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# Effect of Heavy Metals on Soil Nematode Community Structure in Shenyang Suburbs

[1]*Wenju Liang*, [1,2]*Qi Li*, [1,2]*Xiaoke Zhang*, [1]*Siwei Jiang and* [1]*Yong Jiang*

[1]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[2]Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

**Abstract:** The effects of heavy metals (Cu, Zn and Cd) on soil nematode communities were studied along a pollution gradient in Shenyang suburbs. The results showed that highly significant differences in total and available Cu, Zn and Cd contents were found between treatments ($p<0.01$), where Ningguan > Gaoming > Shilihe. Thirty-four genera of nematodes were identified in the investigation and *Acrobeloides*, *Zygotylenchus* and *Helicotylenchus* were found to be the dominant genera. Significant differences in the numbers of total nematodes, bacterivres, fungivores and plant parasites were found between sampling dates ($p<0.01$), treatments ($p<0.01$) and depths ($p<0.01$). The numbers of total nematodes decreased with depth at different sites. The number of total nematodes and the values of nematode richness (S) were negatively correlated with the total and available Cd. The number of omnivores-predators and the values of maturity index (MI) were negatively correlated with total Cu, Zn, Cu and available Cd. MI was found to be a more sensitive indicator for assessing the effects of heavy metals (Cu, Zn and Cd) on soil nematode communities in Shenyang suburbs. These results indicated that the heavy metals (Cu, Zn and Cd) had adverse effects on soil nematode community structure in this study.

**Key words:** Heavy metals · soil nematodes · community structure · maturity index

## INTRODUCTION

Heavy metals can be regarded as stress factors on biological populations [1]. 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 [2]. Their fauna composition together with its ecological indices has emerged as a useful monitor of environmental conditions and ecosystem function in the soil [3]. 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 [4].

In recent years, there have been many reports of the effect of heavy metals on soil nematode assemblages or community structure, sometimes after large applications of heavy metals and assessment of dose responses [1, 4, 5, 6]. These reports indicated significant changes in the qualitative and quantitative characteristics of the nematode communities due to the heavy metal pollution [4].

In Northeast China, little information is available concerning the long-term effects of heavy metals on soil nematode community structure [6]. The objective of this study was to determine the effect of heavy metals (Cu, Zn and Cd) on soil nematode community structure along a pollution gradient in Shenyang suburbs.

## MATERIALS AND METHODS

Shenyang is the capital city of Liaoning Province of China, which is located on the alluvial plains of the Liaohe and Hunhe Rivers. It is an industrial city, named as one of the most polluted cities in the world by the United Nations in early 1980s [7]. The soils in the west suburb of Shenyang have been polluted by heavy metals due to wastewater irrigation during 1960s and 1980s [8]. The Xihe River is a principal wastewater drainage in the west suburb of Shenyang, which collects the industrial wastewater from dozens of factories in Tiexi District of western Shenyang and the living sewage from Huanggu District of northern Shenyang, with an average daily output of $5 \times 10^5$ t. Meanwhile, the Xihe River is also an irrigation drainage which has irrigated the fields of the

**Corresponding Author:** Dr. Wenju Liang, Institute of Applied Ecology, Chinese Academy of Sciences, P.O. Box 417, Shenyang 110016, China

riverside villages and towns for about forty years [7]. The survey was conducted at Ningguan (41°45′ N, 123°15′ E) and Gaoming (41°47′ N, 123°14′ E) in the west suburb and Shilihe (41°31′ N, 123°22′ E) in the south suburb of Shenyang according to a pollution gradient of heavy metals, in which Shilihe was selected as the control site. The above-mentioned three sites are 0.1, 0.5 and 25 km from the Xihe River, respectively. The soil is classified as a Hapli-Udic Argosols in Chinese Soil Taxonomy and the previous crop is soybean (*Glycine max*) at the three sites. Soil samples were collected at the depths of 0-10, 10-20 and 20-30 cm from the three sites (Ningguan, Gaoming and Shilihe). Three random soil samples were taken at each site, placed in individual plastic bags and transported to the laboratory for chemical and biological component determination [9].

