Biologia Serbica, 2016, 38(1):12-17 DOI 10.5281/zenodo.216054

Original paper

Comparative study of the accumulation of metals in the plant *Polygonum aviculare* L. from different sites in the city of Kragujevac

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Recieved for Review: 30 April 2016 / Accepted: 14 June 2016 / Published online: 21 December 2016

Summary. Weedy and ruderal plants can be used in studies of metal pollution of soil, water and air as well as monitoring human mediated degradation of ecosystems. The present study assesses metal concentrations (Fe, Mn, Cu and Ni) in the aboveground parts of *Polygonum aviculare* L. (Polygonaceae) in relation to traffic intensity, human population density and industrial emissions in an area of the city of Kragujevac. To evaluate the bioindicative ability of *P. aviculare*, we chose 5 groups of sites: urban, suburban, rural, industrial and roadside. Mean concentrations of metals in *P. aviculare* were ranked from highest to lowest as follows: Fe > Mn > Cu > Ni. The mean content of Fe and Cu were above toxic concentration limits, while concentrations of Mn and Ni were within the non-toxic range. Compared to other studies conducted with *P. aviculare,* we found higher concentrations of metals, which could be due to our later period of sampling (October). The highest concentrations of Fe, Mn, Cu and Ni were recorded in urban sites, followed by roadside sites. Industrial sites significantly impacted Cu content; although concentrations of Ni were very low at these sites. The lowest concentrations of metals were recorded at rural sites. Statistical analysis revealed significant difference among the sites for Ni, Fe and Mn content. Results from the present study demonstrate that *P. aviculare* possesses differential accumulation ability for these selected metals, and may be useful in remediation projects for reducing the toxicity of Fe and Cu in the environment. Moreover, this species can be used as a bioindicator of metal pollution and contamination in anthropogenic ecosystems.

Keywords: accumulation, bioindication, metals, *Polygonum aviculare.*

INTRODUCTION

Numerous compounds, gases, liquids and solid substances are known to be major environmental pollutants. If their concentrations are higher than accepted levels at a particular location, they can endanger the function and balance of local habitats. Pollution is mainly caused by natural phenomena, or emissions of pollutants from industry, energy production, transport and other anthropogenic factors (Nriagu and Pacyna 1988). Some of the most dangerous pollutants are heavy metals. In living organisms, metals can be found as essential elements, which are very important for growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo and Ni (Langille and MaClean 1976). Plants are able to uptake metals, either from soil, water or air. In addition, certain plants have the ability to accumulate other metals with less clear biological functions, such as Cd, Cr, Pb, Co, Ag, Se and Hg. These elements belong to the group called heavy metals (Hanna and Grant 1962; Baker and Brooks 1989) and are emitted into the atmosphere as aerosols, after which they can be transported up to several kilometers. In this way they can be transferred to the soil as wet or dry deposits and pollute a range of environments.

Elevated concentrations of metals can result in growth inhibition and toxicity in plants. These metals are non-biodegradable and can undergo global ecological cycles. Thus

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heavy metals can enter the food chain, where they have a cumulative effect on animals and humans, manifesting their harmful effects in tissues and organs. In the presence of some pollutants, in plants, different changes may occur, including: leaf necrosis, stomata, epidermis and mesophyll changes (Lorenzini 1999), photosynthesis rate differences, chlorophyll fluorescence and stomatal resistance (Monni et al. 2001), root injuries, morphological and physiological changes in development of the reproductive apparatus and consequently plant fertility (Rezanejad 2007), pollen viability, germination and tube growth (Dickinson 2000; Reznejad 2009).

Some plants can thrive in environments contaminated with heavy metals and appear able to accumulate huge amounts of metals in their organs (De Varennest et al. 1996; Ernst 1996). Because of this ability, these species may play important functions as bioindicators of pollution (Prasad and Freitas 2003).

One of the most frequent sources of pollution is traffic which has become a very important source of heavy metals emission due to a huge increase in the global number of vehicles (Memmon et al. 2001; Fakayode and Olu-Owolabi 2003; Davydova 2005; Stanković et al. 2008).

