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HEAVY METALS CONTENT IN ALFALFA CULTIVATED ON VERTISOLS ALONG THE HIGHWAY E75 FROM BELGRADE TO LESKOVAC (SERBIA)

Zoran DINIĆ[1], Radmila PIVIĆ[1], Jelena MAKSIMOVIĆ[1], Aleksandar STANOJKOVIĆ[2], Dragana JOŠIĆ[1], Aleksandra STANOJKOVIĆ-SEBIĆ[1]

[1]Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia
[2]Institute for Animal Husbandry, Belgrade - Zemun, Autoput 16, 11080 Zemun, Serbia

Corresponding author email: drradmila@pivic.com

Abstract

In order to assess the health and safety of animal feed, in ten samples of soil and plant material, collected along the E75 highway from Belgrade to Leskovac, it was examined the content of heavy metals: Cr, Cu, Ni, Pb, Co, As, and their accumulation in alfalfa (Medicago sativa L.) grown on vertisols. Samples of soil and aerial part of the plant material were collected from the both sides of lanes at locations where the studied plant species was cultivated, at 10, 30, 50 and 400 m perpendicular to the direction of the highway. Soil and plant analyses of the metals content were done according to ICP methodology. Analysis of the soil samples showed the following: the content of total forms of Cr and Ni was above the maximum permissible concentration (MPC) in 50% of samples tested; the content of Pb was above the MPC in 30% of samples tested; in the other soil samples the values of the examined parameters were within permissible limits. In five of the ten tested plant samples Cr content was above the toxic concentrations, and in one sample it was above the maximum tolerance levels for animal feed. In one sample of alfalfa it was determined the contents of As and Pb above the toxic levels. In addition, the concentrations of Co and Pb above the normal levels were registered in one sample of plant material, but they were below the maximum tolerance levels for animal feed. The obtained results suggest a caution in the use of alfalfa, grown near the highway route, for animal feed, because of the potential entry of heavy metals into the food chain. The study also revealed that increased concentrations of analyzed elements occurred at all distances from the route lanes.

***Key words**: heavy metals, vertisols, animal feed, highway.*

INTRODUCTION

In the part of the E75 motorway, section of Belgrade to Leskovac, in the Republic of Serbia, during the 2010, was studied the impact of the highway on the heavy metal accumulation in the alfalfa plants, grown on soil type vertisols. Samples of soil and plant material were taken at a distance of 10, 30, 50 and 400 m from both sides of the lanes (Pivić et al., 2013). According to Jankievicz et al. (2010), the rapid development of industry, the increase in the number of inhabitants and the intensification of road traffic are among the most important causes of ecosystem pollution in urban areas. Heavy metals are found everywhere in the environment, either as a result of natural or anthropogenic activities, which makes the eco system exposed to the pollution in different ways (Wilson and Pyatt, 2007). Environmental risk assessment of soil contamination is particularly important for agricultural areas, due to the fact that heavy metals potentially harmful to human health exist in the soil and can be transferred in significant quantities to the food chain (Szinkovska et al., 2009). Heavy metals are found in fuels, fuel tanks, motors and other components of vehicles, in catalytic converters, tires and brakes, as well as surface materials on roads (Deska et al., 2011) and as such represent potential pollutants. Adoption of microelements depends on the type and age of plants, and their accumulation varies depending on the plant species and the organ in which it accumulates. Zn, Mn, Ni and B are unequally distributed at the root and in the above-ground part of the plant. Cu, Cd, Co, Mo are more represented at the root than in the tree, while the content of Pb, Sn, Ag, Cr is higher at the root and lower in the tree (Kastori et al., 1997). Microelements are required for plants growth in very small quantities, and if supply is inadequate, there may be disorders in physiological-biochemical processes, reduction in growth and yield, and in the food chain may cause disorders of human and animal feeding (Szinkovska et al., 2009; Pivic et al., 2017).

MATERIALS AND METHODS

Field of study and sampling

Sampling of soil and above-ground part of alfalfa plant material was carried out on the section of E75 from Belgrade to Leskovac during the vegetation period during August and September 2010 (Figure 1). Based on the coordinates of the sampling site registered with the GPS device, using the data from the pedological map of the Republic of Serbia (Institute of Land, Mrvić et al., 2013), the locations of the soil type vertisol (WRB, 2014) were determined, from which a plant species, alfalfa was sampled and studied.

