Utilization of Contact Tests for Evaluation of Agricultural Soils

Oľga Šestinová[1*)], Lenka Findoráková[2)], Jozef Hančuľák[3)]

[1*)] Department of Environment and Hygiene in Mining,Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, Košice 040 01, Slovak Republic; email: sestinova@saske.sk; https://orcid.org/0000-0003-1684-7882

[2)] Department of Environment and Hygiene in Mining,Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, Košice 040 01, Slovak Republic; email: findorakova@saske.sk; https://orcid.org/0000-0002-2287-3596

[3)] Department of Environment and Hygiene in Mining,Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, Košice 040 01, Slovak Republic; email: hanculak@saske.sk; https://orcid.org/0000-0002-0374-1555

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Abstract

A soil has been of great concern throughout the world due to increasing environmental awareness and interest in the quality and management of such soils. Košice, the city in eastern Slovakia, is exposed to typical urban contamination sources such, 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 (metallic elements) contamination in Slovakia. Five sampling sites located in the surrounding of U.S.Steel Košice (Slovakia), were selected, where almost all the agricultural soils were polluted by the metallic elements (Fe3+, Al3+, Mn2+, Cu2+, As3+). Agricultural soils toxicity was assessed with the toxicity bioassay -Phytotoxkit. Tests of limit concentrations of the elements (Fe3+, Al3+, Mn2+, Cu2+ and As3+) and Tests of soil concentration series (100-50-25-12.5%) - screening tests mustard Sinapis alba and Lepidium sativum were performed. The testing of the concentration range was performed in order to determine the values of 14d/EC50 and the possibility of comparing the ecotoxicity of metallic elements in agricultural soils (ISO 11269-2 Soil quality). Four concentrations were prepared in test plates: 12.5 - 25 - 50 - 100% soil samples. Concentration of metals in the soil samples ranged from 24400 to 39000 mg/kg for iron; 54000 to 85000 mg/kg for aluminum; 381 to 1035 mg/kg for manganese; 27 to 59 mg/kg for copper; and 7 to 36 mg/kg for arsenic. Based on the median concentration, the metals in the soils were arranged in the following decreasing order: Al3+ > Fe3+ > Mn2+ > Cu2+ > As3+. In the agricultural soils (4USS-PW) showed high contamination values for the iron with a median 35300 mg/kg, aluminum with a median 82500 mg/kg, manganese with a median 1027 mg/kg. The median level of arsenic in the soil (4USS-PW) was 34 mg/kg, this indicate higher concentration as the limit concentration is 25 mg/kg (Law No. 220/2004-2). Agricultural soils 1-3USS and 5USS showed less than 50% inhibition of the seed germination and root growth in the S.alba and L. sativum tests compared to the control, excepting of soil from 4USS plant west (values of the EC50 to 65%). Thus, the results of phytotoxicity tests were consistent with the chemical data. The rapid increase in urbanization, industrialization, human population, and traffic flow has resulted in the environment surrounding farmland ecosystems being critically contaminated by metallic elements.

Keywords: evaluation, metallic elements, agricultural soils, phytotoxkit

Introduction

Environmental pollution remains to rise at an alarming rate as a result of human activities such as urbanization, technological progress, hazardous agricultural practices, and most notably, fast industrialization, etc. [1]. In industrialized areas urban soils are highly modified and intensively managed. Soil pollution is the cause of severe soil degradation in many parts of the world. A soil has been of great concern throughout the world due to increasing environmental awareness and interest in the quality and management of such soils [2, 3]. Potentially toxicity metallic elements (PMEs) (Fe^{3+}, Al^{3+}, Mn^{2+}, Cu^{2+}, As^{3+}), which enter agricultural soils accumulate over time because they are non-biodegradable. In recent years, metal pollution has become a serious ecological problem [4]. Biological tests permit not only measurable determination of a threat (toxic effect) but also take into account the possible interactions among the particular contaminants (antagonism/synergy effect). Moreover, the use of biological tests permits estimation of threats related with the presence of so far unidentified contaminants with potentially toxic effect [5].

In this aspect, the estimation of phytotoxicity of agricultural soils is of particular importance due to its frequent utilization. Moreover, plants are essential primary producers in the terrestrial ecosystem, whereas crop yield and quality are important success criteria in agriculture. The application of phytotoxicity tests, therefore, permits not only to evaluate the applicability of soils for agricultural, but also to identify potential threats for the environment and for human health. Also, pollutants coming from other industrial branches (the technology of steel production), agricultural activities and domestic discharges cause metal pollution [6, 7, 8]. Košice, the city in eastern Slovakia, is exposed to typical urban contamination sources such as municipal sphere, road traffic, and various 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 (metallic elements) contamination in Slovakia [4, 9, 10].

