

# A transplantation study with *Pseudevernia furfuracea* (L.) Zopf about air pollution mapping of Çankırı, Turkey

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## Abstract

For researching of Zn, Cd, Pb, Ni, Mn and Cu concentrations, lichen thallus and airborne particles were investigated in Çankırı city. From an uncontaminated area thalli of fruticose lichen *Pseudevernia furfuracea* (L.) Zopf were taken and exposed to contaminated area by bag (transplant) technique. According to results, existence of heavy metals in particles is considerable for assessing pollution levels in certain areas. The crucial relation between the heavy metals uptaken by lichens and heavy metals in the air is a evidence that lichens reflect atmospheric pollution. In addition, the results indicate that for determining heavy metal pollution lichens can be utilized as a bioindicator organism. The aim of this research was to evaluate the level of air pollution in Çankırı and to constitute air pollution map by using *P. furfuracea* as a bioindicator. Lichen specimens were collected from an unpolluted area in Yapraklı Mountains in Çankırı in July 2002 and carried to 6 different localities in Çankırı. Specimens were re-collected separately in two different periods, 3 and 6 months later. Cd, Cu, Mn, Ni, Pb and Zn concentrations were analyzed with ICP-MS device. Amounts of chlorophyll a and b were measured by DMSO technique. It was reached that with heavy metal content results in *P. furfuracea* to air pollution data in Çankırı. The clear causes of pollution in Çankırı stations were heating activities, vehicle and industrial pollution.

## Keywords

*Biomonitoring, Air pollution, Bag technique, Pseudevernia furfuracea, Çankırı*

## Introduction

Lichens were among the first organisms to be used as bioindicators for assessment of changes in the environment caused by chemical pollution (Bargagli, 1998; Brown, 1984; Conti and Cecchetti, 2001; Garty et al., 1993; Henderson, 1994; Nimis, 1996; Richardson, 1992; Tyler, 1990; Yıldız et al., 2008, 2011, 2018; Işık and Yıldız, 2021, 2022; Işık et al., 2023). Lots of researches indicated that they have the ability to absorb and accumulate pollutants. They are therefore commonly employed to monitor radionuclides, metals,

nonmetals (such as S and F) and other compounds present in the atmosphere (Goldsmith, 1991). Because of air pollution's harmful effects on human health and ecosystem, it has attracted great attention (Abdunnasır et al., 1994; Çayır et al., 2008; Karademir and Toker, 1998). Lichens are used as bioindicators both close to individual pollution sources and for monitoring large areas, either through examination of the spontaneous lichen flora (Garty et al., 1993). Bags technique has been occurred as to observe city air pollution condition in densely populated regions

(Goodman and Roberts, 1971; Yıldız et al., 2008, 2011, 2018). Lichens are supposed that they can accumulate metal ions via two mechanism (Brown and Beckett, 1984). Heavy metal ions are trapped in to cell wall through an ion exchange way (Puckett et al., 1974). Then certain metals can be inserted into the cells, where they are metabolized and bypassed or result in thallus death. The majority of airborne trace elements are found in airborne particulate matter and lichens are highly effective at capturing them (size 0.5 to 1.0  $\mu\text{m}$ ). The existence of particulate matters on the lichen thallus surface and especially in the slack hyphae of the medulla has been indicated by electron microscopy researches (Bargagli, 1998; Adamo, 2003). The aim of this field research is to declare the results of the analysis of existence of Zn, Cd, Pb, Ni, Mn, Cu in airborne particulates over time and impact of the lichen reaction to contamination through identification of the relation between particulate metal concentrations. For this purpose *Pseudevernia furfuracea* (L.) Zopf specimens were transplanted from unpolluted regions to polluted stations innercity. The airborne content of *P. furfuracea* thallus exposed to vehicles, heating and industrial pollution has been identified. This is the first study on the use of lichens for biomonitoring atmospheric pollution in Çankırı, Turkey.

