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RESEARCH NOTE

Correlation Between Chlorophyll Degradation and the Amount of Heavy Metals Found in *Pseudevernia furfuracea* in Kayseri (Turkey)

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Abstract
Air pollution and associated heavy metal pollution, is an important environmental problem. One of the methods used to monitor heavy pollution in the 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 of air pollution heavy metal level in Kayseri 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 10 different stations in Kayseri. Lichen samples were re-collected at two different periods in three month intervals. Inductively coupled plasma (ICP) spectrometry was used to identify the heavy metals, such as Cu, Cd, Mn, Ni, Pb and Zn in the lichen samples. The chlorophyll a and b contents were determined by using the DMSO method. With these values chlorophyll a+b, a/b and b/a were also calculated.
According to the heavy metal analysis results of *Pseudevernia furfuracea*, which worked well as a bioindicator, air pollution in Kayseri due to industry, heating and traffic was observed. The concentration of Cd at the 3rd and 9th station, Cu concentration at the 1st and 8th station, Mn concentration at the 3rd and 10th and Pb concentration at the 3rd, 8th and 10th station were considerably high. In addition to these, the concentration of Zn was observed high at 9th station.
Keywords: Bioindication, Heavy metals, Kayseri, *Pseudevernia furfuracea*, Turkey.

Kayseri İlinde *Pseudevernia furfuracea* Türünde Klorofil Yıkımı ile Ağır Metal Miktarı Arasındaki İlişki

Özet
Hava kirliliği ve buna bağlı ağır metal kirliliği önemli bir çevresel problemdir. Havadaki ağır metal kirliliğini belirlemenin yollarından biri de çanta tekniği ile taşınmış örneklerle yapılan metottur. Bu çalışmada biyomonitör olarak *Pseudevernia furfuracea* kullanılarak, Kayseri ilinin hava kirliliğinin ağır metal seviyesi belirlendi ve kirlilik haritası oluşturuldu. Liken örnekleri 2002 yılında Çankırı ili Yapraklı ilçesi dağlarından toplandı ve Kayseri'de on farklı istasyona yerleştirildi. İstasyonlardaki bu örnekler üçer aylık iki periyotta yeniden toplandı. Inductively couple plasma (ICP) spektrometre yöntemi ile örneklerdeki Cu, Cd, Mn, Ni, Pb ve Zn ağır metalleri tespit edildi. Klorofil a ve b miktarları DMSO metodu kullanılarak tespit edildi. Klorofil a ve b miktarları belirlendikten sonra klorofil a+b, a/b ve b/a oranları hesaplandı.
Biyomonitör özelliği çok iyi olan *Pseudevernia furfuracea*' nin ağır metal analiz sonuçlarına göre, Kayseri ilinde endüstriyel, ısınma ve trafik kaynaklı bir kirlilik görülmektedir. 3. ve 9. istasyonlarda Cd, 1. ve 8. istasyonlarda Cu, 3. ve 10. istasyonlarda Mn, 3., 8. ve 10. istasyonlarda ise Pb konsantrasyonu oldukça yüksektir. Zn ise 9. istasyonda yüksek olarak gözlenmiştir.
Anahtar Kelimeler: Ağır metaller, Biyomonitoring, Kayseri, *Pseudevernia furfuracea*, Türkiye

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INTRODUCTION

In modern cities, air pollution is an important environmental problem. Air pollution has received attention due to the fact that it is harmful for both

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human health and the environment (Abdunnasır et al. 1994, Karademir and Toker 1998, Simonetti et al. 2003, Çayır et al. 2008). Air pollution in urban areas could be caused by many sources and several methods have been developed to determine the sources and the level of this pollution. One of these techniques is using organisms as bioindicators and biomonitors.

A bioindicator contains information on the quality of the environment or a part of the environment. A biomonitor contains information on the quantitative aspects of the quality of the environment (Markert 2007).

