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Original Article

Variations in a Few Macronutrient Elements Depending on the Period in Black Pine (*Pinus nigra* Arnold.) Wood

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What is already known on this topic?

- Elements can accumulate in tree wood.

What this study adds on this topic?

- The exchange of Mg, Ca, and K elements in Pinus nigra wood is limited.
- The S element can be exchanged between tissues in Pinus nigra wood.

ABSTRACT

Nutrient elements are essential for plant growth and development. Magnesium (Mg), calcium (Ca), potassium (K), and sulfur (S), which are macronutrient elements for plants, are necessary for plant development. These nutrient elements are also critical heavy metals, and monitoring the variation in their concentration in the airborne environment is crucial for human and environmental health. In this study, the annual rings of the black pine (*Pinus nigra* Arnold.) tree, which was cut in 2023 and determined to be 356 years old via annual rings, were grouped by 10 years. Then, Mg, Ca, K, and S were analyzed to determine the variations within the last 356 years. The study also determined the relationship of these elements with each other and the wood's age. The results show that the displacement of Mg, Ca, and K elements in *Pinus nigra* Arnold. wood is limited. Therefore, black pine is a proper biomonitor that can be used to observe the variations of these elements' concentrations in the atmosphere. However, it has been determined that S can be displaced within the wood. As a result of the correlation analysis, it was determined that all elements, except Mg and S, had a statistically significant and positive correlation with each other, and S had a negative relationship with the wood age.

Keywords: Annual ring, heavy metal, macronutrient, *Pinus nigra* Arnold.

Introduction

Plants are indispensable living groups for all living things on this planet due to the economic and ecological functions they provide, as well as forming the basis of the food pyramid (Yigit et al., 2023). The most critical environmental aspects in plant enlargement are climatic features, such as precipitation and temperature (Dogan et al., 2023), and edaphic parameters, such as nutrient status, pH, and soil depth (Kuzmina et al., 2023). Among these factors, nutrients play an essential role in plant growth and development. Nine elements (H, C, O, P, N, K, S, Mg, Ca) in plant nutrition are macronutrients, which play an essential role in plant development (Erdem et al., 2024).

This study tried to determine to what extent the macronutrient elements Mg, Ca, K, and S accumulate in black pine wood and how this accumulation has changed from the past to the present. The study's primary objective is to determine the accumulation levels of these essential elements in black pine wood. Black pine lives for many years, and the wood can reach large volumes. Therefore, the elements accumulated in the wood are trapped for a long time and do not join the nutrient cycle.

Another objective of the research is to determine the displacement of these elements, which are nutrients and heavy metals, in wood, their relationships with each other, and the changes in their concentrations over 356 years. Some heavy metals are toxic and destructive to living organisms even at low concentrations (Aydın Uncumusaoğlu & Mutlu, 2021; Erdem, 2023; Sulhan et al., 2023). It is indicated that even those essential nutrient elements for plant species are destructive at elevated concentrations (Key et al., 2022; Yayla et al., 2022). Therefore, determining the variation in the concentration of heavy metals with the help of plants, that is, biomonitoring studies, and reducing heavy metal pollution via plants, that is, phytoremediation studies, have gained significant prominence in recent years (Key et al., 2023; Özel et al., 2024). In this research, the possibilities of using black pine as a biomonitor of Mg, Ca, K, and S elements and their phytoremediation potential were determined.

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Material and Methods

The current research was carried out on a *Pinus nigra* Arnold (Black pine) growing in Kastamonu, Türkiye, and cut (around 50 cm above the soil level) in the late autumn of 2023. First, a log sample (around 10 cm in thickness) was taken, and then, by counting the annual tree rings in the log sample, it was determined that the tree was 356 years old. A total of 108 samples were taken in three directions using a steel drill bit from the wood, which was grouped by 10 years, considering the annual ring widths. The samples were put in glass petri dishes and dried for 15 days at room temperature before spending an additional week drying in an oven at 45°C.