## Materials and Methods

### Study area

Çankırı, with a population of 78,558 (according to the 2000 census), is in the Central Anatolia region of Turkey (Fig. 1) (Anonymous, 2000). The number of vehicles registered to traffic in 2002 was 19,206 (Anonymous, 2003). The major resources of atmospheric pollution in Çankırı are the highways in and around the city and the heating activities in households. Since the rainiest period of the year is spring and early summer, Çankırı's climate shows humid continental weather attributes with dry in summer. The city has four sub-types of continental climate depending upon location and altitude. The city is situated in the transitional area. Summer season is typically warm and dry, and winter season is cold and snowy (Akman, 1999). Climate chart of Çankırı is demonstrated in Figure 2. Predominant winds blow in the NNW, NW, SSW direction (Fig. 3) (Anonymous, 2008). 6 exposure zones were identified in the city of Çankırı as biomonitoring zones (Table 1). All of stations were located in the city centre and the city

(centre and the contamination was originated from similar resources; traffic, industrial and heating activities.

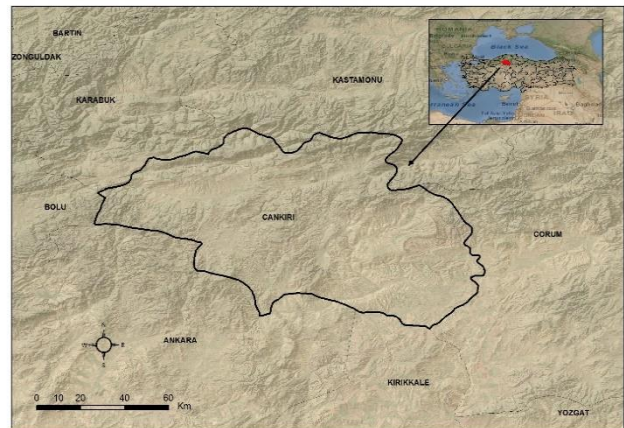


Figure 3. Map of study area

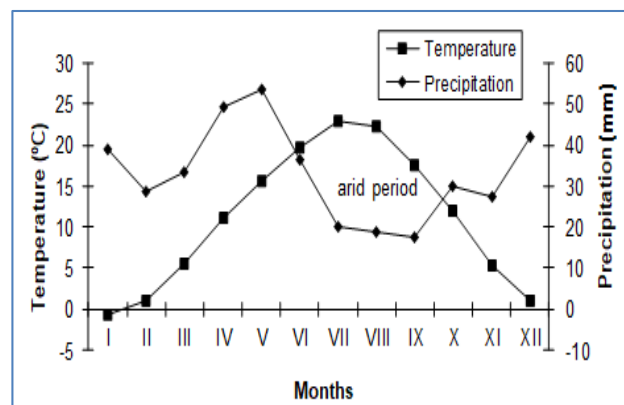


Figure 2. Climate diagram of Çankırı

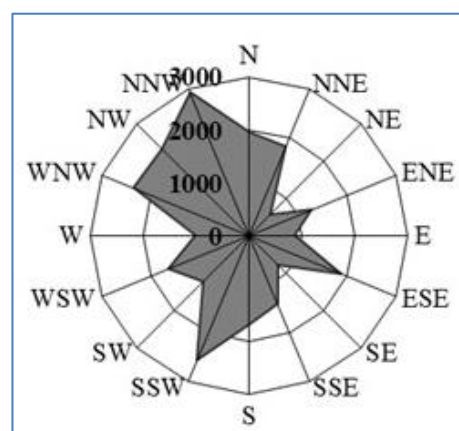


Figure 3  
Weathercock  
of Çankırı

### Biological material

For determining the atmospheric pollution thallus of *P. furfuracea* was utilized. Samples of lichens were taken in the forest near Yapraklı-Çankırı. That area

**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)	<i>Pinus sylvestris</i>	1750 m	N 40° 47' 600" E 33° 46' 818"
C2	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş area (Control group)	<i>Pinus sylvestris</i>	1750 m	N 40° 47' 600" E 33° 46' 818"
1	Çankırı-Yapraklı Road, Fatih Square, in front of Carpet Factory	<i>Pinus nigra</i> subsp. <i>pallasiana</i>	780 m	N 40° 35' 896" E 33° 37' 214"
2	Çankırı-Büyük Hotel, Ankara-Kastamonu Road	<i>Pinus nigra</i> subsp. <i>pallasiana</i>	760 m	N 40° 36' 072" E 33° 36' 400"
3	Çankırı-Çankırı Manucipality, Ministry of Parks and Gardens	<i>Pinus nigra</i> subsp. <i>pallasiana</i>	760 m	N 40° 35' 888" E 33° 36' 902"
4	Çankırı- Garden of Çankırı Ministry of Rural Services	<i>Pinus nigra</i> subsp. <i>pallasiana</i>	780 m	N 40° 36' 990" E 33° 36' 451"
5	Çankırı- Road between Ziraat Bank and Culture Center, Mehmet Çokyılmaz Shop	<i>Fraxinus</i> sp.	760 m	N 40° 36' 005" E 33° 37' 076"
6	Çankırı- Tarım Board of agriculture Residances, Square	<i>Cedrus libani</i>	780 m	N 40° 35' 888" E 33° 36' 902"

