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Effect of heavy metals on soil nematode communities in the vicinity of a metallurgical factory

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Abstract: The influence of Cu and Zn on soil nematode communities was examined along a pollution gradient with increasing distance from a metallurgical factory. Total and available heavy metal contents were used to study the effects of heavy metals on nematode abundance, trophic groups and ecological indices. The results demonstrated significant correlations between the number of total nematodes, bacterivores, plant-parasites and the total and available heavy metals. Bacterivores and plant-parasites were the dominant trophic groups. Significant differences in different sampling sites were found only in the number of bacterivores(P<0.01). The Shannon-Weaver diversity index(H'), trophic diversity index(TD), evenness index(J') and dominance index(λ) were found to be sensitive to soil pH and C/N ratios. Significant correlations were found between the total nematodes (TNEM), some genera (*Acrobeloides, Aphelenchoides, Cephalobus, Ditylenchus, Mesorhabditis, Tetylenchus and Tylenchus*) and distance from the factory.

Keywords: soil nematodes; trophic groups; ecological indices; heavy metals; metallurgical factory

Introduction

The increase in anthropogenic pollution of ecosystems by heavy metals in China has caused severe threats to humans and to the environment(Chen *et al.*, 2005; Xia *et al.*, 2004). Heavy metal contamination can change the functioning of soil ecosystems by disturbing the activities of soil fauna (Cortet *et al.*, 1999) and in some cases can even affect human health through the terrestrial food chain.

Among soil fauna, nematodes possess many characteristics that make them ideal as bioindicators for soil health assessment (Bongers and Ferris, 1999). Various kinds of perturbations to soils, such as the addition of mineral nitrogen fertilizers (Wasilewska, 1989; Liang *et al.*, 2005a), cultivation (Hendrix *et al.*, 1986) and CO_2 enrichment (Li *et al.*, 2005) affect species richness, trophic structure, and the succession status of nematode communities. Their fauna composition, together with its ecological indices, has emerged as a useful monitor of environmental conditions and soil ecosystem function(Neher, 2001).

The influence of heavy metals on nematode communities has been studied in recent years in an agroecosystem treated with sewage sludge(Georgieva *et al.*, 2002; Weiss, 1991), in an agricultural field after being artificially contaminated for seven years(Nagy *et al.*, 2004), in forest ecosystems near a metallurgical factory and Zn smelter (Popovici, 1994; Popovici and Korthals, 1995; Shukuro *et al.*, 2005) and in a pasture polluted with Cu, Cr, As and Ni(Bardgett *et al.*, 1994; Yeates *et al.*, 1994, 1995, 2003). These studies reported significant changes in the characteristics of the nematode communities due to the heavy metal pollution.

Since soil nematodes live in the soil pore water, they are assumed to be exposed to the contaminant concentration in the solution, which offers good perspectives for assessing the effects of contaminants in relation to their bioavailability in the soil(Houx and Aben, 1993). The bioavailability of heavy metals in soils affords valuable information for predicting the transfer of toxic elements in the food chain (Morel, 1997). However, there are relatively few reports on their use for predicting the effect of heavy metal contents on nematode communities in natural environments(Bogomolov *et al.*, 1996; Korthals *et al.*, 2000).

The objective of this study was to investigate the influence of heavy metals (Cu and Zn) on soil nematode communities along a pollution gradient and to evaluate the relationship between the soil nematode communities and the total and available metals. This research may provide a basis for environmental management, remediation and conservation decisions.

