Original Research

Using the Turkish Red Pine Tree to Monitor Heavy Metal Pollution

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Abstract

Turkish red pine is an evergreen tree species widely distributed in the Aegean and Mediterranean regions in Turkey. In the present work, the heavy metal pollution level in Istanbul was investigated using Turkish red pine as a biomonitor. For determining heavy metal concentrations (Cd, Co, Cr, Mn, Ni and Pb) in leaf (unwashed and washed) and bark samples of Turkish red pine and its co-located soil samples we used ICP-OES. Additionally, particulate matter profiles of the research areas were investigated. The samples were collected from 5 different localities: 4 from the Bosporus region (Yildiz Grove, Fethi Pasha Grove, Baltalimani Grove and Mihrabad Grove) and one from Prince Island, also known as Buyukada (as control). According to our measurements, the highest heavy metal accumulations (in mg kg^{-1} DW) in plant part samples were measured between 1.526 ± 0.012 and 1.639 ± 0.015 for Cd, 0.543 ± 0.007 and 0.600 ± 0.009 for Co, 5.110 ± 0.064 and 5.648 ± 0.072 for Cr, 9.347 ± 0.105 and 10.331 ± 0.111 for Mn, 7.483 ± 0.091 and 8.271 ± 0.096 for Ni, and 13.848 ± 0.159 and 14.950 ± 0.167 for Pb, while the highest heavy metal accumulations (in mg kg^{-1} DW) in soil samples were measured between 1.813 ± 0.021 and 1.974 ± 0.029 for Cd, 6.326 ± 0.082 and 6.992 ± 0.091 for Co, 22.017 ± 0.284 and 23.685 ± 0.301 for Cr, 268.333 ± 3.153 and 297.361 ± 3.529 for Mn, 15.194 ± 0.176 and 16.792 ± 0.193 for Ni, and 68.778 ± 0.715 and 74.514 ± 0.883 for Pb. The highest outdoor particulate matter levels (in µg/m³) in research areas were also determined as 27.103 for fine (PM$_{2.5}$) and 67.792 for coarse (PM$_{10}$) aerosols. The findings revealed that Turkish red pine could accumulate noteworthy amounts of heavy metals.

Keywords: heavy metals, particulate matter (PM), *Pinus brutia*, contamination

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Introduction

The levels of heavy metal pollution in the biosphere have substantially increased since the 1900s [1]. The long-term release of heavy metals into the atmosphere has resulted in environmental pollution at ever-increasing rates. As a result, environmental pollution has been a major reason for human health problems globally [2, 3]. The number of studies on heavy metal pollution has extensively increased in the last few decades, which has raised environmental awareness and spurred necessary protective actions [4-6].

Metals with a density greater than 5 g/cm³ are defined as heavy metals [7]. Some heavy metals such as As, Cd, Cr, Hg and Pb are naturally available, but they are not required for living organisms since even their minute amounts could be detrimental [8, 9]. Heavy metal-related environmental pollution, particularly following industrial revolution, have caused grave ecological problems [10]. Once incorporated into biological systems, heavy metals are accumulated to a certain point, and excess levels lead to the toxicity resulting in various symptoms or even loss of viability [11, 12]. Therefore, it is necessary to take measurements on a regular basis for determining the level of environmental pollution, especially for people living in crowded cities. Especially in underdeveloped or developing countries, the application of advanced technologies and implementation of necessary measurements on pollution prevention has been poor due to insufficient environmental regulations, and unplanned and uncontrolled urbanization [13].

The data from WHO attributes to indoor air pollution shows a globally significant number of deaths (more than 1.5 million) and a high percentage of disease existence (2.7% of total). Indoor air pollution as a major risk factor responsible for 3.7% of global disease in developing countries comes after malnutrition, the HIV/AIDS epidemic and lack of safe water and adequate sanitation. Smoke during cooking activities makes women and children most vulnerable to indoor hazardous pollutants [14].

