

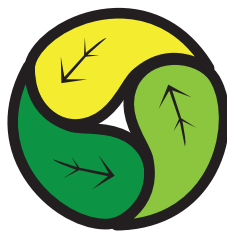
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## СОДРЖИНА CONTENT

Емилија Костадиновска, Саша Митрев, Раде Русевски, Илија Каров и Виолета Димовска Присуство на вирусот на свиткување на листовите кај виновата лоза во Македонија Emilija Kostadinovska, Sasa Mitrev, Rade Rusevski, Ilija Karov, Violeta Dimovska Presence of grapevine leafroll associated virus in the vineyards in the Republic of Macedonia .....	5
Сања Костадиновиќ Величковска, Саша Митрев, Фиданка Илиева, Љупчо Михајлов Сензорна и аналитичка евалуација на ладно-цедени масла од сончоглед Sanja Kostadinović Veličkovska, Saša Mitrev, Fidanka Ilieva, Ljupco Mihajlov Sensory and analytic evaluation of cold-pressed sunflower oils .....	25
Фиданка Трајкова, Лилјана Колева-Гудева и Саша Митрев Култура на зиготски ембриони од кајсија ( <i>Prunus Armeniaca</i> L.) Fidanka Trajkova, Liljana Koleva Gudeva and Sasa Mitrev Zygotic embryos culture from apricot ( <i>Prunus Armeniaca</i> L.) .....	39
Љупчо Михајлов, Весна Зајкова Панова, Биљана Балабанова Фиторемедијација со соја на загадени земјоделски почви со кадмиум Ljupco Mihajlov, Vesna Zajkova Panova, Biljana Balabanova Soybean phytoremediation of cadmium polluted agricultural soils .....	49
Биљана Балабанова, Саша Митрев, Виолета Иванова-Петропулос, Рубин Гулабоски Одредување на изотопи на олово во вино и масло за јадење со примена на индуктивно спрегната плазма со масена спектрометрија Biljana Balabanova, Sasa Mitrev, Violeta Ivanova-Petropulos, Rubin Gulaboski Isotopic lead measurements in wine and edible oil using inductively coupled plasma with mass spectrometry .....	59
Лилјана Колева-Гудева, Фиданка Трајкова и Јулијана Троицки Стимулирање на вегетативното размножување со ауксини кај рузмарин ( <i>Rosmarinus Officinalis</i> L.) и жалфија ( <i>Salvia Officinalis</i> L.) Liljana Koleva Gudeva, Fidanka Trajkova and Julijana Troicki Stimulation of vegetative propagation with auxins in rosemary ( <i>Rosmarinus Officinalis</i> L.) and sage ( <i>Salvia Officinalis</i> L.) .....	69



- Наталија Маркова Руждиќ, Љупчо Михајлов, Верица Илиева,  
Соња Ивановска  
Квалитетно-технолошки својства кај домашни дворедни форми на  
јачмен  
Natalija Markova Ruzdik, Ljupcho Mihajlov, Verica Ilieva, Sonja Ivanovska  
Qualitative - technological properties at domestic two row barley  
genotypes ..... 83
- Биљана Балабанова, Блажо Боев, Саша Митрев, Виолета Иванова-  
Петропулос  
Метод за одредување на содржината на 35 елементи во различни  
примероци со примена на микробранова дигестија и индуктивно  
спрегната плазма со масена спектрометрија (icp-ms)  
Biljana Balabanova, Blazo Boev, Sasa Mitrev, Violeta Ivanova-Petropulos  
Method for determination of 35 elements content in  
various samples with application of microwave digestion  
and inductively coupled plasma with mass spectrometry (icp-ms) ..... 99
- Душан Спасов, Драгица Спасова, Мите Илиевски, Билјана Атанасова  
Влијание на температурата врз интензитетот на појава на  
памуковата совица (*Heliothis obsoleta* FABR. = *Helicoverpa  
armigera* НВ.) како штетник на пиперката во струмичкиот регион  
Dusan Spasov, Dragica Spasova, Mite Ilievski, Biljana Atanasova  
The influence of the temperature at the intensity of the spread of the  
cotton bollworm (*Heliothis obsoleta* FABR. = *Helicoverpa armigera*  
НВ.) as a pest of the peppers in the strumica region ..... 113
- Виолета Иванова-Петропулос, Драгана Петрушева, Саша Митрев  
Методи за определување на SO<sub>2</sub> и редуцирачки шеќери во вина и  
алкохолни пијалаци  
Violeta Ivanova-Petropulos, Dragana Petrusheva, Sasa Mitrev  
Methods for determination of SO<sub>2</sub> and reducing sugars in wines and  
alcoholic beverages ..... 119
- Мите Илиевски, Драгица Спасова, Љупчо Михајлов, Душан Спасов,  
Наталија Маркова Руждиќ, Верица Илиева, Еленица Софијанова  
Производство и застапеност на житните растенија во Република  
Македонија  
Mite Ilievski, Dragica Spasova, Ljupco Mihajlov, Dusan Spasov, Natalija  
Markova Ruzdic, Verica Ilieva, Elenica Sofijanova  
Production and balance among of cereal plants in Republic of  
Macedonia ..... 129



