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Biomonitoring of Heavy Metals Accumulation with *Pseudevernia furfuracea* (L.) Zopf in Kırşehir Province, Türkiye

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ABSTRACT

Today, the problem of air pollution has been highlighted by rapid population growth and urbanisation, along with the development of industry. Over the last fifty years, much attention has been paid to the relationship between lichens and airborne particulate matter (especially heavy metals). The use of living organisms in air pollution studies is now widely accepted in many countries and the results of these biomonitoring studies are very important for future action. The goal of this study was to determine heavy metals in Kırşehir province using the bag technique, a biomonitoring approach, with *Pseudevernia furfuracea* (L.) Zopf lichen and to develop a pollution map of the city. In November 2002, lichen specimens were obtained from an unpolluted region in the Yapraklı Mountains, Çankırı, and transplanted to 4 distinct places in Kırşehir. After 3 and 6 months of exposure, they were collected in order to analyse heavy metals (Cu, Cd, Mn, Ni, Pb and Zn) with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). In addition, chlorophyll-a and chlorophyll-b contents were determined by Dimethyl sulfoxide (DMSO) method. The findings revealed that the heavy metal contents in various stations are the result of industrial, traffic, and heating activities. As a result, *Pseudevernia furfuracea* showed excellent bioindicator ability for detecting air pollution.

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INTRODUCTION

Due to increasing evidence of trace element toxicity on organisms, airborne metal pollution has become a global environmental issue that public authorities must address in recent decades. The impact of human-caused pollutants has surpassed that of natural sources for most metallic contaminants, and there is evidence that airborne particulate matter-bound species, including heavy metals, accumulate in the human body to cause chronic diseases such as neurological degenerative disorders, multiple sclerosis, or muscle dystrophy (Saib et al., 2023). According to studies, even little levels of particulate matter can cause significant mortality in human population. Rapid urbanization is one of the key causes of increased air pollution nowadays. The urbanization process generates prospects for growth in the industrial and commercial sectors, resulting in exaggerated city expansion. Rapid urbanization degrades the environment, creates traffic difficulties, reduces green space, and increases urban imperviousness. All of these factors contribute to dangerous pollutants accumulating and becoming trapped in the atmosphere (Syed and Abas, 2023).

Lichens are commonly utilized in air pollution biomonitoring investigations, either as bioindicators of air quality or as bioaccumulators of atmospheric deposition (Conti and Cecchetti,

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2001; Frati and Brunualti, 2023; Gao et al., 2022; Ramić et al., 2019; Wolterbeek, 2002; Sczepaniak and Biziuk, 2003). Because lichens lack root systems and waxy cuticles, they rely heavily on wet and dry deposition for mineral nutrients. As a result, their elemental composition and physiological condition are heavily influenced by the atmospheric input of air pollutants such toxic chemicals and heavy metals (Bermudez et al., 2009). Thus, heavy metal contents in lichen tissues are thought to mirror atmospheric concentrations or deposition, regardless of any potential hazardous effects (Işık et al., 2023).

In addition, the physical effect of air pollution on lichens is thought to be a decrease in chlorophyll content with increasing pollution level. Heavy metals are known to have an impact with chlorophyll synthesis, either directly by inhibiting an enzyme step or indirectly by causing a nutritional deficit. The degradation of chlorophyll can occur when heavy metals enter the cells of the photobiont (Chettri et al., 1998).

The lichen transplantation technique (Bag Technique), which involves exposing lichen samples collected in a remote area to the study area of pollution, has recently gained popularity because it solves the issue with locating enough lichens in polluted areas; additionally, the initial elemental concentration and exposure length are known (Chahloul et al., 2023). With this technique, lichens are obtained from natural regions, washed, and packed in nylon bags before being hung in strategic locations to monitor air pollution. The hanging technique prohibits any material from being absorbed from their substrate (Boonpeng et al., 2023; Chahloul et al., 2023; Işık et al., 2023, Loppi et al., 2019; Saib et al., 2023; Yıldız et al., 2008, 2011, 2018).

The purpose of this research is to determine the presence of heavy metals (Pb, Cu, Cd, Mn, Ni, Zn) associated with air pollution in Kırşehir province using bioindicator lichen *Pseudevernia furfuracea* (L.) Zopf which is taken from unpolluted areas and transplanted to polluted areas using bag technique and ICP-Mass Spectroscopy and also to generate a heavy metal pollution map of the city.

MATERIALS AND METHODS

Characteristic of study area

The research was conducted in urban areas of Kırşehir (Fig.1) with population of 253.239 habitants according to the 2002 census (DIE, 2002). The total number of registered vehicles in the city is 26,202, according to the 2002 Traffic Statistics Yearbook (DIE, 2002). The city's biggest pollutants include industrial operations, heavy traffic, and heating activities owing to coal use. The annual temperature and precipitation diagram is shown in Fig.2. Prevailing wind direction is northwards as shown in Fig.3. Lichen samples were exposured to pollution in 4 stations in city centre and control stations (2 periods) were selected in Çankırı-Yapraklı Dikilitaş forest area (Table 1.).

Lichen samplings transplantation

Pseudevernia furfuracea lichen samples were collected from the forest area in Yapraklı-Büyükyayla district of Çankırı province (Fig.4). That forested area is located far away from the city's pollution sources and is considered clean (control station) enabling lichen samples to be transplanted to polluted areas. After the lichen samples were washed with distilled water, each 20 g of lichen material was placed in thin nylon bags. 2 of these bags were hung on trees at the stations where biomonitoring will be carried out, at a height of 3 m above the ground. Lichen samples were exposed to air pollution at all biomonitoring stations for two three-month periods (from 5 November 2002 to 6 January 2003). First hanging date of lichen samples was 05.07.2002.

