_{mic}/N_{org}$ | 1.47 ± 0.25b | 1.73 ± 0.08ab | 2.06 ± 0.45ab | 1.92 ± 0.3ab | 2.23 ± 0.34ab | 2.91 ± 1.76a |

*Note*. Microbial number (CB, OB, actinomycete, and fungi): cfus $g^{-1}$ dw, microbial biomass ($C_{mic}$ and $N_{mic}$): $\mu$g $g^{-1}$ dw, BR (basal respiration) and SIR (substrate induced respiration): $\mu$g $CO_2$ $g^{-1}$ dw $h^{-1}$, CM (cellulose mineralization): %, CB/OB: copiotrophic bacteria to oligotrophic bacteria, $C_{org}/C_{org}$: the ratio of microbial biomass C to soil organic C, $N_{org}/N_{org}$: the ratio of microbial biomass N to soil total N.

TABLE IV

Correlations between microbial properties and ratios of available to total heavy metal contents

| Coefficients | $R_{Pb}$ | $R_{Zn}$ | $R_{Cu}$ | $R_{Cd}$ |
|---|---|---|---|---|
| CB | n.s. | n.s. | $-0.853^{*}$ | n.s |
| OB | n.s. | n.s. | $-0.854^{*}$ | $-0.856^{*}$ |
| Actinomycete | $-0.876^{*}$ | n.s | $-0.944^{**}$ | n.s. |
| Fungi | $-0.878^{*}$ | n.s | $-0.854^{*}$ | n.s. |
| $N_{mic}$ | $-0.939^{**}$ | $-0.823^{*}$ | $-0.860^{*}$ | n.s |
| SIR | $-0.988^{**}$ | n.s | $-0.957^{**}$ | n.s |
| CB/OB | $-0.897^{*}$ | n.s | $-0.952^{**}$ | n.s |
| $N_{mic}/N_{org}$ | $-0.957^{**}$ | n.s | $-0.871$ | n.s |

*Note*. $R$ which is labeled with heavy metal subscripts represents the ratio of available to total heavy metal content. $^{*,**}$ indicate the correlations between microbial properties and ratios of available to total heavy metal contents are significant at $P < 0.05$ or 0.01 levels, while n.s. indicated correlations are not significant at $P < 0.05$ or 0.01 levels.

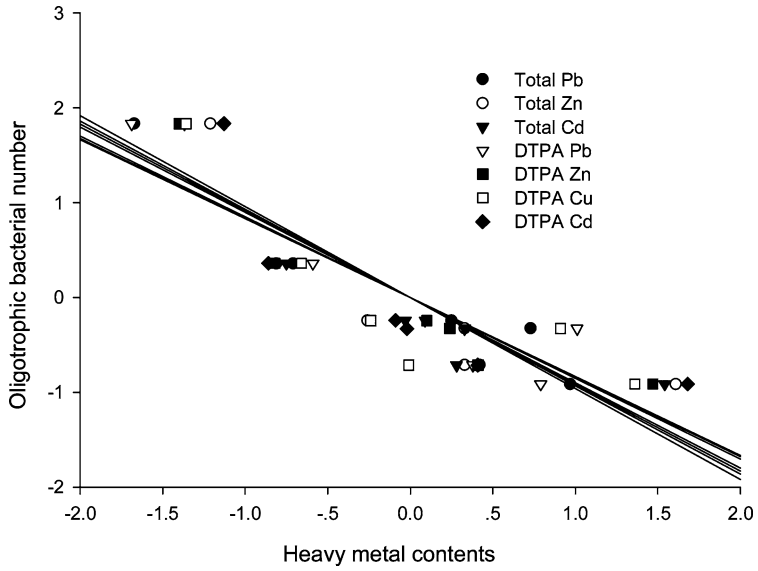


*Figure 3*. Correlation analyses between soil oligotrophic bacterial number and heavy metal contents in six sites. Coefficients of oligotrophic bacteria with heavy metals (total Pb, total Zn, total Cd, available Pb, Zn, Cu and Cd) are $-0.960$ ($P < 0.01$), $-0.852$ ($P < 0.05$), $-0.898$ ($P < 0.05$), $-0.931$ ($P < 0.01$), $-0.917$ ($P < 0.01$), $-0.839$ ($P < 0.05$), $-0.835$ ($P < 0.05$), respectively.

