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Influence of selected heavy metals (As, Cd, Cr, Cu) on nematode communities in experimental soil microcosm

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Summary

In this study, the effects of arsenic, cadmium, copper and chromium treatments were examined on a nematode community structure and proportion of functional groups in the microcosm for 30 days. The toxic effects on the nematode community did not correspond with metals mobility (EDTA extraction) in soil as it was expected. The most toxic element with a significant degradation of community structure was chromium (low mobile), which negatively affected almost all observed ecological parameters (abundance, diversity and ecological indices). On the other hand, cadmium and arsenic influence was negligible even in the plots treated with the highest concentrations and the communities resembled to the control samples. Copper showed a stimulative effect on the community under low concentration (40 $mg.kg^{-1}$), while under higher concentrations the stimulation was replaced by stress responses. The widely used ecological indices, such as the Maturity Index 2-5, Structure Index, and Shannon-Weaver Index and c-p groups showed the best bioindication potential among nematode parameters.
Keywords: nematode; soil; microcosm; heavy metals; contamination

Introduction

Human society depends in many ways on services provided by soil ecosystems. The rapid development in the industry and other aspects of society are closely linked with the release of various waste materials into the environment, where their accumulation increases over time and negatively influences the soil ecosystem. Potential risks of the industrial contamination have often been assessed by a single species toxicity tests in laboratory. However, discrepancies in results of single species laboratory approaches with natural conditions have often been significant, as a result of different interactions among organisms in environment (Korthals *et al.,* 1998). Faunal analysis on the species community level should therefore increase the ecological complexity for the risk assessment and served better as a biotic indicator of soil ecological health. Several studies have shown that nematode communities responded not only to the agricultural practices e.g. ploughing, crop rotation and water management (Kimpinski & Sturz, 2003), but also to the different organic and inorganic pollutants such as polycyclic aromatic hydrocarbons (PAH) or heavy metals (Pen-Mouratov *et al.,* 2008; Chen *et al.,* 2009). Introduction of heavy metals to the soil is even more dangerous due to low degradability and high accumulation in soil horizons. Ministry of Agriculture and Rural Development (MARD) degree No. 531/1994-540 divided the heavy metal contamination of soil into the several categories with threshold limits (A, B and C). However, the contamination of soil is assessed only for the total content of trace elements. This way, the effects of the available fraction, being in direct interaction with soil communities and affecting their biology and behaviour, are generally overlooked (Šalamún *et al.,* 2012).
The present study provides experimental results on the short-term effects of potentially ecotoxic elements (As, Cd, Cr and Cu) with focus on their mobility in the soil. The metal dose and its mobility in the soil matrix were used as the main parameters for assessing the sensitiveness of the nematode community to the pollution. As metal toxicity to nematodes could strongly depend on its solubility and soil-metal interactions, some soil characteristics, which could enhance or inhibit the toxicity, were also observed. For the evaluation

of the impact on nematodes, various nematological indicators, such as generic richness, MI2-5, SI, H' and c-p distribution were used.

Material and Methods

Soil characteristics

Experimental soil was collected in April 2011, from the top soil horizon (upper 20 cm) of a grassland located 13 km north of the city Svidník, Slovakia (49°25'13"N, 21°37'47"E). Soil type was Cambisol with sandy-loam texture. The fresh soil was passed through a 5 mm sieve to remove stones and roots, and then distributed to the experimental vessels. For detailed soil properties (pH, C_{ox}, $N_{anorg.}$, humidity) see Table 1.

Experimental design

Similar as in study Korthals *et al*. (1996a) equivalent portions of the fresh soil (2.5 kg) were placed into vessels and left for 60 days under 3 days irrigation cycle. After the resting period, the soil was treated with adequate amounts of inorganic salts (As_2O_3, $CuSO_4$, $CdSO_4.^8/_3H_2O$, K_2CrO_4) to obtain the required heavy metal concentrations per kg of dry soil. Arsenic (As) was applied at rates of 0, 10, 20, 40 and 80 mg.kg^{-1}, cadmium (Cd) 0, 1, 3, 15 and 40 mg.kg^{-1}, chromium (Cr) 0, 50, 200, 500 and 1,000 mg.kg^{-1} and copper (Cu) at rates of 0, 40, 80, 300 and 750 mg.kg^{-1}, respectively, to obtain contamination levels according to the MARD degree No. 531/1994-540 limit values (MARD, 1994), (Table 2). Contaminants were applied uniformly on the soil surface, to imitate the aerial deposition, and washed in the soil by 200 ml of water. Each concentration plus a control sample was replicated four times. Duration of the experiment was 30 days. Microcosms were kept under ~20 °C temperature with regular irrigation.

