

Assessment of Heavy Metal Content in Tree Barks: *Picea abies*, *Pinus sylvestris*, and *Pinus nigra*

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Extracts obtained from the bark of woody plants are used for their high content in polyphenolic compounds with various biological activities. Thus, it is important to evaluate the heavy metals in various tree barks, as these pollutants may end up in the final product, affecting its properties and having potential health implications. This study assessed the heavy metal content in the barks of spruce (*Picea abies*) and pine (*Pinus sylvestris* and *Pinus nigra*) collected from different areas in Romania. After collecting the vegetal material from different sites, the bark samples were appropriately processed and analyzed via inductively coupled plasma optical emission spectroscopy (ICP-OES). The results showed that 6 out of 8 tested samples exceeded the normal values of selenium (Se). Arsenic (As) and cadmium (Cd) were not detected in any sample, while other metals (chromium (Cr); lead (Pb); nickel (Ni)) were detected in variable amounts. Moreover, relatively high levels of heavy metals were recorded in a spruce bark sample collected from the seemingly most unpolluted site. Although further studies are needed to determine the effects of these heavy metals on extraction yields and their transfer into the final extracts, their presence might indicate a potential problem.

Keywords: Heavy metals; Pollution; Bark; *Pinus sylvestris*; *Picea abies*; *Pinus nigra*; ICP-OES

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INTRODUCTION

Pollution is one of the main environmental concerns of modern society; it is caused by the fast urbanization rate, industrial development, and other anthropogenic activities, mostly in urban areas (Sawidis *et al.* 2011; Rodríguez Martín *et al.* 2015; Steindor *et al.* 2016; Ozturk *et al.* 2017). The heavy metal contamination of environmental matrices including air, water, and soil is a serious environmental issue (Sawidis *et al.* 2011; Dogan *et al.* 2014; Rodríguez Martín *et al.* 2015; Huber *et al.* 2016; Matin *et al.* 2016; Steindor *et al.* 2016; Vardhan *et al.* 2019; Yousaf *et al.* 2020), directly or indirectly (via accumulation in various foodstuff) affecting human health (Sawidis *et al.* 2011; Rodríguez Martín *et al.* 2015; Matin *et al.* 2016; Edelstein and Ben-Hur 2018; Vardhan *et al.* 2019). After prolonged exposure to high levels of heavy metals, many diseases may occur, such as cancers, renal and gastrointestinal failure, osteoporosis, cardiovascular problems, neurological problems, and endocrine disruption (Zhang *et al.* 2016; Edelstein and Ben-Hur 2018; Vardhan *et al.* 2019). Moreover, heavy metals tend to accumulate in the leaves/needles and barks of various vascular plants, due to their large surface area, which

can become attachment sites for solid particles (Świsłowski *et al.* 2020). Thus, they are used as bioindicators of heavy metal pollution in different areas (Sawidis *et al.* 2011; Ejidike and Onianwa 2015; Steindor *et al.* 2016; Ozturk *et al.* 2017; Rodríguez Martín *et al.* 2018; Świsłowski *et al.* 2020; Yousaf *et al.* 2020).

Recent studies have focused their attention on the biological activities and various health benefits of bark extracts from pine (*Pinus sylvestris*, *Pinus nigra*) and spruce (*Picea abies*) because of their high content in polyphenolic compounds (Nakayama *et al.* 2015; Patel *et al.* 2015; Burčová *et al.* 2018, 2019; Gascón *et al.* 2018; Tanase *et al.* 2018; Mármol *et al.* 2019). For example, the *Pinus nigra* bark extracts exhibited antioxidant, antimicrobial, and antiproliferative effects (Dıġrak *et al.* 1999; Yesil-Celiktas *et al.* 2009), while *Pinus sylvestris* bark extracts were shown to present anti-inflammatory and anti-obesity effects (Karonen *et al.* 2004; Vigo *et al.* 2005; Patel *et al.* 2015). Previous studies have also shown that *Picea abies* bark extracts exhibit antibacterial, antifungal, antioxidant, and antitumoral effects (Salem *et al.* 2016; Coşarcă *et al.* 2019; Metsämuuronen and Sirén 2019). Also, because of the structural characteristics of polyphenolic compounds, they are used as heavy metal chelators (Bianchi *et al.* 2015). Unfortunately, in Romania, these vegetal matrices are still used inefficiently for thermal energy by combustion; however there are emerging studies focusing on the phytochemical characterization of these tree species bark and their valorization potential as medical bioproducts (Talmaciu *et al.* 2015, 2016; Nisca *et al.* 2021).