TABLE V
Correlations between microbial and physico-chemical properties

| Coefficients | SOC | TN | TP | $NH_4$-N | $NO_3$-N | AP | pH | WHC |
|---|---|---|---|---|---|---|---|---|
| CB | 0.992** | n.s. | n.s. | 0.893* | 0.952** | 0.837* | n.s. | 0.952** |
| Actinomycete | 0.921** | n.s. | n.s. | 0.916** | 0.895* | n.s. | n.s. | 0.989** |
| Fungi | 0.875* | n.s. | n.s. | 0.938** | 0.891* | n.s. | n.s. | 0.900* |
| $C_{mic}$ | 0.840* | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| $N_{mic}$ | n.s. | 0.984** | 0.835* | 0.967** | 0.857* | n.s. | 0.811* | 0.812* |
| CB/OB | 0.935** | n.s. | n.s. | 0.952** | 0.954** | n.s. | n.s. | 0.926** |
| BR | 0.837* | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| SIR | n.s. | 0.838* | n.s. | 0.942** | n.s. | n.s. | n.s. | 0.905* |
| CM | n.s. | 0.852* | n.s. | 0.828* | 0.900* | n.s. | n.s. | n.s. |
| $qCO_2$ | −0.837* | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| $C_{mic}/C_{org}$ | 0.842* | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| $N_{mic}/N_{org}$ | n.s. | 0.944** | n.s. | 0.969** | 0.835* | n.s. | n.s. | 0.842* |

SOC: soil organic C, TN: total N, TP: total P, AP: available P, WHC: water holding capacity, BD: bulk density, P: porosity. *, ** indicate correlations between microbial and physico-chemical properties are significant at $P < 0.05$ or 0.01 levels, while n.s. indicated correlations are not significant at $P < 0.05$ or 0.01 levels.

were separated into BG, RII-RIV-RV, RIII and FP patterns, respectively. The RII, RIV and RV sites were grouped together and closed to the BG site; The RIII site was classified into a single group. The separation of the microbial patterns along PC1 (explained 63.45% of the data variation) also revealed a shift of the soil microbial composition and activities to the non-polluted condition. The patterns of DA for soil physico-chemical and microbial data here were not depicted because these patterns were similar to PCA patterns (Figure 4). Further, the regression analyses respectively based on PC1 and DF1 scores indicated significant correlations between microbial and physico-chemical properties (Figure 5, the regression coefficient for PC1 scores: $R^2 = 0.776$, $P < 0.001$; and the regression coefficient for DF1 scores: $R^2 = 0.818$, $P < 0.001$).

## 7. Discussion

Although soil pH and bulk density indicated no significant difference, the revegetation significantly improved levels of the soil nutrient (organic C, total N, total P, $NH_4$-N, $NO_3$-N and available P), the physical conditions (WHC, and porosity), and promoted growths and activities of different microbial groups (except that oligotrophic bacteria decreased), thus showed both the nutrient pools and the stability of the soil ecosystem in the revegetated sites were gradually reestablished (Vangronsveld *et al*., 1996; Smith *et al*., 1997; Kelly *et al*., 2003). Meanwhile, the