Material and Methods

Five sampling sites located in the surrounding of U.S.Steel Košice (Slovakia), were selected, where almost all the agricultural soils were polluted by the metallic elements (Fe^{3+}, Al^{3+}, Mn^{2+}, Cu^{2+}, As^{3+}). The samples were from the localities: 1 Perín, 2 U.S.Steel-Slag Heap, 3 Gomboš, 4 U.S.Steel-Plant West and 5 Pereš. Soils were taken at e depth of 20cm into plastic bags, in 2019. Soil samples were homogenized dried at room temperature; sieved through 2-mm sieve opening. For soil analyses were used control reference soil (CS - MicroBioTests, Belgium). Total concentrations of metallic elements (Fe^{3+}, Al^{3+}, Mn^{2+}, Cu^{2+}, As^{3+}) were determined by the X-ray fluorescence spectrometry method (SPECRO XEPO 3). The results were based on soil dry weight. The certified-reference soil GSS4 was used to validation of data. The assessed values of metallic elements in soils were compared to limit values of Slovak soils [11]. The results of tests of limit concentrations of the metal elements were evaluated using the Polynomial FIT model. All data analysed using StatSoft, v.12.0 statistical software [12]. Statistical significance was accepted at $p \leq 0.05$. The significance level between total and limit concentrations; and between total concentrations of metallic elements in soils was calculated using the Pearson's matrix correlation.

Metallic elements phytotoxicity

Tests in the contact arrangement: Agricultural soils toxicity was assessed with the commercial toxicity bioassay—Phytotoxkit™ Test [13]. The phytotoxkit microbiotest measures the decrease (or the absence) of seed germination and of the growth of the young roots after 3 days of exposure of seeds of selected higher plants to contaminated matrix in comparison to the controls in a reference soil. Ten seeds of each plant were positioned at equal distance near the middle ridge of the test plate on a filter paper placed on top of the hydrated soil. After closing, the test plates were placed vertically in a holder and incubated at 25°C by 3 days. At the end of the incubation period, a digital picture was taken of the test plates with the germinated plants. 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 inhibition (IRG) were calculated with the formula: *ISG*/*IRG* = (A-B/A) x 100, where A is the mean seed germination or root length in the control; and B is the mean seed germination or root length in the test soil (mm). For the evaluation of ecotoxicity in the contact arrangement the following phytotoxicity test were selected: germination and growth of higher plants, *Sinapis alba* and *Lepidium sativum*. Furthermore: Tests of soil concentration series (100-50-25-12.5%) - screening tests mustard *Sinapis alba* and *Lepidium sativum*, and Tests of limit concentrations of the elements (Fe^{3+}, Al^{3+}, Mn^{2+}, Cu^{2+}, As^{3+}), were performed. To determine whether the soil has an ecotoxic effect on the organism (*S. alba*) or not, limit concentrations of metallic elements were tested.

The testing of the concentration range was performed in order to determine the values of 14d/EC_{50} and the possibility of comparing the ecotoxicity of metallic elements in agricultural soils [14, 15, 8]. Four concentrations were prepared in test plates: 12.5 - 25 - 50 - 100% soil samples, which were well mixed with standard reference soil (C- MicroBioTests, Belgium). Eight seeds of each plant were positioned at equal distance near the middle ridge of the test plate on a filter paper placed on top of the hydrated soil. After closing, the test plates were placed vertically in a holder and incubated at 22°C, the light intensity was greater than 7000Lx and the period of incubation was 16 hours light and 8 hours dark by 5 days. On day 5, when 50% of the seeds germinated in the control, the plants were irrigated. The test length from germination of 50% of the seeds in the light was 14 days. On the basis of obtained results ecotoxicity of soils from the concentration range was assessed and were determined of potentially effects of metallic elements on germination and growth of the higher plants (*S.alba* and *L. sativum*).

