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EFFECTS OF DIFFERENT Pb AND Cd CONCENTRATIONS ON BIOAVAILABILITY IN SOME PLANT SPECIES

SUMMARY

Our research aims to evaluate the effect of bunch load variation per unit area on The aim of this study was to examine the effect of different concentrations of heavy metals Pb and Cd on bioavailability in five plant species: nettle (*Urtica dioica* L.), spelt/dinkel wheat (*Triticum spelta* L.), spinach (*Spinacea oleracea* L.), phacelia (*Phacelia tanacetifolia* Benth.) and buckwheat (*Fagopyrum esculentum* Moench). These plant species were sown in containers with substrates contaminated with three different concentrations of Pb and Cd. After removal of the crop,the concentration of these metals was examined in the roots and aboveground organs of the plants. The results showed that with increasing concentration of Pb and Cd in the substrate, their concentration in plants increases, except in spelt where the highest concentration of Pb was recorded in the root of spelt grown on the substrate with the lowest concentration of Pb. The results of phytotranslocation potential showed that buckwheat is a suitable plant species for phytoextraction of both Pb and Cd with all three substrate variants, that is at all tested concentrations of both heavy metals.

Keywords: heavy metals, lead, cadmium, substrate, phytoextraction

INTRODUCTION

Heavy metals are among the most widespread pollutants in soil, water, and the environment. By polluting vast areas around the world, heavy metals are highly reactive and toxic, posing serious risks to human health and ecosystems (Raskin and Ensley, 2000; Wuana and Okieimen, 2011). Heavy metals cannot be degraded and can survive in the environment even after the removal of pollution sources (Babin-Fenske and Anand, 2010). The problem of accumulation of heavy metals in the soil has so far been solved by expensive, abrasive, chemical, and

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physical methods, which due to the lack of universal chemicals that would be used for all metals were not efficient, easily applied, and cost-effective (Radočaj et al., 2020).

In recent years, more and more attention is paid to the application of biological, less obstructive technologies in the domain of the new scientific discipline, the so-called phytoremediation. Numerous studies show that certain plant species have a huge genetic potential that enables efficient accumulation and removal of toxic heavy metals from the soil so that they can play an important role in the phytoremediation process (Jakovljević et al., 2016). To date, about 450 hyper accumulating types of heavy metals from 45 families have been identified (Verbruggen et al., 2009).

Regarding the frequent floods in BiH and the danger of soil contamination with heavy metals after the water withdrawal, attempts are being made to find an environmentally friendly method to clean the land (Ahmetović et al., 2020).

In this paper, the possibility of using plant species was investigated: nettle (*Urtica dioica* L.), spelta (*Triticum spelta* L.), spinach (*Spinacea oleracea* L.), phacelia (*Phacelia tanacetifolia* Benth.) and buckwheat (*Fagopyrum esculentum* Moench.) for remediation of contaminated soil with heavy metals: lead- Pb, cadmium - Cd. The selection of these plants for the experiment was made according to its adaptation to local climatic conditions, large production of green mass, depth to which the root penetrates, growth rate, ease of cultivation, ability to absorb large amounts of water and contaminants (heavy metals) and ability to remove toxins from the soil. The aim of this study was to define an effective and non-destructive method for the selection of native researched plant species and which plant organs show the largest phytoremediation potential based on their spontaneous recovery capacities. Phytoremediation using native species may be effective and efficient than its non-native counterparts, and it is ecologically safer, cheaper, aesthetically pleasing, socially acceptable and easier to cultivate (Heckenroth et al., 2016; Futughe et al., 2020).

Nettle (*Urtica dioica*) belongs to the hyperaccumulating type of heavy metals (Hartley, 2004) but its wider phytoremediation potential is neglected. Nettle, after four months of cultivation in soil contaminated with heavy metals, shows a phytoremediation capacity of 8% for Zn, Cd, and Pb (Viktorova et al., 2016).

