



Determination of Arsenic Amount in Some Trees that Can Be Used as Biomonitors

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Toxic metals/metalloids such as arsenic are environmental pollutants that are damaging to living organisms. Commonly found metals pose a serious threat to human health unless they are controlled. In this study, the accumulation of As metalloid, which has entered the food chain for specific reasons, in plant organs was determined, and it was examined whether plants are hyperaccumulators against As concentration. As a result, *Pinus pinaster* Aiton., *Cupressus arizonica* Greene., *Picea orientalis* (L.) Peterm., *Cedrus atlantica* (Endl.), and *Pseudotsuga menziesii* (Mirb.) species accumulated more than 1 mg/kg As concentration. The highest average As concentration (7.91 mg/kg) was found in the northern bark of *P. menziesii*. The highest As concentrations in wood were found in the eastern direction for *P. pinaster* (5.21 mg/kg), *C. arizonica* (4.99 mg/kg), and *P. orientalis* (4.02 mg/kg), and in the western direction for *C. atlantica* (3.56 mg/kg) and *P. menziesii* (3.88 mg/kg). Additionally, it was determined that As concentration varied depending on location, direction, species, and year.

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INTRODUCTION

Today, environmental pollution is one of the most critical problems on a global scale. In particular, air pollution has become a global problem that causes more than 9 million people to die yearly (Fuller *et al.* 2022; Worldbank 2024). Heavy metals are the most dangerous components of air pollution, and it is frequently emphasized that they have fatal effects on human and environmental health (Ghoma *et al.* 2023; Istanbulu *et al.* 2023). Therefore, the priority of studies is monitoring and reducing heavy metal pollution in the atmosphere (Savas *et al.* 2021; Özel *et al.* 2024).

Arsenic (As) is a harmful heavy metal, even at very low concentrations. It is usually found in arsenopyrite minerals and can be released into the environment through human activities such as mining, industrial processes, pesticides, and the combustion of fossil fuels (Farooq *et al.* 2016). Arsenic, which can be found everywhere, is toxic to all life forms. It is mainly found in inorganic forms (Arsenite (AsIII) and Arsenate (AsV)) (Tripathi *et al.* 2007). Arsenates and arsenites are found in soil, water, and foods (Jomoya *et al.* 2011). Arsenic exposure is known to cause various types of cancer (Miller *et al.* 2002). It is also known that As in the liver causes cardiovascular diseases (Navas-Acien *et al.* 2005), affects insulin resistance by reducing insulin synthesis and secretion, and causes diseases such as diabetes (Díaz-Villaseñor *et al.* 2007). The effects of long-term exposure are known to

vary. Arsenic metabolites can cause neurological disorders, dermal and lung cancer, and hypertension (Vahidnia *et al.* 2007; Mazumder 2008).

The As concentration in soil ranges between 0.1 and 40 mg/kg. This situation may vary depending on geographical regions (Farooq *et al.* 2016). Arsenic accumulates in edible plant parts after being taken from the soil (Finnegan and Chen 2012). There are hypothetical mechanisms responsible for these pathways. Plants are known to take up AsV using phosphate transporters (Pi) and AsIII with the help of aquaporins (NIP). AsV is reduced to AsIII by arsenate reductase (AR) using glutathione (GSH) as a reductant. Inside the cell, AsIII is complexed with glutathione (GSH) and thiol compounds such as phytochelatins (PCs). It is then known to be retained in the central vacuole *via* the ATP-binding cassette (ABCC transporter). AsV and AsIII are transported to the shoot tissue where a similar sequestration and reduction takes place. AsIII in the seed is loaded by the phloem via INT genes (inositol transporter). Thus, it is also transported to the seeds. However, the mechanisms regarding As uptake, transport, and detoxification into the plant remain unclear (Farooq *et al.* 2016).

Plants that accumulate metal in high concentrations (more than 1000 $\mu\text{g metal g}^{-1}$ dry mass) are called “hyperaccumulator” plants (Visoottiviseth *et al.* 2002). These species include plant groups, including *Pteris vittata* and other members of the Pteridaceae, which over accumulate As (Ellis *et al.* 2006). The growth status of these plants is not compromised even when they accumulate excessive levels of As. It is known that, unlike plants that cannot accumulate metalloids, hyperaccumulators do not limit As accumulation to roots. This situation is shown as a critical aspect of the hyperaccumulation phenotype (Finnegan and Chen 2012).

