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CONCENTRATION OF HEAVY METALS AND STAND STATE OF SESILLE OAK (*Quercus petraea (Matt.) Liebl.*) ON AVALA MOUNTAIN (SERBIA)

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Abstract: The research of heavy metals contents in soil and leaves of Sessile oak (*Quercus petraea (Matt.) Liebl.*). Moreover, the paper presents the analysis of the stand state on the Avala Mountain. The Avala area is high-grade protected natural resource located on the territory of Belgrade (Serbia) and its regime of exploitation and protection is clearly defined. It is assumed that the human factor is the primary cause of degradation of the protected areas of Avala. Therefore, the current inadequate stand composition and the impact of traffic pollution can be considered the decisive causes of degradation on mountain, as a natural area under special protection. Determining the degree of loading of soil and plants with heavy metals and the analysis of stand conditions of such valuable protected areas such as the area of Avala, are of great importance, based on the results obtained, to take appropriate timely precautionary measures, in order to preserve, improve nature conservation, the environment in general, and especially health conditions. The research of the contents of heavy metals (Pb, Ni, Fe, Zn i Mn) in soil showed the load of soil especially with Pb and Ni. In third locaction the concentration of Ni (amounting to 7.0 μg/g) in the plants leaves is significantly higher which indicates the existance of chemical contamination of soil, before all, with this pollutant. The measured concentrations of all examined heavy metals (Pb, Ni, Zn, Mn and Fe) in the leaves of Sessile oak on Avala fall within the maximum allowed values in accordance with the legal regulations in Serbia.

Key words: Avala, heavy metals, plants, soil, degradation, sessile oak, stand condition.

1. INTRODUCTION

One of the major factors of degradation of some ecosystem components is environmental pollution by chemical substances, so it is extremely important to determine accumulation and toxic effects of heavy metals in plants from an ecological standpoint, since they are entering the food chain in that way (Kastori, 1997; Kramer & Kozlovski, 2006; Maksimović & Pajević, 2002; Pallardy, 2008).

Forests may be degraded in terms of loss of any of the values and services that they provide (fibre, food, habitat, water, carbon storage and other protective, socio-economic and cultural values), and changing of this process can be caused by nature or man (FAO, 2011). Considering elements of sustainable forest management and multifunctional approaches of forest management are defined by suitable indicators for measuring forest degradation. Common indicators for monitoring and assessing forest degradation can be developed for the following key elements: biodiversity, biomass, forest health, productive functions and protective functions (FAO, 2009).

Sessile oak forests in Serbia, covering an area of 173.000 ha, which, according to the presence in the growing stock, makes sessile oak the most important and most common tree species, after beech and oak. Sessile oak forests are predominantly of coppice origin (74.1%), and in terms of preservation are diluted and devastated stands covering 26.3% of the total area of sessile oak forests (Banković et al., 2008), i.e. stand state under Sessile Oak forests is influenced by various degrees of degradation. Therefore, the effects of current production, percentage of technical, quality wood, environmental, functional and economic value of Sessile oak forests are substantially reduced. One of the strategic goals of forestry in Serbia is conversion and melioration of these forests to high structural forms (Banković et al., 2009).

Figure 1. Position of measuring points in Sebian map

Avala is the mountain near Belgrade (Fig. 1). It is the largest forest complex near the urban areas of the city. Preserved as part of nature, this area has a permanent ecological, scientific, cultural, educational, medical, recreational and tourist value. Therefore, it is declared as protected natural area, as a region of outstanding features of the recommendation of the Institute for Nature Conservation of Serbia. The total protected area is 489.13 hectares, and it is located with the following coordinates in the state system of coordinates y = 7462425 (44˚37'33"), x = 4949675 (20˚30'01"), at the altitude of 210-506 m. The most represented tree species is sessile oak, which accounts for about 20% of all forests and about 25% of the total timber volume. During the First and Second World War forests on the mountain were largely cut down, after which they spontaneously regenerated. For that reason, almost all the oak forests on mountain have coppice origin - unfavorable to the status and functions of the forests.

Pollution and pollutants cause further degradation of the protected area of Mountain Avala, due to the presence of the traffic. Unsatisfactory stand state that is reflected in: the coppice origin, reduced production capacity and quality, unfavorable age structure and species composition of trees result in less stability and vitality, and directly significantly influence on the reduction of all forest functions (Kurfurst, 1989).

