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ACCUMULATION OF HEAVY METALS AND TRACE ELEMENTS IN *MEDICAGO SATIVA* L. GROWN ALONG THE E75 ROUTE SECTION BELGRADE-LESKOVAC

Radmila Pivić[1], Zoran Dinić[1], Aleksandar Stanojković[2], Jelena Maksimović[1], Dragana Jošić[1], Aleksandra Stanojković-Sebić[1]

[1]Institute of Soil Science, 11000 Belgrade, Serbia
[2]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
Corresponding author: Aleksandra Stanojković-Sebić, astanojkovic@yahoo.com
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Abstract. The contents of heavy metals and trace elements and their accumulation in *Medicago sativa* L., cultivated on Eutric cambisol along the E75 route section Belgrade-Leskovac, were examined in order to assess the health and safety of animal feed. The samples of soil and aerial parts of the plant material were collected from both sides of lanes 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. The results showed that at the locality L 14, a distance of 30 and 50 meters away from the lanes, the content of total forms of Cr, Ni and Pb in soil was above the maximum permissible concentration. In the plant biomass it was determined the following: in a sample from the location L 14 at a distance of 50 meters from the lanes concentrations of Ni and Co were higher than normal values, and concentrations of Fe and Pb were above toxic levels or maximum tolerance levels for animal feed; determined Fe content in the sample of alfalfa at location L 11, 400 m away from the lanes, and Ni in the sample from the site D 12 at a distance of 50 m from the lanes, was above the normal values, while in the sample from D12 location, at a distance of 30 m from the lanes, the content of Pb was above the toxic levels or maximum tolerance levels for animal feed. The 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.

Key words: Eutric cambisol, highway, pollution, animal feed

Introduction

Risk assessment of threats to the environment caused by pollution of soil is particularly important in rural areas due to the fact that the metals potentially harmful to animal and human health exist in the soil and can be transferred into the food chain in significant amounts *(Szinkovska et al., 2009)*. The rapid development of the industry, increased number of inhabitants and an intensification of road transport are one of the most significant causes of pollution of ecosystems in urban areas *(Jankievicz and Adamczyk, 2010)*. Heavy metals are found everywhere in the environment, whether as a result of natural or anthropogenic activities, to which a wildlife is exposed in different ways *(Wilson and Piatt, 2007)*. Urban roadside soils are the 'recipients' of large amounts of heavy metals from a variety of sources including vehicle emissions, coal burning waste and other activities *(Saeedi et al., 2009; Acosta et al., 2010)*. Heavy metals are found in fuels in the walls of fuel tanks, engines and other vehicle components, in catalytic converters, tires and brakes, as well as in the surface material on the roads *(Deska et al., 2011)* and as such represent the potential pollutants. The mobility and availability of heavy metals in the soil are generally low, especially when the soil is high in pH, clay and organic matter (*Petrotou et al., 2010)*. Heavy metal accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metals, evaluated by either plant uptake or soil to plant transfer factors of the metals *(Rattan et al., 2005)*.

Some of the elements are necessary for growth and development of crops. Some of them have stimulating effect on plant growth, while a group of elements at high concentrations affects very toxically on the plants. An assessment of the environmental risk caused by soil contamination is especially important for agricultural as well as non-cultivated areas due the fact that metals potentially harmful to animal and human health persist in soils for a relatively long time and may transfer into the food chain in considerable amounts *(Szynkowska et al., 2009)*.