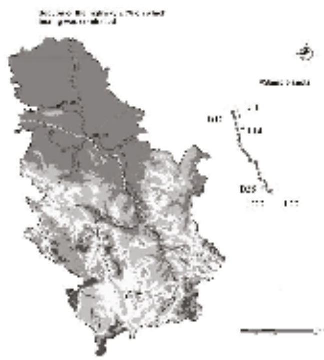

Figure 1. Soil and plant sampling spots in the section of the study with corresponding distances and coordinates

Sample spots (m from route lanes)	Coordinates	
	X	Y
L11(400)	7501072	4931838
D12(30)	7503516	4923959
D12(50)	7503495	4923944
L14(30)	7507509	4909703
L14(50)	7507529	4909705
D26(50)	7544419	4836338
D27(30)	7548003	4830378
D27(50)	7547990	4830558
L27(30)	7548175	4830567
L27(30)	7548193	4830583

Ten soil samples in the disturbed state were sampled to a depth of 30 cm at a different distance from the highway, at 10, 30, 50 and 400 m from the road lanes. The alfalfa plant material, the above-ground part was sampled, with the remark that the year in which it was planted was not recorded. The average sample consisted of 15 to 20 individual samples, where by the cut was carried out by hand cutting at a height of 3-5 cm of the plant.

Preparation and analysis of tested soil samples

In ten composite soil samples prepared in accordance with SRPS ISO 11464: 2004 - Pretreatment of samples for physical-chemical analyzes, sieved through a sieve of 2 mm in diameter, the pH is determined in 1M KCl, potentiometrically (SRPS ISO 10390: 2007 - Determination of pH), calcium carbonate by volumetric method SRPS ISO 10693: 2005- Determination of carbonate content, total contents C, N, S was analyzed on elemental CNS analyzer Vario EL III (Nelson et al, 1996). SOM (soil organic matter) was calculated using the formula:

SOM content (%) = organic C content (%) x factor 1.724 (Džamić et al., 1996)

Available P_2O_5 (determined using spectrophotometry) and K_2O (determined using flame emission photometry) were analyzed by AL-method according to Egner-Riehm (Riehm, 1958). Ca and Mg were extracted by ammonium acetate and determined with an atomic adsorption analyzer SensAA Dual (GBC Scientific Equipment Pty Ltd, Victoria, Australia) (Wright and Stuczynski, 1996). The total contents of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and As in soil samples were determined by inductively coupled plasma-atomic emission spectrometry - THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES, after the digestion of the samples with aqua region (ISO 11466: 1995 Soil quality - Extraction of trace elements soluble in aqua region; ISO 22036: 2008 Soil quality - Determination of trace elements in extracts of soil by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The concentration of trace elements Hg was determined by a flame atomic adsorption spectrophotometer (AAS, GBC, SENSA DUAL HG), method by hydration after the so-called "wet" combustion of samples, i.e. boiled in the mixture of concentrated acids:

HNO_3 and H_2O_2, with filtration and the necessary dilution (AA Hydride system HG 3000, EHG 3000 & MC 3000 Operation & Service manual, 1995).
Reference soils NCS ZC 73005, Soil Certificate of Certified Reference Materials approved by China National Analysis Center Beijing China, and reagent blanks were used as the quality assurance and quality control (QA/QC) samples during the analysis.

Collection, preparation and analyses of the plant material

Medicago sativa L. (Alfalfa) is a perennial leguminous crops, which is regarded as the leading and most important forage crop for the production of high quality feed, and is used in the fresh state and conserved as well as hay, haylage, silage, meal, pellets and pasta (Vučković, 2004; Jakšić et al., 2013). The samples of plant material are air-dried and milled. The sampled plant material were dried at 105^{o}C for a period of 2 hours, using gravimetric method for determination of dry matter contents of plant tissues (Miller, 1998). The contents of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and As in aerial parts were determined in triplicates with THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES after the digestion of the samples with concentrated HNO_3 and redox reaction with H_2O_2 for total forms extraction (Soltanpour et al., 1996). Calibration standards were in the range of 0-10 ppm, except for iron (0-25 ppm).