Among all the chemical pollutants, heavy metals are of particular ecological, biological and medical significance. As the main sources of pollution, there are traffic, industry and a high degree of urbanization. Traffic, in addition to the indisputable benefits, is very strong and extraneous source of heavy metals and other pollutants in soil and plants (Fidora, 1972; Őpik & Rolf, 2005; Primault, 1958; Stanković, 2006; Stanković, 2008).

Kurfurst (1989) and Guthner (1989), pointed out that heavy metals due to their adverse effect on the environment into biosphere attracted more and more attention of researchers especially due to their harmful effect on living organisms which increases because of longterm exposure and cummulative effect. Heavy metals are accumulated in organism and deposited through food chain.

High concentrations of heavy metals cause various anatomic, morphological and physiological changes in plants. Heavy metals may affect metabolism of phytohormones, intensity of photosynthesis and water regime of plants which affects their growth and causes reduction of aggregation of organic matter as well as reduced transpiration.

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 (Kramer & Kozlovski, 2006; Krstić et al., 2007; Stanković 2008; Stanković et al., 2009; Stanković, et al. 2011). Accumulation level of heavy metals in plant tissue is determined by numerous biotic and abiotic factors. Some plant species are capable to survive in the habitats with increased concentrations of heavy metals in soil.

Monitoring of heavy metals is especially important due to the fact that their toxicity and accumulation is huge. 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), Pacyna & Munch (1987).

Potentially, each plant species may be used as bioindicator of environmental condition. The necessary precondition is knowledge of biology as well as ecology (ideoecology) of each individual species which is to be used as bioindicator. It is also necessary to know the width of ecological species valence for each individual factor of the environment (temperature, humidity, light, ph of soil etc.). The ecological species valence of each species for any environmental factor may be narrow or wide. That principle may be applied also for the concentration of polluting substances in the environment.

2. MATERIALS AND METHODS

For this survey there were selected three sites in the area of region of outstanding features "Avala", (Fig. 1) from which samples were taken for analysis of concentrations of heavy metals in the stands of oak:

- Location 1 - at the upward road to the landscape of outstanding features "Avala"
- Location 2 - on the top of the Avala mountain (near the Avala Tower)
- Location 3 - on the downward road from the Avala mountain (Stari Majdan)

Bearing in mind the fact that the quantity of heavy metals in plants directly depends on the distance from the road on each location the plants were taken in the length of 200-300 m along the road and up to 15 m in the depth from the road. Only plant leaves were sampled. The samples of plant material were collected on each of the mentioned locations in the middle of their vegetation period, at the beginning of July 2009. From all sites 1-2kg of material was taken.

The samples were dried at room temperature without prewashing until airy dry mass. Airy dried leaves were then further dried in the dry-kiln at 105º C. The analysis of chemical composition of plants was performed in accordance with the standard procedure (APHA, 1995). The concentrations of heavy metals were determined after dry burning at 450°C under treatment with HCl. The concentrations of (Pb, Ni, Fe, Zn and Mn) were determined by AAS from the master solutions obtained in the described manner. Three independent replications were done for each sample. (Maksimović & Pajević, 2002; Petković et al., 2005).

Soil sampling was performed in layers on two depths: 0-10 cm and 10-20cm. Heavy metals contents in the soil and leaves was determined by atomic absorption spectrophotometry method using „Varian AA-10" device.

The conservation and preparation of soil samples for "pseudo" total contents of Pb, Ni, Zn, Fe and Mn was performed in accordance with UN/ECE Method 9190SH and Method 9109 SA (the soil was treated with the mixture of concentrated HCl, HNO_3 and H_2O_2 in the ratio 3:1:2).

The level of soil load with lead, nickel and zinc was determined on the basis of Brüne & Ellinghaus (1981): very low 1-5%; low 5-10%; middle 10-25%; high 25-50%, and very high 50 - 100% of the maximum allowable limit of a particular heavy metal concentrations in soil.

