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Study of mercury behavior and earthworm bioassays in three solid environment components from selected areas of Eastern Slovakia

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Abstract

A 28-day bioassay with the earthworm (*Dendrobaena veneta*) was used to assessing the ecotoxic effect of mercury in study soils and sediments. This article deals with quality evaluation of the soils, sediments and plants (assimilation organs) from three localities, Rudňany, Krompachy, and water reservoir of Ružín, Eastern Slovakia (Europe) in consideration of their toxic effect on the environment. These areas are well - known for its mercury mining and metallurgical activities for several centuries. Within the frame of evaluation it was found that the concentrations of mercury exceeded some of the MPC (Max. Tolerable Risk) and IV (Serious Risk) values. The samples Rudňany tailing-SED (188.5mg/kg), Krompachy a-SED (69.4 mg/kg), and Krompachy b-SED (93.4 mg/kg) were the most polluted by mercury, which is evident according to it is the highest mortality on the earthworm (*Dendrobaena veneta*). The high mercury concentrations were obtained in the soils of the Rudňany-tailing-S (82.5 mg/kg), Rudňany-tailing, valley-S (57.8 mg/kg), and Krompachy 4KO-S (20.6 mg/kg). A significant positive correlation is found between highest concentrations of mercury Rudňany-tailing SED Hg=188.5 mg/kg (r=0.87) with the highest mortality of *Dendrobaena veneta* after 28 days bioassay.

Keywords: Soil, sediment, plant, mercury, earthworm.

1. Introduction

Soil contamination can seriously and negatively impact soil life, especially persistent soil contaminants such as mercury. The presence of mercury and other contaminants hampers basic soil ecosystem processes such as organic matter degradation. Plant growth may also be hampered by the high contamination either directly, by the impact of mercury, or indirectly, by the changed soil and litter environment (Eijsackers, 2010). Mercury from the source of pollution is transported by soil, water in river and accumulated in sediments. The anthropogenic mercury input into the environment is more miscellaneous compared to the natural one. The main sources are for example: mining dumps, ore transport, aerosols, agriculture, electronics, batteries, and waste facilities. Subsequently, mercury and mercury compounds enter into atmosphere, hydrosphere and pedosphere, where they change during it is cycles and the result of these processes is the increase of toxicity (Hinton and Veiga, 2009; Zhang *et al.*, 2016). High ecological risk of mercury comes from its specific properties. Mining operations with the metallurgical processing of complex metals and copper ores left negative effects on the region Eastern Slovakia, Krompachy and water reservoir of Ružín. In the river basin Hornád and Hnilec, there are several old, abandoned, and flooded mining works as well as mining dumps resulting as mining, treatment and metallurgical processing of Cu, Fe, and Hg ores containing impurities of toxic elements. Siderite deposit of Rudňany belongs to risk localities (Šestinová *et al.*, 2015). A better understanding of ecosystem functioning and bioavailability to buffer the negative impacts of mercury contamination could be obtain by more various screening methods. The majority of bioassays applied to contaminated sediments and soils are based on the toxic effects of sediment solutions or sediment itself on a living organism (e.g. animals, plant and bacterial bioassays). Because bioassays are a direct measure of functional responses, they should have more impact on the decision-making process than criteria based on the concentrations of chemicals alone (Czerniawska-Kusza and Kusza, 2011; Das and Chakrapani, 2011). Earthworms are distributed widely in terrestrial ecosystem and are very active contributors to soil formation, giving shape to physico-chemical and biological properties of soil, thus enhancing soil fertility (Maity *et al.*, 2018). Earthworms are known for their bioaccumulation capacity of organic or inorganic contaminants (De Vaufleury *et al.*, 2013). Also, authors Nannoni *et al.* (2014), the bio-concentration of metal trace elements in earthworms at urban, peri-urban and garden levels in the City of Siena (Italy) has been measured. In study (Mariyadas *et al.*, 2018) were examined silver nanomaterials cause toxicity to earthworm, with effects on survival, reproduction and enzyme activity. Some works has attempted to link bioaccumulation with the so-called “bioavailable” fraction in soils on this type of organism for trace elements (Coelho *et al.*, 2018).

