

Heavy Metals Content in Foliar Litter and Branches of *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Observed at Two ICP Forests Monitoring Plots

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ABSTRACT

Background and Purpose: Due to the ability to uptake and accumulate heavy metals (HM) in their aboveground tissues, trees may be used for phytoremediation purposes, but also as bioindicators of environmental pollution. The aims of the present study were: a) to investigate the content and temporal variation of the studied HMs in different plant organs during the period of intensive leaf falling (September-October), in two species from genus *Quercus*; b) to evaluate the observed HMs content relative to plausible ranges of element concentration in foliar litter, as recommended by ICP Forests Foliar Co-ordinating Centre (IFFCC).

Material and Methods: The contents of zinc (Zn), manganese (Mn), copper (Cu) and iron (Fe) were assessed in the litterfall (i.e. leaves and branches) of *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. grown at two ICP Forests Level II monitoring plots (Fruška Gora and Odžaci, Serbia). Plant material was sampled during September-October 2018 and analyzed by atomic absorption spectrophotometry.

Results: Comparing the content of investigated HMs in foliar litter and branches of *Q. robur* and *Q. petraea*, notably higher concentrations of Mn and Fe have been observed in the foliage of both species, whereas Zn and Cu concentrations were higher in collected branch material of both of the above-mentioned tree species. The results further showed that, when compared to plausible ranges of element concentrations in foliar litter, given by ICP Forests Foliar Co-ordinating Centre (IFFCC), average concentrations of Fe and Zn in the leaves were within suggested limits, whereas certain concentrations of Mn and Cu exceeded the proposed ranges. Furthermore, the content of the studied HMs in plant material of both *Quercus* species significantly varied during sampling period.

Conclusions: Although it was observed that concentrations of certain HMs were beyond plausible limits recommended by IFFCC, our results were in accordance with the findings of other authors who studied HMs content in *Quercus* species grown in natural forests. For that reason, these limits should be considered optionally, i.e. in the cases when the results significantly deviate from the suggested values. Moreover, we believe that the observed variation in the HMs content during sampling period is related to the sampling procedure commonly applied at ICP Forests monitoring plots, which is indirectly associated with the capacity of trees to accumulate HMs and their phenological properties.

Keywords: *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., heavy metals content, litterfall

INTRODUCTION

Recent studies have evidenced that trees can be efficiently used as bioindicators of heavy metals (HMs) contamination in diverse environments, such as urban areas, mining sites,

natural forests, etc. [1-4]. Trees are able to uptake HMs throughout their root and leaves and accumulate it in various organs over time [5]. However, due to different uptake and accumulation paths of different HMs in trees, identification of the principal source of accumulated elements is rather

difficult [6]. For that reason, certain authors believe that for the purpose of air pollution monitoring, lower plants (e.g. mosses and lichens) are more suitable [7, 8]. Nevertheless, using trees for such purpose has a number of advantages. For example, unlike moss and lichens, a clear separation/distinction among different parts (e.g. leaves, branches, fruit, etc.) is possible in plant species [9]. Leaves usually have large surface area, thus contributing to the removal of airborne inorganic and organic pollutants, which might be either sedimented on the leaf blade or translocated into the leaf [10]. Likewise, as long-lived organisms, trees can reflect the cumulative effect of HMs polluting elements from both soil and ambient air [11]. Finally, the same tree species are widely distributed throughout many countries, thus providing the opportunity for establishing international monitoring networks, such as International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests), which enables continent-wide comparison and modeling of data across Europe [12].

Functioning of forest ecosystems largely depends on soil nutrient status, which is, in turn, highly correlated with litterfall amount and its chemical composition, as well as the vicinity of different pollution sources (e.g. highways, mines, etc.). Litterfall has a key role in the global biogeochemical cycles, being an important source of elements (including HMs) and organic matter for soils [13]. Besides macro elements in litterfall, forest production is greatly influenced also by the quantity of micro-components, among which HMs contribute to a large extent [14]. For example, Fe, Mn, Cu and Zn have been described as essential elements for physiological processes in trees [15]. However, although being reported as important micronutrients for plant growth and development regulations, these elements might be also very toxic in high concentrations [16]. Likewise, excessive amounts of HMs in litterfall can reduce the rate of litter decomposition, thus disturbing biological processes in the soil [17]. For these reasons, one of the principal activities within ICP Forests programme is sampling and analysis of litterfall, due to the importance of litter biomass and its chemical content in cycling processes of elements and organic matter along the forest canopy to the soil and vice versa [13].

