



Ecotoxicity Findings in Agricultural Soils of the Suburban and Industrial Areas of Košice

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Abstract

The input of anthropogenic external pollutants is the main cause of environmental problems in agricultural soil. The main objective of the present study was to evaluate the potentially ecotoxicity of heavy metals in suburban and industrial agricultural soils from Košice, using biological tests (Phytotoxicity on *Sinapis alba*, acute and avoidance tests with the *Dendrobaena veneta*). The agricultural areas were selected, namely soils around the Košice, is exposed to suburban and industrial contamination sources such as municipal sphere (domestic waste), road traffic, coal burning and various industrial sources (U.S.Steel, TEKO). The total Fe, Mn, Cu, Zn, As, Pb, Cr, Ni and Co concentrations and ecotoxicological tests of agricultural soils around the Košice were determined in 8 sampling sites, in the year 2024. Exceeded heavy metal concentration limits according to law No. 220/2004 coll. were in order Cu > As > Cr in study soils marked as Ľahanovce village (1). The highest median concentrations were in the case of Cu approximately 8.3 times; for As (1.2 times); and for Cr (1.1) more than limit value. Similarly, exceed median values of metals in soils of the Krásna (2), were in the order Cu > Cr > Zn, for copper (7.5) times more than limit; and also high median was in the case sample Barca (3) for chromium (3.6 time) more than limit value. The obtained results of the contact arrangement phytotoxicity showed that all sample soils around Košice (1 - 8) had less than 50% inhibition of the seed germination and root growth in the mustard *Sinapis alba* compared to the control soil. Low mortality effects were recorded in the acute tests with *Dendrobaena veneta*. The percentage of avoidance response of the earthworms found in the double control was within the range -40 % to 90 % for the all agricultural soils around Košice. The significant ($P < 0.05$) avoidance by *Dendrobaena veneta* were 90% in soils from Gomboš (5) and from main Plant west of the U. S. Steel (7), Košice. Results indicate that the terrestrial pollution mainly comes from the vicinity of U.S. Steel Košice and agricultural activities have a certain impact and motor vehicle exhaust act too. Environmental monitoring and pollution assessment on the whole region are highly important to figure out the contamination level and spatial distribution of pollution.

Keywords: Heavy metals, agricultural soils, phytotoxicity, avoidance test, pollution index

1. Introduction

The increase in metal concentrations in agricultural soil severely threatens the environment. This is a serious worldwide problem owing to their toxicity [1]. Consequently, health of suburban and industrial areas residents are affected by this deteriorated environment. By the authors, those potentially toxic metals in agricultural soils, particularly As, Pb, Cr, Ni and Co, pose significant threats to human health and the environment [2–3]. As a neutral attribute of suburban activities, surface soil is the primary sink of all pollutants, including potential toxic metals (PTMs) [4]. Both natural processes, such as the weathering of rocks, and human activity, such as industrial emissions, motor vehicle exhaust, mining, and inappropriate waste management, can release these toxic elements into the environment. Also, it has been established that brake and tire wear is a primary cause of toxic metal contamination in areas by the authors adjacent to road traffic [5]. Heavy metals have a specific importance in ecotoxicology owing to their long bio-accretion in the food chain and they are non-degradable and unceasingly augmenting in the environment [6]. The main objective of this work was support the interpretation of the eco-toxicological data and to enhance our understanding of potential ecological risk for suburban and industrial agricultural soils, namely soils around Košice. The impact of PTMs (Fe, Mn, Cu, Zn, As, Pb, Cr, Ni and Co) on human health varies depending on the particular element, its concentration, the length of exposure, and each person's vulnerability [7]. In recent decades, with the emergence of various chemical pollutants and their entry into the food chain, concerns about the increase in disease have increased; together with other variables has increased risk of cancers. The soils with lower (sub-lethal) pollutant concentrations require more sensitive test methods such as behavioral tests (Avoidance behavior response) in their risk assessment [8-9]. An earthworm avoidance test has potential advantages for study of evaluation of hazardous soils sites and proved as a quick approach to determining the presence of contaminants. The earthworm (*Dendrobaena veneta*) takes up and retains metals from soil containing of potential toxic elements. Earthworms are widely recognized for their ability to enhance the decomposition of organic matter and play a critical role in nutrient turnover. Moreover, these key soil organisms are influenced by abiotic soil components such as organic matter [10-12]. Phytotoxkit is an alternative test procedure that enables determination of the biological effects of chemical compounds on plants. Assessment of soil phytotoxicity is based on germination and seedling growth of the terrestrial plant (mustard *Sinapis alba*) [13]. The purpose of this study was to evaluate the ecological risk related to the heavy metals in suburban and industrial soils around Košice, using biological tests (Phytotoxicity on *Sinapis alba*, acute and avoidance tests with the *Dendrobaena veneta*).

