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IMPACT OF CHROMIUM, ARSENIC AND SELECTED ENVIRONMENTAL VARIABLES ON THE VEGETATION AND SOIL SEED BANK OF SUBSIDENCE BASINS

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Abstract: As a result of hard coal mining, specific areas of subsidence basins, which are often waterlogged, were created in the Czech part of the Upper Silesian Coal District. These areas may not have a high ecological value or, on the contrary, promising biotopes may arise here under suitable conditions, which should be our goal. As part of this work, the above-ground vegetation, and the soil seed bank (using the cultivation method) were investigated. The concentration of the risk elements chromium and arsenic, which are usually increased in mining areas, was determined by neutron activation analysis. Other environmental variables that can affect both the vegetation and the soil seed bank of subsidence basins (fine earth, slope, biotopes, and their representation in the vicinity) were also determined. Using multivariate DCA analysis, a statistically significant influence of chromium concentration and other variables on above-ground vegetation, characterized by the occurrence of many metallophytes and their high coverage, was found. The soil seed bank, which can impact the further development of vegetation, is mainly influenced by forest and wetland biotopes and the representation of areas covered by tailings in the vicinity of sampling sites. It is evident that there are species capable of resisting or accumulating chromium and arsenic pollution in both the above-ground vegetation and the soil seed bank, which can lead to the gradual rehabilitation of subsidence basins. This research can lead to a better understanding of the development of subsidence basins to increase their future ecological values.

Keywords: chromium, arsenic, subsidence, soil seed bank, vegetation

1. INTRODUCTION

Czech parts of the Upper Silesian Coal Basin (Ostrava – Karviná Coal District) is significantly affected by hard coal mining and metallurgical industry. The Karviná coalfield is characterized by a large thickness of coal seams, which is reflected in the thickness of subsidence basins, reaching a depth up to 25 m. In floodplains of rivers and in areas with high groundwater levels, these subsidence basins are flooded. Results of intense anthropogenic disturbance are not only natural terrain destruction and contamination of environment, but also creation of very promising biotopes in terms of landscape productivity and biodiversity.

The gradual development of biocenoses on the mine heaps and subsidence basins that have not been reclaimed has the character of a primary succession. It is closely related to the physicochemical properties of the tailing's substrate, the method of forming the heap and the available sources of diaspores. Mostly carboniferous tailings mined in Ostrava – Karviná Coal District have relatively favourable physicochemical properties with sufficient nutrients, good porosity, and a suitable water regime due to the existence of a condensation layer (Štýs, 1981). The content of sulphides (pyrite, rarely arsenopyrite) can be problematic, since they are oxidized and with seeping water forms the sulphuric acid which lowers the pH and leads to leaching of ecotoxic elements (Makowska et al., 2019; Pesek et al., 2005).

In terms of vegetation development prediction, in addition to the state of above-ground vegetation, it is necessary to monitor the state of the soil seed bank as a supply of viable, non-germinated seeds in each location (Baskin & Baskin, 2014; Fenner & Thompson, 2005). Soil seed bank analysis is an important source of data in the study of succession in disturbance-affected areas and is extremely important in landscape restoration (Prach et al., 2017; Prach & Walker, 2019; Řehounková et al., 2018).

The development of vegetation in anthropogenic habitats is significantly influenced by environmental gradients, where they are very frequent and diverse. Significant gradients include environmental humidity, diaspore sources, their distances, and stress factors (Prach et al., 2014; Prach & Hobbs, 2008). The main stress factors that affect species composition of plants and its development in the monitored area include presence of heavy metals and other risk elements in the substrate, or occurrence of invasive plants. In terms of risk elements, we focused mainly on the content of chromium and arsenic, which occurs in the substrate of the monitored subsidence basins above the limit concentration.

Chromium occurs naturally in form of $FeCr_2O_4$, primarily in ultrabasic rocks and serpentinites together with Pb, Mg and Al (Katz & Salem, 1994). It enters the environment mainly through anthropogenic activities such as metallurgical production of stainless steel, galvanic industry, catalytic production, production of refractory materials and often in form of dust from roads, cooling towers and others (Babula et al., 2008; Oliveira, 2012; Shtiza et al., 2008). It occurs mainly in trivalent form Cr (III), hexavalent form Cr (VI). Hexavalent chromium is very toxic to plants (Shanker et al., 2005), animals and humans (WHO 1988) due to its high oxidation potential, solubility, and mobility among the cell membranes. Trivalent chromium in form of oxides, hydroxides and sulphates binds to organic soil compounds, making it less mobile and toxic (Babula et al., 2008; Gupta et al., 2022; Oliveira, 2012). In low concentrations, it acts similarly to an essential element and supports plant growth (Peralta-Videa et al., 2009), at higher concentrations, however, it can stop growth (Srivastava et al., 2021). However, in the case of high oxygen concentration or higher manganese content in soil, Cr (III) may oxidize to Cr (VI) (Peralta-Videa et al., 2009). Thus, it can be said that the availability and potential toxicity of chromium to living organisms depends on its oxidation number, soil type, precipitation in the area, ability of Cr to create organic complexes and its binding to colloidal structures (Losi et al., 1994; Zayed & Terry, 2003). Chromium uptake by plant roots is also affected by the presence of mycorrhizal fungi, which can increase it (Davies et al., 2002). In terms of Cr accumulation in tissues (shoot / root ratio), higher concentration is in plant roots (Maruthi Sridhar et al., 2010).

