Distribution of soil nematode communities along a section of Shen-Ha Highway

V. V. S. TOMAR^{1,2}, X. K. ZHANG¹, Q. LI¹, Y. JIANG¹, W. J. LIANG^{1,*}

¹Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China, *E-mail: liangwj@iae.ac.cn; ²Nematode Biodiversity Research Laboratory, Aligarh Muslim University, Aligarh 202002, India

Summary

The effect of lead on soil nematode communities at the 0 – 20 cm depth was investigated along an increasing distance from a highway in Northeast China. The results showed that the abundance of total nematodes significantly increased from 40 m and then decreased eventually and the similar trend was also found in most indices studied. Thirty seven genera of nematodes belonging to 8 orders and 24 families were observed in our study, and *Helicotylenchus* was eudominant genus at all the sampling sites. The numbers of total nematodes significantly correlated with total and available Pb ($P < 0.05$), while Σ MI had a significantly negative correlation with TOC, Total N ($P < 0.01$) and C/N ratio ($P < 0.05$). Total and available Pb in soil had positive correlation with the numbers of total nematodes and bacterivores. The concentration of Pb also had an effect on abundance of *Helicotylenchus*, which increased in moderate concentration of Pb. NCR could be effectively used as indicators, as it was sensitive to concentration of Pb.

Keywords: soil nematodes; community structure; ecological indices; highway; lead

Introduction

The contribution of vehicular exhaust to the global emission of atmospheric pollutants is increasing at alarming rate. The pollutants that mainly include Pb, Cd and Zn have an impact on environment. The exhausts from vehicles contaminate the atmosphere, water and soil due to deposition. Many studies have shown contamination by these elements, at the vicinity of highways (Zhang *et al.*, 1999; Turner & Maynard, 2003). The concentration of heavy metals in the roadside soils may be influenced by factors like wind (Piron-Frenet *et al.*, 1994) or traffic intensity (Garcia & Millan, 1998) and by soil physiochemical characteristics. Heavy metals in this exhaust are usually dispersed in relatively higher concentrations in the vicinity

of a highway, with a gradual depletion as distance from the source increases (Sezgin *et al.*, 2003; Nabulo *et al.*, 2006). Most vehicles burn leaded gasoline, which is well established source of Pb contamination to environment. Mielke *et al.* (1997) observed that accumulation of Pb in soil due to leaded gasoline is directly proportional to highway traffic flow. Nematodes are important component of soil ecosystems and any changes in the soil characteristics may result in change in community dynamics of the nematodes, which can be assessed with the help of ecological indices. Nematode community structure is widely used as a tool in ecological studies, to assess the functioning of soil and biomonitoring. Nematode fauna composition along with its indices, has emerged as a useful monitor for environmental conditions and soil ecosystem function and also helpful in assessing the effects of various soil disturbances (Bongers & Ferris, 1999; Neher, 2001; Berkelmans *et al.*, 2003).

In recent years, there are many reports of the impact of heavy metals on soil ecosystems. Sezgin *et al.* (2003) studied contamination of highway soils with heavy metals in Turkey and reported heavy concentration of lead in the soils near highway. Viard *et al.* (2004) assessed the bioaccumulation of heavy metals (Pb, Cd, Zn) in soil, plants of graminaceae and garden snails; and reported that lead efficiently described traffic impact and it was the most adapted tracer of highway environment. Nabulo *et al.* (2006) assessed the contamination of roadside soils with heavy metals in Uganda and found concentration of heavy metals decreased with increasing distance from roads. Wang *et al.* (2006) compared the distribution pattern of lead in rural and urban Shenyang city and found that east sides of the road were more contaminated as compared to west sides. Many studies have been performed to evaluate nematode responses to heavy metals from sources like industrial complex (Shukurov *et al.*, 2005), metallurgical factory in China (Li *et al.*, 2006; Zhang *et al.*, 2007), however, there is little information on soil nematode responses to heavy

metal pollution from a highway. The objective of this study was to investigate the distribution of soil nematodes along a Pb pollution gradient with increasing distance from a highway in Northeast China.

