Soil nematode responses to heavy metal stress

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Summary

The effect of Cu and Zn on soil nematode communities at the depth of 0–20 cm was investigated along a pollution gradient with increasing distance from a copper smelter in Northeast China. The results showed that the abundance of total nematodes significantly increased with increasing distance from the smelter, and nematode taxon richness also exhibited an increasing trend. Twenty genera were observed in our study, and *Paratylenchus* was the dominant genus at all the sampling sites. The numbers of total nematodes, bacterivores, plant parasites, omnivores-predators and the values of NCR (Nematode Channel Ratio) negatively correlated with total Cu ($P < 0.01$), total and available Zn ($P < 0.01$). The values of SI (Structure Index) negatively correlated with total Cu ($P < 0.05$). Among the nematode ecological indices examined, NCR and SI were found to be sensitive indicators for assessing the effect of heavy metals on soil nematode communities in this study.

Key words: soil nematode; community structure; ecological indices; copper smelter; heavy metals

Introduction

Emission of toxic substances from metal smelters not only causes air pollution but also soil contamination (Tobor-Kaplon *et al.*, 2006). These substances, including Cu, Zn, Pb and Cd, are usually dispersed in relatively higher concentrations in the vicinity of a smelter, with a gradual depletion as distance from the source increases (Shukurov *et al.*, 2005). Heavy metal contamination can change the functioning of soil ecosystems by disturbing the activities of soil fauna (Cortet *et al.*, 1999). Because nematodes occupy key positions as primary and intermediate consumers in soil food webs, evaluation and interpretation of the abundance and function of their faunal assemblages or community structure could offer an in situ assessment of

stress factors (Bongers & Ferris, 1999). Changes in soil nematode community structure have been used very successfully in assessing the ecotoxicological effects of different soil disturbances including heavy metal pollution and fertilization (Neher, 2001; Li *et al.*, 2006; Liang *et al.*, 2006).

In recent years, there have been many reports of the impact of heavy metals on soil nematode assemblages (Yeates *et al.*, 2003). Georgieva *et al.* (2002) found that Cu and Zn had a negative effect on various parameters of a nematode assemblage on a sandy loam in England. Nagy *et al.* (2004) reported that increased Se and Cr concentrations resulted in a significant decrease in nematode density, generic richness and maturity index (MI2-5) on a calcareous chernozem soil in Hungary. Shukurov *et al.* (2005) studied the effect of heavy metals on soil nematodes along the emission gradient of the Almalyk Industrial Complex, Uzbekistan, and showed a significant correlation between the number of total nematodes and the distance from the pollution source. Similar results were also observed by Li *et al.* (2006) in the study of the effect of heavy metals on soil nematode communities in the vicinity of a metallurgical factory in Northeast China. However, little information is available concerning soil nematode responses to heavy metals near a smelter in Northeast China (Li *et al.*, 2006). The objectives of this study were to investigate soil nematode responses to heavy metals along a pollution gradient with increasing distance from a copper smelter in Northeast China, and to evaluate the relationship between nematode ecological indices and concentrations of heavy metals in soil.

Materials and Methods

This study was conducted at corn fields along a pollution gradient from the Hongtoushan Copper Smelter (41°59' N,

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104°31' E), the largest enterprise in the Mountain Area of East Liaoning, Northeast China. The smelter produces crude copper and has a capacity for processing approximately 11500 tons per year. Due to a lack of air-treatment facilities for copper smelting, the smelter is also a major source of air pollution. This study area is located in the continental temperate monsoon zone, with a dry-cold winter and a warm-wet summer. The annual mean temperature is 5.1°C, annual precipitation averages 863 mm. The soil at the study site is a Cambisol (FAO classification) and the previous crop is corn (*Zea mays* L.).

Soil samples were taken from corn fields along a pollution gradient, in a downwind direction from the smelter emission sources. The soil samples were collected from 0–20 cm layer at 50 (site A), 150 (site B), 500 (site C) and 1000 (site D) m along the pollution gradient in November 2006. At each sampling site, four plots (10 × 10 m² for each plot) were randomly selected; four replications were collected in each plot by coring techniques (5 cm diameter × 20 cm depth). Each replication was composed of 5 soil cores. Soil samples were placed in individual plastic bags and transported to the laboratory for chemical and nematode analysis.