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## ФИТОРЕМЕДИЈАЦИЈА СО СОЈА НА ЗАГАДЕНИ ЗЕМЈОДЕЛСКИ ПОЧВИ СО КАДМИУМ

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**Апстракт:** Загадувањето на почвата со кадмиум се должи на засилениот индустриски развој, особено во области на ископување, искористување и обработка на минерални суровини. На територијата на Република Македонија има неколку области со значајно високи содржини на кадмиум во почвата, меѓу кои е и околината на Рудникот за олово и цинк „Злетово“, во околината на градот Пробиштип. Фиторемедијацијата е една од најпогодните техники за ремедијација на тешки метали од загадени почви. Целта на ова истражување беше да се утврди ефикасноста на неколку сорти соја за фиторемедијација на земјоделски обработливи почви со повисока содржина на кадмиум. За таа цел беа користени три сорти соја со кратка вегетација: *Pella*, *Avigea* и *OW* во симбиоза со ризобактеријата *Bradyrhizobium japonicum*. Вкупната и достапната содржина на кадмиум беше одредувана во одделни делови на растението (корен, стебло, лист, семе и мешунка). Дополнително беа направени и физичко-хемиски анализи на почвата. Од направените анализи се утврди дека сортите *Pella* и *OW* покажуваат висок потенцијал за фитостабилизација/фитоекстракција на кадмиум од почвата.

**Клучни зборови:** кадмиум, соја, фиторемедијација, почва, загадување.

## SOYBEAN PHYTOREMEDIATION OF CADMIUM POLLUTED AGRICULTURAL SOILS

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**Abstract.** Soil pollution with cadmium is a result of the strengthened industrial development, especially in the areas of drilling, exploitation and processing of mineral raw materials. On the territory of the Republic of Macedonia there are several areas with significant higher content of cadmium in the soil, including the vicinity of the mine lead and zinc “Zletovo” near the town of Probištip. Phytoremediation is one of the most convenient techniques

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for remediation of heavy metals from contaminated soils. The main purpose of the present study was to determine the effectiveness of soybean varieties for phytoremediation of agricultural soils with higher content of cadmium. For that purpose, three soybean varieties with short vegetation were used: *Pella*, *Avigea* and *OW* in association with rhizobacterium *Bradyrhizobium japonicum*. The total and available content of cadmium were determined in separate parts of the plant (root, stem, leaf, seed and pod). Additionally, physicochemical analyses were conducted for determination soil properties. The analysis showed that varieties *Pella* and *OW* had higher potential for phytostabilization/ phytoextraction of cadmium from the soil.

