NICKEL CONTENT IN PLANTS AND SOIL
IN THE AREA OF THE PROTECTED NATURAL RESOURCE “AVAŁA” – BELGRADE

Abstract: The purpose of this research is above all focused on the aim of determining the load of ecosystem or rather soil and plants with the heavy metal Ni and the level of accumulation in this area so that on the basis of the obtained results adequate protection measures could be timely taken. The research of nickel (Ni) content in the leaves of herbs and woody plants growing under urban conditions in comparison with the nickel concentrations in the plant leaves in the protected natural resource “Avala” (16 km away from Belgrade) indicates that the recorded values of nickel concentrations by locations are statistically significantly different and range from A to F on locations 1, 2 and 3 while on location 4 in urban conditions the values range from A to B in accordance with Duncan’s Test.

Key words: nickel, concentrations, urban conditions, traffic arteries, leaf, soil

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and their accumulation on this area, which would provide, if necessary, timely protection measures based on the obtained results. The investigation of nickel (Ni) concentration in the leaves of woody and herbaceous plants growing under urban conditions compared to the concentration of nickel in leaves of plants in the protected natural reservoir “Avala” (16 km from Belgrade) suggests that the nickel concentrations at different locations are statistically significant and range from A-F at locations 1, 2 and 3 in urban conditions, while at location 4 the nickel concentration is A-B, as determined by the Duncan test.

Keywords: nickel, concentration, urban conditions, traffic accidents, leaf, soil

1. INTRODUCTION

Belgrade is one of the greenest capital cities in Europe. The protected natural resource „Avala“ (or rather the selected part of the unit which is declared the landscape of outstanding features) is located on the territory of the city of Belgrade and attracts the attention of researchers of various profiles. The research for the purpose of determination of the level of load of this area with pollutants has special significance. It should be emphasized that the Avala area was bombed during 1999. On that occasion the Avala Tower was pulled down.

On the basis of the obtained results timely protection measures could be taken with the ultimate aim of preservation, improvement and protection of nature and the environment in general and above all human health. Due to their adverse effect on biosphere, heavy metals attract great attention of researchers especially on account of their harmful effect to living organisms which increases due to long term exposure and cumulative effects (Guthner, 1989, Kurfurst, 1989).

It is well known that plants accumulate microelements (heavy metals) from soil through their root system and from air through photosynthetic organs. Owing to that fact, plants appear as specific receptors through which heavy metals from soil and partly from atmosphere enter humans and animals.

Nickel is a microelement necessary for the regular functioning of plants, animals and humans. However, in higher concentrations it has toxic effects. Increased quantities of this element may be a result of natural processes or anthropogenic factors. Those anthropogenic factors include industrial processes, extraction and processing of ores, traffic etc.

For instance, the total nickel emission from air for the area of California amounts to 23-360 t a year (1991), and for Croatia it amounts to 20-46 t yrs⁻¹ (Official Gazette № 48/95). According to the data from the Municipal Institute of Public Health of Belgrade, based on the official annual report for the state of air pollution in Belgrade area, exceeding of the limits is not registered for nickel in total suspended particles and particle size to 10 microns, or in sediment materials (dust).
Out of the total nickel emission in the atmosphere, 20% is a consequence of traffic. According to California Air Resources Board and Department of Health Services the data show that the vehicles using fuels like gasoline and gasoil also contribute to the overall nickel emission into the atmosphere with 54-72 t a year (1991). Nickel concentrations in gasoil from exhaustion pipes range from 500 to 10.000 mg·L⁻¹ (Frey, Corn, 1967).

Nickel (Ni) is a heavy metal belonging to VIII B group of the Periodic Table of elements with 7 radioisotopes. Although physiological role of Ni is not fully determined there is a prevailing opinion that it is a necessary microelement in the metabolic processes of higher plants (vascular plants) (Mengel, Kirkby, 1982). On the contrary, toxicity of Ni for plants is very well known. The symptoms include chlorosis, cessation of root system growth and sometimes interneural necrosis (Uren, 1992). Increased quantities of this element may be a result of natural processes or anthropogenic factors. The main anthropogenic source of nickel emission is combustion of fossil fuels and traffic (Stanković, 2008, Krstić et al., 2007).

