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ORCHID SPECIES ANACAMPTIS MORIO AS A POTENTIAL BIOREMEDIATOR OF As, Cd AND Pb

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In this study concentration of toxic elements As, Cd, and Pb were determined in different soil types and belonging orchid species Anacamptis morio vital parts, in order to examine accumulation patterns and provide new insights about the potential use of this orchid in bioremediation technology. Soils developed on limestone, serpentine, and the chert were subjected to the BCR sequential extraction. Samples of orchid roots and tubers, as underground parts, and stems, leaves, and inflorescences, as above-ground organs, were also analyzed for the content of As, Cd and Pb. During this research, it was observed that metal content in soil is directly proportional to its content in the plant, more specifically in roots, which suggests that A. morio can potentially be used in the phytostabilization of contaminated sites. Values for BCF factors showed Cd immobilization in roots regardless of the soil type. A certain level of arsenic was transferred from roots to leaves indicating the potential for accumulation of this element into above-ground organs. Assessment of the phytoremediation potential of this orchid or another plant species from diverse environments is important as it provides information about the possibility of their future application in environmental remediation programs.

Key words: toxic metals, orchid, bedrock, bioremediation, BCR fractions

INTRODUCTION

According to the sustainable development in terms of treatment of contaminated area and a fact that plants have the nutrients uptake ability from a soil where they grow, many studies regarding the investigation of the correlation between the metals concentrations in soil and plant, and the mechanism of the absorption, have been carried out [1]. Soil's oversaturation with pollutants originated from anthropogenic or lithogenic factors, is considered as one of the main concern nowadays [2]. Metals and metalloids as a group of hazardous substances with approved mutagenicity and carcinogenicity that negatively affect human health require continuous monitoring in the environment. Although some elements are necessary for plant metabolism, an excessive amount may cause toxicity and contaminate food chain, water and soil resources [3]. Element transfer from soil to plant is affected by various natural and anthropogenic factors of a diverse origin. Yet, the bioremediation can not ensure the decomposition of the toxic metal, but it can influence their mobility and concentrate them so they can be easily removed from the environment by the bioaccumulation process [4]. Some of the natural metals uptaking mechanisms can be used for contaminated soil phytoremediation [5]. Phytoremediation is an environmental restoration technology that uses living plants along with microorganisms to remediate pollutants from sediments, soils, sludge, waste and groundwater, utilizing diverse plant processes and their physical characteristics to assist within contaminated sites remediation. [1]. The basic

phytoremediation forms are: phytoextraction, phytostabilization, phytodegradation, phytofiltration and phytovolatilization. Some of the best known and used processes are phytoextraction (uptake of contaminants and their transportation to the aboveground parts, that are later removed by harvesting) [6] and phytostabilization (reducing the mobility of contaminants through accumulation in roots or immobilization within the rhizosphere, thereby reducing off-site contamination) [7].

The amount of the metals that may be absorbed by the plant roots systems is not only dependent on the concentration in the rhizosphere soil, but also of the bioavailability of the metals [6]. Availability for uptake and metals mobility, and the plant accumulation capabilities are the crucial factors defining plant efficacy in the phytoremediation process and is mostly conditioned by the soil physicochemical properties, such as pH, cation exchange capacity, organic matter content, redox potential [8]. Metals can be classified as water-soluble, exchangeable, oxide-bound, carbonate-bound, organic matter-bound, and residual that is bounded to the resistant minerals (silicates). Water-soluble and exchangeable fractions are bioavailable as well as the oxide- and carbonate-bound fractions; potentially bioavailable is organic matter phase, while the residual matter is considered unavailable for plants [9]. The hazardous effects of toxic metals in soils are in close relation with speciation and elemental content in the liquid and solid phases of the soils [10]. Pseudo-total soil content of heavy metals (estimated by hot plate aqua regia digestion (3:1, v/v, HCl : HNO₃) [11]) is considered to be adequate for the assess

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ment of the maximum potentially soluble or mobile elements. Many sequential extraction schemes are developed so far, concerning the number of involved stages, operating conditions and used extraction reagents. However, selective extraction methods may bring information about mobile and potentially mobile species, which may correlate with phytoavailable content; these methods are giving useful data in land use assessment [12] and are suitable for the evaluation of the remediation potential utilizing and the elemental distribution assessment after the soil amendment with potential risk elements. Two of the most commonly used protocols in this regard are the BCR extraction procedure (Community Bureau of Reference) and the procedure proposed by Tessier et al. (1979) [13].