Lichens are a symbiotic association of fungi and green and blue-green algae. They have no roots or waxy cuticles and they mainly depend on the atmospheric input of nutrients. Lichens were recognized as potential indicators of air pollution as early as the 1860's in Europe (Hawksworth and Rose 1996, Markert 1996, Markert 2001, Carreas and Pignata 2002). Especially in the last decades, their usage in urban areas as an indicator of air quality has increased. Lichens accumulate heavy metals in their tissues depending on their structural and physiological properties. Both their dependence on the atmospheric input of nutrients and their bioaccumulation capacity make them good bioindicators of air pollution (Garty et al. 2003, Loppi and Printsos 2003). Several studies have been carried out about the accumulation and biomonitoring of heavy metals by lichens (Sloof 1995, Loppi et al. 1997, Carreras et al. 1998, Carreras et al. 2001, Simonetti et al. 2003, Carreras et al. 2009, Yildiz et al. 2008). In studies carried out about accumulation and biomonitoring of heavy metals with lichens, the elemental concentrations in lichen tissues are considered to reflect atmospheric concentrations or deposition (Van Dobben et al. 2001). If the amount of pollutants reaches a threshold level for lichens, their physiological, morphological, and chemical structures will change and their numbers will decrease. If the pollution continues to increase, they will be eliminated from the polluted area (Jahns 1973).

A special method called "bags technique" has been developed to monitor air pollution in urban areas (Goodman and Roberts 1971). In this technique, lichens collected from natural areas are transplanted to cities where the air pollution levels are going to be monitored, since they are often scarce or even absent in the urban areas. The advantage of this technique is that only climatic and environmental conditions can influence the accumulation of the contaminants (Garty et al. 1993), since the hanging process prevents absorption of any material from their substrate (Adamo et al. 2003).

In addition to studying the level of contaminants, analysing the chlorophyll content also leads to information about the level of air pollution. The chlorophyll content of the lichen decreases with increasing pollution. 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, Fields and St Clair 1984).

The aim of this study was to analyse the heavy metal contents of *Pseudevernia furfuracea* (Pb, Cu, Cd, Mn, Ni and Zn) and the chlorophyll a and b levels for determination of air pollution levels for Kayseri. For this purpose, lichens collected from unpolluted areas were transplanted to 10 stations in Kayseri. The composition of airborne elements in the thalli of *Pseudevernia furfuracea*, which were subjected to vehicular and industrial pollution, was determined.

MATERIAL AND METHODS

Study Area

Kayseri is located in Central Anatolia with a population of 732,354 inhabitants (according to the 2000 Census) (Anonymous 2000). The total number of vehicles registered was 147.128 in 2002 (Anonymous 2003). The main air pollution sources of Kayseri is smoke from factories, heavy traffic and heating of homes. The climate diagram of Kayseri is given in Fig. 1. The prevailing winds are from the South (S) (Anonymous 2008) (Fig. 2). Ten (10) exposure sites located in the city were selected as monitoring stations (Table 1) and 2 additional stations from Çankırı were used as control stations.

Biological Material

Lichen specimens were collected from a forest near Yapraklı-Çankırı. This region is far from pollution sources and is thought to be relatively clean in terms of air pollution. All the samples were collected with their twigs and the thalli of the *Pseudevernia furfuracea* was used for the monitoring of air pollution.

The samples were rinsed with distilled water and 20 g of fresh material was packed loosely in a fine nylon net and each lichen bag included several thalli. At each of the monitoring stations two of these bags were tied with a nylon rope and hung on two different trees approximately 3 meters from the ground. All the lichen samples were exposed to air pollution for two periods at 3 months intervals (totally 6 months) from 03 July 2002 to 09 January 2003. The hanging date was 03-04 July 2002, the first collection date was 04-05 October 2002 and the last collection date was 07-09 January 2003.

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 unwanted 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. Dried specimens were pounded in a mortar to make the distribution of heavy metals homogenized (shredded to achieve homogeneity).

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 they 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 preparation and also for dilutions, and double distilled water were used. HNO_3 was used for dissolving plant parts, which is very common in such processes (Halici et al. 2005).

1 g of the dried 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 (Inductively Coupled Plasma) in this study.

Chlorophyll Measurement

Chlorophyll was extracted from 20 mg of the air-dried plant material using pure DMSO (Dimethyl sulphoxide (for synthesis) 99% purity, Merck 8.02912). Then 5 mL DMSO was added to the thalli for extraction. Tubes with DMSO and plant material were incubated at 65°C for 40 min in the

Table 1. Locations of the stations.