Processing results

The results of the conducted soil analyze represent the arithmetic means of three replicates of each sampling, their ranges and standard deviations values. The data on microelements and heavy metal concentrations in the studied plant species are presented by figures as bar charts with standard deviation values.

RESULTS AND DISCUSSIONS

Soil type Vertisol WRB (2014) on which was grown tested plant material alfalfa were formed on the substrates containing more than 30% of clay predominantly montmorilonite type.
Vertisols are soils with unfavorable water-air and thermal regime, and some of their properties are the characteristics of hydrogen soils. It swells in a wet state, and in dry cracks with the formation of cracks that can reach one meter in depth. The chemical properties of these soils are considerably more favorable. They are characterized by neutral to low alkaline reaction with high adsorption capacity. Base saturation is up to 90%. Humus, total nitrogen and easily accessible potassium are generally well provided, and in terms of availability of easily accessible phosphorus vertisols are quite poor. These type of soils in terms of benefits for plant production belongs to the third rating class.
Interpretation of the obtained results was carried out on the basis of the Ordinance on the permitted quantities of hazardous and harmful substances in soil and irrigation water and methods of their examination (Official Gazette of RS, 1994), within which the maximum allowed quantities of hazardous and harmful substances have been defined.
The chemical reaction of the tested soil samples ranges from slightly acidic to neutral. The carbonate content is not registered. In relation to the content of easily accessible phosphorus, the examined soils are medium to very high; while the supply of easily accessible potassium is high. The humus content ranges from medium to high supply. In relation to the content of total forms of heavy metal in five soil samples on the positions D16 at 30 and 50 m away from the route lanes, D17 and L17 at 400 m distance from the route lanes and D19 at 30 m away from the highway is determined the content of Cr, and in three samples the Pb content (position D17 and L17 at 400 and D19 at 30 m distance from the route lanes) higher than the maximum allowed concentration (MAC) in the soil sample (Official Gazette of RS, 1994).
The chemical properties of the soil samples tested are shown in Table 1.

Table 1. Properties and composition of Vertisols (means ± standard deviation and intervals)

Property	Value	Property	Value
pH in 1M KCl	5.87±0.44 (5.10-6.60)	Total content of As (mg kg^{-1})	10.71±5.62 (5.07-20.81)
Total content of $CaCO_3$ (%)	below the detection limit	Total content of Cr (mg kg^{-1})	104.13±52.21 (43.09-186.71)
Available P_2O_5 (mg 100 g^{-1})	17.93±12.13 (5.32-40.27)	Total content of Ni (mg kg^{-1})	88.31±55.83 (35.96-192.40)
Available K_2O (mg 100 g^{-1})	32.99±4.32 (27.60-37.30)	Total content of Pb (mg kg^{-1})	64.24±40.57 (26.66-121.20)
Total content of N (%)	0.25±0.08 (0.10-0.35)	Total content of Hg (mg kg^{-1})	0.12±0.09 (0.05-0.31)
Total content of C (%)	2.23±0.81 (0.91-3.57)	Total content of Zn (mg kg^{-1})	67.32±30.69 (40.86-130.11)
Total content of S (%)	0.04±0.02 (0.02-0.08)	Total content of Cd (mg kg^{-1})	1.20±0.55 (0.41-1.95)
SOM (%)	3.84±1.39 (1.57-6.16)	Total content of Cu (mg kg^{-1})	27.29±7.59 (18.29-40.19)

The content of Fe, Mn, Cu, Zn, Ni, Cr, Cd, As, Co, Pb, Hg is determined in the samples of plant material (above-ground biomass sampled for study purposes).

Figures 2-4 show the mean values and standard deviation of the concentration of microelements and heavy metals in the analyzed samples of plant material.