Today, not only soil, water and air measurements, but also biomonitor species or parts of these biomonitor species are commonly used for the assessment of environmental pollution [15, 16]. However, a biomonitor is an organism that provides quantitative information about environmental quality [17-19]. In pollution studies, plant (root, leaf and bark etc.), animal (liver, heart and lung etc.) and human (skin, hair, blood, milk, urine, mucus, nail, and saliva, etc.) parts could be utilized as bioindicators [20]. In contrast, in recent years various parts of higher plants, lichens, mosses, algae, yeast, and fungal species have been extensively used in biomonitoring activities [21-23]. Particularly evergreen higher plants, rather than annuals, are preferred in biomonitoring studies since they could provide more relevant information on short/long-term toxicity levels [24, 25].

Some studies on Turkish red pine, an evergreen tree from the Pinaceae family widely distributed throughout the Mediterranean and Aegean regions of Turkey, revealed its biomonitoring ability [26-28]. Because of its long-term accumulation capability for heavy metals, Turkish red pine was used as a biomonitor organism in order to investigate pollution levels in Istanbul. In this regard, estimating Cd, Co, Cr, Mn, Ni and Pb concentrations was done using leaf (unwashed and washed) and bark samples of Turkish red pine and its co-located soil samples collected from five different locations. In addition, for short-term estimation, the levels of PM_{10} and $PM_{2.5}$ in research areas were also determined.

Materials and Methods

Study Area

Our study area, Istanbul, is located in northwestern Turkey and covers a total of 5343 km². The city extends both on the Asian (Anatolia) and European (Thrace) sides of the Bosphorus in the Marmara Region [29]. At the end of 2019, it is population reached more than 15 million – making it the most populous city in Turkey with 18.6% of the population [30]. As a financial and cultural center, this megacity has 39 districts administered by Istanbul Municipality. About 20% of industrial labor of Turkey is employed in Istanbul; thus, it contributes 38% of Turkey's industrial workspace [31].

Plant and soil samplings were performed at five different locations: Buyukada (as control), Yildiz Grove, Fethi Pasha Grove, Baltalimani Grove and Mihrabad Grove (Fig. 1). Buyukada covers an area of 5.4 km² and Maltepe shore is the nearest terrestrial location to the island at a distance of 2300 m. The island is less populated in comparison with other research areas. It has almost no motor vehicle traffic, especially in winter [31]. Yildiz Grove is located in Besiktas, a historic district of Istanbul, covering an area of about 500000 m² [31]. Fethi Pasha grove, situated in Uskudar District between Kuzguncuk and Sultantepe neighborhoods on the Asian side, covers 16 hectares of green area. Baltalimani Grove, located on the European side of Istanbul, extends from Hisar Kemal Street to Kemal Caglar Street, covering the hills between these streets. Mihrabad Grove, situated in Kanlica District on the Asian side on a vast area of 200 acres, harbors many plant and tree species [32].

Sample Collection, Preparation and Analysis

Samples were collected from the above-mentioned locations. Leaf samples were divided into two groups: for performing analysis, one half was rinsed with de-ionized distilled water for removing dust particles on leaves and the other half was used without using de-ionized distilled water. The isolated plant parts
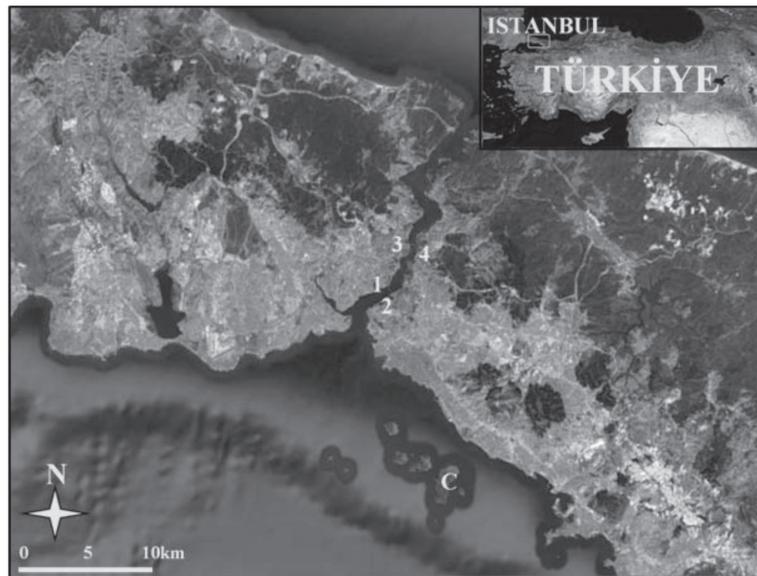
Fig. 1. Satellite map showing Istanbul Province of Turkey (modified from Google Earth); sampling sites are indicated in yellow: C for Buyukada (as control), 1 for Yildiz Grove, 2 for Fethi Pasha Grove, 3 for Baltalimani Grove, and 4 for Mihrabad Grove.

