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APPLICATION OF MICRO-X-RAY FLUORESCENCE INTO THE PLANT UPTAKE ASSESSMENT OF NUTRIENTS AND POTENTIALLY TOXIC ELEMENTS

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Abstract: The main aim of the article is to investigate the content of nutrients and potentially toxic elements in individual parts of the plant body using the analytical method of energy dispersive micro-X-ray fluorescence spectroscopy (µXRF). As the model species, silver birch (*Betula pendula*) and European spruce (*Picea abies*) were selected and sampled at the abandoned Hg-ore deposit Veľká Studňa on the well-known mining territory of Malachov (Central Slovakia). In order to investigate the bioaccumulation mechanism and test the efficiency of the method, the analyses were focused on the several parts of the plant body individually: roots, stems, branches, leaves/needles. Among all elements, Ca was contained in the highest concentrations in all samples. The concentrations of essential elements decreased as follows: Ca > Si > Mg > P > S > Mn; and of potentially toxic elements in order: Zn > Fe > Al > Hg > As > Cu. The article evaluates the efficiency of the presented analytical method for application in plant matrices.

Keywords: bioavailability, phytoextraction, potentially toxic elements, plant nutrients, µXRF

1. INTRODUCTION

Extraction and processing of ore-raw materials leave extensive long-term changes on the landscape after the end of the mining activity. This primarily includes a changed relief, hydrological regime, chemical composition of the soil and, finally, a change in the species composition of the vegetation, which must be adapted to the extreme conditions of the given habitat. The ongoing stages of voluntary or controlled succession can mitigate serious negative impacts, which plays an important role in ecosystem restoration.

The entry of potentially toxic elements (PTEs) into the natural environment and the subsequent accumulation in organisms and landscape components have a fundamental impact on the development of vegetation and the overall functioning of the local ecosystem. The bioavailability of PTEs is conditioned by several factors, on which the magnitude of the negative impact depends – mineral composition of the soil, soil reaction, degree of anthropic intervention, the presence of microorganisms, etc. The toxic effect of PTEs represents a stress factor for plants that can be tolerated only by metallophytes. Different physiological metamorphoses of less resistant ones can be observed very often. PTEs mainly affect enzyme activity, metabolic processes, growth, respiration, and the photosynthesis apparatus.

Total concentrations of elements and their assessment are one of the keystones in modern environmental research. Along with the other methods, like mass spectrometry (MS) and optical emission spectroscopy (OES) with inductively coupled plasma (ICP), X-ray fluorescence (XRF) is also popular due to its practicality and simple application (Andráš, et al., 2017; Buccheri, et al., 2018; Bílková, et al., 2023). This study investigates the content of nutrients and potentially toxic elements in individual parts of plant body using the analytical method of energy dispersive micro-X-ray fluorescence spectroscopy. As the model species, silver birch (*Betula pendula*) and European spruce (*Picea abies*) were selected and sampled in area of well-known Hg-ore deposit Veľká Studňa in Malachov (Central Slovakia). This site has been long-windedly described by many authors (Dadová, et al., 2016; Andráš et al., 2021; Maťová, et al., 2008) for its negative effects on the surrounding ecosystem due to contamination with mercury and PTEs as a result of cinnabar mining.

2. MATERIAL AND METHODS

Within the field survey, samples of juvenile trees were taken (one of each species) and stored in paper bags. The samples were subsequently prepared ex situ. Soil and other impurities that could distort the results during analysis were removed from the roots with distilled water. After drying, the samples were placed in plastic bags to avoid contamination and loss of material, such as needles from branches. Before the actual analysis itself, thin sections from the root, stem, branch, and leaf/needle were cut from the samples using a scalpel. The prepared material was fixed on a glass slide (Figure 1).

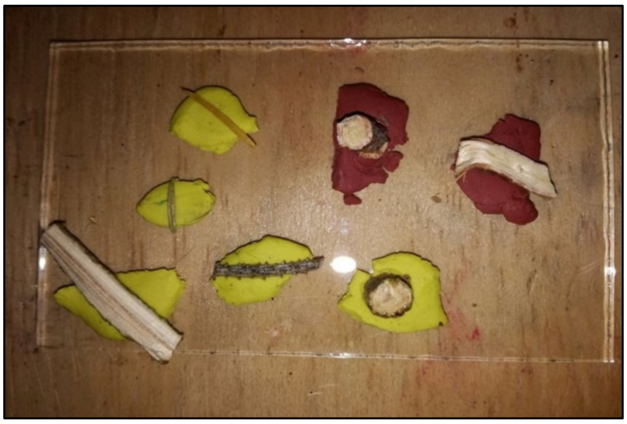

Figure 1. Samples of the plant material – prepared for analyses.

