BIOMONITORING OF HEAVY METALS DEPOSITION WITH PSEUDEVERNIA FURFURACEA (L.) ZOPF IN ÇORUM CITY, TURKEY

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ARTICLE INFO	ABSTRACT
	Heavy metal air pollution is an important environmental problem. One of the methods used to monitor pollution in air is the method of transplanting lichen samples by the "bag technique". In this study, Pseudevernia furfuracea was used as a bioindicator to determine the heavy metal level in the air of Çorum and to generate an air pollution map of the city. The lichen samples were collected from the Yapraklı Mountains in Çankırı in 2002 and transplanted to 8 different stations in Çorum. Lichen samples were retrieved at two different periods in three month intervals. Inductively Coupled Plasma (ICP) spectrometry (Varian Liberty ICP-OES Sequential) was used to identify the heavy metals, such as copper (Cu), cadmium (Cd), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) in the lichen samples. The chlorophyll a and b contents were determined by using the modified DMSO (dimethyl sulfoxide) method. With these values chlorophyll a+b, a/b and b/a were also calculated. According to the results of the heavy metal analysis by the use of P. furfuracea, air pollution levels in Çorum was detected. The reasons of pollution can be stated as heavy traffic, industrial activities and heating processes in the city. P. furfuracea can be used as a bioindicator for pollution studies.

1. INTRODUCTION

Lichens are symbiotic organisms that occur in almost all terrestrial ecosystems and by virtue of their ability to tolerate long periods of drought may even colonise areas with extreme environmental conditions. Because of their high surface: volume ratio, the simple anatomy and absence of a cuticle, they accumulate heavy metals, concentrating them in thalli. Due to this ability, they may show an elemental composition which reflects, over the long term, the

dissolved gases, particulate matter and metal ions of the atmosphere, and can be considered important biomonitors of environmental pollution (Yıldız *et al.* 2011). Air pollution has received attention due to the fact that it is detrimental for human health and the environment (Abdunnasır *et al.* 1994, Karademir and Toker 1998, Çayır *et al.* 2008).

Lichens and mosses have been widely used for more than 20 years for assessing the atmospheric deposition of heavy metals and radionuclides in urban areas (Adamo *et al.* 2003). Lichens occurring naturally in the area as well as those transplanted have been used as biomonitoring organisms in a large number of studies (Bargagli 1998, Brown 1984, Conti and Cecchetti 2001, Henderson 1994, Nimis 1996, Richardson 1992, Tyler 1990, Yıldız *et al.* 2008, Yıldız *et al.* 2011).

In urban areas, where lichens are often scarce or even absent, the ''bags technique'' has been set up and developed in order to monitor air pollution (Goodman and Roberts 1971). Bags consist of a mesh or grid, generally made of nylon, containing lichens. This technique has the following advantages: uniformity of entrapment surface and exposure period, flexibility both in site selection and in the number of stations that can be chosen, known original concentrations of contaminants in the biomonitors and greater collection efficiency for most elements. In addition, the bag techniques eliminate the possibility of contamination via root uptake and, in comparison with dust fall jars or bulk samplers, offer lower cost and higher efficiency (Adamo *et al.* 2003). The duration of exposure is another critical aspect of biomonitoring by bags (Bargagli 1998). Biomonitors may reach a saturation point for the uptake of an element and biomonitoring performance may also be altered by climatic and environmental conditions (Garty *et al.* 1993). The comparability of results obtained with different cryptogamic organisms is another problem associated with biomonitoring studies (Schmid-Grob *et al.* 1992) as a consequence of differences in ecophysiology and mechanisms of metal bioaccumulation. In areas with widespread geochemical natural and anthropogenic sources of metals, epiphytic lichens seem more reliable biomonitors of atmospheric deposition of trace elements (Bargagli and Mikhailova 2002).

