

THE CHANGE OF SOME HEAVY METAL CONCENTRATIONS IN SCOTCH PINE (*PINUS SYLVESTRIS*) DEPENDING ON TRAFFIC DENSITY, ORGANELLE AND WASHING

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Abstract. Increasing population and industrialization have caused air pollution and air pollution in some cities has increased to such an extent that it has started to threaten human health and has become one of the most important agenda topics of our day. Many pollutants arise in cities due to exhaust gases, car wheels, vehicles and vehicle wear. Heavy metals are one of the most infamous pollutants, because they can remain in nature for a long time without degradation and the amount of heavy metal concentration in the environment is constantly increasing. Heavy metals also tend to bioaccumulate. Therefore, the determination of heavy metal concentration is of great importance in terms of identifying risk regions and risk levels. Determination of heavy metal concentrations in plants is important both for determining plants' ability to remove heavy metals from the air and thus to improve air quality, as well as for monitoring air quality. Bio-indicators are the most important indicators of the change in the concentration of heavy metal in the atmosphere. This study aims to determine the usability potential of Scotch Pine (*Pinus sylvestris*) in monitoring the traffic related heavy metal concentration. For this purpose, samples of Scotch Pine individuals were collected from one of the busiest highways of Turkey, at the route of Ankara-Istanbul, from refuges, at roadsides and at distances of 3 m, 10 m, 30 m, 50 m and 100 m from the roadside, some of the branch and needle samples were washed and the change of Cu, Pb and Cd concentrations on these samples was determined. As a result of the study, the change of the concentrations of these heavy metals depending on distance to the road, organelle and washing conditions all seem to indicate that the Scotch Pine is a good bio-monitor which could be used especially to monitor the change of Cd concentration.

Keywords: *bioaccumulate, bio-monitor, highway, plant, pollution, Cd concentration*

Introduction

In addition to the rising population of the world, the increasing population density in city centers has brought various problems with it (Cetin and Sevik, 2016a, b; Gülgün et al., 2014; Cetin et al., 2017a, b, 2018a, b, c, d). This process causes destruction of nature, pollution of air, water and soil, and deterioration of the ecological balance (Mutlu, 2016; Mutlu et al., 2016; Kaya et al., 2018). This process brings many problems with it, but one of the biggest problems is the pollution of environment and especially air (Sevik et al., 2018). Air pollution has become such a big problem that approximately 30 million people die every year due to air pollution related reasons (Cetin et al., 2018).

Air pollution can be defined as the existence of one or more contaminants in the air in amounts and durations which could be harmful to humans, animals and plant life and commercial or personal properties and environmental quality (Cetin et al., 2018).

While there are many components responsible for air pollution, heavy metals are one of the more significant among them. Heavy metals do not deteriorate and disappear in nature.

There are various methods that are used to determine the air pollution. Direct measurement of air pollution via these methods requires both expensive measuring instruments and has a higher risk of contamination compared to bioindicators. Plants have many environmental, social and economic contributions to the environment in which we live, one of which is their use as a bio-indicator in the detection and monitoring of air pollution (Gülgün et al., 2015; Cetin et al., 2018b). Using bioindicators is one of the most effective methods of determining air pollution.

This method is inexpensive and easy to provide, as well as its ability to provide more accurate data on the periodic change of heavy metal concentration. Therefore, many studies have been conducted to determine the change of heavy metal concentrations via bioindicators (Sevik et al., 2018; Turkyilmaz et al., 2018b).

However, different heavy metals can accumulate at different levels in the organelles of the plants. Therefore, it is very important to determine the accumulation level of each heavy metal in each organelle to enable the usage of the right organelle of the right plant as biomonitors in order to get more reliable results from the studies. In this study, it was aimed to determine the change of some heavy metals concentrations depending on the plant organelles and the distance to the road with heavy traffic in Scotch Pines (*Pinus sylvestris*).

Materials and methods

Materials

The study was conducted on the samples collected from the Scotch Pines from the roadside on the Ankara-İstanbul highway between Kaynaşlı province of Düzce city and Bolu city. The highway that was chosen as the case of this study is one of the busiest highways in Turkey. Samples were especially taken from the closest branches to the road. 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 half-meter length of branch samples collected from the road side parts of seven Scotch Pines constitute the materials of this study (*Table 1*). The samples used in the study were collected in the last week of September following the end of the vegetation season of 2018.