**Key words:** *Cadmium, soybean, phytoremediation, soil, pollution.*

## 1. Introduction

Phytoremediation basically refers to use of plants and associated soil microbes for reducing the concentration or toxic effects of contaminants in the environment [1, 2]. It can be used for removal of heavy metals as well as for organic pollutants [3, 4]. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility with inputs of organic matter [5]. The term “phytoremediation” is a combination of two words: Greek “phyto” (meaning plant) and Latin “remedium” (meaning to correct or remove an evil). Green plants have an enormous ability to uptake pollutants from the environment and accomplish their detoxification by various mechanisms. Phytoremediation technology is a relatively recent technology with research studies conducted mostly during the last two decades. The concept of phytoremediation (as phytoextraction) was suggested by Chaney [6]. It is suitable for application at very large field sites where other remediation methods are not cost effective or practicable [7]. Phytoremediation has low installation and maintenance costs compared to other remediation options [8]. The establishment of vegetation on polluted soils also helps prevention of erosion and metal leaching [9]. Furthermore, fast-growing and high-biomass producing plants could be used for both phytoremediation and energy production [10]. Phytoremediation also enjoys popularity with the general public as a “green clean” [11]. This method is not hazardous to human health, it is environment friendly and from economic point of view is very cost effective. There are different types of phytoremediation: phytoextraction, phytovolatilization, phytostabilization and rhizofiltration. The most suitable method for Cd extraction of soil is phytoextraction. This method is based on excluding cadmium from soil by plant through water received from the root system, sorption or through some other mechanism. A great number of plant species have been studied in their ability for remediation of Cd from



soil, including several varieties of soybean. Wei et al. [12] reported *S. nigrum* as hyper-accumulator of Cd. Ishikawa et al. in 2006 published results of the research made on two varieties of soybean (cv. Enrei and cv. Suzuyutaka), two varieties of rice (cv. Nipponbare and cv. Milyang 23) and one variety of maize (cv. Gold dent) in their ability for remediation of cadmium from soil [13]. Studies have shown that there are differences in the phytoremediation ability among different varieties of the same culture. Soybean variety “Suzuyutaka” and rice variety “Milyang 23” have been proved as most effective in removing cadmium from the soil, but in soybean falling of leaves after 60 days was observed, at concentration of cadmium from 4.29 mg/kg. Similar studies were made with three varieties of soybean (Enrei, Tsurunoko and Tsukui) [14]. The ideal plant used for phytoremediation should have fast growth, produce large quantities of biomass and have tolerance and ability to accumulate high concentration of metals in the root system or branches. Plants that have been known as good remediators have poor growth and generally produce reduced amounts of biomass when the concentration of available metals in soil is high. One of the alternatives that are offered as a solution is to use a certain plant species that have a low capacity for accumulation of metals, and on the other hand have a high growth, such as *Brassica juncea* [13]. Another alternative is to use plants that have rapid growth in symbiosis with rhizobacteria [15]. This kind of combination between plant and rhizobacteria is expected to show high efficiency for remediation [16].

Soil pollution with cadmium is a major problem in the world. In Republic of Macedonia there are approximately 1000 ha in the region along the Zletovska river contaminated with cadmium [17]. Therefore, the main purpose of this study is to evaluate the efficiency of three soybean varieties with short vegetation in incorporation with rhizobacterium *Bradyrhizobium japonicum* for cadmium phytoextraction/phytostabilization.

## **2. Materials and methods**

### **2.1. Field settings**

The experiment was set up in an open field conditions with selected soybean varieties with short vegetation (*Pella*, *Avigea* and *OW*). The experiment was performed in appropriate pots for growing soybeans set according to the method of randomized block system, with duration from June to October, 2011. The soil, where the soybean seeds were planted, was collected from the surface layer (0-15 cm) downstream from the potentially polluted area from Zletovska river. Five locations were selected, wherefrom the soil was collected (Z1, Z2, Z3, Z4 and Z5). This is an area where the Pb-Zn mine “Zletovo” is operating for more than 20 years. Many studies have shown that this is an area with polymetallic pollution [17]. Four seeds of each variety were sown in each



container and reduced into two plants after growing. Before the sowing, seeds were treated with a suspension of rhyzobacterium *Bradyrhizobium japonicum*.

