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Nematode community structure in the vicinity of a metallurgical factory

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Abstract Soil nematode communities (taxa composition, trophic structure, ecological indices) in the area of metallurgical factory (Oravské ferozliatinárske závody) in Široká, Northern Slovakia were investigated in 2009. The factory belongs to main sources of emissions originated by ferroalloy production in this region. Four sites (meadows) were selected in a downwind direction from the factory: site A was located 0.85 km far from the factory, and the other sites were maintained in approximately 2-km intervals from each other. Chemical analysis of soil samples showed low concentrations of heavy metals (As, Cd, Cr, Cu, Ni, Pb and Zn), with all values being under Slovak limit concentrations of heavy metals in soils. Only the Cd content in the soil sample from site A slightly exceeded the allowable threshold, but it was decreasing with the distance from the factory, similarly as remaining metals except Cr, with slightly increasing trend of concentration. Within 64 identified nematode genera, the *Helicotylenchus*, *Paratylenchus*, *Pratylenchus*, *Acrobeloides*, *Cephalobus* and *Rhabditis* were most common and eudominant. This was clearly reflected on the trophic structure of nematode communities, where plant feeding nematodes and bacteriovorous prevailed. Significant negative correlation ($P < 0.05$) was observed between the abundance of bacteriovores and the concentration of Cu in the soil. On the other hand, fungivores showed significant correlation with Ni and Cr ($P < 0.05$) as well as predators with Cd, Pb and Zn contents in the soil ($P < 0.01$). The highly significant correlation ($P < 0.05$; $P < 0.01$) was found between As, Cd, Ni, Pb and Zn and Maturity Index 2–5. A negative relationship was detected between Maturity Index and the concentration of Cr in the soil ($P < 0.01$). On the other hand, Cu was in positive correlation with MI values. The MI, reflecting the degree of disturbances and changes in the structure and function of the soil ecosystem, was found to be the most sensitive indicator among all used indices.

Keywords Soil nematode · Community structure · Heavy metals · Pollution · Metallurgical plant

Introduction

In recent years, the increase of heavy metal pollution from the industry represents serious threat to the environment and human health. Emissions of toxic substances releasing to the atmosphere cause soil contamination (Tobor-Kaplon et al. 2006), which can disturb the natural soil processes and

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destroy the fauna, essential for healthy soil ecosystems. Among soil organisms, nematodes possess numerous characteristics which makes them good indicator of the soil health (Bongers and Ferris 1999). Nematodes are the most abundant metazoan organisms in the soil; approximately 7.6×10^5 and 2.9×10^7 individuals per square meters inhabit desert and deciduous forest ecosystems, respectively (Volz 1951; Sohlenius 1980). Due to their prominent role in the soil processes, as a decomposition of organic matter and energy flows (Sohlenius 1980; Yeates et al. 2003), they represent irreplaceable components of the soil food web (Sochová et al. 2006). It has been estimated that approximately 40% of nutrient mineralisation in ecosystem is currently coming from nematodes and other soil microbe-feeding fauna (De Ruiter et al. 1993).

Various physical and chemical perturbations influence soil and also affect the species richness, trophic structure, succession status and structure of nematode communities (Li et al. 2006). These features of nematode communities are often used in an assessment of soil disturbances caused by heavy metal pollution, acidification, pesticide treatments, liming and fertilization (Georgieva et al. 2002) as well as by natural disasters like windfall or wildfire in forest ecosystems (Čerevková and Renčo 2009). An impact of different agricultural practices, application of the heat or soil compaction by farm machinery on nematode communities was studied by Yeates and Bird (1994), Bouwman and Arts (2000) or Kools et al. (2008). Introduced chemicals may also cause deterioration of soil and thus may have an adverse effect on nematode communities (Ekschmitt and Korthals 2006; Yardim and Edwards 1998). Other studies describe nematode community changes after fertilizing (Verschoor et al. 2001) or using sewage sludge contaminated with heavy metals (Weiss and Larink 1991; Georgieva et al. 2002). Šály (1983), Korthals et al. (1996b), Nagy et al. (2004), Zhang et al. (2007) and Pen-Mouratov et al. (2008) studied alterations in nematode community structure resulted from heavy metal pollution in natural and laboratory conditions. All these studies confirmed significant differences in the structure of soil nematode communities under stress conditions.

Several nematode classifications were developed to facilitate the interpretation of changes in the species structure (Korthals et al. 1996b). The classifications based on different feeding modes of nematodes (Yeates et al. 1993; Neher et al. 2004) and on nematode life-history strategies (Bongers 1990; Neher et al. 2004) are most frequently used to assess environmental quality through indices of ecosystem function (Wilson and Kakouli-Duarte 2009). Among these indices belong Maturity Indices (Maturity Index, Plant Parasitic Index, etc.), which placed nematode taxa on colonizer-persister continuum where particular taxa are divided into groups with different colonizers–persisters (*c*–*p*) values ranging from 1 (*r*-strategist) to 5 (*K*-strategist). Indices of ecosystem function together with diversity indices (Species Richness, Shannon's Index, Simpson's Index, etc.) provide a powerful tool for reliable interpretation of pollution-induced changes in nematode communities (Korthals et al. 1996b).

The aims of the present study were to determine the structure of nematode communities in the vicinity of metallurgical works in Široká (SK), where we expected heavy metal contamination originated from emissions and to evaluate the impact of this contamination on nematode communities.

Material and methods

The OFZ a.s. manufacturing works (Oravské ferozliatinárske závody) is located in Široká, the central part of the Orava district, near the Dolný Kubín city, North Slovakia (49°14′48″ S and 19°20′26″ E). The region is hilly with the typically continental climate (mean annual temperature is 7°C; annual precipitation usually 750–860 mm) and mostly east and northeast winds. Cambisol and Rendzic Leptosol soils prevail at the study area. The factory began its production in 1965, and to present, it represents one of the most important sources of emissions in the region. For example, as many as 164.6 tons of solid particles were emitted from the factory during the year 1996 (Kropitz and Pivarči 1998). According to Fargašová (2009), heavy metals participated in emissions releasing from factory are mainly Cr, Cu, Ni and Pb.