is remote from the pollution sources and considered to be clean in terms of air pollution.

Each of 20 g lichen material stacked flaccid in a thin nylon mesh. All bags contained several lichen thallus. At each surveillance station, the bags are attached to a nylon rope and suspended from trees more than 3 meters from the ground. Specimens of *P. furfuracea* were exposed to air pollution during two 3-month intervals (6 months in total) between July 28, 2002 and February 1, 2003. The date of hanging was July 28-30, 2002; the first date of gathering was November 02, 2002; the last date of gathering was February 1, 2003.

#### Sample preparation and heavy metal determinations

Following the collection of the transplanted lichens, they were first washed with tap water and twice distilled water to eliminate undesirable substances. Lichen samples were desiccated in paper bags at 80 °C for 24 hours to save them against microbial breakdown and to supply reference values for dry weight. The desiccated lichen samples were crushed with a mortar to homogenize the heavy metals (shredded to

obtain homogeneity). All glass, plastic and porcelain materials were placed in detergent water and left overnight. Then, bathed in tap water, lay down in 20% of nitric acid and waited again during the night. After these orders, they were washed with double pure water and left to dry in etuve at 60 °C.

All standards and solutions were prepared using 65% w/w (Merck reagent) nitric acid and 35% w/w aqua regia (Merck reagent) HCl. And further in all stages of standard preparation and solution and also dilutions, double distilled water was used. The utilization of HNO<sub>3</sub> to dissolve plant parts is a widely used process (Halıcı et al., 2005). 1 g of dried specimens were placed in a porcelain melting pot and burned at 460°C during 24 hours in the oven. Specimens transformed into ash were placed in a 100 ml beaker and then 65% 100 m HNO<sub>3</sub> added on. Beakers placed in the sand bath were heated and left to evaporate HNO<sub>3</sub> before all the HNO<sub>3</sub> vaporized, the beaker removed from the sand bath and left for refrigerating. After the evaporation process, residual part placed in the centrifugation tube and the volume was supplemented to 15 ml with 1% HNO<sub>3</sub>. Specimens were centrifuged at 3000 rpm

(3000 rpm = 1157 g (relative centrifuge velocity)) for 20 minutes after centrifugation of the supernatant transported in a 25 ml graduated flask. Heavy metal concentrations in these mixtures analyzed by using ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) in our research (Halıcı et al., 2005).

**Chlorophyll analysis**

Chlorophyll was obtained from 20 mg of air-dried lichen material using pristine DMSO. 5 ml of DMSO have been added to the thallus for extracting. Tubes containing DMSO and lichen material were incubated at 65°C for 40 minutes in darkness and then left to cool to room temperature. The lichen extracts were filtered using Whatman no 3 filter paper. The spectrophotometer was calibrated at 750 nm. Optical density of the extracts was measured at 665 and 648 nm. Calculations were performed according to DMSO (Dimethyl sulphoxide, for synthesis, ≥ 99% purity Merck 8.02912) 100% (pure solvent). Amount of chlorophyll calculated according to following equations [1], [2] and [3] of Barnes et al. (1992);

$$C_a = 14,85A^{665} - 5,14A^{648} \quad [1]$$

$$C_b = 25,48A^{648} - 7,36A^{665} \quad [2]$$

$$C_{a+b} = 7,49A^{665} + 20,34A^{648} \quad [3]$$

where Ca = Chlorophyll a; Cb = Chlorophyll b

**Results and Discussion**

The values of Cd, Cu, Mn, Ni, Pb and Zn and the chlorophyll a and chlorophyll b levels of the *P. furfuracea* specimens, which were suspended at 6 stations in the city of Çankırı and two as check stations in Yapraklı-Çankırı, are shown in Table 2. All lichen specimens were exposed to atmospheric pollution for two periods of time and every three months. Control samples were also collected in 3 months intervals from Yapraklı-Çankırı at the same time with the samples hanged in Çankırı city. Pollution maps were drawn for Çankırı city. Using these maps, it is easy to establish and compare the pollution situation in the first and second periods. Pollution maps of Çankırı city according to the analysis are given in Figure 4, 5 and 6.

