Arch. Biol. Sci., Belgrade, 66 (1), 331-336, 2014 DOI:10.2298/ABS1401331M

LEAD AND NICKEL ACCUMULATION IN *IRIS PUMILA*: CONSIDERATION OF ITS USEFULNESS AS A POTENTIAL BIOINDICATOR IN THE NATURAL PROTECTED AREA OF DELIBLATO SANDS, SERBIA

DANIJELA MILJKOVIĆ[1*], S. AVRAMOV[1], VUKICA VUJIĆ[2], L. RUBINJONI[2],
NATAŠA BARIŠIĆ KLISARIĆ[1], U. ŽIVKOVIĆ[1] and A. TARASJEV[1]

[1] *Institute for Biological Research "Siniša Stanković", University of Belgrade,* 11000 Belgrade, Serbia
[2] *Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia

Abstract - In this work, we investigated the suitability of the threatened species, *Iris pumila* L., as a possible bioindicator of traffic pollution in its natural habitats (mostly protected areas and natural reserves). We examined its potential to accumulate lead (Pb) and nickel (Ni) in polluted habitats, and the genetic variability for this capability, since it is an important facet of bioindicator suitability. We estimated the concentrations of Pb and Ni in the leaves of 17 *I. pumila* clones (genotypes) grown in one of their natural habitats, the unpolluted semi-arid habitat of the protected Deliblato Sands Special Natural Reserve, and in the leaves of 18 *Iris pumila* full-sib families grown in an experimental plot in a heavily polluted urban location in Belgrade, Serbia. Comparison of the contrasting habitats by one-way *A*NOVA analysis showed that both Pb and Ni concentrations were significantly higher (six-fold) in the *I. pumila* leaves collected from plants grown in the polluted urban habitat. Two-way ANOVA (randomized block design) analysis performed on the full-sib families grown in the urban location failed to detect significant genetic variation for metal accumulation in *I. pumila* leaves. A significant block effect on the concentration of Ni in leaves was detected, indicating responsiveness to microenvironmental variability. These results suggest that *I. pumila* can serve as a good indicator of traffic pollution in protected areas. The response is stable since genetic variability of *I. pumila* populations does not appear to influence its role as an indicator greatly.

Keywords: Traffic atmospheric pollution, bioindicators, trace elements accumulation, *Iris pumila*, genetic variability, Deliblato sands.

INTRODUCTION

The anthropogenic influence on ecological conditions in natural habitats can have various forms and can affect various ecological attributes (Angermeier and Karr, 1994; Dale et al., 2002). In natural areas that are or should be protected, these influences can include road construction, tourism and agriculture. One of the consequences of these activities is pollution due to increased traffic. Higher plant leaves are increasingly used as biomonitors for atmospheric pollution (Akguz, 2011), and the utilization of species that already inhabit these habitats can significantly contribute to monitoring of pollution levels (Khaniki and Zazoli, 2005). The concentrations of heavy metals in plants near roads are expected to be higher than in plants from unpolluted locations with no traffic, since the emissions of heavy metals highly correlate with traffic intensity (Yunis and Iqbal, 1996; Gajić et al., 2009). In particular, higher concentrations of Pb in the air are closely related to the density of traffic (Yunus and Iqbal, 1996; Van der Gon and Appelman, 2009; Abdollahi et al., 2011), as are Ni concentrations, however, the latter is not only present in automobile exhaust but also in other fossil fuels or the metallurgical industry (Carreras and Pignata, 2002; Akguc et al., 2010). Accumulation of heavy metals in plant tissues can depend on variations in pollution levels and numerous abiotic factors, on the species of plant and organ (Saygideger et al., 2004), as well as on the plant genotype (Wang et al., 2006; Zeng et al., 2008 and references therein). Therefore, studies of indicator suitability should include genotypic and environmental variation in the intake rates of toxic heavy metals, including Pb and Ni.

In this work we used naturally growing clones of the threatened species, *Iris pumila* L., in the protected Deliblato Sands Natural Reserve, as well as plants belonging to different full-sib families that were planted and grown for a couple of years in a heavily polluted habitat in Belgrade, Serbia (Fig. 1). One aim of this study was to test the extent to which the concentrations of Pb and Ni in *I. pumila* leaves change as a result of environmental pollution. The extent of change can suggest whether I. pumila can serve as a bioindicator of traffic pollution in previously uncontaminated habitats. Another aim of this study was to determine whether there is genetic and microenvironmental variability for the accumulation of heavy metals in *I. pumila* leaves in polluted locations.

