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An investigation on determining heavy metal accumulation in plants growing at Kumalar Mountain in Turkey

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Abstract

Background: Biomonitoring approach has been widely used to evaluate the environmental quality and detect the presence of inorganic and organic pollutants that are not routinely measured by conventional monitoring in the air.

Material and Methods: Twenty-five plant samples were obtained from twenty-three species used as biomonitors and found at two different altitudes in Kumalar Mountain with the aim of examining the levels of heavy metals. The concentrations of these elements were determined by inductively coupled plasma optical emission spectrometry. The levels of the heavy metals Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn ($\mu g\ g^{-1}$, dry weight) in plant samples supplied from different altitudes of Kumalar Mountain were assessed.

Results: As a result of this study, the following mean concentrations were determined at different altitudes of Kumalar Mountain: The contents of Al, Ba, Cu, Fe, Mn and Zn ($\mu g\ g^{-1}$, dry weight) ranged from 51.902 to 2960.650, 4.247 to 194.646, 0.927 to 21.024, 113.938 to 4289.115, 26.832 to 635.724 and 4.424 to 75.822, respectively. No Cd, Cr, Pb, Ni and Sn values were determined in the samples collected from both heights.

Conclusions: The accumulation of heavy metals such as iron (Fe) in some plant samples was found to be significantly higher than the normal accumulation levels.

Keywords: Biomonitoring, heavy metals, ICP-OES, Kumalar Mountain, plants, Turkey.

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INTRODUCTION

Progressive and uncontrolled industrialisation, as well as rapid urbanisation in some developing countries, causes serious environmental problems due to inadequate emission control and shortcomings in the environmental legislation (Sanchez-Chardi 2016). As a consequence of emissions from industrial plants and exhaust fumes in the environment, significantly elevated levels of heavy metals are present in the atmosphere and soil (Kleckerová and Dočekalová 2014). Circulation and the migration of metals in the natural environment are mainly associated with processes such as rock decay, volcanic eruptions, evaporation of oceans, forest fires, and soil formation (Dogan et al. 2010, Ugulu et al. 2012). Having high levels of toxicity and environmental persistence, heavy metals may distribute throughout the ecosystem and affect human health through the water supply and food chain (Micó et al. 2006).

A biomonitoring approach has been used widely to evaluate the environmental quality and detect the presence of inorganic and organic pollutants that are not routinely measured by conventional monitoring in the air (Durkan et al. 2011, Unver et al. 2015). This methodology is cost-effective compared to the physical-chemical approach, and can be applied with a flexible experimental design and a higher number of sampling points counterbalancing the relatively lower precision of each single measurement (Capozzi et al. 2016). For this reason, emphasis was given to the use of natural

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bioindicators to monitor air quality in both urban and rural environments in order to assess, minimise, and avoid the detrimental effects of toxic metals (Ng et al. 2005, Ugulu 2015).

Plants are essential components of ecosystems as they transfer elements from the abiotic environment to the biotic environment (Hu et al. 2014, Martínez-López et al. 2014). The main source of trace elements in plants is their growth media. A positive correlation was identified between heavy metal accumulation in the air and heavy metal concentrations in plants (Ugulu et al. 2012), and many plant species are able to absorb and accumulate significant amounts of potentially toxic substances (Piczak et al. 2003). Therefore, efficient plant species and planting designs to protect vulnerable areas in urban settlements from pollution can be used with the aim of mitigating human exposure to anthropogenic pollutants (Sæbø et al. 2012). Knowledge about the efficiency of plant species and cultivated plants in filtering and channelling polluted air and their resistance to urban environments is essential for the designation of measures to improve the air quality in cities (Dzierżanowski et al. 2011, Hu et al. 2014).