Laboratory analysis

Soil analyses (steps 1 – 4) were performed by the certified Laboratory of the Central and Testing Institute in Agriculture in Košice, according to the certified methods (MP SR, 2005).

1) Soil moisture was measured gravimetrically by drying to a constant weight at 105 °C (MP SR, 2005).
2) Organic matter (OM) was calculated from C_{ox} determined by a titration with $K_2Cr_2O_7/H_2SO_4$ (MP SR, 2005).
3) $N_{anorg.}$ was calculated as a sum of NH_4^+ and NO_3^-:
   a. NH_4^+ value was established by spectrophotometry with Nessler reagent (MP SR, 2005).
   b. NO_3^- value was determined by an ion-selective electrode (MP SR, 2005).
4) pH value was determined in $CaCl_2$ solution (ISO/DIS 10390).
5) Concentrations of trace elements were measured by an AAS method with ZEEnit 700P analyser according to the following standards STN ISO 8288 (Cd, Cr, Cu) and STN EN ISO 15 586 (As).

Before the analysis (step 5), approximately 150 g of soil from each replicate was air-dried in petri dishes for 4 days, pounded in agate mortar and sifted through 2 mm and 0.2 mm analytical sieves. For total concentrations, metals were extracted by 2 M nitric acid for 6 hours and 250 rpm at orbital shaker PSU-10i (10 g soil : 50 mL HNO_3) (Sabienë *et al.,* 2004). The available fraction was extracted by 0.05 M Na_2EDTA solution (pH 7) for 1 hour and 240 rpm (5 g soil: 50 ml Na_2EDTA) (Sabienë *et al.,* 2004). After extraction,

Table 1. Soil parameters under different treatments

Parameter	Element	Control	Dose 1	Dose 2	Dose 3	Dose 4
pH ($CaCl_2$)	As	5.93 ± 0.63	5.2 ± 0.18	6.23 ± 0.55	6.13 ± 0.59	5.6 ± 0.78
	Cd		5.5 ± 0.08	5.58 ± 0.1	6.18 ± 0.53	5.3 ± 0.14*
	Cr		5.33 ± 0.32*	5.1 ± 0.23*	5.93 ± 0.13	6.18 ± 0.43
	Cu		6.53 ± 0.13	6.5 ± 0.27	5.38 ± 0.79	5.3 ± 0.71
C_{ox} (%)	As	2.93 ± 0.21	2.55 ± 0.23	2.7 ± 0.34	2.98 ± 0.29	2.84 ± 0.17
	Cd		2.76 ± 0.25	2.8 ± 0.12	2.93 ± 0.36	2.89 ± 0.12
	Cr		2.75 ± 0.18	2.51 ± 0.42*	2.79 ± 0.13	2.77 ± 0.18
	Cu		2.96 ± 0.09	3.1 ± 0.23	3.05 ± 0.18	2.81 ± 0.38
$N_{anorg.}$ (mg.kg^{-1})	As	57.88 ± 14.4	133 ± 20.64**	131.5 ± 48.31**	91.85 ± 36.33	99.58 ± 9.67
	Cd		154.75 ± 88.57*	97.65 ± 25	63.48 ± 26.41	99.43 ± 26.26
	Cr		91.45 ± 24.1	70.7 ± 6.43	114.4 ± 28.95	176.78 ± 83.81**
	Cu		75.08 ± 26.75	64.8 ± 15.75	74.55 ± 12.73	58.23 ± 9.74
Soil humidity (%)	As	19.07 ± 3.26	21.54 ± 8.13	23.23 ± 2.59	25.48 ± 3.11	26.31 ± 2.39
	Cd		20.96 ± 4.61	21.5 ± 4.71	23.03 ± 6.26	20.27 ± 2.43
	Cr		24.2 ± 1.96**	24.42 ± 1.7**	23.42 ± 1.74*	25.32 ± 2.5**
	Cu		24.49 ± 2.69	27.19 ± 3.17	28.21 ± 2.32	22.45 ± 2.34

*P<0.05 and **P<0.01 significance level compared to the control sample

Table 2. Limit values for the Slovak republic (MARD degree No. 531/1994-540)

Element	A	B	C
As	5	30	50
Cd	0.3	5	20
Cr	10	250	800
Cu	20	100	500

A – maximum value for the uncontaminated soil,
B – value, where the contamination was analytically proved,
C – soil remediation value

soil samples were filtered through a 150 mm KA4 filter paper.

