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Using Douglas Fir and European Larch Needles for the Assessment of Their Retention Capacity for Atmospheric Heavy Metals

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Abstract: Among numerous global problems, one of the most significant is air pollution. In this paper, unwashed (U) and water-washed (W) needles of two conifers—European larch and Douglas fir—were used to assess their capacity for the retention and accumulation of heavy metals. The needle samples were used to represent the atmospheric deposition of heavy metals located on the surface of the needles. The sampled European larch and Douglas fir plantations were situated at three locations in Serbia: a least polluted (Kučevo), a moderately polluted (Avala), and a very polluted (Lazarevac) site. The content of five heavy metals (Ni, Cu, Co, Cd, Pb) was investigated in the study. The concentration of cadmium (Cd) was higher in the European larch needles compared to Douglas fir, while the differences in the content of the other heavy metals between the species studied were insignificant. For both species, the following trend applied with respect to the heavy metal content in their needles: Ni > Cu > Co > Pb > Cd. Based on the results obtained, we deduced that the concentrations of all investigated heavy metals at all three locations for both species were within the allowed limits, except for nickel (Ni) content, which was over the predicted limit values for both species in the highly polluted area (Lazarevac). A PCA (principal component analysis) undertaken suggests that European larch has a greater ability to accumulate Co than Douglas fir on sites contaminated with heavy metals. The predictive foliar metal accumulation index (MAI) value was slightly higher in Douglas fir (4.14) than in European larch (3.76); therefore, the results suggest that this species would be a good planting choice, particularly in urban and industrial environments.

Keywords: European larch; Douglas fir; heavy metals; needles; retention capacity; Serbia

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1. Introduction

Heavy metals pose a great risk to the environment as a whole, taking into account the numerous negative impacts they cause [1,2]. Heavy metals have a toxic effect on all living organisms [3]. Different morphological, physiological, and anatomical changes in plants are caused by heavy metals and, as a result, reduced production of organic matter often occurs [4]. The aforementioned factors cause problems in plant metabolism, ion absorption, water regime, and the development of photosynthesis and transpiration [5].

Herbaceous plants are often used for heavy metals phytoextraction, but they also have some disadvantages, such as low biomass and a reduced root system, which is not suitable for cleaning deeper soil layers, unlike wood species [6,7]. Conifers play a very important role as biomarkers of industrial pollution and they can also record different types of atmospheric contamination, such as metallic trace elements and radioelements [8,9]. Scots pine (*Pinus sylvestris* L.), for example, has been used for detecting metallic trace element content in needles for the purpose of creating pollution maps [10].

One of the most important indicators of atmospheric pollution is tree leaves [11]. Leaves on wood plants are in contact with pollutants originating from wet and dry atmospheric deposition and accumulate metals from the soil and the atmosphere [12,13]. Metal is usually taken up from the soil by plants through the root system and transported to the above-ground parts of the tree [14]. The degree of retention depends on many factors, such as the climate, type of pollutant, plant surface size, leaf morphology, and anatomy [11].

Orlano et al. [15] investigated the content of different metallic trace elements (Cd, Cr, Cu, Ni, Pb) in the annual rings of European larch (*Larix decidua* Mill.) in the western Italian Alps to evaluate the degree of pollution and record the gradual increase in MTE (metallic trace element) pollution since 1930. Some authors [16,17] have considered the drought tolerance of conifers compared to hardwoods and deduced that some conifers, such as pines, are more resistant than aspen to water deficit, which can be related to the retention capacity for heavy metals of these species. The authors of [18] investigated the efficiency of hybrid poplar and European larch for the accumulation and uptake of cadmium (Cd), copper (Cu), and zinc (Zn). They found that the growth rate of European larch at the end of the experiment was much reduced compared to that of poplars, suggesting that the adsorptive capacity for the heavy metals investigated is comparatively much higher in European larch.

The various heavy metals of concern have different sources and effects. The largest anthropogenic sources of Cu originate from metallurgy, as well as during iron (Fe) and steel production processes and coal combustion. The mean concentration of Cu in dry matter in plant tissue is 2–20 mg kg^{-1} [19,20]. Cd is a non-essential element for plant nutrition which is poorly mobile in the soil and mostly found in the surface, humus-accumulative horizon [21].

The content of lead (Pb) and its compounds is a significant indicator of air pollution originating from traffic, and atmospheric deposition is thought to be the main source of Pb in forest ecosystems [22]. Nickel (Ni) is an essential element for plant growth, characterized by great mobility, with a normal range in plant tissues of between 0.05 mg kg^{-1} and 5.00 mg kg^{-1} [23]. Cobalt (Co) has toxic effects at high concentrations and plays a specific role in plant metabolism [24,25].

De Nicola et al. [26] investigated the difference in content of some elements between washed and unwashed leaves. The chemical nature and structural characteristics of heavy metals determine the effect of the water washing method [27]. In urban fine particulate matter, certain heavy metals are frequently found in organically bound forms [28], which are not effectively removed through water washing.

