A STUDY ON DETECTING HEAVY METAL ACCUMULATION THROUGH BIOMONITORING: CONTENT OF TRACE ELEMENTS IN PLANTS AT MOUNT KAZDAGI IN TURKEY

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Abstract. The purpose of this study is to determine the present levels of atmospheric heavy metal pollution in the area of the Kazdagi Mountain in the Aegean region in the western part of Turkey. Twenty-five different plants were selected as potential biomonitors of trace elements including nickel, iron, zinc, lead, cadmium, and manganese (μg g^{-1}, dry weight). The samples were collected from two different heights of Mt. Kazdagi. Atomic absorption spectrometry was used to determine the concentrations of trace elements. 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 found at either altitude. For the determination of the existence of any differences between the averages of the herbaceous and woody plants, comparisons were made in the independent sample *t*–test. In the statistical analysis, comparison of heavy metal pollution values of herbaceous and woody plants for Ni, Fe and Mn was significant ($P < 0.05$), while it was not for Pb and Zn.
Keywords: *Mt. Kazdagi, biomonitoring, trace element*

Introduction

Increasing anthropogenic influences on the environment have caused negative changes in natural ecosystems and altered the normal biogeochemical cycling (Ugulu et al., 2008; Dogan, 2012; Ugulu, 2012; Dogan et al., 2013). Among the anthropogenic activities, heavy metal contamination of the biosphere has increased sharply since 1900 and it poses major environmental and human health problems worldwide (Prasad & Freitas, 2003). The circulation and migration of metals in the natural environment are mainly related to such processes as rock decay, volcanic eruptions, evaporation of oceans, forest fires and soil formation. The sources of anthropogenic contamination or pollution of the environment by heavy metals include different branches of industry such as the power industry, transport, municipal waste management, waste dumping sites, fertilisers and waste used to fertilise soil (Aksoy et al., 2000; Karaca et al., 2005; Osma et al., 2012). The heavy metals from these sources are dispersed in the environment and they contaminate soil, water and air (Elekes et al., 2010). They also get into human and animal bodies, directly or indirectly, through plants (Szyczewski, 2009; Yasar et al, 2012a).

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Biological monitoring within a quality control programme involves the systematic use of living beings for obtaining quantitative information on changes in the environment, often due to anthropogenic activities (Bargagli, 1998). Biological responses can be considered more representative than data supplied by chemical or physical detectors, in that they are spatially and temporally extensive; moreover, they allow for estimating both the levels of pollutants and, even more importantly, the impact on biological receptors (Calzoni et al., 2007). For this reason, in order to evaluate, minimise and avoid detrimental effects of toxic metals, there has been an emphasis on the use of natural bioindicators to monitor atmospheric quality in both urban and rural environments (Szczepaniak & Biziuk, 2003; Ng et al., 2005).

Biomonitoring has been used to detect the deposition, accumulation and distribution of trace metals in the ecosystems. By using different types of vegetation with this method, the levels of atmospheric trace metallic concentrations have been successfully monitored (El–Hasan et al., 2002; Baslar et al., 2003; Dogan et al., 2007; Onder et al., 2007). Biomonitoring provides the cheapest and simplest method for monitoring trace metals elements in the atmosphere (Kaya & Yaman, 2008; Cayir et al., 2008).

Recently, many studies have been devoted to showing the distribution of heavy metal pollution in the urban and ruderal habitats in Turkey (Baslar et al., 2005; Yilmaz et al., 2006; Dogan et al., 2007; Cayir et al., 2008; Ozturk et al., 2008; Huseyinova et al., 2009; Akguc et al., 2010; Durkan et al., 2011; Ugulu et al., 2012; Yasar et al., 2012b). In some of these studies, mountainous areas, assumed to be unpolluted, have been used as control groups (i.e. Baslar et al., 2003, 2005, 2009; Yilmaz & Zengin, 2004; Dogan et al., 2007; Dogan et al., 2010). The present study is significant in that it aims to determine heavy metal levels in mountainous areas which are considered to be free of heavy metals, and, therefore, uses them as a reference.

The purpose of this study was to investigate and present the concentrations of lead, cadmium, nickel, zinc, iron and manganese by using plant samples from Mt. Kazdagi.

Materials and methods

Sampling area

Mt. Kazdagi (Mt. Ida) is the highest mountain on the Biga Peninsula, which is situated in the south-western part of Anatolia (*Fig.1*). It separates the Aegean and Marmara regions. It has three summits and the highest among them is 1774 m. Mt. Kazdagi possesses various climatic features due to its geographical position. On its south slopes, it is possible to see the characteristic Mediterranean climate because of its proximity to the Aegean Sea, whereas a cooler and more humid terrestrial climate is observed on its northern slopes.

