EFFECT OF ENVIRONMENTAL LOAD ON THE TOXICITY OF BOTTOM SEDIMENTS

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Abstract: This study is devoted to Ecotoxicity tests, Terrestrial Plant Test (modification of OECD 208), Phytotoxkit microbiotest on *Sinapis alba* and chronic tests of Earthworm (*Eisenia veneta*), modification of OECD Guidelines for the testing of chemicals 317, Bioaccumulation in Terrestrial Oligochaetes on polluted sediments. Earthworms can accelerate the removal of contaminants from soil. The study materials are river sediments, which were obtained from a monitoring station - the Water reservoir the Ružín No.1 particularly, the river Hornád, Hnilec and sample from sludge bed Rudňany. The samples of sediment were used to assess of the potential phytotoxic effect of heavy metals on higher plants. Total mortality was established in earthworms using chronic toxicity test after 7 and 28 exposure days. Based on the phytotoxicity testing, phytotoxic effects of the metals contaminated sediments from the sludge bed Rudňany on *S. alba* seeds was observed. The largest concentration differences were recorded in the sample R7 after 7 days earthworms exposure. The earthworms mortality was not influenced by sediment neither after 7 nor 28 exposure days The spectra of samples H, HO and R showed broad peak at 1 419 - 1 512 cm^{-1} characteristic for carbonate radical. In the spectra of the samples (R and R7) the vibration of C-H groups at 2 926 and 2 921 cm^{-1}, respectively were also observed, demonstrating the presence of organic matter. Our research will continue with determination of metals concentration in earthworms.

Key words: heavy metals, sediments, phytotoxkit test, test of earthworm, XRF, FTIR

1. Introduction

Ecotoxicity testing of complex materials, intended for application on soil is necessary because bioassays (compared with pure chemical assessments) reveal possible interactions between the pollutants in a mixture and integrate the effects of the environmental matrix and bioavailability (Phytotoxkit microbiotest, Bioaccumulation in Terrestrial Oligochaetes). Negative effects of contaminants on the ecosystems and humans are characterized by their environmental toxicity.

Earthworms are often used as terrestrial model organisms for ecotoxicity testing, because of their importance for the structure and function of soil ecosystems (PIOLA *et al.,* 2009). Ecological tests, such as avoidance behaviour, based on the earthworm's ability to detect a toxicant and move away, may be sensitive and fast (LEVEQUEA *et al.*, 2014). Earthworms change the physical and chemical properties of soil by mixing it with organic material and through their burrowing they improve aeration and render contaminants available for microorganisms. The presence of earthworms in contaminated soil indicate that they can survive a wide range of different organic contaminants, such as pesticides, herbicides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and crude oil, at least when concentrations of the contaminant are not too high. The improvement of the soil due to their activity

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and the microorganisms in their digestive track can contribute to the accelerated removal of contaminants from soil, but sometimes their casts adsorb the pollutant so that its dissipation is delayed (CAMPOSA *et al.,* 2014). Effect assessment using ecotoxicological bioassays with organisms at different trophic levels can provide valuable pieces of information on the risk of chemical substances in the ecosystem (NEMCOVÁ *et al.,* 2013).

Sediments in aquatic ecosystems are often contaminated with metals as a result of natural background or anthropogenic activities. Anthropogenic activities, especially mining activity are source of heavy metals contamination in Central Spiš. The highest concentrations of these pollutants are concentrated to the sediments water reservoirs of Ružín No.1, branches of the Hornád and Hnilec Rivers. Numerous precious metals, including Fe and Cu ores, were exploited in the Smolník area, and Cu, Fe, Hg, and Ba ores were mined in the Rudňany area (MACINGOVA *et al.,* 2012; JENCAROVA *et al.,* 2013). One of the mercury polluted area is sludge bed Rudňany, which is contaminated from the former mining activities. Additionally, Cu ores from home production and import (87 %) were treated in Krompachy (JUNAKOVÁ and BÁLINTOVÁ, 2012). These activities, as the long-term monitoring of the metal content in the area has confirmed, influenced the quality of the bottom sediments in the water reservoir Ružín No.1 and sludge bed Rudňany. These mining operations and the metallurgical processing of complex metals negatively impacted this region.