Sampling and sample analysis

Four sites marked as A, B, C and D were selected in a downwind direction from the factory emission source (Fig. 1).

Locality A located 0.8 km far from the factory, 49°14′ N 19°20′ E, altitude 541 m, samples were taken from windward side of old meadow (moderately mown) with plant cover composed mainly by *Festuca rubra* L., *Poa pratensis* L., *Agrostis stolonifera* L., *Acetosa pratensis* L., *Trifolium* L., *Taraxacum officinale* Web. in Wiggers and others; soil is classified as Stagnic-Eutric Cambisol according to Granec and Šurina (1999), pH ($CaCl_2$) 5.25, Cox 2.93%, N (total) 299.2 mg.kg^{-1}, sampling date: May 2009.

Locality B located 2.7 km far from the factory, 49°13′ N, 19°18′ E, altitude 503 m, samples were taken from windward side of old meadow (moderately mown) with plant cover composed mainly by *F. rubra* L., *P. pratensis* L., *Dactylis glomerata* L., *Cichorium intybus* L., *Lolium perene* L., *T. officinale* Web. in Wiggers and others; soil is classified as Stagnic-Eutric Cambisol according to Granec and Šurina (1999), pH ($CaCl_2$) 7.3, Cox 2.47%, N (total) 99.4 mg.kg^{-1}, sampling date: May 2009.

Locality C located 4.9 km far from the factory, 49°13′ N, 19°17′ E, altitude 543 m, samples were taken from windward side of old meadow (moderately mown) with plant cover composed mainly by *F. rubra* L., *P. pratensis* L., *Bromus erectus* L., *Achillea millefolium* L., *Trifolium* L., *Potentilla fruticosa* L., *T. officinale* Web. in Wiggers and others; soil is classified as Stagnic-Eutric Cambisol according to Granec and Šurina (1999), pH ($CaCl_2$) 5.95, Cox 2.1%, N (total) 102 mg.kg^{-1}, sampling date: May 2009.

Locality D located 7.7 km far from the factory, 49°12′ N, 19°14′ E, altitude 510 m, samples were

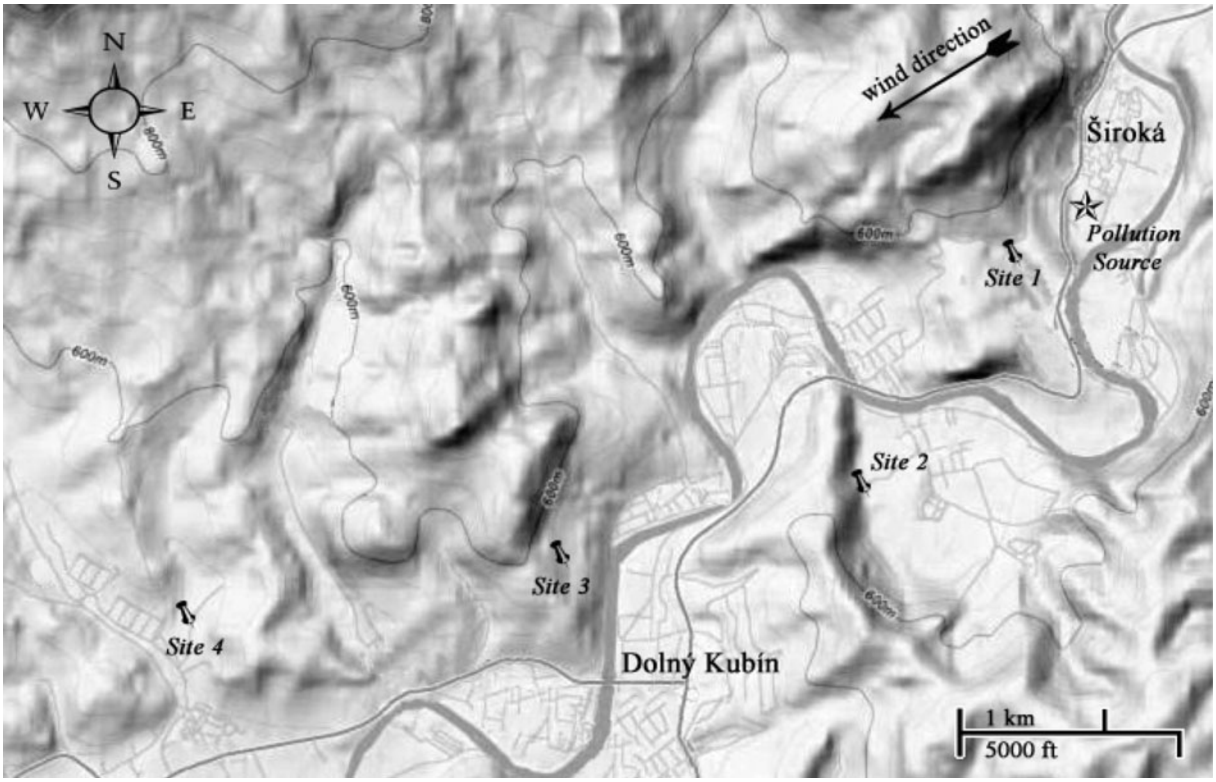

Fig. 1 Location map of the study area with pollution source and soil sampling points

taken from windward side of old meadow (moderately mown) with plant cover composed mainly by *F. rubra* L., *P. pratensis* L., *B. erectus* L., *A. millefolium* L., *Trifolium* L., *C. intybus* L., *D. glomerata* L., *Betonica officinalis* L., *Alopecurum pratensis* L. and others; soil is classified as Stagnic-Eutric Cambisol according to Granec and Šurina (1999), pH ($CaCl_2$) 5.6, Cox 3.41%, N (total) 157.3 $mg.kg^{-1}$, sampling date: May 2009.