Based on the results obtained in a paper that dealt with heavy metal content in the soil, bark, and annual rings of Douglas fir (*Pseudotsuga menziesii*/Mirbel/Franco) (DF) and European larch (*Larix decidua* Mill.) (EL) investigated at the same localities [29], we classified these sites as follows: least polluted (Kučevo), moderately polluted (Avala), and most polluted (Lazarevac).

The main goal of the paper was to establish the content of Ni, Cu, Co, Cd, and Pb in unwashed needles and needles washed with water of two allochthonous conifers (DF and EL) in areas with different levels of pollution in Serbia. Based on the results obtained, we can evaluate the retention capacity of the needles of the two species investigated. It can also help us better detect heavy metal pollution in the atmosphere.

2. Materials and Methods

2.1. Study Area

The first location where EL and DF were sampled was Mountain Avala (Figure 1, N 44°41′57.73″ E 20°30′57.87″ for DF; N 44°41′60.49″ E 20°30′21.39″ for EL). The mean altitude of these sampling points was 372 m above sea level (a.s.l) for DF and 299 m a.s.l. for EL. Avala belongs to the Belgrade municipality of Voždovac and is the northernmost mountain of the Šumadija ridge, which belongs to the low island mountains. It has the shape of an irregular elongated compartment, and the area is about 500 ha. There are many deposits of mineral raw materials on Avala, as evidenced by abandoned mining shafts. The Belgrade-Niš highway passes on the eastern side of the mountain, on the northern side is the Ring Road, and on the western side is the regional road to Mladenovac. The ceno-ecological group at the location of the larch sampling is a climatogenic forest association of Hungarian oak and Turkey oak (*Quercion frainetto* Ht. 1954), while at the site of the Douglas fir sampling, there is a hornbeam forest (*Carpinion betuli illyrico-moesiacum* Ht. 1963). Based on the aforementioned ecological conditions (proximity to roads, but also the fact that the European larch and Douglas fir plantations are isolated to a certain extent), this location was classified as moderately polluted.

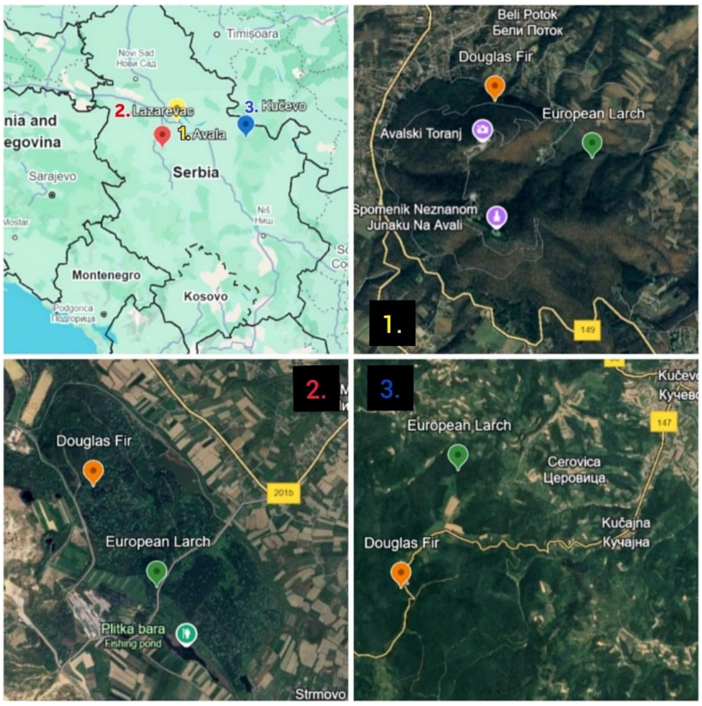

Figure 1. Sampling locations of DF and EL in Serbia: 1—Avala (yellow), 2—Lazarevac (red), 3—Kučevo (blue). The satellite image was obtained via Google Earth.

The second locality of EL and DF sampling was the Kolubara mining basin—Lazarevac (Figure 1, N 44°25′47.4″ E 20°22′42.4″ for DF; N 44°25′15.1″ E 20°23′11.1″ for EL). The mean altitude of this sampling point was 219 m above sea level (a.s.l) for DF and 173 m a.s.l. for EL. In the Kolubara basin, these exotics are most often used for recultivation by afforestation of deposol—mechanically damaged soil. Among the conifer species used for the afforestation of sites damaged by human activity, EL and DF were selected due to their very fast height growth and high degree of adaptability. The soils where afforestation was carried out were created by the disposal of tailings from surface lignite mines. In the vicinity of the investigated location is the Veliki Crljeni thermal power plant with a capacity of 270 MW. The ceno-ecological group to which EL and DF belong in this locality is artificially established plantations. Considering the proximity of the mining pit and the thermal power plant, which produce a large number of pollutants, we can consider this location to be very contaminated.