Spelt (*Triticum spelta*) is a crop that better absorbs nutrients from the soil compared to common wheat. In the study of the impact of contaminated Cd soil on edible parts of the spelt/ dinkel wheat, a high translocation of Cd from the soil to the edible part of the plant was observed, although the concentration of Cd in the soil was below the threshold for agricultural land. (Radovanović et al., 2017).

Studies on the phytoremediation potential of spinach (*Spinacea oleracea*) show the accumulation of large amounts of heavy metals in tissues, but without visible signs of toxicity. Since the content of Cd and Pb in the edible part of the plant was above the safe limits, the research indicates environmental dangers that may arise when growing spinach for agricultural purposes in contaminated soil (Chatuverdi et al., 2019).

Studies of phytoremediation properties of buckwheat (*Fagopyrum esculentum*) on different types of soil contaminated with Cd or Pb show that plants growing in organic acidic soil show the highest sensitivity to Cd toxicity. Based on the translocation factor, we can conclude that buckwheat has a high phytostabilization potential with Pb, while for Cd it is low. This plant can be considered a candidate for phytostabilization in soils contaminated with Pb (Domaska et al., 2021).

This study investigated the possibilities and abilities of accumulation, conversion of heavy metals into insoluble forms and their removal from the soil by the five investigated plant species, and determining whether the concentration and availability of pollutants in soil decreased in an environmentally friendly way, thus creating opportunities for usage listed plants for remediation of contaminated lands.

MATERIAL AND METHODS

The experiment was set up in the greenhouse of the Agro-Mediterranean Faculty at the "Džemal Bijedić" University in Mostar in controlled conditions. The bioavailability of heavy metals Cd and Pb was tested on 5 plant cultures:

1.nettle (*Urtica dioica* L.)
2.spelt/ Dinkel wheat (*Triticum spelta* L.)
3.spinach (*Spinacea oleracea* L.)
4.phacelia (*Phacelia tanacetifolia* Benth.)
5.buckwheat (*Fagopyrum esculentum* Moench.).

The plants were sown in plastic containers measuring 10 x 10 cm, which were filled with the substrate Terra brill - soil for flowers, which is suitable for selected plant species. Each plant was sown separately in special containers with three different concentrations of Pb and containers with three different concentrations of Cd, three times, which is a total of 90 containers in the experiment (Figure 1).

Fig. 1. Growing plants on a substrate contaminated with heavy metals

The minimum concentration added to the substrate in 15 containers (3 containers for each plant) is the maximum allowed amount of Pb and Cd determined by the Law on Agricultural Land ('Official Gazette of F BiH' No. 52/09) and the Rulebook on determining permitted quantities of harmful and dangerous substances in the soil and methods of their testing ('Official Gazette of the F BiH' No. 72/09). In the next 15 containers, Pb was added in concentrations that were 50% higher than the maximum allowed, and in 15 other containers, Cd was added in concentrations that were 50% higher than the maximum allowed. In the remaining 15 containers, Pb was added in 100% higher concentrations than the maximum allowed and Cd in 100% higher concentrations than the maximum allowed. After removal of the crop, sampling of plant material was performed by manually separating the root from the stem of each plant culture. A total of 180 plant samples were obtained (30 samples from each variant). Analyses for Pb and Cd content of all 180 samples was performed by the method of Atomic Absorption Spectrophotometers. The analyzes were performed in the reference laboratory of the Federal Institute of Agriculture in Sarajevo. The obtained analyses results were statistically processed using the statistical program SPSS 22. The influence of plant species and concentration of heavy metals Pb and Cd in the substrate was determined by a two-factor analysis of variance, and the differences between plants and the substrate concentration ($p < 0.05$). Based on the obtained analysis results, phytoremediation and phytoaccumulation potential were calculated for each observed plant and each concentration of Pb and Cd.

