Chronological Levels of As, Pd, V, and Sr in 356-year-old *Pinus nigra* Annual Rings in Northern Türkiye

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The accumulation and allocation of heavy metals—arsenic (As), strontium (Sr), palladium (Pd), and vanadium (V)—were measured in *Pinus nigra* wood over more than three centuries. Contrary to expectations, the results did not show a correlation between increasing air pollution in recent decades and the concentrations of these metals in annual rings. Thus, *P. nigra* wood appears unsuitable as a biomonitor for tracking long-term heavy metal pollution in air. However, metals deposited in specific annual rings remained restricted within those rings, supporting the hypothesis of limited radial migration over time. The study specified *P. nigra* as a promising candidate for phytoremediation due to its capacity to accumulate high concentrations of As, Sr, Pd, and V. Additionally, these metals exhibited robust positive correlations with other toxic elements (tin, antimony, niobium, silver, thallium, selenium, and lead), suggesting that *P. nigra* could serve as an indicator of regions with multiple heavy metal pollutants. These findings highlight the complexity of metal uptake and allocation processes in trees and underscore the need for further research in controlled environments to clarify mechanisms of metal transfer and toxicity. Despite its limitations relative to its usage as a biomonitor, *P. nigra* demonstrated potential for mitigating heavy metal contamination in polluted environments.

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INTRODUCTION

The industrial revolution and advancement in the last era has expanded the need for raw materials, energy, and labor. This process caused irreversible extreme shifts on a global scale. Migration from rural regions to urban areas to meet the workforce needed in industrial communities has revealed the trouble of urbanization (Guoru *et al.* 2022; Anestis and Stathakis 2024). The consumption of fossil fuels to provide the needed energy has also considerably elevated the CO_2 level in the atmosphere and has become the main driver for global climate change (Tekin *et al.* 2022; Arıcak *et al.* 2024). Thus, climate change and urbanization have become irreversible global challenges that people must face (Erturk *et al.* 2024; Gür *et al.* 2024).

Environmental problems, which have arisen in this context have a global impact, especially concerning air pollution (Yayla *et al.* 2022). The extraction of elements needed as raw materials for industrial production from underground reserves and their release into nature has made air pollution a global concern threatening environmental and people's health (Istanbullu *et al.* 2023; Sulhan *et al.* 2023). Air pollution, especially in metropolis or big cities, has extended such severe points that an estimated 90% of the world's residents breathe dirty air, and air smog causes approximately 3.8 to 4.3 million premature childbirth and the death of roughly 7 million individuals universally per year (Ghoma *et al.* 2023; Isinkaralar *et al.* 2023).

The most detrimental components of air contamination are heavy metals. Heavy metals are among the most dangerous and harmful environmental pollutants in terms of human and environmental health, with their concentrations continuously increasing anthropogenically in nature in the last century. Studies conducted to date reveal that the most significant heavy metal pollution sources are industrial activities, traffic, mining activities, agricultural activities, and urbanization (Mutlu and Aydın Uncumusaoğlu 2017; Aricak *et al.* 2019; Demir *et al.* 2021; Şevik *et al.* 2024). Some of the metals are toxic, carcinogenic, and fatal to people and environmental health, even in tiny amounts (Key *et al.* 2022; Özel *et al.* 2024). Considering the potential hazards, priority pollutant heavy metals have been determined by international organizations such as EPA (U.S. Environmental Protection Agency) or ATSDR (The Agency for Toxic Substances and Disease Registry). Many studies have been done on heavy metals such as Cr, Pb, Cu, Zn, and Al (Savas *et al.* 2021; Key *et al.* 2022). However, although elements such as As, Sr, V, and Pd are also included in these lists and can be harmful even at much lower concentrations, studies on these elements are much more restricted.

Arsenic (As), one of these heavy metals, is generally found in the arsenopyrite mineral in nature and can be released into nature as a result of anthropogenic actions, such as mining actions, industrial processes, using pesticides, and the burning of fossil fuels (Farooq *et al.* 2016). As, which can be found everywhere, is a toxic element for all life forms (Tripathi *et al.* 2007).

Palladium (Pd), another detrimental element, is a rare and precious metal with strong catalytic activity. Demand for Pd is high due to its use in automobile catalysts, dental materials, and electrical equipment. Due to its increasing use in automobile catalysts, Pd concentrations in the air are increasing. Exposure to Pd can generally occur through inhalation of respirable particles. Observed symptoms include chronic urticaria, swelling of the cheeks and lips, asthma, itching, stomatitis, dizziness, and oral lichen planus. (Umemura *et al.* 2022).

Vanadium (V) is the fifth most abundant transition element in the earth's crust (Imtiaz *et al.* 2015). It enters nature primarily *via* anthropogenic actions, such as industry, mining, fossil fuel burning, and composting, and high V concentrations pose potential health threats to plants, microbes, humans, and animals (Altaf *et al.* 2021; Chen *et al.* 2021; Hao *et al.* 2021). V causes troubles in humans, such as congestion, vasoconstriction, focal bleeding in the adrenal cortex and lungs, congestion, fatty liver and cardiac disorders, dehydration, diarrhea, weight loss, or reduced food intake (Altaf *et al.* 2021). Long-term exposure to V causes an increased possibility of functional lesions in the nervous system, bones, and spleen. (Yu and Yang 2019). It also harms the immune and respiratory systems, kidneys, digestive organs, skin, and liver (Hao *et al.* 2021).

