Mobility Behavior and Environmental Implications of Trace Elements Associated With Suburban Soils from the Steel Industry

Olga Sestinova ¹, Jozef Hanculak ¹, Tomislav Spaldon ¹, Gesperova Danka ¹
¹ Slovak Academy of Sciences, Institute of Geotechnics, Watsonova 45, Košice 04 001, Slovak Republic
sestinova@saske.sk

Abstract. Our work was aimed on the study of mobility of steel trace elements (STEs), using single-step extraction procedure (Na₂EDTA) and ecotoxicity in suburban soils from the Košice, Slovakia. The total element contents were determined by X-ray fluorescence (XRF) analysis, Zn, Pb, Cd, Ni, and As in extracts and elements contents in earthworms tissues by the atomic absorption spectrometry (AAS) after 7 days bioassays (chronic tests of mortality). An ecotoxicological study was performed with earthworms (Dendrobaena veneta) for the screening evaluation of element mobility of urban grass and agricultural soils samples. Elements concentrations (Zn, Pb, Cd, Ni and As) in the suburban soils of Košice, indicating the significant contamination with Cd, and As were measured at sites located south of the ironworks. From the results of Pearson correlation analysis of yields, it can be seen that Pb - Zn, Pb, Cd, Ni and As in extracts and elements contents in earthworms tissues by the atomic absorption spectrometry (AAS) after 7 days bioassays (chronic tests of mortality). An ecotoxicological study was performed with earthworms (Dendrobaena veneta) for the screening evaluation of element mobility of urban grass and agricultural soils samples. Elements concentrations (Zn, Pb, Cd, Ni and As) in the suburban soils of Košice, indicating the significant contamination with Cd, and As were measured at sites located south of the ironworks. From the results of Pearson correlation analysis of yields, it can be seen that Pb - Zn, Cd, As; Cd - Zn, As; and As - Zn have obvious correlation, and the correlation between chemical parameters and nickel is not relevant.

1. Introduction
Steel trace elements constitute a significant part of soil contaminants, which, at some concentrations, may be toxic to the urban ecosystem. Total concentration of elements might could be suitable as useful indicators for assessment of soil contamination. However, the total concentration of elements is not a suitable indicator of mobility, bioavailability, or toxicity. Element characteristics are mainly depending on the different chemical forms of binding between trace elements and solid phases of the samples [1]. The study of investigate the trace element availability and mobility in soils, a number of extraction methods have been extensively used [2, 3, 4]. The application of the single-step extraction procedure (with Na₂EDTA) is very interesting from a time-saving point of view, and as the first screening information about the most mobile portions (trace elements) in the soil samples. The single-step extractions into the strong chelating agents (e.g. Na₂EDTA) are suitable for the evaluation of mobile and potentially mobile element contents.

Košice has about 239 000 inhabitants and is located in the Košice basin, in the river valley Hornád in the eastern part of Slovakia (Europe). The technology of steel production has a significant impact on the urban environment. Annual raw steel production capability is 4.5 million metric tons. Also, the agricultural and industrial activities contribute to contamination of the soil in this region [5,6].

Many authors have shown that bioassays provide a general indication of metal toxicity in soils [7-8]. Earthworms play important role in soil ecotoxicological risk assessment [9] as the metal content in earthworm tissues is partly dependent on the metal content of soils. The activity of earthworms can be
affected by exposure to contaminants. These tests can be successfully used to assess the trace element mobility on soil pollution, and to understand the ecological risk, transport processes of elements and the correlations with the soil characteristics. The aim of this paper was the investigated of element mobility in soil collected from around the plant U. S. Steel Košice by using a single-step extraction; to used bioassays of earthworms (*Dendrobaena veneta*) to evaluate toxicity of soil, and to analyze a possible relationship between observed toxicity and mobility. The obtained information may provide better understanding of environmental risks of steel trace elements in urban soils.

