Original Research

The Usability of Scotch Pine (*Pinus sylvestris*) as a Biomonitor for Traffic-Originated Heavy Metal Concentrations in Turkey

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Abstract

Heavy metals are one of the most infamous air pollutants. They do not deteriorate easily in nature and they tend to bioaccumulate in nature. Because of their significance in terms of potential damage to human and environmental wellbeing, the monitoring of heavy metal pollution and identifying risk-prone regions is of great importance. Bioindicators are the most important determinants of the change in the concentration of heavy metals in the atmosphere. While plants play the biggest and most important role in reducing pollution in all of its types, they are also the ideal bioindicators. However, some plant species are better equipped to detect heavy metal accumulation than others. This study aims to determine the usability potential of Scotch pine (*Pinus sylvestris*) in monitoring traffic-based heavy metal concentrations. For this purpose, samples of Scotch pine individuals were collected from one of the busiest highways in Turkey (along the Ankara-Istanbul route) from refuges at roadsides and at distances of 3m, 10m, 30m, 50m, and 100m from the roadside. Some of the branches and needles of the samples were also subjected to washing processes and the changes of Ni, Cr and Zn concentrations were determined for these samples. The change of Ni, Cr and Zn concentrations depending on the distance to the road, washing conditions and organelle were evaluated separately. We determined that Scotch pine is a good biomonitor – especially for monitoring changes in Cr concentrations.

Keywords: heavy metal, biomonitor, traffic, Scotch pine, *Pinus sylvestris*

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Introduction

The population of the world has increased more than ever in the last 150 years, and this increase combined with the rural-urban emigration has caused an extreme population density in cities [1-3]. It is estimated that 60-90% of the world's population will live in urban areas by 2030 [4].

Increasing population in urban centers, developing industry and technological developments have caused environmental pollution, which has become one of the biggest problems of the modern world [5-10]. It is stated that approximately 6.5 million people die every year due to air pollution. It has been reported that even in Turkey – which is considered one of the countries with the least amount of air pollution – 29,000 people died of air pollution-related causes in 2016 [11].

Heavy metals are one of the main culprits for causing air pollution. Although micronutrients such as Mn, Zn, Cr, Cu, Fe, and Ni are necessary for living organisms, including plants, they can cause harmful effects at high levels. Metals such as Hg, Cd, As and Pb have serious toxic impacts on organisms – even at low levels [12-14]. In addition, heavy metals do not deteriorate easily in nature and tend to bioaccumulate [4, 10, 13].

It is known that vehicles have a significant share in increasing the heavy metal pollution. The biggest sources of heavy metal emissions are industrial activities and traffic [12, 15]. It has been determined in many studies that there is a significant relationship between traffic density and heavy metal pollution. It has been determined that there is an exact correlation between the amount of some heavy metal concentrations in plant leaves and the distance of the plant to the traffic source [4, 13, 16].

Monitoring heavy metal pollution and identifying risk-prone regions is of great importance. Plants accumulate some of the heavy metals in their bodies via soil or air; and by determining the level of this accumulation, data on heavy metal pollution in soil or air can be obtained [4, 12]. Therefore, the leaves of large plants [16-19], body shells [20], woods [21] and fruits [22] are used as biomonitors.

However, different heavy metals can accumulate at different levels in organelles. Therefore, it is very important to determine the level of each heavy metal accumulation in different organelles, and to use these plants and organelles as biomonitors in order to obtain more reliable results from research. This study aimed to determine the change of some heavy metals in *Pinus sylvestris* depending on the plant organelle and the distance from the main road where the traffic is heavy.

Materials and Methods

Our study was carried out on samples collected from Scotch pine (*Pinus sylvestris*) trees on the highway between Kaynaşlı Disrict of Düzce and Bolu. The highway we chose is one of the busiest highways in Turkey. The samples were collected from the branches that were on the highway side of the trees. The Scotch pine individuals were selected on a specific direction on the highway between Kaynaşlı District of Düzce Province and Bolu Province from the refuge, roadside and at 3 m, 10 m, 30 m, 50 m and 100 m distances. The 50 cm length of the branch samples collected from the roadside parts of seven Scotch pines constitutes the materials of this study.

