DETERMINATION OF HEAVY METAL ACCUMULATION IN AIR THROUGH ANNUAL RINGS: THE CASE OF *MALUS FLORIBUNDA* SPECIES

YIGIT, N.

Department of Forest Engineering, Faculty of Forestry, Kastamonu University Kastamonu Turkey
(email: nyigit@kastamonu.edu.tr; phone: +90-366-280-1747; fax: +90-366-280-1700)

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Abstract. Air pollutants can be observed all over the world. Sulfur dioxide and nitrogen oxides are the most worrying ones among them. The growth rates of many forested areas in the world decrease at high altitudes, and air pollutants are more abundant as the cause of this. The leaf surface constitutes the interface between plants and the worsening atmospheric environment. Therefore, it is the first point of contact between plants and air pollutants and constitutes an effective barrier to the pollutant input. The outer surfaces of leaves are covered with a thin membrane called the cuticle. This membrane layer has many basic functions, such as preventing the excessive water loss of the plant, regulating the intake of dissolved substance and protecting photosynthetic tissues, preventing of harmful irradiation, such as improved UV-B caused by stratospheric ozone depletion. The wood structure also provides us with information on the pollutants to which they are exposed to and their levels due to the fact that trees are stationary. In this study, the accumulations of Co, Cu, Cd, Pb, Ni, Cr, Mn, Fe, Al, Zn, Na, Ca, Ba, Mg, and As elements, accumulated on the annual rings of *Malus floribunda* species in Ankara-Yenimahalle between 1962 and 2017, by years were determined using the GBC Integra XL–SDS-270 ICP-OES device. In conclusion, it is observed that amounts of all elements in the wood in different age ranges are statistically different at a confidence level of at least 99.9%.
Keywords: *wood, air pollutant, tree species, bioindicator, elements*

Introduction

Toxic pollutants have increased considerably in the last century and adversely affect the lives of most living beings around them (Wolz et al., 2003; Tang et al., 2013). Trees in the settlements contribute to preventing air pollution; reducing noise, wind, dust and greenhouse effect; balancing temperature; providing energy savings and moisture; and creating habitats for fauna and flora (Cetin, 2018a, b, c, d). Environmental pollution includes the mixture of organic and inorganic compounds. Although organic components decompose harmlessly and as fully mineralized, the separation of inorganic compounds from the medium is technically difficult since do not change with a physical process (Meagher, 2000). Inorganic pollutants are basically heavy metals, such as As, Ag, Hg, Sb, Cd and Pb, containing the elements with biological functions (such as Fe, Mo, Mn, Zn, Ni, Cu and Co), which are harmful when they are present in excessive amounts and which do not play a known role in living organisms (DalCorso et al., 2013). Inorganic pollutants may arise from natural conditions such as the exposure of rocks to weather conditions, soil erosion, and volcanic activities or human-induced factors such as industrial or agricultural activities (Fasani et al., 2018).

Human-induced factors can be defined by the high concentration of primary pollutants such as SO_2 in the air and initiate local pollution. These activities can be the cause of tree deaths, and the high level of air pollutants in neighboring areas (Garrec, 1993).

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Secondary pollutants are characterized by physiological effects, such as the yellowing of leaves and a decrease in the viability of trees as a result of the interaction of secondary pollutants with trees. These pollutants, usually along with climatic stress, initiate forest decline with a very irregular localization on a regional scale. It is necessary to consider the nature and level of these pollutants to determine the plant stress caused by air pollution. As an example of this, a summary of the scientific literature on the effects of air pollutants on epidermis characteristics during two periods was created (*Table 1*) (Garrec, 1993).

Table 1. *The effects of air pollutants on epidermis characteristics from the scientific literature*

Air pollutant	**Cuticular characteristics**				
	Wax erosion	**No wax erosion**	**Increasing wax amount**	**Decreasing wax amount**	**No effect on wax amount**
SO2	*******	****	**	**	**
O3	*****	*****		**	**
Acid rain	******	***	*	*	**
Acid mist	**	**			**
NH3	**	**			

*Shows the density of pollutants

The data presented in *Table 1* show the variability of the response of the plant surface and/or the inhomogeneity of the action of these pollutants. Nevertheless, based on these data, primary air pollutants such as SO_2 are likely to have a detrimental effect on the epidermis. Unlike the concentrated primary pollutants, secondary air pollutants such as O_3 or acid deposits seem to have a less significant effect on the cuticle layer. In fact, most of the results show that the effect of these less concentrated secondary pollutants is similar to the aging process and does not have a polluting characteristic (Gunthardt-Goerg and Keller, 1987; Lutz et al., 1990).

