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Full Length Research Paper

Woody plants and herbs as bioindicators of the current condition of the natural environment in Serbia

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Being the inhabitants of all parts of the environment (water, air, soil) plants can very precisely point to the ecological conditions of the environment. Vascular plants are often used for biomonitoring of heavy metals. The analyses are most often performed on plant leaves. The results of this research clearly indicate that pollution with lead on the locations of Avala (locations 1, 2 and 3), although being of an anthropogenic origin, do not result only from traffic, while on location 4 due to the frequency of traffic, the greatest agent of lead pollution is traffic. The accumulation of heavy metals in plants in higher concentrations indicates the relative increase and pollution spread all over the habitat.

Key words: Protected natural resource, belgrade, plants, lead concentrations.

INTRODUCTION

The current condition of the ecological system on the Earth originates from unreasonable utilisation of natural resources by humans, which came as a consequence of heavy economic and political crises on the global level before the massive warfare. The preserved, healthy environment now has a "strategic" importance which will even increase in the near future. This process moves people from 'contaminated' to quality 'sanatogenous' environments – the so called green or 'eco' destinations among which the preferrential place is taken in the front of every one by the protected natural resources. Belgrade stands for one of the greenest capital cities in Europe, exactly due to the fact that it has those natural oases nearby the city centre. The protected natural resource, or rather one selected part which is declared the landscape of outstanding features is located on the territory of the city of Belgrade, Vozdovac municipality and it includes parts of cadastral municipalities: Beli Potok, Ripanj, Zuce and Pinosava. The total area of the protected natural resource amounts to 489.13 ha out of which 74.35 ha (15.2%) is privately owned and 414.78 ha (84.8%) is under other forms of ownership. The location of the protected natural resource has the following coordinates in the state coordinate system, y = 74 62 425 (44˚ 37' 33"), x = 49 49 675 (20˚ 30' 01"), with the altitude of 210 to 506 m (Special basis of management of forests, 2008 to 2017). The implementation of vascular plants for indication of soil pollution is based on their ability to 'absorb' metals (especially heavy metals) and other toxic substances from the soil and transport them through their organism or to accumulate them in a specific part. The term 'bioindicators' was first used by Clements (www.patentlens. net/daisy/Bioindicators.html) in order to signify the organisms, which by their presence in a particular habitat, clearly indicated the ecological conditions of that habitat are. The unavoidable segment of the monitoring system is the biological monitoring, which implicates the implementation of living organisms as bioindicators of changes in the environment in space and time (http://www.15-bioloski-monitoring-z.ppt).

Beside chemical measurements, it is desirable to perform the biological assessment of the ecological condition. For this purpose, we use the biological

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indicators. Accumulation of heavy metals in plant tissue indicates the very important role of certain plants as (bio)indicators of environmental pollution (Prasad et al., 2003; Ten Houten, 1983).

Kurfurst (1989) and Guthner (1989) point out that heavy metals, due to their adverse effect on the environment to biosphere, attract the attention of researchers more and more, especially due to their harmful effect on living organisms which increases because of longterm exposure and cummulative effect. Heavy metals are accumulated in the organism and are deposited through the food chain. A very important source of heavy metals and other pollutants in soil and plants is passenger traffic (Primault, 1958; Fidora, 1972; Johnson, 1980; Memon et al., 2001; Stanković et al., 2008, 2009; Stanković, 2008).

Monitoring of heavy metals is especially important due to the fact that their toxicity and accumulation is high. The harmful effects calculated on an annual basis of all those metals exceed the overall harmful effect of radioactive and organic waste produced each year as claimed in the works of Nriagu (1979) and Pacyna (1989). The uptake of heavy metals by plants is performed constantly during the vegetation period and all over the year reaching, as a rule, the highest value at the end of the vegetation period (Krstić et al., 2007; Stanković, 2006; Stanković et al., 2011).

Potentially, each plant species may be used as a bioindicator of the environmental condition. The necessary precondition is knowledge of biology, as well as ecology (ideoecology) of each individual species which is to be used as a bioindicator.

