Ecotoxicological Evaluation of Mining Dump in the Ostrava Region

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Abstract

The aim of this study is to assess the suitability of use of a selected battery of biological tests to evaluate ecotoxicity of mine tailings before biological recultivation of mining dumps. Samples of mine tailings were collected at mining dump Jeremenko in Kunčice nad Ostravicí. For determination of acute toxicity, contact test using earthworms Eisenia foetida, germination and growth inhibition test using seeds of Sinapis alba, luminescence test using bacteria Vibrio fischeri and enzyme acetylcholinesterase inhibition assessment were used. Results showed clearly that indicator organisms reacted sensitively to the presence of toxicological substances found in mine tailings samples. These samples were collected at five locations at mining dump of Jeremenko mine. In three of these locations, a toxicological effect could be observed in all indicator organisms. This study proved the suitability of application of ecotoxicological evaluation for determination of possible use of biological recultivation at mining dumps.

Keywords: recultivation, mine tailings, mining dump Jeremenko, acute toxicity tests, acetylcholinesterase

Introduction

An accompanying phenomenon of black coal deep mining and treatment is the production of tailings, which, based on their composition, are not suitable for greater industrial use. The unused part of tailings is stored close to the mining area in the form of mining dumps, creating extensive ridges often disrupting the natural landscape. Integration and restoration of areas negatively affected by mineral resource mining are realized by recultivation processes, which usually consist of two parts: technical recultivation including area shaping and biological recultivation, such as planting of bushes, trees and therefore creating a suitable habitat for animal and plant species (Gremlica, 2013). At some locations, the biological part of recultivation is not successful. Reasons why grassing and afforestation of surface are unsuccessful could include climatic effects, for example temperature fluctuation at the mining dump's surface, water regime, the colour of substrate but also the presence of substances with toxicological effect in mining tailings (Tordoff et al., 2000). Before executing a biological part of recultivation, it is fitting to verify the presence of toxic substances in substrate of dumps, which could negatively affect planted plants. An evaluation of risks of substances found in mining substrate of dumps to living organisms could be realized by ecotoxicological biotests using selected indicator organisms.

There are approximately 50 mining dumps in broader Ostrava, differentiating in shape, area and volume of carried tailings. The mining dump of Jeremenko mine lies in the area of town Kunčice nad Ostravicí. The mine tailings from Jeremenko mine were stored there up to the height of 7 m in the years 1965 to 1990. In the whole area of mining dump, there are dilapidated tailings without significant coverage by arable and sub-arable soil. In 2002 and 2003, the recultivation works started by planting seeds of selected trees, which were later found dead in massive numbers. During the following years, the surface was covered by substrate and tree seeds were planted again, but the plants withered and died again (Kotyza, 2004, 2006; Latová, 2007). In order to figure out the cause of failure of the repeated efforts of reforestation, soil samples were collected at selected locations on the mining dump and tested by selected biotests in aim to evaluate the possibility of using the selected battery of ecotoxicological tests to figure out the cause of repeated failure of biological recultivation.

Materials and methods

Sample characteristics

Soil sample collection at mining dump of Jeremenko mine was carried out on five locations in total during three years. Locations were chosen by vegetation density. First and second location represented areas with very sparse growth and distinct mine tailings substrate, the third location was in the area with denser growth and last two locations were selected to include places with highest vegetation density including smaller shrubs and trees. Samples were labelled 1–5 with the first location being labelled 1. Following locations were labelled accordingly.

Sample collection and pre-treatment

Before collection itself, all plant coverage was removed from selected locations. Soil samples were collected into plastic containers in a minimal amount of 2 kg from a depth of 30 cm and area of 1m2. Collected samples were dried under room temperature for 2 weeks. After a drying period, samples were sieved through stainless steel sieve with 2 mm holes. The pH values, the proportion of dry matter and soil WHC of sieved samples were determined by ČSN ISO 10390, ČSN ISO 11465 and ČSN ISO 11274 respectively. In order to successfully carry out a test using *Sinapis* alba seed and bacteria *Vibrio fischeri*, water leachates were prepared according to Methodological Instruction of the Waste Department of the

Tab. 1. Physical characteristics of soil from selected locations of Jeremenko mining dump
Tab. 1. Fizyczne cechy gleby z wybranych lokalizacji składowiska górniczego Jeremenko

Numbers of samples	pH values soil samples	pH values of leachates of soil samples	Dry matter in samples [%]	WHC [ml/g]
1	7,69	8,15	93,58	0,56
2	8,82	8,02	93,05	0,50
3	8,49	7,49	90,10	0,52
4	8,32	7,68	78,55	0,83
5	8,25	7,59	91,57	0,72

Ministry of Environment to evaluate the leachability of waste (2007).

