This version of the article has been accepted for publication, after peer review and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections.

The Version of Record is available online at: http://dx.doi.org/10.1007/s10661-022-10732-2

All Rights Reserved

© 2022, The Author(s),
under exclusive licence
to Springer Nature
Switzerland AG

The evaluation of hazardous elements content in the needles of the Norway spruce (*Picea abies* L.) that originated from anthropogenic activities in the vicinity of the native habitats

Vladan Popović[1¶], Darka Šešlija Jovanović[2], Zoran Miletić[1], Jelena Milovanović[3], Aleksandar Lučić[1], Ljubinko Rakonjac[1], Danijela Miljković[2¶*]

[1]Institute of Forestry, Department of Genetics, Plant Breeding, Seed and Nursery Production, Belgrade 11000, Serbia. [2]University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Department of Evolutionary Biology, Belgrade 11000, Serbia; [3]Environment and Sustainable Development, Singidunum University, Belgrade 11000, Serbia

[¶]These authors have contributed equally to this work and share first authorship

*Corresponding author:
Danijela Miljković, Ph.D.
orcid.org/0000-0003-1781-6658
Email: danijela.miljkovic@ibiss.bg.ac.rs
Department of Evolutionary Biology
Institute for Biological Research “Siniša Stanković”
National Institute of Republic of Serbia
University of Belgrade
Bulevar despota Stefana 142
11060 Belgrade, Serbia

Vladan Popović 0000-0003-4326-3364,

Darka Šešlija Jovanović 0000-0003-3031-8426,

Zoran Miletić 0000-0001-9044-3275,

Jelena Milovanović 0000-0001-5310-5779,

Aleksandar Lučić 0000-0002-4473-1791,

Ljubinko Rakonjac 0000-0002-8736-6771,

Danijela Miljković 0000-0003-1781-6658.

Abstract The aim of this research was to quantify the content of hazardous elements in the needles of Norway spruce (*Picea abies* L.) in the natural habitats that were accumulated from thermal power plants, mines and metal processing industry. Fifteen natural populations of the Norway spruce were sampled from the mountain ranges in Southeastern Europe (Dinaric Alps and Balkan Mountains). In two-year-old spruce needles were evaluated the content of the following hazardous elements: heavy metals cadmium, mercury, nickel, lead and zinc, and metalloid arsenic. The effect of the distance between air pollution emitters and the Norway spruce natural habitats on the hazardous elements content in needles was also evaluated. The results of the analysis of variance confirmed interpopulation differences in the content of all analyzed hazardous elements. The effect of the air pollution source (thermal power plants, mines and industry) on the content of hazardous elements in the spruce needles was also assessed. Significant correlation was found between the distance of air pollution emitters and the amount of zinc. This study could serve as the startup point of future monitoring programs and provide new prospect of using Norway spruce needles as the bioindicator of air pollution with hazardous elements on Balkan Peninsula since the fact that the Norway spruce natural populations inhabit wide geographic range of the continental Europe, from the Balkan Peninsula, over European Alps to Scandinavia and a large-scale of altitude from 980 to 1860 meters above sea level.

Key words Anthropogenic pollutants, hazardous elements profile, needles, *Picea abies* L., Southeastern Europe.

Introduction

Anthropogenic activities in various industrial branches (energy - heating plants and thermal power plants, combustion of fossil fuels, construction, metal processing industry, mining, and road and air traffic) change the areas of natural habitats, impair air quality by emitting air pollution particles (PM). The limits of the range of anthropogenic influence depend on the proximity and number of industrial facilities and are positively correlated with the endangerment of natural habitats (Ippolitova, 2019; De La Cruz et al., 2019). In the Western Balkans, the emission of suspended particulate matter was 1.6 times higher in 2019. than in the previous period, according to the National Emission Reduction Plan (NERP) (https://ec.europa.eu/environment/air/cleaner_air/). To reduce air pollution was launched the Clean Air Regions Initiative (CARI) and at the same time was signed the declaration on measures aimed at reducing air pollution in the most critical areas of the Western Balkans (https://balkangreenenergynews.com).

The top ten biggest air pollutants in the Western Balkans (https://www.env-health.org/wp-content/uploads/2020/06/Chronic-Coal-Serbia.pdf) are thermal power plants ‘Kostolac A and B’ and ‘Nikola Tesla A and B’ in Serbia, ‘Ugljevik’, ‘Kakanj’ and ‘Tuzla’ in Bosnia and Herzegovina, ‘Bitola’ in Northern Macedonia and ‘Maritsa East 2’ in Bulgaria. Thermal power plants in the Balkans produce 16 times more suspended particles than the whole of Europe, which directly endangers human health (https: //www.env-health.org/wp-content/uploads/2019/02/Chronic-Coal-Pollution-report.pdf). Also, air pollution (high emissions of sulfur dioxide, ammonia, ozone) affects the ecosystem (EEA 2014) and directly damages vegetation (Guerreiro et al., 2014).

Hazardous elements that most often occur as pollutants and contaminants of air and soil are metals cadmium (Cd), chromium (Cr), copper (Co), mercury (Hg), lead (Pb) and zinc (Zn) and metalloid arsenic (As). Monitoring the concentration of these hazardous chemical elements is very important and necessary for estimating the ecological conditions of the specific plant habitat. Plants can accurately indicate the presence and intensity of various pollutants (heavy metals, chemicals, etc.) in the air and soil, both in natural ecosystems and in urban areas (Lüttge & Buckeridge 2020; Tanase et al., 2021). Leaves and tree bark are plant organs that are usually analyzed in biomonitoring heavy metals and metalloids (Dogan et al., 2014). The accumulation of heavy elements in high concentrations in plants indicates relative increase and expanding of pollution in the habitat. Accumulation potential depends on different conditions and features so that several types of plants can be used as bioindicators in the detection of air pollution by heavy metals (Sawidis et al., 2011; Hoodaji et al., 2012; Sharma et al., 2015). In theory, any plant species can be used as a bioindicator under the condition of good understanding of the biology and ecology of the species (Stanković et al., 2011a) and that the adoption of toxic elements depends on the concentration of pollutants in the environment (Čeburnis & Steinnes, 2000). Besides the types of pollutants and particle size, the accumulation is also affected by leaf morphology, anatomy, size and orientation, as well as environmental conditions such as wind direction and speed, particle solubility and the possibility of leaf washing-off by rain. Evergreen trees are better indicators of pollution, primarily due to the needles longevity, i.e. longer exposure to pollutants (Hoodaji et al., 2012; Sharma et al., 2015).

The large spatial distribution of different plant groups allow an inexpensive and fast way of passive biomonitoring of the heavy metal concentration in the atmosphere (De La Cruz et al., 2019). For instance, a large study on 48 locations in Lithuania revealed differences between gymnosperms needles (spruce (*P. abies* L.) and juniper (*Juniperus communis*)) and Bryophyta moss in As, Cd, Cr, manganese (Mn), lead (Pb) and Zn concentration, as well as between the spruce needles within and under the tree canopy (Čeburnis & Steinnes, 2000). Evergreen trees extensively used in environmental monitoring programs such as *Phoenix dactylifera* (Naderizadeh et al., 2016; Al-Khashman et al., 2011), *Cedrus libani* (Onder & Dursun, 2006), as well as deciduous trees *Fagetum montanum* and *Quercetum -Fagetum* (Knežević et al., 2000), lemon trees (*Citrus limon*) (De La Cruz et al., 2019) and *Populus alba* (Madejón et al., 2004).

Pine needles are suitable for determining air contamination with heavy metals (Hoodaji et al., 2012). It was found that the concentration of heavy metals in needles increased with increasing tree age in the most of studied conifers (*Pinus nigra*, *Picea pungens*, *Pinus sylvestris* and *Abies bornmülleriana*). Still, the accumulation of heavy metals was species specific. *P. nigra* exhibited the highest concentration of iron (Fe), *P. pungens* had the most of Zn and *P. sylvestris* contained the most of Pb. *A. bornmülleriana* proved to be particularly useful as a bioindicator, because its high ability to bind heavy metals from the air that resulted in the highest concentrations of the most hazardous elements (Türkyilmaz et al., 2018). On the other hand, heavy metal analysis of spruce bark from unpolluted locations revealed contradictorily its increased concentration indicating that bark was not a good choice for biomonitoring of heavy metal pollution (Tanase et al., 2021).

Plant responses to the presence of heavy metals are regulated by the process of metal homeostasis. The researchers compared different molecular mechanisms of Zn and Cd homeostasis between metal-sensitive and metal-adapted plant species. Lin & Aarts (2012) listed different groups of plants depending on the strategy they applied in the response to stress caused by the presence of harmful elements: metal sensitive species, metal resistant excluder species, metal tolerant non-hyperaccumulator species and metal hypertolerant hyperaccumulator species.