Four mixed soil samples (each sample consists from four subsamples) were taken by spade from the surface horizont (0–20 cm) of each site. Samples were placed in individual plastic bags until processed. After analyzing of each sample separately, there was calculate average for each site, which is represented in the tables.

Before chemical analysis, soil samples were air-dried, passed through a 2-mm sieve and digested by 2 mol/L HNO_3. Total concentration of monitored heavy metals (As, Cd, Cu, Cr, Ni, Pb and Zn) was determined by Agilent 7500 C ICP-MS. The nematodes were extracted from 100 g soil (2× 50 g; fresh weight) using Baermann funnel method (Southey 1986), counted and identified to the generic level. Several ecological and diversity indices were calculated: (1) maturity index (MI) for free living nematodes and the (2) plant parasitic index (PPI) for plant parasitic nematodes were calculated according to Bongers (1990). (3) MI2–5 indices according to Yeates (1994) [all with Bongers (1990) *c*–*p* values for the nematodes where $c\text{–}p = 1$ represented r-strategists or colonizers, $c\text{–}p = 5$ represented K-strategists or persister] were calculated as measures of functional diversity. (4) The PPI/MI ratio: proportion of plant parasitic index to maturity index (Bongers and Korthals 1995), (5) B/F ratio: proportion of bacterivorous and fungivorous nematodes (Wasilewska 1997). (6) Shannon–Weaver Index (H') calculated for genera according to Shannon and Weaver (1949), (7) Richness S, which divided taxa into eudominant (>10%), dominant (5–10%) and subdominant (2–5%) groups (Losos et al. 1984); (8) Simpson Index (λ) according to Simpson (1949). Because not all data were normally distributed, Spearman's correlation coefficient was calculated to test relationship between nematode characteristics and heavy metals values at study sites. For testing the correlation, software STATISTICA version 9.0 was used. Differences and correlations obtain at levels $P < 0.05$ and $P < 0.01$ were considered being significant.

Results

Soil chemical properties and heavy metal concentrations

The mean values of abiotic environmental parameters in the different sites are presented in Table 1. Mean organic C ranged from 21 to 34.1 g/kg. The highest value was found at site D, the lowest value was at site C. Mean total N changed in wide range among different sampling sites, with the highest value at site A and lowest value at site B. pH of the soils range from acidic to slightly alkali, with mean pH values ranging from 5.25 to 7.3. Site D had the lowest pH and site 1 had the highest.

The concentrations of the heavy metals in the meadow top soils samples in the different distances from metallurgical factory were low. As shown in Table 1, the average values for particular heavy metals per sampling site did not exceed limits stated by the Decree of the Ministry of Land Management of the Slovak Republic No. 531/1994–540 on the highest admissible values of harmful substances in land. The only exception occurred in the case of Cd, where the concentration slightly surpassed the allowable threshold value in site A (0.322 mg.kg), but its content gradually decreased with the increasing distance from the pollution source. Even if the general decreasing tendency between sites A and D was observed in the majority of metals, their concentrations in the sampling site C was significantly higher in comparison with site B. The chrome content was surprisingly found to increase from the sampling sites A to D.

Abundance and dominance of nematode species

The list of nematode genera, their allocation to different trophic groups, average abundance and dominance are shown in Table 2. Altogether 64 nematode genera were identified; of them, 20 taxa belonged to the bacteriovore trophic group, 12 to plant feeding nematodes, 11 to omnivorous and

Table 1 Soil chemical properties (±s.d.) at sampling sites with the different distance from the pollution source (May 2009)

Parameter	Sites				
	A	B	C	D	X
pH ($CaCl_2$)	5.25 ± 0.3a	7.3 ± 0c	5.95 ± 0.84a	5.6 ± 0.59a	–
C_{ox} (%)	2.93 ± 0.41b	2.47 ± 0.04b	2.1 ± 0.17c	3.41 ± 0.6a	–
Total nitrogen ($mg.kg^{-1}$)	218.78 ± 68.35a	99.4 ± 11.86b	101.98 ± 17.84b	157.3 ± 54.44ab	–
As ($mg.kg^{-1}$)	0.877 ± 0.215a	0.464 ± 0.078c	0.561 ± 0.091bc	0.406 ± 0.081c	5
Cd ($mg.kg^{-1}$)	0.322 ± 0.350a	0.122 ± 0.014bc	0.112 ± 0.005c	0.102 ± 0.004c	0.3
Cr ($mg.kg^{-1}$)	2.526 ± 0.294bc	1.651 ± 0.038d	2.133 ± 0.317cd	3.897 ± 0.577a	10
Cu ($mg.kg^{-1}$)	5.789 ± 0.720b	4.132 ± 0.427c	6.879 ± 0.230a	3.065 ± 0.132d	20
Ni ($mg.kg^{-1}$)	2.134 ± 0.179bc	2.925 ± 0.148a	1.703 ± 0.167d	1.910 ± 0.088cd	10
Pb ($mg.kg^{-1}$)	1.766 ± 0.082a	0.926 ± 0.118c	1.345 ± 0.047b	0.815 ± 0.471c	30
Zn ($mg.kg^{-1}$)	4.137 ± 0.877a	1.397 ± 0.208c	1.586 ± 0.095bc	1.389 ± 0.061c	40

Means followed by the same letters on the same rows are not statistically different according to Least Significant Difference Test ($P = 0.05$), $n = 16$

X limits posted by The Decree of the Ministry of Land Management of the Slovak Republic No. 531/1994–540 on the admissible values of harmful substances in land

seven to fungivores, predators and root-fungal feeders each. The highest number of nematode genera (51) was found at site A, located nearest to the pollution source. Then the number of genera gradually decreased with the increasing distance from the factory, with 45 genera at site B, 44 at site C and 37 at the sampling site D, respectively.

