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Influence of industrial heavy metal pollution on soil free-living nematode population

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Trophic structure and sex ratio of soil nematode population are sensitive tools for monitoring industrial pollution.

Abstract

The effect of distance from a heavy metal pollution source on the soil nematode community (trophic structure, sex structure, and taxa composition) was investigated along a 15-km transect originating at the Almalyk Industrial Complex, Uzbekistan (pollution source). The soil nematode community was exposed to heavy metal influence both directly and through soil properties changes. Pollution effect on the density and biomass of soil free-living nematodes was found to be highest at pollution source, with fungivores and plant parasites dominating at the upper and deeper soil layers next to the pollution source. These groups decreased along the transect, yielding domination to bacteria- and fungi-feeders. The sex ratio of nematode communities was found to be dependent on heavy metal pollution levels, with the juveniles being the most sensitive nematode group. The Maturity and modified Maturity Indices, reflecting the degree of disturbance of the soil ecosystem, were found to be the most sensitive indices.

Keywords: Heavy metal; Soil; Nematode; Diversity

1. Introduction

Soil free-living nematode communities in general, and their structural changes in particular, have been found to be among the best biological tools for assessing soil disturbances in terrestrial systems (Gupta and Yeates, 1997; Neher et al., 1998), including heavy metal pollution (Bongers et al., 2001; Georgieva et al., 2002). According to Korthals et al. (1996b), Kammenga et al. (2000) and Ellis et al. (2002), Cu, Pb, Cd, and Zn are among the most common heavy metals found to have an ecological effect on biological activity. Moreover, it is known that in addition to influencing total abundance and number of taxa, heavy metals have a negative effect on trophic group composition (Korthals et al., 1996b, 1998). Bongers and Bongers (1998) and Georgieva et al. (2002) have found that the omnivore-predator nematodes (K-strategist; suborders Mononchina and Dorylaimina) are among the trophic groups most sensitive to different disturbances in ecosystems, including heavy metals.

According to Anderson et al. (2001), heavy metals can affect the reproduction, sex ratio, survival, and development of juveniles (van Straalen and van Gestel, 1993), and *Aphelenchus* juveniles exposed to heavy metals were found to be more sensitive than adult nematodes (Camargo et al., 1998).

Numerous researches have demonstrated the use of multiple ecological indices, such as trophic diversity, genus dominance, Shannon, Maturity, Evenness, and Richness Indices, as useful tools for the assessment of changes occurring in nematode assemblages under environmental disturbances (Yeates and Bird, 1994; Wasilewska, 1997; Pen-Mouratov et al., 2004). Furthermore, it is believed that the Maturity Index is a one-sided mean of individual colonizer–persisters (CP), representing different life strategies and ecological requirements as a promising index of soil biological health (Bongers, 1990; Bongers et al.,

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1997). Different opinions exist regarding the use of the Maturity Index for the assessment of the influence of heavy metals on the soil free-living nematode population. Nagy (Nagy, 1999; Nagy et al., 2004) found a positive response to an increase in heavy metal concentration, while Yeates (2003) reported no significant effect of heavy metals (particularly Cu, Ni and Zn) on the Maturity Index.

Our previous study demonstrated that metal mining and smelting activities negatively influence soil microbial activity (Shukurov et al., 2005).

The aims of the present study were to determine the extent of the effect of the metallurgical industry at the Almalyk Mining and Metallurgical Complex on the structure of the soil free-living nematode population. The Almalyk Mining and Metallurgical Complex (AMMC) is the main source of environmental pollution in this area. This complex is the second largest mining company in Uzbekistan, and is known to be one of the main sources of air pollution, responsible for 13% of all of Uzbekistan's air emissions from stationary sources (UNESCO, 2001).

The metallurgical complex produces chemical elements such as copper, gold, silver, lead concentrate, metal zinc, etc., with an annual cumulative production of some metals, such as Cu, Zn, and Pb, amounting to 130,000, 40,000, and 80,000 t/yr, respectively (Richard, 1999).

We hypothesized the following.

(1) Nematodes at the juvenile stage are more sensitive to industrial pollution.
(2) Trophic diversity and sex ratio, along with the nematode species, are useful tools for examination of ecological condition of industrial area.
(3) The Maturity Index along with other common ecological indices may be very useful for an industrial pollution evaluation.

2. Material and methods

2.1. Study site

The fieldwork of this study was conducted at the southeast part of the Tashkent region of the Republic of Uzbekistan, in the Almalyk Mining and Metallurgical Complex area (40°85′N and 69°69′E) near the city of Almalyk. The Almalyk Mining and Metallurgical Complex was set up in 1949.

The climate in the study area is continental, with a minimal temperature range between −25 and −30 °C in February and maximal temperatures of 42–47 °C in July. The annual rainfall ranges between 100 and 200 mm, with most of the precipitation falling during spring and winter (Information Agency Jahon of the Ministry of Foreign Affairs of the Republic of Uzbekistan, 2003).

The type of soil at the study site along the transect is leptosol with high levels of $CaCO_3$, contributing to a stable accumulation of heavy metals on top of the soil layer (FAO, 2003). The vegetation of the study area mainly consisted of various meadow plants with separate trees belonging to *Salicaceae* and *Ulmaceae* families.

2.2. Sampling

Soil samples were collected from four sites: sampling stations (ST) I, II, III, and IV (0, 5, 10 and 15 km, respectively), along the deposition gradient, beginning at the source of pollution (Almalyk Industrial Complex), and continuing in a downwind direction. A total of 80 soil samples were collected from two soil layers (0–10 and 10–20 cm). The sample plots were set up on the bare soil as far as minimum 15 m from shrubs or trees. The size of sample plots was approximately 4 m^2 (2 m × 2 m^2) each. Ten replicate soil samples, 1 kg each, were taken from the upper (0–10 cm) and deeper (10–20 cm) soil layers of each plot (10 replicates × two layers × four stations) on 25 July 2003. The soil samples were placed in individual plastic bags and transported to the laboratory in an insulated container. They were kept in cold storage at 4 °C and sieved through a 2-mm mesh sieve before biological and chemical analyses.

2.3. Sample analysis

All soil samples were subjected to the following analyses.