The air quality in an environment is directly linked to the proximity of air pollutants (thermal power plants, smelting plants,...). Concentrations of As, Cd, Hg, Ni, Pb and Zn can be registered even at large distances from the smelting plants, up to 217 km (Canadian smelting plant Flin Flon). The concentration of heavy metals depends on the direction and speed of the wind, the terrain configuration, and the size of the suspended particles in the air (Nikolić et al., 2011).

The aim of this study was to assess the content of hazardous elements As, Cd, Hg, Ni, Pb and Zn in two-year-old needles of Norway spruce (*P. abies* L.) in order to determine interpopulation differences of fifteen populations of two mountain ranges, the Balkan Mountains (Mts.) and Dinaric Alps, in relation to the distance from the main sources of air pollution (thermal power plants and mines) and precipitation as an important climatic factor.

Materials and methods

Study habitats and species

The research was conducted in fifteen natural, geographically distant, spruce populations located at the altitudes ranging from 980 to 1860 m a.s.l., in two major mountain ranges of the Southeastern Europe, the Balkan Mts. and the Dinaric Alps (Table 1, Fig. 1). The natural distribution of this species covers the most of the continental Europe, from the Balkan Peninsula, over European Alps to Scandinavia (Stojnić et al., 2019; Goczał et al., 2020; Popović et al., 2022). At high altitudes Norway spruce distribution is reduced as a result of climate change and air pollution. Mean annual precipitation (MAP) data in this area was valued for the period from 1961 to 2020, according to Climate EU v4.63 software package, available at http://tinyurl.com/ClimateEU (Hamann et al., 2013).

Determination of heavy metal concentration in needles

Each population was represented by 30 mature trees, which were located at a distance of at least 50 m. The samples of assimilation organs (needles) aged two years were collected from the upper third of the tree canopy (Rautio et al., 2016). The collected samples were dried at 40° C and ground to a powder. Moisture was determined by drying the samples at 105° C. A weight of 0.2 g of ground material of known humidity was poured in 8 ml of concentrated nitric acid (HNO_3, Zorka "Pharma" a.d. Šabac) and 2 ml of hydrogen peroxide (H_2O_2, Zorka "Pharma" a.d. Šabac). Digestion was performed in a microwave digester "ETHOS EASY" (Digestion System - Milestone Ethos LEAN). Determination of total amount of hazardous elements in the spruce needles was performed by the ICP-OES spectrometer VISTA PRO Varian. Certified standard solutions were used to calibrate the ICP spectrometer. The wavelengths measured for the elements were the following: As =193.69 nm, Cd = 228.82 nm, Hg = 194.16 nm, Ni = 231.60 nm, Pb = 182.14 nm and Zn = 213.86 nm. Factory solutions were stabilized with HNO_3, so that the elements were in the form of nitrate (the carrier anion is NO_3-). The limit of detection was 0.13 mg/kg, and the limit of quantification was 0.4 mg/kg. In this study a total of 16 pollution emitters was followed (Table 2).

Statistical analyses

The content of As, Cd, Hg, Ni, Pb and Zn in needles was evaluated by PROC MEANS procedure and expressed by the basic parameters of descriptive statistics, mean values and standard errors (SE), in each population. The statistical significance differences in the spruce needle content of hazardous elements between populations and trees (nested in populations) were estimated by analysis of variance (ANOVA, procedure PROC GLM in SAS software) (SAS 2011). Testing of selected variation factors (population as fixed factors and tree as a random factor) was performed by F-test, using RANDOM options in PROC GLM procedure (Table 3). We performed procedure PROC CORR to estimate Pearson's correlation coefficients between altitudes, distances of air pollution emitters and hazardous element content.

The Principal Component Analysis (PCA) multivariate method was applied for the analysis of population variability and was derived directly from the analyzed needle traits. PCA based on the correlation matrix (Pearson’s method) was performed in order to examine the patterns of population variability in relation to the needle content of hazardous elements and annual precipitation as a climatic condition. The corresponding principal component can be considered as the main factor of the obtained variability if the eigenvalue is greater than 1.0. We used biplot graphs to visually present results both on populations and hazardous elements.

The Microsoft Excel XLSTAT add-in software package Agglomerative Hierarchical Clustering (AHC) (Ward’s method) was performed on standardized mean values of needle traits using Euclidean distance, which provided the optimal classification of the analyzed populations into homogeneous groups, so-called clusters. The cluster analysis was visually presented by a dendogram. Statistical data analyses were performed using the appropriate procedures in the software package SAS 9.1.3 (SAS 2011). Graphic presentations of the obtained results were performed by XLSTAT in Microsoft Excel add-in software package.

Results and Discussion

Assessing the impact of pollutants, such as heavy metals and metalloids, allows the evaluation of stress on individual organisms and also on entire ecosystems due to their toxicity, persistence and bioaccumulation. This assessment of the response of organisms and eco-systems to the concentration of environmentally hazardous elements in relation to the spatial distribution of the source of pollution, presents biomonitoring (Lodenius, 2013).

The present study confirmed significant difference between populations in the content of all analyzed hazardous elements, excluding Hg content. The lowest values of hazardous element concentration (< 0.5 µg/g needle dry mass) in *P. abies* in two years old needles from 15 populations (four from the Balkan Mts. and eleven from the Dinaric Alps) were obtained for Hg (< 0.3 µg/g), Cd (< 0.5 µg/g) and As (< 0.5 µg/g), except in the populations of Kopaonik and Golija (approximately 0.8 µg/g) and Tara (1.5 µg/g). Values of Ni content were up to 5 µg/g. Rila population had the highest Ni concentration (4.17 µg/g) in the Balkan Mts, whereas Kopaonik, Golija, and Tara populations (1.87 µg/g, 3.50 µg/g, and 4.11 µg/g, respectively) had the highest values of Ni content in the Dinaric Alps. The content of Pb in the Norway spruce needles had different pattern, the highest values up to 12 µg/g were registered in the populations of Stara Planina (Balkan Mts.), Mojstir (10.06 µg/g), Javor (11.79 µg/g) and Zlatar (11.75 µg/g) (Dinaric Alps). The variability of Zn content in the populations of both mountains ranged similarly from 18 to 35 µg/g. The only exception was recorded in the spruce needles in Lisina population (Dinaric Alps), almost 60 µg/g (Figure 2). However, populations were significantly different in the concentration of all analyzed hazardous elements (all $p < 0.0001$), except in Hg ($p > 0.05$) (Table 3). The results of the analysis of variance on the content of hazardous elements in the spruce needles revealed no statistically significant difference between trees within populations (all $p > 0.05$), indicating no genetic variability, as an interindividual difference between trees within populations (Table 3).

Evergreen plants play a significant role in the biomonitoring of air pollution, due to their wide geographical distribution, the needle shape and the wax layer on them. Accumulation of harmful elements also depends on the needle age. One-year-old needles of the black pine had higher concentrations of harmful elements compared to the fresh buds, which concentration also depended on the exposure of trees to traffic pollution (Zeiner et al., 2021). The evaluation of unpolluted and polluted habitats in Poland, revealed that the content of heavy metals (Cd, Fe, Mg, Pb and Zn) increased simultaneously with the needle age and the level of pollution in one– and two-year-old needles of the Scots pine (*P. sylvestris*) (Kandziora-Ciupa et al., 2016). Wax structures on their surface reduced the intake of pollutants into the leaf tissue, which could cause low heavy metal concentrations in needles even in contaminated environments (Lodenius, 2013; Matin et al., 2016). Heavy metals such as Pb, Cd, Cr and Hg are potentially highly toxic to plants, for they lead to growth damage, reduced biomass and plant death. However, some heavy metals are essential elements for normal plant growth and metabolism (such as Co, Fe, Mn, Ni, Zn and Cu), but in higher concentrations they become toxic (Matin et al., 2016). Studies have shown that Norway spruce needles and bark were suitable as bioindicators of the environment pollution. Statistically significant differences were found in the content of N, P, Ca, Zn, Mn, Fe, Ni, Cu and Cd in two-year-old needles and in the content of N, K, Ca, Zn, Mn, Fe, Ni, Cu and Cd in one-year-old needles of two spruce species (*P. abies* and *Picea omorika*) in the forests of Slovakia. The bark of both *Picea* species proved to be acidic, for *P. abies* accumulated Ni and Mg, whereas *P. omorika* accumulated Fe and Ca (Parzych et al., 2018). High concentrations of heavy metals Se, Cd, Cr, Pb and Ni and metalloid As were detected in the bark of Norway spruce (*P. abies*), Scots pine (*Pinus sylvestris*) and black pine (*P. nigra*) in Romania, in locations that were not characterized as highly polluted (Tanase et al., 2021). Investigating ecosystems with different level of pollution in Poland was found that locations with high level of pollution had high concentrations of Zn, Pb and Cd in the upper layers of the soil, forest litter and pine needles (Pająk et al., 2015). Genetic variability in heavy metals Pb, Mn, Fe and Zn was also confirmed in the linden leaves (*Tilia* spp.) at the unpolluted location of the National Park 'Fruška gora', whereas the content of Ni in the leaves was under the direct influence of environmental conditions (Šijačić-Nikolić et al., 2012).

