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# Heavy metal tolerance of nematode-trapping fungi in lead-polluted soils

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**Abstract**

A study was conducted to establish whether the diversity of nematode-trapping fungi in Pb-polluted soils increases or decreases with increasing degree of soil contamination, and whether the fungi from polluted soils exhibit higher tolerance to Pb toxicity than those from unpolluted soils. Five genera containing 28 nematode-trapping fungi were recorded in total from five collection sites highly contaminated by Pb, with the concentration ranging from 306 to 4907 mg $kg^{-1}$. These fungi fell into seven groups according to their trapping mechanisms. In this area, the most frequent group was the net former of which 16 species were recorded and its occurrence frequency (61.15%) was higher than those of the others. Fungal diversity of NTF was slightly positively correlated with the Pb pollution levels ($r = 0.29$), which suggested the distribution of nematode-trapping fungi was not restricted by the heavy metal at these sites. The mycelial growth of nematode-trapping fungi which derived from either Pb-polluted soils or from unpolluted soils was completely inhibited by 1.8 mmol of Pb. At the Pb concentration of 1.2 mmol, the inhibition growth rates varied between 18.50 and 22.57% and there was no significant difference in the Pb tolerance of nematode-trapping fungi as to whether the strains derived from Pb pollution soils or unpolluted soils.


*Keywords:* Nematode-trapping fungi; Lead; Fungal diversity; Tolerance

## 1. Introduction

Nematophagous fungi consist of a wide and diverse range of fungi able to infect and digest nematodes. These fungi can be divided into four categories: endoparasitic fungi, nematode-trapping fungi (NTF), fungi which parasitize eggs and females, and toxin-producing fungi (Barron and Thorn, 1987; Dackman et al., 1992). NTF are unique, however, in their morphological adaptation to the predacious habit and in their ability to capture and consume prey. These fungi have a more complex relationship with their nematode host, since they also have an ability to live saprophytically. They form different hyphal structures (adhesive nets, knobs, branches or hyphae, constricting rings or non-

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constricting rings) in order to capture nematodes (Barron, 1977). NTF have been known for over a century. Ecological surveys on the occurrence indicate that this group is found throughout the world, in all types of climate and in all the habitats examined (Gray, 1987). Gray (1983) surveyed the distribution of nematophagous fungi, including NTF, in 10 broad terrestrial habitats in Irish. He found that these fungi were abundant in all the habitats examined, although most widely in temporary agricultural pasture, coniferous leaf litter and coastal vegetation, with over 90% of the samples containing these fungi. He also found the distribution of some species, such as *Arthrobotrys musiformis*, *A. robusta*, *Dactylella lobata* and *Stylopage hadra*, is largely independent of habitat or soil conditions. Pollution of the biosphere with toxic metals due to man-made activities poses a major environmental and human health problem. Also, toxicity of heavy metals to soil microorganisms in terms of number, diversity, and microbial activity is of primary importance (Dumestre et al., 1999). Heavy metals in soils have been widely reported to exert an adverse effect on microorganisms and microbial processes (McGrath et al., 1995a,b). Soil Pb pollution has attracted considerable attention since the 1980s, when neuropsychological effects of Pb ingestion in children were identified (Needlemann et al., 1979, 1990). Elevated Pb in soils may compromise soil productivity, and a very low Pb concentration may inhibit some vital plant processes, such as photosynthesis, mitosis, and water absorption, with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short roots (Kabata-Pendias and Pendias, 2001). The toxicity of Pb also affected the growth and physiology of microorganisms. Inorganic Pb inhibited growth and photosynthesis of marine algae and cyanobacteria (Malanchuk and Gruendling, 1973), nitrogen fixation of cyanobacteria (Henriksson and DaSilva, 1978), germination of spores and mycelial growth of fungi (Somers, 1961; Smith, 1977), and the growth, trap formation and collagenase activity of NTF (Rosenzweig and Pramer, 1980).

