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Heavy Metals in Soil and Application of New Plant Materials in the Process of Phytoremediation

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Abstract

The aim of this paper is to determine the concentration of Pb, Cd, and Zn in: I) alluvial soil; and II) plants: a) vegetable: lettuce (*Lactuca sativa*) and common onion (*Allium cepa*); b) legumes: bird's- foot trefoil (*Lotus corniculatus L.*) and red clover (*Trifolium pretense L.*); and c) grasses (weed plants): zubach (*Cynodon dactylon*) and tall fescue (*Festuca arundinaceous* Schreb.); as well as III) determination of new plant species suitable for bioaccumulation and phytoremediation processes.

The experimental work was carried out through vegetation experiments, and the concentration of metal was determined by potentiometric stripping analysis (PSA). The obtained results show that: I) the concentration of metals in the analyzed soil is above the allowed values at all measuring points; II) a) concentration of Cd in leaf and Pb at the root of the lettuce is increased; b) the concentration of the metal in the bird's-foot trefoil and the red clover is lower than the critical value for the plants; c) in grasses (weed plants), tall fescue and couch grass, the concentration of metal is higher than critical in all measuring points, and in some places it has a value that is toxic for plants; and e) weed plants: tall fescue and couch grass contain high concentrations of heavy metals indicating a certain degree of tolerance and the possibility of using these plants in the bioaccumulation and phytoremediation process.

Keywords: Heavy metals; Soil; Plants; PSA; Phytoremediation

Introduction

Due to the production method [1], toxicity, plant adoption and inclusion in the food chain, heavy metals increasingly appear as dominant carriers of environmental pollution [2,3]. In addition to the polluters, in the northern part of Kosovo and Metohija, they represent a good indicator of the effects of the production processes and the existing passive and active ore dumps of the Trepca mine located in this area.

Due to the process in these complex formations, erosion, the effects of watercourses and atmospheric precipitation, heavy metals come into the air, water, and soil. Processes in the soil and the presence of substances that are very important for plants dictate product properties and fertility of the soil [4]. Pollution of the soil by heavy metals, i.e., knowledge of the mechanism of adoption, distribution, metabolism, and accumulation in plants is of great importance [5,6]. Plants react differently to elevated metal concentrations. The presence of heavy metals affects the life processes of plants through nutrition, water regime, photosynthesis, respiration, or through all physical-biochemical processes [7]. The effects of heavy metals on plants are the reduction in the production of organic matter and changes in the chemical composition of plants [8,9]. Plants adopt heavy metals in the form of ions or organic complexes [10]. The process of adoption depends on the characteristics of the soil itself, the content of organic matter, accessible nutrients, the processes in the rhizosphere, and the application of phosphate, lime, and pH, the intensity of light and temperature, as well as cultivated genotype [11-14]. There is a frequent occurrence of damage to the mechanisms of regulation and adoption of ions by plants. Some authors have found that among metals there are antagonisms and a different reaction of plants to the presence of heavy metals [15,16]. By basic soil analysis (in order to evaluate interdependence and computation of correlation matrices), the dominant role of certain ions was also determined [17].

The scope of research in this paper is to determine the content (concentration) of Pb, Cd, and Zn in alluvial soil and plants. The land for analysis was taken in the villages of Rudare, Grabovac, Srbovac and Gornji Krnjin in the northern part of Kosovo and Metohija, and the analysed plants are: *Lactuca sativa*, common onion (*Allium cepa*) as a plant that is intensively cultivated in the area; bird's-foot trefoil (*Lotus corniculatus L.*) and red clover (*Trifolium pretense L.*), plants used in animal nutrition; as well as grasses (weed plants): *Cynodon dactylon* and *Festuca arundinaceous* Schreb. selected to examine the possibility of applying these plants in the process of phytoremediation-cleaning of contaminated soil using plants that can accumulate larger amounts of heavy metals [18-21]. The concentration of the tested metals was determined at the beginning and at the end of the experiment using potentiometric stripping analysis [22-27].