Arsenic, in terms of rocks or soil types, most often occurs in localities with coal reserves (range 0.3 - 35,000 mg / kg) (Smedley & Kinniburgh, 2002). On a European scale, As is also represented in common rock, forming minerals as sulphides, sulphates, oxides (especially Fe oxides), phosphates. Higher concentration is also found in igneous rocks (basalt), sedimentary rocks (limestones, sandstones) and metamorphic rocks (slates, phyllites) (Smedley & Kinniburgh, 2002). Arsenic is also widely used in industry (mining and subsequent processing activities, burning of fossil fuels) (Alderton et al., 2014; Angelovičová et al., 2015), from where it enters the environment, very often by atmospheric deposition. Arsenic is most often found in terrestrial ecosystems as pentavalent and trivalent forms. As (III) is typical for soils with anaerobic conditions (e.g., flooded), As (V) is the main state in aerobic soils. In aerobic soils, As is strongly absorbed by oxides / hydroxides of iron and aluminum, it is minimally present in the soil solution and its bioavailability is therefore relatively low, however it depends on other substrate properties like pH (Moschner et al., 2020). In flooded anaerobic soils, its bioavailability is higher (Zhao et al., 2010). The bioavailability of arsenate is also affected by the phosphate content. These two compounds can replace each other in chemical reactions. This biochemical competition controls the fate of As in the environment (Strawn, 2018; Tripathi et al., 2012; Zhao et al., 2010). However, in the reducing environment, different biochemical reactions of arsenic and an increase in its solubility and mobility occurs, which is not observed for phosphorus (Strawn, 2018).

The plant accumulates As especially in roots and only very small amounts are transported to the shoots. With increasing As concentration in the soil, the As accumulation in terrestrial plant increases, but in case of submerged plants, accumulation of As may be high even at low As content in sediment, probably due to foliar uptake of As. The root-stem ratio is not clear in the case of As. Tolerant plants generally translocate As into aboveground biomass, in plants that do not tolerate increased concentration of As, accumulation in roots predominates, but this process has not yet been fully studied (Quaghebeur & Rengel, 2003; Zemanová et al., 2021). Arsenic toxicity in plants manifests itself as morphological, physiological, and biochemical changes (Abbas et al., 2018). However, arsenic is needed at low concentrations for the normal physiological and biochemical functioning of plants (Mirza et al., 2014).

In this study, we aim to quantify the content of the risk elements of arsenic and chromium in soils of subsidence basins and to determine, using a multivariate analysis, whether these elements, together with the environmental variables of slope, biotope, and their area representation in the vicinity of the sampling sites, have an impact on the overall distribution of plant species in the aboveground vegetation and soil seed bank.

2. MATERIAL AND METHODS

2.1. Study area

The two monitored subsidence basins are in the Karviná region. U cesty (49.8129506N, 18.4779606E), located in the north of the Horní Suchá municipality, is the endorheic subsidence basin of 7900 square meters and maximum depth of 4.5 m with a western bank covered with tailings (Pierzchala, 2012). To determine the soil seed bank, vegetation, and analysis of elements (Figure 1) a total of fifteen sampling sites were defined here. Sampling sites 1 and 2 corresponds to a non-reclaimed habitat without tree coverage, sampling sites 3-8 to a wetland habitat with herbaceous and shrub vegetation with local occurrence of trees and sampling sites 9-15 to a forest habitat. The second research area is the subsidence basin Kozinec located near the Doubrava municipality. It is an extensive basin (approx. 0.49 square kilometers), with the western part formed by the Karviná stream and the eastern by the endorheic Kozinec Lake, which is divided by the anthropogenically created peninsulas and partly subsidized by saline mine-water. Fifteen sampling sites were defined here as well, locally focused on the northern part of the Karviná stream with a significant slope leading to the water body (Figure 1). Sampling sites 16-18 corresponds here to ecotonal vegetation of the forest edge and forest-free areas, sites 19-21 have a wetland character and sites 22-30 are forests with significant synanthropisation as remains of settlement.