Physico-chemical characteristics of the substrates used are shown in Table 1. (taken from declaration).

Table 1. Physio-chemical characteristics of the used substrates

White peat	Black peat	NPK-fertilizer g/m^3	Trace elements	Salts mg/dm^3	N mg/dm^3	P_2O_5 mg/dm^3	K_2O mg/dm^3
25%	75%	1500	100 g/m^3 + Wetting agent	1,2– 1,7	130– 290	140– 340	170– 390

Heavy metals Pb and Cd in different concentrations were added to the containers with the substrate. Experiment variants with concentrations of added Pb and Cd are shown in Table 2.

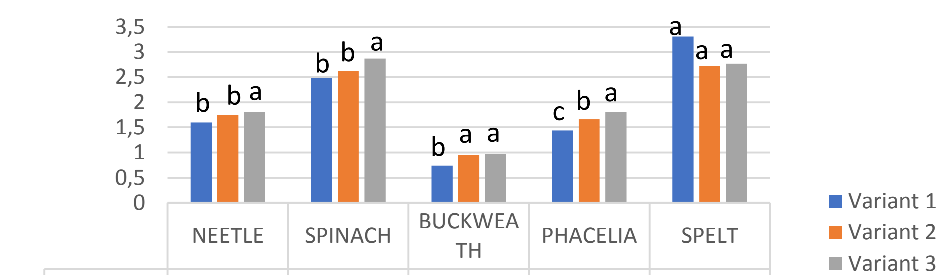
Table 2. Concentrations of metals in soil substrate in containers

Pb concentrations	Cd concentrations
80 mg/kg - Variant 1	1 mg/kg - Variant 1
120 mg/kg - Variant 2	1,5 mg/kg - Variant 2
160 mg/kg – Variant 3	2 mg/kg - Variant 3

RESULTS AND DISCUSSION

The results of research of Pb and Cd contents accumulated in the tested plant cultures, and depending on their concentration in the substrate during cultivation under controlled conditions, are presented in tabular form through

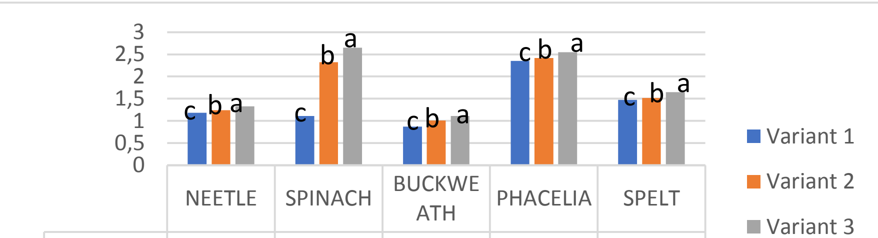
average values of heavy metal content and through statistical elements of all tested variants.
The lead content in the roots of the tested plants is shown in Graph 1.



	NEETLE	SPINACH	BUCKWEATH	PHACELIA	SPELT
Variant 1	1,6	2,48	0,74	1,44	3,31
Variant 2	1,75	2,62	0,95	1,66	2,72
Variant 3	1,81	2,87	0,97	1,8	2,77

Graph 1. Pb content (mg/kg) in the root of plants depending on the concentration in the substrate

The Pb content in the root of all observed plant species increased with increasing Pb concentration in the substrate. The exception to this is the spelled root whose root has the highest Pb concentration on the substrate with the lowest Pb concentration, which is also the highest Pb content in the root in Variant 1. In Variant 2 the highest content was recorded in the spelt compared to all other observed plants, while in Variant 3 the highest Pb content was recorded in spinach roots. The Pb content in the aboveground parts of the tested plants is shown in Graph 2.