The third locality where the investigated species were sampled is Kučevo. The municipality of Kučevo is located in northeastern Serbia and includes the middle course of the River Pek. It covers an area of 721 km^2, and the samples were taken from the Management Unit "Donji Pek" (Figure 1, N 44°26′23.5″ E 21°35′47.4″ for DF; N 44°27′39.2″ E 21°36′39.3″ for EL). The mean altitude of this sampling point was 460 m above sea level (a.s.l.) for DF and 314 m a.s.l. for EL.

The ceno-ecological group to which EL and DF in this area belong is mountain beech forest (*Fagenion moesiaca submontanum*). Bearing in mind that anthropogenic influences here have been reduced to a minimum and that the artificially established plantations of the two researched species are situated within natural stands of beech, we defined this location as a least polluted area.

2.2. Sample Collection and Processing

The average age of the sampled trees grown in the DF and EL plantations is 35–40 years. One-year-old needles of both species were sampled, bearing in mind that European larch is a deciduous conifer, so needles of the same age were used for air quality biomonitoring. Ten European larch and ten Douglas fir trees were selected in each of the three localities, totaling 30 trees of each species. The sampled trees were of similar height and diameter at breast height, in satisfactory health condition, located at distances of 15–20 m from each other. Needles were collected at 2 m and 4 m from the ground surface from all four cardinal directions (north, south, east, and west). Then, the needles from different heights were mixed, and a balanced sample was taken using the quartering method. The needles collected from all ten trees of one species per locality, combined from both heights, were pooled to form one sample. Since there were two species and three localities, the total number of samples was six. After collection, the needles were transported to the laboratory and treated in two ways: without washing (U) and by extended rinsing with tap water, followed by three rinses with distilled water and a final rinse with deionized water. The treated needle samples were placed in an oven (total of 12 samples), denatured at 105 °C for 20 min, and dried at 65 °C until the weight was constant. We then ground the needles into powder and passed them through a 65-mesh sieve in preparation for further analysis.

For the determination of heavy metals, the needle samples were digested in a microwave oven, Milestone ETHOS EASY (Milestone Srl, Sorisole, Italy). The digestion of the needle samples was performed using carefully selected acids based on the nature of the sample to achieve complete breakdown of the matrix and release of heavy metals for further analysis [30–33]. A 0.3 g sample was weighed using a Kern balance (KERN & SOHN GmbH, Balingen, Germany) and transferred to Teflon vessels for microwave digestion. Subsequently, 5 mL of 65% concentrated nitric acid and 1 mL of 30% hydrogen peroxide were added to the vessels. The Teflon vessels were then sealed with caps to ensure no leakage during the digestion process. The rotor holding the Teflon vessels was placed in a microwave oven and the appropriate program turned on to ensure complete digestion of the sample. The samples were digested according to the following program: 10 min to reach 160 °C, then 15 min at 210 °C, and 10 min at 210 °C, with a microwave output capacity of 1800 W. After complete digestion, the samples were left for 24 h and then analyzed for heavy metal content.

The total content of heavy metals in each sample was measured in triplicate using an inductively coupled plasma optical emission spectrometer, ICP OES (Spectro Genesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany), according to the methodology of Rautio et al. (2020) [30]. Before ICP-OES analysis, all samples were diluted with deionized ultrapure water to a final volume of 25 cm^3 and filtered through 0.45 μm cellulose filters. The quality of this test procedure was assured by using Certipur® Certified Reference Material ICP multi-elements standard solution IV in diluted nitric acid (Merck KGaA, Darmstadt, Germany, Art. No.: 1.11355.0100), which contains 23 elements (Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Ti, Zn) at a concentration of 1000 mg kg^{-1}. Multistandard IV standard solution was used for the preparation of the calibration solutions. The results were arranged according to the appropriate standards and wavelengths for each metal.

Since tree leaves accumulate various elements concurrently, the total capacity of the tree related to the heavy metal accumulation was estimated using the predictive foliar metal accumulation index (MAI) [34].

$$MAI = \left(\frac{1}{N}\right)\sum_{j-1}^{N} I_j \quad (1)$$

where N is the total number of analyzed heavy metal elements, and Ij is the ratio of the mean value to the standard deviation of the concentration of a certain heavy metal in the leaves. In this study, N = 5.

2.3. Statistical Analysis

The numerical data obtained in this study were processed using descriptive, univariate, and multivariate statistical methods. For the descriptive statistics, the following parameters were calculated: mean, standard deviation (SD), and coefficient of variation (CV). An analysis of variance (ANOVA) and the post hoc Fisher's least significant difference (LSD) test were used in order to determine the significance of differences between the mean values. The presence of heavy metals in the sampled needles was also analyzed using principal component analysis (PCA) to show the variation and relationships between needles of the two species sampled at three sites and treated by different washing methods. The statistical analyses were preceded by testing the normality and collinearity of the data. All statistical analyses were performed in Statgraphics Centurion, ver. XVI.I (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results

The heavy metal content in the unwashed needle samples was first analyzed to establish the difference in the needle heavy metal content of DF and EL between different sampling sites in Serbia. Based on this analysis, the descriptive statistics and ANOVA results were presented (Table 1), with a visual representation of the basic statistical parameters of the variables with the highest F-ratio values (Figure 2).