Analyses are made in the laboratories of the Faculty of Forestry, University of Belgrade, Faculty of Sciences, University of Novi Sad and the Institute of Lowland Forestry and Environment in Novi Sad. Stand state was analyzed by placing the sample plot at the location 3 on the Mountain Avala. Researching of stand state at the experiment field was based on the data that included measurement of basic taxation elements to determine stand condition, collecting ecological characteristics necessary to define the site conditions and typology. All trees with diameter over 7.5 cm were measured, and heights are measured, and cores are taken from only a certain number of trees in each diameter level of 5 cm wide. Assessment of the biological status of trees, tree trunks and foliage quality were done in order to determine the quality of the stand as a criterion for determining the degree of degradation (Krstić, 2006; Krstić et al, 2011). Elements of stand conditions were calculated using Prodan's functions for the design height curve, Schumacher-Hall's function to calculate the volume and diameter increment method to calculate the current volume increment. Also, mean diameters of the basal area of stands (d_g) and the dominant tree (D_g) were calculated, and diameter structure elements are shown by the following statistical indicator: arithmetic mean (d_a), standard deviation (s_d), coefficient of variation (c_v), variation width ($v_š$), asymmetry coefficient (α_3) and the coefficient of kurtosis (α_4) (Stamenković & Vučković, 1988).

The data were analyzed by using standard statistical methods: descriptive statistics, cluster analysis, and analysis of variance and significance testing of mean values were determined by using Duncan's test. All tests were performed with the level of significance $p \leq 0.05$. The results are presented in an appropriate manner, in tables and graphs. Statistical analysis was performed using software SPSS 17.0 and Statistica 10.

3. RESULTS

3.1. The concentration of heavy metals in sessile oak leaves and soil

Based on these results we can conclude that the average values of Pb, Ni, Zn, Fe and Mn in the soil, shown, by Brüne & Ellighauss (1981), a very high load especially Pb and Ni.

Lead content in soil on Mountain Avala is in the range from 34.68 μg/g (site 1) to 108.65 μg/g (site 2). The average value (for three sites) of lead in soil on Mountain Avala was 61.95 μg/g which indicates a very high load of soil lead (61.95%), by Brüne & Ellighauss (1981).

Value exceeds the MAC values (Maximum Allowed Concentration) only by Pb content on the site 2, near the Avala tower.

The average values of nickel on the study sites vary within the range from 16.8 μg/g to 46.34 μg/g. (Table 1).

Table 1. Contents of heavy metals (Pb, Ni, Zn, Fe and Mn) in soil on the analyzed locations

Loc.	**Pb** (μg/g)	**Ni** (μg/g)	**Zn** (μg/g)	**Fe** (μg/g)	**Mn** (μg/g)
1	34.68	46.34	88.74	24,388.81	627.83
2	108.65	16.68	74.66	22,185.62	669.55
3	42.54	44.23	70.07	30,850.00	1046.65
Avrg. val.	61.96	35.75	77.82	25,808.14	781.34
Crit. val.*	25-100	10-85	60-150 (200)	100 - 100.000**	500-1.000***

* Multifunctional use possibilities: De Vries and Bakker (1998):

** Average values: Vanmechelen et al., (1997):

*** Average values: Adriano (1986)

Soil in the area of Avala, according to Brune & Ellighauss (1981), shows high load with Pb and Ni, and a medium-load with Zn, Mn and Fe, (Table 1).

Biological recovery of contaminated soil represents an effective method of decreasing the risk for human health as well as ecosystem. Beside chemical measurements it is desirable to perform also biological assessment of ecological situation. For that purpose biological indicators are used. The accumulation of heavy metals in plant tissue points out to a very important role of certain plant species as (bio)indicators of environmental pollution (Ten-Houten, 1983; Prasad & Freitas, 2003).

Since the analysis of variance for all sites showed that the $P < 0.05$, it can be, with a probability of 95%, concluded that there are significant differences in the content of heavy metals in oak leaves.

All locations were examined by Duncan's test for statistically significant differences (with a probability of 95%) between the mean values of Pb, Ni, Mn, Fe and Zn.

Although, in general, there are significant differences between mean values of heavy metals in all three locations (probability level of 95%), it can be argued that for Pb, sites 2 and 3 constitute a homogenous group (A-A) or that only these two sites show no statistically significant differences between mean values of Pb, while the site 1 in relation to these two sites differ statistically (B-A-A).

For all other heavy metals Duncan's test showed significant differences on any of the studied sites, and a range of values is the A-B-C (Fig. 2-6). Kabata-Pendias & Pendias (1989) indicate that the natural concentration of lead in plants ranging from 5-10 mg/kg.

