Carpathian Journal of Earth and Environmental Sciences, February 2017, Vol. 12, No. 1, p. 69 - 75

THE CONCENTRATION OF Zn, Mn AND Fe IN LEAVES OF *Ulmus laevis* Pall. AT VELIKO RATNO OSTRVO ISLAND (BELGRADE, SERBIA)

Jovana DEVETAKOVIĆ[1], Dragica STANKOVIĆ[1], Vladan IVETIĆ[1], Bojan MITROVIĆ[1] & Nebojša TODOROVIĆ[2]

[1]*Faculty of Forestry-University of Belgrade, jovana.devetakovic@sfb.bg.ac.rs, dragica.stankovic@sfb.bg.ac.rs, vladan.ivetic@sfb.bg.ac.rs, bmitrovic05@gmail.com*

[2]*RB "Kolubara", Lazarevac, nebojsa.todorovic@rbkolubara.rs*

Abstract: Considering the importance of environment quality, especially in protected natural areas, we are conducted research of heavy metals contents (Zn, Mn and Fe) in plants and soil at protected natural area "Veliko ratno ostrvo" in Belgrade (Serbia). European white elm (*Ulmus laevis* Pall.) is native on the Veliko ratno Ostrvo Island and research were conducted in the aim to confirm possibility for using as bioindicator. The Veliko ratno ostrvo island represent unpolluted area, only two samples (total of 5 soil samples) show slightly exceed the value of Zn concentration (site 5 - 288.37 $mg.kg^{-1}$ and site 2 - 263.75 $mg.kg^{-1}$) in soil, while concentrations of Mn and Fe are in normal range. Concentration of heavy metals in plant tissue is in the normal range, except Fe where there are concentrations over optimal range at 10 trees (up to 415.14 $mg.kg^{-1}$) from total 13 tested trees. There are oscillations between different trees i.e. genotypes, which confirmed differences into absorption of heavy metals within species.

Keywords: Zn, Mn, Fe, European white elm, bioindicator, pollution

1. INTRODUCTION

The accumulation and concentration of heavy metals in plant tissue indicate importance of certain species as (bio) indicators of environmental pollution (Ten-Houten, 1983; Prasad & Freitas, 2003). There is an increasing attempt to woody plants used in the monitoring of environmental pollution, but the problem are critical values for certain heavy metals, plant resistance to their presence, cumulative and possible synergistic action, as well as many other aspects that are today still little known.

About that how important is concentration and accumulation of heavy metals in some area, it can be clearly considering if we know that harmful effects of heavy metals, calculated annually, exceed the total harm of radioactive and organic waste (Nriagu, 1979; Pacyna & Munch, 1989).

There are differences in the uptake of heavy metals between the different plant species as well as in plants of the same species, which primarily depends on their genetic characteristics, from the influence of the surface of the root system and their capacity to absorb ions, form of root secretions and speed evapotranspiration (Alloway, 1995), then climatic conditions, different regimes and the sensitivity of the plant to the effect of heavy metals and others.

Veliko ratno Ostrvo Island was protected as Landscape of exceptional quality. It is located in Belgrade, on the territory of Zemun municipality, with a total area of 211 ha 36 a 78 m^2 (Veliko ratno ostrvo 210 ha 64 a 38 m^2, and Malo ratno ostrvo 72 a 40 m^2). Island is very attractive area for visitors; beach Lido on Danube River is very popular in summer time. Population of European white elm (*Ulmus laevis* Pallas, *Ulmus effusa* Wild., *Ulmus pedunculata* Fogg) is native at Veliko ratno ostrvo island and occurs in the forest types of the White willow (Salicetum alba Issl.26) at the recent wet and layered alluvial forests and White and Black poplar (Populetum albo nigrae Slav.52) in a mosaic of different alluvial soil.

