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Biomonitoring of heavy metals by *Pseudevernia furfuracea* (L.) Zopf in Aksaray city, Turkey

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Abstract
Mixed air pollutants are considered a major cause of damage in living organisms. Air pollution and associated heavy metal pollution is a serious environmental problem. One of the methods used to monitor heavy metal pollution is the method of transplanting lichen samples by the 'bag technique'. The objective of this study was to determine the air pollution level of Aksaray and to generate the air pollution map of this city by using *Pseudevernia furfuracea* (L.) Zopf as a bioindicator. The study area was characterized by the presence of numerous industrial, heating activities and traffic. Lichen samples were collected from unpolluted area at Yapraklı Mountains, Çankırı in November 2002 and transplanted to 7 different localities in Aksaray. They were retrieved following an exposure of 3 and 6 months. Heavy metal, Cu, Cd, Mn, Ni, Pb and Zn, contents were determined by using inductively coupled plasma mass spectrometry (ICP-MS). The chlorophyll a and b contents were determined by using DMSO method. According to the heavy metal analysis results of *P. furfuracea*, air pollution in Aksaray owing to industrial activities, heating and traffic showed various changes in different stations and periods studied. Heavy metal concentrations in various stations showed obvious differences according to determination methods applied in the experiments. The results indicated that lichens had a great potential of bioindicator capacity.

Keywords
Heavy metals, biomonitoring, Pseudevernia furfuracea, Aksaray, Turkey

Introduction

Air pollution is an important environmental problem in modern cities for both human and environmental health. There are so many sources of air pollution in urban areas and also many techniques have been developed for the determination of the levels and sources of this pollution. One of these methods is the usage of organisms for determination and monitoring of pollution. Lichens which are symbiotic associations of fungi and green and blue-green algae have no roots or waxy cuticles and depend mainly on the atmospheric input of mineral nutrients.

These features and also their bioaccumulation capacity make them good bioindicators of air pollution (Garty et al., 2003). Lichens were recognized as potential indicators of air pollution as early as the 1860's in Europe (Hawksworth and Rose, 1996; Carreas and Pignata, 2002; Yıldız et al., 2008, 2011, 2018; Işık and Yıldız, 2021). "Bags technique" was developed in order to monitor air pollution in urban areas (Goodman and Roberts, 1971).

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Lichens are often scarce or even absent in urban areas so they have to be transplanted to cities with bags technique. Lichens are collected from natural areas, washed and placed in nylon bags, then hanged in appropriate places for monitoring air pollution. The hanging process prevents absorption of any material from their substrate (Adamo et al., 2003; Yıldız et al., 2008, 2011, 2018).

Climatic and environmental conditions can influence the accumulation capacity of the lichens and also the lichen specimen chosen for biomonitoring can reach a saturation point for contaminants, these factors are sensitive points of the bags technique (Garty et al., 1993).

The emissions and atmospheric dispersal of heavy metals and the pollution caused by them have received much attention due to the fact that they are unsafe for both human and environment health (Simonetti et al., 2003). Numerous studies have been carried out about accumulation and biomonitoring of heavy metals by lichens (Sloof, 1995). In these studies, the elemental concentrations in lichen tissues are considered to reflect atmospheric concentrations or deposition, irrespective or possible toxic effects.

It was known that the chlorophyll content of the lichen decrease with increasing pollution which is the physiological effect of pollution on lichens. 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; Yıldız et al., 2008, 2011, 2018; Işık and Yıldız, 2021, 2022).

The purpose of this study is to analyze Pb, Cu, Cd, Mn, Ni and Zn heavy metals and the chlorophyll a and b contents of *Pseudevernia furfuracea* (L.) Zopf for determination of air pollution levels of Aksaray. For this purpose lichens from unpolluted areas were transplanted to the appropriate locations in Aksaray. The composition of airborne elements in thalli of *P. furfuracea* which was subjected to vehicular and industrial pollution was determined.