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It is known that the chlorophyll content of the lichen decrease with increasing pollution which is the physiological effect of pollution on lichens (Yıldız *et al.* 2011). This may be due to the inhibition of "the novo synthesis" and (or) an increase in amino acid degradation (Godzik and Linskens 1974). It has been shown that air pollutants ultimately reduce both photosynthetic and respiration rates in lichens (Pearson and Skye 1965, Puckett *et al.* 1974, Beekley and Hoffman 1981).
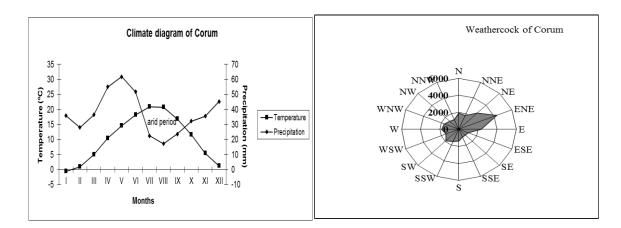
The purpose of this study is to determine Pb, Cu, Cd, Mn, Ni and Zn concentrations and chlorophyll a and b contents in *Pseudevernia furfuracea* (L.) Zopf. For this purpose, lichens from an unpolluted area were transplanted to the selected locations in Çorum.
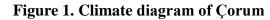
2. MATERIAL AND METHODS

2.1. Study Area

The study was performed in the urban area of Çorum. Çorum is located at Central Anatolia (Figure 3) with a population of 221,699 inhabitants (according to the 2000 census) (Anonymous 2000). The total number of vehicles registered was 82,487 in 2002 (Anonymous 2003). The main air pollution sources of Çorum are urban motorway, industrial and domestic heating. The climate of the city is semi-arid continental climate with cold winters (Akman 1999) with mean annual temperature of 8,5 °C. The climate diagram of Çorum is given in (Figure 1). The prevailing winds are from ENE (Anonymous 2008) (Figure 2). Eight exposure sites were

selected in the city of Çorum as monitoring stations (Table 1). All the stations in Çorum are at the city centre and the pollution sources are urban motorway traffic, industrial and domestic heating.
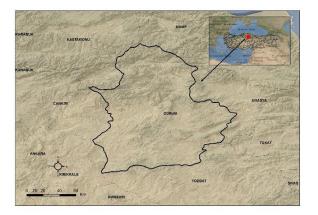

Figure 1. Climate diagram of Çorum   Figure 2. Weathercock of Çorum

Figure 3. Map of study area

2.2. Biological Material

The thalli of *P. furfuracea* lichen samples were collected from a forest near Yapraklı Büyük Yayla Forest Çankırı. This region is far from the pollution sources and thought to be unpolluted as compared to the selected city centre stations. About 20 g of fresh material was packed loosely in a fine nylon net. Each lichen bag included several thalli. At each monitoring stations two of these bags was tied on a nylon rope and hanged on two different trees above 3 meters from the ground. All the lichen samples were exposed to air pollution for two periods of 3 months (totally 6 months) from 04 July 2002 to 09 January 2003. Hanging date was 04-05 July 2002, first collection date was 05 October 2002 and the last collection date was 09 January 2003.

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Table1. Locations of the stations

Station no	Station	Substrate of the specimen	Altitude of the station (GPS)(m)	Coordinates
C1	Çankırı-Yapraklı, Yapraklı Büyük Plateau, Dikilitas area (control group)	*Pinus sylvestris*	1750 m	N 40º 47' 600" E 33º 46' 818"
C2	Çankırı-Yapraklı, Yapraklı Büyük Plateau, Dikilitas area (control group)	*Pinus sylvestris*	1750 m	N 40º 47' 600" E 33º 46' 818"
1	Çorum - Centrum, Hurriyet Park	*Pinus nigra* subsp. *pallasiana*	801 m	N 40º 33' 005" E 34º 57' 287"
2	Çorum- Velidedeoglu Park, Samsun Road, Inonu Street, Gazi Street.	*Pinus brutia*	800 m	N 40º 33' 275" E 34º 57' 995"
3	Çorum- Samsun road, İnönü street, Gazi Street, in front of Cement Factory	*Fraxinus* sp.	820 m	N 40º 33' 857" E 34º 58' 796"
4	Çorum- Binevler vicinity, In front of Old Governor Hall	*Fraxinus* sp.	840 m	N 40º 34' 482" E 34º 58' 032"
5	Çorum- Iskilip cros road , Garden of Eser tile manufactory	*Salix alba*	774 m	N 40º 33' 226" E 34º 55' 675"
6	Çorum- Samsun Highway, Burun Farm Vicinity, Emiroğlu Flour Mill	*Salix* sp.	743 m	N 40º 31' 839" E 34º 55' 114"
7	Çorum- Yenikent, Mimar Sinan Square, Mimar Sinan Mosque	*Populus alba*	805 m	N 40º 31' 907" E 34º 56' 900"
8	Çorum- İpekli Baglari, 19th Street, No:7, House garden	*Malus* sp.	874 m	N 40º 32' 326" E 34º 58' 721"

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2.3. Sample Preparation and Heavy Metal Determination

After the collection of the transplanted lichen samples, they were first washed with tap water and distilled water twice to remove any dirty substances. Specimens were dried in paper bags at 80°C for 24 hours to protect them against microbial decomposition and to provide reference values for dry weight. The dried lichen samples were ground into powder using mortar and pestle.

