

Turkish Red Pine as a Biomonitor: A Comparative Study of the Accumulation of Trace Elements in the Needles and Bark

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Abstract

The increase in industrial activities and human population and the high urbanization in the study area have made the area one of the most rapidly developing areas in Turkey. However, because of this, environmental problems have rapidly increased in Western Anatolia. The *Pinus brutia* (Pinaceae) (Turkish red pine) is a widespread evergreen tree in the Mediterranean and Aegean regions of Turkey. The needles and bark of *P. brutia* were tested as a possible biomonitor of trace element pollution in Western Anatolia. Samples were collected from 30 different stations in the industrial, suburban, roadside, rural and control areas. Throughout the region, an area covering approximately 1/8th of Turkey was investigated. The concentration of lead, cadmium, chromium, iron, nickel and copper ($\mu\text{g g}^{-1}$, dry weight) in the needles and bark of the red pine was analyzed by atomic absorption spectrometry. The highest concentration of all these elements was measured in the industrial areas. The maximum values of Pb, Cd, Cr, Fe, Ni, and Cu for the needles of *P. brutia* in these areas was 12.1, 0.54, 12.12, 595.5, 10.4, and 10.1 $\mu\text{g g}^{-1}$ in dry weight, respectively and for the bark of *P. brutia* in these areas was 20.03, 0.71, 3.17, 1274.04, 18.87, and 15.30 $\mu\text{g g}^{-1}$ in dry weight, respectively. This study demonstrates that the bark of *P. brutia* can be used as a biomonitor for detecting the heavy metal pollution in a specific area instead of the needles.

Keywords: Bark, needle, *Pinus brutia*, trace element, Western Anatolia.

Bir Biyolojik İndikatör Olarak Kızılçam: Yaprak ve Kabuklarındaki İz Element Birikimlerinin Karşılaştırılması

Özet

Endüstriyel aktiviteler, yüksek nüfus yoğunluğu ve hızlı kentleşme Batı Anadolu'yu Türkiye'nin en hızlı gelişen bölgelerinden biri haline getirmiştir. Bütün bu nedenlerden dolayı da, bu bölge çevre problemlerinin en hızlı arttığı bölgelerden biridir. Çalışmada, Ege ve Akdeniz bölgelerinde doğal olarak yayılış gösteren herdem yeşil bir ağaç olan kızılçamın (*Pinus brutia*, Pinaceae) yaprak ve kabuk örnekleri kullanılmıştır. Bu amaçla, Türkiye'nin yaklaşık 1/8'lik kısmına karşılık gelen bölgeden; endüstriyel, kırsal, yol kenarı, banliyö ve kontrol noktaları olmak üzere toplam 30 farklı noktadan örnekler alınmıştır. Alınan kızılçam yaprak ve kabuk örneklerindeki kurşun, kadmiyum, krom, demir, nikel ve bakır birikim değerleri atomik absorpsiyon spektrometresi ile belirlenmiştir. Bu ölçümlere göre en yüksek ağır metal birikim değerlerinin endüstriyel bölgelerden alınan örneklerde ortaya çıktığı görülmüştür. Bu bölgelerden alınan bitki örneklerindeki ağır metal (Pb, Cd, Cr, Fe, Ni ve Cu) birikim değerleri yaprak için sırasıyla; 12.1, 0.54, 12.12, 595.5, 10.4 ve 10.1 $\mu\text{g g}^{-1}$ ve kabuk için sırasıyla; 20.03, 0.71, 3.17, 1274.04, 18.87 ve 15.30 $\mu\text{g g}^{-1}$ şeklindedir. Yaprak ve kabuk örneklerine birikim değerleri karşılaştırıldığında, çam kabuğunun yaprağa göre daha iyi bir biyolojik indikatör olabileceği anlaşılmıştır.

Anahtar Kelimeler: İz element, Batı Anadolu, kabuk, *Pinus brutia*, yaprak.

