

IMPACT OF SUBSTRATE ACIDIFICATION ON THE PLANT AVAILABILITY OF SOME TRACE ELEMENTS IN A COAL WASTE MATERIAL

Serhii KRASOVSKYI¹, Oleksandr KOVROV¹, Iryna KLIMKINA¹ & Oliver WICHE²

¹Department of Ecology and Environmental Protection Technologies, Dnipro University of Technology, 19, Dmytra Yavornytskoho ave., Dnipro, Ukraine, 49005. e-mail: Krasovskiyi.Se.A@nmu.one; kovrov.o.s@nmu.one; klimkina.i.i@nmu.one

²Institute for Biosciences, Biology/Ecology Group, Technische Universität Bergakademie, Freiberg, Leipziger Str. 29, D-09599 Freiberg, Germany. e-mail: oliver.wiche@ioez.tu-freiberg.de

Abstract: Coal waste dumps present potential sources of toxic elements, which may enter surrounding soils, water bodies, and can be transferred into plants. Acidification of the dump material as a consequence of sulphide mineral oxidation can foster element mobilization and plant uptake. In the present study we explored the elemental composition (Co, As, Cu, Pb, Mn, Zn and Cr) of substrate from the waste heap of the coal mine "Heroiv Kosmosy", Ukraine and investigated effects of substrate acidification on leaching and plant accumulation of the elements, considering two native grass species wall barley (*Hordeum murinum*) and japanese brome (*Bromus japonicus*). Both species were cultivated in a greenhouse experiment on substrate from the waste dump. The plants were watered with acidic treatment solutions at a pH range from pH 2–7. Element concentrations in leachate solutions, plant biomass and aqua regia extracts of the substrate were analyzed by ICP-MS. The pH of the substrate collected from the coal dump was 7.68 ± 0.02 the value and the electrical conductivity was 1200 ± 25 $\mu\text{S}/\text{cm}$. The substrate contained low concentrations of plant available (mineral) N (0.09 ± 0.01 mg/kg), and low concentrations of DL-extractable phosphate (0.016 ± 0.01 mg/kg). The aqua regia extractable concentrations of the investigated elements exceed the maximum allowable concentration in 59; 38; 47; 11.5; 2.5; 25 and 64 times, respectively. Decreasing the pH in the substrate significantly increases the concentrations of all studied elements in leachate solutions. The growth experiment showed that both *H. murinum* and *B. japonicus*, respectively germinated and grew on the substrate. After 21 days of plant growth *H. murinum* developed 7 ± 2 cm roots, and average shoot size was 20 ± 1 cm. In contrast, *B. japonicus* showed a root length 4 ± 0.5 cm and shoots were 8 ± 0.5 cm (pH=2). Decreasing the pH-values in the substrate led to increased element abortion in both plant species. However, *H. murinum* preferably accumulated the investigated elements in the roots while *B. japonicus* showed a substantially higher root–shoot transfer. The calculated translocation factors ranged between TF = 0.4–6.6 (*B. japonicus*) and between TF = 0.1–1.7 in *H. murinum*. We could demonstrate that substrate acidification strongly impacts plant availability in coal waste material. Both plant species are meaningful candidates for a phytoremediation of soil waste heaps in the Western Donbas.

Keywords: Physicochemical parameters, heavy metals, pH, ICP-MS, phytoremediation, ruderal plants, translocation factor.

1. INTRODUCTION

The coal industry is currently playing an essential role in the economic and energy sectors. World coal reserves exceed 860 million tons, and it is estimated that this may be enough for the next 112 years of active use (BP Global Group 2012 BP statistical review of world

energy 2012). Although coal remains an essential source of energy, and its operation contributes to the development of several national economies, the coal industry leaves behind many adverse changes in the environment (Zhengfu et al., 2010). These changes apply to all spheres of the planet. In the formation of coal dumps under the main influence line is the lithosphere.

Large areas are needed to construct coal dumps, during which the atmosphere and the hydrosphere are polluted in parallel. The data show that biological activity is also declining in the areas of coal dumps (Sun et al., 2011). Besides many other negative environmental impacts, coal dumps are potential sources of potentially toxic elements that can threaten the surrounding soil, surface, and groundwater (Shim et al., 2005). The chemical and physical characteristics of coal are determined by the nature of precursor plants, the amount of inorganic materials, as well as the nature, intensity and duration of biochemical and geochemical processes responsible for the formation of coal (Finkelman, 1993). Coal heaps are affected by various factors during which solubilization processes occur, sometimes converting almost insoluble metals into soluble substances, leading to water and soil pollution (Thurdin et al., 2006). There are several mechanisms for mobilizing chemical elements: 1) Mobilization due to pH changes. This can be caused by neutralization processes between alkaline ash and weakly acidic atmospheric water (pH 5-6), or acid rain (pH 4 or below), (Otero-Rey et al., 2005); 2) Mobilization through changes in the redox potential. The deposition of coal ash on dumps in the presence of organic matter (derived from the surrounding soil or surface water) can cause oxygen consumption by microbes and can lead to a decreasing redox potential with increasing depth. In consequence, oxides of Fe, Mn and related potentially toxic trace elements might dissolve due to the reduction and formation of soluble chemical element species, (Hamilton-Taylor et al., 1995); 3) Mobilization through ion-exchange reaction. (Abbas et al., 2003); 4) Mobilization through complexation and ligand exchange reactions. The presence of natural complexing agents, such as humic substances in water and dissolved organic carbon from microbial decomposition of organic matter in the heap body, can significantly increase the amount of leaching elements (Janos et al., 2002). (Popovic et al., 2009) experimented on the potential leaching of chemical elements from coal dumps, near Belgrade (Serbia). To assess the leaching potential, the ash samples of the heap were extracted with solutions with decreasing pH values (8.5, 7.0, 5.5 and 4.0), simulating the reactions of alkaline ash particles with possible alkaline, neutral and acidic waters (e.g. acid rain). The authors could demonstrate that the greatest environmental threat is recently deposited "fresh" ash, which releases significant quantities of potentially toxic elements even at the lowest pH level. In contrast, "aged" ash has already lost its potential for pollution due to constant leaching in landfills (Popovic et al., 2009). Various bio fertilizers and organic substances were added to reduce the mobilization of heavy metals to increase the pH level (Asha et al., 2008). The oxidation of pyrite and the