2.2. Sampling and analysis

The aboveground vegetation was monitored in 2019-2021, three times a growing season according to Braun-Blanquet (Braun-Blanquet, 1964). Each relevé had a uniform shape and size – a square with an area of one hundred square meters and plant species and their abundance were determined within each square. On each basin, fifteen sampling sites were selected within the area of relevant phytosociological relevé for soil seed bank sampling, depending on the habitats and, in the case of Kozinec,

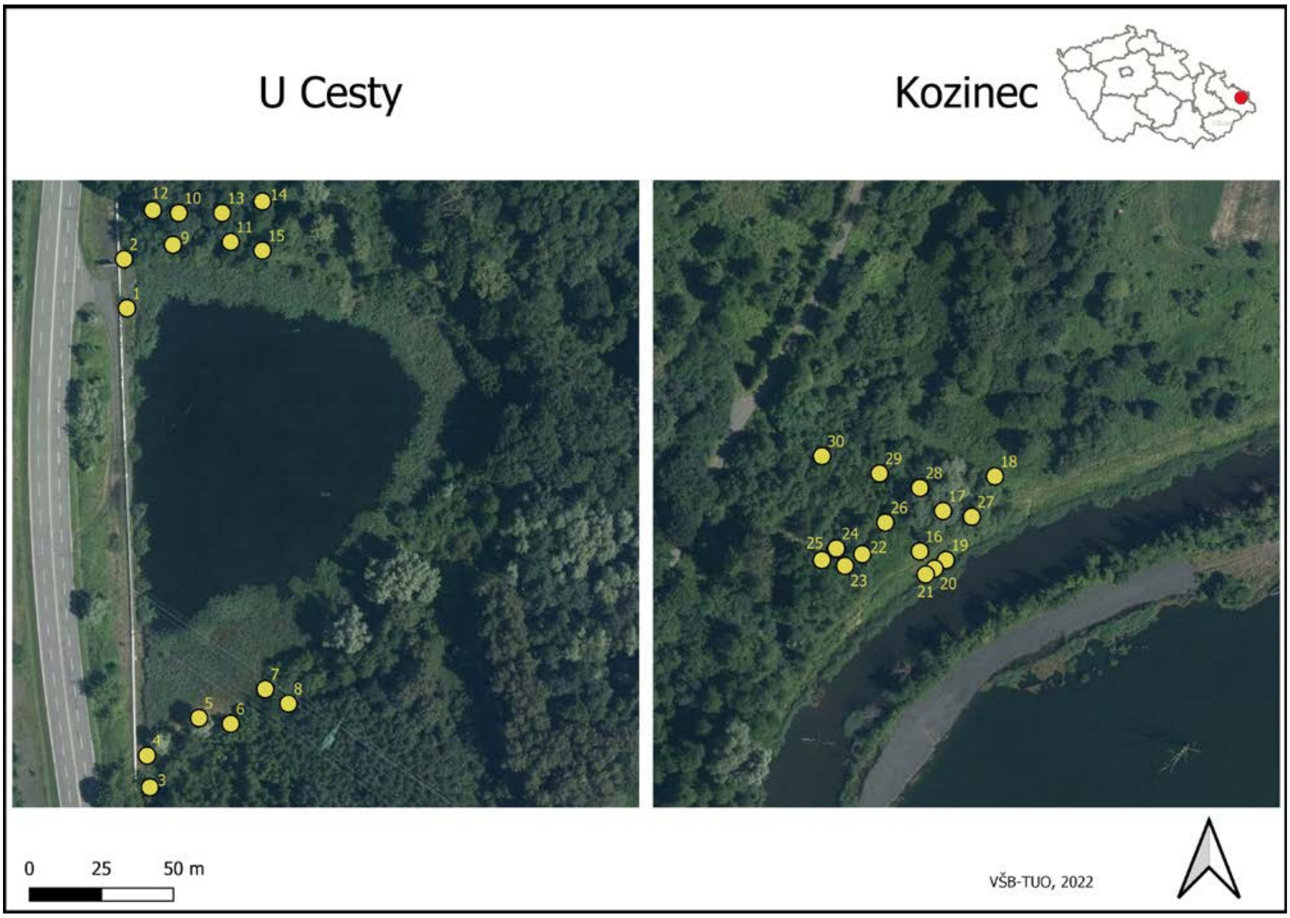

Figure 1. Map of sampling localization

also slope. Soil samples for soil seed bank determination were taken from selected spots in the form of a mixed sample. In each selected area, five samples (5.3 cm deep and 5.3 in diameter) were taken by Kopecký cylinders at random from a 2 m diameter circle, which were then mixed. Thus, the total area of one composite sample was 88,247 cm^2. The samples were taken twice a year - in spring and autumn - and the results of both samples were fused together for a complete view on the soil seed bank. Samples 19, 20, 21 were subsequently combined due to their mutual proximity and connection to the same habitat. In the autumn of 2020, samples 3-8 were not taken for the cultivation method of soil seed bank analysis, due to increased precipitation leading to flooding of the area. For this reason, an average of seedling grown in prior sampling was added to the spring samples to approximate the data. The assessment of biodiversity and equitability of aboveground vegetation and soil seed bank was calculated using the Shannon-Wiener index (Shannon, 1948).

Sample processing was performed according to Heerdt et al., (1996) - the mixed samples were sieved in the laboratory through a 0.2 mm sieve to separate even small seeds. The supernatant phase was then planted in pots with a substrate suitable for sowing. The pots were left in a convenient environment and watered regularly. Grown seedlings were identified and removed from the pots. To control possible seed contamination from substrate or different sources, one control pot was grown with substrate only. The numbers of cultivated seedlings were finally recalculated per m^2 according to the sampling area.