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INTRODUCTION

Global industrialization and human social and agricultural activities have altered global biogeochemical cycling of heavy metals, resulting in a progressive increase in the flux of bioavailable chemical forms into the atmosphere (Ng et al. 2005). Although heavy metals are natural components of the environment, they have been the object of many studies because they are being added

to soil, water, and air in increasing amounts as a result of rapid growth in population, increased urbanization, and expansion of industrial activities (Baslar et al. 2005). The main sources of these pollutants in the atmosphere are stack and exhaust emissions in the form of particles released from industrial plants, power stations, domestic heating systems, and motor vehicles (Karaca et al. 2005, Uyar et al. 2009).

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Biomonitoring is an indicator of environmental pollution that includes trace element analyses of plants. This method provides valuable information about the quantity and quality of pollutants in the atmosphere (Cayir et al. 2007, 2008, Kaya and Yaman 2008). In this method, the levels of atmospheric trace metal concentrations have been successfully monitored by using different types of vegetation (El-Hasan et al. 2002, Baslar et al. 2003, Dogan et al. 2007, Onder et al. 2007). Botanical materials have been used to detect the deposition, accumulation, and distribution of trace metals in the ecosystems. They provide the cheapest and simplest method for monitoring trace metals elements in the atmosphere (Baslar et al. 2009a, Kula et al. 2010).

Plant biomonitoring is being increasingly used, especially in Europe, as an alternative to the traditional methods of studying regional deposition of natural and anthropogenic pollutants from the atmosphere to the terrestrial environment (Pacheco et al. 2001, Dogan et al. 2007). Because it is an easy, inexpensive, and practical method of determining air quality when compared with conventional monitoring methods (Cayir et al. 2007).

Because the accumulation of pollutants vary from plant to plant and also among various parts of a plant species (Baycu et al. 2006, Singh et al. 2005, Huseyinova et al. 2009), proper selection of plant species for biomonitoring plays an important role in the determination of the extent of toxic metal pollution in the environmental media such as soil, water, and air (Kaya and Yaman 2008). In order to overcome these problems, increased attention is being paid to the use of plant parts such as the leaf, shoot, and bark for biomonitoring (Bargagli 1998, Smodis et al. 2004, Tomasevic et al. 2004, Mertens et al. 2005, Singh et al. 2005, Baycu et al. 2006, Baslar et al. 2009b, Samecka-Cymerman et al. 2009).

In related literature, there are many studies focusing on using the bark and needles of the pine species as a biomonitor. Some of them are as follows: Galuszka (2005) used the needles and bark of the Scots pine (*Pinus sylvestris* L.) with some other species, Dmuchowski and Bytnerowicz (1995), Yilmaz and Zengin (2004), used the needles of the Scots pine, Pyatt (1999) used the needles of the Corsican pines (*Pinus nigra* Arnold. subsp. *laricio* (Poir.) Maire), and Baslar et al. (2003) used the needles of the Turkish red pine (*Pinus brutia* Ten.) to monitor heavy metal pollution. On the other hand,

some other researchers, like Grodzinska (1971), Huhn et al. (1995), Poikolainen (1997), Schulz et al. (1999), Pacheco et al. (2001), Harju et al. (2002), studied the Scots pine bark; Laaksovirta et al. (1976), Takala et al. (1994), Loppi et al. (1997), Van Dobben et al. (2001), used the pine bark and lichens living on the bark and Lippo et al. (1995) and Samecka-Cymerman et al. (2006) used the pine bark and mosses living on them. However, in the aforementioned studies, the bark and needle samples were evaluated independently and the question of which part is more useful in terms of biomonitoring was left unanswered.

The aim of present study is to determine the pollution levels of Pb, Cd, Cr, Fe, Ni, and Cu by using the bark and the needles of the *P. brutia* as a bioindicator in the Western Anatolian part of Turkey and to compare the accumulation levels of the bark and needles of the pine samples collected from different localities in the study area.

