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Using Bio-Monitors to Determine the Mercury Air Pollution in a Former Mining Area †

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Abstract: Total mercury air pollution was evaluated in the former mining area of Gelnica (Slovakia) using tree bark, mosses (*Climacium* sp., *Pleurosium* sp.), and lichen (*Pseudevernia* sp.). Samples were collected (tree bark) and exposed (moss and lichen bags) on the heaps and near the mines. Additionally, the internal parts of the mines were evaluated. The mercury content in the bio-monitors was evaluated using an AMA-254 analyzer. The results showed significant differences in tree bark mercury content between the mines and heaps. The Hg content in mosses and lichens was not influenced by the type of mining work. The lichen *Pseudevernia* sp. was found to be the best Hg accumulator compared with mosses.

Keywords: tree bark; moss and lichen bag technique; environmental monitoring; open mining pits; heaps of waste material

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1. Introduction

Air pollution is currently understood as an urgent regional and global problem. The large number of pollutants released from various emission sources contributes to the deterioration of air quality, soil quality, purity of resources, agricultural production, and human health [1]. The high content of hazardous elements in the air, when absorbed into the human body, can lead to direct poisoning or chronic intoxication, depending on the exposure [2]. Mercury is considered the most toxic non-essential metal in humans. Its presence in the environment is associated with several industrial activities including mining [3]. Mercury has extremely dangerous effects on the human body and causes a wide range of diseases [4]. Among all anthropogenic activities, mining and industrial activities that focus on the processing of ore materials are among the most important producers of dust and aerosol emissions [5]. Underground mining and the blasting of the upper layers retain solid particles, which are similar in composition to the substrate of the igneous, sedimentary, or mineral upper layers [6]. However, it is not only the areas where mining and processing activities are currently taking place that are problematic, since the dust particles produced during mining operations are carried by wind and rain, it is easy to pollute the wide-ranging surroundings of mining areas [7]. Methods based on bioindicators have become popular to evaluate the state and quality of air in areas with different types of environmental loads [8–10]. This method uses the ability of living organisms (both plants and animals) to respond sensitively to stress caused by changes in their natural environment. Undoubtedly, these changes include an increased content of risk elements in environmental components. A changed or disturbed environment manifests as changes (disruptions) in the physiological and biochemical reactions of the bioindicators [11]. Owing to their excellent accumulation capabilities, mosses and lichens are considered to be among the most suitable and frequently used bioindicators of air quality [12]. According to previous studies, the ability of these organisms to accumulate hazardous elements in their insoles is much higher than that of other organisms [13]. Biomonitoring methods based on mosses and lichens have several advantages over classical methods. They are cheap, available, highly sensitive, can also be used in different areas of the world, and are very suitable for monitoring [14]. Moreover, it is possible to simultaneously evaluate several hazardous substances in the air [15]. Thus far, mosses and lichens have been successfully used to monitor air quality in various types of environments, such as traffic, parking lots, urban areas, mining areas, and the interior spaces of buildings [16–20]. Bioindicator methods aimed at assessing soil and air pollution, as well as monitoring environmental changes, include tree bark. Because the outer parts of the bark are no longer physiologically active and do not have disruptive growth cycles or metabolic processes, tree bark is an ideal bioindicator [21]. Different types of trees can serve as bioindicators of different types of pollutants. In European countries, oak, pine, plane tree, ash, and elm are most often used [22]. Studies using tree bark as a bioindicator have the potential to contribute to the better understanding and monitoring of environmental problems and issues in the formulation of environmental protection measures.

The aims of this study were (i) to compare the accumulation capacity of mosses and lichens, (ii) to evaluate the suitability of tree bark for mercury air pollution, (iii) to compare ambient air pollution depending on the type of mine work, and (iv) to evaluate the state of mercury air pollution in different internal parts of the mining pits.

2. Material and Methods

2.1. Collection of Tree Bark Samples and the Methods of Their Evaluation

Tree bark was taken mainly from deciduous trees (oak, beech) at a height of approximately 1.5–2.0 m. Using a chisel, four bark samples 1 × 1 cm in size were taken from each tree, each from a different cardinal direction. From the four samples collected from each tree, one mixed sample was created and placed in a PE bag. Bark samples were collected from the vicinity of five mines (with three samples collected from the vicinity of each mine) and from the vicinity of four heaps (two samples from each heap). Tree bark was maintained in a Memmert UF 110 m forced-air laboratory furnace (Memmert GmbH & Co. KG; Schwabach, Germany) at 40 °C for 22 h. The samples were homogenized in an IKA A 10 basic rotary homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany) and stored in resealable PE bags before analysis. The total mercury content was determined using an AMA-254 instrument (AlTec Spol. s r.o., Prague, Czech Republic).