According to the De Agostini, [14] rare plants, such as orchids, may occur in modified habitats and on soils containing heavy metals, yet their ecological and physiological responses to heavy metals are still poorly understood. Some orchids, such as *Epipactis atrorubens*, have the ability to adapt to nutrient-poor and dry substrates on different bedrock types, as stated by Filimonova et al. (2019) [15]. It is assumed that orchid mycorrhizae contribute to increased tolerance to unfavorable soil conditions, such as high concentrations of toxic metals [16]. However, there is still a considerable lack of studies on metal tolerance and the remediation potential of orchid species.

Few studies provide information about plant communities and geological bedrock types preferred by the specific orchid species in Serbia [17]. *Anacamptis morio* L. R. M. Bateman, Pridgeon & M. W. Chase belongs to the group of generalist taxa, which occurs across a wide range of habitat types. It inhabits serpentine habitats possibly due to low nutrients content in the soil, which orchids are quite sensitive to, or due to the lack of strong agricultural impact and less competitiveness among plants [17]. Geological bedrock was highlighted as one of the most important gradients with a huge impact on orchids distribution and ability of *A. morio* to adapt to serpentine harsh conditions as a refuge from anthropogenic pressure recorded in the surrounding limestone areas was stated by Đorđević et al (2014). Moreover, in their research *Anacamptis morio* was found to form significantly larger populations on serpentine compared with non-serpentine bedrock types. Another study proved the existence of *Anacamptis morio* on 8 different bedrock types in western Serbia [18]. Herein, an investigation of the capacity of *A. morio* to uptake toxic metals from habitats on different bedrock types was carried out.

The primary aims of this research were to examine: i) As, Cd and Pb bioavailability in soils developed on different bedrock type and the correlation between the concentration of elements in the soil and the plant, ii) the uptake patterns of As, Cd and Pb in different parts of orchid *Anacamptis morio* on soils with different bedrock type iii) potential of the analyzed orchid species to be used for bioremediation purposes.

EXPERIMENTAL

Site description

Soil and orchid samples were collected from a total of 9 sampling points of unpolluted areas in western Serbia (Fig. 1). Sampling sites were located on different bedrock types: limestone, serpentine and chert. Each bedrock type was represented three times among the investigated localities. Sampling points on limestone were marked K1-K3 (Figure 1b,c). Mt. Blagaja (K1) is situated in western Serbia, near the town of Požega, and the altitude range of sampling sites was between 620 - 730 m. Mačkat (K2) is a village located on the northern slopes of Mt. Zlatibor. The sampling site is located at 700 m a.s.l. Buradja (K3) is located in southwestern Serbia, next to Kokin Brod. The sampling site is located at about 900 m a.s.l. The prevailing habitat types of collected *A. morio* on limestones are perennial calcareous grasslands and basic steppes, whereas vegetation type is represented by the community *Festucetum valesiaca* (*Festucion valesiaca*). Sampling points on serpentine bedrocks (S1-S3) were placed in the area of mountain Zlatibor (Figure 1b,d), situated in the western part of Serbia, known for the significant presence of ultramafics. All sampled sites are located on ultramafics (two sites on serpentinites and one site on harzburgites), at an altitude of 1000 to 1100 m, while the habitats can be described as serpentine steppes. The corresponding vegetation type is *Bromo-Danthonietum calycinae* (*Chrysopogono-Danthonion calycinae*). Sampling points on cherts (R1-R3) were placed in the area of mountain Zlatar (Figure 1b, e), located in southwestern Serbia. All sampled sites are located at an altitude of around 1110 m, in habitats defined as closed non-Mediterranean dry acid and neutral grassland. The corresponding vegetation type is *Festuco-Agrostietum capillaris* (*Nardo-Agrostion tenuis*).