Exhaust gases which result from combustion of gasoil contain more than 40 air polluters (2003) and among others nickel compounds (nickel-oxides, nickel-sulphates and nickel subsulphides). All these compounds are harmful to plants and may be genotoxic and cancerogenic for animals and humans (2005).

Bioremediation of contaminated soil represents an effective method of minimizing the risk for human health and ecosystem. Beside chemical measurements it is desirable to perform biological assessment of the ecological status. For that purpose we use biological indicators. The accumulation of heavy metals in plant tissue indicates a very important role of certain plant species as (bio)indicators of environmental pollution (Ten-Houten, 1983, Prasad, Freitas, 2003).

2. MATERIALS AND METHODS

The selection of representative locations for sampling of plants and soil for analysis was performed very carefully bearing in mind that it is necessary for each of the selected locations to contain the same plant species. It was also important to determine the key parameters from the point of view of emission and imission of the observed heavy metals, the distance from the road, as well as many other previously specified relevant criteria.

Three locations for sampling in the area of the landscape of outstanding features - Avala Mountain were selected.

The selected locations in accordance with the specific forest management plan for the management unit “Avala” (2008-2017) belong to FMU “Avala”:
- Location 1 - on the upward road to the landscape of outstanding features „Avala”;
- Location 2 - on the top of Avala mountain (near the Avala Tower);
- Location 3 - on the downward road from Avala mountain (Stari Majdan).
Upon the examination of several potential locations we selected the fourth one - the so called control location in the centre of Belgrade in Bulevar despota Stefana street (former street name 29. Novembra), where there is high frequency of traffic.

– Location 4 (control) - Bulevar despota Stefana street in the centre of the city of Belgrade.

For conducting the detailed research of the concentration of pollutants the contents of heavy metals were analyzed in vegetative parts of 8 selected plant species. Potentially any plant species may be used as a bioindicator of the status of the environment. A necessary precondition for this is knowledge of biology as well as ecology of each individual species which is used as a bioindicator. It is also necessary to know the range of ecological valence of a species for each factor of the environment (temperature, humidity, daylight, soil pH, etc.).

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. We selected the following 8 plant species:

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;
7. Quercus petraea (Matt.) Liebl. - Sessile Oak;
8. Pseudotsuga menziesii (Mirb.) Franco - common Douglas-fir.

For control purposes 4 plant species were chosen as representative samples on the fourth location in the centre of the city.

Bearing in mind the fact that the quantity of heavy metals in plants is directly dependent on the distance from the road at each location the plants were taken in the length of 200-300 m along the road and up to 15 m in depth from the roadside.

The samples of plant material were collected at each of the mentioned locations in the middle of their vegetation period, at the beginning of July 2009. Only the leaves were sampled and for each species 1-2 kg of material from all locations were taken. The samples were dried to airy dry mass without previous washing. Airy dry leaves were further kiln-dried at 105°C, ground and used for laboratory analyses.

Considering the importance of the quality of the environment and especially in high-grade areas and in areas dedicated to recreation within the previously mentioned locations the research of nickel (Ni) content in soil was conducted.

Heavy metals contents in soil were determined by atomic absorption spectrophotometry using a ,Varian AA-10" device. The conservation and preparation of samples for "pseudo" total contents of Ni were performed in line with EC-UN/ECE Method 9190SH and Method 9109 SA (soil samples were treated by a mixture of concentrated HCl, HNO₃.
and H₂O₂ in the following ratio 3:1:2). The obtained data were processed by the computer application “Statgraphic Plus” whereby we performed descriptive statistics, Variance Analysis and LSD Test. The level of soil load with lead, nickel and zinc was determined on the basis of Brune-Ellinghaus (1981): very low 1-5%; low 5-10%; average 10-25%; high 25-50%, very high 50-100% of the maximum allowable limit of heavy metal concentrations in soil.

The sampling of soil was performed in layers at two depths: 0-10 cm and 10-20 cm at all four locations (Table 1).