2. Material and methods

2.1. Material

2.1.1. Study area
Urban grass soils (UGS) and agricultural soils (AS) were collected in the Košice suburban, in surrounding of U. S. Steel Košice, which is located about 10km south-westwards from the centre of Košice. Soils were taken in 2016 - 2017. In these samples the pH was measured. Then the representative samples marked as Ke-01-12 (Table 1) were dried at laboratory temperature, quartered and sieved for chemical and earthworms bioassay. Urban grass soils (UGS) were from the following localities: Ke-01 Perín, Ke-02 U.S.Steel-slag heap, Ke-03 Gomboš, Ke-04 U. S. Steel-plant (main gate), Ke-05 Pereš-south, Ke-06 U.S.Steel-north-west and Ke-07 U.S.Steel- north-eastern. The five samples of agricultural soils (AS) were from the localities: Ke-08 Perin, Ke-09 U.S.Steel-slag heap, Ke-10 Gomboš, Ke-11 U.S.Steel-plant west and Ke-12 Pereš-south. The soils were sampled at a depth of 20 – 40 cm into plastic bags.

2.1.2. Bioassays with *Dendrobaena veneta*

The earthworms were purchased from a local supplier. Three replicates were performed for each test (100 g of dry weight sediment) with 10 earthworms. The distilled water was added to each box for the purpose of obtaining 30% moisture of sediment. After that, the boxes with soils were kept for 7 days at room temperature in dark room. The similar results with earthworms bioassay we obtained in our previous works [10, 11].

2.2. Methods

2.2.1. Analysis of single-step extraction

A single-step extraction with 0.05 mol L-1 Na$_2$EDTA [10] was used for the soils from the studied area. The single-step extractions into the strong chelating agents (e.g. EDTA) are suitable for the evaluation of mobile and potentially mobile element contents [12]. The solid soil/solution extraction ratio (weight/volume ratio) of 1:150 (0.05 mol L-1 Na$_2$EDTA (75 mL) was added to the soil sample (0.5 g) in a 100 mL polyethylene vessel). Tightly closed vessels were shaken for 6 h. The extract was separated by filtration and stored in a polyethylene vessel. This solution was used for the determination of the extractable portions of Zn, Pb, Cd, Ni and As in the soil samples. The total concentrations of the steel trace elements (Zn, Pb, Cd, Ni and As) and the element determinations of soil residues in soil samples were measured using SPECTRO XEPOS X-ray fluorescence spectrometer (XEPOS 3). The concentrations of trace metals after single-step extraction (Na$_2$EDTA) and after 7 days bioassay earthworms in the studied soils were measured by XRF method.

2.2.2. Analysis of bioassays and soils

The experiments were carried out in the OECD Guidelines 317 for the testing of chemicals relating to environmental fate, chronic tests. The earthworms tissue were lyophilized (at -50°C and 50Pa), then wet acid digestion (HNO$_3$/HF/H$_2$BO$_3$ (5:2:20)) to total destruction of samples was used to prepare samples for measurement by the atomic absorption spectrometry (AAS-Variant, Australia). The physico-chemical parameters of soils were determined (pH value in H$_2$O and KCL solutions (ISO/DIS 10390)), organic matter (OM) (STN EN 12879). 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).

2.2.3. Statistical analysis
The certified river sediment LGC6187 was used to validation of data. All data analysed using SPSS v.9.0 statistical software. The statistical dependence between chemical parameters (and elements yield of single-step extraction (Na\textsubscript{2}EDTA)) and total trace element concentrations were evaluated by Pearson matrix correlation. Principal correlation analysis was used comparison of the potential relationships among the data. As control, earthworms feeded in non-contaminated soil were used CRM.

3. Results and discussions
The recent years, (STEs) pollution has become a serious ecological problem. Industrial activities related to ore processing cause the main source of trace element pollution. The pollutants entering the soil gradually accumulate and form a layer (i.e. the industrialized soils) [12]. 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, about the quality of the soil).