release of oxidation products from low-grade coal waste heaps into the groundwater have been investigated by geochemical and hydro geochemical methods at the Alborz Sharghi Coal Flushing Plant, Shahrud, North-east Iran. The analysis showed that at a standard pH of 7.4 to 7.5, the concentration of leached elements such as Fe, S, Cu, Zn and Co was not altered, but began to increase sharply in the sludge with a decrease in pH (Faramarz et al., 2011). A direct relationship between a decrease in pH and an increase in the concentration of Fe, Cu, Mn and Ni was observed during a physicochemical experiment in wastewater taken from different mines in India (Tiwary, 1999). Although many heavy metals are essential components of pigments and enzymes (mainly Cu, Ni, Zn, Co, Fe and Mo) for algae and plants, all metals/metalloids, especially cadmium (Cd), lead (Pb), mercury (Hg) and copper (Cu) are toxic in higher concentrations because they disrupt enzymes, replace the essential metals in pigments and foster production of oxygen species (Petr et al., 2009). The similarity of some non-essential elements to essential elements (for example, Cd-Zn, Se-S or AsO_4^{3-} - PO_4^{3-} pairs) causes their high uptake rates and high toxicity to plants due to the possibility of replacing essential metals in enzymatic systems (Petr et al., 2009). There are many different approaches to removing potentially toxic elements from contaminated substrates; however, most methods are restricted to small areas and off-site remediation of the contaminated material. A possible on-site approach to remove potentially toxic elements from heaps and mine dumps is phytoremediation (Heilmeyer & Wiche 2020). Phytoremediation is an innovative, cost-effective and environmentally friendly "green" technology to remove or stabilize contaminants in soil (McGrath et al., 2002). In the course of phytoextraction, plant-available elements are accumulated in plant biomass, which can be subsequently removed from the site after harvest. In contrast, phytostabilization deploys the ability of certain plants to convert dissolved element species in soil into immobile forms (McGrath et al., 2002). In this regard, knowledge on the accumulation potential of different plant species, their heavy metal tolerance, growth characteristics on a specific substrate and soil-associated factors and plant-associated factors influencing the phytoextraction efficiency is decisive. Concerning phytoremediation of fresh coal heaps the use of pioneer plant species characterized by fast germination and fast development of dense plant cover offers numerous benefits, including erosion stability of the heap, reduction of rainwater infiltration and removal of potential leaching of compounds into neighboring water bodies. At the same time, the plant species can be used for element extraction or enhanced stabilization of the elements in the root-zone which predominantly depends

on species-specific functional and morphological traits and physicochemical substrate properties such as the distribution of elements in mobile element fractions, soil pH and organic matter contents (Wiche et al., 2017b). The increase in water-soluble forms of Cd with lowering the pH level in the substrate was observed by (Eriksson et al., 1989). However, in this experiment, no excessive extraction properties of perennial ryegrass (*Lolium perenne*) and rapeseed (*Brassica napus*) could be measured. In contrast, (Iqbal et al., 2012), observed a direct relationship between a decrease in pH and an increase in the concentration of Cd and Zn in the plants dandelion (*Taraxacum officinale*) and ribwort plantain (*Plantago lanceolata*). Additionally, (Reddy et al., 1977) reported a significant negative relationship between substrate pH, the concentration of water-soluble forms of Pb and Cd, and accumulation of the elements in rice (*Oryza sativa*). To our best knowledge, there is no report available in the literature dealing with the effects of soil pH on element mobilization in coal mine tailings and availability of the elements to ruderal grass species native in Western Donbass.

Thus, in the present study, wall barley (*Hordeum murinum*) and Japanese brome (*Bromus japonicus*) were cultivated on a substrate from a coal waste heap in the Western Donbass, Ukraine under conditions of decreasing soil pH. Element concentrations in leachate solutions and element uptake by plants were measured by ICP-MS (Co; As; Cu; Pb; Mn; Zn and Cr) to explore the fate of elements in the substrate with decreasing pH and evaluate the prospects for the reclamation of heaps in the Western Donbass.

2. MATERIALS AND METHODS

2.1. Study area

The object of the study was a coal waste heap in the Western Donbass, Ukraine. The Western Donbass is a coal basin within the Dnipropetrovsk region, part of the Donetsk coal basin. In 2020, Ukrainian miners received about 18.9 million tons of coal. This, in turn, is accompanied by the formation of approximately 60 million tons of rocks, which are stored in special areas forming dumps (The coal mining industry, 2021). Sampling for the study was performed on the basis at the mine "Heroiv Kosmosy", village Verbky, Ukraine. The samples were freshly extracted rock from the mine.