**Table 2.** Results of lichen material analysis

Elements	Periods	Cu	Cd	Ni	Pb	Mn	Zn	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a/b	Chlorophyll b/a
		µg.g <sup>-1</sup>						µg chl.mg air-dry wt thallus <sup>-1</sup>				
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	0,48493	0,02345	0,50634	0,52688	3,21164	0,19379	0,673	0,308	0,981	2,185	0,458
	2	0,34342	0,02616	0,49354	0,46808	2,80063	0,41186	0,443	0,124	0,567	3,573	0,280
2	1	0,39257	0,02180	0,36653	0,51861	2,56067	0,61576	1,591	0,198	1,789	8,035	0,124
	2	0,43676	0,02564	0,42534	0,64116	3,07125	0,30030	0,325	0,045	0,37	7,222	0,138
3	1	0,28887	0,02607	0,33477	0,45538	3,20539	0,30761	0,119	0,01	0,129	11,9	0,084
	2	0,23324	0,02229	0,24581	0,42836	1,92065	0,67450	0,599	0,345	0,944	1,736	0,576
4	1	0,29915	0,02596	0,28253	0,49587	1,72037	0,14647	7,189	1,001	8,18	7,182	0,139
	2	0,38530	0,02562	0,29653	0,57435	1,63302	0,22361	5,207	0,951	6,158	5,475	0,183
5	1	0,24479	0,02749	0,19759	0,40300	2,06471	0,14355	4,614	0,718	5,332	6,426	0,172
	2	0,31063	0,03140	0,29355	0,69184	2,87703	0,31241	1,8	0,553	2,353	3,255	0,307
6	1	0,20611	0,02717	0,21529	0,44613	1,22481	0,13267	5,55	0,712	6,262	7,795	0,128
	2	0,26540	0,02250	0,26189	0,51414	1,33302	0,38473	0,201	0,13	0,331	1,546	0,647