Members of the genus *Helicotylenchus* were generally found to be the most common nematode species group (dominance up to 22.2%) oc-

Table 2 *c–p* values, average abundance (±s.d.) and dominance of nematode genera per 100 g soil

Nematode genera	*c–p*	A		B		C		D	
		Abundance	D%	Abundance	D%	Abundance	D%	Abundance	D%
Bacterial feeders									
Acrobeles	2	–	–	–	–	–	–	0.25 ± 0.50	0.04
Acrobeloides	2	11.00 ± 10.61	2.83	19.50 ± 6.40	4.84	13.75 ± 1.26	2.45	100.25 ± 69.61	15.47
Alaimus	4	2.00 ± 1.41	0.51	1.25 ± 1.89	0.31	0.75 ± 0.50	0.13	3.00 ± 3.46	0.46
Amphidelus	4	0.25 ± 0.50	0.06	–	–	–	–	–	–
Aulolaimus	3	–	–	0.50 ± 1.00	0.12	1.25 ± 1.89	0.22	–	–
Cephalobus	2	28.25 ± 22.54	7.26	29.75 ± 12.45	7.38	26.50 ± 10.25	4.72	71.50 ± 22.58	11.03
Cervidellus	2	–	–	0.75 ± 1.50	0.19	–	–	–	–
Diplogaster	1	–	–	0.25 ± 0.50	0.06	–	–	–	–
Eucephalobus	2	2.00 ± 1.41	0.51	5.25 ± 4.50	1.30	4.50 ± 2.89	0.80	14.75 ± 20.82	2.28
Eumonhystera	2	–	–	–	–	0.75 ± 1.50	0.13	0.50 ± 1.00	0.08
Heterocephalobus	2	7.00 ± 14.00	1.80	5.75 ± 3.30	1.43	4.75 ± 3.50	0.85	3.00 ± 3.46	0.46
Mesorhabditis	1	–	–	0.25 ± 0.50	0.06	–	–	–	–
Monhystera	2	0.75 ± 0.50	0.19	0.50 ± 1.00	0.12	29.25 ± 34.33	5.21	0.75 ± 0.96	0.12
Panagrolaimus	1	0.25 ± 0.50	0.06	0.25 ± 0.50	0.06	0.25 ± 0.50	0.04	1.75 ± 1.26	0.27
Plectus	2	8.00 ± 1.15	2.06	21.75 ± 13.60	5.40	11.75 ± 9.18	2.09	9.00 ± 10.98	1.39
Prismatolaimus	3	2.75 ± 1.71	0.71	1.00 ± 1.41	0.25	5.00 ± 8.72	0.89	24.50 ± 26.46	3.78
Protorhabditis	1	0.75 ± 1.50	0.19	–	–	–	–	–	–
Rhabditis	1	63.25 ± 19.62	16.26	35.25 ± 30.48	8.75	10.25 ± 4.57	1.83	61.00 ± 72.09	9.41
Teratocephalus	3	–	–	–	–	0.5 ± 1	0.09	–	–
Wilsonema	2	0.25 ± 0.50	0.06	–	–	–	–	–	–
Fungal feeders									
Aphelenchoides	2	17.00 ± 8.68	4.37	15.25 ± 8.69	3.78	27.50 ± 9.00	4.90	38.00 ± 35.88	5.86
Aphelenchus	2	9.50 ± 8.06	2.44	5.25 ± 4.72	1.30	38.00 ± 18.13	6.77	50.75 ± 24.61	7.83

Table 2 (continued)