MATERIALS AND METHODS

Study species

A perennial clonal plant, *I. pumila* (Iridaceae) has large hermaphroditic flowers and exhibits great flower-color polymorphism that enables the detection of different genotypes (Tucić et al., 1988). *I. pumila* is an allotetraploid with a chromosome number of $2n = 36$ (Fehmiye, 2003). It inhabits steppe-type habitats (Stjepanović-Veseličić, 1953) and is classified as a threatened species in Serbia. It has been previously demonstrated that *I. pumila* shows significant responses to environmental variation in various traits (Tarasjev et al., 2012), including phenology (Tarasjev, 1997), morphology (Tarasjev et al., 2009) and developmental stability (Miljković, 2012).

Locations

The natural populations of *I. pumila* used in this study are located in the semi arid area of the Deliblato Sands Natural Reserve (44°57’42” N, 21°01’46” E), an unpolluted sandy location situated 50 km northeast of Belgrade (Gajić, 1983) and one of the most important centers of biodiversity in Serbia and Europe. The populations are 7.5 km removed from the nearest settlement, and 15 km from the regional road and railway line. Parts of the Deliblato Sands are used for tourism and agriculture and there are a several local roads running through the whole complex.

The selected polluted site was located in an urban area of Belgrade (44°99’03” N, 20°29’14” E), capital of Serbia and largest city in the Western Balkans, with a population of over 1.6 million inhabitants (Census data, 2011). Experimental plots were located approximately 25 m from the main road leading to the Belgrade-Pančevo Bridge, with over 60 000 vehicle crossings per day (Transport Master Plan of Belgrade, 2012). This urban area is considered one of the most heavily polluted areas in Belgrade (Grubačević et al., 2009; Sawidis et al., 2011). There are many old vehicles on the streets, and leaded gasoline (0.4 g Pb l^{-1}) was widely used until 2011 (Aničić et al., 2011). Leaded gasoline was put out of use after the experiment ended. Belgrade has much higher annual bulk deposition fluxes of heavy metals compared to many other urban areas in the world (Mijić et al., 2010; Sawidis et al., 2011) and much higher deposition fluxes of Pb and Ni in particular (21.7 and 11.3 mg m^{-2} yr^{-1} respectively - Mijić et al., 2010).

Experimental procedures

During the blooming phase of *Iris pumila*, 17 clones were chosen for analysis from the natural unpolluted habitat. Thirty-six simultaneously flowering clones were chosen for hand-pollinations and 18 full-sib families were produced. Seedlings were grown un-

Table 1. The results of analyses of variance (ANOVA) for lead and nickel concentrations in leaves of *Iris pumila* plants: (A) one-way ANOVA on plants from two locations (unpolluted natural and polluted urban); (B) two-way ANOVA (randomized block design without replications) on families grown in two blocks in urban polluted environment.

A	Lead			Nickel	
Source of variation	df	MS	F value	MS	F value
Locality	1	3.81	114.21^{****}	6.32	309.57^{****}
Error	33	0.03		0.02	

B	Lead			Nickel	
Source of variation	df	MS	F value	MS	F value
Family	17	0.52	1.22^{ns}	0.32	0.55^{ns}
Block	1	0.99	2.54^{ns}	8.42	14.60^{**}
Remainder	17	0.39		0.58	

ns – non significant, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

der controlled conditions in a growth chamber and after one year, the plants were transferred to pots and moved to the experimental plot. Pots were arranged in two blocks with 6 plants (genotypes) from each family randomly positioned within each block**.** The pots were placed on black plastic sheets that prevented the assimilation of heavy metals from the soil.

After a couple of years of acclimation, fully-grown leaves from full-sib plants grown in the urban polluted environment were collected, as well as fully grown leaves from 17 clones grown in the unpolluted natural habitat (two samples per clone).