Although numerous surveys on the occurrence of NTF have been undertaken, only one study has been performed to examine the heavy metals including Pb associated with the distribution of the major types of NTF or individual species (Gray, 1988), and one in vitro to investigate the influence of several heavy metals on growth, trap formation and collagenase activity of NTF (Rosenzweig and Pramer, 1980). The two reports remain the only limited information on distribution and metal tolerance of NTF. For this reason, in this study we addressed the following two questions:

1. Does the diversity of NTF in the area contaminated by Pb increases or decreases with the increase of degrees of Pb contamination?
2. Do the fungi from lead-polluted soils exhibit higher tolerance to Pb toxicity than those from lead-unpolluted soils?

## 2. Materials and methods

### 2.1. Site description and soil sampling

The study site is located in LaoChang Pb Mine area in the southeast of Yunnan Province, China (Fig. 1). Soils in this region are heavily polluted by lead due to mining and smelting the metalliferous ores. This area has a humid frigid climate with an annual average temperature of 11.8 °C and rainfall of 1013.6 mm. Since 1980s the Pb Mine has been operating in a conventional ground operation in a mountain with an altitude of 2270 m. Consequentially, the surrounding farmlands, about 200 ha, including the study area, are seriously affected by the mining activities such as the continuous discharge of mining effluents and the dispersion of mine tailing dust. Five sites with about 5–10 km distance from each other were selected along, but 100 m away, the road from Mengzi County to Gejiu City. Site 1 (23°23′N, 103°19′E, altitude 1292 m), Site 2 (23°35′N, 103°27′E, altitude 1495 m), Site 3 (23°29′N, 103°25′E, altitude 2054 m) and Site 5 (22 °34′N, 103°16′E, altitude 1765 m) were vegetable fields, and Site 4 (23°30′N, 103°21′E, altitude 2270 m) was mining area where the mining activities are taking place. At each sampling site, 50 soil samples, each about 200 g from a depth of 0–10 cm, were collected over an area of about 100 $m^2$. The distribution of NTF is affected by habitant (Gray, 1987), to reduce the affect when considering sampling sites, we selected the sites all planting

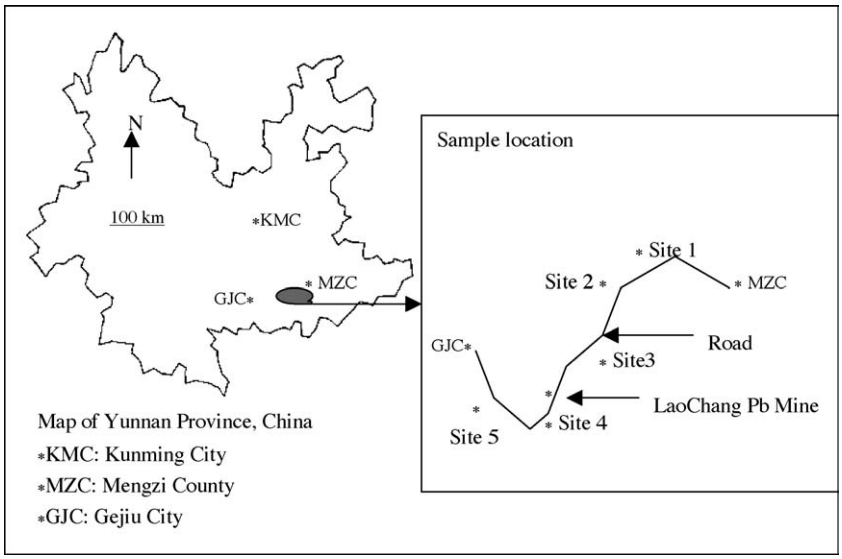


Fig. 1. Sampling location map of the study area.

vegetable but Site 4, and the distances of each site to the road are almost equal.

### 2.2. Quantification of living nematodes in soil

All the 50 soil samples from each site were mixed thoroughly and 100 g subsample was used for nematode quantification by the flask recovery method (McKenna, 1999). Briefly, a single piece of paper tissue was folded over the soil sample, skewered with a piece of wire and suspended in a 100 ml conical flask filled with water. After standing for 36 h, the soils were discarded and the fluid in the flask was reduced to a final volume of 3–5 ml for examination using a j-shaped tube attached to a water vacuum pump. The nematodes were counted following the procedure as McKenna (1999) described.

