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Earthworms as useful bioindicator of soils contamination around Košice city, Slovakia

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Abstract

This study was conducted to investigate heavy metals bioaccumulation in industrialized soils in surrounding of Košice city (Slovakia), using earthworms. In the present research, we used ecotoxicity tests with *Dendrobaena veneta* (7 and 28-day bioassays) to infer about potential toxic risks to the agricultural (A) and permanent grass vegetation (PGV) of soils around the plant U. S. Steel Košice. The total Fe, Cu, Zn, Pb, Cd, Cr and As contents and eco-toxicological tests of industrialized soils from the Košice area were performed for 12 sampling sites in years 2016 – 2017. An influence of the sampling sites distance from the largest steel producer plant on the total concentrations of heavy metals was determined for Fe, Cd, Cr and As. It was found that earthworms (*Dendrobaena veneta*) in some cases caused a decrease of metals concentration in contaminated soils, the largest metal concentration differences were recorded in the samples PGV (4) U. S. Steel-plant-main gate. The results of the bioaccumulation factors of heavy metals in *D. veneta* (BAFs/7-28 d) are < 1 for the studied metals order in the sequence: Cr < Fe < Pb < Cu < As and > 1 for Zn > Cd.

Introduction

Urban environmental quality is vital to be investigated as large parts of human populations live in cities. However, continuous urbanization and industrialization in urban areas pose a great threat to environment as well as human. Soils are susceptible to anthropogenic contamination with metallic trace elements that generate risks to ecosystems and human health. In industrialized areas urban soils are highly modified and intensively managed. Košice, the city in eastern Slovakia, is exposed to typical urban contamination sources such as road traffic, municipal sphere, and small industrial sources. Furthermore, being the largest steel producer in Central Europe, it is long-term environmentally loaded by the iron and steel works that represent the largest source of (metal) contamination in Slovakia (Hančuľák *et al.* 2015). The transport of heavy metals in soil is the result of processes between soil and metal components, which include processes of physical, chemical, and biological nature (Violante *et al.* 2008). Their monitoring and studying should be complex to provide relevant picture on their effects and impacts. The combination of chemical measurements with the calculation of the bioaccumulation factor (BAF) and Person matrix correlations (r) can be a useful tool in risk assessment. Earthworms by virtue of their habitation participate in the formation of soil structure and regulate dynamics of soil organic matter, therefore (directly or indirectly) can modulate the transfer of inorganic and organic

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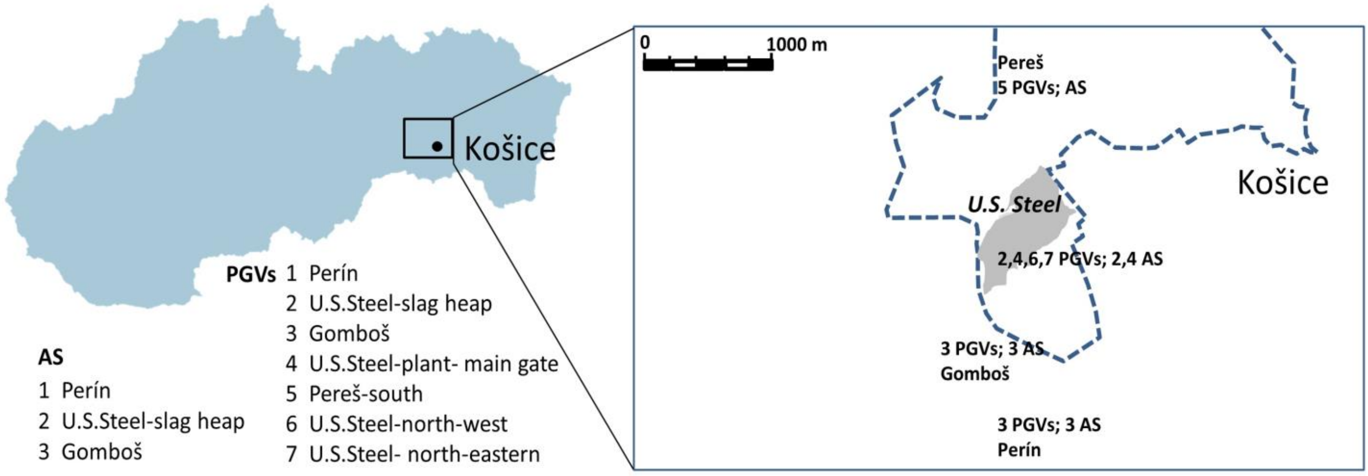