General Distribution of Turkish Red Pine

The Turkish red pine (*Pinus brutia*) (Pinaceae) is a native pine in the eastern Mediterranean region. In Turkey, *P. brutia* forms extensive forests, especially in regions where the Mediterranean climate prevails. Ecologically and economically, it is one of the most important forest tree species of the country and is dominantly distributed in Turkey. It can be seen from sea level to 600 m, and up to 1500 m in the southern parts. *P. brutia* generally occurs in the Mediterranean-type climate of hot and dry summers and mild and rainy winters. *P. brutia* occurs most abundantly in the semi-arid and sub-humid zones. The species is also extends to the East Aegean Islands, Crete and Greece, the Crimea, Armenia, Georgia, Azerbaijan, Northern Iraq, Jordan, Western Syria, Lebanon, and Cyprus. According to IUCN Red List of Threatened Species, this species is LC (Least concern) (Frankis 1999, Quézel 2000, Boydak 2004, Anonymous 2009).

MATERIAL AND METHODS

Site Description

The study was carried out in the western region of Turkey. The study area, which includes the cities of Izmir, Manisa, Aydin, Denizli, Mugla, Balikesir, Canakkale, and Kutahya and this area, covers approximately 1/8th of Turkey (Fig. 1). There are many small and medium sized industries, predominantly metallurgical, textile, petroleum, cement, and electronic factories in the study area.



Fig 1. Geographical location of the study area.

The mountains of Akdag, Bozdag, Murat Dagı, and Kaz Dagı were selected as control sites, and the samples were collected, at a distance of 35–40 km from the city center with a negligible traffic and pollution load. The urban road side sampling material was collected about 2 m from the main road.

Sample Preparation

The bark and needles of the *Pinus brutia* Ten. (Pinaceae) were collected from different sites during July and August. The numbers of sites from each category sampled are as follows: industrial 8, road side 6, suburban 6, rural area 6, and control 4.

The bark was carefully removed with a stainless steel pen knife at an average height of about 2 m. About 200 g of the upper part of the bark of the *P. brutia* from each side (west, east, south, and north) of the trees was collected. The samples were then dried in an oven at 80°C for 24 h, milled in a microhammer cutter, and fed through a 2 mm sieve.

The needles were carefully removed with a stainless steel pen knife at an average height of about 3 m above the ground. Five needles from each side (west, east, south, and north) of the trees were collected. The middle parts of the needles (about 3 years old) were used for sampling. The collected samples were dried in the shade for one month. The dried material was then ground and sieved (0.2 mm). The bark and needle samples were stored in clean self-sealing plastic bags with a silica gel desiccant. Contamination from the microhammer cutter was negligible during the grinding because it was washed after every grinding, first with absolute alcohol then with triple distilled water. In this step,

the samples were not digested. The digestion was realized just before the analysis.

Chemical Analyses

The method used for plant digestion was according to that described by the Perkin Elmer Corporation (Anonymous 1996). The digested samples were aspirated into an air-acetylene flame and the metals were determined by flame atomic absorption spectrometry (FAAS). The reproducibility of the method used in decomposing the plant samples was checked by carrying out triplicate analyses. All of the samples were analyzed immediately after preparation.

All chemicals used were of analytical reagent grade unless otherwise specified. Triple distilled water was used throughout the experiments. The working metal standard solutions were prepared just before use, by diluting the stock standard solution with water. Determination of the metals was performed with a Perkin Elmer Analyst 700 model flame atomic absorption spectrometer equipped with a deuterium background correction, hollow cathode lamps (HCl), and acetylene burner.

Data Analyses

Concentration values are given as mean $\pm$ standard error (SE). Statistical significance was determined by the One-sample t-test. In the independent t-test, comparisons were made in order to determine whether there were any differences between the averages of the sites assumed as polluted and the control region. Differences at $P < 0.05$ were considered to be significant. A Statistical Package was used in the analysis of t-test for the data collected.

RESULTS

In this study, the bark and needle samples of *P. brutia* collected from 30 different stations in the industrial, suburban, roadside, rural, and control areas throughout nearly 100,000 km² of the Western Anatolian region in Turkey was investigated. By chemical analysis, the levels of Cd, Pb, Cr, Fe, Ni, and Cu ($\mu\text{g g}^{-1}$, dry weight) in the bark and needles of *P. brutia* collected from the sampling stations was determined and are given in Table 1.

