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Soil Nematode Response to Heavy Metal Pollution of Industrial Origin in Bulgaria

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Abstract

The effect of heavy metal pollution on the soil nematode community was evaluated on five sampling sites along a pollution gradient transect originating at the KCM 2000-Plovdiv (pollution source). Soil samples were collected twice (spring and autumn) in 2013 at 0-10 and 10-20 cm depth. Twenty-eight nematode genera were identified and *Paratylenchus, Eucephalobus* and *Tylenchus* were found to be the dominant genera. The number of total nematodes decreased with the depth at different sites. Significant differences in the number of plant parasites and bacterivores were observed between sampling sites ($p<0.01$) and depths ($p<0.01$). The number of fungivores and omnivores-predators were significantly different between sampling sites ($p<0.01$), dates ($p<0.01$) and depths ($p<0.01$). The number of plant-parasites, fungivores and omnivores-predators were negatively correlated with Cu, Zn, and Cd, but bacterivores had a positive correlation with Pb. The values of nematode richness (S), maturity index (MI_{2-5}) and structure index (SI) were negatively correlated with the traced heavy metals. MI_{2-5} and SI indices proved to be sensitive indicators for assessing the effects of heavy metals on soil nematode community structure.

Key words: Community structure, ecological indices, industry, pollution, soil nematodes

Introduction

In recent years, there is an unmistakable deterioration of the soil ecosystems due to enormous development of human society associated with a great increase in production of wastes and various contaminants, including heavy metals (Šalamún et al., 2012). Soil as a primary source of food and inhabitance for many microorganisms, is one of the most endangered habitat. Since the beginning of 1990s, several chemical and biological approaches have been proposed to assess the deterioration of the soil (Edwards, 2002).

Soil nematodes as the most abundant microfauna in the soil directly influence the main soil processes (nutrient cycling, decomposition, etc.) (Neher, 2001). They possess several attributes that make them useful ecological indicators: do not rapidly migrate from stressful conditions and the community structure is indicative of conditions in the soil horizon that it inhabits; have transparent body, and their diagnostic internal features can be seen without dissection; respond rapidly to disturbance and enrichment (Bongers and Ferris, 1999).

Nematodes have been used for monitoring the polluted soils with heavy metals (Yeates et al., 1993; Zhang et al., 2006; Shao et al., 2008). The effects of heavy metals on soil nematode communities have been studied in recent years in agro-ecosystems treated with sewage sludge (Georgieva et al., 2002), in a pasture polluted with Cu, Cr, As and Ni (Yeates et al., 2003), in agricultural fields after being artificially contaminated for several years (Nagy et al., 2004), in forest and agroecosystems near a metallurgical factory (Li et al., 2006; Pen-Muratov et al., 2008) and in agricultural fields near a highway (Han et al., 2009). These studies have been reported significant changes in the qualitative and quantitative characteristics of the nematode communities. Some of this evaluations indicated that nematode community was affected by heavy metals positively, and other studies showed negative influence of heavy metals on soil nematodes.

Two species classifications based on life strategy and trophic preference that enable to interpret the changes in nematodes communities are currently available. The c-p classification, which describes the nematode life strategies ranges from one (colonizers, tolerant to stress) to five (persisters, sensitive to stress) (Bongers, 1990). Feeding-type classification divides nematodes into different groups: plant feeders, predators, bacterivores, fungivores and omnivores according to their feeding preference (Yeates et al., 1993).

Heavy metals pollution in the region of Plovdiv is well known and widely discussed problem. However, no information exists on the effect of heavy metals on the nematode assemblages in this region. The objectives of this study were to determine the structure of soil nematode communities using the indices (nematode richness, maturity index, structural index) based on c-p classification and feeding-type classification and to evaluate the nematode response to heavy metals in the polluted region.