2. Material and methods

2.1 Soil sampling and laboratory analysis

Samples of subsoil (10–30 cm) marked as 1 - 8, were taken from the Košice industrial locations, in the year 2024 (table 2). The agricultural soils were from the localities: 1/ Ľahanovce village-north, north, near the former Slovak magnesite plant (SMZ), 2/Krásna, tailings pond-south, 3/ Barca, behind the airport of Košice, 4/ Hýľov garden, 5/ Gomboš, 6/ surrounding U.S. Steel, slag heap, 7/ surrounding U.S. Steel, plant west an 8/ Hýľov – background (BG), (table 2). As contaminated ecosystem was choose surroundings of Košice, Slovakia, and as a control, was used the relatively undisturbed ecosystem in the Hýľov (agricultural soil) situated near Košice, Slovakia, where relatively unpolluted biotopes might be expected. Control and contaminated ecosystems, were approximately 17 km apart. Total concentrations of metals: Fe, Mn, Cu, Zn, As, Pb, Cr, Ni, Co, were determined by the X-ray fluorescence spectrometry method (SPECRO XEPO 3). The certified-reference soil Eutric Cambisols S-VM was used to validation of data.

2.2. Phytotoxicity tests:

The phytotoxkit measures the decrease (or the absence) of the seed germination and growth of the roots after 3 days (at 25°C) of seeds exposure of higher plants to contaminated matrix in comparison to the control soil [13]. Ten seeds of plant (*Sinapi alba*) were positioned at the test plate on a filter paper placed on top of the soil. The analyses and the length measurements were performed using the Image Tool 3.0 for Windows. The bioassays were performed in six replicates. The percent inhibition of seed germination (ISG) and root growth (IRG) was calculated according to the equation 1:

$$ISG / IRG (\%) = \frac{(A - B)}{A} \times 100 \quad (1)$$

where A is the mean seed germination or root length in the control soil; and B is the mean seed germination or root length in the test soil (mm).

2.3. Avoidance and Acute tests:

The avoidance test with *D. veneta* has numerous advantages (short test period, comparatively lower work expense, and good sensitivity). Earthworm (*D. veneta*) avoidance tests were based on ISO 17512-1/2008 [11]. The test containers were incubated at 20±2°C and a 16:8 h light: dark photoperiod for 48 h. The tests were run in 5 replicates. At the end of the test period the earthworms were counted in each side of the replicates. The amount of earthworms counted was converted to a percentage of avoidance based on equation 2:

$$R (\%) = \frac{[(C - T)]}{N} \times 100 \quad (2)$$

where R = avoidance; C = number of worms in control soil (CRM); T = number of worms in each soil; N = total number of worms.

The used control reference soil (CRM, MicroBioTest, 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 7 day acute tests were based for the testing of chemicals related to environmental fate, and mortality. Ten earthworms were added to each plastic box with 100 g of dry soil sample. The boxes with soils were kept at laboratory temperature for 7 days. The mortality of earthworms in soils (replicated tests I-V) was determined and compared with the data from control reference soil.

2.4. Ecological risk assessment

For ecological risk assessment was used with the equation 3:

$$Pi = \frac{Ci}{Si} \quad (3)$$

where Pi is the environmental quality index of pollutant i in the soil; Ci = concentration of pollutant i in the soil (mg/kg) and Si is background value [5]. The classification standard of the environmental quality index:

$$\begin{aligned} Pi \leq 1 & \text{ means non-contamination} \\ 1 < Pi \leq 2 & \text{ means slight contamination} \\ 2 < Pi \leq 3 & \text{ means medium contamination} \\ Pi > 3 & \text{ means heavy contamination} \end{aligned}$$

All data were analyzed using StatSoft, v.12.0 statistical software [14]. Data from avoidance behavior response tests (*D. veneta*) and toxic metal concentrations were analyzed using the Pearson's matrix correlation.