The analyses of plant and soil samples were performed in the laboratories of the Department of biology and ecology of the Faculty of Natural Sciences in Novi Sad, laboratories of the Faculty of Forestry of Belgrade University and the Institute for lowlands Forestry and Environmental Protection in Novi Sad.

The heavy metals concentrations in plants and soil were determined by the atomic absorption spectrophotometry method and the measurement was performed with three repetitions.

Table 1. Labels and description of sampling locations for plants and soil.

| Location name | Description | Location label |
|---------------|-------------|----------------|
| Avala 1       | Upward part of the road towards the Tower | 1              |
| Avala 2       | Nearby the Tower                          | 2              |
| Avala 3       | Downward part of the road from the Tower  | 3              |
| Control Location 4 | Bulevar Despota Stefana street in the centre of Belgrade | 4              |

The obtained data on the accumulation of heavy metals were processed through the implementation of standard statistical methods, Variance Analysis, LSD Test for the level of significance $p = 0.05$ and the examination of average values significance was performed using Duncan’s Test.

All tests were performed with the level of significance $p \leq 0.05$.

The obtained results were presented in a suitable manner - in tables and charts.

3. RESULTS

The research results as may clearly be seen in the presented charts and on the basis of the previously mentioned toxic values show that in accordance with the average values all of the locations show the values below the critical value.

However, in charts 3 and 4 it is also clearly presented that plants already proven in numerous other researches to be accumulators of heavy metals in this research also show
and even exceed the critical values of accumulation of nickel. Namely, at location 3 on Avala mountain, wild cherry shows the value of 12.29 μg·g⁻¹ and at the control location in Belgrade (location 4) nickel accumulation in Dandelion is close to critical values and amounts to 9.15 μg·g⁻¹.

We obtained very interesting results with a huge range of values on different locations for the accumulation of nickel by wild cherry. The values range from 1.04 μg·g⁻¹ which is the lowest value for all plant species in all locations to the highest value in the same plant species - wild cherry amounting to 12.29 μg·g⁻¹ at location 3.

Diagram 1. Nickel contents on the first location distributed by plant species

Diagram 2. Nickel contents on the second location distributed by plant species

Diagram 3. Nickel contents on the third location distributed by plant species

Diagram 4. Nickel contents on the fourth location distributed by plant species
The least concentration of nickel in plants was recorded on locations 1 and 2, on the top of Avala and the upward road to Avala.

Comparing the average nickel contents in four plant species in different locations (Diagram 6) it can be stated that there is a huge range of values and that average values range from 2.32 to 6.60 μg·g⁻¹.

The significant difference in concentrations of nickel measured on different locations undoubtedly points to the fact that there was contamination on the location of high frequency of traffic. Also, there were high values of Ni concentration on location 3 on Avala which points to the fact that traffic is not the only polluter of plants by this heavy metal.

The presence of significantly higher concentrations of nickel in plant leaves taken from location 3 on Avala in comparison to the control location 4 in the centre of Belgrade.
indicates the existence of chemical contamination of soil with this pollutant. These conclusions are also confirmed by our research where on locations 3 and 4 there are relatively high concentrations of nickel in soil amounting to 44.44 μg·g⁻¹ (although they do not exceed maximum allowable values).

The research and comparison of nickel contents in the leaves of the four different plant species growing in urban (location 4) and suburban conditions (locations on Avala 1, 2 and 3) show that the recorded Ni concentration in Dandelion amounting to 4.85 μg·g⁻¹ on location 3 is two times less than the one recorded in the extreme urban conditions amounting to 9.15 μg·g⁻¹ on location 4 (Diagram 5).

When the four plant species at all four analyzed locations are compared it is clear that Dandelion seems to be a better accumulator than nickel in relation to plant species examined in our research and that the values on the control location (although not exceeding critical value) are still by almost 50% higher than the values recorded on the locations on Avala mountain.

| Min | Max | Average | Std. deviation |
|-----|-----|---------|----------------|
| 1   | 16.11 | 60.11 | 41.52 | 16.63 |
| 2   | 16.22 | 61.94 | 42.37 | 17.27 |

Table 2. Summary statistics and variance analysis of Ni contents in soil on different depths (1 and 2)

The obtained results show that the concentrations of heavy metals adsorbed and absorbed in the leaves of the analyzed plants may be used as a very reliable indicator of chemical pollution of soil.