Strong impact of Dutch elm disease pandemics on elm populations across Europe combined with the changes of natural habitats of European white elm (Collin, 2003) resulted reduction of populations and some kind of vulnerability of the species. European white elm is recognized as rare and endangered species in some countries, for example in Serbia (Banković et al., 2009). Considering the importance of environment quality, especially in protected natural areas, we are conducted research of heavy metals contents (Zn, Mn and Fe) in plants and soil at Protected natural area "Veliko ratno ostrvo" in Belgrade (Serbia).

2. MATERIAL AND METHODS

Analysis of heavy metals content in the soil was performed at 5 sites (S), and on this occasion, sample of plant material (leaves) was taken a total of 13 trees which grow in these locations (S1- trees 29 and 31; S2-trees 18, 19 and 21; S3 - trees 33, 34, 35, 36 and 37; S4 - trees 13 and 14; S5 - tree 32). The spatial distribution of sites and trees is shown in figure. 1. Site 1 is on the forest edge on the elevated terrain where flooding is least frequently, while site 5 is in the river coast. Site 2, 3 and 4 are typical stands for European white elm, where trees exist in the second or first floor of crowns, on alluvial soils with higher percent of clay.

For collecting samples were selected healthy European white elm trees of different ages (Table 1). Leaves and soil sample are collected on September 2014th and samples were dried on air temperature and prepared for laboratory analysis. The milled soil (3g) was digested with aqua regia under reflux for 2 hours with water-cooled condensers on a digestion block using the standard procedure (ISO, 1995).

Table 1. Diameter, height and age of selected trees

MARK ON THE FIELD	DIAMETER (cm)	HEIGHT (m)	AGE
13	60	26	26
14	32	21	17
18	41	17	27
19	37	18	16
27	20	5	14
29	47	13	24
31	24	12	17
32	46	18	23
33	37	17	17
34	43	18	12
35	39	18	12
36	49	15	15
37	19	7	12

Figure 1. Distribution of sites and trees at Veliko ratno ostrvo island

The leaves are milled into powder. The Zn, Mn and Fe contents were determined using the same procedure as applied for soil samples.

Analyses were performed in the laboratories of the Faculty of Forestry, University of Belgrade. The contents of Zn, Mn and Fe were determined by flame atomic absorption spectrophotometer.

The obtained values were processed using STATISTICA 7.0, and descriptive statistic, analysis of variance, post-hoc Tukey HSD test and correlation. The results are presented in tables and figures. Bioaccumulation factor was calculated as the ratio of metal concentration in leaves over the metal content in soil.

3. RESULTS

3. 1. Soil analysis

According average concentrations of Zn, site 5 (288.37 $mg.kg^{-1}$) and site 2 (263.75 $mg.kg^{-1}$) with the values over 250 $mg\ kg^{-1}$ have higher content than other sites where the values are ~150 $mg.kg^{-1}$ (Fig. 2).

Average concentration of Mn is the highest on the site 2 (804.91 $mg.kg^{-1}$), slightly lower content there are on site 5 (732.25 $mg.kg^{-1}$) and site 3 (660.19 $mg.kg^{-1}$). Content of Mn is somewhat over 500 $mg.kg^{-1}$ on site 4 (516.09 $mg.kg^{-1}$) and site 1 (531.28 $mg.kg^{-1}$) (Fig. 3).

Average concentration of Fe is the highest on the site 3 (37440.57 $mg.kg^{-}1$). Site 2 and site 5 have similar values of Fe content, about 31900 $mg.kg^{-1}$, also as site 1 and site 4 with values about 21000 $mg.kg^{-1}$ (Fig. 4).

In the case of Zn and Mn content of metals decreased with increase of soil depth on all sites. The iron showed something different, on site 2 content of Fe is higher on deeper layers of soil and on the site 3 there are slightly descending on middle layer and then re-increase. Content of heavy metals Zn, Mn and Fe on the site 1 do not show differences between soil layers.