3. Results and discussion

3.1. Soil properties

In study area according to the Slovakia soil types [15] the soil types were established as Litozems. The values of the soils reaction pH (KCl/H₂O), oxidation-reduction potential (Eh), organic portion, and grain size (%) measured in the Košice suburban and industrial areas are listed in table 1. The research showed that the all investigated soils are neutral to slightly alkaline (pH 6.54 to 8.20). Table 1 shows that dust particles as well as mild silt are the most represented in samples that had the more sand particles. Organic matter (OM) of the soils ranged from 4.09 to 7.33 % and oxidation-reduction potential (Eh) of soils ranged from 422 to 522 mV, according to table 1. Basic statistical parameters such as minimum, maximum and median of the metal values were carried out (table 2).

Tab. 1. Selected properties of soil samples from Košice.

	1	2	3	4	5	6	7	8 BG
pH/KCl	7.46	7.13	6.54	7.44	7.33	6.89	7.44	5.75
pH/H ₂ O	8.20	7.93	7.81	8.08	7.96	7.74	7.80	7.08
Eh(mV)	451	481	519	424	522	441	422	550
OM(%)	4.13	7.32	6.87	7.33	4.99	4.38	4.09	6.78
Grain size (%)								
Sand	39.32	19.21	14.93	22.79	45.30	42.44	25.01	25.38
Silt	53.59	70.62	71.65	69.37	32.53	33.79	38.60	66.45
Clay	7.09	10.16	12.43	7.95	22.17	23.77	36.39	8.16

pH: KCl/H₂O (ISO/DIS10390), OM (%) Organic matter dry weight (STN EN 12879),
(Eh) Oxidation–reduction potential,
Grain size: Sand (50 µm – 2 mm), Silt (10 - 50 µm), Clay (< 2 µm).

The obtained data shows the wide ranges of the concentration of metals (table 2). Exceeded heavy metal concentration limits according to law No. 220/2004 coll. [22] were in order of Cu > As > Cr in study soils of the marked as Ťahanovce village (1). The highest median concentrations were in the case Cu approximately (8.3 times); for As (1.2 times); and for Cr (1.1 times) more than limit value. Similarly, exceed median values of metals in soils of the Krásna (2), were in the order of Cu > Cr > Zn, for copper of (7.5 times) more than limit; and also high median was in the case of sample Barca (3) for chromium (3.6 time) more than limit value.

Road transport can significantly affect the accumulation of potential metals in soil, such as the suburban and industrial agricultural soils. Traffic seems to be one of the sources of these metals also, but the influence of other factors cannot be excluded (iron and steel plants – U.S. Steel, Košice and TEKO). Also, the heavy metals can leach into the surrounding soils through rainwater runoff and dust dispersion, there by increasing the concentration of corresponding heavy metals in the soil. Improper handling or storage of tailings can lead to environmental issues, including contamination of nearby agricultural lands. Dust emissions from exposed tailings piles can also contribute to atmospheric deposition, further exacerbating soil pollution [16 -18].

Tab. 2. Statistical analyses of metals and mortality after 7-days bioassay in soils around of Košice.