Before analysis, soil samples were air-dried, 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 $HNO_3$-$HClO_3$ (3:1, v/v) and determined using AAS (WFX120A) with detecting limit of 0.01 mg $L^{-1}$ for Cu, Zn and Cd. The concentrations of available heavy metals in soil samples were determined after extraction with 0.1 mol $L^{-1}$ HCl (solid: liquid =1:5) [6].

The nematodes were extracted from 100 g soil (fresh weight) using the sugar flotation and centrifugation method [10]. All extracted nematodes in each sample were counted and expressed per 100 g dry weight soil. The nematodes from each sample were identified 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 habitats or stoma and esophageal morphology [11]. Two ecological indices of nematode communities were calculated: (1) nematode richness (S), S is the total number of genera [12]; (2) Maturity index (MI), $MI = \Sigma v(I) \bullet f(I)$, where v(I) is the c-p value of taxon I, f (I) is the frequency of taxon I in a sample [13].

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.

## RESULTS AND DISCUSSION

Highly significant differences in total and available Cu, Zn and Cd contents were found between treatments ($p<0.01$), where Ningguan > Gaoming > Shilihe (Table 1). These results indicated that Ningguan site was heavily polluted by Cu, Zn and Cd and Gaoming site was also contaminated by these elements. Significant differences in total and available Cu and Zn contents were also observed between depths ($p<0.01$), while no significant differences in total and available Cd content were found between depths (Table 1).

Thirty-four genera of nematodes were identified in the investigation, belonging to 21 families (Table 2). The number of genera in our study was higher than that reported by Li *et al.* [6] in the vicinity of a metallurgical factor, south suburb of Shenyang. The highest number of genera was 14 found at 10-20 cm depth in Shilihe site. The lowest number of genera was 4 found at 20-30 cm depth in Gaoming site. *Acrobeloides*, *Zygotylenchus* and *Helicotylenchus* were found to be the dominant genera in our study, their relative abundance was 10.3, 11.0 and 15.5%, respectively.

Table 1: Soil chemical properties of different sites in spring, 2005 (mg $kg^{-1}$)

| | | Total | | | Available | | |
|---|---|---|---|---|---|---|---|
| Site | Depth | Cu | Zu | Cd | Cu | Zu | Cd |
| Ningguan | L1 | 49.74±3.66 | 231.68±18.49 | 2.66±0.32 | 8.17±2.77 | 9.39±1.73 | 1.05±0.08 |
| | L2 | 52.63±6.99 | 238.62±10.46 | 3.22±0.08 | 11.23±1.92 | 10.00±0.81 | 1.00±0.16 |
| | L3 | 37.68±5.48 | 168.99±21.96 | 3.80±0.82 | 7.05±2.52 | 8.18±1.25 | 1.15±0.15 |
| Gaoming | L1 | 27.17±1.07 | 94.36±9.72 | 2.06±0.17 | 2.88±0.25 | 4.69±0.51 | 0.48±0.08 |
| | L2 | 25.98±0.20 | 92.19±2.07 | 2.37±0.18 | 6.17±0.47 | 3.94±0.45 | 0.39±0.03 |
| | L3 | 24.18±0.77 | 69.91±11.01 | 1.75±0.02 | 2.75±0.14 | 2.54±0.46 | 0.31±0.08 |
| Shilihe | L1 | 19.64±0.12 | 50.96±2.81 | 0.75±0.02 | 2.63±0.23 | 1.40±0.26 | 0.13±0.01 |
| | L2 | 18.23±0.71 | 39.47±5.08 | 0.75±0.20 | 2.59±0.34 | 0.89±0.06 | 0.13±0.02 |
| | L3 | 18.22±0.45 | 37.46±4.26 | 0.72±0.04 | 2.00±0.05 | 0.73±0.01 | 0.10±0.01 |
| ANOVA (p value) | | | | | | | |
| | Treat | ** | ** | ** | ** | ** | ** |
| | Depth | ** | ** | Ns | ** | ** | Ns |
| | Treat×Depth | ** | ** | ** | Ns | Ns | Ns |