3. 2. Plant analysis

The measured concentrations of heavy metals in leaves of European white elm (*Ulmus laevis* Pall.) is shown in Table 2. The concentration of Zn is in the range of 15.11 $mg.kg^{-1}$ to 42.94 $mg.kg^{-1}$. According to the average values of Mn are in range of 13.98 $mg.kg^{-1}$ to 34.84 $mg.kg^{-1}$. Concentration of Fe according to the average values ranges from 86.66 $mg.kg^{-1}$ to 415.14 $mg.kg^{-1}$. Bioaccumulation factor is low for all three metals; values are lower than 1.

Correlation analysis was presented to the interaction between the content of heavy metals in soil and plant material (Table 3). The results show that there is a direct correlation between the content of heavy metals in soil and leaves of European white elm at Mn ($r = 0.6995$, $p <0.01$) and Zn ($r = 0.6946$, $p <0.01$), while the strongest correlation exists with the content Mn and Zn in soil ($r = 0.7842$, $p <0.01$). A positive correlation was recorded between the Fe content in leaves and Zn in soil ($r = 0.6859$, $p <0.01$).

4. DISSCUSION

In relation to critical limits that apply in the European Union, in accordance with the concept of providing a multifunctional land use, as well as by De Vries & Bakker (1998), only Zn concentration in soil of sample 5 and 2 have slightly exceed the value. Other sites have normal values of Zn concentration for soil. The concentration of Zn in soil is increased as a result of human activity in many industrial countries, but great influence has native substrate.

Manganese contents of worldwide soils vary from 411-550 $mg.kg^{-1}$ (Kabata-Pendias & Pendias, 2000), and reported a concentration of 1500 $mg.kg^{-1}$ of manganese at which can appear toxic symptoms. According to Adriano (1986) a "normal" Mn content for most of land is in the range of 500-1000 $mg.kg^{-1}$, which indicates that the concentration of Mn is in the range of normal concentrations.

Iron is present in soils in higher concentrations than any other nutrient, and concentrations in soil layers varies from no more than 100 to almost 100 000 $mg.kg^{-1}$ (Vanmechelen et al., 1997). Plants adopt iron from the soil in the form of ferrous ions (Fe^{2+}), ferric ions (Fe^{3+}) and in the form of iron chelate. Physiologically is active Fe^{2+} ion. A reduction in the adoption of iron is caused by high pH, high concentrations of phosphate and calcium ions (Stanković et al., 2009).

When talking about the concentration of heavy metals in woody plants, we emphasize that these are data obtained in a short period of lifecycle. Accumulation of heavy metal do not always uniformly between shoot and root. Zinc and manganese are uniformly distributed between shoot and root but iron usually accumulated in roots rather than in shoots (Siedlecka, 1995).

We have to take into account the time when the research was conducted and, because although the uptake of heavy metals by plants occurs constantly during the year as a rule, the highest concentration is on the end of the vegetation period (Stanković et al., 2011, 2015a, 2015b).

A slight increase of Zn in the locality of 5 and 2, do not causes morphological and physiological changes and does not lead to an increase of this element in leaves of European white elm. The value of 35.41 $mg.kg^{-1}$ at tree 32 at site 5 and 42.94 $mg.kg^{-1}$ at tree 21 at site 2 is within the optimal range of values, but also it concentration is higher than other trees (at site 2 and total). Values of bioaccumulation factor indicate this species as a potentially excluder (Baker & Walker 1990; Oliva & Espinosa, 2007; Mehes-Smith et al., 2013).

Keeping in mind importance of zinc in plant nutrition, the attention of researchers in the world is more dedicated to his absence than its surplus. Zinc in plants, occurs at the relatively low concentrations of 20-50 ppm. A value less than 20 ppm in dry matter is a critical value for most of plant species (Carol & Loneragan, 1969).