Trees are regarded as the sensors that record environmental disturbances since they live for a long time, that remain in the same place throughout their lives and thus display chronology, and that are geographically common (Kord et al., 2009; Balouet et al., 2007). The fact that trees accumulate the heavy metals they receive from the air with the components in and above the soil makes them good indicators. Therefore, various organelles such as trunks, branches, and leaves of trees have been used for many years to determine the concentration of heavy metals in them. Leaves are the most commonly used organelles. The main reasons for this can be listed as the fact that leaves incorporate heavy metals through their stomata during photosynthesis and accumulate them, the fact that the collection of leaves does not cause permanent damage to the tree, and the fact that how long the accumulated heavy metal accumulates is known since the ages of leaves are known. Furthermore, herbarium collections (Herpin et al., 1997), the annual growth rings of trees (Watmough, 1999) also provide historical examples for bio-analysis. In fact, trees are not better indicators compared to fungi, algae, and moss. However, the use of trees throughout cities and the fact that they live longer compared to other indicator plants provide more information to scientists about the increase in heavy metal pollution in the air from past to present in the studies carried out on them (Sawidis et al., 2011; Turkyilmaz et al., 2018a; Shahid et al., 2017; Shcerbenko et al.,

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2008). In recent years, many authors have reported the temporal and spatial reconstructions of heavy metals recorded on tree rings of different tree species (Aznar et al., 2008; Lageard et al., 2008; Nabais et al., 1999; Orlandi et al., 2002; Vives et al., 2005; Türkyılmaz, 2018b).

Materials and methods

The study was carried out on the wood materials obtained from *Malus floribunda* tree located in Yenimahalle region, which is one of the regions of Ankara province with the heaviest traffic. Ankara province has an area of 26.897 km^2 and is located between 39.57 N latitude and 32.53 E longitudes. Its altitude above sea level is approximately 890 m. Ankara province is located in B4 square (Grid) according to P. H. Davis's Turkey map grid system (Davis, 1965-1985). In terms of biogeography, it is located in the Irano-Turanian floristic region. The Irano-Turanian floristic region covers the central and eastern Anatolia regions of Turkey. These regions are cold and snowy in winters and usually dry and without rain in summers (Atalay and Efe, 2014; Saya and Güney, 2014). The average temperature on a provincial scale is 11.7 °C, and the annual average precipitation is 389.1 mm. The highest and lowest temperature values were determined to be 40.8 °C and -24.9 °C, respectively. Upon examining the provincial center and the wind status of stations in general, the dominant wind is observed to vary depending on the land structure. Accordingly, the dominant wind blows in the northeast direction in Ankara (center), Esenboğa and Yenimahalle districts where the study area is located. The highest wind speed determined in Ankara is 29.2 m/s (URL 1) (*Fig. 1*).

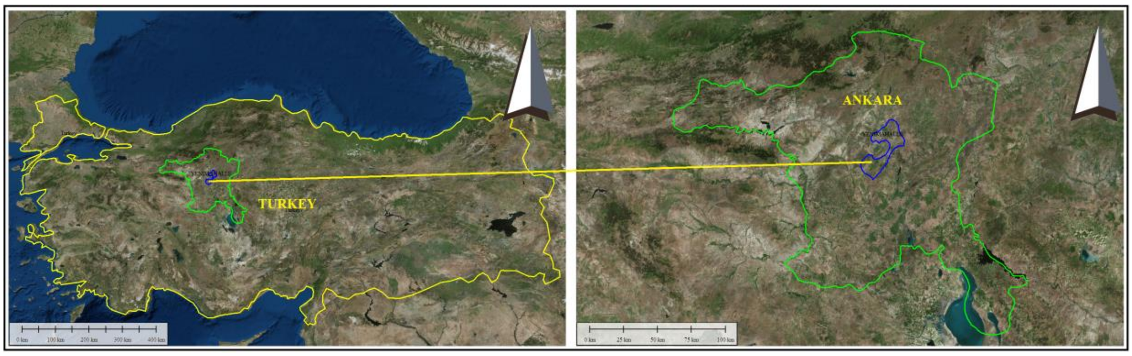

Figure 1. *A map of Ankara*

The samples were cut from *Malus floribunda* in Ankara-Yenimahalle (Turkey) in December, when the vegetation season ends, in 2017. The cut samples were brought to the laboratory and separated into pieces in the form of 1 cm-thick discs in the laboratory environment. The sectional surfaces were made smooth by sandpapering so that annual rings could be observed clearly. During the macroscopic observation, it was determined that the tree was 55 years old, and it was decided that it was appropriate to perform grouping into five age ranges by considering the widths of annual rings. The annual rings were divided into five-year sections, and the samples taken from two opposite sides of the tree were classified. Thus, 11 samples including five-year annual rings and barks were obtained.