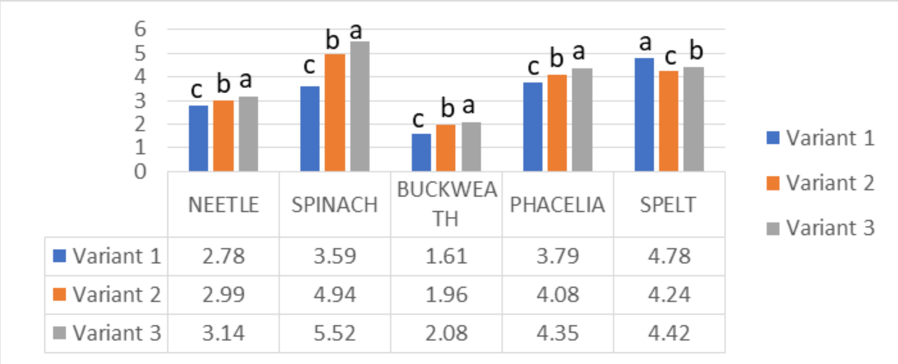


	NEETLE	SPINACH	BUCKWEATH	PHACELIA	SPELT
Variant 1	1,18	1,11	0,87	2,35	1,47
Variant 2	1,24	2,32	1,01	2,42	1,52
Variant 3	1,33	2,65	1,11	2,55	1,65

Graph 2. Pb content (mg/kg) in the aboveground part depending on the concentration in the substrate

The Pb content in the aboveground parts of the observed plants increased with increasing concentration in the substrate. In Variant 1 and Variant 2 it was the highest in phacelia plant, while in Variant 3 it was the highest in spinach.

Average total Pb content depending on the concentration in the substrate is shown in Graph 3.

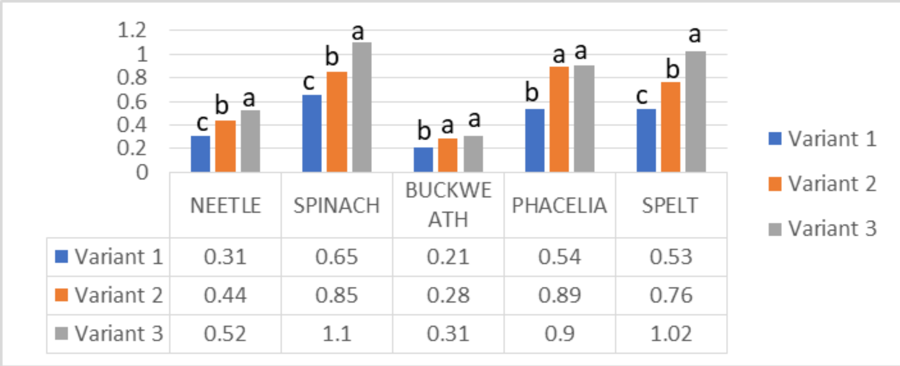


Graph 3. Average total Pb content (mg/kg) depending on the concentration in the substrate

The total Pb content in all plants increased with increasing Pb concentration in the substrate except for spelt, where the total highest Pb content was recorded at the lowest Pb concentration in the substrate. For Pb, the normal concentration in plants is from 0.5 to 10 mg/kg of plant mass, and phytotoxic from 30 to 300 mg/kg (Baker and Brooks 1989), which means that in all tested plants the concentration of Pb was within normal limits. The threshold for Pb batteries was 100 mg/kg and for hyperaccumulators 1000 mg/kg (Boyd R.S. 2011), which means that, according to the obtained results, none of the tested plant species can be considered as either accumulator or a Pb hyperaccumulator.