The steel trace element total concentration of the studied soils along with their TS, pH and OM are listed in Table 1. All soils have weakly alkaline pH value (5.67-7.95) and low OM range (4.1-10.3 %). The highest concentrations of studied elements were measured at sites located south of the ironworks in their vicinity. At the main gate of the U.S. Steel-plant (locality Ke-04) high concentrations Zn, Pb, Cd and As (exceed of 5% for Zn; 3% for Pb; 21% for Cd; 3% for As) were detected for UGV soils. The highest values of elements from the areas were determined in AS- soils: U.S.Steel-slag heap (Ke-09) for Cd exceeds of 9% and Pereš-south (Ke-12) exceeds of 6.9% (Table 1).

| Soil Type | TS  | pH (H\textsubscript{2}O) | pH (KCl) | OM (%) | Zn (mg/kg) | Pb (mg/kg) | Cd (mg/kg) | Ni (mg/kg) | As (mg/kg) |
|-----------|-----|-----------------|----------|--------|------------|------------|------------|------------|------------|
| Ke-01     | UGS | 6.80            | 6.88     | 10.3   | 88.7       | 28.9       | 2.0        | 67.9       | 10.6       |
| Ke-02     | UGS | 7.18            | 7.13     | 7.1    | 156.7      | 50.1       | 1.6        | 54.1       | 13.4       |
| Ke-03     | UGS | 6.86            | 7.45     | 6.6    | 65.9       | 29.9       | 1.9        | 50.1       | 12.5       |
| Ke-04     | UGS | 6.10            | 7.83     | 5.4    | 1084       | 379.7      | 21.4       | 68.3       | 93.8       |
| Ke-05     | UGS | 7.01            | 5.67     | 4.8    | 64.4       | 30.5       | 3.3        | 52.2       | 9.9        |
| Ke-06     | UGS | 6.89            | 6.34     | 6.5    | 86.5       | 36.8       | 1.2        | 56.7       | 10.8       |
| Ke-07     | UGS | 7.71            | 7.22     | 7.8    | 95.5       | 46.3       | 2.7        | 63.0       | 12.7       |
| Ke-08     | AS  | 7.20            | 6.21     | 4.7    | 60.2       | 26.3       | 2.1        | 52.6       | 7.3        |
| Ke-09     | AS  | 7.74            | 6.99     | 4.3    | 83.8       | 30.4       | 9.1        | 52.3       | 15.9       |
| Ke-10     | AS  | 7.95            | 7.21     | 4.9    | 67.9       | 32.9       | 1.8        | 38.6       | 10.8       |
| Ke-11     | AS  | 7.80            | 7.45     | 4.1    | 121.3      | 69.3       | 4.4        | 47.6       | 34.8       |
| Ke-12     | AS  | 6.68            | 5.62     | 4.4    | 62.4       | 29.5       | 6.9        | 54.3       | 9.0        |
| CRM       |     | 6.12            | 5.93     | 4.7    | 439.2      | 77.4       | 2.7        | 34.7       | 24.0       |

Statistical results of trace elements

Type soil- TS, Limit value-LV, Urban grass soil- UGS, Agricultural soil-AS, Control soil-CS, Organic matter-OM (dry weight STN EN 12879), Standard deviation-St.Dev, Minimum-Min, Maximum-Max

|       | CS  | -    | 6.56 | 6.24 | 5.2 | 7.5 | 26.6 | 1.5 | 13.1 | 1.8 |
|-------|-----|------|------|------|-----|-----|------|-----|------|-----|
| Type  | LV  | -    | -    | -    | 200 | 115 | 1    | 60  | 30   |     |
| Min   | -   | 6.10 | 5.39 | 4.1  | 60.2 | 26.3 | 1.2  | 38.6 | 7.3  |     |
| Max   | -   | 7.95 | 7.83 | 10.3 | 1084 | 379.7 | 21.4 | 68.3 | 93.8 |     |
| Median | -   | 7.17 | 6.85 | 5.4  | 85.2 | 31.7 | 2.4  | 53.4 | 11.7 |     |
| St.Dev| -   | 0.64 | 0.78 | 1.2  | 289.5 | 99.6 | 5.7  | 8.4  | 24.3 |     |
Effect of elements on mortality *D. veneta* after 7-days of exposure at the end of the tests showed that the earthworms’ mortality was influenced by industrialized soils after exposure (average of mortality for the UGV soils range 1-4%, and for the AS soils range 1-3%). Mortality was not statistically significantly influenced. On the other hand, earthworms in some cases caused decrease of metals concentration in the soils, between 2-40% for Zn, between 1-17% for Pb, between 1-5% for Cd, between 5-30% for Ni, and between 2-20% for As for all studied soils (Table 2).