Samples brought to the laboratory were first divided into groups and some of the samples were washed. The samples were then separated into organelles and the bark of the wood was peeled. Washed needles, unwashed needles, washed bark, unwashed bark and wood samples were obtained. The samples were labeled and kept for 15 days until they got air dried. Air-dried samples were put in glass containers and dried in a drying oven at 50°C for one week.

The dried samples were pulverized by a steel blender in the laboratory. The pulverized samples were made to weigh 2 gr each in 10 ml concentrated HNO_3 at room temperature for 1 day in the fume cupboard, and then boiled at 180ºC for 1 hour. 20 ml of distilled water were added to the prepared solutions and the solutions were filtered through a 45 µm filter paper. The prepared solutions were numbered in order to prevent any mix-ups and prepared for analysis. In the solutions obtained from the filtrate, heavy metal analysis was performed with a GBC Integra XL ICSDS-270 ICP-OES device.

The obtained data were evaluated with the help of the SPSS package program, variance analysis was applied to the data and homogeneous groups were obtained by applying the Duncan test to the values having at least 95% confidence level differences statistically. The obtained data was simplified and tabulated and interpreted.

Results and Discussion

The change in Ni concentration due to organelle and distance to the traffic source was determined separately, and F value and significance level obtained by variance analysis and homogeneous groups resulting from Duncan test are shown in Table 1.

As the result of variance analysis we determined that the change of Ni concentration was significant at 99.9% confidence level for all factors. When the change of Ni concentration due to distance was examined, no significant change was observed in proportion to distance. For example, in the unwashed needle samples, the highest values were obtained in the samples that were collected from closest and furthest distance to the road. A similar situation was also noticeable in wood samples. The lowest values were obtained from the samples that were collected from the roadside and the longest distances.

Table 1. Change of Ni concentration depending on organelle and distance from the road.

Distance	Needle		Bark		Wood	F Value
	Washed	Unwashed	Washed	Unwashed		
0	1015,2 Bd	2394,3 De	939,3 Ab	1597,0 Cd	1039,8 Bc	1737,837***
1	345,7 Aabc	674,3 Bb	924,7 Cb	1143,7Dc	306,1 Aa	54,897***
3	550,7 Ac	6793,6 Eg	4316,6 Dd	973,6 Bb	1294,5 Cd	7462,612***
10	220,7 Aab	197,6 Aa	1236,8 Cc	1695,0 De	1073,2 Bc	240,999***
30	137,7 Aa	1647,8 Cd	599,5 Ba	745,2 Ba	2425,1 De	354,015***
50	386,6 Abc	959,9 Bc	568,2 Aa	1706,0 Ce	4446,2 Df	559,206***
100	1871,4 De	2394,3 Df	1128,0 Bc	1612,9 Cd	540,5 Ab	88,828***
	78,558***	2968,363***	1102,578***	219,551***	614,329***	

When the changes of the Ni concentration depending on organelles were examined, the difference between the washed and unwashed samples was noteworthy. Concentrations in washed samples in almost all samples on both needles and barks were lower than the concentrations in the unwashed samples. The organelle and distance-related changes of Cr concentrations in organelles were determined separately and mean values, F value and significance level obtained as a result of analysis of variance and homogeneous groups as a result of Duncan test are given in Table 2.

We determined that the change of Cr concentration was significant at 99.9% confidence level for all factors. When the change of Cr concentration depending on distance was examined, it was seen that the amount of Cr concentration decreases in most of the samples as distance increases. When the values obtained from 50 and 100 m distances, which were the furthest distances, were examined it was seen that the majority of the data were in the first homogeneous group as a result of Duncan test.

When the change of Cr concentration depending on the organelle was examined, we noted that all the wood samples take place in the first homogenous group resulting from Duncan test. Therefore, the lowest values were obtained in wood samples. Moreover, the difference between wood and other organelles can be five times more in the samples taken from the same branch. It is also noteworthy that the values obtained from the bark were significantly higher than the values obtained from the needles.

The organelle and distance-related changes of Zn concentrations in organelles were determined separately and mean values, F value and significance level obtained as a result of analysis of variance and homogeneous groups as a result of Duncan test are given in Table 3.