1. Soil moisture was determined gravimetrically (105 °C, 48 h).
2. Organic matter was determined by oxidization with dichromate in the presence of H_2SO_4, without application of external heat (Rowell, 1994).
3. Soil pH was determined in H_2O (soil solution ratio 1:2:0) with a potentiometric glass electrode.
4. Soluble cations (Ca^{2+}, Na^{+}, and K^{+}) were determined by a flame photometer (Rhoades, 1982).
5. Heavy metal concentrations were determined using the atomic absorption spectrometry (AAS) method. Subsamples from each sample were air-dried and manually ground using an agate mortar. The metals were extracted by digestion with three parts of concentrated HNO_3 and one part of concentrated $HClO_3$. The concentration was determined using AAS (Zeien and Brummer, 1989; Zeien, 1995).
6. The nematode population was extracted from 100-g aliquots of the soil samples using the Baermann funnel procedure (Cairns, 1960). The recovered organisms were counted and preserved in formalin (Steinberger and Sarig, 1993). A maximum of 100 individuals from every soil sample were identified according to order, family, genus level (if possible) and sex, using a compound microscope. Maximum body width (±1 μm) and 'length' (±5 μm) were also measured in order to determine the biomass of the extracted population using the Andrassy (1956) and Yeates (1972) method. Therefore, the 'length', which is the distance from the lips to the anus plus a conical extension equal in volume to the volume of the tail and biomass, calculated correctly to three places, is given by $(W^2 \times L)/16 \times 100$ and is in microgram when W (maximum body width) and L (body 'length') are in micrometer. Drawings from which 'length' was measured were normally magnified 216×, and width 840×.

2.4. Ecological indices and statistical analysis

The characteristics of the nematode communities were described by means of indices: (1) absolute abundance of individuals per 100 g dry soil; (2) abundance of omnivore-predator (OP), plant parasitic (PP), fungal-feeding (FF) and bacterial-feeding (BF) nematodes (trophic structure) (Steinberger and Loboda, 1991; Steinberger and Sarig, 1993; Pen-Mouratov et al., 2003, 2004); (3) trophic diversity, $T = 1/\Sigma P_i^2$, where P_i is the proportion of the i-th trophic group (Heip et al., 1988); (4) Simpson's dominance index, $\lambda = \Sigma P_i^2$ (Simpson, 1949); (5) Shannon–Weaver Index, $H' = -\Sigma P_i \ (\ln P_i)$, where P is the proportion of individuals in the i-th taxon (Shannon and Weaver, 1949); (6) Maturity Index, $MI = P_i v_i$, where v_i, is the CP (colonizer-persistor) value assigned by Bongers (1990) of the i-th genus in the nematode and P_i is the proportion of the genus in the nematode community. The CP values describe the nematode life strategies, and range from 1 (r-selected or colonizer with short generation times, large population fluctuations, high fecundity and tolerant to disturbance) to 5 (K-selected or persisters, produce few offspring, appear later in succession and sensitive to disturbance); (7) modified maturity index (MMI), including plant-feeding nematodes (Yeates and Bird, 1994); (8) Evenness, $J' = H'/\ln(S)$, where S is the number of taxa; (9) Richness, $SR = (S-1)/\ln(N)$, where S is the number of taxa and N is the number of individuals identified (Yeates and King, 1997); (10) number of males, females, and juveniles of each trophic group; and (11) biomass of males, females, and juveniles of each trophic group (Yeates, 1972, 1979).

The data presented in this study are reported as oven-dried weights. All data were subjected to statistical analysis of variance using the SAS model (GLM, Duncan's multiple range test and Pearson correlation coefficient) and were used to evaluate differences between separate means. GLM, followed by Tukey's HSD test, was performed to establish the significance of differences between plot areas using the statistical package, Statistica 4.3. Differences obtained at levels of $p < 0.05$ were considered significant.

3. Results

3.1. Soil characteristics

The mean soil moisture content ranged between 0.67 and 1.99% (Table 1). Soil moisture was found to be significantly ($p < 0.001$) higher in the deeper (10–20 cm) soil layer than in the upper (0–10 cm) soil layer, with no significant differences between sampling sites I, II, and III (Table 1). Soil moisture at sampling site IV was significantly higher ($p < 0.0001$), reaching values that were 3.5 and 2.5 times higher than in the other sampling sites at the 0–10 and 10–20 cm soil layers, respectively.

Unlike soil moisture content, organic matter content was found to be significantly ($p < 0.001$) higher in the upper (0–10 cm) soil layer than in the deeper (10–20 cm) soil layer along the sampling sites (Table 1). Organic matter content was significantly different, increasing beginning at sampling site II ($p < 0.01$, $n = 80$) (Table 1), and negatively correlated with As and Pb (Table 2).

No significant differences in soil moisture and pH levels were found between the two first sampling sites. The soils were weakly alkaline, with a pH ranging from 8.15 to 8.11 at the two first stations and then slightly decreasing along the transect (Table 1).

The soluble cations Ca^{2+}, Na^{+}, and K^{+} were significantly different between sampling locations, with minimal values next to the source of pollution (Table 1). Sodium and potassium were found to be negatively correlated with As, Cu, Pb, while Ca^{2+} showed a positive correlation with Zn (Table 2).

3.2. Heavy metals

The heavy metal content along the transect (Table 1) was highest near the pollution source (ST I), decreasing along the downwind direction. Contents of As, Cu, and Pb were found to be maximal at ST I, decreasing significantly toward ST II, with no significant difference between the other downwind sampling stations. However, for Cd (Table 1), no significant differences in the level of contamination were obtained for the sampling sites, while Zn (Table 1) was found to have a relatively high value at the first two sampling sites, decreasing significantly toward the last sampling location.

Table 2
Correlation coefficients between soil biological activity and soil conditions along the 15 km downwind transect from Almalyk Mining and Metallurgical Complex (Bold values indicate $p < 0.05$)

	As	Cd	Cu	Pb	Zn
SM	NS	NS	NS	NS	NS
C_{org}	**−0.78***	NS	NS	**−0.71***	NS
pH	NS	NS	NS	NS	NS
Ca^{2+}	NS	NS	NS	NS	**0.60****
K^{+}	**−0.52***	NS	**−0.54***	**−0.58****	NS
Na^{+}	**−0.50***	NS	**−0.52***	**−0.58****	NS

3.3. Nematode community structure

Twenty-nine nematode taxa were identified in the present investigation: 12 taxa belonged to the bacterivore trophic group, five were fungivores, seven were plant parasites and five were omnivores-predators (Table 3). The mean density of the soil free-living nematodes increased with distance from the pollution source in both soil layers (Table 4), negatively correlated with pH and observed heavy metals and positively correlated with C_{org}, Na^{+} and K^{+} (Table 5). Moreover, nematode density in the upper (0–10 cm) soil layer showed a gradual increase in TNEM population between STs I and III, yielding significantly high $R^2 = 0.98$ values (Fig. 1), a value which decreased to a level of $R^2 = 0.69$ with the increase in distance (STs I–IV). In the deeper (10–20 cm) soil layer, the R^2 values obtained for a similar distance were found to be similar ($R^2 = 0.8$) (Fig. 1). The nematode density was higher in the upper soil layer (from 1.2 to 2.0 times at the different stations) than in the deeper soil layer ($p < 0.02$) (Tables 3 and 4).