(barks and leaves) were dried in an oven at 80ºC for 24 h. After that, weighted 0.2 g of each plant part sample was transferred into Teflon vessels and then 8 ml 65% HNO_3 (Merck) was added to each sample. Soil samples (~500g each) taken from a depth of about 10 cm using a shovel were dried by air and sieved using a 2mm sifter. Then, 6 ml HNO_3 (65%), 3 ml HCl (37%) and 2 ml HF (48%) (Merck) were added to 0.250 g of each soil sample. Sample mineralization was done using a microwave oven (Berghof-MWS2) at 145ºC for 5 min, 165ºC for 5 min, and 175ºC for 20 min. After cooling, the samples were passed through a 1-2 μm filter paper and filled up to 50 ml volume with ultrapure water in falcon tubes [8]. Using multi-element stock solutions, 1000 ppm (Merck), standard solutions were prepared. The concentrations of Cd, Co, Cr, Mn, Ni and Pb were determined using inductively coupled plasma optical emission spectroscopy (PerkinElmer-Optima 7000 DV). An aerosol monitor (TSI DustTrak II-8530) was also employed for measuring the mass fraction concentrations of PM_{10} and $PM_{2.5}$ [33]. The field measurements were done for three days during sampling periods.

Statistical Analysis

Statistical analysis and other calculations were performed using the data from heavy metal concentration measurements on a dry weight (DW) basis. Multivariate analysis of variance (MANOVA) with Tukey's post-hoc HSD, Pearson correlation and hierarchical cluster analysis were done using IBM SPSS Statistics 20 software. The levels of statistical significance were expressed as 0.01 (**) and 0.05 (*) levels (2-tailed). Tukey's post-hoc test was applied for localities.

Results and Discussion

The concentrations of Cd, Co, Cr, Mn, Ni and Pb in barks and leaves of Turkish red pine and its co-located soil samples from five different Istanbul locations were determined using ICP-OES. In addition, the terms "washed" and "unwashed" will be abbreviated hereafter as "w" and "uw."

The average concentrations of heavy metals (Cd, Co, Cr, Mn, Ni and Pb) in plant parts and soil samples showed the highest values at station 1, whereas the lowest were found at the control station. In the plant samples the highest values and lowest values (in mg kg^{-1}) were recorded as: 1.526±0.012-1.639±0.015 (in bark) and 0.297±0.003-0.316±0.005 (in w leaf) for Cd; 0.543±0.007-0.600±0.009 (in uw leaf) and 0.060±0.001-0.067±0.001 (in bark) for Co; 5.110±0.064-5.648±0.072 (in uw leaf) and 0.819±0.011-0.907±0.013 (in bark) for Cr; 9.347±0.105-10.331±0.111 (in uw leaf) and 3.543±0.051-3.921±0.055 (in w leaf) for Mn; 7.483±0.091-8.271±0.096 (in uw leaf) and 0.829±0.010-0.949±0.013 (in w leaf) for Ni; and 13.848±0.159-14.950±0.167 (in bark) and 3.718±0.048-4.285±0.057 (in w leaf) for Pb, respectively (Fig. 2), while in the soil samples the highest values and lowest values (in mg kg-1) were recorded as: 1.813±0.021-1.974±0.029 and 0.744±0.009-0.844±0.011 for Cd; 6.326±0.082-6.992±0.091 and 3.358±0.044-4.328±0.052 for Co; 22.017±0.284-23.685±0.301 and 11.931±0.159-13.194±0.181 for Cr; 268.333±3.153-297.361±3.529 and 140.694±2.046-145.556±2.225 for Mn; 15.194±0.176-16.792±0.193 and 7.042±0.092-7.782±0.102 for Ni; and 68.778±0.715-74.514±0.883 and 29.991±0.305-32.479±0.411 for Pb, respectively (Fig. 3).