Before analysis, soil samples were air-dried and ground to pass through a 2 mm sieve for determination of available metals. Subsamples were then ground and passed through a 149 µm sieve for determination of total metals. For total metals, soil samples were digested with HNO₃—HClO₃ (3:1, v/v) and determined using AAS (WFX120A) with a detecting limit of 0.01 mg/L for Cu and Zn. The concentrations of available heavy metals in soil samples were determined after extraction with 0.1 mol/L HCl (solid: liquid = 1:5) (Li *et al.*, 2006; Liang *et al.*, 2006). Soil physicochemical properties at different sites were showed in Table 1. Nematodes were extracted from 100 g (fresh weight) of soil from each sample using sugar flotation and centrifugation (Hua *et al.*, 2006; Liang *et al.*, 2006; Liu *et al.*, 2006), the nematode abundance was expressed per 100 g dry weight soil (Liang *et al.*, 2007). All extracted nematodes in each sample were counted and identified to genus level using an inverted compound microscope. The classification of trophic groups was assigned to: (1) bacterivores; (2) fungivores; (3) plant-parasites; and (4) omnivores-predators, based on known feeding habitats or stoma and esophageal morphology (Yeates *et al.*, 1993; Renčo, 2004; Hua *et al.*, 2006; Liu *et al.*, 2006; Meng *et al.*, 2006; Liang *et al.*, 2007).

Four ecological indices of nematode communities were calculated: (1) nematode taxon richness (S), S is the total number of genera (Ekschmitt *et al.*, 2001); (2) Maturity index (MI25), $MI25 = \sum v(i) \cdot f(i)$, where $v(i)$ is the c-p value of taxon i (excluding c-p 1 group), $f(i)$ is the frequency of taxon i in a sample (Bongers, 1990; Korthals *et al.*, 1996; Nagy *et al.*, 2004). (3) Structure index (SI), SI is a measure of the stable and structured status of a soil nematode community, was calculated according to the method of Ferris *et al.* (2001). (4) Nematode channel ratio (NCR), $NCR = B / (B + F)$, where B and F are the proportions of the nematode

fauna allocated to bacterivorous and fungivorous groups (Yeates *et al.*, 2003).

All the data were subjected to statistical analysis of variance (ANOVA) in the SPSS statistical package. Differences with $P < 0.05$ were considered significant.

Results

Significant differences in total and available Cu and available Zn concentrations were found between sampling sites ($P < 0.05$), where $A > B > C > D$ (Fig. 1 and Fig. 2). These

Fig. 1. Concentrations of total and available Cu (mean ± SD) at different sampling sites along a pollution gradient (A, 50 m; B, 150 m; C, 500 m; D, 1000 m from Hongtoushan copper smelter)

Fig. 2. Concentrations of total and available Zn (mean ± SD) at different sampling sites along a pollution gradient

Fig. 3. Abundance of total nematodes (mean ± SD) at different sampling sites along a pollution gradient

Table 1. Soil physicochemical properties at different sampling sites

Site	SM (%)	Bulk Density (g cm ⁻³)	pH	EC (μs cm ⁻¹)	TOC (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)	C/N
A	12.1	1.42	5.25	158.9	14.6	1.3	12.0
B	15.9	1.37	5.35	120.0	11.4	1.2	9.8
C	16.5	1.36	5.41	126.9	12.4	1.3	11.0
D	18.2	1.10	5.19	167.9	14.2	1.2	11.9

Explanations: SM – soil moisture; TOC – total organic carbon; EC – electrical conductivity

Table 2. The proportion (%) of nematode genera and trophic groups at different sampling sites