Because of its climatic properties, geological structure and location, Mt. Kazdagi is ecologically and floristically diverse, containing a number of plant species endemic to Turkey. There are about 800 taxa growing naturally in the area and 79 of them are endemic to Turkey (Satil, 2008).

The location of the sampling points on steep slopes provides results that depend on altitude rather than on horizontal distance (Zechmeister, 1995). The samples were collected from altitudes of 600 m and 1100 m above sea level and 35–40 km away from the city centre.

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***Figure 1**. Geographical location of the study area*

Sample collection and preparation

The samples were collected from 600 m and 1100 m during the months of July–August, 2006. Twenty-five plant species in total were collected, 11 of which were from 600 m and 14 from 1100 m. The taxonomy of the plant samples was determined according to Davis (1965–1985), Davis et al. (1988) and Guner et al. (2001).

For analysis purposes, about 100 g of aboveground parts of bushy species and well-developed leaves of other plants were collected. The samples were dried in an oven at 80°C for 24 h, milled in a micro–hammer cutter and fed through a 0.2 mm sieve. The samples were stored in clean self–sealing plastic bags under silica gel desiccant. In order to eliminate contamination from the micro–hammer cutter during the grinding, it was washed after every grinding, first with absolute alcohol then with distilled water. Therefore, the contamination was negligible.

Wet digestion procedure

The method described by the Perkin Elmer Corporation was used for plant digestion (Anonymous, 1996). The digested samples were aspirated into an air–acetylene flame and the existence of metals was determined by flame atomic absorption spectrometry (FAAS). The reproducibility was ensured by carrying out triplicate analyses. All samples were analysed immediately after digestion.

Reagents

Unless otherwise specified, all the chemicals used were of analytical reagent grade. Triple-distilled water was used throughout the experiments. By diluting the stock standard solution with water, working metal standard solutions were prepared just before use.

Instrumentation

The existence of the metals was determined with the Perkin Elmer Analyst 700 model flame atomic absorption spectrometer equipped with deuterium background

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correction, hollow cathode lamps (HCL) and acetylene burner. The absorption measurements of the metals were performed under the conditions recommended by the manufacturer. A Cole–Parmer microfiltration apparatus with membrane filter (0.45 µm pore size manufactured by Micro Filtration Systems, MFS) was used for the filtration of the aqueous phase before metal determination.

Data analysis

Statistical significance was determined by the independent sample *t*–test. In the independent sample t– test, comparisons were made in order to determine whether there were any differences between the averages of the herbaceous plants and woody plants. 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 and discussion

The plants, used as biomonitors to investigate the levels of the trace elements, were sampled with 25 different species at two different levels of altitude on Mt. Kazdagi. The concentrations of elements were determined by atomic absorption spectrometry. The levels of the trace elements Cd, Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) in plant samples from different altitudes of Mt. Kazdagi are given in *Tables 1* and *2*. As a result of experiments carried out, the following mean concentrations were determined for 600 m altitude: The contents of Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) 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, respectively (*Table 1*). As for the average for 1100 m altitude, the contents of Ni, Zn, Fe, Pb and Mn (µg g^{-1}, dry weight) 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, respectively (*Table 2*). No Cd values were determined in the samples collected from both heights.

Table 1. *Trace element contents in plants growing in the Mt. Kazdagi (µg g–1 dry weight) (600m)*

Plant	Ni	Zn	Fe	Pb	Mn
		woody			
Celtis australis L.	0.170	0.598	2.347	0.504	0.877
Cydonia oblanga Miller.	0.138	0.521	1.432	0.523	0.226
Juglans regia L.	0.222	0.619	2.633	0.357	0.531
Malus sylvestris Miller.	0.134	0.414	1.449	0.503	0.230
Pinus brutia Ten.	0.133	0.337	0.873	0.352	1.429
Quercus cerris L. subsp. *cerris*	0.169	0.305	2.731	0.498	2.823
		herbaceous			
Dryopteris filix–mas (L.) Schott	0.107	0.269	1.332	0.398	0.925
Epilobium angustifolium L.	0.442	0.432	9.030	0.453	1.525
Hypericum lydium L.	0.196	0.538	3.179	0.454	0.519
Juncus inflexus L.	0.208	0.407	1.067	0.376	0.589
Verbascum sp.	0.150	0.366	0.897	0.338	0.143
Min.:	0.107	0.269	0.873	0.338	0.143
Max.:	0.442	0.619	9.030	0.523	2.823
Mean:	0.18±0.02	0.43±0.03	2.45±0.70	0.43±0.02	0.89±0.23