Materials and methods

Study area

The study was performed in the urban area of Aksaray. Aksaray is located at Central Anatolia with a population of 396084 inhabitants (according to the 2000 census) (DIE (Turkish Statistical Institute), 2000 Census) (Fig. 1). The total number of automobiles was recorded as 42260 in 2002 (Statistical Almanac of Traffic). The main air pollution sources of Aksaray are urban motorway and domestic heating.

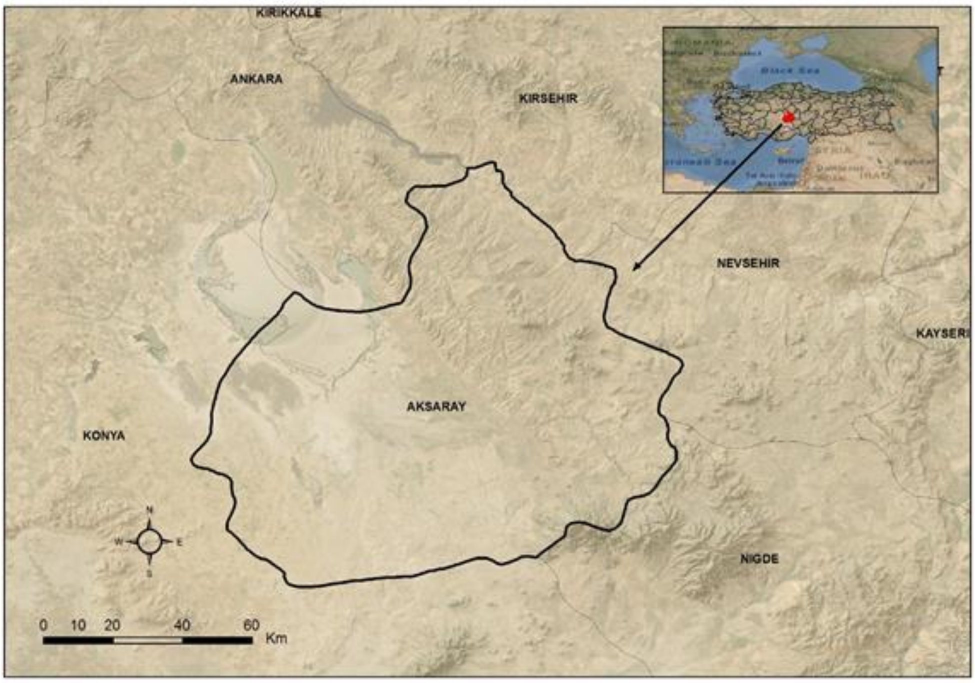

Figure 1
Map of study area

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The climate of the city is semi-arid continental climate with cold winters (Emberger, 1955) with an average annual rainfall is 346.1mm concentrated in Sp.W.F. Su and a mean annual temperature of 12°C. The climate diagram of Aksaray was given in Figure 2. The prevailing winds are from SW (Turkish State Meteorological) (Fig. 3). Six exposure sites were selected in the city of Aksaray as monitoring stations (Table 1). All the stations in Aksaray were at the city centre and the pollution sources were the same; urban motorway traffic and domestic heating.