Due to the tendency of heavy metals to accumulate in the bark of different species, the potential use of these vegetal materials in healthcare products, and the property of polyphenols to form complexes with different heavy metals, these heavy metals may influence the extraction yields from different tree barks or remain in the final product that might have negative effects on human health.

This study assessed the heavy metal (As, Cd, Cr, Ni, Pb, Se) content in the barks of spruce (*Picea abies*) and two different pine species (*Pinus sylvestris* and *Pinus nigra*) from different locations in Romania.

EXPERIMENTAL

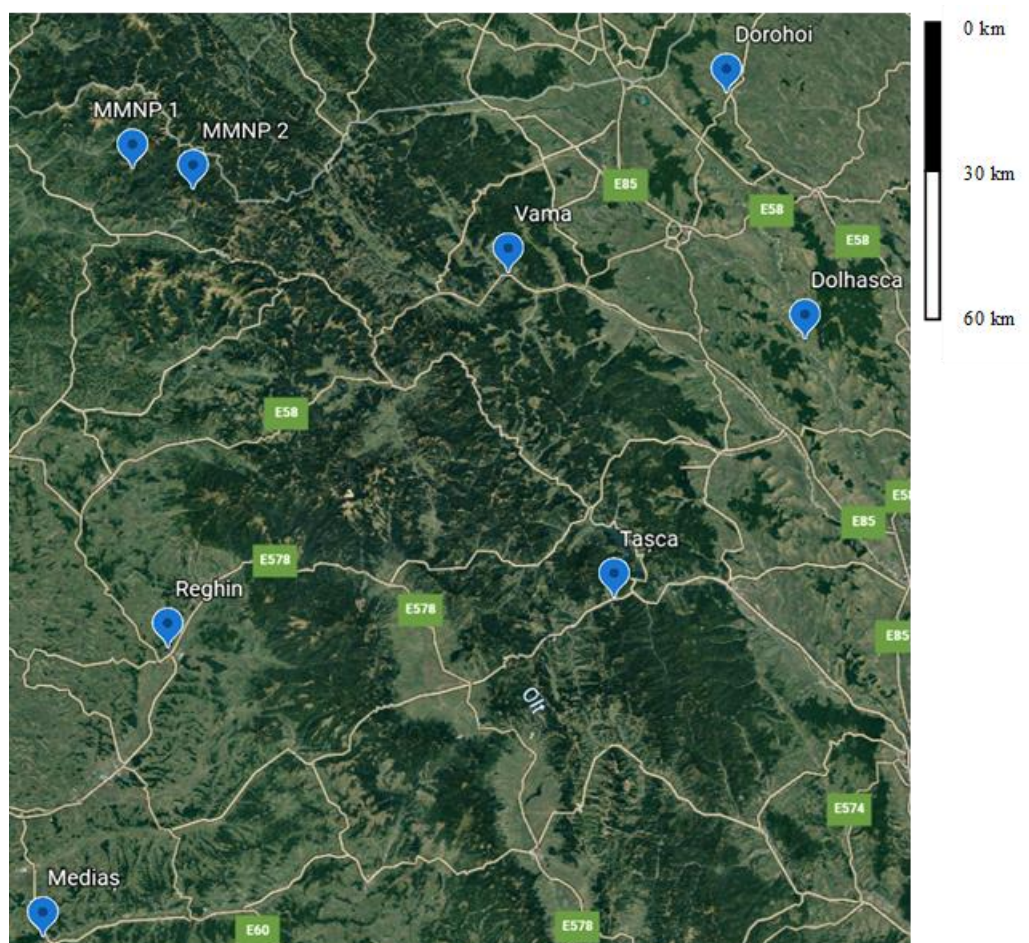
Materials and Sources of Contamination

Bark samples from *Pinus sylvestris*, *Pinus nigra*, and *Picea abies* were collected from different locations in Romania, as presented in Table 1. A map indicating the collection sites is presented in Fig. 1. All of the bark samples were collected from up to 5 randomly selected specimens of each species, from each location, using the itinerary method. These samples were collected at trunk heights ranging between 1 and 1.5 m, from 20 to 35 years old trees. The vegetal material was ground, after the collection and drying processes, with a GRINDOMIX GM 200 mill (Retsch GmbH, Haan, Germany). In the end eight experimental variants resulted (Table 1).