Experimental Part

Apparatus

Determination of Pb, Cd, and Zn was performed using the Striping analyzer M1 (Faculty of Technology, Novi Sad, and Symmetry, Leskovac, Serbia) [28-30]. The investigated metals were determined in the same analytical step at a constant electricity of -48.90 μA over a time of 300 seconds and the potential of separation -1.40 V [22].

Chemicals

To perform the experimental part of this work, solutions from high purity chemicals (suprapur) manufactured by Merck (Darmstadt, Germany) were prepared. he basic solutions were prepared from the standards of lead, cadmium, zinc and mercury (1.000 g dm^{-3}) while the working solutions were prepared from the bases in the concentration range of 50-90 mg dm^{-3}. In addition to the standard solutions, acid solutions were used: chloride (HCl, 30%), nitrate (HNO_3, 65%); Salt solutions: potassium chloride (KCl), copper sulphate ($CuSO_4$), gallium chloride ($GaCl_3$), and acetone (CH_3COCH_3, 99.5%). he solutions are stored in polyethylene bottles.

Sampling and preparation of samples

Soil samples were taken using a system of concentric circles from a depth of up to 30 cm using a hand probe. After drying (105°C), shredding and screening, the gram of powder obtained that way was converted into a solution by digestion with concentrated nitric and chloric acid, after which evaporation was carried out. The residual mass was dissolved in a 2% solution of chloric acid and stored in measuring vessels of 100 cm^3 until analysis [22].

To determine the concentration of Pb, Cd, and Zn, the following plants were used: *Lactuca sativa* (lettuce) and Allium cloves (onion); *Lotus corniculatus* L. (bird's-foot trefoil), *Trifolium pretense* L. (red clover); *Cynodon dactylone* (zubach) and *Festucea arundinacea* (tall fescue). After washing, drying, and grinding (500°C), the gram of ash was soluble in nitric acid (5 cm^3, conc.) and evaporated. Evaporation was repeated after adding a few drops of concentrated chloric acid. The residual white matter was dissolved with the chloric acid (5 cm^3, 2% solution), and prepared for analysis in 100 cm^3 measuring bottles [22,25,26].

Tests in this work were carried out through vegetation experiments, in six series with four tests per 2 kg of soil, Figure 1.

Figure 1: The vegetation experiment.

In one series, we have planted a lettuce (*Lactuca sativa*), in the second onion (*Allium cepa*), in the third bird's-foot trefoil (*Lotus corniculatus* L.), in the fourth red clover (*Trifolium pretense* L.) in the fifth couch grass (*Cynodon dactylonea*), and in the sixth tall fescue (*Festucea arundinaceae*). Experiments were exhibited in the same conditions (sunlight, sprayed deionized water, without the addition of nutrients and atmospheric effects) from March to June.

Results and Discussion

The results of determining the concentrations of Pb, Cd, and Zn in alluvial soil samples in the northern part of Kosovo and Metohija are shown in Table 1.

X µg g^{-1}	Measuring location				MDK µg g^{-1}
	Rudare	Grabovac	Srbovac	Gornji Krnjin	
Pb	169.48	212.88	12.41	370.46	100
Cd	4.85	5.98	4.04	13.03	3
Zn	300.4	479.34	291.6	567.89	300

Table 1: The concentrations of Pb, Cd and Zn in the analysed soil samples. Number of measurements, n=5.