2.2. Physicochemical analysis of substrate quality

The analyses were conducted in the laboratories

of the Institute of Biosciences at the Technische Universität Bergakademie Freiberg. The soil reaction (pH) and electrical conductivity (EC) were measured according to Sobek et al., (1978). Five grams of fresh soil samples were mixed with 50 ml deionized water and shaken for 1 h. The pH and EC values were measured directly from the centrifuged solutions using pH- and EC-electrodes. For analysis of mineral N, NO_3^- and NH_4^+ were extracted from soil samples with deionized water and 1 M KCl, respectively, and spectrophotometrically determined according to Bolleter et al., (1961) and Hartley et al., (1963). Plant available (mineral) N in soil was calculated as the sum of NH_4^+ -N and NO_3^- -N. For analysis of mineral P, phosphate was extracted from soil samples with double lactate solution and photometrically determined according to (Egnér et al., 1960). All analysis of substrate samples were done in three replicates.

2.3. Plant growth experiment

Wall barley (*Hordeum murinum*) and Japanese brome (*Bromus japonicus*) were used as phytoindicators of the typical ruderal vegetation of the steppe region of Ukraine. Thirty-six pots (size of each pot: height was 10 cm; diameter was 9 cm) were filled with 0.4 kg of the substrate. Half of the pots (18 pots) were used to cultivate *H. murinum* while the other half contained *B. japonicus*. Thirty seeds of each plant species, were allowed to germinate and the seedlings were transferred to the pots. The pots were incubated in a growth chamber (20°C, 600 $\mu\text{M}/\text{m}^2$ s PAR) in a fully randomized design. After two weeks, the plants were continuously watered with weekly doses of 100 mL of tap water with pH values adjusted at pH 2–7. For pH adjustment, a 1:10 diluted H_2SO_4 was used which mimics a substrate acidification by oxidation of sulfide minerals. The humidity of the growth substrate level was maintained at 70% of field capacity throughout the experiment. Each of the pH treatments per plant species was threefold replicated. Leachate solutions were collected weekly at the bottom of the pots using a micropipette. The experiment lasted 21 days. After this time, the shoots of plants were cut 2 cm above the substrate. The roots were carefully separated from the substrate, thoroughly washed with deionized water. The plant parts were weighed and dried at 60°C for 48 h.

2.4. Analysis of trace element concentrations by ICP-MS

The total content of heavy metals, other potentially toxic elements in the substrate, leachate solutions and plant samples was determined by inductively coupled plasma mass spectrometry (ICP-

MS). For measurement with ICP-MS, all solid samples (plant, substrate) were dissolved by wet acid digestion according to Kurtz et al., (2002) and Wiche et al., (2017a). For the determination of total element concentrations in the heap material, representative subsamples of the substrate used for the growth experiment were collected and dried at 105°C for one day. The samples were sieved (2 mm) and ground to a fine powder using an agate mortar and pestle. The dried plant material from each pot was ground in a bullet mill (Retsch) to obtain homogeneous samples for later analysis. For digestion of heap material samples 100 mg of the dried substrate were transferred into Teflon vessels and mixed with 200 µL deionized water, 300 µL of nitric acid (65%), 900 µL hydrochloric acid (36%) and 600 µL hydrofluoric acid (4,9%). The digestion vessels were sealed and placed in the microwave workstation ETHOS.lab (MLS). The samples were heated to 200°C at a rate of 10°C·min⁻¹. This temperature was maintained for 20 min. The samples were removed when the system cooled down to room temperature. In the case of plant samples, 100 mg were transferred into Teflon vessels with 200 µL deionized water, 1900 µL of nitric acid (65%) and 600 µL hydrofluoric acid (4,9%). The digestion vessels were sealed and placed in the microwave workstation ETHOS.lab (MLS). The samples were heated to 200°C at a rate of 10°C·min⁻¹. This temperature was maintained for 20 min. The samples were removed when the system cooled down to room temperature. Concentrations of Co, As, Cu, Pb, Mn, Zn and Cr of the diluted digestion solutions and leachates were determined by ICP-MS with 10 µg/L rhodium and rhenium as internal standards.

3. RESULTS AND DISCUSSION

3.1. Substrate properties of the heap material

The pH of the substrate collected from the coal dump was 7.68 ± 0.02 , the value of the electro conductivity was 1200 ± 25 µS/cm. The results of spectrophotometric analysis of the substrate supply of nutrients showed that plant available (mineral) N in substrate was 0.09 ± 0.01 mg/kg, and DL-extractable phosphate was 0.016 ± 0.01 mg/kg, indicating extremely low concentrations of these macronutrients compared to Ukrainian soils (EuroSOMNET). ICP-MS analysis of aqua regia dissolvable of trace elements in samples taken from the coal dump (Fig. 1) revealed that the concentration of all measured elements exceeds the maximum allowable concentration (MAC), (Ministry of Health of Ukraine) in 59 (Co); 38 (As); 47 (Cu); 11.5 (Pb); 2,5 (Mn); 25 (Zn) and 64 (Cr)

times (Fig. 1). Since MAC values give a threshold for the environmental risk assessment the heap material must be clearly considered as a source of potentially toxic elements. In contrast, results of the important mineral plant nutrients nitrogen and phosphorus showed that this substrate was clearly depleted in plant nutrients. Thus our results show that typical mining rock of Western Donbas and heaps containing the material are especially problematic for the restoration and phyto-assisted remediation. Their remediation requires the use of plant species with low demands for resources and particularly high metal resistance.