General data about this type of contamination, regarding the whole territory of the county in which the collecting sites are located, were identified. For example, Suceava's county council reported that the contamination of air with specific heavy metals (Pb, As, Cd, Ni) did not exceed the normal limits established by the law, in previous years. The same report suggested that the main sources of contamination are represented by the energy sector (heating of residential and institutional buildings; industrial activity) and car traffic (APM Suceava 2020).

Table 1. Collecting Locations and Experimental Variants

Vegetal material	Plant species	Location	Coordinates	Experimental variants
Bark	<i>Pinus sylvestris</i>	Vama	47°34'19" N, 25°42'55" E	PSV
		Reghin	46°47'04" N, 24°41'20" E	PSR
		Mediaș	46°10'21" N, 24°19'37" E	PSM
	<i>Pinus nigra</i>	Dorohoi	47°56'53" N, 26°23'12" E	PND
	<i>Picea abies</i>	MMNP1/MMNP2	47°47'05" N, 24°34'20" E	M1/M2
		Tășca	46°53'34" N, 26°02'05" E	MT
		Dolhasca	47°25'49" N, 26°36'34" E	MD



MMNP1/MMNP2 - Maramureșului Mountains National Park site 1/2; PSV - *P. sylvestris* bark (Vama); PSR - *P. sylvestris* bark (Reghin); PSM - *P. sylvestris* bark (Mediaș); PND - *P. nigra* bark (Dorohoi); M1 - *P. abies* bark (Maramureșului Mountains National Park site 1); M2 - *P. abies* bark (Maramureșului Mountains National Park site 2); MT - *P. abies* bark (Tășca); MD - *P. abies* bark (Dolhasca)

Fig. 1. Bark sample collecting locations indicated by the blue pins. MMNP1/MMNP2 – Maramureșului Mountains National Park site 1/2

In Reghin, one of the main sources of heavy metals is represented by a fiberboard factory. The facility needs high amounts of energy produced through natural gas combustion, as it produces 2.3 million m³ of different types of fiberboards. The traffic load is also high, considering the population and the fact that three national roads (highly transited roads) intersect in the locality.

Mediaș is a more industrialized locality, compared to Reghin. Also here is located one of the biggest natural gas producers in Romania, Romgaz SA, producing over 5000 million m³ of natural gas per year. The processing of this natural resource may lead to emissions of Pb, Cd, As, Se, and Hg. However, the reports indicate that the air levels of Pb, Cd, As and Ni are lower than the normal levels set by the law, despite the active status of the region (APM Sibiu 2021).

In Dorohoi, the main source of heavy metal pollution should be the combustion of fuels for building heating and the traffic load as 2 national roads cross this locality. There is no heavy industrial activity to be mentioned. However, a glass factory functioned until 2007, which could lead to higher levels of heavy metals, especially Se as it can be used as a glass decolorizer or pigment (Kavлак and Graedel 2013). Also, reports suggest that atmospheric levels of Pb, Cd, As, and Ni are much lower than the limit values (APM Botoșani 2014; CJ Botoșani 2018).

Tășca is a small locality with a small population. A potential source of heavy metal contamination is the active cement factory, which produces 3 million t of cement every year, using alternative fuels such as used tires and wastes from forestry, industry, and domestic sources. The combustion of these materials could lead to the heavy metal contamination of the area if not managed correctly (Hasselriis and Licata 1996; Nzihou and Stanmore 2013). The website of the company reports that measurements of the heavy metal emissions are regularly performed and the levels emitted are under the limits, but no specific data are shown.

As for the collecting sites from Maramureșului Mountains National Park, the found reports were not considered relevant, as the facility that measured the parameters of the air quality is located further away and near a highly populated locality. Minimum anthropogenic activity is conducted in this area.