In recent years, many biomonitoring studies have been conducted to assess the effects of air pollutants in ecosystems, including approaches using several parameters in local plant species (Baslar et al. 2003, 2005, Yilmaz and Zengin 2004, Yilmaz et al. 2006, Dogan et al. 2007, Huseyinova et al. 2009, Sanchez-Chardi 2016). The samples collected in some of these studies have used mountains as a control group based on the assumption that these areas are not affected by pollutants (i.e. Baslar et al. 2003, 2005, Yilmaz and Zengin 2004, Dogan et al. 2007). This study is essential for determination of the levels of heavy metals in mountains which are considered to be free of heavy metals and therefore taken as a reference. Accordingly, the main purpose of this study is to present and examine the accumulation of Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn, and Zn by using plant species obtained from Kumalar Mountain.

MATERIALS AND METHODS

Sampling area

Located in the transition zone between the Aegean Region and the Central Anatolia Region, the sampling area is located between 38° 7' N and 38° 35' E, within the provincial borders of Afyon. Dividing the provincial area into two large catchment basins in the direction of Afyon-Sandıklı-Dinar, i.e. North-South direction, Kumalar Mountain is a mountain range that is 50 km to 60 km length, and 30 km to 35 km wide (Fig. 1). The Afyon-Sandıklı-Dinar highway constitutes the western borders of the sampling area, and the sampling area is surrounded by Sandıklı plain in the west, Dombay plain, Gül plain, and Çamur plain in the south, and Şuhut plain in the east. In terms of phytogeography, the sampling area is located in the transition zone of the Mediterranean and Irano-Turanian phytogeographical regions, according to Davis (1965-1988). The sampling area is located in B3 square according to the square system of Davis (1965-1988).

Sample collection and preparation

The plants were collected from two different altitudes (800-1200 m and 1200-1900 m) of Kumalar Mountain.

A total of twenty-five plant samples (twenty-three species) were collected. The taxonomic determination of the plant samples was carried out according to Davis (1965-1988).

The plant samples were exposed to open air to dry and then ground in a porcelain mortar into fine dust. The samples were stored in polyethylene storage containers. An analytical balance was used to measure approximately 0.3000 g of the samples to be transferred into a Teflon container; then, 10 mL of concentrated HNO_3 was added to the container and it was left for 12 h. Afterwards, the samples were exposed to microwave at 180°C for 10 min after heating the microwave digestion system to 180°C within 15 min. After cooling, the samples were transferred to a 25 mL volumetric flask and the remaining volume was made up with deionised water; the samples were then transferred to a polyethylene storage container.

Instrumentation

Plant samples were measured using an analytical balance (Sartorius, Germany) and digested by a