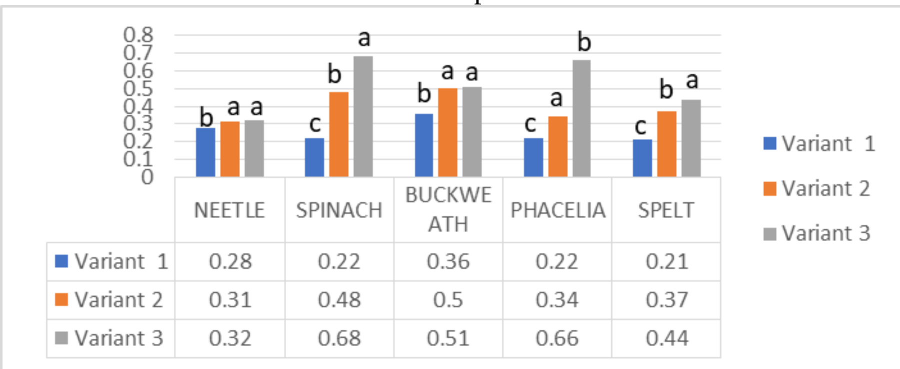
The results of the analysis of variance showed that the plant species, and different substrate concentrations, and their interaction had a statistically significant effect on the total Pb content. The LSD test showed that spinach had a statistically significantly higher value compared to the other observed plant species, and that buckwheat had a statistically significantly lower total Pb content. Spelt had a higher value of total lead intake Pb compared to phacelia and nettle. Phacelia had a significantly higher total Pb content compared to nettle. All differences in the total Pb content in plants between substrates are significant. Significantly the highest Pb content in the aboveground parts of plants was measured on the substrate of Variant 3, and significantly the lowest value was registered on the substrate from Variant 1. The only deviation was registered in spelt with statistically higher total Pb content in the plant.

In all observed plant species, the Cd content in the root increased in parallel with the increase in the Cd concentration in the substrate. In Variant 1 and Variant 3, it was the highest in spinach, and in Variant 2 in phacelia. The content of Cd in the root of the examined plant species depending on its concentration in the substrate is shown in Graph 4.



Graph 4. Cd content (mg/kg) in plant roots depending on the concentration in the substrate

The Cd content in the aboveground parts of the plant from variously contaminated substrates is shown in Graph 5 .

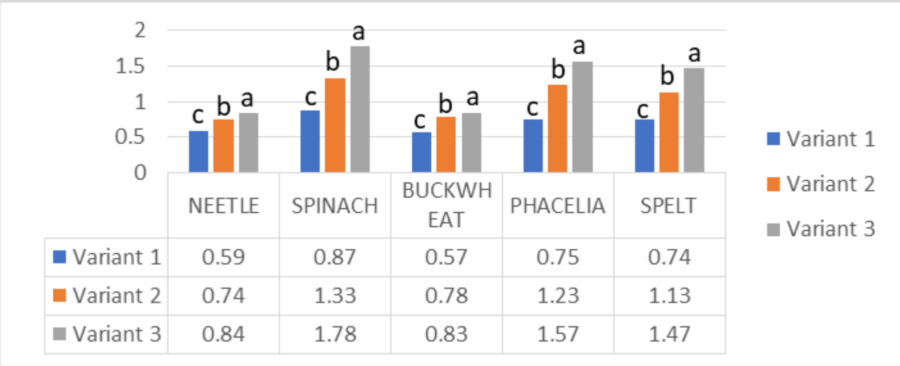


Graph 5. Cd content (mg/kg) in the aboveground parts depending on the concentration in the substrate

The Cd content in the aboveground parts of all plants increased with increasing Cd concentration in the substrate. The highest in Variant 1 and Variant 2 was recorded in buckwheat, and in Variant 3 in spinach.