1 Materials and methods

1.1 Soil sampling

Soil samples were taken from a soybean field (about 20 hm^2) south of a metallurgical factory founded in 1952, located in the southern suburb of Shenyang, northeast China. According to Jiang *et al.* (2003), the heavy metals Cu and Zn were the main pollutants near the metallurgical factory. The field was planted rice before 2003, and soybean has been sown since 2004. Fertilizers were applied at a rate of 225 kgN/hm^2, 60 kgP/hm^2 and 112 kgK/hm^2. The mean total organic C is 16.98 g/kg, total N is 1.47 g/kg, pH is 5.55, and bulk density is 1.56 g/cm^3. The available Ca, Mg, K, and Na are 1535 mg/kg, 470 mg/kg, 169 mg/kg and 186 mg/kg, respectively. Twelve sites, numbered 1 to 12, were selected in a downwind

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direction from the factory emission sources, with site 1 being closest (100 m) to the factory, and the other sites at 80 m intervals from each other. Six replicates were taken from the surface horizon (0—10 cm) of each site in November 2004. These soil samples were placed in individual plastic bags and kept in cold storage at 4℃ until processed.

1.2 Soil chemical analysis

Before analysis, soil samples were air-dried, ground to pass through a 2-mm sieve for determination of available metals and soil pH. Subsamples were then ground and passed through a 149 μm sieve for determination of total nutrients and metals. For total metals, soil samples were digested with HNO_3:$HClO_3$ (3:1, v/v) and determined using AAS (WFX-120A, BRAIC) with a detection limit of 0.01 mg/L for both Cu and Zn. The available heavy metal concentrations in the soil samples were determined after extraction with 0.1 mol/L HCl(solid: liquid = 1:5) (Page, 1982). Soil total organic C was analyzed by dry combustion using a TOC 5000 Total C Analyzer (Shimadzu, 1993). Soil total N was determined by Kjeldahl digestion, followed by NaOH distillation, and measured by titration with 25 mmol/L H_2SO_4 in a boric acid indicator (Bremner, 1996). Soil pH was determined using a glass electrode in 1:2.5 of soil:water suspensions.

1.3 Nematode extraction and identification

The nematodes were extracted from 100 g soil (fresh weight) using the sugar flotation and centrifugation method (Liang *et al.*, 2002). All extracted nematodes in each sample were counted and expressed per 100 g dry weight soil. The nematodes from each sample were identified, mainly to genus level, using an inverted compound microscope. The classification of trophic groups was assigned to: (1) bacterivores (BF); (2) fungivores (FF); (3) plant-parasites (PP); and (4) omnivore-predators (OP), based on known feeding habits or stoma and esophageal morphology (Yeates *et al.*, 1993; Liang *et al.*, 2002, 2005b).

Several ecological indices of nematode communities were calculated: (1) Shannon-Weaver diversity $H' = -\Sigma p_i(\ln p_i)$; (2) evenness $J' = H'/\ln(S)$; (3) dominance $\lambda = \Sigma p_i^2$ and (4) trophic diversity $TD = 1/\Sigma p_i^2$, where p_i is the proportion of individuals in the i-th taxon and S is the number of taxa(Yeates and Bongers, 1999); (5) maturity index $MI = \Sigma v(i) \cdot f(i)$, where $v(i)$ is the c-p value of taxon i, $f(i)$ is the frequency of taxon i in a sample(Bongers, 1990).

All the data obtained in the study were subjected to statistical analysis of variance (ANOVA). Differences at the $P < 0.05$ level were considered significant.

2 Results

2.1 Soil chemical properties

The mean values of abiotic environmental parameters in the different sites are presented in Table 1. Mean total organic C ranged from 15.19 g/kg to 21.68 g/kg. The highest value was found at site 1, the lowest value was in site 7. Mean total N changed little at different sampling sites, with the highest value at site 12 and the lowest value at site 8. The C/N ratios exhibited a decreasing trend with increasing distance from the factory. Site 1 had the highest C/N ratio and site 11 had the lowest ratio. The soils were weakly acidic, with mean pH values ranging from 5.46 to 5.77. Site 4 had the lowest pH and site 1 had the highest.

