

Terrestrial Eco-Toxicological Tests as Screening Tool to Assess Soil Contamination in Kropachy Area

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Abstract. In this study, we present screening tool of heavy metal inputs to agricultural and permanent grass vegetation of the soils in Kropachy. This study is devoted to Ecotoxicity tests, Terrestrial Plant Test (modification of OECD 208, Phytotoxkit microbiotest on *Sinapis Alba*) and chronic tests of *Earthworm (Dendrobaena veneta)*, modification of OECD Guidelines for the testing of chemicals 317, Bioaccumulation in Terrestrial *Oligochaetes*) as practical and sensitive screening method for assessing the effects of heavy metals in Kropachy soils. The total Cu, Zn, As, Pb and Hg concentrations and eco-toxicological tests of soils from the Kropachy area were determined of 4 sampling sites in 2015. An influence of the sampling sites distance from the copper smeltery on the absolutely concentrations of metals were recorded for copper, lead, zinc, arsenic and mercury. The highest concentrations of these metals were detected on the sampling sites up to 3 km from the copper smeltery. The samples of soil were used to assess of phytotoxic effect. Total mortality was established at earthworms using chronic toxicity test after 7 exposure days. The results of our study confirmed that no mortality was observed in any of the study soils. Based on the phytotoxicity testing, phytotoxic effects of the metals contaminated soils from the samples 3KR (7-9) *S. alba* seeds was observed.

1. Introduction

To assess soil quality and risk assessment, bioassays can be useful tools to gauge the potential toxicity of contaminants (heavy metals). The purpose of the present study was to use Phytotoxkit test and chronic test of earthworm (*Dendrobaena veneta*) as useful screening methods to evaluate toxicity of the polluted soils from Eastern Slovakia (concretely from the Kropachy area). The mentioned tests represent simple, quick, inexpensive, direct soil phase toxicity testing procedure. Mining operations with the metallurgical processing of complex metals and copper ores left negative effects on the region Eastern Slovakia [1]. An influence of the sampling sites distance from the copper smeltery on the absolutely concentrations of metals were recorded for copper, zinc, lead, arsenic and mercury. Soil contamination comes from industry, agricultural practical, combustion of fossil fuels and mining activities, which together with mining related industries became the largest sources of environmental pollution by heavy metals [2-4]. In recent decades there has been increasing interest on long-term effect of heavy metals at high concentration in the environment due to their persistent in the soil for tens of thousands years. Physical, chemical and biological soil properties are very important for its behaviour and fertility. Many authors have used the soil enzymatic activities as bio-indicators to determine toxicological influences of various pollutants on soil quality [5-8].



2. Materials and methods

2.1. Material and method for soils testing

In the year 2015, 12 soil samples were collected from the village Kropachy in Eastern Slovakia. The four sampling sites were localised on area of the villages: 1KL - Kluknava: PGV-permanent grass vegetation, 2KL- Kluknava: A-agricultural soil, 3KR -Kropachy 1,2 km of the smeltery (PGV) and 4KO- Kolinovce (PGV). The soils were sampled at a depth of 20cm to 40cm into ground plastic bags. Soil samples were homogenized, dried at room temperature (25°C); sieved through 2-mm sieve opening and stored in plastic bags until analyzes.

All the analyzed samples were conducted in triplicate and the data were based on soil dry weight. Total concentrations of heavy metals (Cu, Zn, Pb, As and Hg) were determined by the X-ray fluorescence spectrometry method (SPECRO XEPO3), also after phytotoxicity testing and testing of earthworm. The used control soil contains: 85% quartz, 10% kaolin and 5% peat.