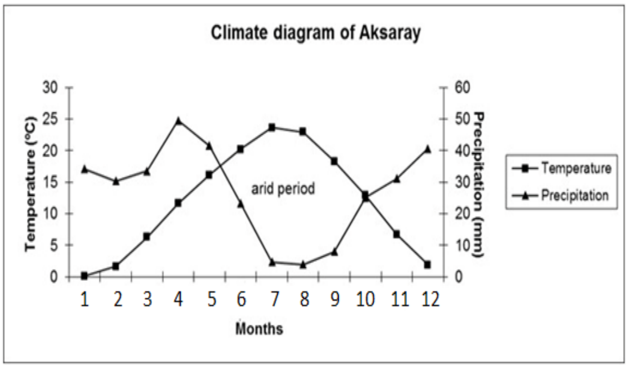

Figure 2. *Climate diagram of Aksaray*

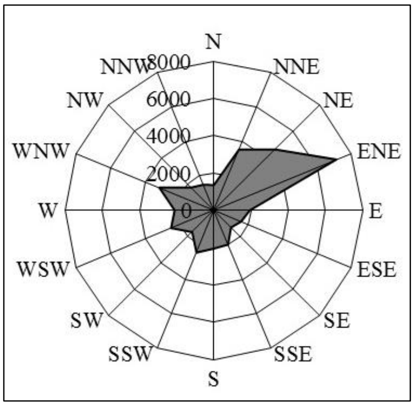

Figure 3. *Weathercock of Aksaray*

Table 1. *Locations of the stations*

Station no	Station	Substrate of the specimen	Altitude of the station (GPS) (m)	Coordinates
C1	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş area (control group)	Pinus sylvestris	1750 m	N 40° 47' 600" E 33° 46' 818"
C2	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş area (control group)	Pinus sylvestris	1750 m	N 40° 47' 600" E 33° 46' 818"
1	Aksaray- Nevşehir road, by Ağaçlı Recreational Facility cross road	Eleagnus angustofolia	964 m	N 38° 23' 113" E 33° 59' 769"
2	Aksaray Manicupality, in front of Aksaray High School, centerpoint	Cupressus arizonica	970 m	N 38° 22' 464" E 34° 01' 688"
3	Aksaray Bedr-i Muhtar Cemetery, opposite of Aksaray Manicupality, by the Fountain	Pinus nigra subsp. pallasiana	1030 m	N 38° 23' 025" E 34° 02' 494"
4	Aksaray Atatürk Street and Cumhuriyet Street Centerpoint, Esenkayalar Mall	Cupressus arizonica	992 m	N 38° 22' 940" E 34° 01' 693"
5	Aksaray Industry site, by the Fountain	Fraxinus sp.	958 m	N 38° 22' 434" E 33° 59' 863"
6	Aksaray- Konya -Adana Intersection, Özeller Recreational Facility	Fraxinus sp.	951 m	N 38° 21' 394" E 33° 58' 336"

Biological material

The thalli of *P. furfuracea* lichen samplings were collected from forest near Yapraklı Büyük Yayla Forest-Çankırı. This region is far from the pollution sources and thought to be unpolluted in compared to performing stations. The specimens were rinsed with distilled water and 20 g of fresh material packed loosely in a fine nylon net. Each lichen bag included several thalli. At each monitoring stations two of these bags tied on a nylon rope and hanged on two different trees above 3 meters from ground. All the lichen samples 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.

Sample preparation and heavy metal determination

After the collection of the transplanted lichen samples, they were first washed with tap water and

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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. 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 specimen parts, which is very common in such processes (Halıcı 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-MS (Inductively Coupled Plasma-Mass Spectrometry) (Halıcı et al., 2005).

Chlorophyll measurement

Chlorophyll was extracted from 20 mg of the air dried lichen material using pure DMSO (Dimethylsulphoxide (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 dark and then allowed to cool to room temperature.

The extracts were filtered through a Whatman no 3 filter paper. The spectro-photometer was calibrated at 750 nm with DMSO.

Absorbance of the extracts was read at 665 and 648 nm. Calculations of the amount of chlorophyll were calculated according to the following equations [1], [2] and [3] of Barnes et al. (1992).:

$$\text{Chlorophyll-a} = 14.85A^{665} - 5.14A^{648} \quad [1]$$

$$\text{Chlorophyll-b} = 25.48A^{648} - 7.36A^{665} \quad [2]$$

$$\text{Chlorophyll (a+b)} = 7.49A^{665} + 20.34A^{648} \quad [3]$$

Chlorophyll extractions were done according to Barnes et al. (1992).