About 0.5 g of the dried specimens were taken, and 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added before the specimens were put into the microwave oven. The temperature in the microwave oven reached 200°C in 15 minutes, and that temperature was maintained for another 15 minutes. The dissolved samples were taken into volumetric flasks and made up to 50 mL with ultrapure water. The prepared samples were analyzed with the ICP-OES device, and the concentrations of Mg, Ca, K, and S elements were calculated by considering the dilution factor. In recent years, the study's methodology has been frequently applied to research on this topic (Isinkaralar et al., 2023; Key et al., 2022). The data were subjected to variance analysis (ANOVA), and factors with statistically significant differences of at least a 95% confidence interval ($p < .05$) were subjected to the Duncan test. In addition, correlation analysis was applied with the help of the IBM SPSS (Statistical Package for the Social Sciences) software program to determine the level of relationship between the age periods of the elements in the wood and each other.

Results

The following table provides average concentrations and the results of statistical analysis about the variations in Mg, Ca, K, and S element concentrations in *Pinus nigra* Arnold. wood by period (Table 1).

As seen in Table 1, the period-based change of all the elements is significant at the 99.9% confidence interval ($p < .001$). According to the Duncan test results, there are 23 homogeneous groups in Mg, 27 in Ca, and 17 in K and S. Based on the period, Mg concentration varies between 59.50 ppm and 214.07 ppm, Ca concentration is between 295.57 ppm and 1203.30 ppm, K concentration is between 30.43 ppm and 174.57 ppm, and S concentration is between 147.07 ppm and 185.60 ppm. The highest values were approximately 3.6 times the lowest for Mg, 4.0 for Ca, and 5.7 for K. In S, it was determined that there was an approximately 26% alteration between the lowest value and the highest value.

When the values are evaluated, it is seen that the transfer of elements other than S into the wood is limited. When the values are analyzed based on period, for instance, Mg concentration was calculated as 78.8 ppm in the 1794-1803 period, while it was calculated as 214.04 ppm in the adjacent 1784-1793 period and 83.83 ppm in the adjacent 1774–1783 period. Similarly, K concentrations in three consecutive periods between 1724 and 1753 were calculated as 46.93 ppm, 109.47 ppm, and 48.33 ppm, respectively. These outcomes can be inferred as the transfer of Mg, Ca, and K elements in wood is limited. However, the range of differences in S is relatively narrow, and S concentrations obtained in subsequent periods are pretty close. This outcome can be interpreted as S can be transferred within the wood.

Correlation analysis was implemented to define the relationships of the elements subject to the study with each other and the age of the wood, and the outcomes are presented in Table 2.

When the outcomes of the correlation analysis are evaluated, it is noticed that there is no statistically significant relationship between wood age and Ca. However, a positive and weak relationship exists between wood age and Mg and K concentrations. A statistically significant, negative, and relatively robust relationship exists between wood age and S. The relationship between Mg and S is not statistically significant. The relationships between other elements are statistically significant and positive. The most robust relationship was determined between Ca and Mg.

Discussion

In the current study, it was determined that Mg, Ca, K, and S elements accumulated in very high concentrations in *Pinus nigra* Arnold. wood. Studies show that many elements accumulate in diverse concentrations in the plant organs of different species (Guney et al., 2023; Koç et al., 2024). For example, it was found that the Mg concentration varied between 59.50 ppm and 214.07 ppm in the current research. Erdem et al. (2024) stated that the average Mg concentrations in 5 different types of wood vary between 157.8 ppm and 242.2 ppm. Similarly, it has been determined that the accumulation of many elements in the same organs can differ significantly based on the species (Yayla et al., 2022).

Research has indicated that the organ-based accumulation of various metal elements in the same individuals varies significantly, and that the minimum accumulation is generally in wood (Isinkaralar et al., 2022). For example, Karacocuk determined that the average Mn concentration in *Robinia pseudoacacia* wood, 1.2 ppm in areas without traffic in Samsun, exceeded 10 ppm in the bark and 35 ppm in the leaves. Similarly, the boron concentration in four different types of wood was at very low concentrations and remained below detectable limits (Erdem et al., 2023). Other studies obtained similar results (Cobanoglu et al., 2023a).