2. Material and methods

2.1 Characterization of sample

This study is devoted to two tests of ecotoxicity, Terrestrial Plant Test: Phytotoxkit microbiotest with *Sinapis alba* and chronic tests of earthworm (*Eisenia veneta*). Ecotoxicity and bioavailability levels of heavy metals were studied in contaminated sediments collected in Eastern Slovakia. The materials are river sediments, which were obtained from the Water reservoir the Ružín No.1 particularly, the river Hornád (HO), Hnilec (H) and sample from sludge bed Rudňany (R). The sediments were sampled in October/2012 at a depth of 50 cm. The samples were first dried at room temperature, and the sediments were thoroughly mechanically homogenized immediately prior to the experiments and then quartered. The sediments consist of sand, silt and clay fractions. The sediment reference material LGC6187 (CRM) was used as control soil (C) in experiments. The x-ray fluorescence spectrometry was used to determine concentrations of metals (Cu, Zn, As, Pb, Cr, Ni, Cd and Hg) in sediment fraction 63 µm. On the basis of obtained XRF and FTIR results, the sediment contamination was investigated. One way of sediment utilization could be its application into agglomerate using the sintering process which is used for processing of fine-grained materials (LEGEMZA *et al.,* 2011; FINDORÁK *et al.,* 2014).

2.2 X-ray fluorescence spectrometry

The concentration of heavy metals in sediments and sediments after 7 and 28 days earthworms exposure was measured through the use of SPECTRO XEPOS X-ray fluorescence spectrometer (range of elements: Na(11)-U(92), SPECTRO Analytical Instruments, Germany). The sample material for XRF measurement was dried and homogenized, then powder sample (5 g) was homogenized with Clariant micropowder C (1 g) and then pressed with 15 tons to pellet with 32 mm diameter.

2.3 Infrared spectroscopy

The infrared spectra were recorded on FTIR spectrometer BRUKER TENSOR 27, (Germany) equipped with DTGS KBr detector. For each sample 64 scans were measured in the 4 000-400 cm^{-1} spectral range in the transmission mode with a resolution of 4 cm^{-1}. The KBr pressed-disc technique was used for preparing a solid sample for routine scanning of the spectra. Samples of approximately 0.1 mg were dispersed in 200 mg of KBr to record optimal spectra in the regions 4 000-400 cm^{-1}. A diameter of the pellets, pressed from samples, was 10 mm. For FTIR measurements total samples and samples after 7 and 28 days earthworms exposure were used.

2.4 Toxicity testing with Phytotoxkit

Phytotoxkit is an alternative test procedure that enables determination of the biological effects of chemical compounds on plants. The sediment was covered with the filter plate and ten seeds of *S. alba* were placed on top of the filter in a single row. After closing the test plates with the transparent cover, they were placed vertically and incubated for 72 h at 25 °C. Three replicates were performed for each sample set. Root growth inhibition was measured after 72 h. The test was considered to be valid if the number of germinated seeds in the control was at least 90 %. Pictures of the test plates were analyzed with the free image analysis program Image Tools. The sediment samples were used to evaluate potential phytotoxic effects using the parameters of the percentage inhibition of seed germination (ISG) and percentage inhibition of root growth (IRG) in the test sediment.