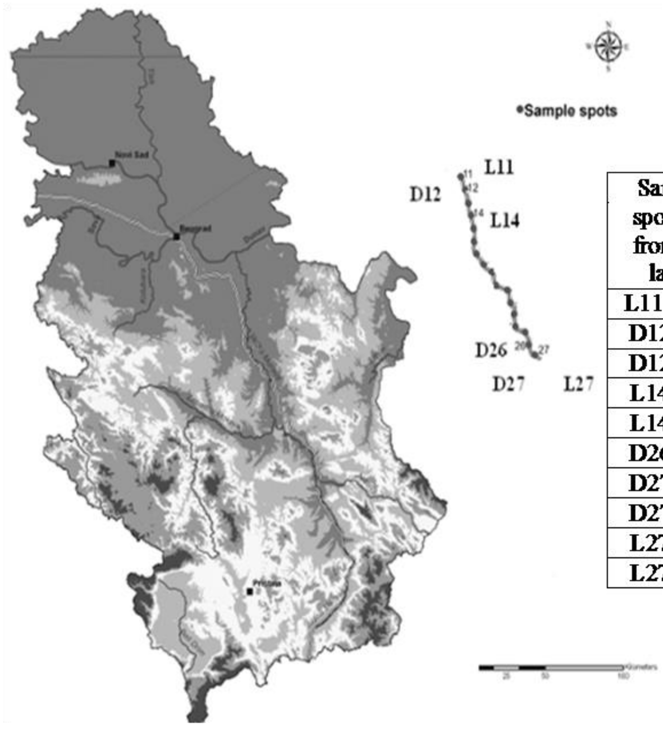
The highway presents the highest class of traffic routes. It is designed for fast motor traffic and consists of two physically separated lanes. In 2010, on Belgrade-Leskovac section of the highway E75, it was conducted the research of its impact on accumulation of heavy metals and trace elements in alfalfa cultivated on Eutric cambisol. The soil along the highway mostly belongs to the agricultural area. Thus, the study was aimed to determine whether there was a pollution of Eutric cambisol and cultivated crop (alfalfa) in the research area, and the level of pollution. A type of soil was determinated based on pedological map of Institute of Soil Science, Belgrade *(Mrvić et al., 2013)*.

Material and Methods

Study area

The area of study included the highway route E75, section from Belgrade to Leskovac, where the soil and plant samples were taken from each side of the lane at a distance of 10, 30, 50 and 400 m perpendicular to the direction of the highway (Figure 1). The sampling was carried out during August and September in 2010.

According to *WRB (2014)* classification studied soil was Eutric cambisol *(Mrvić et al., 2013)*. These are mainly medium-heavy soils, with a pronounced texture differentiation within the profile. Chemical properties vary depending on the intensity of use, degree of erosion, chemical properties of the parent material and level of development. The soil does not contain carbonates, belonging to the soils of high ecological and productive value.



Sample spots (m from the lane)	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

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

In the framework of the Rule book of permissible concentrations of dangerous and hazardous materials in soil and in water for irrigation and methods for analysis *(Official Gazette of RS, 1994)* the maximum permissible levels of dangerous and hazardous matters were used in the interpretation of the results of the analyzed soil samples.

Soil sampling, preparation and analysis

Ten samples of soil were taken in disturbed state in three repetitions from the depth of 0-30 cm. Composite soil samples were carried to the laboratory, dried and passed through a 2-mm sieve *(SRPS ISO 11464:2004, 2004)*. Soil pH in H_2O and 1M KCl was analyzed potentiometrically with glass electrode *(SRPS ISO 10390:2007, 2007)*. The content of $CaCO_3$ was determined volumetrically, according to the standard *SRPS ISO 10693:2005 (2005)*. The contents of total N and C were determined using elemental CNS analyzer, Vario model EL III *(Nelson and Sommers, 1996)*, whereby on the basis of organic C content, the content of SOM (soil organic matter) was calculated using the formula: SOM content (%) = organic C content (%) * factor 1.724 *(Džamić et al., 1996)*. Available P_2O_5 and K_2O were analyzed by Al-method according to Egner-Riehm *(Riehm, 1958)*, where 0.1M lactate (pH 3.7), was used for extraction. After extraction, K_2O was determined by flame emission photometry and P_2O_5 by spectrophotometry after color development with ammonium molybdate and stannous chloride. Ca and Mg were extracted by ammonium acetate and determined with an atomic absorption analyzer SensAA Dual (GBC Scientific Equipment Pty Ltd, Victoria, Australia) according to *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 regia *(ISO 11466:1995, 1995; ISO 22036:2008, 2008)*. Reference soil 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 legume which is considered to be the leading and most important forage crop for the production of high quality feed, used in fresh and conserved state as hay, haylage, silage, meal, pellets and paste *(Vučković, 2004; Jakšić et al., 2013)*. The aerial parts of the alfalfa plant material, grown at selected locations, have been taken in the period August-September in 2010, in flowering stage during the third cutting. Average sample consisted of 15 to 20 individual plant samples, whereby the swath was carried out manually by cutting the plant at a height of 3-5 cm. Samples of the plant material were air-dried and milled using grinding mill. Then, they 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 nitric acid and redox reaction with hydrogen peroxide for total forms extraction *(Soltanpour et al., 1996)*. Calibration standards were in the range of 0-10 ppm, except for iron (0-25 ppm).