2.2. Moss and Lichen Collection, Preparation, Exposure, and Evaluation

Two mosses (*Climacium* sp. and *Pleurosium* sp.) and one lichen (*Pseudevernia* sp.) were collected from the Slanské Vrchy Mountains in places that were free of environmental burdens, at least 1 km from main roads, and at least 0.5 km from forest roads. Approximately 500 g of material was collected from each taxon, stored in a paper bag, and transported to the laboratory, where the samples were manually cleaned of plant parts, needles, and soil particles. The mosses and lichen were washed three times in deionized water for 5, 10, and 20 min (approximately 10 L of deionized water per 100 g dry weight of mosses and lichen). After washing, the samples were manually wrapped and dried at 40 °C for 24 h (Venticell 111; BMT, Czech Republic). Approximately 5 g of each taxon was wrapped and tied in nylon mesh (2 mm) and cut into pieces (10 × 10 cm). Each taxon was stored in the laboratory as a control sample (to determine its initial condition). Subsequently, each taxon was exposed to sites of interest in two replications. The samples were exposed to the internal environment of five mines (always at the beginning, middle, and end of the mine, and one series outside in front of the mine) and four heaps of mining material. The mosses and lichen bags were exposed for 6 weeks. The analysis of the samples for the presence of Hg was carried out in the same way as in the case of the tree bark samples, as described above. The relative accumulation factor (RAF) was used to evaluate the Hg content in moss and lichen samples, which was calculated as follows:

$$RAF = (C_{exp} - C_{cont})/C_{cont}$$

where C_{exp} is the mercury content measured after exposure, C_{cont} is the mercury content measured before exposure (in the control sample).

2.3. Statistical Evaluation of the Obtained Data

All statistical analyses were performed using the PAST program [23]. All data were logarithmically transformed prior to analysis. The non-parametric Mann–Whitney U test was used to compare the values of Hg (in the bark) and RAF (for moss and lichen bags) between the types of mine works (mines, heaps). The non-parametric Kruskal–Wallis test was used to compare the accumulation abilities of individual taxa and to compare the content or use in moss and lichen bags depending on the place of exposure (beginning, middle, end, and external environment of the mine).

3. Results and Discussion

3.1. Content of Mercury in Tree Bark

The mercury content (min-max (average ± standard deviation)) in tree bark samples ranged between (0.009–0.166 (0.041 ± 0.06) mg/kg). Preasetia et al. [24], who evaluated the mercury content in tree bark in Indonesia around gold mines, found that the average mercury content in *T. catappa*, *M. indica*, and *S. aromaticum*, and *L. domesticum* bark samples reached an average value of 0.0662, 0.0424, 0.0261, and 0.0154 mg/kg, respectively. Near mining areas focused on gold mining in Myanmar, the mercury content in tree bark ranged from 0.002 to 0.417 mg/kg [25]. Comparing two mining bodies it was found that the content of mercury in tree bark was significantly higher ($p < 0.05$) in the bark of trees growing next to (on the) heaps than in those growing next to the open mining pits (Figure 1). In earlier studies, it was found that the surface of mine heaps is more disturbed by weather [13] than open mine pits, which are more stable in terms of pollution.

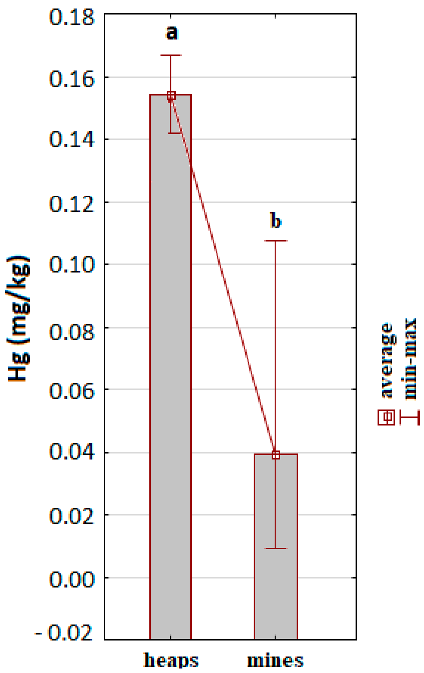

Figure 1. Comparison of tree bark mercury content between trees growing on different mining works and Mann–Whitney U test results expressing statistical differences (various letters)/similarity (same letters) between mining works in tree bark mercury content.