Fig. 1. The study area and sampling localities.

chemicals (toxicants) in soils. For this reason they are considered as useful biological indicator of several metals in soils (Suthara *et al.* 2008). Furthermore, they relatively efficiently accumulate certain essential and non-essential metals thus also can affect soil processes and local biodiversity directly by transferring metals to other organisms (Morgan *et al.* 2001).

Bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can be transported into the cell across the cell membrane and accumulate intracellularly. The accumulated metals are detoxified through cell metabolic cycle (Coelho *et al.* 2018). Earthworms have already been used in several studies on environmental contamination by heavy metal, and usually were considered a good bioindicator for soil pollutants. Their biological activities can also influence the accumulation and/or biodegradation of inorganic contaminants including heavy metals (Lingxiangyu *et al.* 2010).

Many authors have shown that bioassays provide a general indication of metal toxicity in soils cycle (Das *et al.* 2011; Yu *et al.* 2012; Xu *et al.* 2014; Findoráková *et al.* 2017; Maity *et al.* 2018). These tests can be successfully used to evaluate potential toxicity and to assess the environmental risks of heavy metals. The aim of this study was evaluation of the impact of anthropogenic heavy metals in urban soils from the Košice area, using bioassays on earthworms, and by means of the bioaccumulation factor and Person matrix correlations.

Experimental

Soil sampling and analysis

Košice city has about 239 000 inhabitants and is located the Košice basin in the river valley Hornád in the eastern part of Slovakia (Europe). In the period of 2016 – 2017, 12 soil samples were collected in the Košice suburban, in surrounding of U. S. Steel Košice, which is located about 10 km south-westwards from the centre of the city (Fig. 1). Annual raw steel production capability is 4.5 million metric tons. The technology of steel production has a significant impact on the urban environment. Furthermore, the agricultural and industrial activities contribute to contamination of the soil in this region. The seven sampling sites were localised on area of the U. S. Steel Košice. Permanent grass vegetation soils (PGVs) were sampled on following localities (Fig. 1): 1 – Perín, 2 – U. S. Steel-slag heap, 3 – Gomboš (Veľká Ida), 4 – U. S. Steel-plant (main gate), 5 – Pereš-south, 6 – U. S. Steel-north-west and 7 – U. S. Steel-north-eastern. The five samples of agricultural soils (AS) were collected in localities (Fig. 1): 1 – Perín, 2 – U. S. Steel-slag heap, 3 – Gomboš (Veľká Ida), 4 – U. S. Steel-plant west and 5 – Pereš-south. The soils were sampled at a depth of 20 – 40 cm into plastic bags. Soil samples were homogenized, dried at room temperature (25 °C) and sieved through 2-mm sieve. For analysis of soils were used the mixed samples of total weight of 5 kg. The concentrations of the heavy metals (Fe, Cu, Zn, Pb, Cd, Cr and As) in soil samples were measured using SPECTRO XEPOS X-ray fluorescence spectrometer model XEPOS 3 (range of elements: Na(11) – U(92), scattering targets: Mo, Co, Al_2O_3, Pd, HOPG-crystal, X-ray lamp (type VF50): Pd with Be window, resolution: 145 keV on line Kα Mn). All the analysed samples were conducted in triplicate and the data were expressed to soil dry weight unit. The used control reference soil (CRM - TM52, MicroBioTests, Belgium) contained: 85 % (w/w) quartz, 10 % (w/w) kaolin, 5 % (w/w) peat and calcium carbonate (pH of 6±0.5) (OECD soil). The certified river sediment LGC6187 was used for validation of data. All data were analysed using SPSS ver. 9.0 statistical software. Statistical correlations at the levels $P < 0.05$ and $P < 0.01$ were considered significant. Soil physico-chemical parameters were determined (pH value in H_2O and KCl solutions and oxidation–reduction potential (Eh) (ISO/DIS 10390), organic matter (OM) (STN EN 12879).