Nematode genera	*c–p*	A		B		C		D	
		Abundance	D%	Abundance	D%	Abundance	D%	Abundance	D%
Diphtherophora	3	6.25 ± 5.44	1.61	–	–	1.25 ± 1.50	0.22	1.75 ±0.96	0.27
Dorylaimellus	5	0.25 ± 0.50	0.06	–	–	1.50 ± 1.91	0.27	–	–
Nothotylenchus	2	–	–	–	–	0.25 ± 0.50	0.04	0.50 ± 1.00	0.08
Tylencholaimellus	4	1.75 ± 3.50	0.45	–	–	–	–	–	–
Tylencholaimus	4	0.25 ± 0.50	0.06	–	–	0.75 ± 1.50	0.13	1.00 ±2.00	0.15
Omnivores									
Aporcelaimellus	5	20.75 ± 6.18	5.33	20.75 ± 10.53	5.15	24.50 ± 5.26	4.36	25.25 ± 18.28	3.90
Axonchium	5	5.75 ± 7.59	1.48	5.75 ± 3.40	1.43	2.00 ± 2.16	0.36	2.25 ± 3.30	0.35
Campydora	4	0.25 ± 0.50	0.06	2.00 ± 4.00	0.50	0.25 ± 0.50	0.04	–	–
Dorylaimoides	4	1.00 ± 1.41	0.26	–	–	–	–	–	–
Dorylaimus	4	4.00 ± 4.08	1.03	13.75 ± 15.69	3.41	44.75 ± 14.75	7.97	24.25 ± 11.18	3.74
Enchodelus	4	2.00 ± 3.37	0.51	–	–	–	–	–	–
Eudorylaimus	4	5.50 ± 9.11	1.41	22.25 ± 19.14	5.52	5.00 ± 2.94	0.89	12.25 ± 12.74	1.89
Mesodorylaimus	5	2.50 ± 1.29	0.64	2.00 ± 1.63	0.50	0.75 ± 0.96	0.13	3.50 ± 1.29	0.54
Oxydirus	5	8.50 ± 6.40	2.19	3.75 ± 2.99	0.93	4.50 ± 2.89	0.80	4.25 ± 2.22	0.66
Prodorylaimus	4	0.50 ± 1.00	0.13	0.50 ± 0.58	0.12	–	–	–	–
Paraxonchium	4	0.25 ± 0.50	0.06	–	–	–	–	–	–
Predators									
Anatonchus	4	2.25 ± 2.87	0.58	1.75 ± 3.50	0.43	3.75 ± 2.87	0.67	4.00 ± 2.16	0.62
Clarkus	4	8.00 ± 2.16	2.06	8.75 ± 4.11	2.17	7.50 ± 4.43	1.34	8.50 ± 2.65	1.31
Mononchus	4	1.00 ± 1.41	0.26	0.75 ± 0.50	0.19	2.75 ± 4.27	0.49	1.75 ± 0.96	0.27
Mylonchulus	4	4.00 ± 3.46	1.03	5.75 ± 7.23	1.43	–	–	–	–
Nygolaimus	5	3.25 ± 2.75	0.84	1.00 ± 2.00	0.25	0.75 ± 1.50	0.13	–	–
Prionchulus	4	8.25 ± 8.26	2.12	5.25 ± 6.40	1.30	–	–	–	–
Tripyla	3	18.50 ± 9.75	4.76	0.25 ± 0.50	0.06	–	–	–	–
Plant feeding									
Bitylenchus	2	1.25 ± 1.26	0.32	6.00 ± 6.68	1.46	2.25 ± 2.06	0.40	6.25 ± 9.91	0.96
Criconema	3	–	–	–	–	3.75 ± 6.85	0.67	–	–
Geocenamus	3	–	–	–	–	–	–	1.00 ± 2.00	0.15
Helicotylenchus	3	62.25 ± 29.71	16.00	85.50 ± 85.36	21.22	124.75 ± 85.20	22.22	47.25 ± 25.25	7.29
Heterodera	3	0.50 ± 0.58	0.13	0.75 ± 0.96	0.19	3.00 ± 5.35	0.53	–	–
Merlinius	3	1.00 ± 2.00	0.26	0.25 ± 0.50	0.06	0.50 ± 1.00	0.09	–	–
Paratylenchus	2	7.50 ± 1.29	1.93	31.00 ± 25.94	7.69	68.50 ± 49.63	12.20	66.50 ± 38.73	10.26
Pratylenchus	3	11.00 ± 7.75	2.83	29.25 ± 20.39	7.26	19.25 ± 10.28	3.43	23.50 ± 28.34	3.63
Rotylenchulus	3	4.25 ± 8.50	1.09	–	–	–	–	–	–
Rotylenchus	3	–	0.00	0.75 ± 0.96	0.19	29.25 ± 45.04	5.21	10.75 ± 12.23	1.66
Trophurus	3	–	0.00	0.50 ± 0.58	0.12	–	–	–	–
Tylenchorhynchus	3	1.25 ± 1.89	0.32	–	–	1.50 ± 3.00	0.27	1.50 ± 3.00	0.23
Root-fungal feeders									
Aglenchus	2	13.50 ± 9.54	3.47	5.25 ± 4.79	1.30	4.00 ± 3.92	0.71	5.50 ± 3.87	0.85
Boleodorus	2	12.00 ± 15.38	3.08	0.25 ± 0.50	0.06	21.50 ± 15.02	3.83	3.00 ± 6.00	0.46
Coslenchus	2	0.25 ± 0.50	0.06	–	–	–	–	–	–
Filenchus	2	3.25 ± 1.26	0.84	0.25 ± 0.50	0.06	0.25 ± 0.50	0.04	–	–
Malenchus	2	12.25 ± 23.19	3.15	4.75 ± 3.59	1.18	0.75 ± 1.5	0.13	2.25 ± 3.20	0.35
Psilenchus	2	–	–	0.25 ± 0.50	0.06	–	–	–	–
Tylenchus	2	1.00 ± 0.82	0.26	1.50 ± 2.38	0.37	7.25 ± 7.85	1.29	12.00 ± 11.17	1.85
Number of genera		51		45		44		37	
Abundance per sample[a]		389		403		561.5		648	

D% dominance

[a] Number of total abundance divided by number of samples (4) at each sampling sites

curring at sites A, B and C. The abundance of *Helicotylenchus* increased depending on the distance from factory and subsequently decreased at the most distant site D. *Paratylenchus* was found as another very common genus with the highest dominance (12.2%) at site C. Analogous to *Helicotylenchus*, the abundance of *Paratylenchus* nematodes increased from site A to C. The genera *Monhystera*, *Plectus*, *Aphelenchoides*, *Aphelenchus*, *Aporcelaimellus*, *Dorylaimus*, *Eudorylaimus*, *Pratylenchus* and *Rotylenchus* were classified as dominant, most often at only one or two sampling sites and *Acrobeloides*, *Cephalobus* and *Rhabditis* were eudominant at only one site. Their proportion within total amount of nematode genera varied without any dependence on the distance of sampling sites from the pollution source. Totally 31 nematode genera occurred sporadically and each contributed less than 1% of individuals to nematode abundance in the studied area (Table 2). Contrary to number of genera, the abundance showed the opposite trend. The lowest mean abundance was stated at site A—389 individuals per 100 g and was increasing toward site D—648 nematode individuals per 100 g.

Trophic groups and nematode community structure

Among nematode trophic groups, the most abundant were bacteriovorous and plant feeding nematodes, involving approximately 55% to 67% (depending on the site) of the total nematode fauna (Table 3). The proportion of bacteriovores was decreasing from site A to C but increased again in the most distant site D. The relatively low abundance of bacteriovores at site C compared to sites A and D was found being significant ($P <$ 0.05). The proportion of plant-feeding nematodes, on the other hand, increased from site A to C and was repeatedly diminished at site D (Table 3). The occurrence of omnivorous nematodes, representing the third most frequent trophic group, was almost equal at all four sampling sites. The proportion of fungal feeders increased from sites A to D, but root-fungal feeders and predators, contrary to fungal feeders, showed decreasing trend with the increasing distance from the works. Whilst differences among proportion of root-fungal feeders were not significant in individual sampling sites ($P > 0.05$), variations in abundance of

Table 3 Percentage of individual nematode trophic groups and average indices values (±s.d.) calculated for each sampling site