Samples for NAA were taken according to the IAEA methodology (*IAEA*, 2004) as part of the soil seed bank collection. These samples were sieved in the laboratory through a 2 mm sieve to separate the fine soil, which was sent to the Institute for Nuclear Research (JINR) in Dubna (Russia) to the NAA. This method is based on neutron activation, which is designed to determine the chemical elements in a sample. The sample to be analysed is bombarded with neutrons in a nuclear reactor. Through nuclear reactions, stable nuclei are transformed into other, radioactive nuclei and gamma radiation is released and recorded. A different specific value of gamma radiation applies to each element. It is possible to distinguish individual elements and their concentration in the sample (Greenberg et al., 2011)

Selected and assessed environmental variables which may affect state and properties of vegetation and soil seed bank of subsidence basins are:

a. concentration of heavy metals As and Cr - determined by neutron activation analysis in the IBR-2 reactor FLNP at the Institute for Nuclear Research (JINR) in Dubno. These hazardous substances were evaluated according to the Methodological guideline of the Ministry of the Environment: Pollution indicators (MŽP 2013), for industrially used areas (based on RSL values for Industrial soils).

b. slope – expressed as the presence/absence.

c. percentage of soil particles smaller than 0.2 mm as a main part of soil with ecotoxic elements content

d. area up to 100 m - surrounding habitats (forest, non-forest, waterbody, tailings cover, and anthropogenic structures), important for seed distribution according to Prach and Hobbs (2008) – calculated in geoinformation system QGIS (QGIS.org., 2022).

e. affiliation of individual sampling areas to forest, non-forest, and wetland habitats.

We determined the impact of these environmental variables on vegetation in R Software (R Core Team, 2020) using the multivariate DCA method with the Vegan package (Oksanen et al., n.d.).

3. RESULTS AND DISCUSSION

3.1. Chromium and arsenic contamination

Based on the NAA results of the neutron activation analysis, the risk elements were assessed according to the Methodological guideline: Pollution indicators (MŽP 2013). The values of indicators for As (limit 2.4 mg/kg) and Cr (limit 5.6 mg/kg) were exceeded at all monitored sampling sites. In the case of chromium, the values of indicators for Cr (VI) were used, as the NAA does not allow to determine the valence of the element. The NAA results are shown in Figure 2.

The average chromium concentration as seen in Figure 2 at the U cesty site was 78.13 ± 23.5 mg/kg with a maximum of 129 mg/kg at the tailings site. In the case of Kozinec, the Cr content reached 79.47 ± 13.31 mg/kg with a maximum of 100 mg/kg in a wetland habitat with reeds. The average concentration of chromium in soils varies considerably depending on the type and texture of soil, the WHO reports an average concentration in the range of 14 - 17 mg/kg, but it can reach up to 1000 mg/kg (World Health Organization. Regional Office for Europe, 2000). In the case of arsenic, the average concentration in the soil of the U cesty locality was 9.8 ± 4.4 mg/kg with a maximum of 24.7 mg/kg at site 3 corresponding to the wetland on the tailings. In Kozinec, the average concentration of

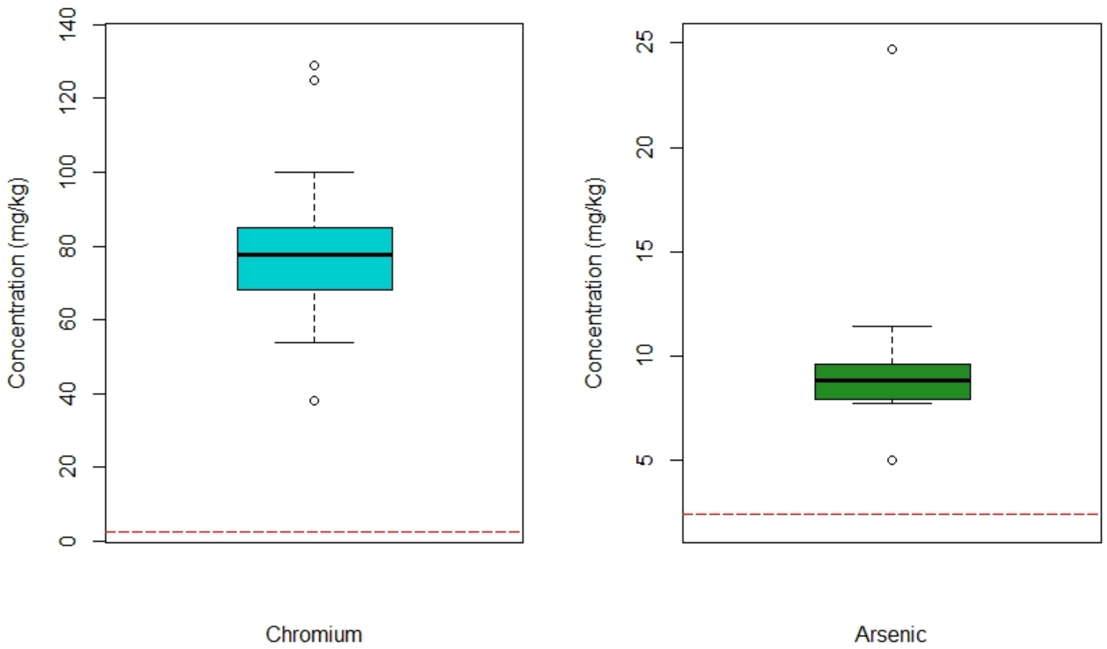

Figure 2. Concentration of Cr and As in soil. Explanation: The dashed line represent limit according to Methodological guideline (MŽP 2013)