Sampling, analysis and procedures

Soil samples were taken from the rhizosphere soil (up to 10 cm depth) of all collected orchid individuals. From each sampling locality, three samples of *A. morio* (Figure 1f) and its corresponding soil were sampled in May. Soil samples were air-dried at room temperature for three weeks. Plant samples were separated into tubers, roots, stems, leaves and inflorescences thoroughly washed with tap and distilled water and then air-dried at room temperature for several weeks.

In this research, soil samples developed on the limestone, serpentine and chert were subjected to the Community Bureau of Reference (BCR) sequential extraction procedure in order to investigate the metals partitioning. Four-step soil analysis included i) exchangeable phase, for which it was used 0.11 M acetic acid, ii) reducible fraction (with 0.5 M hydrochloride at pH 1.5), iii) oxidizable fraction (with 8.8 M hydrogen peroxide and 1 M ammonium acetate, at pH 2) and iv) residual fraction (using aqua regia, 37% HCl and 65% HNO₃ at 80°C, during 5h) (Table 1).

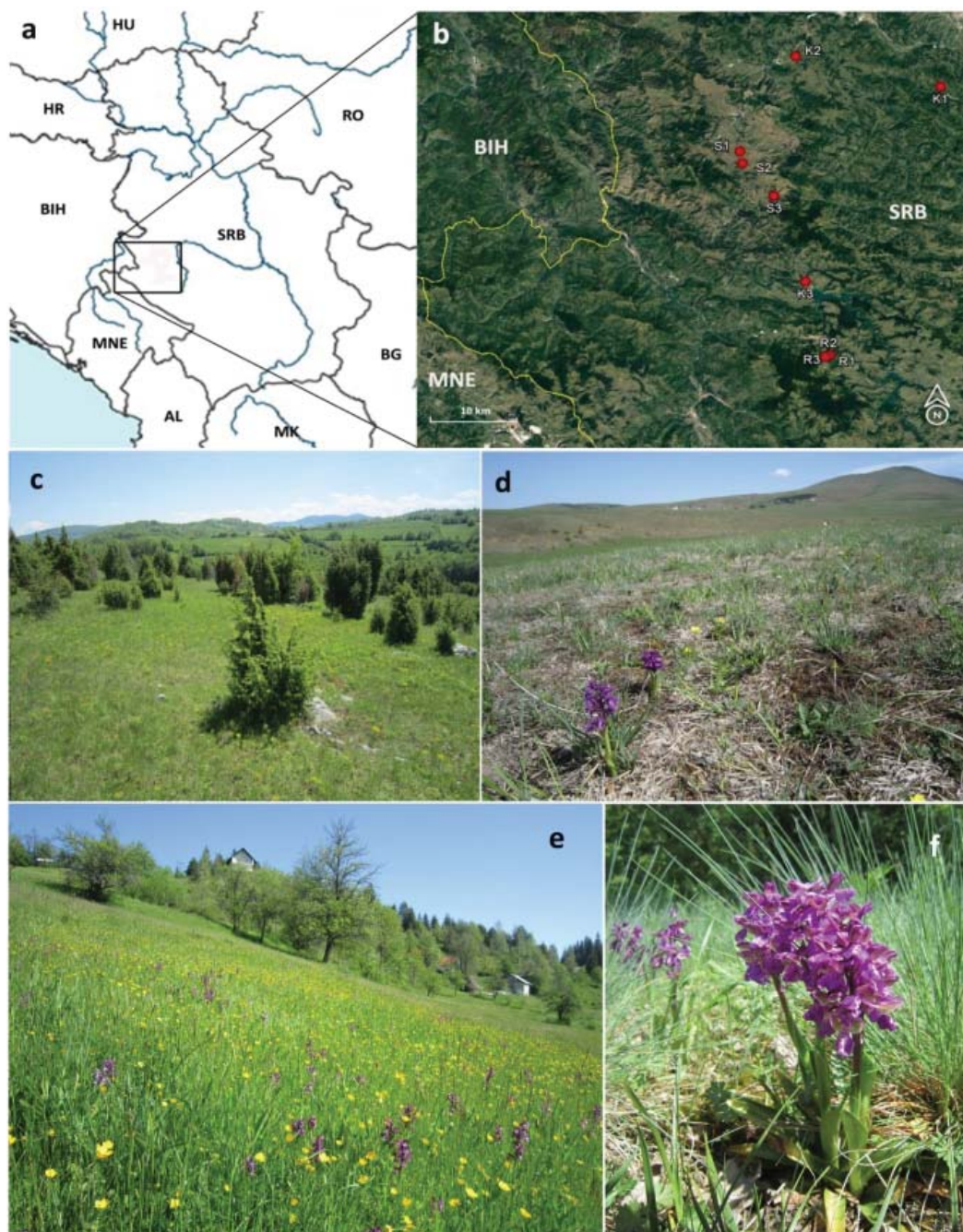


Figure 1: a) map of Serbia with marked sampling area in western Serbia; b) sample points for *Anacamptis morio* on different bedrock types (K – limestone, S – serpentine, R – chert); c) grassland habitat of *A. morio* on limestone; d) specimens of *A. morio* on serpentine steppe; e) grassland habitat of *A. morio* on chert; f) orchid species *Anacamptis morio*

Table 1: Sequential extraction steps and used reagents

Extraction step	Extraction agent	Fraction
1	0.11 mol/l CH_3COOH	Exchangeable, water, and acid-soluble phase - fraction bounded by carbonates
2	0.1 mol/l $\text{NH}_2\text{OH} \cdot \text{HCl}$, pH 2	Fe-Mn hydroxide/oxide
3	8.8 mol/l H_2O_2 ,	Organic/sulphide matter
	1mol/l $\text{CH}_3\text{COONH}_4$	
Residual fraction, aqua regia or aqua regia with HF		Residual or/and silicate