Results and Discussion

The heavy metals, Ni, Pb, Zn, Cu and Mn, and chlorophyll a and chlorophyll b contents of *P. furfuracea* specimens which were hanged at 6 points in Aksaray and 2 points in Çankırı as a control were determined (Table 2).

The specimens were collected 2 times with the time intervals of 3 months. It was observed that the accumulations of all heavy metals that were examined and decrease in the contents of chlorophyll.

For many years lichens have been known to be sensitive to air pollution due to the impact of pollutants on primary metabolic functions in both symbionts (Brodo et al.. 2001). The most sensitive species may be affected negatively in urban areas or near industrial regions. while a few. pollution resistant species will survive (Riddell et al., 2008). In the current study transplant technique which was developed by Brodo (1961) was used in Aksaray province.

When the Cu accumulation analysed. it could easily be observed that there was a significant Cu accumulation at the station 5 especially during second period that falls into winter period. which was a period of high fossil fuel consumption for heating activities (Fig. 4a). Station 5 was located in industrial region of the city. It was analysed that other stations had closer results to the control samples. According to Nirel and Pasquini (2010). copper is a widely used metal employed in many fields: transportation. manufacturing. currency. transportation of electricity. construction (roofing and decoration) and agriculture (fungicide. herbicide). Copper pollution from urban origin is accompanied by Zn contamination (Nirel and Pasquini. 2010).

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Table 2. *Results of lichen material analysis (Values for Cu, Cd, Ni, Pb, Mn and Zn are in µg.g⁻¹chlorophyll a and chlorophyll b are in µg chl.mg air-dry wt thallus⁻¹)*

Elements	Periods	Cu	Cd	Ni	Pb	Mn	Zn	Chlorophyll				
								a	b	a+b	a/b	b/a
C1	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	0.24848	0.01334	0.22659	0.42005	1.94694	0.17223	6.122	0.796	6.918	7.691	0.130
	2	0.27010	0.01736	0.25015	0.47583	1.70162	0.36917	4.834	0.814	5.648	5.939	0.168
2	1	0.32656	0.01280	0.27353	0.38396	2.14510	0.27650	3.955	0.471	4.426	8.397	0.119
	2	0.27236	0.01831	0.21039	0.39631	1.80953	0.17278	3.416	0.623	4.039	5.483	0.182
3	1	0.29840	0.01800	0.36497	0.51012	1.68731	0.17971	5.721	0.856	0.856	6.683	0.150
	2	0.34097	0.01192	0.41418	0.47842	1.91209	0.38777	4.799	0.855	5.654	5.613	0.178
4	1	0.28318	0.01817	0.23496	0.39449	1.90798	0.16128	3.934	1.238	5.172	3.178	0.315
	2	0.30808	0.01418	0.26176	0.40735	2.32452	0.43990	1.495	0.474	1.969	3.154	0.317
5	1	0.53771	0.01351	0.24980	0.70859	2.21149	0.24642	4.899	0.76	5.659	6.446	0.155
	2	0.66798	0.01619	0.27216	0.80989	2.15918	0.34744	2.255	0.35	2.605	6.443	0.155
6	1	0.37566	0.01539	0.29873	0.40129	2.24749	0.17818	3.148	0.963	4.111	3.269	0.306
	2	0.46963	0.01573	0.29128	0.46060	2.36205	0.62013	5.382	0.756	6.132	7.176	0.139