2.5 Toxicity testing with earthworms

The experiments were carried out as described in the OECD Guidelines 317 for the testing of chemicals relating to environmental fate, tests of mortality. Contact bioassays are important for testing the ecotoxicity of solid materials (KOBETIČOVÁ *et al.,* 2010). The reaction to the earthworm (*E. veneta)* was used for chronic tests in the sediments. The earthworms (*E. veneta)* 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 sediment samples was in the range 6.25 - 7.35. Three replicates were performed for each test (of the sediment 100 g dry weight) with ten earthworms added to each boxes. Then distilled water was added for purpose to obtain 30 % moisture of sediment. After that, the boxes with sediments were kept for 7 and 28 days at 20 °C. A small part of chippings oats was added every week in each box as a source of food as recommended. The results were evaluated as the percentage inhibition of mortality and compared to the control soil. Total mortality was observed at earthworms after 7 and 28 exposure days.

3. Results and discussion

Table 1 summarizes the results of the chemical analyses of the metals in the sediments, revealing significant contamination with Cu and As for sample Hnilec (**H**) and with all the metals for sample Rudňany **(R)** according to the laws of the Methodological Instruction of the Ministry of Environment of the Slovak Republic No. 549/1998-2 for Assessment of Risks from Pollution of Sediments of Streams and Water Reservoirs.

Table 1. Concentrations of heavy metals in the total sediments H, HO, R, C and in sediments after 7 and 28 days earthworms exposure (H7, H28, HO7, HO28, R7, R28, C7, C28).

	Concentration (mg/kg d. w.)							
	Cu	**Zn**	**As**	**Pb**	**Cr**	**Ni**	**Cd**	**Hg**
H	408.5 ± 2.2	399.5 ± 12.3	56.7 ± 1.4	94.3 ± 1.3	54.3 ± 4.2	45.8 ± 2.9	2.8 ± 0.3	2.6 ± 0.3
H7	397.7 ± 4.7	397.4 ± 5.4	49.5 ± 4.6	90.3 ± 1.0	53.3 ± 9.6	46.0 ± 3.9	2.1 ± 0.1	2.1 ± 0.2
H28	404.1 ± 3.2	404.0 ± 15.9	54.8 ± 2.1	93.0 ± 2.2	61.8 ± 8.9	45.6 ± 2.5	2.5 ± 0.4	2.5 ± 0.2
HO	161.2 ± 1.9	263.0 ± 14.9	32.8 ± 3	47.1 ± 1.9	95.9 ± 3.8	76.5 ± 6.6	6.5 ± 0.7	6.7 ± 0.4
HO7	150.1 ± 0.9	264.5 ± 6.7	35.6 ± 2.8	50.1 ± 4.3	82.9 ± 6.4	67.9 ± 4.8	4.2 ± 0.3	5.9 ± 0.3
HO28	144.0 ± 2.2	255.6 ± 8.6	34.5 ± 2,1	49.2 ± 2.4	84.9 ± 8.3	67.6 ± 4.5	3.8 ± 0.4	5.7 ± 0.3
R	1429 ± 5.7	1853 ± 31.8	133.5 ± 5.4	645.5 ± 9.1	481.7 ± 16.4	155.1 ± 4.7	67.7 ± 2.1	188.5 ± 1.1
R7	1269 ± 24.5	1574 ± 17.5	124.7 ± 6.3	547.9 ± 6.7	359.1 ± 17.3	136.9 ± 9.5	58.5 ± 3.9	137.9 ± 8.4
R28	1415 ± 33.3	1756 ± 21.2	144.4 ± 9.6	617.9 ± 10.0	476.7 ± 13.6	150.4 ± 4.2	63.7 ± 2.6	187.0 ± 4.9
C	<0.5 ± 0.1	8.0 ± 1.2	0.7 ± 0.3	31.7 ± 0.9	175.5 ± 4,3	7.4 ± 0.9	8.4 ± 1.1	<0.5 ± 0.2
C7	<0.5 ± 0.2	5.9 ± 1.5	0.2 ± 0.4	21.4 ± 1.0	86.7 ± 7.4	6.3 ± 0.7	4.3 ± 1.0	0.2 ± 0.1
C28	<0.5 ± 0.2	7.1 ± 0.2	0.4 ± 0.3	20.1 ± 0.9	95.0 ± 4.9	8.4 ± 0.3	9.6 ± 1.2	0.4 ± 0.3
	Norm used for comparison (mg/kg d.w.)							
TV	36	140	29	85	100	35	0.8	0.3
MPC	73	620	55	530	380	44	12	10
IV	190	720	55	530	380	210	12	10