Strontium (Sr) is one of the most lethal heavy metals for the environment and human health. Certain Sr compounds are detrimental to human health, even at low concentrations. They can induce lung cancer and build up in the body throughout a lifetime, creating severe complications that could even compel sudden death (Erdem 2023a).

Humans can be exposed to these elements in various ways. Mushrooms, parsley, black pepper, cereals, dill seed, leafy vegetables (spinach, lettuce), fruits (strawberries, apples, pears), shellfish, and wine and beer are some familiar sources of V, and V is taken into the human body by consuming them as food. However, V is poorly absorbed by the gastrointestinal tract (Aihemaiti *et al.* 2020). As can enter the human body through food and beverages and inhalation due to polluted air and cigarette smoke (Joseph *et al.* 2015). Sr enters the human body mainly through food and drinking water (Peng *et al.* 2021). Pd can be exposed by inhaling respirable particles in the environment from automobile catalytic converters containing Pd (Umemura *et al.* 2022).

These four elements are hazardous for people and environmental health, even at small amounts. Due to these menaces, they have been included in the checklist of priority pollutants by the ATSDR (Agency for Toxic Substances and Disease Registry) (Savas *et al.* 2021). It is noted that breathing in heavy metals can cause much more severe health concerns (Ghoma *et al.* 2023). Thus, tracking the shifts in these atmospheric metal element amounts and diminishing contamination is vital. Biomonitors are generally used to observe the variation in atmospheric heavy metal pollution (Karacucuk *et al.* 2022; Kuzmina *et al.* 2023). In particular, tree annual rings provide essential information about the long-term shifts in atmospheric concentrations of heavy metals (Koc *et al.* 2024).

In addition to tracking heavy metal pollution, reducing pollution is also important. One of the most compelling arguments used in reducing heavy metal pollution is plants. In this application called "phytoremediation," plants can significantly reduce environmental heavy metal pollution by accumulating heavy metals in their organs. However, the plants to be used for this purpose should not die as a result of the heavy metal uptake (Savas *et al.* 2021; Şevik *et al.* 2024). Heavy metal pollution is one of the most critical stress factors that limit plant development and even cause plant death (Yayla *et al.* 2022; Koc *et al.* 2024).

The metal elements considered in this study are also among the elements that can cause serious harm to plants. Exposure of plants to As causes growth and physiological disorders. As prevents root elongation and reproduction. When it is translocated to shoots, it seriously affects plant growth by slowing down or stopping growth and biomass. It can endanger the plant's reproductive capacity due to losses in fruit production, yield, and reproduction of the same plant (Stoeva and Bineva 2003; Garg and Singla 2011).

Pd is considered a metal biotoxic. It has been determined that Pd causes DNA damage in plants, delays plant development, causes a decrease in root mass, causes shells to be deformed and seed production to be absent, and disrupts photosynthesis (Ronchini *et al.* 2015).

Vanadium (V) toxicity in plants can cause poor root growth and plant death. High V concentrations disrupt material cycling and energy metabolism; hinder key enzymes that mediate ion transport, protein synthesis, energy production, and other critical physiological functions, leading to shoot and root abnormalities, growth retardation and even plant death. Also, V concentrations above 2000 ppb cause growth inhibition, leaf yellowing and necrosis, oxidative stress, coralloid root network, and suppression of the uptake of distinct necessary elements, causing toxic effects in plants and shoot and root abnormalities and deformation of plant cells (Aihemaitia *et al.* 2019; 2020).

Sr can reduce cell wall plasticity and thus inhibit cell elongation. It causes a decrease in chlorophyll content in higher plants and visible toxicity symptoms, such as chlorosis and necrosis. It causes stress in plants, increases amino acids, such as proline and free amino acids, decreases protein pool content, and also significantly increases leaf nitrate (Burger and Lichtscheidl 2019).

This research aimed to specify the usability of *P. nigra* in order to monitor the changes and mitigate the As, Pd, V, and Sr amounts in the environment. In addition, the study examined the correlation of As, Pd, V, and Sr with other elements. The selected species is frequently preferred in landscape studies due to its high durability. It can contribute significantly to the reduction of pollution by collecting heavy metals. It is frequently used in areas such as afforestation, erosion control, rehabilitation areas, and landscaping works due to its contentment, not being selective about soil, and being resistant to climate conditions such as drought and frost (Yigit *et al.* 2023; Cantürk *et al.* 2024; Şevik *et al.* 2024). In addition, it is a long-lived species, which can prevent the heavy metals it accumulates in its wood from returning to nature for hundreds of years. It is well known that a tree mainly draws in water through the its roots and that the main upward flow takes place within the most recent, outermost annual rings, which are part of the sapwood. For instance, it has been shown that specific conductivity within the sapwood of lodgepole pine declines from a maximum at the living cambial layer to lowe values adjacent to the heartwood at the core of a mature tree (Reid *et al.* 2003). Due to this feature, there is potential for it to provide information about past periods of hundreds of years. Due to these features, the study was carried out on *P. nigra*, and the pollution of selected heavy metals over more than three centuries was evaluated. In line with the objectives of the study, the first hypothesis of the study is the concentrations of heavy metals As, Pd, V, and Sr in *P. nigra* wood have been increasing in parallel with the increase in air pollution in recent years. The second hypothesis is that increased levels of such metals in the air will have affected their concentrations in the soil of forested areas, and that the metals will have been conducted upwards mainly through the outer layers of sapwood, where some of it will remain adsorbed. The third hypothesis is that metals deposited within specific annual rings will tend to remain within those rings, rather than migrate over time to other groups of annual rings.