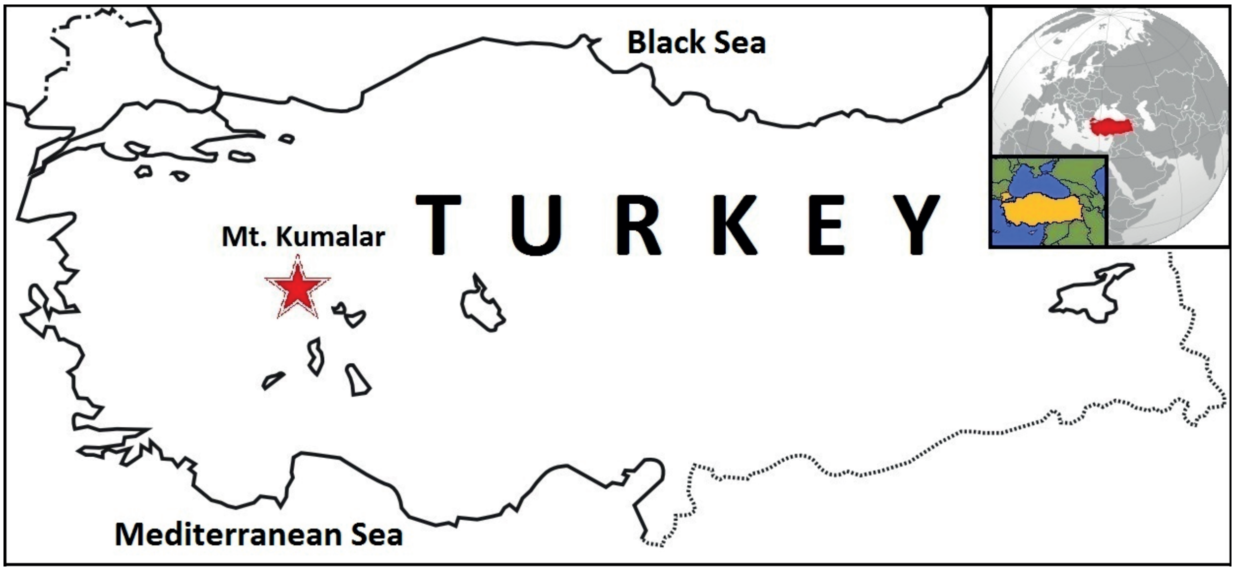

Fig. 1. Geographical location of the Mt. Kumalar.

microwave digestion system (CEM MARS 5, USA) in Teflon containers (HP 500, CEM MARS 5, USA). Inductively-coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 3100 XL, USA) was used for the determination of Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn, and Zn elements.

Reagents

Here, 18.2 MΩ cm deionised water (Sartorius, Germany) was used in experimental processes. Plant samples were digested using HNO_3 (Merck, Germany). Stock solutions of 1000 mg L^{-1} element (Merck, Germany) were used to prepare standard solutions.

Analysis process

Operating conditions for ICP-OES instrument for the measurement of Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn, and Zn elements contained in the plant samples were as follows: plasma gas flow: 15 L min^{-1}; auxiliary gas flow: 0,5 L min^{-1}; nebulisation gas flow: 0.5 L min^{-1}; view height: 15 mm; wavelengths (nm): 308.215 (Al), 233.527 (Ba), 228.802 (Cd), 267.716 (Cr), 327.393 (Cu), 238.204 (Fe), 257.610 (Mn), 231.604 (Ni), 220.353 (Pb), 189.927 (Sn) and 206.200 (Zn). Limit of Quantification (LOQ) for each element was calculated based on the calibration chart for each element.

Statistical data analysis

Statistical significance was determined by analysis of variance (ANOVA). ANOVA comparisons were made in order to determine whether there was any difference in terms of mean values between herbaceous plants and woody plants as well as any difference in terms of mean values between the plants collected between altitudes of 800 m and 1200 m and those collected between 1200 m and 1900 m altitudes, respectively. Variances determined as $p<0.05$ were considered significant. The Statistical Package for Social Sciences (SPSS) was used in the analysis of variance for the data collected.

RESULTS AND DISCUSSION

The samples were obtained from 23 plant species used as biomonitors and found at two different altitudes in Kumalar Mountain with the aim of examine the levels of heavy metals. The concentrations of these elements were determined by inductively coupled plasma optical emission spectrometry. The levels of the heavy metals Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn (µg g^{-1}, dry weight) in plant samples supplied from different altitudes of Mt. Kumalar are given in Table 1. As a result of this study, the following mean concentrations were determined at different altitudes of Mt. Kumalar: The contents of Al, Ba, Cu, Fe, Mn and Zn (µg g^{-1}, dry weight) ranged from 51.902 to 2960.650, 4.247 to 194.646, 0.927 to 21.024, 113.938 to 4289.115, 26.832 to 635.724 and 4.424 to 75.822, respectively (Table 1). No Cd, Cr, Pb,

Table 1. Al, Ba, Cu, Fe, Mn, Zn contents in plants growing at Mt. Kumalar (μg g^{-1}, dry weight).