Materials and Methods

Study area

The sampled sites are located in 10-14 kilometres south from Plovdiv, nearby the KCM 2000-Plovdiv (a metallurgical factory – main source of pollution). The region has a flat topography, with small hills reaching up to 180 m above sea level. The soil type is Cinnamon Forest Soils (BST classification), and the landscape is dominated by agricultural land use. According to the climatic data (National Institute of Meteorology and Hydrology, BAS – branch in Plovdiv), the average air temperature from April through October 2013 was 23.2°C. The average rainfall for the investigation period was 395 mm.

The five sites (named A, B, C, D, and E) were chosen according to previous information about contamination of heavy metals in the soil. The soil samples were collected along a pollution gradient (site A - 42°30' N, 24°49' E, site B - 42°24' N, 24°49' E, site C - 42°20' N, 24°50' E, site D - 42°35' N, 24°48' E, site E - 42°40' N, 24°49' E).

Soil sampling

The soil samples were collected from the five sites in spring (May) and in autumn (October) in 2013. Plant cover in the study sites was removed before sampling and only soil was taken. Four plots (10 × 10 m^2 for each plot) were randomly selected at each sampling site. Four replications were collected in each plot by coring technique (5 cm × 5 cm, 0-10 and 10-20 cm depth). Each replication was composed of four soil cores, which were mixed together and placed in individual plastic bags, and transported to the laboratory for chemical and biological analyses. After analyzing each replicate separately, an average was calculated for each site and depth.

Heavy metal analysis

Total concentration of heavy metals (Cu, Zn, Pb and Cd) was determinate by ICP-MS method (Table 1). Before analysis, soil samples were air-dried, ground to pass through 150 µm sieve for determination of total metals. Metals were extracted by digestion with 2 mol/L HNO and the concentration was determined using ICP-MS.

Table 1. Total heavy metal content in the soil of different sites in spring 2013 (mg kg^{-1})

Site	Depth (cm)	Cu	Zn	Pb	Cd
A	0-10	550.68±72.12	6498.96±976.33	6403.15±903.22	104.99±20.05
	10-20	513.74±48.45	6413.71±777.44	6432.98±1011.49	110.68±15.37
B	0-10	181.42±20.31	2829.18±411.16	1755.24±375.78	35.82±4.81
	10-20	162.03±23.17	2797.66±694.84	1692.33±317.24	37.00±6.17
C	0-10	64.28±6.35	942.75±187.97	150.45±36.66	3.52±0.44
	10-20	61.19±4.18	950.04±165.02	167.28±29.03	3.19±1.12
D	0-10	101.25±19.28	2120.96±566.71	2496.19±500.09	39.04±3.99
	10-20	95.14±11.34	2074.84±605.01	2407.79±688.13	32.11±7.62
E	0-10	32.00±5.22	150.35±27.56	75.17±17.18	1.51±0.13
	10-20	20.35±1.97	141.12±18.00	68.23±22.65	1.33±0.09

Nematode extraction and analyses

The nematodes were extracted from 100 g (2 × 50) soil from each replicate using Bearmann funnel procedure (Barker, 1985). The extracted nematodes were killed by heat and fixed in 4% formalin (Southey, 1986). Nematodes from each replicate were counted under stereomicroscope and the first 150 individuals were further identified to family and genus level using a compound microscope. All nematodes were identified when the nematode were fewer than 150 individuals in a replicate. Nematodes were separated into four trophic groups: (1) bacterivores (BF); (2) fungivores (FF); (3) plant-parasites (PP); (4) omnivore-predators (OP); based on their feeding preferences or stoma and esophageal morphology (Yeates et al., 1993). The nematode communities were separated into five groups according c-p (colonizer-persister) model of Bongers (1900): from c-p 1 (colonizers, tolerant to stressc) to c-p 5 (persisters, sensitive to stress).