Norm No. 549/1998-2: TV-Target Value (Negligible Risk), MPC–Maximum Permissible Concentration (Max. Tolerable Risk), IV-Intervention Value (Serious Risk), Control soil (C, C7, C28), sediment of Hnilec (H, H7, H28) and Hornád (HO, HO7, HO28) River, sludge bed of Rudňany (R, R7, R28), ± SD

From the obtained XRF results it is evident (shown in Table 1) that in all study samples without sample **H** was recorded decrease in metal concentrations after 7 days earthworms exposure, mainly the concentration of As, Cd for sample **H7**; Cu, Cr, Ni Cd, Hg for sample **HO7**; Cu, Zn, As, Pb, Cr, Ni, Cd, Hg for sample **R7**; Zn, As, Pb, Cr, Hg for sample **C7.** Other concentrations were higher than basic concentration.

After 28 days earthworm exposure decrease of concentrations Cu, As, Cd for sample **H28**; Cu, Zn, Cr, Ni, Cd for sample **HO28**; Cu, Zn, Pb, Cr, Hg for sample **R28**; Cu, Zn, As, Pb, Cr for sample **C28** was found. The largest concentration differences were recorded in the sample **R7** after 7 days earthworms exposure. It was found that earthworms in some cases caused decrease of metals concentration in contaminated sample. The earthworms mortality was not influenced by sediment neither after 7 nor 28 exposure days.

The FTIR spectra of studied sediments (total samples and after 7 and 28 days earthworm exposure) are shown in Fig.1. The wavenumbers and associated vibration types of dominant minerals are given in Table 2 - 3. It was found that the absorption bands in the range 3 620-3 695cm^{-1} were due to asymmetric valence vibration of structurally bound OH group from clay minerals. The peaks around 3 423 and 1 630cm^{-1} correspond to the H-O-H stretching and bending vibrations of the adsorbed water, respectively. In the spectra of the samples (**R** and **R7**) the vibration of C-H groups at 2 926 and 2 921cm^{-1}, respectively were also observed, demonstrating the presence of organic matter (FARMER, 1974; MADEJOVÁ, 2003). But in the sample **R28** the vibration around 2 920cm^{-1} was missing. This is probably related to the viability and metabolic activity of earthworms.