EXPERIMENTAL PROCEDURES

Considering the fact that the heavy metal lead (Pb) shows an extremely harmful effect than the others, the main source of Pb are the exhaust gases from vehicles, as well as the heavy metals concentration. Particularly, lead concentration in plants is a specific kind of ability for certain plant species to accumulate lead. This paper has the task to present the analysis of contents of heavy metal lead (Pb) in the leaves of 8 plant species, growing in the protected natural resource area "Avala", and one more location in urban conditions in the centre of the city of Belgrade in the street with the highest traffic frequency.

The selection of the locations was, before all, conditioned by the situation in the field. The selection of representative locations for sampling of plants and soil for analysis was performed very carefully, bearing in mind that it was necessary that each of the selected locations contained the same plant species. It was also important to determine the key parameters from the point of view of emission and immision of the observed heavy metals, the distance from the road and many other relevant criteria.

Three locations were selected for sampling in the area of the landscape of outstanding features – the Avala Mountain. The selected location in accordance with the special basis for management of forests for management unit “Avala” (2008 to 2017) belong to MU “Avala”:

Location 1 – At the upward road to the landscape of outstanding features "Avala".
Location 2 – On top of the Avala Mountain (near the Avala Tower).
Location 3 – On the downward road from Avala Mountain (Stari Majdan).

After examination of several potential locations, we selected the fourth location - the so called control location, in the centre of Belgrade in Bulevar despota Stefana street (former street name was 29. novembra), where there is high frequency of traffic.

Location 4 – Control: Bulevar despota Stefana street in the centre of Belgrade city.

For conducting the detailed research of the concentration of pollutants, the contents of heavy metals were analyzed in the vegetative parts of 8 selected plant species.

In the process of selecting plant species for the analysis, we performed a very detailed inventory list of all plant species present in this area. As such, we selected the following 8 plant species that were present on all three locations on Avala:

1. *Tilia tomentosa Mnch*. - Silver Lime
2. *Pinus nigra Arn*. - European Black Pine.
3. *Plantago media L*. - Hoary plantain
4. *Taraxacum officinale Web*. - Common Dandelion
5. *Acer campestre L*. - Field Maple
6. *Prunus avium L*. - Wild cherry, Gean
7. *Quercus petraea (Matt.) Liebl.* - Sessile Oak
8. *Pseudotsuga menziesii (Mirb.) Franco* - Coast Douglas-fir, common Douglas-fir

For the purpose of control on the fourth location in the centre of the city, 4 plant species were chosen as the representative sample. On location 1, we also sampled the eighth plant species, that is, Douglas fir.

Bearing in mind the fact that the quantity of heavy metals in plants directly depended on the distance from the road on each location, the plants were taken with a length of 200 to 300 m along the road and up to 15 m in the depth from the road. However, only plant leaves were sampled. The samples of the plant material were collected on each of the mentioned locations in the middle of their vegetation period at the beginning of July 2009. Only leaves were sampled, and for each species, 1 to 2 kg of the material was taken from all locations. The samples were dried to airy dry mass without previous washing. Airy dry leaves were further dried in dry-kiln at 105°C, and were ground and used for laboratory analyses.

The analyses were performed in the laboratories of the Department of Biology and Ecology, Faculty of Natural Sciences in Novi Sad, and the Institute for Lowlands Forestry and Environmental Protection.

Preparation of samples for plant analysis

The preparation of samples for the analysis of heavy metals contents commenced by measuring approximately 5 g of the homogenous metals, after which dry plant material is crushed in a beaker and placed on a hotplate where it is heated until it is totally charred. Then, it is brought into a furnace for burning at the temperature of 450°C. After complete combustion and cooling, 2 to 3 ml of 30% hydrogen peroxide is added to the sample and is further steamed on the hotplate until it becomes dry. The burning in the furnace continues on a temperature of 450°C up to one hour. The dry sample is then added to the 10 ml of 25% solution of hydrochloric acid and is slowly steamed until it becomes 1/3 of its volume, after which the sample is quantitatively transferred by boiling distilled water from beaker into a measurement cup through filter paper. After cooling, the measurement cup is filled with distilled water (Maksimovic et al., 2002). The concentrations of heavy metals in plants are determined by the atomic absorption spectrophotometry method.