Table 1
Chemical characteristics of the soil samples

Sampling locations	SM (%)	C_{org} (%)	pH	Ca (mg kg^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
ST I	0.67^{b}	0.41^{b}	8.15^{a}	20.15^{b}	180.60^{c}	4.095^{c}	32.17^{a}	2.84^{a}	1209.25^{a}	249.05^{a}	52.23^{a}
ST II	0.88^{b}	0.89^{a}	8.11^{a}	27.75^{a}	412.35^{b}	42.73^{a}	17.15^{b}	2.47^{ba}	202.50^{b}	73.93^{b}	44.00^{ab}
ST III	0.89^{b}	0.98^{a}	7.91^{b}	21.82^{b}	486.20^{a}	49.33^{a}	10.55^{b}	1.44^{ba}	131.75^{b}	62.98^{b}	19.50^{bc}
ST IV	1.99^{a}	0.87^{a}	7.60^{c}	11.49^{c}	396.60^{b}	32.83^{b}	11.90^{b}	0.26^{b}	93.99^{b}	81.33^{b}	12.18^{c}
GLM data											
F-test	49.7	17.02	22.72	16.76	26.8	33.03	8.68	2.64	5.73	6.98	4.42
p Values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.007	0.18	0.02	0.01	0.04

Different letters indicate significant differences between site sampling, $p < 0.05$, $n = 80$.
ST, station of sampling site.

Table 3
Mean abundance (indices per 100 g dry soil) and standard deviation of nematode genera and trophic groups at different distances of source of industrial pollution

Station	ST I	ST II	ST III	ST IV	ST I	ST II	ST III	ST IV	*p* Value[e]	
	Upper soil layer (0−10 cm)				Deeper soil layer (10−20 cm)				Location	Depth
Trophic groups/genus/family[a]										
TNEM[b]	**70.5 ± 39.3**	**395.4 ± 155.7**	**587.9 ± 227.3**	**488.4 ± 146.9**	**33.7 ± 10.4**	**310.8 ± 136.7**	**340.7 ± 143.2**	**419.8 ± 122.1**		
Bacterivores										
BF[c]	**1**	**28**	**48**	**23**	**2**	**15**	**43**	**40**		
BF′[d]	**4**	**34**	**40**	**23**	**17**	**16**	**42**	**32**		
Acrobeles	0	0	71.7 ± 66.8	0	1.5 ± 1.5	0	9.3 ± 8.9	7.9 ± 7.0	**0.03**	0.23
Acrobeloides	1.4 ± 1.1	3.5 ± 1.9	0	3.5 ± 2.9	0	2.0 ± 1.9	8.4 ± 8.3	4.7 ± 4.5	0.8	0.63
Alaimus	0	3.7 ± 3.1	0	0	0	0	0	0	0.4	0.31
Cephalobus	0	6.0 ± 3.9	5.0 ± 3.8	0	0	6.3 ± 6.1	12.8 ± 9.9	21.5 ± 19.0	0.15	**0.05**
Cervidellus	0	9.6 ± 9.0	4.1 ± 4.0	0	1.1 ± 1.0	4.5 ± 4.3	30.1 ± 28.9	0	**0.004**	0.14
Chiloplacus	1.4 ± 1.3	28.1 ± 24.5	125.5 ± 79.6	75.9 ± 41.9	2.6 ± 1.9	36.1 ± 32.5	70.0 ± 68.6	45.0 ± 36.6	**0.0001**	0.25
Diplogaster	0	0	0	0	0.5 ± 0.5	0	0	0	0.46	0.32
Heterocephalobus	0	28.9 ± 23.0	0	0	0	2.1 ± 1.8	0	3.7 ± 2.9	0.07	0.2
Mesorhabditis	0	34.9 ± 26.4	0	5.6 ± 4.8	0	0	0	15.8 ± 15.3	0.31	0.39
Panagrolaimus	0	0	0	0	0	0	0	3.7 ± 4.3	0.37	0.32
Pelodera	0	15.6 ± 13.1	4.9 ± 4.7	3.4 ± 3.0	0	0	4.3 ± 4.3	0	0.33	0.11
Plectus	0	6.0 ± 5.7	20.7 ± 16.1	23.8 ± 21.9	0	0	8.3 ± 8.0	30.9 ± 29.6	**0.006**	0.6
Fungivores										
FF	**17**	**28**	**42**	**13**	**1**	**78**	**12**	**9**		
FF′	**53**	**16**	**15**	**6**	**5**	**36**	**5**	**3**		
Anguinae	0	0	0	0	0	11.4 ± 9.7	0	0	0.41	0.36
Aphelenchoides	26.3 ± 26.1	34.7 ± 27.1	90.3 ± 81.4	5.7 ± 5.6	0.6 ± 0.5	37.3 ± 32.5	17.0 ± 16.5	9.5 ± 9.4	**0.007**	**0.02**
Aphelenchus	2.5 ± 2.1	3.7 ± 2.8	0	12.4 ± 11.9	0	40.4 ± 38.0	0	3.5 ± 3.2	0.06	0.31
Nothotylenchus	8.1 ± 7.7	23.4 ± 21.0	0	5.4 ± 5.3	0	21.5 ± 18.0	0	0	0.11	0.58
Tylencholaimellus	0.6 ± 0.5	0	0	4.3 ± 3.8	1.0 ± 0.8	0	0	0	0.45	0.48
Plant parasites										
PP	**8**	**40**	**41**	**11**	**5**	**28**	**30**	**37**		
PP′	**42**	**40**	**27**	**9**	**72**	**40**	**39**	**40**		
Filenchus	0.7 ± 0.6	10.1 ± 8.8	31.9 ± 27.1	15.1 ± 12.5	0	7.1 ± 6.3	6.7 ± 6.6	3.6 ± 2.9	**0.05**	0.06
Meloidogyne	2.5 ± 2.3	0	0	0	2.2 ± 1.7	5.1 ± 4.7	0	0	0.29	0.66
Pratylenchus	1.8 ± 1.6	0	17.0 ± 13.8	0	4.1 ± 3.8	15.3 ± 14.0	49.9 ± 49.0	5.6 ± 5.3	**0.003**	0.10
Telotylenchus	7.0 ± 5.9	0	0	0	4.9 ± 4.6	0	0	0	**0.01**	0.97
Tetylenchus	7.6 ± 5.6	49.4 ± 28.4	8.9 ± 5.0	0	2.2 ± 1.9	22.7 ± 19.0	3.9 ± 3.2	0	**0.05**	0.53
Tylenchorhynchus	7.2 ± 6.2	33.3 ± 24.7	43.6 ± 38.6	0	9.9 ± 8.9	9.1 ± 8.1	50.0 ± 47.0	0	**0.002**	0.58
Tylenchus	2.8 ± 4.3	63.4 ± 61.4	56.1 ± 35.5	29.0 ± 27.4	0.9 ± 0.8	63.5 ± 60.4	20.7 ± 20.3	157.7 ± 120.7	**0.004**	0.27
Omnivores-predators										
OP	**0.1**	9.1	**23.8**	**67.0**	**1**	**14**	**27**	**58**		
OP′	**1**	10	**18**	**62**	**6**	**8**	**14**	**25**		
Dorylaimus	0	19.4 ± 19.1	30.7 ± 23.5	143.9 ± 111.4	1.0 ± 0.9	6.3 ± 5.8	24.1 ± 23.3	51.5 ± 47.2	**0.001**	0.19
Eudorylaimus	0.6 ± 0.5	11.4 ± 8.8	49.3 ± 39.5	30.9 ± 28.3	1.1 ± 0.8	17.6 ± 16.0	16.1 ± 15.3	32.6 ± 30.2	**0.006**	0.28
Leptonchus	0	2.9 ± 2.4	0	0	0	0	0	0	0.4	0.28
Mesodorylaimus	0	3.7 ± 3.4	0	96.6 ± 90.7	0	0	0	7.0 ± 6.5	**0.020**	**0.03**
Nygolaimus	0	3.7 ± 2.8	28.3 ± 24.2	33.4 ± 26.1	0	2.5 ± 2.5	9.1 ± 8.8	15.6 ± 11.7	**0.02**	**0.05**