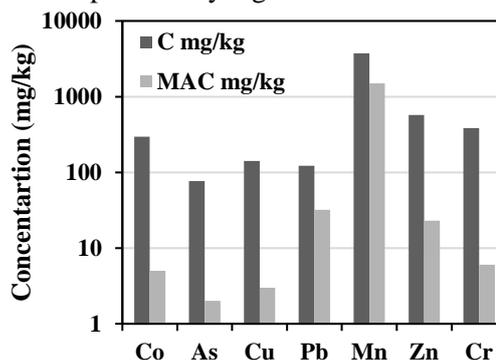


Figure 1. Concentration of heavy metals and As (aqua regia dissolvable trace elements) in comparison with the MAC.

3.2. Elements in leachate solutions affected by plant growth and substrate acidification

The treatment of the substrate with solutions with different pH levels led to increasing concentrations of all investigated elements of the leachate solutions with decreasing pH level. In pots with *H.murinum* the concentrations of all measured elements such as: Co, As, Cu, Pb, Mn, Zn and Cr increased by 21; 2; 4; 25; 73; 16 and 62 times respectively (Table 1). In pots with *B.japonicus* the concentrations of elements: Co, As, Cu, Pb, Mn, Zn and Cr increased by 48; 3; 10; 55; 84; 21 and 43 times respectively (Table 2). In generally, the concentration of soluble forms of elements were increased in the order $\text{Cu} < \text{Zn} < \text{Co} < \text{Pb} < \text{Cr} < \text{Mn}$, which may be interpreted by dissolution of the corresponding mineral phases and increased removal of the element from ion exchange sites (Kabata-Pendias 2011). In addition, the observed pattern might reflect the corresponding stability constants of the metal sulfides. Under sulfidic conditions these inorganic complexes are relatively stable (Calmano et al., 1992). When fresh heap material is in contact with atmospheric or dissolved oxygen, the sediment is oxidized and sulfidic compounds are dissolved by a formation of sulfuric acid, which mobilizes, a large portion of the potentially toxic elements. Depending on their chemical attributes, some species remain in solution or/are re-adsorbed on

freshly precipitated iron hydroxides and oxyhydrates which are formed during oxidation (Calmano et al., 1992). Our results suggests that weakly acidic atmospheric water (pH 5-6), or acid rain (pH 4 or below) can already mobilize chemical elements in the heap material, highlighting their high potential risk for the environment (Otero-Rey et al., 2005). As it can be seen in Table 1 and Table 2 the concentrations of soluble forms of elements were increased with decreasing pH level with a maximum effect at pH 2.

3.3. Plant growth on the substrate depending on pH

The growth experiment showed that both plant species germinated and showed good plant growth on the heap substrate, which was irrigated with solutions of different pH levels. Under strongly acidic conditions, *H. murinum* showed better growth results, than *B.japonicus*. Compared to *B. japonicus*, *H. murinum* developed longer roots and higher shoots length (Table 3). When the plants were watered with

solutions adjusted to the lowest pH value (pH = 2), the length of the roots were 7 ± 2 cm, shoots were 20 ± 1 cm in *H. murinum*, while *B. japonicas* developed smaller root systems (4 ± 0.5 cm), and smaller shoots 8 ± 0.5 cm. The total biomass at pH=2 of roots was 0.6 ± 0.01 g and shoots biomass was 0.6 ± 0.02 g for *H. murinum*. In contrast, under these strongly acidic conditions, roots of *B. japonicus* were 0.6 ± 0.01 g and shoots were 0.5 ± 0.02 g, which could indicate a higher stress resistance in *H. murinum* compared to *B. japonicus* and highlights the applicability of *H. murinum* for the remediation of sulfidic mineral environments. Relationships between the investigated plant growth parameters, the biomass and the pH levels of the watering solutions are presented in Table 3.

3.4. Element concentrations in plants

ICP-MS analysis of aqua regia substrate extracts (Tables 1 and 2) and elements accumulated in plant biomass (Tables 4 and 5) allowed the evaluation of

Table 1. The concentration of aqua regia extractable elements and element concentrations in soil leachates of pots with Wall barley (*Hordeum murinum*)