Biological assay with D. veneta

Earthworms (*Dendrobaena veneta*) are often used as bio-monitoring organisms for assessment of soil pollution, because they are widespread in their terrestrial distribution and have a capacity to accumulate and concentrate large quantities of inorganic and organic pollutants. The experimental design was based on OECD guideline 317/2010 for the testing of chemicals related to environmental fate, and mortality. Adult earthworms were purchased from a local supplier, and were allowed to acclimatize for one week in the experimental conditions. Ten earthworms (also C.w. – control worms) were added to each plastic box (9 cm x 9 cm x 3 cm) with 100 g of dry soil sample. As reference, C.w. was added to 100 g of CRM soil. Then distilled water was added to the boxes to obtain 30 % (w/w) moisture of soil. The boxes with soils were kept at laboratory temperature for 7 and 28 d (respectively). The mortality of earthworms in soils (replicated tests A – D) was determined and compared with the data from control reference soil. The earthworms from individual boxes were collected and lyophilized at -50 °C and 50 Pa (íl Shin-Bio Base, the Netherlands). The concentrations of heavy metals in the *D. veneta* tissue were determined after mineralization with a mixture of acids $HNO_3/HF/H_3BO_3$ (5:2:20) in the microwave system MWS33 (Germany) by atomic absorption spectrometry (AAS- Variant, Australia).

Bioaccumulation of heavy metals in D. veneta *tissues*

Bioaccumulation factor for earthworms (BAFs) were calculated according to OECD (2010): BAFs = (metal) earthworm / (metal) soil. The relationship between mortality of earthworms (ANDE - Absolute Number of Dead Earthworms) and total metal concentrations were evaluated by Pearson matrix correlation. As control, earthworms fed in non-contaminated (CRM) soil were used.

Table 1. Soil physico-chemical parameters considered in this study.

Soil type	**Locality**	**pH [KCl]**	**pH [H_2O]**	***Eh* [mV]**	**OM [%]**
PGV	**1** Perín	6.88	6.81	462	10.3
	2 U. S. Steel-slag heap	7.18	7.13	472	7.90
	3 Gomboš	7.45	7.55	443	6.60
	4 U. S. Steel-plant- main gate	7.83	7.72	391	5.39
	5 Pereš-south	5.67	6.10	534	4.80
	6 U. S. Steel-north-west	6.34	6.89	476	6.50
	7 U. S. Steel- north-eastern	7.22	7.38	369	7.74
AS	**1** Perín	6.20	7.20	490	4.65
	2 U. S. Steel-slag heap	6.99	7.74	441	4.34
	3 Gomboš	7.23	7.95	522	4.94
	4 U. S. Steel-plant west	7.47	7.80	422	4.11
	5 Pereš-south	5.43	6.68	457	4.36

Table 2. Metal concentration (average ± SD) in soil (DW) at locality U. S. Steel Košice.

Locality	**Fe [%]**	**Cu [mg.kg^{-1}]**	**Zn [mg.kg^{-1}]**	**Pb [mg.kg^{-1}]**	**Cd [mg.kg^{-1}]**	**Cr [mg.kg^{-1}]**	**As [mg.kg^{-1}]**
Permanent grass vegetation soils							
1	3.3±2.1	27.1±3.9	88.7±4.3	28.9±0.9	2.0±1.1	91.7±4.1	10.6±1.8
2	3.4±2.7	27.2±2.7	157±6	50.1±2.3	1.6±0.8	85.8±3.4	13.4±2.9
3	2.5±1.8	22.5±2.8	65.9±6.9	29.9±1.5	1.9±1.2	68.8±2.8	12.5±2.1
4	10.5±3.8	64.9±6.1	1,084±11	379±14	21.4±2.9	278±14	94±13
5	2.5±1.5	24.7±2.2	64.4±5.7	30.5±1.4	3.3±1.4	106±9	9.9±2.3
6	2.9±1.7	30.8±4.3	86.5±4.6	36.8±2.2	1.2±0.1	248±11	10.8±1.8
7	3.6±2.9	28.0±3.0	95.5±6.2	46.3±2.9	2.7±0.8	264±12	12.7±1.1
Agricultural soils							
1	2.5±2.0	30.5±2.6	60.2±4.7	26.3±1.2	2.1±0.7	61.6±3.1	7.3±0.9
2	3.5±2.6	28.4±3.2	83.8±6.3	30.4±1.7	9.1±2.3	55.3±2.9	15.9±4.5
3	2.6±1.4	54.0±5.8	67.9±5.1	32.9±2.3	1.8±0.6	160±10	10.8±3.1
4	3.6±1.9	32.3±4.6	121±9	69.3±5.8	4.4±1.2	69.1±4.6	34.8±8.8
5	2.7±1.8	29.9±2.7	62.4±3.9	29.5±2.7	6.9±2.1	83.3±6.2	9.0±5.6
CRM	2.4±0.9	83.6±1.5	439±12	77.2±3.3	2.7±1.3	84.0±5.3	24.0±3.4
Low No. 220/2004-2 [mg.kg^{-1}DW]							
Limit	-	70	200	115	1	90	