As reached 8.9 ± 0.9 mg/kg with a maximum of 10.3 mg/kg in a wetland with reeds. Bowell et al., (2013) states that in soils affected by mining activities, the arsenic concentration can reach values of 4 to 9000 mg/kg. However, it must be added that in the case of arsenic, in the Czech Republic, due to geochemical conditions in the rock environment and intensive industrial activities, its higher natural concentrations are common. In the case of the Ostrava and Karviná regions, an increased concentration of As was recorded in the upper parts of the soil horizon, mainly due to atmospheric deposition (Sucharová & Suchara, 1998).

3.2. Aboveground vegetation

In total 115 plant species belonging to 43 families were recorded in the aboveground vegetation of the subsidence basins, among which *Asteraceae* (15.7 %), *Poaceae* (10.4 %) and *Rosaceae* (9.6 %) families prevailed, which are generally the most common families within Europe (Večeřa et al., 2021). The diversity reaches an average value of 1.84 on the subsidence basins combined with a minimum of 0.78 at Kozinec and a maximum of 2.86 U cesty. In post-mining areas of Europe, the diversity of aboveground vegetation is in general medium or lower (Kondratenko et al., 2022), the diversity of plants is higher on more skeletal soils within Upper Silesia (Kompała-Bąba et al., 2019). This is the case of U cesty subsidence basin, with average H´ = 2.16 (Kozinec – average H´ = 1.51). Also, the average equitability of plant communities is higher U cesty (0.74) than at Kozinec (0.68). Most of the vegetation of subsidence basins was determined as the initial communities very likely leading to these associations: *Dauco carotae-Melilotion*, *Fragarion vescea*, *Phragmition australis*, *Tilio platyphylli-Acerion*, *Alnion incanae*, *Quercion roboris*, *Arrhenatherion elatioris* or *Aegopodion podagrariae*. Among the important factors that can significantly affect the species composition and may block the succession is a dominance of expansive *Calamagrostis epigejos*, possible spread of invasive *Reynoutria japonica* and *Solidago canadensis*. Many of metallophytes were found among the identified plant species of the subsidence basins – e.g., *Typha latifolia* (Sasmaz et al., 2008), *Agrostis stolonifera* (Štofejová et al., 2021), *Reynoutria bohemica* (Širka et al., 2016), *Cirsium vulgare* (Dökmeci & Adiloğlu, 2020), *Juncus effusus* (Syranidou et al., 2017), *Lysimachia nummularia* (Singh & Tripathi, 2007). These species were most diverse and with the highest coverage in the relevés of the area covered with tailings and the southern wetlands of the U cesty basin (samples 1-8). Of the metallophytes found here, *Lysimachia nummularia* had the highest coverage (50-75 % of the relevé) in the sampling site 3. Other high coverage (up to 50%) metallophytes species recorded were *Poa pratensis*, *Calamagrostis epigejos* and *Juncus effusus*.

The influence of most selected environmental variables can be determined from the ordination diagram via multivariate analysis of aboveground vegetation (Table 1). Except of As and NF_100m variables, the relation of the of aboveground vegetation scores on the selected variables is manifested. Strong relation ($p < 0.001$) was manifested for the variables A_100m (including the highest correlation coefficient), F, F_100m and slope (Sl) at the threshold of significance 0.05. The data of the ordination diagram show that the first axis DCA1 represents a combined gradient of nature-friendly habitats (forests, wetlands, forests up to 100m, water areas up to 100m) and captures 12.056% of the total variation in species composition. DCA2 then combines a gradient of strongly anthropically impacted habitats (anthropic areas up to 100m, tailings areas up to 100m, fine earth) and captures 10.824% of the total variation in species composition. Both axes show a correlation with slope, non-forest vegetation and chromium concentration.

Table 1. Variation and significance of multiple regression

	Vegetation		Soil seed bank	
Env. Var.	r2	Pr(>r)	r2	Pr(>r)
Cr	0.2074	0.042 *	0.1166	0.210
As	0.0131	0.786	0.0176	0.809
A_100m	0.7466	0.001 ***	0.2942	0.017 *
F_100m	0.6726	0.001 ***	0.2005	0.053
WB_100m	0.3093	0.007 **	0.0331	0.638
OB_100m	0.2341	0.029 *	0.4574	0.002 **
NF_100m	0.1334	0.153	0.2766	0.012 *
M_0.2	0.3603	0.009 **	0.1007	0.257
Sl	0.4541	0.001 ***	0.0897	0.313
F	0.6818	0.001 ***	0.3798	0.001 ***
NF	0.2763	0.009 **	0.0378	0.620
WL	0.2917	0.011 *	0.2973	0.010 **