Acute toxicity test using Vibrio fischeri

In order to determine acute toxicity of water leachate samples of soil samples, luminescence test using bacteria *Vibrio fischeri* was carried out according to ČSN EN ISO 11348-1 – Water Quality – Determination of Inhibitory Effect of Water Samples on Light Emission of *Vibrio fischeri* (Luminescent Bacteria Test) – Part 1: Method using freshly prepared bacteria.

Parts of every test were negative and positive controls using a standard substance, potassium dichromate (0,4-100 mg/l). Water leachate samples were diluted geometrically, the highest measured concentration of concentration series was 50% solution. Luminescence inhibition evaluation was carried out after exposition times of 15 and 30 minutes using luminometer with measuring chamber tempered to 15 ± 1°C (LUMIStox, Hach-Lange GmbH). From obtained results, EC20 and EC50 values were calculated, determining concentration necessary for 20% (50% respectively) light emission inhibition in bacteria *Vibrio fischeri* compared to control. The results are stated in the form of mg of soil that was needed to be leached in 1 litre of distilled water to achieve respective light emission inhibition in bacteria *Vibrio fischeri*.

Acute toxicity test using Sinapis alba

Test using seeds of *Sinapis* alba was performed according to the instructions in Methodological guideline of the Waste Department to determine waste ecotoxicity (2007). Water leachates were evaluated without using concentration series. The test included negative and positive control with standard potassium dichromate (100 mg/l). The test was carried out in glass Petri dishes of 12 cm in diameter. From every water extract, 4,5 ml was pipetted on the surface of the filter paper in a Petri dish. On moistened filter paper, 30 seeds of *Sinapis* alba were placed there. Incubation took place in a thermostat without light for 72 ± 2 hours with temperature 20 ± 2 °C. Mean root length of seeds was an observed parameter. After 72 hours, the percentage of growth inhibition was calculated.

Acute toxicity test using Eisenia foetida

Acute toxicity test using Eisenia foetida was performed according to OECD No. 207 (1984). Actual solid soil samples collected at each location were evaluated. The test included artificially prepared artificial soil, composed from peat (10% by weight), kaolin clay (20% by weight) and quartz sand (70% by weight) as a negative control. A positive control was also included by using standard potassium dichromate (1 g/kg). For testing, 2 months old earthworms from synchronous breeding were selected. Testing was performed in 1000 ml beakers. Each beaker contained 500 g of artificial soil moistened to 50% WHC (positive and negative control) or 500 g of samples (sample 1–5) also moistened to 50% WHC. 10 earthworms were put onto the surface of each beaker. The test was performed for 14 days at temperature 20 ± 2 °C. After the exposure time, earthworm mortality was evaluated. Earthworms were considered dead if they did not respond to any mechanical stimulus. Percentage of mortality was calculated from a number of dead earthworms.

Inhibition assessment of enzyme acetylcholinesterase

Activity assessment of enzyme AChE was performed on earthworms Eisenia foetida that were exposed to samples for 14 days. Afterwards, earthworms were homogenized and centrifuged. In formed supernatant, the amount of protein was evaluated using the Bradford method (1976). AChE activity was evaluated spectrophotometrically according to Ellman et al. (1961). The test inclided negative and positive control with O,O-diethyl hydrogen phosphorothioate (100mg/kg).

Results and discussion

Collected samples from selected locations at mining dump of Jeremenko mine were evaluated using physical assessments. The proportion of dry matter and WHC of actual soil samples, as well as the pH values of soil and leachates were measured. The pH values of soil samples from different locations varied from 7,69 to 8,82, the pH values of leachates of soil samples were similarly from 7,59 to 8,15. The proportion of dry matter in samples from most locations was from 90,10% to 93,58%, the only sample from location number 4 showed lower value (78,55%). Water capacity WHC values were lower in samples 1–3 (0,50–0,56 ml/g) in comparison to WHC of samples 4–5 (0,72–0,83 mg/l). Results are stated in Table 1.

Toxicity assessment

Water leachates prepared from collected samples were tested using bacteria *Vibrio fischeri* and seeds of *Sinapis* alba. Acute toxicity test using *Vibrio fischeri* showed inhibition of light emission in all samples, though the level of inhibition varied. The highest inhibition effect after 30 minutes of exposure was observed in sample 2, the lowest inhibition effect and thus the lowest toxicological effect was observed in sample 5. Sample 4, collected from the location with grassy vegetation, showed raised values of luminescence inhibition. Positive control using potassium dichromate gave value 30minEC50 = 2,72 mg/l. This value matches standardized values, therefore confirms the validity of results. EC20 and EC50 values after exposure of 30 minutes are shown in Figure 1.