Results and Discussion

Earthworms play important role in soil ecotoxicological risk assessment (Spurgeon *et al.* 2003; Dai *et al.* 2004) as the metal content in earthworm tissues is partly dependent on the metal content of soils. This is determined by several factors including physio-chemical edaphic interactions and factors such as pH, concentration of different cations and of organic matter (C : N ratio) and cation-exchange-capacity (Kizilkaya 2005). The soil types examined here were of silty-clay texture from various areas to keep representativeness, and their quality was established with reference to law (220/2004, No.2, Slovak Republic). Table 1 summarizes the results of basic physico-chemical parameters of the permanent grass vegetation soils and agricultural soils (PGV and AS, respectively) sampled from the Košice area close to U. S. Steel Košice (Fig. 1). The soil samples (both types) presented a slightly alkaline reaction, and pH/KCl and also pH/H_2O were in the range from 6.34 to 7.83 (Table 1). Exceptions were the PGV and A-soils at locality Pereš-south (5) that were slightly acidic (5.43 – 6.68). Organic matter (OM) content of the PGV-soils ranged between 4.8 to 10.7 %, in contrast to the A-soils that were relatively low (4.11 to 4.94 %).

The results summarized in Table 2 indicate to significant contamination with cadmium, chromium and arsenic comparing to the limits defined by the Slovak law No. 220/2004-2 about the quality of the soil. The highest concentrations of studied metals were measured in vicinity of sites located south of the ironworks. At the main gate of the U. S. Steel-plant (locality **4**) high Fe, Zn, Pb, Cd, Cr and As concentrations were detected for PGV soils (Table 2). As for the A-soils were determined the highest values of metals from the areas: U. S. Steel-plant west (locality **4**) – mainly for As, and Gomboš (locality **3**) for Cr (Table 2). The lowest average concentration of heavy metals in both the soil types were found in localities distant from metallurgical industry, as are Pereš-south (locality **5**) and Perín (locality **1**) (Table 2).

Bioaccumulation factors (BAFs) are used in the risk assessment to estimate trophic transfer of contaminants such as metals from soil and can be helpful to the prediction of risks associated with this transfer. BAF can be derived from laboratory studies through the determination of steady-state concentrations or kinetic estimation methods. The BAF is considered as the equilibrium ratio between the metal concentration in any organ of an organism and its concentration in the ambient media (soil). In this study, bioaccumulation tests were performed in the collected industrialized soils to determine uptake of Fe, Cu, Zn, Pb, Cd, Cr and As by earthworms *D. veneta*.

Table 3. Metal concentration in soil (DW) of U. S. Steel Košice after 7-days bioassay (average ± SD).