The elements subject to the current study are both macronutrients that are necessary for plants. Deficiencies of these elements in the soil adversely affect plant development (Erdem et al., 2024). The greatest portion of the plant is its wood, especially in trees such as black pine, which is the species subject to study. Since these trees are long-lived, unlike organs such as leaves and cones, the elements accumulated in the wood do not participate in the nutrient cycle for many years (Koç et al., 2024; Sevik et al., 2024). The plantation of these trees, especially on agricultural lands, may cause the soil to become poor by removing some of the nutrients from the soil. Therefore, it is recommended that trees with this feature are not used on agricultural lands.

The elements subject to study are also heavy metals. Studies show that heavy metals harm living things in high concentrations, even though they are necessary as nutritional elements (Isinkaralar et al., 2023; Mutlu et al., 2021). For example, sulfur dioxide causes irritation to the eyes, mucous membranes, skin, and respiratory tract. Additionally, high exposure may cause pulmonary edema, bronchospasm, laryngeal spasm, and bronchial inflammation (Türk Toraks Derneği., 2016). In addition, the ingestion of heavy metals or trace elements into the human cell, primarily through inhalation from the air, poses a tremendous health risk (Ghoma et al., 2022).

Table 1.
Variation in Mg, Ca, K, and S Concentrations (ppm) During Age Period

Age period	Mg (ppm)	Ca (ppm)	K (ppm)	S (ppm)
2014–2023	59.5a	295.57a	90.73ij	170.8hijklmn
2004–2013	70.4c	657.57k	109.97o	175.23lmno
1994–2003	76.8fg	702.87no	70.03g	172.87klmno
1984–1993	72.33cd	608.2h	63.67efg	169.2ghijk
1974–1983	106.93qr	912.17w	68.8fg	178op
1964–1973	104.97pq	868.8v	62.83efg	175.4mno
1954–1963	106.23q	1013.8y	56.87e	181.17pr
1944–1953	102.67op	1184.6z	77.53h	185.6r
1934–1943	75.93efg	542.5e	36.47ab	168.83ghijk
1924–1933	92.37lm	607.83h	39.33bc	166.1fghi
1914–1923	94.97n	628.1i	56.03e	169.4ghijk
1904-1913	122.37s	794.17r	90.33ij	173.63klmno
1894–1903	82.7ij	599.27h	107.2no	168.67ghijk
1884–1893	91.63lm	639.9j	96.27jkl	172jklmn
1874–1883	95.33n	635.27ij	111.6o	172.07jklmn
1864–1873	88.67k	573.37f	105.3mno	175.97no
1854–1863	62.23b	513.7d	30.43a	167.07fghij
1844–1853	60.73ab	413.93b	86.4i	166.3fghi
1834–1843	80.77hi	608.83h	91.73ijk	168.13fghijk
1824–1833	90.13kl	721.9p	99.9lmn	172jklmn
1814–1823	95.07n	840.1t	98.7klm	172.5jklmn
1804–1813	83.43j	736.37q	61.27ef	169.9ghijklm
1794–1803	78.8gh	645.7j	67.5fg	165.83fgh
1784–1793	214.07x	798.23r	79.53h	169.7ghijkl
1774–1783	83.83j	825.43s	59.27e	171.53ijklmn
1764–1773	74.6def	413.63b	44.93cd	147.07a
1754–1763	74de	441.13c	56.8e	156.8bc
1744–1753	93.57mn	677.53l	46.93d	159.03bcd
1734–1743	129.73t	852.43u	109.47o	164.63efg
1724–1733	83.87j	584.13g	48.33d	154.5b
1714–1723	101.03o	693.8mn	48.6d	160.4cde
1704–1713	106.5q	685.93lm	92ijk	162.7def
1694–1703	109.13r	712.83op	127.9p	159.97cde
1684–1693	145.73v	991.1x	105.5mno	166.03fghi
1678–1683	185.43w	1203.3é	174.57r	172.63jklmno
F values	1421.2***	3163.5***	156.0***	20.5***

Note: According to Duncan's test results, digits followed by the lowercase letters (a, b, c, etc.) show noteworthy differences among age periods within each element.
***$p \le .001$.