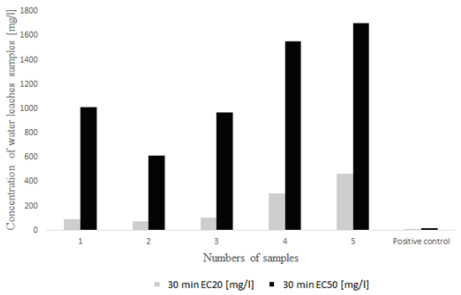

Fig. 1. EC_{20} and EC_{50} values after 30-minute exposure of water leachates samples to bacteria *Vibrio fischeri*
Rys. 1. Wartości EC_{20} i EC_{50} po 30-minutowej ekspozycji próbek odcieków wody na bakterie *Vibrio fischeri*

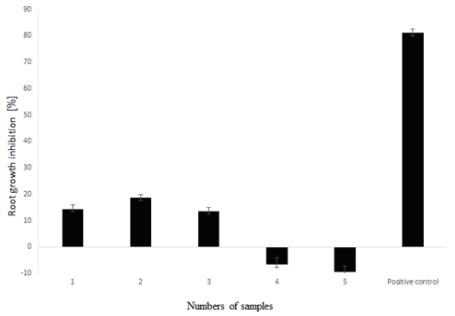

Fig. 2. Root growth inhibition of *Sinapis alba* at chosen locations at mining dump of Jeremenko including the positive control
Rys. 2. Hamowanie wzrostu korzenia *Sinapis alba* w wybranych lokalizacjach na hałdzie kopalni Jeremenko, w tym kontrola pozytywna

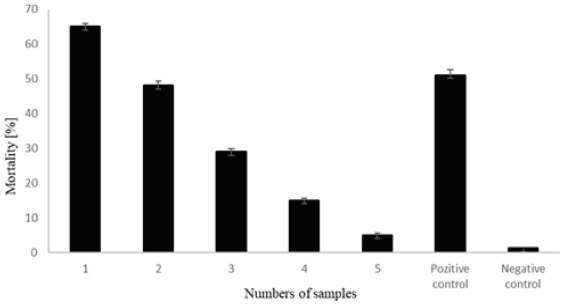

Fig. 3. Mortality of earthworms *Eisenia foetida* in selected locations at mining dump of Jeremenko including positive and negative controls
Rys. 3. Śmiertelność dżdżownic *Eisenia foetida* w wybranych lokalizacjach na hałdzie Jeremenko, w tym kontrole pozytywne i negatywne

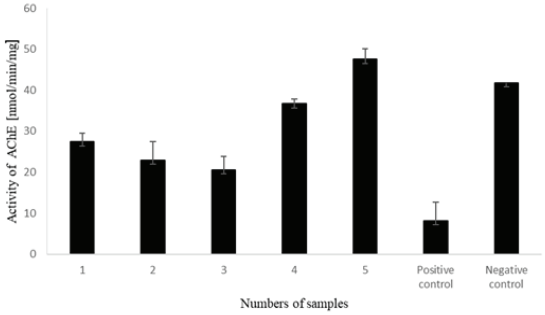

Fig. 4. Average activity of enzyme AChE in earthworms after exposure to soil samples from mining dump Jeremenko
Rys. 4. Średnia aktywność enzymu AChE u dżdżownic po ekspozycji na próbki gleby z wysypiska Jeremenko

Tab. 2. Summary of results of acute toxicity tests using chosen indicator organisms. Toxic effect (+), weaker toxic effect (+/-), no toxic effect (-)
Tab. 2. Podsumowanie wyników badań toksyczności ostrej z wykorzystaniem wybranych organizmów wskaźnikowych. Działanie toksyczne (+), słabsze działanie toksyczne (+/-), brak działania toksycznego (-)

Number of samples	*Vibrio fischeri*	*Sinapis alba*	*Eisenia foetida*	Inhibition of AChE
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+/-	-	+/-	+/-
5	+/-	-	-	-

Results of acute toxicity test using *Sinapis* alba confirmed the toxic effect on samples from locations 1–3, which resulted in root growth inhibition from 13,58% to 18,59%. The toxic effect in samples 4 and 5 was not observed, on the contrary, growth stimulation up to 10% was recorded. Positive control showed growth inhibition values to be 81±1,6 %, thus confirmed the validity of the test. The final of root growth inhibition of *Sinapis* alba is shown in Figure 2.