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The wood samples were shredded and turned into sawdust. During these processes, attention was paid to not using any tool made of the elements discussed in the study. The wood samples were first kept for 30 days until they became dry. Then, they were dried in the drying oven at 50 °C for one week. 0.5 g of the dried samples were taken, 6 ml of 65% HNO_3 and 2 ml of 30% H_2O_2 were added to them, and they were placed in a microwave oven. The program of the microwave device was set to 15 min at 200 °C. After burning the samples in the microwave oven, the samples which were dissolved into solution were taken into balloons and completed to 50 ml with ultrapure water and made ready for Fe, Co, Ni, Zn, Cd, Hg and Pb analyses with the GBC Integra XL–SDS-270 ICP-OES device (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) For the analysis of the samples, the plasma of the ICP device was burned, and ultra-pure water was passed through the system for 15 min to equilibrate. The calibration chart was created by preparing standard solutions according to the elements to be analyzed. After constructing the calibration chart, the samples were given to the system and read. Since the sample was taken by 0.5 g and completed to 50 g with water, the analysis results were multiplied by 100. Different calibration charts were created at the ppm or ppb level according to the analysis results that did not fall into the calibration chart, and reading was performed again. The detection limits for the GBC device were determined to be (Pb---> 0.377 ppb, Cu---> 0.639 ppb, Ca---> 0.00208 ppm, Mg--->0.00758 ppm, Cd---> 0.063 ppb, Cr---> 0.311 ppb, Ni---> 0.171 ppb, Fe---> 0.00068 ppm, Mn---> 0.00015 ppm, Zn---> 0.00634 ppm). In the study, all measurements were performed in three replicates. Then, the data were evaluated by the analysis of variance, Duncan's test, and correlation analysis using SPSS package program (Anonim, 1998; Eymen, 2007).

Results

A total of 15 elements were discussed for the determination of heavy metal accumulations in the wood and bark. The analysis of variance and Duncan's test were applied to the results obtained in terms of the studied elements. The average values obtained for wood and bark and the F values calculated as a result of the analysis of variance are presented in *Table 2*.

Based on the results summarized in *Table 2*, it was determined that there was no significant difference among Cu, Fe, Ca and As values at a confidence level of at least 95%. For the rest of the elements, statistically significant differences were found between the wood and bark. According to the F values calculated, the values obtained for bark and wood were significantly different at a confidence level of 99.9% for all elements (*Table 2*). However, Duncan's test was not applied to determine the homogeneous group since it was performed on 2 characters including bark and wood.

The analysis of variance was performed using SPSS according to all the elements studied on annual rings in the wood sample, and the analysis result, F value, and Duncan's test results are presented in *Table 3*.

Upon examining *Table 3*, it was observed that there were significant differences at a confidence level of at least 99.9% in terms of all the characters subjected to the analysis of variance. It was determined that Cu value was divided into 9 different homogenous groups and that the highest value (26674.13) was reached between the years 1967-1972. Similarly, it was determined that Ni (1907.5), Al (2327.9), Mg (907.56) and Zn (14033.33) also had the highest values between those years. It was determined that As

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value was divided into 2 different homogenous groups and that Na, Ba, and B values were divided into 10 different homogenous groups. Na value was determined to be the highest (2016.6) between the years 2000 and 2005. It was determined that Mg element reached its highest value between 1955 and 1960.

Table 2. *Average values obtained for wood and bark and the analysis of variance results*

Elements	Wood	Bark	F value
Co ppb	**529.6**	1229.4	21.807***
Cu ppb	**7779.2**	11767.2	0.728 ns
Cd ppb	**176**	330.5	63.992***
Pb ppb	**2077.4**	4532.9	48.949***
Ni ppb	**1132.8**	2977.7	86.53***
Cr ppb	**453.2**	2154.3	623.793***
Mn ppb	**1058**	30294.2	4179.785***
Fe ppb	**18442.2**	29312.2	0.595 ns
Al ppb	**770.4**	28813.1	4381.278***
Zn ppb	**3763**	8640.8	2.648***
Na ppm	**912.1**	511.3	1.661***
Ca ppm	**2097.5**	3218.9	4.385 ns
Ba ppm	**9.1**	56	138.572***
Mg ppm	**417.2**	1767.5	44.028***
As ppm	**1.6**	2	11.656 ns

ns: Not significant
***Significant at 0.001 level

Table 3. *Average values by age in wood samples and the analysis of variance results and F value*