RESULTS

Comparing the obtained results with the analyzed plant species, it is notable that there are significant differences among plant species irrespective of location. The greatest lead concentrations on all locations are determined in Dandelion (Taraxacum officinale), depending on the location that the lead contents range from 3.07 µg/g on the first location to 20.01 µg/g on the fourth location (Figures 1 to 4).

The results indicate that out of the analyzed plant species, Dandelion (*Taraxacum officinale*) and Plantain *(Plantago lanceolata*) are hyperaccumulators of lead in comparison to other analyzed plant species.

Kabata-Pendias and Pendias (1989) discovered that lead contents ranged from 0.19 to 9 mg/kg of dry substance in the overground parts of herbs in the unpolluted soil, while lead contents in the polluted soil amounted from 63 to 232 mg/kg. The same authors stated that ordinary concentrations of lead in plants ranged from 5 to 10 mg/kg.

The measured lead concentrations in plants on Avala when compared to the maximum allowable concentrations, in accordance with legal regulations in Serbia, ranged within allowable values or do not often exceed the maximum allowable concentrations. Lead concentrations in plantain, on the second location, stand out with the value of 31.30 µg/g. Such high value of lead accumulation on this location does not originate from traffic, yet the influence of bombing and destruction of the old Avala Tower is vast. This assumption may be further confirmed by the results of the research of lead concentrations in soil on this location where we recorded high values amounting to 114.06 mg/kg in the first layer and in the second soil layer, the values were to a certain extent lower, yet they exceeded the maximum allowable concentrations and amounted to 103.24 mg/kg.

The recorded lead concentrations in plants on locations 1, 2 and 3 are proportionally less than concentrations on location 4, which can be explained by the fact that those three locations are situated on Avala, yet location 4 is situated in the centre of the city where traffic frequency is substantially higher. Lead concentrations in woody plants included in this analysis range from 1.12 µg/g in oak to about 10.00 µg/g in lime.

As a very interesting indicator appears in the recorded lead concentrations in the conifer, Pinus nigra are higher than the case of deciduous trees. An exception is the Wild Cherry on the second location with the value of 8.65 µg/g, and on location 4, the values are approximately the same as in lime. The value of lead contents in Black Pine is 9.85 µg/g, and in the other woody species (lime), it is a bit higher and amounts to 10 µg/g on location 4 (Figure 4).

Considering the four plant species (Lime, Black Pine, Plantain and Dandelion) present on all four locations as is clearly shown in the chart, Dandelion and Plantain on all locations in comparison to all analyzed plant species in most cases shows higher values of lead contents (Figure 5).

Comparing the plant species on locations situated on Avala, beside the highest lead concentration on the second location, the extremely greatest concentration of lead accumulation in Plantain (*Plantago lanceolata,* 31.3 µg/g) clearly stands out.

These data indicate among other things that this value is significantly above allowable concentrations and that, in plantain, it certainly causes reduction of physiological activities. Moreover, plantain may be classified into plants, that is, the hyperaccumulators of heavy metal lead, since it shows high values on all locations as shown in the Chart and especially on locations 2 and 4.

DISCUSSION

One of the greatest polluters (lead) enters human organisms through plants in different ways causing series of disturbances in the organism where it is deposited as cummulative retarded toxin.

In humans, lead causes general intoxication and it especially endangers the nerve cells changing the permeability of the cell membrane. Organic compounds of lead (tetraethyl and tetramethyl lead) show greater affinity towards fatty acids and are attached to the brain or nerve walls rather.

Uptake of lead by plants is in the form of ions (Pb^{2+}) or in the form of organic compounds: tetramethyl and tetraethyl lead. However, the very mechanism of lead uptake is not fully uncovered. The maximum concentrations of these compounds in gasoline is regulated by law in many countries and must not exceed 1.12 g/l.

Due to the importance of this problem, many countries in the world (USA, Japan, Brazil, Austria, European Union and others) limited the maximum contents of lead additives in gasoline to the values less than 0.15 g/l or they totally exclude it.

In comparison to EU countries, in Serbia, we consume fuel with high content of lead additives (approximately 0.5 g/l), while the share of unleaded gasoline in total consumption amounts to only 2%.

