iForest Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor4611-017
vol. 17, pp. 370-377

Bioaccumulation of long-term atmospheric heavy metal pollution within the Carpathian arch: monumental trees and their leaves memoir

Diana Vasile [(1)], Raluca Enescu [(2)], Andrei Apafaian [(1)], Simona Coman [(1)], Virgil Scarlatescu [(3)], Vlad Crisan [(1)]

Atmospheric pollution is a major problem in urban environments. Pollutants with various sizes of particulate matter result from long-term anthropogenic sources, including industrial emissions and the combustion of fossil fuels by vehicles and commercial and residential buildings. Pollutants affect both humans and green areas. Trees are good indicators of pollution due to their longevity, easy identification, and geographical distribution in urban areas. Recently, due to climate change, all EU member states have agreed to measure heavy metal pollution in urban areas using plants as bioindicators. This study aimed to analyze heavy metal concentrations (Pb, Cd, Mn, Fe, Cu, Zn) in the leaves of monumental trees (both evergreen coniferous and deciduous) in the Carpathian Arch. Leaf samples were collected in the summer of 2022 from 37 specimens across 12 sites in nine counties – Alba, Brasov, Harghita, Mures, Sibiu, Satu Mare, Bistrita Nasaud, Maramures, and Cluj. The leaf heavy metal contents correlated with a low level of atmospheric pollution. Of the six heavy metals analyzed, only four were highly accumulated in the leaf samples and exceeded the toxicity threshold. Of the nine counties sampled, heavy metal pollution above the toxicity threshold was detected only in two sites – the city of Baia Mare, Maramures county (Zn and Cd), and the city of Cluj-Napoca, Cluj county (Fe, Zn, Cd, and Cu). The response of the tree species to changes in atmospheric heavy-metal concentrations confirmed that trees are suitable bioindicators of air pollution in urban areas. Monumental trees can also be used for environmental restoration and to promote sustainable urban development, decrease pollution, and increase urban environmental health.

Keywords: Atmosphere Pollution, Urban Environments, Heavy Metals, Monumental Trees, Samples

Introduction

In the last decade, strategies and policies have been developed worldwide to improve air quality by addressing the factors that increase air pollution, aiming to protect the environment and human health. According to the World Health Organization (WHO 2023), almost the entire global population (around 99%) breathes air with high concentrations of pollutants, and in some cases, the pollution exceeds WHO guideline levels. Ambient and household pollution is estimated to have caused 6.7 million premature deaths per year worldwide. Moreover, by the 2030s, the WHO has cautiously anticipated 250,000 additional annual deaths due to climate change and its consequences (https://www.who.int/news-room/fact-sheets/detail/climate-change-and-health). Due to pollution, 85% of annual deaths worldwide are premature deaths of people from low- and middle-income countries (Newell et al. 2017). However, people from high-income countries are also affected by pollution (Juginović et al. 2021).

For this reason, many countries have rallied to reduce the effects of pollution. A turning point was the 1997 Kyoto Protocol, recognized as the most noteworthy environmental treaty ever negotiated for reducing greenhouse gas emissions (Böhringer 2003). Actions like this are needed when voluntary agreements cease to have an effect. Such treaties provide some opportunities but mostly constrain the signatory countries (Swinton & Sarkar 2008). Strategies and policies have also been developed and implemented regionally. For example, the European Green Deal is an effort to reduce the net greenhouse gas emissions of the EU member states by more than half by the year 2030, compared to the levels reported for the year 1990 (https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en). Additionally, action plans like the "Towards Zero Pollution" for air, water, and soil plan are helping European Green Deal members achieve their targets (https://environment.ec.europa.eu/strategy/zero-pollution-action-plan_en). According to the European Environment Agency's publication "Air Quality in Europe 2022", at least 238,000 people died prematurely in

(1) Department of Ecology, "Marin Dracea" National Institute for Research and Development in Forestry, 13 Closca Street, 50004 Brasov (Romania); (2) Transilvania University of Brasov, Faculty of Silviculture and Forest Engineering, Brasov (Romania); (3) National Institute for Research and Development in Forestry "Marin Dracea", Principala Str., 117470 Mihaesti (Romania)

@ Andrei Apăfăian (apafaian.andrei@gmail.com)

Received: Mar 29, 2024 - First Accepted: Jul 19, 2024 - Final Acceptance: Nov 13, 2024

Citation: Vasile D, Enescu R, Apafaian A, Coman S, Scarlatescu V, Crisan V (2024). Bioaccumulation of long-term atmospheric heavy metal pollution within the Carpathian arch: monumental trees and their leaves memoir. iForest 17: 370-377. - doi: 10.3832/ifor4611-017 [online 2024-11-27]

Communicated by: Daniela Baldantoni

the EU that year due to exposure to pollutants (https://www.eea.europa.eu/publications/air-quality-in-europe-2022). Common health impacts of pollution are stroke, ischemic heart disease, and primary cancer of the trachea, bronchus, and lung (Juginović et al. 2021). In the spring of 2023, Romania enacted Law No. 97/2023 for the protection of remarkable trees (Official Gazette 2023). The law defines a remarkable tree as being at least 160 years old and having a minimum diameter measured at breast height (https://legislatie.just.ro/Public/DetaliiDocument/267143). WIthin the EU, the Commission serves as a guardian to member states that do not comply with the relevant regulations and directives. For example, Germany, France, Belgium, Spain, Poland, and Romania have all recently been sent to the EU Court of Justice by the European Commission for failing to comply with relevant legislation on air quality. Several member states have also been fined (EC 2023).

According to a report from the Romanian National Environment Agency, published in 2022, Law No. 104/2011 is responsible for evaluating air quality in Romania and endorses/transposes all the directives of the European Parliament and the Council. The network for air-quality monitoring comprises 163 stations that monitor traffic and industrial emissions, urban and suburban pollution, and regional/rural pollution (National Agency for Environmental Protection 2022).

In urban environments, atmospheric pollutants with different sizes of particulate matter come from long-term anthropogenic sources. These sources include industrial emissions and fossil fuels combusted by vehicles and commercial and residential buildings. They affect the lives of urban residents and their green areas. Of these sources, fossil fuels used in vehicles are responsible for releasing most of the toxic trace elements, especially Pb (Duong & Lee 2011, Fujiwara et al. 2011, Sawidis et al. 2011).