Depth: 0-10 cm (L1); 10-20 cm (L2); 20-30 cm (L3), The values are means±SD, **: Significant at 0.01 and Ns: No significant

Table 2: Nematode genera and families identified in this investigation

| Trophic group | Family | Genus | c-p |
|---|---|---|---|
| BF | Alaimidae | *Alaimus* | 4 |
| | Cephalobidae | *Acrobeles* | 2 |
| | | *Acrobeloides* | 2 |
| | | *Cephalobus* | 2 |
| | | *Chiloplacus* | 2 |
| | | *Eucephalobus* | 2 |
| | | *Heterocephalobus* | 2 |
| | Panagrolaimidae | *Panagrolaimus* | 1 |
| | Plectidae | *Plectus* | 2 |
| | Prismatolaimidae | *Prismatolaimus* | 3 |
| | Rhabditidae | *Mesorhabditis* | 1 |
| | | *Protorhabditis* | 1 |
| | Teratocephalidae | *Metateratocephalus* | 3 |
| FF | Anguinidae | *Ditylenchus* | 2 |
| | Aphelenchidae | *Aphelenchus* | 2 |
| | Aphelenchoididae | *Aphelenchoides* | 2 |
| | Leptonchidae | *Dorylaimoids* | 4 |
| | | *Tylencholaimus* | 4 |
| PP | Criconematidae | *Macropothonia* | 3 |
| | Heteroderidae | *Heterodera* | 3 |
| | Hoplolaimidae | *Helicotylenchus* | 3 |
| | Paratylenchidae | *Paratylenhus* | 2 |
| | Pratylenchidae | *Pratylenchus* | 3 |
| | | *Zygotylenchus* | 3 |
| | Psilenchidae | *Psilenchus* | 2 |
| | Tylenchidae | *Filenchus* | 2 |
| | | *Malenhus* | 2 |
| | | *Tylenchus* | 2 |
| OP | Aprocelaimidae | *Aprocelaimellus* | 5 |
| | Belondiridae | *Axonchium* | 5 |
| | Qudsianematidae | *Eudorylaimus* | 4 |
| | | *Epidorylaimus* | 4 |
| | | *Microdorylaimus* | 4 |
| | | *Thonus* | 4 |

Trophic group: Bacterivores (BF); Fungivores (FF); Plant parasites (PP); Ominivores-predators (OP)
c-p values for nematode genera were based on Bongers [13]

Significant differences in the numbers of total nematodes were found between sampling dates ($p<0.01$), treatments ($p<0.01$) and depths ($p<0.01$) (Table 3). The number of total nematodes was negatively correlated with total ($p<0.01$) and available Cd ($p<0.05$) (Table 4). The numbers of total nematodes in this study ranged from 15 to 383 individuals per 100 g dry soil. This result is lower than that observed by Li *et al*. [6] for a soybean field polluted by Cu and Zn in south Shenyang suburb. The highest number of total nematodes was found at the 0-10 cm depth at Shilihe site and the lowest number was found at the 20-30 cm depth at Ningguan site in autumn (Table 3). The numbers of total nematodes decreased with depth in different sites. This result was in accordance with that observed by Ou *et al*. [14] in a corn field at Shilihe site [14].