In the coming period monitoring will to a large extent contribute not only to control the conditions but to scientifically explain such a huge range of values of nickel.
accumulation in wild cherry as a species considered a hyperaccumulator of heavy metals and nickel among others.

The energy of adsorption of heavy metals differs depending on the physical and chemical properties of soil. Nickel in soil is present both in organic and inorganic matter.

The average nickel concentration in soil worldwide amounts to 40 $mg \cdot kg^{-1}$ of soil. However, that concentration varies depending on the soil type. Coal combustion is a significant source of nickel, followed by ore mines, ironworks, forest fires and waste incineration.

Nickel contents at the analyzed locations vary within the range from 16.68 to 61.02 $mg \cdot kg^{-1}$. Since the allowable values range up to 50 $ppm$, it may be concluded that only on location 4, the recorded contents exceed the maximum allowable concentrations. On all locations on Avala the measured values were below the maximum allowable limit.

Table 3. Summary statistics, Variance Analysis and LSD-Test for heavy metals contents (Zn, Ni, Fe, Pb and Mn) in soil on different locations (1-4)

|     | Min | Max | Average | Std. deviation |
|-----|-----|-----|---------|---------------|
| 1   | 45.37 | 47.31 | 46.34 | 1.062 |
| 2   | 16.11 | 16.22 | 16.68 | 0.059 |
| 3   | 44.01 | 44.46 | 44.23 | 0.233 |
| 4   | 60.10 | 61.94 | 61.02 | 1.00 |

Variance Analysis

| Between locations | $F$-ratio | $p$-value |
|-------------------|-----------|-----------|
| 3832.32 | 0.0000 |

LSD-Test

| Average | Homogeneous groups |
|---------|--------------------|
| 2       | ×                  |
| 3       | ×                  |
| 1       | ×                  |
| 4       | ×                  |

Considering the nickel content on locations 1 to 4 it can be concluded that they are getting close to maximum allowable concentrations (50 $mg \cdot kg^{-1}$) which indicates that this load originates from vehicles consuming fuel oil, such load being caused by traffic at these locations.

On the basis of the presented data on nickel content in soil the following conclusions can be drawn:
The obtained differences between Ni content at different soil depths (0-10 cm and 10-20 cm) are not statistically significant whereby there is the decreasing trend with the increase in soil depth;

- There are statistically significant differences between the average values of nickel contents in soil among the four analyzed locations.

The average values of Ni contents in soil for the first three locations on Avala are less than the average values of the heavy metal Ni on location 4 (the centre of the city of Belgrade).

4. DISCUSSION

It is important to point out and it can be claimed with certainty that the importance and role of plants and especially forests is huge and such a role encompasses different aspects of action (Ban kovič et al., 2009).

Level of accumulation of heavy metals in plant tissue is determined by numerous biotic and abiotic factors and the characteristic genotype is one of the dominant ones (Pajević et al., 2008, Nikolić et al., 2008). Interspecies calibration is also applied for certain heavy metals (Berlizov et al., 2007).

Numerous non-standard effects of heavy metals depend on their direct and indirect effect in certain metabolic processes; for example high content of Ni in endoderm and parenchim cells blocks cell division in parenchim and results in the prevention of root branching (Serigin, Kozhevnikova, 2006).

Moreover, according to many other authors (Rubio et al., 1994, Ahmad et al., 2007), heavy metals uptake and accumulation in plants results in a reduction of contents of K, Ca and Mg in plants, especially in scions which points to the fact that heavy metals hinder not only intake of nutrients but also their distribution into different parts of plants.

Nickel shows high mobility in xylem as well as phloem and it significantly accumulates in fruits and seeds. Plant species differ by level of accumulation of Ni and the ones that in dry matter contain more than 1 mg·g⁻¹ of Ni are considered hyperaccumulators. Moreover, it is also confirmed that plant species show different potential for nickel uptake under the same environmental conditions which is also in line with the results of Guo and Marschner (1995).