An agglomerative hierarchical clustering (AHC) analysis of the hazardous element content (As, Cd, Hg, Ni, Pb and Zn) in the spruce needles of fifteen populations revealed four clusters on the dendrogram (with a dendrogram cut at 114.57 Euclidean distances). Pirin, Murtenica, Rila, Golija and Tara populations formed one cluster, Mojstir, Sokolac, Osogovo, Bioška, Kopaonik and Kovač - the second, Javor, Zlatar and Stara planina - the third. The population of Lisina formed one separate cluster (Figure 3) probably due to the highest Zn content (Figure 1), the lowest altitude (below 1000 m a.s.l.) and the highest MAP value (1100 mm, Table 1). No consistency could be observed relative to the altitude and the MAP value for the other clusters. The optimal classification of the analyzed populations into homogeneous groups - clusters was obtained on the basis of large differences between (95.19%) and within (4.81%) homogeneous groups. The Balkan Mts. populations were not clearly separated from the Dinaric Alps populations, since the populations of both mountain ranges were classified in all three clusters (Figure 3).

Concentration and type of pollutants in the air and ground, characteristics of the plant species, climate conditions like precipitation, wind direction and speed, all influence the accumulation of pollutants in plants. During dry period, there is dry deposition of heavy metal particles in leaves and needles. These deposits reduce the growth of buds, change the color of leaves, cause changes in cell membranes and chlorophyll, reduce resistance to drought, frost, fungi and insects, and lead to drying the parts or the whole plant (De La Cruz et al., 2019). Due to precipitation, atmospheric hydrometeors scavenge aerosol particles by wet deposition (gravitational, Brownian and/or turbulent droplet coagulation). There are two ways of wet deposition by below-cloud scavenging when rain or snow droplets collide with aerosol particles and in-cloud scavenging where aerosol particles get into cloud droplets and get to the ground surface where plants can absorb them (Hoodaji et al., 2012; Alexandrino et al., 2020).

In our study, we obtained no significant relationship between the hazardous element content and the MAP, as an important climatic parameter. But, the MAP vector made an acute angle with the Zn content vector, indicating their positive correlation. Lisina population had the highest Zn content in the spruce needles (approximately 60 µg/g), which could be the outcome of its assimilation from the ground where it got by high precipitation. This population had the highest MAP, about 1100 mm (Table 1), which facilitated the deposition of the suspended particles from the air into the ground.

At the locations of two forest ecosystems Crni vrh (beech forest, *Fagetum montanum*) and Fruška gora (beech and sessile oak forest, *Quercetum-Fagetum*), the content of Zn and Cu in the soil and plant tissue was within the average values according to ECCE (International Union of Biological Sciences 1994 Element concentration cadasters in ecosystems. in: Progress report, 25th General Assembly, Paris) (EEA 2014). The content of Cd was increased in the bark and leaves of sessile oak and beech, whereas Pb content was increased only in the bark of beech on Fruška gora (Knežević et al., 2000). The needle samples from the polluted area of Obrenovac, near the thermal power plant "Nikola Tesla A", in almost all cases exhibited higher concentrations of As, Fe, Hg, Mn and Pb in the white poplar *P. alba* growth rings, in the period from 1979 to 2005, in relation to the samples from the urban area of Novi Sad (Marković et al., 2008; Marković et al., 2011). The concentration of heavy metals of Ni and Pb in the leaves of *Paulownia elongata* S. Y. Hu and *Paulownia fortunei* Hems. was twice as high in urban area trees comparing to the rural ones (Stanković et al., 2009a). The study of phytoextraction of heavy metals in *Paulownia elongata* in the area of the City of Belgrade revealed the increasing concentrations of Fe, Pb, Ni, Cr and Cd in the plant leaves going from rural to urban surroundings (Stanković et al., 2009b). Some studies have confirmed the antagonism between Fe and Mg, as well as the different pattern of hazardous element content in woody and herbaceous plant species. The research on hazardous element concentrations in woody (*Tilia tomentosa* Mnch., *Pinus nigra* Arn., *Prunus avium* L., *Quercus petraea* (Matt.) Liebl, *Pseudotsuga menziesii* (Mirb.) Franco) and herbaceous plants (*Plantago media* L., *Taraxacum officinale* Web.), sampled at three locations on Mount Avala near Belgrade and one location in the center of Belgrade, revealed that the largest accumulation of Mn was obtained in oak and linden, and Fe in hoary plantain and dandelion, regardless of the location. This research specified that the concentration of Mn was higher at unpolluted Avala locations than in the center of Belgrade, which clearly indicated that the increase of Mn concentration in plants was not directly related to traffic as its primary source, but to soil (Stanković et al., 2011b). Close correlations were obtained between Mn and Pb in the bark and wood of large-leaved linden (*Tilia platyphyllos*) at four locations in Serbia (Marković et al., 2012). The study on the air pollution near Bor copper mine revealed that the leaves of birch, as a deciduous species, had higher accumulation of Cu, Zn, Pb and Mn elements compared to needles of spruce, as an evergreen species (Serbula et al., 2014).

Based on the results of PCA analysis, the obtained first principal component axis (PC1) described 41.36% of the total variability, and the second axis (PC2) 19.83% (Figure 4a). The eigenvalues were > 1 for the first two axes, which indicated that the variability of the needles could be clearly separated and these axes described 60.74% of the population variability in hazardous element content (Figure 4b). The distribution of the analyzed populations (white and blue circles) exhibited that all the Balkan Mts. populations (Rila, Pirin, Osogovo and Stara planina) and some of the Dinaric Alps populations (Mojstir, Javor and Zlatar) were separated along the first axis from the other populations (Figure 4a). The Pb content in the spruce needles of Mojstir, Javor, Zlatar and Stara planina populations was significantly higher (10-12 μg/g) than in the other populations (< 1 μg/g) (Figure 2). Along the PC2 axis on the presented biplot, the Dinaric Alps populations (Kopaonik, Golija and Tara) were separated from the other analyzed populations (Figure 4a). The concentration of As, Cd and Ni (Figures 2 and 4a) contributed the most to this separation, but also did the amount MAP (it was less than 1000 mm in Kopaonik and Tara populations comparing the other populations from the Dinaric Alps, unlike Lisina population where MAP was over 1100 mm). Generally, all populations had higher MAP in the Dinaric Alps (ranging from 937.88 mm to 1100.64 mm) compared to the Balkan Mts. (ranging from 594 to 797.17 mm, Table 1). Hazardous elements As, Cd and Ni contributed the most to the separation of populations according to the first axis (all p values > 0.8) (Table 4), as well as they were the closest to the first axis (make sharp angle < 90°), which also contributed to the mentioned population separation (Figure 3a). The biplot diagram also showed the relationship between the variables As, Cd and Ni that were clustered, i.e. they overlapped the sharp angle between the vectors (< 90°), which indicated that they were positively correlated. The statistically significant correlations were confirmed by Pearson's coefficients between As, Cd and Ni content (As vs. Cd $r = 0.94$, $p = 0.0001$; As vs. Ni $r = 0.67$, $p = 0.0065$; Cd vs. Ni $r = 0.64$, $p = 0.0106$). Zn and Pb vectors were opposite to each other and in relation to the other variables, they formed an obtuse angle between the vectors (> 90°, Figure 3a), i.e. were negatively correlated but non-significant ($r = -0.30$, $p = 0.2863$). The Hg vector was the shortest, which indicated that it was poorly described by the first two principal components. Precipitation as an important climatic factor led to the separation of the populations Kopaonik, Golija and Tara according to the second principal component axis (PC 2) (Figure 4a).

Defining changes in the quality of the environment is enabled by the properties of plant organisms, which can assimilate and accumulate trace metals from the environment. Therefore, plants present a convenient and inexpensive way to monitor air quality (Mancheno et al., 2021). Some natural processes and human activities produce the particulate matters (PMs), which contained hazardous elements Hg, Cd, Pb and As (Briffa et al., 2020). The metallurgical industry and fossil fuels are, besides traffic, the main source of Ni in the air (Miljković et al., 2014). The location of populations with higher concentrations of some heavy elements is in vicinity of thermal power plants and surface coal mines, the main air pollutants on the Balkan Peninsula. The result of various combustion processes and industrial activities is the emission of toxic metals Cd, Pb, Hg and Ni and metalloid As that pollute air, land and water. There is a relatively small number of stations in Europe that measure the concentration of hazardous elements on a daily basis. The concentrations of As, Cd, Pb and Ni in the air are often below the lower limit set by the European Union law (Guerreiro et al., 2014).