Ages / Elements	**1955-1960**	**1961-1966**	**1967-1972**	**1973-1978**	**1979-1984**	**1985-1987**	**1988-1993**	**1994-1999**	**2000-2005**	**2006-2011**	**2012-2017**	**f**
Co ppb	800.67c	775.23c	1149.4d	412.67b	387.07ab	377.87ab	429.2b	419.07b	325.2a	356.93ab	392.3ab	127.727
Cu ppb	15719.4g	16152.93h	26674.13ı	8459.5f	4631.2e	2409.57c	2168.8b	3273.4d	1561.03a	2172.9b	2348.63c	35580.948
Cd ppb	204.33d	203.27d	245.23e	193.47d	175.63c	167.17c	166.13c	163.2c	148.77c	137.2ab	131.63a	63.162
Pb ppb	2769.13c	2814.73c	3208.7d	2037.67b	1965.03b	1817.43b	1943.1b	1874.1b	1516.43a	1448.97a	1456.33a	50.877
Ni ppb	1486.5h	1419.1g	1907.5ı	1019.9d	1141.4e	1200.57f	865.23b	956.2c	791.8a	846.9b	825.5ab	563.292
Cr ppb	523.83e	657.57f	628.2f	485.47de	463.3cde	404.73bc	432.43cd	406.47bc	330.17ab	314.83a	338.2ab	21.291
Mn ppb	2925.93ı	1069.3f	2079.73h	663.77d	511.8c	278.47a	515.03c	1276.5g	423.57b	957.6e	936.47e	3790.982
Fe ppb	7350.6c	33867f	78524h	40929.83g	27887.5e	33867d	3710.66b	41.37a	386.06a	226.26a	254.36a	46162.103
Al ppb	1837.1h	1284.73g	2327.9ı	602.03e	865.23f	461.53d	87.83a	349.47c	58a	421.17d	179.1b	1357.294
Zn ppb	13135.63h	6938.7g	14033.33ı	3663.83f	1992.53e	371.3c	61.03a	653.76d	375.2c	147.6b	20.1a	75236.672
Na ppm	521.06c	422.23b	868.5f	841.33e	1016.8g	1198.26h	1716.03ı	360.36a	2016.6j	719.6d	352.3a	28471.922
Ca ppm	3250.4g	2914.96e	3136.86f	1955.93d	1956.7d	1982d	1974.83d	3247.1g	1073.93c	823.36b	756.7a	12064.255
Ba ppm	25.9j	10.93h	17.7ı	10.2g	7.8f	4.66d	6.13e	6.033e	4.4c	3.36b	2.8a	21305.217
Mg ppm	1227.3k	499ı	907.56j	174.4h	466g	220.4f	174.4d	186.33e	119.03a	144.7b	160.2c	54894.807
As ppm	1.9b	1.83b	1.83b	1.5a	1.53a	1.46a	1.46a	1.53a	1.5a	1.46a	1.53a	9.745

The graphics, which were prepared to make the changes associated with heavy metals according to the ages more clearly understandable, are presented in *Figure 2*.

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Figure 2. *Annual changes in heavy metal concentrations over years (A changes in Co concentrations, B changes in Cu concentrations, C changes in Cd concentrations, D changes in Pb concentrations, E changes in Ni concentrations, F changes in Cr concentrations, G changes in Mn concentrations, H changes in Fe concentrations, I changes in Al concentrations, J changes in Zn concentrations, K changes in Na concentrations, L changes in CA concentrations, M changes in Ba concentrations, N changes in Mg concentrations, O changes in As concentrations*

As seen in *Figure 2,* especially Pb, Cu, Co, Mg and Ba elements were found to decrease after 1979-1984 years. One of the reasons for this could be the use of natural gas in Ankara since the 1980s.

As a result of the study, correlation analysis was performed to determine the relationships between the elements and the results are presented in *Table 4*. The studied relationship that was not measured in the correlation analysis is related to the linear part of the relationship between the variables. The correlation coefficient calculated as a result of the correlation analysis is indicated with r and takes values between –1 and +1. The fact that the coefficient is close to +1 indicates that there is a good correlation

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between the two variables, and the fact that it is close to -1 indicates that there is a good but inverse correlation; in other words, one of the variables increases while the other one decreases. Upon evaluating the results in this respect, it is observed that the level of relationship between some elements is really high.