The maximum values of Pb, Cd, Cr, Fe, Ni, and Cu for the needles of *P. brutia* in these areas are 12.1, 0.54, 12.12, 595.5, 10.4, and 10.1 $\mu\text{g g}^{-1}$ in dry weight (d.w.), respectively. On the other hand, in the control sites, the mean values of the contents of these metals in the needles of *P. brutia* in these areas

Table 1. The mean concentrations of trace elements ($\mu\text{g g}^{-1}$, dry weight) and statistical evaluation in the bark and needle of the Turkish red pine collected from Western Anatolia.

Site	N	Part	Element concentration ^a ($\mu\text{g g}^{-1}$, dry weight)					
			Pb	Cd	Cr	Fe	Ni	Cu
Control	4	Bark	4.59±0.57*	0.68±0.06 *	0.44±0.12	486.35±130.95*	3.56±0.52*	3.52±0.35*
		Needle	1.40±0.30*	0.43±0.10*	0.78±0.20	57.28±4.60*	0.88±0.10*	1.10±0.20*
Rural	6	Bark	7.32±2.05	0.63±0.05*	0.84±0.12*	526.21±160.80	5.11±1.60	4.57±0.82
		Needle	2.80±0.60	0.42±0.20*	0.83±0.10*	93.13±9.70	6.99±1.00	3.91±0.40
Suburban	6	Bark	9.02±1.41	0.68±0.04*	0.99±0.34	598.70±170.10*	4.26±0.66	5.78±1.18
		Needle	3.60±0.90	0.54±0.20*	0.90±1.50	236.40±13.70*	10.40±0.90	3.10±0.30
Roadside	6	Bark	16.15±8.53*	0.60±0.02*	1.24±0.54*	594.58±154.12	3.72±0.91*	5.36±1.45
		Needle	12.10±1.10*	0.45±0.40*	6.40±0.40*	500.90±36.80	4.10±0.30*	5.30±0.30
Industry	8	Bark	20.03±10.50	0.71±0.02*	3.17±1.26*	1274.04±263.20*	18.87±10.60	15.30±7.87
		Needle	8.30±0.80	0.37±0.20*	12.12±1.30*	595.50±43.30*	5.90±1.20	10.10±1.80

^aThe mean of N determinations and standard error of mean.*Indicates significant difference from reference values ($\alpha=0.05$, One-sample t-test).

was determined as 0.43, 1.4, 0.78, 57.28, 0.88, and $1.1 \mu\text{g g}^{-1}$ in d.w., respectively. Trace metal accumulation for the bark of *P. brutia* in these areas is 0.71, 20.03, 3.17, 1274.04, 18.87, and $15.30 \mu\text{g g}^{-1}$ in d.w., respectively. The mean values of the contents of those metals ($\mu\text{g g}^{-1}$ d.w.) were determined as 0.68, 4.59, 0.44, 486.35, 3.56, and 3.52, respectively.

The results indicate that the bark of *P. brutia* can accumulate high heavy metal concentrations. The industrial and urban roadside samples experiencing the highest human activity, together with higher vehicular density show the highest accumulation in regard to heavy metal concentrations.

In the statistical analysis; the Cd values in the control and other polluted areas, Pb values in the control and roadside areas, Cr values in the rural, roadside and industrial areas, Fe values in the control, suburban, and industrial areas, Ni values in the control and roadside values, and the Cu value in the control area were statistically meaningful ($P<0.05$). The other correlations between assumed as polluted sites and the control region were found not to be significant.

DISCUSSION

The increase in industrial activities, human population, and the high urbanization in the study area and its environs has made it one of the most rapidly developing areas in Turkey. Along with it, the environmental problems have rapidly increased in Western Anatolia. This study investigated the pollution level in the region by analyzing the density of some trace elements in the needle and bark samples of *P. brutia* which is the most widespread evergreen tree in this region of Turkey.