Genus	c-p	A	B	C	D
BF		37.7	57.1	17.0	42.5
<i>Acrobeles</i>	2	0.0	0.0	0.0	2.0
<i>Acrobeloides</i>	2	21.8	28.8	8.6	13.3
<i>Eucephalobus</i>	2	8.5	6.8	0.0	6.8
<i>Plectus</i>	2	0.0	0.0	1.0	0.2
<i>Prismatolaimus</i>	3	3.0	10.1	3.7	1.5
<i>Protorhabditis</i>	1	4.4	9.5	0.7	15.6
<i>Wilsonema</i>	2	0.0	0.0	3.0	0.0
FF		15.7	26.7	1.5	3.2
<i>Dorylaimoides</i>	5	3.1	4.9	0.0	0.0
<i>Tylencholaimus</i>	4	0.0	0.0	0.0	0.2
<i>Paraphelenchus</i>	2	12.6	21.8	1.5	3.2
PP		46.6	18.3	72.6	54.3
<i>Bitylenchus</i>	3	2.1	0.0	16.7	3.0
<i>Coslenchus</i>	2	0.0	0.0	3.2	0.0
<i>Filenchus</i>	2	7.5	0.2	16.7	3.0
<i>Macroposthonia</i>	3	9.1	1.0	0.0	0.5
<i>Paratylenchus</i>	2	14.0	17.1	13.2	39.9
<i>Pratylenchus</i>	2	13.9	0.0	28.9	7.9
OP		0.0	0.0	8.2	2.9
<i>Aporcelaimellus</i>	5	0.0	0.0	8.2	1.7
<i>Discolaimus</i>	5	0.0	0.0	0.0	0.3
<i>Epidorylaimus</i>	4	0.0	0.0	0.7	0.0
<i>Thonus</i>	4	0.0	0.0	0.0	0.9

Explanations: BF – bacterivores; FF – fungivores; PP – plant parasites; OP – omnivores-predators. c – p values for nematode genera were based on Bongers (1990)

results indicated that total and available Cu and available Zn concentrations decreased with increasing distance from the smelter. According to the China environmental quality standard for soils (GB 15618 – 1995), total Cu contents at site A and B belong to quality class III, while those at site C and D belong to quality class II; total Zn contents at all the sites belong to quality class II. These results indicated that soil at site A and B were unsuitable for agricultural use, and site C and D were suitable for agricultural use.

Twenty genera of nematodes were identified in our investigation (Table 2), and *Paratylenchus* was dominant genus at all the sampling sites. The number of *Paratylenchus* negatively correlated with total and available Cu ($P < 0.05$) and Zn ($P < 0.01$). *Acrobeloides* and *Paraphelenchus* were found to be dominant genera at A and B, while *Bitylenchus* and *Pratylenchus* were dominant at site C.

Significant differences in the number of total nematodes were found between sampling sites ($P < 0.01$), where $A < B < C < D$ (Fig. 3). The number of total nematodes negatively correlated with total Cu ($P < 0.01$), total and available Zn ($P < 0.01$) (Table 3).

Significant differences in the numbers of bacterivores, plant parasites and omnivores-predators were found between sampling sites ($P < 0.05$). The number of plant parasites exhibited a similar trend to those of total nematodes. Omnivores-predators were the least abundant trophic group, averaging from 0.0 % to 8.2 % of the nematode community (Table 2). The numbers of bacterivores, plant parasites and omnivores-predators negatively correlated with total Cu ($P < 0.05$), total and available Zn ($P < 0.05$) (Table 3).

Table 3. Correlation coefficients for soil nematodes and soil chemical properties at different sampling sites

Indicator	pH	EC	Available Cu	Available Zn	Total Cu	Total Zn
TNEM	0.001	0.414	- 0.281	- 0.868**	- 0.878**	- 0.885**
BF	0.132	0.120	- 0.499	- 0.687**	- 0.725**	- 0.770**
FF	0.324	- 0.358	- 0.381	- 0.026	- 0.131	- 0.100
PP	- 0.015	0.165	0.015	- 0.863**	- 0.886**	- 0.888**
OP	0.367	0.038	0.188	- 0.574*	- 0.669*	- 0.649*
S	0.263	0.427	0.285	- 0.398	- 0.404	- 0.492
MI2-5	0.387	- 0.036	0.291	- 0.451	- 0.534	- 0.501
SI	0.460	- 0.095	0.163	- 0.504	- 0.585*	- 0.546
NCR	- 0.075	0.203	- 0.101	- 0.752**	- 0.737**	- 0.745**

Explanations: *, ** – Correlations are significant at $P < 0.05$ and 0.01 levels, respectively

Significant differences in the selected ecological indices were found between sampling sites ($P < 0.05$). The highest value of S (number of genera) was 16 at site D, and the lowest 9 at site B. The lowest values of MI2-5 and SI were found at site A. The values of NCR showed the same trend with those of S (Table 4). The values of SI negatively correlated with total Cu ($P < 0.05$), and those of NCR negatively correlated with total Cu ($P < 0.01$), total and available Zn ($P < 0.01$) (Table 3).