EXPERIMENTAL

This research was carried out on black pine (*P. nigra* Arnold.) growing in Kastamonu region, Türkiye. As of 2023, approximately 389 thousand people live in the province, and the number of vehicles registered in traffic is about 159 thousand (TUİK 2024). Traffic density in the region is relatively low. The livelihood of people living in the province is generally based on forestry and agriculture. According to the employment rates of industrial establishments, they are listed as textile products (29.1%), wood and mushroom products (24.5%), metal ore (11.8%), and food products (10.8%) sectors. There are 29 mining facilities in the province (KTB 2024). As an outcome of the investigations carried out in the province, many metallic mineral and industrial raw material deposits and occurrences have been revealed, the most important of which are lead, zinc, copper, and quartzite deposits. Mercury and manganese are found in the region in metallic minerals, and clay, graphite, quartz sand, cement raw materials, kaolin, and phosphate formations are found in industrial raw materials (MTA 2024).

Studies on air pollution in Kastamonu emphasize that air pollution in the city center increases significantly, especially in winter. The increase in air pollution in winter is due to the city center's location in a valley, where air circulation is relatively limited (Sevik *et al.* 2015). Studies conducted on heavy metals throughout the province determined that the pollution level of some heavy metals (Ni, Cd, Zn, Cr, and Mn) is high, especially in the city center. The primary source of this pollution is traffic density (Sevik *et al.* 2019; Savas *et al.* 2021).

A 10 cm thick log sample was taken from the tree cut in the autumn of 2023, approximately 50 cm above the soil surface. In order to clearly see the annual tree rings in the log sample, the log sample was sanded, the annual tree rings were counted, and it was found that the tree was 356 years old. The annual tree rings were grouped by ten years from outside (bark) to inside (pith) (from newest to oldest), taking into account the annual ring widths. Sawdust samples were taken from woods of all age clusters within three different spots using a steel drill tip.

The obtained samples were first subjected to pre-drying. At this stage, the sawdust samples were dried at room temperature (20 to 22 °C) and in an oven (45 °C) for 15 and 7 days, respectively. After the pre-drying process, the samples were pre-combusted. At this stage, 0.5 g dried samples, 6 mL of 65% HNO_3, and 2 mL of 30% H_2O_2 were mixed in a volumetric flask and microwaved (200 °C) for 15 min. The applied pre-combustion process has been used in multiple studies where wood samples were analyzed (Cesur *et al*. 2021; Canturk *et al*. 2024; Sevik *et al*. 2024). After the pre-combustion process, the volumetric flask was filled up to 50 mL with ultra pure water. After running pure water through the system, the samples were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; GBC Scientific Equipment Pty Ltd., Melbourne, Australia).

The As, Pd, V, and Sr amounts were computed by multiplying the obtained concentrations with the dilution factor.

During digestion, a blank sample was used for each element for quality assurance/quality control (QA/QC). Five precision standards for the extraction and metal assessment process were used to plot the calibration curve simultaneously. Each sawdust sample experienced three replicates and evaluation.

In addition, other element analyses were also carried out in the research to clarify the correlation levels of these four metal elements with others.

This method has often been used in various studies conducted on this topic in recent years (Key *et al.* 2022; Isinkaralar *et al.* 2022; Canturk *et al.* 2023; Key *et al.* 2023; Koc *et al.* 2024).

Analysis of variance (ANOVA) was utilized to the obtained concentration by using the IBM SPSS 22.0 software, and the Duncan test was applied for factors with noteworthy differences of at least a 95% confidence interval ($P < 0.05$). In addition, correlation analysis was applied to explain the correlation level of the elements (A, Pd, V, Sd) with other elements. The As, Pd, V, and Sd metal concentration detection limits were measured at 27, 16, 57, and 210 ppb, respectively.

RESULTS AND DISCUSSION

The averages and ANOVA and Duncan analysis outcomes regarding the change in the concentrations of the metal elements rch in *P. nigra* wood on a period basis are in Table 1. The As concentration ranged between 1150 and 3350 ppb. As shown by ANOVA, there was a significant difference ($P < 0.001$) between the periods, and because of the Duncan test, 17 groups were formed. The highest As concentration is roughly 2.92 times the lowest As concentration. The smallest As amounts were obtained among 1744 - 1753 and 1994 - 2023. The maximum As was found in the period 1914-1923.