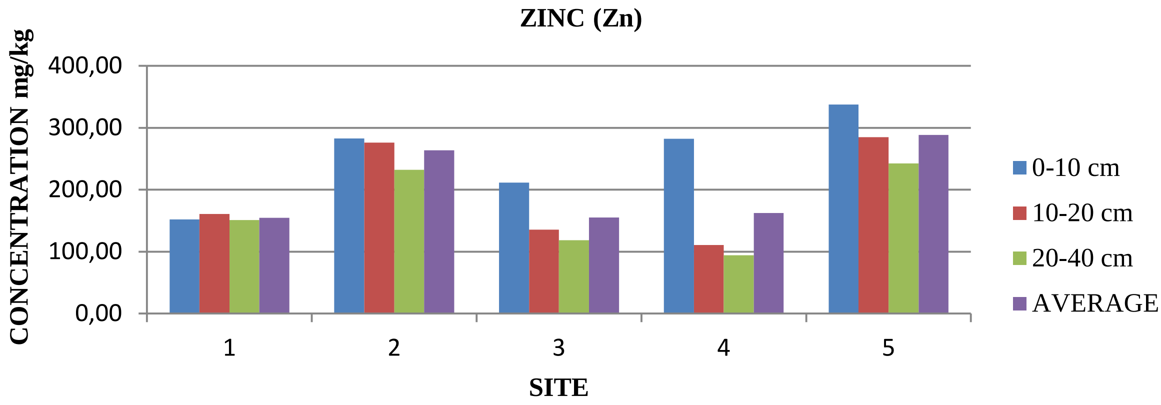

Figure 2. Concentration of Zn on 5 sites at Veliko ratno ostrvo island

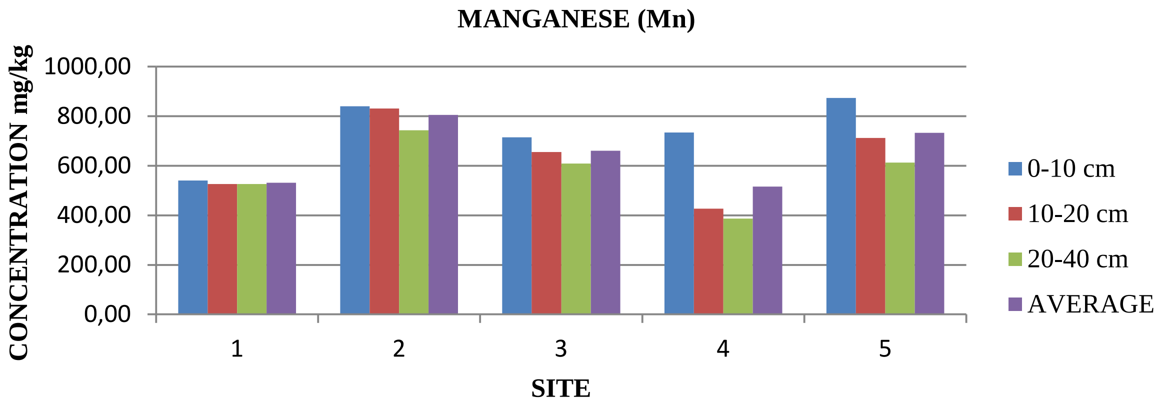

Figure 3. Concentration of Mn on 5 sites at Veliko ratno ostrvo island

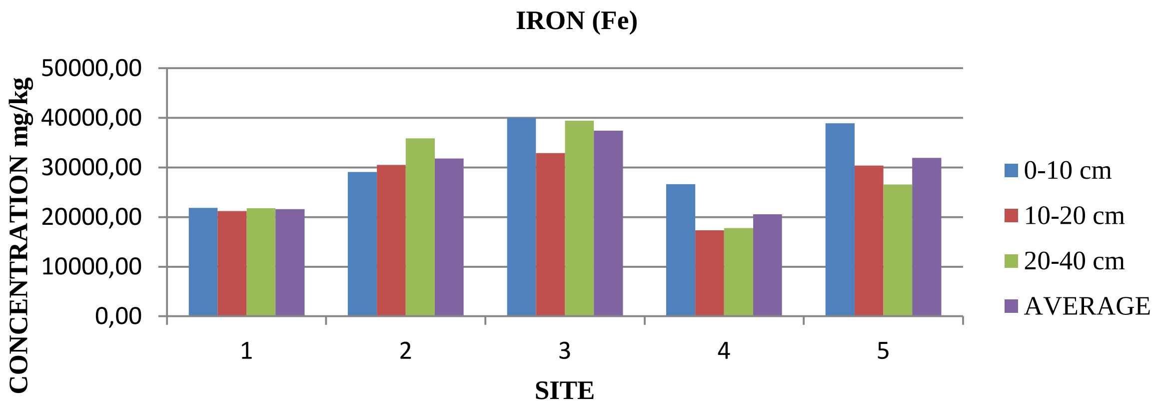

Figure 4. Concentration of Fe on 5 sites at Veliko ratno ostrvo island

Table 2. Concentration (bioaccumulation factor) of Zn, Mn and Fe in leaves of European white elm

TREE	Zn	Mn	Fe
	average concentration mg/kg		
13	24.70[d] (0.10)	25.18[g] (0.04)	269.24[h] (0.01)
14	22.43[c] (0.14)	21.32[e] (0.04)	229.37[e] (0.01)
18	26.89[ef] (0.14)	29.61[i] (0.03)	268.94[h] (0.01)
19	27.12[fg] (0.15)	34.84[l] (0.05)	415.14[k] (0.01)
21	42.94[i] (0.10)	32.43[j] (0.04)	248.39[f] (0.01)
29	15.11[a] (0.18)	13.98[a] (0.04)	86.62[a] (0.01)
31	26.42[ef] (0.16)	16.54[b] (0.04)	162.47[b] (0.01)
32	35.41[h] (0.16)	23.76[f] (0.04)	412.46[j] (0.01)
33	24.39[d] (0.14)	23.34[f] (0.05)	268.66[h] (0.01)
34	22.37[c] (0.12)	18.70[c] (0.03)	205.99[d] (0.01)
35	28.11[g] (0.10)	27.63[h] (0.03)	256.86[g] (0.01)
36	21.21[b] (0.10)	33.93[k] (0.04)	271.92[i] (0.01)
37	25.89[e] (0.14)	20.52[c] (0.04)	188.13[c] (0.01)
average	26.38 (0.14)	24.75 (0.04)	252.62 (0.01)

*different letters significant differences between means at 95% (Tukey HSD post hoc test)

Table 3. Correlations between contents of heavy metal in plant and soil

		PLANT			SOIL		
		Zn	Mn	Fe	Zn	Mn	Fe