The plant material was dried at 60°C for 48 h and then pulverized. Microwave Digestion – EPA Method 3052 on the Multiwave 3000, was used to digest plant samples. The samples were analyzed by EPA 6010c (inductively coupled plasma-atomic emission spectrometry). According to the calibration curve, the reporting limit (RL) for Pb was 0.03 mg/kg, and 0.15 mg/kg for Ni.

Statistical analyses

Untransformed data were used for the analyses as normality was confirmed *by* Shapiro-Wilk test (Shapiro and Wilk, 1965). *One-way ANOVA* was used to test the differences between concentrations of Pb and Ni in the leaves of the I. pumila plants. Comparison within the polluted environment was performed by two-way ANOVA (randomized block design without replication) (Zar, 1999) with full-sib family and block as main factors. All statistical analyses were performed using SAS version 9.1.3 (*SAS* Institute, 2003) with PROC UNIVARIATE and PROC GLM procedures.

RESULTS AND DISCUSSION

In this pilot study of heavy metal accumulation in *Iris pumila*, the estimated value of Pb concentration in *Iris pumila* leaves (1.24 ± 0.11 μg/g) in the urban area was lower than in most samples of *Aesculus hippocastaneum* leaves in Belgrade urban areas (Aničić et al., 2011) or samples from *Platanus orientalis* and *Pinus nigra* collected at a nearby site (Sawidis, 2011), and to a lesser degree lower then the values measured in the leaves of *Tilia* sp. (Aničić et al., 2011). However, these concentrations were more than six times higher than those detected in *I. pumila* leaves from the unpolluted habitat (0.17± 0.11 μg/g); this difference was highly statistically significant (Table 1A).

This is consistent with findings indicating that the accumulation of Pb in plants in urban areas is higher in tree species then in herbaceous ones, and fits well in the range of reported herbaceous species (*Lyco-*