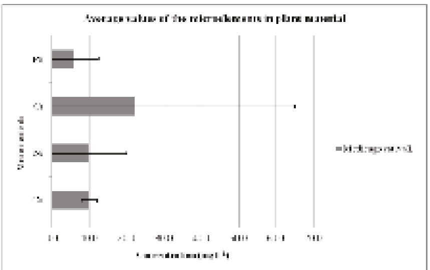

Figure 2. Concentrations of copper (Cu), nickel (Ni), chromium (Cr) and lead (Pb) in the plants aerial parts (mg kg^{-1})

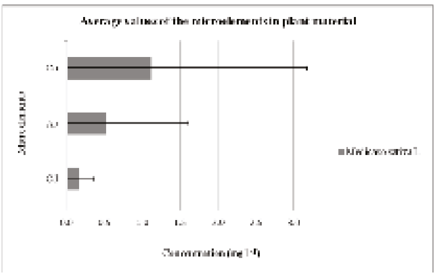

Figure 3. Concentrations of cadmium (Cd), arsenic (As) and cobalt (Co) in the plants aerial parts (mg kg^{-1})

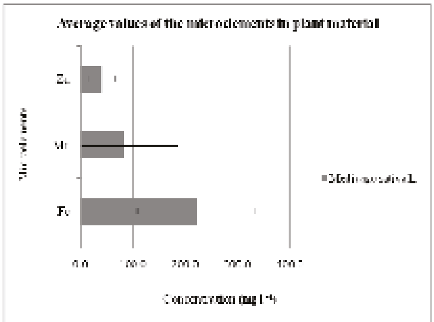

Figure 4. Concentrations of iron (Fe), manganese (Mn) and zinc (Zn) in the plants aerial parts (mg kg^{-1})

The table 2 shows the reference values of the content of microelements in plants relative to normal and toxic concentrations.

Table 2. Reference values for trace elements content in plants according to literature sources

Element	Normal concentrations	Toxic concentrations	Maximum tolerant level for fooder
	(mg kg^{-1})		
Cu	3-15[a]	20[b]	12-50[g]
Ni	0.1-5[a]	30[b]	50[g]
Pb	1-5[a]	20 [b]	40[g]
Cr	<0.1-1[a]	2[b]	
Cd	<0.1-1[a]	10[b]	1[g]
Mn	15-100[e]	400[b]	
Zn	15-150[a]	200[b]	2000[g]
Co	0.05-0.5[e]	30-40[d]	
Fe	50-250[f]	(>500)[f]	1250[g]
As	10-60[c*]	<2[c]	4[g]

μg kg^{-1}; reference values: [a] *Kloke et al. (1984)*; [b] *Kastori et al. (1997)*; [c]*Kabata-Pendias and Mukherjee (2007)*; [d] *Kabata-Pendias (2011)*; [e]*Misra and Mani (1991)*, [f]*Schulze et al. (2005)*; [g]*NRC* (2005), *Adams (1975)*

Iron (Fe) can be accumulated in plants without any harmful effects (Marić et al., 2013; Simić et al., 2015; Pivić et al., 2017), so it is not uncommon that the contents of this element could be higher than the MPC. The concentrations of Fe adopted by plants in addition to the species and stages of growth, depends on the soil properties. In all tested samples of alfalfa grown on vertisol, the toxic contents of this element were not registered.

The most important role of Mn is reflected in participation in oxidation-reduction processes. Due to rapid transmission through the plant, it is most accumulated in young plant organs and less in the root. In organs of plants, Mn occurs in excess when there are high levels of this element in the soil, low pH and high redox potential (Misra and Mani, 1991; Kastori et al., 1997). No toxicity of this element (>400 mg kg^{-1}) was detected in nine tested alfalfa samples except in sample D33 at a distance of 50 m from the highway route in which the concentration of Mn in the plant material was 277.88 mg kg^{-1} and above normal concentrations.

Copper belongs to the category of micronutrients that have an important role in photosynthesis, respiration, carbohydrate metabolism, nitrogen reduction and fixation, protein metabolism etc. The rate at which the plant adopts the copper largely depends on the type of plant, but also on the origin of the copper present. Young plant organs are particularly exposed to copper deficiency. The surplus of this element usually occurs in acidic soils, and as copper is one of the most vulnerable elements in the acidic environment, its accessibility for plants is growing. Sensitive plant species on copper toxicity are cereals, legumes and spinach. For normal development, copper is required by plants in small amounts of 5-20 mg kg^{-1}, less than 4 mg kg^{-1} is considered a deficit, and more than 20 mg kg^{-1} may cause toxicity (Kloke et al., 1984; Kastori et al., 1997). In the tested alfalfa samples, the toxic value of copper was not registered.