Common knotgrass, *Polygonum aviculare* L. (Polygonaceae) is a cosmopolitan plant, sometimes considered to be a weed, that lives mostly in trodden places, near diverse substrates (Josifović 1972). It can accumulate metals from the ground and air very efficiently (Polechońska et al. 2013). This plant is an important biological indicator of local heavy metal concentrations because it represents the most widespread plant in almost all habitats in human settlements, especially in nitrified or polluted habitats.

The aim of the present study was to investigate levels of environmental pollution in the city of Kragujevac associated with traffic, through analysis of metal concentrations (Fe, Cu, Mn and Ni) in the aboveground parts of the plant *P. aviculare*.

MATERIALS AND METHODS

Investigated area

The city of Kragujevac is located in the Kragujevac basin, the central part of the Republic of Serbia (Fig. 1), with coordinates lat 44°22′N and long 20°56′E. The city covers an area of 835 km^2 and is situated at an altitude of 180 m (Stepanović 1974). Local geological substrate is made up of neogenic sediments of middle and upper Miocene age, sandstone, clay and marl. Cambisoil and cambisoil in the process of browning are the most common types of soil in the city of Kragujevac (Tanasijević 1957). The local hydrographic network is composed of numerous small rivers and streams, with the largest Lepenica River and its tributaries. There are four reservoirs (Stepanović 1974). The climate is temperate continental and characterized by cold and moderate wet winters, with hot and dry summers (Stepanović 1974). Vegetation consists of communities of characteristic oak forest, ass.

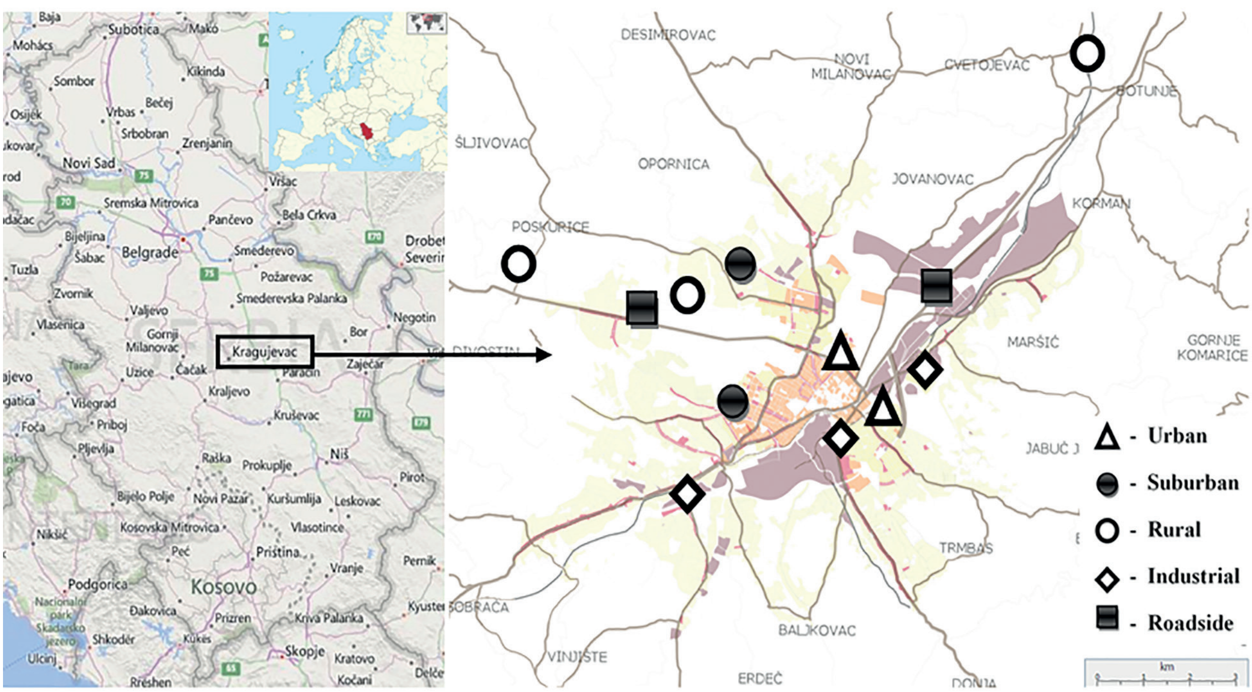

Fig. 1. Map of the locations of *Polygonum aviculare* sampling sites in the city of Kragujevac.