Material and Methods

This study was conducted at a maize field that was distributed near a highway. Soil samples were collected from the maize field along the section of Shen-Ha highway between Shenyang and Tieling, which are all the large cities in Liaoning, Northeast China. The section suited in the Puhe town (41°57' N, 123°36' E) far from the centre of Shenyang city. The section of highway is characterized by a traffic level of 50,000 vehicles and 300,000 passenger flow per day. The study site is located in the continental monsoon zone with a dry-cold winter and a warm-wet summer. The annual temperature is 7.3 °C, mean annual precipitation is 667 mm. The test soil is classified as a Hapli-Udic Argosol in Chinese soil taxonomy (Hua *et al.*, 2006), with 12.75 g kg⁻¹ total C, and 1.11 g kg⁻¹ total N, pH (H₂O) 6.3, 38.0 % sand, 41.4 % silt and 20.6 % clay at the 0–20 cm depth.

Soil samples at the 0–20 cm depth were taken from the maize field (100 m × 320 m) along a pollution gradient with distance at 5, 20, 40, 80, 160 and 320 meters in a downwind direction from the east side of highway on October 5, 2007. Five replications were collected by coring techniques (5 cm diameter). 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 chemical analysis, soil samples were air-dried, ground to pass through a 2 mm sieve for determination of available Pb. Subsamples were then ground and passed through a 149 µm sieve for determination of total Pb. Soil samples were digested with HF-HNO₃-HClO₄ (3:1, v/v) and determined using AAS (WFX120A) with detecting limit of 0.01 mg/L for total Pb. The concentrations of available Pb in soil samples was determined after extraction with 0.1 mol/L HCL (solid: liquid =1:5) (Li *et al.*, 2006; Qin *et al.*, 2009).

Nematodes were extracted from 100 g (fresh weight) of soil from each sample using modified Baermann's funnel technique (Tomar *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 (Ba); (2) fungivores (Fu); (3) root-fungal feeders (RFF); (4) herbivores /plant-parasites (H); (5) omnivores (OM) and (6) carnivores/predators (Ca), based on known feeding habitats or stoma and esophageal morphology (Yeates *et al.*, 1993; Háněl, 2004; Renčo, 2004; Okada *et al.*, 2005).

Following ecological indices of nematode communities were calculated: (1) Dominance $\lambda = \sum p_i^2$; (2) Shannon-Weaver diversity $H' = -\sum p_i \ln p_i$, where p_i is the proportion

of individuals in the i th taxon; (3) Species richness $SR = (S-1)/\ln N$, where N is the number of nematodes; (4) Evenness(J') = $H'/\ln S$, S (nematode taxon richness) is the total number of genera (Yeates & Bongers, 1999); (5) Σ maturity index $\Sigma MI = \sum v(i) \cdot f(i)$, where $v(i)$ is the c-p value of taxon i , $f(i)$ is the frequency of taxon i in a sample; (6) MI2-5 (excluding c-p 1 group) (Bongers, 1990; Korthals *et al.*, 1996; Yeates *et al.*, 2003; Nagy *et al.*, 2004); (7) 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). Three dominances were divided according to the proportions of nematode genera: "eudominant" is over 10 %, "dominant" 5–10 % and "subdominant" 2–5 %. 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 Pb concentrations were found among sampling sites ($P < 0.05$), with increase and subsequent decrease in concentrations (Fig. 1). The highest values of total and available Pb were found at 20 m and lowest value at the 320 m from the highway. These results indicated that total and available Pb concentrations decreased with increasing distance from the highway. Exceptionally, very low amount of total and available lead was present at 5 m from highway. Based on the Standards for Soil Environmental Quality of China (1995), the allowed value (II grade) for Pb is 150 mg kg⁻¹ when soil pH value was less than 6.5. The average concentrations of Pb at the sampling sites from 20, 40 and 80 cm were above the allowed values of Pb (Fig. 1).

Fig. 1 Concentrations of total and available Pb (mean ± SD) at different sampling sites along a pollution gradient from the Highway

Table 1. The proportion (%) of nematode genera and trophic groups at different sampling sites from the Highway