| Table 2. Element concentration in soils of U.S.Steel Košice after 7-days bioassay. |
|---------------------------------|---------|---------|---------|---------|---------|
|       | Zn/7d. (mg/kg) | Pb/7d. (mg/kg) | Cd/7d. (mg/kg) | Ni/7d. (mg/kg) | As/7d. (mg/kg) |
| Ke-01 | 49.5          | 27.9       | 1.98       | 53.1       | 7.8       |
| Ke-02 | 106.3         | 48.7       | 1.5        | 32.7       | 9.1       |
| Ke-03 | 61.2          | 28.1       | 1.9        | 42.2       | 8.7       |
| Ke-04 | 1026          | 358        | 19.5       | 48.7       | 67.3      |
| Ke-05 | 48.7          | 29.9       | 3.1        | 22.5       | 8.4       |
| Ke-06 | 74.6          | 35.4       | 1.1        | 29.8       | 9.8       |
| Ke-07 | 92.0          | 46.2       | 2.8        | 51.1       | 10.6      |
| Ke-08 | 55.8          | 25.3       | 1.9        | 33.1       | 6.1       |
| Ke-09 | 79.8          | 29.1       | 8.7        | 44.4       | 11.8      |
| Ke-10 | 67.1          | 32.0       | 1.5        | 34.8       | 8.4       |
| Ke-11 | 110           | 64.3       | 4.1        | 27.2       | 30.3      |
| Ke-12 | 62.0          | 24.7       | 5.9        | 35.8       | 57.5      |
| CRM   | 421.0         | 76.1       | 2.6        | 26.2       | 21.5      |

The concentrations of trace metals after single-step extraction (Na$_2$EDTA), after 7 days bioassay earthworms and the elements value of residual in the studied soils are shown in Table 3. In this study, the single-step extraction was applied to define toxicity and mobility (bioavailability) of five soil trace elements (Zn, Pb, Cd, Ni, and As) in surrounding of U. S. Steel Košice suburban soils. The comparison of the elements after extraction and after bioassays with residual showed the lower results of elements after extraction of 7-days bioassays (Table 3.).

The yields of the single-step extraction, after 7-days bioassay are between 5-73% for Zn, between 2.1-47% for Pb, between 1-34% for Cd, between 3-17% for Ni, and between 6.8-67% for As in studied industrialized soils, see Figure 1.-3. The maximum yields are ranged 67-73% for Zn and As. The potentially mobility of the elements in the soils was in followed sequence: Zn > As > Pb > Cd > Ni. Relative high mobility of Zn, As, moderate mobility of Pb, Cd, and minimal mobility of Ni were observed in our experimental and local geological conditions. It was confirmed that relative content of Ni and Cd are not present in mobile form which is bio-accessible to plants, but elements are bound in crystal lattices and mineral matrices.
Table 3. Relative bioassays, distribution of elements obtained by the single-step extraction, comparison of elements after extraction and after 7-days bioassays of soils extraction, and elements value of residual.