[a] By classification Yeates and King (1997).
[b] TNEM, number of total nematodes.
[c] Comparative changes of abundance of trophic groups on the different distances from the source pollution (%).
[d] Contribution of separated trophic group in trophic composition of observed nematode population (%).
[e] By 'GLM' statistical analysis, where bold values indicate $p < 0.05$.

Table 4
Statistic analysis by 'GLM' for the nematode population along the emission gradient (p values)

Trophic groups/genus/family[a]	Number		Biomass	
	Location	Depth	Location	Depth
TNEM	0.0001	0.02	0.0004	0.01
Trophic and sex structure				
BF	0.0001	NS	0.007	NS
FF	0.02	0.0001	0.004	NS
PP	0.0006	0.002	0.0004	NS
OP	0.0001	0.007	0.0008	0.0009
Total male	0.004	NS	0.03	NS
Total female	0.0001	0.003	0.002	0.008
Total juveniles	0.0001	NS	0.004	NS
Ecological indices				
T	0.0004	NS	–	–
λ	0.04	NS	–	–
H'	0.005	NS	–	–
SR	NS	NS	–	–
MMI	0.0001	NS	–	–
MI	0.0001	NS	–	–

TNEM, number of total nematodes; BF, bacterivores; FF, fungivores; PP, plant parasites; OP, omnivores-predators; T, trophic diversity; λ, genus dominant; H', Shannon–Weaver Index; SR, Richness; MMI, Maturity Index modification; MI, Maturity Index.
Values of $p < 0.05$ were considered significant.
[a] By classification Yeates and King (1997).

3.4. Trophic groups and nematode species

The percentage of each trophic group out of the whole population was found to be affected by the distance from the industrial complex (Fig. 1). Trophic group density increased with distance; however, these increases were not always similar. Bacterivores (BF) and plant parasites (PP) in the deeper soil layer and omnivore-predators (OP) in both soil layers, increased consistently from the pollution source to the edge of the study area, whereas BF, PP and fungivores (FF) in the upper soil layer increased to ST III and then decreased to ST IV (Tables 3 and 4). In contrast, the density of FF in the deeper soil layer increased rapidly to ST II, with a further decrease to the edge (Tables 3 and 4; Fig. 1) of the study area. The BF were negatively dependent on AS, Cu, Pb and Zn and positively dependent on C_{org}, K^+, and Na^+ (Table 5). The PP showed a positive correlation with potassium (Table 5). The OP were positively dependent on SM, C_{org}, K^+, and Na^+ and negatively dependent on pH and Ca^{2+} (Table 5).

Almost all observed nematode species tended to decrease under the load of pollution (Fig. 2a). However, only a few demonstrated indicator properties. Among the bacterivore trophic group, only two (*Chiloplacus* and *Plectus*) were found to be sensitive to industrial pollution (Fig. 2), resulting in an increasing number of individuals for each sample and sampling location from ST I toward ST IV (Table 3). Although GLM statistical analysis indicated significant p values for *Acrobeles* and *Cervidellus* along the emission gradient (Fig. 2), a correlation with sampling location was only found there for the deeper layer ($R^2 = 0.6106$ and $R^2 = 0.8366$, for the two above-mentioned species, respectively). Among the omnivore-predators, only three (*Dorylaimus*, *Eudorymaimus*, and *Nygolaimus*) were closely correlated with distance and demonstrated either direct or indirect (e.g., lack of prey) dependence on industrial pollution, with quantitative changes along the transect (Fig. 2d–f; Table 3). Among the fungivore trophic group, only *Aphelenchoides* were found to be significantly correlated with location (Table 3), decreasing with the decrease in heavy metal content along the soil gradient. However, this regression ($R^2 = 0.8466$) was observed only for the upper soil layer of the first three stations. Among the plant parasite trophic group, only *Pratylenchus*, *Tylenchorhynchus*, and *Tylenchus* were found to be significantly correlated in the two soil layers along the three first stations from the pollution source, where the upper and deeper soil layers accounted for $R^2 = 0.6623$ (0.92), 0.9409 (0.745), and 0.6488 (0.6264), for the three nematode species, respectively, *Telotylenchus*, *Tetylenchus*, and *Filenchus* had high regression ($R^2 = 0.95$), but only in the upper soil layer of the first three stations.

3.5. Ecological indices

The mean trophic diversity values (T) at all observed sites ranged from 0.7 to 0.4, with maximal values at the pollution source (ST I) (Fig. 3A, A′). The severe decrease in T values between ST I and the other three stations was significant ($p < 0.01$), whereas no significant differences were found between the two soil layers along the sampling sites (Table 4).

The calculated indices, such as the Simpson's dominance index (λ), Shannon–Weaver Index (H'), and Richness (SR), were not found to reflect changes in distance from the pollution source (Fig. 3B–E′). However, the MI and MMI exhibited a gradual ($p < 0.05$) increase in the upper soil layer, with increasing distance from the pollution source (Fig. 3F, F′; Table 4).

Table 5
Correlation coefficients between soil biological activity and soil conditions along the 15 km downwind transect from Almalyk Mining and Metallurgical Complex

	SM	C_{org}	pH	Ca^{2+}	K^+	Na^+	As	Cd	Cu	Pb	Zn
TNEM	NS	**0.47****	**−0.23***	NS	**0.41****	**0.36****	**−0.68****	**−0.52***	**−0.60***	**−0.58****	**−0.53″**
BF	NS	**0.46*****	NS	NS	**0.38*****	**0.40*****	**−0.53***	NS	**−0.46***	**−0.50***	**−0.60***
FF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PP	NS	NS	NS	NS	**0.26***	NS	NS	NS	NS	NS	NS
OP	**0.30***	**0.44*****	**−0.40*****	**−0.28***	**0.37****	**0.35****	NS	NS	NS	NS	NS

*, **, *** Correlation coefficients significant at $p < 0.05$, 0.01 and 0.0001, respectively ($n = 80$).