PLANT	Zn	1.0000					
	Mn	0.4601	1.0000				
	Fe	0.4852	0.6831	1.0000			
SOIL	Zn	0.6946*	0.5286	0.6859*	1.0000		
	Mn	0.6188	0.6995*	0.6063	0.7842*	1.0000	
	Fe	0.2141	0.4061	0.3043	0.0847	0.6294	1.0000

*marked correlations are significant at p<0,01

According to literature data, deficit limit can take place at a concentration of 20-25 $mg.kg^{-1}$, and the toxicity at the concentration of about 400 $mg.kg^{-1}$ (Kárpáti et al., 1967).

According ECCE (1994) content of Zn is in range 15-150 $mg.kg^{-1}$, Mn from 1-700 $mg.kg^{-1}$ and Fe is in range 5-200 $mg.kg^{-1}$ in plant material, which indicate that only trees 29, 31 and 37 have content of Fe in optimal range.

Concentrations of heavy metals Zn, Mn and Fe in leaves of different trees show a statistically significant difference. These results indicate that the effect of trees genotype has influence on the level of accumulation of heavy metals, which confirms earlier research about differences into absorption of heavy metals within species (Kraljević-Balalić et al., 2009; Borišev, 2010; Šijačić-Nikolić et al., 2012).

Zn, Mn and Fe are minerals necessary for plant metabolism, but also they may become toxic if their supply exceeds the plant needs (Krstić et al., 2011). Interaction between mineral nutrients are very important and can either induce deficiencies or toxicities and can modify growth response (Krupa et al., 2002).