3.2. Content of Mercury in Moss and Lichen Bags and Comparation between Taxa

The results showed that the mercury content in moss and lichen bags (regardless of the taxa), expressed through RAF (Hg) range from (0.01–0.16 (0.09 ± 0.08)). The samples exposed near the mines reached values of (0.01–0.16 (0.09 ± 0.02)), and the samples exposed on the heaps reached values of (0.07–0.10 (0.08 ± 0.01)). The results of the non-parametric Mann–Whitney U test showed that there was no significant difference between the evaluated mining works. Because the difference between heaps and mines was confirmed for tree bark, we assume that this is because the exposure time of mosses and lichens was negligible compared to that of tree bark. Open mining pits are a source of environmental pollution, particularly during active mining. After completion, they are much more stable and are not disturbed by external factors (weather, people), as in the case of heaps. Heaps with loose surfaces are susceptible to erosion. Surface erosion can occur when natural elements, such as rainfall and wind, interact with the heaps. This can lead to the spread of contaminated material into surrounding areas and waterways.

There were some differences between the individual taxa. As shown in Table 1, the ability of lichen *Psuedevernia* sp. to accumulate mercury was significantly higher ($p < 0.05$) than that of the mosses *Climacium* sp. and *Pleurosium* sp. Several studies worldwide have compared the accumulation abilities of mosses and lichens and found that epiphytic lichens are primarily used as bio-monitors for qualitative indication as well as for the spatial and quantitative assessment of atmospheric metal contamination [26–28]. Bargagli et al. [29] compared the advantages and disadvantages of using mosses and lichens. The advantage is that, compared to mosses, epiphytic lichens are less affected by snow cover in winter, but the disadvantage is their high sensitivity to sulfur oxides in polluted areas. Lippo et al. [30] concluded that mosses more easily reflect regional differences in heavy metal deposition compared to lichens.

Table 1. The content of mercury in individual taxa expressed by RAF and the results of the non-parametric Kruskal–Wallis test expressing a statistically significant difference (various letters)/similarity (same letters) between taxa (* standard deviation).

RAF (Hg)	*Climacium* sp. [a]	*Pleurosium* sp. [a]	*Pseudevernia* sp. [b]
min	0.068	0.072	0.010
max	0.130	0.093	0.164
average	0.085	0.084	0.113
median	0.081	0.084	0.113
SD *	0.015	0.006	0.035

3.3. Comparation of Mercury Content in Moss and Lichen Taxa between Different Parts of the Mine

Moss and lichen bags exposed to different parts of the mine showed different results. The highest RAF (Hg) values were determined at the beginning of the mine and the lowest at the external part of the mine. The results of the Kruskal–Wallis test showed that RAF (Hg) values statistically differed only between the beginning (B) and the external part (EX) of the mine, as well as between the end of the mine (E) and the external part (EX) (Figure 2). The pollutant content can vary in different parts of the mining complex owing to several factors. Geological conditions, the type, and method of mining in a given part, as well as various natural and geological processes, can influence this.

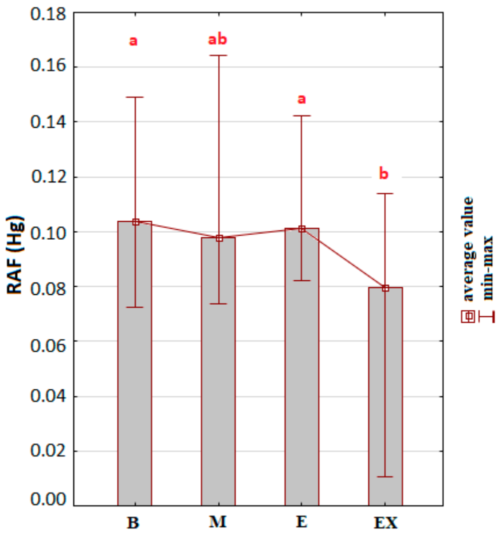

Figure 2. Mercury content in moss and lichen bags exposed in different parts of the mine (B-beginning, M-middle, E-end, EX-external part of the mine) expressed through RAF (Hg) and the results of the non-parametric Kruskal–Wallis test expressing statistical differences (various letters)/similarity (same letters) between different parts of the mine in RAF values.

4. Conclusions

Mercury is considered one of the most toxic heavy metals with a serious impact on human and ecosystem health. Its monitoring in various components of the environment as well as the implementation of measures to limit its spread mainly in risk areas should be the priority of every state. Former mining areas, which are characterized by a high number of unrehabilitated mining bodies, are very risky from the point of view of the spread of a wide spectrum of contaminants.

The use of bioindicators such as tree bark as well as moss and lichen bags has proven to be an effective alternative to traditional monitoring methods. While tree bark showed statistically significant differences between mining works in terms of mercury content, the moss and lichen bags did not differ by exposure location (different mining works). A difference was noted between different taxa. The lichen *Pseudevernia* sp. Was found to be a significantly better Hg accumulator when compared to the mosses. The difference in the mercury in the air in different parts of the mine corridors was interesting, and it was probably caused by a wide range of factors, such as geological processes, the type of mining in the given part of the mine, air flow, and many others.

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