Quercetum frainetto-cerris moesiacum Rudski ap. Ht. 1946 (Horvat et al. 1974), submountain meadows, all. *Festucion valesiacae* Klika 1931 and valley meadows that belong to the two alliances: *Deschampsion caespitosae* Horvatić 1930 and *Arrhenatherion elatioris* Koch 1926 (Veljović 1967).

Site selection

Twelve sampling sites were selected in the Kragujevac metropolitan area (Table 1), based on differences in traffic influences and industrial emissions. Sites were divided into 5 groups: urban, suburban, rural, industrial and roadside. Urban sites included densely populated localities with high traffic density. Samples from suburban sites were taken in moderately populated parts of the city of Kragujevac, while rural sites included low populated localities with small traffic intensity. Industrial sites were chosen from the green parts of industrial zones and roadside sites included highways to nearby towns.

Material preparation

Aboveground plant parts of *P. aviculare* were collected at the end of the growing season in October 2012. Collected plant materials were washed with distilled water, dried at room temperature, and completely dried in an oven (Binder/Ed15053) at a temperature of 105 °C. The dried plant material was milled and prepared for chemical analysis by standard procedures (Radulović et al. 2014).

Metals analyzed were iron (Fe), copper (Cu), manganese (Mn) and nickel (Ni). Measurements of metal concentrations in plant tissues were performed using an atomic absorption spectrophotometer (Perkin-Elmer Company Model 3300/96) with an MHS-10 hydride system and PC. Concentrations of metals in plant materials are expressed in $mg \cdot kg^{-1}$ dry weight.

Table 1. List of researched sites in city of Kragujevac.

Site number	Site name	GIS coordinates	Altitude (above sea level)
I – Urban sites			
1.	Clinical center Kragujevac	44°01′7.5″N 20°54′49.8″E	196 m
2.	Kragujevac Bus station	44°00′39.6″N 20°55′39.6″E	178 m
II – Suburban sites			
3.	Vinogradi	44°02′17.9″N 20°53′7.7″E	301 m
4.	Bagremar	44°00′29.6″N 20°53′10.5″E	248 m
III – Rural sites			
5.	Cvetojevac village	44°04′4.9″N 20°58′50.2″E	162 m
6.	Divostin village	44°02′5.6″N 20°49′50.3″E	301 m
7.	Šumarice lake	44°01′47.1″N 20°52′47.2″E	228 m
IV – Industrial sites			
8.	Industrial zone (Ilijina voda)	44°01′9.3″N 20°56′20.6″E	182 m
9.	Factory “Energetika”	44°00′14.7″N 20°55′03.1″E	193 m
10.	Grošnička stanica (near Fiat cooperants)	43°59′41.8″N 20°52′35.7″E	198 m
V - Roadside sites			
11.	Road to Gornji Milanovac (near Botanical garden)	44°01′20.8″N 20°53′11.7″E	228 m
12.	Highway to Batočina (near Cross)	44°01′40.5″N 20°56′19.8″E	164 m

Statistical analysis

For each metal, minimum and maximum concentrations were determined. Mean and standard deviations were determined for the entire sample and for each group of sites separately. Differences among the 5 groups of sites (urban, suburban, rural, industrial and roadside) in terms of concentrations of metals in aboveground parts of *P. aviculare* were evaluated by one-way ANOVA. The post hoc Dunnett 3 test was used because homogeneity of variance was not assumed. All data were statistically analyzed using the software package SPSS for Windows, version 22.