	Aqua regia extractable elements in the substrate with <i>Hordeum murinum</i> (mg/kg)							Element concentrations in leachate solutions of pots with <i>Hordeum murinum</i> (mg/L)						
	Co	As	Cu	Pb	Mn	Zn	Cr	Co	As	Cu	Pb	Mn	Zn	Cr
pH2	17.6 ±0.1	8.1 ±0.05	20.8 ±0.2	12.5 ±0.1	318 ±0.5	68.9 ±0.2	90.5 ±0.2	12.4 ±0.1	0.2 ±0.05	1.7 ±0.02	1 ±0.01	286.7 ±0.1	14.1 ±0.02	0.5 ±0.02
pH3	16.2 ±0.1	7.8 ±0.05	20.4 ±0.2	12.3 ±0.1	309 ±0.5	70.1 ±0.2	87.4 ±0.2	1.6 ±0.05	0.1 ±0.05	1 ±0.02	0.08 ±0.001	21.2 ±0.1	1.9 ±0.02	0.008 ±0.002
pH4	14.4 ±0.1	6.5 ±0.05	15.6 ±0.2	16.3 ±0.1	261 ±0.5	55.5 ±0.2	68.7 ±0.2	1.5 ±0.06	0.2 ±0.05	0.2 ±0.02	2.8 ±0.01	29.7 ±0.2	0.6 ±0.02	0.02 ±0.002
pH5	16.2 ±0.1	18.2 ±0.05	18.2 ±0.2	12.3 ±0.1	285 ±0.5	65.1 ±0.2	110.1 ±0.2	0.9 ±0.05	0.1 ±0.05	0.5 ±0.02	0.1 ±0.01	9.8 ±0.1	1.6 ±0.02	0.02 ±0.002
pH6	19.2 ±0.1	8.5 ±0.05	23.2 ±0.2	14.0 ±0.1	392 ±0.5	81.5 ±0.2	131.8 ±0.2	0.9 ±0.05	0.1 ±0.05	0.3 ±0.02	0.03 ±0.001	5.1 ±0.08	1.4 ±0.02	0.01 ±0.002
pH7	16.1 ±0.1	7.7 ±0.05	19.0 ±0.2	11.4 ±0.1	287 ±0.5	62.9 ±0.2	98.4 ±0.2	0.6 ±0.01	0.09 ±0.005	0.4 ±0.02	0.04 ±0.001	3.9 ±0.1	0.9 ±0.02	0.008 ±0.002

Table 2. The concentration of aqua regia extractable elements and element concentrations in soil leachates of pots with Japanese brome (*Bromus japonicus*)

	Aqua regia extractable elements in the substrate with <i>Bromus japonicus</i> , mg/kg							Element concentrations in leachate solutions of pots with <i>Bromus japonicus</i> , mg/L						
	Co	As	Cu	Pb	Mn	Zn	Cr	Co	As	Cu	Pb	Mn	Zn	Cr
pH2	17.6 ±0.1	9.2 ±0.04	18.4 ±0.2	13.0 ±0.05	339 ±0.1	71.9 ±0.1	78.1 ±0.1	9.5 ±0.1	0.2 ±0.04	1.9 ±0.1	1.1 ±0.05	200.7 ±0.1	10.4 ±0.1	0.3 ±0.02
pH3	15.9 ±0.1	9.3 ±0.04	16.7 ±0.2	11.8 ±0.05	289 ±0.1	62.3 ±0.1	66.6 ±0.1	0.8 ±0.05	0.08 ±0.004	0.9 ±0.05	0.07 ±0.005	12.9 ±0.1	0.8 ±0.02	0.01 ±0.002
pH4	16.2 ±0.1	8.6 ±0.04	20.9 ±0.2	13.5 ±0.05	322 ±0.1	71.6 ±0.1	80.5 ±0.1	0.9 ±0.01	0.1 ±0.04	0.3 ±0.02	4.4 ±0.05	5.6 ±0.1	1.1 ±0.02	0.007 ±0.001
pH5	17.5 ±0.1	10.0 ±0.04	20.1 ±0.2	17.1 ±0.05	332 ±0.1	71.0 ±0.1	95.8 ±0.1	2 ±0.02	0.3 ±0.04	0.2 ±0.02	0.04 ±0.005	13.6 ±0.1	1.4 ±0.1	0.007 ±0.001
pH6	11.9 ±0.1	8.9 ±0.04	13.9 ±0.2	9.1 ±0.05	242 ±0.1	49.0 ±0.1	65.6 ±0.1	1.1 ±0.1	0.1 ±0.04	0.2 ±0.02	0.03 ±0.005	4.1 ±0.1	1.2 ±0.05	0.01 ±0.001
pH7	17.9 ±0.1	8.6 ±0.04	20.1 ±0.2	12.4 ±0.05	444 ±0.1	70.2 ±0.1	89.6 ±0.1	0.2 ±0.02	0.06 ±0.004	0.2 ±0.02	0.02 ±0.005	2.4 ±0.1	0.5 ±0.02	0.007 ±0.001

the phytoremediation potential of the two plant species at different pH levels and the calculation translocation factors (Table 6). Generally, the total concentration of elements in *H. murinum* were increased with increasing pH levels (Table 4).

Table 3 - Correlation between their biomass and plant growth at different pH levels (HM = *H. murinum*, BJ = *B. japonicus*)

	Root of HM $\frac{g}{cm}$	Shoot of HM $\frac{g}{cm}$	Root of BJ $\frac{g}{cm}$	Shoot of BJ $\frac{g}{cm}$
pH2	$\frac{0.6 \pm 0.01}{7 \pm 2}$	$\frac{0.6 \pm 0.02}{20 \pm 1}$	$\frac{0.6 \pm 0.01}{4 \pm 0.5}$	$\frac{0.5 \pm 0.02}{8 \pm 0.5}$
pH3	$\frac{1.1 \pm 0.02}{4 \pm 2}$	$\frac{1.7 \pm 0.03}{15 \pm 3}$	$\frac{0.9 \pm 0.02}{5 \pm 1}$	$\frac{0.7 \pm 0.01}{10 \pm 1}$
pH4	$\frac{1.1 \pm 0.01}{7 \pm 1}$	$\frac{1.9 \pm 0.02}{15 \pm 2}$	$\frac{1.3 \pm 0.01}{4 \pm 1}$	$\frac{1.6 \pm 0.02}{10 \pm 1}$
pH5	$\frac{0.9 \pm 0.01}{4 \pm 2}$	$\frac{1.4 \pm 0.01}{15 \pm 2}$	$\frac{0.2 \pm 0.01}{4 \pm 1}$	$\frac{1.6 \pm 0.01}{10 \pm 2}$
pH6	$\frac{0.3 \pm 0.02}{5 \pm 2}$	$\frac{1.2 \pm 0.02}{17 \pm 2}$	$\frac{0.9 \pm 0.01}{4 \pm 1}$	$\frac{0.8 \pm 0.01}{10 \pm 2}$
pH7	$\frac{0.3 \pm 0.02}{6 \pm 3}$	$\frac{0.9 \pm 0.01}{19 \pm 1}$	$\frac{0.3 \pm 0.02}{2 \pm 0.5}$	$\frac{0.8 \pm 0.02}{10 \pm 1}$