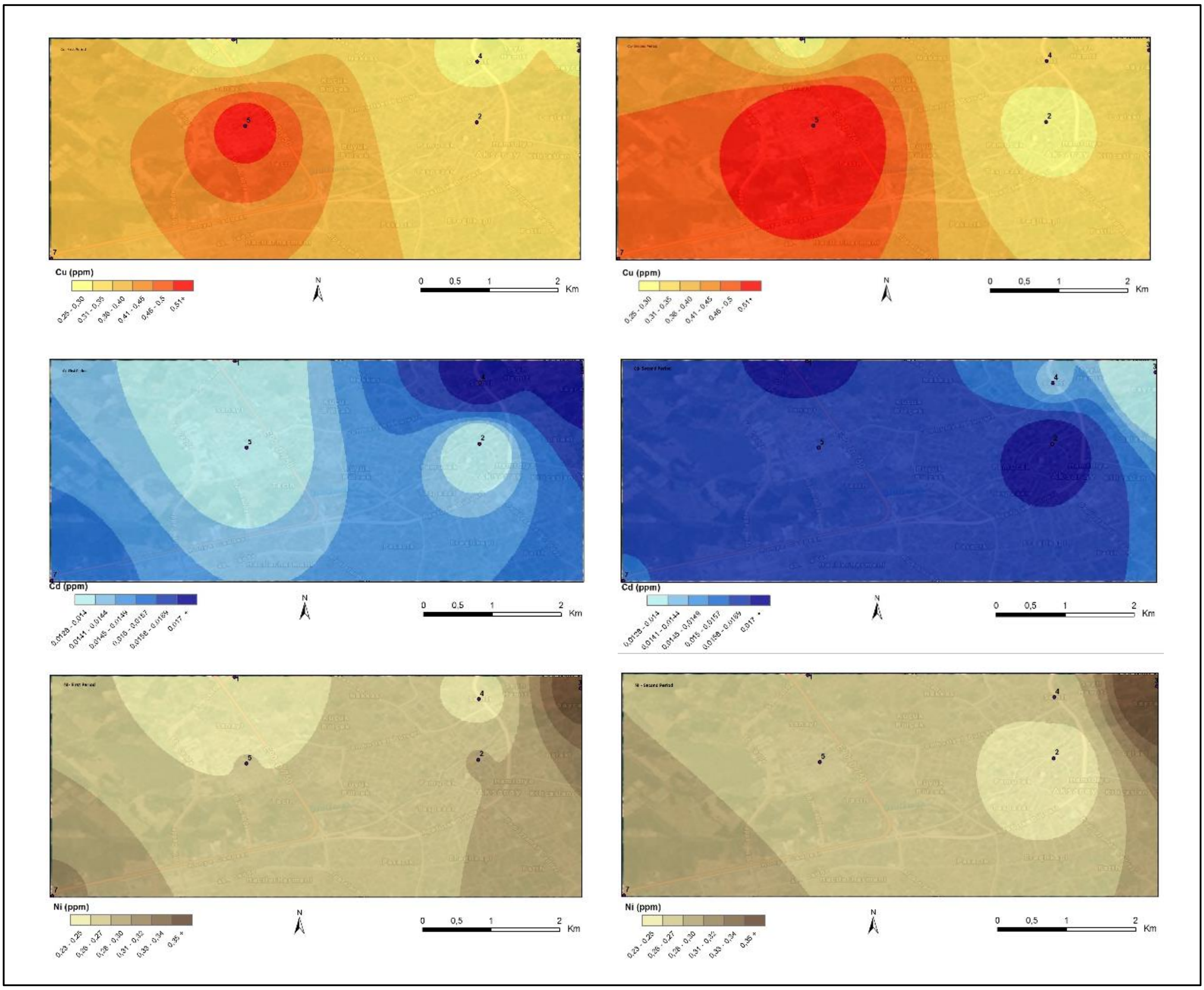

Figure 4a. *Pollution maps of Aksaray according to the heavy metals Cu, Cd and Ni*