Tests of the limit concentrations of the elements (Fe^{3+}, Al^{3+}, Mn^{2+}, Cu^{2+} and As^{3+}) were pursued according to the modified standards [13, 15]. For the elements (Fe^{3+}, Al^{3+}, Mn^{2+}) concentration of solutions were prepared into plates of the standard reference soil: 100, 200, 300, 400, 600, 800, 1000 mg/L, and for Cu^{2+}: 10, 20, 30, 40, 50, 60 mg/L, and As^{3+}: 1, 3, 5, 10 mg/L. The test conditions were the same as for phytotoxicity testing in contact arrangement with mustard (*S.alba*) [13] and the total length of tests was 3 days. The Screening Test of Plant Germination [15] was carried out in order to confirm or refute the ecotoxic properties of the tested soils; which are recommended as a supplementary test to evaluate the ecotoxicity of soils.

Results and Discussions

Metals Concentration Levels

Physicochemical properties of the soils are listed in table 1. The soil reaction (pH/H2O and pH/KCl), oxidation-reduction potential (ORP), organic matter, and texture; and the results of the granulometric analysis published in [16], were used for the evaluation of the environmental risk. Table 1 shows that dust particles as well as mild sand fractions are the most represented in samples (1-5USS).

The pH / (KCl and H_2O) of all soil samples was in the range of 6.13-7.95 indicating the neutral to alkaline nature of agricultural soils. Organic matter (OM) of the soils ranged from 4.10 to 4.90 % and oxidation-reduction potential (ORP) of soils ranged from 422 to 490 mV, according to table 1. Basic statistical parameters such as mean, minimum, maximum, median and standard deviation of the element values were also carried out (Table 1). Metallic elements in study soils (area U.S. Steel Košice) show high variation in terms of concentration levels. Concentration of metals in the soil samples ranged from 24400 to 39000 mg/kg for iron; 54000 to 85000 mg/kg for aluminum; 381 to 1035 mg/kg for manganese; 27 to 59 mg/kg for copper; and 7 to 36 mg/kg for arsenic. Based on the median concentration, the metals in the soils were arranged in the following decreasing order: $Al^{3+} > Fe^{3+} > Mn^{2+} > Cu^{2+} > As^{3+}$. In the agricultural soils (4USS-PW) showed high contamination values for the iron with a median 35300 mg/kg, aluminum with a median 82500 mg/kg, manganese with a median 1027 mg/kg. The median level of arsenic in the soil (4USS-PW) was 34 mg/kg, this indicate higher concentration as the limit concentration is 25 mg/kg according of the Law No. 220/2004-2 [11]. Metallic elements could be released from the soils or precipitated in accordance with the changes in the physicochemical properties of the environment, i.e., pH, ORP, dissolved oxygen, presence of organic chelae's, etc. Most likely, such contamination high levels resulted from the long-term environmental loaded by the iron and steel production. This shows on the anthropogenic pollution of agricultural soils in surrounding of U. S. Steel Košice.

Tab. 1. Physicochemical properties and element concentrations of the soil samples (*N=40)*.

Samples (T)	pH (KCl)	pH (H_2O)	ORP (mV)	OM (%)		Fe^{3+} (%)	Al^{3+} (%)	Mn^{2+} (mg/kg)	Cu^{2+} (mg/kg)	As^{3+} (mg/kg)
1Perín (P) <u>Dust sandy</u>	6.20	7.20	490	4.70	*Mean*	2.44	5.97	635.90	31.50	7.34
					Min	2.00	5.70	622.00	29.00	7.00
					Max	2.70	6.30	646.00	34.00	7.60
					Median	2.50	5.95	637.50	31.50	7.30
					St.Dev.	0.24	0.21	7.80	1.43	0.20
2USS (HS) <u>Dust sandy</u>	6.99	7.74	441	4.30	*Mean*	3.50	8.21	717.36	28.60	15.73
					Min	3.20	7.90	708.00	27.00	15.10
					Max	3.90	8.50	730.00	30.00	16.20
					Median	3.45	8.20	718.00	28.75	15.80
					St.Dev.	0.23	0.22	7.54	0.99	0.35
3Gomboš (G) <u>Dust sandy</u>	7.23	7.95	522	4.90	*Mean*	2.48	5.70	392.54	55.30	10.54
					Min	2.10	5.40	381.00	52.00	10.00
					Max	2.80	5.90	399.00	59.00	11.00
					Median	2.50	5.75	394.00	55.00	10.50
					St.Dev.	0.22	0.21	5.85	2.45	0.31

Control reference soil (MicroBioTests, Belgium) – **C**, Oxidation-reduction potential – **ORP**, Organic matter **- OM (**dry weight STN EN 12879), Texture - **T**.