Heavy metals, such as Cu, Fe, Pb, and Cd, are present in particulates of different sizes depending on the emission source. These particulates spread into the atmosphere of urban environments (De Vives et al. 2006, Anagnostatou 2008). Even if cities banned cars from city centers, invested in pedestrian travel, cycling, and public transportation, or redesigned urban streets, it will take a significant amount of time before pollution levels decrease.

Recently, plants have been used as biomonitors or bioindicators to detect environmental pollution of the soil in both urban areas and forest ecosystems (Latwal et al. 2023, Zheng et al. 2023). Trace elements are often present in leaves in higher quantities than in the soil (Ma et al. 2001). In particular, trees are good indicators of pollution due to their longevity, easy identification, and geographical distribution in urban areas (Aksoy et al. 2000, Berlizov et al. 2007). Their longevity allows research/measurements to be repeated every few years over long periods. Heavy metals deposited on leaf surfaces can be absorbed and then spread into intercellular spaces, entering the channels, cuticles, and stomata. Moreover, toxic elements can be transported through plant xylem and phloem (White 2023). The assimilation of heavy metals in trees is regulated by various factors, including the plant water deficit and leaf size, the light intensity, wind direction, and speed, and gas solubility in water.

Tree leaves are good indicators of heavy-metal pollution at a given time, reflecting what was recently absorbed from the atmosphere. Conversely, heavy metal pollution in the soil can take several years to build up and become detectable (Bonneau 2010).

Many centuries-old, monumental trees are vital in the urban environment. They provide several benefits, such as carbon storage and sequestration and air pollution removal, and they help define the local identity of urban areas (Cannizzaro & Corinto 2014). Monumental trees often have more significant social and environmental benefits than smaller trees (Nowak & Dwyer 2000) due to the large number of leaves in their crowns. Indeed, monumental trees with a diameter of 76 cm can remove 30 to 65 times more air pollution in a year than smaller trees (Nowak 2004). Due to climate change effects, all European states have recently agreed to measure urban atmosphere heavy-metal pollution using plants as bioindicators (Sawidis et al. 2011). Various tree species have been used as bioindicators, such as *Robinia pseudoacacia* (Serbula et al. 2012), *Aesculus hippocastanum* (Tomašević et al. 2008, 2011), *Eleagnus angustifolia, Fraxinus excelsior*, and *Juglans regia* (Dogan et al. 2014), *Quercus ilex* (Ugolini et al. 2013), *Pinus brutia, Citrus aurantium*, and *Olea europaea* (Sawidis et al. 2012), and *Terminalia catalpa* (Olajire & Ayodele 2003).

The main aim of this study was to provide a comprehensive overview of heavy-metal accumulation (Pb, Cd, Mn, Fe, Cu, Zn) in the leaves of (evergreen coniferous and deciduous) monumental trees from different cities located in nine counties in the Carpathian arch.

Materials and methods

Study sites

To analyze the effects of heavy metal pollution and its bioaccumulation in the leaves of monumental trees, twelve types of urban areas were selected across nine counties (Fig. 1).

The degree of exposure to pollutants in the studied urban areas is different due to the past and present factors. According to the Romanian National Institute of Statistics (https://insse.ro/cms/en), almost 1 million inhabitants live in the study areas overall; the lowest number is in Jucu (5,349) while the highest is in Cluj Napoca (286,598). Also, a relevant criterion for the selection of trees to be sampled was their age and health status, two factors that can lead to variations in pollutant storage besides anthropogenic activities.

In total, leaf samples were collected from twelve sites: 2 from Alba Iulia county, 6 from Brasov county, 3 from Harghita county, 4 from Mures county, 2 from Sibiu county, 4 from Satu Mare county, 4 from Bistrita county, 3 from Maramures county and 9 from Cluj county (Tab. 1).

The majority of sampled trees were selected for their large size, while two specimens are from historical trees for that area (Fig. 2). The age varied between 100 and 600 years, the oldest trees being two *Q. robur* individuals from Blaj and Sibiu. The height ranges between 13 and 43 m, and the diameter between 0.68 and 2.13 m. The highest and the thickest tree was the monumental plane tree from Carei.

Sample collection and preparation

During the summer of 2022 (June-August), 10-20 g of fresh leaves (5-10 g of dry material) were collected from each of the 37 monumental trees selected. According to Cools & De Vos (2010), mature leaves were sampled from the upper third of the crown. For the deciduous species, samples were collected during the second half of the growing season and before the onset of autumn yellowing and senescence.

The leaves were cut off the branches at a

Fig. 1 - Location of the monumental trees analyzed in this study (source: Google Earth™).

height of approximately 3 m using stainless steel scissors. Leaves were randomly selected from different areas of the crown, totaling about 30-40 fully developed leaves collected from each tree. The leaves were stored and transported in paper bags to the laboratory, where they remained until they were processed under laboratory conditions. In the laboratory, the leaf samples were left unrinsed and were oven-dried at 70 °C for 1 week. The dried samples were milled to uniform size using a laboratory mill, packed in polyethylene bags, and kept under stable conditions until their chemical analysis. The mill was washed after each grinding, first with alcohol and then with distilled water, to avoid any contamination between samples (Celik et al. 2005).

Fig. 2 - Examples of the monumental trees selected for the study.

Tab. 1 - The monumental tree species from which the samples were collected. (CBH): Circumference at breast height.