As a result of the variance analysis, we determined that the change of Zn concentration depending on distance and organelle was significant at least at 95% confidence level for all factors. When the change of Zn concentration due to distance was examined, no significant change was observed in proportion to distance. From the needles, the lowest and highest values of washed samples were obtained at the nearest distance to the road, while the lowest values of unwashed samples were also obtained at the nearest distance to

Table 2. Change of Cr concentration depending on organelle and distance.

Distance	Needle		Bark		Wood	F Value
	Washed	Unwashed	Washed	Unwashed		
0	419,3 Ac	508,9 Bc	1355,8 Dc	1443,7 Ce	414,2 Ab	449,106***
1	323,3 Ab	536,8 Bc	1093,5 Cb	1165,0 Cd	244,0 Aa	103,365***
3	438,2 Bc	541,0 Cc	677,1 Da	849,7 Ebc	265,2 Aa	69,784***
10	282,0 Aab	418,2 Bb	1428,7 Dc	1210,0 Cd	285,7 Aa	757,441***
30	278,3 Aab	275,8 Aa	717,6 Ba	701,9 Ba	284,3 Aa	46,041***
50	241,4 Aa	585,6 Bc	705,0 BCa	769,1 Cab	365,7 Ab	28,742***
100	274,9 Aab	395,7 Ab	585,5 Ba	932,5 Cc	274,7 Aa	49,678***
	15,763***	13,235***	58,617***	34,260***	14,426***	

Table 3. Change of Zn concentration depending on organelle and distance.

Distance	Needle		Bark		Wood	F Value
	Washed	Unwashed	Washed	Unwashed		
0	5,2 Aa	12,5 Ba	27,9 Db	42,6 Ef	13,3 Cc	83799,042***
1	**30,1 Bf**	34,6 Cg	39,4D c	49,7 Eg	11,3 Ab	10150,938***
3	22,5 Cc	22,5 Cc	19,2 Ba	25,1 Db	17,6 Af	758,679***
10	**44,5 Eg**	31,5 Be	39,5 Dc	35,9 Ce	13,6 Ad	11907,906***
30	22,1 Db	23,9 Ed	18,4 Ca	17,2 Ba	16,1 Ae	1829,296***
50	24,8 Cd	20,7 Bb	27,5 Eb	25,6 Dc	8,7 Aa	6571,769***
100	27,4 BCe	33,7 Cf	18,9 Aa	31,6 Cd	21,3 ABg	6,997**
	12146,249***	8886,543***	20,422***	15090,224***	3441,771***	

the road. A similar situation is also noticeable in wood samples. The highest and lowest values of wood samples were obtained at the longest distances to the road.

As for the organelle-related change of Zn concentration, it is noteworthy that almost all of the wood samples were in the first homogenous group resulting from Duncan test. In addition, the concentration of Zn measured in most of the unwashed samples is higher than that measured in the washed samples.

Ni, one of the elements examined within the scope of the study, is a carcinogenic element for mammals and other animals [12, 23]. Ni is used in coal, petroleum, steel, alloy production, galvanization and the electronics industry [24]. As a result of the study, it was determined that the concentration of Ni on unwashed samples was higher than the concentrations on washed samples in both needle and bark samples. This situation has also been revealed in various studies because heavy metals can hold on to various particles in the atmosphere after spreading [11, 12]. Thus, the heavy metal concentrations on unwashed samples are measured higher compared to the washed samples due to the higher amount of particulate matter on these samples [25].

In this study and similar to former studies, it was determined that Ni concentration in plant organelles can reach up to 4446 ppb. Turkyilmaz et al. [19] stated that Ni concentrations in plant organelles were 4,381 ppm in the non-traffic areas, 5,840 ppm in areas with low dense traffic and up to 10,745 ppm in areas with heavy traffic. Mossi [25] revealed that Ni concentrations in *Buxus sempervirens* reached up to 1639.1 ppb; while Turkyilmaz et al. [19] calculated 4,571 ppm in *Tilia tomentosa*, 4,746 ppm in *Eleagnus angustifolia*, 5,224 ppm in *Ailanthus altissima* and 13,413 ppm in *Prunus cerasifera*.

