

CONTENTS OF THE HEAVY METALS NICKEL AND LEAD IN LEAVES OF *PAULOWNIA ELONGATA* S. Y. HU AND *PAULOWNIA FORTUNEI* HEMS. IN SERBIA

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Abstract — A comparative study was carried out on the capabilities of the two species *Paulownia elongata* S. Y. Hu and *Paulownia fortunei* Hems. as biomonitors of atmospheric (and soil) heavy-metal pollution. Research on content of the heavy metals nickel (Ni) and lead (Pb) in leaves of the species *Paulownia elongata* growing under urban and suburban conditions with the comparison to the concentration of these elements in leaves of the species *Paulownia elongata* and *Paulownia fortunei* on an experimental plot in Bela Crkva showed average nickel concentrations of 2.7 µg/g on the experimental plot in Bela Crkva, Serbia, twice as low as the concentrations measured under extreme urban conditions (6.62 µg/g, or 4.54 µg/g in the immediate vicinity of suburban traffic lines). Inasmuch as *Paulownia elongata* endures urban conditions well, it can be recommended for cultivation in parks, tree alleys, and wind-protection zones along urban and regional traffic lines.

Key words: *Paulownia*, urban conditions, traffic lines, leaf, concentration, nickel, lead, exploitation, tree alleys, wind-protection zones

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INTRODUCTION

The quality of air in the urban environment is conditioned by emissions of sulfur dioxide (SO₂), nitrogen dioxide (NO₂), carbon dioxide (CO₂), soot, powders, and other substances originating from thermal power plants, industry, traffic, burning in individual furnaces, etc. The list of very specific substances that pollute the air includes lead, cadmium, manganese, arsenic, nickel, chrome, zinc, and other heavy metals and organic compounds resulting from different activities (Marković et al., 2007). Lately, the poor quality of atmospheric air in the urban environment is a consequence of increase in the number of motor vehicles. Huge air pollution is a result of exploitation of leaded gasoline and gas oil with high content of sulfur.

High concentrations of heavy metals cause numerous anatomical, morphological and physiological changes in plants (Veselinović, 2002). Heavy metals affect the metabolism of

phytohormones and further division and elongation of cells, i.e., they affect plant growth. Moreover, they reduce the intensity of photosynthesis, inhibiting transport through the electron transport chain and synthesis of photosynthetic pigments (Veselinović, 2006). As a result, the accumulation of organic matter in plants is reduced. Heavy metals also affect the water regime of plants by reducing absorption of water and its transport into the overground organs, increasing diffusion resistance of the stomata, and thus reducing transpiration.

The emission of polluting fuels and heavy metals is regulated by rules stipulating marginal values of content as measured by methods in accordance with the effective Republic Law on Environmental Protection (Official Gazette No. 66/91 and Official Gazette No. 54/92). The EU allows a concentration of 0.5 µg/g of lead in the air on an annual basis, while WHO standards prescribe 0.05 µg/g of lead in water. Regulations of the Republic of Serbia allow 1 µg/g

of air over 24 h in both populated and unpopulated areas.

Accumulation of heavy metals in plants occurs constantly during vegetation and all year round, reaching a peak as a rule at the end of the growing season (Krstić et al., 2007).

With respect to lead (Pb), a large number of tested vegetable species showed a higher value of accumulation (from 50 to 100%) at the end in comparison with the beginning of the growing season (Stanković, 2006).

There has been huge interest lately in the species *Paulownia elongata* and *Paulownia fortunei*, which in urban conditions are being cultivated in parks or tree alleys on account of their decorative characteristics. Owing to the large surface of their leaves, which on the underside are densely overgrown with hairs, these species absorb significant quantities of sulfur dioxide in combination with particles of dust from the air (Šijačić-Nikolić et al., 2008). The most important characteristic of these species is their extremely rapid growth, which puts them among the fastest growing trees in the world (Vilotić, 2005).

According to Wang et al. (2009), Pb contents in leaves of *Paulownia fortunei* significantly increased during the growing season and were higher than in stems and roots, indicating an important pathway of internal Pb and Zn transport from the roots and stems to the leaves, thus confirming a common mechanism whereby plants protect themselves from toxicity of Pb (and some other heavy metals), i.e., through higher metabolic activity of their leaves.