No.	County	City	Species	Total height (m)	CBH (m)	Estimated age (years)	Health status
1	Alba	Blaj	*Quercus robur* L.	26.5	6.50	600	Very good
2			*Tilia tomentosa* Moench.	17.5	3.80	200	Very good
3	Brasov	Brasov	*Aesculus hipocastanum* L.	22.6	3.49	200	Good
4			*Ginkgo biloba* L.	25.5	3.14	100	Very good
5			*Acer pseudoplatanus* L.	29.6	3.42	200	Good
6			*Platanus hybrida* Brot.)	33.5	5.02	300	Very good
7		Codlea	*Salix babylonica* L.	22.9	4.93	100	Poor
8			*Acer platanoides* L.	27.7	3.89	200	Good
9	Harghita	Miercurea Ciuc	*Ulmus glabra* Huds.	23.3	4.30	200	Good
10			*Populus nigra* L.	41.5	4.46	200	Good
11			*Salix babylonica* L.	14.8	4.14	150	Poor
12	Mures	Sovata	*Acer campestre* L.	16.1	2.26	150	Very good
13			*Thuja plicata* Don.	24	2.14	150	Very good
14		Sighisoara	*Aesculus hipocastanum* L.	15.2	3.86	100	Good
15			*Taxus baccata* L.	13.1	3.58	150	Very good
16	Sibiu	Sibiu	*Quercus robur* L.	17.5	5.81	600	Poor
17			*Platanus hybrida* Brot.	39.5	4.02	100	Very good
18	Satu Mare	Carei	*Platanus hybrida* Brot.	43.4	6.69	300	Good
19			*Sophora japonica* L.	31.8	4.58	200	Very good
20			*Acer pseudoplatanus* L.	27.5	3.80	200	Very good
21			*Ginkgo biloba* L.	27.5	4.40	200	Very good
22	Bistrita Nasaud	Bistrita	*Acer negundo* L.	17.2	2.67	150	Very good
23			*Fraxinus excelsior* L.	28.6	4.58	200	Very good
24			*Acer platanoides* L.	19.3	3.08	150	Good
25			*Acer pseudoplatanus* L.	33	3.49	150	Very good
26	Maramures	Baia Mare	*Sophora japonica* L.	26	3.58	200	Very good
27			*Camaecyparis lawsoniana* (A. Murr) Parl.	31.3	3.58	200	Very good
28			*Pinus strobus* L.	38.8	3.08	200	Very good
29	Cluj	Cluj	*Alnus glutinosa* (L.) Gaertn.	27.9	3.05	100	Good
30			*Gleditschia triacanthos* L.	22	2.54	100	Very good
31			*Juglans regia* L.	16.9	3.17	100	Poor
32			*Populus nigra* L.	26.5	5.28	150	Good
33			*Sophora japonica* L.	25.2	3.49	100	Very good
34			*Quercus robur* L.	25.4	5.50	200	Very good
35		Jucu	*Juglans nigra* L.	24.7	2.73	100	Very good
36			*Quercus robur* L.	27.6	5.84	200	Good
37			*Ulmus laevis* Pall.	19.3	3.30	100	Very good

Chemical analysis

For the chemical analysis, 0.4 g (dry weight) of leaves was digested in 3 ml of 65% HNO_3 and 2 ml of 30% H_2O_2 in a microwave oven (SpeedwaveTM MWS-3, Berghof, Germany). After digestion, the solution was diluted with distilled water to a total volume of 25 ml. Cd (trace element) concentration was analyzed by inductively coupled plasma-mass spectrometry. Fe, Cu, Mn, Zn, and Pb concentrations were analyzed by inductively coupled plasma-optical-emission spectrometry (Rautio et al. 2016).

Results

Lead

High lead (Pb) concentrations can negatively influence plant growth and development (*e.g.*, root blackening), and can eventually cause plant death (Nas & Ali 2018). The current acceptable Pb concentration for plants is 2 ppm (Ogütücü et al. 2021), having previously been 3 ppm (Allen 1989).

The lead concentrations in the monumental tree leaves ranged between 0 and 8.44 ppm (Fig. 3a). This range is below the toxicity threshold, but the acceptable threshold was exceeded in many trees. For example, in Miercurea Ciuc, the leaves of *Ulmus glabra* had a concentration of 2.8 times the threshold, with the leaves of *Salix babylonica* having a concentration of 1.8 times the threshold. In Blaj, the leaves of *Tilia tomentosa* had a concentration 1.7 times the threshold, whereas in Codlea city, the leaves of *Acer platanoides* had a concentration 2 times the threshold.

Iron

An iron (Fe) concentration of 40 to 300 ppm in tree leaves is normal, with less than 40 ppm being considered deficient and above 300 ppm being considered toxic, causing chlorosis (the abnormal reduction or loss of the green coloration in plant leaves – Bergmann 1983, 1992). However, the median Fe concentration in leaves is about 150 ppm (Bergmann 1992).

High Fe levels were found in only two cities: Bistrita, in the leaves of *Acer negundo* (405.3 ppm, 1.3 times the toxicity threshold), and Cluj, in the leaves of *Sophora japonica* (320.7 ppm), *Alnus glutinosa* (430.3 ppm, 1.4 times the threshold), and *Quercus robur* (595.1 ppm, 1.9 times the threshold – Fig. 3b). These results indicate that Bistrita and Cluj had high levels of Fe pollution.

Manganese

Similar to lead Pb pollution, manganese (Mn) pollution is related to traffic density and vehicular emissions, although some researchers have indicated that airborne manganese Mn originates from soil (Oliva & Rautio 2005). The Mn concentration in unpolluted natural environments should lie between 50 and 500 ppm (Bergmann 1992).

The Mn concentration in the leaf samples ranged between 9.49 and 401.7 ppm, which is below the toxicity threshold (Fig. 4a).

Copper

According to Bergmann (1992) and Kabata-Pendias (2011), tree leaf copper (Cu) concentrations of 5 to 20 ppm are normal, whereas less than 4 ppm is considered deficient. This can cause considerable changes to the biochemical processes in plants. Concentrations above 20 ppm are considered toxic for plant growth and, similarly to iron deficiency, can cause chlorosis (Bergman 1983).

The copper concentrations in the monumental tree leaves were low, ranging between 3.18 and 15.24 ppm (Fig. 4b). Only a single value exceeded the toxicity threshold (by 1.1 times) from an *Alnus glutinosa* in Cluj.

Zinc

The zinc (Zn) toxicity threshold in plants is 150 ppm. Levels above this threshold may cause production loss, while levels below 10 ppm may cause leaf deformation (Bucher & Schenk 2000, Norouzi et al. 2015).