Station no	Station	Samples hanged on	Altitude of the station (GPS)	Coordinates
C1	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş (Control Station)	*Pinus sylvestris*	1750 m	N 40° 47' 600" E 33° 46' 818"
C2	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş (Control Station)	*Pinus sylvestris*	1750 m	N 40° 47' 600" E 33° 46' 818"
1	Kayseri- Ankara- Sivas- Malatya Yolu, Sanayi Odası Kavşağı	*Pinus nigra* subsp.*pallasiana*	1060 m	N 38° 44' 067" E 35° 29' 742"
2	Kayseri- Ankara- Sivas- Malatya Yolu, Kumarlı Yolu - Sivas Cad. kavşağı, Atlas Halı Fabrikası	*Robinia pseudoacacia*	1070 m	N 38° 44' 939" E 35° 32' 938"
3	Kayseri- Kumarlı, Kılıçaslan Spor Stadı karşısı, Orta refüj	*Pinus nigra* subsp.*pallasiana*	1070 m	N 38° 46' 411" E 35° 34' 291"
4	Kayseri- Sivas Cad., Alemdar- Belediye kavşağı, Atatürk Stadyumu	*Pyrus* sp.	1180 m	N 38° 43' 476" E 35° 29' 921"
5	Kayseri- Talas, Talas Cad., Marmaris Sitesi, Halit Bey Sitesi, Talas Belediyesi karşısı	*Acer* sp.	1160 m	N 38° 41' 608" E 35° 32' 854"
6	Kayseri- Talas, Yukarı Talas, Kayısı Sekileri karşısı	*Eleagnus angustifolia*	1280 m	N 38° 40' 538" E 35° 33' 649"
7	Kayseri-Hisarcık, Hisarcık yolu, Polisevini geçince, Orta refüj	*Robinia pseudoacacia*	1240 m	N 38° 40' 148" E 35° 30' 311"
8	Kayseri- Organize Sanayi İçi. 1.Cad., Başkent Metal Sanayii karşısı, Göbek, Orta refüj	*Pinus nigra* subsp.*pallasiana*	1060 m	N 38° 44' 016" E 35° 22' 416"
9	Kayseri- Boğazköprü, Göbek, Niğde tarafı	*Pinus nigra* subsp.*pallasiana*	1040 m	N 38° 45' 104" E 35° 18' 537"
10	Kayseri- Niğde Yolu, Çinkur Fabrikası, Orta refüj	*Robinia pseudoacacia*	1040 m	N 38° 42' 609" E 35° 15' 710"

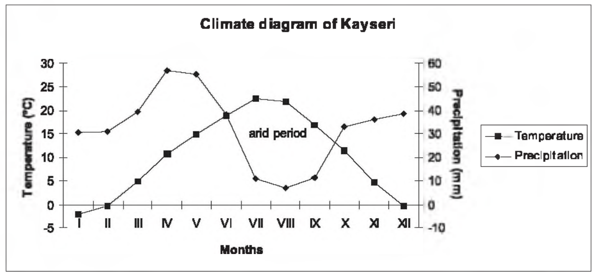

Fig. 1. Climate diagram of Kayseri after Walter and Lieth.

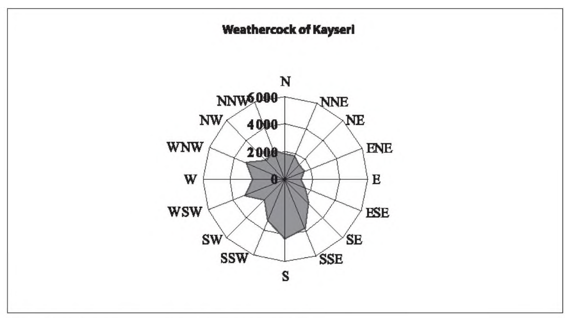

Fig. 2. Weatherclock of Kayseri.

dark and then allowed to cool to room temperature. The extracts were filtered through a Whatman no 3 filter paper. The spectrophotometer 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 Barnes et al. (1992).

RESULTS AND DISCUSSION

The results for the heavy metals, Ni, Pb, Zn, Cu, Cd and Mn, and chlorophyll a and chlorophyll b contents of *Pseudevernia furfuracea* samples which were hung at 10 points in Kayseri and 2 points as control stations in Çankırı are given in Table 2.