2.2. Material and method for soils phytotoxicity testing

Three replicates were done for each sample set. Phytotoxkit is the alternative test procedure, which allows the determining of the biological effect of heavy metals on plants. The soil was covered with the filter plate, and ten seeds of *Sinapis alba* were placed on top of the filter in one row. After closing the test, the plates with the transparent cover, were placed vertically and incubated for 72 h at 25°C. Pictures of the test plates were analyzed with the free image analysis program (Image Tools). The phytotoxic effects of soils were establish using the parameters of the percentage inhibition of seed germination (ISG) and percentage inhibition of root growth (IRG) in the test soil [1]. All experimental bioassay results were expressed in terms of the inhibition of plant and /or root growth (EC₅₀) caused by test soils sample and then compared relative to the growth observed in control experiment, cultivated in the reference medium.

2.3. Material and method for soils testing of earthworm

Earthworms are often used as terrestrial model organisms for ecotoxicity testing, because of their importance for the structure and function of soil ecosystems. The experiments were carried out as described in the OECD Guidelines 317 for the testing of chemicals relating to environmental fate, tests of mortality. The reaction to the earthworm (*Dendrobaena veneta*) was used for chronic tests in the soils. The earthworms were purchased from a local supplier. Prior to the start of the experiment, the earthworms were allowed to acclimatize for one week in the experimental conditions.

The adult worms were used in the tests. The pH of the soil samples was in the range 5.23 - 7.95. Three replicates were performed for each test (of the soil 100 g dry weight) with ten earthworms added to each boxes. Then distilled water was added for purpose to obtain 30% moisture of soil. The results were evaluated as the percentage inhibition of mortality and compared to the control soil. After that, the boxes with soils were kept for 7 at laboratory temperature. Testing of earthworms was used also after phytotoxicity testing of soils sampling.

3. Results and Discussions

3.1. Physical and chemical parameters

Physicochemical properties of the soils of all sampling sites are listed in Table 1, soil reaction (pH/H₂O and pH/KCl), oxidation-reduction potential (ORP), dry mass and organic matter, were used for the evaluation of the risk caused by the heavy metals in the soils.

The soil samples were study by grain size analysis. The samples contain from 94.3 to 98.7% silt fraction, from 21.8 to 35.4% clay fraction and from 4.5 to 0.1% sand fraction. Soil types were silty-clay texture for all the soil samples.

Table 1. Physicochemical property of the soils from the area Krompachy

Samples	pH/H ₂ O	pH/KCl	ORP (mV)	Dry mass (%)	Org. matter (%)	
1KL	1	6.82	5.53	498	97.46	6.25
	2	6.75	5.46	504	98.11	6.19
	3	6.44	5.44	489	97.50	6.40
	4	6.48	5.19	543	97.28	5.59
2KL	5	6.40	5.29	582	97.91	5.93
	6	6.58	5.51	539	98.05	5.68
	7	6.68	5.12	556	97.77	7.50
3KR	8	6.82	5.17	563	97.65	7.25
	9	6.77	5.23	602	97.48	7.60
	10	7.95	7.20	512	94.30	7.18
4KO	11	7.63	7.30	582	95.00	7.31
	12	7.42	7.15	541	94.85	7.40

Table 2 summarizes the results of the chemical analyses of the metals in the soils, revealing significant contamination of all the metals (Cu, Zn, As, Pb and Hg) according with permissible limit values for Slovak soils (Supplement No. 220/2004 Coll of Laws).

Table 2. Total metal contents of the samples of the area Krompachy (average ± standard deviation)