Table 1. The exact locations of sampling indicated by GPS coordinates

Distance (m)	Coordinates DMS (degrees, minutes, seconds)	
	Latitude N	Longitude E
0	40°46'32.225"	31°19'41.524"
1	40°46'45.450"	31°19'25.086"
3	40°46'48.105"	31°19'53.024"
10	40°47'09.660"	31°18'22.076"
30	40°47'36.155"	31°18'39.323"
50	40°48'07.464"	31°17'23.096"
100	40°48'23.124"	31°17'15.233"

Determination of heavy metal concentrations

Samples brought to the laboratory were firstly 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; and 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 taken into glass containers and dried in 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 g each in 10 ml concentrated HNO₃ at room temperature for 1 day in the fume cupboard, and then boiled at 180 °C for 1 h. 20 ml of distilled water were added to the prepared solutions and the solution was 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 the GBC Integra XL ICSDS-270 ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) device (Sevik et al., 2018; Turkyilmaz et al., 2018b).

Statistical analysis

The obtained data were evaluated with the help of 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 is simplified and tabulated and interpreted (Sevik et al., 2018; Turkyilmaz et al., 2018b).

Results

The change in Cu 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 2*.

As the result of the variance analysis, it was determined that the change of Cu concentration was significant at a 99.9% confidence level for all factors. When the change of Cu concentration due to distance was examined, no significant change was observed. For example, in the unwashed bark samples, some of the lowest and the

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

Table 2. The change of Cu (ppm) concentration depending on organelle and on distance from the road

Distance (m)	Needle		Bark		Wood (ppm)	Value (ppm)
	Washed (ppm)	Unwashed (ppm)	Washed (ppm)	Unwashed (ppm)		
0	1642.8 Ce	783.7 Ac	1274.6 Bc	6094.9 Df	6461.1 Eg	3615.463***
1	786.5 Bb	220.4 Aa	2956.5 De	1620.2 Cc	128.7 Aa	897.041***
3	1034.6 Bc	559.3 Ab	1295.5 Cc	1020.1 Bb	2728.2 Dd	294.611***
10	1909.5 Cg	1124.9 Ad	1686.0 Bd	1880.3 Cd	3170.6 De	193.553***
30	1787.9 Df	1572.0 Ce	503.1 ABa	332.0 Aa	561.2 Bb	127.001***
50	1418.0 Dd	1482.4 De	712.5 Bb	194.3 Aa	834.8 Cc	203.899***
100	550.2 Aa	1805.6 Cf	1321.8 Bc	2759.7 De	5296.4 Ef	1390.201***
	237.794***	174.617***	209.164***	1024.679***	3880.312***	

*Significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level

The letters a, b, c, etc. means according to Duncan test results; show that the group is located. It is statistically different from the values contained in different groups, starting with the letter a, the numerical value grows

Capital letters in rows; lower case letters show groupings in columns. For example, the concentration of Cu in the washed needle is also as the distance of the fifth; C is in the third homogeneous group

When examining the change of Cu concentration depending on the organelles; it is noteworthy that the concentration amounts in wood samples were considerably high. It was observed that the amount of the Cu concentration in wood samples were higher than the values obtained from the other organelles.

The change in Pb 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 3*.

When the results of the analysis of variance in Pb are examined, it is noteworthy that Pb concentration in wood does not differ statistically significant at least at 95% confidence level depending on distance. Apart from this, it can be said that the change of Pb concentration due to distance is statistically meaningless. However, when the change of Pb concentration is examined depending on organelle, it is observed that all values obtained from the wood samples are quite low and all the values obtained from the wood samples are in the first two homogenous groups that were formed as the result of Duncan test. Furthermore, it was observed that the values obtained from needles were lower than the values obtained from the bark samples, while the values of washed samples were also lower than the values of unwashed samples.

The change in Cd 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 4*.

As the result of the variance analysis, it was determined that the change of Cd concentration was significant at a 99.9% confidence level for all factors. When the

change of Cd concentration due to distance was examined, it was seen that Cd concentration decreases generally as the distance increases.

Table 3. The change of Pb (ppm) concentration depending on organelle and distance

Distance (m)	Needle		Bark		Wood	Value (ppm)
	Washed (ppm)	Unwashed (ppm)	Washed (ppm)	Unwashed (ppm)	Unwashed (ppm)	
0	267.9 Abc	337.1 Ab	1246.2 Bb	1304.1 Bbc	361.6 A	41.008***
1	93.6 Aa	765.6 Ccd	1592.2 Dc	1766.1 Dd	421.7 B	122.962***
3	371.4 Ac	1042.4 Be	1110.1 Bb	1023.0 Ba	273.1 A	22.517***
10	258.2 Abc	458.5 Ab	2131.8 Dd	1778.7 Cd	404.7 A	82.814***
30	150.1 Aab	74.3 Aa	947.6 Cb	1070.8 Cab	581.6 B	39.022***
50	321.2 Ac	656.2 Bc	1089.1 Cb	1196.7 Cab	371.2 A	33.357***
100	219.4 Aabc	942.3 Cde	395.2 Aa	1455.2 Dc	581.2 B	70.513***
	3.936*	31.334***	27.357***	15.223***	2.096ns	

*Significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level

The letters a, b, c, etc. means according to Duncan test results; show that the group is located. It is statistically different from the values contained in different groups, starting with the letter a, the numerical value grows

Capital letters in rows; lower case letters show groupings in columns. For example, the concentration of Cu in the washed needle is also as the distance of the fifth; C is in the third homogeneous group

Table 4. The change of Cd (ppm) concentration depending on organelle and distance

Distance (m)	Needle		Bark		Wood	Value (ppm)
	Washed (ppm)	Unwashed (ppm)	Washed (ppm)	Unwashed (ppm)	Unwashed (ppm)	
0	40.8 Ac	65.9 Ac	172.4 Ce	157.0 BCc	138.8 Bb	36.731***
1	14.7 Aa	32.8 Bab	81.5 Cc	69.9 Ca	45.4 Ba	35.040***
3	25.9 Ab	41.1 Bb	80.6 Dc	53.9 Ca	43.2 Ba	95.119***
10	17.3 Aa	25.0 Aa	114.0 Cd	95.0 Cb	46.0 Ba	45.881***
30	19.0 Aa	25.2 Aa	73.6 Dbc	50.9 Ca	39.9 Ba	31.902***
50	26.6 Ab	26.0 Aa	61.4 Cab	57.5 Ca	40.4 Ba	103.357***
100	19.3 Aa	36.4 Bab	46.9 Ca	65.2 Da	39.5 BCa	27.582***
	30.335***	14.796***	49.714***	24.435***	58.942***	

*Significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level

The letters a, b, c, etc. means according to Duncan test results; show that the group is located. It is statistically different from the values contained in different groups, starting with the letter a, the numerical value grows

Capital letters in rows; lower case letters show groupings in columns. For example, the concentration of Cu in the washed needle is also as the distance of the fifth; C is in the third homogeneous group

When the change of Cd concentration depending on the organelles was examined, it was determined that the lowest values were obtained from the needle samples, the highest values were obtained from bark samples, and the values obtained from wood samples were higher than the values of needle samples while lower than the values of bark samples. Apart from that, the Cd concentration amount calculated in unwashed

needle samples were generally higher than the washed needle samples, and the Cd concentration amount calculated in washed bark samples were higher than the unwashed bark samples.

In addition, the Cd concentrations obtained from the unwashed samples in the hands are generally higher than the washed samples and the Cd concentrations in the washed samples are higher than the Cd concentrations obtained in the washed samples.

Discussion

Plants are often used as biomonitors in monitoring the heavy metal concentration. Some of the species that are used as biomonitors of traffic related air pollution are; *Aesculus hippocastanum* (Anicic et al., 2011), *Elaeagnus angustifolia* (Aksoy and Şahin, 1999), *Robinia pseudoacacia* (Celik et al., 2005), *Tilia* sp. (Tomasevic and Anicic, 2010), *Quercus ilex* (Gratani et al., 2008) and some other species (Ozturk and Bozdogan, 2015; Mossi, 2018).

Cu, one of the heavy metals that were evaluated within the scope of this study is quite an important element for plant organisms due to its involvement in enzyme activation, carbohydrate and lipid metabolism (Asri and Sönmez, 2006). It has been determined that copper plays an important role in physiological events such as photosynthesis, respiration, carbohydrate degradation, nitrogen use and storage, cell wall metabolism, and that it regulates xylem permeability, controls the production of DNA and RNA and plays an important role in resistance mechanism against diseases. In case of copper deficiency, it is stated that plant growth stopped (Okcu et al., 2009).

Although plant species need different amounts of it, copper is a highly toxic metal. Some effects of copper poisoning include tissue damage, deterioration of roots and darkening of plant color. Other effects of copper poisoning are, loss of ion as a result of deterioration of membrane permeability in the stem cells and disruption of photosynthesis process as a result of DNA damage (Okcu et al., 2009). Acute copper intoxication may cause abdominal pain, nausea, vomiting and diarrhea in humans (Asri and Sönmez, 2006). While taking low amounts of copper ions may cause liver cirrhosis, Wilson's disease, systemic rheumatic diseases, kidney diseases; taking high amounts of copper ions may cause blood cancer (Mossi, 2018). Therefore, many studies have been conducted to determine Cu concentration in plants and to correlate with traffic density (Turkyilmaz et al., 2018a, b).