The normal worldwide distributions of the studied heavy metals in mg kg^{-1} in soils are in ranges of:

0.06-1.1 with 0.5 global average for Cd; 4.5-12 with 10 global average for Co (in surface soils); and 54 (global average) for Cr [34]. Generally, the Mn contents (in mg kg^{-1}) in soil lie between 270 (podzols) and 525 (cambisols), but overall the worldwide average is found to be 437 [35]. However, the Mn contents (in mg kg^{-1}) in soils are highly diverse worldwide and range from 10 to 9000. Ni is one of the micronutrients and also a heavy metal, and it occurs in soils (in mg kg^{-1}) in the range of 0.2-450 with the average between 19-22. The natural Pb concentrations (in mg kg^{-1}) originated from the main rocks in regions between 10 and 40. with a grand average of 25 [34].
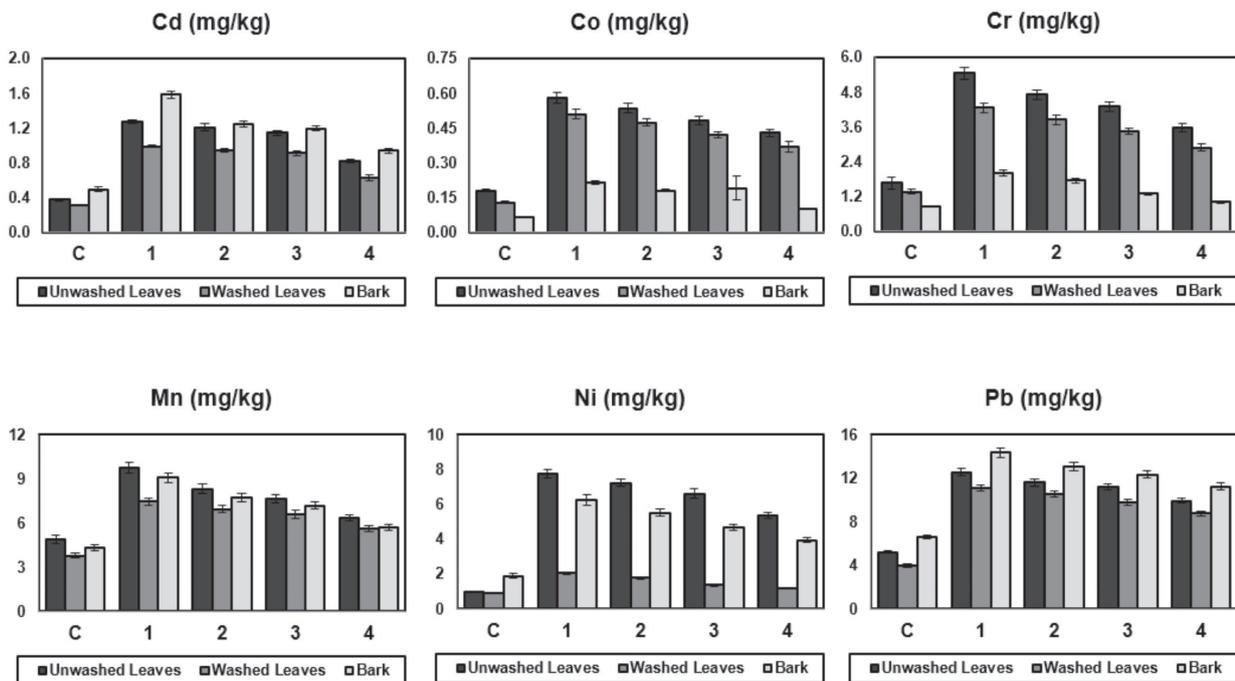