Before BCR analysis pH values were measured with pH-meter ADWA AD 1000 in deionized water in 1:5 soil/ solution. Organic matter content was measured by oxidation with potassium permanganate and retitration with oxalic acid. The metals concentrations in soil fractions and orchid underground and above-ground organs were determined with inductively coupled plasma with optic emission spectroscopy - ICP-OES (Thermo Scientific, UK, model 6500 Duo). General characteristics of the apparatus are concentric nebulizer, cyclonic spray-chamber, detector type CID86. Source parameters at which measurements were made were: the strength of the RF generator: 1150 W, axial gas flow speed: 0,5 l/min, nebulizer gas flow speed: 0,5 l/min, and cooling gas flow speed: 12 l/min. For sample dissolving, 65 % nitric acid and 30 % hydrogen peroxide were used.

Samples were air-dried at room temperature for three weeks. The specimen was torn down through wet digestion with 65% nitric acid and 30% hydrogen peroxide. Multi-elemental standard with a concentration of 10 mg/l was used as standards during the analysis with ICP-OES. After the measuring (0.5 g) samples were placed into plastic tubes. In each one of them, it was added 7ml of nitric acid (HNO₃) and 1 ml hydrogen peroxide (H₂O₂). Digestion was carried out in a water bath, during 6h, at the temperature of 85°C. After the cooling period, samples were replaced in a normal court of 50 ml and diluted with deionized water. Metals concentration was determined with the ICP-OES technique. Selected wavelengths for As, Cd, and Pb were 193.7 nm, 214.4 nm, and 220.3 nm, respectively. For ICP-OES measurements, it was used a multi-elemental stock solution 1000 g/L. Measurements were carried out in triplicate. Absorption coefficients, bioconcentration, and translocation factors [19, 20] of researched metals were calculated according to the formulas given down below. All values were expressed in µg/g.