Also, morphological and histopathological analyses have been used to identify tissue damage caused by exposure to sewage sludge in terrestrial invertebrates (*Eisenia fetida*), in order to gain a better understanding of adverse effects caused by sewage sludge and to analyse its impact on the production of reactive oxygen species and lipid peroxidation in earthworms, were measured (Nogarol and Fontanetti, 2010; Christofoletti *et al.*, 2012; Babić *et al.*, 2016).

The research was aimed to mercury distribution and their toxic effects in the soils, sediments and plants ((spruce, pine, birch) using bioassays, tests of mortality of *Dendrobaena veneta*.

2. Materials and methods

2.1. Soil, sediment and plant sampling

The soil samples from two areas (Krompachy and Rudňany) and the sediment samples from three areas (water reservoirs Ružín No.I and Krompachy, and tailing Rudňany) were used for the analysis (Figure 1). The Rudňany deposit is situated in the northern part of the Spišsko-Gemerské Rudohorie Mountains (Spišská Nová Ves). The location of the water reservoir Ružín is situated in the Valley of Volvos’ Mountains and Hornád basin. In the years 2014-2015 sediments from two sites at the water reservoir Ružín NoI. (Hornád and Hnilec River), and two sites at the water reservoir Krompachy (a, b) were collected. In the year 2015, the four sampling sites (depth was 0.05–0.20 m) were localised on area of the villages Krompachy: 1KL (Kluknava), 2KL (Kluknava), 3KR (Krompachy) and 4KO (Kolinovce). The highest concentrations of Cu, Pb, Zn, As and Hg were detected on the sampling sites up to 3 km from the plant Kovohuty a.s., Krompachy, (Šestinová *et al.*, 2015; Angelovičová *et al.*, 2015). The plant Kovohuty a.s. represents one of the most important sources of emissions. Soil samples were sampled from the top soil horizon (upper 20 cm) and sediments at a depth of 50 cm. The samples were air dried at room temperature, and the mixed samples were thoroughly mechanically homogenized. Soil samples were fine sieved (<2 mm) and sediments (<1 mm) to minimize compositional heterogeneity before chemical analysis.

Presented sort of tree species (spruce, pine, birch) were selected from the reason of their most frequent occurrence in the monitored areas (outdoors) as well as for a possibility of the comparison of mercury content and its mobility in the soils, sediments and plant (assimilation organs). These tree samples (fresh leaves of birch; and spruce and pine needles) were collected in each of the autumn seasons of the years 2006-2015. For analysis of biota were used the mixed tree samples of 1 kg weight. The tree samples were washed by distillate water, and then dried for one week, at 25°C, homogenized and was used on chemical analyses. The total content of mercury in the samples was analyzed by the trace mercury analyzer (DMA-80) without mineralization. All the analyzed samples were conducted in triplicate and the data were based on samples dry weight.

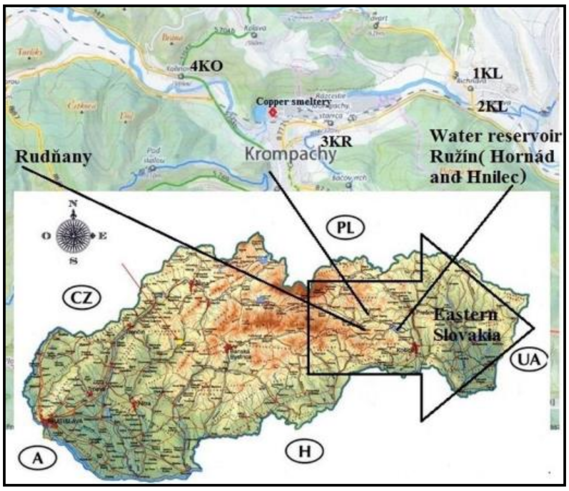

Figure 1. Studied areas

Granulometric analysis was performed on a set of soil and sediment samples. Firstly, the samples were wet sieved to separate the coarser particles (>63 μm), then a decantation process was carried out (gravity settling in deionized water) to characterize the finer fraction. All sediment samples contained the sand, silt and clay fractions. The silt and clay fractions were determined as the percentage of the sediments passing through a sieve with an opening size of 63 μm. Soil types were by silty-clay texture. The used control soil contained: 85% quartz, 10% kaolin and 5% peat. The quality of the soil was established with reference to law (220/2004, No.2, Slovak Republic) from various areas to keep representativeness. The quality of the sediment was established with reference to methodical instructions of the Ministry of Environment of the Slovak Republic 549/1998-2 and by law 203/2009 for assessing risks from pollution of sediments streams and water reservoirs. The values obtained were compared with the test values (TV), the maximum permissible concentrations (MPC) and the intervention values (IV), which are listed in Table 2.