µXRF is an elemental analysis technique that allows spot analyses – examination of very small sample areas (Figure 2). Like conventional XRF instruments, this method uses direct X-ray excitation to induce characteristic fluorescence emission from a sample in order to analyse the elemental content. Unlike conventional XRF, which typically has a spatial resolution ranging from a few hundred micrometers to a few millimeters, µXRF uses X-ray optics to limit the size of the resulting beam or focus the excitation beam onto a small spot on the sample surface.

After the application of this method, in each of the samples taken, the contents of PTEs (Al, Fe, As, Hg, Cu, Zn) in plants were determined, as well as the concentrations of essential elements that are necessary for plant growth and reproduction (Mg, Si, P, Ca, Mn). The contents were evaluated according to XRF spectra (Figure 3).

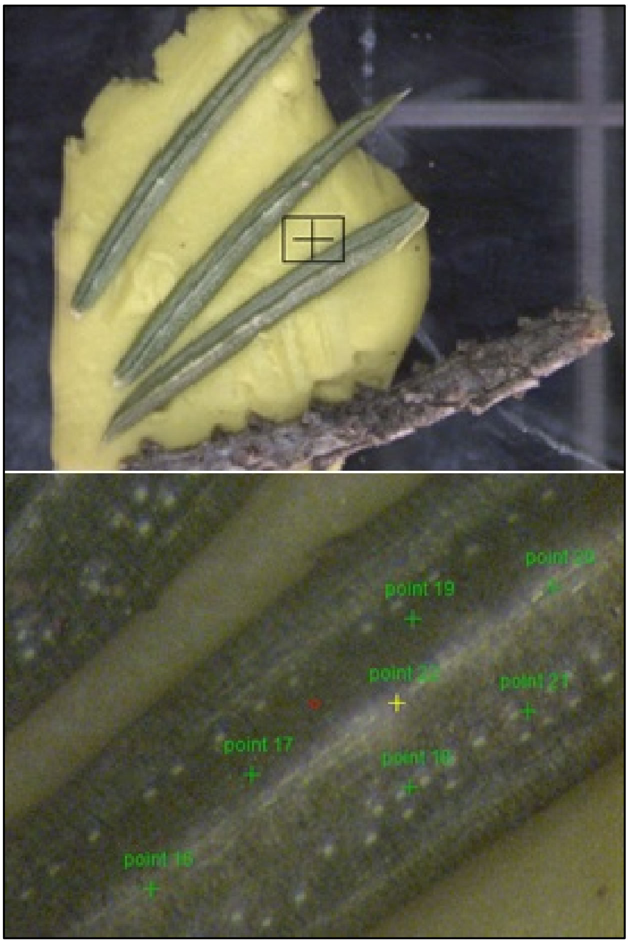

Figure 2. Location of the analysed points on the surface of the needle (*Pcea abies*).

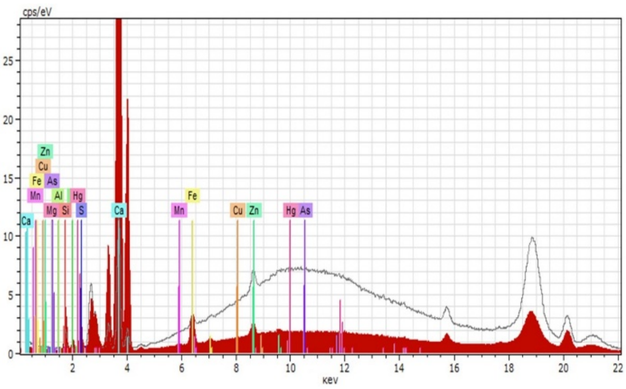

Figure 3. Spectrogram of µXRF (*Betula pendula*).

3. RESULTS

The contents of selected PTEs and plant nutrients are presented in tables 1 and 2. The application of µXRF method shows it efficiency in detecting the contents of key elements.

Table 1. Concentrations of essential elements in plant tissues. Abbreviations: c-c (cross-cut section), l-c (longitudinal-cut section), f-s (facial side), r-s (reverse side), n.d. (not detected).

	Mg	Si	P	S	Ca	Mn
Betula pendula						
roots						
c-c	5.05	2.28	1.74	1.13	79.29	0.21
l-c	10.07	0.38	6.03	3.85	61.78	0.42
stem						
c-c	2.54	1.11	1.10	0.50	88.19	0.10
l-c	7.48	3.53	7.50	4.56	59.32	0.32
branch						
l-c	1.41	25.20	0.99	1.24	63.30	0.19
leaves						
f-s	7.24	19.43	1.24	0.74	68.90	0.13
r-s	4.65	2.24	1.73	1.81	86.58	0.13
Picea abies						
roots						
	6.15	1.83	1.91	n.d.	82.98	0.24
stem						
c-c	10.01	8.46	1.78	0.82	70.06	0.34
l-c	2.15	4.68	0.58	0.40	87.43	0.17
branch						
l-c	1.45	25.33	1.00	1.25	64.04	0.20
needles						
	0.95	n.d.	0.70	0.42	93.37	0.40

Table 2. Concentrations of PTEs in plant tissues. Abbreviations: c-c (cross-cut section), l-c (longitudinal-cut section), f-s (facial side), r-s (reverse side).