The greatest quantities of lead are emitted in the combustion of gasoline (more than $270*10^6$ kg a year) where lead content amounts to 0.45 g/l. There are a lot of published works elaborating this topic and indicating the substantial accumulation of lead in plants, especially nearby highways (Heilenz, 1970; Sommer, 1976), as well as dynamics of lead in soil and its uptake by plants (Höll et al., 1975).

In accordance with the research of Janjatovic et al. (1991), one of the consequences of greater concentrations

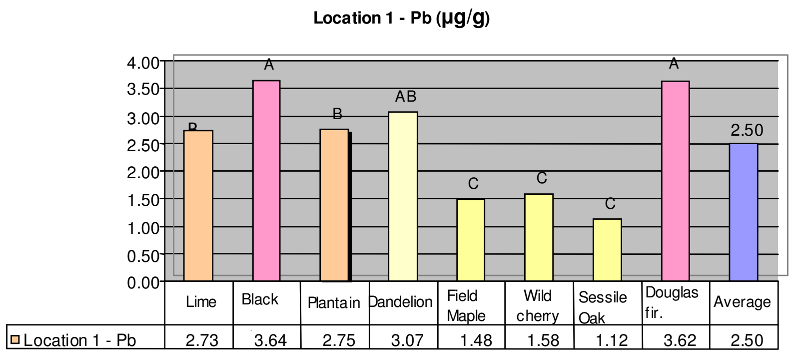


Figure 1. Lead contents on the first location distributed by plant species.

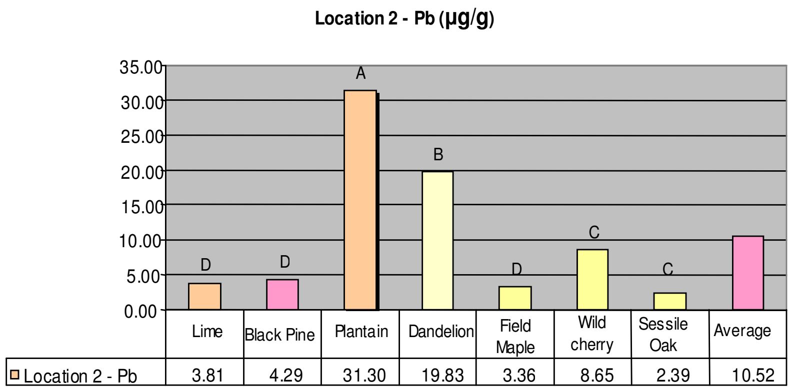


Figure 2. Lead contents on the second location distributed by plant species.

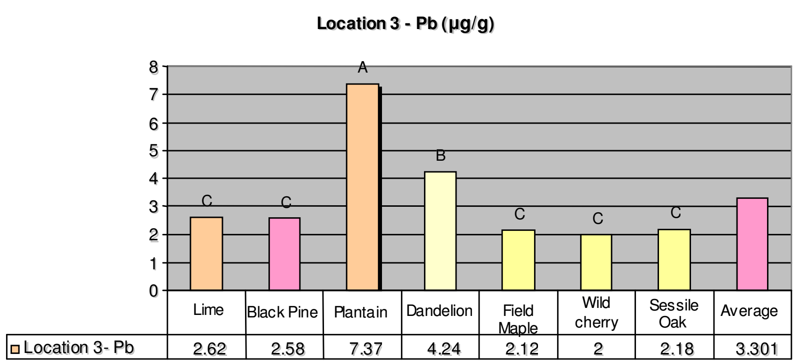


Figure 3. Lead contents on the third location distributed by plant species.