The values of the coefficient of variation (CV%) of the studied variables ranged from low (<10%) to very high (>50%), depending on the species and site, but most of the variables had a low variability level. The ANOVA showed that there were statistically significant differences ($p < 0.05$) between the means of the Co contents in the DF needles and the contents of all the studied metals in the EL needles from different sites, with a significant contribution of these variables to the general differentiation of sites, which can be seen from the F-ratio values (Table 1; Figure 1). Based on the homogeneous groups in the LSD (least significant difference) test, Co accumulated to the greatest extent in the needles from Lazarevac compared to the other sampling sites of DF. With respect to the EL samples, Ni accumulated to the greatest extent in the needles from Lazarevac, less in the needles from Avala, and to the least extent in the needles from Kučevo. The same applied to the Pb contents, but there was no statistically significant difference between the mean values for Avala and Kučevo. In contrast, Cd accumulated to the greatest extent in the needles from Kučevo, less in the needles from Avala, and to the least extent in the needles from Lazarevac. The same applied to the Cu contents, except there was no statistically significant difference between the mean values for Avala and Lazarevac. Finally, Co accumulated to the greatest extent in the needles from Kučevo, less in the needles from Lazarevac, and to the least extent in the needles from Avala (Table 1). The results obtained show that the mean values were mostly higher for Lazarevac compared to the other two sampling sites.

Table 1. Descriptive statistics (mean, SD—standard deviation, CV—coefficient of variation) and analysis of variance (ANOVA) for heavy metal content (mg kg^{-1}) in the unwashed needles of two conifer species sampled on three sites in Serbia.

Site		Douglas Fir					European Larch				
		Ni	Cu	Co	Cd	Pb	Ni	Cu	Co	Cd	Pb
Lazarevac	Mean [1]	14.11 a	3.33 a	0.67 a	0.19 a	0.46 a	17.50 a	3.13 b	0.56 b	0.16 c	0.50 a
	SD	5.98	0.42	0.08	0.07	0.40	0.95	0.06	0.06	0.01	0.12
	CV (%)	42.39	12.49	11.25	38.75	87.16	5.45	1.84	10.40	6.25	23.70
Avala	Mean	6.09 b	3.43 a	0.16 b	0.13 a	0.16 a	13.70 b	4.07 a	0.19 c	0.19 b	0.04 b
	SD	0.42	0.06	0.04	0.03	0.24	0.15	0.12	0.01	0.01	0.07
	CV (%)	6.89	1.68	25.80	20.35	153.24	1.06	2.84	5.26	2.99	173.21
Kučevo	Mean	8.43 ab	3.67 a	0.14 b	0.16 a	0.00 a	7.36 c	4.07 a	0.76 a	0.27 a	0.00 b
	SD	0.11	0.29	0.03	0.01	0.00	0.11	0.12	0.02	0.01	0.00
	CV (%)	1.35	7.87	22.43	3.69	0.00	1.46	2.84	2.28	3.70	0.00
ANOVA	F-ratio	4.26	1.01	97.86	1.21	2.50	250.10	87.11	196.81	122.71	37.01
	p-value [2]	0.0706	0.4178	**0.0000**	0.3613	0.1234	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0004**

[1] Means with different letter designations within a column are significantly different from each other at the 95% confidence level. [2] Variables with $p < 0.05$ (ANOVA) are marked with bold numbers.