$$BCF = \frac{C_{root}}{C_{soil}}$$

$$TF = \frac{C_{stem}}{C_{root}}$$

$$TF = \frac{C_{leaf}}{C_{root}}$$

$$TF = \frac{C_{inflorescence}}{C_{root}}$$

$$AC = \frac{C_{plant}}{C_{soil}}$$

Data about metals concentrations were analyzed with the nonparametric Kruskal-Wallis test in order to find statistically significant differences among soil types for soil and plant samples with Statistica Statsoft package (2007) [21]. Test values are given within Tables 3 and 4, together with average values for metals concentrations in soil in extraction phases and plant organs. Values for p, which are lower than one, indicate significant differences (in tables are marked with an asterisk).

RESULTS AND DISCUSSION

Prior to BCR analysis, pH values and organic matter content was determined in soil samples (Table 2). All soil samples had features of a slightly acidic medium, mostly close to neutral values. The content of organic matter was the highest on soils developed on limestone and lowest on soils developed on chert (Table 2). The solubility of metals and its bioavailability in the soil is highly influenced by pH and organic matter content [22].

Table 2: pH values (ranges) and organic matter content (in %, average values ± standard deviation) for investigated soil types

Bedrock type	Locality	pH	Organic matter (%)
Limestone	K1	6.11 - 6.26	1.93 ± 0.53
	K2	6.89 - 7.11	0.72 ± 0.10
	K3	6.78 - 6.96	1.10 ± 0.37
Serpentine	S1	6.72 - 7.23	0.82 ± 0.03
	S2	6.04 - 6.26	0.85 ± 0.07
	S3	6.18 - 6.30	0.92 ± 0.15
Chert	R1	5.92 - 6.10	0.69 ± 0.06
	R2	5.68 - 5.94	0.70 ± 0.02
	R3	5.58 - 5.97	0.63 ± 0.07

By the insight at the achieved results of concentration of the metals in soils (Table 3), considerable variability in obtained values can be noticed. Differences appeared by the sequential phases and bedrock type. In all investigated soils, the lead was the most represented metal out of all analyzed elements. Average lead concentration of around 30 µg per g of soil was in the range of average lead concentrations for European unpolluted soils, with negligible differences regarding bedrock type. Cadmium concentration was several times higher than the European average of 0.284 µg/g on all investigated soils (Table 3). Total arsenic content covered a wide range from 0.09 µg/g for the serpentine to 6.31 µg/g for the limestone, and it was lower than average for European unpolluted soils [23]. In our study, according to the concentrations of detected metals in analyzed soils on different bedrock types cadmium and the lead showed similar features regarding bioavailability – approximately more than 50% of the total content was potentially bioavailable. Arsenic bioavailability was much lower (in a range of 7.82 – 22.22%, with the highest value for the serpentine), keeping in mind that about 60% of As showed up in residual phase. Including the fact that the third BCR phase may be partially bioavailable, those percentages may be even higher.

The first extraction phase showed statistically significant values for the content of Cd on different bedrock types; the rest of the analyzed phases showed a significant difference in regards to bedrock types for all measured elements (Table 3). The most represent metal in the exchangeable phase was the lead, with around 80% on all

Table 3: Concentrations of As, Cd and Pb ($\mu\text{g/g}$) by phases of sequential extraction (given as mean value \pm standard deviation)