2.2. Earthworm bioassay

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 and sediments. Earthworms are often used as terrestrial model organisms for ecotoxicity testing, because of their importance for the structure and function of soil ecosystems (Eijsackers, 2010). 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. 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. After that, the boxes with soils (sediments) were kept for 28 at laboratory temperature. The earthworms were lyophilized (at temperature -50°C and pressure 50 Pa) and the concentrations of mercury after 28 days earthworms exposure were measured direct by trace mercury analyzer DMA-80. The results were evaluated as the percentage inhibition of mortality and compared to the control soil.

Table 1. Main physicochemical parameters and granulometric distribution in the soils and sediments

Area	pH/H_2O	Eh (mV)	Organic matter d.w. (%)	Grain size (µm)			
				>100 (%)	>63 (%)	>40 (%)	<40 (%)
Hornád River ***SED***	7.2	148	12.8	1.4	3.3	20.4	77.9
Hnilec River ***SED***	7.5	288	10.5	2.1	5.2	24.6	68.1
Krompachy 1KL - PGV ***S***	6.7	504	6.2	2.8	11.9	19.5	65.8
Krompachy 2KL - A ***S***	6.5	582	5.6	3.7	11.5	18.7	66.1
Krompachy 3KR - PGV ***S***	6.8	602	7.6	0.7	6.7	19.8	72.8
Krompachy 4KO - PGV ***S***	7.6	582	7.3	4.5	6.5	18.2	70.8
Krompachy - *a* ***SED***	7.7	259	14.6	2.8	5.2	34.9	57.1
Krompachy - *b* ***SED***	7.3	129	16.2	1.8	3.6	33.8	60.8
Rudňany-Markušovce ***S***	5.6	686	9.8	4.2	12.3	14.9	68.6
Rudňany - tailing ***S***	4.9	673	5.4	3.1	7.2	15.8	73.9
Rudňany - tailing, valley ***S***	5.7	651	8.7	2.9	10.5	17.1	69.5
Rudňany - tailing ***SED***	7.1	547	13.5	0.1	5.0	9.5	85.4

S–*Soils,* ***SED***–*Sediments,* ***PGV***-*permanent grass vegetation soils,* ***A***-*agricultural soils,* ***Organic matter*** *dry weight (d.w.) - (according to STN EN 12879) organic proportion determination of dry matter losses during combustion*