Leaded fuel is still a main source of lead

pollution; other atmospheric lead sources are, metal production, waste incineration, and mining activities. In addition, coal as a fossil fuel may also contain considerable amounts of lead (Uyar et al. 2009). Fig. 2 indicates that the amount of accumulation increases parallel to the aforementioned lead sources. It is clearly seen that lead levels rise especially in rural and suburban areas where coal is used for heating and that they become very high because of tools and industrial facilities considered as fundamental. As in cadmium, lead accumulation is higher in the bark compared to the needles. The concentration of lead was found higher in the industrial areas and roadside areas. Other than the industrial activities, lead in the petroleum caused the roadside areas to be polluted by the lead. Similar results were reported by other researchers in Turkey. For example, Baslar et al. (2005) come to the conclusion, as a result of their study carried out in the same study area on *Populus nigra* L., that industry and the dense traffic on the roads are the source of heavy metal pollution in Turkey. This opinion is also shared by some other researchers in the field in Turkey (Aksoy et al. 2000, Soylak et al. 2001).

Among heavy metals, cadmium is one of the elements of greatest environmental concern (Dogan et al. 2007). When the concentrations of cadmium in the *Pinus* needles and bark were investigated, no significant difference was observed between the control and rural, suburban, roadside, and industrial areas (Fig. 3). However, when the needles and bark were compared, it was clearly seen that Cd concentrations in the bark of the sampled trees in all areas were higher than those in the needles. The reason for this might be because of the fact that all

the needles are shed every 3 years, but the bark remains on trees. This is important data in terms of indicating the amount of Cd which the trees accumulated or are exposed to during their lives. However, the accumulations observed in the bark were lower than the values reported by Bowen (1979). This clearly shows that the Cd accumulation in the area is below dangerous levels.

Chromium is mainly emitted by the iron and steel processing industry and from coal combustion (Cucu-Man et al. 2004). The other emission sources may be the intensive traffic, especially transportation near the intercity road. The maximum chromium concentrations were measured at the sites closest to the most urbanized area, because this element is emitted when coal is burned (Ruhling and Steinnes 1988). Therefore, similarities of Cr concentrations between suburban and industrial areas can be explained by the usage of coal stoves. Fig. 4 indicates that, contrary to other metals, the chromium concentration is higher in the needles. This may be due to the accumulation of Cr especially in plant needles as a result of atmospheric pollution, whose basic source of emission is coal consumption.

Iron is one of the principle elements in the Earth's crust (Baslar et al. 2005). It is usually thought that this element is bound to soil particles and thus the content is not solely due to atmospheric deposition (Uyar et al. 2009). The main emission sources can be the steel industry, coal burning, and intensive traffic; there may also be an influence from soil dust, especially in the agricultural regions (Ruhling and Steinnes 1988). The comparison of iron levels between the contaminated and control sites shows that in the contaminated sites (industrial areas) iron contamination is much higher than the control sites (Fig. 5). This is also observed in the concentration levels in the bark. Concentration levels in the needles clearly show that the levels outside the control area increase closer to the industrial areas.

Nickel mainly originates from oil and coal burning, the steel industry, and smelters (Otvos et al. 2003). This metal is absorbed easily and rapidly by plants (Gune et al. 2004). The critical level for Ni in the soil is around $2.0\text{--}50\ \mu\text{g g}^{-1}$ as shown in the studies of Bergman (1992), Shallari et al. (1998), and Madejon et al. (2002). According to the results of our study, there is no critical nickel pollution in the investigation area. The highest Ni content was

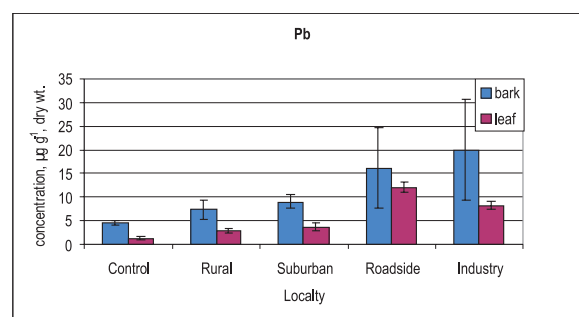


Fig 2. Lead concentrations in the bark and leaves of *P. brutia*.