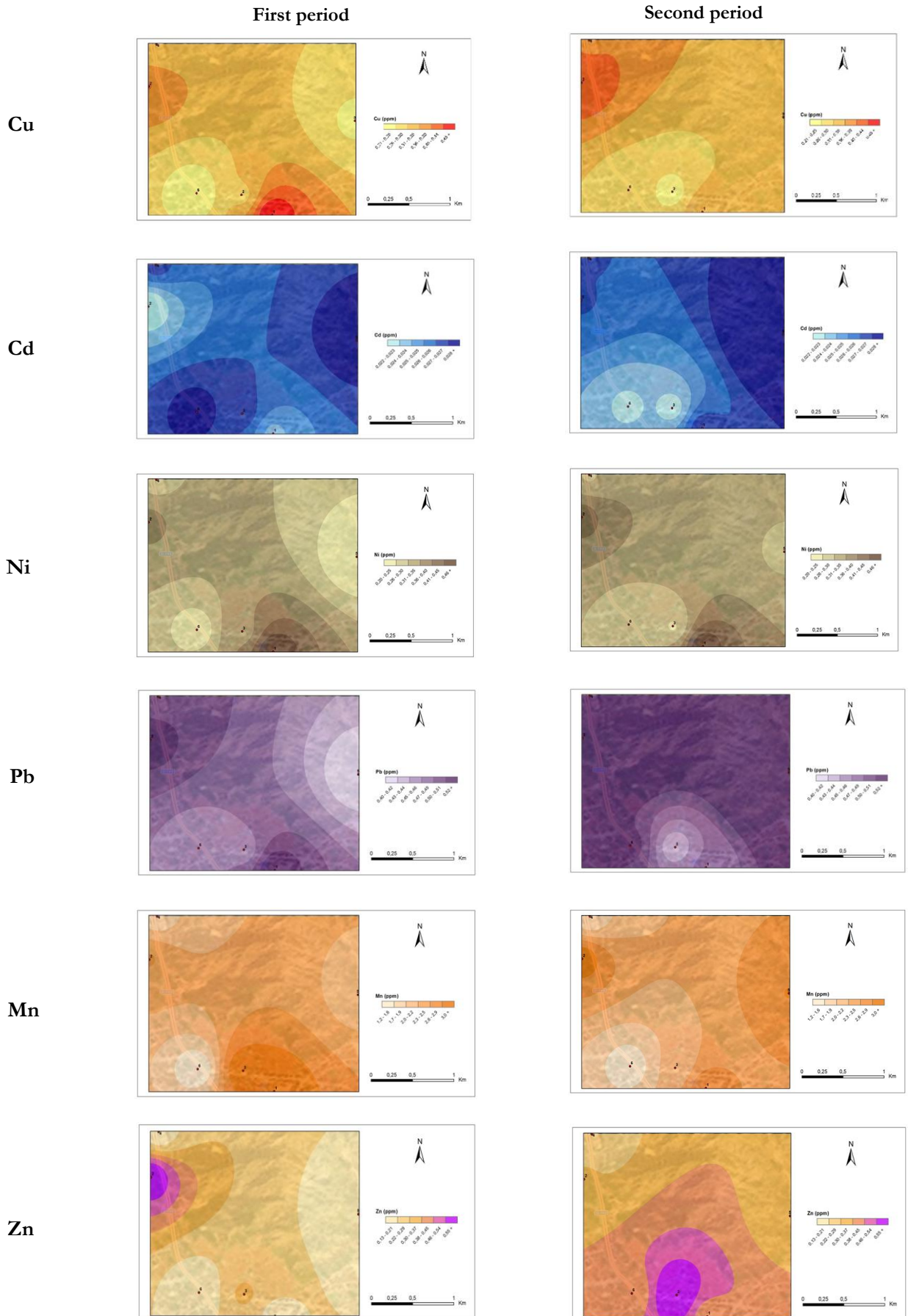


Figure 4. Pollution maps of Çankırı according to the heavy metals

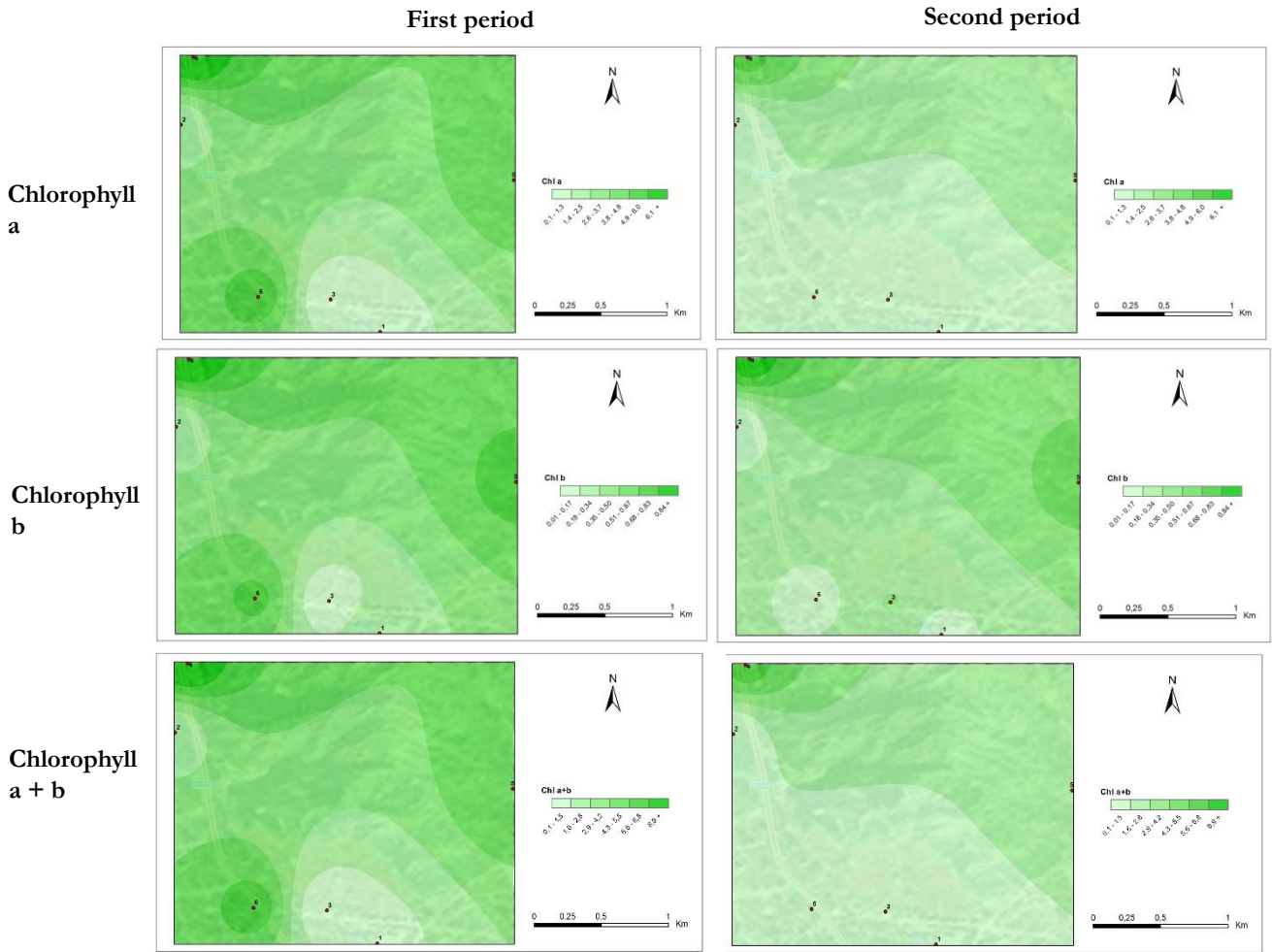


Figure 5. Pollution maps of Çankırı according to chlorophyll a, b and a+b

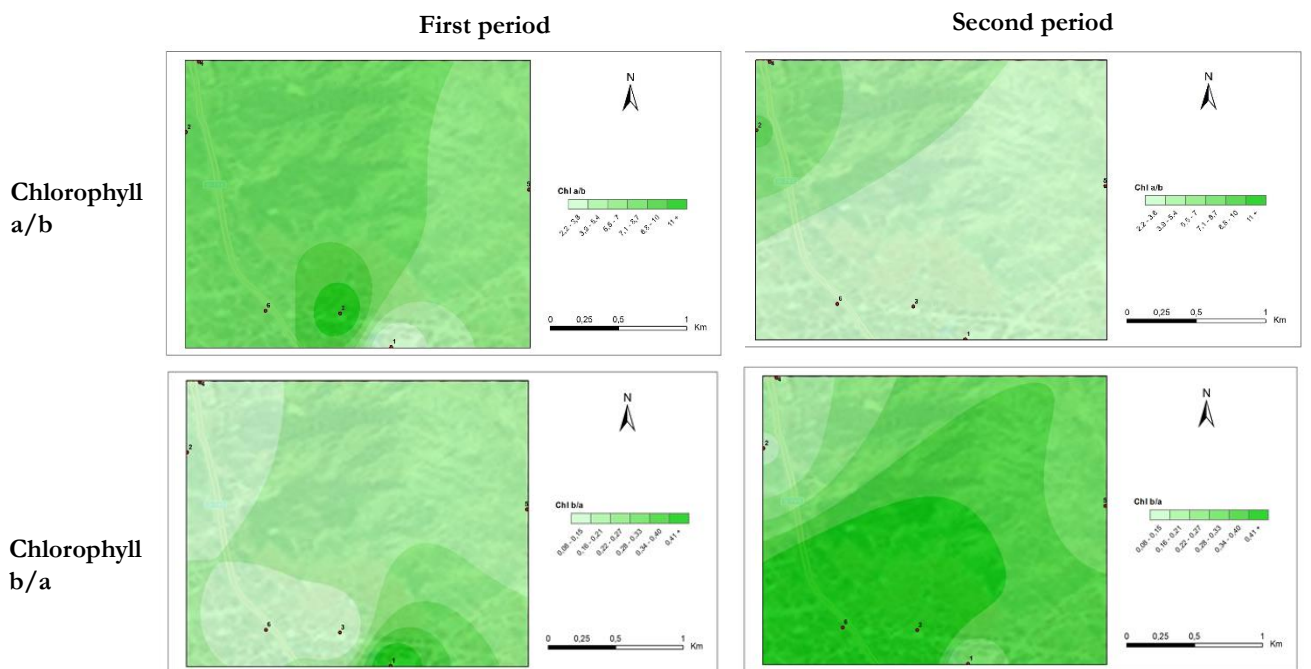


Figure 6. Pollution maps of Çankırı according to chlorophyll a/b and b/a

It is clearly seen that the organism, named *P. furfuracea*, accumulated heavy metals as a result, it has functioned better as a "bioindicator" in all maps.

Pollutants release in the atmosphere through various sources and activities. Sources causing air pollution are mainly stationary sources such as smoke of power plants, factories and heating appliances usage with fossil fuels (natural gas, coal and oil) and mobile resources such as automobiles (Markert, 1993).

When the Cu and Cd accumulation maps were reviewed, it was easy to see that there was no significant change at all stations for both periods.

For all stations in second period heavy metal accumulation levels are higher than the first period.

When air pollution charts for Ni were assessed, it was easy to construe the alteration in level Ni between two periods. High accumulation level of Ni in station-1 was observed in both periods. The major pollution from Ni in Çankırı comes from mobile sources and waste from carpet facilities. Station-1 was close to the carpet factory. In similar studies in Ankara and Aksaray provinces, the source of pollution in stations with high Ni levels was reported as heavy traffic and coal combustion (Yıldız et al., 2008; Işık et al., 2023).