No statistically significant correlation was obtained between the elevation, distance of the air pollutant emitters and the hazardous element content in the Norway spruce needles (all $p > 0.05$), except the correlation between the emitter distance and Zn content ($r = 0.56$, $p = 0.0280$). The values of the Pb content in needles were higher in populations Mojstir (10.06 µg/g), Javor (11.79 µg/g) and Zlatar (11.75 µg/g) in the Dinaric Alps and Stara planina (9.76 µg/g) in the Balkan Mts., compared to the other populations (less than 0.45 µg/g) (Figure 2). The highest content of As, Cd and Ni was recorded in the spruce needles of Tara, Kopaonik and Golija populations. In the vicinity of these populations are located several mines. Trepča is the Pb and Zn mine on the south side of Kopaonik. North of Kopaonik and northeast of Golija and Tara are located Kolubara mining basin of coal, the thermal power plant Kolubara (150 km away) and the Ni mine on the mountain Rudnik (80 km away). On the northwest side of Tara are positioned the mines and the thermal power plants Tuzla, Kakanj and Ugljevik (Figure 5). In this area the most polluted cities are Sarajevo, Zenica and Tuzla. According to the wind rose, there are several wind directions from north to east, all on the west side of Tara. Northeast of Tara and north of Golija is located the Pb and Zn mine Veliki Majdan, where the wind rose indicates wind direction towards Golija (available at https://www.meteoblue.com/en/weather/maps).

The proximity of the city of Sarajevo on the west side of these mountains, south-west of Pljevlja thermal power plant and a little further on Gacko thermal power plant, all might have caused high Pb values in the needles of Zlatar population (11.25 µg/g), because the wind direction towards this location is from the west and the north (Figures 2 and 5). Population Mojstir is near Trepča mine and thermal power plant on the east side, and since winds are the most common from the northeast side it might be the reason for the increased concentration of Pb in this population spruce needles.

The Balkan Mts. populations Rila, Pirin and Osogovo are located close to Bobov Dol thermal power plant and the Pernik surface coal mine. The content of Ni in Norway spruce needles in Rila population was 4.2 µg/g (Figures 2 and 5). All these populations are located southeast of the mentioned pollutants and according to the wind rose diagram the most representative winds blow from the north in Rila and Osogovo populations, whereas more western wind blows in the Pirin population (htts://www.meteoblue.com/en/weather/maps). The reason of high Ni concentration in Rila population needles might be the direction of the wind coming from the north where the mentioned pollutants are located. According to the report of the European Environment Agency (https://www.eea.europa.eu/data-and-maps/explore-interactive-maps/up-to-date-air-quality-data) the concentration of suspended particulates PM_{10} in this region amounts up to 100 µg/m3. The spruce needles in Stara Planina population contained high Pb concentration, which might be the consequence of PM emission from Aleksinac mine on the west side and from Bor mining and smelting basin on the north-west side of the location (both pollutants are about 80 km away) (Figure 5). The majority of air pollution emitters are located less than 200 km away from the populations: Kopaonik and Golija (15 - sum of emitters), Zlatar, Tara, Mutrenica, Javor, Kovač (13), Mojstir and Bioška (12), Sokolac (9), Lisina and Stara planina (5), Osogovo (4), and Rila and Pirin (3) (Figure 5).

The concentration of Zn in the spruce needles in the Balkan Mts. populations were similar to those in the Dinaric Alps. The highest values of Zn content were recorded in the spruce needles of Lisina, Sokolac and Kopaonik populations. Southeast of Lisina (at low altitude 980 m a.s.l.) are located thermal power plants Kakanj, Tuzla and Ugljevik from where the wind partly blows towards this population. The prevailing wind blows from these thermal power plants towards Sokolac population, located on the north. South of Kopaonik is located Trepča thermal power plant and mine, which could contribute to the high concentration of Zn in this population spruce needles.

The content of elements in the leaves can reveal whether air pollutants are deposited on the leaf surface or in the leaf tissue, partly by assimilation through the leaf surface from the air or partly by uptake from the ground, indicating the composition and quality of soil (Giordano et al., 2021). Conifers are better indicators of air pollution than deciduous trees, because they accumulate hazardous elements throughout the year or even several years due to the longer life-span of needles then leaves. The advantage of needles is primarily in their different morphology and anatomy comparing to the leaves, and also in better adaptation to acid soil that allows better solubility of hazardous elements and thus its assimilation. Also, variability of coniferous species, such are *P. nigra, P. sylvestris,* *Abies bornmulleriana,* and *Picea pungens,* make them useful in environmental quality biomonitoring projects (Alexandrino et al., 2020).

The concentration of pollutants on or inside the leaf surface indicates the way the pollutants are absorbed from the air or from the ground (Giordano et al., 2021). Absorption of heavy metals occurs in two ways: actively through stomatal structures or passively through deposition of heavy metal particles on the needle surface, so that the concentration of pollutants on the surface is a direct indicator of pollutants in the surrounding air (Matin et al., 2016). The impact of anthropogenic pollutants on terrestrial ecosystems in wildlife requires the study of many different species. The response to pollutants depends on various factors such as the type of organism and tissue, duration of exposure, interaction with other stressors, climate change, deficiency of food etc. (Rhind 2009).

Conclusion

The main purpose of this study was to test the Norway spruce needles as the possible bioindicator of accumulated hazardous elements in their native habitats in the vicinity of air pollution emitters (power plants, mines and industry). Besides conventional methods of monitoring the air quality, it is necessary to investigate the use of plants as an alternative due to their suitability for temporal and spatial detection of air pollution, as well as a reliable and inexpensive method. Our results on the content of hazardous elements, determined direct connection with the vicinity of pollutant emitters, except in the content of Zn. Although it would be expected that at high altitude was an unpolluted area, the highest Ni content was obtained in spruce needles at an altitude of 1860 m a.s.l. Besides employing plant species as bioindicators it is also necessary to include other aspects into research such as proximity to air pollution emitters, relief features, precipitation, soil type, etc.). The study on the Norway spruce (*P. abies*) needles proved to be a significant contribution to the variety of vegetation species that can be used as bioindicators. and make a valuable starting point of future monitoring programs in the mountain ranges of Southeastern Europe.

Funding This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant numbers 451-03-68/2022-14/ 200027 and MPNTR-IBISS contract number 451-03-68/2022-14/ 200007.

Conflict of interest The authors declare no conflict of interest.

Author contributions Conceived the idea and designed the study V.P. and D.M.; conducted fieldwork, conceptualization, investigation, V.P.; substantive translation and correction of the final version of the manuscript, D.Š.J.; heavy metal screening via ICP-OES spectrometer Z.M.; contribution to the draft version of manuscript J.M.; project management responsibility for research, Project administration, Funding acquisition, A.L., LJ.R., D.Š.J. and D.M.; methodology, validation, supervision, performed statistical analyses, data curation, visualization results, writing - original manuscript, D.M.

Data availability The data that support the findings are available on request from the corresponding author.

References

Alexandrino, K., Viteri, F., Rybarczyk, Y., Guevara Andino, J. E., & Zalakeviciute, R. (2020). Biomonitoring of metal levels in urban areas with different vehicular traffic intensity by using araucaria heterophylla needles. *Ecological Indicators, 117*, 106701. https://doi.org/10.1016/j.ecolind.2020.106701

Al-Khashman, O. A., Al-Muhtaseb, A. H., & Ibrahim, K. A. (2011). Date palm (*Phoenix dactylifera* L.) leaves as biomonitors of atmospheric metal pollution in arid and semi-arid environments. *Environmental Pollution, 159*(6), 1635-1640. https://doi.org/10.1016/j.envpol.2011.02.045

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6*(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Čeburnis, D., & Steinnes, E. (2000). Conifer needles as biomonitors of atmospheric heavy metal deposition: comparison with mosses and precipitation, role of the canopy. *Atmospheric Environment, 34*(25), 4265-4271.