Nematode handling
During nematode handling, the soil was mixed by hand and 100 g were taken for nematodes extraction using the modified Baermann funnel method (Southey, 1986). The extracted nematodes were fixed in Ditlevsen's solution (Formalin–Acetic–Alcohol), (van Bezooijen 2006) and counted under microscope. All nematodes in the samples were identified to the order, family and genus levels.
The following parameters were scored: (1) total abundance per 100 g (A); (2) nematode generic richness; (3) colonizers-persisters (c-p) structure of the nematode community and (4) Maturity Index 2-5 (MI2-5), (Bongers & Korthals, 1993); (5) Shannon-Weaver Index (H'), (Shannon-Weaver, 1949); (6) Structure Index (SI) and (7) Channel Index (CI), (Ferris *et al.,* 2001). Nematode taxa were assigned to c-p and feeding types sensu Nemaplex (http://plpnemweb.ucdavis.edu/nemaplex/).

Statistical analysis
For statistical evaluation, nonparametric Spearman's correlation coefficient (r_s) was calculated to test a relationship between the nematode community characteristics values and obtained available trace elements concentrations at individual sites using STATISTICA v. 9.0 software. Statistical correlations at the levels $P<0.05$ and $P<0.01$ were considered significant. Differences in nematode community mean traits and indices among individual sites were tested by Duncan Test at the levels $P<0.05$ or $P<0.01$. For the analysis of the ecological distance among treatments, a constrained ordination redundancy analysis (RDA) was used; data were processed by CANOCO 5 software. The significance of the axis was tested by Monte Carlo permutation test (ter Braak & Šmilauer, 2012). RDA figures for As and Cd were not included due to insignificant results with nematode parameters.

Results

Soil characteristics and heavy metals
According to Table 1 only in the Cu treatment the soil parameters were similar to the control, while in the Cr treatment all soil parameters showed significant variances ($P<0.05$) compared to the control at the end of the experiment. In the Cd treatment pH and $N_{anorg.}$ differed significantly ($P<0.05$) in the samples treated with 1 mg.kg^{-1} and 40 mg.kg^{-1} dose. Values for $N_{anorg.}$ were significantly higher ($P<0.05$) in 10 mg.kg^{-1} and 20 mg.kg^{-1} As dose. The total concentrations of applied elements are shown in Table 3. Data showed remarkable differences in the element mobility, which was substantially lower in As and Cr when compared to Cd and Cu with high mobility in soil horizon. Independently to the mobility the increasing concentration in treatments was clearly recognizable in both extraction modes for all elements.

Nematode community
Based on the nematode community reactions, elements could be split into two groups – the first, with strong effects on nematodes, represented by Cr and Cu and the second one with negligible effects, represented by As and Cd. The Cr and Cu application had significant impact ($P<0.01$) on the generic richness (Table 5), and in samples with Cr treatment the total density of nematodes was reduced as well ($P<0.01$), (Table 4). Moreover Cr altered the distribution of c-p 5 nematodes in the concentration of 500 mg.kg^{-1} and above. Surprisingly, the c-p 4 nematodes with relatively high sensitivity to disturbances were represented by quite high proportion under 1000 mg.kg^{-1} (about 25 %), with positive correlation to the increasing Cr concentration (Fig. 1A).
In copper treatment, the nematode responses varied more than under chromium application. Stimulation of the nematode community (SI, MI 2-5) under lowest Cu concentration of 40 mg.kg^{-1} (Table 4), was replaced by a clear stress responses and decline

Table 3. Available fraction and total concentration of applied elements in soil; average values (±S.D.)