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Some plant species are useful for biomonitoring of the atmospheric deposition of pollutants (Kaya & Yaman 2008; Baycu et al., 2006; Mertens et al., 2005; Singh et al., 2005; Smodis et al., 2004). In this respect, the aggregation of investigated trace elements in plants collected from the 600 m height on Mt. Kazdagi was presented in *Table 1*. It can be seen from the table that Ni content was the highest in *Epilobium angustifolium* (0.442 µg g^{-1}), and the lowest in *Dryopteris filix–mas* (0.107 µg g^{-1}). Zn content was the highest in *Juglans regia* (0.619 µg g^{-1}), and the lowest in *Dryopteris filix–mas* (0.269 µg g^{-1}). It was determined that Fe content was the highest in *Epilobium angustifolium* (9.030 µg g^{-1}), while the lowest was in *Pinus brutia* (0.873 µg g^{-1}). In terms of Pb content, *Cydonia oblanga* (0.523 µg g^{-1}) was the highest, and *Verbascum* sp. (0.338 µg g^{-1}) was the lowest. Mn content was the highest in *Quercus cerris* subsp. *cerris* (2.823 µg g^{-1}), and the lowest in *Verbascum* sp. (0,143 µg g^{-1}). At 1000 m, the highest values were recorded as Ni and Fe in *Epilobium angustifolium,* Zn in *Juglans regia,* Pb in *Cydonia oblanga* and Mn in *Quercus cerris* subsp. *cerris.*

The results of analysis of trace element values in plants collected at 1100 m are presented in *Table 2*. The table shows that Ni content was highest in *Plantago holosteum* (1.806 µg g^{-1}), and the lowest in *Rubus idaeus* (0.119 µg g^{-1}). In terms of Zn content, it was the highest in *Fragaria vesca* (0.792 µg g^{-1}), and the lowest in *Erica manipuliflora* (0.232 µg g^{-1}). Fe content was highest in *Fragaria vesca* (5.720 µg g^{-1}), and the lowest in *Abies nordmanniana* subsp. *equi–trojani* (0.618 µg g^{-1}). It was seen that Pb content was highest in *Abies nordmanniana* subsp. *equi–trojani* (0.534 µg g^{-1}), and the lowest was in *Origanum vulgare* (0.371 µg g^{-1}). The highest Mn content was observed in *Quercus cerris* subsp. *cerris* (4.782 µg g^{-1}), while the lowest Mn content was in *Celtis australis* (0.766 µg g^{-1}). At 1600 m, Ni content was highest in *Plantago holosteum*, Zn and Fe were highest in *Fragaria vesca,* Pb was highest in *Abies nordmanniana* subsp. *equi–trojani* and Mn was highest in *Quercus cerris* subsp. *cerris.*

Sources of pollution that cause accumulation of various heavy metals have been reported by some researchers. For instance, Pb and Zn originate mainly from anthropogenic activities (Alfani et al., 2000; Blok, 2005; Oliva & Rautio, 2005). The burning of coal and oil cause the production of Cu, Ni and Pb, and mining operations, steel works and the cement industry are major anthropogenic sources of Ni (Nriagu & Pacyna, 1988). Plants have been reported to be highly affected by contamination of the soil by Fe and Mn in the Mediterranean climate zone, although airborne Mn originates mainly from soil (Loppi et al., 1999; Bargagli et al., 2003; Oliva & Rautio, 2005). Fe originates both from anthropogenic and natural sources (Oliva & Rautio, 2005).

Various researchers in different parts of the world, as well as in Turkey, investigated the accumulation of trace elements in plant parts. Some of those are given below for the purpose of comparison with our findings: Dijingova et al. (1995) (Cd: 0.10–31.20 µg g^{-1}, Ni : 0.50–4.9 µg g^{-1}, Zn: 7–302 µg g^{-1}, Fe: 100–283 µg g^{-1}, Pb: 0.80–21.30 µg g^{-1}, Mn: 44–405 µg g^{-1}), Baslar et al. (2003) (Ni: 0.88 µg g^{-1}, Fe: 57.28 µg g^{-1}, Pb: 1.4 µg g^{-1}), Dogan et al. (2007) (Ni: 3.56 µg g^{-1}, Fe: 486.35 µg g^{-1}, Pb: 4.59 µg g^{-1}), Baslar et al. (2005) (Cd: 1.7 µg g^{-1}, Zn: 63.4 µg g^{-1}, Fe: 182.6 µg g^{-1}, Pb: 2.3 µg g^{-1}) and Kapusta et al. (2005) (Cd: 6.44 µg g^{-1}, Pb: 5.64 µg g^{-1}, Zn: 304 µg g^{-1}).

Trace elements, which are an intrinsic part of nature, appear in the composition of plants. Bowen (1979) has reported the normal natural concentration intervals for land plants as 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}, Mn: 20–700 µg g^{-1}. Comparison of our results with these findings (*Tables 1* and *2*) clearly shows that our values are well below the accepted range. Hence, the

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area studied is free from the contamination of heavy metal pollution in terms of the trace elements investigated. The accumulation levels obtained are soil orientated.