Woody species with long life forms are extremely useful in monitoring long-term changes in heavy metal pollution in the air and reducing pollution (Key *et al.* 2023; Sulhan *et al.* 2023). In order for these species to be used for biomonitoring and phytoremediation purposes, information is needed regarding their potential to accumulate heavy metals in the wood and the ability of heavy metals to transfer within the wood (Yayla *et al.* 2022; Koc *et al.* 2024). By analysing annual growth rings, researchers can gather essential information about the presence and distribution of heavy metals over time (Chen *et al.* 2021; Cobanoğlu *et al.* 2023). Numerous studies highlight the potential of tree rings in tracing heavy metal contamination (Cuciurean *et al.* 2024; Ozturk Pulatoglu *et al.* 2025). Evidence shows a connection between the levels of certain elements in growth rings and environmental pollution (Key *et al.* 2022; Sevik *et al.* 2024a). However, the way elements move within wood differs by species, emphasizing the need for further research to evaluate long-term As levels in the air-soil-plant system for better risk assessment. Monitoring urban air quality is crucial for assessing atmospheric pollution and its effects. Consequently, selecting the right tree species for detecting specific heavy metals is essential. This study aimed to monitor the As change in the air and determine the most suitable woody species that can be used to reduce pollution. The study was carried out in Düzce, among the most polluted cities in Türkiye, regarding air pollution.

EXPERIMENTAL

The species used within the scope of the study were selected from the species frequently preferred in landscape studies. These types included *Pinus pinaster* Aiton. (Maritime pine), *Cupressus arizonica* Greene (Arizona cypress), *Picea orientalis* (L.)

Peterm., *Cedrus atlantica* (Indl.) Manetti ex Carr. (Atlas cedar), and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) species. These species used in the study were taken in 2022 from Düzce city center, which is among the most polluted cities in Türkiye. Samples were taken from trees as bottom logs, with their coordinates and directions marked on them. Samples were taken using an increment auger, and after the samples were taken, the wound was covered with medicated paste. Thus, the trees were not harmed.

Annual ring age ranges were determined on the samples taken and divided into groups. Samples were taken from each determined age range using a steel-tipped drill. After taking samples from the wood part, samples were taken from the log's inner and outer bark surfaces. The study was conducted in 3 replications. Thus, 10 wood and bark samples from each species (8 wood, 1 outer bark, 1 inner bark) * 4 directions * 3 replicates, 120 samples were analyzed. Since there were 5 species in the study, a total of 600 samples were analyzed.

The samples were placed in glass petri dishes and ground. The samples were ground into sawdust and air-dried in the laboratory for approximately one week. The room-dried samples were kept at 45 ± 2 °C for two weeks using an oven. At the end of 2 weeks, 0.5 g of the dried samples were weighed, and 6 mL of 65% HNO₃ and 2 mL of 30% H₂O₂ were added. The samples were microwaved and set to rise to 200 °C for the first 15 min and to remain at 200 °C for the second 15 min. After the samples were burned, they were placed in a volumetric flask and ultrapure water was added to make up to 50 mL.

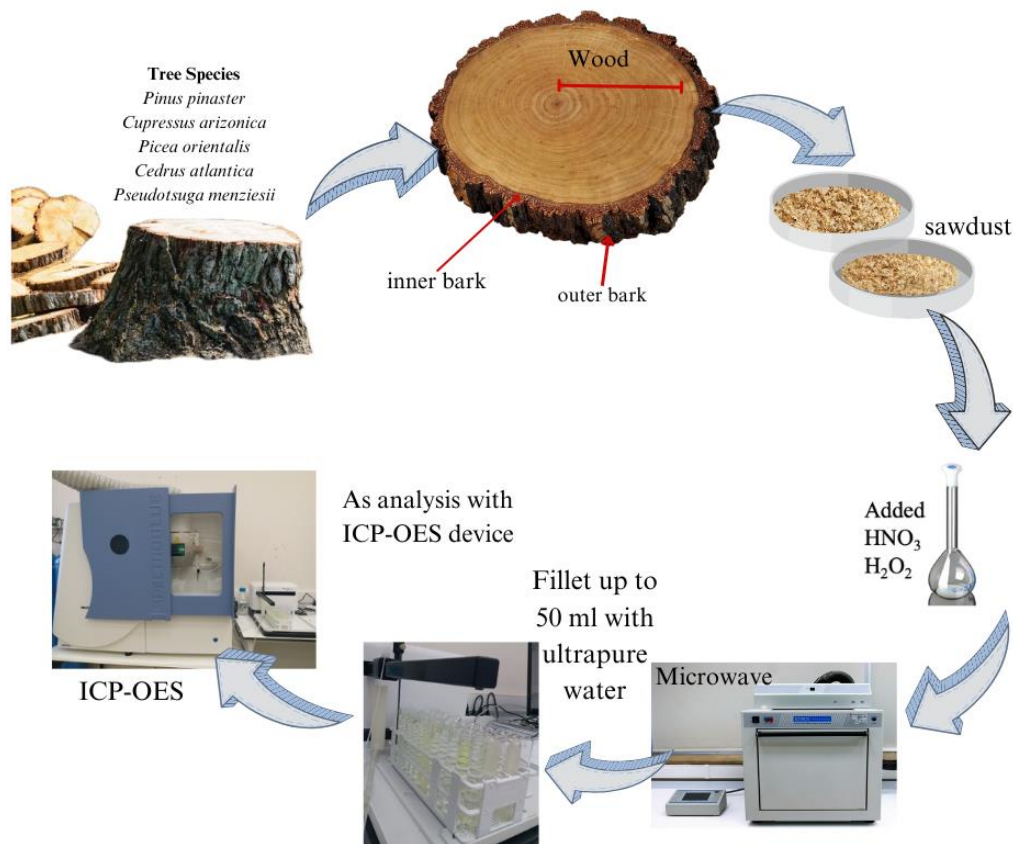


Fig. 1. Method Flow Chart. ICP-OES (Inductively coupled plasma-optical emission spectrometry); As (Arsenic)