RESULTS AND DISSCUSION

Concentrations of Fe, Cu, Mn and Ni measured in the aboveground plant parts of *P. aviculare* are presented in Table 2. The mean values of the concentrations of metals in *P. aviculare,* obtained for all sampling sites, decreased in the following order: Fe > Mn > Cu > Ni.

The mean values of the concentration of metals in the aboveground parts of *P. aviculare* from different sites decreased (Fig. 2) in the following order:

Fe: urban>roadside>industrial>suburban>rural;
Cu: urban>industrial>roadside>suburban>rural;
Mn: urban>roadside>industrial>suburban>rural;
Ni: urban>roadside>suburban>rural>industrial sites.

Analysis of variance (ANOVA $p < 0.05$) indicated that there was a statistically significant difference among the 5 groups of sites (urban, suburban, rural, industrial and roadside) for Fe ($p = 0.006$), Ni ($p = 0.001$) and Mn ($p = 0.05$). There was not statistically significant difference among the sites for content of Cu.

The post hoc Dunett T3 test indicated that a significant difference for Fe exists between the urban and rural sites ($p = 0.007$). For Ni significant differences exist between urban and industrial ($p = 0.026$), rural and roadside ($p = 0.018$) and between industrial and roadside sites ($p = 0.015$). Significant differences were found between urban and rural sites ($p = 0.027$) for Mn.

Concentrations of metals were highest in urban sites (Fe, Mn, Ni and Cu). Roadside sites had a high influence on Ni, Fe and Mn content, while industrial sites showed greater impact on Cu content. The lowest concentrations of Fe, Cu and Mn were found in rural sites, while the lowest concentrations of Ni were found in industrial sites.

Table 2. Concentrations of metals in aboveground plant parts of *Polygonum aviculare* (mg·kg^{-1} dry weight).

Site/Metal	Mean ± SD (Min-Max)	F-test
Fe	1721.83 ± 685.65 (822-2960)	9.266*
Cu	18.81 ± 10.95 (8.25-41.75)	1.376
Mn	79.23 ± 28.24 (44.75-135)	4.115*
Ni	7.67 ± 3.38 (3.75-13)	15.446*

*Significance of comparison of means by ANOVA (F-test) was indicated ($p < 0.05$).

Urban sites had the greatest impact on Fe content. In fact, Fe was found to be below 1000 mg·kg^{-1} only at rural sites. Stanković et al. (2011b) reported that iron content in dry plant matter varies in the range from 50 to 1000 mg·kg^{-1}. Some authors consider that Fe concentrations of 40-500 mg·kg^{-1} (Allen 1989) and 5-200 mg·kg^{-1} (Markert 1992) are toxic to plants. In relation to this data, concentrations of iron measured in the present study in the aboveground part of *P. aviculare* were higher than normally observed values and this species can accumulate excess iron.

Cu concentrations in plant tissues were highest at industrial sites. Several important sources of copper in the soil are fertilizers, sewage sludge, manure, agrochemicals, industrial waste and irrigation water. Some plant species have a high tolerance for elevated concentrations of copper and can accumulate extremely high amounts of this metal in their tissues. The concentration of copper in the ashes of various plants that grow under diverse living conditions has been reported to be between 5 and 1500 mg·kg^{-1} (Kabata-Pendias 2011). However, copper content in aboveground parts of the plant often does not exceed 20 mg·kg^{-1}; thus values between 20 to 100 mg·kg^{-1} suggest excessive content of this metal (Kabata-Pendias 2011). In the present study, mean values of Cu concentrations at urban, industrial and roadside sites were above 20 mg·kg^{-1}. Thus our results indicate that *P. aviculare* accumulated larger amounts of copper at these sites and that this species can tolerate larger amounts of copper.

High Mn content in aboveground parts of *P. aviculare* was found at roadside sites influenced by heavy traffic. The main anthropogenic source of manganese is municipal water, sewage sludge and byproducts of the process of melting metal. Since 1970, manganese is also used in the production of gasoline, in the form of organic substances – MMT (Kabata-Pendias 2011). Markert (1992) reported that a concentration of Mn above 700 mg·kg^{-1} is toxic for plants. However, in some plants its levels may be as high as 1500 mg·kg^{-1}, without any harmful effects (Pais and Jones 2000). Results from our research show that Mn concentrations from all investigated sites were within the non-toxic range, and that although *P. aviculare* can tolerate higher concentrations of manganese it is not an accumulator of this metal.