Table 2. Nematode genera and families identified in this study

Trophic group	Family	Genus	c-p
PP	Criconematidae	*Criconemoides*	3
	Dolichodoridae	*Tylenchorhynchus*	3
	Hoplolaimidae	*Hoplolaimus*	3
	Meloidogynidae	*Meloidogyne*	3
	Paratylenchidae	*Paratylenchus*	2
	Pratylenchidae	*Pratylenchus*	3
	Longidoridae	*Longidorus*	5
	Tylenchidae	*Filenchus*	2
		Tylenchus	2
BF	Cephalobidae	*Acrobeles*	2
		Acrobeloides	2
		Cephalobus	2
		Eucephalobus	2
	Diplogasteridae	*Rhabdontolaimus*	1
	Plectidae	*Plectus*	2
	Rhabditidae	*Caenorhabditis*	1
		Mesorhabditis	1
		Rhabditis	1
		Pelodera	1
FF	Anguinidae	*Ditylenchus*	2
	Aphelenchidae	*Aphelenchus*	2
	Tylencholaimidae	*Tylencholaimus*	4
OP	Belondiridae	*Belondira*	5
	Discolaimidae	*Discolaimus*	5
	Dorylaimidae	*Dorylaimus*	4
	Qudsianematidae	*Eudorylaimus*	4
		Microdorylaimus	4
		Thonus	4

Trophic group: PP – plant parasites; FF – fungivores; BF – bacterivores; OP – omnivores-predators
c-p values of nematode genera

Two features and three ecological indices of nematodes communities were determined: (1) total number of nematodes in 100 g soil; (2) number of nematodes into a trophic group; (3) nematode taxon richness (S), S is the total number of genera (Ekschmitt et al., 2001); (4) maturity index (MI) for nematodes communities (based on c-p value of free-living nematodes (MI_{2-5}) excluding c-p_1 group) (Bongers 1990); (5) structure index (SI) is based on the relative weighted abundance of description-sensitive guilds representing structure (Ferris et al., 2001).

Statistical analysis

All the data obtained in the study were analyzed by ANOVA using SPSS 15.0 statistical software (SPSS Inc., Chicago IL). Differences at the $p<0.05$ and $p<0.01$ levels were considered as significant. Differences in means of nematode trophic groups and ecological indices among study sites were tested by Least Significant Differences test (LSD). Spearman's correlation test was used to assess the correlation between nematode groups and indices, and heavy metals in the soil.

Results

Twenty-eight genera of nematodes belonging to nineteen families and fife orders (*Aphelenchida, Dorylaimida, Plectida, Rhabditida* and *Tylenchida*) were identified in this study (Table 2). *Paratylenchus* was dominant genus (14.3% abundance) at all the sampling sites. The genera *Eucephalobus* and *Tylenchus* were found at four of five sites and their relative abundance was 11.2 and 8.1%, respectively. The most abundant trophic group was those of bacterivores represented by eleven genera with low c-p value (c-p_1 and c-p_2), but the only three nematode genera with c-p_2 and c-p_4 values were observed in fungivores trophic group.

Significant differences in the number of total nematodes were observed between sampling sites ($p<0.01$), dates ($p<0.01$) and depths ($p<0.01$) (Table 3). The number of total nematodes ranged from 29.5 to 855.3 individuals/100 g soil. The highest number of nematodes was found at the 0-10 cm depth at site E in autumn. The least number of nematodes was observed at site A at 10-20 cm depth in spring.

The nematodes identified in this study belonged to four groups: plant-parasites, bacterivores, fungivores and omnivores-predators (Table 2). Significant differences in the number of fungivores and omnivores-predators were found between sampling sites ($p<0.01$), dates ($p<0.01$) and depths ($p<0.01$) (Table 3). Significant differences in the number of plant parasites and bacterivores were observed only between sampling sites ($p<0.01$) and depths ($p<0.01$). Plant-parasites were the dominant group, averaging 47.5% of the nematode community. Bacterivores were the second largest trophic group, representing more than 40.0% of the nematode communities at C and D sampling sites.