The samples were analyzed by using an ICP-OES (Inductively Coupled Plasma-Optic Emission Spectrometer, GBC Scientific Equipment Pty Ltd., Melbourne, Australia) device. The results were calculated by multiplying by 100 (dilution factor) as it was made up to 50 mL by adding pure water (Fig. 1). This method has been frequently used in recent years to determine heavy metal concentrations in wood (Key *et al.* 2022; Guney *et al.* 2023; Erdem *et al.* 2024). Analysis of Variance (ANOVA) was applied to the data obtained as a result of the study using the SPSS (Statistical Package for the Social Sciences Statistics Software 21.0) package program. According to the results of variance analysis, the DUNCAN test was applied to the data with a statistical difference according to $p < 0.05$. Using this analysis, the variation in the concentration of the element As according to plant type, direction, year, and organ was determined and evaluated.

RESULTS AND DISCUSSION

The changes in arsenic (As) concentration in woods depending on direction and year are shown in Table 1. There were statistically significant differences in the directional change of As concentration in all species, except for the southern and northern directions ($p < 0.05$). The change in As concentration in the north and south directions in all years was not significant ($p > 0.05$). Significant differences in As concentration were only in the east and west directions ($p < 0.05$). In general, As concentration decreased in the east direction after 2002. In the western direction, while As concentration was at its lowest levels in 2003 to 2007 compared to other years, it increased again after 2007. In general, when the directions are compared, it is seen that As concentration is higher in the east direction compared to other directions.

Table 1. Change of Arsenic (As) Concentration in Woods by Year and Direction (mg/kg)

Years	North \pm Std. Er.	East \pm Std. Er.	South \pm Std. Er.	West \pm Std. Er.	F Value
2018-2022	3.2 \pm 0.41 a	2.92 \pm 0.59 a	1.83 \pm 0.33	2.96 \pm 1.10 ab	1.808 ns
2013-2017	3.12 \pm 0.39 Aa	3.59 \pm 0.43 Aab	2.03 \pm 0.34 A	3.11 \pm 0.06 Aab	2.920*
2008-2012	3.03 \pm 0.38 Ca	4.69 \pm 0.42 Bbc	1.40 \pm 0.11 A	2.89 \pm 0.02 Bab	14.390***
2003-2007	2.61 \pm 0.47 a	3.03 \pm 0.47 a	1.58 \pm 0.03	2.41 \pm 0.81 a	1.873ns
1998-2002	4.08 \pm 0.40 Ba	5.28 \pm 0.22 Cc	1.49 \pm 0.12 A	1.81 \pm 0.60 Aa	31.384***
1993-1997	3.03 \pm 0.19 ABa	4.15 \pm 0.48 Babc	2.01 \pm 0.35 A	1.97 \pm 0.65 Aa	6.887***
1988-1992	3.72 \pm 0.30 Ba	4.88 \pm 0.77 Bbc	2.01 \pm 0.35 A	5.01 \pm 0.30 Bb	6.722***
1983-1987	3.38 \pm 0.39 Ba	4.12 \pm 0.48 Babc	1.95 \pm 0.38 A	1.80 \pm 0.43 Aa	7.200***
F Value	1.490 ns	2.811 *	0.730 ns	2.598 *	
Ave.	3.27 C	4.19 D	1.81 A	2.59 B	43.3***

Columns show differences between years; Rows show differences between directions. According to Duncan test, letters a, b, and c show statistical differences between years. Capital letters (A, B and C) show differences between directions horizontally. *** Statistically significant at confidence level; $p < 0.001$, * $p < 0.05$. ns: not significant. UL: Below the limit.

When the changes in As concentration depending on organ and direction were examined, no statistically significant difference was found in all organs in the east direction (Table 2). There were statistically significant differences in As concentration in all other aspects. According to the table values, it has been observed that the outer bark and inner bark values are generally at high levels in the north direction. However, when the wood part was examined, the As value was highest in the east direction. The lowest value was in

the south direction. The change in As concentration according to species, organ, direction, and year is shown in the tables below.