Element	Control	Dose 1	Dose 2	Dose 3	Dose 4
			Available fraction (Na_2EDTA)		
As	0.03 ± 0.01	0.25 ± 0.07	1.09 ± 0.49	1.98 ± 0.93	3.93 ± 2.01
Cr	0.35 ± 0.19	1.76 ± 0.32	5.47 ± 1.00	12.07 ± 2.25	41.19 ± 8.87
Cd	0.11 ± 0.07	1.98 ± 1.14	7.82 ± 3.24	25.53 ± 4.83	49.87 ± 9.28
Cu	2.58 ± 0.28	67.47 ± 12.27	110.93 ± 3.83	298.29 ± 39.21	737.74 ± 104.54
			Total concentration (HNO_3)		
As	0.58 ± 0.27	0.65 ± 0.79	3.21 ± 1.16	7.1 ± 0.93	18.29 ± 8.31
Cr	11.57 ± 11.63	30.09 ± 5.92	80.36 ± 8.74	217.39 ± 13.2	445.63 ± 48.96
Cd	0.18 ± 0.01	1.82 ± 0.8	6.48 ± 2.62	24.89 ± 4.33	39.59 ± 2.04
Cu	3.73 ± 0.23	68.21 ± 10.63	108.97 ± 9.55	295.00 ± 43.48	748.49 ± 69.04

Table 4. Effects of concentration gradients on ecological indices in nematodes; average values (± SD)

Index	Element	Control	Dose 1	Dose 2	Dose 3	Dose 4
Generic Richness	As	26.25 ± 6.4[a]	23 ± 2.16[a]	25.25 ± 1.71[a]	25.75 ± 2.87[a]	25.25 ± 1.5[a]
	Cd	26.25 ± 6.4[ab]	24 ± 2.71[ab]	22.75 ± 1.26[ab]	28.5 ± 1.91[a]	22 ± 3.83[b]
	Cr	26.25 ± 6.4[a]	22.75 ± 2.22[a]	16.25 ± 4.92[b]	11 ± 1.63[b]	2.25 ± 0.96[c]
	Cu	26.25 ± 6.4[ab]	29.5 ± 3.11[a]	26.75 ± 1.26[ab]	20.75 ± 3.3[bc]	18.25 ± 7.59[c]
Abundance	As	398.3 ± 278.92[a]	389.5 ± 99.1[a]	261 ± 43[a]	445.8 ± 35.2[a]	456 ± 138.1[a]
	Cd	398.3 ± 278.92[a]	410 ± 73[a]	283.3 ± 45.5[a]	555.3 ± 312.3[a]	281 ± 126.7[a]
	Cr	398.3 ± 278.92[a]	265.3 ± 120[a]	143.8 ± 124.4[b]	68.8 ± 21.6[b]	7.5 ± 8.6[b]
	Cu	398.3 ± 278.92[a]	438.8 ± 109.4[a]	521.3 ± 215.5[a]	296.3 ± 86.4[a]	224.3 ± 152.4[a]
H'	As	2.42 ± 0.3[a]	2.56 ± 0.12[a]	2.34 ± 0.33[a]	2.21 ± 0.28[a]	2.48 ± 0.14[a]
	Cd	2.42 ± 0.3[a]	2.51 ± 0.11[a]	2.35 ± 0.07[a]	2.45 ± 0.06[a]	2.19 ± 0.25[a]
	Cr	2.42 ± 0.3[a]	2.39 ± 0.14[a]	2.24 ± 0.04[ab]	1.75 ± 0.4[b]	0.64 ± 0.46[c]
	Cu	2.42 ± 0.3[a]	2.59 ± 0.13[a]	2.31 ± 0.2[ab]	2.15 ± 0.28[ab]	2.05 ± 0.45[b]
MI2-5	As	2.77 ± 0.36[ab]	2.96 ± 0.25[a]	3.09 ± 0.36[a]	3.19 ± 0.21[a]	2.95 ± 0.37[a]
	Cd	2.77 ± 0.36[a]	2.84 ± 0.32[a]	2.89 ± 0.33[a]	2.99 ± 0.19[a]	2.96 ± 0.3[a]
	Cr	2.77 ± 0.36[ab]	2.72 ± 0.14[ab]	2.91 ± 0.38[ab]	2.34 ± 0.28[a]	n.c.
	Cu	2.77 ± 0.36[b]	3.25 ± 0.12[a]	2.68 ± 0.19[b]	2.51 ± 0.22[b]	2.59 ± 0.16[b]
SI	As	67.62 ± 13.56[a]	76.66 ± 8.03[a]	79.23 ± 9.96[a]	82.47 ± 4.44[a]	74.56 ± 12.18[a]
	Cd	67.62 ± 13.56[a]	70.91 ± 14.52[a]	73.55 ± 11.17[a]	77.61 ± 5.21[a]	74.61 ± 11.8[a]
	Cr	67.62 ± 13.56[a]	67.85 ± 7.08[a]	74.06 ± 12.06[a]	39.02 ± 29.21[b]	n.c.
	Cu	67.62 ± 13.56[a]	85.26 ± 2.56[b]	65.54 ± 9.78[a]	55.5 ± 13.4[a]	61.53 ± 9.25[a]
CI	As	41.61 ± 39.7[a]	41.92 ± 12.39[a]	35.91 ± 6.26[a]	41.86 ± 17.76[a]	46.75 ± 17.47[a]
	Cd	41.61 ± 39.7[ab]	50.06 ± 28.33[ab]	38.01 ± 34.28[ab]	18.54 ± 7.33[b]	66.08 ± 19.64[a]
	Cr	41.61 ± 39.7[a]	28.25 ± 6.17[a]	32.01 ± 45.46[a]	42.28 ± 40.99[a]	27.5 ± 48.56[a]
	Cu	41.61 ± 39.7[ab]	22.84 ± 9.13[b]	44.73 ± 31.67[ab]	71.75 ± 34.02[a]	11.19 ± 7.91[b]