Soils N=25 (0-30cm)		Fe	Mn	Cu	Zn	As	Pb	Cr	Ni	Co	Mor. 7 D. (%)
		(%)		(mg/kg)							
1 Ťahanovce	Min	3.30	0.10	150.00	143.00	37.00	37.00	95.00	55.00	19.00	1
	Max	3.00	0.13	172.00	153.00	44.00	39.00	102.00	57.00	26.00	4
	Median	3.10	0.12	166.00	150.00	40.00	38.00	99.00	56.00	23.00	2
2 Krásna	Min	3.40	0.11	141.00	140.00	16.00	39.00	87.00	64.00	21.00	1
	Max	3.60	0.13	152.00	147.00	18.00	42.00	115.00	66.00	29.00	3
	Median	3.50	0.12	150.00	144.00	17.00	41.00	95.00	65.00	25.00	2
3 Barca	Min	2.80	0.07	28.00	68.00	9.00	26.00	259.00	51.00	11.00	1
	Max	3.10	0.10	45.00	75.00	10.00	31.00	346.00	55.00	13.00	4
	Median	3.00	0.09	34.00	70.00	9.50	29.00	320.00	52.00	12.00	3
4 Hýľov garden	Min	2.70	0.11	57.00	98.00	14.00	20.00	82.00	43.00	14.00	0
	Max	3.00	0.12	59.00	102.00	16.00	22.00	90.00	46.00	17.00	2
	Median	2.80	0.11	58.00	101.00	15.00	21.00	88.00	44.00	16.00	1
5 Gomboš	Min	2.60	0.08	54.00	68.00	11.00	33.00	155.00	55.00	22.00	1
	Max	2.80	0.10	59.00	73.00	13.00	35.00	161.00	57.00	32.00	4
	Median	2.70	0.09	56.00	70.00	12.00	34.00	158.00	56.00	28.00	3
6 Slag heap U.S.S	Min	3.40	0.08	29.00	84.00	16.00	30.00	65.00	51.00	30.00	1
	Max	3.70	0.10	36.00	91.00	19.00	32.00	78.00	53.00	32.00	3
	Median	3.50	0.09	34.00	88.00	18.00	31.00	70.00	52.00	31.00	2
7 Plant west, U.S.S.	Min	3.60	0.12	33.00	121.00	35.00	69.00	69.00	59.00	28.00	2
	Max	3.90	0.14	39.00	130.00	42.00	75.00	74.00	62.00	31.00	5
	Median	3.80	0.13	35.00	128.00	40.50	72.00	72.00	61.00	30.00	4
8 Hýľov -BG	Min	2.50	0.10	31.00	84.00	14.00	38.00	42.00	23.00	21.00	0
	Max	2.80	0.11	37.00	89.00	17.00	40.00	49.00	31.00	23.00	1
	Median	2.60	0.09	34.00	86.00	15.00	39.00	45.00	28.00	22.00	0
LV		-	0.30	20	140	33	115	90	60	20	

(LV) Limit value of SR Law 220/2004, Mor.7 D. mortality after 7-days bioassay, ANDE-abs. number of dead *D.veneta*

3.2. Data of Phytotoxicity tests

The obtained results of the contact arrangement phytotoxicity (Fig. 1 - 2) showed that the all agricultural soil samples 1-8 (9 - 45 %) had less than 50% inhibition of the seed germination and root growth in the mustard *S. alba* compared to the control soil. The acute toxicity classes was used to estimate the soil toxicity: ATC (percent acute toxicity) < 20% no acute toxicity (class I), 20% < ATC < 40% slight acute toxicity (class II), ATC < 50% acute toxicity (class III), ATC < 100% (class IV) high acute toxicity; ATC > 100% (class V), very high acute toxicity [19 - 21]. The potential acute toxicity (ATC) associated with heavy metals due contamination of agricultural soils were in the order: 8 BG < 4 Hýľov < 6 U.S.S < 2 Krásna < 7 U.S.S < 5 Gomboš < 1 Ťahanovce < 3 Barca. The concentrations of Cu, As, and Cr were higher in Ťahanovce (1) and also other municipalities with more intense flows of vehicles (Barca (3), Gomboš (5) and Plant west, U.S.S. (7)). These soils were classified to ATC < 50% acute toxicity (class III), the soils from the sites Hýľov garden (4) and 8 (BG) were in (class I) < 20% no acute toxicity; and soils from the sites Krásna (2) and Slag heap U.S.S (6) were in < 40% slight acute toxicity (class II). The transfer of potential toxic metals within the soil-plant chain is a part of the biochemical cycling of metals, and it is a metal flow from nonliving to the living compartments of the biosphere. There

are many factors that might have an impact on this plant response. Since metals with similar physico-chemical properties may replace one another in enzymatic pathways and receptor proteins, they compete for absorption, transport, and accumulation.