Table 2. Change of As Concentration Based on Organ and Direction (mg/kg)

	North \pm Std. Er.	East \pm Std. Er.	South \pm Std. Er.	West \pm Std. Er.	F Value
Outer Bark	5.05 \pm 0.39 Cb	4.55 \pm 0.63 BC	3.33 \pm 0.49 Bc	1.39 \pm 0.22 Aa	15.614***
Inner Bark	4.81 \pm 0.45 Cb	3.94 \pm 0.66 BC	2.54 \pm 0.17 Ab	3.24 \pm 0.52 ABb	5.122**
Wood	3.27 \pm 0.13 Ca	4.19 \pm 0.19 D	1.81 \pm 0.10 Aa	2.60 \pm 0.27 Bb	43.361***
F Value	14.901***	0.224 ns	12.376***	4.427*	
Ave.	3.27 C	4.87 d	1.81 A	2.60 B	43.361***

Columns show differences between organs; Rows show differences between directions. According to Duncan test, letters a, b and c show statistical differences between organs. Capital letters (A, B and C) show differences between directions horizontally. *** Statistically significant at confidence level; $p < 0.001$, * $p < 0.05$. ns: not

The variation of As concentration in the north direction in woods depending on species and year is also shown in Table 3. According to the results of ANOVA (Table 3), there were significant differences at the 99.9% confidence level in the As concentration change in all species according to years. Among the species, it is seen that *C. arizonica* accumulated the highest level of As units as of 2018-2022. In general, the species with the lowest As concentration was *P. orientalis*. However, the species were taken from the same area, 5.51 mg/kg As concentration was detected in *C. arizonica*, while 1.67 mg/kg in *P. pinaster*, 1.75 mg/kg in *P. orientalis*, and 2.77 mg/kg in *C. atlantica*, and 3.90 mg/kg in *P. menziesii* between 2013 and 2017. According to the table values, the responses of different species in the same direction to the metal concentrations they were exposed to in the same years were at different levels. Therefore, we can say that different species facing the same direction accumulate the same element concentration at different levels.

Table 3. Change of As Concentration in Woods in the North Direction by Year and Species (mg/kg)

Years	<i>P. pinaster</i>	<i>C. arizonica</i>	<i>P. orientalis</i>	<i>C. atlantica</i>	<i>P. menziesii</i>	F value
2018-2022	2.86 Cd	6.17 Dg	2.53 Be	2.66 Ba	1.77 Ab	1440.065***
2013-2017	1.67 Abc	5.51 De	1.75 Acd	2.77 Ba	3.90 Cd	1621.285***
2008-2012	1.63 Ab	3.49 Cb	1.65 Abc	5.45 Df	2.89 Bc	693.069***
2003-2007	0.60 Aa	3.70 Cbc	1.63 Bb	5.40 Df	1.60 Ba	1831.039***
1998-2002	3.92 Bf	5.91 Ef	4.12 Cg	5.01 Dd	1.45 Aa	917.496***
1993-1997	3.23 Ce	3.16 Ca	2.91 Bf	4.02 Db	1.83 Ab	136.153***
1988-1992	3.83 BCf	4.01 Cd	1.77 Ad	5.22 De	3.76 Bd	409.903***
1983-1987	1.90 Bc	3.74 Cc	1.46 Aa	4.82 Dc	4.96 De	266.623***
F Value	220.4***	291.4***	643.6***	329.6***	692.3***	
Ave.	2.46 A	4.46 B	2.22 A	4.57 B	2.77 A	23.129***

Columns show differences between years; Rows show differences between species. According to Duncan test, letters a, b and c show statistical differences between years. Capital letters (A, B and C) show differences between species horizontally. *** Statistically significant at confidence level***; $p < 0.001$, * $p < 0.05$. ns: not significant. UL: Under limit.

The variation of As concentration in woods in the south direction depending on species and year is also shown in Table 4. According to the results of the ANOVA performed on a species basis in the wood samples taken from the south, there were significant differences in the change of As concentration depending on the years ($p < 0.001$). However, the As concentration in *P. pinaster* was below the limit between 2003 and 2008. Except for *P. pinaster*, it was observed that samples taken from the south of other species accumulated up to 4.5 mg/kg values at least once between 1983 and 2022 and even exceeded this value in some years. However, the periods in which each plant species accumulates maximum As concentrations vary.

Table 4. Variation of As Concentration in Woods in the South Direction based on Year and Species (mg/kg)

Years	<i>P. pinaster</i>	<i>C. arizonica</i>	<i>P. orientalis</i>	<i>C. atlantica</i>	<i>P. menziesii</i>	F Value
2018-2022	1.10 Ab	1.43 Ba	4.27 Ce	1.43 Bb	0.93 Aa	593.417***
2013-2017	0.67 Aa	4.47 Ec	1.86 Dd	1.51 Bb	1.74 Cc	3337.308***
2008-2012	UL	1.68 Bb	1.59 Bc	0.78 Aa	1.56 Bb	58.512***
2003-2007	UL	1.65 Cb	1.70 Cc	1.43 Ab	1.55 Bb	15.579**
1998-2002	0.61 Aa	1.72 BCb	1.72 BCc	1.64 Bc	1.76 Cc	281.819***
1993-1997	1.26 Bc	4.54 Ec	0.78 Ab	1.84 Dd	1.65 Cbc	695.214***
1988-1992	1.49 Bd	1.73 Cb	0.60 Aa	1.71 Cc	4.51 De	1904.494***
1983-1987	0.59 Aa	1.67 Cb	0.87 Bb	4.61 Ee	1.99 Dd	2637.928***
F Value	112.8***	827.3***	780.8***	761.0***	733.6***	
Ave.	0.95 A	2.36 D	1.67 B	1.92 BC	1.96 BC	4.915**

Columns show differences between years; Rows show differences between species. According to Duncan test, letters a, b and c show statistical differences between years. Capital letters (A, B and C) show differences between species horizontally. *** Statistically significant at confidence level***; $p < 0.001$, * $p < 0.05$. ns: not significant. UL: Under limit.