When the change in Sr concentration was examined, as a result of ANOVA, it was determined that there was a significant difference ($P < 0.001$) between periods. As a result of the Duncan test, 22 groups were formed, and the last ten-year period in which the lowest value (1950 ppb) was obtained and the 1774-1783 period in which the highest value (6870 ppb) was obtained formed a separate group. There was approximately a 3.52 times difference between these two values.

Table 1. Changes in As, Sr, Pd, and V Concentrations by Period

Age group	As (ppb)	Sr (ppb)	Pd (ppb)	V (ppb)
2014-2023	1155.7a	1950.4a	25999.8c	5639.8bc
2004-2013	1293.2ab	2577.8e	27401.3d	5988.4cd
1994-2003	1227.3a	2772.4gh	28410.7ef	6151.4de
1984-1993	1574.5c	2401.3c	31175.7ijk	6977.2ijk
1974-1983	1597.0c	3329.4n	30759.1hij	6835.3hij
1964-1973	1569.3c	2818.1h	30764.6hij	6931.9ijk
1954-1963	1712.3cd	3754.2o	31218.0jkl	6909.9hijk
1944-1953	2076.2g	4508.0s	30440.2hi	7281.1klmn
1934-1943	2305.3h	2310.2b	31389.7jkl	7000.2ijk
1924-1933	3148.2op	3798.5o	32413.8mnop	7248.0klmn
1914-1923	3344.9r	2900.9i	32553.6nop	7025.2ijkl
1904-1913	2712.9kl	3373.2n	33352.3r	7416.8lmn
1894-1903	2830.7lm	2630.0f	32313.7mnop	6900.5hijk
1884-1893	2692.9jkl	3131.3l	32282.3mnop	7036.4ijklm
1874-1883	2660.5jk	2733.1g	32696.0opr	7132.1jklmn
1864-1873	3238.4pr	2663.5f	32589.1nopr	6983.4ijk
1854-1863	3182.1p	2414.9c	32616.2nopr	7425.4mn
1844-1853	3126.5op	2292.3b	31986.0lmno	7127.3jklmn
1834-1843	2713.3kl	2467.3d	31116.9ijk	6941.7ijk
1824-1833	2505.6i	3116.4kl	31827.7klmn	7228.1jklmn
1814-1823	2539.2ij	3353.1n	32645.2opr	7275.5klmn
1804-1813	2900.6mn	3076.2k	31704.4klm	7224.5jklmn
1794-1803	2931.7mn	3251.3m	32375.0mnop	7247.5klmn
1784-1793	3000.4no	3253.0m	31454.8jkl	7022.3ijkl
1774-1783	2670.9jkl	6866.1w	32883.0pr	7524.1n
1764-1773	1408.7b	3204.0m	21763.1a	4894.1a
1754-1763	1404.4b	3021.9j	23714.1b	5086.9a
1744-1753	1146.7a	4028.8r	24253.4b	5234.5a
1734-1743	1703.6cd	5342.1u	26256.9c	5586.4b
1724-1733	1850.4def	3937.2p	27675.5de	5978.8cd
1714-1723	2001.4fg	4630.8t	28470.9f	6227.5def
1704-1713	1799.3de	3986.3r	28281.2ef	6423.3efg
1694-1703	2015.2g	3752.4o	28085.8def	6127.4de
1684-1693	1942.1efg	5384.0u	29505.8g	6678.6ghi
1678-1683	2325.2h	5696.9v	30215.5h	6551.1fgh
F-value	173.409***	3946.882***	145.483***	35.592***

Means followed by the different letters (a, b, ...*etc.*) indicate they are statistically dissimilar for each element separately. *** = P ≤ 0.001.

The Pd amount ranged from 21800 to 33400 ppb. The ANOVA indicated that there was a significant variation (P < 0.001) between the periods. As an outcome of the Duncan test, 17 groups were formed. While the lowest Pd concentrations were obtained in three periods covering 1744-1773, the highest Pd concentration was obtained in 1904-1913. The maximum Pd amount was approximately 1.53 times the lowest Pd concentration.

There were statistically signficant differences ($p<0.001$) at the 99.9% confidence intervals between the periods in terms of V concentration. As an outcome of the Duncan test, the data formed 14 groups. The lowest values were obtained from 1744 through 1773. The highest values were obtained immediately after this period, that is, in the period 1774-1783. Notably, there is a difference of approximately 1.53 times between the two consecutive periods in which the lowest value (4894.1 ppb) and the highest value (7524.1 ppb) were obtained. This result shows that there may be a relationship between the elements. Therefore, the relationship among the metal elements was defined, and the results of the correlation examination are given in Table 2.