### 2.3. Soil sample preparation and total Pb concentration analysis

All the 50 soil samples from each site were mixed thoroughly and 200 g subsample was used for chemical analysis. The soil samples were air-dried and passed through 2 mm mesh sieve, then pulverized and passed through 180 μm mesh sieve. After being dried for 2 h at 105 °C, the samples were digested in aqua regia following the method of McGrath and Cunliffe (1985) and the total Pb concentrations were analyzed by using an atomic absorption spectrophotometer (model Z-8000, Hitachi, Japan). Quality control was assured by the use of reagent blanks, duplicates and standard reference material (NIST 2709).

### 2.4. Quantification of nematode-trapping fungi in soil

In order to accurately quantify NTF in a site, accurately, 1 g soil from a sample was spread on a Corn Meal Agar (extract of 20 g cornmeal, agar 18 g, adding water to the final volume of 1000 ml, CMA) plate and approximately 200 nematodes (*Panagrellus redivivus*) in 0.1 ml distilled water were added as a bait for NTF to the plate. Each sample was plated on three replicated petri dishes and which will result in 150 plates for 50 samples of a site. After storage at room temperature (about 20–28 °C) for 20 days, the entire surface of the petri dish was observed under a dissecting microscope and the presence of NTF was recorded for each petri dish. These fungi were identified according to the keys provided by Li et al. (2000).

### 2.5. Effect of lead on mycelial growth of nematode-trapping fungi

Eight species, each with 1–2 strains, from Pb-polluted soil were tested for their susceptibility to Pb. For every species, 1–3 strains from soil without Pb contamination were also used as the contrast strains (Table 3). The soils in which the contrast strains were isolated contained totally 11.23 mg $kg^{-1}$ Pb and were regarded as substance without pollution. The effects of Pb on the mycelial growth were investigated on CMA medium supplemented with $Pb(NO_3)_2$ in a concentration of 1.2 mmol or without Pb addition. The agar plates were inoculated with agar disks (5 mm diameter) cut from the colony margin of the plates covered by the growing mycelium. After incubation of the plates at 28 °C for 4–10 days, the diameters of the mycelial colonies were measured. Each experiment for a strain was carried out in triplicate. To compare the effect of lead on the mycelial growth, the inhibition growth rate (IGR) was introduced and calculated using the following formula:

$$\mathrm{IGR} = \frac{\text{The balance of growth rate per24 h on plate without and with Pb}}{\text{Growth rate per24 h on plate without Pb}} \times 100\%$$

### 2.6. Data analysis

The individual numbers of each/all species and the occurrence frequencies of a species ($F$) were recorded and calculated for each/all sites. The individual number of a species was defined artificially as the number of soil samples in which the species occurred. For example, as mentioned above, a soil sample was plated on three replicated dishes, when a species was checked in any one of the three replicated plates or all the three, its individual was recorded as 1. Fungal taxa whose occurrence frequencies were higher than 10% were classified as frequent species. Shannon–Weiner index (H′) was applied to evaluate the diversities of nematode-trapping fungi in soils contaminated by Pb. Sørensen's index of similarity ($S'$) was plotted to evaluate different fungal communities and expressed with values between 0 (no similarity) and 1 (absolute similarity). The above data were calculated using the following formulas:

$$F = \frac{\text{Individual number of a species}}{\text{Individual number of all species}} \times 100\%$$

$$H\prime = -\sum_{i=1}^{s} P_i \log_e P_i, \quad \text{where } P_i = \frac{N_i}{N}$$

$$S' = \frac{2c}{a+b}$$

$N_i$ is the individual number of $i$th species; $N$ is the individual number of all species; $P_i$ is the proportion of $i$th species; $\log_e P_i$ is the natural logarithm of $P_i$; $S$ is species number; $a$, $b$ are number of species in community $a$, $b$, respectively; $c$ is number of species found in both community $a$ and $b$.

Data were analyzed by ANOVA with SPSS software package (SPSS 11.01 Inc., Chicago, USA). Least significance differences (LSD) at 0.01 were used for comparisons between treatments.