Locality	Fe [%]	Cu [mg.kg^{-1}]	Zn [mg.kg^{-1}]	Pb [mg.kg^{-1}]	Cd [mg.kg^{-1}]	Cr [mg.kg^{-1}]	As [mg.kg^{-1}]
Permanent grass vegetation soils (PGVs)							
1	3.1±1.7	15.3±3.5	49.5±4.2	27.9±1.1	1.9±0.8	90.3±3.9	7.8±0.6
2	2.9±1.8	22.6±2.2	106±4	48.7±2.2	1.5±0.7	87.8±2.9	9.1±1.9
3	2.0±1.9	16.4±1.9	61.2±2.9	28.1±0.6	1.9±0.6	66.9±2.4	8.7±1.3
4	9.1±2.9	55.7±7.9	1,022±13	358±12.6	19.5±1.7	258±12	67.3±7.8
5	1.9±1.1	19.9±2.1	48.7±5.1	29.9±1.5	3.1±1.1	106±7	8.4±1.7
6	2.3±0.8	23.1±3.4	74.6±6.3	35.4±1.9	1.1±0.1	248±11	9.8±2.3
7	2.8±1.5	26.2±2.7	92.0±8.4	46.2±3.0	2.8±0.9	264±13	10.6±2.8
Agricultural soils (AS)							
1	2.3±0.5	18.9±1.6	55.8±3.6	25.3±0.6	1.9±0.9	60.1±2.8	6.1±0.9
2	3.1±1.3	20.5±1.4	79.8±4.1	29.1±1.2	8.7±2.2	52.9±3.0	11.8±1.4
3	2.5±0.5	50.3±4.3	67.1±2.3	32.0±1.5	1.5±1.3	152±12	8.4±2.1
4	3.8±1.4	31.1±2.6	110±10	64.3±3.6	4.1±2.6	64.3±4.5	30.3±2.8
5	2.3±1.2	25.8±1.9	62.0±4.1	24.7±1.3	5.9±3.7	82.8±6.3	7.5±0.7
CRM	2.1±2.0	80.5±6.3	421±12	76.1±9.3	2.6±0.7	84.0±8.8	21.5±1.6
C.w.	0.05±0.8	4.2±1.3	49.8±2.4	1.7±1.9	0.5±0.4	1.0±0.1	0.7±1.4
C.w./7d.	0.06±0.6	4.1±1.2	49.2±1.6	1.7±0.8	0.4±0.8	0.9±0.7	0.6±0.9

CRM (control reference soil); C.w. – Control worms; C.w./7d. – Control worms after 7 d.

Effect of metals on mortality of *D. veneta* after periods of 1 – 7 and 1 – 28 d of exposure (respectively) at the end of the tests (replicates A-D) showed that the earthworms' mortality was influenced by industrialized soils already after 7 d of exposure (Fig. 2). On the other hand, earthworms in some cases caused significant decrease of metals concentration in the soils (Table 3). The largest metal concentration differences were recorded in the PGV soil samples at U. S. Steel-plant-main gate (locality **4**) (As = 67.3 mg.kg^{-1}, Cr = 259 mg.kg^{-1}, Cd = 19.5 mg.kg^{-1}, Pb = 368 mg.kg^{-1}, Zn = 1,022 mg.kg^{-1} and Fe = 9.1 mg.kg^{-1}), and

Table 4. Metal concentration in soil (DW) of U. S. Steel Košice after 28-days bioassay (average ± SD).

Locality	Fe [%]	Cu [%]	Zn [mg.kg^{-1}]	Pb [mg.kg^{-1}]	Cd [mg.kg^{-1}]	Cr [mg.kg^{-1}]	As [mg.kg^{-1}]
Permanent grass vegetation soils (PGVs)							
1	3.2±1.5	18.1±2.9	62.1±3.5	28.1±1.8	2.0±0.9	90.9±2.3	9.1±0.8
2	2.9±1.1	23.5±3.1	126±6	49.3±4.4	1.5±1.0	87.8±2.9	12.7±3.2
3	2.1±0.8	19.7±2.7	64.8±3.2	28.9±1.5	1.9±0.5	67.5±3.4	10.2±5.3
4	9.6±1.9	56.1±5.8	1,066±10	369±14	20.7±8.4	270±10	82.1±8.8
5	1.9±0.6	21.9±2.6	59.2±3.7	31.1±1.9	3.2±1.2	106±6	8.9±2.5
6	2.3±0.9	25.7±2.9	80.9±6.0	35.9±2.5	1.2±0.6	248±9	9.9±2.9
7	2.8±1.3	27.1±1.7	94.5±9.1	46.8±2.8	2.8±1.1	265±11	11.2±4.1
Agricultural soils (AS)							
1	2.3±0.9	19.5±1.3	59.3±4.1	26.0±0.9	2.0±0.7	61.4±1.3	7.1±1.2
2	3.1±1.1	22.4±0.9	82.9±6.2	29.1±1.2	9.1±2.1	54.1±3.3	13.4±5.1
3	2.5±0.8	52.7±2.8	66.4±2.8	31.7±2.5	1.7±1.8	159±9.5	9.1±2.8
4	3.8±0.9	31.8±4.1	119±8	67.2±4.2	4.2±2.5	66.1±3.9	33.1±6.1
5	2.3±1.0	27.4±2.7	61.9±3.8	29.1±2.3	6.4±2.1	82.9±5.7	8.7±1.4
CRM	2.1±1.7	80.8±5.6	424±11	77.0±10	2.7±0.5	84.6±5.8	23.4±2.5
C. w	0.05±0.8	4.2±1.3	49.8±2.4	1.7±1.9	0.5±0.4	1.0±0.1	0.7±1.4
C.w/28	0.06±0.6	4.1±1.2	49.2±1.6	1.7±0.8	0.4±0.8	0.9±0.7	0.7±0.9
Low No. 220/2004-2 (mg/kg DW)							
Limit	-	70	200	115	1	90	30