Data analysis

The obtained data on trace elements and heavy metals concentration in the soil studied represent the arithmetic means of three replicates of each sampling, their ranges and standard deviations values. The data on trace alements and heavy metal concentrations in the alfalfa are presented by figures as the bar charts with standard deviation values.

Results and Discussion

Chemical characteristics of the studied soil

Chemical properties of the studied soil are shown in Table 1.The reaction of the analyzed soil samples ranged from acidic to slightly acidic. In relation to the content of available phosphorus, the values ranged from very low to very high, while the supply with available potassium goes from high to very high values. The humus content in the analyzed samples ranged from medium to high level of provision. Regarding the total content of heavy metals, in two tested soil samples from the L14 position at 30 m, and as well from the position L14 at 50 m distance from the highway route, the concentrations of Cr and Ni were higher than the maximum permissible concentration (MPC) in soil *(Official Gazette of RS, 1994)*.

Ni and Cr are mainly found in the basic and ultrabasic rocks, which is the main source of geochemical origin of these metals in the soil. Anthropogenically, they may enter the soil mostly through atmospheric deposition of the coal, oil and diesel combustion, which presumably could be the source of these metals high level in soil studied. For agricultural soils is typical the Ni pollution from the use of organic sludge, and to a lesser extent, it is contained in mineral fertilizers. Liming, as an ameliorative measure, could also be the source of Ni in the soil *(Kabata-Pendias and Mukherjee, 2007)*.

In addition, above the MPC was also the content of Pb in one soil sample from the position L14 at 50 m from the highway route. Unlike Ni and Cr, the soil contamination with Pb occurs in nature only anthropogenically. The most important anthropogenic sources of Pb are mines, smelters and the exhaust gases from vehicles, and in agriculture - industrial sludges and pesticides *(Kabata-Pendias, 2011)*.

Table 1. Chemical properties of the studied Eutric Cambisol (means ± standard deviation and intervals)

Property	Value	Property	Value
pH in 1M KCl	5.2±0.3 (4.7-5.6)	Total content of As (mg kg $^{-1}$)	6.6±2.6 (3.9-11.6)
Total content of $CaCO_3$ (%)	below the detection limit	Total content of Cr (mg kg $^{-1}$)	74.9±40.0 (37.4-141.6)
Available P_2O_5 (mg 100g $^{-1}$)	8.2±10.5 (0.6-35.2)	Total content of Ni (mg kg $^{-1}$)	49.7±25.3 (33.0-97.7)
Available K_2O (mg 100g $^{-1}$)	27.5±3.2 (24.2-33.7)	Total content of Pb (mg kg $^{-1}$)	44.5±34.6 (25.0-122.8)
Total content of N (%)	0.2±0.1 (0.1-0.4)	Total content of Zn (mg kg $^{-1}$)	67.3±30.7 (40.9-130.1)
Total content of C (%)	2.1±0.7 (1.1-3.5)	Total content of Cd (mg kg $^{-1}$)	0.8±0.5 (0.3-1.5)
SOM (%)	3.6±1.8 (2.0-6.0)	Total content of Cu (mg kg $^{-1}$)	23.6±4.9 (17.5-31.6)

Trace elements and heavy metals content in alfalfa biomass

Figure 2 shows the mean and standard deviation values of the concentration of trace elements and heavy metals in the analyzed samples of the plant material. In addition, Table 2 displays the reference values for trace elements and heavy metals content in plants as compared to normal and toxic concentration.