The zinc concentrations in the monumental tree leaves were low in most locations (Fig. 5a), ranging from 5 to 111 ppm. This indicates that most of the locations were not polluted with this heavy metal. The highest zinc concentrations were found in Baia Mare, in the leaves of *Camaecyparis lawso-*

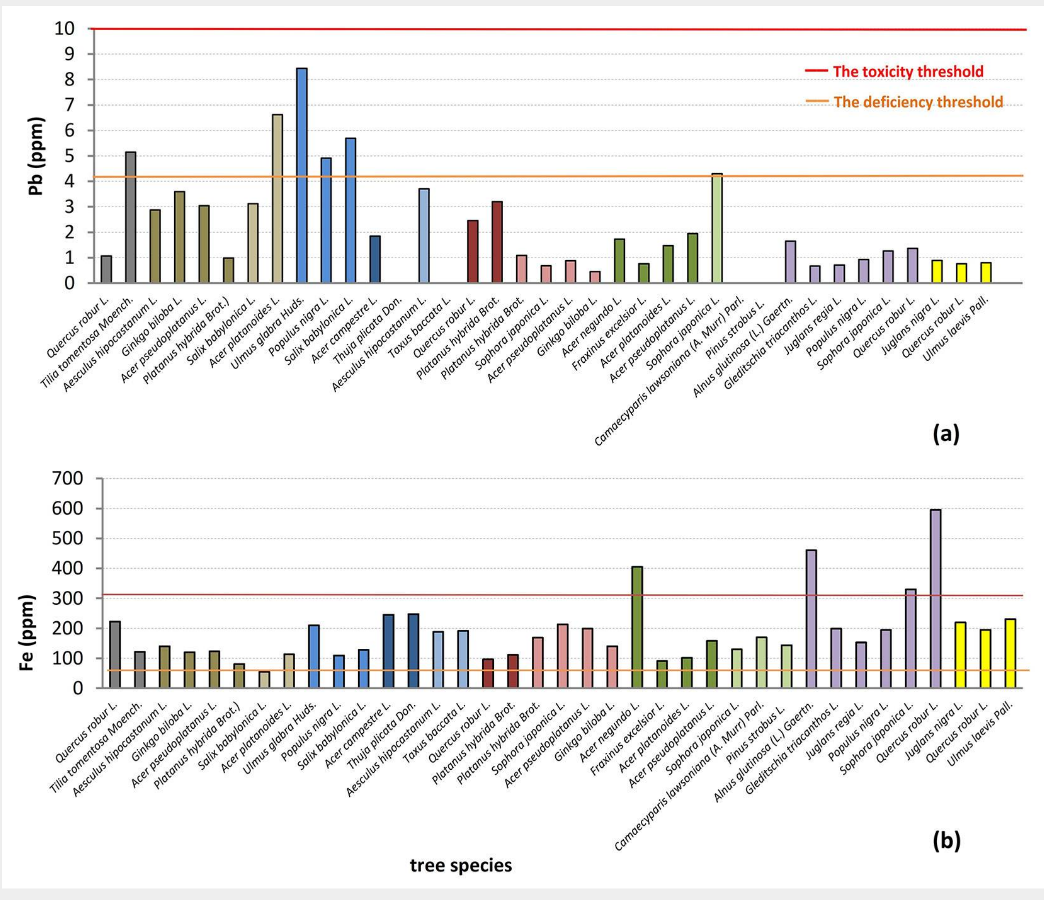

Fig. 3 - The metal content of monumental tree leaves. (a): Lead; (b): Iron.

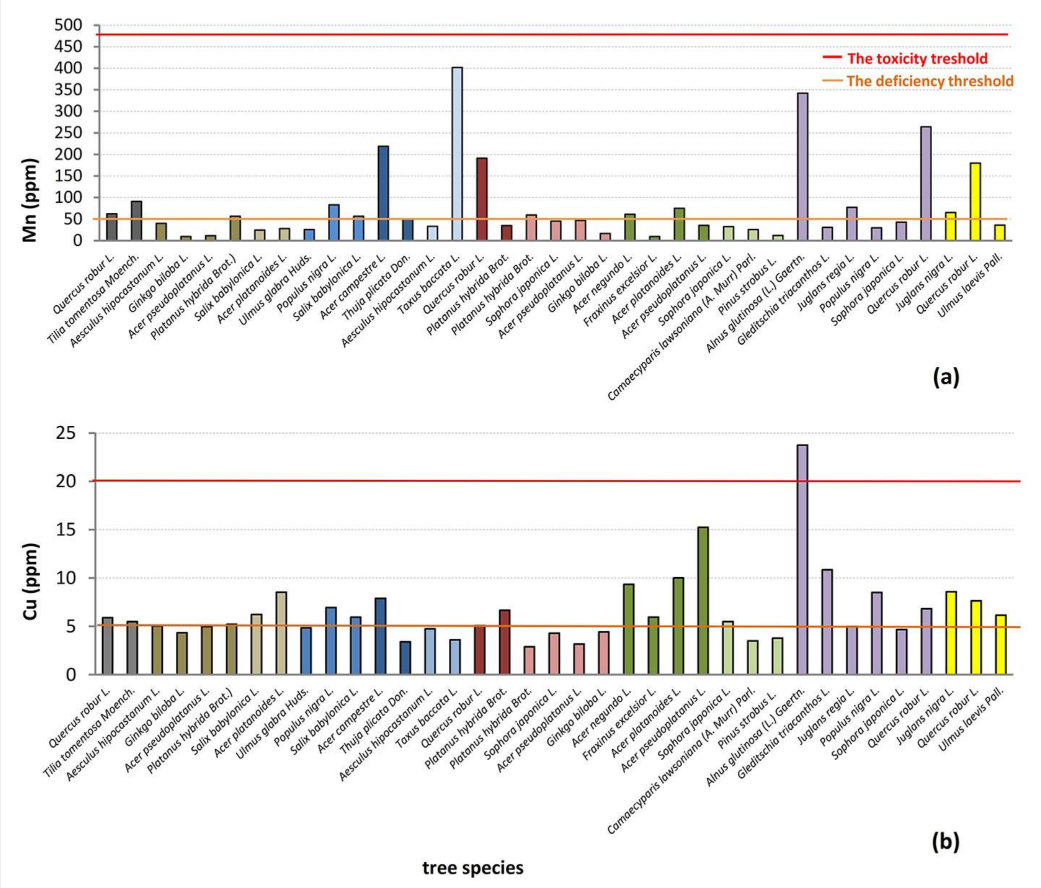

Fig. 4 - The metal content of monumental tree leaves. (a): Manganese; (b): Copper.