Although trace elements are extremely harmful to health, their amounts in the atmosphere are continuously rising. According to research, anthropogenic sources—particularly mining operations (Kuzmina et al., 2023), industrial actions (Istanbullu et al., 2023), and traffic—are the main sources of trace elements (Ucun Ozel et al., 2019). Research has demonstrated that the most efficient methods of mitigating heavy metal contamination are plants, particularly large-mass trees (Koc et al., 2024). Yet, research has indicated that the concentrations of trace elements in many types of wood remain below detectable limits (Key et al., 2022). Thus, in phytoremediation research, species that may build up high amounts of heavy metals in the wood portion are significant.

Table 2.
Correlation Analysis Results

	Mg	Ca	K	S
Age	0.397**	0.136	0.218*	−0.591**
Mg		0.655**	0.435**	0.134
Ca			0.356**	0.512**
K				0.243*

*$p \leq .05$.

The current study determined that the displacement of Mg, Ca, and K elements in wood was limited. This feature is essential for a species to be used as a biomonitor. In various studies, it has been claimed that different species are suitable biomonitors for different heavy metals. For example, *Cedrus deodora* is proper for observing the pollution of Cu (Zhang, 2019), while *Cupressus arizonica* is suitable for Fe, Ni, Cd, Zn, Cr, and Tl (Canturk, 2023; Cesur et al., 2021; 2022; Cobanoglu et al., 2023a; Koc et al., 2024), *Cedrus atlantica* is suitable for Cr, Mn, and Ni (Koç, 2021; Savas et al., 2021), *Picea orientalis* is suitable for Tl (Canturk, 2023), *Pseudotsuga menziesii* is suitable for Cr (Koc et al., 2024), *Corylus colurna* is suitable for Al, Cd, Fe (Key et al., 2022), Zn, Cr, Pb (Key & Kulaç, 2022), Mn, Co, and Ni (Key et al., 2023), that is, the motion of these metal elements in the species' wood is restricted. However, research has shown that Co in *Cedrus atlantica* (Koç, 2021), Zn and Pb in *Cedrus deodora* (Zhang, 2019), Cr, Li, and Bi in *Cupressus arizonica* can be replaced in wood, and these species are not suitable as biomonitors of these elements (Cesur et al., 2021; 2022; Cobanoglu et al., 2023a; Zhang, 2019). In this study, it was determined that the use of black pine as a biomonitor of the S element was not appropriate.

The movement of heavy metals within the species' wood portion is predominantly linked to the cell structure and particularly the cell wall (apoplastic pathway). The apoplast is a flexible form found in plants that senses and signals metal/metalloid stress. It is situated between the plasma membrane (CWPM) and the cell wall. Different kinds of abiotic stressors activate cell wall proteins (CWPs) (Wani et al., 2018). Trees often face strain facets, particularly abiotic strains, in their lives. The tree's most familiar stress factors are climatic pressure factors such as high and low temperatures (Koc & Nzokou, 2023; Arıcak et al., 2024). Tree growth relies on the relationship between genetic design (Kurz et al., 2023) and environmental constraints (Ertugrul et al., 2021; Sevik et al., 2021). Global climate change causes temperature anomalies and strain parameters such as elevated drought, UV-B, and radiation (Cobanoglu et al., 2023b). For this reason, heavy metal accumulation in plants may vary depending on the effects of stress-related factors.

Conclusion and Recommendations

The study's findings showed that every element had a statistically noteworthy and positive correlation with every other element, except for Mg and S. Studies reveal that many elements (Kravkaz-Kuscu et al., 2018) and heavy metals correlate (Istanbullu et al., 2023). A study determined that S has a statistically influential and positive relationship with elements highly harmful to human and living health, such as Se, Ag, Tl, and Sb (Sevik et al., 2024). In this study, it was also found that S had a negative relationship with the wood age. That is, as the age of the wood increases, the S concentration decreases. This variation shows that the S concentration in wood formed in recent years is higher. This finding can be interpreted as S contamination in the air, which has increased in recent years. Many studies indicate that air contamination has elevated significantly in recent years due to anthropogenic factors (Cobanoglu et al., 2023a; Koc et al., 2024).

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

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