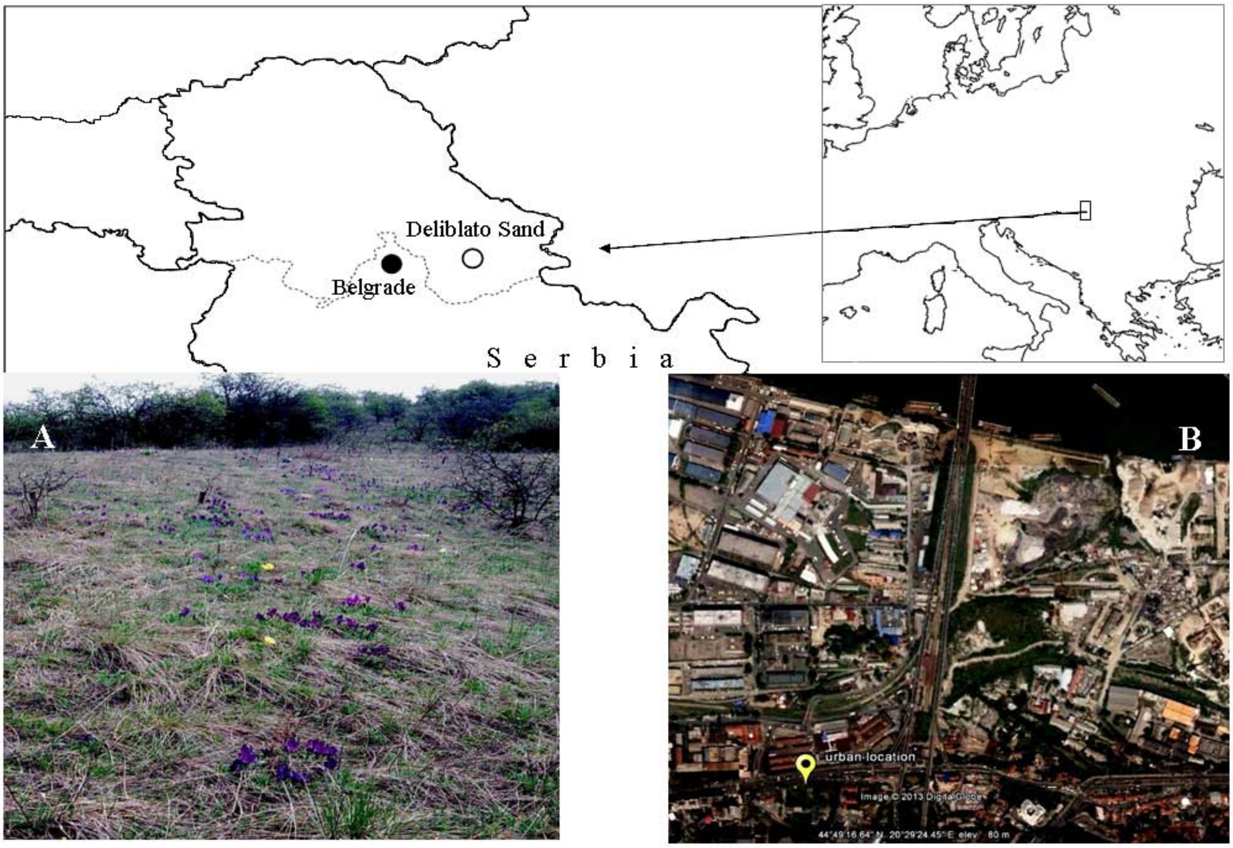

Fig. 1. The unpolluted (The Deliblato Sands Natural Reserve – white circle and picture A) and polluted (Belgrade, urban area – black circle and picture B) localities in Serbia utilized in this study.

persicum esculentum, Capsicum annuum, Solanum melan, Phaseolum vulgaris, Solanum tuberosum and *Petroselinum crispum*) in Yerevan, Armenia (Hovhannisyan and Nersisyan, 2009). On the other hand, the detected levels of Ni concentration in *I. pumila* leaves in the polluted urban location in Belgrade (1.85±0.14 μg/g) were higher than the concentrations reported for both *A. hippocastaneum* and *Tilia* sp. in the Belgrade urban area (Aničić et al., 2011). The concentration of Ni was also more than six-fold higher in the polluted than in the unpolluted natural habitat in the Deliblato Sands (the value for the unpolluted habitat was 0.27±0.12 μg/g). In addition, the concentration of Ni in *I. pumila* leaves responded to microenvironmental variability (significant Block effect in Table 1B). The histogram (Fig 2) shows the values of Pb and Ni concentrations in the leaves of *I. pumila* grown in polluted and unpolluted habitats.

In order to serve as a good indicator, the utilized species should show low variability in their responses to changes in environmental factors (Dale and Beyeler, 2001; Kurtz et al., 2001). Genetic variability for heavy metal accumulation has previously been shown, with the variability for Pb and Ni accumulation being greater than for other metals (Wang et al., 2006; Zeng et al., 2008 on rice). This variability can have a significant impact on the species' suitability to serve as a bioindicator. However, in this study we failed to detect significant between-family variations in the accumulation of Pb and Ni ($p = 0.2862$ and $p = 0.8827$ for Pb and Ni, respectively) (Table 1B), indicating stability of the plant's response. Therefore, the genetic variability of *I. pumila* populations should not greatly influence its usefulness as an indicator. These results demonstrate that *I. pumila* can serve as a reliable indicator