## 3. Results

### 3.1. Pb pollution level and nematode total numbers in the collection sites

The soils collected from the five sites were heavily polluted by lead with a concentration varying between 306 and 4907 mg $kg^{-1}$ (Table 1). These concentrations are obviously higher than the average normal background value (23.6 mg $kg^{-1}$) of Chinese soil. Site 4, located in LaoChang Pb Mine, showed the highest pollution level, in which the Pb concentration amounted to 4907 mg $kg^{-1}$, followed by Site 3 (1463 mg $kg^{-1}$), Site 5 (740 mg $kg^{-1}$), Site 1 (313 mg $kg^{-1}$) and Site 2 (306 mg $kg^{-1}$). Except Site 2, which showed a similar Pb concentration as that of Site 1, the pollution levels in the rest of the collection sites increased significantly ($P < 0.01$) with their locations being closer to the LaoChang Mine. The

Table 1
The concentration of total Pb (mg $kg^{-1}$) and nematode amount of 100 $g^{-1}$ soil in the five collection sites

| Collection sites | Concentration of Pb (mean ± S.E., $n = 3$) | Multiple comparisons | Nematode number (mean ± S.E., $n = 3$) | Multiple comparisons |
|---|---|---|---|---|
| Site 1 | 306 ± 2.96 | D | 761 ± 1.15 | A |
| Site 2 | 313 ± 2.08 | D | 764 ± 2.03 | A |
| Site 3 | 1463 ± 3.61** | B | 438 ± 1.76** | C |
| Site 4 | 4907 ± 2.52** | A | 395 ± 3.22** | D |
| Site 5 | 740 ± 0.57** | C | 615 ± 3.22** | B |

fluctuation of nematode numbers in the five sites showed a similar pattern as that of Pb concentration (Table 1). Except Site 2, which had a similar amount as that of Site 1, the nematode numbers in soils decreased significantly ($P < 0.01$) according to the increasing Pb concentration. In the five sites, the number of active nematodes was negatively correlated with the Pb concentration ($r = -0.81$).

### 3.2. NTF distribution in Pb-polluted soils

Totally, 28 NTF taxa belonging to five genera were identified from 206 soil samples collected at five sites with lead pollution levels of 306–4907 mg $kg^{-1}$ (Table 2). The plates of the rest 44 samples were not been included due to their agar media were decomposed by some unknown eelworms, in which the fungi not grow normally. The highest fungal diversity was found in Site 2 ($H' = 2.43$; $S = 16$; $N = 86$), followed by Site 4 ($H' = 2.32$; $S = 14$; $N = 58$), Site 5 ($H' = 2.30$; $S = 12$; $N = 64$), Site 3 ($H' = 1.93$; $S = 11$; $N = 48$) and Site 1 ($H' = 1.71$; $S = 13$; $N = 66$). The observed fungi were classified into seven groups according to their trapping mechanisms. The most frequent trapping mechanism recorded was adhesive nets ($S = 16$), followed by adhesive knobs ($S = 3$), non-constricting rings combined with adhesive knobs ($S = 3$) and those species without trap formation ($S = 3$). Furthermore, two species with unmodified adhesive hyphae, two with adhesive branches and one with constricting rings also were recorded. In this Pb-polluted area, three net forming predators, *Monacrosporium thaumasium* ($F = 18.94\%$), *Arthrobotrys oligospora* ($F = 16.77\%$) and *M. rutgeriense* ($F = 11.18\%$) were the frequent species. They were recorded in all sites except *M. thaumasium* which did not occur in Site 5, and showed higher occurrence frequencies than the others. But for a given site, the frequent taxa varied: *M. thaumasium* ($F = 46.97\%$) and *Dactylella clavata* ($F = 24.24\%$) for Site 1; *M. thaumasium* ($F = 17.44\%$), *A. oligospora* ($F = 16.28\%$), *M. rutgeriense* ($F = 13.95\%$) and *M. candidum* ($F = 11.63\%$) for Site 2; *A. oligospora* ($F = 35.42\%$), *M. thaumasium* ($F = 16.67\%$), *M. rutgerisense* ($F = 14.58\%$) and *M. gephyrophagum* (10.42%) for Site 3; *A. oligospora* ($F = 22.41\%$), *M. gephyrophagum* (13.79%), *M. megalosporum* ($F = 12.69\%$) and *M. thaumasium* ($F = 12.69\%$) for Site 4; *M. rutgeriense* ($F = 15.63\%$), *Stylopage hadra* ($F = 15.63\%$), *A. oligospora* ($F = 12.5\%$), *D.* sp1 ($F = 12.5\%$) and *M. ellipsosporum* ($F = 10.94\%$) for Site 5. There were low similarity indices ($S' = 0.19$–$0.33$) between the communities of NTF distributed in Pb-polluted soils (Table 3). The lowest similarity ($S' = 0.19$) was found between Site 1 and Site 4, and the highest similarity ($S' = 0.33$) was found between Site 2 and Site 3. The diversity of NTF in Pb-polluted soils was surprisingly found to correlate positively with the Pb concentration ($r = 0.29$) and negatively with the number of nematodes ($r = -0.11$).