Recent studies have shown that the average Pb concentration in the alluvial soil is 15-20 µg g^{-1}, Zn 30-40 µg g^{-1}, while according to the literature the average content in the uncontaminated soil is 2- 200 µg g^{-1} Pb; 0.01-0.7 µg g^{-1} Cd and 10-300 µg g^{-1} Zn [9,31]. The obtained results (Table 1) show that at all measuring points the concentration of metals in alluvial soil exceeded the limits of maximum allowable values [31]. Such results indicate that all analyzed soil samples belong to the category of contaminated soils. The cause of the increased concentration of tested heavy metals in the alluvial soil in the northern part of Kosovo and Metohija is the influence of the production processes and the Trepca mines, located in the immediate vicinity along the Ibar River. Heavy metals from ore deposits under the influence of the wind and atmospheric conditions, water course and process waters reach most of the land. The highest concentration of investigated metals was determined in the sample at the measuring site of Gornji Krnjin, located in the vicinity of the active and passive mines of the Trepca. Srbovac is one of the farthest places in terms of landfills, so the lowest concentration of tested metals has been determined in this soil sample.

The results of the determination of the concentration of Pb, Cd, and Zn in vegetable plants: lettuce (*Lactuca sativa*) and common onion (*Allium cepa*), grown on the test soil, are shown in Table 2.

	X/µg g^{-1} SM										
Measuring location		Rudare		Grabovac		Srbovac		Gornji Krnjin			
		koren	list	koren	list	koren	list	koren	list		
	Pb	14.27	7.86	15.34	8.03	12.48	7.18	18.29	9.71	10	20
	Cd	4.15	6.45	4.56	6.96	3.94	5.01	4.85	8.38	5	10
Lactuca sativa	Zn	68.45	109.78	73.12	114.23	65.17	101.32	94.73	132.45	150	200
	Pb	9.14	4.53	9.58	6.93	8.73	4.29	9.99	8.43	10	20
	Cd	3.42	4.07	3.58	4.18	3.06	3.98	3.99	4.33	5	10
Allium cepa	Zn	61.99	111.13	69.2	121.14	48.28	107.07	63.28	133.97	150	200

Table 2: The concentrations of Pb, Cd and Zn in *Lactuca sativa* and *Allium cepa*. X-average of the measurements; number of measurements, n=5; SM-dry matter; Cc-critical concentration; Ct-toxic concentration.

Adoption and accumulation of tested metals by plants depend to a great extent on the nature and the kinetics of enzymatic reactions, as well as on the concentration of metals in the soil [9-11,13]. Based on the results shown (Table 2), the plants mostly accumulated zinc (as the essential element), where the concentration of Zn in the leaves is higher in relation to the root (observed for all measuring points), which is in correlation with the content of Zn in the soil and in consistency with the range of concentrations shown in the literature [9,32].

The plants easily adopt Zn over the roots and move it further into the above-ground organs. The process of adopting, moving and accumulating Zn in plants depends on a number of factors: soil pH, temperature [2,9], the presence of bicarbonate [33], phosphate [34], ion exchange capacity, organic matter content, redox conditions, chloride ion content [35]. In addition, Zn promotes the adoption of Cd, so that both plants: *Lactuca sativa* and *Allium cepa* largely absorb Cd over the root and transport it to vegetative over ground organisms [8]. The amount of Cd in the leaf of *Lactuca sativa*, at the measuring site of Gornji Krnjin, is higher (8.38 µg g^{-1} SM) than the amount of Cd at the root (4.85 µg g^{-1} SM), which is in agreement with the results of other researchers that plants that have a developed leaf surface accumulate more Cd [8,9,13]. The concentration of Cd in the *Lactuca sativa* is higher in relation to the *Allium cepa* observed for all the analyzed soil samples. The leaves of *Lactuca sativa* have mostly accumulated cadmium from the soil from the measuring sites of Rudare, Grabovac and Gornji Krnjin. The adoption of Cd has a great influence on the concentration of Zn in the soil. At lower concentrations of Cd and Zn in the soil, plants (*Lactuca sativa* and *Allium cepa*) accumulate Cd more, and where the concentrations of Cd and Zn are larger, plants accumulate Zn more.