|  | Zn (mg/L) | Pb (µg/L) | Cd (µg/L) | Ni (µg/L) | As (µg/L) |
|---|---|---|---|---|---|
| Ke-01 | 0.59 | 193 | 7.0 | 453 | 60 |
| E-ex. | 0.40 | 78.6 | 1.7 | 74.2 | 27.9 |
| 7d-ex. | 0.28 | 72.7 | 1.5 | 53.1 | 25.7 |
| Residual | 0.30 | 120 | 5.5 | 400 | 34 |
| Ke-02 | 1.05 | 333 | 10 | 360 | 90 |
| E-ex. | 0.43 | 158 | 3.4 | 37.2 | 60.4 |
| 7d-ex. | 0.42 | 154 | 2.9 | 19.8 | 59.5 |
| Residual | 0.63 | 178 | 7.1 | 340 | 31.0 |
| Ke-03 | 0.44 | 198 | 12 | 334 | 84 |
| E-ex. | 0.19 | 74.3 | 1.3 | 56.8 | 16.8 |
| 7d-ex. | 0.15 | 72.3 | 0.9 | 42.2 | 18.1 |
| Residual | 0.29 | 126 | 11 | 292 | 67 |
| Ke-04 | 7.23 | 253 | 143 | 455 | 62 |
| E-ex. | 0.83 | 89.6 | 18.2 | 48.8 | 30.5 |
| 7d-ex. | 0.75 | 82.5 | 17.2 | 28.4 | 28.7 |
| Residual | 6.5 | 170 | 126 | 427 | 33 |
| Ke-05 | 0.43 | 203 | 22 | 348 | 70 |
| E-ex. | 0.13 | 67.0 | 0.9 | 32.0 | 11.8 |
| 7d-ex. | 0.10 | 60.6 | 0.8 | 22.5 | 9.6 |
| Residual | 0.33 | 142 | 21 | 325 | 60 |
| Ke-06 | 0.58 | 247 | 8 | 378 | 73 |
| E-ex. | 0.16 | 69.7 | 1.2 | 24.3 | 10.6 |
| 7d-ex. | 0.20 | 69.9 | 1.1 | 30.4 | 10.7 |
| Residual | 0.42 | 177 | 6.9 | 354 | 63 |
| Ke-07 | 0.64 | 309 | 18.0 | 420 | 65 |
| E-ex. | 0.23 | 82.5 | 0.9 | 24.7 | 10.9 |
| 7d-ex. | 0.22 | 78.9 | 0.8 | 19.4 | 11.8 |
| Residual | 0.42 | 230 | 17 | 401 | 54 |
| Ke-08 | 0.40 | 175 | 13.0 | 351 | 48.7 |
| E-ex. | 0.09 | 36.5 | 0.5 | 33.1 | 7.2 |
| 7d-ex. | 0.11 | 33.9 | 0.5 | 29.6 | 6.5 |
| Residual | 0.39 | 141 | 12 | 321 | 43 |
| Ke-09 | 0.56 | 203 | 60.7 | 349 | 106 |
| E-ex. | 0.13 | 46.4 | 0.8 | 24.4 | 12.4 |
| 7d-ex. | 0.41 | 44.1 | 0.6 | 34.8 | 9.7 |
| Residual | 0.43 | 159 | 60 | 325 | 96 |
| Ke-10 | 0.45 | 219.0 | 12.0 | 257 | 72 |
| E-ex. | 0.13 | 47.5 | 0.5 | 24.8 | 23.7 |
| 7d-ex. | 0.13 | 46.8 | 0.7 | 18.1 | 21.8 |
| Residual | 0.32 | 171 | 11 | 232 | 50 |
| Ke-11 | 0.81 | 462 | 29.3 | 317 | 232 |
| E-ex. | 0.21 | 118.5 | 1.2 | 14.2 | 46.5 |
| 7d-ex. | 0.30 | 115.3 | 1.2 | 9.7 | 45.2 |
| Residual | 0.60 | 347 | 28 | 307 | 187 |
| Ke-12 | 0.41 | 197 | 46 | 362 | 60 |
| E-ex. | 0.17 | 31.4 | 0.5 | 15.8 | 4.1 |
| 7d-ex. | 0.09 | 30.6 | 0.5 | 12.6 | 5.3 |
| Residual | 0.24 | 166 | 46 | 349 | 56 |
| CRM | 2.93 | 514 | 18 | 231 | 12 |
| E-ex. | 0.15 | 13.8 | 0.5 | 16.2 | 4.3 |
| 7d-ex. | 0.23 | 10.6 | 0.5 | 11.2 | 3.8 |
| Residual | 2.78 | 500 | 17.5 | 220 | 8 |
Figure 1. Yields of Zn and Pb in industrialized soils determination after single-step extraction and after 7-days bioassay.