In all observed plant species, the total Cd content increased with increasing Cd concentration in the substrates. The highest total Cd content in all three substrate variants was recorded in spinach. The lowest was recorded in variant 1 in buckwheat, variant 2, and variant 3 in nettle. The normal amount of Cd in plants is 0.005 to 2 mg/kg, while phytotoxic ranges from 5 to 700 mg/kg (Baker, A. J. M., Brooks, R . R., 1989), so according to the results of this study, no plant is phytotoxic. The threshold for accumulators in Cd is 20, and for hyperaccumulators, it is 100 mg/kg (Boyd 2011), which means that, according to the obtained results, none of the tested plant species can be considered as an accumulator or hyperaccumulator of Cd. The results of the analysis of variance showed that the plant species, different concentrations of Cd in the substrate and their interaction had a statistically significant effect on the total Cd content. The

LSD test showed that spinach had a statistically significantly higher total Cd content compared to the other observed plant species and that nettle and buckwheat had a statistically significantly lower total Cd content. Phacelia had a significantly higher value compared to spelt. All differences in total Cd content among differently contaminated substrates are significant. Significantly the highest total Cd content was measured on the Variant 3 substrate, that is with the highest Cd concentration, and significantly the lowest value was registered on the Variant substrate, with the lowest Cd concentration. Average total Cd content in plants depending on the concentration in the substrate is shown in Graph 6.



	NEETLE	SPINACH	BUCKWHEAT	PHACELIA	SPELT
Variant 1	0.59	0.87	0.57	0.75	0.74
Variant 2	0.74	1.33	0.78	1.23	1.13
Variant 3	0.84	1.78	0.83	1.57	1.47

Graph 6. Average total Cd content (mg/kg) in plants depending on the concentration in the substrate

Based on the value of phyto - accumulation potential, the ability of the plant to accumulate metals from the soil can be determined, and based on the value of the translocation potential, the ability of the plant to translocate the adopted metals from the roots to aboveground organs can be determined. Plants with this index equal to or greater than one are considered suitable for phyto-extraction of heavy metals from the soil. The values of phyto-translocation and phyto-accumulation potential are shown in Tables 3 and 4.

Based on the obtained results, it can be concluded that nettle is suitable for phyto-extraction of Pb if its concentration is 160 mg/kg. Buckwheat is suitable for phyto-extraction of both Pb and Cd at all concentrations. Phacelia is suitable for phyto-extraction at all Pb concentrations.

Research on the possibility of using native plants in the process of phytoremediation is often conducted due to the possibility of simpler and environmentally friendly ways of cleaning the soil from heavy metals.

In the study conducted in Western Serbia (Dimitrijević *et al.*, 2016) of bioaccumulation of heavy metals using nettle the results of investigation have shown that nettle has a tendency to accumulate Pb which may be used in phytoremediation of polluted soil which is in line with our research.

Table 3. Phyto-translocation potential values

PLANT SPECIES		Lead - Pb conc. mg/kg			Cadmium – Cd conc. mg/kg		
	Plant part	**80**	**120**	**160**	**1**	**1,5**	**2**
Neetle	Root	1,60	1,75	1,81	0,31	0,44	0,52
	Aboveground part	1,18	1,24	2,55	0,28	0,31	0,32
	TF index	**0,74**	**0,71**	**1,41**	**0,90**	**0,70**	**0,62**
Spinach	Root	2,48	2,62	2,87	0,65	0,85	1,10
	Aboveground part	1,11	2,32	2,65	0,22	0,48	0,68
	TF index	**0,45**	**0,89**	**0,92**	**0,34**	**0,56**	**0,62**
Buckwheat	Root	0,74	0,95	0,97	0,21	0,28	0,31
	Aboveground part	0,87	1,01	1,11	0,36	0,50	0,51
	TF index	**1,18**	**1,06**	**1,14**	**1,71**	**1,79**	**1,65**
Phacelia	Root	1,44	1,66	1,80	0,54	0,89	0,90
	Aboveground part	2,35	2,42	2,55	0,22	0,34	0,66
	TF index	**1,63**	**1,46**	**1,42**	**0,41**	**0,38**	**0,73**
Spelt	Root	3,31	2,72	2,77	0,53	0,76	1,02
	Abveground part	1,47	1,52	1,65	0,21	0,37	0,44
	TF index	**0,44**	**0,56**	**0,60**	**0,40**	**0,49**	**0,43**