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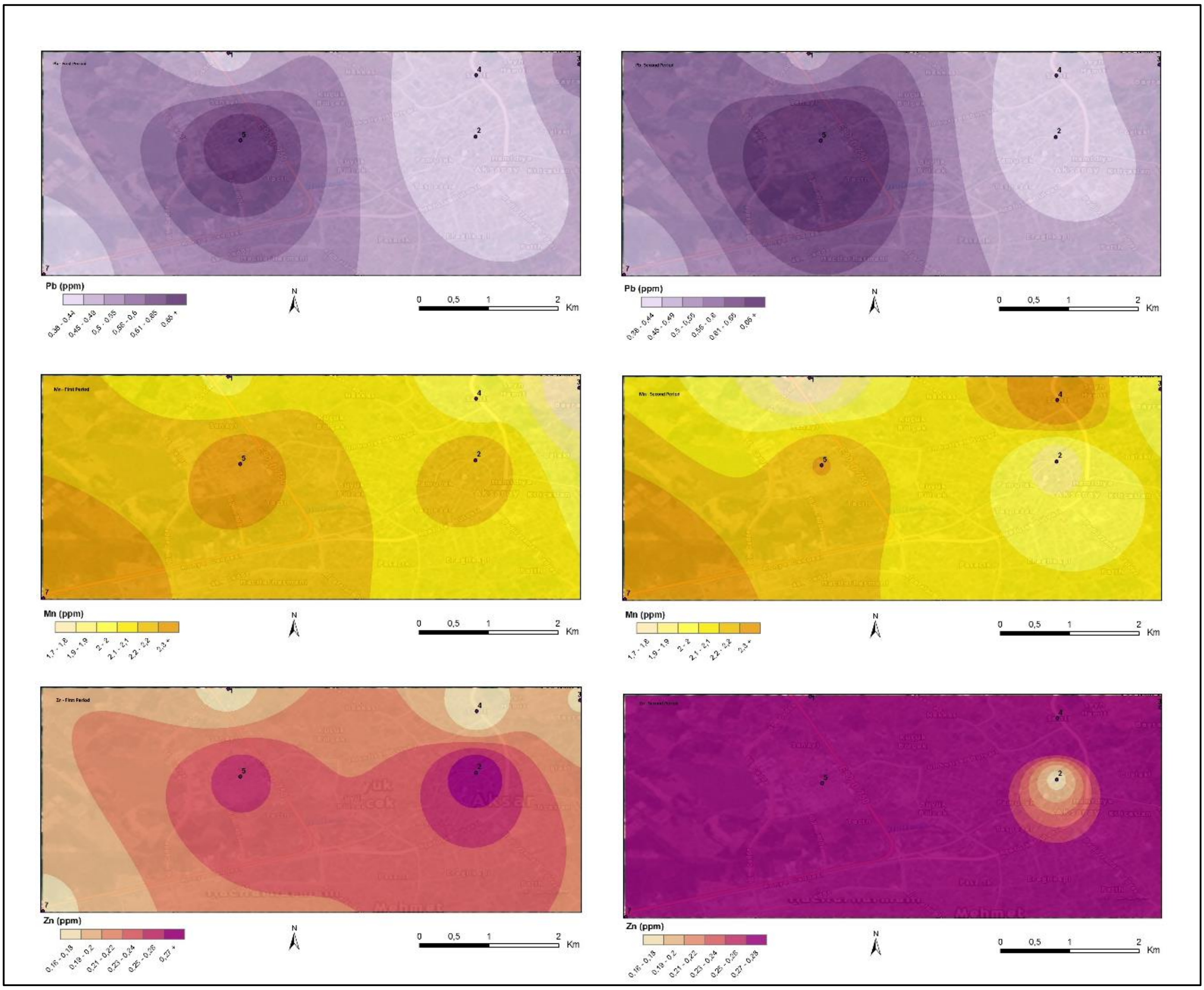

Figure 4b. *Pollution maps of Aksaray according to the heavy metals Pb, Mn and Zn*

The high concentrations of trace elements in *P. furfuracea* may be explained by the heavy traffic and heating activities. Similar results were found for Cu by (Önder and Dursun, 2006). Although Cd levels had known to be associated with automobile exhaust Zschau et al. (2003). it was not observed any considerable changes in the Cd concentrations in transplanted *P. furfuracea* samples of with compared to control samples. When the air pollution maps for Cd analysed it was not easy to interpret the change in the Cd level between two periods (Fig, 4a).