Table 2. *Trace element contents in plants growing in the Mt. Kazdagi (µg g–1 dry weight) (1100m)*

Plant	Ni	Zn	Fe	Pb	Mn
woody					
Abies nordmanniana (Steven) Spach subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen	0.171	0.631	0.618	0.534	2.601
Castanea sativa Miller.	0.214	0.704	1.902	0.414	2.246
Celtis australis L.	0.223	0.615	1.816	0.404	0.766
Erica manipuliflora Salisb.	0.200	0.232	4.065	0.440	2.634
Pinus nigra Arn. subsp. *pallasiana* (Lamb) Holmboe	0.237	0.486	1.272	0.417	2.623
Quercus cerris L. subsp. *cerris*	0.187	0.321	1.833	0.420	4.782
Rubus idaeus L.	0.119	0.393	1.045	0.470	3.820
herbaceous					
Dactylis glomerata L. subsp. *hispanica* (Roth) Nymas	0.201	0.384	0.997	0.460	1.154
Dryopteris filix–mas (L.) Schott	0.179	0.510	0.812	0.434	0.892
Euphorbia sp.	0.175	0.659	1.632	0.390	0.823
Fragaria vesca Coville	0.678	0.792	5.720	0.512	3.352
Origanum vulgare L.	0.314	0.625	5.165	0.371	1.339
Plantago holosteum L.	1.806	0.732	4.823	0.519	2.474
Verbascum sp.	0.161	0.377	1.472	0.445	1.466
Min.:	0.119	0.232	0.618	0.371	0.766
Max.:	1.806	0.792	5.720	0.534	4.782
Mean:	0.34±0.11	0.53±0.04	2.36±0.47	0.44±0.01	2.21±0.32

Baslar et al. (2009) have obtained the following results in a study they conducted on Mt. Honaz, which is 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 ($\mu g\ g^{-1}$, 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($\mu g\ g^{-1}$, dry weight) of Pb, Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. Additionally, Kula et al. (2010) have studied trace element concentrations of plants on Mt. 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 ($\mu g\ g^{-1}$, 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 ($\mu g\ g^{-1}$, dry weight) for Ni, Zn, Fe, Pb and Mn, respectively. No Cd was detected at either altitude. The similarities of the results obtained from Mt. Honaz, Mt. Akdag and Mt. Kazdagi show the validity and credibility of all studies concerned.

In the statistical analysis, comparison of heavy metal pollution values of herbaceous and woody plants for Ni, Fe and Mn was significant ($P < 0.05$), while it was not for Pb

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and Zn (*Table 3*). Kula et al. (2010) found the difference between the values of Fe, Pb and Mn from herbaceous and woody plants of Mt. Akdag statistically significant, while Yildiz et al. (2010) found the values of Fe and Mn statistically significant in their work on Mt. Bozdag. When we compare our results with those of the above two studies, it can be concluded that the concentration of Fe, particularly, displays a statistically significant difference between herbaceous and woody plants of the area.

Table 3. *Statistical analysis values of herbaceous and woody plants*

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ni	Equal variances assumed	6,856	,015	-1,564	23	,131	-,206519	,132036	-,479656	,066617
	Equal variances not assumed			-1,501	11,139	,161	-,206519	,137598	-,508911	,095872
Zn	Equal variances assumed	,023	,881	-,516	23	,611	-,032506	,063001	-,162835	,097822
	Equal variances not assumed			-,514	22,433	,612	-,032506	,063201	-,163430	,098417
Fe	Equal variances assumed	12,325	,002	-1,502	23	,147	-1,163115	,774471	2,765230	,438999
	Equal variances not assumed			-1,454	13,522	,169	-1,163115	,800065	2,884797	,558566
Pb	Equal variances assumed	,331	,570	,842	23	,408	,019756	,023450	-,028753	,068266
	Equal variances not assumed			,846	23,000	,407	,019756	,023366	-,028579	,068092
Mn	Equal variances assumed	4,524	,044	1,460	23	,158	,701558	,480539	-,292513	1,695629
	Equal variances not assumed			1,487	20,271	,152	,701558	,471746	-,281643	1,684759

In the present study, the plants used as biomonitors to investigate the levels of the trace elements Cd, Ni, Zn, Fe, Pb and Mn (μg g^{-1}, dry weight), were sampled with 25 different species at two different heights (600 m and 1100 m) on Mt. Kazdagi. The values of the trace elements we obtained were below the values obtained from control samples of other studies of clean areas, and therefore low values are concluded to be soil orientated. We are convinced that this study will contribute to future studies on pollution.

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