All the lichen samples were exposed to air

Table 2. Results of plant material analysis (Values for Cu, Cd, Ni, Pb, Mn and Zn are in $\mu g.g^{-1}$.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
C1	1	0,28423	0,02621	0,27508	0,51637	1,89763	0,15076	1,845	0,462	2,307	3,994	0,250
	2	0,38909	0,02757	0,28306	0,55338	1,94752	0,57671	0,573	0,473	1,046	1,211	0,825
C2	1	0,25191	0,03153	0,20229	0,52883	1,91850	0,18884	3,155	0,523	3,678	6,033	0,166
	2	0,34413	0,02832	0,31485	0,56882	1,98790	0,58973	3,035	0,72	3,755	4,215	0,237
1	1	0,82038	0,02601	0,55103	0,86731	2,79080	0,44295	2,249	0,514	2,763	4,375	0,229
	2	1,09930	0,02830	0,55365	0,98566	2,55684	1,02865	2,525	0,468	2,993	5,395	0,185
2	1	0,33935	0,01187	0,54543	0,52644	2,01642	0,22597	4,49	0,663	5,153	6,772	0,148
	2	0,24923	0,06071	0,16732	2,04383	0,61652	0,680853	3,273	0,74	4,013	4,423	0,226
3	1	0,34999	0,01370	0,76529	0,64150	2,32811	0,27559	5,301	0,95	6,251	5,58	0,179
	2	0,54279	0,01763	0,85468	0,71478	2,85750	0,35772	4,297	0,759	5,056	5,661	0,177
4	1	0,40625	0,02161	0,64889	0,67471	2,18860	0,35979	3,646	0,738	4,384	4,94	0,202
	2	0,63578	0,02331	0,66068	0,58210	2,63596	0,83708	5,11	0,907	6,017	5,634	0,177
5	1	0,46493	0,02436	1,05051	0,90723	3,46755	0,41077	6,165	0,912	0,912	7,077	0,148
	2	0,62077	0,02363	0,70155	0,90081	2,37255	0,61485	2,775	0,62	3,395	4,476	0,223
6	1	0,30156	0,02586	0,57662	0,67631	2,44974	0,29192	5,81	0,812	6,622	7,155	0,140
	2	0,39707	0,01462	0,46431	0,65450	2,42488	0,29852	0,59	0,189	0,779	3,122	0,320
7	1	0,43491	0,01897	0,31236	0,55538	1,75735	0,21349	3,476	0,593	4,069	5,862	0,171
	2	0,68315	0,01794	0,25098	0,53681	1,90331	0,41409	3,45	0,721	4,171	4,785	0,209
8	1	0,42406	0,02963	0,31973	0,42071	1,71560	0,39013	0,856	3,841	4,697	0,223	4,487
	2	1,18072	0,03327	0,50187	1,05705	2,41628	1,00600	4,097	0,63	4,727	6,503	0,154
9	1	0,48896	0,01717	0,35021	0,81282	2,04156	0,69982	1,845	0,462	2,307	3,994	0,250
	2	0,35588	0,05272	0,29528	1,04716	2,13159	1,52556	0,573	0,473	1,046	1,211	0,825
10	1	0,51410	0,02265	0,86017	0,58925	2,84310	0,62878	3,155	0,523	3,678	6,033	0,166
	2	0,36796	0,03407	0,96864	0,62228	3,29936	0,65482	3,035	0,72	3,755	4,215	0,237