Results of the contact arrangement

Results of in the contact arrangement phytotoxicity tests are shown in figure 1. The effect of the soils studied on seed germination and root growth was a little different and depended on the plant species (Figure 1). Agricultural soils 1-3USS and 5USS showed less than 50% inhibition of the seed germination and root growth in the *S.alba* and *L. sativum* tests compared to the control, excepting of soil from 4USS plant west (values of the EC_{50} to 65%). Thus, the results of phytotoxicity tests were consistent with the chemical data. No seed germination and root growth inhibition was observed in control soils.

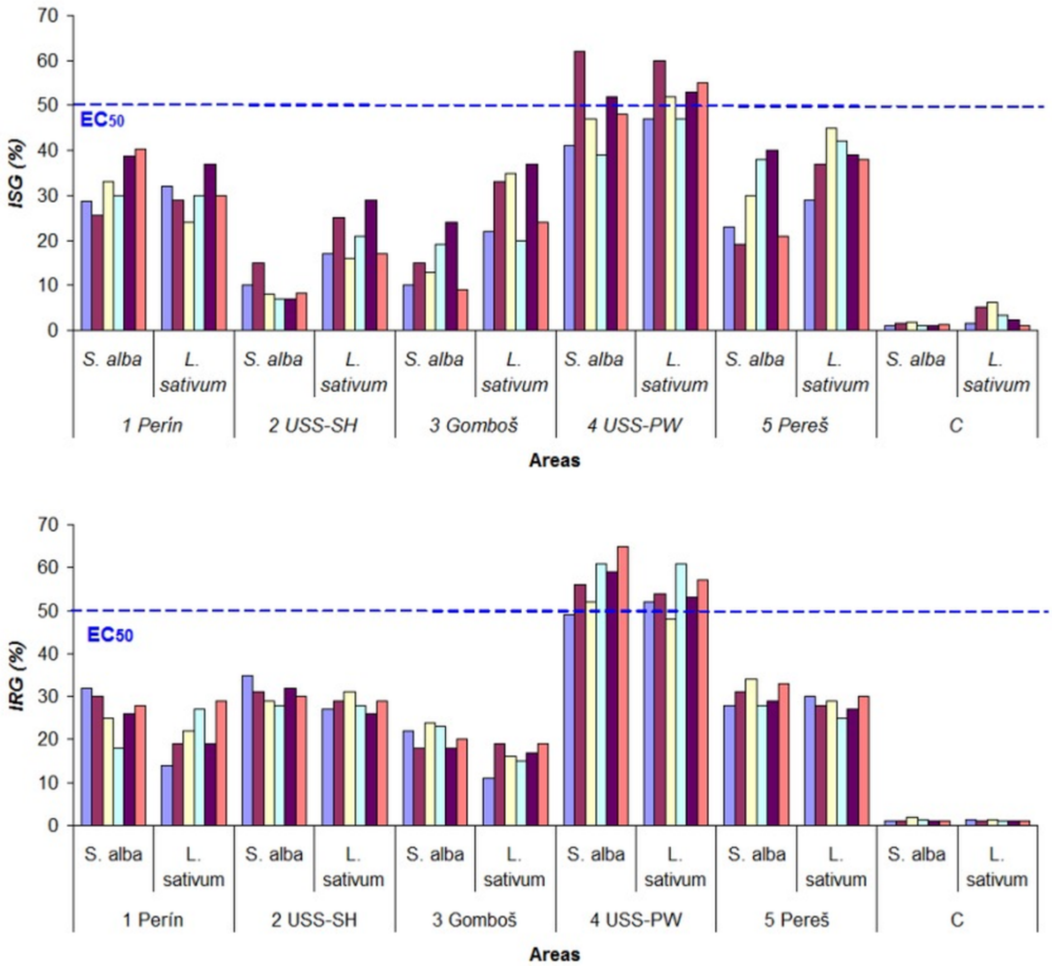

Fig. 1. Germination index (*ISG* and *IRG*) values and effects of plant reaction (EC_{50}) in agricultural and control soils (C).

Results of the concentration series test (100-50-25-12.5%)

The results of the testing of the concentration range in the soils 1-5 U.S.Steel showed diversified effects, which ranged from growth inhibition to growth stimulation. Comparison of the ecotoxic effects of the tested soils (1-5USS) showed that only soil samples from the 4USS site (PW) had the highest inhibition effect on higher plants of *S.alba* and *L. sativum*, ranging from 29% to 59%, for the tests of concentration series 100% (Figure 2). Soil properties, however, can mitigate phytotoxicity.