The variation of As concentration in woods in the eastern direction depending on species and year is also shown in Table 5. According to the results of ANOVA in the eastern direction, there were significant differences in the year-by-year change in As concentration in all species ($p < 0.001$). Based on the results, the As concentrations of *P. orientalis* and *P. menziesii* species in the eastern direction were at below-limit values between 2013 and 2022. On the other hand, it was observed that *C. arizonica* accumulated 5.25 and 4.33 mg/kg As metal between these years. It was observed that *P. pinaster* species accumulated 4.57 mg/kg As metal between 2013 and 2017. In addition, it is observed that *C. arizonica* species accumulated 4 mg/kg of As metal between 1983-2022, except for 2003-2007. Another species, *P. pinaster*, accumulated As concentrations above 3 mg/kg in all years except 2018-2022. In fact, between 1988 and 1992, the accumulation reached 9.26 mg/kg, approximately 2.5 times the previous years. In this case, we can say that *C. arizonica* and *P. pinaster* accumulate more As metal in the eastern direction than other species.

Year-by-year changes in As concentration in woods according to plant species in the western direction are shown in Table 6. According to the results of ANOVA, As concentration remained at below-limit values in the western direction for many years. The

As concentration in *P. pinaster* species remained below the limit in all years except 1983-1987, 1993-2007, and 2018-2022. All years except 1998-2002 are below the limit values in *C. arizonica* species. In *P. orientalis* species, As concentration was below the limit in all years. The As concentration of *C. atlantica* was below the limit in all years except 1983-1988. However, compared to other species, *P. menziesii* accumulates As concentration at above-limit values in all years. The highest As concentration accumulation was between 2018-2022, and the lowest As accumulation was between 2013-2017.

Table 5. Variation of As Concentration in Woods in the Eastern Direction by Year and Species (mg/kg)

Years	<i>P. pinaster</i>	<i>C. arizonica</i>	<i>P. orientalis</i>	<i>C. atlantica</i>	<i>P. menziesii</i>	F Value
2018-2022	1.63 Aa	5.25 Bc	UL	1.86 Acd	UL	575.550***
2013-2017	4.57 Cc	4.33 Bb	UL	1.89 Ad	UL	817.590***
2008-2012	6.43 Ed	4.37 Bb	5.13 Ce	1.83 Abcd	5.71 DCc	968.415***
2003-2007	6.24 Cd	2.10 Aa	3.47 Bb	1.79 Abc	1.57 Aa	73.745***
1998-2002	5.13 Bc	6.16 Ed	3.76 Ac	5.50 Ce	5.83 Dc	405.960***
1993-1997	4.68 Bc	6.13 Dd	4.04 Cd	1.75 Aab	UL	605.152***
1988-1992	9.26 Ce	5.81 Bcd	5.85 Bf	1.68 Aa	1.79 Ab	109.691***
1983-1987	3.72 Bb	5.81 Dcd	1.86 Aa	5.49 Ce	UL	1816.771***
F Value	82.2***	52.8***	564.2***	3148.2***	2684.8***	
Ave.	5.21 E	4.99 CD	4.02 BC	2.73 A	3.73 AB	7.972***

Columns show differences between years; Rows show differences between species. According to Duncan test, letters a, b and c show statistical differences between years. Capital letters (A, B and C) show differences between species horizontally. *** Statistically significant at confidence level***; $p < 0.001$, * $p < 0.05$. ns: not significant. UL: Under limit.

Table 6. Variation of As in Woods in the West Direction by Year and Species (mg/kg)

Years	<i>P. pinaster</i>	<i>C. arizonica</i>	<i>P. orientalis</i>	<i>C. atlantica</i>	<i>P. menziesii</i>	F Value
2018-2022	0.51 a	UL	UL	UL	5.41 e	1907.553***
2013-2017	UL	UL	UL	UL	3.11 b	
2008-2012	UL	UL	UL	UL	2.89 a	
2003-2007	0.60 b	UL	UL	UL	4.22 d	1740.202***
1998-2002	0.64 Ab	0.57 A	UL	UL	4.21 Bd	3051.110***
1993-1997	0.51 a	UL	UL	UL	3.43 c	8840.637***
1988-1992	UL	UL	UL	5.67 c	4.34 d	1140.821***
1983-1987	0.51 Aa	UL	UL	1.46 Ba	3.44 Cc	698.155***
F Value	5.3*			3998.3***	164.3***	
Ave.	0.55 A	0.57 A		3.42 B	3.88 B	42.961***

Columns show differences between years; Rows show differences between species. According to Duncan test, letters a, b and c show statistical differences between years. Capital letters (A, B and C) show differences between species horizontally. *** Statistically significant at confidence level***; $p < 0.001$, * $p < 0.05$. ns: not significant. UL: Under limit.