Table 1 Soil chemical properties at different sites*

Site	pH	Total N, g/kg	Organic C, g/kg	C/N
1	5.77±0.4	1.43±0.16	21.68±2.33	15.28±1.64
2	5.61±0.11	1.39±0.23	17.57±1.78	12.82±1.81
3	5.51±0.05	1.39±0.22	18.35±1.50	13.32±1.37
4	5.46±0.13	1.47±0.14	16.57±1.87	11.29±0.43
5	5.57±0.09	1.41±0.12	16.22±1.14	11.50±0.88
6	5.52±0.08	1.41±0.17	16.47±1.50	11.72±0.73
7	5.60±0.18	1.35±0.16	15.19±1.32	11.35±1.01
8	5.62±0.13	1.34±0.15	15.22±2.26	11.36±0.83
9	5.52±0.17	1.41±0.14	15.64±1.77	11.08±0.34
10	5.49±0.16	1.62±0.14	16.47±1.55	10.18±0.84
11	5.50±0.15	1.66±0.18	16.59±1.71	10.01±0.51
12	5.51±0.09	1.72±0.17	17.79±1.15	10.39±0.90
P-value	ns	<0.01	<0.01	<0.01

Note: * The values are means ±SD(n=6)

2.2 Total and available metals

Total Cu and Zn varied considerably with distance from the factory, with respective values ranging from 413.6 to 159.3 mg/kg for Zn, 96.7 to 38.2 mg/kg for Cu. Available Zn ranged from 17.9 to 8.2 mg/kg, and available Cu from 18.5 to 5.9 mg/kg. Significant correlations were found between the total and available heavy metal contents (Cu and Zn) and distance from the factory(Fig.1).

2.3 Nematode composition and ecological indices

Sixteen nematode genera were identified in the study, belonging to 12 families (Table 2). The highest number of genera was 14 in site 1. The lowest number of genera was 9 found in sites 2, 9, 10 and 12. The *TD* index (Table 3) showed that the trophic diversity was the highest at site 8. The Shannon-Weaver diversity index H' also indicated the same trend, i.e. that species diversity was the highest at site 8. The TD, H', λ and J' indices all indicated that the lowest species diversity; disproportionate species composition and simplest trophic structure occurred at site 11, whereas the highest mean maturity index was found at site 11.

2.4 Total nematodes

Total nematodes(TNEM) ranged from 180 to 720 individuals per 100 g dry soil (Table 3). The highest

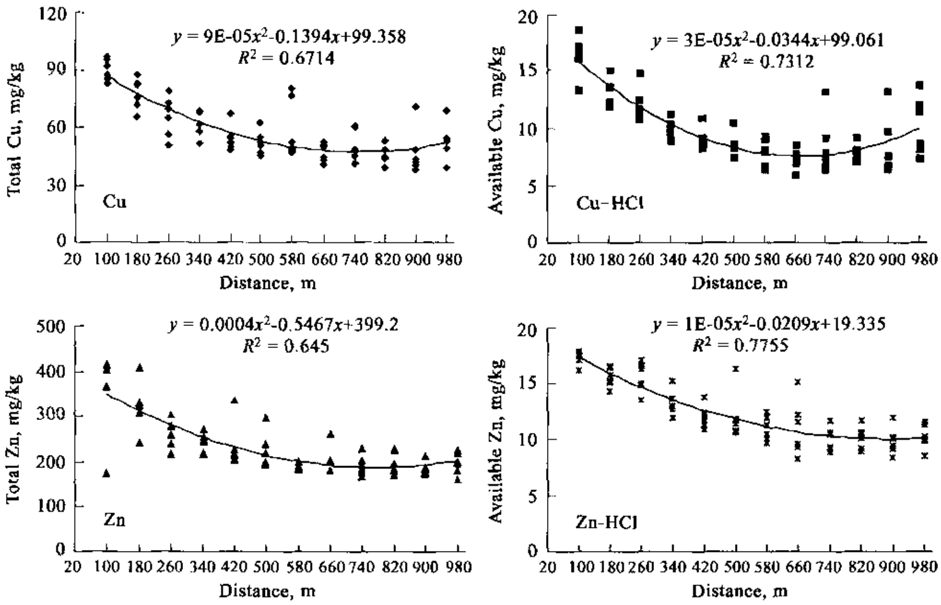

Fig.1 Relationships between total and available metal contents (Cu and Zn) and distance from the factory