MATERIALS AND METHODS

For the purpose of determining concentrations of the heavy metals nickel (Ni) and lead (Pb) in leaves of *Paulownia elongata* and *Paulownia fortunei* growing in urban conditions, samples (leaves) were collected at three locations near the end of the growing season:

Tree mark	Location	Location description
K	Bela Crkva	Control trees on an experimental plot of an agricultural land project

1	Mali Mokri Lug	Trees in suburban conditions 10 m away from a main traffic line
2		Trees in suburban conditions 1 m away from a main traffic line
3	Bulevar Kralja Aleksandra	Trees in urban conditions along a main traffic line
4		Trees in urban conditions in the green zone between two lanes of a main traffic line

At the Bela Crkva location (control), samples were collected from trees of two different species, viz., *Paulownia elongata* and *Paulownia fortunei*, from two different places on the trees (at the bottom and top of the tree crown):

Tree mark	Species	Place of sampling
E1	<i>Paulownia elongata</i> S. Y. Hu	Top of tree crown
E2		Bottom of tree crown
F1	<i>Paulownia fortunei</i> Seem. Hems.	Top of tree crown
F2		Bottom of tree crown

At all other locations, samples were taken from the middle of the crown of trees of the species *Paulownia elongata*.

Samples were dried at room temperature without prior washing until they became an air-dry mass. Air-dry leaves were then dried in a dry-kiln at 105°C.

After sampling, the vegetable matter was classified and dried at 105°C. Analysis of chemical composition of the plants was performed in accordance with standard procedure (APHA, 1995). Concentrations of Ni and Pb were determined by AAS after dry burning at 450°C and treatment with HCl.

The obtained data were statistically processed using Statgraph software. Influence of the type and place of sampling on the concentrations of Ni and Pb in leaves from the location of Bela Crkva was measured by means of two-factorial analysis of variance with interactions. The influence of location on the concentration of Ni and Pb in leaves of *Paulownia elongata* was measured by means of single-factor analysis of variance.

Testing for the presence of significant differences between two average values in both cases was

performed by Duncan's test for a significance level of 95%.

RESULTS AND DISCUSSION

Nickel (Ni) is a heavy metal of the VIII-B group of the periodic table of elements with seven radio isotopes. Although the physiological function of Ni is not completely clear, there is a prevailing opinion that this microelement is necessary in metabolic processes of higher plants (Mengel and Kirkby, 1982). On the other hand, toxicity of Ni for plants is very well-known. Symptoms of toxicity include chlorosis, the cessation of root system growth, and occasionally inter-neural necrosis (Uren, 1992). Increased quantities of Ni can be a result of natural processes or anthropogenic factors. The main anthropogenic source of nickel emission is combustion of fossil fuels and traffic (Krstić et al., 2007). Exhaust gases resulting from the combustion of gas oil contain more than 40 air pollutants (OEHHA and ALAC Reports, 2003), nickel compounds among others (nickel-oxide, nickel-sulfate, and nickel-subsulfide). All these compounds are harmful to plants and may also be genotoxic or cancerogenic for animals and humans (U.S. Environmental Protection Agency, 2005).

Lead is a heavy metal that has an extremely harmful effect on plants as well as on animals and humans, since it has a cumulative retarded effect. The main source of Pb are the exhaust gases from vehicles that use gasoline with tetraethyl lead and tetramethyl lead, which are antidetonators in gasoline for internal combustion engines. Tetraethyl lead and tetramethyl lead are mixed with chloride and bromide hydrocarbons in order to be transformed into inorganic compounds of lead in the process of combustion in engines; however, after combustion these organic compounds of lead appear as exhaust gases. Their negative influence on the environment results from the harmful effect of lead, which settles along roads on soil, vegetation, and agricultural crops. Lead has a distinctly toxic effect on plants, as well as on animals and humans.

Biological remediation of contaminated soil is an effective method of reducing the risk to

human and ecosystem health. Apart from chemical measurement, biological evaluation of the ecological situation is desirable. Biological indicators are used for this purpose. Accumulation of heavy metals in plant tissues creates an important role for different plant species as (bio)indicators of environmental pollution (Ten-Houten, 1983; Prasad and Freitas, 2003).

In plants lead is absorbed through root and foliar absorption. While inorganic lead is poorly absorbed, organic compounds of lead, especially the side products of lead from gasoline, are absorbed well and are very quickly transported from roots to overground parts (Kastori, 1997). The physico-chemical properties of heavy metals, together with their distribution and bioavailability in soil and air, are factors that exert significant effects on bioremediation. It has been demonstrated that lead inhibits plant growth (Mukherji and Maitra, 1977), sprout growth (Lane and Martin, 1977), elongation of cells (Lane et al., 1978), synthesis of chloroplast pigments (Kastori et al., 1996), and photosynthesis (Bazzaz et al., 1974). The numerous nonspecific effects of heavy metals depend on their direct and indirect action in specific metabolic processes; for example, high Ni content in endoderm and pericycle cells blocks cell divisions in the pericycle and results in the inhibition of root branching (Seregin and Kozhevnikova, 2006).