Turkyilmaz et al. (2018c) stated that Cu concentration amount in plant organelles differ depending on traffic density and that while they calculated the Cu concentration at 69.615 ppb in areas with none-traffic, it was 71.096 ppb in areas with low dense traffic and it reached up to 110.441 ppb level in areas with heavy traffic. Suzuki et al. (2009) determined that the concentration of Cu could reach up to 22.22 mg kg⁻¹ in the leaves of *Rhododendron pulchrum* in Okayama, Japan; while Demirayak et al. (2011) reported that Cu concentration was averagely at 35 ppm level in the leaves of *M. grandiflora* in Samsun city. Li et al. (2007) stated that the amount of Cu concentration in *Sophora japonica* L. leaves were higher in the individual trees on roadside than the ones in the parks.

Sawidis et al. (2011) determined that the Cu concentration in *Pinus nigra* leaves in the control group was calculated at 3.182 µg/g in Salzburg; 3.263 µg/g in Belgrade; and 2.432 µg/g in Thessaloniki; while it increased up to 4.875 µg/g in Salzburg; 25.391 µg/g in Belgrade and 16.486 µg/g in Thessaloniki in contaminated regions.

Erdem (2018) in his study on different species that the Cu concentration between 0.16 and 24.66 ppm and depending on the intensity of the traffic varies depending on a high level of values, for example, *Ailanthus altissima* in the areas of heavy traffic in the Cu concentration of the determined concentration of Cu in areas where the approximately 80 times. Pınar (2019) in his study of five species on the Cu concentration of 3 ppm to 286.5 ppm ranged between the highest value of the traffic is concentrated in the linden seeds.

Cu concentration in wood samples was higher than in the other organelles. This result is generally consistent with the literature. Cu concentration varies in different species, depending on species and organelles (Mossi, 2018; Turkyilmaz et al., 2018d).

In his study of four different types, Erdem (2018) states that the Cu concentration changes significantly on the basis of the organelle. In some species, the highest values are determined on the leaf and in some species on the branches and seeds. Similar results were obtained by Özel (2019) and Pınar (2019). Pb is one of the other heavy metals that were evaluated within this study. Pb concentration is of great importance among the other heavy metals. Pb, which is a widely used element in industrial and agricultural activities, is a heavy metal that is emitted to the atmosphere as a metal or compound and in each case has toxic properties. Pb is one of the heavy metals that cause the most damage to the ecological system by human activities (Mossi, 2018). Therefore, a large number of studies have been performed on the change of Pb due to traffic density (Lei et al., 2015; Assirey et al., 2015; Galal et al., 2015).

In the study, it was determined that the values obtained in the wood samples were quite low, and the values obtained from the needle samples were lower than the values obtained from the bark samples and the values obtained from the washed samples were lower than the values obtained from the unwashed samples. Mossi (2018) found similar results in different studies; he determined that the lowest values of Pb concentrations were obtained from washed samples while the highest values were calculated in the unwashed samples in areas with heavy traffic.

Pb with more than the normal levels can be found in the foods of animal and vegetable origin that are grown in areas close to the city centers and industrial regions (França et al., 2017; Mossi, 2018). In addition, lead-containing gasoline is an important source (Okcu et al., 2009). Therefore, there are numerous studies documenting the relationship between Pb and traffic density (Qing et al., 2015; Begum et al., 2017).

Erdem (2018) stated that different types of Pb concentrations vary significantly depending on the traffic density, whereas the average Pb concentration in areas where there is no traffic is 346 ppb is 635 ppb in areas with low traffic and 1782 ppb in high traffic areas. Pınar (2019) stated that Pb concentration increased with traffic density and this level was quite high in some organelles.

There are many studies on the change of Pb in organelle and species. In studies, it is stated that Pb changes significantly on organelle basis. For example, Akarsu (2019) stated that the Pb concentration in *Cedrus arizonica* wood was 1619 ppb and this value increased to 5902 ppb in the outer shell. Ozel (2019) indicates that the concentration of Pb in the mulberry shells in the areas where there is no traffic is increased to 2630 ppb in the leaves. The same figures in the areas where traffic is 5352 ppb in the shell, 11582 ppb level in the leaf (Ozel, 2019).