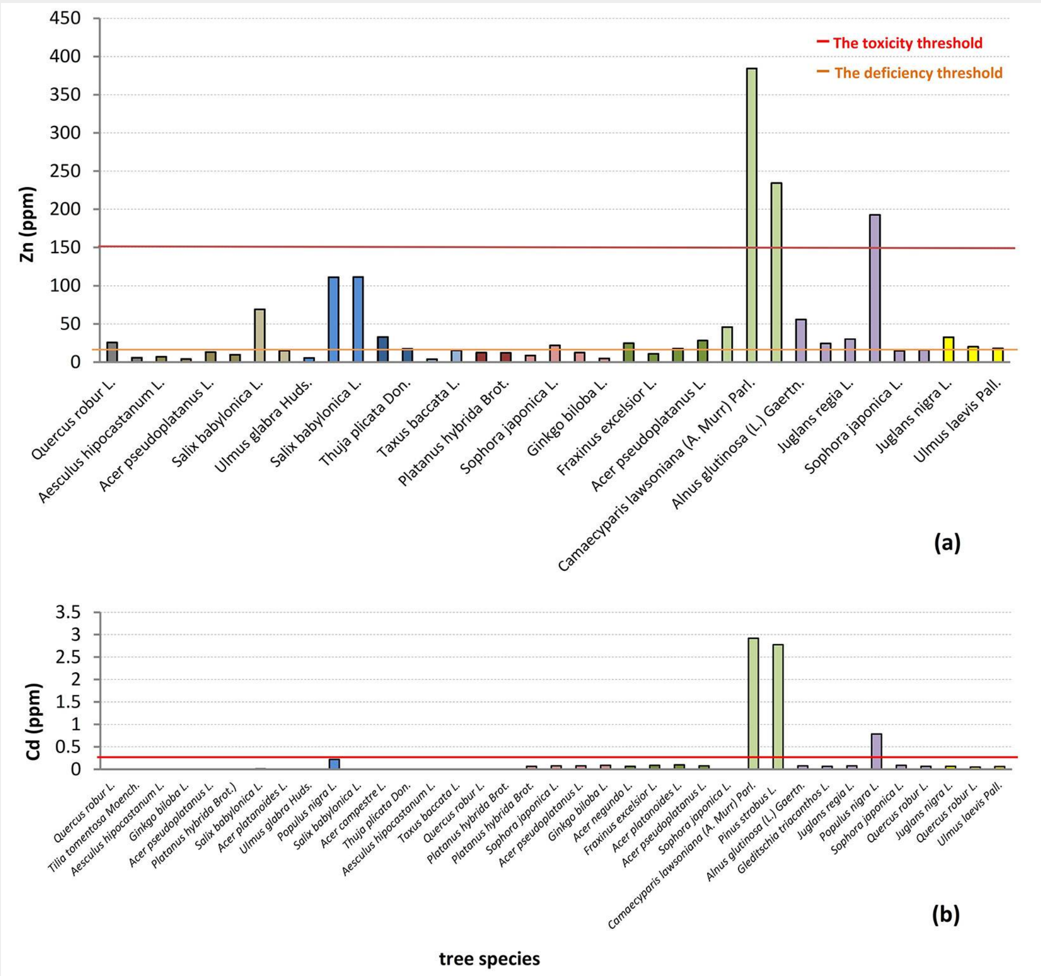

Fig. 5 - The metal content of monumental tree leaves. (a): Zinc; (b): Cadmium.

niana (384.3 ppm) and *Pinus strobus* (234.3 ppm), and in Cluj, in the leaves of *Populus nigra* (192.7 ppm). These concentrations exceeded the toxicity threshold by 2.5 times, 1.5 times, and 1.2 times, respectively.

Cadmium

The cadmium (Cd) concentrations in unpolluted natural environments should lie between 0.03 and 0.37 ppm (Allen 1989, Bergmann 1992).

The cadmium concentrations in the monumental tree leaves were generally similar to the unpolluted levels, with most samples ranging between 0 and 0.22 ppm (Fig. 5b). Only three high values were recorded--in Baia Mare (2.92 and 2.78 ppm) and Cluj (0.79 ppm). These values exceeded the toxicity threshold by 8 times in Baia Mare and 2 times in Cluj.

Discussion

Lead

Lead has been used in different forms from ancient times. However, when it reaches high concentrations, the exposure to humans will impact almost all organs, especially the central nervous system, kidneys, and blood. Excessive levels will cause even death (Tong et al. 2000).

In the last decades, Lead pollution has been caused by anthropogenic activities and industrial emissions, but most is caused by emissions from road vehicles using fossil fuels (Oliva & Rautio 2005). In general, lead appears on plant leaves by aerial deposition, especially along the roads, depending on traffic intensity and the distance from the road (Muzychenko et al. 2017). The quantity of lead decreases in the leaves of plants with increasing distance from roads (Scerbo et al. 2002).

According to Kabata-Pendias & Piotrowska (1984) in an urban environment, the normal concentrations of Pb in tree leaves are less than 10 ppm, this value is considered to be the toxicity threshold. According to WHO, a safety limit of lead for plants is 2 ppm (Ogütücü et al. 2021) however in the past, an acceptable value of lead for plants can be also 3 ppm (Allen 1989).

In Artvin, Turkey, no significant lead pollution was detected, as the Pb content in the leaves of *Juglans regia* ranges between 0.158-0.665 ppm (Dogan et al. 2014) while in Serbia (Hall Pioneer Park, Belgrade), the highest Pb concentration found in plane leaves is of 13.748 ppm (Sawidis et al. 2011), exceeding of 1.3 times the toxicity threshold. Therefore, we concluded that there is no lead pollution in the cities where the samples were taken.