Accumulation of lead in plants along roads depends on the distance of the plants from the road, the level of coverage of soil with vegetation, the direction and intensity of wind, frequency of passenger traffic, and the time of idling of vehicle motors (Stanković, 2008). Lead concentration in plants progressively decreases with distance from the road (Milošević et al., 2001).

The level of accumulation of heavy metals in vegetable tissue is determined by numerous biotic and abiotic factors, one of the most dominant of which is particularity of the genotype (Nikolić et al., 2008; Pajević et al., 2008). For certain heavy metals, there is also interspecies calibration (Berlizov et al., 2007). According to numerous published data, species of *Paulownia* are efficient excluders of heavy

metals (Shaw, 1990; Wang et al., 2009).

Also, according to many authors (Rubio et al., 1994; Ahmad et al., 2007), metal uptake and accumulation in plants induced a decrease in K, Ca, and Mg contents in the plants, particularly in their shoots, indicating that heavy metals, Ni especially, interfered not only with nutrient uptake, but also with nutrient distribution into the different plant parts.

Results of the performed research indicate that lead concentration values in leaves of the species *Paulownia elongata* range from 1.00 (control tree) to 3.16 (tree 4) $\mu\text{g/g}$. Nickel concentration values range from 2.70 (control tree) to 6.62 (tree 4) $\mu\text{g/g}$ (Table 1).

The results clearly show that contents of the analyzed elements are highest in leaves of trees growing in the green zone between two lanes of the main traffic line, which indicates the causality of Ni and Pb concentrations on the one hand and traffic density or frequency on the other.

Increased concentrations of Pb and Ni are probably to a large extent the consequence of atmospheric deposition of particles with adsorbed and absorbed pollutants, since there are data which show that in some plant species (*Fraxinus* for example) the root uptake and xylem transport of elements are generally not intense enough to hide the atmospheric flux of mineral contaminants (Catinon et al., 2008).

Table 1. Concentrations of Ni and Pb in leaves of *Paulownia elongata* S. Y. Hu ($\mu\text{g/g}$).

Tree mark	Ni ($\mu\text{g/g}$)	Pb ($\mu\text{g/g}$)
K	2.70	1.00
1	3.23	1.09
2	4.54	0.92
3	3.11	2.28
4	6.62	3.16

Table 2. Concentrations of Ni and Pb in leaves of *P. fortunei* and *P. elongata* ($\mu\text{g/g}$) at the Bela Crkva location.

Tree mark	Ni ($\mu\text{g/g}$)	Pb ($\mu\text{g/g}$)
E1	2.59	0.74
E2	2.81	1.26
F1	3.49	0.80
F2	2.69	1.06

Comparative analysis of Ni and Pb in leaves of the plant species *Paulownia elongata* and *Paulownia fortunei* at the Bela Crkva location (Table 2) indicates higher concentrations of analyzed elements in samples taken at the bottom of the tree crown in both tested species.

These data also indicate that the atmospheric deposition of pollutants on plant leaves is a very important factor in determining the absorption route and metabolism of heavy metals in plant cells. However, there are many studies which suggest that the route of Pb uptake through roots and transport into leaves is also a very frequent metabolic mechanism whereby plants protect themselves from toxicity of Pb, increased metabolic activity of leaves making detoxication possible (Wang et al., 2009). The bioavailability of heavy metals is an important factor and according to Doumett et al. (2008), the distribution of Cd, Cu, Pb, and Zn between contaminated soil and the tree species *Paulownia tomentosa* was significantly influenced by the presence of a specific chemical form of metal. They obtained significant enhancement of metal uptake and translocation to leaves in response to application of a complexing organic agent.

Two-factorial analysis of variance for plant species (*P. fortunei* and *P. elongata*) and the place of sampling (the top of the tree crown) clearly indicates that there are statistically significant differences in the average concentrations of nickel in leaves of *Paulownia elongata* and *Paulownia fortunei*, since the level of significance is $p = 0.0000 < 0.05$. Moreover, it can be concluded that there is an interaction between factors A and B (species and sampling place), which means that influence of the species on average Ni concentrations in the leaves is not independent of the sampling location or rather that the concentration of the analyzed element in leaves from the bottom and top of the crown is affected by the species (Table 3). Furthermore, variance analysis indicates the existence of statistically significant differences in average concentrations of Ni at the top and bottom of the tree crown in both analyzed species.