The value of the accumulation of lead in the locality 2, although in the MAC values (2.39 μg/g) (Maximum Allowed Concentration / MDK), was higher compared to sites 1 (1.12 μg/g) and 3 (2.18 μg/g) on the Mountain Avala. This is consistent with the results of the concentration of lead in soil at this site, where we get high values of 108.5 μg/g.

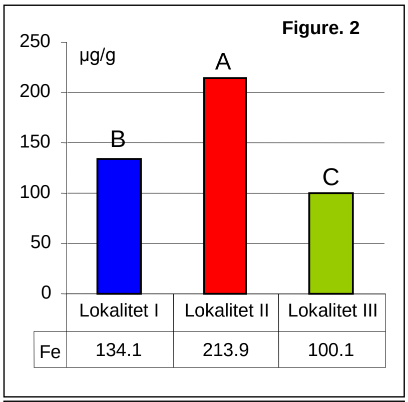
Lead is a heavy metal that shows extremely harmful effect on plants as well as on animals and humans since it has a cumulative retarded effect. The main source of Pb are the exhaust gases from vehicles which use gasoline with tetraethyl lead and tetramethyl lead which represent antidetonators in gasoline for internal combustion engines.

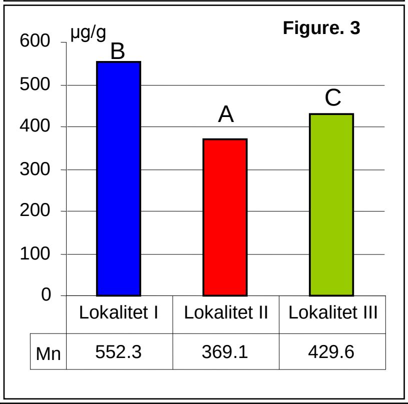
The greatest or toxic concentrations of Ni, in our region, were observed in Sessile Oak stands (*Quercus petraea* Matt. Liebl. 16.16 μg/g) in National Park "Fruška Gora" and Silver Lime stands (*Tilia tomentosa* Monch. 15.39 μg/g), also in Fruška Gora (34.35). On examined sites in Avala concentration of nickel in oak trees range from 2.95 to 7.00 μg/g (Fig. 5).

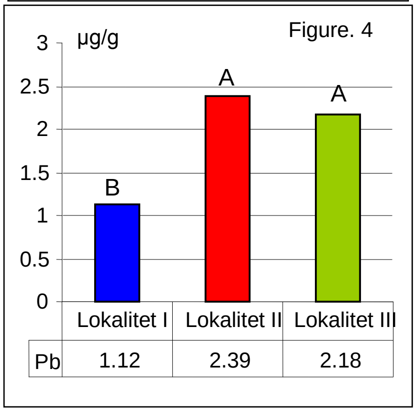
The presence of significantly higher concentrations of nickel in leaves of plants taken from the locality 3 on mountain points to the existence of chemical contamination of soil by this pollutant. These findings are confirmed by the data on the site 3 where the relatively high concentrations of nickel in the soil of 44.23 g/g were found, but not exceeded the MAC values (Maximum Allowed Concentration) (Table1).

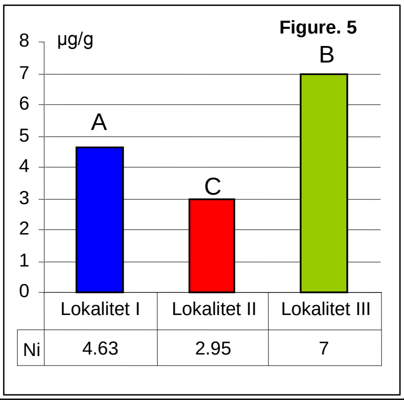
Starting from the fact that one of the goals of cluster analysis is the formation of such groups where in one group will be objects more similar to each other than with objects from other groups, and for a clearer examination of relations and the accumulation of the studied heavy metals in oak at all three sites, cluster analysis was done. The similarity is usually operationalized as some sort of distance.

In this study, dendrogram of cluster analysis clearly shows the grouping of sites 1 and 3 (Fig. 7). Squared Euclidean distance at all three sites is about 14,000 to 33,000.