Throphic group (%), index	Sites			
	A	B	C	D
Bacterial feeders	32.10 ± 5.40a	30.70 ± 3.40ab	19.00 ± 5.80b	42.00 ± 16.20a
Fungal feeders	9.10 ± 1.90ab	5.50 ± 2.00b	13.30 ± 6.00a	15.60 ± 6.40a
Omnivores	13.30 ± 4.90a	17.90 ± 6.00a	15.20 ± 2.50a	11.60 ± 4.80a
Predators	11.30 ± 4.30a	8.40 ± 7.00ab	3.20 ± 2.10b	2.40 ± 1.00b
Plant feeders	23.10 ± 8.90b	33.40 ± 12.90ab	42.10 ± 10.80a	24.70 ± 10.00b
Root-fungal feeders	11.10 ± 11.20a	4.00 ± 3.90a	7.20 ± 4.90a	3.90 ± 2.30a
Maturity index	2.65 ± 0.22ab	2.85 ± 0.17a	2.85 ± 0.09a	2.42 ± 0.21b
Plant parasitic index	2.63 ± 0.29a	2.65 ± 0.09a	2.61 ± 0.09a	2.45 ± 0.19a
MI2–5	3.20 ± 0.11a	3.15 ± 0.22ab	2.93 ± 0.09b	2.61 ± 0.12c
PPI/MI	0.99 ± 0.04a	0.93 ± 0.09a	0.92 ± 0.06a	1.02 ± 0.15a
B/F	3.70 ± 1.23ab	6.20 ± 2.21a	1.81 ± 1.21b	3.61 ± 3.28ab
Genera richness	34.25 ± 6.25a	28.50 ± 3.20a	29.75 ± 2.04a	29.25 ± 1.08a
Shannon–Weaver index	1.44 ± 0.06a	1.35 ± 0.12a	1.30 ± 0.12a	1.29 ± 0.18a
Simpson index	0.27 ± 0.02a	0.30 ± 0.04a	0.33 ± 0.05a	0.33 ± 0.10a
Mean abundance	389.00 ± 53.40a	403.00 ± 230.20a	561.50 ± 218.70a	648.00 ± 222.40a

Means followed by the same letters on the same rows are not statistically different according to Least Significant Difference Test ($P = 0.05$)

predacious and fungivorous nematodes were significant ($P < 0.05$; Table 3).

Significant correlations were recognized among concentrations of several heavy metals and nematode trophic groups (Table 4). Trophic groups could be divided into several groups depending on the effects of heavy metals. Into the first group with only negative effect belong bacteriovorous nematodes with their negative correlation with Cu ($P < 0.05$). Similar one-way influence of heavy metals, but with positive effect, was observed among trophic group of root-fungal feeders and surprisingly predators with Pb, Zn ($P < 0.05$) and Cd ($P < 0.01$). Mixed effects on trophic group have Cr with positive and Ni with negative influence on fungal feeders ($P < 0.05$). In the last group with neutral respond to different loads of heavy metals in the soil among studied trophic groups were plant feeders and omnivorous nematodes.

Individual nematode community structure was characterized by ecological and diversity indices (Table 3). PPI and PPI/MI ratio values found within four sampling sites were not significant ($P > 0.05$), but the decreasing tendency of MI and MI2–5 indices from sites A to D was significant ($P < 0.05$). Due to a low proportion of fungivorous nematodes, the highest B/F ratio was calculated at site B and the difference between this site and site C, where the lowest B/F ratio was recorded, was statistically significant ($P < 0.05$). The values of Richness (S) and Shannon–Weaver index (H') showed the non-significant decreasing trend along a decreasing pollution gradient. Contrary to that, the Simpson index (λ) slightly increased depending on the distance from the pollution source (Table 3).

Statistical analyses between indices and contents of heavy metals show also some interesting correlation. Most of the correlations were positive as it is in case of MI2–5 with all of metals except Cr and Cu. Same positive correlation was observed between PPI and Cd ($P < 0.05$). Only one negative correlation was found. It was between MI and Cr ($P < 0.01$), where Cr is one of the main elements origin from the emissions (Fargašová 2009). Decomposition of organic matter resp. B/F ratio, according to the results, was influenced mainly by nickel and its concentration in the soil ($P < 0.05$). From diversity indices used in this study, only H' responds to different contents of heavy metals at sites, where the positive correlation ($P < 0.05$) was observed between H' and content of Zn in the soil. Other indices did not exhibit any significant correlation with respect to altering environment conditions and the presence of heavy metals in soils.

Table 4 Spearman's correlation coefficient between individual trophic groups of nematodes, ecological indices, Cox, total Nitrogen and contents of heavy metals in the soil

Trophic groups/Indices	As	Cd	Cr	Cu	Ni	Pb	Zn	C_{ox}	N
Bacterial feeders	−0.218	−0.079	0.435	−0.615*	0.303	−0.212	−0.05	0.748**	0.474
Fungal feeders	0.053	−0.405	0.597*	0.05	−0.615*	0.215	0.147	0.084	0.262
Omnivores	0.029	0.152	−0.382	0.194	0.229	0.118	0.012	−0.278	−0.268
Predators	0.426	0.634**	−0.218	0.35	0.497	0.526*	0.591*	0.028	0.271
Plant feeders	0	−0.127	−0.409	0.276	−0.226	−0.282	−0.315	−0.485	−0.588*
Root-fungal feeders	0.385	0.141	0.115	0.397	−0.121	0.5*	0.518*	−0.214	0.338
Maturity index	0.118	0.25	−0.632**	0.509*	0.165	0.153	0.182	−0.667*	−0.444
Plant parasitic index	0.271	0.5*	−0.432	0.309	0.129	0.209	0.097	−0.184	−0.159
MI2–5	0.559*	0.843**	−0.453	0.444	0.644**	0.562*	0.559*	−0.196	0.203
PPI/MI	0.085	0.05	0.238	−0.253	−0.071	−0.094	−0.1	0.37	0.226
B/F	−0.182	0.21	−0.388	−0.3	0.544*	−0.324	−0.262	0.138	−0.147
Genera richness	0.425	0.208	0.272	0.169	−0.162	0.262	0.221	0.304	0.318
Shannon–Weaver index	0.353	0.459	−0.044	0.218	0.371	0.482	0.529*	0.091	0.3
Simpson index	−0.121	−0.328	−0.1	−0.05	−0.362	−0.371	−0.356	−0.287	−0.335
Abundance	−0.203	−0.468	0.271	−0.141	−0.526*	−0.312	−0.397	0.162	−0.147

$*P < 0.05$ and $**P < 0.01$, $n = 16$

Discussion

The aerial deposition coming from manufacturing works caused heavy metal contamination of different level in investigated soils. The highest concentrations of heavy metals were found mainly at site A (the site closest to the factory) and the lowest at site D (the most far site). The pollutants are spreading to a certain distance from the factory, where they deposit and accumulate in soil (Viard et al. 2004; Han et al. 2009). The further dispersion of contaminants depends mostly on local conditions, like climatic factors (wind direction, rainfall), landscape patterns (topography, altitude) and the industrialization of the area (Promeyrat 2001).