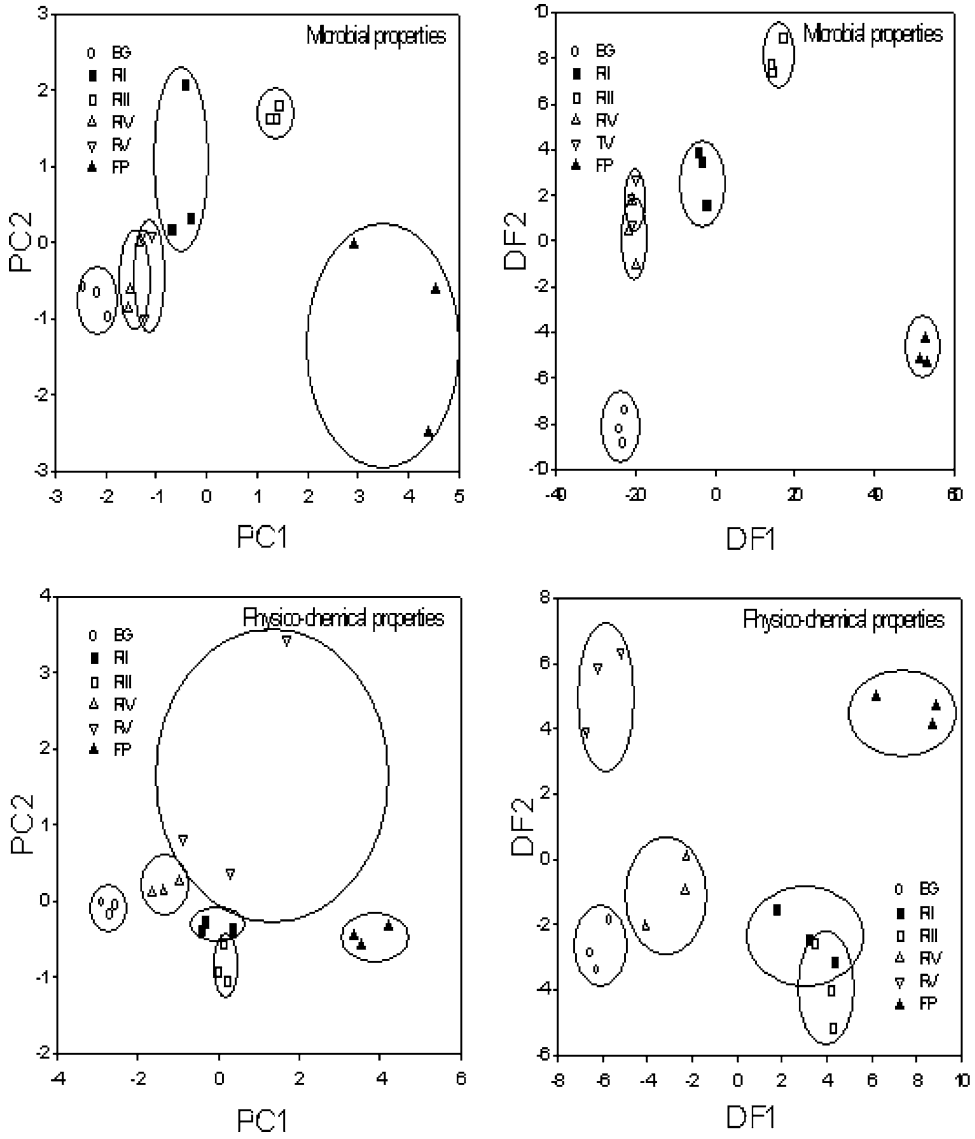


*Figure 4*. Score plots resulted from PCA and DA analyses on the soil physico-chemical and microbial properties datasets in the six studied sites. PCs represent principal components, and DFs represent discriminant functions.

revegetation resulted in accumulation of total or available metals on surface soil, thus limited dispersion of heavy metals into surroundings. Our results confirmed the fixing and stabilizing effects of revegetation on the soil heavy metals through plant roots and exudates suggested by Dupuy and Douy (2001) and Zimmermann and Frey (2002).

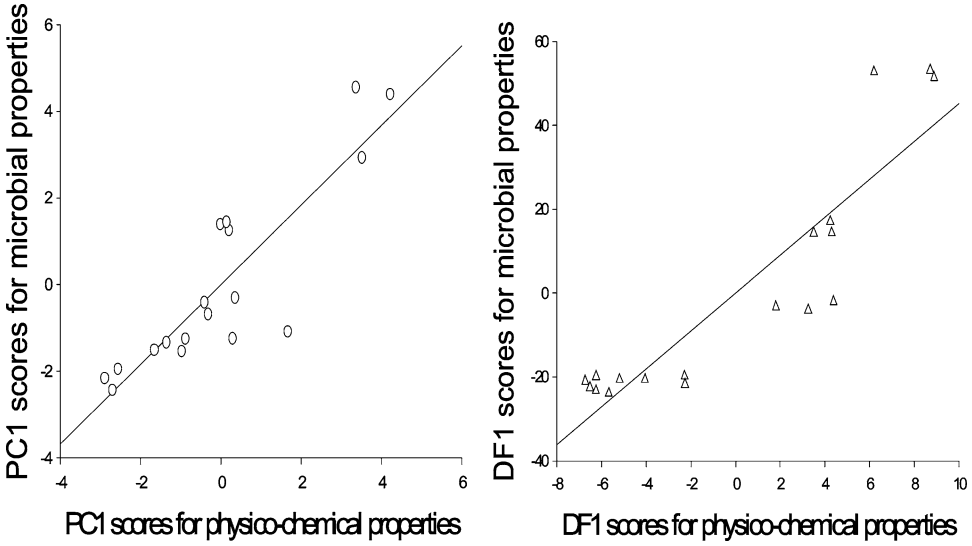


*Figure 5*. Regression analyses between microbial and physico-chemical patterns on PC1 and DF1scores in six sites. PC1: $R^2 = 0.776$, $P < 0.001$; DF1: $R^2 = 0.818$, $P < 0.001$.