Methods

Plant sample digestion

To perform the analysis, 1 g of each sample was treated with 7 mL of HNO₃ and 21 mL of HCl as extraction agents. Once the mixture was ready after 24 h under a fume hood, the sample was placed in a sand bath to boil for the removal of vapors and internal gases. Then, the sample was cooled and transferred in a flask with 100 mL of pure water, having a conductivity less than approximately 18.2 μS/m. At the same time, a blank sample was used in the same way to follow the real traceability of the heavy metals from spruce (*Picea abies*) and pine (*Pinus sylvestris* and *Pinus nigra*) barks.

ICP-OES analysis

For the determination of heavy metals, the ICP-OES model PlasmaQuant PQ 9000 Elite (Analytik Jena GmbH, Jena, Germany) was used. Based on the type of material and its nature, the recommended method for extraction/ mineralization is the European Standard EN 13650:2001 (British Standards Institution 2001). This European Standard specifies a method for the routine extraction of aqua regia soluble elements from soil improvers or growing media. Materials containing more than about 28% (m/m) organic

matter will require treatment with additional nitric acid. With high solute concentrations in extract solutions, spectral interferences and background enhancement should be expected. According to the standard above, the extraction method to apply to this kind of material should be done in aqua regia (HNO₃ and HCl with 1:3 molar ratio).

To calibrate the ICP-OES, certified reference materials such as ICP multi-element Standard VIII and Standard IV (Merck KGaA, Darmstadt, Germany) were used. The first calibration step consisted of the dilution of the multi-elemental standard solution (ICP multi-element Standard IV) from 1000 ppm (concentration of each element) to concentrations of 0.5, 1, 10, and 20 ppm. The dilution was performed in a 1% (v/v) HNO₃ solution. The verification step was performed by diluting the ICP multi-element Standard VIII from 1000 ppm to a 1 ppm solution. All the measured values were included in the ICP sensitivity interval (0.9 to 1.10 ppm). Both reference materials were measured applying high precision ICP-OES and were directly traceable to the corresponding NIST Standard Reference Materials®. As for the actual analysis, the following operation conditions were used: 1200 W RF Power, gas flow of 15 L/min, auxiliary gas flow of 1 L/min, and nebulizers gas flow of 0.5 L/min. The sample pump flow intake rate was set at 1 mL/min. Four points of background correction and three replications were performed for each bark sample to measure the analytical signal. The limits of detection (LODs) and limits of quantitation (LOQs) for each metal are presented in Table 2.

Table 2. LODs and LOQs of the Analyzed Heavy Metals with the Described Method

Heavy Metal	LOD (ppm)	LOQ (ppm)
As	0.002936	0.0088
Cd	0.000066	0.0002
Cr	0.000265	0.0008
Ni	0.000174	0.0005
Pb	0.00001001	0.0030
Se	0.002700	0.0081

LOD – Limit of Detection; LOQ – Limit of quantitation

Statistical analysis

To assess the statistical significance of the differences between the heavy metal contents of the bark samples, a one-way ANOVA test was used to compare the variances of the analyzed data series. If a significant difference between the variances occurred, then a *post hoc* (Tukey) test was used to evaluate which variants differed significantly (significance level $\alpha = 0.05$, $p \leq 0.05$). The PAST statistical software was used to perform these statistical tests.

RESULTS AND DISCUSSION

The heavy metal analysis of the spruce and pine barks indicated that As and Cd levels were under the method's LODs in all of the experimental variants. Also, measurable Pb (1.001 ± 0.055 mg/kg bark) and Ni (1.077 ± 0.018 mg/kg bark) levels were found only in the PSM variant among all the pine bark variants. Lead (1.159 ± 0.043 mg/kg bark) was also found only in the MD variant among all the spruce bark experimental variants. The normal levels of the analyzed heavy metals in plants used to compare our results were:

0.0006 to 18 mg/kg for Cr, 0.1 to 3.7 mg/kg for Ni, less than 3 mg/kg for Pb, and 0.002 to 0.08 mg/kg for Se (Hajar *et al.* 2014).