Sample	Al	Ba	Cu	Fe	Mn	Zn
900-1200 m						
Herbaceous Plants						
Adonis aestivalis L.	1139.231	56.744	7.850	837.192	61.553	24.188
Asperula involucrata Wahlenb.	127.793	7.795	1.812	153.872	44.489	5.595
Cynanchum acutum L. subsp. *acutum*	51.902	116.681	1.591	120.445	174.649	10.448
Epilobium hirsutum L.	860.299	33.562	8.506	878.461	64.420	34.843
Inula heterolepis Boiss.	1295.299	23.975	9.278	1051.959	40.191	20.636
Lonicera etrusca Santi var. *etrusca*	336.946	67.785	9.209	157.853	59.488	19.993
Onobrychis hypargyrea Boiss.	726.039	14.625	2.550	491.245	225.887	4.424
Ranunculus ficaria L. subsp. *ficariformis* Rouy & Foucaud	225.351	4.247	12.275	283.463	60.858	52.941
Reseda lutea L. var. *lutea*	882.940	82.879	5.193	732.915	51.470	15.642
Salvia frigida Boiss.	2700.846	92.702	10.984	1882.256	96.218	36.638
Woody Plants						
Rhamnus lycioides L. subsp. *oleoides* (L.) Jahandiez & Maire	139.883	162.861	1.927	139.171	60.217	8.790
Rhamnus rhodopea Velenovsky	249.343	194.646	0.927	230.907	44.070	7.728
Ulmus glabra Huds.	1559.306	47.574	14.010	1469.766	61.458	38.653
1200-1900 m						
Herbaceous Plants						
Chenopodium botrys L.	2960.650	58.009	10.586	4289.115	249.543	75.822
Linaria genistifolia (L.) Miller subsp. *genistifolia*	366.591	37.633	4.597	248.917	182.917	31.083
Thalictrum minus L. var. *minus*	108.258	53.135	6.228	149.352	65.243	21.389
Veronica multifida L.	1568.302	58.883	13.134	1309.972	49.576	66.830
Vincetoxicum canescens (Willd) Decne subsp. *pedunculata* Browicz	768.561	43.520	8.838	737.254	56.615	10.593
Vincetoxicum canescens (Willd) Decne subsp. *pedunculata* Browicz	331.732	11.970	7.464	327.402	65.771	22.630
Vincetoxicum tmoleum Boiss.	106.986	129.991	21.024	113.938	226.383	33.688
Viola sieheana W. Becker	718.699	21.712	4.830	777.491	144.886	16.498
Viola sieheana W. Becker	2887.616	89.803	13.734	2061.019	111.180	48.297
Woody Plants						
Mespilus germanica L.	463.261	105.026	3.266	528.889	26.832	6.979
Pyrus elaeagnifolia Pallas subsp. *elaeagnifolia*	300.803	194.602	4.860	339.856	101.791	23.221
Quercus cerris L. var. *cerris*	159.315	55.476	6.526	155.908	635.724	25.291
Min.:	51.902	4.247	0.927	113.938	26.832	4.424
Max.:	2960.650	194.646	21.024	4289.115	635.724	75.822
Mean:	841.438 ±175.981	70.633 ±10.905	7.647 ±0.968	778.744 ±183.703	118.457 ±25.184	26.513 ±3.755

Ni and Sn values were determined in the samples collected from both heights.

Some plant species are useful for biomonitoring of the accumulation of pollutants in the atmosphere (Baslar et al. 2009). Accordingly, all heavy metals examined in plant samples collected from Mt. Kumalar were presented in Table 1. From the Table, it can be seen that Al content was the highest in *C. botrys* (2960.650 μg g^{-1}), and the lowest in *C. acutum* subsp. *acutum* (51.902 μg g^{-1}). Ba content was the highest in *R. rhodopea* (194.646 μg g^{-1}), and the lowest in *R. ficaria* subsp. *ficariiformis* (4.247 μg g^{-1}). It was determined that Cu content was the highest in *V. tmoleum* (21.024 μg g^{-1}), whereas the lowest value was recorded in *R. rhodopea* (0.927 μg g^{-1}). In terms of Fe content, *C. botrys* (4289.115 μg g^{-1}) was the highest, and *V. tmoleum* (113.938 μg g^{-1}) was the lowest. Mn content was the highest in *Q. cerris* var. *cerris* (635.724 μg g^{-1}), and the lowest in M. germanica (26.832 μg g^{-1}). Finally, it was determined that Zn content was the highest in *C. botrys* (75.822 μg g^{-1}), whereas the lowest value was recorded in *O. hypargyrea* (4.424 μg g^{-1}).