The Pb accumulation maps make it clear that there is obviously Pb pollution throughout the city that can be resulted by stationary and mobile sources in the downtown area. The results about Pb accumulation for station 2 and 5 has presented interesting results. As the station 5 was located very close to city center and had very heavy traffic, it was logical that the lead level in the atmosphere was quite higher than the other stations. Compared to the studies conducted in Kayseri, Ankara and Aksaray provinces, similar sources of lead pollution were identified as mobile sources, vehicle emissions and heating activities (Yıldız et al., 2008, 2011; Işık et al., 2023). Looking at the Zn pollution map, it could be seen that Zn accumulation was occurring in the city during the first period, which was caused by stationary and mobile resources (Markert, 1993). In Kayseri study, Zn pollution source was cited as mobile sources and prevailing winds from the refining plant (Yıldız et al., 2011). Levels of photosynthetic pigments such as chlorophyll a and b could be easily measured and commonly used to monitor the stress of metals on plants. It is reported that heavy metal exposure prevent the biosynthesis of the chlorophyll a and b pigments and inhibit the enzymes required for chlo-

rophyll biosynthesis. Woolhouse (1983) showed that Zn caused a decrease in the photosynthetic pigments by replacing itself with Fe which was required for chlorophyll biosynthesis pathways. Liu et al. (2000) stated that Cu concentration decreased chlorophyll content.

The accumulation of heavy metals in plant tissues causes the deterioration of chlorophyll pigments (Garty et al., 1985; Ra et al., 2005). A comparison of the heavy metal accumulation maps with the chlorophyll reduction maps shows an apparent inverse relationship. In terms of total accumulation of heavy metals, chlorophyll a and b levels diminished over the two periods. Especially high decrease was observed in city centers obviously, considerably. This finding could also be confirmed by the chlorophyll a+b map which shows the total decline of photosynthetic pigments. Chlorophyll a/b maps showed that chlorophyll a was more damaged than chlorophyll b by atmospheric pollution, which was predicted when photosynthetic pigments were deteriorated by pollution. In addition that consequence was confirmed through maps for chlorophyll b/a. All these results in chlorophyll a and b concentrations and their proportions could be clarified by pollution from various resources. Climatic weather conditions, seasons, light intensity and the lichen itself could affect the deterioration of chlorophyll (Backor et al., 2003). We can state in conclusion that this type of diagnosis is useful not only for developing contamination models but also, and more importantly, for assessing the overall cost of transport-related pollution. *P. furfuracea* can be utilized for biomonitoring in ecosystem in safety.

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