	Al	Fe	Cu	As	Hg	Zn
Betula pendula						
roots						
c-c	0.82	1.49	0.09	0.17	0.95	6.79
l-c	2.15	2.48	0.34	0.51	2.69	9.31
stem						
c-c	0.61	0.96	0.07	0.10	0.52	4.17
l-c	1.87	1.79	0.17	0.44	1.54	11.47
branch						
c-c	2.98	2.68	0.04	0.04	0.08	0.87
leaves						
f-s	0.15	1.49	0.03	0.01	0.14	0.49
r-s	0.42	1.22	0.02	0.03	0.09	1.01
Picea abies						
roots						
c-c	1.36	1.88	0.16	0.23	1.12	1.99
stem						
c-c	2.39	1.82	0.13	0.30	1.51	2.39
l-c	0.42	1.34	0.09	0.19	1.06	1.51
branch						
l-c	2.99	2.70	0.04	0.04	0.08	0.88
needles						
	0.30	2.64	0.05	0.03	0.22	0.37

3.1. Silver birch (*Betula pendula*)

Calcium reached the highest concentration in roots along the essential elements in the cross-section (in longitudinal-cut, as well as in cross-cut). Other elements were contained only in minimal quantities. Calcium reached the highest concentration among all elements in the longitudinal section, and it also occurred here along with a lower Mg content. The most toxic element in the cross-cut section was Zn. The contents of Fe, Al and Hg are also slightly increased. The highest concentration values in the longitudinal section are achieved for Zn as well. Hg, Fe and Al have reach approximately similar contents. The concentration of essential elements in the cross-cut section decreased in the order: Ca > Mg > Si > P > S > Mn; in the longitudinal-cut: Ca > Mg > P > S > Mn > Si; of PTEs in the cross section: Zn > Fe > Al > Hg > As > Cu, in the longitudinal-cut: Zn > Hg > Fe > Al > As > Cu.

Similarly, as in the roots, Ca showed the highest contents in the stem among essential elements in both cross-cut and longitudinal-cut sections, while other elements were detected only in low concentrations. Manganese reached the lowest content. Zn reached the highest content among the PTEs; however, the amounts of Fe, Al and Hg could also be considered noticeable. In the longitudinal-cut section, Zn had the highest content. Aluminium, iron, and mercury were detected in lower amounts. The concentration of essential elements in the cross-cut section decreased in the order: Ca > Mg > Si > P > S > Mn; in the longitudinal-cut section: Ca > P > Mg > S > Si > Mn; of the toxic elements in the cross-cut section; Zn > Fe > Al > Hg > As > Cu; and in the longitudinal-cut section: Zn > Al > Fe > Hg > As > Cu. Very similar situation can be observed for branch, where the contents decreased as follows: Ca > Si > Mg > S > P > Mn (nutrients); Al > Fe > Zn > Hg > Cu > As (PTEs).

In leaves, the most contained element is also Ca. Its quantity was higher on the underside of the leaves (Figure 4). The second high-contained element was Si, the content of which is, on the contrary, higher on the facial side of the leaf. The third element showing higher concentrations was magnesium. The highest concentration among PTEs was detected for Fe, and even here it shows differences in the facial and reverse sides of the leaf. Other notably represented elements were Zn, Al, and Hg. The concentration of nutrients decreased in the facial side in order: Ca > Si > Mg > P > S > Mn; in the reverse side: Ca > Mg > Si > S > P > Mn; and the PTEs contained in the facial side were as follows: Fe > Zn > Al > Hg > Cu > As; and in the reverse side: Fe > Zn > Al > Hg > As > Cu.