In the present paper we studied the content and temporal variation of four HMs (Mn, Fe, Cu and Zn) in litterfall fractions (i.e. leaves and branches) of *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L., grown at two ICP Forests monitoring plots, in Serbia. The mentioned oak species are among the most important tree species in Serbia, both from economic and ecological point of view. According to National Forest Inventory of the Republic of Serbia [18], *Q. petraea* accounts for 5.9% of volume and 6.1% of volume increment, being the third most abundant tree species in Serbia (after *Fagus sylvatica* and *Quercus cerris*). On the other hand, although it

covers only 2.5% of volume and 1.7% of increment, *Q. robur* is economically the most valuable tree species in Serbia [19]. The aims of the study were: 1) to investigate the pattern of HMs accumulation in different plant organs, in two species from genus *Quercus*, 2) to determine the content of HMs in relation to plausible ranges of element concentration in foliar litter, given by ICP Forests Foliar Co-ordinating Centre [13], and 3) to study temporal variation in the concentrations of studied HMs during the period of leaf falling (September-October).

MATERIAL AND METHODS

Plant material was collected from *Q. petraea* and *Q. robur* grown at two ICP Forests Level II monitoring plots situated in Serbia, respectively. The first plot was established in 2009 at Fruška Gora Mountain, in a mixed forest of *Quercus petraea*, *Fagus sylvatica* and *Tilia platyphyllos*, whereas the second plot was founded in 2011, in a pure forest of *Q. robur* on meadow-black soil. Both plots have been founded and maintained by the University of Novi Sad, Institute of Lowland Forestry and Environment (Novi Sad, Serbia). More information about monitoring plots are given in Table 1.

According to ICP Forests manuals [13], 20 fixed litterfall traps have been evenly distributed across the plot to represent the whole area and allow the collection of all tree species plant material. A top height of litterfall traps is approx. 1 m, while the catching area (i.e. the area of trap upper frame) amounts to 0.25 m². Canopy leaves and other litterfall inputs were collected in nylon nets, 0.5 m deep, which were attached to trap frames.

Sampling of plant material was performed five times at Fruška Gora and seven times at Odžaci plot during September and October 2018. At both sites, litterfall was collected from each trap (20 per single plot) and pooled in each sampling period, respectively. After collection, plant material was transported to the laboratory and temporarily stored at 4°C [13]. Prior to chemical analyses any litter collected was sorted out into foliar and non-foliar fractions (i.e. branches and fruit) and dried at 70°C for 24 hours [13].

Determination of selected microelements (Zn, Mn, Cu and Fe) was done in digested solutions that were made from powdered litterfall samples using standard method of microwave assisted digestion (D series; Milestone, Bergamo, Italy), while the quantification of metals was performed by Atomic Absorption Spectrophotometer in a flame regimen (model FS AAS240/GTA120, Varian – AAS, California, US). Approximately 0.3 g of oven-dried material were ground and homogenized in a laboratory mill and then digested in 10 ml of nitric acid and 2 ml 30% (w/v) hydrogen peroxide using a microwave-assisted digestion system (D series; Milestone,

TABLE 1. Description of ICP Forests monitoring plots Fruška Gora and Odžaci.

Locality	Latitude (Deg.Min)	Longitude (Deg.Min)	Altitude (m a.s.l.)	Soil type	Mean air temperature (°C)	Annual sum of precipitations (mm)
Fruška Gora	45°09'	19°48'	490	Cambisol	11.2	795
Odžaci	45°27'	10°10'	86	Meadow-black	10.9	577

Bergamo, Italy) for 45 min at 180°C (900 W) [20]. Filtrates were then diluted to 25 ml with deionizer water. Pre-treated samples were processed by AAS using the acetylene/air burner flame technique (with an atomization temperature of about 2300°C). Concentrations of Zn, Mn, Cu and Fe were determined by using single element hollow-cathode lamps at 213.9 nm (Zn), 324.8 nm (Cu), 248.3 nm (Fe) and 279.5 nm (Mn) respectively, and expressed in $\mu\text{g g}^{-1}$ dry weight (d.w.) of plant material.