Samples	Concentration mg/kg d. w.					
	Cu	Zn	As	Pb	Hg	
1KL	1	176.9±2.8	257.0±2.9	53.3±2.1	80.9±5.2	1.3±0.1
	2	188.0±3.4	275.2±1.9	54.9±1.7	84.5±4.2	1.8±0.3
	3	178.5±2.9	267.4±2.1	54.0±1.4	82.9±2.6	2.4±0.3
	4	148.2±1.5	215.5±3.7	48.0±1.3	67.9±3.4	2.4±0.1
2KL	5	163.0±4.2	221.0±2.6	52.1±1.6	72.3±2.1	1.7±0.2
	6	158.1±3.5	229.2±1.5	51.4±2.0	71.3±1.9	1.4±0.3
	7	649.2±9.1	716.6±7.6	144.2±2.0	177.0±4.1	4.5±0.2
3KR	8	667.1±7.3	721.1±6.6	149.1±1.8	181.0±3.2	4.8±0.3
	9	642.3±6.9	699.7±5.9	139.2±2.1	179.2±4.8	4.3±0.1
	10	295.7±5.7	453.0±4.9	69.0±3.7	96.8±5.5	19.8±0.2
4KO	11	309.7±6.0	469.0±3.7	74.1±2.1	103.5±3.9	21.5±0.1
	12	307.9±4.9	458.9±4.0	71.5±2.4	102.6±4.1	20.6±0.3
Control soil	<0.5 ± 0.1	8.0 ± 1.2	0.7 ± 0.3	31.7 ± 0.9	<0.5 ± 0.2	
Laws used for comparison mg/kg d. w.						
Limit value	70	200	30	115	0.75	

3.2. Toxicity testing with *Phytotoxkit*

According to Figure 1 the percentage inhibition of seed germination (ISG) was of 1.8 to 57% in the all the soil samples and higher value was of 12% to 57% in the soils 3KR (7-9). The tests are considered to be valid if the number of germinated seeds in the control was at least 90%. The percentage inhibition of root growth (IRG) was of 7.5-69 % in the all samples and 22–69% in the soils 3KR (7-9) (Figure 2) and the highest concentrations of the metals were detected on the sampling sites up to 3 km from the copper smeltery, Krompachy (Table 2). According to the *Phytotoxkit* microbiotest, the experimental concentration at which growth inhibition rises above 50% after 72 hours can be considered as the effective concentration 72/EC₅₀.

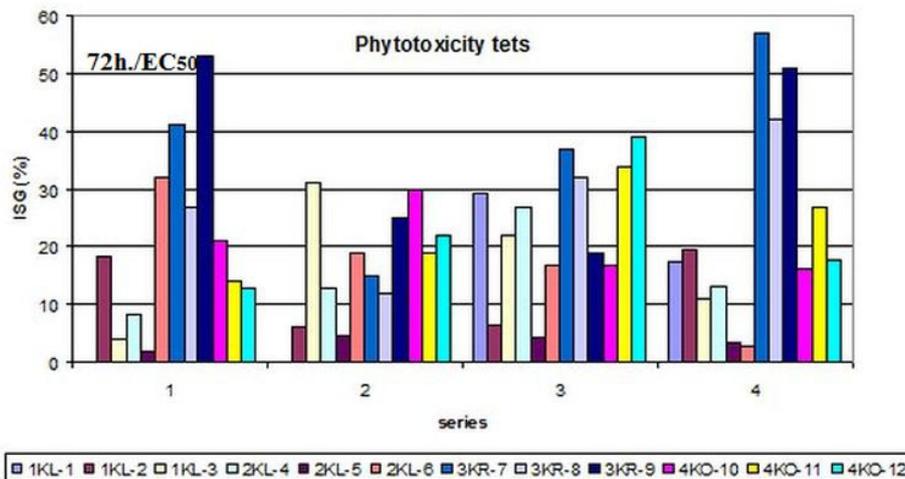


Figure 1. Index ISG (%) of the *S. alba* in the 4 series of the soils from the area Krompachy