Table 2 Absolute abundance(individuals per 100 g dry soil) of nematode taxa at different sites*

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	c-p
Acrobeloides	81±18	41±15	26±9	106±45	144±36	64±15	36±8	25±7	29±13	22±11	11±4	31±17	2
Cephalobus	165±76	13±8	14±6	5±3	30±12	11±4	9±5	7±5	5±3	-	3±1	-	2
Eucephalobus	25±24	-	-	-	-	-	-	-	-	-	-	-	2
Heterocephalobus	50±31	104±27	79±21	21±4	77±31	80±21	73±22	55±12	69±12	84±35	84±11	95±31	2
Mesorhabditis	65±16	14±10	31±13	23±11	18±5	52±22	33±16	26±10	23±6	10±8	6±2	10±5	1
Panagrolaimus	25±12	1±1	1±1	-	-	8±6	2±2	1±1	-	6±3	-	-	1
Aphelenchus	4±4	4±4	3±2	2±2	-	-	5±2	2±1	-	-	-	2±2	2
Aphelenchoides	43±23	10±4	9±3	12±9	27±9	28±12	10±4	4±1	11±3	7±3	5±2	6±2	2
Ditylenchus	31±30	3±1	11±7	8±5	13±5	-	3±3	5±2	4±3	5±2	2±1	1±1	2
Helicotylenchus	56±51	-	59±51	12±9	10±10	152±130	274±129	28±13	1±1	3±2	3±3	17±12	3
Longidorus	1±1	-	-	-	-	-	-	-	-	-	-	-	5
Psilenchus	33±24	-	1±1	1±1	15±13	2±2	3±2	2±2	1±1	-	2±1	-	2
Tetylenchus	-	-	2±2	-	1±1	8±8	10±8	9±7	-	5±5	1±1	1±1	2
Tylenchus	136±119	201±65	102±59	183±68	126±20	84±38	68±28	16±4	105±38	58±30	65±32	18±4	2
Tylenchorhynchus	3±3	-	-	-	1±1	-	-	-	-	-	-	-	2
Tripyla	-	-	-	-	-	-	-	1±1	-	-	-	-	3
Number of genera	14	9	12	10	11	10	12	13	9	9	10	9	

Note: * The values are mean±*SE*(n=6)

number was at site 1 and the lowest at sites 8, 11 and 12. No significant differences were found in the total nematodes at the different sampling sites. A significant correlation was found between the total nematodes and the distance from the factory(R^2=0.64, P< 0.01).

2.5 Trophic groups

The omnivore-predators population was the least trophic group. Only *Tripyla* was found at site 8. The bacterivores and plant parasites were dominant trophic groups at all sites (Table 3). Significant differences were found in the number of bacterivores at different sites (P<0.01). Significant correlations were observed between the numbers of some genera (*Acrobeloides, Aphelenchoides, Cephalobus, Ditylenchus, Mesorhabditis, Tetylenchus* and *Tylenchus*) and the distance from the factory (R^2=0.42, P<0.05; R^2 = 0.41, P<0.05; R^2 =0.68, P<0.01; R^2 = 0.36, P<0.05; R^2=0.48, P<0.05; R^2 =0.42, P<0.05 and R^2=0.51, P<0.01; respectively).

2.6 Correlations between nematodes and abiotic parameters

Significant correlations were observed between

Table 3 Nematodes abundance, trophic groups (individuals per 100 g dry soil) and ecological indices at different sites*