Biomonitoring studies using plant samples were conducted on many mountains located in the Western Anatolia with the aim of determining heavy metal pollution in the atmosphere. Dogan et al. (2014) obtained the following results in their study conducted on Kazdagi, another important mountain of the same region: The mean concentrations determined at 600 m altitude ranged from 0.107 to 0.442, 0.269 to 0.619, 0.873 to 9.030, 0.338 to 0.523 and 0.143 to 2.823 (μg g^{-1}, dry weight), for Ni, Zn, Fe, Pb and Mn, respectively. At 1100 m altitude, the values ranged from 0.119 to 1.806, 0.232 to 0.792, 0.618 to 5.720, 0.371 to 0.534 and 0.766 to 4.782 (μg g^{-1}, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. Ugulu et al. (2012) investigated heavy metal accumulation in the plant samples in the study they conducted on Mt. Murat: The mean concentrations determined at 1000 m altitude ranged from 0.139 to 4.518, 0.223 to 0.986, 0.359 to 6.930, 0.443 to 0.727 and 0.077 to 3.222 (μg g^{-1}, dry weight), for Ni, Zn, Fe, Pb and Mn, respectively. At 1600 m altitude, the values ranged from 0.191 to 6.248, 0.302 to 1.008, 2.387 to 8.896, 0.345 to 0.570 and 0.195 to 3.502 (μg g^{-1}, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was found at either altitude. Kula et al. (2010) have studied trace element concentrations of plants in Akdag and obtained the following results: The mean concentrations determined at 1000 m altitude ranged from 0.011 to 0.882, 0.241 to 0.714, 0.532 to 9.396, 0.329 to 0.487, and 0.155 to 3.439 (μg g^{-1}, dry weight), for Ni, Zn, Fe, Pb and Mn, respectively. At 1600 m altitude, the values ranged from 0.092 to 0.600, 0.272 to 0.834, 1.130 to 8.021, 0.263 to 0.889 and 0.076 to 0.508 (μg g^{-1}, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. Baslar et al. (2009) obtained the following results in their study conducted on Honaz, another important mountain of the same region: The mean concentrations determined at 1000 m altitude ranged between 0.273 to 0.488, 0.099 to 0.488, 0.306 to 0.682, 1.017 to 3.744, and 0.148 to 0.674 (μg g^{-1}, dry weight), of Pb, Ni, Zn, Fe, and Mn, respectively. At 1600 m altitude, the values ranged between 0.225 to 0.534, 0.150 to 0.842, 0.234 to 0.905, 1.082 to 3.864 and 0.023 to 0.982 (μg g^{-1}, dry weight) of Pb, Ni, Zn, Fe, and Mn, respectively. No Cd was detected at either altitude.

Based on the comparison between the results obtained from the abovementioned studies and the findings of this study, we can conclude that the accumulation levels of Fe, Mn, and Zn metals, commonly examined in the aforementioned studies and this study, were found to be significantly high in Mt. Kumalar. The variance between the results of the aforementioned studies and this study may be due to differences in the sample plant species, and soil composition where these sample plant species were cultivated as well as sample digestion methods and spectrometric methods adopted in this study. On the other hand, the findings were similar in all studies in terms of inability to determine the level of Cd.