In general, when the changes in As concentrations in woods of the species depending on the years were analyzed, the same species generally accumulated As concentrations in different years. Accordingly, plants of different species have different levels of metal concentrations even though they are found in the same directions and places. This situation may be related to the effects of different environmental factors and the plants' morphological, physiological, and genetic structures.

Table 7 shows data on organs (outer bark, inner bark, and wood) taken from different species in different directions. The wood concentration in all directions was at the lowest levels in *P. pinaster* species. The highest value was generally in the inner bark. In *C. arizonica* species, there was no considerable difference in the change of As concentration between organs in the south direction ($p>0.05$). In the eastern direction, other organs remained below the limit, except for wood concentration. The lowest concentration in the north direction was in the outer bark and wood (4.12 and 4.46 mg/kg, respectively), and the highest concentration was in the inner bark. The lowest As concentration (0.57 mg/kg) was in wood in the western direction, while the highest As concentration was 3.96 mg /kg in the inner bark.

Table 7. Variation of As Concentration according to Direction, Species and Organ (mg/kg)

Direction	Organ	<i>P. pinaster</i>	<i>C. arizonica</i>	<i>P. orientalis</i>	<i>C. atlantica</i>	<i>P. menziesii</i>	F Value
North	OB	4.46 Bb	4.12 Aa	4.61 Bb	4.16 Aa	7.91 Cb	536.969***
	IB	5.92 Cb	6.29 Cb	3.83 Bb	6.06 Db	1.97 Aa	1056.168***
	W	2.46 Aa	4.46 Ba	2.23 Aa	4.43 Ba	2.77 Aa	23.129***
	F Value	17.7***	4.3*	15.2***	3.6*	28.0***	
	Ave.	3.00 A	4.61 B	2.63 A	4.57 B	3.20 A	12.622***
South	OB	1.53 Ab	3.79 Ca	3.10 Ba	1.63 Aa	6.58 Db	930.781***
	IB	1.87 Ab	3.70 Da	2.27 Ba	2.65 Ca	2.20 Ba	303.968***
	W	0.95 Aa	2.36 Ca	1.67 Aa	1.87 BCa	1.96 BCa	4.915**
	F Value	11.9***	3.3 ns	2.8 ns	0.9 ns	31.6***	
	Ave.	1.14 A	2.64 C	1.88 B	1.92 B	2.45 BC	6.424***
East	OB	6.90 Ca	UL	UL	2.62 Aa	4.13 B	6028.643***
	IB	6.56 Ca	UL	2.41 Aa	2.86 Ba	UL	1916.304***
	W	5.21 Ea	4.99 CD	4.02 BCa	2.72 Aa	3.73 AB	7.972***
	F Value	1.4 ns		4.3 ns	0.0 ns	0.0 ns	
	Ave.	5.51 C	4.99 C	3.79 B	2.73 A	3.81 B	12.873***
West	OB	0.85 Ab	3.00 Cb	0.94 ABa	0.93 ABa	1.24 Ba	89.184***
	IB	1.00 Ac	3.96 Bc	0.87 Aa	5.65 Db	4.73 Cb	1172.116***
	W	0.55 Aa	0.57 Aa	UL	3.56 Bab	3.88 Bb	42.961***
	F Value	61.9***	2884.3**	0.4 ns	5.6*	20.4***	
	Ave.	0.66 A	2.51 B	0.91 A	3.42 BC	3.70 C	22.039***

Columns show differences between organs according to direction; Rows show differences between species. According to Duncan test, letters a, b and c show statistical differences between organs. Capital letters (A, B and C) show differences between species depending on direction. *** statistically significant at confidence level; $p<0.001$, * $p<0.05$. ns: not significant
Sig.: Significant. OB: Outer Bark, IB: Inner Bark, W: Wood. Sig.: Significant. OB: Outer Bark, IB: Inner Bark, W: Wood.

There were statistically significant differences in *P. orientalis* species only in the northern direction. The lowest As concentration in this species was determined to be in the wood part with 2.23 mg/kg, while the highest value was in the outer bark with 4.61 mg/kg. In *C. atlantica*, according to the ANOVA, As concentrations in the north and west directions were determined to be statistically significant. The lowest As concentration was in the outer bark, while the highest As value was in the inner bark. While the lowest values were generally seen in the inner bark and wood in the north and south directions, they were seen in the outer bark in the west direction. The highest As concentrations were found in the outer bark in the north and south directions and the inner bark and wood in the west.

In the *P. menziesii* species, there were no statistically significant differences based on organs only in the eastern direction ($p > 0.05$). However, there were significant differences in all other aspects at the 99.9% confidence level on an organ basis. Generally, the lowest As concentrations were in the wood part in the north and south directions.

Environmentally, it is common for soils to be contaminated with As, which is both toxic and carcinogenic (Ma *et al.* 2001). This study examined concentration changes depending on the direction in different organs of 5 plant species (*P. pinaster*, *C. arizonica*, *P. orientalis*, *C. atlantica*, *P. menziesii*). In addition, it was aimed to determine how the As concentration changed from 1983 to 2022, with samples taken from the wood part for specific years. As a result of the study, the usability of these plant species as both biomonitors and As hyperaccumulators were evaluated.