Based on the results shown in Table 2, it is noted that the concentration of Pb is higher at the root than in the leaf regarding both analysed plants. Plants adopt Pb most often in an inorganic form, where the degree of adoption is low as well as the mobility in the above-ground organisms [8-10,13]. Similar to Cd, it could be expected that the concentration of Pb is higher in the leaf than at the root of the plants with a developed leaf surface, which was not the case for the examined lettuce samples. The obtained results are in agreement with the fact that lead mobility increases with the increase of acidity of the soil (soil samples had pH values between 6.80 and 7.10) [36]. In addition, it has been observed that the concentration of metals in cultivated plants is positively correlated with the concentration of metals in the soil. The highest concentration of metals in *Lactuca sativa* and *Allium cepa* plants, both at the root and in the leaf, is found in samples grown on the soil from Gornji Krnjin, and the smallest on samples of the plants grown on the soil from Srbovac. The results of determining the concentration of metals in leguminous plants (fodder plants) and grasses (weed plants) grown on the analysed soil are shown in Table 3.

X µg g^{-1} SM					
Number of series		Rudare	Grabovac	Srbovac	Gornji Krnjin
	Pb	9.34	8.12	5.24	3.46
Lotus corniculatus L.	Cd	/	0.97	/	1.5
	Zn	108.48	144.97	115.72	119.29
	Pb	7.51	3.64	3.52	3.63
Trifolium pretense L.	Cd	0.99	1.32	1.09	1.07
	Zn	145.01	147.96	141.92	149.81
	Pb	116.09	145.87	87.3	253.84
Cynodon dactylonea	Cd	0.94	1.25	0.85	3.16
	Zn	129.17	206.12	125.4	244.22
	Pb	45.98	57.76	34.57	100.51
Festucea arundinacea	Cd	1.03	1.7	1.15	4.27
	Zn	183.85	293.36	178.46	347.57

Table 3: The results of determination of the concentration of metals in plants grown on the investigated soils. X-average of the measurements; number of measurements, n=5.

Determination of the concentration of Pb, Cd, and Zn in the listed plants (Table 3) was performed in the total biomass (leaf and root) in order to determine the total accumulation potential. Plants of *Lotus corniculatus* L. and *Trifolium pretense* L. represent legumes used as fodder plants. The amount of Pb and Zn in these plants does not exceed the values of critical concentrations in plant tissue in which there may be up to 10% loss of biomass (10-20 μg g^{-1} Pb, 100-500 μg g^{-1} Zn), while for most of the analyzed samples, the amount of Cd is approximately or above the maximum tolerable level for feeding animals (1 μg g^{-1}) [37]. The amount of Zn in total biomass of red clover was around 145 μg g^{-1} SM for all measuring points, which means that the total amount of Zn in the analyzed soil and the characteristics of the soil itself do not affect to the greatest extent on the sorption of this element. Pb was most accumulated in the couch grass in Gornji Krnjin (253.84 μg g^{-1} SM). The tendency of accumulation in this plant also has Zn (206.12 μg g^{-1} SM in Grabovac; 244.22 μg g^{-1} SM in Gornja Krnjina). And the tall fescue accumulated Pb and Zn at all measuring points, in values above tolerant. When comparing these two plants, the couch grass accumulated a larger amount of lead and a tall fescue a higher amount of Zn. Otherwise, the accumulation of Pb and Zn for both analyzed plants is in correlation with the amount of the analyzed metals in the soil. The higher concentration of Pb and Zn in the total biomass corresponds to a higher concentration of metals in the analyzed soil.

The amount of the metal in the soil was determined also after the removal of the plant material. Results of determination of metals in the analyzed soil after planting: *Lactuca sativa, Allium cepa, Lotus corniculatus L., Trifolium pretense L., Cynodon dactylonea* i *Festucea arundinaceae* are shown in the Table 4.