Table 4. *Correlation analysis results*

	Cu ppb	Cd ppb	Pb ppb	Ni ppb	Cr ppb	Mn ppb	Fe ppb	Al ppb	Zn ppb	Na ppm	Ca ppm	Ba ppm	Mg ppm	As ppm
Co ppb	.839**	.906**	.940**	.914**	.754**	.675**	.649**	.686**	.867**	-.365*	.714**	.830**	.877**	.867**
Cu ppb		.648**	.691**	.615**	.330*	.208	.826**	.227	.945**	-.345*	.681**	.482**	.649**	.744**
Cd ppb			.973**	.968**	.904**	.835**	.597**	.850**	.710**	-.266	.701**	.929**	.917**	.755**
Pb ppb				.957**	.881**	.803**	.556**	.815**	.755**	-.334*	.735**	.917**	.919**	.846**
Ni ppb					.924**	.875**	.523**	.889**	.692**	-.332*	.650**	.943**	.925**	.769**
Cr ppb						.979**	.294	.986**	.428**	-.273	.486**	.939**	.833**	.635**
Mn ppb							.154	.998**	.341*	-.259	.389*	.927**	.799**	.560**
Fe ppb								.195	.673**	-.148	.463**	.322	.452**	.448**
Al ppb									.349*	-.246	.394*	.926**	.801**	.563**
Zn ppb										-.350*	.720**	.642**	.805**	.818**
Na ppm											-.392*	-.312	-.370*	-.429**
Ca ppm												.603**	.671**	.674**
Ba ppm													.961**	.745**
Mg ppm														.815**

Upon examining *Table 4*, it is observed that there is a positive correlation in general with respect to the measured characters. When the results are examined in this context, the relationship between some elements is observed to be quite high. For example, the correlation coefficients between Cr and Al (0.986), Mn and Al (0.998) and Cr and Mn (0.979) are quite high. Similarly, the correlation coefficient calculated between Na and As (-0.429) is negative but very strong. Furthermore, very strong relationships were observed among many elements (*Table 4*).

Discussion and conclusion

The results of the study reveal that the concentration of all heavy metals that adversely affect human health increases with age. It can be said that the increase in the amount of heavy metals in tree rings is due to the morphological structure of the tree and ecological impacts, which does not exhibit a linear increase, high in some years and low in other years. The morphological structure of the epidermis layer varies by age and polluted air (Huttunen and Laine, 1983; Mengel et al., 1989; Turunen and Huttunen, 1990). Similar results were also obtained in the studies carried out on different species (Beramendi-Orosco et al., 2013; Türkyılmaz et al., 2018c, 2019). In the study carried out by Beramendi-Orosco (2013): on *Prosopis julifora* annual rings, the researchers ported that the amount of Cu, which was 1.09 ppm during 1988-1992, was 1.27 ppm during 2003-2007 and that the amount of Pb, which was 0.35 ppm during 1998–2002, was 0.46 ppm during 1993-1997. Similar results were also obtained in the studies in which changes were determined by months (Norouzi et al., 2015).

When tree annual rings were examined, it was determined that heavy metal accumulation was intense in the early years in elements such as Cd, Pb, Al, Zn, Mg, Ca and B. However, there is no clear evidence on whether this intensity was due to the accumulation during the first years of the tree or the accumulation of all years. As seen

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in *Figure 2*, especially Pb, Cu, Co, Mg and Ba elements were found to decrease after 1979-1984. One of the reasons for this could be the use of natural gas in Ankara since the 1980s.

Although some elements such as K, S, Mg, CI, and P can be transported to the plant's metabolic activities, the transport of certain elements in the phloem (B, Ba, Ca, Cu, Fe, Li, Mn, Mo, and Zn) is more limited (Perone et al., 2018). In recent years, the nature and intensity of the concentration of elements in nature have increased due to the increase in human effects and vehicle traffic. Some data indicate that climate change is affected not only by the traces of pollution but also by plant metabolism and by the distribution of elements in plants (Cui et al., 2013; Li et al., 2004). Nowadays, in the world, motor vehicles exhausts, industrial activities, mineral deposits and enterprises, use of urban wastes as fertilizers, chemical fertilizers and pesticide applications, and reach a significant amount of heavy metal soil. The accumulation of heavy metals in the soil not only affects soil fertility and ecosystem activities, but also affects many metabolic events such as photosynthesis, respiration, growth and development within the plant and therefore affects animal health and human health significantly due to deteriorated food chain. In the soils where heavy metal accumulation is observed, heavy metals reach the human body through the skin contact of people, breathing, growing vegetables and fruits. For this reason, heavy metal that can be harmful to human health, etc. the means of transmission and prevention of all kinds of substances should be investigated.

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