Soil microorganisms play a crucial role in increasing soil fertility and accelerating revegetation process through their activities in disturbed soils (Visser *et al*., 1983). In the present study, rapid increase in most of microbial parameters determined except for the $qCO_2$ mainly occurred in sites RII and RIII and after that, gradual decrease occurred in sites RIV and RV, thus indicating microbial parameters did not varied with revegetation ages. Some studies have also indicated that accumulation of microbial biomass primarily occurred in the earlier stage of vegetation succession (Roy and Singh, 2003; Jia *et al*., 2005), while Wardle *et al*. (2004) also emphasized that microbial biomass was optimized at the intermediate stages of the succession. The correlation analysis indicated that microbial parameters were predominantly and positively related to soil organic C, $NH_4$-N, $NO_3$-N and WHC, which indicated the soils carbon, nitrogen and humidity might be important factors in mediating microbial process (Bauhus and Khanna, 1994; Pankhurst *et al*., 1995; Rampazzo and Mentler, 2001; Hofman *et al*., 2004). The soil oligotrophic bacteria, which represent less nutrient demanding microorganisms indigenous to soil (Filip *et al*., 2000), were not correlated to the nutrient improvement. The microbial biomass C was merely related to the soil organic C, which confirmed the results from Ekblad and Nordgren (2002) who studied the relationships between the microbial biomass and availabilities of soil C and N. Böhme (2005) studied effects of plant growth on soil microbial biomass C and enzyme activities, results indicated that all three enzyme activities and $C_{mic}$ were significantly and positively correlated with $C_{org}$. The microbial biomass N, which resulted from ninhydrin reactive N measurement, was significantly correlated with soil nutrient components such as total

N, total P, $NH_4$-N, $NO_3$-N, pH and WHC, indicating the interrelationships of the microbial biomass N with soil physico-chemical properties was different from the biomass C (Wardle, 1992; Ross *et al*., 1993; Powlson, 1994; Sparling, 1997). The basal respiration was significantly correlated with soil organic C, whereas substrate induced respiration showed positive relations to the total N, $NH_4$-N and WHC, respectively, which indicated soil nitrogen and WHC were possibly key factors controlling SIR fluctuation when enough carbon substrate was added. The cellulose mineralization was correlated to the organic C, $NH_4$-N and $NO_3$-N, which supported the results observed by Mendelssohn *et al*. (1999) and Griffiths *et al*. (2001) in which cellulose mineralization was mainly determined by soil fertility such as organic C, inorganic N and P. The ratio of copiotrophic bacteria to oligotrophic bacteria was significantly correlated to soil carbon, nitrogen and WHC. This showed the CB/OB was a sensitive indicator in reflecting a shift of soil nutrient pool from oligotrophic to copiotrophic condition because the former actively responses to more nutrients (Hattori and Hattori, 1980; Ohta and Hattori, 1983). The soil metabolic quotient ($qCO_2$), e.g. specific respiration of microbial biomass, is often regarded as a physiological indicator in reflecting environmental stress (Odum, 1985; Anderson and Domsch, 1990). In the present study, $qCO_2$ was higher in BG, RIV and RV sites than the levels in RII, RIII and FP sites, and was negatively correlated to soil organic C, thus indicating stress effects of soil organic C in BG, RIV and RV sites on soil microorganisms. Higher ratios of $C_{mic}$ to $C_{org}$ and $N_{mic}$ to $N_{org}$ in the revegetated sites indicated the microbial biomass played an important role in restoring soil nutrient pools.

The soil oligotrophic bacteria were negatively correlated to individual heavy metal contents (Figure 3), and not to soil nutrient levels and physical conditions. This was in line with the results obtained by Tada and Inoue (2000) in which oligotrophic bacteria were sensitive to Pb, Zn, Cu, Cd, Cr and Ag, and also indicated the soil oligotrophic bacterial number were more sensitive to heavy metals than to soil nutrient improvement. Other microbial measurements were not significantly related to the heavy metal contents, which was possibly either due to decrease of heavy metal toxicity with increases of soil organic matter and pH (Oste *et al*., 2001; Clemente *et al*., 2003; Walker *et al*., 2003), or the long-term adaptation of soil microorganisms to the presence of high contents of soil heavy metals which masks the possible inhibition caused by the increase in soil heavy metals (Insam *et al*., 1995; Bech *et al*., 1997; Chew *et al*., 2001; Madejón *et al*., 2001). Therefore, the soil oligotrophic bacteria will be a more sensitive indicator for monitoring the heavy metal pollutions than the other microbial measurements (Tada *et al*., 2001). The ratios of available (DTPA-extracted) to total heavy metal contents reflect the potential availability of soil heavy metals (Singh *et al*., 1998; Remon *et al*., 2005). It was interesting to note that the ratio of Pb was negatively correlated with actinomycete, fungi, $N_{mic}$, SIR, CB/OB and $N_{mic}/N_{org}$, (Table IV), while the ratio of Cu was negatively correlated with all microbial parameters in Table IV. This might be related to the cute toxicity of Pb and Cu to microbial community because some