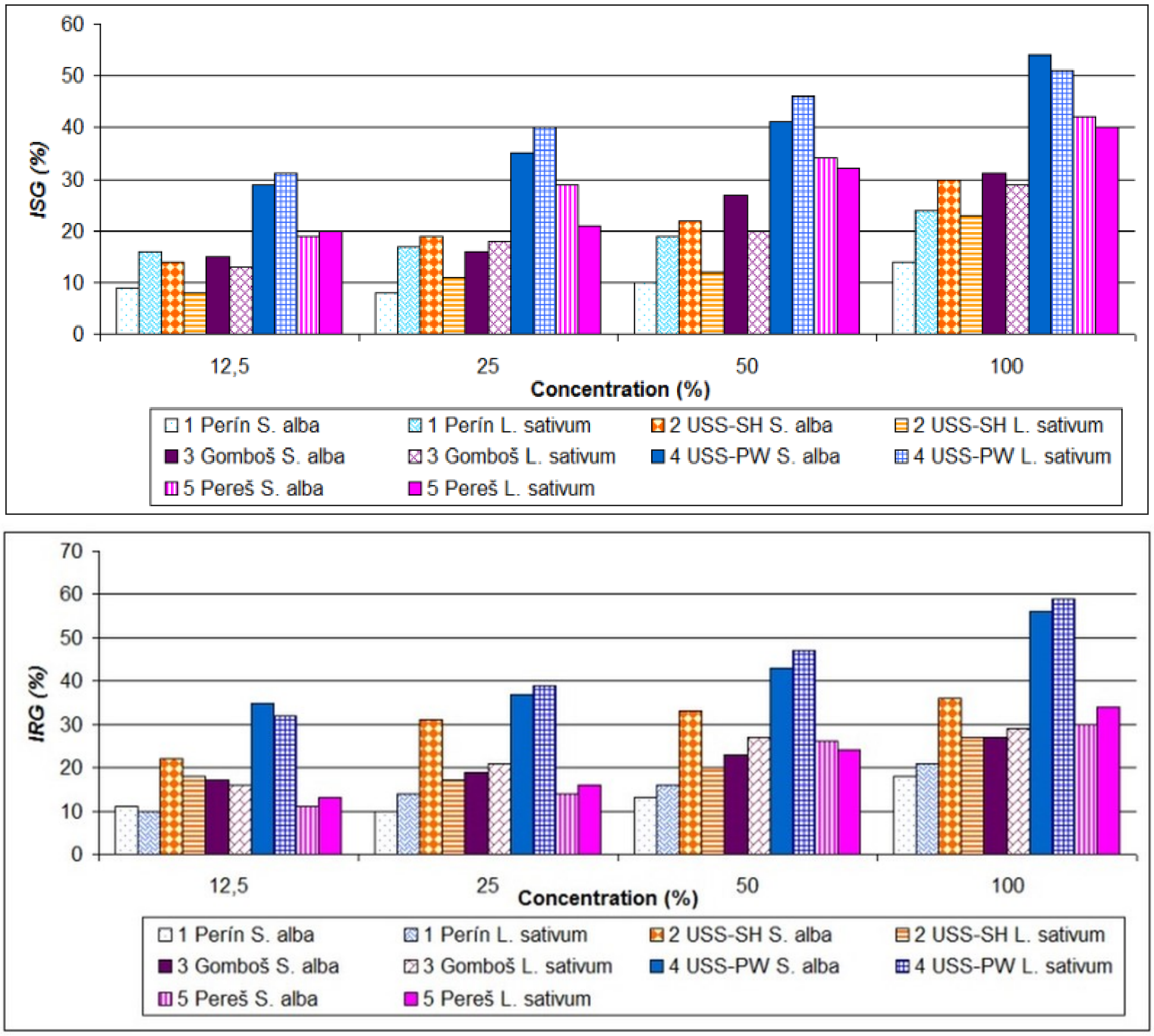

Fig. 2. Comparison ecotoxic-effects of soils (1-5USS) in concentration series test (100-50-25-12.5%).

Results of the limit concentrations test of the Fe, Al, Mn, Cu and As

To evaluate the toxicity of soils in the contact arrangement, phytotoxicity tests were selected to determine the EC_{50} values and the possibility of comparing the ecotoxic effects of the tested soils (1-5USS Košice**)**. Figures 3 show the calculated EC_{50} values of the monitored metallic element using the Polynomial FIT (Origin) statistical model. The calculated effective EC_{50} concentrations at the chosen time (72 hours) determined very low concentration limits (mainly for As^{3+}) for the studied elements. For aluminum the limit value is EC_{50} = 340mg/kg and the correlation coefficient R=0.9411, for copper EC_{50}=25mg/kg and R= 0.9697, for iron EC_{50}=260mg/kg and R=0.9411, for manganese the EC_{50}=235mg/kg and R= 0.9635, and for arsenic the EC_{50}=1.95mg/kg and R=0.9916.