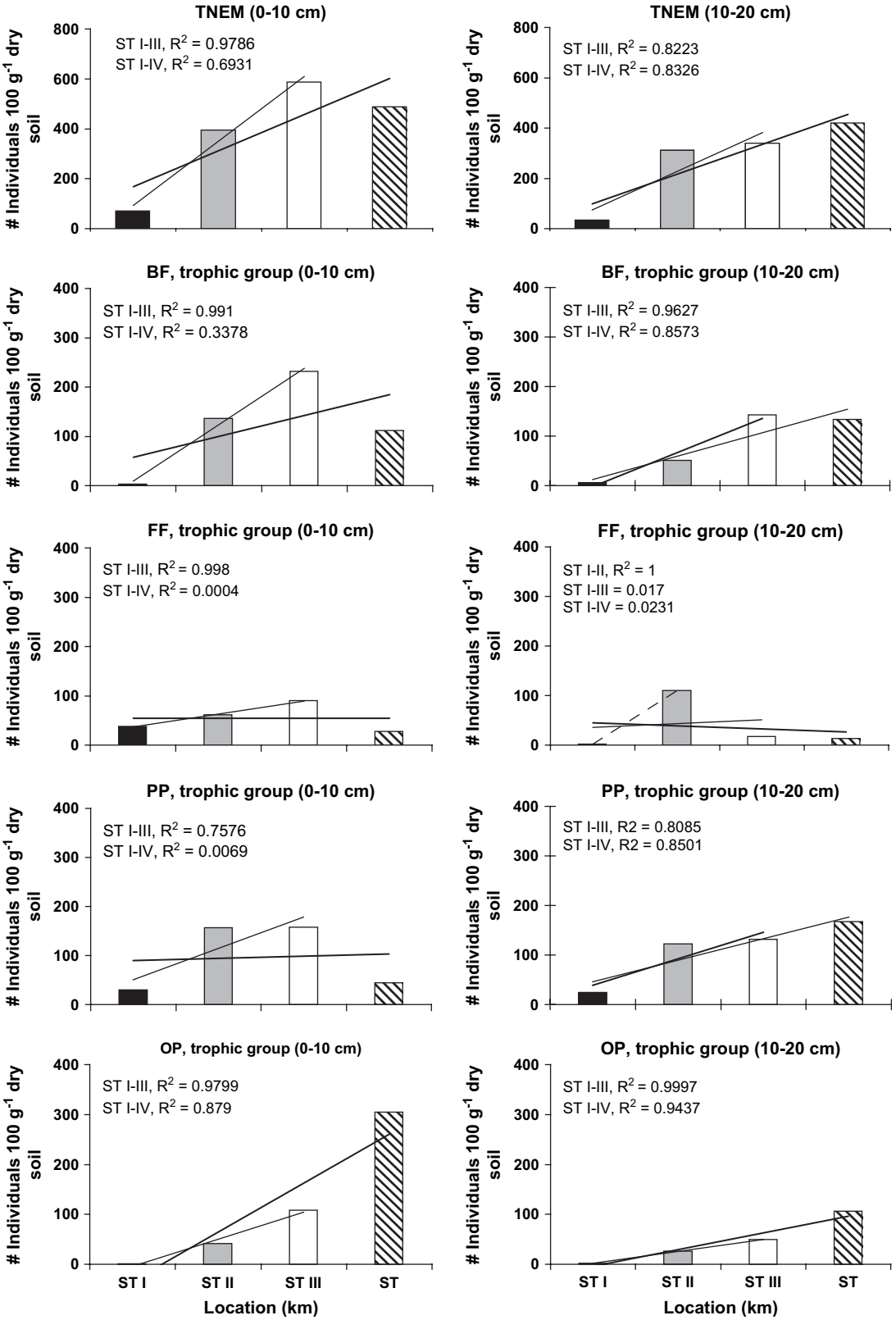

Fig. 1. Changes in the total soil free-living nematodes (TNEM) and their trophic distribution (BF – bacterivore, FF – fungivore, PP – plant parasitic, OP – omnivores-predators) as percentage of the total population along the deposition gradient in two soil layers (0–10 cm and 10–20 cm) at the Almalyk Industrial Site. R^2, regression values between the pollution source station (ST) I and ST III, as well as between ST I and ST IV sampling stations, are represented. Different letters indicate significant differences ($p < 0.05$, $n = 80$).