Iron (Fe) can be accumulated in plants without any harmful effects *(Marić et al., 2013; Simić et al., 2015)*, so it is not uncommon that the contents of this element could be higher than the MPC. It is an essential element required in many physiological and biochemical processes. The concentration of Fe, assimilated by plant, besides the type and stage of its development, depends on the soil properties. High pH value and high concentrations of phosphate and calcium ions reduce its assimilation. Normal iron content in plant material, according to *Kabata-Pendias (2011)*, ranges from 18-1000 mg kg^{-1} of dry matter. In plants an excess of this element is rare, and its deficiency occurs when its content in the dry matter of leaves is less than 50 mg kg^{-1}. From the total of ten samples of alfalfa biomass, only in one sample from the location L14 at a distance of 30 m from highway lane it was identified iron content above the MPC for animal feed, which is 1250 mg kg^{-1} of dry matter. The mentioned indicates that, in addition to the possible impact of the highway traffic on the iron content, the reason of undesirable Fe content occurrence in plant material may be an increased content of this element that entered the soil otherwise than atmospheric (use of pesticides, fertilizers, etc.).

Manganese (Mn) is actively assimilated and quickly transported through the plants, in which process a passive absorption occupies a special place. Due to the rapid transfer through the plant, it mostly accumulates in the organs of young plants and less in the root. In plants organs Mn appears in excess when there is a high concentration of this element in the soil, together with low pH values and high redox potential *(Misra and Mani, 1991; Kastori et al., 1997)*. In all tested samples of alfalfa a toxic content of this element (>400 mg kg^{-1}) was not registered, which indicates that the proximity of the road did not cause an increased concentration of this element in the plant material.

Copper (Cu) belongs to the category of micronutrients. The rate at which the plant assimilates the copper is largely dependent on the type of plant and the origin of the copper present. The species sensitive to the toxicity of copper are grains, legumes and spinach. For normal development, copper is required in the plants in small quantities (5-20 mg kg^{-1}), and less than 4 mg kg^{-1} is considered as a deficiency, while more than 20 mg kg^{-1} can cause the occurrence of toxicity *(Kloke et al. 1984*; *Kastori et al., 1997).* In the tested samples of alfalfa a toxic value of copper was not registered.

Zinc (Zn) is an essential nutrient for plant growth and is involved in important metabolic processes. Soluble forms of zinc are easily available to the plants and assimilation of this element is in a linear relation with the content of this element in the nutrient solution or in soil. The composition of the nutrient solution, especially of calcium content, is of great importance to the assimilation of zinc *(Kastori et al., 1997)*. Registered zinc content in the analyzed samples of plant material was not above the toxic levels (>200 mg kg^{-1}).