Table 4 – Concentrations in shoot/in root in *H. murinum*

Elements	Concentration in shoot mg/kg					
	Concentration in root mg/kg					
	pH2	pH3	pH4	pH5	pH6	pH7
Co	4.6	6.0	4.6	2.5	11.5	4.2
	10.9	22.9	15.8	11.2	18.5	36.3
As	1.5	1.7	1.2	0.4	3.2	11.8
	3.5	4.6	4.7	4.8	3.8	48.8
Cu	11.0	12.6	10.4	11.6	18.9	12.4
	12.4	19.6	16.5	11.3	12.9	25.4
Pb	5.1	2.8	6.2	1.0	5.7	1.3
	12.5	8.5	12.8	2.8	6.5	7.1
Mn	173	183	204	320	247	222
	179	483	276	274	359	705
Zn	47.8	48.6	38.9	50.8	72.9	49.5
	39.6	65.2	63.1	40.5	41.9	71.0
Cr	13.3	11.5	11.8	4.1	46.2	5.9
	19.4	49.4	38.1	10.5	38.7	38.8

The lowest concentrations of all elements in shoots and roots were observed when plants were irrigated with solutions pH=7. *Hordeum murinum* accumulated higher concentration all elements in roots than in shoots. Surprisingly, our data shows that the elements concentration in leachate solutions and concentrations in the plant parts of *H. murinum* were not directly related to each other. Possibly, the pH value and resulting increase in the concentration of toxic elements in soil solution negatively affected nutrient homeostasis and plant growth through metals toxicity which can negatively affect the accumulation of the studied elements (Schulz et al., 2012).

Compared to *H. murinum*, *B. japonicus* accumulated higher concentrations of the heavy metals and other toxic elements in roots and in shoots. Our study shows that concentrations of Co; As; Mn; Zn and Cr in plants increased sharply when plants were irrigated with acidic solution showed highest concentration at pH of 4-5. This is in good agreement to the increased element concentrations found in leachates (Tables 1 and 2) and seems to be the optimal pH-range for a phytoremediation of coal waste dumps. Most probably at this pH element acquisition and accumulation is optimal while plant growth and health was not significantly affected.

Table 5 – Concentrations in shoot/in root in *B. japonicus*

Elements	Concentration in shoot mg/kg					
	Concentration in root mg/kg					
	pH2	pH3	pH4	pH5	pH6	pH7
Co	8.5	6.7	16.4	4.9	4.4	6.6
	7.2	7.9	11.3	6.7	9.3	9.1
As	1.7	2.8	13.9	2.5	8.6	2.2
	2.1	3.7	3.1	1.9	1.3	21.9
Cu	14.6	12.4	29.6	6.6	12.5	15.0
	11.7	8.2	15.2	7.8	7.5	7.7
Pb	2.7	4.9	20.9	4.0	3.2	4.4
	5.2	4.2	11.1	3.5	3.2	3.6
Mn	280	174	496	139	151	234
	153	253	301	246	217	281
Zn	80.7	48.7	145	29.0	70.0	69.8
	38.5	40.5	87.1	54.5	50.4	37.8
Cr	11.4	23.6	44.8	19.7	11.4	19.3
	26.9	20.5	33.9	11.1	10.5	15.4

3.5. The translocation factor

The roots/shoots concentration ratio was calculated for each element. This quotient shows the translocation factor (TF) of heavy metals and As from roots to shoots (Table 6). The translocation factor was higher in the *B. japonicus* than in *H. murinum* for all elements. For *B. japonicus* TFs ranged between 0.4 - 6.6, while at the same time it ranged between 0.1 - 1.7 in the *H. murinum*. The highest TF values were observed under near neutral and slightly acidic conditions (Table 6) representing the typical pH values of fresh or slightly aged dump material (see Section 3.1). Higher TF values in *B. japonicus* suggest the high value of this plant species for a phytoremediation in a sense of phytoextraction which requires the elements to be transferred into the shoot biomass which can be easily harvested. Due to higher growth rates of roots and shoots of *H. murinum*, especially under acidic conditions *H. murinum* must be considered as a meaningful candidate for a phytoremediation by phytostabilization where the elements are stored in roots which prohibits their movement into neighboring

sites or water bodies.