Ni pollution mainly depends on the mobile sources. The highest result of Ni was obtained at the station 3 in period 2 (Fig. 4a). This station contains high vehicular traffic causing high rate of pollution.

Pb accumulation maps clearly indicates that there is Pb pollution in station 5 which can be caused by vehicular sources (Fig. 4b). The data about Pb accumulation for station 5 has presented high contamination value in both periods. As stated by Kınalıoğlu et al. (2010) the contamination from this element is closely related to vehicle emissions and fuel combustion. According to the air pollution maps for Mn since vehicular emissions and industrial facilities caused a considerable bioaccumulation. It was also easy to predict an increase in the accumulation of Mn for stations 2 and 4 (City centre) and station 5 which is located in industrial area (Fig, 4b).

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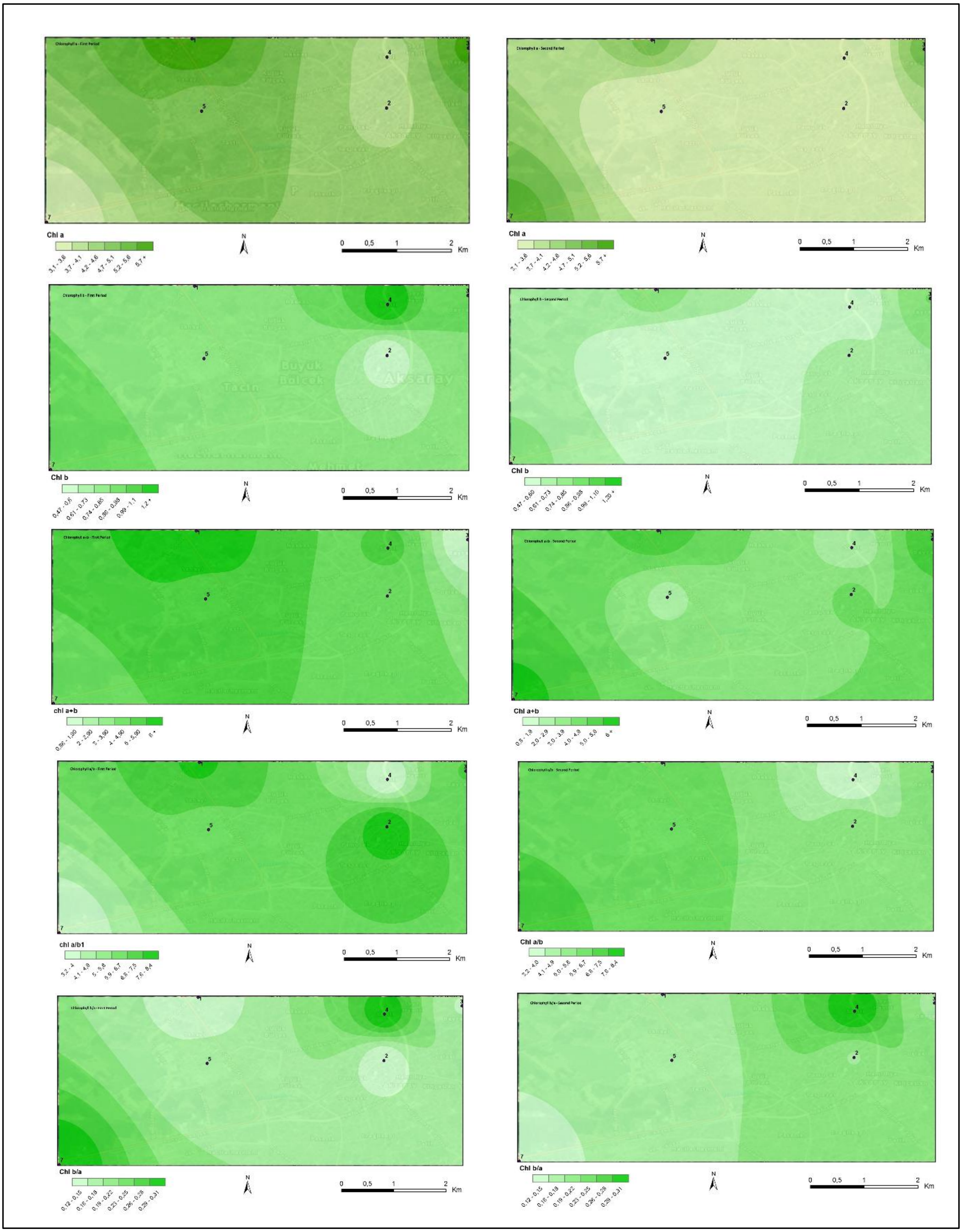