Genus	Guild	5 m	20 m	40 m	80 m	160 m	320 m	P-value
<i>Acrobeles</i>	Ba ₂	0.19	0.19	1.43	0.42	4.82	4.23	NS
<i>Acrobeloides</i>	Ba ₂	0	0.85	0.52	3.3	0.18	1.67	NS
<i>Cephalobus</i>	Ba ₂	8.74	6	5.89	4.9	10.23	3.47	NS
<i>Chiloplacus</i>	Ba ₂	0	0	0.91	1.49	3.29	2.22	NS
<i>Eucephalobus</i>	Ba ₂	7.07	6.98	2.26	11.19	6.63	2.03	NS
<i>Mesorhabdtis</i>	Ba ₁	0	4.18	4.11	0.36	0.83	0.91	<0.05
<i>Monhystera</i>	Ba ₁	0	0.19	0	0.69	0.17	1.65	NS
<i>Panagrolaimus</i>	Ba ₁	0	0	0.32	0.37	0	0.36	NS
<i>Prismatolaimus</i>	Ba ₃	0.18	3.56	1.35	0.82	1.59	0.72	NS
<i>Rhabditis</i>	Ba ₁	0	0	0	0	1.25	0	NS
<i>Teratocephalus</i>	Ba ₃	0	0	0	0	0.18	0	NS
<i>Wilsonema</i>	Ba ₂	0.37	0	0	0.18	0	0.36	NS
<i>Aphelenchoides</i>	Fu ₂	8.25	1.87	2.53	4.14	5.91	9.41	<0.05
<i>Aphelenchus</i>	Fu ₂	0.58	0.74	0.52	2.41	1.54	0.72	NS
<i>Diptherophora</i>	Fu ₃	0.37	0.34	0	0	0	0	NS
<i>Dorylaimoides</i>	Fu ₄	0.37	1.54	1.85	0.39	1.4	1.37	NS
<i>Tylencholaimus</i>	Fu ₄	0	0	0.35	0	0	0	<0.05
<i>Aglenchus</i>	RFF ₂	0	0	0	0.18	0	0	NS
<i>Boleodorus</i>	RFF ₂	18.45	6.02	6.7	2.59	5.58	13.02	<0.05
<i>Filenchus</i>	RFF ₂	8.65	3.24	2.66	6.39	2.56	2.76	NS
<i>Psilenchus</i>	RFF ₂	0.39	0.67	0	0.46	0	0	NS
<i>Tylenchus</i>	RFF ₂	0	4.31	2.15	0.18	2.15	3.51	NS
<i>Epidorylaimus</i>	Om ₄	0	0	0	0.2	0	0	NS
<i>Eudorylaimus</i>	Om ₄	0.37	0.75	3.79	5.61	1.52	2.19	<0.01
<i>Thornenema</i>	Om ₄	0	0.75	0.36	0.22	0.73	0	NS
<i>Axonchium</i>	Om ₅	0.99	0.54	1.51	0.64	0	3.32	<0.05
<i>Dorylaimellus</i>	Om ₅	0	1.14	0	0.44	0.59	0.92	NS
<i>Aporcelaimellus</i>	Ca ₅	0	0.33	0.74	0.22	1.76	0	NS
<i>Discolaimus</i>	Ca ₅	0	0	0	0	0.18	0	NS
<i>Iotonchus</i>	Ca ₄	0	0	0	0	0.18	0	NS
<i>Mylonchulus</i>	Ca ₄	0.97	0.37	0	0	0	0	<0.01
<i>Criconemoides</i>	H ₃	0	0.38	0	0	0	0.18	NS
<i>Helicotylenchus</i>	H ₃	37.19	44.82	45.9	39.49	36.42	29.29	NS
<i>Heterodera</i>	H ₃	0	6.12	0	0	1.54	3.28	<0.01
<i>Hirschmaniella</i>	H ₃	0	2.4	1.25	7.82	4.06	0.75	<0.01
<i>Pratylenchus</i>	H ₃	11.51	6.38	3.24	7.54	0.92	9.79	NS
<i>Trophurus</i>	H ₃	2.49	0	3.16	1.77	11.54	0.18	<0.01

Guild designation is the composite of feeding habit and cp value: Ba – bacterivore; Fu – fungivore; RFF – root-fungal feeders; Om – omnivore; Ca – carnivore (predator); H – herbivore. Numbers following the letters indicate the cp value of each taxon based on Bongers (1990, 1999), Ferris & Matute (2003).

Thirty seven genera of nematodes from eight orders and twenty-four families were identified in our investigation (Table 1), and *Helicotylenchus* was eudominant genus at all the sampling sites with highest dominance (46 %) at 40 m. *Pratylenchus* was eudominant only at 5 m while *Boleodorus* was eudominant at 5 m and 320 m. *Eucephalobus* and *Trophurus* were eudominant at 80 m and 160 m, respectively. *Cephalobus* was present at all sites, with eudominance at the distance of 160 m from the highway. *Aphelenchoides* was dominant at 5 m, 160 m and 320 m, respectively. The dominance of *Aphelenchoides* and *Boleodorus* were all higher at 5 m and 320 m than at other sites. *Mesorhabdtis*, *Aphelenchoides*, *Tylencholaimus*, *Boleodorus*, *Axonchium* ($P < 0.05$) and *Eudorylaimus*,

Mylonchulus, *Heterodera*, *Hirschmaniella*, *Trophurus* ($P < 0.01$) were significantly different at different sampling distances.