The data were processed in Statistica 13 software [21], using Analyses of Variance (ANOVA) and methods of descriptive statistics (mean value and standard deviation). Significant differences in concentration of HMs in the leaves and branches of two oak species were determined at $p < 0.05$.

RESULTS

Descriptive statistics of HMs concentrations in *Q. petraea* and *Q. robur* litterfall from ICP Forests monitoring plots at Fruška Gora and Odžaci are reported in Table 2. Notably higher

concentrations of Mn and Fe have been detected in leaves than in branches of *Q. petraea* and *Q. robur*, respectively. On the other hand, the content of Zn and Cu was higher in collected branch of the above-mentioned tree species.

The ratio between the content of four HMs in leaves and branches, based on the average concentration during the sampling period, is presented in Figure 1. The highest ratio in the leaves of both species was reached by Fe (*Q. petraea* = 74.2%; *Q. robur* = 74.3%), which was followed by Mn (*Q. petraea* = 71.8%; *Q. robur* = 66.7%). In contrast, slightly higher ratios of Cu (*Q. petraea* = 52.1%; *Q. robur* = 54.3%) and Zn (*Q. petraea* = 64.1%; *Q. robur* = 56.8%) were calculated for branches of the studied tree species.

The results showed that the average detected amount of Mn in the leaves and branches of *Q. petraea* was $788.6 \mu\text{g g}^{-1}$ (ranging between 630.7 and $976.8 \mu\text{g g}^{-1}$) and $309.9 \mu\text{g g}^{-1}$ (229.4–408.0 $\mu\text{g g}^{-1}$), respectively (Figure 2). In *Q. robur*, average concentrations of Mn were found to be $602.9 \mu\text{g g}^{-1}$ (455.1–753.3 $\mu\text{g g}^{-1}$) and $301.4 \mu\text{g g}^{-1}$ (137.2–460.3 $\mu\text{g g}^{-1}$) in the leaves and branches, respectively (Figure 3).

TABLE 2. Average concentration of heavy metals (mean value \pm standard deviation) in litterfall of *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. observed on two ICP Forests monitoring plots.

Heavy metal concentration ($\mu\text{g g}^{-1}$)	<i>Quercus petraea</i> (Matt.) Liebl.			<i>Quercus robur</i> L.		
	Leaves	Branches	F-test	Leaves	Branches	F-test
Mn	788.6 \pm 141.5	309.9 \pm 72.9	45.2***	602.9 \pm 110.4	301.4 \pm 116.2	24.8***
Fe	165.2 \pm 24.5	57.4 \pm 21.0	55.7***	145.3 \pm 17.5	50.2 \pm 17.5	103.6***
Cu	8.6 \pm 0.7	9.4 \pm 2.1	0.61 ^{ns}	10.1 \pm 1.4	12.0 \pm 2.1	4.1 ^{ns}
Zn	14.0 \pm 1.7	24.4 \pm 8.8	7.2*	18.9 \pm 2.9	24.8 \pm 3.1	13.9**

* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; ns – non-significant

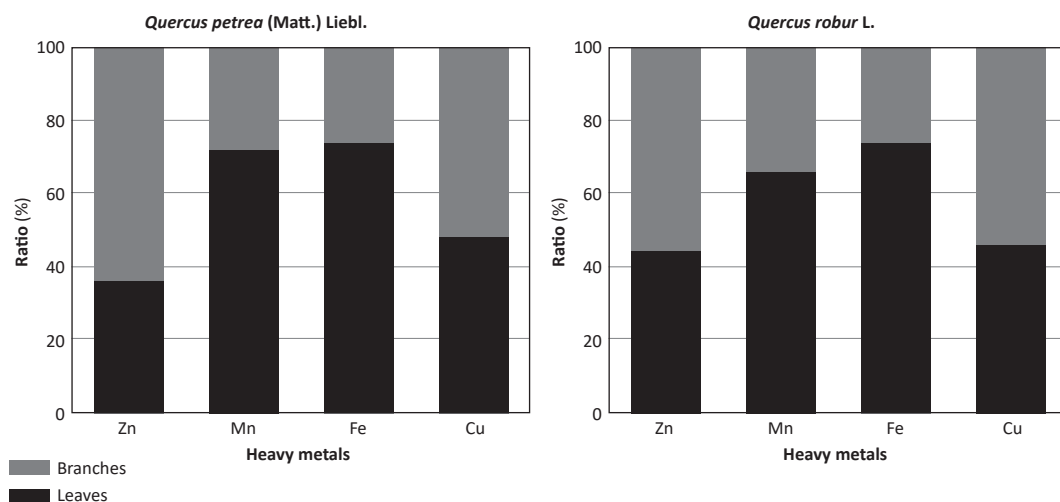


FIGURE 1. The ratio between the content of four heavy metals (Zn, Mn, Fe and Cu) in the leaves and branches, based on the average concentration ($\mu\text{g g}^{-1}$) during the sampling period.

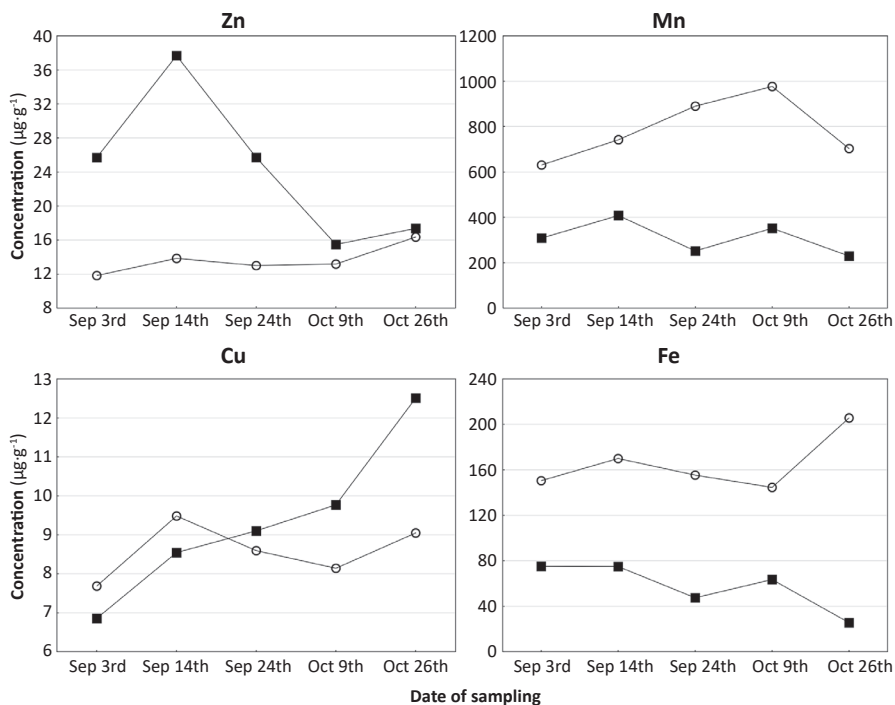


FIGURE 2. Temporal variation of heavy metals (Zn, Mn, Fe and Cu) content in the leaves (□) and branches (■) of *Quercus petraea* (Matt.) Liebl.