Table 6 – The translocation factor in *H. murinum* and *B. japonicus*

Element	TF in the <i>Hordeum murinum</i>					
	TF in the <i>Bromus japonicus</i>					
	pH2	pH3	pH4	pH5	pH6	pH7
Co	0.4	0.3	0.3	0.3	0.6	0.1
	1.2	0.8	1.4	0.7	0.5	0.7
As	0.4	0.4	0.2	0.1	0.8	0.2
	0.8	0.7	4.5	1.3	6.6	0.1
Cu	0.8	0.6	0.6	1	1.5	0.5
	1.2	1.5	1.9	0.8	1.7	1.9
Pb	0.5	0.3	0.5	0.3	0.9	0.2
	0.5	1.2	1.9	1.1	1	0.8
Mn	0.9	0.4	0.7	1.2	0.7	0.3
	1.8	0.7	1.6	0.6	0.7	0.8
Zn	1.2	0.7	0.6	1.2	1.7	0.7
	2.1	1.2	1.6	0.6	0.7	0.8
Cr	0.7	0.2	0.3	0.4	1.2	0.1
	0.4	1.1	1.3	1.8	1.1	1.2

We could demonstrate, that Wall barley (*H. murinum*) and Japanese brome (*B. japonicus*) can be used like pioneer plants. They showed resistance to germination on a substrate from a coal waste heap under conditions of decreasing soil pH.

4. CONCLUSION

We could demonstrate that dump material from the coal waste dump of "Heroiv Kosmosy", contains considerable amounts of potentially toxic elements, while it is impoverished by most macronutrients for plants. Thus, a phytoremediation of coal waste dumps is particularly challenging. The initial pH of the dump material is slightly alkaline but typically decreases with ongoing sulfide oxidation. We have demonstrated that a lowering in pH beyond a value of pH 4 strongly mobilizes Co, As, Cu, Pb, Mn, Zn and Cr, which can threaten the environment. Both pioneer grass species *H. murinum* and *B. japonicus*, respectively showed good germination rates on the substrate and are meaningful candidates for a phyto-assisted remediation of the waste heaps. In direct comparison, *H. murinum* was characterized by better growth properties (longer roots and shoots) compared to *B. japonicus*, especially under strongly acidic conditions. Concomitantly, *H. murinum* preferably accumulated the investigated elements in the roots while *B. japonicus* showed a substantially higher root–shoot transfer. This highlights that both plant species are meaningful candidates for a phytoremediation of coal waste dumps. On fresh coal wastes with an initial pH of 7.8 both grasses can improve erosion stability of

the dumps and could reduce leaching of toxic elements by a reduction of rainwater infiltration. With ongoing sulfide oxidation and substrate acidification our results suggest that *H. murinum* could be used for a phytostabilization of contaminants characterized by low TFs, while *B. japonicus* is a meaningful candidate for phytoextraction of toxic elements in mining areas, in the Western Donbas. Future studies will explore the benefits of intercropping of both species to increase the remediation efficiency on the coal dump and evaluate the remediation efficiency under field conditions.

Acknowledgements

The authors express special thanks to Prof. Dr. Hermann Heilmeyer for the support and possibility to use the technical equipment of the Institute of Bioscience, TU Bergakademie Freiberg. Presented research was supported in the frame of the DAAD project "EcoMining: Development of Integrated PhD Program for Sustainable Mining & Environmental Activities" (2019–2022) and cooperation between Technische Universität Bergakademie Freiberg, Germany, and Dnipro University of Technology, Dnipro, Ukraine (DAAD-Ostpartnerschaften 2021).

REFERENCES

- Abbas, Z., Moghaddam, A. P. & Steenari, B. M., 2003. Release of salts from municipal solid waste combustion residues. Waste Management. 23:291–305.
- Asha A. Juwarkar, Hemlata P. & Jambhulkar, 2008. Phytoremediation of coal mine spoil dump through integrated biotechnological approach, Environmental Biotechnology Division, National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur 440 020, India.
- Bolleter W.T., Bushman C.J. & Tidwell P.W., 1961. Spectrophotometric determination of ammonium as indophenol. Analytical Chemistry 33:592–594.
- BP Global Group, 2012. BP statistical review of world energy 2012, 48 p. http://www.bp.com/assets/bp_internet/globalbp/globalbp_uk_english/reports_and_publications/statistical_energy_review_2011/STAGING/local_assets/pdf/statistical_review_of_world_energy_full_report_2012.pdf.
- Calmano W., Ahlf W., Baade H. & Forstner U., 1992. Transfer of heavy metals from polluted sediments under changing environmental conditions. In: Heavy Metals in the Hydrological Cycle, Hydrobiologia 235, 605–610.
- Egnér, H., Riehm, H. & Domingo, W.R., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. Kungliga Lantbrukshögskolans Annaler, 26, 199–215.