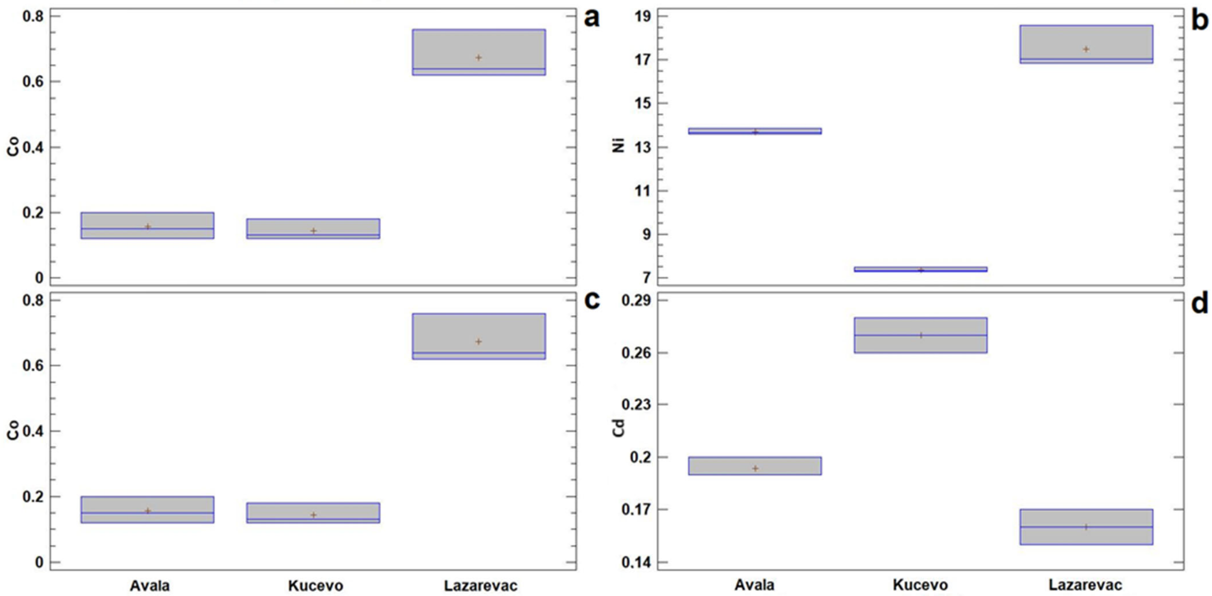

Figure 2. Box and whisker plots of basic statistical parameters for heavy metal content (mg kg^{-1}) in the unwashed needles of two conifer species sampled on three sites in Serbia. Variables with the highest F-ratio values: (**a**) Co in the needles of Douglas fir; (**b**) Ni in the needles of European larch; (**c**) Co in the needles of European larch; and (**d**) Cd in the needles of European larch. Legend: middle sign—mean, middle line—median, box—lower and upper quartile.

The differences between the studied species in terms of heavy metal contents of unwashed needles sampled in Serbia were represented by the predictive foliar metal accumulation index (MAI), descriptive statistics, and analysis of variance (ANOVA) (Table 2). The ANOVA revealed that there were statistically significant differences ($p < 0.05$) between the mean values of Cd content in the unwashed needles of the studied species, with a significant contribution of this variable to the differentiation of species, which can be seen from the F-ratio. According to the LSD test, the mean content of Cd in the unwashed needles of EL was significantly higher than the mean content of this heavy metal determined for the unwashed needles of DF. The values of MAI were somewhat higher for DF compared to EL (Table 2).

Table 2. Metal accumulation index (MAI), descriptive statistics, and analysis of variance (ANOVA) for heavy metal content (mg kg^{-1}) in the unwashed needles of two conifer species sampled in Serbia.

Species		Ni [1]	Cu	Co	Cd	Pb	MAI
Douglas fir		9.54 ± 4.40 a	3.48 ± 0.28 a	0.32 ± 0.25 a	0.16 ± 0.04 b	0.31 ± 0.29 a	4.14
European larch		12.85 ± 4.21 a	3.76 ± 0.45 a	0.50 ± 0.24 a	0.21 ± 0.05 a	0.18 ± 0.24 a	3.76
ANOVA	F-ratio	2.37	2.22	2.17	4.46	0.93	--
	p-value [2]	0.1434	0.1553	0.1602	**0.0407**	0.3493	--

[1] Data are expressed as mean ± standard deviation. Means with different letter designations within a column are significantly different from each other at the 95% confidence level. [2] Variables with $p < 0.05$ (ANOVA) are marked with bold numbers.

The differences between the washing methods for the heavy metal contents in the DF and EL needles from Serbia were obtained by descriptive and univariate (ANOVA) statistical analyses (Table 3). The CV% values of the variables ranged from a low (<10%) to a very high variability level (>50%), depending on the species and washing method, but most of them were very variable. The ANOVA showed that there were statistically significant differences ($p < 0.05$) between the mean values of the Cu and Pb contents in the DF needles and the Pb contents in the EL needles treated with different washing methods, with a significant contribution of these variables to the general differentiation of washing methods, which can be seen from the F-ratios. According to the LSD test, the unwashed needles of DF had a much higher concentration of Cu and Pb compared to the washed needles. With respect to the Pb content, the same pattern was obtained for EL—a significantly higher concentration was recorded in the unwashed compared to washed needles (Table 3). As can be seen from these results, the mean values were mostly higher in the unwashed needles than in the washed needles of both studied species.

The differences between the sites, species, and applied methods for the heavy metal contents in the needles of DF and EF from Serbia were established by principal component analysis (PCA) (Table 4) and presented using a PCA scatterplot (Figure 3). The first two principal components, with eigenvalues >1, explained 71.47% of the total variation. Most of the variation was explained by the first axis (PC1) (41.36%) and 30.11% by the second one (PC2). The variables Cu and Cd, with factor loadings >0.70, influenced PC1, whereas PC2 was influenced by the variable Co (Table 4). The PCA resulted in the separation of samples along PC1. Corresponding to the sampling sites, three groups of samples were formed, as shown in Figure 3. The samples from Lazarevac formed a group mainly at the negative part of PC1, whereas the samples from Kučevo were mostly separated from the samples from Avala as a group at the positive part of the same axis (Figure 3), suggesting significant differences between the sampling sites in terms of the Cu and Cd contents (Table 4). In addition, most of the EL samples were separated from the Douglas fir samples as two groups from Lazarevac and Kučevo at the negative part of PC2; an indication of differentiation within these three groups was also visible along PC2 related to the washing method used (Figure 3), which indicates significant differences between the sampled species regarding their ability to accumulate Co (Table 4). Specifically, the results of this analysis suggest that EL has a greater ability to accumulate Co than DF on sites contaminated with heavy metals.