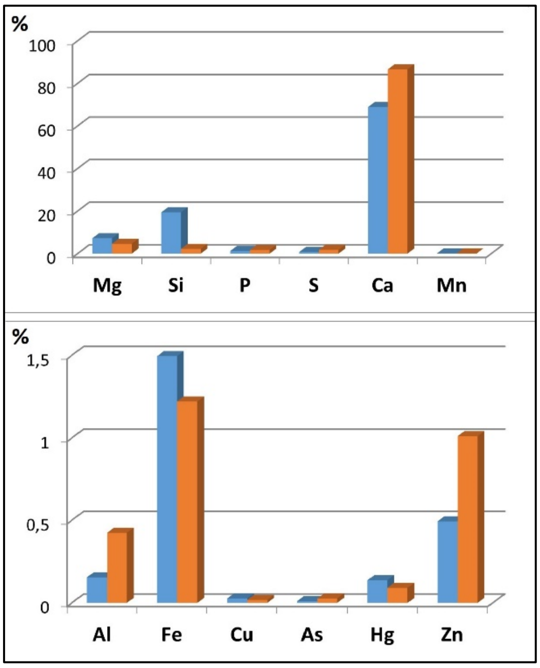

Figure 4. The concentrations of essential elements and PTEs in leaf of *Betula pendula*. Blue columns stand for facial side and orange for reverse side.

3.2. European spruce (*Picea abies*)

Along with Ca, Mg was another most-contained element in the root, but with notably lower contents. The elements P, Si, and Mn are present only minimally. The PTEs occurring in higher concentrations are Zn, Fe, Al, and Hg. The concentration of essential elements decreased in the following order: Ca > Mg > P > Si > Mn; and PTEs: Zn > Fe > Al > Hg > As > Cu.

The detected amounts of plant nutrients in the cross-cut section as well as in the longitudinal-cut section of the stem were highest for Ca and slightly lower for Mg and Si. Regarding the PTEs and their contents in the cross-cut section, increased amounts of Zn, Al, Fe, and Hg were detected. The highest concentrations of Zn, Fe and Hg were found in the longitudinal-cut section. Aluminium, arsenic, and copper are present only in minimal amounts. The concentration of essential elements in the cross-cut section decreased in the order: Ca > Mg > Si > P > S > Mn; in the longitudinal section: Ca > Si > Mg > P > S > Mn; and of PTEs in the cross section: Zn > Al > Fe > Hg > As > Cu; and in the longitudinal-cut section: Zn > Fe > Hg > Al > As > Cu. A similar situation can be described for results in nutrient contents in branch samples. PTEs are characterized by a higher content of Al and Fe, and slightly less Zn. The concentration of nutrients decreased as follows: Ca > Si > Mg > S > P > Mn; and of PTEs in order: Al > Fe > Zn > Hg > Cu > As.

In needles, the only noticeable element among the nutrients is Ca, the other detections were very low. Similarly, in the case of PTEs, there was a higher concentration only for Fe. The amounts of Zn, Al, and Hg were slightly higher. The content of essential elements decreases in the following order: Ca > Mg > P > S > Mn; and PTEs as follows: Fe > Zn > Al > Hg > Cu > As.

4. DISCUSSION

Among all the essential elements, Ca was contained in the highest concentrations in all parts of the plant body for both *Picea abies* and *Betula pendula*. The well-known fact is that this element has a structural function in cell walls and membranes (Jones, 1999); similarly, as Mg, which has also general importance as it is a necessary component in the process of photosynthesis, as well as an activator of transport reactions of enzymes and substrates (Bergmann, 1992). Among the PTEs, the most abundant element in plant tissues is Zn. The insoluble fraction usually represents up to 90% of the total concentration of Zn in soils (Broadley et al., 2007; Barak & Helmke, 1993) and in comparison, with other PTEs, Zn ranks among the most available elements for plants (Kabata-Pendias & Pendias, 2001). The most extraordinary is, however, the high content of Hg, which is contained predominantly in roots and stems. Since the locality has Hg-mining history (Maťová, et al., 2008) and the Hg obviously leaks into surrounding ecosystem components (Dadová, et al., 2016 Andráš, et al., 2022) this description could be suspected. The high ability to accumulate Hg in above ground parts of *Picea abies* at this locality was proved in previous research (Midula et al., 2023), where the concentration in plant tissues was analysed by ICP-MS and reached 24.15 mg kg^{-1}. The results presented in this study, however, shows the content in stem up to 1.51 % which is substantially higher. It is clear that the presented analytical method is able to detect an expected content of element, nevertheless, the accuracy according to exact concentrations is questionable. The difference that confirms the limited accuracy can also be observed within the comparison of two samples representing the same part of the plant body (cross-cut and longitudinal-cut sections). The method itself, however, can be used (with a proper concentration reference) for the detection of trace elements in micro-scale plant organs, which brings a substantial advantage to modern environmental research.

5. CONCLUSIONS

The higher concentrations of essential elements and PTEs in plant tissues were proven. It is clear that μXRF technology is able to detect trace elements even in plant matrices, however, since the spectra cover only the surface area, the representativeness of the whole sample is limited. The efficiency of application could nonetheless be very effective in tracing elemental contents in specific plant organs, however, with the proper repetition in order to obtain the highest possible accuracy. The consequent comparison with other relevant analytical methods (ICP-MS; ICP-OES) should also be considered.

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