Urban soil pollution, such as the aluminum, iron, copper, manganese, arsenic, is an ongoing environmental concern. The results of total concentrations (Tables 1) and results of the limit concentrations of the metallic elements (Figure 3) confirmed that metallic elements total concentrations were significantly ecotoxic positive and correlated s limit concentrations of the elements (namely soil from area 4UUS).

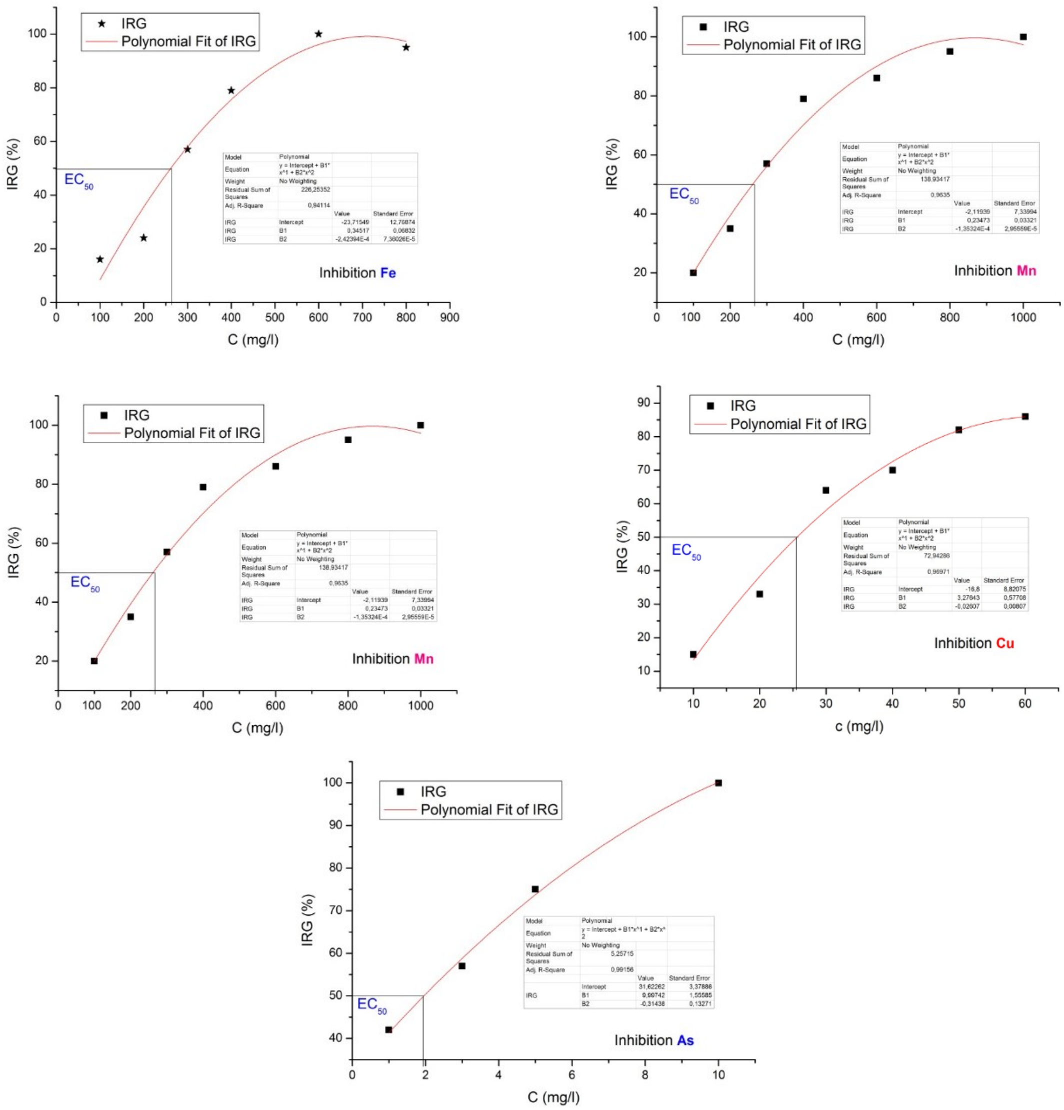

Fig. 3. Calculation of 3d/EC_{50} *S.alba* values for tested matrices (Fe^{3+}, Mn^{2+} , Al^{3+}, Cu^{2+} and As^{3+}) according to the Polynomial FIT model.