Measurements of Ni content showed the highest variance between sites. High Ni were found in plant materials collected from urban and roadside sites, while industrial sites showed a lower concentration of this metal. Rural sites had a higher impact on Ni content in *P. aviculare* than industrial sites. Nickel is considered a serious pollutant, which is released from metallurgical factories and increased burning of coal and oil (Kabata-Pendias 2011). In addition, sewage sludge and phosphate fertilizers can be a significant source of nickel in agricultural soil. Of the total Ni emissions in the atmosphere, approximately 20% is attributed to traffic

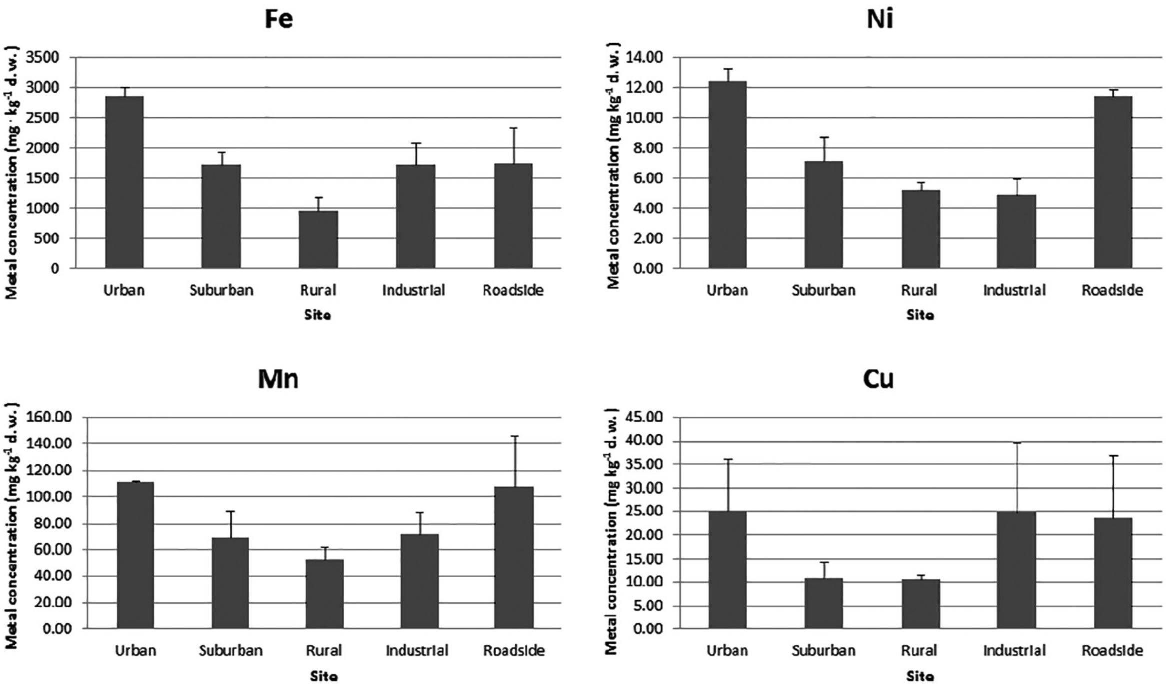

Fig. 2. Fe, Cu, Mn and Ni mean concentrations in aboveground parts of *Polygonum aviculare* from different sites in the studied area (mg·kg^{-1} dry weight ± SD) in the city of Kragujevac.

(Stanković et al. 2011a). Phytotoxic Ni concentrations range widely among plant species and cultivars, and are reported for various plants to be from 40 to 246 mg·kg^{-1} (Kabata-Pendias 2011). However, there are a great number of plants (317 taxa of 37 families) which have the ability to accumulate Ni concentrations over 1000 mg·kg^{-1} (Greger 1999). Results from our research indicate that concentrations of Ni in the aboveground parts of *P. aviculare* from different sites were within expected limits.