Plant samples are often used for ecosystem quality assessment due to their vulnerability to chemical changes in environmental composition and the fact that they accumulate pollutants (Ugulu 2015). On the other hand, like all living organisms, plants are often vulnerable to both deficiency and excess availability of some heavy metal ions as essential micronutrients, whereas the same at higher concentrations and other ions such as As, Cd,

Table 2. Statistical analysis values of herbaceous and woody plants.

		df	F	Sig.
Al	Between Groups	1	1,362	,255
	Within Groups	23		
	Total	24		
Ba	Between Groups	1	12,264	,002
	Within Groups	23		
	Total	24		
Cu	Between Groups	1	2,012	,170
	Within Groups	23		
	Total	24		
Fe	Between Groups	1	,844	,368
	Within Groups	23		
	Total	24		
Mn	Between Groups	1	,656	,426
	Within Groups	23		
	Total	24		
Zn	Between Groups	1	1,488	,235
	Within Groups	23		
	Total	24		

Table 3. Statistical analysis values of plant species collected from 900-1200 m and 1200-1900 m.

		df	F	Sig.
Al	Between Groups	1	,082	,777
	Within Groups	23		
	Total	24		
Ba	Between Groups	1	,008	,931
	Within Groups	23		
	Total	24		
Cu	Between Groups	1	1,221	,281
	Within Groups	23		
	Total	24		
Fe	Between Groups	1	,535	,472
	Within Groups	23		
	Total	24		
Mn	Between Groups	1	2,646	,117
	Within Groups	23		
	Total	24		
Zn	Between Groups	1	1,945	,176
	Within Groups	23		
	Total	24		

and Hg are highly toxic to metabolic actions (Anonymous 2004). Bowen (1979) has reported the normal natural concentration intervals for land plants as some heavy metals like Cd: 0.2-2.4 μg g^{-1}, Ni: 1-5 μg g^{-1}, Zn: 20-400 μg g^{-1}, Fe: 70-700 μg g^{-1}, Pb: 1-13 μg g^{-1}, and Mn: 20-700 μg g^{-1}. Comparing our results with these findings shows that our results are well below the accepted range for Zn and Mn. On the other hand, Fe accumulation in some plant samples was found to be significantly higher than the normal accumulation levels.

It may be useful to identify the sources of contamination caused by the accumulation of heavy metals, as demonstrated by various researchers, in order to evaluate the findings obtained from the results of the study. For instance, Pb and Zn mainly originate from anthropogenic actions (Alfani et al. 2000, Blok 2005, Oliva and Rautio 2005). Major anthropogenic sources of Ni are burning of coal and oil, production of Cu, Ni, and Pb, mining operations, steel works and the cement industry (Nriagu and Pacyna 1988). Loppi et al. (1999) reported that plants were highly affected by soil contamination by Fe and Mn in the Mediterranean climate zone although airborne Mn mainly originates from soil (Bargagli et al. 2003, Oliva and Rautio 2005) whereas Fe originates from both anthropogenic and natural sources (Oliva and Rautio 2005).

In the statistical analysis, comparison of heavy metal pollution values of herbaceous and woody plants for all heavy metals was not significant except for Ba ($p>0.05$) (Table 2). Similar to the findings of this study, Dogan et al. (2014) were unable to determine a statistically significant difference in terms of heavy metal accumulation in herbaceous and woody plants based on the results of their study conducted in Kazdagi. On the other hand, Kula et al. (2010) determined the variance of Fe, Pb, and Mn levels between herbaceous and woody plants to be statistically significant based on the studies conducted on Bozdag.

Based on the analysis of the values of heavy metal accumulation in plants cultivated at altitudes between 900 m and 1200 m and between 1200 m and 1900 m, accumulation values for each heavy metal found in plant samples obtained between 1200 m and 1900 m were concluded to be higher than those cultivated at lower altitudes (Table 1).

These high levels of accumulation observed at higher altitudes of Mt. Kumalar may be due to pollution in the atmosphere. On the other hand, analyses conducted to determine whether there was a significant difference between the plant samples found in higher and lower altitudes demonstrated that there was no statistically significant difference (Table 3).

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