Figure 5. *Pollution maps of Aksaray according to Chlorophyll a and b degradation*

As stated by Conti et al. (2008) increasing Zn concentrations indicate high atmospheric deposition and vehicular traffic. In contrast to their studies any considerable change was not observed and recorded at transplanted samples in various stations (Fig. 4b).

The concentrations of the photosynthetic pigments could be easily calculated and generally it is used to monitor the metal stress on plants (Küpper et al.. 1996).

It is reported that heavy metals prevent the biosynthesis of the chlorophyll pigments and inhibit enzymes required for chlorophyll biosynthesis (Aggarwal. 2011).

Woolhouse (1983) reported that Zn caused considerable decrease in the photosynthetic pigments by replacing itself with iron which was required for chlorophyll production in plant cells. Lu et al. (2000) stated that Cu concentration has decreased chlorophyll concenrations of various samplings. In addition. the key enzyme. protochlorophyllide reductase. which is involved in the reduction of protochlorophyll to chlorophyll. is well known to be inhibited by heavy metals (De Filippis and Pallaghy. 1994). According to studies and our results. heavy metal accumulation in plant tissues results in degradation of chlorophyll molecule. When the maps for the heavy metal accumulation and the maps for the decrease in the chlorophyll content were compared. an obvious reverse correlation could be observed (Fig. 5). Maps for chlorophyll a/b presented that chlorophyll a was affected more than chlorophyll b from the air pollution. which was expected when the photosynthetic pigments were degraded by pollution. This result was also supported by the maps for chlorophyll b/a. All of these decreases and increases in chlorophyll a and b contents and the ratios of them could be explained by the environmental stress like pollution. The amount of chlorophyll in the lichen thalli is often related to the levels of environmental stress; with greater amounts of chlorophyll under stressed conditions than under non-stressful conditions (Wakefield and Bhattacharjee. 2012).

Conclusions

According to the heavy metal analysis results of *P. furfuracea*. air pollution in Aksaray owing to industrial activities. heating and traffic showed various changes in different stations and periods studied. *P. furfuracea* can be utilized safely in biomonitoring researches. And baq technique of biomonitoring study here in Aksaray indicated that lichens couldn't absorp any material from their substrat. they can only be exposured to air polluted area. Heavy metal concentrations in various stations showed obvious differences according to determination methods applied in the experiments. The data from the current study support that the metal accumulation of lichens is strongly dependent the location of stations and periods of exposure.

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Author statements

Volkan IŞIK: Visualization. Data cCuration. Writing - Original Draft. Validation. writing - Review and editing. Çiğdem VARDAR: Validation. Formal analysis. Writing - Original Draft. Visualization. Writing - Review and Editing. Ahmet AKSOY: Data Curationt administration. Supervision. Writing - Review and Editing. Atila YILDIZ: Project administration – Supervision – Writing – Review and Editing – Resources – Conceptualization - Methodology. Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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