Concentrations of iron, copper and manganese in the aboveground parts of *P. aviculare* from different sites in the city of Kragujevac were significantly higher than those recorded for *P. aviculare* in Wroclaw, Poland (Polechońska et al. 2013) and Kraljevo in the Republic of Serbia (Radulović et al. 2014). Differences between concentrations of these four metals in the cities of Wroclaw and Kraljevo were negligible. Also, differences in the concentration of nickel measured in the cities of Kragujevac, Kraljevo and Wroclaw were very small.

A possible cause of these differences may be the sampling month - in our study plants were sampled in October, and in Wroclav (Polechońska et al. 2013) sampling was performed over 4 months (June, July, August, September). In the city of Kraljevo (Radulović et al. 2014) plant material was sampled in September. The warmer climate in Serbia vs. Poland results in a longer growing period. Thus a longer period of exposure could impact accumulation of heavy metals from water, soil and air.

CONCLUSIONS

Based on concentrations of these metals in the aboveground parts of *P. aviculare,* results from the present study indicate that traffic intensity and human population density have a very high impact on Fe, Cu, Mn and Ni content in the environment. In particular, levels of Cu were highly influenced by industrial emissions, while concentrations of Ni were low at these sites. Measured levels of Fe and Cu were above toxic concentration limits, while concentrations of Mn and Ni were within non-toxic limits. Differences in the timing of plant material sampling may have resulted in higher concentrations of metals in aboveground parts of *P. aviculare* in the present study vs. other studies using the same species.

Based on our results, *Polygonum aviculare* can accumulate excess Fe and Cu, and can be used for reducing the toxic effects of these metals in environment. In addition, this plant is a good bioindicator and can be used in environmental pollution monitoring studies in semi natural and urban ecosystems.

Acknowledgments

This research was part of a larger project „Ecological and physiological characteristics of plant species as indicators of environmental conditions in the city of Kragujevac" (No. 400-900/12-III), financed by Department of Environmental Protection, City of Kragujevac, during 2013. Project owner was the Ecological research association „Mladen Karaman".

REFERENCES

Allen SE. 1989. Analysis of Ecological Materials. 2nd Ed. Oxford: Blackwell Scientific Publication. p. 380

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements - A review of their distribution, ecology and phytochemistry. Biorecovery. 1:81–126.

Davydova S. 2005. Heavy metals as toxicants in big cities. Microchemical Journal. 79:133–136.

De Varennest A, Torres JF, Coutinho MM, Rocha SG, Neto MP. 1996. Effects of heavy metals on the growth and mineral composition of a nickel hyperacumulator. Journal of Plant Nutrition. 19:669–676.

Dickinson HG. 2000. Pollen stigma interactions: So near yet so. Trends in Genetics. 16:373–376.

Ernst WHO. 1996. Phytotoxicity of heavy metals. In: Rodriguez-Barrueco C, editor. Fertilizers and Environment. Dordrecht (NL): Kluwer Academic Publishers. p. 423–430.

Fakayode SO, Olu-Owolabi BI. 2003. Heavy metal contamination of roadside topsoil in Osogbo, Nigeria: its relationship to traffic density and proximity to highways. Environmental Geology. 44:150–157.

Greger M. 1999. Metal availability and bioconcentration in plants. In: Prasad MN, Hagemeyer J, editors. Heavy Metal Stress in Plants. Berlin: Springer. p. 1–27.

Hanna WJ, Grant CL. 1962. Spectrochemical analysis of the foliage of certain trees and ornamentals for 23 elements. Bulletin of the Torrey Botanical Club. 89:293–302.

Horvat I, Glavač V, Ellenberg H. 1974. Vegetation Südosteuropas (Vegetation of Southeast Europe). Stuttgard: Geobotanica selecta, Band IV, Gustv Fischer Verlag, p. 768. German.