The number of total nematodes increased at 40 m and subsequently decreased to 320 m. The highest abundance of nematodes was at 40 m while lowest at 5 m. Significant differences in the number of total nematodes ($P < 0.05$) were found among sampling sites (Table 2). Except fungal feeders, which were highest at 320 m, the number of bacterial feeders, root-fungal feeders, herbivores and omnivores-predators also showed the same trend as total nematodes. Bacterial feeders ($P < 0.01$) were significantly different with distance.

Significant differences in the ecological indices including

Table 2. Abundance (individuals per 100 g dry soil) of soil nematodes and ecological indices at different sampling sites from the Highway

	5 m	20 m	40 m	80 m	160 m	320 m	P-value
Abundance							
TNEM	274.0±97.0	399±123.7	543.8±123.2	421.0±166.4	307.6±94.3	318.8±45.0	<0.05
Ba	42.7±35.1	81.7±45.6	81.3±45.0	71.8±15.8	79.1±33.2	52.0±14.3	<0.01
Fu	26.6±19.8	19.5±15.9	28.3±23.9	24.1±8.4	23.2±17.3	35.7±18.5	NS
RFF	58.0±14.7	48.3±10.7	92.2±40.0	39.4±9.2	29.0±5.7	52.1±13.3	NS
H'	139.0±20.2	231.7±29.7	309.6±45.2	258.4±73.9	161.0±30.2	159.2±34.2	NS
Om+Ca	7.6±3.3	18.0±7.0	32.5±8.5	27.4±10.9	15.2±5.4	19.9±3.8	NS
Ecological index							
λ	0.23±0.05	0.25±0.07	0.36±0.20	0.23±0.13	0.19±0.05	0.21±0.14	<0.01
H'	1.72±0.23	1.86±0.27	1.65±0.64	1.95±0.39	2.05±0.26	2.06±0.43	NS
SR	1.39±0.40	1.97±0.40	1.98±0.65	2.08±0.39	2.22±0.72	2.47±0.54	NS
J'	0.80±0.03	0.73±0.08	0.63±0.17	0.75±0.13	0.80±0.09	0.75±0.13	<0.01
Σ MI	4.25±0.50	4.93±0.65	5.24±0.46	4.88±0.24	5.20±0.25	4.69±0.39	NS
MI2-5	2.24±0.07	2.47±0.17	2.74±0.22	2.37±0.11	2.46±0.13	2.33±0.16	NS
NCR	0.59±0.17	0.81±0.06	0.75±0.17	0.75±0.06	0.79±0.14	0.60±0.15	<0.01

dominance (λ), evenness (J'), MI2-5 and nematode channel ratio (NCR) ($P < 0.01$) were found at sampling sites. The lowest values of MI2-5, NCR and Σ MI were found at 5 m from the highway. The values of λ ranged from 0.19 to 0.36, with high and low values at distance of 40 m and 160 m from the highway (Table 2). H' was highest at 320 m, while MI2-5 and NCR followed the same trend as the number of total nematodes. The obtained values of NCR ranged from 0.59 to 0.81 with highest value at 20 m and lowest at 5 m.

The number of total nematodes positively correlated with total and available Pb, while bacterial feeders correlated with total Pb ($P < 0.05$). SR and J' negatively correlated with total nitrogen and total Pb ($P < 0.05$), while Σ MI negatively correlated to TOC, Total N, C/N ($P < 0.01$) and NCR with pH ($P < 0.01$) (Table 3).

concentrations were found on east side at site 40 m and the lowest at 360 m in our study. These results were in line with those of Wang *et al.* (2006) in Shenyang, who found that east sides of the highways are more contaminated than west sides and Nabulo *et al.* (2006) in Uganda, who reported that generally highest concentrations of Pb were found at 30-40 m from highway. Increase in soil Pb concentration at 40 m may be reasoned that impact distance for Pb is 30 m to 40 m from a highway (Viard *et al.*, 2004; Nabulo *et al.*, 2006). Presence of low concentration of Pb nearest to highway (5m) can be explained that wind is mainly responsible for deposition of lead from vehicular exhaust, and Pb particles travel certain distance in air before settling down in soil (Viard *et al.*, 2004).