De La Cruz, A., Ferreira, L., Andrade, V., & Gioda, A. (2018). Biomonitoring of toxic elements in plants collected near leather tanning industry. *Journal of the Brazilian Chemical Society, 30*, 256-264. https://doi.org/10.21577/0103-5053.20180174

Dogan, Y., Unver, M. C., Ugulu, I., Calis, M., & Durkan, N. (2014). Heavy metal accumulation in the bark and leaves of *Juglans regia* planted in Artvin city, Turkey. *Biotechnology & Biotechnological Equipment, 28*(4), 643-649. https://doi.org/10.1080/13102818.2014.947076

EEA, (2014). Exposure of ecosystems to acidification, eutrophication and ozone (Indicator CSI 005), European Environment Agency

Giordano, S., Spagnuolo, V., & Capozzi, F. (2021). Biomonitoring of air pollution. *Atmosphere, 12*(4), 433. https://doi.org/10.3390/atmos12040433

Goczał, J., Oleksa, A., Rossa, R., Chybicki, I., Meyza, K., Plewa, R., Landvik, M., Gobbi, M., Hoch, G., Tamutis, V., Balalaikins, M., Telnov, D., Dascălu, M., & Tofilski, A. (2020). Climatic oscillations in Quaternary have shaped the Co-evolutionary patterns between the Norway spruce and its host-associated herbivore. *Scientific Reports, 10*(1). https://doi.org/10.1038/s41598-020-73272-0

Guerreiro, C. B., Foltescu, V., & De Leeuw, F. (2014). Air quality status and trends in Europe. *Atmospheric Environment, 98*, 376-384. https://doi.org/10.1016/j.atmosenv.2014.09.017

Hamann, A., Wang, T., Spittlehouse, D. L., & Murdock, T. Q. (2013). A comprehensive, high-resolution database of historical and projected climate surfaces for western North America. *Bulletin of the American Meteorological Society, 94*(9), 1307-1309. https://doi.org/10.1175/bams-d-12-00145.1

Hoodaji, M., Ataabadi, M., & Najafi, P. (2012). Biomonitoring of airborne heavy metal contamination. *Air Pollution - Monitoring, Modelling, Health and Control. 21*, https://doi.org/10.5772/32963

Kandziora-Ciupa, M., Ciepał, R., Nadgórska-Socha, A., & Barczyk, G. (2016). Accumulation of heavy metals and antioxidant responses in *Pinus sylvestris* L. needles in polluted and non-polluted sites. *Ecotoxicology, 25*(5), 970-981. https://doi.org/10.1007/s10646-016-1654-6

Knežević, M., Belanović, S., Košanin, O., & Kadović, R. (2000). Sadržaj teških metala u šumi bukve na Crnom vrhu i kitnjaka na Fruškoj gori. *Acta biologica Iugoslavica-serija A: Zemljište i biljka, 49*, 19-28.

Ippolitova, N. A. (2019). Anthropogenic impact of industrial production on the environment (on the example of the Siberian region). In IOP Conference Series: Earth and Environmental Science (Vol. 381, No. 1, p. 012035). IOP Publishing.

Lin, Y., & Aarts, M. G. (2012). The molecular mechanism of zinc and cadmium stress response in plants. *Cellular and Molecular Life Sciences, 69*(19), 3187-3206. https://doi.org/10.1007/s00018-012-1089-z

Lodenius, M. (2013). Use of plants for biomonitoring of airborne mercury in contaminated areas. *Environmental Research, 125*, 113-123. https://doi.org/10.1016/j.envres.2012.10.014

Lüttge, U., & Buckeridge, M. (2020). Trees: Structure and function and the challenges of urbanization. *Trees*, 1-8. https://doi.org/10.1007/s00468-020-01964-1

Madejón, P., Marañón, T., Murillo, J. M., & Robinson, B. (2004). White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests. *Environmental Pollution, 132*(1), 145-155. https://doi.org/10.1016/j.envpol.2004.03.015

Mancheno, T., Zalakeviciute, R., González-Rodríguez, M., & Alexandrino, K. (2021). Assessment of metals in PM10 filters and *Araucaria heterophylla* needles in two areas of Quito, Ecuador. *Heliyon, 7*(1), e05966. https://doi.org/10.1016/j.heliyon.2021.e05966

Marković, D. M., Milošević, I. R., & Vilotić, D. (2012). Accumulation of Mn and PB in Linden (*Tilia platyphyllos* scop.) bark and wood. *Environmental Science and Pollution Research, 20*(1), 136-145. https://doi.org/10.1007/s11356-012-1024-8

Marković, D. M., Novović, I., Vilotić, D., & Ignjatović, L. (2008). Determination of as in tree-rings of poplar (*Populus alba* L.) by U-shaped DC arc. *Environmental Monitoring and Assessment, 151*(1-4), 377-382. https://doi.org/10.1007/s10661-008-0279-5

Marković, D. M., Novović, I., Vilotić, D., & Ignjatović, L. (2007). Determination of Fe, Hg, Mn, and Pb in three-rings of poplar (*Populus alba* L.) by U-shaped DC arc. *Russian Journal of Physical Chemistry A, 81*(9), 1493-1496. https://doi.org/10.1134/s0036024407090282

Matin, G., Kargar, N., & Buyukisik, H. B. (2016). Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. *Ecological Engineering, 90,* 331-335. https://doi.org/10.1016/j.ecoleng.2016.01.035

Miljković, D., Avramov, S., Vujić, V., Rubinjoni, L., Klisarić, B., Živković, U., & Tarasjev, A. (2014). Lead and nickel accumulation in *Iris pumila*: Consideration of its usefulness as a potential bioindicator in the natural protected area of Deliblato sands, Serbia. *Archives of Biological Sciences, 66*(1), 331-336. https://doi.org/10.2298/abs1401331m

Naderizadeh, Z., Khademi, H., & Ayoubi, S. (2016). Biomonitoring of atmospheric heavy metals pollution using dust deposited on date palm leaves in southwestern Iran. *Atmósfera*. *29*(2), 141-155 https://doi.org/10.20937/atm.2016.29.02.04

Nikolić, D., Milošević, N., Živković, Z., Mihajlović, I., Kovačević, R., & Petrović, N. (2011). Multi-criteria analysis of soil pollution by heavy metals in the vicinity of the copper smelting plant in Bor (Serbia). *Journal of the Serbian Chemical Society, 76*(4), 625-641. https://doi.org/10.2298/jsc100823054n

Onder, S., & Dursun, S. (2006). Air borne heavy metal pollution of Cedrus libani (A. Rich.) in the city centre of Konya (Turkey). *Atmospheric Environment, 40*(6), 1122-1133. https://doi.org/10.1016/j.atmosenv.2005.11.006

Pająk, M., Gąsiorek, M., Cygan, A., & Wanic, T. (2015). Concentrations of Cd, Pb and Zn in the top layer of soil and needles of scots pine (*Pinus sylvestris* L.): A case study of two extremely different conditions of the forest environment in Poland. *Fresenius Environmental Bulletin*, *24*(1), 71-76.

Parzych, A., Mochnacký, S., Sobisz, Z., Polláková, N., & Šimanský, V. (2018). Needles and bark of *Picea abies* (L.) H. Karst and *Picea omorika* (Pančić) Purk. as bioindicators of environmental quality. *Folia Forestalia Polonica, 60*(4), 230-240. https://doi.org/10.2478/ffp-2018-0024

Popović, V., Nikolić, B., Lučić, A., Rakonjac, L., Šešlija Jovanović, D., & Miljković, D. (2022). Morpho-anatomical trait variability of the Norway spruce (*Picea abies* (L.) Karst.) needles in natural populations along elevational diversity gradient. *Trees*. https://doi.org/10.1007/s00468-022-02277-1

Rautio, P., Fürst, A., Stefan, K., Raitio, H., & Bartels, U. (2016). Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests: Part XII Sampling and analysis of needles and leaves. https://www.icp-forests.org/pdf/manual/2016/ICP_Manual_2017_01_part12.pdf

Rhind, S. M. (2009). Anthropogenic pollutants: A threat to ecosystem sustainability? *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1534), 3391-3401. https://doi.org/10.1098/rstb.2009.0122

SAS Institute Inc. The SAS System for Windows, release 9.3. 2011, SAS Institute, Cary, NC.

Sawidis, T., Breuste, J., Mitrovic, M., Pavlovic, P., & Tsigaridas, K. (2011). Trees as bioindicator of heavy metal pollution in three European cities. *Environmental Pollution, 159*(12), 3560-3570. https://doi.org/10.1016/j.envpol.2011.08.008

Serbula, S. M., Radojević, A. A., Kalinović, J. V., & Kalinović, T. S. (2014). Indication of airborne pollution by birch and spruce in the vicinity of copper smelter. *Environmental Science and Pollution Research, 21*(19), 11510-11520. https://doi.org/10.1007/s11356-014-3120-4

Sharma, P., Kumar, S., Garg, A., & Ghosh, C. (2015). Analyzing the effect of atmospheric trace elements on selected plant species. *Environmental Earth Sciences, 74*(12), 7793-7800. https://doi.org/10.1007/s12665-015-4452-1

Sijačić-Nikolić, M., Stanković, D., Krstić, B., Vilotić, D., & Ivetić, V. (2012). The potential of different lime tree (*Tilia* spp) genotypes for phytoextraction of heavy metals. *Genetika, 44*(3), 537-548. https://doi.org/10.2298/gensr1203537s

Stanković, D., Igić, R., Sijačić-Nikolić, M., Vilotić, D., & Pajević, S. (2009a). Contents of the heavy metals nickel and lead in leaves of *Paulownia elongata* S.Y. Hu and *Paulownia fortunei* hems. in Serbia. *Archives of Biological Sciences*, *61*(4), 827-834. https://doi.org/10.2298/abs0904827s

Stanković, D., Krstić, B., Orlović, S., Trivan, G., Poljak, L. P., & Nikolić, M. S. (2011a). Woody plants and herbs as bioindicators of the current condition of the natural environment in Serbia. *Journal of Medicinal Plants Research*, *5*(15), 3507-3511.