Interaction of nickel pollution (Ni) with plant species and place of sampling on the experimental

Table 3. Analysis of variance for nickel (Ni). All F-ratios are based on the residual mean square error.

Source	Sum of squares	Df	Mean square	F-Ratio	P-Value
Main effects					
A: Species	0.444675	1	0.444675	762.30	0.0000
B: Place	0.190008	1	0.190008	325.73	0.0000
Interaction					
AB	0.765075	1	0.765075	1311.56	0.0000

Table 4. Analysis of variance for lead (Pb). All F-ratios are based on the residual mean square error.

Source	Sum of Squares	Df	Mean square	F-Ratio	P-Value
Main effects					
A: Type	0.0075	1	0.0075	0.25	0.6326
B: Place	0.425633	1	0.425633	14.02	0.0057
Interaction					
AB	0.0408333	1	0.0408333	1.34	0.2796

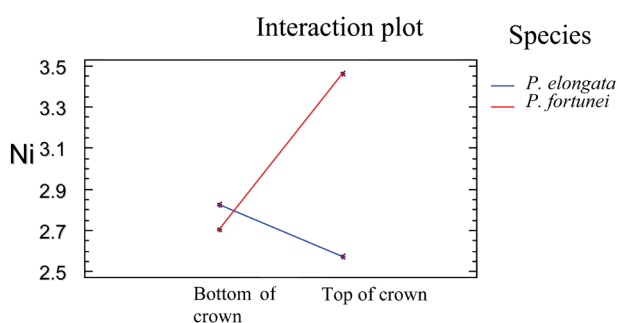


Fig. 1. Interaction of nickel pollution (Ni), between plant species and place of sampling on the experimental plot in Bela Crkva.

plot in Bela Crkva (Chart 1) indicates that Ni concentration rises from the bottom to the top of the tree crown in *Paulownia fortunei*, while the concentration falls in the same direction in *P. elongata*. Nickel shows good mobility in xylem as well as phloem, and it accumulates to a great extent in fruits and seeds. Plant species differ in accumulation of Ni, and ones that in dry matter contain more than 1 mg of Ni g⁻¹ fall into the category of hyperaccumulators.

As for Pb (in contrast to Ni), analysis of the influence of species shows that there are no statistically significant differences of concentrations in leaves of *P. elongata* and *P. fortunei*, since the level of significance is $p = 0.6326 > 0.05$.

In the case of the sampling place factor, it is the same with Pb as with Ni, i.e., there are statistically significant differences in the average concentrations in leaves from the bottom and top of the crown, inasmuch as the level of significance is $p = 0.0057 < 0.05$. Since $p = 0.2796 > 0.05$ with 95% certainty, it can be asserted that Pb analysis shows no interaction between factors A and B (type and place of sampling).

In order to obtain information about significance of the difference in pollution at the analyzed locations, the data were processed by variance analysis and Duncan's test. The object was to establish whether there are statistically significant differences (with a probability of 95%) between average values of pollution at the analyzed locations (Charts 2 and 3).

On the basis of the obtained results of Duncan's test, it can be concluded that concentrations of the analyzed elements (Ni and Pb) differ statistically significantly by locations and range from A to E. The results indicate that the concentration of heavy metals adsorbed and absorbed by *Paulownia* leaves can be used as a very reliable indicator of air (and soil) chemical pollution. The significant difference obtained for the concentration of pollutants at different locations of test plants unquestionably indicates the presence of contamination by nickel and lead in areas of frequent traffic. Moreover, the

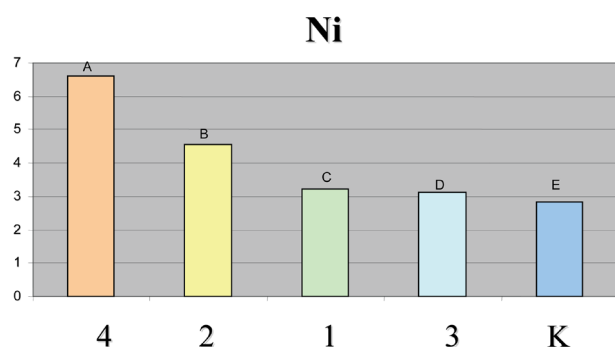


Fig. 2. Nickel content in *Paulownia elongata* at the analyzed locations.

presence of significantly higher concentrations of lead in leaves of *Paulownia elongata* sampled from the control location (Bela Crkva) in relation to the Mali Mokri Lug location indicates the presence of chemical contamination primarily of air, but also of soil by this pollutant.