*Explanations: Environmental variables are: A_100m – anthropic structures coverage up to 100m, F_100m – forests coverage up to 100m, WB_100m – waterbodies coverage up to 100m, OB_100m – coverage of areas covered with tailings up to 100 m, NF_100m – coverage of non-forest habitats up to 100 m, M_0.2 – percentage of soil particles < 0.2 mm, Sl – slope, F – affiliation to the forest habitat, NF – non-forest habitat, WL – wetland habitat; r2 – variation explained by the model of multiple regression, Pr(>r) – the significance of the multiple regression, where signif. codes are: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

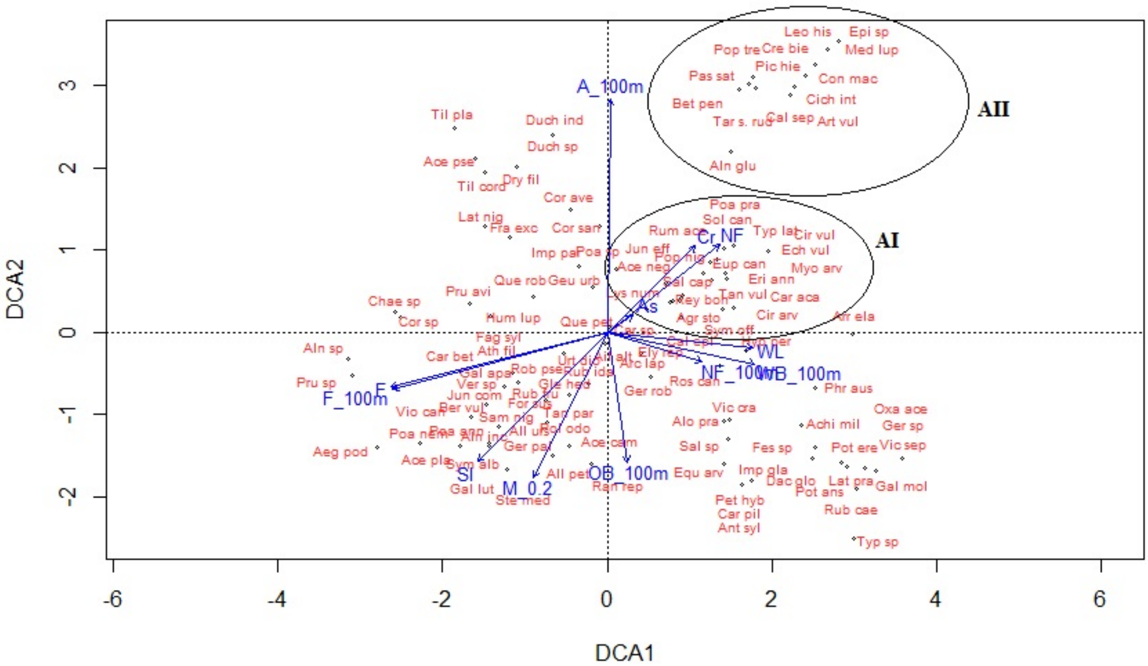

Figure 3. DCA diagram of vegetation

Two clusters appeared in the ordination DCA diagram of vegetation analysis (Figure 3). Cluster AI is in the first quadrant. Even though the environmental variables chromium and arsenic have little or no effect on the data set, correlation to the plant species of this cluster is manifested. Several metallophytes with phytoremediation abilities are in this cluster, like *Reynoutria bohemica* (Širka et al., 2016), *Agrostis stolonifera* (Štofejová et al., 2021 *Juncus effusus* (Syranidou et al., 2017), *Symphytum officinale* (Du et al., 2019), *Lysimachia nummularia* (Singh & Tripathi, 2007), *Eupatorium cannabinum* (González et al., 2019), *Erigeron annuus* (Zhang et al., 2021), *Calamagrostis epigejos* (Ranđelović et al., 2018) and others. This part of the ordination diagram corresponds with wetlands and its ecotone habitats, areas covered with tailings and forest areas of U cesty basin.

Cluster AII, which consists of pioneer and ruderal species in drier non-forest habitats is situated far from the centre of the ordination diagram. Sites covered with tailings are located on the ordination diagram between these two clusters. The species of the second quadrant of the ordination diagram do not form a compact cluster, however, synanthropic species with a connection to the presence of anthropogenic relief up to 100 m can be observed here, such as *Duchesnea indica*, *Cornus sanguinea*, *Corylus avellana*, *Geum urbanum*, *Impatiens parviflora*. Sampling sites of forest parts of the U cesty basin are in this part of diagram. Variables of forest habitat, representation of the forests up to 100 m, slope, and content of the fine earth are in the third quadrant of the ordination diagram, to which common forest trees such as *Fagus sylvatica*, *Carpinus betulus*, *Alnus incana*, *Acer platanoides* are correlated to. Sciophytes *Athyrium filix-femina*, *Urtica dioica*, *Galium aparine*, *Allium ursinum* of the slopes bordering the Kozinec subsidence basin are in this part of diagram. There are also fruity shrubs of recultivation or urban plantings tied to more inclined positions. It may be a residue of the former vegetation of residential areas before the decline and subsequent flooding of the area.