I phase					
	Limestone	Serpentine	Chert	KW	p
As	0.002 \pm 0.003	0.007 \pm 0.008	0.008 \pm 0.008	4.095	0.129
Cd	0.169 \pm 0.024	0.038 \pm 0.007	0.007 \pm 0.039	22.014	0.000*
Pb	0.162 \pm 0.068	0.098 \pm 0.073	0.073 \pm 0.044	2.554	0.279
II phase					
	Limestone	Serpentine	Chert	KW	p
As	0.492 \pm 0.127	0.000 \pm 0.000	0.100 \pm 0.119	21.835	0.000*
Cd	0.471 \pm 0.157	0.423 \pm 0.042	0.271 \pm 0.077	11.432	0.003*
Pb	24.949 \pm 8.510	26.786 \pm 1.702	17.495 \pm 1.648	7.714	0.021*
III phase					
	Limestone	Serpentine	Chert	KW	p
As	2.127 \pm 0.449	0.000 \pm 0.000	0.606 \pm 0.589	24.023	0.000*
Cd	0.101 \pm 0.022	0.203 \pm 0.019	0.080 \pm 0.025	17.908	0.000*
Pb	3.680 \pm 1.242	5.282 \pm 0.958	2.309 \pm 0.797	16.617	0.000*
IV phase					
	Limestone	Serpentine	Chert	KW	p
As	3.688 \pm 0.997	0.034 \pm 0.056	0.717 \pm 0.582	23.108	0.000*
Cd	0.182 \pm 0.024	0.193 \pm 0.015	0.123 \pm 0.046	11.877	0.003*
Pb	0.673 \pm 0.133	0.003 \pm 0.007	0.749 \pm 2.019	17.016	0.000*
Total					
	Limestone	Serpentine	Chert	KW	p
As	6.308 \pm 1.417	0.041 \pm 0.059	1.431 \pm 1.064	23.150	0.000*
Cd	0.922 \pm 0.197	0.858 \pm 0.061	0.559 \pm 0.115	13.746	0.001*
Pb	29.450 \pm 9.020	32.170 \pm 1.867	20.669 \pm 2.971	8.310	0.016*

*- statistically significant ($p > 0.05$) differences between analyzed soils

observed soil types. Cadmium was present in the first fraction with 18.32% on the limestone bedrock, 4.43% on the soil developed on the serpentinite, and 15.21% on the chert bedrock. The contribution of As in the exchangeable phase was less than 1%. Similarities in percentages of the second phase regarding different soils may be noticed. Cadmium was also present in the second phase with high percentages: 51.05%, 49.36%, and 48.48% of total content for limestone, serpentine, and chert bedrock, respectively. Metals associated with the third phase are considered to be bounded to the organic matter and as they remain longer in soil, assumed to be more hardly extracted. However, by decomposition processes, they may become bioavailable to plants. In between 30 to 40% of arsenic was extracted in soils developed on limestone and chert. Cadmium and the lead contribution in the third fraction were around 20% or below. The residual fraction is not available neither for plants nor microorganisms, due to the strong association of the metals with soil minerals. Arsenic was dominant in extracted metals in the fourth phase, with more than 50% in all types of an analyzed pad. More than 19% of

Cd was also present in the residual phase.

Concentrations of the metals in orchid vital parts - tubers, roots, stems, leaves, and inflorescences in for localities with soils formed on three different bedrock types are shown in Table 4. As stated by the results, the lead was the best-absorbed analyte, its concentrations were the highest on all bedrock types, and it was the most absorbed element among the investigated metals in all analyzed orchid organs. Content of lead in orchid parts corresponds to the lead content in the soil samples (Table 3); comparing these concentrations, noticeable regularity may be noticed. Similar results in terms of metals (Pb and Zn) accumulation in the plants from the contaminated soil have already been reported [24]. Differences in concentration of lead in the tubers among soils on different bedrocks were not significant, unlike arsenic and cadmium (Table 4). Results for the amount of the uptaken metals by plants generally followed concentrations in the soil. Soils with a high content of certain metal generated a large amount of the same element in the plant, mainly accumulated in the roots.