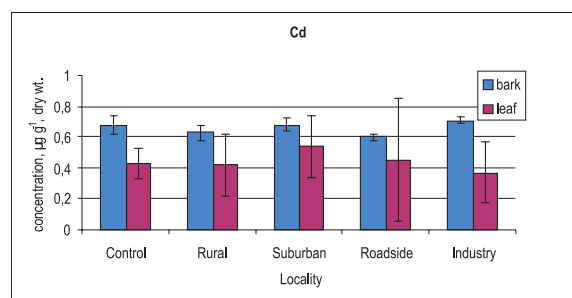


Fig 3. Cadmium concentrations in the bark and leaves of *P. brutia*.

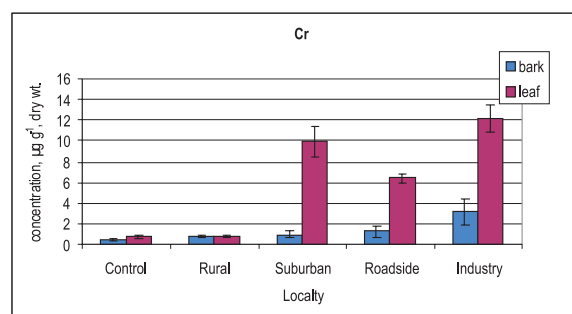


Fig 4. Chromium concentrations in the bark and leaves of *P. brutia*.

measured in the industrial areas (Fig. 6). The Ni concentration levels in the bark and needles differ from other metals in the roadside samples. This may be due to the fact that exhaust gases are not among the primary emission sources of Ni.

Copper mainly originates from the metal industry, mining, coal-fired plants, traffic, copper-containing fungicides, and fertilizers used by agriculture and even from the soil (Ruhling and Steinnes 1988, Otvos et al. 2003). As seen in Fig. 7, in the pines of the rural, suburban and roadside areas that were affected by these factors, the copper concentration was elevated in both the bark and needles, and this reached a maximum in the industrial areas. The samples we used in our study,

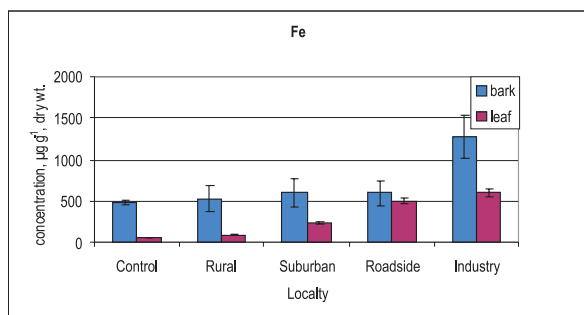


Fig 5. Iron concentrations in the bark and leaves of *P. brutia*.

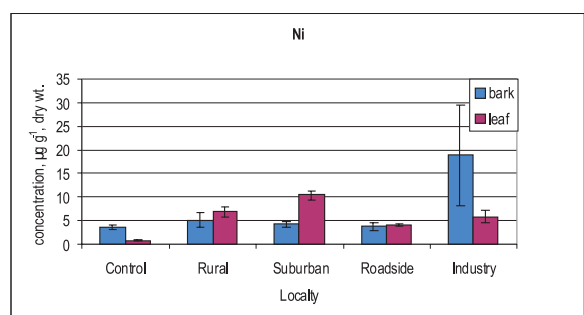


Fig 6. Nickel concentrations in the bark and leaves of *P. brutia*.