Site	Total nematodes	Trophic groups				Ecological indices				
		BF	FF	PP	OP	*H'*	λ	*J'*	*TD*	*MI*
1	720±269	412±118	78±51	229±118	-	1.71±0.07	0.23±0.02	0.83±0.03	4.52±0.39	1.77±0.06
2	393±104	174±79	17±9	201±65	-	1.25±0.08	0.37±0.04	0.72±0.04	2.82±0.24	1.92±0.03
3	339±85	152±22	23±7	164±66	-	1.49±0.10	0.31±0.04	0.74±0.04	3.50±0.46	1.84±0.06
4	372±105	154±46	22±12	196±63	-	1.21±0.07	0.40±0.03	0.67±0.03	2.61±0.27	1.86±0.07
5	462±76	269±50	40±13	153±29	-	1.55±0.12	0.27±0.04	0.80±0.03	4.02±0.54	1.94±0.03
6	489±176	215±49	28±12	246±126	-	1.57±0.08	0.26±0.04	0.83±0.03	4.10±0.47	1.78±0.05
7	526±146	152±40	19±6	355±111	-	1.42±0.15	0.35±0.05	0.70±0.04	3.18±0.49	1.82±0.05
8	181±44	115±28	11±2	55±16	1±1	1.79±0.04	0.21±0.01	0.82±0.02	4.81±0.33	1.83±0.04
9	248±41	126±24	15±4	107±40	-	1.32±0.08	0.34±0.04	0.76±0.04	3.05±0.27	1.81±0.06
10	239±74	146±48	14±2	79±31	-	1.41±0.11	0.31±0.04	0.65±0.04	3.51±0.49	1.85±0.05
11	182±35	103±12	8±3	71±36	-	1.10±0.08	0.45±0.03	0.64±0.02	2.28±0.16	1.95±0.01
12	180±48	136±50	8±3	36±14	-	1.23±0.14	0.38±0.06	0.74±0.05	2.96±0.42	1.94±0.03
P value	ns	<0.01	ns	ns	ns	<0.01	<0.01	<0.01	<0.01	ns

Note: * is the same as Table 2

the total and available metal contents(Cu and Zn) and the number of total nematodes, bacterivores and fungivores; and between the number of plant parasites and the total Cu, Zn and available Zn. Significant correlations were also found between C/N ratio and the total nematodes, bacterivores and fungivores; and between the ecological indices(TD, *H'*, λ) and the soil pH and C/N ratio(Table 4).

Table 4 Correlation coefficients between soil nematodes, ecological indices and abiotic parameters

Index	Cu	Cu-HCl	Zn	Zn-HCl	pH	C/N
TNEM	0.403**	0.309**	0.405**	0.416**	0.026	0.310**
BF	0.388**	0.388**	0.428**	0.417**	0.073	0.387**
FF	0.319**	0.357**	0.375**	0.324**	-0.031	0.330**
PP	0.323**	0.150	0.282*	0.320**	-0.006	0.160
OP	-0.126	-0.163	-0.059	-0.184	0.094	-0.036
S	0.170	0.141	0.152	0.212	0.299*	0.316**
TD	0.087	0.110	0.099	0.146	0.242*	0.274*
λ	-0.098	-0.146	-0.146	-0.166	-0.254*	-0.294*
H'	0.110	0.135	0.148	0.183	0.268*	0.322**
MI	-0.162	-0.080	0.006	-0.173	-0.184	-0.182
J'	0.043	0.115	0.111	0.088	0.196	0.215

Notes: * and ** represent significant level at $P<0.05$ and $P<0.01$, respectively

3 Discussion

The background values of elements for the paddy soil of Shenyang are 21.7 mg/kg for Cu and 56.7 mg/kg for Zn (Jiang *et al.*, 2003). The heavy metal contents in the study field were significantly higher than the background values. The total and available Cu and Zn exhibited a significant correlation with the distance from the factory, and the same trend was found in C/N ratios, which may act as a source and sink of nutrients in the soil, triggering the soil biota community (Shukurov *et al.*, 2005).

The results demonstrate that Cu and Zn had positive effects on the nematode communities and trophic structure. These results are in partial agreement with the results of Korthals *et al.* (1998), who reported that the absolute abundance of certain taxa appears to be stimulated at intermediate Cu or Zn concentrations (25 to 200 mg/kg). The discrepancy may due to the lower concentrations of available Cu and Zn in the study. Based on short-term exposure periods, Cu and Zn may have stimulated the hatching of nematode eggs (Clarke and Shepherd, 1966). However, the study field had been contaminated for fifty years, and the results are more likely due to less food competition or predation experienced by the tolerant taxa (Hendrix and Parmelee, 1985).