Fig. 2. Estimated concentrations of Cd, Co, Cr, Mn, Ni and Pb in leaf (washed and unwashed) and bark samples of Turkish red pine; estimations are given as mg kg^{-1} DW and sampling sites are indicated as: C for Buyukada (as control), 1 for Yildiz Grove, 2 for Fethi Pasha Grove, 3 for Baltalimani Grove, and 4 for Mihrabad Grove.
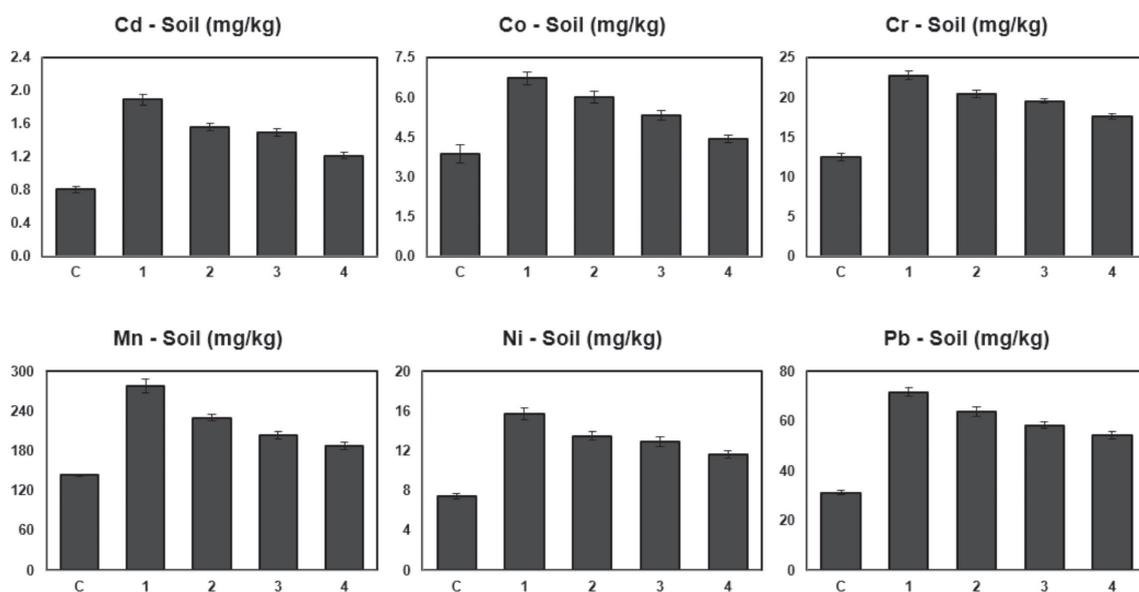

Fig. 3. Estimated concentrations of Cd, Co, Cr, Mn, Ni and Pb in soil samples in localities; estimations are given as mg kg^{-1} DW and sampling sites are: C for Buyukada (as control), 1 for Yildiz Grove, 2 for Fethi Pasha Grove, 3 for Baltalimani Grove, and 4 for Mihrabad Grove.

The normal worldwide distributions of the studied heavy metals (in mg kg^{-1}) in the plant bodies are in ranges of: 0.05-0.5 for Cd [36]; 0.005-0.270 in cereal grains, 0.100-0.570 in clovers and 0.060-0.270 in grasses for Co (therefore, the contents of Co in plants could be in the range of 0.005-0.570) [34]; 0.006-18 in plants for Cr [37]; 30-300 in plant parts for Mn [35, 38]; 0.5-5 in plant tissues for Ni; and 5-10 and above 30 is considered as toxic in plants for Pb [35, 38], respectively.

When the results obtained in our study are compared with the abovementioned values, the concentrations of Cd in soil and plant part samples were found to be higher than the normal limits in all stations except Buyukada (as control), and the concentrations of Co in soil samples were found to be within the normal ranges, whereas the contents of Ni in uw and w leaf samples were detected as higher than the normal ranges in all stations except Buyukada (as control); however, the contents of Ni in bark samples were found to be within normal ranges. The concentrations of Cr and Mn in the plant and its co-located soil samples were found to be in normal ranges. Ni concentrations in soil samples were determined within the normal limits. Although Ni concentrations in the uw leaf samples were detected as higher than the normal ranges in all stations except Buyukada, Ni concentrations in the w leaf samples were found to be within the normal ranges. This shows that the higher Ni concentrations in uw leaf samples are due to the dust and debris containing Ni in the air adhering to the leaves. Finally, the concentrations of Pb in soil and plant part samples were determined as higher than the normal limits in all stations except Buyukada, not reaching the toxic level.