3. Results and discussions

3.1. The physicochemical parameters and mercury concentrations in different types of samples

The physicochemical properties of the sediment from the Hornád and Hnilec Rivers, the pH were in the range 7.2-7.5 indicate near-neutral, likely due to a higher content of carbonates in the bottom sediments (Table 1). The organic matter of studied sediments ranged from 10.5 to 16.2%. Krompachy soils and sediments measurements indicated similar pH, with values ranging from 6.5-7.7. The pH of the soils from the area Rudňany was in the range 7.1-7.7 and for the sediments was in the range 7.1-7.8. This pH indicates slightly alkaline samples because the acidity generated by decomposition of the sulfides is efficiently neutralized by the abundant carbonate minerals. The most frequent primary minerals are siderite, quartz, barite, and muscovite (Kučerová *et al.*, 2014). The organic matter of soils ranged from 5.4 to 9.8%. Total mercury concentrations, mercury concentration of the samples after bioassay and accumulated in *Dendrobaena veneta* earthworm tissues during a 28-day bioassay in soil and sediment are shown in Table 2. From the Table 2 it is evident that the most contaminated sediment is sediment Rudňany tailing-SED (188.5 mg/kg), Krompachy a-SED (69.4 mg/kg), and Krompachy b-SED (93.4 mg/kg). The high mercury concentrations were obtained in the soils of the Rudňany-tailing-*S* (82.5 mg/kg), Rudňany-tailing, valley-*S* (57.8 mg/kg), and Krompachy 4KO-*S* (20.6 mg/kg). Also it was found that earthworms decrease the mercury concentration after 28 days earthworms exposure mainly in the Rudňany tailing. In Table 3, there are the results of the mercury concentration in the plant samples (Rudňany). This metal is released into aquatic environments in response to changes in redox due to oxidation and subsequent degradation of organic substances and decomposition of sulfides by changing physicochemical conditions. According to the authors (Šestinova *et al.*, 2015; Aydin *et al.*, 2018), it was found that sequential extraction revealed high bioavailability of Hg in the sediments, which are primarily of anthropogenic origin and organic bound metals extracted in organic-sulfide fraction. It is considered, that mercury is bound with stable humic substances. Also, when pollution due to metals enters the lake environment, it is incorporated into sediments with organic matter, sulfides, clay and iron/manganese (Fe/Mn) oxides. Study (Yin *et al.*, 2016) showed also that different cultivation practices can largely change the distribution and speciation of Hg in agricultural soils. In the assimilation organs trees were measured very low values of mercury (0.031–0.270 mg/kg). Maximum of mercury concentration was detected in the birch leaves in the area Rudňany – tailing, valley. The mercury from the anthropogenic human activity (like the Rudňany area) is in such environment superimposed to its geochemical background, while high concentration of mercury in the local scale may arise with its dangerous impacts on the environment.

3.2. Earthworm bioassay and correlation coefficients

The results of the toxicity tests are shown in Table 4. The largest mercury concentration differences were recorded in the samples Rudňany–tailing, valley *S* (18.6mg/kg)*,* Rudňany–tailing *SED* (14.2 mg/kg) and Rudňany–tailing, *S* (7 mg/kg), after 28 days earthworms exposure. It was found that earthworms (*Dendrobaena veneta*) in some cases caused decrease of mercury concentration in contaminated soils and sediments. The Pearson correlation coefficients for the mercury in the studied soil and sediment samples are summarized in Table 4. A significant positive correlation was found between highest concentrations of mercury Rudňany-tailing SED Hg=188.5 mg/kg (r=0.87) with the highest mortality of *Dendrobaena veneta* after 28 days bioassay. From Table 4 it is evident that samples with the low concentrations of Hg did not influence significantly the mortality of earthworms. The correlation (positive and negative) found between the studied metals may show nearly similar levels and sources of contamination in the study area (Tang *et al.*, 2013).

Table 2. Mercury concentration in different types of samples from Eastern Slovakia (total mercury concentration of the samples, mercury concentration of the samples after bioassay and accumulated in *Dendrobaena veneta* earthworm tissues during a 28-day bioassay (average ± standard deviation)

Area	Hg Total concentration in sample (mg/kg)/d.w.	Hg Concentration in sample after bioassay (mg/kg)/d.w.	Hg Concentration in worms (mg/kg)/d.w.
Hornád River ***SED***	8.7±2.2	6.3±1.9	0.17±0.02
Hnilec River ***SED***	2.9±1.6	2.1±1.2	0.18±0.01
Krompachy 1KL - PGV ***S***	1.9±2.1	1.7±1.1	0.09±0.002
Krompachy 2KL - A ***S***	1.7±1.6	1.7±2.1	0.12±0.01
Krompachy 3KR - PGV ***S***	4.8±3.3	4.5±2.9	0.29±0.09
Krompachy 4KO - PGV ***S***	20.6±7.4	19.9±9.1	0.54±0.05
Krompachy - *a* ***SED***	69.4±5.4	69.9±4.1	0.24±0.04
Krompachy - *b* ***SED***	93.4±8.2	89.9±6.5	0.98±0.06
Rudňany - Matejovce ***S***	6.5±3.2	5.5±2.9	1.12±0.93
Rudňany - tailing ***S***	82.5±6.1	75.5±9.3	11.3±5.0
Rudňany- tailing, valley ***S***	57.8±4.1	39.2±7.3	19.1±3.3
Rudňany - tailing ***SED***	188.5±8.2	174.3±9.3	14.5±3.9
Control ***S***	0.5±0.2	0.08±0.04	0.420±0.003
Control worms		-	0.036±0.002
Norm used for comparison (mg/kg) Sediments			
TV	-	-	0.3
MPC	-	-	10
IV	-	-	10
Laws used for comparison (mg/kg) Soils			
Limit value	-	-	0.75