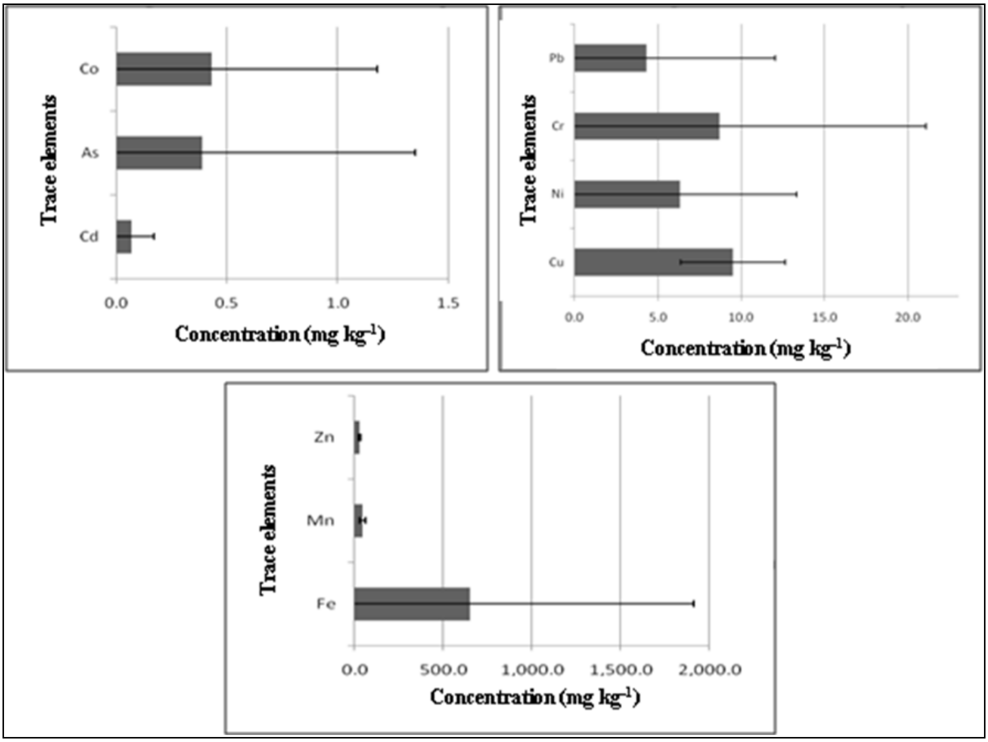


Figure 2. Average values of analyzed trace elements in the aboveground biomass of alfalfa (mg kg^{-1})

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

Element	Normal concentrations	Toxic concentrations	Maximum tolerant level for fodder
	(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), [g]*Adams (1975)*.

Assimilation of nickel (Ni) depends on soil properties and on properties of the plant itself. The most important factor is the pH value of the soil. Its origin is also very important for assimilation of this element, because the studies indicate the occurrence that anthropogenically deposited nickel is more easily assimilated *(Kloke et al., 1984*; *Kastori et al., 1997)*. Since nickel is readily mobile in plants, usually all parts of the plant have high concentrations of this element. In the analyzed samples of plant material the content of nickel was above the normal levels (>5 mg kg^{-1}) in two samples from the positions D12 and L14 at a distance of 50 m from the lanes. Partly, the zones of occurrence of this element within the specified range overlap with the zones where the total content of nickel in the soil was above the MPC (position L14, 50 m from the lane).

Chromium (Cr) content in plants is very different and depends largely on the geological substrate. It is almost always higher in root than in leaves or stems, while the lowest concentrations were registered in the fruits. Chromium content in the tested plant samples does not exceed toxic levels of this element for animal feed (50-3000 mg kg^{-1}).

Cadmium (Cd) is one of the most toxic and dangerous element, which has damaging effects on biological activity of the soil, plant metabolism and the health of humans and animals. It is easily assimilated through the root system and accumulated in the aboveground parts of plants. The pH value of the soil solution is considered to be a major factor in assimilation of cadmium. In addition to this, the contents of clay and carbonate in the soil are also very important. Origin of cadmium is also an important factor that affects the solubility and availability of this elements *(Adams, 1975; Kloke et al., 1984*; *Kastori et al., 1997; NRC, 2005)*. Cadmium content in the analyzed samples of plant material is in the range of normal values (up to 10 mg kg^{-1}), which is a desirable outcome.

Available previous studies suggest that arsenic (As) from the soil into the plant gets through the passive way. Most studies indicate that an increased content of arsenic in the soil leads to the accumulation of arsenic in roots and old leaves. Legumes are sensitive to the effects of arsenic. The most common result of the high content of this element in the soil is a reduction in crops yield *(Kabata-Pendias and Mukherjee, 2007; NRC, 2005)*. The content of arsenic in studied plant material is in critical concentrations only in one sample from the positions L14 at a distance of 50 m from the lanes (3.10 mg kg^{-1}), while in the other tested samples it is present in normal concentrations. The value of arsenic content in mentioned sample is below the maximum tolerable level for animal nutrition.

The studies on cobalt (Co) content in plants have become very important when it was observed that its deficiency in the soil and therefore in the plant biomass causes the diseases in sheep, goats, cattle and other livestock. In the soil this element is commonly found as a companion of iron, nickel and other heavy metals *(Misra and Mani, 1991; Kabata-Pendias, 2011)*. In the tested samples of the plant material only in one sample from the position L14 at a distance of 30 m from the lanes it was determined a value of Co higher than the normal value (2.535

mg kg^{-1}). In the other analyzed samples of alfalfa the value of this element ranged from 0.08 to 0.285 mg kg^{-1}.