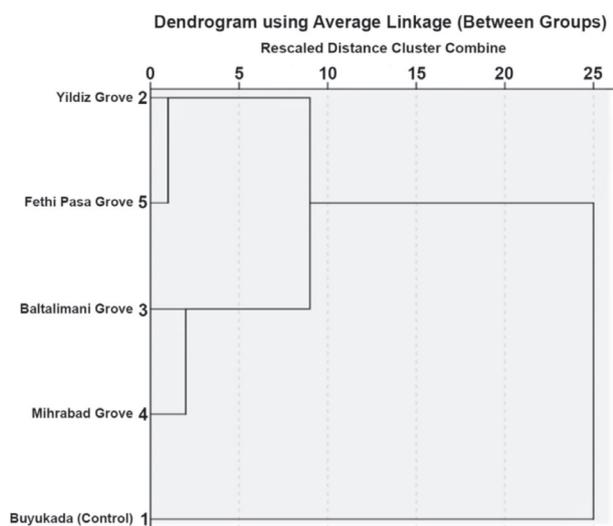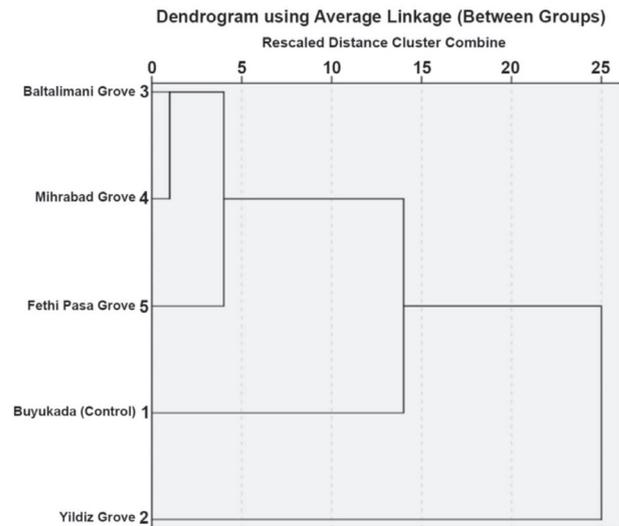The relationships between heavy metal levels in the soil and w leaves of the plant in all localities

Fig. 5. Dendrogram constructed from 5 different locations based on the element compositions of sampled leaves; generated with a hierarchical cluster analysis with average linkage (between localities).

were shown in Figs 4 and 5 with hierarchical cluster analysis. When the comparisons were done between the dendrograms of leaf and soil, some differences were noticed between them. Baltalimani and Mihrabad Groves as a group were relatively close to the control, Yildiz and Fethi Pasha Groves as a group saw high concentrations detected and Buyukada (as control) as a group formed 3 groups all together (Fig. 4). This was also compatible with our graphics. However, when considering the situation on the leaf data, Baltalimani and Mihrabad Groves comprised a group and no such grouping was detected for the other localities (Fig. 5). This situation shows that the groups show similarity in terms of heavy metal contents within the soil samples, and that the choice of sites represent 3 different levels of pollution. Also, as a dynamic entity of the plant, it shows that the synergistic and antagonistic relationships between the elements in conjunction with physiological and biochemical processes of plants affect the heavy uptake and accumulation in the plant.

In addition, the washing procedure also significantly reduced the airborne deposition of heavy metals on plant leaves, and estimated highest to lowest accumulation rates (in %), respectively, were between 23.78 (in station 4) and 18.44 (in control) for Cd, 28.21 (in control) and 11.63 (in station 2) for Co, 21.83 (in station 1) and 18.42 (in control) for Cr and 24.68 (in control) and 9.55 (in station 2) for Pb (Table 1).

Moreover, the highest outdoor PM_{10} value (in μg/m³) was estimated as 67.792 at station, whereas the lowest outdoor PM_{10} value (in μg/m³) was estimated as 37.483 at the control station. On the other hand, the highest outdoor $PM_{2.5}$ (in μg/m³) was recorded as 27.103 at station 1, whereas and the lowest outdoor $PM_{2.5}$ (in μg/m³) was found to be 11.216 at the

Fig. 4. Dendrogram constructed from 5 different locations based on the element compositions of sampled soils; generated with a hierarchical cluster analysis with average linkage (between localities).

Table 1. Percentage change (% removal) of heavy metal contents in leaves before and after washing.