3.3. Soil seed bank

In the soil seed bank assessed by the cultivation method, 1487 seedlings were identified, belonging to 60 plant species and 24 families. The *Asteraceae* (27 %) and *Poaceae* (15 %) families predominated, other families were represented in the range of 2 % to 5 %, which corresponds to aboveground vegetation as a reflection of previous succession (Thompson & Fenner, 2005). The species with the highest average density are synanthropic, mostly autochorous *Urtica dioica* (862 s/m2), *Chenopodium album* (132 s/m^2) and *Poa annua* (105 s/m^2). The diversity of the soil seed bank is usually lower to the aboveground vegetation (He et al., 2016; Kuht et al., 2016; Wang et al., 2021), also in the case of post-mining areas it does not exceed H´ value of 2.8 and equitability E value of 0.7 (Balestrin et al., 2019; González-Alday et al., 2009; Martins et al., 2021). In case of our research, the diversity of soil seed bank is low: min 0.19 (Kozinec – meadow); max 1.81 (Kozinec – ecotone); average 1.24 (Kozinec) and 1.28 (U cesty).

In the ordination diagram of the DCA analysis of the cultivation method, the first DCA1 axis is a combined gradient of the fine earth variable and most habitat components (forests and wetlands) and their representation in 100m area (forests, tailings, anthropogenic areas), which captures 17.292% of total species composition variation. It thus represents a gradient of more nature-close habitats, where the significant impact of nearby anthropogenic habitats with ruderal and pioneer species is manifested. The second axis represents the combined gradient of the content of selected heavy metals (chromium and arsenic) together with the slope variable and the non-forest habitats. This axis captures 13,627 % of variation in species composition. The representation of waterbody in the vicinity of 100 m is correlated by both axes.

According to the results of the multivariate analysis, half of the selected environmental variables impacts the species score. The strongest relation ($p<0.001$) is in the forest habitat variable. Relations of variables of tailings up to 100 m, wetland habitat, representation of non-forest areas in the vicinity of 100 m, anthropogenic areas up to 100 m and forests up to 100 m gradually decreases. Variables of chromium and arsenic content, waterbody areas up to 100 m, fine earth content, slope and non-forest habitats are statistically insignificant. However, the effect of chromium and arsenic content on the cultivated soil seed bank is affected by the medium used for cultivation (a common horticultural substrate). Seed cultivation in the original medium - tailings was unsuccessful, only one species of *Poa annua*, which is potentially bioaccumulate As (Comino et al., 2009), germinated. In case of using a common horticultural substrate, several species germinated. An increased concentration of chromium and arsenic in the soil may affect the soil seed bank by inhibiting seed germination through reducing α-amylase and β-amylase activity leading to a reduction in sugar stores for the developing embryo (Liu et al., 2005; Zeid, 2001). Cases of affecting the structure and function of male gametophytes in the kiwi species, inhibiting pollen germination and pollen tube growth, and inducing alteration in the pollen tube shape (Speranza et al., 2009, 2007), and callose deposition and arabinogalactan protein distribution in the pollen wall (Speranza et al., 2009) have also been reported. The ability of plant species to cope with the effects of chromium and arsenic toxicity on germination depends on their tolerance to these heavy metals, which varies significantly from species to species and source of contamination (López-Luna et al., 2009). For example, increasing arsenite content reduces wheat germination (*Triticum aestivum*) by up to 38% at a concentration of 16 mg/l, and by 24.2% for arsenate (Liu et al., 2005). In the case of chromium, germination has been reduced in the same species at a concentration of 100 ppm Cr (VI) by 63% (Dotaniya et al., 2014). On the other hand, variables that can provide a subsidy of seeds up to a radius of 100 m (apart from waterbody areas) and the immediate source of seeds according to the affiliation to the habitat (apart from forest-free) have a higher impact in our study.

The ordination diagram of sampling sites shows two clusters. The first, BI, lies on the border of the 2nd and 3rd quadrants which are represented by the sites of the forest interior of both Kozinec and U cesty, forest-wetland ecotone and forest-meadow and wetland habitat of Kozinec. The cluster is mainly impacted by tailings coverage, fine earth, and forest habitat variables. The second, looser BII cluster consists of the wetland part of the U cesty basin, where non-forest area up to 100 m and wetland habitat are impactful variables according to the diagram. The sampling sites on tailings of U cesty basin with developed ruderal vegetation, forest site number 10 with a significant development of tree juveniles and an area 16 which is a forest-wetland ecotone are the diagram outliers.