### 3.3. Effect of lead on mycelial growth of nematode-trapping fungi

Totally, eight species of NTF, each with strains from Pb-polluted soils and 1–3 strains from the soils with very low contamination of Pb (11.23 mg $kg^{-1}$) were tested for their susceptibility to Pb (Table 4). Several preliminary tests had indicated that none of the fungi tested was significantly sensitive to less than 0.15 mmol $Pb(NO_3)_2$, but all fungi were significantly sensitive to the heavy metal at a concentration between 1.0 and 1.6 mmol, and the growth of all species could completely be inhibited by the metal at a concentration over 1.8 mmol (data not shown). To

Table 2
Fungal taxa on different sites of Pb contaminated soil

| | Ni (individual number of *i*th species) | | | | | *F* |
|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | |
| Taxa and diversity analysis | | | | | | |
| *A. botroyospora* | | | 1 | 1 | | 0.62 |
| *A. cladodes* | 1 | | | | | 0.31 |
| *A. conoides* | | | | 1 | | 0.31 |
| *A. dactyloides* | | 4 | | 1 | 5 | 3.11 |
| *A. musiformis* | 1 | | | 3 | 3 | 2.17 |
| *A. oligospora* | 2 | 14 | 17 | 13 | 8 | 16.77 |
| *A. robusta* | | | | 1 | | 0.31 |
| *A. superba* | | | | 5 | 2 | 2.17 |
| *A. vermicola* | | 3 | | 1 | | 1.24 |
| *D. clavata* | 16 | | | | | 4.97 |
| *D. leptospora* | 2 | 1 | 1 | | | 1.24 |
| *D.* $sp_1$ | 3 | 1 | | | 8 | 3.73 |
| *D.* $sp_2$ | | 1 | | | | 0.31 |
| *M. candidum* | | 10 | 1 | | | 3.42 |
| *M. cionopagum* | | 2 | 4 | | 6 | 3.73 |
| *M. cystosporium* | 3 | 3 | | | 2 | 2.48 |
| *M. ellipsosporum* | 1 | 4 | 2 | 2 | 7 | 4.97 |
| *M. eudermatum* | | 5 | | | | 1.55 |
| *M. gephyrophagum* | | 3 | 5 | 8 | | 4.97 |
| *M. lysipagum* | | 1 | | | | 0.31 |
| *M. megalosporum* | 2 | | | 7 | 1 | 2.48 |
| *M. phymatopagum* | | | | | 2 | 0.62 |
| *M. rutgeriense* | 2 | 12 | 7 | 5 | 10 | 11.18 |
| *M. sinense* | 1 | | | | | 0.31 |
| *M. sphaeroides* | 1 | | | | | 0.31 |
| *M. thaumasium* | 31 | 15 | 8 | 7 | | 18.94 |
| *S. hadra* | | 7 | 1 | 3 | 10 | 6.52 |
| *T. aphanopaga* | | | 1 | | | 0.31 |
| Sample size | 43 | 44 | 41 | 38 | 40 | |
| *N* (individual number of all species) | 66 | 86 | 48 | 58 | 64 | |
| *S* (species number recorded) | 13 | 16 | 11 | 14 | 12 | |
| *H′*(Shannon–Weiner index) | 1.71 | 2.43 | 1.93 | 2.32 | 2.30 | |