The nematodes identified belonged to four groups: bacterivores, fungivores, plant parasites and omnivors-predators (Table 3). Significant differences in the numbers of bacterivores, fungivors and plant parasites were found between sampling dates ($p<0.01$), treatments ($p<0.01$) and depths ($p<0.01$) (Table 3). Significant differences in the numbers of omnivors-predators were observed between sampling dates ($p<0.01$) and treatments ($p<0.01$), but not depths. Plant parasites were the most dominant group in this study, averaging 45.1% of the nematode community. The numbers of plant parasites exhibited similar trends to those of total nematodes. Omnivors-predators were the least abundant trophic group, averaging 2.4% of the nematode community. These results are comparable to those reported by Ou *et al*. [14] in Shilihe site. The numbers of total nematodes were negatively correlated

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

| | | | Trophic groups | | | | Ecological indices | |
|---|---|---|---|---|---|---|---|---|
| Site | Depth | TNEM | BF | FF | PP | OP | S | MI |
| | | | 5 April 2005 (Spring) | | | | | |
| Ningguan | L1 | 90±27 | 18±10 | 24±15 | 47±22 | 1±1 | 9±1 | 1.80±0.15 |
| | L2 | 39±22 | 12±6 | 8±4 | 19±14 | 0±1 | 9±4 | 1.64±0.24 |
| | L3 | 38±4 | 14±3 | 4±5 | 18±5 | 2±3 | 9±1 | 1.93±0.55 |
| Gaoming | L1 | 66±37 | 46±28 | 13±12 | 6±3 | 1±1 | 9±1 | 1.81±0.14 |
| | L2 | 48±35 | 33±32 | 9±6 | 6±4 | 0±1 | 7±2 | 1.81±0.08 |
| | L3 | 35±7 | 18±9 | 8±3 | 7±5 | 1±1 | 11±3 | 1.88±0.17 |
| Shilihe | L1 | 146±9 | 24±6 | 13±6 | 109±4 | 1±0 | 12±1 | 1.84±0.10 |
| | L2 | 73±21 | 22±12 | 9±7 | 41±8 | 1±1 | 11±1 | 1.83±0.05 |
| | L3 | 51±8 | 18±8 | 8±1 | 24±1 | 1±1 | 12±1 | 2.07±0.22 |
| | | | 5 July 2005 (Summer) | | | | | |
| Ningguan | L1 | 120±60 | 23±13 | 33±17 | 62±40 | 1±3 | 10±1 | 1.79±0.11 |
| | L2 | 71±21 | 42±28 | 8±3 | 21±17 | 0±0 | 11±1 | 1.42±0.19 |
| | L3 | 34±7 | 16±3 | 3±2 | 15±11 | 0±0 | 7±1 | 1.57±0.25 |
| Gaoming | L1 | 130±16 | 84±9 | 22±12 | 22±7 | 3±2 | 13±2 | 1.86±0.11 |
| | L2 | 65±15 | 39±13 | 7±5 | 16±4 | 3±4 | 11±3 | 1.96±0.18 |
| | L3 | 30±16 | 16±7 | 1±2 | 10±7 | 2±2 | 6±2 | 2.17±0.17 |
| Shilihe | L1 | 132±15 | 5±4 | 0±0 | 117±9 | 8±6 | 10±2 | 2.87±0.47 |
| | L2 | 179±48 | 16±8 | 3±4 | 150±46 | 9±6 | 11±5 | 2.69±0.13 |
| | L3 | 36±13 | 6±1 | 5±6 | 20±3 | 5±6 | 11±3 | 2.62±0.10 |
| | | | 5 October 2005 (Autumn) | | | | | |
| Ningguan | L1 | 212±43 | 21±7 | 31±17 | 157±87 | 3±3 | 9±2 | 1.82±0.30 |
| | L2 | 48±11 | 14±9 | 3±1 | 31±2 | 0±0 | 9±3 | 1.79±0.04 |
| | L3 | 15±6 | 5±3 | 2±1 | 7±2 | 0±1 | 7±1 | 1.82±0.07 |
| Gaoming | L1 | 157±31 | 108±4 | 8±5 | 37±25 | 0±0 | 9±2 | 1.87±0.03 |
| | L2 | 69±14 | 54±13 | 8±7 | 7±2 | 1±1 | 8±1 | 1.88±0.05 |
| | L3 | 23±5 | 16±7 | 4±1 | 3±2 | 0±0 | 4±1 | 1.88±0.11 |
| Shilihe | L1 | 383±83 | 49±15 | 70±15 | 266±57 | 1±2 | 11±3 | 1.91±0.09 |
| | L2 | 333±56 | 62±17 | 42±19 | 227±58 | 1±2 | 14±2 | 1.81±0.16 |
| | L3 | 38±5 | 15±4 | 4±1 | 19±6 | 0±1 | 9±2 | 2.00±0.14 |
| ANOVA (p value) | | | | | | | | |
| Date | | ** | ** | ** | ** | ** | Ns | ** |
| Treat | | ** | ** | ** | ** | ** | ** | ** |
| Depth | | ** | ** | ** | ** | Ns | ** | Ns |
| Date×Treat×Depth | ** | * | ** | ** | Ns | Ns | Ns | |