Table 4: Concentrations of As, Cd and Pb ($\mu\text{g/g}$) in orchid parts classified according to the bedrock type (given as mean value \pm standard deviation)

Tubers					
	Limestone	Serpentine	Chert	KW	p
As	0.389 \pm 0.311	0.003 \pm 0.006	0.045 \pm 0.029	22.192	0.000*
Cd	0.500 \pm 0.218	0.156 \pm 0.014	0.248 \pm 0.066	16.004	0.000*
Pb	1.269 \pm 0.628	0.922 \pm 0.182	0.670 \pm 0.186	5.347	0.069
Roots					
	Limestone	Serpentine	Chert	KW	p
As	0.398 \pm 0.391	0.075 \pm 0.127	0.016 \pm 0.039	11.980	0.003*
Cd	2.318 \pm 0.458	1.119 \pm 0.195	2.606 \pm 0.979	17.365	0.000*
Pb	4.471 \pm 1.046	3.312 \pm 0.314	3.943 \pm 3.218	6.603	0.037*
Stems					
	Limestone	Serpentine	Chert	KW	p
As	0.037 \pm 0.064	0.008 \pm 0.011	0.007 \pm 0.016	4.075	0.130
Cd	0.422 \pm 0.166	0.102 \pm 0.022	0.192 \pm 0.081	19.079	0.000*
Pb	0.315 \pm 0.398	0.128 \pm 0.105	0.276 \pm 0.670	1.568	0.457
Leaves					
	Limestone	Serpentine	Chert	KW	p
As	0.187 \pm 0.11	0.032 \pm 0.023	0.024 \pm 0.020	10.175	0.006
Cd	0.241 \pm 0.051	0.078 \pm 0.011	0.103 \pm 0.024	19.651	0
Pb	0.757 \pm 0.122	0.660 \pm 0.269	0.388 \pm 0.449	9.199	0.01
Inflorescences					
	Limestone	Serpentine	Chert	KW	p
As	0.016 \pm 0.02	0.002 \pm 0.006	ND \pm 0	8.314	0.016*
Cd	0.163 \pm 0.061	0.089 \pm 0.018	0.096 \pm 0.026	6.127	0.047*
Pb	0.472 \pm 0.37	0.898 \pm 0.457	0.186 \pm 0.094	14.042	0.001*

*- statistically significant ($p > 0.05$) differences between analyzed soils

On the soil developed on the limestone bedrock, the highest concentration of the total arsenic content (38.80%) was in the roots. Arsenic was also represented in the tubers (37.84%), which indicates the tendency for the accumulation of this element in underground parts of the plant. On the serpentine bedrock, As was present in the roots with 62.52%, while on the chert bedrock it was mostly concentrated in the tubers (48.83%). Metals' immobilization in underground orchid organs turned out to be the prevailing mechanism for all three analytes, regardless of the examined geological bedrock. Their concentrations were significantly different between populations inhabiting soils on different bedrock types. More than 80% of Cd and Pb total content was accumulated in the roots and tubers. Similarly, the accumulation of a high concentration of toxic metals in the roots system of orchid *Epipactis atrorubens*, growing on abandoned mining sites in Italy with a high content of metals, has already been described [14]. Also, within the assessment of the metals bioavailability and its translocation, while calculating absorption coefficients and bioconcentration and translocation factors (Table 5), the presence of accu-

mulation in underground organs was noticed, for all three toxic metals, as well as arsenic transportation to the above-ground orchid organs; between 18.17 – 26.49% of total arsenic was transferred to leaves, showing no significant difference among soils developed on different bedrocks (Table 4). Analyzing the stems, 11.58% of Cd was found on the limestone bedrock. On the soil developed on serpentinite, inflorescences contained about 15% of the total uptaken lead.

For calculating BCF, as a quantitative measure of plant phytostabilisation ability, in this study, the sum of the underground parts (tubers and roots) was used. Cadmium on the chert bedrock had the highest bioconcentration factor among all observed elements – 5.10 (Table 5). In general, Cd had BCF higher than 1 on all soils. Arsenic bioconcentration factor was high, as well, on the serpentine bedrock (BCF = 1.92). The translocation factors were calculated for each above-ground orchid part separately. The leaves also retained the high amount of As in comparison to the soil on the chert bedrock, as the TF value was 1.55. TF values for Cd and Pb were, on the contrary, quite low. TFstems was generally low with the