A: *Arthrobotrys*, D: *Dactylella*, M: *Monacrosporium*, S: *Stylopage*, T: *Tripos*porina.

confirm whether the fungi from lead pollution soils exhibit a higher tolerance to Pb than those from the soils with low concentration of lead, an index, inhibition growth rate (IGR), was introduced and measured. On CMA medium containing 1.2 mmol of Pb, the values of IGR of eight species, 27 strains tested varied between 18.50 and 22.57%. For any given species, the values of all strains were not significantly different ($P > 0.01$) independent of whether the strains derived from habitats with or without Pb pollution. Only three strains, belonging to three different species, showed significant differences ($P < 0.01$) in their tolerance against Pb toxicity (Table 4). These results suggested that NTF from lead-polluted soils exhibit similar levels of tolerance to Pb toxicity as those from the soils with very low concentration of lead.

Table 3
Similarity indices of fungal communities between different sites

| Sites | Sørensen's index (*S′*) | | | |
|---|---|---|---|---|
| | Site 2 | Site 3 | Site 4 | Site 5 |
| Site 1 | 0.24 | 0.21 | 0.19 | 0.28 |
| Site 2 | | 0.33 | 0.27 | 0.29 |
| Site 3 | | | 0.24 | 0.20 |
| Site 4 | | | | 0.32 |

Table 4
Effect of lead on mycelial growth of nematode-trapping fungi

| Species | Strains | Pb concentration of soil the strain from (mg $kg^{-1}$) | IGR (mean IGR ± S.E., $n = 3$) | Multiple comparisons |
|---|---|---|---|---|
| *A. oligospora* | 4–30–1 | 4907 (from Site 4) | 21.20 ± 0.87 | B |
| | 4–2–2 | 4907 (from Site 4) | 22.57 ± 0.60** | A |
| | 1.1435 | 11.23 | 20.80 ± 0.85 | B |
| | 1.1411 | 11.23 | 22.53 ± 0.96 | B |
| *A. superba* | 4–36–1 | 4907 (from Site 4) | 20.50 ± 1.10 | B |
| | 4–13–2 | 4907 (from Site 4) | 20.50 ± 0.85 | B |
| | 1.16 | 11.23 | 18.50 ± 0.87** | C |
| *D. clavata* | 1–25–2 | 306 (from Site 1) | 21.37 ± 0.61 | B |
| | 1–35–1 | 306 (from Site 1) | 20.57 ± 1.16 | B |
| | 1.124 | 11.23 | 20.37 ± 0.98 | B |
| *D. leptospora* | 2–20–2 | 314 (from Site 2) | 19.77 ± 0.88 | B |
| | 3–39–1 | 1465 (from Site 3) | 20.47 ± 0.98 | B |
| | 1.126 | 11.23 | 21.73 ± 0.71 | B |
| | 1.562 | 11.23 | 21.00 ± 0.79 | B |
| *M. candidum* | 2–24–1 | 314 (from Site 2) | 19.77 ± 0.78 | B |
| | 3–34–2 | 1465 (from Site 3) | 20.53 ± 1.20 | B |
| | 1.36 | 11.23 | 20.43 ± 1.10 | B |
| | 1.543 | 11.23 | 20.00 ± 1.08 | B |
| *M. cionopagum* | 3–11–2 | 1465 (from Site 3) | 20.37 ± 0.78 | B |
| | 5–12–1 | 740 (from Site 5) | 19.80 ± 1.07 | B |
| | 1.569 | 11.23 | 20.20 ± 0.67 | B |
| | 1.580 | 11.23 | 21.40 ± 0.72 | B |
| *M. gephyrophagum* | 4–23–1 | 4907 (from Site 4) | 22.33 ± 0.81 | B |
| | 1.123 | 11.23 | 21.57 ± 0.79 | B |
| *M. thaumasium* | 2–9–2 | 314 (from Site 2) | 20.50 ± 0.98 | B |
| | 3–10–2 | 1465 (from Site 3) | 20.30 ± 0.96 | B |
| | 1.1428 | 11.23 | 18.50 ± 0.55** | C |