Heavy Metal	Buyukada (Control)	Yildiz Grove	Fethi Pasa Grove	Baltalimani Grove	Mihrabad Grove
Cd	18.44	22.19	22.08	20.55	23.78
Co	28.21	12.08	11.63	12.45	13.51
Cr	18.42	21.83	18.15	19.82	19.43
Mn	22.50	23.81	16.72	14.13	11.78
Ni	8.97	73.48	75.39	79.46	78.46
Pb	24.68	11.64	9.55	12.74	12.04

Table 2. Outdoor PM_{10} and $PM_{2.5}$ levels (in $\mu g/m^3$) at sampling stations.

PM	Buyukada (Control)	Yildiz Grove	Fethi Pasa Grove	Baltalimani Grove	Mihrabad Grove
PM_{10} ($\mu g/m^3$)	37.483	67.792	57.778	50.419	44.630
$PM_{2.5}$ ($\mu g/m^3$)	11.216	27.103	21.194	18.801	15.237

control station (Table 2). We noticed that the highest metal concentrations were persistently recorded in station 1, which was relatively close to the highly crowded areas. The U.S. EPA's national air quality standards suggest that the measured annual mean (in $\mu g/m^3$) and daily concentration (in $\mu g/m^3$) have to be under 50 and 150 $\mu g/m^3$ for PM_{10}, and 15 and 35 $\mu g/m^3$ for $PM_{2.5}$, respectively [39]. Therefore, particulate matter estimations for PM_{10} on an annual basis were not within the acceptable limits of the EPA at Yildiz, Fethi Pasha and Baltalimani Groves, and particulate matter estimations for $PM_{2.5}$ on an annual basis were found to be at higher than normal limits except Buyukada. However, estimations on a daily basis were within the normal ranges at all stations for PM_{10} and $PM_{2.5}$.

In this work, the results obtained from heavy metal concentration measurements in leaf (uw and w) and bark samples of Turkish red pine and its co-located soil samples were used for statistical analysis by employing multivariate analysis of variance (MANOVA) with Tukey's post-hoc HSD and Pearson correlation, and hierarchical cluster analysis were performed by using IBM SPSS Statistics 20 software. In the tests performed for 6 heavy metals, only the significant differences shown in Table 3 were found for Cd, Ni and Pb. When examined, the localities as 5 regions for Cd in the table were divided into 4 different subset (a, b, c and d), and Fethi Pasha Grove and Baltalimani Grove were found to be in the same subset. For Ni, 2 subsets (a and b) were taking place: Yildiz, Fethi Pasha and Baltalimani Groves were in one while Mihrabad Grove was shown similarity with Buyukada and 3 other localities found in the first subset. And for Pb in two subsets (a and b), the biggest difference was found for Yildiz Grove and Fethi Pasha, while Baltalimani and Mihrabad Groves were found to be shown similarly to Yildiz Grove and Buyukada (as control). All these results were considered with heavy metal concentrations determined; Yildiz

Grove, showing the highest level of heavy metal pollution, was generally found to be dominant to other groups. Buyukada, with the lowest level of heavy metal pollution, showed the actual control group characteristics. In other localities, the heavy metal pollution rates were found to be in close proximity to each other. This situation could be explained by traffic density and the direction of the wind.

In addition, the results from Pearson correlation test performed by using the data obtained from the measurements of heavy metal concentrations in all plant parts and soil samples were given in Table 4. When examined, it was understood that there was no negative correlation between the 6 heavy metals. On the contrary, 6 heavy metals had high positive correlations with each other (>0.59, >0.99).

In previous studies done with Pinaceae family members, Dogan et al. [28] used Turkish red pine barks for estimating heavy metal pollution along control, rural, suburban and roadside areas in Western Anatolia, and the values (in mg kg^{-1}) were found as 0.68, 0.63, 0.68, 0.60 for Cd; 0.44, 0.84, 0.89, 1.24 for Cr; 3.56,

Table 3. Multivariate analysis of variance (MANOVA) with Tukey's post-hoc HSD were performed and means for group in homogeneous subsets were displayed; the levels of statistical significance were expressed as 0.01 (**) and 0.05 (*); Tukey's post-hoc test were for localities.

Localities	Cd	Ni	Pb
Buyukada (Control)	0.496[d]	2.790[b]	11.701[b]
Yildiz Grove	1.434[**a]	7.954[**a]	27.436[*a]
Fethi Pasa Grove	1.240[**b]	7.000[**a]	24.713[ab]
Baltalimani Grove	1.186[**b]	6.393[**a]	22.956[ab]
Mihrabad Grove	0.898[**c]	5.518[ab]	21.063[ab]

Table 4. Correlation coefficients between heavy metals determined in plant and soil samples.