All the glass, plastic and porcelain equipment was put in water with detergent and left over night, washed with tap water and then put into a solution of 20% nitric acid and left

overnight again. After these steps the glassware were washed with double-distilled water and dried at 60°C before use. For the preparation of all standards solutions of 65% w/w nitric acid and aqua regia 35% w/w HCl were used. All the steps of standard and solution preparations and also for dilutions, double distilled water was used. HNO_3 was used for dissolving specimen parts, which is very common in such processes (Halıcı *et al.* 2005). About 1g of the dried lichen sample was put into a porcelain crucible and burned at 460°C for 24 hours in an oven. Samples turned into ash and were put into a 100 mL beaker and then a 65% solution of 10 mL HNO_3 added. Beakers were heated in a sand bath in order to evaporate the excess HNO_3. Just before all the HNO_3 evaporated, the beakers were taken from the sand bath and left to cool at room temperature. After evaporation, the remaining part was placed into centrifuge tubes and the volume adjusted to 15 mL with 1% HNO_3. Samples were centrifuged at 3000 rpm (3000 rpm= 1157 g relative centrifuge acceleration) for 20 min. After centrifugation the supernatant was transferred into 25 mL volumetric flask and the volume was adjusted to 25 mL with 1% HNO_3. Heavy metal contents were determined by using ICP (Varian Liberty ICP-OES Sequential) f(Halıcı *et al.* 2005).

2.4 Chlorophyll Measurement

Chlorophyll was extracted from 20 mg of the airdried lichen material using pure DMSO (Dimethylsulphoxide (for synthesis) 99% purity, Merck 8.02912). Then 5 mL of DMSO was added to the thalli for extraction. Tubes with DMSO and lichen samples were incubated at 65°C for 40 min in the dark and then allowed to cool to room temperature. The extracts were filtered through a Whatman no 3 filter paper. The spectrophotometer (Varian Liberty ICP-OES Sequential) was calibrated at 750 nm with DMSO. Absorbance of the extracts was read at 665 and 648 nm. Calculations were done according to the following equations;

$C_a = 14.85A^{665} - 5.14A^{648}$

$C_b = 25.48A^{648} - 7.36A^{665}$

$C_{a+b} = 7.49A^{665} + 20.34A^{648}$

Chlorophyll extractions were done according to method describe by Barnes *et al.* (1992).
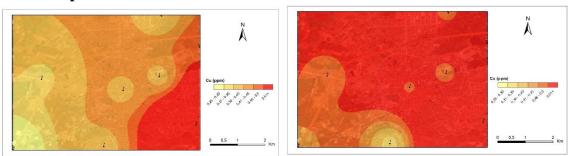
3. RESULTS AND DISCUSSION

The heavy metals, Cu, Cd, Ni, Pb, Mn and Zn and chlorophyll a and chlorophyll b contents of *P. furfuracea* specimens which were hanged at 8 stations in Çorum stations and 2 stations in Çankırı as a control were determined (Table 1). The specimens were collected two times in time intervals of 3 months. According to the analysis, there was an increase in heavy metal accumulation of the Cu, Ni, Zn, Mn and decrease in Chlorophyll a content of the specimens in two following periods (Table 2). It was known that the chlorophyll content of a plant decreases with increasing pollution. If the pollution rates increase, chlorophyll b degradation starts and rises up considerably. Any considerable change was observed in chlorophyll b content at different time intervals.
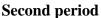
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Table 2. Results of lichen material analysis (Values for Cu, Cd, Ni, Pb, Mn and Zn are in µg.g^{-1}. Chlorophyll a and chlorophyll b are in µg chl.mg air-dry wt thallus^{-1})