n.c. - not calculable
[a,b,c] - means followed by the same letters on the same rows are not statistically different (P < 0.05)

in genus numbers and the proportions of c-p 3-5 groups from 300 mg.kg^{-1} onwards (Table 4, 6). In contrast, the c-p 1 and 2 groups appeared to have relatively high degree of resistance, being dominant in the community structure under 300 and 750 mg.kg^{-1} Cu, respectively (Fig. 1B).

As and Cd application did not increase stress responses of nematode communities. Their parameters were comparable with control samples (Table 4). Although based on the significant increase in the proportion of c-p 5 nematodes under Cd addition, it might have a stimulating effect on c-p group (Table 6).

Ecological Indices

Table 5. Correlations of generic richness and nematode density relating to concentration gradients

Index	As	Cd	Cr	Cu
Generic richness	n.s.	n.s.	-0.932**	-0.805**
Nematode density	n.s.	n.s.	-0.818**	n.s.

** (P<0.01); n.s. – not significant

Ecological indices MI2-5, SI and H' expressed clear negative correlation to the Cr treatments (Fig. 1A). On the contrary, these indices were positively stimulated by low Cu load (40 mg.kg^{-1}) in the samples. Although under higher doses the ecosystem and community structure degradation was evident (Fig. 1B). For As and Cd no ecotoxic effects were visible or detected after the treatment (ecological values MI2-5, SI, H'; Table 4).

The rate of organic matter degradation, described through CI, was found being in positive correlation with the c-p 2 group under Cr and Cu contamination (Fig. 1A, B). Application of Cr, despite its high toxicity, showed a weaker effect on CI in comparison to Cu (Table 4). Nevertheless, due to the low density of nematodes under the highest Cr doses, these results might not reflect the actual degradation of organic matter via bacterial or fungal channels.

For Cu treatment, the significant depression in CI values under the highest dose was in sharp contrast to the development under Cd treatment (Table 4). This could indicate important shifts in decomposers in the food web under the highest doses of Cu and Cd. The

Table 6. Effects of concentration gradients on the c-p group distribution of non-plant feeding nematodes (%); average values

Element	Control	Dose 1	Dose 2	Dose 3	Dose 4
As					
c-p 1	17.16[a]	14.46[a]	9.68[a]	16.94[a]	19.4[a]
c-p 2	55.56[a]	48.86[a]	47.49[a]	41.57[a]	49.09[a]
c-p 3	1.27[a]	0.3[a]	1.55[a]	0.62[a]	0.91[a]
c-p 4	15.88[a]	27.81[a]	26.14[a]	23.98[a]	20.72[a]
c-p 5	10.12[a]	8.56[a]	15.14[a]	16.89[a]	9.88[a]
Cd					
c-p 1	17.16[a]	12.77[a]	21.14[a]	18.6[a]	5.07[a]
c-p 2	55.56[a]	55.73[a]	48.05[a]	45.65[a]	58.52[a]
c-p 3	1.27[a]	0.93[a]	0.99[a]	1.51[a]	0.61[a]
c-p 4	15.88[a]	21.57[a]	21.24[a]	22.89[a]	17.54[a]
c-p 5	10.12[ab]	8.99[a]	8.59[a]	11.36[ab]	18.26[b]
Cr					
c-p 1	17.16[a]	17.05[a]	18.31[a]	11.97[a]	17.31[a]
c-p 2	55.56[a]	56.15[a]	47.58[a]	73.98[a]	32.69[a]
c-p 3	1.27[a]	0.55[a]	1.58[a]	0[a]	0[a]
c-p 4	15.88[a]	20.1[a]	25.57[a]	14.05[a]	25[a]
c-p 5	10.12[a]	6.37[a]	7.4[a]	0[b]	0[b]
Cu					
c-p 1	17.16[ab]	16.69[a]	6.98[a]	3.48[a]	44.89[c]
c-p 2	55.56[ab]	36.34[a]	64.83[b]	74.41[b]	40.45[a]
c-p 3	1.27[ab]	1.76[a]	0.52[bc]	0.79[b]	0[c]
c-p 4	15.88[a]	32.6[c]	20.66[ab]	14.75[a]	13.22[a]
c-p 5	10.12[a]	12.61[a]	7[ab]	6.57[ab]	1.45[b]