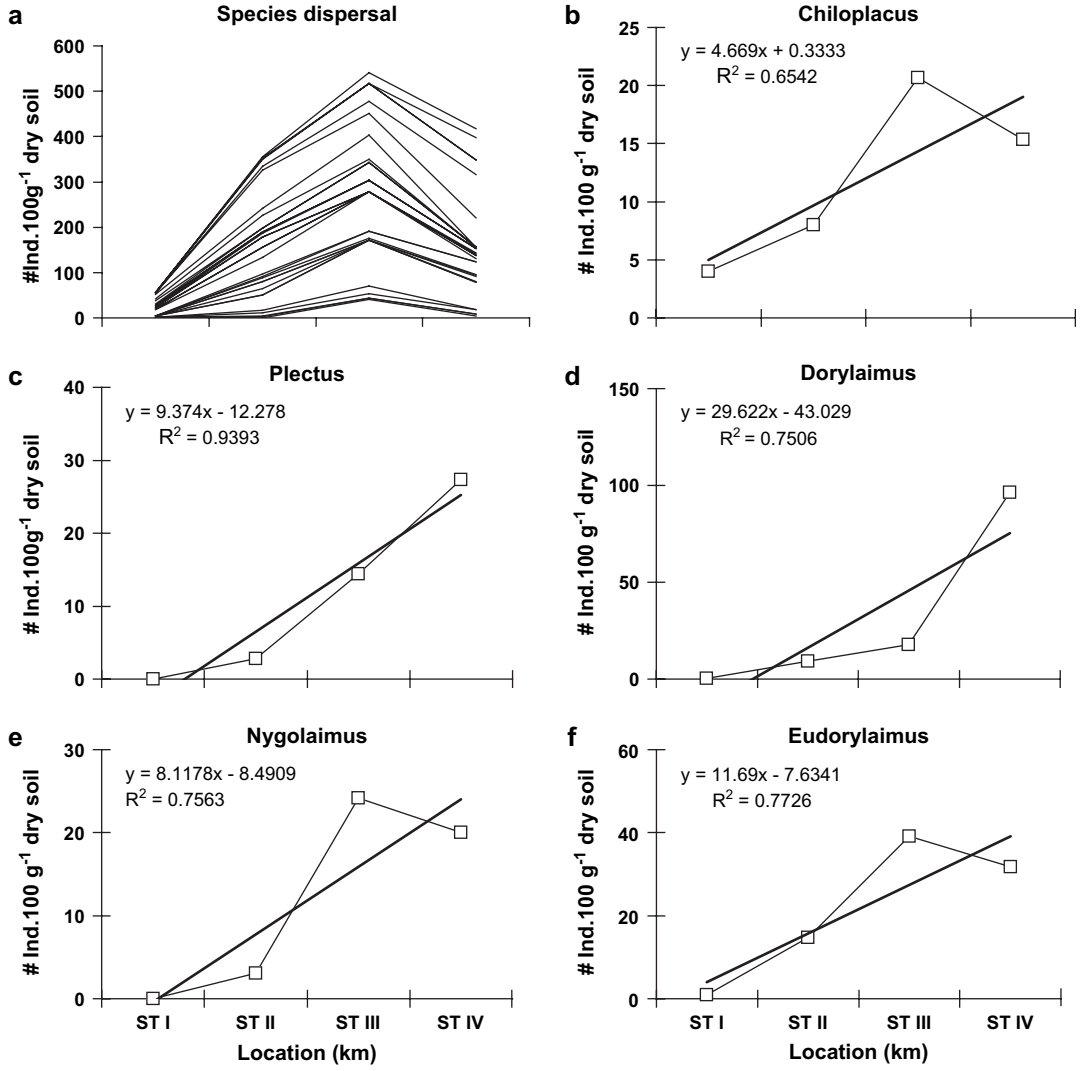

Fig. 2. Changes in mean values of soil free-living nematode species (a) and the five species found to be sensitive to pollution along the deposition gradient in the 0–20 cm soil layer at the Almalyk Industrial Site. R^2, regression values between sampling stations.

3.6. Number of males, females, and juveniles

The mean total numbers of nematode males, females, and juveniles per 100 g dry soil at the observed sites were 57.3, 161.9, and 113.9 individuals, respectively.

Fig. 4 presents the numbers of females, males, and juveniles at each sampling station for both the 0–10 and 10–20-cm soil layers. The total number of all three sex groups of nematodes extracted at ST I (pollution source) was significantly ($p < 0.01$) lower than at the other three locations for both soil layers (Fig. 4; Table 4). The mean values of males and juveniles out of the total population were found to remain unchanged in both soil layers, whereas the female population exhibited a significant depth ($p < 0.003$) effect (Fig. 4; Table 4). The proportion of the three sex groups next to the pollution source was 9:23:1 (male:female:juvenile, respectively). At ST III, this proportion was 1:2:3 and at ST IV it was 1:3:1.5.

3.7. Biomass of soil free-living nematodes

The changes in the total biomass of the soil free-living nematodes (Fig. 5A, A′) exhibited a gradual and significant ($p < 0.01$) increase from 18.3 to 274.9 μg per 100 g dry soil in the upper (0–10 cm) soil layer, with increase in distance from the pollution source. A similar trend was found in the deeper (10–20 cm) soil layer, with a minimum value of 14.2 and a maximal value of 133.3 μg per 100 g dry soil. This was significantly ($p < 0.01$) lower than in the upper soil layer (Table 4). The contribution of the different trophic groups to the total biomass (Fig. 5A, A′) was found to be significantly affected (Table 4) by the sampling site and not by the soil layer, except for the omnivore-predator feeding group.

Fig. 5B, B′ presents the changes in total biomass of males, females, and juveniles along the sampling gradient. Based on the obtained values, the total female biomass (TBf) in the upper (0–10 cm) soil layer was found to be significantly higher