Stanković, D., Krstić, B., Sijačić-Nikolić, M., & Knezević, M. (2011b). Concentrations of manganese and iron in some Woody and herbs plants. *Zbornik Matice srpske za prirodne nauke*, *121*, 39-49. https://doi.org/10.2298/zmspn1121039s

Stanković, D., Nikolić, M., Krstić, B., & Vilotić, D. (2009b). Heavy metals in the leaves of tree Species *Paulownia Elongata* S.Y.Hu in the region of the city of Belgrade. *Biotechnology & Biotechnological Equipment*, *23*(3), 1330-1336. https://doi.org/10.1080/13102818.2009.10817664

Stojnić, S., V. Avramidou, E., Fussi, B., Westergren, M., Orlović, S., Matović, B., Trudić, B., Kraigher, H., A. Aravanopoulos, F., & Konnert, M. (2019). Assessment of genetic diversity and population genetic structure of Norway spruce (*Picea abies* (L.) Karsten) at its southern lineage in Europe. Implications for conservation of forest genetic resources. *Forests*, *10*(3), 258. https://doi.org/10.3390/f10030258

Tanase, C., Nisca, A., & Lopez, A. (2021). Assessment of heavy metal content in tree barks: *Picea abies*, *Pinus sylvestris*, and *Pinus nigra* (Retracted). *BioResources*, *16*(3), 5047-5057. https://doi.org/10.15376/biores.16.3.5047-5057

Turkyilmaz, A., Sevik, H., & Cetin, M. (2018). The use of perennial needles as biomonitors for recently accumulated heavy metals. *Landscape and Ecological Engineering*, *14*(1), 115-120. https://doi.org/10.1007/s11355-017-0335-9

Zeiner, M., & Juranović Cindrić, I. (2021). Accumulation of major, minor and trace elements in pine needles (*Pinus nigra*) in Vienna (Austria). *Molecules*, *26*(11), 3318. https://doi.org/10.3390/molecules26113318

Figure legends

Fig. 1 Map of the analyzed populations and air pollution emitters (1-16; see Table 2). The Balkan Mountains populations are presented with white circles and the Dinaric Alps populations with blue circles. Corresponding symbols present the nearest thermal power plants and mines.

Fig. 2 Mean values of the hazardous element content (As, Cd, Hg, Ni, Pb, and Zn) in the Norway spruce needles in fifteen populations.

Fig. 3 Dendrogram showing the grouping of fifteen populations from two mountain ranges (Balkan Mountains - white circles and Dinaric Alps -blue circles) based on the analysis of hazardous element content (As, Cd, Hg, Ni, Pb, and Zn) in the Norway spruce needles.

Fig. 4 (A) PCA biplot showing the first two principal component axes for hazardous element content (As, Cd, Hg, Ni, Pb, and Zn) in the Norway spruce needles in the Balkan Mountains populations (white circles) and Dinaric Alps populations (blue circles); (B) Eigenvalues of all seven principal components with percentage of cumulative variability.

Fig. 5 Distances of air pollution emitters in the vicinity of fifteen studied populations of Norway spruce. The smallest inner circle with yellow dots in the radar diagram presents the air pollution emitters at the distance of 200 km for each population.

Figure 1

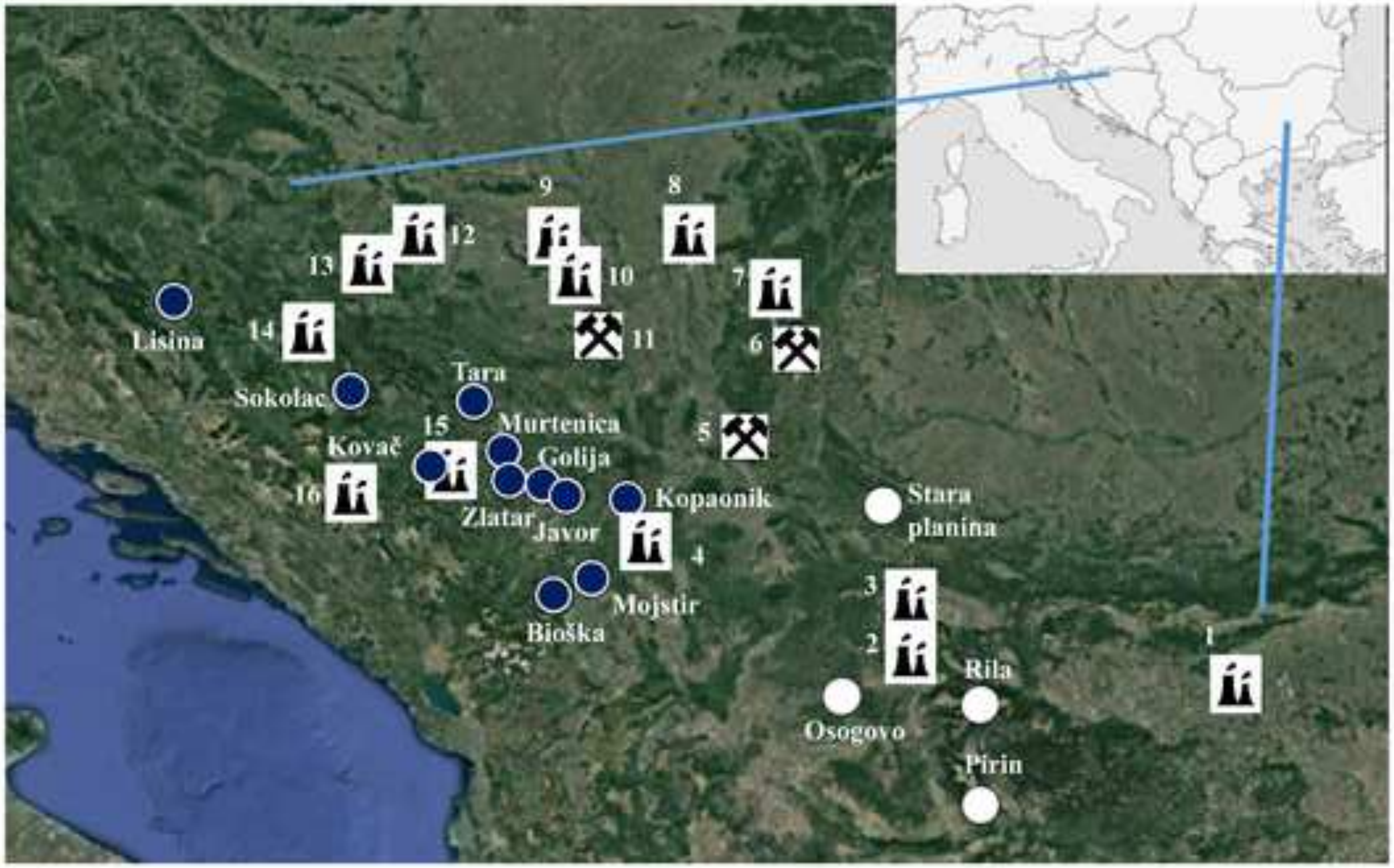
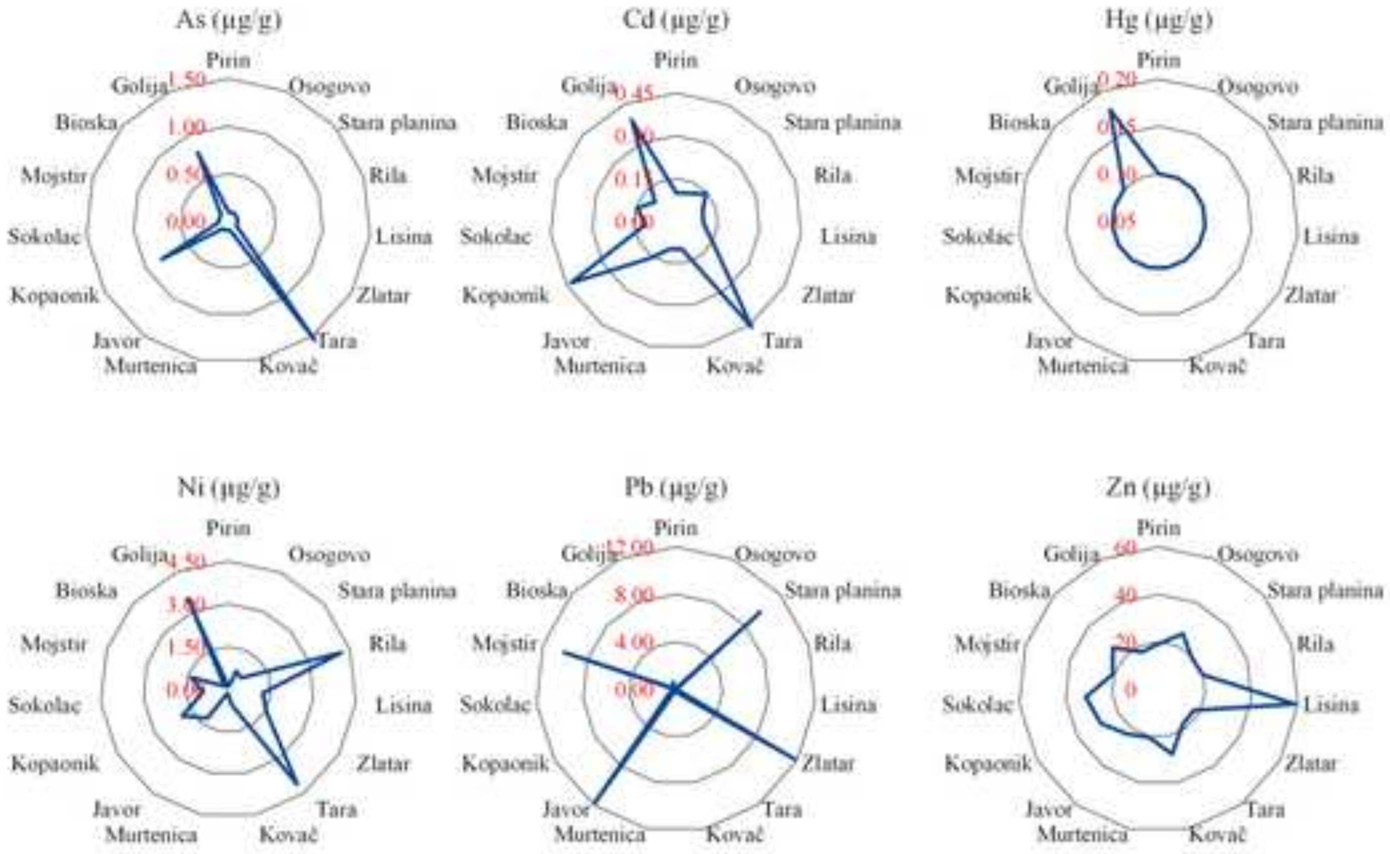