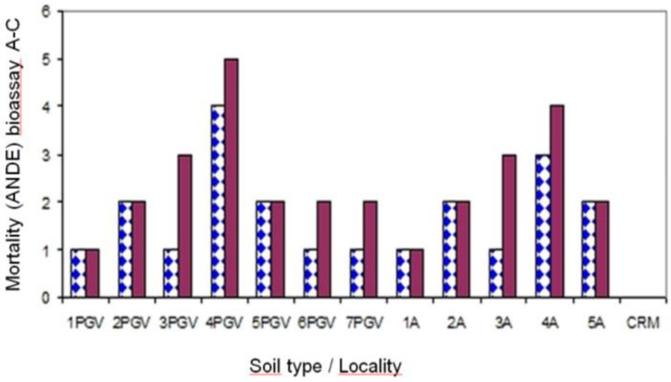

Fig. 2. Effect of metals on mortality of *D. veneta* (ANDE) after periods of 1 – 7 (chequered columns) and 1 – 28 (filled columns) days of exposure at the end of the tests (average values: $n_{/1-7}$ =2 and $n_{/1-28}$ = 3).

in the samples of A-soils at localities (4) U. S. Steel-plant west As = 30.3 mg.kg^{-1} and (3) Gomboš Cr = 152 mg.kg^{-1}, 7 d after earthworms exposure (Table 3). The results show possible to use of earthworms in the remediation of industrialized soils.

After a longer period of 28 d of exposure (Table 4) the earthworms' mortality was much higher than after 7 d. The metal concentration values recorded were higher in the corresponding soil samples, (both PGVs and AS), at simultaneous decrease of metal concentrations in the earthworms. Further research is need about this topic, it can be hypothesized that chemical extractions like NaOAc would be useful to predict the potential bioaccumulation of certain metals considering the matrix physico-chemical properties.

The values of the BAFs/7-28d are < 1 for the studied metals and order in the following sequence: Cr < Fe < Pb < Cu < As, while are > 1 for Zn > Cd (Tables 5 and 6). It is noteworthy that the BAF values in the agricultural soils are greater on day 28 when compared to values on day 7 (Tables 5 and 6). Concentrations of Zn and Cd in the earthworm tissues were higher than that of the values Cr < Fe < Pb < As < Cu, indicating that these elements could be better bio-accumulated and migrated from the studied soils to the earthworm tissues.