Element correlation was expressed as a value between +1 and -1; +1 being a perfectly positive correlation between two elements a negative correlation with -1. More detail, if correlation index value is between 0.1 and 0.3, it means a weak linear relationship between elements, and between 0.3 and 0.7, it means a distinct quantitative correlation, and between 0.7 and 1.0, it means strong linear relationship each other [17].

The results of correlations analyses (Tables 2-6) confirmed that metallic element **total concentrations** were significantly correlated with **limit concentrations** of the soils (1-5 USS Košice). The correlation coefficients, which ranged between -0.05 and 0.90, increased in the order Fe^{3+}<As^{3+}<Mn^{2+} < Al^{3+}<Cu^{2+} for the soils **1Perín (P)** (Table 2). In addition, Cu was significantly correlated with the aluminum value (r=0.84, p=0.05). According of the author Hančuľák of the atmospheric deposition, the highest values of correlation coefficients was calculated by Pearson correlation analysis for the elements whose dominant source is ironworks technologies, namely manganese, iron, chromium and aluminum [4].

Tab. 2. Correlation relationship between total and *limit* concentrations, and between total concentrations of metallic elements in soil 1Perín (P).

Limit	Al^{3+}	Fe^{3+}	Mn^{2+}	Cu^{2+}	As^{3+}
Al^{3+}	**0.62***	0.32	0.08	**0.84****	-0.07
Fe^{3+}		-0.05	**0.75****	0.13	**0.85****
Mn			**-0.59***	-0.11	**0.57***
Cu				**0.90****	-0.20
As^{3+}					**0.51***

** Significant at p=0.05, * significant at p=0.01 level

The correlation coefficients, which ranged between 0.06 and -0.95, increased in the order As^{3+}<Cu^{2+} <Fe^{3+}< Al^{3+}<Mn^{2+} for the soils **2USS (SH)** (Table 3). In addition, Mn^{2+} was significantly positive correlated with the arsenic, aluminum and cooper values (r=0.98-0.80, p=0.05).

Tab. 3. Correlation relationship between total and *limit* concentrations, and between total concentrations of metallic elements in soil 2uss (sh).

Limit	Al^{3+}	Fe^{3+}	Mn^{2+}	Cu^{2+}	As^{3+}
Al^{3+}	***-0.69***	0.23	**0.81**	0.44	**0.82**
Fe^{3+}		*0.44*	**0.62**	**0.94**	**0.64**
Mn^{2+}			***-0.95***	**0.80**	**0.98**
Cu^{2+}				*0.42*	**0.84**
As^{3+}					*0.06*

** Significant at p=0.05, * significant at p=0.01 level

The correlation coefficients, which ranged between -0.83 and 0.92, increased in the order Cu^{2+} <Mn^{2+} <As^{3+}< Al^{3+}<Fe^{3+} for the soils 3Gomboš (G) (Table 4). In addition, Fe^{3+} was significantly positive correlated with the aluminum, manganese and cooper values (r=0.96-0.57, p=0.05).

Tab. 4. Correlation relationship between total and *limit* concentrations, and between total concentrations of metallic elements in soil 3Gomboš (G).

Limit	Al^{3+}	Fe^{3+}	Mn^{2+}	Cu^{2+}	As^{3+}
Al^{3+}	***-0.83***	**0.96**	**0.93**	**0.57**	0.07
Fe^{3+}		***0.92***	**0.91**	**0.74**	-0.22
Mn^{2+}			***-0.52***	**0.74**	**-0.62**
Cu^{2+}				*0.31*	-0.62
As^{3+}					***0.61***

** Significant at p=0.05, * significant at p=0.01 level

The correlation coefficients, which ranged between -1.00 and 1.00 increased in the order Mn^{2+} <Fe^{3+}<Al^{3+}< Cu^{2+} <As^{3+} for the soils **4USS (PW)** (Table 5). In addition, As^{3+} was significantly positive correlated with the iron and cooper values (r=1.00, p=0.05); and As^{3+} was significantly negative correlated with the aluminum and manganese values (r=-1.00, p=0.01).

Tab. 5. Correlation relationship between total and *limit* concentrations, and between total concentrations of metallic elements in soil 4USS (PW).