Table 3. Descriptive statistics (mean, SD—standard deviation, CV—coefficient of variation) and analysis of variance (ANOVA) for heavy metal content (mg kg^{-1}) in unwashed and water-washed needles of two conifer species sampled in Serbia.

Needle Treatment		Douglas Fir					European Larch				
		Ni	Cu	Co	Cd	Pb	Ni	Cu	Co	Cd	Pb
Unwashed	Mean [1]	9.54 a	3.47 a	0.32 a	0.16 a	0.31 a	12.85 a	3.76 a	0.50 a	0.21 a	0.18 a
	SD	4.66	0.29	0.27	0.05	0.31	4.46	0.47	0.25	0.05	0.25
	CV (%)	48.88	8.48	81.90	29.01	100.66	34.70	12.64	50.08	23.80	139.09
Washed with water	Mean	10.84 a	2.91 b	0.26 a	0.12 a	0.00 b	12.11 a	3.74 a	0.41 a	0.17 a	0.00 b
	SD	6.37	0.39	0.26	0.03	0.00	4.20	0.61	0.22	0.05	0.00
	CV (%)	58.76	0.39	100.49	22.40	0.00	34.71	16.37	54.98	26.96	0.00
ANOVA	F-ratio	0.24	12.07	0.31	4.27	8.88	0.13	0.01	0.74	2.83	4.65
	p-value [2]	0.6284	**0.0031**	0.5837	0.0553	**0.0088**	0.7211	0.9403	0.4033	0.1121	**0.0466**

[1] Means with different letter designations within a column are significantly different from each other at the 95% confidence level. [2] Variables with $p < 0.05$ (ANOVA) are marked with bold numbers.

Table 4. Principal component analysis (PCA) for heavy metal content in the needles of Douglas fir and European larch treated with different washing methods after sampling on three sites in Serbia.

Component	Eigenvalue	Percentage	Ni	Cu [1]	Co	Cd	Pb.
PC1	2.07	41.36	−0.64	**0.85**	0.12	**0.85**	−0.45
PC2	1.51	30.11	−0.49	0.06	**−0.94**	−0.50	−0.38

[1] Variables with factor loadings >0.70 are marked with bold numbers.

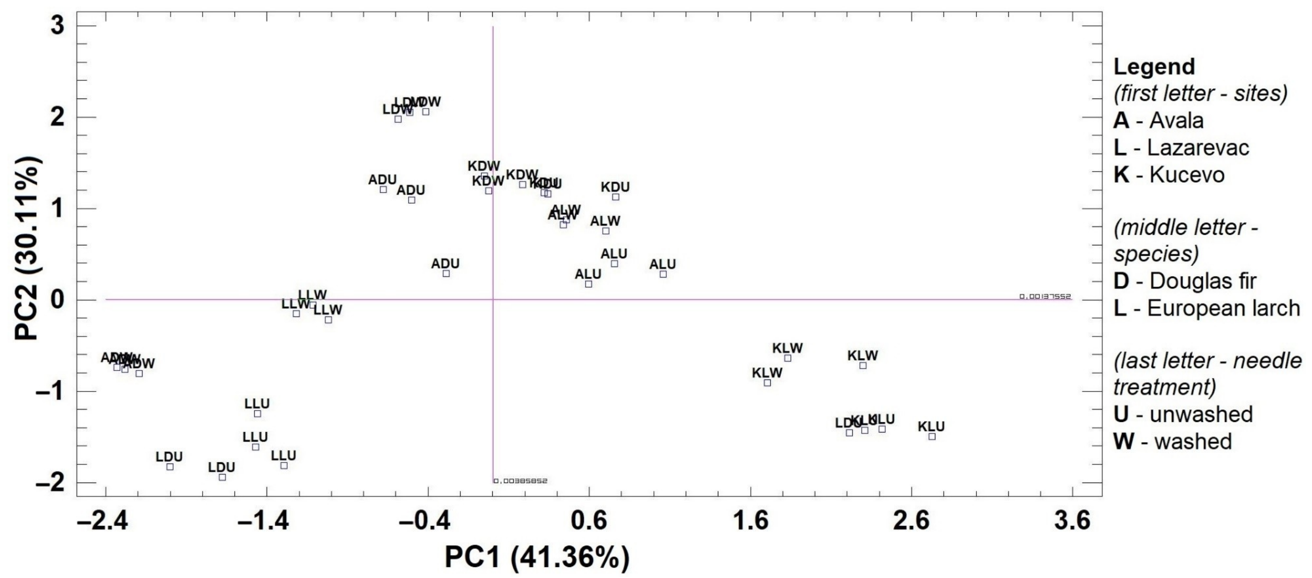

Figure 3. Principal component analysis (PCA) scatterplot for heavy metal content (mg kg^{-1}) in the needles of Douglas fir and European larch treated with different washing methods after sampling on three sites in Serbia.