## 2.2. Soil sampling and analysis

Soil properties [pH, cation exchange capacity (CEC), electrical conductivity (EC), organic matter (OM), soil texture, available potassium and phosphorus, total and available Cd] were examined before planting of soybean seeds. Standard reference methods were applied for soil pretreatment [18-20].

## 2.3. Soybean analysis

Total cadmium content was determined in specific plants parts (root, stem, leaves and seeds), for each soybean varieties: “Pella”, “Avigea” and “OW”. After vegetation, plants were collected and brought to the laboratory. Every plant was washed with distilled water and dry to a constant mass. Each plant part was separated from each other and mild. The total digestion of the samples was performed using open wet digestion method with a mixture of 5 mL hydrogen peroxide ( $H_2O_2$ , 33%w/w, with ultra-trace purity, Sigma Aldrich, Germany) and 10 mL nitric acid ( $HNO_3$ , 69 %w/w, with ultra-trace purity, Sigma Aldrich, Germany). The inductively coupled plasma mass spectrometer (ICP-MS, model 7500cx, Agilent technologies, USA) was used for cadmium content measurements.

## 3. Results and discussion

Large number of factors control the metal accumulation and bioavailability associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer processes, sequestration and speciation, redox states, the type of plant root system and the response of plants to elements in a relation to seasonal cycles. Structure of the sediment has also been considered very important that affect the extent of the metals taken up by the plants. Clay particles also play an important role in availability of the metals. Metals solubility in soils is predominantly controlled by pH, amount of metals cations exchange capacity, organic carbon content and oxidation state of the system [21]. The basic chemical/mechanical parameters determined in this study for the soil composition are given in Table 1. Soil 1 (Z1), was sandy loam with pH of 6.9, EC of 0.4 mS/cm and 1.4% organic matter. Second and third soil (Z2 and Z3) very similar to Z1, were sandy loam with pH of 6.0 and 6.9, with lower cationic exchange capacity (2.5 and 0.3 cm/mol, respectively) compared to Z1 (9.1 cm/mol). The total cadmium content in the first three soils (Z1, Z2 and Z3) was determined as 2.3, 4.2 and 1.3 mg/kg, respectively; while the available content of Cd was 1.1, 2.3 and 0.84 mg/kg, respectively for Z1, Z2 and Z3 soil. Considering this, the calculated



activity ratio for the bioavailable content of cadmium in these soils was 48% for Z1, 54% for Z2 and maximum ratio was obtained for Z3 (64%). Soil of sites 4 and 5 was loamy sand with dominant soil texture class and very similar pH (3.3). Higher pH may restrain the absorbability of elements from the soil solution and translocation into plant tissues [14]. The significant differences occurred for electrical conductivity (EC), observing values of 12.1 mS/cm for Z4 and 2.37 mS/cm for Z5. Maximum value for the organic matter content was obtained for Z4 soil (5.24%). The total cadmium content in Z4 soil was 17.6 mg/kg, while the available content of cadmium was 3.9 mg/kg. Therefore, the activity ratio for the cadmium in Z4 soil was 22%, compared to the first three soils is significantly lower. Minimum value for cadmium content was obtained for Z5 soil (0.97 mg/kg). The available content of cadmium in this soil was 0.32 mg/kg, which gives 32% activity ratio.