The increased concentrations of Ni are to a large extent the consequence of the atmospheric deposition of particles with adsorbed and absorbed pollutants since there are data that with certain plant species like Fracinus, root uptake and transport of elements through xylem is usually not intensive enough to cover the circulation of mineral pollutants in the atmosphere (Cat inon et al., 2008).

According to the existing data (Kastori et al., 1997) the average nickel content in plants ranges from 0.1-5.0 ppm of dry matter and toxic values are higher than 10 ppm of dry matter.
Therefore, this pollution originates from traffic and probably higher concentration of soot and other particles in the air which absorb nickel, which further deposits in vegetation (plants). Air pollution may be dispersed on hundreds and thousands of kilometers depending on weather conditions, especially wind speed and direction. Therefore it is important to establish an overall monitoring of wide areas which would include the defining of values of physical and chemical parameters of the soil and the air as well as the group of biological parameters.

In our region the highest or rather toxic concentrations of Ni have been recorded in Sessile Oak (*Quercus petraea* Matt. Liebl., 16.16 μg·g⁻¹) in the national park of Fruška gora and in Silver Lime also on Fruška gora (*Tilia tomentosa* Monch., 15.39 μg·g⁻¹). (Stanković, 2006, Stanković et al., 2009). At our locations on Avala Ni concentrations in Sessile Oak range from 2.95 to 7.00 μg·g⁻¹.

On the basis of the conducted research it may be concluded that Ni concentrations in the leaves of different plant species growing in urban and suburban conditions show a wide range of values. Some of the values are very close to maximum allowable values. Yet, they are still under the critical level. According to Asher, 1991 the critical concentrations are >10 μg·g⁻¹ in sensitive species and >50 μg·g⁻¹ in middle tolerant species.

High concentrations of nickel have toxic effect. The deficiency symptoms are similar to the symptoms of iron deficiency. In case of high concentrations of nickel in plants the signs of necrosis appear on leaf edges.

Considering nickel contents in soil in accordance with the findings of certain authors the total Ni contents range from 5-100 ppm and in soil types formed on serpentine they amount to 500 ppm and even up to 600 ppm (Ušavić et al., 1993).

The most frequent Ni contents in soil range within 10-50 mg·kg⁻¹, and the allowable limit of Ni concentration in soil is 100 mg·kg⁻¹.

In accordance with Brune-Ellinghouse (1981) the soil in Avala is highly loaded with nickel.

In line with the Rulebook on the permitted quantities of toxic and hazardous substances in soil and water for irrigation the maximum allowable level of nickel in soil is up to 50 mg·kg⁻¹ (Official gazette of RS № 23/93).

5. CONCLUSION

On the basis of the conducted research it may be concluded that Ni concentrations in leaves of different plant species growing in urban as well as in suburban conditions in Belgrade show a very wide range of values where certain values are very close to maximum allowable concentrations, yet they are still below the critical level.

The presence of significantly higher Ni concentrations in plant leaves taken from location 3 on Avala in comparison to the control location 4 in the centre of Belgrade indicates the existence of chemical contamination primarily of air but also of soil with this...
pollutant. These conclusions are further ascertained by the data from locations 3 and 4 showing high concentrations of Ni in soil, amounting to 44.44 μg·g⁻¹ (yet, they do not exceed maximum allowable concentrations).

For the time being, the presence of heavy metal - nickel (Ni) in the soil and plants in the area of the protected natural resource „Avala” does not represent a threat of potential notable damage to forests but since on the other hand it shows the tendency of concentrations increase it should be under intensive monitoring.

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САДРЖАЈ НИКЛА У БИЉКАМА И ЗЕМЉИШТУ НА ПОДРУЧЈУ ЗАШТИЋЕНОГ ПРИРОДНОГ ДОБРА АВАЛА - БЕОГРАДУ

Резиме

Никл је неопходан микроелемент за биљке, животиње и човека. Међутим, при већим концентрацијама делује токсично. Повећане количине овог елемента могу бити резултат природних процеса или антропогенних фактора.