Pb is one of the most studied elements due to the properties of this element. Numerous studies have been conducted on many species to be used as the biomonitor of Pb. Some of the studies that were aimed to determine the change of Pb concentration in

different species depending on traffic density are; Aksoy and Şahin (1999) *Elaeagnus angustifolia*, Tam et al. (1987) *Bauhinia variegata*, Çelik et al. (2005) *Robinia pseudoacacia*, Demirayak et al. (2011) *M. grandiflora* and *A. Cyanophylla*, Tanushree et al. (2011) *Alstonia scholaris*, *Ficus bengalensis*, *Morus alba*, and *Polyalthia longifolia*, Sawidis et al. (2011) *Platanus orientalis* (Ozel et al., 2015), *Pinus nigra*, Li et al. (2007) *Sophora japonica*.

Another heavy metal that has been mostly studied is Cd. Cd is a highly toxic metal that comes to the fore with a variety of usage areas and with its role in the environmental pollution. The carcinogen effect of Cd in human body was identified in 1976 and it was classified as Type 1 carcinogen by IARC (International Agency for Cancer Research) in 1993 (Boğa, 2007). Cd is an element that is toxic for both humans and animals as well as plants (Asri and Sönmez, 2006; Boğa, 2007). Not only is it toxic for the human body even at very low doses, but it also has a long biological half-life.

In addition to industrial activities, cadmium is spread through phosphorous fertilizers used in agriculture, sewage wastes in residential areas and atmospheric deposits. It is estimated that 0.2-1.0 mg/m² of Cd is added annually in the soils of the roadsides in areas with heavy traffic (Asri and Sönmez, 2006). In our study, it was determined that Cd concentration decreased as the distance to the road increased. In addition, the lowest values were obtained in needles while the highest values were obtained in bark samples.

The results of the study revealed that the amount of Cd changed depending on both traffic density and organelle. In a large number of studies, it was determined that the concentration of Cd varies according to plant species, traffic density and plant organelle (Mossi, et al., 2018; Turkyilmaz et al., 2018e). Ozel (2019) Cd concentration, depending on the type, organelle and traffic density, Akarsu (2019) *Cedrus arizonica* facing the road in the average Cd concentration of wood at 157 ppb level of this value is 2601 ppb level in the outer shell.

In this study, the possibility of using pine as a biomonitor to determine the heavy metal pollution in the air was investigated. It is stated in the studies that it can be used as biomonitor in determining the heavy metal pollution of traffic origin of pine in different areas (Turkyilmaz et al., 2018b). In order for a species to be used as a biomonitor for monitoring heavy metal pollution, it must first be able to collect heavy metals within that species (Bat et al., 1999). Plants that can collect heavy metals in the air can also remove heavy metals from the air. Therefore, they can clean the air in terms of heavy metal. Therefore, the plants that can collect heavy metals within the body can also contribute to the cleaning of the heavy metal. This situation was also expressed in other studies (Saleh, 2018; Mossi, 2018; Erdem, 2018). In this study, it was determined that pine can collect some heavy metals within its structure. Therefore, in the environment where yellow pine is grown, it will help to remove heavy metals from the air and contribute to the cleaning of air pollution.

Conclusions

Expanding green areas is one of the most effective methods among the solution proposals to tackle air pollution. In many studies, it has been proved that green areas and the plants used in these areas reduce all kinds of air pollution in different ways (Cetin and Sevik, 2016a, b; Cetin et al., 2017).

Although a large number of plant species have been the subject of studies to date, these studies are not yet sufficient. There is no information about the potential of many

plant species to accumulate heavy metals. However, it has been reported in numerous studies that there are great differences between the heavy metal deposition potentials of plant species. Therefore, it is necessary to use the species that are not examined yet in similar studies and to identify the plants that will be more effective in monitoring and reducing the heavy metal pollution. Therefore, it is suggested to keep similar studies going by diversifying the studied plant species.

In the selection of the plants used in urban centers, their visual qualities are generally prioritized and their functional uses are made to be of secondary importance. However, in order to use the plants in a proper manner; it is vital to determine the more effective species to perform the desired function and the selection of the species in such areas should be made accordingly. Scotch pine is a tree species that can be used for forestation of the urban areas especially due to being an evergreen species as well as being extremely resistant to cold climates. Also, as it has low needs for maintenance, soil and water tree, it is a valuable landscape plant. It is determined that it is also a good biomonitor especially for the monitoring of Cd pollution.

Within the scope of the study, measurements have only been conducted on needles, branches and wood samples of the tree. However, former studies have proved that the heavy metal concentration in different organelles of the plants such as root, fruit, etc. can be higher. Therefore, the low concentration in the needles, branches and wood samples of a tree may not mean that the plant does not accumulate heavy metals as the plant may have a significant amount of heavy metal concentration in its other organelles. The inclusion of other organelles of the plants in the studies to be carried out in this field may provide important results.

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