The number of total nematodes exhibited a gradual increasing trend to a distance and then decreasing with

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

Indicator	pH	Total Pb	Available Pb	TOC	Total N	C/N
TNEM	-0.11	0.48*	0.42*	-0.16	-0.03	-0.10
Ba	-0.38	0.41*	0.29	-0.34	-0.26	-0.31
Fu	0.30	-0.28	-0.28	0.05	-0.09	0.17
RFF	0.09	-0.06	0.19	0.07	0.01	0.14
H'	-0.18	0.32	0.25	-0.16	-0.14	-0.07
Om+Ca	-0.14	0.15	0.04	-0.19	-0.22	-0.04
λ	-0.14	0.33	0.30	0.05	0.07	0.02
H'	-0.09	-0.35	-0.31	-0.24	-0.38	0.02
SR	-0.19	-0.05	-0.24	-0.36	-0.40*	-0.13
J'	0.22	-0.46*	-0.10	0.06	0.02	0.06
Σ MI	-0.27	0.37	-0.17	-0.60**	-0.52**	-0.43*
MI2-5	-0.27	0.27	0.02	-0.07	-0.05	-0.04
NCR	-0.57**	0.20	0.34	-0.24	-0.02	-0.33

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

Discussion

Aerial deposition of Pb emitted from the heavy and light traffic passing on the highway caused an increase in heavy metal concentrations in the investigated soils. The highest

increase in distance from the highway in our investigation, well explained by positive correlation of total number of nematode with total and available Pb, thus Pb had effects on the number of total nematodes.

Response of nematode abundance to heavy metals is vari-

able, the population of nematodes may increase or decrease with heavy metal concentration (Sanchez-Moreno & Navas, 2007). The numbers of bacterivores correlated positively with total Pb in soil in our study. These results indicated that Pb had a positive effect on bacterivorous nematodes although this does not fully agree with Sanchez-Moreno and Navas (2007), who found low populations of bacterial and fungal feeding nematodes in heavy metal polluted soils. It may be due to fact that heavy metal toxic effects may disappear due to adaption of organisms (Haimi & Matasniemi, 2001). Herbivores were most dominant trophic group in our study. The high abundance of herbivores was at the medium Pb concentration which agrees well with Korthals *et al.* (1998) who reported that intermediate pollutant concentrations may be beneficial to plant parasites as due to increase in nutrient availability and a higher leakage of root exudates may occur which plays a role between plant parasites and heavy metal concentration. *Helicotylenchus* was most eudominant genus that can be explained in a crop field presence of plant roots leads to abundant resources for plant parasites and hence they build up their population. The correlation between total and available Pb and *Aphenlenchoides* was negatively significant. Higher dominance of *Aphenlenchoides* appeared at 5 m and 320 m with low concentrations of Pb. However, no significant correlation between the concentration of Pb and *Boleodorus* was found and its distribution was probably better explained by the joint action of soil properties and heavy metals. Omnivore-predators were least abundant in our study and the most sensitive trophic group to heavy metal pollution, which fully agrees with Georgieva *et al.* (2002) and Zhang *et al.* (2006) who reported that the population of omnivore-predators was reduced due to increase in concentrations of heavy metals.

MI2-5 gives a much better response to disturbances than MI (Nagy *et al.*, 2004). The values of MI2-5 in our investigation ranged from 2.46 to 3.19, which agree well with that reported by Nagy *et al.* (2004) in Hungary and Zhang *et al.* (2006) in China. The nematode channel ratio (NCR) is known to be an important indicator of the decomposition pathway in the soil food webs (Yeates *et al.*, 2003). The values of NCR in our study ranged from 0.59 to 0.81 with highest value at 20 m and lowest at 5 m. The values of NCR indicated the bacterial decomposition pathway was relatively more dominant at 20 m. The values of NCR correlated negatively with pH ($P < 0.01$) in our study. The lower NCR values may result from lower pH values. A higher value of NCR may reflect the abundance of mycorrhizal fungi (De Goeda *et al.*, 1993).

It can be concluded from present study that total and available Pb in soil had a positive correlation with the number of total nematodes as well as with that of bacterivores. The concentration of Pb also have effect on abundance of *Helicotylenchus*, which increases in moderate concentration of Pb. NCR could be effectively used as indicators, as it was sensitive to concentration of Pb. Thus nematodes can serve as useful bioindicators of heavy metal

pollution to reflect the condition of soil ecosystem.

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