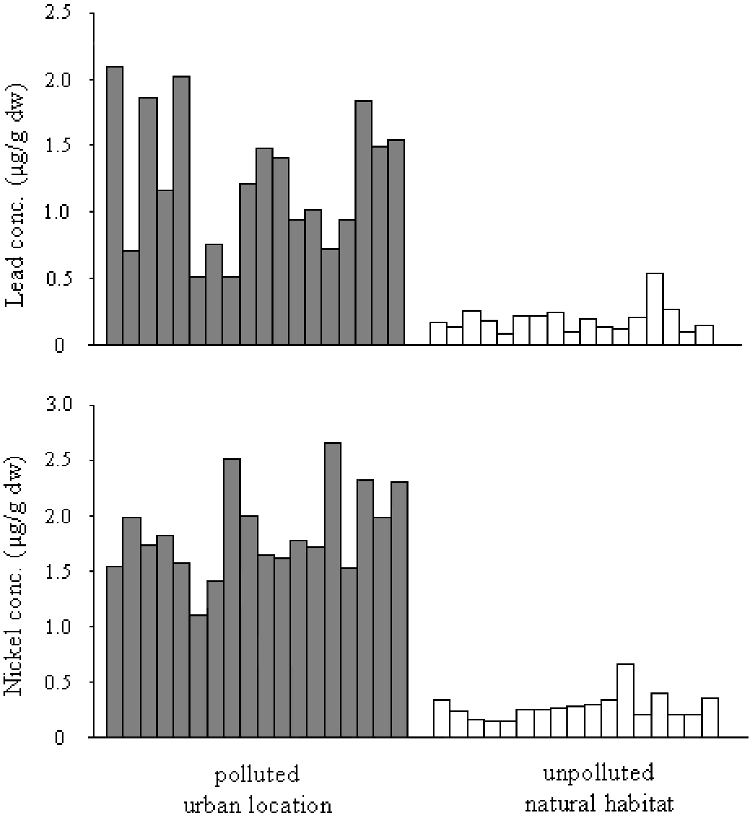

Fig. 2. The lead and nickel concentration (mg/kg) in leaves of *Iris pumila* genotypes grown at unpolluted natural (white bars) and genotypes grown in polluted urban (black bars) habitats.

of possible traffic pollution in protected areas, as in the Deliblato Sands Natural Reserve. Further studies should be conducted.

Acknowledgments – This work was funded by the Ministry of Education, Science and Technological Development of Serbia (project OI 173025 "Evolution in heterogeneous environments: mechanisms of adaptation, biomonitoring and conservation of biodiversity"). The funding agency was not involved in the study design, collection, analysis and interpretation of the data, or in writing and the decision to publish the results. The authors are grateful to Milana Kostić and Isidora Kecojević from AbiotechLab for the spectrophotometric analyses. The initial English language reader was Dr. Krinka Vidaković Petrov.

REFERENCES

Abdollahi, H., Fekeri, M. and *M. Mahmodabadi* (2011). Effect of heavy metals pollution on Pistachio trees. *Int. J. Agri. Biol.* **13**, 599-602.

Akguc, N., Ozyigit, I. I., Yasar, U., Leblebici, Z. and *C. Yarci* (2010). Use of *Pyracantha coccinea* Roem. as a possible biomonitor for the selected heavy metals. *Int. J. Environ. Sci. Tech.* **7,** 427-434.

Angermeier, P. and *J. Karr* (1994). Biological integrity versus biological diversity as policy directives: protecting biotic resources. *Bioscience,* **44**, 690-697.

Aničić, M., Spasić, T., Tomašević, M., Rajšić S. and *M. Tasić* (2011). Trace elements accumulation and temporal trends in leaves of urban deciduous trees (*Aesculus hippocastanum* and *Tilia* spp.) *Ecol. Indic.* **11**, 824-830.

Carreras, H. A. and *M. L. Pignata* (2002). Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. *Environ. Poll.* **117**, 77-87.

Census data (2011), Institute for Informatics and Statistics, City of Belgrade, Statistical Yearbook of Belgrade, 2011. LR

Dale, V. H. and *S. C. Beyeler* (2001). Challenges in the development and use of ecological indicators. *Ecol. Indic.* **1**, 3-10.

Dale, V. H., Beyeler, S. C. and *B. Jackson* (2002). Understory indicators of anthropogenic disturbance in longleaf pine forests at Fort Benning, Georgia, USA. *Ecol. Indic*. **1**, 155-170.

Fehmiye, K. (2003). Morpho-anatomical and karyological studies on *Iris pumila (Iridaceae). Bocconea*, **16,** 625-639.

Gajić, G., Mitrović, M., Pavlović, P., Stevanović, B., Djurdjević, L. and *O. Kostić* (2009). An assessment of the tolerance of Ligustrum ovalifolium Hassk. to traffic-generated Pb using physiological and biochemical markers. *Ecotoxic. Environ. Safety,* **72**, 1090-1101.

Gajić, M. (1983). Flora Deliblatske peščare. Prirodno matematički fakultet OOUR Institut za biologiju, Novi Sad. (in Serbian).

Grubačević, M., Gojković, M., Dudić, V., Pantelić, G., Tanasković, M., Mandić, M., Pajić, D., Majstorović, B., Milutinović, M. and *A. Tanasković* (2009). Kvalitet životne sredine grada Beograda u 2009. godini. Sekretarijat za zaštitu životne sredine; Gradski zavod za javno zdravlje; REC, Beograd.