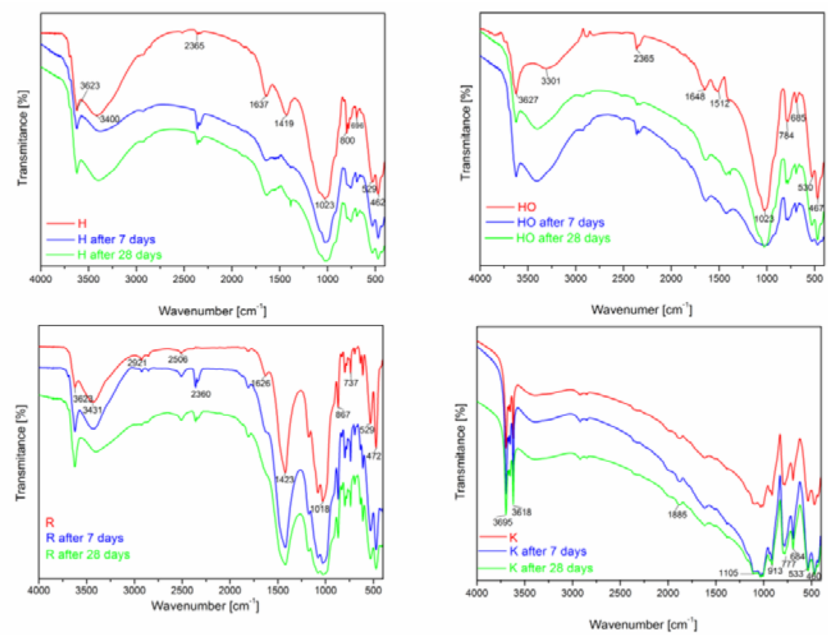

Fig. 1. The FTIR spectra of the total sediments: H, HO, R and K (Control soil) and in sediments after 7 and 28 days earthworms exposure (H7, H28, HO7, HO28, R7, R28, K7, K28).

The spectra of samples **H**, **HO** and **R** showed broad peak at 1 419-1 512cm^{-1} characteristic for carbonate radical. The main absorption band for SiO_2 connected with the stretching vibration of bridging oxygen atoms is known to be in the range 1 000-1 100 cm^{-1} and the position of the peak depend on the structural arrangement of the oxygen atom (GREEN *et al.,* 2001). The absorption bands located at 790 and 690 cm^{-1} are characteristic bands of network Si-O-Si, which correspond to quartz. At 529, 530 and 533 cm^{-1}, respectively was found the absorption band, which corresponds to stretching vibration of Si-O-Si.

In FTIR spectrum the characteristic bands of carbonate vibrations at 1 418 and 1 023 cm^{-1} were also seen (CARNIN *et al.,* 2012), or it could be organic proportion. To recognize the mineral species the positive identification of many characteristic bands of individual minerals as possible in the whole 4 000-400 cm^{-1} range is needed though the OH stretching region which is perhaps the most important diagnostic part of the spectrum. The results of Table 2 and 3 confirm the presence of functional groups that are part of the toxic substances.

Table 2. Characteristic vibrations (ν/cm^{-1}) of the studied sediments.

	ν(AlOH)	**ν (H_2O)**	**ν (C-H)**	**Calcite and Dolomite**	**Carbonate**	**δ(H_2O)**	**ν (C-H) ν (C-C)**
H	3 623	3 400	-	-	2 365	1 637	1 419
H7	3 620	3 369	-	-	2 360	1 642	-
H28	3 623	3 394	-	-	2 365	1 632	1 387
HO	3 627	3 301	-	-	2 365	1 648	1 512
HO7	3 623	3 400	-	-	2 350	1 637	1 423
HO28	3 623	3 410	-	-	2 354	1 637	1 434
R	3 623	3 431	2926	2506	-	1 626	1 419
R7	3 623	3 420	2921	2506	2 360	1 616	1 419
R28	3 623	3 405	-	2506	2 360	-	1 419
C	3 695	3 618	-	-	-	-	-
C7	3 695	3 618	-	-	-	-	
C28	3 695	3 618	-	-	-	-	

Table 3. The characteristic vibrations (ν/cm^{-1}) of studied sediments.

	ν (SiO)	**δ(AlAlOH)**	**Si-O-Si**	**ν (SiOSi)**	**δ(SiOSi)**	**δ(SiOAl)**
H	1 023	-	800	696	529	462
H7	1 013	-	753	687	529	467
H28	1 008	-	747	685	529	462
HO	1 023	-	784	685	530	467
HO7	1 018	-	789	685	530	467
HO28	1 008	-	789	691	530	462
R	1 023	-	862	607	529	472
R7	-	-	867	607	529	472
R28	1 018	-	862	618	529	472
C	1 105	913	777	684	533	460
C7	-	1 105	913	777	684	533
C28	-	1 105	913	777	684	533

According to Fig. 2 the percentage inhibition of seed germination (ISG) was 11.6 – 47.1 % in the H and HO samples and 28.5–60 % in the R. The test is 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 9.7–48.1 % in the H and HO samples and 29.7–70.1 % in the R respectively, which was higher than the inhibition of seed germination (ISG) especially of the sediments from Sludge bed Rudňany. The ISG (%) was 10.9-47.2 % in the H-28 and HO-28 samples and 24.7-50.9 % in the R-28 after 28 exposure days of earthworms. The IRG (%) was 9.8–44.8 % in the H-28 and HO-28 samples and 28.9–66.2 % in the R-28.