**Table 1.** Chemical/mechanical soil composition from Zletovska River region (n=5/5)

Soil parameters	Determined Range	Median
Cation exchange capacity, CEC (cm/mol)	0.3-9.8	5.7
Electrical conductivity, EC (msS/cm)	0.22-12.1	1.72
pH/KCl	3.2-6.9	3.2
Organic matter, OM (%)	0.92-5.24	1.47
K <sub>2</sub> O (mg/100g)	1.4-15.3	4.9
P <sub>2</sub> O <sub>5</sub> (mg/100g)	1.4-12	3.4
Total Cd (mg/kg)	0.97-17.6	2.3
Available Cd (mg/kg)	0.32-3.9	1.1
Loam (%)	0.01-10.2	1.9
Sand (60-2000 μm)	69.5-97.2	78.7

Considering the basic chemical/mechanical parameters for soil composition, bivariate analysis was applied to investigate the correlations between these parameters. For that issue the linear coefficient of correlation was used. Matrix of the dominant correlation coefficients is given in Table 2. The significant correlation data ( $r > 0.50$ ) are given as bolded values. The available content of cadmium is significantly correlated with soil pH and electrical conductivity of soil solution. Increased soil pH may reduce the availability of Cd<sup>2+</sup> to plants through increased adsorption at cation exchange sites. Soil organic matter can either increase or decrease the availability of Cd<sup>2+</sup> by binding it, or increasing its mobility.





**Table 2.** Matrix of correlation coefficients (soil properties)

<b>CEC</b>	1.00								
<b>pH</b>	0.27	1.00							
<b>EC</b>	<b>0.62</b>	-0.40	1.00						
<b>OM</b>	<b>0.91</b>	0.43	0.49	1.00					
<b>Loam</b>	-0.01	0.31	-0.34	-0.15	1.00				
<b>Sand</b>	0.05	0.28	-0.03	0.27	<b>-0.75</b>	1.00			
<b>K<sub>2</sub>O</b>	<b>0.85</b>	<b>0.58</b>	0.31	<b>0.96</b>	-0.01	0.25	1.00		
<b>P<sub>2</sub>O<sub>5</sub></b>	<b>0.86</b>	<b>0.53</b>	0.36	<b>0.97</b>	-0.19	0.39	<b>0.95</b>	1.00	
<b>Av(Cd)</b>	0.01	<b>-0.54</b>	<b>0.67</b>	-0.15	-0.29	-0.04	-0.26	-0.28	1.00
	<b>CEC</b>	<b>pH</b>	<b>EC</b>	<b>OM</b>	<b>Loam</b>	<b>Sand</b>	<b>K<sub>2</sub>O</b>	<b>P<sub>2</sub>O<sub>5</sub></b>	<b>Av(Cd)</b>

Crops vegetate on the Z1, Z2, Z3 and Z5 soils, while seeds planted on Z4 soils didn't sprout. Values for total content of cadmium in separate plants parts (stem, leaf, root, seed and pod) are given in Table 3. In plant roots, the Cd total content was highest in *OW* variety root (5.41 mg/kg) from Z5 soil and lowest in *Avigea* (0.15 mg/kg). In general, total Cd content in all variety samples were greater in shoots (green parts above root) than in roots. Total Cd content in stem ranged between 0.13 to 7.7 mg/kg (Table 3). Maximum value for Cd was obtained for *Pella's* variety leaf (13.5 mg/kg). Very similar data were obtained for *OW's* leaf too (13.4 mg/kg). These varieties were also characterized with higher Cd content in seed and pod compared to *Avigea* variety. Maximum value for Cd content in seed (2.6 mg/kg) was obtained for *OW's* variety from soil Z5. For the same variety 3.52 mg/kg of Cd in pod was obtained.

**Table 3.** Total content of Cd in soybean varieties with short vegetation in different parts of plants (values given in mg/kg)

	<i>Avigea</i>					<i>OW</i>					<i>Pella</i>				
	Root	Stem	Leaf	Seed	Pod	Root	Stem	Leaf	Seed	Pod	Root	Stem	Leaf	Seed	Pod
<b>Z1</b>	0.15	0.37	1.20	0.27	0.36	0.75	0.96	6.02	0.77	1.70	0.57	1.09	4.60	0.49	0.88
<b>Z2</b>	0.33	0.55	1.43	0.39	0.91	3.34	6.29	13.4	0.95	3.35	0.63	0.65	1.72	0.45	0.70
<b>Z3</b>	0.26	0.13	0.84	0.08	0.10	1.27	1.31	2.42	0.61	0.79	4.52	4.72	13.5	1.29	3.52
<b>Z5</b>	0.42	1.45	3.10	0.36	0.93	5.72	7.73	7.60	2.57	5.41	4.92	2.55	7.87	0.94	1.85