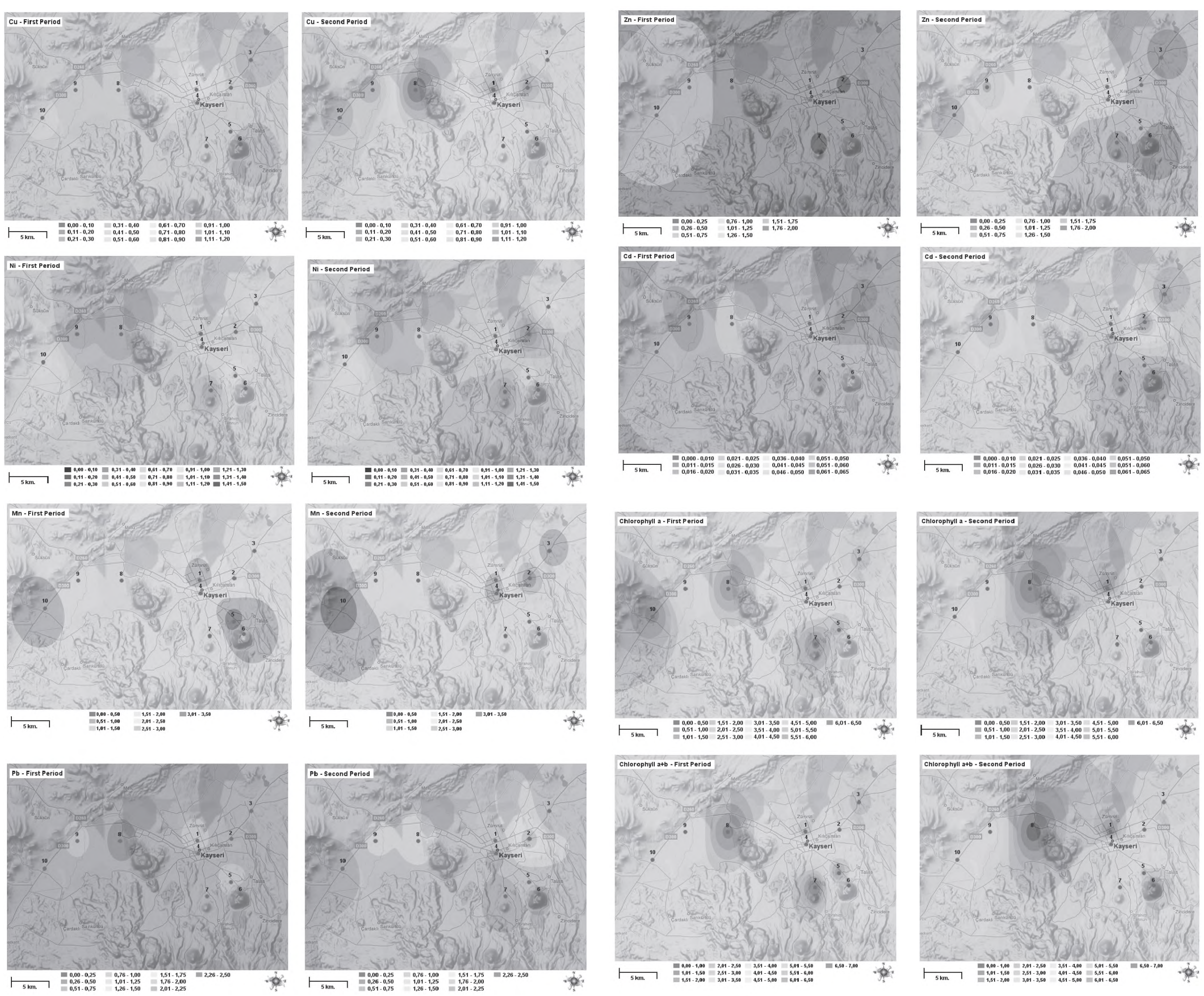

Fig 2. Pollution maps for Kayseri.