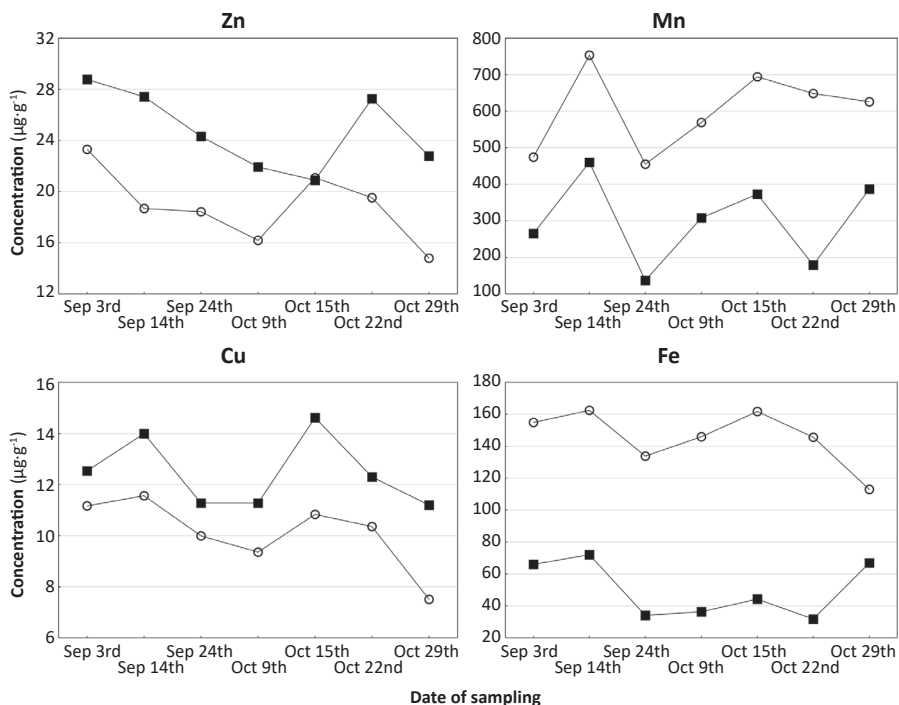


FIGURE 3. Temporal variation of heavy metals (Zn, Mn, Fe and Cu) content in the leaves (□) and branches (■) of *Quercus robur* L.

Average detected concentration of Fe in *Q. petraea* leaves was $165.2 \mu\text{g}\cdot\text{g}^{-1}$, ranging between 144.5 and $205.6 \mu\text{g}\cdot\text{g}^{-1}$, while its concentration in branch material was $57.4 \mu\text{g}\cdot\text{g}^{-1}$ and varied between 25.7 and $75.2 \mu\text{g}\cdot\text{g}^{-1}$. Concerning *Q. robur*, the observed concentration of Fe in the leaves and branches was a bit lower than it was recorded in sessile oak and amounted to $145.3 \mu\text{g}\cdot\text{g}^{-1}$ (ranging between 112.9 and $162.5 \mu\text{g}\cdot\text{g}^{-1}$) and $50.2 \mu\text{g}\cdot\text{g}^{-1}$ (31.8 and $72.0 \mu\text{g}\cdot\text{g}^{-1}$), respectively.

In contrast to Mn and Fe, slightly higher content of Cu was observed in the branch material of both species. In *Q. petraea* average content of Cu amounted to $9.4 \mu\text{g}\cdot\text{g}^{-1}$ (ranging between 6.9 and $12.5 \mu\text{g}\cdot\text{g}^{-1}$) and $8.6 \mu\text{g}\cdot\text{g}^{-1}$ (7.7 – $9.0 \mu\text{g}\cdot\text{g}^{-1}$), in the branches and leaves, respectively. Similar values for Cu content have been also recorded in *Q. robur* branches ($12.0 \mu\text{g}\cdot\text{g}^{-1}$; ranging between 8.3 – $14.6 \mu\text{g}\cdot\text{g}^{-1}$) and leaves ($10.1 \mu\text{g}\cdot\text{g}^{-1}$; ranging between 7.5 – $11.6 \mu\text{g}\cdot\text{g}^{-1}$).

Average content of Zn was almost the same in the branches of both tree species; i.e. $24.4 \mu\text{g}\cdot\text{g}^{-1}$ (17.4 – $37.7 \mu\text{g}\cdot\text{g}^{-1}$) in *Q. petraea*, and $24.8 \mu\text{g}\cdot\text{g}^{-1}$ (21.9 – $28.8 \mu\text{g}\cdot\text{g}^{-1}$) in *Q. robur*. Finally, Zn content in the leaf material of *Q. petraea* and *Q. robur* amounted to $13.6 \mu\text{g}\cdot\text{g}^{-1}$ (11.8 – $16.4 \mu\text{g}\cdot\text{g}^{-1}$) and $18.9 \mu\text{g}\cdot\text{g}^{-1}$ (14.8 – $23.3 \mu\text{g}\cdot\text{g}^{-1}$), respectively.