The source of this pollution is traffic and probably a higher concentration of airborne soot and other particles that absorb lead, which then accumulates on vegetation. An airborne pollutant may spread over hundreds and even thousands of kilometers, depending on meteorological conditions, especially wind direction and speed. It is therefore necessary to establish comprehensive monitoring of a wide area. Such monitoring should involve defining the values of physico-chemical parameters of soil and air, as well as a group of biological parameters.

Average Ni concentrations of 2.7 µg/g on the experimental plot in Bela Crkva are twice as low as the concentrations in extreme urban conditions (6.62 µg/g, sample 4) and in the immediate vicinity of suburban traffic lines (4.54 µg/g, sample 2), which is in accordance with the data given by Frey (1967), who reported higher concentrations of Ni near frequently traveled roads as a consequence of fuel combustion. Nickel concentration in gas oil from exhaust pipes ranges from 500 to 10,000 (mg/dm³). In our region, the greatest (toxic) concentrations of Ni were recorded in sessile oak (*Quercus petraea* Matt. Liebl.) in Fruška Gora National Park (16.16 µg/g) and in silver lime (*Tilia tomentosa* Moench) on Mt. Fruška Gora (15.39 µg/g) (Stanković, 2006). On the basis of the performed research, it can be concluded that Ni and Pb concentrations in leaves of *P. elongata*

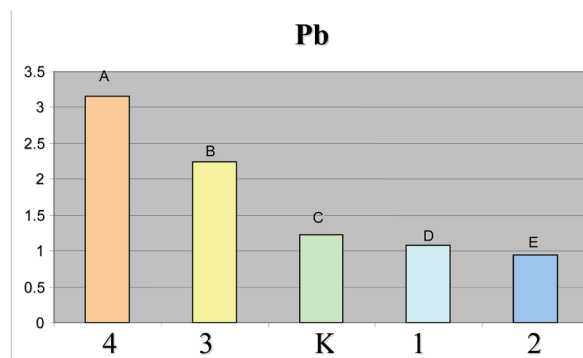


Fig. 3. Lead content in *Paulownia elongata* at the analyzed locations.

growing outside the direct harmful effects of main traffic lines are under the critical level. According to Asher (1991), the critical concentrations are > 10 µg/g for sensitive species and > 50 µg/g for moderately tolerant species.

The performed research showed that the highest concentrations of Ni and Pb are recorded in the leaves of sample 4 (*P. elongata* growing under urban conditions in the green zone between two lanes of one of the most frequently traveled traffic lines in Belgrade). The lowest concentrations are recorded in leaves of the control sample on the experimental plot in Bela Crkva, far from main traffic routes and isolated in a complex of agricultural land.

The average Ni concentrations of 2.7 µg/g recorded on the experimental plot in Bela Crkva are more than twice as low as those recorded under extreme urban conditions (6.62 µg/g) or in the immediate vicinity of suburban traffic lines (4.54 µg/g).

Comparing the two species *Paulownia elongata* and *Paulownia fortunei* growing on the experimental plot in Bela Crkva, we note that average Ni concentrations are lower in leaves of *P. elongata* than in those of *P. fortunei*, while the situation with Pb is the reverse. The obtained differences in concentrations are statistically significant only for Ni.

Because the species *P. elongata* endures urban conditions well, it can be recommended for cultivation in parks, tree alleys, and wind protection zones along urban and regional traffic lines. In addition, it is a highly decorative species that is very

prominent in the blossoming period.

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САДРЖАЈ ТЕШКИХ МЕТАЛА НИКЛА И ОЛОВА У ЛИСТОВИМА *PAULOWNIA ELONGATA* S. Y. HU И *PAULOWNIA FORTUNEI* НЕМС. ИЗ СРБИЈЕ

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Компаративна студија способности две биљне врсте *Paulownia elongata* S. Y. Hu и *Paulownia fortunei* Немс. показује да су оне биомонитори загађења ваздуха (и земље) тешким металима. Истраживања садржаја тешких метала, никла (Ni) и олова (Pb), у листовима стабала врсте *Paulownia elongata* која расту у градским и приградским условима у поређењу са концентрацијом ових елемената у листовима стабала врста *Paulownia elongata* и *Paulownia fortunei* на огледном

пољу у Белој Цркви показују да су евидентиране просечне концентрације никла од 2.7 µg/g на огледном пољу у Белој Цркви, двоструко мање од оних евидентираних у екстремним градским условима 6.62 µg/g или у непосредној близини приградских саобраћајница 4.54 µg/g. С обзиром да *Paulownia elongata* добро подноси градске услове може се препоручити за гајење у парковима, дрворедима и ветрозаштитним појасевима поред урбаних и регионалних саобраћајница.