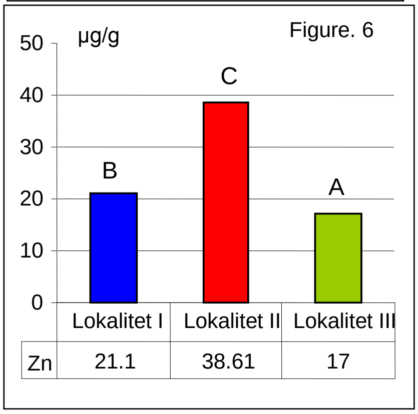


Figure 2-6. The analysis of chemical elements in leaves sesille oak at all locations

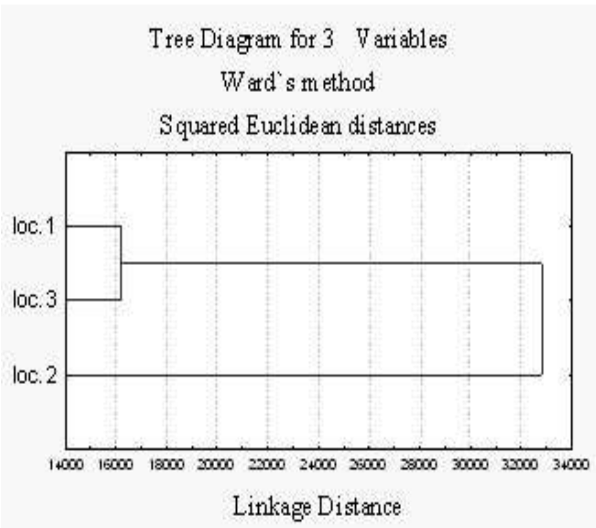


Figure 7. Dendrogram of cluster analysis made on the basis of heavy metals concentration in oak leaves in three locations on the Mountain Avala.

Homogeneity of sites 1 and 3 is clearly separate, and the largest distance of 33,000 is between the site 2 and sites 1 and 3 respectively. This grouping shows very clearly higher concentration of heavy metals on the second site in relation to the other two sites.

3.2 Stand state

Analysis of stand conditions was carried out on experimental fields located at an altitude of 400 m, while its slope is around 10° and east exposure. The stand of coppice origin is about 65 years old. Canopy closure is complete (0.7). The stand is typologically defined as a type of Sessile Oak trees with Turkey Oak (*Quercetum petraeae-cerris* Jov. 1979) on eutric cambisol soil.

Diameter structure of analyzed stand has parameters that characterize typical even-aged structure that is characteristic of sessile oak forests. Basic information on stand condition (Table 2) indicate a number of good production indicators (for coppice) from the basal area of 32.0 $m^2 \cdot ha^{-1}$, volume of 269.3 $m^3 \cdot ha^{-1}$ and annual volume increment of 4.64 $m^3 \cdot ha^{-1}$. Mean stand diameter is 25.6 cm with the average height of 16.8 m. Distribution of trees by diameter indicates the typical even-aged structure. Although stands of good productivity, the analysis of stem and crown that indicates the low share of trees with good timber quality of 5.1% and the share of trees with well-developed crown of 28.2% (Table 3) determined that the investigated oak stands of poor quality due which can be considered as a form of degradation.

Table 2**.** Basic information on the stand state

Age (year)	65
d_g (cm)	25.6
h_g (m)	16.8
D_g (cm)	31.1
H_g (m)	17.4
No tree·ha^{-1}	624
basal area ($m^2 \cdot ha^{-1}$)	32.03
volume ($m^3 \cdot ha^{-1}$)	269.3
mean annual diameter increment (mm)	2.47
mean annual volume increment ($m^3 \cdot ha^{-1}$)	4.64
percentage of annual increment (%)	1.72
s_d (cm)	4.7
c_v (%)	18.86
$V_š$ (cm)	19.4 (14.3-33.7)
α_3	-0.05
α_4	2.64

Table 3. The quality of the stand

	Biological status (%)	The quality of timber (%)	The quality of crown (%)
1 - good	69.2	5.1	28.2
2 - medium	30.8	33.3	41.0
3 - poor	0.0	61.6	30.8

4. DISCUSSION

For proper growth and development of plants biogenic elements are essential. It is established that there are three groups of elements. Some are necessary because without them the plant can not normally complete their life cycle, others may act stimulating, while a group of elements, especially heavy metals, at higher concentrations are toxic to plants.

To date, the plant tissues revealed the presence of about 70 elements. It is reasonable to assume that they contain all 88 elements which, according to current knowledge, participate in the construction of the Earth. Their presence in plants is different and depends on internal and external factors.