## 4. Discussion

Heavy metals are a serious threat to the quality of soils due to their persistence after entering soils. They enter arable soils mainly through the addition of sewage sludge for P and N fertilization. However, there are other important sources of heavy metal contamination, e.g. mining or factory dumps, where the concentrations of heavy metals markedly exceed those of soils amended with sewage sludge (Chander et al., 2001). In this study area, another important source of Pb pollution is the sediments of rainwater from the LaoChang Mine, which increased the pollution level with increasing proximity to the Mine. The total nematode number in this area was found to correlate negatively with the Pb concentration ($r = -0.81$). This result was in accordance with the common responses of the nematode communities in soil contaminated with heavy metals (Georgieva et al., 2002).

Gray (1988) investigated the occurrence of NTF associated with heavy metals in 48 soil samples with Pb concentration of 113.8–254.3 mg $kg^{-1}$. He found that natural levels of heavy metals in the soil restricted the distribution of NTF with their presence being significantly associated with soils containing low levels of Cr ($P < 0.001$), Ni ($P < 0.005$) and Cu ($P < 0.05$). Especially, the knob formers were restricted to soils with very low concentration of all the metals examined. However, in this study, NTF with all kinds of trapping mechanism were recorded to the Pb-polluted habitats, and the knob formers were not found to be restricted in soils with low concentration of Pb, e.g. *M. ellipsosporum*, capturing nematodes by

adhesive knobs, was recorded in all collection sites and showed a higher occurrence frequency ($F = 4.97\%$) than all of the net formers except the three frequent species, *M. thaumasium*, *A. oligospora* and *M. rutgeriense*. The result that the net formers were the most frequent species was in agreement with the findings of numerous surveys on the occurrence of NTF (Gray, 1987). Furthermore, in opposition to common findings that the heavy metals have an adverse influence on microbial population and activities, we found the diversity of NTF in Pb-polluted soils was positively correlated with Pb concentration ($r = 0.29$). These results suggest that the NTF are also abundant in these Pb-polluted soils and the Pb pollution appears not to be the primary factor restricting the distribution of NTF. Chander et al. (2001) also found that the effects of heavy metals on microbial properties were not linearly related to the metal concentrations, so that the extremely contaminated soils still showed considerable microbial activities. This non-linear effect of heavy metals on microbial properties was also observed by Rost et al. (2001).

Heavy metal resistance also results in considerable microbial activities in the extremely contaminated soil. Fungi vary their tolerances to specific heavy metals, e.g. the mycelial growth of *Rhizoctonia solani* and *Aspergillus giganteus* could completely be inhibited by 1.5 mmol of Pb, *Botrytis cinerea* and *Penicillium brefeldianum* exhibited initially reduced growth at the same concentration, whilst *Fusarium solani* and *Aspergillus niger* grew normally even at the stress of 3 mmol of Pb (Babich and Stotzky, 1979). The effect of heavy metals on NTF has been virtually unstudied except for a laboratory experiment carried out by Rosenzweig and Pramer (1980). They measured the effect of Cd, Zn and Pb on mycelial growth, trap formation and collagenase activity of seven pure cultures of NTF and found that the growth varied with species and was dependent on the specific metal tested and its concentration. Cd was found to exhibit the greatest toxicity, followed by Zn and Pb, respectively. In our study, the mycelial growth of all tested species including three strains (4–30–1, 4–36–1 and 4–23–1) from metalliferous mine tailings, was completely inhibited by 1.8 mmol of $Pb(NO_3)_2$. The Pb concentration (1.8 mmol), which stopped fungal growth, is by far lower than that of Site 4 (14.78 mmol). This suggested that only a part of the total Pb in soils exhibited toxicity to fungi. Additionally, we found that there was no significant difference in the Pb tolerance of NTF regardless the strains from soils with or without Pb pollution. This finding appears to explain why the NTF widely distribute in soils extremely contaminated by Pb.

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