Comparing the pine bark samples, the highest content of Cr was identified in the PSM variant (7.302 ± 0.038 mg/kg bark), a significantly higher value compared to the PSV and PSR experimental variants. In the *P. nigra* sample (PND) it could not be detected. In contrast, the Se content among the pine bark samples was the highest in the PND variant (2.35 ± 0.113 mg/kg bark) and the lowest in the PSM variant (1.928 ± 0.090 mg/kg bark). The differences between PND and the other experimental variants were significant. In the PSV variant, Se could not be detected. The results of the pine bark heavy metal content analysis are illustrated in Fig. 2.

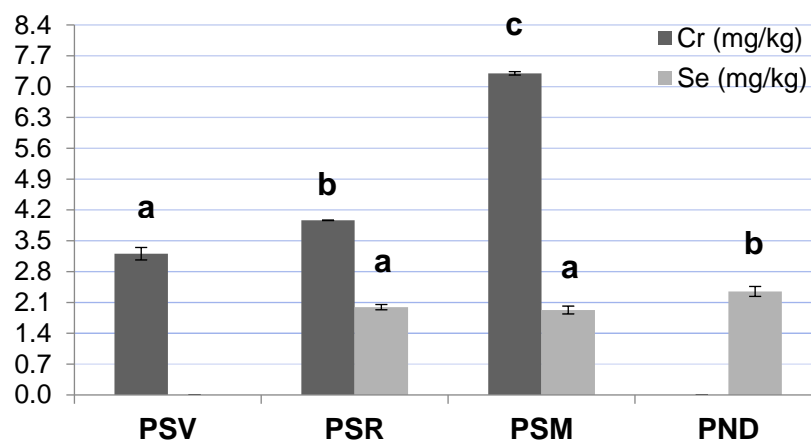


Fig. 2. Cr and Se contents of pine bark samples. Results were expressed as mean \pm standard deviation ($n = 3$). Different letters indicate significant differences (significance level $\alpha = 0.05$). PSV - *P. sylvestris* bark (Vama); PSR - *P. sylvestris* bark (Reghin); PSM - *P. sylvestris* bark (Mediaș); PND - *P. nigra* bark (Dorohoi)

Compared to the normal levels of heavy metals in plants presented in the literature, only the Se content of all the pine bark samples was higher than normal, except for PSV, in which Se was not detected. The other metals' content was lower than the upper limit of the normal range. However, PSM having the highest Cr content, a higher than normal Se content, and being the only pine bark sample to comprise Pb and Ni, might be the result of the pollution caused by the intense industrial activity in the Mediaș area. Also, PND having the highest Se content might be explained by the activity of the glass factory in Dorohoi, Se being used as a glass decolorizer or pigment (Kavlak and Graedel 2013).

In a study conducted on *P. brutia* bark samples collected from different regions of Turkey, lower levels of Cr were recorded (between 0.819 ± 0.011 mg/kg and 0.907 ± 0.013 mg/kg) compared to our *P. sylvestris* bark samples (between 3.206 ± 0.142 mg/kg and 7.302 ± 0.038 mg/kg), but higher compared to the *P. nigra* sample (Cr not detected). However, in the same study Cd was detected in the samples (between 1.526 ± 0.012 mg/kg and 1.639 ± 0.015 mg/kg), and also higher contents of Pb were recorded (between 13.848 ± 0.159 mg/kg and 14.950 ± 0.167 mg/kg) compared to our results (Yalcin *et al.* 2020). Parzych *et al.* recorded higher contents of Ni in *P. sylvestris* (90.6 ± 2 mg/kg) and *P. nigra* (80.5 ± 2 mg/kg) bark samples and also detected Cd in these samples (1.2 ± 0.7 mg/kg for *P. nigra* and 2.1 ± 1.6 mg/kg for *P. sylvestris*) (Parzych *et al.* 2017). Sut-Lohmann *et al.* tested *P. sylvestris* bark samples from different locations and registered a series of heavy

metal ranges: between 1.0 ± 0.4 mg/kg and 1.9 ± 0.7 mg/kg for Ni; between 0.3 ± 0.1 mg/kg and 1.4 ± 0.6 mg/kg for Cd and between 2.9 ± 0.8 mg/kg and 4.0 ± 1.2 mg/kg for Cr (Sut-Lohmann *et al.* 2020). All these results show that the heavy metal contents' vary depending on the collecting location.