According to Slovak soil limits for heavy metals (Ministry Decree No. 531/1994–540), the content of almost all trace elements investigated in the present study did not exceed the permitted limits. Only the content of Cd from site A surpassed the allowable threshold 0.3 mg.kg for uncontaminated soils, but it subsequently decreased with the distance from the factory, similar to As, Cu, Ni, Pb and Zn.

At site A, 51 genera and the 389 nematode individuals per 100 g of soil were found, while only 37 genera but as many as 648 individuals per 100 g of soil were identified at site D (Table 2). This nematode distribution most probably resulted from different condition like distribution of heavy metals, content of nitrogen or C_{ox} in individual sites. At site A, the highest concentrations of almost all metals except Cr were detected, which has the highest concentration at site D. At this site, the nematode community was mainly composed of species insensitive to disturbances (e.g. bacterial feeders of the family Rhabditidae: *Acrobeloides*, *Cephalobus*, *Eucephalobus*), as also stated Bardgett et al. (1994), Yeates et al. (1994) and Georgieva et al. (2002). Most abundant nematode genera identified from surroundings of manufacturing works Široká represented feeding groups of bacteriovorous and plant feeding nematodes, of them namely genera *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Rotylenchus*. These genera were found as dominant or even eudominant in studies of Valocká and Sabová (1997), Liang et al. (2006) and Tomar et al. (2009) as well. The high abundance could be related with availability of food resource—vegetation (Yeates et al. 1994; Kortals et al. 1998). Bacteriovorous nematodes belonging to genera *Acrobeloides*, *Cephalobus*, *Monhystera*, *Plectus* and *Rhabditis* were found being also relatively abundant in our study. Generally, bacteriovores are considered as species insensitive or resistant to various disturbances of environment or even being able to increase their number under changed conditions (Weiss and Larink 1991; Nagy et al. 2004).

Distribution of soil nematodes within six trophic groups reflecting their food-web relations helps to imagine the trophic structure inside nematode community. Analysis of nematode trophic groups often demonstrated their relationships with heavy metals (Li et al. 2006; Han et al. 2009). Studies of Georgieva et al. (2002) or Tomar et al. (2009) showed positive correlation between the abundance of some genera of bacteriovorous (*Eucephalobus*, *Acrobeloides*) and fungivorous (*Aphelenchoides*) nematodes and heavy metals concentrations in the soil. In present study, the bacteriovores were most abundant at the closest site A and also at site D were the highest content of Cr was found. But in case of Cu, significant negative correlation ($P < 0.05$) was proved. Similar phenomenon was also observed by Zhang et al. (2007). Number of fungivores showed significant negative correlation with Ni content ($P < 0.05$) but positive correlation with Cr ($P < 0.05$), which was followed, from sites A to D by a slight rise in fungivorous nematode proportion in the community. These findings support results by Zibilske and Wagner (1982) and Nagy et al. (2004), who found that higher concentrations of heavy metals can initiate a greater production of a fungal and bacterial biomass. This could be due to degradation of sensitive fauna and flora soil communities, which can provide higher intake of organic matter in the soil ecosystem and add more nutrition resources to the food webs for decomposers (microbes, fungi, etc.). Together with the possibility of higher microbes and fungi resistance against pollutants introduced in to the soil they could support indirectly higher organisms, which feed on this source of food (e.g.

bacteriovorous, fungivorous or omnivorous nematodes). We can explain this phenomena also from life history point of view—a relatively high reproduction capacity, genetic variability and ability for rapid recovery of the most abundant bacteriovorous genera (e.g. *Acrobeloides*, *Cephalobus*, *Rhabditis*) belonging to lower *c–p* groups. This allows them to select through relatively short time period populations, which are less sensitive to changes in environment and prosper under inconvenient conditions.

Plant feeding nematodes were abundant at all sites, but the highest abundance and number of genera occurred at site C. They did not exhibit significant correlation with any of traced metals, which might mean that plant feeders are affected by vegetation rather than contents of heavy metals in the soil. Vegetation as a nutrition source is generally sensitive to heavy metals (Banašová and Lackovičová 2004), and in this way, the heavy metal impact on plant feeding nematodes could be indirect, through a loss of primary production (shift from C fixation—photosynthesis to C respiration) and organic matter (decomposition) (Yeates et al. 1994). Also, high contents of nitrogen in the soil could suppress the plant feeding nematodes (Renčo et al. 2009). In our study, samples taken from sites A and D have the lowest proportion of plant feeding nematodes among all monitoring sites that may confirm this assumption.

Predacious nematodes were the only feeding group that answered rather sensitively but surprisingly in the positive way to higher concentrations of Cd, Zn and Pb in the soil. Their abundance was the lowest in comparison with other trophic groups and the number diminished with the distance from sites A to D, what could be cause by more factors. One possibility could be increasing concentration of Cr, but its low level was not high enough to cause statically significant correlation itself, so it probably affect the community together with other factors (nitrogen, pH, etc.). The lowest abundance of predators under conditions with a high concentration of heavy metals reported also Zhang et al. (2007) and Korthals et al. (1996a, b).