Table 2. Correlation Analysis Results of the Elements

	As	Sr	V	Pd
Sr	-0.079			
V	0.718**	-0.04		
Pd	0.773**	-0.09	0.953**	
Age group	0.077	0.588**	-0.268**	-0.285**
Ca	-0.049	0.638**	0.289**	0.240*
Mg	0.094	0.488**	0.078	0.090
K	0.044	0.186	0.029	0.094
S	0.169	-0.054	0.590**	0.590**
Ba	-0.355**	0.394**	-0.18	-0.300**
Al	-0.053	0.357**	0.100	0.023
B	-0.741	-0.918**	-0.782	-0.934**
Bi	0.695**	-0.256**	0.871**	0.900**
Cr	0.048	0.131	-0.006	-0.109
Cd	0.445**	-0.568**	0.631**	0.666**
Cu	-0.477**	-0.409**	-0.207*	-0.201*
Co	0.145	-0.004	0.461**	0.359**
Ga	0.742**	-0.064	0.947**	0.990**
In	0.830**	0.073	0.922**	0.945**
Fe	-0.139	-0.182	-0.095	-0.130
Pb	0.825**	-0.110	0.922**	0.951**
Li	0.514**	-0.237*	0.828**	0.878**
Mn	-0.316**	0.505**	-0.052	-0.193*
Na	-0.370**	0.062	-0.258**	-0.282**
Ni	-0.055	0.232*	0.089	-0.054
Ag	0.779**	-0.096	0.941**	0.987**
P	-0.200*	-0.304**	0.274**	0.251**
Se	0.966**	-0.075	0.818**	0.864**
Si	-0.352**	0.103	-0.326**	-0.411**
Tl	0.948**	-0.109	0.836**	0.877**
Zn	0.019	-0.280**	0.167	0.188
Au	0.755**	0.015	0.936**	0.942**
Ge	0.831**	-0.308*	0.049	0.246
Mo	0.313**	0.185	0.483**	0.362**
Nb	0.769**	-0.056	0.951**	0.982**
Pt	0.790**	-0.240*	0.902**	0.934**
Sb	0.885**	0.107	0.872**	0.890**
Sn	0.957**	-0.129	0.584**	0.656**
Ti	-0.176	0.377**	0.060	-0.043

* Correlation is significant at the 0.05. ** Correlation is significant at the 0.01.

When the relationship levels between the elements are examined, it was noticed that there was a statistically significant relationship between the elements subject to the study, As, V, and Pd. These relationships were robust and positive. It was noted that there was a statistically signficant correlation between the elements (Sr, V, and Pd) and the period. The relationship between Sr and period was positive, while the relationship between V and Pd and period was negative. In other words, while Sr concentration has decreased from past to present, Pd and V concentrations have increased from past to present.

Another point that draws attention to the table is that high levels of positive relationships between elements are extremely harmful to health. For example, it was determined that there was a robust positive relationship between As, Sn, Sb, Nb, Ag, Tl, Se, and Pb. Similarly, there was a generally robust positive correlation between these elements and Pd and V. However, the relationship between Sr and these elements was generally statistically nonsignificant, weak, or negative.

In the current study, As, Sr, Pd, and V elements accumulated within detectable limits in *P. nigra* wood. The determined concentrations of these elements were at very high levels. While heavy metals such as Cr, Pb, Ni, and Cu have frequently been the subject of biomonitor studies conducted to date (Isinkaralar *et al.* 2022; Guney *et al.* 2023), elements that show severe toxicity at much lower concentrations (As, Sr, Pd, V, Tl, Se, Sn and Be) have generally been neglected. However, these elements are highly harmful. For example, V can cause serious health problems (impairment of the nervous system, cardiac diseases, liver and kidney hemorrhage) and even death in humans (Briffa *et al.* 2020). At the same time, Aihemaiti *et al.* (2019) stated that high V concentrations in plants disrupt energy metabolism and matter cycle and hinder key enzymes that mediate energy production, ion transport, protein synthesis, and other critical physiological functions; it also causes root and shoot abnormalities, growth retardation, and even plant death by suppressing mitotic activity. In addition, V metal concentration above 2000 ppb generates poisonous impacts in plant species by causing growth inhibition, oxidative stress, leaf necrosis and yellowing, suppression by favoring the uptake of various fundamental elements, and coralloid root system (Aihemaiti *et al.* 2019). However, they found that the V concentration in the wood part varied between 4900 ppb and 7500 ppb, which shows that the *P. nigra* subject to the study has a high tolerance to V. Similar results apply to other elements.

The toxicity level of Sr for plants is evaluated as 30 thousand ppb (30 ppm) (Burger and Lichtscheidl 2019). The current study's Sr concentration varied between 1950.4 ppb and 6866.1 ppb. Miletić *et al.* (2024) studied *Populus alba*, *Salix alba*, *Populus nigra*, *Juglans regia*, *Ulmus glabra*, *Reynoutria japonica*, *Amorpha fruticosa*, *Impatiens glandulifera*, and *Solidago canadensis*. They obtained the highest Sr concentrations in the leaves of *Salix alba*, *Populus nigra*, and *Impatiens glandulifera* species. It was determined that Sr concentrations in these species reached up to approximately 107, 113, and 81 mg kg^{-1} dry mass, respectively. Bzour *et al.* (2017) determined in their studies that Sr concentrations in different plants ranged from 134 mg kg^{-1} (*Astragalus sparsus*) to 404 mg kg^{-1} (*Maresia pygmaea*). In the study on wood of different species, Erdem (2023a) found that the mean Sr concentration was 3210 ppb in *Cupressus arizonica*, 2340 ppb in *Cedrus atlantica*, 1200 ppb in *Pseudotsuga menziesii*, 1880 ppb in *Pinus pinaster*, and 5320 ppb in *Picea orientalis*. These concentrations show that *P. nigra* can accumulate higher levels of Sr than other coniferous species subject to studies.