Regarding the spruce bark samples, in Fig. 3 can be observed that in the MT variant only Cr was detected, but its level was significantly lower compared to the other spruce bark samples. The highest Cr content was identified in the MD variant (9.188 ± 0.033 mg/kg bark), being significantly higher than all the other samples. The Ni level was significantly higher in the M2 variant (3.736 ± 0.025 mg/kg bark), while the Se level was the highest in the MD variant (1.974 ± 0.057 mg/kg bark) but the differences between the samples were not significant.

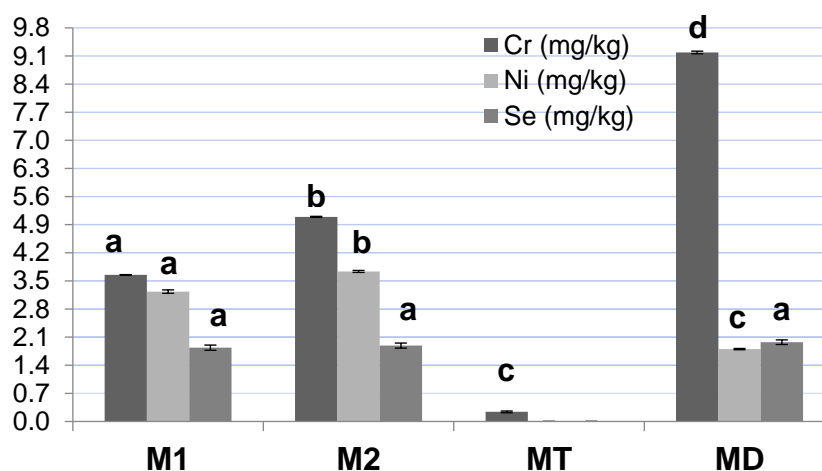


Fig. 3. Cr, Ni, and Se content of spruce bark samples. Results were expressed as mean \pm standard deviation ($n = 3$). Different letters indicate significant differences (significance level $\alpha = 0.05$). M1 - *P. abies* bark (Maramureşului Mountains National Park site 1); M2 - *P. abies* bark (Maramureşului Mountains National Park site 2); MT - *P. abies* bark (Taşca); MD - *P. abies* bark (Dolhasca)

As in the case of the pine bark samples, all of the spruce bark samples exceeded the normal level of Se in plants, except the MT variant in which Se was not detected. Additionally, a slightly higher than normal level of Ni was recorded in the M2 variant, this result being surprising, considering the absence of any major pollution source nearby. Moreover, considering that Dolhasca seems to be the least polluted collecting location out of all the four sites, in the MD variant Cr, Ni, Pb, and Se were all detected, having the highest Cr levels and also being the only variant that contained Pb. Moreover, in the spruce bark sample from Taşca was recorded the lowest content of Cr, none of the other tested metals being detected, even though the area has an active cement factory.

Even though the M1 and M2 bark samples were collected from an unpolluted location as well, relatively high concentrations of Cr, Ni, and Se were quantified. Moreover, significant differences were recorded between the levels of Cr and Ni, when comparing these variants, although they were collected in the same general area. Other studies regarding the metal content of spruce were focused on other species, parts of the plant, or tested metals (Turkyilmaz *et al.* 2018; Cetin *et al.* 2020). In one of these studies, the Ni levels of the unwashed *Picea pungens* bark ranged from 8.67 mg/kg to 13.49 mg/kg, thus indicating a higher level of Ni compared to the present results (Cetin *et al.* 2020).

CONCLUSIONS

1. The tested samples did not exceed the normal ranges of heavy metals in plants, excepting the slightly higher level of Ni in the M2 variant and the Se levels of the M1, M2, MD, PSM, PSR, and PND variants.
2. The safest barks to use for the development of future products might be the *P. abies* bark from Taşca and the *P. sylvestris* bark from Vama, containing only Cr in the lowest amounts out of all the other bark samples, but further studies are needed to assess the effect of the bark heavy metal content on the extraction yields and the levels of heavy metals transferred in the final extracts.
3. Spruce bark may not be a good indicator of the heavy metal pollution of the collecting location, relatively high contents of metals being recorded for the bark sample collected in a relatively unpolluted location. Thus, spruce bark might need special attention from the perspective of heavy metals content if used in different bio-products.

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