9
8
12
13
10
7
14
11
6
Lisina
Tara
Sokolac
15
Murtenica
5
Kovač
Golija
16
Kopaonik
Stara
planina
Zlatar
Javor
4
3
Mojstir
Bioška
2
Rila
1
Osogovo
Pirin

Figure 2



As (µg/g)
1.50
1.00
0.50
0.00
Cd (µg/g)
0.45
0.30
0.15
0.00
Hg (µg/g)
0.20
0.15
0.10
0.05
Ni (µg/g)
4.50
3.00
1.50
0.00
Pb (µg/g)
12.00
8.00
4.00
0.00
Zn (µg/g)
60
40
20
0
Pirin
Osogovo
Stara planina
Rila
Lisina
Zlatar
Tara
Kovač
Murtenica
Javor
Kopaonik
Sokolac
Mojstir
Bioska
Golija

Figure 3

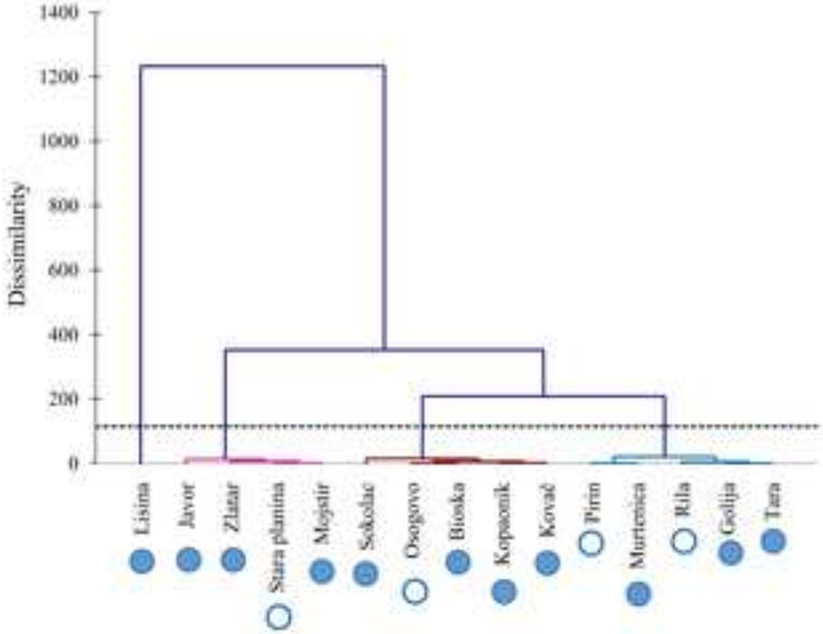

Dissimilarity
1400
1200
1000
800
600
400
200
0
Lisina
Javor
Zlatar
Stara planina
Mojstir
Sokolac
Osogovo
Bioska
Kopaonik
Kovač
Pirin
Murtenica
Rila
Golija
Tara

Figure 4

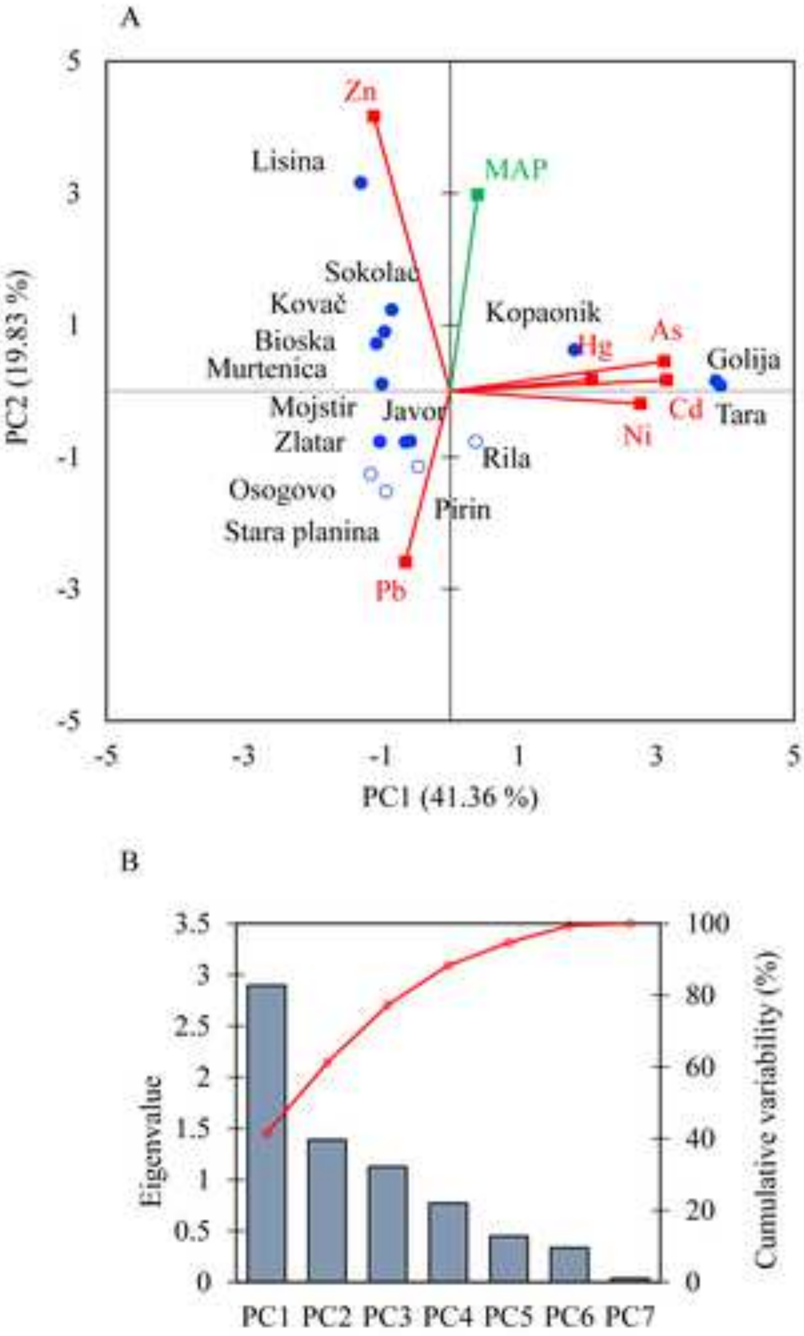

A
Zn
MAP
Lisina
Sokolac
Kovač
Bioska
Murtenica
Kopaonik
Hg
As
Golija
Cd
Tara
Ni
Mojstir
Javor
Zlatar
Rila
Osogovo
Pirin
Stara planina
Pb
PC2 (19.83 %)
PC1 (41.36 %)
5
3
1
-1
-3
-5
-5
-3
-1
1
3
5
B
Eigenvalue
Cumulative variability (%)
3.5
3
2.5
2
1.5
1
0.5
0
100
80
60
40
20
0
PC1
PC2
PC3
PC4
PC5
PC6
PC7