Zinc is an essential nutrient for plant growth and is involved in significant metabolic processes. Soluble forms of zinc are easily accessible for the plant, and the adoption of this element is in linear relationship with the content of this element in the nutrient solution or the soil. The content of zinc in the tested samples of plant material is not registered above toxic values (> 200 mg kg^{-1}).

Adoption of nickel depends on soil properties and the properties of the plant itself. The most important factor is the pH value of the soil. In order to adopt this element, its origin is very important, as studies indicates that anthropogenic deposited nickel is much easier to adopt by plants (Kloke et al., 1984; Kastori et al., 1997). The content of nickel in unpolluted soils varies and depends on ecological and biological factors. Since the nickel is easily mobile in plants, usually all parts of plants show a high concentration of this element. In the tested samples of plant material, nickel content was in five samples above the normal value, but below the toxic level of the above-ground plant mass, for the tested samples from the location D16 (30 and 50 m), L17 (400 m), L23 (30 m) and D33 (50 m) distance from the route lanes. This corresponds to the zones where in the soil has also been determined that the total nickel is above the MPC.

The content of chromium in plants varies and depends largely on the geological substrate. The source of chromium is also a significant factor that affects the solubility and availability of these elements (Adams, 1975; Kloke et al., 1984; Kastori et al., 1997; NRC, 2005). The concentration of chromium is almost always higher in the root than in leaves or trees, while the lowest quantities are recorded in the fruits. The chromium content in the test samples, except for the sample at the location of D33 (at 50 m away from the route lanes) does not exceed the toxicity values of this element for animal feed (50-3000 mg kg^{-1}). In sample D33, the content of chromium in the plant material is 103.35 mg kg^{-1}.

Cadmium is one of the most toxic and harmful elements that adversely affects soil biological activity, plant metabolism and human and animal health. It is easily absorbed through the root system and accumulated in the aboveground plant parts. The pH of the soil solution is cited as the main factor in the adoption of cadmium. The origin of cadmium is also an important factor that affects the solubility and availability of this element. The content of cadmium in the tested samples of plant material ranges in the range of normal values (up to 10 mg kg^{-1}).

Different plant species show a different degree of tolerance in relation to arsenic. Leguminous species are susceptible to arsenic. The most common result of a high content of this element in the soil is a reduced yield of (Kabat Pendias and Mukherjee, 2007; NRC, 2005). In the tested alfalfa samples, the toxic value of the arsenic content was recorded in one sample at the D33 site (50 m from the route lanes).

Measuring the content of cobalt in plant mass has become very important when it was observed that the lack of this element in the soil and consequently in the plant mass causes a disease for sheep, goats and other livestock. In the soil, this element is usually found as a side element of iron, nickel and other heavy metals. In the tested samples of the plant material, a value higher than the normal values was registered, in a sample at D33 (50 m), which was 5.00 mg kg^{-1}, while in other examined alfalfa samples, the value was within normal limits.

Lead is the least mobile element among the microelements of the soil (Kabata-Pendias A., 2011). Lead is poorly adopted and transferred to the above-ground organisms of the plant, except on acidic soils. Plants can accumulate lead either from the soil or absorbed from the air. Most of the lead from the soil is not available to plants.

Non-oganic lead forms become accessible to plants only in acidic soils (Wiklander and Vahtras, 1977). Lead originating from the air is the main source of pollution by this element. According to some studies, about 95% of the total amount of lead in the plant can be originated from the air. In a sample at the D16 site (30 m), the lead concentration was above the toxic values (>20 mg kg^{-1}) and in samples D16 (50 m) and D33 (50 m) above normal values (>5 mg kg^{-1}). Increased content of this element which was in the range of critical concentrations for animal feeding (10-30 mg kg^{-1}) was registered in the sample at the location of D16 (30 m).

Mercury content in all tested samples of alfalfa is below the limit of detection therefore is not presented in the graphic.