The relation between plant species and environmental variables of soil seed bank analysis is shown in Figure 4. The species on the ordination diagram do not form any solid clusters, they are scattered. Most environmental variables affect only a few captured plant species. Species of annual to perennial herbs bound mainly to anthropogenic or wetland vegetation shows a correlation with the representation of anthropogenic areas. These are *Sinapis arvensis*, *Lapsana communis*, *Poa annua*, *Chrysosplenium alternifolium*, *Persicaria hydropiper*, *Artemisia vulgaris* species. In the second quadrant there are slope, fine earth, forest habitat, tailings, and forests variables. The presence of tailings, forests and forest habitats have similar vectors, and they affect similar species - perennial herbs of wetland, forest, often anthropogenic habitats such as *Lamium album*, *Erigeron annuus*, *Plantago major*, *Galium aparine* and a woody species *Alnus glutinosa*. The representation of water bodies correlates with species *Chenopodium polyspermum*, *Rubus idaeus*, *Capsella bursa-pastoris* that requires an environment with sufficient nutrients and occurs in wetland, floodplain forest and anthropic habitats. The species corresponding to the variable of non-forest areas are meadow perennial herbs of at least partially illuminated places (*Daucus carota*, *Eupatorium cannabinum*, *Cirsium arvense*, *Hypericum perforatum*, *Tanacetum vulgare*).

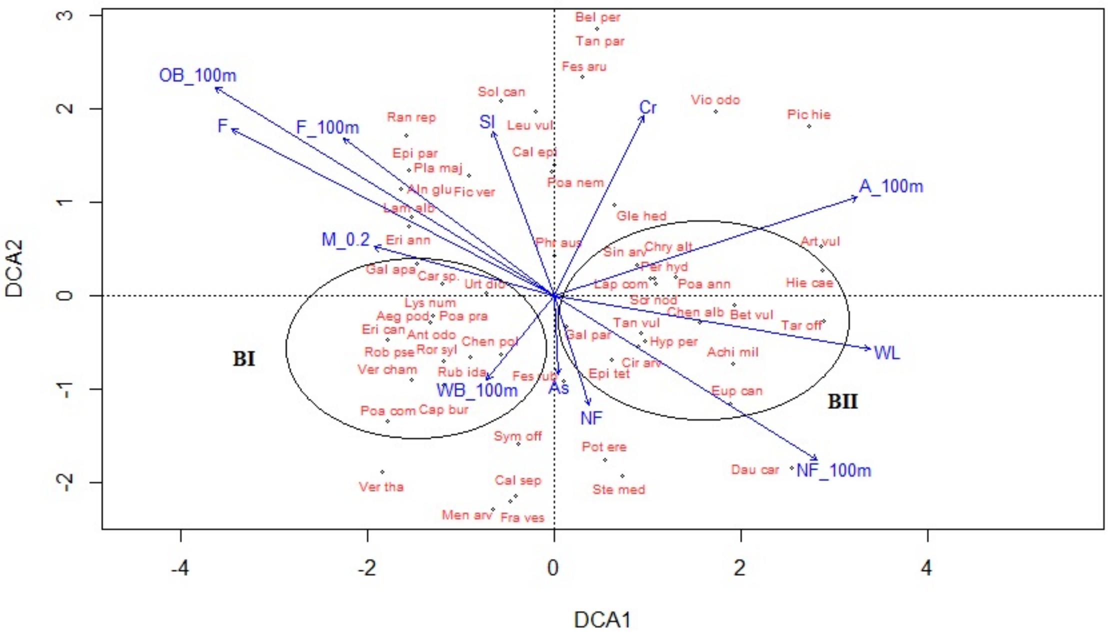

Figure 4. DCA diagram of soil seed bank

In contrast to the aboveground vegetation, the effect of chromium and arsenic content affects fewer plant species. Weak bonds to the chromium content are manifested only in *Glechoma hederacea*, one of the medical plants known as concentrator of chromium (Šeremet et al., 2022) and *Phragmites australis*, which has seeds highly tolerant to chromium pollution (Calheiros et al., 2008). Only *Festuca rubra* is affected by arsenic. This plant is considered an excluder - a plant capable of effective limitation of heavy metal uptake level into its shoots (Dradrach et al., 2020), which is often used in the revegetation of degraded habitats, mine spoils or in the remediation of contaminated soils (Cuske et al., 2016; Simon, 2005). In general, our cultivation analysis of the soil seed bank reveals the predominance of herb species with an optimum or dominant in anthropogenic vegetation.

4. CONCLUSION

Research has shown excessive values of the content of the risk elements chromium and arsenic in the soils of subsidence basins, especially on tailings and wetland areas, therefore further attention should be paid specifically to these parts of the basins. The impact of chromium content on the above-ground vegetation is statistically significant, which is characterized by the presence of various species of metallophytes, often with a high vegetation coverage in the given area. In further research, the content of risk elements in metallophyte biomass should be analysed to assess the development of the pollution of subsidence basins and the possible rehabilitation of these areas by spontaneous succession. Succession can be influenced by the soil seed bank, which is in subsidence basins impacted by forest and wetland biotopes, but also by the presence of anthropogenic structures, areas covered with tailings and non-forested biotopes in their vicinity. The inability of most seeds to germinate on contaminated tailing was apparent in former experiments. However, in more suitable conditions (without contamination) they germinate and grow without problem, which was also proven by DCA, where no statistically significant dependence on As and Cr was demonstrated. The possibility of using this information to potentially rehabilitate subsidence basins and create valuable biotopes should be further explored.

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