Iron

Iron plays an important role in respiration and photosynthesis reactions *via* enzymes and proteins (Ancuceanu et al. 2015). Iron derives from both anthropogenic and natural sources (Oliva & Rautio 2005). In Belgrade, Serbia, high iron contents in pine leaves have been detected in the Hall Pioneer Park (319.411 ppm), whereas in Kosutnjak (also in Belgrade), the iron content was 185.411 ppm (Sawidis et al. 2011). In Artvin, Turkey, high iron concentrations have been reported in walnut leaf samples (332.5-698.2 ppm – Dogan et al. 2014).

In the current study, the iron concentrations exceeded the limits only in the cities of Bistrita and Cluj, indicating Fe contamination in these areas.

Manganese

Manganese is a distinctive component of the photosynthetic oxygen-evolving system in chloroplasts (Celik et al. 2005), and manganese oxides have the role of fixing trace elements (cobalt, copper, zinc, nickel, and even pollutants like lead) in the soil. In medicine and some analyses, the chemical compound permanganate is used because it is a strong oxidizing agent (Allen 1989).

Low levels of manganese, similar to those found in this study (53.6-349.2 ppm), have also been reported for various species, such as *Robinia pseudoacacia* from Denizli, Turkey (Celik et al. 2005), in the walnut leaf samples (1.001-204.6 ppm) from Artvin, Turkey (Dogan et al. 2014) and in the *Quercus ilex* leaf samples (68.4-405 ppm) from Florence, Italy (Ugolini et al. 2013).

The data from all plots show values mostly below the 400 ppm threshold, indicating low pollution levels.

Copper

Copper is present naturally in the environment in the elemental form and is very important for the normal growth and metabolism of all living organisms, but it also can be commercially produced from sulfides and oxide minerals.

Copper is used in the manufacturing of electrical equipment, in construction, in agriculture (pesticides and fungicides), in wood preservation, etc. (Kanoun Boule et al. 2008, Kula et al. 2010).

In Edirne, Turkey, Cu content in the leaves of *A. hippocastanum* has been reported to range between 0.322-0.466 ppm (Yilmaz et al. 2006), indicating a copper deficiency, while the leaves of *Juglans regia* in Artvin, Turkey, showed a Cu content ranging from 0.339 to 13.80 ppm (Dogan et al. 2014). In contrast, in Athens, Greece, the average CU content in the leaves of *Citrus aurantium, Olea europaea*, and *Pinus brutia* was 99.86 ppm, which exceeds the toxicity threshold by 5 times (Sawidis et al. 2011).

In this study, all species except for one (*Alnus glutinosa*) sampled in Cluj city showed values below the maximum limit for copper, indicating low contamination levels.

Zinc

Zinc is an essential plant nutrient that plays an important role in the biosynthesis of enzymes, auxins, and proteins. The normal levels of zinc in plants are between 10 and 150 ppm (Allen 1989, Kabata-Pendias 2011).

Low zinc concentrations have been reported in the leaves of *Aesculus hippocastanum* from Edirne, Turkey (0.374-0.532 ppm – Yilmaz et al. 2006), and *Ficus religiosa* (0.766 ppm) and *Eucalyptus* species (9.290 ppm). All these levels are below the deficiency threshold and could induce leaf deformation. Conversely, optimal zinc concentrations have been found in *Quercus ilex* leaf samples (23.2-48 ppm) from Florence, Italy (Ugolini et al. 2013).

In the current study, zinc concentrations below the threshold were found in most locations. However, the samples from Baia Mare and Cluj, which have polluting industries, had concentrations exceeding the threshold by up to 2.5 times.

Cadmium

The toxic effect of cadmium on plants causes a reduction in plant growth and the appearance of chlorosis (abnormal reduction or loss of the normal green coloration of leaves). It is dangerous due to its great aerial dispersion, small size of particles being able to accumulate at great distances to the source of pollution (De Nicola et al. 2008),

In Athens, Greece, the mean cadmium content in tree leaves has been reported to be 0.62 ppm (Sawidis et al. 2011), exceeding the toxicity threshold 1.6 times. In the city of Edirne, Turkey, even in the roadside samples, the values of cadmium were very low (0.068 ppm – Yilmaz et al. 2006).

Again, in this study, the highest values were found only in the cities of Baia Mare and Cluj, with values respectively 8 and 2 times greater than the toxicity threshold, indicating residual pollution in those areas.

Conclusion

The concentrations of heavy metals (Pb, Fe, Cu, Zn, Cd, Mn) were estimated under standardized experimental conditions in the leaves of 20 monumental trees of various species from urban areas in the Carpathian Arch. Overall, the results indicate low levels of atmospheric pollution. Only four of the six heavy metals analyzed exceeded the toxicity threshold, and only two of the nine counties included in the study had heavy metal pollution. The samples from Maramures County (in the city of Baia Mare) had zinc and cadmium levels above the toxicity threshold, and samples from Cluj County (in the city of Cluj) had iron, zinc, cadmium, and copper levels above the toxicity threshold.

Differences in heavy metal concentrations among tree species could result from their different capacities for heavy-metal storage. However, changes in leaves in response to concentrations of heavy metals in the atmosphere demonstrate that trees are suitable bioindicators of urban air pollution. Monumental trees can also aid in environmental restoration by decreasing pollution, resulting in healthier and more sustainable urban environments.

Acknowledgments

This work was supported by the National Institute of Research and Development in Forestry "Marin Dracea", project no. 19070505 "Health status and conservation of monumental trees in Romania in the conditions of climate change", within the BIOSERV program, financed by the Romanian Ministry of Research and Innovation.

References

Aksoy A, Sahin U, Duman F (2000). *Robinia pseudacacia* L. as a possible biomonitor of heavy metal pollution in Kayseri. Turkish Journal of Botany 24: 279-284.

Allen SE (1989). Chemical analyses of ecological material (2nd edn). Blackwell Scientific Publications, London, UK, pp. 368.

Anagnostatou AV (2008). Assessment of heavy metals in Central Athens and suburbs using plant material. Dissertation, University of Surrey, Guildford, UK, pp. 234.