- EPA.** Introduction to Phytoremediation, 600/R-99/10. February. U.S. Environmental Protection Agency. Cincinnati. Ohio. 2000.
- Eriksson E.**, 1989. *The influence of pH, soil type and time on adsorption and uptake by plants of Cd added to the soil*, Water, Air and Soil Pollution 48: 317-335.
- EuroSOMNET**, 2002 – a European database of long-term experiments on soil organic matter.
- Faramarz D., Ardejani, Behshad Jodieri Shokri, Ali Moradzadeh, Seyed Ziadin Shafaei & Reza Kakaei**, 2011. *Geochemical characterisation of pyrite oxidation and environmental problems related to release and transport of metals from a coal washing low-grade waste dump, Shahrood, northeast Iran*, Environmental Monitoring Assess 183:41–55.
- Finkelman, R. B.**, 1993. *Organic Geochemistry*. New York: Plenum Press.
- Hamilton-Taylor, J., Davidson, W. & Morfett, K.** 1995. *The biogeochemical cycling of Zn, Cu, Fe, Mn and dissolved organic C in a seasonally anoxic lake*. Limnology & Oceanography 41:408–418.
- Heilmeier, H. & Wiche, O.**, 2020. *The PCA of Phytomining: Principles, Challenges and Achievements*. Carpathian Journal of Earth and Environmental Sciences 15 (1), 37-42. DOI:10.26471/cjees/2020/015/106.
- Hartley A.M. & Asai R.I.**, 1963. *Spectrophotometric Determination of Nitrate with 2,6-Xylenol Reagent*. Analytical Chemistry 35:1207—1213.
- Iqbal Muhammad, Markus Puschenreiter & Walter W. Wenzel.**, 2012. *Cadmium and Zn availability as affected by pH manipulation and its assessment by soil extraction, DGT and indicator plants*, Science of the Total Environment 416. 490-500.
- Janos, P., Wildnerova, M. & Loucka, T.**, 2002. *Leaching of metals from fly ashes in the presence of complexing agents*. Waste Management. 22:783–789.
- Kabata-Pendias A.**, 2011, *Trace Elements in Soils and Plants*, CRC Press, Boca Raton.
- Kurtz A. C., Derry L. A. & Chadwick O. A.**, 2002. *Germanium–silicon fractionation in the weathering environment*, Geochimica et Cosmochimica Acta, 66, 1525–1537.
- McGrath, S.P., Zhao, F.J. & Lombi, E.**, 2002. *Phytoremediation of metals, metalloids, and radionuclides*. Advances in Agronomy 75, 1–56.
- Ministry of Health of Ukraine** 2020, (In Ukrainian Language) (Міністерство охорони здоров'я України). *About the statement of Hygienic regulations permissible content of chemicals in the soil*. Article 9 of the Law of Ukraine, № 1595.
- Otero-Rey, J. R., Mato-Fernandez, M. J., Moreda-Pineiro, J., Alonso-Rodriguez, E., & Muniategui Lorenzo, S.**, 2005. *Influence of several experimental parameters on As and Se leaching from fly ash samples*. Analytica Chimica Acta 531:299–305.
- Petr Babula, Vojtech Adam, Radka Opatrilova, Josef Zehnalek, Ladislav Havel & Rene Kizek.** 2009, *Uncommon Heavy Metals, Metalloids and Their Plant Toxicity: A Review*, Organic Farming, Pest Control and Remediation. Environmental Chemistry Letters 6(4):189-213.
- Popovic A. & Djordjevic D.**, 2009. *pH-Dependent Leaching of Dump Coal Ash— Retrospective Environmental Analysis*, Energy Sources, Part A, 31:1553–1560.
- Reddy C.N. & Patrick W.H.Jr.**, 1977. *Effect of Redox Potential and pH on the Uptake of Cadmium and Lead by Rice Plants*, Journal Environmental Quality, Vol. 6, no. 3.
- Shim, Y. S., Rhee, S. W., & Lee W. K.** 2005. *Comparison of leaching characteristics of heavy metals from bottom and fly ashes in Korea and Japan*. Waste Management. 25:473–480.
- Hardy Schulz & Bruno Glaser**, 2012. *Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment*. Journal of plant nutrition and soil science 175(3), 410-422.
- Sobek, A. A., Schuller, A. W., Freeman, R. J., & Smith, M. R.**, 1978. *Field and laboratory methods applicable to overburdens and minesoils*. Environmental protection technology series.
- Sun H, Li M. & Li D.**, 2011. *The vegetation classification in coal mine overburden dump using canopy spectral reflectance*. Computers and Electronics in Agriculture 75:176–180.
- The coal mining industry** 2021. (In Ukrainian Language) Видобуток і збагачення вугілля), *MEU statistical review of coal energy 2021*, 1 p. <http://mpe.kmu.gov.ua/minugol/doccatalog/document?id=245548911>.
- Thurdin, R. T., van Hees, P. A. W., Bylund, D. & Lundstrom, U. S.**, 2006. *Bio fuel ash in a road construction: Impact on soil solution chemistry*. Waste Management. 26:599–613.
- Tiwary R.K.**, 1999. *Environmental impact of coal mining on water regime and its management*. Central Mining Research Institute, Barwa Road, Dhanbad, Bihar, India.
- Wiche O., Tischler D., Fauser C., Lodemann J. & Heilmeier H.**, 2017a. *Effects of citric acid and the siderophore desferrioxamine B (DFO-B) on the mobility of germanium and rare earth elements in soil and uptake in Phalaris arundinacea*, International Journal of Phytoremediation, 19, 746–754.
- Wiche O, Zertani V, Hentschel W, Achtziger R. & Midula P.**, 2017b *Germanium and rare earth elements in topsoil and soil-grown plants on different land use types in the mining area of Freiberg (Germany)*, Journal of Geochemical Exploration 175, 120–129.
- Zhengfu B, Hilary I, John D, Frank O. & Sue S.**, 2010. *Environmental issues from coal mining and their solutions*. Mining Science and Technology 20:0215–0223.

Received at: 09. 02. 2022

Revised at: 12. 02. 2022

Accepted for publication at: 15. 02. 2022

Published online at: 17. 02. 2022