Translocation factor (TF), Biological Accumulation Coefficient (BAC) and Biological Concentration Factor (BCF) values were used to evaluate the potential of plant species for phytoextraction and phytostabilization of the soybean varieties with short vegetation [22, 23]. Biological accumulation coefficient was calculated as a ratio of heavy metal in shoots to that in soil [22]. The BAC for *Avigea* variety ranged from 0.14 to 9.65 for different plant parts (Table 4). The *OW* and *Pella* varieties showed better accumulation ability for Cd, considering these varieties more suitable for phytostabilization. For the *OW*'s stem and leaf BAC was 24.1 and 23.7, respectively, while for *Pella*'s leaf ranged from 0.76-24.5 (Table 4). BCF was also calculated as a metal concentration ratio of plant roots to soil [23]. Phytostabilisation is a process which depends on roots ability to limit the contaminant mobility and bio-availability in the soils which occurs through the sorption, precipitation, complexation or metal valance reduction [21]. Heavy metals tolerant species with high BCF and low TF can be used for phytostabilisation of contaminated soils. The *Avigea* variety showed lower BCF (<1) vs. *OW* and *Pella* varieties.

**Table 4.** Biological Accumulation Coefficient (BAC) and Biological Concentration Factor (BCF) in soybean varieties

	<i>Avigea</i>					<i>OW</i>					<i>Pella</i>				
	Root	Stem	Leaf	Seed	Pod	Root	Stem	Leaf	Seed	Pod	Root	Stem	Leaf	Seed	Pod
<b>Z1</b>	0.14	0.34	1.10	0.25	0.33	0.68	0.88	5.51	0.70	1.56	0.52	1.00	4.22	0.45	0.80
<b>Z2</b>	0.15	0.24	0.63	0.17	0.40	1.48	2.78	5.94	0.42	1.48	0.28	0.29	0.76	0.20	0.31
<b>Z3</b>	0.31	0.15	1.00	0.10	0.12	1.52	1.56	2.89	0.73	0.94	5.39	5.63	16.1	1.53	4.20
<b>Z5</b>	1.31	4.51	9.65	1.11	2.88	17.8	24.1	23.7	8.01	16.9	15.3	7.95	24.5	2.94	5.76

Translocation factor (TF) was described as a ratio of heavy metals in plant shoot to that in root [22, 23]. These soybean varieties had high biomass and based on the high TF values could have enormous potential to be used for phytoextraction of Cd than other species which also showed TF>1 for different metals. Higher cadmium accumulation may be attributed to well develop detoxification mechanism based on sequestration of cadmium ions in vacuoles, by binding them on appropriate ligands and metal exclusion strategies of soybean varieties [21]. For the analysed soybean varieties translocation factor values above 1 was obtained.



**Table 5.** Translocation Factor (TF) in soybean variety (*Avigea*, *OW* and *Pella*)

	Stem	Leaf	Seed	Pod
<b>Min</b>	0.49	1.33	0.19	0.38
<b>Max</b>	3.44	8.05	1.81	2.72
<b>Median</b>	1.28	3.22	0.48	1.00

#### 4. Concluding remarks

The present investigation revealed that soybean varieties with short vegetation, *OW* and *Pella* could be efficient plants for phytoextraction of Cd from contaminated soil. Highest efficiency of Cd phytoextraction occurred when soil was contaminated. The soybean variety *Avigea* showed lower ability for phytostabilization and phytoextraction. Both, BAC and TF values higher than 1 indicated that *OW* and *Pella* are potentially useful for remedying Cd-contaminated soil and can be introduced as a good potential Cd-hyper accumulator plants.