The results in different directions show that As can vary based on plant species and organs. Also, it may be related to the root systems of these plants and the relative uptake of water in different compass directions of the active xylem layers. Elements can enter plants *via* roots from the soil, leaves from the air, or through the stem (Chen *et al.* 2021). Airborne metal ions can also be absorbed by trees through rainfall, which facilitates their uptake by roots and subsequent distribution to leaves and other plant parts. The absorption of elements by plants depends significantly on their concentration, availability, and activity in the soil solution (Erdem *et al.* 2024). Therefore, it is quite difficult to assert that the As concentrations determined in wood from different directions are high due to air pollution. This is because the incorporation of the As element into wood varies depending on numerous factors, including rainfall, humidity, soil characteristics, root structure, wood anatomy, and plant habitus. Thus, the potential of the plants studied to serve as biomonitors for As remains uncertain.

Hyperaccumulator plants are species that accumulate more than 1000 $\mu\text{g/g}$ dry mass (Visoottiviseth *et al.* 2002). The study observed that the amount of As accumulated in the wood parts of the species was more less than 1000 $\mu\text{g/g}$. The highest As value was 9.26 mg/kg in *P. pinaster* species. It was determined as 6.17 mg/kg in *C. arizonica*, 5.85 mg/kg in *P. orientalis*, 5.67 mg/kg in *C. atlantica*, and 5.83 mg/kg in *P. menziesii*. According to the results of this study, it is seen that these plant species are not among the hyperaccumulator plant species. However, one cannot conclude this with certainty. Because when the ages of the plant species at the time of cutting are taken into consideration, it is seen that the ages of the species were young. Therefore, the amount of As in older species should be investigated. It is also thought that this situation may change when the total amount of arsenic accumulated in the biomass of the plants is calculated. Studies conducted on different species have indicated that arsenic (As) concentrations vary significantly across species. In their study, Yaşar Ismail *et al.* (2025) assessed the As concentrations in the bark and wood of five tree species, and found that the As concentrations in *Tilia tomentosa*, *Pseudotsuga menziesii*, and *Fraxinus excelsior* were

below detectable limits. In the same study, the average As concentration in the wood of *Robinia pseudoacacia* was found to be 55.300 ppb, while the average As concentration in *Cedrus atlantica* wood was 27.100 ppb (Yaşar Ismail *et al.* 2025).

In the region where the study was conducted, other heavy metal concentrations that pose significant risks to human health were also found to be at dangerously high levels. The studies in the area showed that the average chromium (Cr) concentration in the wood was 22.300 ppb in *Robinia pseudoacacia* and 11.300 ppb in *Cedrus atlantica* (Ozturk Pulatoglu *et al.* 2025). Additionally, bismuth (Bi) concentrations were found to be 53 mg/kg in *Cupressus arizonica* and 49 mg/kg in *Pseudotsuga menziesii* (Isinkaralar *et al.* 2023), antimony (Sb) concentration was 4.47 µg/g in *Cupressus arizonica* (Canturk *et al.* 2024), palladium (Pd) concentration was 11.100 ppb in *Cupressus arizonica* and 9.130 ppb in *Cedrus atlantica* (Sevik *et al.* 2024b), strontium (Sr) concentration was 5.320 ppb in *Picea orientalis* (Erdem 2023), and tin (Sn) concentration was 15.300 ppb in *Robinia pseudoacacia* (Ozturk Pulatoglu 2024). As can be seen, not only As, but also many other heavy metal concentrations in the study area are at alarmingly high levels. This result is quite natural, as studies have shown a significant correlation between the concentrations of heavy metals in plant organs. For instance, a statistically significant relationship ($p < 0.01$) was found between arsenic (As) and selenium (Se) (0.966), silver (Ag) (0.779), thallium (Tl) (0.948), and antimony (Sb) (0.885) (Sevik *et al.* 2024a).

These values have not been determined in all organs of plants. The As concentrations varied according to species and directions. This situation is thought to occur due to the change in the bioavailability of As in plants. Farooq *et al.* (2016) stated that As bioavailability may vary depending on environmental conditions, changes in the rhizosphere soil, physical properties of the soil, bioaccumulation kinetics, habitat, and chemical properties. It is known that a genotype-dependent proportion of As is transferred to the shoot and other tissues of the plant (Finnegan and Chen 2012). Cobanoğlu *et al.* (2023) stated that heavy metals accumulated in plants may transfer between organs.