However, it has been revealed that the Ni concentration is lower in coniferous species. Mossi [25] stated that Ni concentration was 801,7 ppb in *Juniperus sabina* while Turkyilmaz et al. [13] calculated 225,3 ppb in *Pinus sylvestris*, 312.4 ppb in *Pinus nigra*, 566 ppb in *Picea pungens* and 1119.2 ppb in *Abies bornmülleriana*.

And as for the highest values of Ni concentrations they calculated 1801 ppb in 3-year-old needles of *Abies bornmülleriana*. According to these results, it can be said that the value obtained in our study is quite high compared to the results obtained from coniferous species in previous studies.

Cr, one of the heavy metals subjected to this study, is among the most toxic heavy metals in terms of potential toxicities and exposure to living organisms [12, 26]. Asthma attacks can be seen in people who show allergy to chromium, as well as nasal bleeding, nasal discharge, itching and upper respiratory tract perforations in case they take this heavy metal into their body through the air [24]. The dry matter in plants is also toxic to many tall plants at 100 mg / kg [24]. Non-essential metals such as Cr may enter the leaves of the plant through leaf transfer [27].

As a result of the study, we determined that the amount of Cr concentration in plants varies depending on the distance of plants to the traffic source. Therefore, the concentration of Cr increases in regions where traffic density increases. Similar results were obtained in many studies. Turkyilmaz et al. [19] indicated that the concentration of Cr increased up to 23,716 ppm in the areas with heavy traffic while it was 16,595 ppm in on-traffic areas. Sawidis et al., [20] stated that the Cr concentrations in the control group of *Platanus orientalis* leaves were calculated as 0,227 μg/g in Salzburg, 0,404 μg/g in Belgrade and 0,558 μg/g in Thessaloniki, while reaching up to 0,388 μg/g in Salzburg, 0,472 μg/g in Belgrade and 0,621 μg/g in Thessaloniki in samples that were collected from contaminated regions. Similarly, they stated that the Cr concentration amount in control group of *Pinus nigra* leaves was calculated as 0,386 μg/g in Salzburg, 0,333 μg/g in Belgrade and 0,621 μg/g in Thessaloniki; while it reached up to 0,423 μg/g in Salzburg, 0,576 μg/g in Belgrade and 0,661 μg/g in Thessaloniki in samples that were collected from contaminated regions [28].

Within our study it has been determined that the amount of Cr concentration varies significantly

depending on plant organelle, and the amount of Cr concentration in wood samples were very low so that even in the samples that were collected from the same branch, the difference between the amount of Cr concentrations of other organelles were five times more than the amount calculated in wood samples. Apart from this, the values obtained from the bark samples were noticeably higher that the values obtained from the needle samples.

Cr is reported as one of the elements of which the concentration amounts in plants differ most depending on species and organelle [25]. Turkyilmaz et al. [21] reported that the difference between the amount of Cr concentration in wood and bark is almost 9 times greater. Mossi (2018) revealed that the Cr concentration calculated in leaves was approximately 1,44 times more than the amount calculated in branches. Cr concentration was determined to rise up to 1443 ppb in unwashed bark samples. In the study of Mossi [25], while Cr concentration in *Eonymus japonica* was calculated as 846.2 ppb; it was found to increase up to 2330.9 ppb in *Juniperus sabina*.

Zn, another heavy metal subjected to this study, is an essential element for humans, animals and plants [29]. Zn is involved in protein and carbohydrate synthesis in plants. In addition, it is effective on biological membrane stability as well as enzyme activation, photosynthesis and respiratory activities. Therefore, it directly affects the quantity and quality of the plant product [23].

Although Zn is involved in many vital functions in human and animal organisms, it is toxic in high amounts [25]. Zn is used in many fields such as cosmetics, paints, inks, copy papers, linoleum, rubber, and the metal industry in metal coatings and alloys in industry. It reaches the soil through wastewater released from intensive industrial areas, and through acid rain and sewage water. The observed Zn toxicities usually start after 400 ppm. Root growth slows down, roots get thinner, young leaves get curled, chlorosis appears, cell growth and elongation get precluded, cell organelles get degraded and the synthesis of chlorophyll decreases [24].