Correlation Matrix (R)					
Pearson Correlation	Co	Cr	Mn	Ni	Pb
Cd	.591**	.620**	.590**	.818**	.683**
Co		.989**	.995**	.878**	.982**
Cr			.983**	.895**	.981**
Mn				.877**	.987**
Ni					.913**

**Correlation is significant at the 0.01 level (2-tailed)

5.11, 4.26, 3.72 for Ni; and finally 4.59, 7.32, 9.02, 16.15 for Pb. Sawidis et al. [40] used leaves and barks of *Pinus* sp. in their study in Salzburg, Belgrade and Thessaloniki and the values (in mg kg⁻¹) in the leaves and barks of *Pinus* sp. found for control in 3 cities (Salzburg, Belgrade and Thessaloniki) were 0.39 (in leaves)/0.31 (in barks), 0.33 (in leaves)/0.59 (in barks), 0.31 (in leaves)/0.88 (in barks) for Cr and 2.44 (in leaves)/1.09 (in barks), 2.22 (in leaves)/2.05 (in barks), 2.81 (in leaves)/2.05 (in barks) for Pb, while the values (in mg kg⁻¹) in the leaves and barks of *Pinus* sp. found for polluted areas in 3 cities (Salzburg, Belgrade and Thessaloniki) were 0.42 (in leaves)/0.41 (in barks), 0.58 (in leaves)/0.83 (in barks), 0.66 (in leaves)/0.14 (in barks) for Cr and 2.46 (in leaves)/5.98 (in barks), 14.45 (in leaves)/15.57 (in barks), 12.74 (in leaves)/9.15 (in barks) for Pb. Miri et al. [41] used the leaves of *Pinus eldarica* as study material in their research done in Yazd City in Iran, where the concentrations of Cd and Pb in mg kg⁻¹ were found to be 0.74 and 2.99, respectively. Research done by Coskun [42] in the Thrace region in Turkey using the barkss of *Pinus nigra* found the concentrations of Cd and Pb (in mg kg⁻¹) as 0.16 and 12.9, respectively; and the concentrations of Cd, Mn, Ni and Pb (in mg kg⁻¹) were 0.37, 29.2, 0.54 and 2.1 in a study done by Harju et al. [43] in Merimasku City in Finland using the barks of *Pinus sylvestris*. A comparison of all the data from previous studies performed with *Pinus* sp. mentioned above and the data obtained from our study showed that although there were no big differences, occasional fluctuations were noted, generally higher than previous studies.

In our study, the highest heavy metal concentrations were detected in Yildiz Grove in Besiktas District, whereas the lowest heavy metal concentrations were detected in Buyukada (as control). A previous study done in the same research areas using *Celtis australis* L. and its co-located soil samples as study materials produced similar results showing the lowest values in Buyukada (as control), whereas the highest values were in Besiktas for Cd and Pb, respectively [15]. A particulate matter metal component study was performed in 4 districts of Istanbul by Sahin et al. [44], and its findings showed that the higher heavy metal concentrations were detected in Besiktas, which has heavier traffic congestion than the other districts.

Conclusions

Based on EPA projections, PM_{10} could likely cause adverse health effects because of its particulate capability for reaching the respiratory tract [39]. In this work, PM values were estimated as normal based on the daily amounts. On the other hand, these values at some stations were found to be higher than the EPA's standards based on annual amounts (Table 2). So, when considered together with high heavy metal concentrations, it can be said that people living in Istanbul could suffer from airborne diseases in the future if necessary precautions are not implemented.

The data from our study showed that high concentrations of heavy metals were detected in Yildiz Grove in the soil samples and also in plant part samples, whereas low concentrations for heavy metals were detected in Buyukada in soil samples and also in plant part samples. Compatible results were obtained in other regions. Therefore, overall accumulation levels of different heavy metals (Cd, Co, Cr and Pb) in plant parts suggests that Turkish red pine as a biomonitor organism provided useful information for evaluating pollution levels in Istanbul.

Conflict of Interest

The authors declare no conflict of interest.

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