S*–Soils,* ***SED****–Sediments, PGV-permanent grass vegetation soils, A-agricultural soils,* ***Norm No. 549/1998-2****:* ***TV****-Target Value (Negligible Risk),* ***MPC****–Maximum Permissible Concentration (Max. Tolerable Risk),* ***IV****–Intervention Value (Serious Risk),* ***Low No. 220/2004***

Table 3. Mercury concentration in samples from the area of Rudňany (average ± standard deviation)

Area	Birch (mg/kg)/d.w.	Pine (mg/kg)/d.w.	Spruce (mg/kg)/d.w.
Rudňany - Matejovce	0.064±0.02	0.041±0.03	0.047±0.02
Rudňany - tailing	0.124±0.03	0.120±0.02	0.125±0.04
Rudňany - tailing, valley	0.270±0.05	0.031±0.03	0.063±0.01

Table 4. Effect of mercury on mortality *Dendrobaena veneta* after 28 days of exposure at the end of the tests (A-E) in different types samples from Eastern Slovakia and correlation coefficients between mercury and mortality earthworms (*r* - Pearson matrix correlation)

		Repeats of test					
Area	**Input of worms No.**	**Mortality A (ANDE)**	**Mortality B (ANDE)**	**Mortality C (ANDE)**	**Mortality D (ANDE)**	**Mortality E (ANDE)**	**Pearson Matrix Correlation**
Hornád River **SED**	10	3	1	2	3	2	*r*=0.3669
Hnilec River **SED**	10	1	2	2	1	1	*r*=0.3273
Krompachy 1KL **S**	10	3	3	1	2	2	*r*=0.2182
Krompachy 2KL **S**	10	1	3	1	2	1	*r*=0.2004
Krompachy 3KR **S**	10	2	4	1	3	4	*r*=0.6054
Krompachy 4KO **S**	10	3	5	4	3	3	*r*=0.6394
Krompachy a **SED**	10	5	3	6	5	2	*r*=0.5418
Krompachy b **SED**	10	4	5	3	5	3	*r*=0.5403
Rudňany Matejov. **S**	10	2	2	3	2	3	*r*=0.7206
Rudňany tailing **S**	10	4	5	3	5	4	*r*=0.8501
Rudňany tail.valley **S**	10	3	5	2	4	2	*r*=0.8549
Rudňany tailing **SED**	10	6	4	6	3	6	*r*=0.8747
Control **S**	10	1	0	1	1	1	*r*=0.2500

(ANDE) - Absolute numbers of dead earthworms

4. Conclusions

The aim of this study was the evaluation of mercury distribution and toxic effects in soils, sediments, plants from Eastern Slovakia, using earthworms. The high mercury concentrations were determined in the Rudňany tailing-SED (188.5 mg/kg), Krompachy a-SED (69.4 mg/kg), Krompachy b-SED (93.4 mg/kg), and the soils of the Rudňany-tailing-S (82.5 mg/kg), Rudňany-tailing, valley-S (57.8 mg/kg) and Krompachy 4KO-S (20.6 mg/kg Hg). It was found that earthworms (*Dendrobaena veneta*) in some cases caused decrease of mercury concentration in contaminated soils and sediments. A significant positive correlation was found between highest concentrations of mercury Rudňany-tailing SED Hg=188.5 mg/kg (r=0.87) with the highest mortality of *Dendrobaena veneta* after 28 days bioassay. The highest proportion of mercury from the soil accumulated to the birch leaves in the area Rudňany - tailing, valley. The high concentration of mercury in the study samples may lead to severe environmental and health impact. Therefore, it is important to understand the distribution of mercury in soils and sediments in relation to pollution control.

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