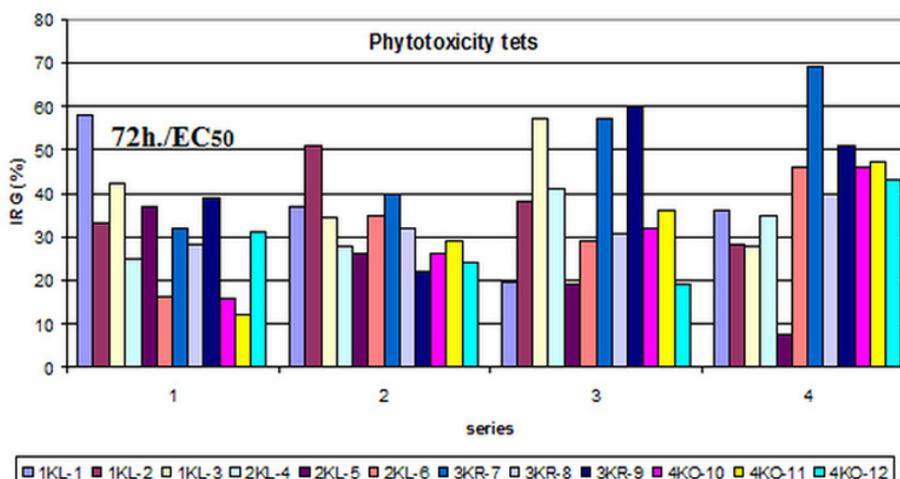


Figure 2. Index IRG (%) of the *S. alba* in the 4 series of the soils from the area Krompachy

3.3. Toxicity testing with earthworms (*Dendrobaena veneta*)

The earthworm's mortality was not influenced by soils after 7 exposure days. The results of tested soil samples at earthworms (Table 3) were evaluated after 72 hours' bioassays (Phytotoxkit) and 7 days' earthworm's exposure without Hg was decrease of the metal concentrations in all the samples, mainly the concentration of Cu and Zn for sample 2KL (4-6) and 3KR (7-9). Concentrations of the mercury were equal than original concentration for all soils. The largest concentration differences were recorded in the sample 3KR after 7 days' earthworm's exposure. It was found that earthworms (*Dendrobaena veneta*) in some cases caused decrease of copper and zinc concentration in contaminated soils.

4. Conclusions

The soils from the area Krompachy were polluted with Cu, Zn, As, Pb and severely Hg. Extremely high and above the limit values of mercury were measured at the soils 4KO (10-12). Based on the phytotoxicity testing, phytotoxic effects of the metals contaminated soils from the samples 3KR (7-9) *S. alba* seeds was observed.

Table 3. Metal contents of the soils the area Krompachy after 72 hours' bioassays (Phytotoxkit tests) and after 7 days' earthworm's exposure (average \pm standard deviation)