previous studies have confirmed that the Pb and Cu contamination may cause larger reduction in microbial biomass and activity than other heavy metals (Zevenhuizen, *et al*., 1979; Lombi *et al*., 2002; Shi *et al*., 2002). Zn and Cd in published literatures were also regarded as important factors in stressing microbial growth and activity (Knight *et al*., 1997; Aceves *et al*., 1999; Simona *et al*., 2004). However, our results showed that other microbial parameters except for $N_{mic}$ and OB did not show any significant stress relationships to ratios of Zn and Cd. This on the one hand might be related to a shift of the microbial community composition in long-term heavy metal-contaminated soils toward more tolerant species to Zn and Cd (Rosen, 1996; Beveridge *et al*., 1997; Almås *et al*., 2004; Simona *et al*., 2004), and on the other hand might reflect biocomplexity in soil microbial community because components of soil microbial community may not respond to perturbation, particularly to context of pollution impacts in similar ways (Anand *et al*., 2003; Vig *et al*., 2003). Despite the mechanism for the relationships between microbial properties and the potential availability of heavy metals in soils was less known, at least in our study it was possibly true that microbial properties were more sensitive to the potential availability of soil heavy metals than the individual heavy metal contents.

PCA and DA were used to evaluate how well soil physico-chemical and microbial parameters distinguished the processive development of the revegetated soils under different revegetation ages. PCAs and DAs based on the soil physico-chemical and microbial data clearly separated the revegetated sites from the control sites (Figure 4). The physico-chemical patterns were divided into four types: BG, RII-RIII, RIV-RV and FP, whereas the microbial patterns were divided into different four types: BG, RII-RIV-RV, RIII and FP. In any case, the physico-chemical or microbial patterns along PC1 axis indicated a shift of the revegetated soils toward non-polluted condition. However, it was noteworthy that the RIV and RV sites along PC1 axis were lagged behind the RII and RIII sites in physico-chemical pattern and in microbial patterns, indicating that soil microbial and physico-chemical parameters did not change with revegetation ages. Similar results were also reported by Kelly *et al*. (2003) who investigated soil microbial community structure (by means of phospholipid fatty acid analysis) in several remediation sites near a Zn smelter, and their results indicated that the more recently remediated sites had phospholipid fatty acid (PLFA) profiles most similar to the least contaminated site, and the sites which had been remediated earlier (10 years) had PLFA profiles more similar to the most heavily contaminated site. Anand *et al*. (2003) studied the effect of contaminant concentrations (soil heavy metals) and environmental variables (soil moisture and vegetation cover) on soil microbial populations and diversity along a contamination gradient, and concluded that recovery of heavy-metal contaminated soil was not necessarily linear since increasing distance from the pollution source did not correlate well with increasing microorganism population or diversity, and metal concentrations also did not correlate with microbial dynamics. Regression analysis on PC1 and DF1 scores revealed that the microbial pattern was well correlated to the

physico-chemical pattern, showing the consistent developments of the microbial and physico-chemical properties in the course of the revegetation.

## 8. Conclusions

Our results showed that soil nutrient and physical conditions in the revegetated sites were improved, thus indicating effects of the revegetation. The higher heavy metal contents in the revegetated sites compared with levels of the BG site indicated that the revegetation effectively prevented the dispersion of the soil heavy metals to surroundings. Similarly, the microbial numbers (except for the oligotrophic bacteria), biomass, activities and biomass ratios also increased in the revegetated sites compared with levels of the BG site and displayed significant correlations with the soil nutrients (organic C, total N, $NH_4$-N, $NO_3$-N) and water holding capacity. The correlation analysis also indicated that the oligotrophic bacterial number was most sensitive microbial indicator in reflecting soil heavy metal stress, but not in reflecting the improved soil fertility. The other microbial properties, despite not showing significantly negative relations to the heavy metal contents, were very sensitive to the potential availability of Pb and Cu. Both PCA and DA revealed shifts of the soil physico-chemical and microbial patterns from bare ground to unpolluted conditions. However, the soil physico-chemical and microbial patterns in RIV and RV sites were relatively lagged behind those in RII and RIII sites, indicating the possible loss of the revegetation effects on soil remediation over time.

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