Table 5: Bioaccumulation factor(BCF), translocation factor (TF) and absorption coefficient (AC) of *Anacamptis morio*

	BCF			TF _{stems}			TF _{leaves}			TF _{inflorescences}			AC		
	L	S	C	L	S	C	L	S	C	L	S	C	L	S	C
As	0.12	1.92	0.04	0.09	0.10	0.44	0.47	0.42	1.55	0.04	0.03	ND	0.16	2.93	0.06
Cd	3.05	1.49	5.10	0.18	0.09	0.07	0.10	0.07	0.04	0.07	0.08	0.04	3.95	1.80	5.80
Pb	0.19	0.13	0.21	0.07	0.04	0.07	0.17	0.20	0.10	0.11	0.27	0.05	0.25	0.18	0.25

*L-limestone, S-serpentine, C-chert

highest value for arsenic on the chert (0.44). TF_{inflorescences} was also low for all metals and soils (maximum value was 0.27 for Pb on the serpentine). As few studies so far revealed [25, 26], certain orchid species can grow in the presence of high concentrations of heavy metals and metalloids in the substratum, and that they can also accumulate and translocate those elements in their organs.

The absorption coefficient (AC) was calculated as a ratio of a sum of all analyzed orchid organs and concentration of the corresponding metal in the soil (Table 5, values higher than 1 are marked bold). AC scores for As and Cd correspond with calculated BCF factors. High AC value appeared for As on the serpentine (2.93). Cadmium AC showed high values on all bedrock types, by following BCF values (3.95, 1.80 and 5.80 for limestone, serpentine and chert, respectively). When it comes to lead, the maximum value was reached on the limestone (0.25). Even though the lead was the well-absorbed element as far as compared to the other two observed metals (Table 4), a much higher amount of the lead in the soil (Table 3) affected low AC value. For the soil developed on the limestone, the total absorbed amount of the lead was 7.283 µg/g, while the lead content in the soil was 29.450 µg/g, whereas 25.097 µg/g was bioavailable (the sum of the first and the second fraction). The same situation was revealed with the chert, with the same absorption coefficient (0.25), where 17.611 µg/g of the lead was bioavailable, and 5.463 µg/g was absorbed. Bedrock type highly influenced the amount of accumulated toxic elements. The highest values of calculated accumulation indicators were achieved on the soils developed on the serpentine bedrock.

CONCLUSIONS

Orchid species *Anacamptis morio* was screened for metal content on soils formed on different bedrock types in order to determine its bioremediation potential. Soils developed on limestone, serpentine, and chert showed the highest content of lead in comparison to the content of cadmium and arsenic and variations in bioavailability of investigated elements. Around 80% of the total lead amount in the soils was potentially bioavailable, while cadmium content varied regarding bedrock type - available content was the highest on the limestone, with around 70%, and the lowest on serpentine with 53.68%

of the total amount. The arsenic bioavailability was significantly lower as more than 40% was determined in the residual fraction. The content of the metals uptaken by plants generally followed their concentrations in the soils and calculated AC corresponded to the BCF values. The highest BCF value was found for Cd on the chert, while the arsenic had BCF higher than 1 on the serpentine. Bioaccumulation factors for cadmium showed low variability and had high values regardless of the bedrock type on which the analyzed soils were developed, which makes *Anacamptis morio* suitable potential bioremediator for cadmium contaminated soils. Each of the analyzed elements was accumulated mostly in the underground orchid parts (tubers and roots); it was the dominant absorption pattern for all three toxic metals. About 20% of total arsenic content in the orchid was found in the leaves, which also indicates a certain level of transport from the underground to the aboveground organs. Although concentrations of the metals in the investigated soils were relatively low, this orchid showed the possibility to stabilize their available fractions naturally, consequently preventing them from spreading in the environment. Despite developed tolerance to extreme conditions, relatively low biomass production of *Anacamptis morio* so far represents the main issue for wider use in phytoremediation. Nevertheless, this species may be inspected as an additional bioremediation option, combined with additional plant species or remediation techniques.

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