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/EC_{50}. The inhibition of the germination rate differs significantly from the controls in our case. Based on the phytotoxicity testing, phytotoxic effects of the metals contaminated sediments from the sludge bed Rudňany on *S. alba* seeds was observed.

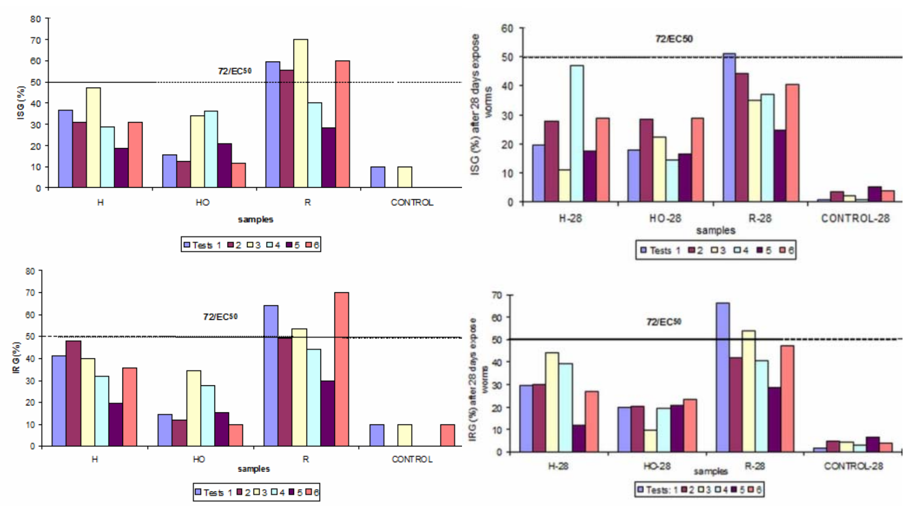

Fig. 2. Indexes ISG and IRG of the *S. alba* in the six series of the sediments (H and H-28), (HO and HO-28), (R and R-28) and (Control and Control-28).

4. Conclusions

This study was aimed to obtain detailed information about the total metals concentrations, mineral species, organic matter and percentage of earthworms mortality after 7 and 28 exposure days in river sediments (Hnilec and Hornád) and sludge bed Rudňany. The results of the chemical analysis of the metals in the sediments, show significant contamination with all the metals for sample Rudňany. It was found that earthworms in some cases caused decline of metals concentration in contaminated samples. FTIR spectroscopy confirmed in samples of R and R7 the presence of vibrations of C-H groups at 2 926 and 2 921 cm^{-1}. It demonstrates the presence of organic matter. The absence of that vibration in sample R28 probably related to the viability and metabolic activity of earthworms.

The results of our study confirmed that no mortality was observed in the studied sediments. Based on the phytotoxicity tests, no phytotoxic effects of the metal contaminated sediments from the water reservoir Ružin No.I, Hnilec (H, H-28) and Hornád (HO, HO-28) on *S. alba* seeds was observed. Potential phytotoxic effects of the metals contaminated sediments from the sludge bed Rudňany (R, R-28) on *S. alba* seeds was observed. The inhibition of the germination rate (phytotoxic and mortality tests) of sludge bed Rudňany was probably the result of their high contamination by heavy metals and of their physico-chemical properties.

This is the first study to use *E. veneta* as an indicator species to assess the risk of sediment contamination by heavy metals. However, more toxicity data for various species are needed to evaluate the environmental risks of heavy metals in sediments. Our research will continue with determination of metals concentration in earthworms. The obtained results might be useful in the planning of future sediment utilization.

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