X/ μg g^{-1}					
Broj serije		**Rudare**	**Grabovac**	**Srbovac**	**Gornji Krnjin**
I	Pb	152.53	191.51	114.67	333.42
Lettuce	Cd	4.26	5.26	3.56	11.46
	Zn	264.59	422.52	258.68	497.84
II	Pb	154.23	194.28	116.43	337.86
Allium cepa	Cd	3.35	5.21	3.52	11.33
	Zn	261.34	417.02	253.07	494.06
III	Pb	150.83	189.46	113.39	329.73
Lotus corniculatus L.	Cd	3.27	5.08	3.43	10.97
	Zn	255.43	408.94	247.68	483.65
IV	Pb	151.68	190.52	114.03	330.45
Trifolium pretenseu L.	Cd	3.29	5.11	3.45	11.14
	Zn	256.84	409.82	249.32	485.54
V	Pb	53.35	67.05	40.15	116.82
Cynodon dactylon	Cd	2.91	4.51	3.05	9.83
	Zn	17123	273.52	166.82	323.97
VI	Pb	11.64	149.6	89.2	259.95
Festucea arundinaceae	Cd	2.82	4.28	2.89	10.76
	Zn	117.55	186.98	116.64	221.32

Table 4: The results of determination of the concentration of metals in the soil after plant crops. X-average of the measurements; number of measurements, n=5.

Based on the results shown in Table 4, the reduction in the amount of metal in the analyzed soil after the planting is noticeable. Analyzed vegetable plants (lettuce and onion) and legumes (bird's-foot trefoil and red clover) accumulate 9-11% of the concentration of Pb, 12-15% of the Cd concentration and 12-15% of the Zn concentration in the soil. The couch grass can accumulate up to 70% of Pb, about 25% of Cd and 43% of zinc. The tall fescue from the soil can reduce Zn up to 61%, Cd up to 27% and Pb up to 30%, which indicates a high degree of tolerance and possible application of these plants as a bio accumulator in the process of soil phytoremediation. As the couch grass and tall fescue in different relationships accumulate Pb, Cd, and Zn, sowing their mixture can affect the total increase in a number of accumulated metals.

Conclusion

The results shown in this study indicate that in all analyzed samples of alluvial soil the concentration of Pb, Cd, and Zn is increased. Based on the prescribed maximum allowable values, the analyzed land belongs to the category of contaminated soil, which is a direct consequence of the influence of the production process and the ore mines of the Trepca. Cultivated vegetable crops, lettuce, and onion to some extent accumulate the investigated metals, especially in quantities that are found in edible parts of plants. The concentration of Pb and Zn in bird's-foot trefoil and red clover does not exceed the values of critical concentrations in plant tissue, and for most analyzed samples, Cd amount is approximately near or above the maximum tolerant level for animal feeding. The amount of Zn in total biomass of red clover was around 145 μg of g^{-1} SM at all measuring points.

The most accumulated Pb was found in the bird's-foot trefoil, depending on the measurement point, the amount of accumulated Pb ranged from 87.3-253.84 μg g^{-1} SM. The Zn (125.4-244.22 μg g^{-1} SM) has a tendency to accumulate in the bird's-foot trefoil. At all measuring points, Pb was accumulated in a tall fescue in values above tolerant ones. In this plant, the accumulation of Zn at all measuring points was more intense and ranged from 178.46-347.57 μg g^{-1} SM.

Planting reduces the amount of metal in the soil. Analysed vegetable plants (lettuce and onion) and legumes (bird's-foot trefoil and red clover) accumulate 9-11% of the concentration of Pb, 12-15% of the Cd concentration and 12-15% of the Zn concentration in the soil. The couch grass can accumulate up to 70% of Pb, about 25% of Cd and 43% of zinc. The tall fescue from the soil can reduce Zn up to 61%, Cd up to 27% and Pb up to 30%, which indicates a high degree of tolerance and possible application of these plants as a bio accumulator in the process of soil phytoremediation. As the couch grass and tall fescue in different relationships accumulate Pb, Cd, and Zn, sowing their mixture can affect the total increase in a number of accumulated metals.

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