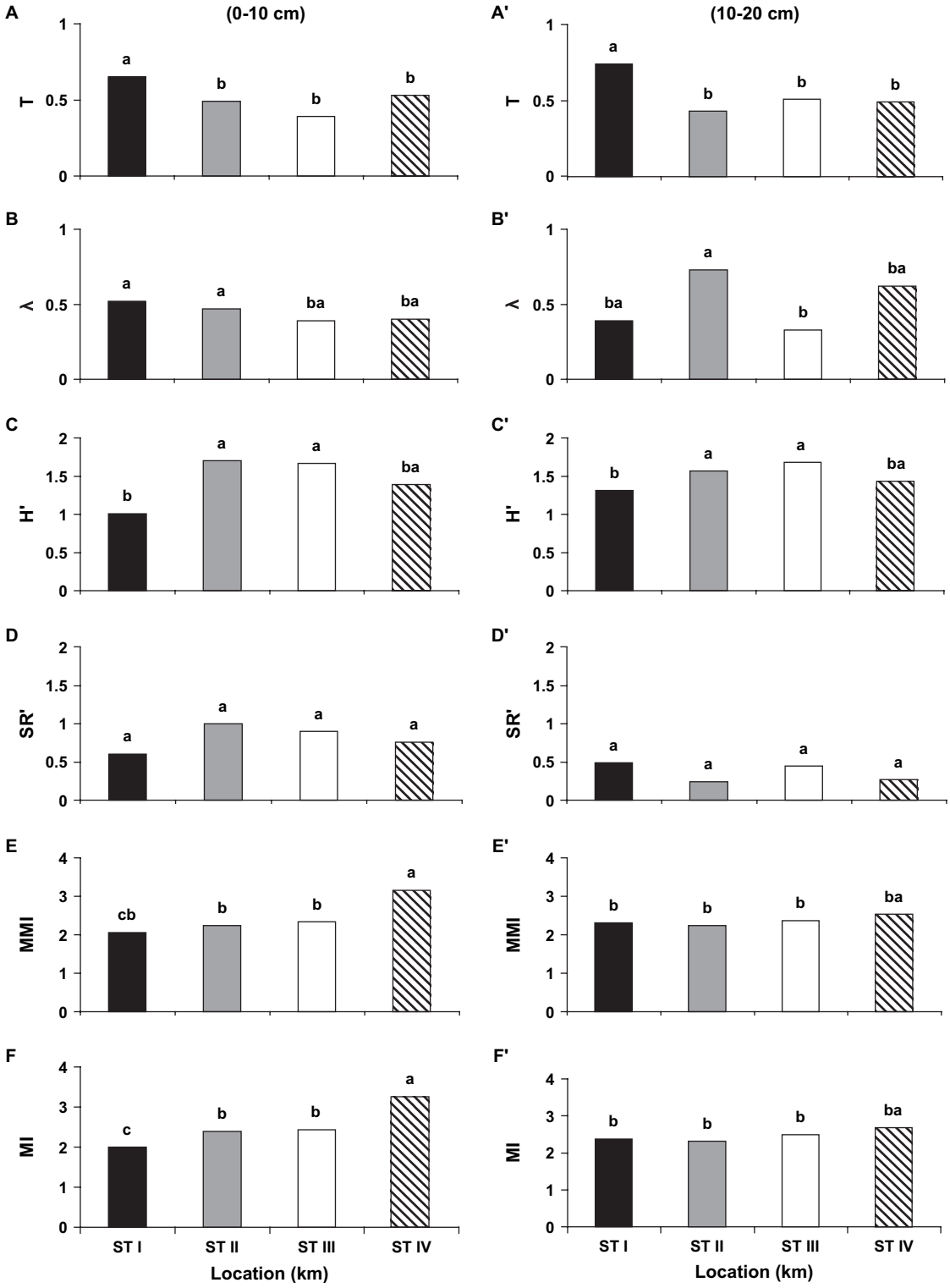

Fig. 3. Variations in soil free-living nematode ecological indices [T – trophic diversity index (A, A′); λ – genus dominance (B, B′); H' – Shannon–Weaver Index (C, C′); SR – Richness (D, D′); MMI – Maturity Index modification (E, E′); and MI – Maturity Index (F, F′)], along the deposition transect in the 0–10 and 10–20 cm soil layers. Different letters indicate significant differences ($p < 0.05$, $n = 80$) using Duncan's multiple range test.

($p < 0.008$) in comparison to the deeper layer (Table 4) and significantly ($p < 0.002$) affected by location (Table 4). The male as well as juvenile biomasses were found to be significantly affected only by sampling sites along the gradient.

4. Discussion and conclusions

Statistical analysis showed no correlation between the population and soil water availability as an independent trigger

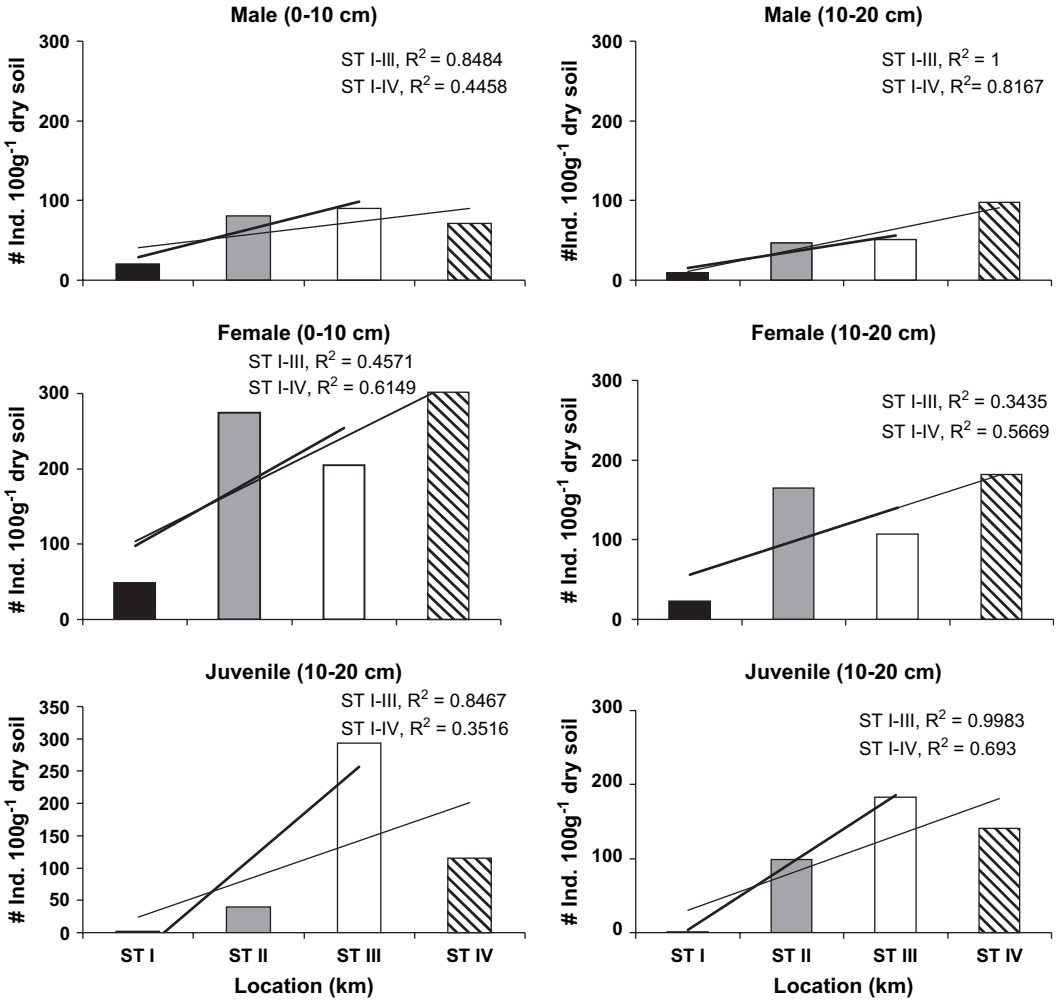

Fig. 4. Changes in the total number of soil free-living nematode sex groups along the deposition gradient in two soil layers (0–10 cm and 10–20 cm) at the Almalyk Industrial Site. R^2, regression values between the pollution source station (ST) I and ST III, as well as between ST I and ST IV sampling stations, are represented. Different letters indicate significant differences ($p < 0.05$).

along the emission gradient, whereas the addition of a heavy metal gradient effect, together with organic matter levels, was found to explain the nematode population response. These results are supported by Lal and Stewart (1992), Vig et al. (2003), and Klumpp et al. (2003), who worked on system health, soil organic matter, plant and soil microbiological properties, heavy metal air pollution environments, and who suggested that soil population densities are correlated with multivariate detrimental ecosystem changes.

Our results, which used food consumption characteristics in order to understand the soil free-living nematode function in the system as a response to heavy metal pollution, indicate that plant parasites, followed by fungi-feeding nematodes, were the most dominant trophic groups at the pollution source, while, with distance, the dominance was replaced by bacteria-feeding and omnivore-predators nematodes. The contribution of BF, FF, PP, and OP trophic groups to the trophic composition of the nematode population near the pollution source accounted for 4, 53, 42, 1 and 17, 5, 72, 6% in the upper and deeper soil layers, respectively (Table 3). The contribution of the observed trophic groups at the end of the study area accounted for 23, 6, 9, 62 and 32, 3, 40, 26% in the upper and deeper soil layers, respectively (Table 3). However, the OP, BF, FF, and PP were found to correlate with the changes in heavy metal content in the soil along the emission transect. Our data are in agreement with other studies (Parmelee et al., 1993; Korthals et al., 1996b), that showed that the addition of Cu, Ni, and Zn up to 1600 mg kg^{-1} significantly affected many parameters of the nematode community structure, such as the populations of certain omnivorous and predatory nematodes with a K-strategist type of life history (Bongers and Bongers, 1998; Bakonyi et al., 2003). Moreover, the populations of several nematode taxa were significantly affected by the concentration of Cu, Ni, and Zn (Korthals et al., 1996b).