DISCUSSION

Previous studies demonstrated that HMs content within *Quercus* genus is species- and organ-dependent [22, 23], although environmental conditions [5] and tree lifetime have certain effects on HMs accumulation as well [24]. Since HMs content in two oak species occurring at separated sites and, therefore, growing under different environmental conditions was examined, direct comparison of absolute values of HMs content in their organs was not possible. For that reason, the ratio was used to evaluate accumulation pattern of different HMs in leaves and branches. Similar approach was applied by Wegiel *et al.* [25] for studying of HMs accumulation in Scots pine stands of different densities. It was found that HMs content ratio in the leaves and branches of *Q. petraea* and *Q. robur* was similar in both species. Concerning Mn and Fe notably higher concentrations, and therefore higher ratios of these elements, were recorded in the foliage. Considering the time frame of sample collection (i.e. end of the growing season), the results obtained in this study are in accordance with the findings of Santa Regina *et al.* [24]. Namely, the authors continually reported seasonal accumulation of these two elements in the leaves of *Quercus pyrenaica*, whereas only slight increase in accumulation, but not statistically significant, was observed to have occurred in the branches. In contrast, the ratios of Zn and Cu were higher in branch material of the studied oak species. Similar results have been reported by Serbula *et al.* [4], who found slightly higher concentrations of Zn and Cu in branches than in leaves of *Robinia pseudoacacia* trees growing on unpolluted area.

Considering plausible range of element concentrations in foliar litter, given by ICP Forests Foliar Co-ordinating Centre [13], our results evidenced that concentrations of Fe and Zn in the studied tree species leaf material were within suggested limits. According to Pitman *et al.* [13]

foliar concentration of Fe should range between 50 and $200 \mu\text{g}\cdot\text{g}^{-1}$ in *Q. petraea*, and 90 – $150 \mu\text{g}\cdot\text{g}^{-1}$ in *Q. robur*, which is also confirmed by the results of other authors (e.g. [26–28]). Unlike these findings, Aboal *et al.* [29] and Santamaría and Martín [30] recorded Fe concentration outside the given ranges. In case of Zn, recommended foliage content should vary between 14 and $25 \mu\text{g}\cdot\text{g}^{-1}$ in *Q. petraea*, and 15 – $25 \mu\text{g}\cdot\text{g}^{-1}$ in *Q. robur*. Similar values have been reported by other authors [30, 31], although certain authors reported the content of Zn in the leaves of studied oak species that was beyond these limits [26, 27, 29].

In contrast to Fe and Zn, certain concentrations of Mn and Cu were outside of plausible limits recommended by Pitman *et al.* [13]. Namely, while the previously mentioned authors stated that concentration of Mn in the leaf material should vary between 700 and $1700 \mu\text{g}\cdot\text{g}^{-1}$ for *Q. petraea*, and 1000 – $1200 \mu\text{g}\cdot\text{g}^{-1}$ for *Q. robur*, our results showed that average concentration of Mn in the leaves of the latter species was much lower ($602.9 \mu\text{g}\cdot\text{g}^{-1}$). Lower values were also reported by other authors: i.e. Kovács *et al.* [26] and Santamaría and Martín [30] reported that average foliar concentration of Mn in *Q. robur* amounted to 817 and $821 \mu\text{g}\cdot\text{g}^{-1}$, respectively. Likewise, studying HMs content in the leaves of *Q. robur* trees grown across rural areas of Galicia, Aboal *et al.* [29] evidenced that average concentration of Mn was approximately $305 \mu\text{g}\cdot\text{g}^{-1}$. Therefore, it is likely that upper and lower limits should be considered only conditionally. For example, Stanković *et al.* [32] showed that concentration of Mn in the leaves of *Q. petraea* varied over a broad range (434.5 – $722.0 \mu\text{g}\cdot\text{g}^{-1}$), depending on properties of the growing site. Intriguingly, De Visser [22] observed various foliage concentration of Mn in three *Q. robur* populations (i.e. 180 , 420 and $1180 \mu\text{g}\cdot\text{g}^{-1}$, respectively).