Nematodes have been assigned to four trophic groups based on their food source (BF, FF, PP and OP), which reflect the trophic structure in the soil food web. Trophic analysis of a nematode community by Parmelee *et al.* (1993) demonstrated that certain trophic groups are more sensitive to copper than the total nematode population. This is in agreement with our results, that no significant differences were observed in the total nematodes and that the bacterivores was the only trophic group sensitive to different heavy metal concentrations. The bacterivores was the dominant trophic group among the identified nematodes, which included extreme colonizers and enrichment opportunists (*Mesorhabditis*, *Panagrolaimus*) and general opportunists (Cephalobedae).

No persisters were found in the study. Some bacterivorous persisters, similarly to omnivore-predators, are sensitive to disturbance, and were not observed in the study. These results are in agreement with Georgieva *et al.* (2002), who found that some persisters(*Alaimus, Bastiania*) were obviously reduced or completely eliminated in soil treated with sewage sludge.

The omnivore-predators population was sensitive to heavy metal pollution and other disturbances. In this study, the population of omnivore-predators was the least trophic group; only one genus was found in site 8. Similar results were found by Georgieva *et al.* (2002), who reported that the omnivore-predators were reduced or eliminated from the nematode communities in soil with high Cu and Zn concentrations. Popovici (1994) and Korthals *et al.* (1996a) also found negative long-term effects of heavy metals on these trophic groups in field studies. The increased abundance of plant-parasites and bacterivores may have led to the disappearance of the omnivore-predators in this study.

Changes in the abundance of plant-parasites are usually related to primary production and plant susceptibility, bacterial and fungal populations or a combination of these factors. In our investigation, some genera (*Tetylenchus* and *Tylenchus*) were significantly correlated with distance from the factory. Similar results were found by Korthals *et al.* (1998), who found that intermediate pollutant concentrations may be beneficial to herbivores or plant-associated organisms. For example, due to an increase of N-availability, a higher leakage of root exudates or a breakdown of the plant's defense strategy may occur (White, 1984). This may play a role in the relation between some plant-parasites and the different heavy metals concentrations.

Aphelenchoides belong to the fungivores and have been reported to be tolerant in heavy metal contaminated soil (Weiss and Larink, 1991; Popovici and Korthals, 1995; Korthals *et al.*, 1996b; Georgieva *et al.*, 2002). In the present study, the number of *Aphelenchoides* exhibited a significant correlation with distance from the factory. Nagy (1999) and Korthals *et al.*(1996c) also found that *Aphelenchoides* reacted to Cu with increasing abundance. These results were probably caused by increased fungal biomass, reduced food competition with other nematodes, reduced predation pressure or combinations of these factors (Korthals *et al.*, 1996c).

The *MI* indices have been successfully used as indicators for heavy metal pollution (Popovici, 1994; Yeates *et al.*, 1995; Korthals *et al.*, 1998; Georgieva *et al.*, 2002). The *MI* values (1.77 to 1.95) in our investigation were lower than those (2.05 to 2.58) observed by Ou (2005) in an unpolluted paddy soil. The lower *MI* values indicated a disturbed environment due to the heavy metal pollution. Other indices, such as *H'*, *TD* and λ, showed that site 8 had the highest trophic and species diversity. However, site 11 had a disproportionate species composition and a simpler trophic structure. Since soil characteristics affect not only the bioavailability of pollutants, but also the nematode community structure, a significant correlation between *TD* and λ and soil pH and C/N ratios may contribute to changes in these indices.

In conclusion, the total nematodes and some tolerant genera were significantly correlated with distance from the factory, and the heavy metals in the study were found to positively affect the nematode communities and their trophic structure. Further study is needed to uncover the relationship between soil nematodes and heavy metal pollution.

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