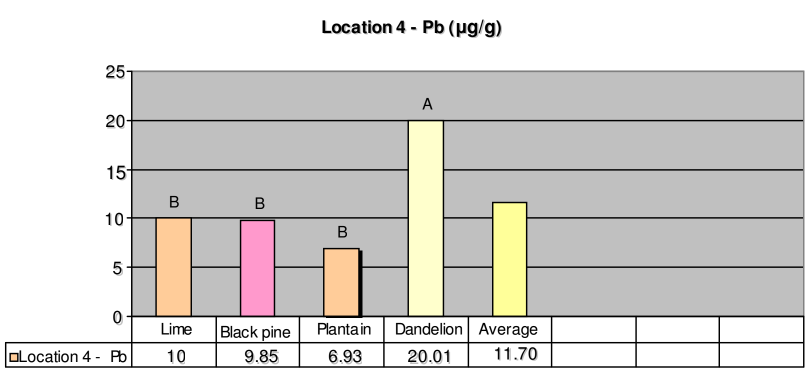
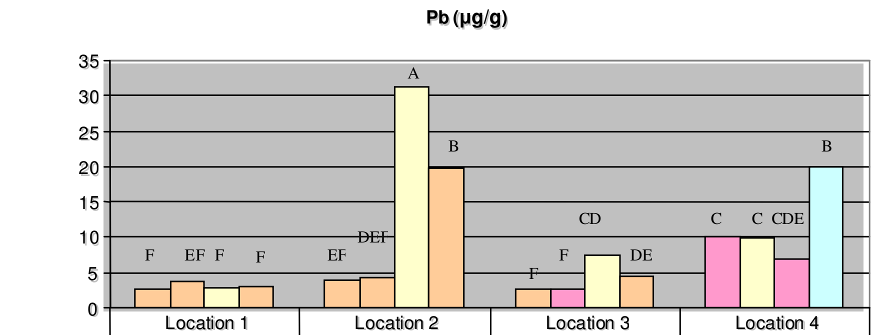


Figure 4. Lead contents on the fourth location distributed by plant species.



	Location 1	Location 2	Location 3	Location 4
Lime	2.73	3.81	2.62	10
Black pine	3.64	4.29	2.58	9.85
Plantain	2.75	31.3	7.37	6.93
Dandelion	3.07	19.83	4.42	20.01

Figure 5. Lead concentrations by plant species on all four locations.

of lead in plants is the reduction of leaf area. It is determined that lead inhibits plant growth (Mukherji et al.,1977), scion growth, elongation of cells (Lane et al., 1978) and photosynthesis (Bazzaz et al., 1974). As for photosynthesis, a large number of experiments show that heavy metals primarily have effect on photosystem II, phosphorilation and of course electrons transport. The lead that was added to the nutritive solution significantly reduced the quantity of chlorophyll in sunflower plant in comparison to control plants (Kastori et al., 1996). It is considered that the reduction of chlorophyll comes as a result of its enzymic decomposition. Moreover, synthesis of ATP is reduced in oxidation and photosynthetic phosphorilation.

In accordance with Pählosson-Balsberg (1989), lead concentrations in plant tissue of forest trees, which do not cause damaging effects amount to Pb<15 µg/g, and in the case of higher lead concentration between 20 and 70 µg/g, it causes a reduction of physiological activities and growth.

Conclusions

On the basis of the performed research, the following conclusions may be drawn:

1. In general, we can conclude that lead pollution of locations on the Avala mountain (locations 1, 2 and 3), although mostly of an anthropogenic origin, is not a consequence of pollution only but of traffic, while on location 4, bearing in mind the frequency of traffic, the greatest agent of pollution with lead is traffic;
2) According to the average values of lead contents on all four locations, it can be noted that the value of location 2 (on top of the Avala), which is 10.52 µg/g, is close to the values of pollution for location 4, which shows that the value of 11.70 µg/g is located in the centre of Belgrade.
3) The content of these heavy metals in woody plants in individual cases is far lower that in herbs, when analyzed in the area of Avala;
4) Herbs show far higher concentrations of lead. Moreover, dandelion and plantain stand out as hyperaccymulators of this heavy metal;
5) The recorded concentrations of lead in plantain on location 2 are above the maximum allowable values, while in all other analyzed plants, they are below or on the limit of allowable levels as prescribed by law;
6) Immisions of polluting fuels and heavy metals are regulated by Rulebook on limiting values, measurement methods in line with the Serbian effective Law on environmental protection (Official gazette 66/91, 54/92);
7) As EU regulations are far stricter than the existant domestic ones, it is necessary to harmonize the legal regulations referring to this subject matter;
8) Concentrations of heavy metal lead in plants in the area of the protected natural resource "Avala" for the time being do not represent danger for occurence of visible damage to forests, yet it indicates that the situation should be under intensive monitoring.

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