As is generally poorly soluble in soil and poorly taken up by plants. The above-ground parts of plants generally contain As in small concentrations (<1000 ppb = 1 mg kg^{-1}). The permitted As level in dry feed was 4 mg kg^{-1} (Dradrach *et al.* 2020). The current study's As concentration ranged between 1150 and 3350 ppb. Khan *et al.* (2024) determined that As concentration in goosefoot, coriander, mint, green amaranth, potato, alfalfa, and cucumber species varied between 1.38 and 11.1 mg kg^{-1} on average. Bzour *et al.* (2017) determined in their study that As concentrations in different plants ranged from 0.7 mg kg^{-1} (*Arthocnemum mucronatum*) to 2.7 mg kg^{-1} (*Astragalus* sp.). Dradrach *et al.* (2020) reported that total As concentrations in the above-ground parts of plants growing in an old mine site varied in a wide range of 0.5 to 509 mg kg^{-1}, with the median value determined being 8.9 mg kg^{-1}. With regard to shoot As concentration, the highest median value (47.5 mg kg^{-1}) was found in *Equisetum* spp., a genus known to be an As accumulator.

Trace amounts of V (1 to 10 ppb) can promote plant growth, while concentrations above 100 ppb are harmful (Roychoudhury 2020). The current study determined that the V amounts varied between 4890 ppb and 7520 ppb. Aihemaiti *et al.* (2018) compared the V concentrations in *Setaria viridis*, *Kochia scoparia*, and *Chenopodium album* species in their study in the V minefield and obtained the highest value (157 mg kg^{-1}) in *Setaria viridis* species. Bzour *et al.* (2017) determined that the V concentrations in different plants varied between 3.6 mg kg^{-1} (*Convolvulus arvensis*) and 21.8 mg kg^{-1} (*Astragalus* sp.). Erdem (2023b) determined that the V concentration in wood was 1105.3 ppb in *Abies bornmüllerina*, 2110 ppb in *Pinus sylvestris*, and 2930 ppb in *Fagus orientalis*. The V concentrations obtained in the current study are much higher than these values.

The Pd values obtained from the study were much higher than those of other elements. The current study's Pd concentration ranged between roughly 21800 and 33400 ppb. Sevik *et al.* (2024) stated that the mean Pd concentration was approximately 7220, 11100, 4770, 9130, and 6010 ppb in *P. pinaster*, *C. arizonica*, *P. orientalis*, *C. atlantica*, and *P. menziesii*, respectively. These concentrations show that *P. nigra* can accumulate higher levels of Pd than other coniferous species subject to studies. Clement *et al.* (2015) determined that Pd concentrations in plant communities at different distances from the road varied between 10 and 15.5 ppb, and the highest values were obtained in plants close to the road. While the concentrations of other elements were up to 7500 ppb, it was determined that the concentration of Pd varied between 21700 ppb and 33400 ppb. These results show that the *P. nigra* subject to the study can accumulate these elements at high levels in the wood portion. The most key feature in phytoremediation research is that the plant can accumulate metals in its wood. This is important because the wood part is the biggest organ of plants in terms of volume (Key *et al.* 2023; Koc *et al.* 2024). However, studies have generally determined that the most negligible element accumulation is in wood (Koç et al. 2024). The current research outcomes indicate that the element concentrations are pretty high in *P. nigra* wood, which shows that this tree species is an appropriate species for phytoremediation research to decrease the contamination of these metal elements.

Heavy metals penetrate the plant cell through roots from the soil, leaves from the atmosphere, and direct adsorption from stem parts (Erdem *et al.* 2023a; Cobanoglu *et al.* 2023). V enters the plant body mainly through the roots. It is stated that the V concentration in the roots is 2 to 1000 times higher than in other organs of the plant (Aihemaiti *et al.* 2020). As acts as a phosphate analog. Thus, it is transported across the plasma membrane *via* phosphate transport systems. After entering the cytoplasm, its competition with phosphate begins (Stoeva and Bineva 2003). Sr is not a necessary component for plant development. Due to the similar chemical characteristics of Sr and Ca, plants that absorb excessive amounts of Sr may have disruptions in their regular metabolism, namely in the uptake of Ca and other nutrients. According to research, Sr can enter different kinds of plant cells through non-selective cation channels, which are part of the routes for the uptake of K^+ and Ca^{2+} (Yan *et al.* 2022).

Due to the different entry pathways, heavy metal accumulation in each organ is at different levels (Erdem *et al.* 2023a,b). However, in both phytoremediation and biomonitoring studies, the stem wood is of particular prominence because it contains the largest part of the plant in terms of mass. In addition, unlike organs, such as leaves and fruits, it keeps heavy metals away from environment for several years. Because organs, such as fruits and leaves, fall to the ground and rot within a year or a few years, the heavy metals in them return to nature. However, the wood part keeps heavy metals away from environment for several years. Therefore, the wood part has a unique essence in phytoremediation studies (Şevik *et al.* 2024). However, it is vital to determine the species that can collect heavy metals in high amounts in the wood section. Because studies generally show that the organ where heavy metals accumulate the least is wood (Karacocuk *et al.* 2022; Erdem *et al.* 2024; Koç *et al.* 2024).