Fungivores, as relatively tolerant to ecological disturbances were observed at approximately 22.5% of the nematode community. Omnivores-predators were the most affected by the high content of heavy metals in the soil of A and D sampling sites. Their trophic group was the least abundant, averaging 2.7% of the nematode community.

The numbers of total nematodes were negatively correlated with total Cu, Zn, Cd ($p<0.01$) and Pb ($p<0.05$) at the five investigated sites (Table 4). The group of bacterivores was the least affected by the heavy metals and only this group was found to be in positively correlation with Pb (Table 4). These results indicate that Cu, Zn, Cd and partly Pb had significant adverse effect on nematode trophic structure.

Significant differences in the values of nematode richness (S) were observed between sampling sites ($p<0.01$) (Table 3). The values of S had a decreasing trend with increasing of heavy metal content. There were seven genera of nematodes in the most polluted site A. Nematode richness was negatively correlated with all four traced heavy metals, but Cd had the most adverse effect.

Significant differences in the values of maturity index (MI) were observed between sampling sites ($p<0.01$) and dates ($p<0.01$), but not depths ($p<0.01$) (Table 3). The highest values of MI were observed at site E with 2.48 and 2.12 in spring and autumn, respectively. The lowest values of MI were found at site A with 1.47 and 1.62, in spring and autumn, respectively. Similar trend was observed in the values of structural index (SI), which had significant differences between sampling sites ($p<0.01$) and dates ($p<0.01$). The values of MI and SI were negatively correlated with total Cu, Zn, Cd and Pb in the soil of the five sites.

Discussion

According to Park et al. (2011), heavy metals negatively influenced all aspects of soil ecosystems, including community of soil nematodes. The results obtained in this study indicated that heavy metal pollution originating from emissions releasing by KCM 2000-Plovdiv negatively affected the composition of soil nematode communities. The highest concentration of Cu, Zn, Pb and Cd were found at the site A (site closest to the factory) and the lowest at site E (the most distant site). The number of total nematodes showed a gradual increasing trend with increasing distance from the metallurgical factory. This result was in agreement with that of Zhang et al. (2007), who it observed in agricultural field near a copper smelter in Northeast China. The number of total nematodes decreased with depth in different sites. Similarly, Ou et al. (2005) and Liang et al. (2006) reported lower number of nematodes at 10-20 cm depth than 0-10 cm. In addition, Li et al. (2008) reported that the number of total nematodes depend on land use and soil properties.

Different responses of trophic group to heavy metal levels were observed in this study. The number of plant parasites, bacterivores, and omnivores-predators correlated negatively with total Cu, Zn and Cd. The most resistant to pollution were fungivores, in which even a positive correlation with Pb was observed. Conversely, in some other studies (Li et al., 2006; Shukurov et al., 2006) bacterivores and plant-parasites showed to be the most resistant groups to high concentration of heavy metals. On the other hand, several other studies (Zhang et al., 2006; Pen-Muratov et al., 2010; Park et al., 2011) reported about opposite effect of heavy metals on bacterivores and plan-parasites. The results could be explained either by different physical and chemical properties of the soil such as the soil moisture, pH and soil texture (de Goede and Bongers, 1994) as well as by absence of emphatic pressure of the predator nematodes (Allen-Morley and Coleman, 1989).

Table 3. Nematode abundance (individuals per 100 g soil), trophic groups (individuals per 100 g soil) and ecological indices at different depths and dates in different sites