Results of acute toxicity test using earthworms Eisenia foetida proved that the highest toxic effect was manifested in samples 1–3, in which mortality values varied from 29% to 65%. The lowest mortality was observed in sample 5 (5%). These results matched levels of vegetation density of each location. Sample 1 was collected from the location with minimal growth and sample 5 from the location with the highest vegetation density including smaller shrubs and trees. Mortality in positive control was 51±1,54%, which responds to the amount of added standard. The negative control, consisting only of artificial soil, showed negligible mortality 1,25±2,18%. Results of both positive and negative control confirmed the validity of the test. Comparison the mortality of earthworms after exposure to the samples, including positive and negative control, is shown in Figure 3.

Evaluation of enzyme acetylcholinesterase activity was part of sample assessment too. It appropriately completes traditional acute toxicity test by being a more sensitive biomonitoring tool, thus allowing the discovery of risks of contaminants in the environment sooner than using standard ecotoxicological tests. Activity assessment of enzyme acetylcholinesterase (AChE) was performed using earthworms Eisenia foetida, which were exposed to the samples for 14 days. The final enzymatic activity of AChE is shown in Figure 4.

The results of enzyme acetylcholinesterase activity in earthworms after exposure to soil samples from mining dump Jeremenko show that the enzyme activity was decreased the most in samples from the first three locations (samples 1–3). The highest inhibition was observed in sample 3, in which the enzyme activity was reduced by 50% compared to control. Sample 5 did not show any signs of the presence of substances inhibiting the amount of enzyme AChE in tissues of earthworms Eisenia foetida.

Conclusion

In the area of mining dump Jeremenko, five locations were selected there based on vegetation density and were then evaluated by the selected battery of acute toxicity tests. Based on results determined by contact acute toxicity test using earthworms Eisenia foetida, water leachates of soil samples test using seeds of white mustard *Sinapis* alba and bacteria *Vibrio fischeri* as well as inhibition assessment of enzyme acetylcholinesterase, the presence of substances or mixtures of substances inducing toxic effects on chosen indicator organisms was proven in some of the monitored locations. This occurrence was found most often in locations 1–3, in which negative effects on all chosen indicator organisms were observed. In location 4, the resulting effect on live organisms was weaker there as no toxic effect on seeds of *Sinapis* alba was observed. Sample collected from location 5 was non-toxic for all chosen indicator organisms. Summary of results is shown in Table 2.

Detected toxic effect at each location where a sample was collected correlates to its vegetation density. Locations without any vegetation show a toxic effect on indicator organisms unlike locations with high vegetation density. During investigations of the place, extreme conditions were determined. Mining dump's soil is porous and very dark, which especially during summer and sunny months causes overheating of the surface and subsequent drying, which negatively affects plants growing there. Lack of success of tries of afforestation of mining dump Jeremenko is not caused only by such extreme conditions but also because of the observed presence of toxic substances or mixture of toxic substances which prevent or limit the presence of plants.

Based on obtained results we can state that the use of battery of tests including representative of producers (*Sinapis* alba), consumers (Eisenia foetida) and decomposers (*Vibrio fischeri*) completed by early warning test (assessment of AChE) was appropriately chosen and presents an important tool for evaluation of suitability of application of biological part of recultivation of mining dumps.

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Ekotoksykologiczna ocena hałdy górniczej w regionie ostrawskim

Celem przeprowadzonych badań jest ocena przydatności zastosowania wybranej baterii testów biologicznych do oceny ekotoksyczności odpadów kopalnianych przed biologiczną rekultywacją hałd górniczych. Próbki odpadów kopalnianych pobrano na składowisku Jeremenko w Kunčicach nad Ostrawicą.

W celu określenia ostrej toksyczności zastosowano test kontaktowy z wykorzystaniem dżdżownic Eisenia foetida, test zahamowania kiełkowania i wzrostu z wykorzystaniem nasion Sinapis alba, test luminescencji z wykorzystaniem bakterii Vibrio fischeri oraz ocenę hamowania enzymu acetylocholinesterazy.

Wyniki wykazały wyraźnie, że organizmy wskaźnikowe reagowały wrażliwie na obecność substancji toksykologicznych w próbkach odpadów kopalnianych. Próbki te pobrano w pięciu lokalizacjach na hałdzie kopalni Jeremenko. W trzech z tych miejsc można zaobserwować efekt toksykologiczny we wszystkich organizmach wskaźnikowych. Badanie to potwierdziło przydatność zastosowania oceny ekotoksykologicznej do określenia możliwego zastosowania rekultywacji biologicznej na hałdach górniczych.

Słowa kluczowe: rekultywacja, odpady kopalniane, wysypisko Jeremenko, testy ostrej toksyczności, acetylocholinesteraza