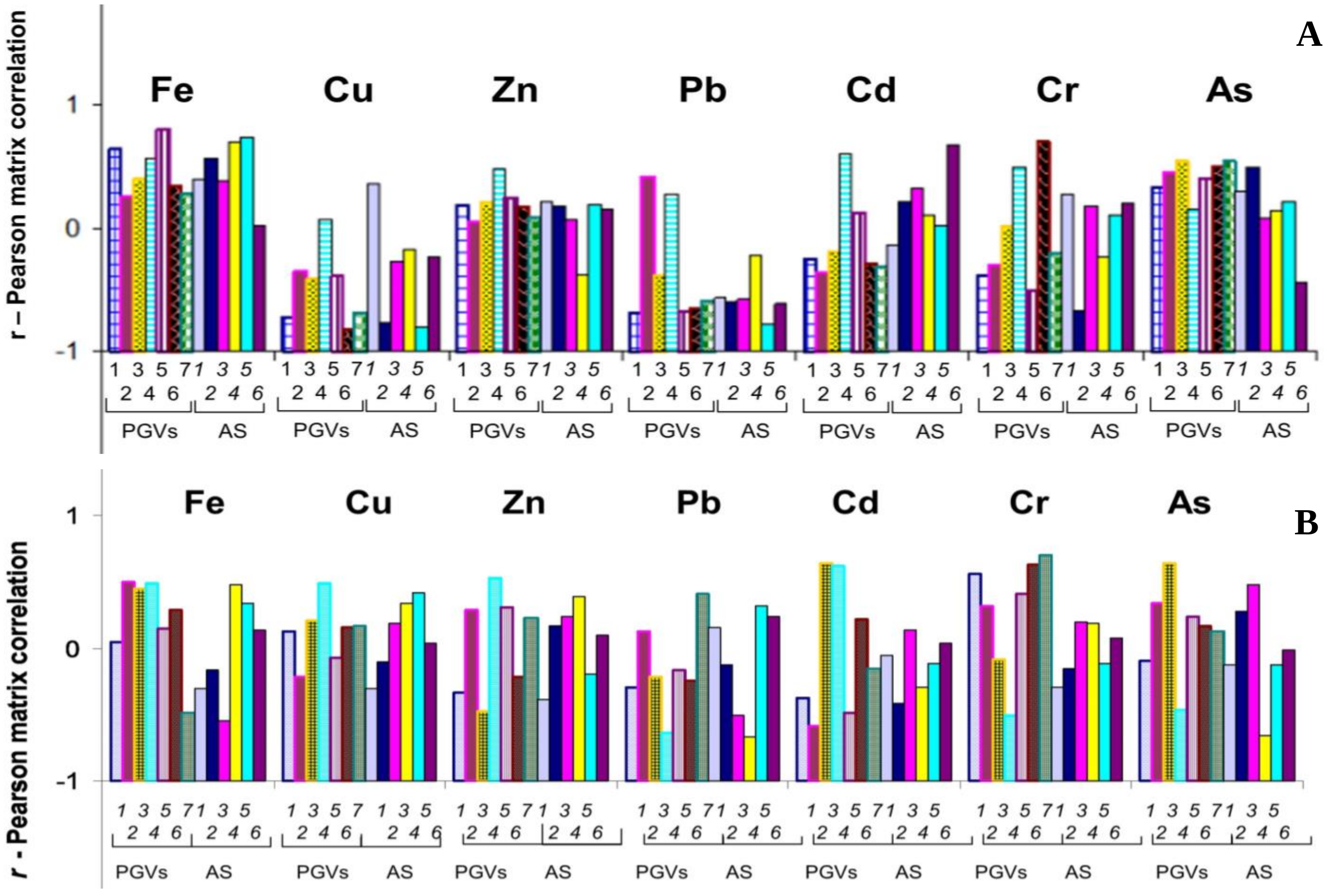

Fig. 3. Correlation between metal concentrations and mortality of earthworms after 7 d (A) and 28 d (B) exposure to soils from localities with permanent grass vegetation (PGV 1 – 7) and agricultural soils (AS 1 – 5) (CRM-6).

Table 5. Bioaccumulation factors for metals in earthworm tissues fed in AS (localities 1 – 5) and PGV (localities 1 – 7) (DW) in surrounding of Košice after 7 d bioassays.

Locality	Fe	Cu	Zn	Pb	Cd	Cr	As
Permanent grass vegetation soils (PGVs)							
1	0.06	0.86	1.35	–	0.95	0.04	0.41
2	0.06	0.29	0.66	–	1.56	0.04	0.25
3	0.22	0.41	1.37	–	0.79	0.11	0.57
4	0.07	0.15	0.12	0.03	0.19	–	2.14
5	0.16	0.82	2.33	0.01	0.36	0.03	0.26
6	0.14	0.27	1.16	0,01	0.92	–	0.22
7	0.16	0.25	1.9	0,02	0.56	0.02	0.24
Agricultural soils (AS)							
1	0.13	0.23	1.41	–	0.47	0.02	0.21
2	0.11	0.27	1.7	–	0.22	0.03	0.13
3	0.14	0.26	1.33	–	0.44	–	0.11
4	0.08	0.21	0.79	–	1.65	–	0.19
5	0.09	0.24	1.63	0.08	0.21	–	0.01
CRM	0.03	0.05	0.18	0.01	0.07	–	–

Table 6. Bioaccumulation factors for metals in earthworm tissues fed in AS (localities 1 – 5) and PGV (localities 1 – 7) (DW) in surrounding of Košice after 28 d bioassays.