Ancuceanu R, Dinu M, Hovanet MV, Anghel AI, Popescu CV, Negres S (2015). A survey of plant iron content - A semi-systematic review. Nutrients 7 (12): 10320-51. - doi: 10.3390/nu7125535

Bergmann W (1992). Nutritional disorders of plants. Gustav Fischer Verlag, Jena, Germany, pp. 52. [online] URL: http://www.cabidigitallibrary.org/doi/full/10.5555/19932336963

Bergman W (1983). Farbatlas Ernahrungsstorungen bei Kulturpflanzen fur den Gebrauch im Feldbestand [Color atlas of nutritional disorders in crops for use in the field]. VEB Gustav Fischer Verlag, Jena, Germany, pp. 254. [in German]

Berlizov AN, Blum OB, Filby RH, Malyuk IA, Tryshyn VV (2007). Testing applicability of black poplar (*Populus nigra* L.) bark to heavy metal air pollution monitoring in urban and industrial regions. Science of the Total Environment 372: 693e706. - doi: 10.1016/j.scitotenv.2006.10.029

Böhringer C (2003). The Kyoto protocol: a review and perspectives. Oxford Review of Economic Policy 19 (3): 451-466. - doi: 10.1093/oxrep/19.3.451

Bonneau M (2010). Les analyses de sol et le diagnostic foliaire comme indicateurs de gestion durable [Soil analysis and foliar diagnosis as indicators of sustainable management]. Forestry and Cynegetics Journal 26: 22. [in French]

Bucher AS, Schenk MK (2000). Toxicity level for phytoavailable zinc in compost peat substrates. Scientia Horticulturae 83 (3-4): 339-352. - doi: 10.1016/S0304-4238(99)00086-2

Cannizzaro S, Corinto GL (2014). The role of monumental trees in defining local identity and in tourism. a case study in the Marche region. Geoprogress Journal 1: 29-48.

Celik A, Kartal AA, Akdogan A, Kaska Y (2005). Determining the heavy metal pollution in Denizli (Turkey) by using *Robinia pseudacacia* L. Environment International 31: 105-112. - doi: 10.1016/j.envint.2004.07.004

Cools N, De Vos B (2010). Part X: Sampling and analysis of soil. In: "Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests". UNECE ICP Forests Programme Co-ordinating Centre, Hamburg, Germany, pp. 209. [online] URL: http://icp-forests.net/page/icp-forests-manual

De Nicola F, Maisto G, Prati MV, Alfani A (2008). Leaf accumulation of trace elements and polycyclic aromatic hydrocarbons (PAHs) in Quercus ilex L. Environmental Pollution 153 (2): 376-383. - doi: 10.1016/j.envpol.2007.08.008

De Vives AES, Moreira S, Boscolo Brienza SM, Silva Medeiros JG, Tomazello Filho M, Zucchi OLAD, Nascimento FV (2006). Monitoring of the environmental pollution by trace element analysis in tree-rings using synchrotron radiation total reflection X-ray fluorescence. Spectrochimica Acta 61 (10-11): 1170-1174. - doi: 10.1016/j.sab.2006.08.007

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

Duong TTT, Lee BK (2011). Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. Journal of Environmental Management 92 (3): 554-62. - doi: 10.1016/j.jenvman.2010.09.010

EC (2023). Environment: Commission takes Poland to Court over air quality and marine policy legislation and urges compliance with the Nitrates Directive. European Commission, Press Release, web site. [online] URL: http://ec.europa.eu/commission/presscorner/detail/en/IP_11_1434

Fujiwara FG, Gómez DR, Dawidowski L, Perelman P, Faggi A (2011). Metals associated with airborne particulate matter in road dust and tree bark collected in a megacity (Buenos Aires, Argentina). Ecological Indicators 11: 240-247. - doi: 10.1016/j.ecolind.2010.04.007

Juginović A, Vuković M, Aranza I, Bilos V (2021). Health impacts of air pollution exposure from 1990 to 2019 in 43 European countries. Scientific Reports 11: 22516. - doi: 10.1038/s41598-021-01802-5

Kabata-Pendias A, Piotrowska M (1984). Zanieczyszczenie Gleb i Roslin Uprawnych Pierwiastkami Sladowymi [Contamination of soils and crops with trace elements]. CBRopracowanie Problemowe, Warszawa, Poland, pp. 403. [in Polish]

Kabata-Pendias A (2011). Trace elements from soils to plants (4th edn). CRS Press, Francis and Taylor Group, Boca Raton, FL, USA, pp. 520.

Kanoun Boule M, De Albuquerque MB, Nabais C, Freitas H (2008). Copper as an environmental contaminant: phytotoxicity and human health implications. In: "Trace Elements as Contaminants and Nutrients: Consequences in Ecosystems and Human Health" (Prasad MNV ed). John Wiley and Sons, Cambridge, MS, USA, pp. 653-678. - doi: 10.1002/9780470370124.ch25

Kula I, Yildiz D, Dogan Y, Ay G, Baslar S (2010). Trace element contents in plants growing at Mt. Akdag, Denizli. Biotechnology and Biotechnological Equipment 24 (1): 1587-1591. - doi: 10.2478/V10133-010-0010-X

Latwal M, Sharma S, Kaur I, Nagpal AK (2023). Global assessment of air pollution indices of trees and shrubs for biomonitoring and green belt development - A tabulated review. Water, Air, and Soil Pollution 234 (3): 7977. - doi: 10.1007/s11270-023-06194-y

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kenelley ED (2001). A fern that hyperaccumulates arsenic. Nature 409: 579. - doi: 10.1038/35054664

Muzychenko I, Jamalova G, Mussina U, Kazulis V, Blumberga D (2017). Case study of lead pollution in the roads of Almaty. Energy Procedia 113: 369-376. - doi: 10.1016/j.egypro.2017.04.010

Nas FS, Ali M (2018). The effect of lead on plants in terms of growing and biochemical parameters: a review. Environmental Science and Ecotechnology 3 (4): 265-268. - doi: 10.15406/mojes.2018.03.00098