Table 4. Nematode ecological indices at different sampling sites

Site	S	MI2-5	SI	NCR
A	11	2.23	33.30	0.73
B	9	2.26	37.92	0.65
C	13	3.10	78.57	0.92
D	16	2.32	43.65	0.93
P-values	0.037	0.023	0.037	0.017

Explanations: S – nematode taxon richness; MI2-5 – maturity index (2-5); SI – structure index; NCR – nematode channel ratio

Discussion

Aerial deposition of Cu and Zn emitted from the adjacent smelter caused an increase in heavy metal concentrations in the investigated soils. The highest concentrations were found at site A (site closest to the smelter) and the lowest at site D (the most remote site) in our study. These results were partly in agreement with those of Tobor-Kapłan *et al.* (2006), reported from the vicinity of a zinc smelter in Budel, the Netherlands. The number of total nematodes exhibited a gradual increasing trend with increasing distance from the copper smelter in our investigation. This result was in agreement with that of Shukurov *et al.* (2005), who observed it in the vicinity of an industrial complex in Uzbekistan. However, it was inconsistent with the finding of Li *et al.* (2006) that the Cu and Zn had positive effects on soil nematodes near a metallurgical factory in the lower reaches of Liao River Plain, Northeast China. The discrepancy may be a consequence of the lower concentrations of Cu and Zn in our previous study (Li *et al.* 2006), since the lower concentrations of Cu and Zn in soil could stimulate the hatching of nematode eggs (Clarke & Shepherd, 1966). Significant correlations with the total contents of Cu and Zn were found in this study, but not or weak correlations with available contents of Cu and Zn. The reason for the latter was that soil temperature was very low during this sampling time and would decrease the contents of available Cu and Zn.

The numbers of bacterivores, plant parasites and omnivores-predators correlated negatively with total Cu, total and available Zn in soil in our study. These results indicated that Cu and Zn had negative effects on most trophic groups. Omnivores-predators were in our study the least abundant and the most sensitive trophic group to heavy metal pollution; no genus was found at site A and B.

Similar results were found by Georgieva *et al.* (2002), who re-reported that omnivores-predators were reduced or eliminated from the nematode communities in soil with high Cu and Zn concentrations. However, positive effects due to a moderate level of Cu and Zn application on nematode abundance and taxon richness were reported by Nagy (1999); and in the Zn treated plots even MI and the proportion of omnivores-predators increased compared with the control (Nagy, 1999). Moreover, some stimulative effects of Cu on many genera at low concentrations were found in a laboratory experiment added to different CuSO_4 solutions in The Netherlands (Bongers *et al.*, 2001). There is evidence for comparable effects of Cu and Zn to the findings of present study.

Nematode richness (S), as indicated by the number of genera, proved to be a very suitable tool showing a consistent correlation with mass and microflora in the grassland soils (Ekschmitt *et al.*, 2001). The values of S in our study ranged from 9 to 16, which were consistent with those observed by Li *et al.* (2006) in the vicinity of a metallurgical factory in the lower reaches of Liao River Plain. MI measured disturbances with a lower value indicating a more disturbed environment (Bongers, 1990). MI2-5 value (excluding c-p 1 group) gave a much better response to disturbances than the MI (Nagy *et al.*, 2004). The values of MI2-5 in our investigation ranged from 2.23 to 3.10, which showed a similar trend with those reported by Nagy *et al.* (2004) in an agricultural field in Hungary. Nematode channel ratio (NCR) reflects the soil decomposition pathway. The values of NCR ranged from 0.67 to 0.93 with higher values found at site C and D compared with site A. The values of NCR indicated the bacterial decomposition pathway was relatively more dominant at site C and D. The values of NCR correlated negatively with total Cu, total and available Zn in our study. These results were in agreement with those found by Yeates *et al.* (2003) in New Zealand.

In conclusion, soil heavy metals (Cu and Zn) near a copper smelter had adverse effects on the numbers of total nematodes, bacterivores, plant parasites and omnivores-predators. Among the nematode ecological indices examined, SI and NCR were found to be sensitive indicators for assessing the effect of heavy metals on soil nematode communities in this study.

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