Locality	Fe	Cu	Zn	Pb	Cd	Cr	As
Permanent grass vegetation soils (PGVs)							
1	0.02	0.61	1.53	0.06	0.77	0.03	0.58
2	0.03	0.45	0.75	0.05	1.20	0.03	0.37
3	0.03	0.42	1.37	0.05	0.98	0.04	0.68
4	0.00	0.19	0.15	0.01	0.10	0.03	0.10
5	0.09	0.46	3.3	0.09	0,29	0.04	0.82
6	0.04	0.35	1.19	0.08	0.65	0.02	0.57
7	–	0.30	1.6	0.03	0.30	0.01	0.30
Agricultural soils (AS) dw							
1	0.15	0.49	1.42	0.08	1.10	0.09	1.11
2	0.09	0.39	1.9	0.10	0.29	0.12	0.75
3	0.14	0.17	1.32	0.07	0.51	0.04	0.90
4	0.07	0.27	0.82	0.08	1.90	0.10	0.36
5	0.12	0.33	1.64	0.09	0.34	0.07	0.80
CRM	0.05	0.07	0.20	0.01	0.42	0.04	0.25

Previously, efficient regulation of Zn uptake level has been demonstrated in some organisms by mechanism of either increased exclusion or decreased uptake from environmental media (Heikens *et al.* 2001; Demuynck *et al.* 2007; Leitgib *et al.* 2007; Tang *et al.* 2013). According to Coelho *et al.* (2018) Zn is probably the most likely metal that often limits earthworm populations. The study indicates that earthworms have efficient potential for bioaccumulation of metals in their tissues which can be used as an ecological indicator of soil contaminations.

Pearson matrix correlation coefficients can point on relation between toxicity to organisms and metal concentration in the soils (Shuai *et al.* 2019). Correlation between mortality of earthworms (ANDE) after 7 and 28 d exposure to metals in soils (respectively) were calculated (Fig. 3A, B). The results show the possible positive correlation between mortalities of earthworms and Fe concentrations after 7 d (r = 0.25 to 0.80 at individual localities). Lack of correlation was identified for concentration of Zn, As and Pb, and the lowest values were detected in case of Cd and Cr after 7 d bioassays (Fig. 3A). According to the correlation coefficients, the metal contamination level in the soils followed the sequence: of PGVs at localities (5) > (1) > (4) > (3) > (6) > (7) > (2) and AS at localities (5) > (4) > (2) > (1) > (3). After 28 d exposure of earthworms to metals in soil samples (Fig. 3B) we revealed possible positive correlation between earthworms mortality and metal concentrations in the order Cd > Cr > Fe > Zn > Cu. The high concentrations of metals have no significant effect on the mortality of earthworms; our bioassays results exhibited high earthworms tolerance to contaminated soils probably because of distinct detoxification mechanisms developed.

Conclusions

Local industry located in the region influences the quality of the environment, including soils. The Košice area, in addition to typical urban contamination sources is long-term environmental loaded by the iron and steel. The total concentrations of selected metals as well as ecotoxicological impact of industrialized soils from Košice area were determined and revealed significant contamination with Fe, Cd, Cr and As. For certain metals, significant concentration differences in time were recorded in bioassays for the soils sampled from both permanent grass vegetation (locality at U. S. Steel-plant-main gate) and agricultural fields (U. S. Steel-plant west and Gomboš) suggesting that earthworms can remove part of polluting metal from samples. The BAFs indicated positive correlation between toxicity and concentration of metals for Zn > Cd. Correlation has been found between mortality and certain metals as well. The obtained results may provide a baseline data for soil (contamination) condition and information about ecosystem in urban regions subjected to emission of industrial contamination sources.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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