Limit	Al^{3+}	Fe^{3+}	Mn^{2+}	Cu^{2+}	As^{3+}
Al^{3+}	***-0.94***	**0.86**	**0.92**	**-1.00**	**-1.00**
Fe^{3+}		***0.75***	**0.64**	**1.00**	**1.00**
Mn^{2+}			***-0.64***	**-1.00**	**-1.00**
Cu^{2+}				***-1.00***	**1.00**
As^{3+}					***1.00***

** Significant at p=0.05, * significant at p=0.01 level

The correlation coefficients, which ranged between -0.76 and 0.87 increased in the order Mn^{2+} <Cu^{2+} <As^{3+}< Al^{3+} <Fe^{3+} for the soils **5Pereš (PS)** (Table 6). In addition, Fe was significantly positive correlated with the arsenic, cooper, aluminum, manganese values (r=0.96-0.62, p=0.05).

Tab. 6. Correlation relationship between total and *limit* concentrations, and between total concentrations of metallic elements in soil 5Pereš (PS).

Limit	Al^{3+}	Fe^{3+}	Mn^{2+}	Cu^{2+}	As^{3+}
Al^{3+}	**-0.76**	**0.81**	0.13	**0.53**	**0.68**
Fe^{3+}		**0.87**	**0.62**	**0.85**	**0.96**
Mn^{2+}			0.09	**0.57**	**0.73**
Cu^{2+}				**0.50**	**0.92**
As^{3+}					**-0.60**

** Significant at p=0.05, * significant at p=0.01 level

The fate of potentially toxic metallic elements in agricultural soil depends on the forms in which they enter, the species of plants and organisms living in the soil. These factors influence the processes that determine their transformation when interacting with soil components. The main factors include mechanical composition, redox potential, soil reaction pH, quality of clay and organic matter, oxides (oxyhydroxides) of iron, manganese and aluminum, carbonates and salts. The transfer of trace elements within the soil-plant chain is a part of the biochemical cycling of chemical elements-it is an element flow from nonliving to the living compartments of the biosphere. Several factors control the processes of mobility and availability of elements; in general, they are of geochemical, climatic, biological, as well as of anthropogenic origin. The accumulation of the metallic elements in the ecosystem is continually being fueled by the agricultural use of fertilizers and pesticides, as well as industrial inputs and metal-contaminated sewage [5, 7]. Furthermore, environmental toxic metals pollution pose a grave threat to human life as well as the other biotic elements of the ecosystem. There are many studies in the world focusing on these factors and parameters [2, 4, 16, 18, 19].

Conclusion

The aim of this study was utilization of contact tests for evaluation the metallic elements anthropogenic impact in agricultural soils from the Košice area, using bioassays Phytotoxkit, and to identify potential threats for the environment and for human health. Metallic elements in study soils (area U.S. Steel Košice) show high variation in terms of concentration levels. Based on the median concentration, the metals in the soils were arranged in the following decreasing order: $Al^{3+} > Fe^{3+} > Mn^{2+} > Cu^{2+} > As^{3+}$. In the agricultural soil (4USS-PW) showed high contamination values for the iron with a median 35300 mg/kg, aluminum with a median 82500 mg/kg, manganese with a median 1027 mg/kg. The median level of arsenic in the soil (4USS-PW) was 34 mg/kg.

The obtained results of the contact arrangement phytotoxicity show that the agricultural soils 1-3USS and 5USS showed less than 50% inhibition of the seed germination and root growth in the *S.alba* and *L. sativum* tests compared to the control, excepting of soil from 4USS plant west, values of the EC50 to 65%. Comparison of the ecotoxic effects of the tested soils (1-5USS) showed that only soil samples from the 4USS site (PW) had the highest inhibition effect on higher plants of *S.alba* and *L. sativum*, ranging from 29% to 59%, for the tests of concentration series 100%. The calculated effective EC_{50} concentrations determined very low concentration limits for the studied elements (mainly for As^{3+}). For aluminum the limit value is EC_{50} = 340mg/kg, for copper EC_{50}=25mg/kg, for iron EC_{50}=260mg/kg, for manganese the EC_{50}=235mg/kg, and for arsenic the EC_{50}=1.95mg/kg.

Most likely, such contamination high levels resulted from the long-term environmental loaded by the iron and steel production. This shows on the anthropogenic pollution of agricultural soils in surrounding of U. S. Steel Košice. Toxicity contact tests allowed us to carry out a preliminary screening of the contaminated areas ecotoxicological risk.

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