4. Discussion

Heavy metals present in the unwashed needles may originate from different sources. One component is related to the transport of heavy metals from the soil to the leaves by the root system through transpiration [35], while another component is connected with atmospheric particulate matter deposition. PM (particulate matter) containing heavy metals can be trapped by the epidermal layers of the leaves and diffuse into the leaves via different lipophilic or hydrophilic routes [36]. These substances may also enter the leaves directly via the stomata and become an integral part of the leaves. The uptake and accumulation of Ni and Cu were much higher for both studied species compared to the other three elements (Co, Cd, and Pb), which is in accordance with the fact that Ni and Cu are essential elements for plant nutrition and are transported much faster from the roots to the leaves [37–39]. Ni and Cu are also much more prevalent in the atmosphere compared to the other three elements [40]. Some authors have established that between different conifer species, there are significant differences in MAI values due to the morphological structure of the crown and leaf structure, as well as due to the developmental and bioecological properties of the species [41,42]. However, in our study, these differences in MAI values were insignificant, even though there is a difference between DF and EL in terms of the anatomical structure of their needles (one is evergreen, the other a deciduous conifer; one is round, the other is flat). Based on the obtained results, we can infer that the needles of both species can accumulate and take up heavy metals occurring in urban environments. The leaf structure and crown morphology allow the species to retain more dust particles, which is related to a greater accumulation of heavy metals, and this is very important for the selection of wood species in polluted sites [34]. The authors of [43] concluded that Cu and Cd in particulate matter deposition originate mostly from anthropogenic sources. Reflecting the properties of Cd, the greatest proportion of this metal remains on the leaf surface in a residue state, while a very low concentration of this element reaches the leaf interior [44]. Based on literature data, it was inferred that the Cd and Cu on the blade surface of leaves may originate from different sources, such as exhaust gases from vehicles, mechanical wear caused by engines, and friction of tires on the ground [45]. The same authors [45] suggest that some essential elements may also originate from steel smelting and the alloy industry. One of the ways to reduce the level of pollution in big cities would be to adopt an appropriate long-term strategy [46,47] that would involve the selection of adaptable species.

In this study, we applied two treatments (unwashed and water-washed needles) to determine the retention capacity of the species investigated for atmospheric heavy metals, bearing in mind that water washes all the additional substances, such as dust particles, atmospheric deposition of environmental pollutants of different origin, etc. from the surface of the needles. In DF, insignificantly greater content of Ni was obtained in washed compared to unwashed needles. On the other hand, in EL, a slightly higher amount of Ni was recorded in unwashed needles, which is connected to the anatomical properties of the leaf itself, i.e., its degree of permeability and the thickness of the surface and protective layer. For the remaining elements, the same patterns were obtained in both species, i.e., slightly higher values were recorded in unwashed compared to water-washed needles. It should be noted that Pb was not detected after washing the needles, possibly as a consequence of its properties and the fact that it hardly diffuses through the membranes of the leaf to its interior, which is the reason it is present in the surface layer. In addition, it should be noted that the assumption that the Kolubara mining basin (Lazarevac) is the most polluted area was confirmed, because the highest average content of almost all the heavy metals in both species was recorded there. Based on the LSD test, it was determined that the average content of Cd in unwashed EL needles was significantly higher compared to the average concentration of this metal in unwashed DF needles. This could be related to the fact that the anatomical structure of EL needles is characterized by a much lower permeability to Cd than in the case of DF. EL has a more powerful protective layer and rougher cuticle texture [48], so, for these reasons, the needle permeability for the entry of some elements, such as Cd, which penetrate into the interior with difficulty, is much less compared to that of DF. As a result, most of the Cd remains on the surface of the EL needles. Using the LSD test, a significantly higher content of Cu and Pb was recorded in unwashed compared to washed DF needles. The same applied to the Pb content in the EL needle. This is related to the fact that Cu and Pb, due to their properties, have difficulty entering the interior of the needle and mostly remain on the surface of the leaf tissue [34]. Some studies [49] confirm that these elements are often present in their residue in an organic state, and that water treatment does not produce satisfactory results in terms of moving these fractions of heavy metals into the interior of the needle.

Based on the obtained results, we can conclude that for Co, Cu, Cd, and Pb, cleaning with water was very effective.

The authors of [11] investigated the concentrations of Cd, Cu, Pb, and Zn in two tree species (one conifer and one deciduous) in washed and unwashed leaves and obtained similar results. Comparing the values of the concentrations obtained, Zn was found to have the highest concentration as an essential element, while there was the least content of Cd, which is consistent with our results.

The authors of [50] note that most woody species can accumulate significant concentrations of Cu in natural and anthropogenically altered site conditions. The normal content of Cu in plant tissue is between 2 mg kg^{-1} and 20 mg kg^{-1}, while levels over 30 mg kg^{-1} are phytotoxic for this element [51,52].