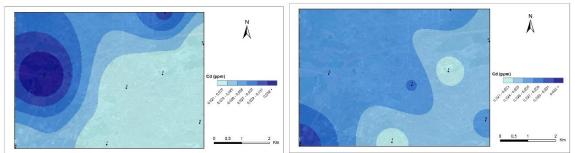| Elements	Periods	Cu	Cd	Ni	Pb	Mn	Zn	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a/b	Chlorophyll b/a
1	1	0,28423	0,02621	0,27508	0,51637	1,89763	0,15076	7,7827	1,945	9,7277	5,0007	0,312
	2	0,38909	0,02757	0,28306	0,55338	1,94752	0,57671	9,252	3,013	12,265	4,5167	0,3337
C2	1	0,25191	0,03153	0,20229	0,52883	1,91850	0,18884	4,9797	1,109	6,0887	5,7143	0,2017
	2	0,34413	0,02832	0,31485	0,56882	1,98790	0,58973	4,8937	1,036	5,9297	5,9523	0,1983
1	1	0,38453	0,02075	0,26365	0,48536	1,73946	0,18607	2,184	0,499	2,683	4,377	0,228
	2	0,49511	0,02657	0,26200	0,50564	1,73805	0,26309	3,541	0,997	4,538	3,552	0,282
2	1	0,37696	0,02140	0,28538	0,52764	1,66436	0,15476	6,615	1,331	7,946	4,976	0,201
	2	0,46015	0,02205	0,30775	0,53300	1,80570	0,17035	0,711	0,519	1,23	1,37	0,730
3	1	0,49033	0,02142	0,39869	0,59078	1,88175	0,14193	4,006	0,743	4,749	5,392	0,185
	2	0,68991	0,02411	0,45526	0,69311	2,40114	0,26192	0,185	0,206	0,391	0,898	1,114
4	1	0,35153	0,02894	0,51852	0,61448	2,17770	0,17050	2,849	0,605	3,504	4,792	0,209
	2	0,48507	0,02527	0,67293	0,55186	2,31232	0,42495	2,809	0,717	3,526	3,918	0,255
5	1	0,34388	0,03312	0,86758	0,53409	3,22150	0,26841	1,243	0,453	1,696	2,744	0,364
	2	0,48547	0,02465	0,96167	0,49614	3,26384	0,59104	0,113	0,104	0,217	1,087	0,920
6	1	0,20476	0,02328	0,19107	0,35280	1,29833	0,60275	4,465	0,653	5,118	6,838	2,916
	2	0,42504	0,02971	1,23956	0,61766	2,83451	0,15157	1,889	0,508	2,397	3,719	0,269
7	1	0,34959	0,03057	0,40533	0,46533	2,25299	0,18544	2,189	0,613	2,802	3,571	0,280
	2	0,33478	0,02234	0,39414	0,41925	2,04386	0,28176	0,582	0,491	1,073	1,185	0,844
8	1	1,30756	0,02268	0,75375	0,59376	2,82091	0,22735	4,332	0,788	5,12	5,497	0,182
	2	2,09217	0,02473	0,40454	0,54071	2,17521	0,85283	1,839	0,556	2,395	3,308	0,302

In the view of heavy metal analysis, it can be said that *P. furfuracea* accumulated heavy metals and worked well as a bioindicator organism for biomonitoring. With respect to the maps, the pollution status of the city can be easily observed and compared. The changes in the first and second period can be examined from these maps (Figure 4, Figure 5). The examination of the Cu maps showed that there were differences between the first and the second periods in all 8 stations. Based on the Cu analysis results it can be said that there is a continuous high level of Cu around 8th station and the difference occurred at the other stations could be explained with the climatic circumstances. Station 8 was a house garden but it was so close to the main street. The reason of high Cu accumulation might be the main street traffic and crowd.

Figure-4 Pollution maps of Çorum according to the heavy metals Cu, Cd, Mn, Ni, Pb and Zn
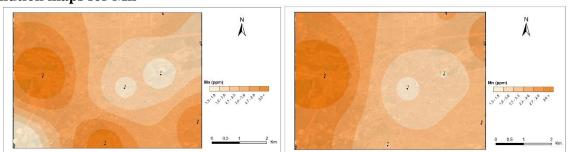**Pollution maps for Cu**
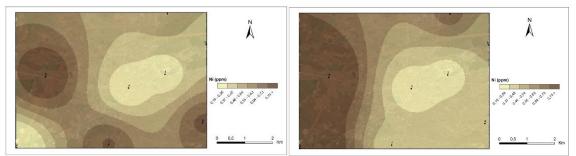