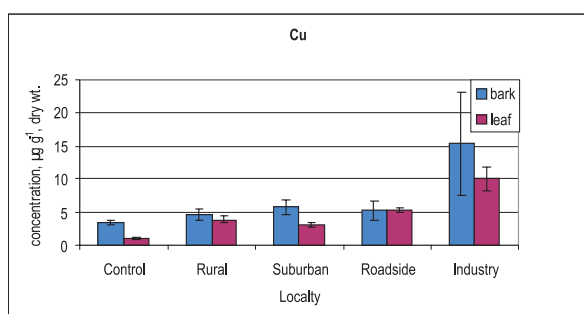


Fig 7. Copper concentrations in the bark and leaves of *P. brutia*.

when evaluated in terms of this element, it can be said that the needle of the *Pinus* is a better biomonitor than the bark.

Bioaccumulation of substances, including heavy metals, has been reported by a number of workers. Pyatt (1999) reported the heavy metal concentrations in the soil as $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu}$, while the concentrations in the Corsican pine needles was $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu}$. Pyatt (2001) and as also reported in another study the heavy metal concentrations in the acacia tree (*Acacia retinoides* Schltr.) from Cyprus as the control leaves holding the order of $\text{Fe} > \text{Mn} > \text{Cu} > \text{Pb}$. Celik et al. (2005) used leaves of the *Robinia pseudo-acacia* L. in their studies ranked

heavy metals as $\text{Fe} > \text{Mn} > \text{Zn} > \text{Pb} > \text{Cu} > \text{Cd}$. In our study, the accumulation values of heavy metals in the bark and leaf samples were observed respectively as: $\text{Fe} > \text{Pb} > \text{Ni} > \text{Cu} > \text{Cr} > \text{Cd}$ and $\text{Fe} > \text{Cr} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$. In general, other than Cr, no difference was observed. In that respect, it can be concluded that *Pinus* needles are better suited for the determination of Cr accumulation.

El-Hasan et al. (2002) investigated the concentrations in the bark of the *Cupressus sempervirens* L. in order to measure the atmospheric heavy metal pollution in the Amman City-Jordan area. They found the concentrations of Pb, Cd, and Cu in the bark samples collected from the polluted area (industrial and heavy traffic) in the range of 238-445, 0.11-0.51, and 6-82.7 mg kg respectively. The results correspond to our results in terms of Cd concentrations, while differing in terms of other values.

Huhn et al. (1995), Schulz et al. (1999), and Harju et al. (2002) said that pine tree bark is an excellent adsorbent of airborne pollutants, including anthropogenic heavy metals. Pacheco et al. (2001) reported that the olive tree bark as a suitable biomonitor as lichens or bryophytes. Similarly, Kuang et al. (2007) reported that the potential of the bark of the Masson pine for indicating typical elements from industrial sources seems promising as well. Studies of Mulgrew and Williams (2000) with conifer and those of Coskun (2006) and Samecka-Cymerman et al. (2006) with *P. nigra* and *P. sylvestris* species indicated that the bark of these trees are useful materials for the determination of air pollution. Also, Samecka-Cymerman et al. (2009) reported in a newer study from Poland that the bark of the *Robinia pseudoacacia* L. seems to be a better bioindicator of long-term cumulative traffic pollution in the investigated area instead of needles. All these studies support our conclusion that pine bark is a good biomonitor.

CONCLUSION

The increase in industrial activities and human population and the high urbanization in the study area have made the area one of the most rapidly developing areas in Turkey and parallel to these developments; environmental problems have also rapidly increased in the region. In this study, the needles and bark of the *P. brutia* were tested as a possible biomonitor of trace element pollution in the area. The discussed results of this study showed

that investigating the heavy metal pollution with *P. brutia* bark and needles lead us to the conclusion that there is no heavy metal pollution in most of the study area for all trace elements, except for the industrial areas and some roadside areas. That means that investigating the heavy metal accumulation of an area with the bark and needles of

the *P. brutia* has positive results only in identifying heavy metal pollution. Hence, this study demonstrates that the needles and bark of the Turkish red pine can be used as effective biomonitors for detecting heavy metal pollution in the study area. However, the trace element accumulation in the bark samples was observed to be relatively higher.

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