By means of lab experiments, Donkin and Williams (1995) learned that the presence of potassium and sodium salts in the medium significantly reduced the toxicity effect of heavy metals on the free-living nematodes. Our results coincided with previous research and showed that in the metallurgical area, the soil free-living nematodes positively correlated with sodium and potassium. On the other hand, sodium and potassium were found to be in a negative correlation with heavy

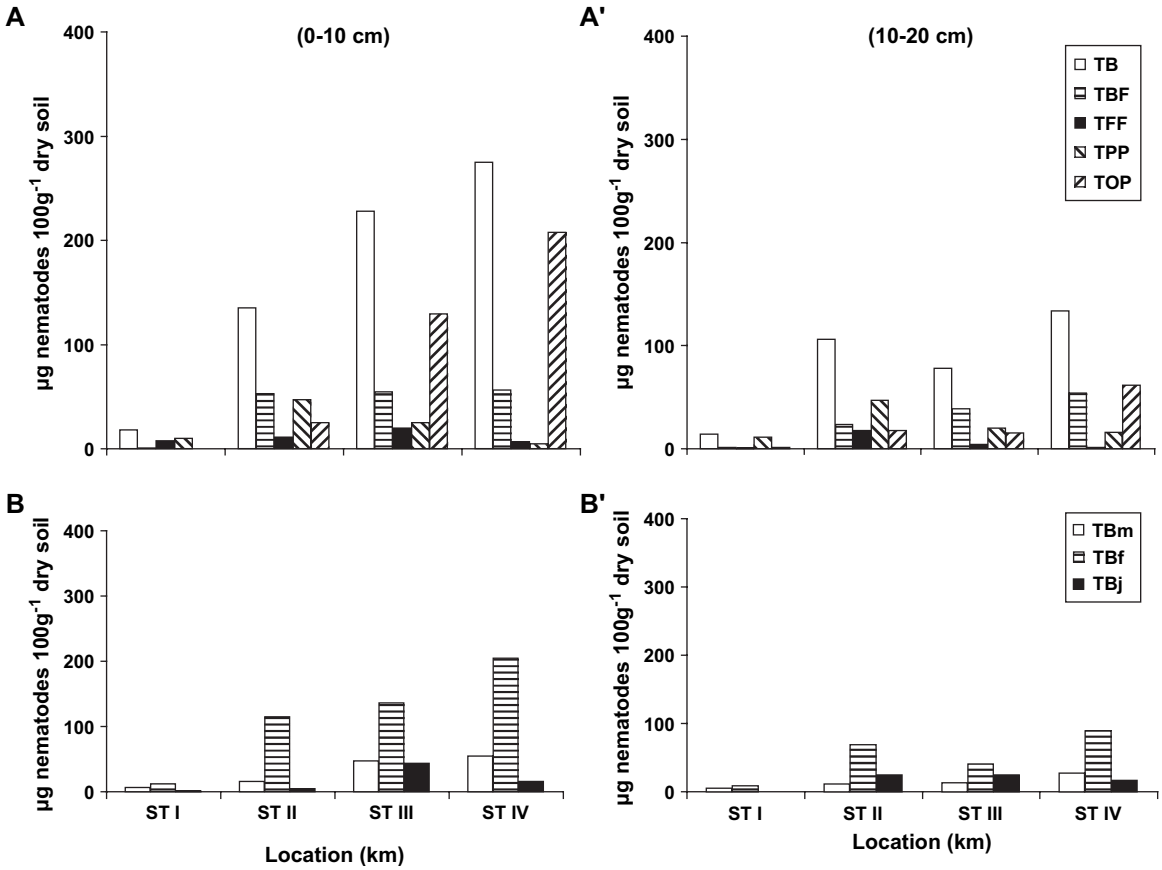

Fig. 5. Changes in total biomass of soil free-living nematodes (A, A′) and the different sex group total biomass (B, B′) along the deposition transect in the 0–10 and 10–20 cm soil layers (TB – total biomass of nematodes, TBF – total biomass of bacterivore trophic group, TFF – total biomass of fungivore trophic group, TPP – total biomass of plant parasites trophic group, TOP – total biomass of omnivores-predators trophic group, TBm – total biomass of males, TBf – total biomass of females, TBj – total biomass of juveniles). Different letters indicate significant differences ($p < 0.05$) using Duncan's multiple range test. R^2, regression values between the pollution source station (ST) I and ST III, as well as between ST I and ST IV sampling stations, are represented.

metals that, in turn, indicated a negative influence of heavy metals on the observed soil cations.

In our study, the Maturity and modified Maturity Indices were found to be a useful tool for assessing metallurgical-industry pollution in soil systems along an air pollution emission gradient. The use of trophic diversity was found to show a mild response to changes along the pollution transect while the Shannon–Weaver, Richness and Evenness Indices were not found to respond numerically to any changes in the study sites.

The MI and MMI values at STs II, III, and IV along the emission gradient (ranging from 2.00 to 3.26) were found to be comparable to the values reported in other studies (ranging from 1.80 to 3.54), as reported by Wasilewska (1994), Yeates and Bird (1994) and Porazinska et al. (1997) for different systems.

Korthals et al. (1996a) and Nagy et al. (2004) showed that fungivore nematodes (dominated by *Aphelenchus* and *Aphelenchoides*) were quite insensitive to most pollutants, including Cu, while *Acrobeles* (Korthals et al., 1996b) appeared to be the most sensitive taxon. In the present study, fungivores, such as *Aphelenchoides*, *Aphelenchus* and *Nothotylenchus* and plant parasites such as *Meloidogyne*, *Telotylenchus*, *Tetylenchus*, *Tylenchorhynchus*, and *Tylenchus* were more numerous near the pollution source than other species, while the majority of bacteria-feeding species, including *Acrobeles*, were relatively rare or completely absent near the pollution source as opposed to the dominant nematode species.

The sex ratio demonstrated significant change along the emission gradient, with juveniles being the most sensitive to industrial pollution. The low number of juvenile nematodes in the present research could be explained using the view of researchers who believe that juvenile nematodes are more sensitive to pollution than are the adults (Kammenga et al., 1996; Camargo et al., 1998), and that the sex proportion changes under the effects of pollution (Anderson et al., 2001); or using the view of researchers (Vranken and Heip, 1986) who believe that nematode reproduction is more sensitive to heavy metal pollution than survival and development.

The present study elucidates the possibility as well as the importance of using nematode density, biomass, activity, and community structure as indicators of ecosystem health. The biomass of soil organisms, together with their number dynamics, has been found to be a useful indicator of environmental pollution by Ingham et al. (1986a,b), Paul and Clark (1989), Nannipieri et al. (1990), and Yeates et al. (2003), in their studies of different systems. Moreover, in monitoring soil organism dynamics, we can detect detrimental ecosystem changes and possibly prevent further degradation (Lal and Stewart, 1992).

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