[a,b,c] - means followed by the same letters on the same rows are not statistically different ($P < 0.05$)

rate of decomposition under As treatment was similar to the control samples, i.e. without any obvious change (Table 4).

Discussion

The present study was originally started as an experiment focusing on nematode communities under different contamination levels according to the MARD degree No. 531/1994-540. The degree defines soil on four different contamination levels from uncontaminated (below limit A) to heavily polluted and suitable for remediation (above limit C). However, this pollution assessment deals only with the total concentration of heavy metals (extraction in 2M HNO_3), and it does not take into account the available fraction. The dissolved contaminants in the soil solution have more intense interactions with soil biota and their solubility might be a crucial factor for the intensity of toxic effects (Schultz & Joutti, 2007).

According to the mobility, the elements used in our study could be split into groups with high (Cd, Cu) and low (As, Cr) mobility in the soil profile. The difference in mobility and the resulting toxicity could be influenced by various soil parameters such as pH, organic matter content, transformation, etc. (Fijałkowski *et al.*, 2012). The low mobility of chromium in our study, might be caused by high pH and reduction of the highly toxic and mobile Cr^{6+} to the significantly less mobile Cr^{3+} (Kabata-Pendias, 2011). This process occurs mainly in the upper soil horizon (Stewart *et al.,* 2003), from which the soil for our experiment was taken, and where electron-rich sources for Cr reduction are available (clay, organic matter, Fe-oxides). Nevertheless, despite the low Cr mobility in soil matrix, the impact on nematodes was obvious and did not correspond with our hypothesis. The nematode abundance and generic richness decreased clearly and indicated severe impact of Cr treatment, despite their limited indication value (Šalamún *et al.,* 2012). Similar outcomes for the environment could be deduced from other parameters, as well. The c-p 3 nematodes and the most sensitive c-p 5 nematodes were eliminated from the community, followed by depression in several ecological indices (MI2-5, SI, H') under the highest doses 500 and 1000 $mg.kg^{-1}$, respectively. The only exception in this negative trend were c-p 4 nematodes, considered also as K-strategists (Wilson & Kakouli-Duarte, 2009), with a share of 25 % in population under the highest Cr dose. This