Hovhannisyan, H. A. and *G. S. Nersisyan* (2009). A study of lead pollution of vegetation in Yerevan (Armenia). Book of proc. "Environmental Accounting and Sustainable Development Indicators", Czech, Prague, April, 23-24, 2009, http://ea-sdi.ujep.cz/en/conference-2009/full-paper-submissions.html.

Khaniki, G. R. J. and *M. A. Zazoli* (2005). Cadmium and Lead Contents in Rice (*Oryza sativa*) in the North of Iran. *Int. J. Agri. Biol.* **7,** 1026-1029.

Kurtz, J. C., Jackson, L. E. and *W. S. Fisher* (*2001).* Strategies for evaluating indicators based on guidelines from the Environmental Protection Agency's Office of Research and Development. *Ecol. Indic.* **1**, 49 - 60.

Mijić, Z., Stojić, A., Perisić, M., Rajsić, S., Tasić, M., Radenković, M. and *J. Joksić* (2010). Seasonal variability and source apportionment of metals in the atmospheric deposition in Belgrade. *Atmosph. Environ.* **44**, 3630-3637.

Miljković, D. (2012). Developmental stability of *Iris pumila* flower traits: a common garden experiment. *Arch. Biol. Sci*. **64**, 123-133.

SAS Institute Inc. (2003). SAS/STAT User's Guide, version 9.1.3, Cary, N.C., SAS Institute Inc.

Sawidis, T., Breuste, J., Mitović, M., Pavlović, P. and *K. Tsigaridas* (2011). Trees as bioindicator of heavy metal pollution in three European cities. *Environ. Pollut.* **159**, 3560-3570.

Saygideger, S., Dogan, M. and *G. Keser* (2004). Effect of Lead and pH on Lead Uptake, Chlorophyll and Nitrogen Content of *Typha latifolia* L. and *Ceratophyllum demersum* L. *Int. J. Agri. Biol.* **6,** 168-172.

Shapiro, S. S. and *M. B. Wilk* (1965). An analysis of variance test for normality (complete samples). *Biometrika,* **52,** 591-611.

Stjepanović-Veseličić, L. (1953). Vegetacija Deliblatske peSciare. - SANU, Beograd.

Tarasjev, A. (1997). Flowering phenology in natural populations of *Iris pumila. Ecography,* **20**, 48-54.

Tarasjev, A., Avramov, S. and *D. Miljković* (2012). Three decades of evolutionary biology studies on *Iris pumila* clonal plant: advantages of a good model system, obtained results and directions for further research. *Arch. Biol. Sci.* **64,** 159-174.

Tarasjev, A., Barisić Klisarić, N., Stojković, B. and *S. Avramov* (2009). Phenotypic plasticity and between population differentiation *Iris pumila* transplants between native open and anthropogenic shade habitats. *Russ. J. Genet.* **45,** 944-952.

Transport Master Plan of Belgrade (2012), Belgrade Land Development Public Agency, PTV, DDC, TREDIT, PNZ, GISTEC, 2008. LR. Pravilnik o tehničkim i drugim zahtevima za tečna goriva naftnog porekla, Službeni glasnik RS, 64/11. LR

Tucić, B., Milojković, S., Vujčić, S. and *A. Tarasjev* (1988). Clonal diversity and dispersion in *Iris pumila. Acta Oecol./Oecol. Plant.* **9**, 211-219.

Van der Gon, H. D. and *W. Appelman* (2009). Lead emissions from road transport in Europe. *Sci. Total Environ.* **407**, 5367-5372.

Wang, C., Zhang, G., Yao, H., Wu, W. and *M. Xu* (2006). Genotypic and environmental variation in cadmium, chromium, arsenic, nickel and lead concentrations in rice grains. *J. Zhejiang Univ - Sci. B.* **7**, 565-571.

Yunus, M. and *M. Iqbal* (1996). Plant Response to Air Pollution. Wiley, Chichester, UK.

Zar, J. H. (1999). Biostatistical analysis. Prentice Hall, New Jersey, **4.** ed.

Zeng, F., Mao, Y., Cheng, W., Wu, F. and *G. Zhang* (2008). Genotypic and environmental variation in chromium, cadmium and lead concentrations in rice. *Environ Pollut.* **153,** 309-14.