CONCLUSIONS

The total content of As, Cd, Zn, Co, and Hg in all tested samples of soil type vertisol were within the limits of maximum permissible concentration (MPC). In the samples from sites D17 (400 m), L17 (400 m) and D19 (30 m) and on the site D16 at a distance of 30 and 50 meters from the route lanes, the total contents of Cr and Ni was above the MPC. The content of lead above MPC was determined in samples D17 and L17 (400 m) and D19 (30 m). In addition, anthropogenic pollution, which is reflected in excessive use of the preparation of pesticides and fertilizers, as well as the air pollution originating from motor vehicles, in some sections of the tests, it is evident geochemical pollution of soil.

In the tested biomass of alfalfa, content of increased concentrations of certain elements with respect to the normal value, is registered in a certain location and at a distance usually 30-50 m from the motorway route. In the sample at the site D33 (50 m) the chromium content exceeds the toxicity values of this element for animal feed (50-3000 mg kg^{-1}). The content of arsenic above toxic concentrations was registered in only one sample, also at D33 (50 m from the route lanes). In a sample at the D16 site (30 m), the lead concentration is above the toxic values (>20 mg kg^{-1}) and in samples D16 (50 m) and D33 (50 m) above the normal values (>5 mg kg^{-1}). It is to be expected that this element is most present in the immediate vicinity of roads as a product of exhaust gases of motor vehicles. The increased content of critical concentrations of lead for animal feed (10-30 mg kg^{-1}) was recorded in the sample at D16 (30 m).

The obtained results indicate caution for cultivation of alfalfa in near vicinity of route lanes for the animal feeding due to the possible entry of heavy metals into the food chain.

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REFERENCES

Adams R.S., 1975. Variability in mineral and trace element content of dairy cattle feeds. Journal of Dairy Science, 58 (10), 1538-1548.

Deska J., Bombik A., Marcinivik-Kuska A., Rzymuza K., 2011.Trends in lead and cadmium content in soils adjacent to European highway E30. Polish Journal of Environmental Studies, 20 (2), 317-325.

Džamić R., Stevanović D., Jakovljević M., 1996. Agrochemistry Manual. Faculty of Agriculture, University of Belgrade, Serbia.

Jakšić S., Vučković S., Vasiljević S., Grahovac N., Popović V., Šunjka D., Dozet G., 2013. Accumulation of heavy metals in *Medicago sativa* L. and *Trifolium pratense* L. at the contaminated fluvisol. Chemical Industry, 67 (1), 95-101.

Jankiewicz B., Adamczyk D., 2010. Assessing heavy metal content in soils surrounding a power plant. Polish Journal of Environmental Studies, 19 (4), 849-853.

Kabata-Pendias A., Mukherjee A.B., 2007. Trace Elements from Soil to Human. Springer-Verlag, Berlin, Heidelberg.

Kabata-Pendias A., 2011. Trace Elements in Soils and Plants, 4th edition. CRC Press, Boca Raton, Florida, USA.

Kastori R., Petrović N., Arsenijević-Maksimović I., 1997. Heavy Metals and Plants. In: Heavy Metals in the Environment. Ed. Kastori R. Institute of Field and Vegetable Crops, Novi Sad, Serbia, 196-257.

Kloke A., Sauerbeck D.R., Vetter H., 1984. The Contamination of Plants and Soils with Heavy Metals and the Transport of Metals in Terrestrial Food Chains. In: Changing Metal Cycles and Human Health. Ed Nriagu J.O. Dahlem Konferenzen, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 113-141.

Marić M., Antonijević M., Alagić S., 2013. The investigation of the possibility for using some wild and cultivated plants as hyperaccumulators of heavy metals from contaminated soil. Environmental Science and Pollution Research, 20 (2), 1181-1188.

Miller R.O., 1998. Determination of Dry Matter Content of Plant Tissue: Gravimetric Moisture. In: Handbook of Reference Methods for Plant Analysis. Ed Kalra Y. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA, 51-52.

Misra S.G., Mani D., 1991. Soil Pollution. Ashish Publishing House, Punjabi Bagh, New Delhi, India.

Mrvić V., Antonović G., Čakmak D., Perović V., Maksimović S., Saljnikov E., Nikoloski M., 2013. Pedological and pedogeochemical map of Serbia. Proceedings of the 1st International Congress on Soil Science and XIII National Congress in Soil Science „Soil-Water-Plant“, Plenary lectures, September 23-26, Belgrade, 93-104.