Figure 2. Yields of Cd and Ni in industrialized soils determination after single-step extraction and after 7-days bioassay.

Figure 3. Yields of As in industrialized soils determination after single-step extraction and after 7-days bioassay.

The correlation between elements can reflect whether they are from the same source, and the highly correlated elements may have the same source [5]. From the results of Pearson correlation analysis (Table 4), it can be seen that Cd and Zn, Pb, As have highest obvious correlation ($r = 0.89$ to $0.90$). The results show that Zn, Pb, Cd and OM have obvious correlation (Tables 4.). The results show ($r = 0.78$ to $0.79$ for Pb and Cd, As) the relatively highest positive correlation between yield (CMY) and risk steel trace (Table 5.). Statistical correlations at the levels $P<0.05$ and $P<0.01$ were considered.
significant. Low correlation was identified for concentration of Cd, Ni, Pb, Zn, As in case pH/H₂O in Table 4-5. According to the correlation coefficients, the trace element contamination level in the studied soils was followed: As > Cd > Pb > Zn > Ni. The elements show strong correlation coefficients and it indicates the degree of urban contamination (i.e. As, Cd, Zn and Pb). It shows on the anthropogenic pollution of industrialized soils in surrounding of U. S. Steel Košice.

**Table 4.** Correlation matrix of (CM) between chemical parameters and risk steel trace elements (n=30).

|        | Zn   | Pb   | Cd   | Ni   | As   |
|--------|------|------|------|------|------|
| pH/H₂O| -0.58| -0.55| -0.49| -0.63| -0.46|
| pH/KCl | 0.46 | 0.48 | 0.30 | 0.11 | 0.54 |
| OM (%) | 0.96*| 0.97*| 0.89*| 0.42 | -0.16|
| Zn     | 0.99*| 0.90*| 0.51 | 0.96*| 0.42 |
| Pb     | 0.89*| 0.48 | 0.97*| 0.97*| 0.43 |
| Cd     | 0.43 | 0.89*| 0.96*| 0.97*| 0.42 |
| Ni     |      |      |      | 0.42 |      |
| As     |      |      |      |      |      |

*Correlations are significant at 0.05 levels; CM - elements content of single-step extraction (Na₂EDTA) / Total element content (%)*

**Table 5.** Correlation matrix of yield (CMY) between chemical parameters and risk steel trace elements (n=30).

|        | Zn   | Pb   | Cd   | Ni   | As   |
|--------|------|------|------|------|------|
| pH/H₂O| -0.48| -0.15| -0.24| -0.01| 0.15 |
| pH/KCl | -0.21| 0.39 | 0.14 | 0.31 | 0.52 |
| OM (%) | 0.73*| 0.62*| 0.70*| 0.59 | 0.49 |
| Zn     | 0.40 | 0.59 | 0.50 | 0.20 |      |
| Pb     | 0.78*| 0.67*| 0.79*|      |      |
| Cd     | 0.45 | 0.71*|      |      |      |
| Ni     |      |      |      |      | 0.48 |
| As     |      |      |      |      |      |

*Correlations are significant at 0.05 levels; CMR - elements yield of single-step extraction (Na₂EDTA) / Total element content (%)*

4. **Conclusions**

In this study, the single-step extraction was applied to define toxicity and mobility (bioavailability) of five soil trace elements (Zn, Pb, Cd, Ni, and As) in surrounding of U. S. Steel Košice suburban soils. The present investigation reported that the soil of surrounding of U. S. Steel Košice is highly contaminated by trace elements Cd and As. Relative high mobility of Zn, As, moderate mobility of Pb, Cd, and minimal mobility of Ni were observed. The better understand the mobility and environmental risk of these elements, it would be useful to determine their geochemical speciation in soils.

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