In biomonitor studies, non-evergreen leaves can only provide data for one vegetation season, while the leaves of some evergreen species, such as spruce and fir, can provide data for a maximum of 8 to 10 years. However, data for tens or even hundreds of years can be obtained with monitoring studies using annual rings (Cesur *et al.* 2022; Key *et al.* 2023). One of the study's aims is to determine the species' suitability as a biomonitor. The most critical trait sought in species whose annual rings will be used as biomonitors is the ability to accumulate adequate amounts of heavy metals in their wood. Another essential feature is that the transport of heavy metals in wood is restricted. Studies show that only some species may be suitable biomonitors for some heavy metals.

As can be seen in Table 3, studies have shown that not every species will be a suitable biomonitor for every heavy metal. Therefore, while some elements can be used as biomonitors in some tree species (the transportation of elements in plant wood is restricted), the same elements are not suitable for use as biomonitors in other tree species (the transportation of elements in plant wood is increased) (Table 3).

Table 3. Suitable and Unsuitable Metals as Biomonitors in Some Tree Species' Woods

Species	Metal element(s)	Suitable biomonitor (Transport in wood)	Author(s)
Picea orientalis	Tl	Yes	Canturk 2023
Cedrus deodora	Cu	Yes	Zhang 2019
Cedrus atlantica	Cr, Ni, Mn	Yes	Koç 2021; Savas *et al.* 2021
Cupressus arizonica	Zn, Cd, Tl, Cr, Ni, Fe	Yes	Cesur et al. 2021; Cesur et al. 2022; Canturk, 2023; Cobanoglu *et al.* 2023; Koc *et al.* 2024
Corylus colurna	Ni, Zn, Cd, Co, Pb, Mn, Cr	Yes	Key *et al.* 2022; Key and Kulaç 2022; Key *et al.* 2023
Cupressus arizonica	Bi, Li, Cr	No	Zhang 2019; Cesur *et al.* 2021; Cesur *et al.* 2022;

			Cobanoglu *et al.* 2023
Pseudotsuga menziesii	Cr	Yes	Koc *et al.* 2024
Cedrus deodora	Pb ve Zn	No	Zhang 2019
Cedrus atlantica	Co	No	Koç 2021
Pinus nigra	Sb, Ag, Se, Tl	No	Şevik et al. 2024

In the current study, the allocation of the elements in the wood was limited because there was a difference between the minimum and maximum values of 2.92 times in As, 3.52 times in Sr, and 1.53 times in Pd and V. More notably, there was a large difference between successive periods that occur in subsequent periods. For example, one of the lowest Pd values (21800 ppb) was obtained in the 1764-1773 period, while one of the highest values (32900 ppb) was acquired in the instantly next period of 1774-1783. Similarly, while the lowest V concentration (4890 ppb) was obtained in the 1764-1773 period, the highest V concentration (7520 ppb) was obtained in the immediately following period (1774-1783). These outcomes indicate that the metal does not diffuse between the adjacent tree rings.

The extent to which metal ions drawn up into the sapwood remain present in those tissues is likely to depend on the xylem's varying ion exchange capabilities in various tree species. The ion exchange capacity (IEC) controls the quantity of metal ions adsorbed onto biomaterials (Hubbe *et al.* 2011). For one mole of a divalent metal element absorbed, one mole of Ca element is relocated (Crist *et al.* 2003). In other words, depending on the valence of the metal species, each type of adsorbed metal ion is expected to displace many other ions, such as hydrogen or sodium (Hubbe 2013). Positively charged ions can relate to most cellulosic compounds (Hubbe *et al.* 2022). The plant cell wall is an intricate and multifunctional system that is a part of the apoplast. Due to the presence of ion exchange groups, the plant cell wall regulates the composition of the periplasmic media and the movement of metabolites and ions throughout the plasma membrane (Meychik *et al.* 2017).

As an outcome, As, Pd, and V elements exhibited a very robust and positive correlation. Moreover, these elements had a positive and robust interaction with Sn, Sb, Nb, Ag, Tl, Se, and Pb, which are perilous and detrimental to individual and environmental health. Similar results were stated in other studies. For instance, a study specified that Se, Ag, Tl, and Sb have a robust correlation with each other and other elements, such as V, As, In, and Pb (Şevik *et al.* 2024). The strong relationship between these elements can provide a great advantage in identifying risky areas. They mentioned trace metals in the study can pose a primary menace to the health of humans and other living things. Therefore, Al, Ag, Be, Ba, Cd, Cu, Co, Cr, Mn, Pb, Se, Pd, Hg, Sb, Ni, Pu, Tl, Sr, U, Th, Zn, V and As in ATSDR, As, Sb, Be, Cd, Cu, Cr, Hg, Pb, Ni, Ag, Se, Ti and Zn are included in the priority contaminants checklist of the EPA (U.S. Environmental Protection Agency) (Elajail and Sevik 2024).