Strong correlations between Fe and Zn content in plant is known (Siedlecka, 1995), and verified with correlation between content of Zn in soil and Fe in plant ($r = 0.6859$, $p < 0.01$).

The total amount of zinc in the soil are grouped into several factions (Wietes, 1966), the plants are accessible to only those forms in which zinc is potentially soluble. By applying the higher doses of phosphorus (P), for example. phosphorus fertilizers can reduce the accumulation of zinc or lead to a deficiency of Zn. Also, high levels of Mn as well as the high pH value can lead to a deficiency of Zn (Kabata-Pendias & Pendias, 2000). Irregularity in the distribution of the concentration of Fe is probably the result of the organic layer of soil. Organic layers with few mineral materials are very low in Fe, because most Fe is in mineral form. There is a negative correlation between Fe concentration and organic content in the layer (Vanmechelen et al., 1997).

5. CONCLUSIONS

There was a difference in the degree of adoption of the heavy metals between and within locality and genotype. The strongest correlation between the content of heavy metals in soil and leaves of European white elm is in the case of Mn and Zn, but also there are strong correlations between content of Zn in soil and Fe in plant and content of Mn and Zn in soil. European white elm is tolerant on slightly exceeded concentrations of zinc, but also accumulate of iron exceed normal ranges which require additional research in the aim of possibility of using on contaminated soils.

We concluded that the area of Veliko ratno ostrvo island is still ecologically preserved, which is in accordance with the declaration of the protected area, but it must be emphasized necessity of constant monitoring of pollution in this area in order to observe negative effects timely and take appropriate precautionary measures.

Acknowlegments

This paper was realized as a part of the project "Establishment of wood plantations intended for afforestation of Serbia" (31041) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the period 2011-2014.

REFERENCES

Adriano D C., 1986. *Trace elements in terrestrial environment.* Springer-Verlag, New York, 1-533.

Alloway B J., 1995. *Heavy Metals in Soils*. Second edition: Blackie Academic & Professional, London – Glasgow, 1-354.

Banković S., Medarević M., Pantić D., Petrović N., Šljukić B. & Obradović S., 2009. *Šumski fond Republike Srbije - stanje i problemi*. Glasnik Šumarskog fakulteta 100, 7-30. /*Forest Fund of the Republic of Serbia – situation and problem*s. Gazette of the Faculty of Forestry 100, 7-30/.

Baker, A.J.M. & Walker P.L., 1990. *Ecophysiology of metal uptake by tolerant plants: Heavy metal uptake by tolerant plants.* In: Shaw, A.J. (Editors). Evolutionary Aspects. CRC, Boca Raton, 156-173.

Borišev M., 2010. *Potential of willow (Salix spp.) clones for heavy metal phytoextraction*. Doctoral thesis. Faculty of Science-University of Novi Sad, pp 187.

Carol M.D. & Loneragan J.F., 1969. *Response of plant species to ceoncentration of zink in solutuion.* II rate of zink absorption and their relation to growth. Austrl. J. Agric. Res. 20, 457-163.

Collin E., 2003. EUFORGEN: *Technical Guidelines for genetic conservation and use for European white elm (Ulmus laevis).* International Plant Genetic Resources Institute, Rome, Italy. 6 pages.

De Vries W. & Bakker DJ., 1998. *Manual of calculating critical loads of heavy metals for terrestrial ecosystems: Guidelines for critical limits, calculation methods and input data*. DLO Winnand Starting Centre, Wageningen, Report 166, 144.

Element Concentration Cadasters in Ecosystems (ECCE), 1994. Progress Report. Presented at the 25th General Assembly of International Union of Biological Sciences (IUBS), Paris, France, 1-25.

International Organization for Standardization ISO 1995. 11466:1005 Soil quality. In: Extraction of Trace Elements Soluble in Aqua Regia, BSI, London, 1-12.

Kabata-Pendias A. & Pendias H., 2000. *Trace elements in soils and plants*, 3rd edition. CRC Press, Boca Raton, FL, 1-413.