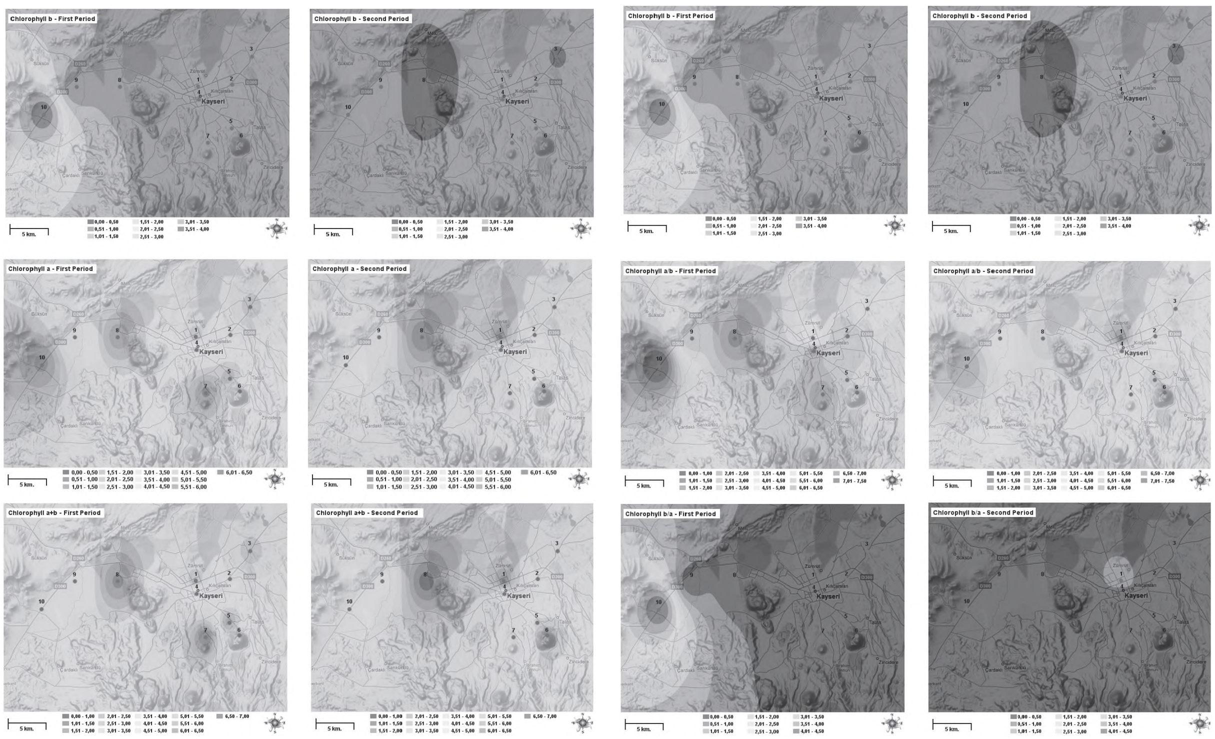

Fig 2. Pollution maps for Kayseri.

pollution for the two periods of time having 3 month intervals. The T-test (SPSS 11.5) was used in order to determine the changes statistically.

According to the analyses, the air pollution map of Kayseri based on the heavy metal accumulation in *Pseudevernia furfuracea* and the chlorophyll a and b degradation was constructed by using ESRI ArcMap 9.2.

The T-test (SPSS 11.5) was used in order to determine the statistical significance of the changes. Pollution maps of Kayseri according to the analysis are given in Fig. 3.

By using these maps, the pollution status of the city in the first and second periods can be easily observed and compared.

The main sources of the air pollution in Kayseri are the smoke of factories, heating devices using fossil fuels (coal and oil) and mobile sources such as motor vehicles and aircraft. According to Valkovic (1983) coal, the main fossil fuel used in Kayseri for heating activities, yields several elements in air including Cu (1.3 ppm), Cd (51 ppm), Pb (650 ppm), Mn (430 ppm) and Zn (5900 ppm).

Accumulation of Cd at stations 3 and 9 are obvious, which was caused by mainly traffic and heating devices using fossil fuels.

When the maps for Cu accumulation were analysed, it could easily be observed that there was a significant Cu accumulation at the 1st and 8th stations especially during the second period which falls into the winter period, which is a period of high coal consumption for heating activities. In addition to the heating activities the factories around the 8th station are the main reason for the Cu accumulation.

When the air pollution maps for Ni were analysed, it is not easy to interpret the change in the Ni level between the two periods. But Valkovic (1983) stated that coal contains 16 ppm Ni but it does not yield Ni in air after burning activities, which means the main Ni pollution in Kayseri depends on the mobile sources.

According to the air pollution maps for Mn, it is also easy to predict an increase in the accumulation of Mn for the 3rd and 10th station, which depends on the mobile sources and the heating activities.

The Pb accumulation maps clearly shows that there is significant Pb accumulation in the two periods of time. The Pb pollution for the entire city could be caused by both stationary and mobile sources (Markert 1993, Markert 2008). The accumulation of Pb is clear for the 3rd, 8th and 10th stations. The sources of this accumulation are the mobile sources and heating activities.

As the maps for the Zn pollution are compared, it can be observed that there was Zn accumulation inside of the city during the two periods of time, which is caused by both stationary and mobile sources (Markert 1993). An interesting result is observed for station number 9. Although a high amount of Zn accumulation is expected for the 10th station, as it is close to a Zinc refining factory, the accumulation is observed at the 9th station. This accumulation could be caused by the prevailing winds from the South.

The concentrations of photosynthetic pigments can be easily calculated and generally it is used to monitor metal stress on plants (Stevenson et al. 1996). Heavy metal accumulation in plant tissues results in degradation of chlorophylls (Garty et al. 1985, Ra et al. 2005). When the maps for heavy metal accumulation and the maps for the decrease in the chlorophyll content are compared, an obvious reverse correlation could be observed. This result could be also double checked with the map for chlorophyll a+b which shows the total degradation of the photosynthetic pigments.

Maps for chlorophyll a/b present that chlorophyll a was affected more than chlorophyll b from the air pollution, which was expected when the photosynthetic pigments were degraded by pollution (Backor et al. 2003). This result is also supported by the maps for chlorophyll b/a.

Analysing all the maps overall it can be said that the lichen, *Pseudevernia furfuracea*, accumulated the heavy metals and it worked well as a biomonitor.

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