Samples	Concentration mg/kg d. w.					
	Cu	Zn	As	Pb	Hg	
<i>1KL after 72 hours bioassays</i>						
1KL	1	175.6 \pm 3.1	251.9 \pm 3.7	51.8 \pm 1.1	78.9 \pm 2.4	1.2 \pm 0.1
	2	182.6 \pm 5.4	264.0 \pm 6.2	50.4 \pm 1.5	81.0 \pm 1.7	1.7 \pm 0.1
	3	174.9 \pm 2.8	263.3 \pm 2.6	52.3 \pm 2.1	80.1 \pm 1.3	2.1 \pm 0.2
1KL after 72 hours bioassays + 7 days earthworms exposure						
1KL	1	174.6 \pm 2.1	255.9 \pm 3.0	53.0 \pm 2.1	79.9 \pm 3.1	1.3 \pm 0.1
	2	180.6 \pm 4.3	269.0 \pm 5.1	52.2 \pm 1.4	83.2 \pm 2.7	1.6 \pm 0.1
	3	175.8 \pm 1.6	267.3 \pm 2.4	53.8 \pm 1.9	80.9 \pm 1.0	2.2 \pm 0.1
<i>2KL after 72 hours bioassays</i>						
2KL	4	146.1 \pm 2.4	211.3 \pm 1.4	46.7 \pm 1.7	63.5 \pm 2.4	1.9 \pm 0.1
	5	155.1 \pm 3.1	219.0 \pm 3.1	50.0 \pm 2.0	70.1 \pm 1.9	1.5 \pm 0.2
	6	156.8 \pm 2.0	220.5 \pm 2.0	49.2 \pm 1.6	68.2 \pm 1.1	1.3 \pm 0.1
2KL after 72 hours bioassays+ 7 days earthworms exposure						
2KL	4	140.1 \pm 4.1	210.3 \pm 2.1	45.2 \pm 1.5	60.5 \pm 1.4	2.0 \pm 0.1
	5	150.9 \pm 2.0	212.0 \pm 5.7	48.0 \pm 3.3	65.1 \pm 3.5	1.6 \pm 0.2
	6	153.8 \pm 3.7	218.5 \pm 3.8	49.7 \pm 1.2	69.2 \pm 1.9	1.5 \pm 0.1
<i>3KR after 72 hours bioassays</i>						
3KR	7	639.5 \pm 4.9	687.1 \pm 6.1	138.5 \pm 2.2	175.0 \pm 3.2	4.0 \pm 0.1
	8	662.6 \pm 3.1	635.7 \pm 8.9	142.3 \pm 4.1	177.8 \pm 4.1	4.3 \pm 0.1
	9	634.1 \pm 7.1	645.8 \pm 5.6	135.7 \pm 3.5	170.9 \pm 3.3	4.1 \pm 0.1
3KR after 72 hours bioassays+ 7 days earthworms exposure						
3KR	7	625.9 \pm 7.9	678.1 \pm 7.1	136.5 \pm 1.2	172.0 \pm 2.5	4.0 \pm 0.1
	8	652.3 \pm 4.2	629.8 \pm 6.9	140.9 \pm 3.5	175.7 \pm 3.7	4.5 \pm 0.2
	9	640.1 \pm 2.1	635.2 \pm 5.9	133.2 \pm 2.1	170.9 \pm 2.3	4.3 \pm 0.1
<i>4KO after 72 hours bioassays</i>						
4KO	10	292.7 \pm 5.1	450.7 \pm 2.1	62.3 \pm 3.1	92.5 \pm 2.5	19.2 \pm 0.1
	11	300.7 \pm 6.7	466.0 \pm 2.8	70.0 \pm 4.3	100.0 \pm 1.6	21.4 \pm 0.1
	12	301.1 \pm 4.4	449.2 \pm 4.1	68.8 \pm 1.9	99.5 \pm 3.0	20.4 \pm 0.1
4KO after 72 hours bioassays+ 7 days earthworms exposure						
4KO	10	289.8 \pm 6.0	449.6 \pm 1.7	61.5 \pm 3.0	90.5 \pm 2.3	18.5 \pm 0.1
	11	301.2 \pm 4.7	465.0 \pm 3.5	70.5 \pm 1.3	99.7 \pm 1.9	20.4 \pm 0.1
	12	300.1 \pm 2.4	452.1 \pm 3.1	67.5 \pm 1.5	98.5 \pm 4.8	20.1 \pm 0.1
Control soil	3.2 \pm 0.1	8.0 \pm 1.2	1.8 \pm 0.3	31.7 \pm 0.9	0.5 \pm 0.2	
<i>C. soil/ after 72 hours bioassays</i>						
	2.9 \pm 0.2	7.5 \pm 1.5	1.7 \pm 0.4	30.4 \pm 1.0	0.5 \pm 0.1	
<i>C. soil/7 days earthworms</i>						
	2.5 \pm 0.2	7.1 \pm 0.2	1.4 \pm 0.3	26.1 \pm 0.9	0.4 \pm 0.1	
Laws used for comparison mg/kg d. w.						
Limit value	70	200	30	115	0.75	

The results of tested soil samples at earthworms were observed that on in some cases caused decrease of copper and zinc concentration in contaminated soils. Heavy metals accumulation in the soil can cause abiotic stress. Such stress disrupts plant growth, especially biomass production and seed yield. Every metal interacts in different, specific way, depending on soil type, metal form and other ions presence.

These results are good indicator of long-term soil contamination by heavy metals and could be useful for monitoring changes in agricultural ecosystems.

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