Kárpáti I., Kárpáti V. & Tölgyesi G., 1967. *Manganese content in aquatic plants*. Acta Bot. Hung. 13, 95-112.

Kraljević-Balalić M., Mladenovo N., Balalić I. & Zorić M., 2009. *Variability of leaf cadmium content in tetraploid and hexaploid wheat*. Genetika, 41, 1, 1-10

Krstić B., Oljača R. & Stanković D., 2011. *Fiziologija drvenastih biljaka*. Univerzitet u Banjoj Luci, Univerzitet u Novom Sadu, Banja Luka, 1-352 /*Phisiology of woody plants*. University of Banja Luka, University of Novi Sad, Banja Luka, 1-352, (in Serbian).

Krupa Z., Siedlecka A., Skórzynska-Polit E. & Maksymiec W., 2002. *Heavy Metal Interactions with Plant Nutrients*. in Prasad MNV and Strzalka K. (eds.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer Academic Publishers, 287-301.

Mehes-Smith M., Nkongolo K.K., Narendrula R. & Cholewa E., 2013. *Mobility of heavy metals in plants and soil: a case study from a mining region in Canada.* American Journal of Environmental Science, 9, 6, 483-493.

Nriagu J.O., 1979. *Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere*. Nature 279, 409-411.

Oliva S.R. & Espinosa A.J.F., 2007. *Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to identify possible contamination sources*. Microchemical Journal 86, 131-139.

Pacyna J. & Munch J., 1989. *European Inventory of Trace Metal Emissions to the Atmosphere.* Heavy metal emissions Prague 24-26.10. Vol. 1.

Prasad M.N.V. & Freitas H.M.O., 2003. *Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology*. Electronic Journal of Biotechnology, 6, 3, 225-321.

Siedlecka A., 1995. *Some aspects of interactions between heavy metals and plant mineral nutrients.* Acta Soc. Bot. Pol., 64, 265-272.

Stanković D., Šijačić-Nikolić M., Krstić B. & Vilotić D., 2009. *Heavy metals in the leaves of tree species Paulownia elongata S.Y.Hu in the region of the city of Belgrade*. Biotechnology & Biotechnological Equipment 23, 1330-1336.

Stanković D., Medarević M., Krstić B., Bjelanović I., Šljukić B., Karić D. & Janić M., 2011.

Concentration of heavy metals and stand state of Sesille oak (Quercus petraea (Matt.) Liebl.) on Avala mountain (Serbia). Carpatian Journal of Earth and Environmental Sciences, 8, 3, 59 - 66.

Stanković D., Jokanović D., Veselinović M., Letić Lj., Jović Đ. & Karić D., 2015 (a). *Zinc concentration in woody and herbaceous plants at Kosmaj area*. Fresenius Environmental Bulletin, 11, 24, 3672-3675.

Stanković D., Ivetić V., Ocokoljić M., Jokanović D., Oljača R. & Mitrović S., 2015 (b). *Manganese concentration in plants of the protected natural resource Kosmaj in Serbia*. Archives of Biological Sciences 67, 1, 251-255.

Šijačić-Nikolić M., Stanković D., Krstić B., Vilotić D. & Ivetić V., 2012. *The potential of different lime tree (Tilia spp) genotypes for phytoextraction of heavy metals*. Genetika 44, 3, 537-548.

Ten Houten JG., 1983. *Biological indicators of air pollution*. Environmental monitoring and assessment, 3-4, 257-261.

Vanmechelen L., Groenemans R. & Van Ranst E., 1997. *Forest Soil Condition in Europe*. Results of the Large-Scale Soil Survey, Brussels Geneva, 1-279.

Wietes J., 1966. *Zinc deficiency in the soil –plant system.* In Prasad A (ed.): Zink metabolism. C.C.Thomas, Springfiel, 90-128.

Received at: 01. 04. 2016
Revised at: 03. 08. 2016
Accepted for publication at: 26. 08. 2016
Published online at: 05. 09. 2016