Regarding Cu content in the leaves, slightly higher concentration of this element in case of *Q. petraea* was observed, whereas its concentration in *Q. robur* foliage significantly exceeded the upper limit ($7 \mu\text{g}\cdot\text{g}^{-1}$) given by Pitman *et al.* [13]. Similar results to Pitman *et al.* [13] have been reported by Kovács *et al.* [26] and Aboal *et al.* [29]. On the other hand, Santamaría and Martín [29] have found that average concentration of Cu in *Q. robur* leaves observed across 14 mixed stands in Spain amounted to $9.8 \mu\text{g}\cdot\text{g}^{-1}$.

Our results showed that the content of all HMs in litterfall of studied oak species varied between sampling dates, although sampling was performed in a quite short period of time (Figures 2 and 3). Possible explanation for this phenomenon might be the sampling procedure applied at ICP Forests plots. Namely, according to ICP Forests procedures [13], HMs content in plant fractions is estimated from the bulk samples consisting of litterfall collected from all traps on the plot. As sampled litterfall originates from different oak genotypes spanned randomly across the sampling plot area, the results probably depended on both the ability of different genotypes to accumulate HMs in their tissues and the amount of certain genotypes of litterfall collected in the traps. Indeed, previous studies evidenced that HMs content in the plant material may vary over a broad range even among trees within a single population. For example, studying the concentration of Zn, Mn and Fe in the leaves of *Ulmus laevis* Pall. trees, Devetaković *et al.* [33] reported that their contents greatly varied between different trees grown

on a small and isolated island. Similarly, studying the content of Pb, Mn, Zn, Ni and Fe in different lime genotypes grown across several sites at Fruška Gora Mountain, Šijačić-Nikolić *et al.* [34] concluded that genotype (i.e. genetic architecture of each sampled tree) was the main driver influencing the concentration of the studied HMs in the leaves (i.e. since metal transporters are genetically coded proteins).

In addition to different capacity of genotypes to accumulate HMs in their tissues, the observed differences in HMs content in litterfall during the sampling period may be also the consequence of phenological characteristics of oak trees on the plots. It is well known that plant phenology depends on a number of factors, such as air temperature, soil moisture, day length, activities of herbivores and pollinators, etc. [35]. Giving an overview of spring and autumn phenology in *Q. petraea* and *Q. robur* at current ICP Forests monitoring plots in the six-year period (2011-2016), Pekeč *et al.* [36] reported that leaf fall phases may take between 8 and 29 days, depending on tree species and year of observation. However, phenological stages may also be influenced by genotype properties within single tree species [37]. In the case of *Q. robur* the existence of four varieties occurring along Danube and Sava River (*precox*, *typica*, *tardiflora* and *tardissima*) was even documented, which are distinguished based on spring phenology [38]. In this sense, it might be assumed that phenological phases, and, therefore the intensity of leaf falling in autumn, differ among various trees on the plots, thus contributing to unequal amount of each genotype litterfall in the bulk sample.

CONCLUSIONS

The results of this study showed that concentrations of Mn and Fe were significantly higher in the foliage of both oak species, whereas slightly higher content of Zn and Cu was observed in the collected branch material. Considering plausible range of element concentrations in foliar litter, suggested by ICP Forests Foliar Co-ordinating Centre [13], it was observed that average concentrations of Fe and Zn in the leaves were within suggested limits, whereas certain concentrations of Mn and Cu were beyond these limits. Nevertheless, our results correspond to the findings of other authors who studied HMs content in natural and unpolluted areas, and therefore we believe that these limits should be considered mainly in the cases when the results significantly deviate from the suggested values. Moreover, it was also observed that the content of HMs in the leaves and branches of the studied tree species varied during the sampling period (September – October 2018), probably as the consequence of sampling procedure commonly applied at ICP Forests monitoring plots, as well as biological characteristics of sampled trees (i.e. the capacity to accumulate HMs and phenological features).

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