			Trophic groups				Ecological indices		
Site	Depth (cm)	TNEM	PP	FF	BF	OP	S	MI	SI
16 May (Spring)									
A	0-10	91.25±70.39	30.06±32.17	7.02±2.04	21.43±9.94	0.00±0.00	6.25±0.76	1.47±0.04	21.42±11.13
	10-20	29.50±16.26	10.44±11.04	2.45±1.13	12.67±16.45	0.00±0.00	6.00±0.54	1.42±0.08	23.75±9.40
B	0-10	395.50±178.37	82.28±73.14	28.88±21.77	37.50±46.52	1.34±0.28	16.25±2.18	1.93±0.07	42.17±12.35
	10-20	151.25±144.28	64.17±26.17	31.74±20.56	53.17±22.18	0.92±0.34	15.50±2.27	1.93±0.11	39.82±22.88
C	0-10	337.25±101.35	50.24±10.02	48.04±25.72	50.56±17.13	1.16±0.16	13.00±1.56	1.91±0.13	46.42±15.27
	10-20	172.00±88.36	45.57±31.42	43.27±12.32	61.16±22.88	0.00±0.00	13.00±1.95	1.88±0.06	51.07±19.46
D	0-10	175.25±98,42	92.04±42.12	1.78±4.27	46.18±13.18	0.00±0.00	8.00±0.92	1.69±0.09	29.13±17.43
	10-20	98.00±29.45	58.16±17.45	4.12±1.83	21.36±37.17	0.00±0.00	8.00±0.74	1.66±0.06	26.94±21.28
E	0-10	776.50±277.18	41.18±28.75	24.33±11.18	77.03±14.73	7.46±2.72	19.00±2.01	2.48±0.24	67.81±39.27
	10-20	152.25±75.12	37.15±14.24	30.25±10.43	74.21±66.21	8.39±3.04	19.25±1.85	2.42±0.18	64.02±41.35
13 October (Autumn)									
A	0-10	124.50±73.14	33.07±21.17	18.46±7.55	19.07±19.38	0,00±0,00	7.00±0.42	1.62±0.07	26.24±13.42
	10-20	58.00±21.36	14.12±12.37	24.02±21.49	16.23±7.07	0,00±0,00	6.50±0.88	1.58±0.05	25.98±19.37
B	0-10	351.50±157.29	79.01±25.05	30.49±14.27	38.83±57.18	6,67±1,42	16.50±1.12	2.16±0.04	46.28±25.35
	10-20	186.00±75.17	61.07±17.35	38.02±29.77	42.38±19.46	4,53±1,09	16.50±1.34	2.19±0.07	42.05±26.98
C	0-10	401,25±197.77	47.82±84.03	41.65±31.44	52.75±15.35	7,78±1,14	13.00±1.45	2.27±0.21	57.72±17.35
	10-20	146.25±68.28	41.08±52.74	31.09±12.17	56.04±47.32	2,84±0,27	13.00±0.98	2.14±0.17	59.93±12.94
D	0-10	214.25±143.17	87.93±99.04	19.25±7.35	42.82±38.12	0,00±0,00	9.25±1.03	1.90±0.06	34.73±15.22
	10-20	121.00±104.22	66.22±33.16	13.07±8.99	26.05±46.28	0,00±0,00	9.00±1.17	1.85±0.13	33.06±31.49
E	0-10	855.25±294.55	42.95±57.49	29.87±13.76	74.11±62.16	3,07±0,48	21.25±2.75	2.12±0.19	74.73±61.88
	10-20	183.25±86.87	38.78±11.34	33.03±18.12	74.97±79.88	3,21±0,97	20.50±1.54	2.07±0.12	75.12±52.17
ANOVA (p value)									
Date		**	NS	**	NS	**	NS	**	**
Site		**	**	**	**	**	**	**	**
Depth		**	**	**	**	**	NS	NS	NS
Date x Depth x Site	**	*	NS	*	NS	**	NS		

*, ** Significant at 0.05 and 0.01, respectively; Ns: No significant; TNEM – number of total nematodes;
PP – plant parasitic; FF – fungivores; BF – bacterivores;
OP – omnivores-predators; S – taxon richness; MI – maturity index; SI – structure index.