Nelson D.W., Sommers L.E., 1996. Total Carbon, Organic Carbon, and Organic Matter. In: Methods of Soil Analysis. Part 3. Ed Sparks D.L. SSSA, Madison, Wisconsin, USA, 961-1010.

Official Gazette of RS, 1994. Rule book of permissible concentrations of dangerous and hazardous materials in soil and in water for irrigation and methods for analysis, No. 23.

Pivić R., Stanojković-Sebić A., Jošić D., 2013. Assessment of soil and plants contamination with selected heavy metals along route in the section Belgrade-Preševo. Polish Journal of Environmental Studies, 22 (5), 1465-1472.

Pivić R., Dinić Z., Stanojković A., Maksimović J., Jošić D., Stanojković-Sebić A., 2017. Accumulation of heavy metals and trace elements in *Medicago sativa* L. Grown along the E75 route section Belgrade-Leskovac. Biotechnology in animal husbandry 33 (3), 361-374.

Riehm H., 1958. Die Ammoniumlaktatessigsaure-Methode zur Bestimmung der leichtloslichen Phosphorsure in Karbonathaltigen Boden. Agrochimica, 3, (1), 49-65.

Saeedi M., Hosseinzadeh M., Jamshidi A., Pajooheshfar S.P., 2009. Assessment of heavy metals contamination and leaching characteristics in highway side soils. Environmental Monitoring and Assessment, 151 (1), 231-241.

Simić A., Dželetović Ž., Vučković S., Sokolović R., Delić D., Mandić V., Anđelković B., 2015. Usability value and heavy metals accumulation in forage grasses grown on power station ash deposit. Chemical Industry, 69 (5), 459-467.

Schulze E.D., Beck E., Müller-Hohenstein K., Lawlor D., Lawlor K., Lawlor G., 2005. Plant Ecology. Springer-Verlag, Berlin, Heidelberg.

Soltanpour P.N., Johnson G.W., Workman S.M., Bentonjones J.J., Miller R.O., 1996. Inductively Coupled Plasma Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry. In: Methods of Soil Analysis. Part 3. Ed Sparks D. L. SSSA, Madison, Wiskonsin, USA, 91-139.

Szynkowska M.I., Pawlaczyk A., Leśniewska E., Paryjczak T., 2009.Toxic metal distribution in rural and urban soil samples affected by industry and traffic. Polish Journal of Environmental Studies, 18 (6), 1141-1150.

Vučković S., 2004. Pašnjaci, Poljoprivredni fakultet, Univerziteta u Beogradu, Srbija.

Wilson B., Pyatt F.B., 2007. Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, UK. Ecotoxicology and Environmental Safety, 66 (2), 224-231.

Wright R.J., Stuczynski T., 1996. Atomic Absorption and Flame Emission Spectrometry. In: Methods of Soil Analysis. Part 3. Ed Sparks D.L. SSSA, Madison, Wisconsin, USA, 65-90.

Wiklander L., Vahtras K., 1977. Solubility and uptake of heavy metals from a Swedish soil. Geoderma, Elsevier, Vol. 19 (2), 123-129.

***ISO 11466: 1995. Soil quality - Extraction of trace elements soluble in aqua regia. International Organization for Standardization, Geneva, Switzerland.

***ISO 22036: 2008. Soil quality - Determination of trace elements in extracts of soil by inductively coupled plasma-atomic emission spectrometry (ICP-

AES). International Organization for Standardization, Geneva, Switzerland.
***NRC - National Research Council, 2005. Mineral Tolerance of Animals, 2nd revised edition. National Academies Press,Washington DC.
***SRPS ISO 11464: 2004. Soil quality - Pretreatment of samples for physico-chemical analyses. Institute for Standardization of Serbia, Belgrade.
***SRPS ISO 10390: 2007. Soil quality - Determination of pH. Institute for Standardization of Serbia, Belgrade.
***SRPS ISO 10693: 2005. Soil quality - Determination of carbonate content. Institute for Standardization of Serbia, Belgrade.
***WRB, 2014. World Reference Base for Soil Resources - International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. Food and Agriculture Organization of the United Nations, Rome, http://www.fao.org/3/a-i3794e.pdf.