According to the data obtained from the study, Zn concentration was determined to be lower in wood samples than in other organelles. Similar results have also been revealed in different studies. Turkyilmaz et al. [21] reported that the average Zn concentration measured in wood samples of *Acer platanoides* was 3.59 ppb, while this amount increased to 14.79 ppb in bark samples. Similar results were also obtained in different studies [4, 13, 14, 19, 21].

We also determined that the concentration of Zn measured in unwashed samples was higher than the amount measured in washed samples. Numerous studies have been conducted on the change of Zn concentration depending on plant organelle [19, 30], traffic density [31-33], location [34-36] and plant species [4, 13].

In this study, we determined that the amount of Zn concentration in chosen plants increased up to 49.7 ppm. Similar values were obtained by previous studies. Mossi [25] states that the concentration of Zn reached up to 25,13 ppm in areas with heavy traffic. Similarly, Demirayak et al. [37] stated that they calculated Zn concentration in *L.vulgare* leaves at a level of 70 ppm in Samsun; Tanushree et al. [38] reported 83 mg kg^{-1} of Zn concentration in *Morus alba,* 59 mg kg^{-1} in *Polyalthia longifolia,* 49 mg kg^{-1} in *Ficus bengalensis,* and 42 mg kg^{-1} in *Alstonia scholaris* in India. However, Aksoy and Şahin [39] reported the average amount of Zn concentration in unwashed leaf samples of *E. angustifolia* as 231,26 µgg^{-1} in industrial regions, 83,52 µgg^{-1} in roadside regions, 69,14 µgg^{-1} in a city center, 38,16 µgg^{-1} in suburbs and 22,08 µgg^{-1} in rural areas. Serbula et al. [40] determined that the amount of Zn concentration could reach up to 192,7 mgkg^{-1} in branches of *Robinia pseudoacacia*. Celik et al. [41] stated that they calculated the amount of Zn concentration in *Robinia pseudoacacia* L. samples that were collected from Denizli Province as 456,88 µg g^{-1} in industrial regions, 456,88 µg g^{-1} in a city center, and 81,23 µg g^{-1} in suburbs.

Reducing environmental pollution has become an important agenda in today's urban life quality. Green areas, which are an important part of urban ecosystems, are important implementation tools because they reduce airborne pollution and thus increase quality of life. Various pollutants (industrial, domestic, traffic, etc.) exist in and around cities. Air pollution is quite high in urban roads, which are also open-green areas. The polluted gases in the air are 5-25 times and the dust concentration and particles are 10 times higher in urban areas than the surrounding rural areas [42, 43]. In developing cities of Turkey, while the number of vehicles involved into urban or suburban traffic is rising day by day, the traffic-related pollution has also started to increase [25] (Mossi, 2018). This negatively affects the quality of the environment and increases the importance of urban planting.

It has been determined in studies aimed at determining air pollution in cities that air pollution varies according to many factors such as traffic density, climate change, wind direction and precipitation [2, 44, 45]. Therefore, the concentration of heavy metals in the air may vary depending on many parameters. In order to monitor this change and to effectively use plants in order to reduce heavy metal concentrations, studies on this subject are of great importance.

Conclusions

In the selection of the plants used in urban centers, visual qualities are generally prioritized and their functional uses are of secondary importance. However, plant species should be determined primarily by considering which plant species is more effective at performing the desired function.

Scotch pine is an appropriate plant species to use in urban plantations for being quite resistant even to cold climate conditions as well as being evergreen. Also, being an evergreen coniferous and of low maintenance, requiring a plant with low needs of soil and water makes this species a valuable landscape plant. In addition to all these advantages this study revealed that this species is a good biomonitor of Cr pollution.

Currently, air pollution in city centers is one of the most important problems of cities. Therefore carrying on this type of research by dissemination and diversification is of great importance in order to determine the most effective species for the removal of heavy metals from the air. Beside determining appropriate species for biomonitoring, organelle and air conditioning-based studies must also be conducted in order to reveal the heavy metal accumulation depending on plant organelle and air conditions in future studies.

Conflict of Interest

The authors declare no conflict of interest.

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