Josifović M. 1972. Flora SR Srbije 3 (Flora of SR Serbia Vol. III.) Srpska akademija nauka i umetnosti, Beograd, p. 56. Serbian.

Kabata-Pendias A. 2011. Trace Elements in Soil and Plants, 4th Edition. Washington, D.C: CRC press. p. 201–268.

Langille WM, MacLean KS. 1976. Some essential nutrient elements in forest plants as related to species, plant part, season and location. Plant and Soil. 45:17–26.

Lorenzini G. 1999. Le piante e l'inquinamento dell'aria (The plants and air pollution). Bologna, Edagricole 1, 335. Italian

Markert B. 1992. Presence and significance of naturally occurring chemical elements of the periodic system in the plant organism and consequences for future investigations on inorganic environmental chemistry in ecosystems. Vegetatio. 103:1–30.

Memmon A, Aktoprakligil D, Özdemir A, Vertii A. 2001. Heavy metal accumulation and detoxification mechanisms in plants. Turkish Journal of Botany. 25:111–121.

Monni S, Uhlig C, Hansen E, Magel E. 2001. Ecophysiological responses of Empetrum nigrum to heavy metal pollution. Environmental Pollution. 112:121–129.

Nriagu JO, Pacyna JM. 1988. Quantitative assessment of worldwide contamination of air, water and soil by trace metals. Nature. 333:134–139.

Pais I, Jones JB, Jr. 2000. The Handbook of Trace Elements. Boca Raton (FL): St. Lucie Press.

Polechońska M, Zawadzki K., Samecka-Cymerman A, Kolon K, Klink A, Krawczyk J, Kempers AJ. 2013. Evaluation of the bioindicator suitability of *Polygonum aviculare* L. in urban areas. Ecological Indicators. 24:552–556.

Prasad, MNV, Freitas HM. 2003. Metal hyperaccumulation in plants biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology. 6:286–290.

Radulović M, Stanković S, Simić Z, Radaković M, Topuzović M. 2014. The accumulation of metals in *Polygonum aviculare* L. in area of the Kraljevo city. Kragujevac Journal of Science. 36:175–184.

Rezanejad F. 2007. The effect of air pollution on microsporogenesis, pollen development and soluble pollen proteins in *Spartium junceum* L. (Fabaceae). Turkish Journal of Botany. 31:183–191.

Rezanejad F. 2009. Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). Grana. 48:205–213.

Stanković D, Krstić B, Nikolić N. 2008. Effect of traffic on the soil contamination with polycyclic aromatic hydrocarbons (PAHs). Journal of Biotechnology & Biotechnological Equipment. 22:736–741.

Stanković D, Knežević M, Krstić B, Šijačić-Nikolić M, Vilotić D. 2011a. Nickel content in plants and soil in the area of the protected natural resource „Avala" - Belgrade. Bulletin of the Faculty of Forestry. 103:131–146.

Stanković D, Krstić B, Šijačić-Nikolić MT, Knežević MN. 2011b. Concentrations of manganese and iron in some woody and herbs plants. Nature conservation and land resources. Proceedings for Natural Sciences, Matica Srpska, Novi Sad. 121:39–49.

Stepanović Ž. 1974. Hidrološke karakteristike Kragujevačke kotline sa posebnim osvrtom na snabdevanje Kragujevca vodom (Hydrological characteristics of Kragujevac valley with special emphasis on water supply of the City of Kragujevac). Fond za finansiranje visokoškolskih ustanova, naučne i naučno izdavačke delatnosti Skupštine opštine Kragujevac. Serbian.

Tanasijević D. 1957. Pedološko-agrohemijske osobine zemljišta Sreza Kragujevac (Pedological-agrochemical characteristics of soil in the Kragujevac Region). Institut za pedologiju i agrohemiju, Beograd – Topčider. Serbian.

Veljović V. 1967. Vegetacija okoline Kragujevca (Vegetation of Kragujevac). Glasnik Prirodnjačkog muzeja, Ser. B. 22:1–180. Serbian.