Relatively high proportions without any correlation with heavy metals showed the trophic group of omnivorous nematodes (*Aporcelaimellus*,*Dorylaimus*,*Eudorylaimus*). Their abundance was surprisingly relatively constant, oscillating around 15% at all sites, even if this trophic group usually respond to heavy metal or other disturbing stress by decreasing in their abundance or disappearing from the samples (Zullini and Peretti 1986; Georgieva et al. 2002; Tomar et al. 2009).

Useful tools, which are widely used for assessing the intensity of deterioration of soil are ecological and diversity indices (Porazinska et al. 1999; Valocká and Sabová 1997; Urzelai et al. 2000; Zhang et al. 2007) as indicators of ecosystem quality (e.g. diversity, stability and resilience) and important processes (decomposition, energy flow and nutrient cycles) in ecosystem. There were used several indices for characterization of soil ecosystem and nematode communities in our study. Not all of the used indices, however, could reflect changes in the communities.

We presumed that with increasing distance from the pollution source, the deterioration of soil ecosystem and nematode community structure will decrease and ecosystem complexity, generally associated with stability (Urzelai et al. 2000) and more complex relationships in food webs, will increase. Then, stability of ecosystem could be characterized also from diversity point of view. For assessing diversity, we used three different indices—Richness, Shannon–Weaver index and Simpson index. Richness (S) reflects biodiversity of soil habitat and ecological resilience (Peterson et al. 1998). There was found no significant differences ($P > 0.05$) in number of genera among particular sites and no significant correlation with any of traced heavy metals. This could be explain by incapability to reflect changes in the structure of nematode communities through S, what makes this index insensitive to changes in community structure and inappropriate for environmental assessment (Porazinska et al. 1999). Reason of insensibility could be theoretically in the relative long time of exposition to heavy metals or other stressors during which, the ecological functions and ecological niches occupied by original extinct genera were transposed by ecologically similar but less sensitive genera.

Shannon–Weaver index (H') differs in its characterization of sites from Simpson diversity index (λ). According to H', site with the highest diversity was site A and with the lowest diversity was site D. According to λ, the value for diversity has the exact opposite trend, with the highest value at sites D and C and with the lowest value for diversity at site A. This discrepancy has possibly its origin in different approaches of calculating the diversity, where Shannon–Weaver diversity index is more sensitive to rare taxa in the ecosystem but Simpson index is more sensitive to common taxa (Neher 2001), what could indicate differences in the genera composition between sites.

The bacteriovorous-to-fungivorous nematode ratio (B/F) is known to be an important indicator of the decomposition pathway of organic matter in the soil food webs (Yeates 2003). Values of this ratio suggest that organic matter was turnover by decomposers (fungi, bacteria), which proportionally correspond with the abundance of the above trophic groups (Wardle and Lavelle 1997). High values of the ratio indicate higher contribution of bacteria to the decomposition and relatively quick breakdown of available organic matter in the soil (Wasilewska 1997). Decreasing trend of the ratio indicates relatively slower rate of organic matter turnover due to fungal pathway of decomposition (Porazinska et al. 1999). The lowest values in our study were found at site C. Besides this, there was found significant positive correlation ($P < 0.05$) between B/F and concentration of Ni, what could be cause by different influence of Ni on assemblages of fungivorous and bacteriovorous nematodes or their food source. Because the concentration did not exceed threshold limits, we could assume that in this study there was rather positive effect of Ni than negative.

As a useful tool for assessing metallurgical-industry pollution in the soil, Maturity Index and indices derived from it, based on the *c*–*p* groups, were applied in our study. It seems that MI indices offer better possibility to reflect and sufficiently illustrating changes in the soil environment (Bongers 1990; Yeates 1994). In contrast with measures of diversity, which include only quantitative aspects of ecosystems, maturity indices contain in addition except quantitative also biological–ecological aspects of the individual nematode genera of a community. The *c*–*p* scale corresponds to *r*–*K* strategy, where fundamental *r*-strategists are presented as colonizers or opportunists and are assign to *c*–*p* 1 group. Genera, with life history similar to *K*-strategists or persisters belong to *c*–*p* group with value 5. Groups among these two extremes have features, which are continuously changing along the *c*–*p* scale, from colonizers to persisters as the value of groups is raising from 1 to 5 (Neher 2001).

Ecosystem is expressed using the following indices: Maturity Index—MI (Bongers 1990), Maturity Index (2–5), Plant Parasite Index—PPI, and the PPI/MI ratio. Whilst, PPI and PPI/MI ratio did not show any significant correlation between sites along downwind transect, MI2–5 index continuously decreased with increasing distance from pollution source and reached together with MI the lowest value at site D. The decreasing trend is connected with different proportion of opportunistic nematodes and persisters at sites and negative correlation of MI ($P < 0.01$) with the content of C_{ox} as a source of organic matter and increasing concentration of Cr. These two facts together cause probably the lowest MI and MI2–5 values at site D among all sites. Similar respond under enriched condition described Bongers (1990) and decrease after Cr application was experimentally proved by Nagy (1999) and Nagy et al. (2004). Similar decreasing trend was observed also in the case of PPI. Although, the decreasing was not significant, it was cause probably due to two possible different way of stress. The first stress could be the loads of heavy metals in the soil and their negative impact on vegetation (Yeates et al. 1994) and the second could be relative high levels of nitrogen in the soil, which has negative influence on plant feeders and enhanced their suppression (Renčo et al. 2009). According to these results, site D was evaluated as the most disturbed site among all. Although, the content of heavy metals was relatively low in the investigated area, significant changes in nematode communities were registered. Number of nematode genera, proportion of different trophic groups and ecological indices were influenced in both, positive and negative way with respect to the distance from the pollution source and concentration of individual heavy metals. Index MI sensitively responded to changeable concentrations of Cr. It is supposed that higher heavy metal load will evoke more conspicuous adverse effect on soil nematode communities. But as showed this study and studies of other authors dealing with this actual environmental problem, variations in nematode communities indicate even small changes in the environment and could be used as bioindicators and thus prevent further ecosystem degradation.

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