Емисија никла из ваздуха за подручје Калифорније износи 23-360 тона по години (1991), а подручје Хрватске од 20-46 тона по години (Official Gazette, № 48/95). Од укупне емисије никла у атмосфери око 20% је последица саобраћаја.

Према подацима California Air Resorces Board и Department of Helth Services показује се да возила која користе моторна горива као што су бензин и дизел гориво, такође доприносе емисији никла у атмосфери са 54-72 тона годишње (1991). Концентрација никла у дизелу из издувних цеви креће се од 500-10.000 mg L⁻¹ (Frey, Corn, 1967).

Симптоми токсичности биљака никлом укључују хлорозу, престанак раста кореновог система и понекад, међунервну некрозу (Uren, 1992). Повећане количине овог елемента могу бити резултат природних процеса или антропогенних фактора. Главни антропогени извор емисије никла је сагоревање fosilних горива и саобраћај (Krstić, et al., 2007). Повећане количине овог елемента могу бити резултат природних процеса или антропогенних фактора. Главни антропогени извор емисије никла је сагоревање fosilних горива и саобраћај (Stanković, 2008, Krstić, et al., 2007).

Потенцијално, свака биљна врста може бити употребљена као биоиндикатор стања животне средине. Неопходан предуслов за то је познавање како биологије, тако и екологије (идиоекологије) сваке појединачне врсте која се користи као биоиндикатор. Потребно је, такође, познавати и ширину еколошке валенце врсте за сваки појединачан фактор спољашње средине (температуру, влажност, светлост, pH земљишта, итд.).

При избору врста биљака за анализу, веома прецизно је извршена инвентаризација свих врста биљака на овом подручју. Одабрана су три локалитета на Авали и један локалитет у тзв. транзитној зони ( zona II) у центру Београда.
За истраживање је одабрано 8 врста биљака које су заступљене на локалитетима на Авали, док је на четвртом локалитету у центру града одабрано четири врсте биљака, које су послужиле за компаративну, а и као биоиндикатриста стања у урбаној средини. Узорковани су само листови биљака.

На свим локалитетима, спроведена су и истраживања садржаја никла (Ni) у земљишту. Узорковање земљишта је вршено слојевито, на две дубине: 0-10 см и 10 до 20 см на сваком локалитету.

Концентрација тешких метала у биљкама и земљишту одређена је атомском апсорбционом спектрофотометријом.

Резултати истраживања, показују да према просечним вредностима ни један локалитет не прелази критичне вредности од 10 μg g⁻¹.

Најмања концентрација никла у биљкама је на локалитетима 1 и 2 односно на улазном путу до Авале и на врху Авале.

Упоређујући просечан садржај никла код четири биљне врсте по локалитетима, можемо констатовати да се просечне вредности крећу од 2.32 до 6.60 μg g⁻¹. Такође, потврђена је констатација да биљне врсте показују различит потенцијал за никл при истим стаништима, а што је у сагласности са резултатима Guo и Marschner-a (1995).

Значајна разлика добијена у концентрацији никла са различитих локалитета, управо указује, на постојање контаминације на локалитету фреквентног саобраћаја. Високе вредности концентрације никла су се показале и на 3. локалитету на Авали, а то указује да саобраћај није једини загађивач биљака овим тешким металом.

Присуство сигнификантно већих концентрација никла у листовима биљака узетим са локалитета 3. на Авали у односу на контролни локалитет 4 у центру Београда, указује на постојање хемијске контаминације земљишта овим полутантом. Ове констатације потврђују и добијени подаци, где су на локалитету 3 и 4 релативно високе концентрације никла у земљишту од 44,44 μg g⁻¹ (иако не премацају MDK вредности).

Садржај никла у земљишту на испитиваним локалитетима варира у границима од 16,68-61,02 mg kg⁻¹, како му је дозвољен садржај до 50 ppm, може се констатовати да је утврђен садржај изнад MDK само на 4. локалитету. Садржај испод MDK био је на свим локалитетима на Авали.

Тешки метал Ni у земљишту и биљкама на подручју заштићеног природног добра „Авали“ за сада не представља опасност за настанак видљивих оштећења шума, али показује тенденцију повећања концентрација, те их треба интензивно пратити.