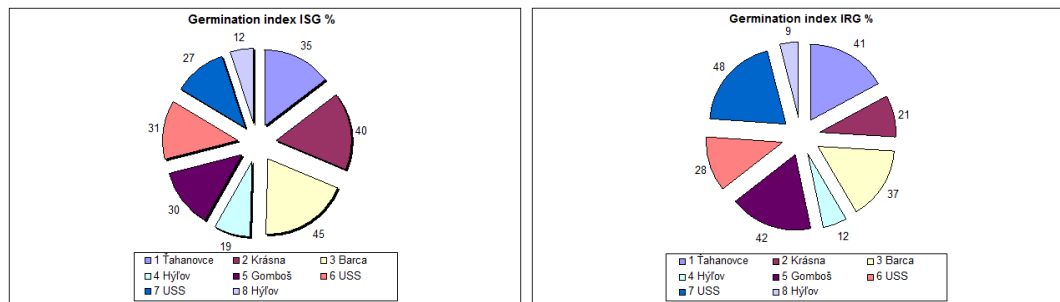


Fig. 1. - 2. Average germination indexes ISG / IRG values in the suburban and industrial soils of Košice.

3.3. Data of Acute and Avoidance tests

A low mortality (1 - 3%) was observed in all of 7 days acute tests, except of the sample (7) plant west U.S.Steel (was 4%). Acute effect of metals on mortality of *D.veneta* (ANDE) after period of 7 days of exposure (table 2), at the end of the tests showed that the earthworms mortality was potential allow influenced by industrialized soils ((west of factory U.S.Steel, Gomboš and Barca). The Košice area, in addition to typical urban contamination sources is long-term environmental loaded by the iron and steel. Road transport can significantly affect the accumulation of potential toxic metals in soil and plants, such as suburban and industrial soils of Košice (Tahanovce - train transport, Krásna - field behind the tailings pond and Barca - field behind the airport of Košice). Traffic seems to be one of the sources of these metals also, but the influence of other factors cannot be excluded. Copper-based fungicides contribute to Cu accumulation in agricultural soils, furthermore, phosphate fertilizers may contain minor amount of Pb [5, 16].

The earthworm (*Dendrobaena veneta*) takes up and retains metals from soil containing of heavy metals. No mortality was recorded in the avoidance tests with *D. veneta*. The percentage of avoidance response of the earthworms found in the double control was within the range -40 % to 90 % for the all agricultural soils around Košice, after 48h (Fig. 3). The significant avoidance by *D. veneta* was 80 - 90% in soils of areas Gomboš and from Plant west of the U. S. Steel, Košice. Positive values account for avoidance of earthworms in test soil, while neutral or negative responses represent indifference or preference of the test substance, such as in the sample soil of Hýľov background (8 BG) – 40%. Results showed that heavy metals are toxic in different concentration ranges for these organisms.

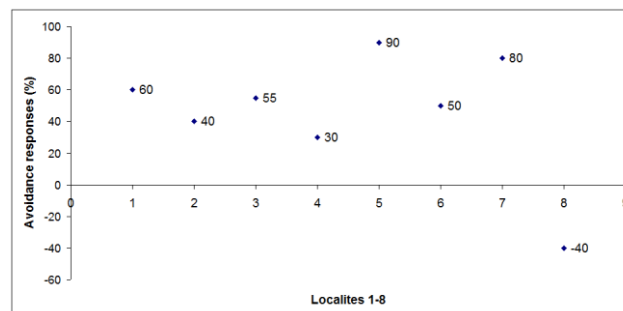


Fig. 3. Avoidance responses of *D. veneta* in suburban soils of Košice.

The highest correlation coefficients among percentage of avoidance responses (*D. veneta*) and toxic metal concentrations (figure 3 and table 3), which ranged between -0.59 and 0.42, decreased in the order Cr>Fe>As>Co for the soils: (7) Plant west U.S.Steel of the Košice. In addition, Ni, Co and As was significantly positive and negative correlated ($r=0.48$, -0.59 and -0.47, $p=0.05$) with avoidance responses (*D. veneta*) for the sample soil (5) Gomboš. Also, the samples (1-Tahanovce, 2-Krásna and 3-Barca) had the high correlation coefficients for Co>Cr>Pb>As>Cu>Mn>Zn.

Avoidance behavior offers a highly relevant information as it reveals the ability to avoid (or not) possible toxic compounds in the field, hence it provides information about reasons for the presence/absence in the field [8, 11].

Tab. 3. Correlation matrix of the agriculture soils in Košice, among percentage of avoidance responses (*D. veneta*) (Fig. 3) and metal concentrations (table 2).