Heavy metals in the air can be absorbed by plants either through respiration or by adhering to plant organs along with particulate matter. Some metals, under the influence of rain and gravity, can dissolve into the soil and water. Heavy metals are absorbed into root cells from the soil and stored in the root tissues, after which they are transported upward through the xylem and downward through the phloem. Plants generally take up heavy metals along with water and nutrients (Ozturk Pulatoglu 2024). Research suggests that heavy metals are effectively transported within the root symplasm, loaded into the xylem vessels, and then moved to the above-ground tissues through transpiration flow (Zeng *et al.* 2013; Deng *et al.* 2016). Thus, while heavy metals can enter the plant through various pathways, determining the exact proportion of heavy metals taken up via each pathway is quite difficult. This is because these pathways can work together in the uptake process of heavy metals (Shahid *et al.* 2017; Ozturk Pulatoglu *et al.* 2025).

The entry and transfer of heavy metals within plants is not yet fully understood. Studies have generally found that the lowest concentrations of heavy metals are present in the woody parts of the plant (Erdem *et al.* 2023). The wood part does not have direct contact with soil or air, and heavy metals must be transported and accumulated within the plant structure. Therefore, wood is generally the organ with the lowest concentration of heavy metals (Sevik *et al.* 2024a). This variation may be attributed to the different ion exchange capacities (IECs) of the xylem in various tree species. The adsorption of metal ions onto biomaterials is influenced by their ion exchange capacity (IEC) (Hubbe *et al.* 2011). For every mole of metal adsorbed, one mole of Ca is displaced (Crist *et al.* 2003). In other

words, each metal ion that is adsorbed is expected to displace other ions, such as sodium or hydrogen, in proportion to the metal's valence (Hubbe 2013). Most cellulosic materials are capable of binding with positively charged ions (Hubbe *et al.* 2022). The plant cell wall, a complex and multifunctional structure, is part of the apoplast. It plays a crucial role in regulating the composition of the periplasmic medium and controlling the movement of ions and metabolites across the plasma membrane due to the presence of ion-exchange groups (Meychik *et al.* 2017).

The amount of As in plants remained at sub-limit levels in some organs and aspects. It is thought that this situation occurs for two different reasons. First, it is thought that it may be related to the exposure levels to As sources in the direction where the plants are located. Secondly, it is thought that this may be due to the level of development of decomposer reduction and detoxification mechanisms in plants. Ma *et al.* (2001) discovered that fern, a fast-growing species, quickly removes arsenic from the soil. They also stated that fern was the first known As hyperaccumulator. Accordingly, considering the amount of As accumulated by the species used in the present study, it is thought that they may be effective in reducing the As toxicity in their environment. Plants can reduce toxic metals/metalloids such as As to limited levels by using some mechanisms (for example, by synthesizing metal-binding proteins) (Degola *et al.* 2015; Abbas *et al.* 2017).

Generally, As phytotoxicity is commonly attributed to the chemical properties of two inorganic species (Talano *et al.* 2013). It is thought that the uptake of As(III) into the plant occurs passively through membrane aquaporins, as it does not dissolve when the pH is below 8 (Smith *et al.* 2010). It is known that arsenite is more mobile in the soil than arsenate and is instead absorbed into the plant body through mechanisms that are independent of phosphate. Arsenate is a phosphate analog, and its uptake into the plant is known to compete with it for uptake by roots via membrane phosphate transporters (Degola *et al.* 2015). AsV is taken up and translocated in higher plants through the high-affinity phosphate carrier system due to its chemical similarity with phosphate (Talano *et al.* 2014; Bali and Sidhu 2021).

According to the results of the study, plants of different species have different levels of metal concentrations, even though they are found in the same directions and places. This situation may be related to the effects of different environmental factors and the plants' morphological, physiological, and genetic structures. All phenotypic traits of plants, including their potential to accumulate heavy metals, are shaped by the interaction between genetic structure and environmental conditions (Erturk *et al.* 2024; Aricak *et al.* 2024; Sevik *et al.* 2025). Therefore, even when plants grow under the same environmental conditions, their phenotypic traits may differ due to differences in genetic makeup (Özdikmenli *et al.* 2024). In fact, trees of the same species may exhibit different phenotypic characteristics due to differences in their genetic structures (Tandogan *et al.* 2023; Yigit *et al.* 2023). As a result, the potential of plants to accumulate heavy metals may vary depending on their genetic composition. It is not clearly understood by which means of transport As and other metals and metalloids are transferred between plant organs in higher plants. As a result of the studies, it has been stated that the transition between organs such as wood, bark (inner and outer) (Çobanoğlu *et al.* 2022; Erdem *et al.* 2024), branches, needles, broad leaves, and fruits (Koç *et al.* 2024) remain unclear.

CONCLUSIONS

1. The study should investigate in detail whether *P. pinaster*, *C. arizonica*, *P. orientalis*, *C. atlantica*, and *P. menziesii* are hyperaccumulator trees by conducting studies on older species because they accumulate high concentrations of arsenic. If a choice is to be made among these species, it is suggested that the order of priority should be *P. pinaster*, *C. arizonica*, *P. orientalis*, *C. atlantica*, and *P. menziesii*.
2. The differences in As concentration in plant organs may be due to transport between organs, reducing toxicity to certain levels, and, most importantly, the proximity and contact of the plant to the main source. This situation needs to be examined in more detail in woody plant species. Using these plant species as biomonitors in areas exposed to metal toxicity is recommended.

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