#### 5. References

- [1] Greipsson, S. (2011). Phytoremediation. *Nature Education Knowledge*, 3(10), 7.
- [2] Ali, H., Khan, E., Sajad, M.A. (2013). Phytoremediation of heavy metals concepts and applications. *Chemosphere*, 91(7): 869-881.
- [3] Sarma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, 4(2), 118-138.
- [4] Vithanage, M., Dabrowska, B. B., Mukherjee, A. B., Sandhi, A., Bhattacharya, P. (2012). Arsenic uptake by plants and possible phytoremediation applications: a brief overview. *Environmental Chemistry Letters*, 10(3), 217-224.
- [5] Mench, M., Schwitzguébel, J. P., Schroeder, P., Bert, V., Gawronski, S., Gupta, S. (2009). Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. *Environmental Science and Pollution Research*, 16(7), 876-900.
- [6] Chaney, R.L.(1983). Plant uptake of inorganic waste constituents. In: Parr, J.F.E.A. (Ed.), Land Treatment of Hazardous Wastes. Noyes Data Corp., Park Ridge, NJ, 50-76.
- [7] Garbisu, C., Alkorta, I. (2003). Basic concepts on heavy metal soil bioremediation. *European Journal of Mineral Processing and Environmental Protection*, 3(1), 58-66.
- [8] Van Aken, B. (2009). Transgenic plants for enhanced phytoremediation of toxic explosives. *Current Opinion in Biotechnology*, 20(2), 231-236.
- [9] Chaudhry, T. M., Hayes, W. J., Khan, A. G., Khoo, C. S. (1998).



- Phytoremediation-focusing on accumulator plants that remediate metal-contaminated soils. *Australasian Journal of Ecotoxicology*, 4(1), 37-51.
- [10] Abhilash, P. C., Powell, J. R., Singh, H. B., Singh, B. K. (2012). Plant-microbe interactions: novel applications for exploitation in multipurpose remediation technologies. *Trends in Biotechnology*, 30(8), 416-420.
- [11] Pilon-Smits, E. (2005). Phytoremediation. *Annual Reviews of Plant Biology*, 56, 15-39.
- [12] Wei, S., Q. Zhou, and P.V. Koval. 2006. Flowering stage characteristics of cadmiumhyperaccumulator *Solanum nigrum* L., and their significance to phytoremediation. *Science of the Total Environment*, 369, 441-446.
- [13] Ishikawa, S., Noriharu, A. E., Murakami, M., Wagatsuma, T. (2006). Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?–Possibility of using other plant species for Cd-phytoextraction. *Soil Science and Plant Nutrition*, 52(1), 32-42.
- [14] Li, M.S., Luo Y.P., Su, Z.Y. (2007). Heavy metal concentrations in soils and plant accumulation in a restored manganese mine land in Guangxi, South China. *Environmental Pollution*, 147:168-175.
- [15] Glick, B. R. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances*, 21(5), 383-393.
- [16] Abou-Shanab, R. A., Angle, J. S., Delorme, T. A., Chaney, R. L., Van Berkum, P., Moawad, H., et al., Ghozlan, H. A. (2003). Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. *New Phytologist*, 158(1), 219-224.
- [17] Boev, B., & Lepitkova, S. (2004). Tertiary intrusive rocks in the central part of the Vardar zone. In *5th International Symposium on Eastern Mediterranean Geology*, 3, 1095-1097.
- [18] ISO 11464:1994, Soil quality- Pretreatment of samples for physico-chemical analysis.
- [19] ISO 11464:2006, Soil quality - Pretreatment of samples for physico-chemical analysis.
- [20] ISO 14869-1:2001 Soil quality - Dissolution for the determination of total element content - Part 1: Dissolution with hydrofluoric and perchloric acids.
- [21] Ghosh, M., Singh, S.P. (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research*, 3, 1-18.
- [22] Cui, S., Zhou, Q., Chao, L. (2007). Potential hyper-accumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. *Environmental Geology*, 51, 1043-1048.
- [23] Yoon, J., Cao, X., Zhou, Q., Ma, L.Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, 368, 456-464.