This outcome shows that heavy metal sources, especially industry, and traffic, can simultaneously produce many detrimental elements. Studies reveal that industrial actions, mining, transportation, and mining are the most influential sources of metal elements (Yayla *et al.* 2022; Istanbullu *et al.* 2022; Kuzmina *et al.* 2023).

As was stated earlier, the first hypothesis of the study is that "the concentrations of heavy metals As, Pd, V, and Sr in *P. nigra* woods have been increasing in parallel with the increase in air pollution in recent years." As it is known, air pollution has been continuously increasing due to anthropogenic sources in the last century, and studies have emphasized that heavy metal pollution in the air has also been continuously increasing (Canturk *et al.* 2024; Sevik *et al.* 2025). In this case, a regular increase is expected in the concentrations of As, Pd, V, and Sr in the *P. nigra* woods subject to the study in recent years. However, the study's results show no such increase. That is, there was no correlation between the metal levels in the woods and the increase in heavy metal concentrations due to industrial development and traffic increases. According to these results, the hypothesis of the study can be rejected. The second hypothesis, regarding the expectation of pollutants being drawn up into and retained, at least in part, especially in the xylem of recently formed annual rings, was not clearly demonstrated in the present work. That is because there was no statistically significant rise in metal contents over the passage of years, despite the expectation of increasing levels of pollutants in the air and soil.

On the other hand, the third hypothesis appears to be well supported by the present work. That hypothesis stated that "metals deposited within specific annual rings will tend to remain within those rings, rather than migrate over time to other groups of annual rings." This was shown most clearly by a case in which the lowest values of V were measured in xylem layers from years 1744 through 1773 and then, immediately thereafter, the highest values were measured in layers from years 1774 to 1983. Related, but not always so striking contrasts were found in various periods for many different metal elements.

Due to the rejection of the first and second hypotheses, other possible explanations can be sought. As a general answer, the results are consistent with an expectation that the entry of heavy metals into the plant body and the subsequent reactions will be under the influence of many factors that affect each other. Numerous studies have shown that traffic, urbanization, mining, agricultural, and industrial activities are important sources of heavy metals (Istanbullu *et al.* 2023; Kuzmina *et al.* 2023; Ozturk Pulatoglu *et al.* 2025). However, a more thorough investigation reveals that an extremely intricate process controls the entry of heavy metals into the plant body. According to Cobanoglu *et al.* (2023), heavy metals can enter the leaves through the stomata or be absorbed from the roots through the stem. After diffusion or penetration, metals are typically moved within the cell by active transport through the symplastic pathway. Heavy metals' active transport within plants largely depends on plant biochemical and metabolic processes (Shahid *et al.* 2017). The simultaneous occurrence of two absorption pathways in places close to industrial and metropolitan locations makes it quite difficult to distinguish whether the metal concentration is taken from the soil root cells in the internal tissues of the plant or from the atmosphere through the leaf surfaces (Ozturk Pulatoglu 2024). The primary hypothesis of the study is that the distribution of the studied metals in *P. nigra* woods is due to heavy metal pollution in the air; that is, it has increased remarkably, especially in the last century. However, in recent years, there has been no significant increase in the concentrations of the metals in question in *P. nigra* woods. Since the facts reject the hypothesis, this situation demonstrates that the entrance of metals into tree rings varies depending on other conditions. Water, for instance, is known to flow upward from a tree's roots through the xylem of the previous year. The hypothesis of the current study was rejected since no empirical evidence was found that the hypothesized mechanism leads to consistent directionality.

CONCLUSIONS

1. The As, Sr, Pd, and V elements were allocated in *P. nigra* wood in notable amounts, and the concentrations of these elements in wood were quite high when compared to similar research. These results implied that *P. nigra* is a suitable tree species that can be used in phytoremediation applications to mitigate the As, Sr, Pd, and V concentrations in the environment. *P. nigra* could be used intensively in regions where these elements contamination exists.
2. The As, Sr, Pd, and V elements allocation within the *P. nigra* wood was quite limited. Therefore, the species is a suitable biomonitor to track the change of these element concentrations in the air. Since *P. nigra* are long-lived trees, decades-long shifts in any area can be determined using the method utilized in the current study.
3. The As, Pd, and V exhibited a robust positive relationship with each other and other elements, such as Sn, Sb, Nb, Ag, Tl, Se, and Pb. This outcome indicates that where these metal elements are elevated, other elements detrimental to health are also very high. Still, the quantity of research on this subject is inadequate. Research on the subject be carried out multifacetedly.
4. Studies have emphasized that heavy metal pollution in the air has increased significantly, especially in the last century, and that this increase has accelerated in the last two or three decades. However, the study results for the content of metals in different groups of annual rings do not reflect this increase. In this case, it is seen that the heavy metal accumulation in *P. nigra* wood was not significantly related to heavy metal concentrations in the air and presumably also in the soil, due to rain. Therefore, it can be said that *P. nigra* wood is not a suitable biomonitor for monitoring heavy metal pollution in the air.
5. It has been observed that the most significant information gaps in studies on heavy metals still concern the entry of metal elements into the plant cell, their transfer between organs, and toxicitys. Therefore, it is recommended that studies be conducted in controlled environments for each heavy metal in these areas to clarify these issues.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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