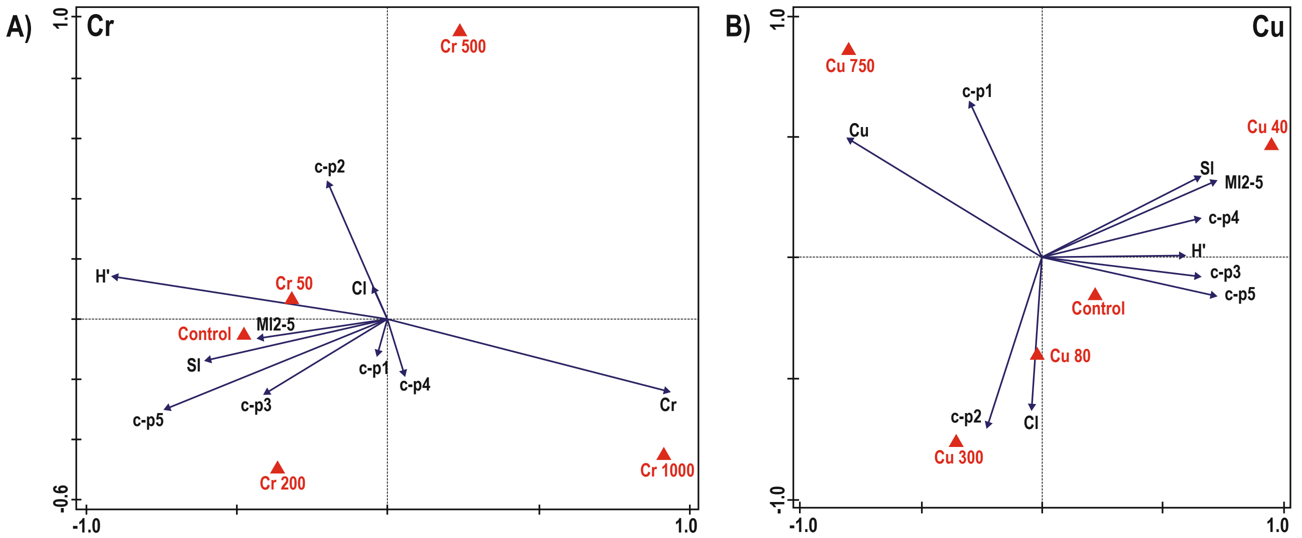

Fig. 1. RDA analysis of ecological indices and c-p groups expressed for Cr (A) with 34.9 % explained variance (axis: 0.278; 0.056; F=2; P=0.008) and Cu (B) with 57.2 % explained variance (axis: 0.319; 0.222; F=5; P=0.002)

high variation however, has to be seen in the context of extremely low nematode densities in this treatment. Nevertheless, the depression in all parameters confirmed the destructive character of this element, observed also in other experimental and field studies (Nagy, 1999; Bakonyi *et al.*, 2003; Chen *et al.*, 2009).

Regarding to the Cu treatments, different responses were seen under the applied doses. According to Nagy *et al.* (2004), nematodes showed resistance to copper up to the 130 mg.kg^{-1}. This is in agreement with our results, where under 110 mg.kg^{-1} of Cu the community exhibited no significant alterations in comparison with control samples and furthermore, low Cu contamination (40 mg.kg^{-1}) showed positive effects on the structure complexity (SI), maturity (MI2-5) and diversity (H') of the nematode assemblage. Under the higher doses however, significant stress in ecosystem with deleterious influence on nematodes, similar as described by Georgieva *et al.* (2002) was detected. The stimulating effect and relatively high tolerance to Cu may be the consequence of its importance in physiological processes and could have common base as motility stimulation by copper sulphate described by Bongers *et al.* (2001).

The As and Cd application, despite their evident contrast in mobility, showed relatively low impact on soil nematodes. Although, arsenic is one of the most common soil contaminants (Yang *et al.*, 2002), its relatively strong binding to the soil components greatly limited its mobility (Kabata-Pendias, 2011). The immobilization of As was clearly visible during our experiment as well, where less than 5 % were extracted in available fraction. The reactions to the As may differ throughout the various soil animal groups. For example, earthworms under As pollution showed a decrease in both reproduction and survival. Meanwhile the enchytraeids were stricken only by the lower reproduction rate (Schultz & Joutti, 2007). For nematodes, only few studies have dealt with the impact of As (Nagy, 1999; Pen-Mouratov *et al.*, 2008) and their outcomes were contradictory. The negligible effect, without any clear tendencies under different levels of contaminations are probably due to combination of several factors such as limited mobility, low applied dose (in comparison e.g. with Cr), and with that related low uptake or effective elimination from body tissues (Schwartz *et al*., 2010). Similar results were found for relatively mobile Cd, which even showed positive influence on the most sensitive nematodes from the c-p 5 group. Korthals *et al.*, (1996b) described the tolerance of nematodes to Cd up to 160 mg.kg^{-1} without any signs of substantial changes in community structure. In our study, the soil Cd content was only about 40 mg.kg^{-1}. However, this is two times higher than the MARD degree remediation limit. Since the other components of soil ecosystem, e.g. plants, springtails or mites may have better response to the contamination and are far more sensitive to Cd (Korthals *et al.*, 1996b) the use of nematodes to Cd biomonitoring is questionable.

As showed in this paper, the ecotoxicity of some elements may considerably differ as could be predicted from the total element concentrations or available concentration, which is usually considered as one of the major factors contributing to the final strength of toxicity (Schultz & Joutti, 2007). The other factors, such as soil binding capacity or organisms' selective sensitivity to different heavy metals seem to have a significant influence on resulting toxicity for the communities and their role in environmental biomonitoring.

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