Soils	Fe	Mn	Cu	Zn	As	Pb	Cr	Ni	Co
1	0.17	0.46*	0.47*	-0.45*	-0.04	0.23	-0.13	0.34	0.39
2	0.02	0.28	0.33	0.32	-0.48*	0.51*	0.23	-0.03	0.60*
3	0.14	0.05	0.32	0.28	0.22	0.55*	-0.58*	-0.31	0.20
4	0.51*	0.12	0.53*	0.17	-0.23	-0.17	0.31	0.07	0.25
5	0.27	0.15	-0.15	0.16	-0.47*	0.41	-0.34	-0.59*	0.48*
6	-0.18	-0.20	-0.07	-0.53*	-0.09	0.06	-0.21	-0.28	-0.36
7	-0.48*	0.13	0.15	0.18	-0.43*	-0.32	-0.59*	0.30	0.42*
8 BG	0.24	0.08	0.25	0.03	0.25	0.22	-0.03	0.31	0.05

*Correlations are significant at over $p=0.5$ and - 0.5 levels

3.4. Data of Ecological risk assessment

On the basis of the single factor pollution index (P_i) (table 4) it was found that the contamination of samples by heavy metals is as follow: $Cr < Ni < Cu < As < Zn < Co < Mn < Fe < Pb$. The potential ecological risk was means heavy contamination for Cr (risk factor of 7.11 for the Barca (3) and 3.51 for the Gomboš (5)); results indicate that probable the terrestrial pollution also comes from the plant of U.S. Steel Košice. According to pollution factor it is evident that sampling sites Ťahanovce (1), Krásna (2) and Plant west, U.S.S. (7) was the slight-medium contaminated mainly with Cu, Cr, Ni and As. In the case Fe, Mn, Zn, Pb and Co there was slight contamination also non-contamination in all studied samples. This assumption was confirmed by XRF spectrometry as it is seen from table 2.

Tab. 4. The single factor pollution index (P_i) of heavy metals in the suburban and industrial soils of Košice.

(P_i)	Fe	Mn	Cu	Zn	As	Pb	Cr	Ni	Co
1	1.19	1.33	4.88	1.74	2.67	0.97	2.20	2.00	1.05
2	1.35	1.33	4.41	1.67	1.13	1.05	2.11	2.32	1.14
3	1.15	1.00	1.00	0.81	0.63	0.74	7.11	1.86	0.54
4	1.08	1.22	1.71	1.17	1.00	0.59	1.96	1.57	0.73
5	1.04	1.00	1.65	0.81	0.80	0.87	3.51	2.00	1.27
6	1.35	1.00	1.00	1.02	1.20	0.79	1.56	1.86	1.41
7	1.46	1.44	1.03	1.49	2.70	0.91	1.60	2.18	1.36

4. Conclusion

In recent years, heavy metals pollution has become prominent issues in the field of agricultural soil contamination. This research endeavoured to assess the impact of potentially ecotoxicity in industrial agricultural soils from the Košice, using tests of the Phytotoxicity on *Sinapis alba*, acute and avoidance tests with the *Dendrobaena veneta*, collected in the 2024 year. Exceeded heavy metal concentration limits were in order of $Cu > As > Cr$ in study soils marked as Ťahanovce village (1). The potential acute toxicity (ATC) associated with heavy metals due contamination of agricultural soils were in the order: $8\text{ BG} < 4\text{ Hýľov} < 6\text{ U.S.S} < 2\text{ Krásna} < 7\text{ U.S.S} < 5\text{ Gomboš} < 1\text{ Ťahanovce} < 3\text{ Barca}$.

The concentrations of Cu, As, and Cr were higher in Ťahanovce (1) and also other municipalities with more intense flows of vehicles (Barca (3), Gomboš (5) and Plant west, U.S.S. (7)). The results revealed that the potential ecological risk was means heavy contamination for Cr (risk factor of 7.11 for the Barca (3) and 3.51 for the Gomboš (5)); results indicate that probable the terrestrial pollution also comes from the plant of U.S. Steel Košice. According to pollution factor it is evident that sampling sites Ťahanovce (1), Krásna (2) and Plant west, U.S.S. (7) were slight - medium contaminated, mainly with Cu, Cr, Ni and As. In the case of Fe, Mn, Zn, Pb and Co there was slight contamination also non-contamination in all studied samples. This study could contribute to environmental and public health protection in the Košice region.

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