*, **: Significant at 0.05 and 0.01, respectively; Ns: No significant

Table 4: Correlation coefficients between soil nematodes, ecological indices and heavy metals

| Index | Total Cu | Total Zn | Total Cd | Available Cu | Available Zn | Available Cd |
|---|---|---|---|---|---|---|
| TNEM | -0.176 | -0.18 | -0.387** | -0.183 | -0.214 | -0.249* |
| BF | -0.12 | -0.139 | -0.058 | -0.136 | -0.079 | -0.135 |
| FF | 0.018 | 0.026 | -0.198 | -0.026 | -0.015 | -0.058 |
| PP | -0.073 | -0.076 | -0.318** | -0.117 | -0.12 | -0.15 |
| OP | -0.227* | -0.226* | -0.309** | -0.188 | -0.251* | -0.242* |
| *S* | -0.136 | -0.152 | -0.331** | -0.139 | -0.192 | -0.249* |
| MI | -0.487** | -0.487** | -0.526** | -0.456** | -0.510** | -0.492** |

*, **: Significant at 0.05 and 0.01, respectively

with total Cd ($p<0.01$), while those of omnivors-predators were negatively correlated with total Cu, Zn and Cd and available Zn and Cd ($p<0.05$) (Table 4). These results indicated that Cu, Zn and Cd had significantly adverse effects on nematode trophic structure.

Nematode richness (S), as indicated by the number of genera, reflects biodiversity of soil habitat [14]. Significant differences in the values of S were observed between treatments ($p<0.01$) and depths ($p<0.01$) (Table 3). The values of S exhibited a decreasing trend at each depth along a pollution gradient, where Shilihe > Gaoming > Ningguan. Nematode richness was negatively correlated with total and available Cd ($p<0.05$) (Table 4). The result showed that Cd had an adverse effect on nematode diversity in our study.

The maturity index (MI) has been successfully used as indicators to heavy metal pollution [4, 6]. MI measured disturbances with a lower value indicating a more disturbed environment [11]. Significant differences in the values of MI were found between sampling dates ($p<0.01$) and treatments ($p<0.01$), but not depths (Table 3). The values of MI showed a decreasing trend at each depth along a heavy metal pollution gradient (Table 3). The values of MI were negatively correlated with total and available Cu, Zn and Cd ($p<0.01$) (Table 4). These results demonstrated that MI was a sensitive indicator for assessing the effects of heavy metals (Cu, Zn and Cd) on soil nematode communities in Shenyang suburbs.

In conclusion, the heavy metals (Cu, Zn and Cd) had adverse effects that were correlated with the changes in the nematode community structure, i.e., decreases in the numbers of total nematodes, the numbers of four trophic groups, nematode richness and maturity index.

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