Lead (Pb) is a non-essential element for plants, although in lower concentrations has a stimulative effect. Lead could be accumulated through the food chain and become toxic to humans or animals. It is the least moveable element within trace elements in soil *(Kabata-Pendias, 2011)*; its assimilation and transfer to the aerial plant parts are low, except in acid soils. Plants can accumulate lead either from soil or from the air. Most of the lead from the soil is not available to plants. The inorganic forms of lead become available to plants only in acid soils *(Wiklander and Vahtras, 1977)*. Lead originating from the air is the main source of pollution from this element. According to some studies, about 95% of the total content of lead in the plant can be derived from the air *(Kloke et al., 1984*; *Kastori et al., 1997)*. It is evident that, on the location where the plant material with increased concentrations of this element was sampled, it was also determined the total content of lead in the soil above the MPC (position L14, 50 m from the lanes). Increased content of this element, which is in the range of critical concentrations for animal nutrition (10-30 mg kg^{-1}), was also recorded in the sample from the position D12 at a distance of 30 m from the lanes. This could result in contamination of animals through ingesting the polluted forage. Lead has a toxic effect and oncological action to animals, leading to hepatic, cutaneous and pulmonary cancer and changed haematological parameters *(Kochare and Tamir, 2015)*.

Conclusion

In addition to anthropogenic pollution, which is reflected in the excessive use of plant protection preparations and fertilizers, as well as the impact of air pollution from motor vehicles originating from the certain sections of study, the presence of geochemical pollution of soil cover is evident. The results showed that at the locality L 14, a distance of 30 and 50 meters away from the lanes, the content of total forms of Cr, Ni and Pb in soil was above the maximum permissible concentration. In the plant biomass it was determined the following: in a sample from the location L 14 at a distance of 50 meters from the lanes concentrations of Ni and Co were higher than normal values, and concentrations of Fe and Pb were above maximum tolerant level for animal feed; determined Fe content in the sample of alfalfa at location L 11 400 m away from the lanes, and Ni in the sample from the site D 12 at a distance of 50 m from the lanes, was above the normal values, while in the sample from D12 location, at a distance of 30 m from the lanes, the content of Pb was above the toxic levels or maximum tolerance levels for animal feed. The 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.

Akumulacija teških metala i mikroelemenata u lucerki (*Medicago sativa* L.) gajenoj uz deonicu autoputa E75 Beograd-Leskovac

Radmila Pivić, Zoran Dinić, Aleksandar Stanojković, Jelena Maksimović, Dragana Jošić, Aleksandra Stanojković-Sebić

Rezime

U deset uzoraka zemljišnog i biljnog materijala duž trase autoputa E 75 kroz Republiku Srbiju, na deonici od Beograda do Leskovca, ispitan je sadržaj teških metala i mikroelemenata i njihova akumulacija u *Medicago sativa* L. gajenoj na eutričnom kambisolu, radi ocene zdravstvene ispravnosti stočne hrane. Uzorci zemljišta i nadzemnog dela biljnog materijala uzorkovani su sa obe strane kolovoznih traka i to na 10, 30, 50 i 400 m upravno na pravac autoputa. Analiza zemljišnih uzoraka pokazala je da je na lokalitetu L 14, na udaljenosti 30 i 50 metara od kolovoznih traka sadržaj ukupnih formi Cr, Ni i Pb bio iznad maksimalno dozvoljenih koncentracija. U biljnoj masi u uzorku sa lokacije L14 na udaljenosti 50 metara od kolovoznih traka koncentracije Ni i Co su bile više od normalnih vrednosti, a koncentracije Fe i Pb bile su iznad toksičnih vrednosti odnosno maksimalno tolerantnog nivoa za ishranu životinja. Utvrđeni sadržaj Fe u uzorku lucerke na lokaciji L11 udaljenoj 400 m od kolovoznih traka, kao i Ni u uzorku sa lokacije D12 na udaljenosti 50 m od kolovoznih traka, bio je iznad normalnih vrednosti, dok je u uzorku D12 na udaljenosti 30 m od kolovoznih traka sadržaj Pb, bio iznad toksičnih vrednosti, odnosno maksimalno tolerantnih nivoa za ishranu životinja. Dobijeni rezultati upućuju na oprez pri korišćenju lucerke gajene pored trase autoputa za ishranu životinja zbog mogućeg ulaska teških metala u lanac ishrane.

Ključne reči: Eutrični kambisol, autoput, zagađenje, stočna hrana

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