Certain types of phytotoxic elements, for example, heavy metals (Pb, Ni, Fe, Zn, Mn) of the precipitation in the form of dust in the soil, root system shall be adopted and transmitted in the upper part, and then further into the food chain. Pollution of heavy metals are mainly of anthropogenic origin (transport - Pb, Ni and PAHs – Polycyclic Aromatic Hydrocarbons or by metallurgical complex) and their allowable concentrations are regulated by the legislation of individual countries.

Maximum amount of lead is released during combustion of gasoline (over 270•106 kg per year) in which it was lead content near 0:45 g/l. In this regard, a numerous papers were published that show a significant accumulation of lead in plants, especially near the highways (Heilenz, 1970; Sommer & Stritesky, 1976; Stanković et al., 2011), as well as the dynamics of lead in soil and on its adoption by the plant (Höll & Hampp, 1975).

Between different plant species, there are differences in the adoption of heavy metals, which depends on various factors: their genetic characteristics, the influence of the surface of the root system and their capacity to absorb ions, from the form of root secretions and rate of evapotranspiration. However, differences in the adoption of heavy metals exists in plants of the same species, but different genotypes, which is due, above all, to different climatic conditions and different regimes, as well as the sensitivity of the plant itself to the action of heavy metals. Plants as well as soil, are often polluted by a combination of different pollutants (Oljača et al., 2006; Pallardy, 2008; Stanković et al., 2009; Stanković et al., 2011).

Saturation of soil and plants by heavy metals, on one hand, and analysis of stand conditions, on the other hand, are two important elements that can serve for accurately determination the degree of degradation of researched area.

Data on growing stock, annual volume increment, age structure, quality of wood and other elements of production functions are potential indicators of forest degradation (FAO, 2009).

The difference between the potential above ground biomass and the actual present biomass can be used as an degradation indicator. Inventory data available at national level with estimations of growing stock per hectare for each forest type, usually are indicator of how well or poorly stocked the forests are, and indications on the trends in the quality of the forest resources (FAO, 2011).

The amount of volume and volume increment of concrete stands were compared with the optimal estimated values for the exact type of trees and with average values at the national level (Krstić, 2006). For oak forests in Serbia optimal estimated values are: V = 200 m^3 ha^{-1} and Iv = 5.0 m^3 ha^{-1} (Jović et al., 1977). According to the NFI 2009 (Banković et al., 2008; Banković et al., 2009) average values for the oak high forests in state ownership are: V= 236 $m^3 \cdot ha^{-1}$ and Iv= 5.2 $m^3 \cdot ha^{-1}$. According to these production indicators (Table 2) as indicators of degradation can be concluded that the oak forests on Mountain Avala currently using production potential to a large extent, and that there is no indication in terms of degradation.

Quality stands as a criterion for determining the degree of degradation (Krstić, 2006) indicates the poor quality of studied stands (Table 3) and is used to determine future process of land reclamation.

5. CONCLUSIONS

In regard to fact that the given concentration values of heavy metals in soil showed a very high amount, especially for Pb and Ni, so they accumulate in plants to such an extent that they endanger the growth and development. It can be concluded that much more dangerous is their entry from the soil through plants into the food chain.

Measured concentrations of all studied heavy metals in leaves of sessile oak in Avala, are in the MAC values by Serbian legislation.

Heavy metal concentrations in oak leaves at the sites in Avala, is mainly of anthropogenic origin and is not due only pollution from traffic. This is confirmed by the values of the concentration of lead in soil from 108.65 μg/g at site 2, which exceeded the MAC, so that, of all examined heavy metals in Avala, lead can be described as predominant or even as a factor that can cause harmful effects.

According to comparative data on current productivity, these stands are not consider as degraded by these values as indicator of degradation.

However, coppice origin and inadequate management in the past, resulted that the current poor quality of the stand reduces the percentage of technical wood, stability and vitality of the stand, so these forests can be considered as a form of degradation.

Although presence of heavy metals in soil and plants in the protected area "Avala" is evident, this is not a current risk for the occurrence of visible damage to forests. It must be emphasized necessity of constant monitoring of pollution in this area in order to observe negative effects timely and take appropriate precautionary measures.

Overall, we concluded that the area of Avala is still ecologically preserved, which is in accordance with the declaration of the protected area of Avala.

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