Figure 5

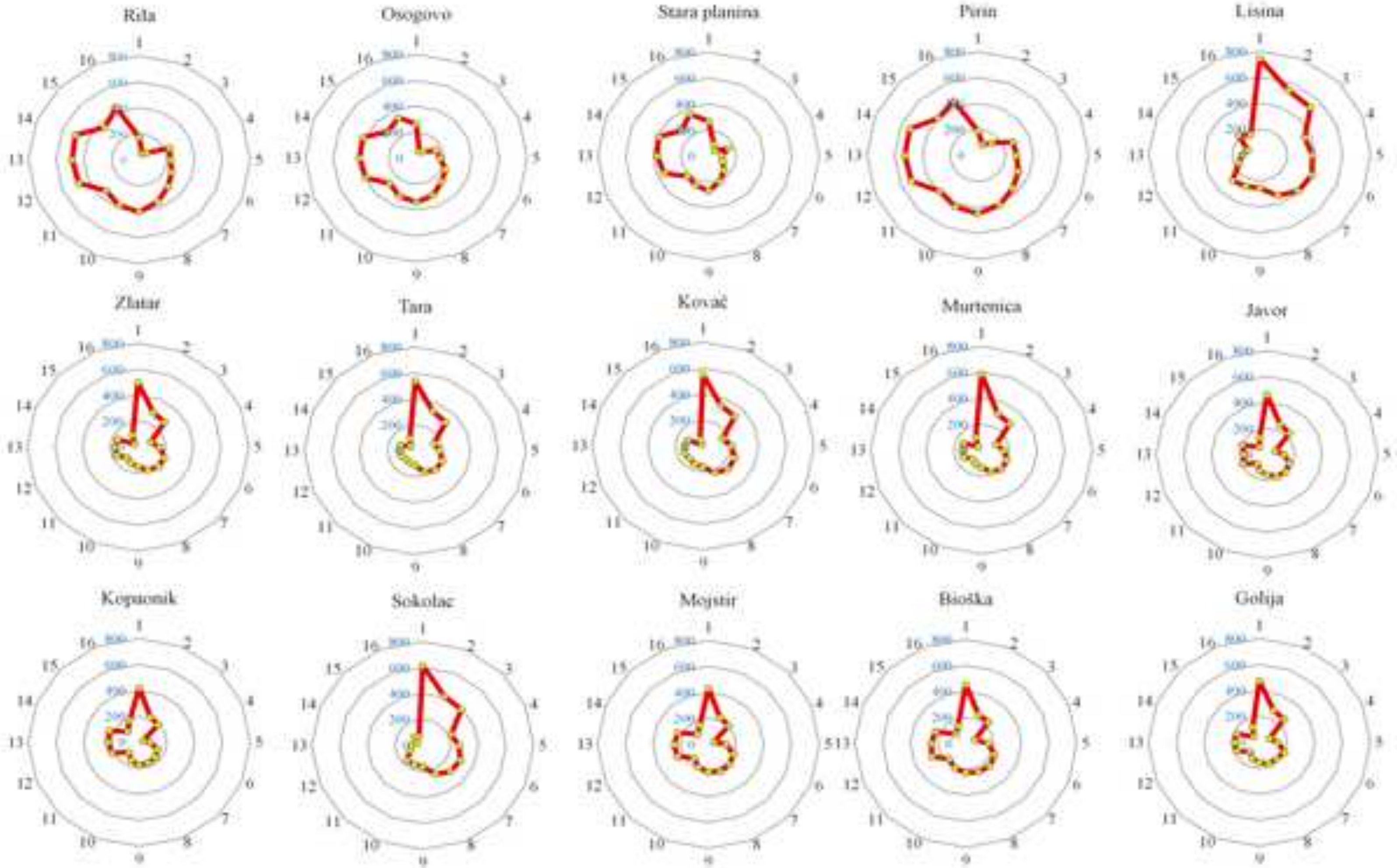

Rila
Osogovo
Stara planina
Pirin
Lisina
Zlatar
Tara
Kovač
Murtenica
Javor
Kopaonik
Sokolac
Mojstir
Bioška
Golija
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
0
200
400
600
800

Table 1 Geographic information (latitude, longitude, altitude, MAP – mean annual precipitation) of fifteen *Picea abies* (L.) Karst. populations from Balkan Mts. (four) and Dinaric Alps (eleven).

Population	Latitude WGS84	Longitude WGS84	Altitude	MAP
Balkan Mts				
Pirin	41.56889	23.55833	1100	594.17
Osogovo	42.21444	22.48250	1260	606.31
Stara planina	43.31932	22.80120	1500	797.17
Rila	42.15167	23.55972	1860	764.31
Dinaric Alps				
Lisina	44.40381	17.00266	980	1100.19
Zlatar	43.44348	19.81033	1070	1025.02
Tara	43.89713	19.50234	1220	983.76
Kovač	43.50333	19.16667	1250	1089.86
Murtenica	43.60668	19.76124	1255	996.76
Javor	43.41643	20.08705	1320	1011.62
Kopaonik	43.34000	20.76000	1360	937.88
Sokolac	43.92973	18.49639	1380	1100.64
Mojstir	42.88177	20.48378	1470	1017.36
Bioška	42.78056	20.18861	1520	1065.62
Golija	43.36243	20.26954	1530	1022.38

Table 2

Table 2 Geographic coordinates (latitude and longitude) of air pollution emitters (thermal power plants and mines) in the vicinity of *Picea abies* (L.) Karst. natural populations in Southeastern Europe.

	Air pollution emitters	Latitude	Longitude
1	Maritsa 3 - coal power plant	42°02'52.34"N	25°37'27.97"E
2	Bobov Dol - thermal power plant	42°16'55.71"N	23°02'01.30"E
3	Pernik - thermal power plant	42°36'13.85"N	22°59'30.82"E
4	Trepča - mine	42°56'13.28"N	20°55'18.40"E
5	Aleksinac - mine	43°33'27.42"N	21°41'30.64"E
6	Bor - mining and smelting complex	44°04'33.90"N	22°06'29.99"E
7	Majdanpek - mine	44°24'19.83"N	21°56'17.46"E
8	Kostolac - thermal power plant	44°43'37.70"N	21°12'49.71"E
9	“Nikola Tesla” - thermal power plant	44°40'17.36"N	20°09'22.77"E
10	RB Kolubara - coal mining and smelting complex	44°28'22.09"N	20°14'00.97"E
11	Rudnik - mine and flotation	44° 08'02.64"N	20°29'52.91"E
12	Ugljevik - coal mine	44° 40'04.44"N	18°59'47.24"E
13	Tuzla - thermal power plant	44° 31'04.13"N	18°36'05.55"E
14	Kakanj - thermal power plant	44° 05'19.56"N	18°06'49.36"E
15	Pljevlja - thermal power plant	43°20'04.33"N	19°19'39.72"E
16	Gacko - thermal power station and coal mine	43°17'01.79"N	18°30'55.72"E

Table 3

Table 3 Results of two-way ANOVA with factors population and tree (nested in population) for the hazardous element content (μg/g of dry needle mass): arsenic, cadmium, mercury, nickel, lead and zinc in the *Picea abies* (L.) Karst. needles**.**

		Arsenic		Cadmium		Mercury	
Source of variation	df	MS	F-value	MS(x10-2)	F-value	MS	F-value
Population	14	5.44	15.6****	51.30	203.09****	0.01	1.09
Tree (Population)	29	0.39	1.11	0.24	0.94	0.01	1.00
Error	406	0.35		0.25		0.01	

		Nickel		Lead		Zinc	
Source of variation	df	MS	F-value	MS	F-value	MS	F-value
Population	14	56.83	3.67****	715.28	88.00****	3183.29	19.81****
Tree (Population)	29	14.57	0.94	7.66	0.94	137.16	0.85
Error	406	15.47		8.13		160.70	

**** P < 0.0001

Table 4

Table 4 The Eigenvectors table with linear coefficients for the seven principal component axes (PC 1-7) and hazardous elements content in needles (µg/g of dry needle mass) of arsenic (As), cadmium (Cd), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn), and mean annual precipitation (MAP in mm). The highest absolute values are presented in bold.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
As	**0.918**	0.092	-0.067	-0.310	-0.167	0.047	-0.133
Cd	**0.928**	0.034	0.071	-0.200	-0.183	0.210	0.124
Hg	0.607	0.035	0.084	0.775	0.001	0.151	-0.027
Ni	**0.816**	-0.039	-0.008	-0.077	0.543	-0.181	0.016
Pb	-0.192	-0.529	0.765	-0.133	0.144	0.241	-0.029
Zn	-0.329	**0.851**	-0.041	-0.102	0.233	0.317	-0.012
MAP	0.118	0.609	0.723	0.035	-0.131	-0.273	0.008