| **First period** | **Second period** |

Pollution maps for Cd

| **First period** | **Second period** |

Pollution maps for Mn

| **First period** | **Second period** |

Pollution maps for Ni

First period   **Second period**

Pollution maps for Pb

First period   **Second period**

Pollution maps for Zn

First period   **Second period**

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Figure-5 Pollution maps of Çorum according to Chlorophyll a and b degradation
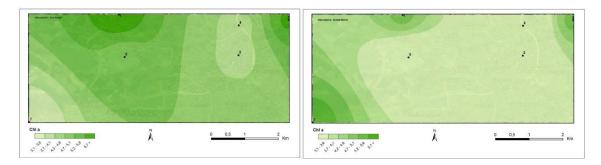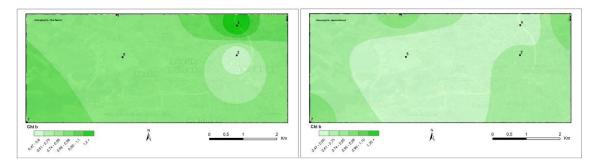

First period   **Second period**

First period   **Second period**

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First period   **Second period**
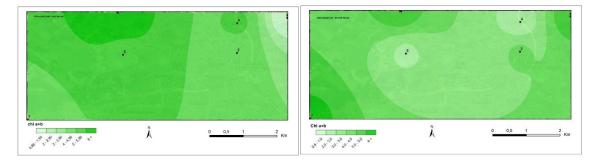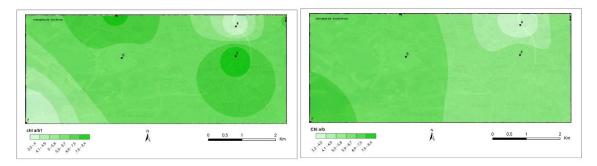

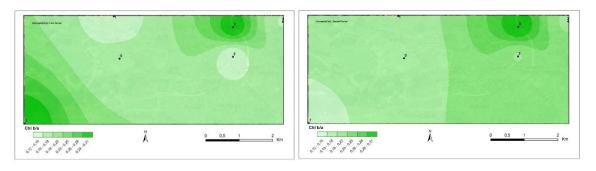**First period**   **Second period**

First period   **Second period**

As figured on the maps, it can be said that the pollution level of Zn decreased in the second period, although at first period there were some high values observed.

For the heavy metal Mn, there were fluctuations throughout the sampling period. But it was obvious that stations 3, 4, 5,6,7,8 illustrated high Mn levels. These stations were close to the main roads and the pollution around these stations was mainly caused by heavy traffic and factories established. Also station 3 was closed to an industrial area. The area was the only cement factory in the city. At the second periods it has shown obvious pollution values for almost all heavy metal types.

The Pb accumulation did not show a considerable change in samplings. The Zn pollution which was also originated from vehicles (Markert 1993) were found higher around station 8, it was under the influence of heavy traffic. Heavy metal accumulation in plant tissues results in degradation of chlorophylls (Garty *et al.* 1985, Ra *et al.* 2005). At the maps of chlorophyll concentration, there was a decrease in the chlorophyll a and b contents of the samples. The ratio of chlorophyll b to a (Chlorophyll b/a) is the sign of decreasing rates in photosynthesis pathways. Because of the small changes in the values of chlorophyll b, the changes in chlorophyll b/a were dependent on chlorophyll a and the decrease in chlorophyll a results in increase in chlorophyll b/a. The highest increase in chlorophyll b/a was observed around the stations 3 and 6. It was expected that, high pollution results in decrease in chlorophyll a (Backor *et al.* 2003). So the chlorophyll a/b content of the samples decreased and these changes can be observed from the maps. All changes in chlorophyll a and b contents and the ratios could be explained by the environmental stress like pollution but it is hard to say that the only reason of these changes were the pollution, also climatic conditions, seasons, strength of the light and the lichen itself effective on these changes. The chlorophyll a+b content of the samples shows similarities with the maps of chlorophyll a and chlorophyll b as expected. Chlorophyll a+b content of the tissues depended mostly on chlorophyll a content because of the great change in time. But the small decrease in chlorophyll b content was also effective. The results are also supported by the maps for chlorophyll b/a. Analysing all the maps overall it can be said that the lichen species, *P. furfuracea*, accumulated the heavy metals and it worked well as a biomonitor organism.

4. CONCLUSION

1. Atomic absorption method used in this study provides remarkable results on bioaccumulation caused by heavy metal pollution.

2. The results of ICP method also provide an early warning system with a higher sensitivity than the other techniques. The system is not only sensitive but also economic.

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3. Lichens can be used as biomonitors of heavy metal pollution.

ACKNOWLEDGEMENTS

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