National Agency for Environmental Protection (2022). Preliminary report regarding air quality. Web site. [in Romanian] [online] URL: https://apmbuc.anpm.ro/ro/raportare-anuala

Newell K, Kartsonaki C, Lam KBH, Kurmi OP (2017). Cardiorespiratory health effects of particulate ambient air pollution exposure in low-income and middle-income countries: a systematic review and meta-analysis. Lancet Planet Health 1 (9): 368-380. - doi: 10.1016/S2542-5196(17)30166-3

Norouzi S, Khademi H, Faz Canob A, Acosta JA (2015). Using plane tree leaves for biomonitoring of dust borne heavy metals: a case study from Isfahan, Central Iran. Ecological Indicators 57: 64-73. - doi: 10.1016/j.ecolind.2015.04.011

Nowak DJ, Dwyer JF (2000). Understanding the benefits and costs of urban forest ecosystems. In: "Urban and Community Forestry in the Northeast" (Kuser J ed). Plenum Publishing Corporation, New York, USA, pp. 11-25.

Nowak DJ (2004). Assessing environmental functions and values of veteran trees. In: Proceedings of the International Congress "The trees of history: protection and exploitation of veteran trees" (Nicolotti G, Gonthier P eds). Torino (Italy) 1-2 Apr 2004. Regione Piemonte and Università di Torino, Turin, Italy, pp.45-49.

Official Gazette (2023). Law no. 97/2023 on the protection of remarkable trees. No. 320, Romanian Parliament, Bucharest, Romania. [in Romanian] [online] URL: http://legislatie.just.ro/Public/DetaliiDocument/267143

Ogütücü G, Ozdemir G, Acararicin Z, Aydin A (2021). Trend analysis of lead content in roadside plant and soil samples in Turkey. Turkish Journal of Pharmaceutical Sciences 18 (5): 581-588. - doi: 10.4274/tjps.galenos.2021.45389

Olajire AA, Ayodele ET (2003). Study of atmospheric pollution levels by trace elements analysis of tree bark and leaves. Bulletin of the Chemical Society of Ethiopia 17 (1): 11-17. - doi: 10.4314/bcse.v17i1.61724

Oliva SR, Rautio P (2005). Spatiotemporal patterns in foliar element concentrations in *Ficus microcarpa* L. f. growing in an urban area: implications for biomonitoring studies. Ecological Indicators 5: 97-107. - doi: 10.1016/j.ecolind.2004.08.003

Rautio P, Fürst A, Stefan K, Raitio H, Bartels U (2016). Part XII: Sampling and analysis of needles and leaves. In: "Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests". UNECE ICP Forests Programme Co-ordinating Centre, Thünen Institute of Forest Ecosystems, Eberswalde, Germany, pp. 19. [online] URL: http://icp-forests.net/page/icp-forests-manual

Sawidis T, Krystallidis P, Veros D, Chettri M (2012). A study of air pollution with heavy metals in Athens city and Attica basin using evergreen trees as biological indicators. Biological Trace Element Research 148: 396-408. - doi: 10.1007/s12011-012-9378-9

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: 3560-3570. - doi: 10.1016/j.envpol.2011.08.008

Scerbo R, Possenti L, Lapugnami L, Ristori T, Barale R, Barghigiani C (2002). Lichen (*Xantoria parietina*) biomonitoring of trace element contamination and air quality assessment in Livorno province (Tuscany, Italy). Science of the Total Environment 241 (1-3): 91-106. - doi: 10.1016/S0048-9697(99)00333-2

Serbula SM, Miljkovic DJ, Kovacevic RM, Ilic AA (2012). Assessment of airborne heavy metal pollution using plant parts and topsoil. Ecotoxicology and Environmental Safety 76: 209–214. - doi: 10.1016/j.ecoenv.2011.10.009

Swinton JR, Sarkar A (2008). The benefits of the Kyoto Protocol to developing countries. Environment, Development and Sustainability 10: 731-743. - doi: 10.1007/s10668-007-9082-7

Tomašević M, Vukmirović Z, Rajšić S, Tasić M, Stevanović B (2008). Contribution to biomonitoring of some trace metals by deciduous tree leaves in urban areas. Environmental Monitoring Assessment 137: 393-401. - doi: 10.1007/s10661-007-9775-2

Tomašević M, Anicić M, Jovanović LJ, Peric-Grujić A, Ristić M (2011). Deciduous tree leaves in trace elements biomonitoring: a contribution to methodology. Ecological Indicators 11: 1689-1695. - doi: 10.1016/j.ecolind.2011.04.017

Tong S, Von Schirnding YE, Prapamontol T (2000). Environmental lead exposure: a public health problem of global dimensions. Bulletin of the World Health Organization 78 (9): 1068-1077. [online] URL: http://www.scielosp.org/pdf/bwho/v78n9/v78n9a03.pdf

Ugolini F, Tognetti R, Raschi A, Bacci L (2013). *Quercus ilex* L. as bioaccumulator for heavy metals in urban areas: effectiveness of leaf washing with distilled water and considerations on the trees distance from traffic. Urban Forestry and Urban Greening 12: 576-584. - doi: 10.1016/j.ufug.2013.05.007

White PJ (2023). Chapter 3 - Long-distance transport in the xylem and phloem. In: "Marschner's Mineral Nutrition of Plants" (4th edn). Academic Press, S. Diego, USA, pp. 49-70. - doi: 10.1016/B978-0-12-384905-2.00003-0

WHO (2023). Climate change. World Health Organisation, UN, New York, USA, web site. [online] URL: http://www.who.int/news-room/fact-sheets/detail/climate-change-and-health

Yilmaz R, Sakcali S, Yarci C, Aksoy A, Ozturk M (2006). Use of *Aesculus hippocastanum* L. as a biomonitor of heavy metal pollution. Pakistan Journal of Botany 38 (5): 1519-1527.

Zheng J, Hou R, Tang X, Xu Z, Huang Z, Wang Z, Zhang W, Yang C, Li T (2023). A comparative study of air pollution tolerance capabilities of four tree species in Xi'an City, China. International Journal of Environmental Science and Technology 21: 665-674. - doi: 10.1007/s13762-023-04970-1