Table 4. Phyto-accumulation potential values

PLANT SPECIES		Lead - Pb conc. mg/kg			Cadmium – Cd conc. mg/kg		
	Total plants/ substract	Variant 1	Variant2	Variant 3	Variant 1	Variant 2	Variant 3
Neetle	total	2.78	2.99	3.14	0.59	0.74	0.84
	substract	80	120	160	1	1,5	2
	TT index	**0.034**	**0.025**	**0.019**	**0.59**	**0.493**	**0.420**
Spinach	total	3.59	4.94	3.52	0.87	1.33	1.78
	substract	80	120	160	1	1,5	2
	TT index	**0.044**	**0.041**	**0.022**	**0.87**	**0.886**	**0.890**
Buckwheat	total	1.61	1.96	2.08	0.57	0.78	0.83
	substract	80	120	160	1	1,5	2
	TT index	**0.020**	**0.016**	**0.013**	**0.57**	**0.52**	**0.415**
Phacelia	total	3.79	4.08	4.35	0.75	1.23	1.57
	substract	80	120	160	1	1,5	2
	TT index	**0.047**	**0.009**	**0.027**	**0.75**	**0.82**	**0.785**
Spelt	total	4.78	4.24	4.42	0.74	1.13	1.47
	substract	80	120	160	1	1,5	2
	TT index	**0.059**	**0.035**	**0.028**	**0.740**	**0.750**	**0.735**

A pot study (Chaturvedi *et al.,* 2019) was conducted to assess the phytoremediation potential of Spinach plants along with their physiological and biochemical response when grown in soil contaminated with heavy metal(loid)s (HMs). Plants were grown under different doses of Pb, Cd and As; and their metal(loid) accumulation efficiency was studied upon harvest. Despite of accumulating high amount of HMs in tissues, no visible signs of toxicity were seen; and hence the efficient survival and defense mechanism shown by spinach plants conclude that they are a viable option to be used for phytoremediation of sites contaminated with Cd and Pb which is in line with our research.

Possibilities for phytoremediation of facelia have been very little researched and there are no results that would be comparable to our research

Buckwheat has relatively high biomass productivity is adapted to many areas of the world, therefore buckwheat is widely used for the phytoremediation process. After harvest, Cd and Zn concentrations of plant biomass and translocation factors for Zn and Cd were determined. Cadmium accumulation in biomass significantly increased in dose-dependent manner (Kaplan and Akan 2018). In the Tamara *et al.* (2005) experiment, the possibility of buckwheat to absorb haevy metals was investigated. In the investigation common buckwheat grown in Pb-contaminated soil was found to accumulate a large amount of Pb in its leaves, stem and roots, without significant damage. The results of both these studies correspond to the results of our research that buckwheat is suitable for phyto-extraction of Cd and Pb.

CONCLUSIONS

In the root of all tested plant species, the Pb content increased with its content in the substrate, except in spelled in which the highest Pb content in the root was on the substrate with the lowest Pb concentration. This also affected the results of the total Pb content in plants, so in spelled it was the highest on the substrate with the lowest Pb concentration. The results on the Pb content in the aboveground organs of all five plant species show that the Pb content increased with increasing concentration in the substrate. The content of Cd in both the root and aboveground organs, as well as in plants in all five examined plant species, grew with increasing concentration of Cd in the substrate. The highest total content in Cd in all Variants, i.e. at all concentrations in the substrate, was recorded in spinach. The values of phyto-accumulation potential in all tested plant species were less than 1.

The results of phyto-translocation potential showed that buckwheat is a suitable plant species for phyto-extraction of both Pb and Cd with all three substrate variants, i.e. at all tested concentrations of both heavy metals. Nettle is suitable for phyto-extraction of Pb at its extremely high **c**oncentration in the substrate, 160 mg/kg. Phacelia is suitable for phyto-extraction of Pb at all Pb concentrations in the substrate.

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