With respect to Pb, its typical concentration in plants is below 10 mg kg^{-1} [53,54], with the normal concentration range for this element in plants being 5–10 mg kg^{-1}, while the toxic concentration is above 30 mg kg^{-1}. Thus, we can note that the amounts of Pb obtained in our paper are negligible. Plants take up lead from the atmosphere, which [54] is then deposited on the leaf surface; however, transport of Pb from the roots to the leaves is not the main route for uptake of this element [55,56]. The authors of [57] concluded that for poplar plantations along a road, the main route of Pb was through open stomata, and the capacity of stomata to respond to these environmental conditions was greatly reduced due to the emission of large amounts of exhaust gases [58].

Ejidike and Onianwa [59] found an average Cd concentration of 0.10 mg kg^{-1} in the bark of trees from 65 different habitats affected by intensive human activity. The authors of [60] found a substantial content of Cd in the wood and bark of DF plantations grown on contaminated soil. The acceptable natural concentration of Cd is between 0.2 and 2.4 mg kg^{-1} [60], which means that our values are within a tolerable range for both species. There are various sources of Cd, such as the burning of fossil fuels, friction of car tires, and burning of municipal solid waste [54]. Cd is characterized by good mobility, and it is not an essential element, while the largest content of Cd is located in the cell wall in a form that cannot enter the interior of the plant [3]. Arsenov [61] found that the phytotoxic concentration of Cd is between 5 mg kg^{-1} and 10 mg kg^{-1} in sensitive plant species, while Macnicol and Beckett [62] suggest a range of 10 mg kg^{-1} to 20 mg kg^{-1} as representing critical Cd levels.

Various anthropogenic sources contribute to the release of nickel into the environment [63]. It plays an essential role in plant nutrition, but its content in many plants is very low (0.05–10 mg kg^{-1} dry weight). At the very polluted site in Lazarevac, we obtained slightly higher values of Ni concentrations compared to usual values. A much higher content of Ni affects mitotic activities, plant growth dynamics, and the growth of trees [64].

Phytotoxic Ni concentrations range widely among plant species and cultivars and have been reported for various plants to be from 40 mg kg^{-1} to 246 mg kg^{-1} [65].

The results of the PCA suggest that EL has a greater ability to accumulate Co than DF on sites contaminated with heavy metals. Co is a transition metal with seven oxidation states and is not an essential element for plants. The toxicity of Co excess is linked to oxidative stress, inhibition of photosynthesis, and Fe deficiency [66]. It disrupts Fe homeostasis and competes with it in various transportation processes [67].

Based on a number of factors, such as their wide geographical distribution, morphological and anatomical needle structure, and the protective layer on the leaf surface, conifers are very important for the assessment of air pollution [68]. The retention and accumulation capacity of the needles is closely related to needle age; for instance, one-year-old needles of black pine (*Pinus nigra* J.F.Arnold) contain far more toxic elements compared to the fresh buds, the degree of pollution of which is highly dependent on the position of trees and their distance to traffic [33]. Some studies carried out in Poland [69] found that the heavy metals content increased with the needle age of Scots pine. Protective layers on the needle surface, such as wax structures, reduce the content of pollutants that enter the leaf tissue. As a result, low heavy metals content in needles has been recorded even in polluted areas [70]. Popović et al. [68] found that Norway spruce (*Picea abies* H. Karst) needles and bark were very good indicators for air pollution assessment, and also found very significant differences in heavy metals accumulation between one-year-old and two-year-old Norway spruce needles. The retention capacity of the needles for heavy metals accumulation depends on many factors, i.e., the concentration and type of pollutants in the air and ground; developmental features of the plant species; climate conditions, such as rainfall, wind direction and speed; and the proximity of the pollution source [71].

5. Conclusions

Based on the heavy metal (Ni, Cu, Co, Cd, Pb) content in unwashed and water-washed needles of European larch (EL) and Douglas fir (DF), sampled at three locations with different degrees of pollution in Serbia, the following conclusions can be drawn:

- Considering the content of almost all the analyzed heavy metals, Lazarevac was the most polluted site for both species studied, confirming the main hypothesis of this study;
- The concentrations of all the heavy metals analyzed at the researched sites for both species were within the maximum allowable limits, apart from nickel (Ni), the concentration of which at the very polluted area of Lazarevac was slightly over the usual limit values;
- Higher contents of almost all the heavy metals analyzed, apart from (Ni), were found in the unwashed compared to the washed needles;
- The content of cadmium (Cd) was higher in the unwashed EL needles than in the unwashed DF needles;
- The content of copper (Cu) and lead (Pb) was higher in the unwashed compared to the washed DF needles, and the same applied to the Pb content in the EL needles;
- EL has a greater potential to accumulate cobalt (Co) than DF on polluted sites;
- Considering the calculated values of the predictive foliar metal accumulation index (MAI), DF could be a good planting choice in urban areas where heavy metals contamination is significant.

Finally, to have more reliable information, it would be necessary to sample another segment of the above-ground part of the tree (wood and bark) and to obtain soil samples, which would contribute to the implementation of comprehensive air quality biomonitoring.

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