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

Index	Cu	Zn	Pb	Cd
TNEM	-0.641**	-0.719**	-0.473*	-0.693**
PP	-0.587*	-0.613**	-0.201	-0.626**
FF	-0.424*	-0.583**	-0,191	-0.414**
BF	-0.198	-0.164	0,022	-0.297*
OP	-0.603**	-0.526*	-0.493*	-0.637**
S	-0.218	-0.191	-0.138	-0.513*
MI_{2-5}	-0.462*	-0.501*	-0.423*	-0.704**
SI	-0.402*	-0.322	-0.208	-0.501*

*, ** Significant at 0.05 and 0.01, respectively

TNEM – number of total nematodes; PP – plant parasitic; FF – fungivores; BF – bacterivores; OP – omnivores – predators; S – taxon richness; MI_{2-5} – maturity index (excluding c-p_1 group); SI – structure index

Omnivores-predators were the most sensitive trophic group to heavy metal pollution in the present study. Genera of this group were no found at the sites (A and D) with highest heavy metal content. Pavao-Zuckerman and Coleman (2007) compared abundance of functional groups in urban forest soils, and found that urban soils had lower abundances of omnivores and carnivores, which had the most sensitive response to heavy metals. Similar results were reported by Georgieva et al. (2002). Authors observed that omnivores and predators were reduced or eliminated from the nematode communities in soil with high concentrations of Cu and Zn. Their results suggest that omnivores and predators classified as persisters are the most sensitive nematodes to heavy metal pollution.

Nematode richness (S), as indicated by the number of genera, reflects biodiversity of soil habitats (Ekschmitt et al., 2001). The values of S in this survey ranged from 6 to 19, which were consistent with those reported by Zhang et al. (2007) in a land near a copper smelter. Although, the diversity was mostly in negative correlation with the degree of pollution, nematode abundance increased in sites with low and medium contaminations. These results might be because of the increase in some tolerant genera, such as bacterivores and fungivores of c-p_2 (Yeates et al., 2003).

Maturity index (MI) measured disturbances and usually used to indicate ecosystems stability (Bongers, 1990). Generally, MI with lower value indicates a more disturbed environment. MI_{2-5} value (excluding c-p_1 group) gave a much better response to disturbances than MI (Nagy et al. 2004). The values of MI_{2-5} in our evaluation ranged from 1.42 to 2.48, which show a similar trend with those reported by Shao et al. (2008) in fields in Hunan Province, China. Georgieva et al. (2002) found the negative impact of the sewage sludge containing 160 mg kg^{-1} Zn or 170 mg kg^{-1} Cu on MI_{2-5} index. According to Korthals et al. (1996) nematode community showed relatively high sensitivity to Cd without any significant changes up to 160 mg kg^{-1}. Nagy et al. (2004) observed even positive correlation between Cd (190 mg kg^{-1}) and MI_{2-5}. In contrast, the results obtained in our study showed negative correlation between Cd (110 mg kg^{-1}) and MI_{2-5}. Although the toxicity of some elements above limits is considers as negligible, their toxic effect may increase in combination with other heavy metals and may have negative impact (Georgieva et al., 2002) on nematode diversity and abundance.

According to Ferris et al. (2001), SI index provide strong interpretation tool of environmental disturbances. The values of SI were significantly different at five sampling sites. These results showed that heavy metals were disturbed the soil food web by decreasing the abundance of nematode of c-p_5 group. Sanchez-Moreno and Navas (2007) reported that total C, Zn, Pb and Cd significantly decreased the values of SI and EI in the Guadiamar river basin owing to lower abundance of predators of c-p_5 and omnivores of c-p_4 groups. According to Georgieva et al. (2002) lower values of SI indicates a reduction of the complexity of the soil food web and a shortening of the food chains.

Conclusion

Soil heavy metal pollution induced by the industrialization had adverse effect on the structure of soil nematode communities. Different features of community (abundance, proportion of the nematodes in trophic groups) as well as ecological indices (S, MI_{2-5}, SI) showed degradation of soil near a pollution source. Nematodes proved to have adequate characteristics as bioindicators and might be used as assessment tool of new spots of soil environment disturbances in the future.

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