Experimental design

Samplings preparation and analysis of metal concentration in lichens

The lichen samples were cleaned twice with distilled water after 3 and 6 months of exposure

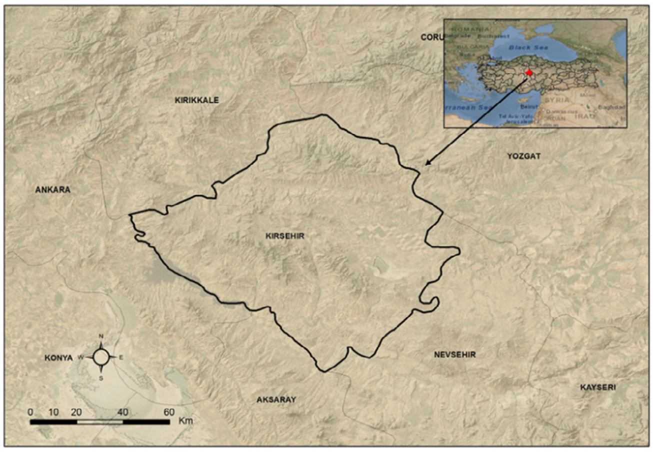


Fig. 1. Map of study area.

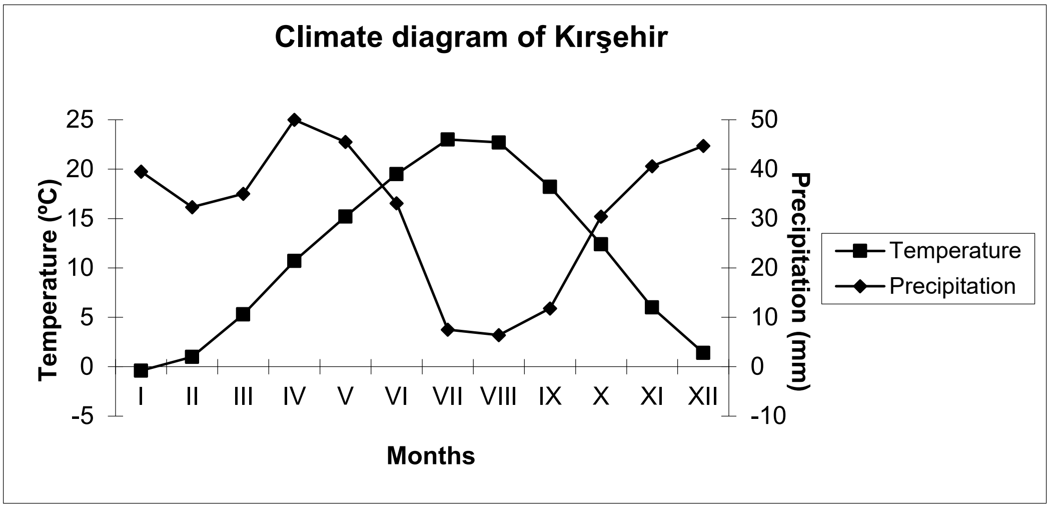


Fig. 2. Climate diagram of Kırşehir.

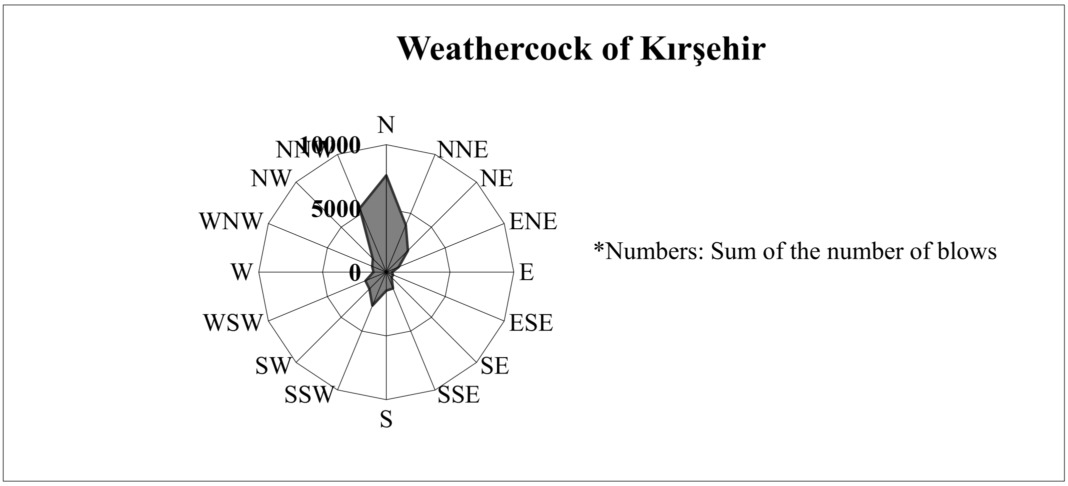


Fig. 3. Weathercock of Kırşehir

Table 1. Locations for collecting of lichen samples.

Station no	Stations	Substrate of the specimens	Altitude of the stations (GPS) (m)	Coordinates
C1	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş area (control group)	*Pinus sylvestris*	1750 m	N 40º 47' 60" E 33º 46' 81"
C2	Çankırı-Yapraklı, Yapraklı Büyük Yayla, Dikilitaş area (control group)	*Pinus sylvestris*	1750 m	N 40º 47' 60" E 33º 46' 81"
1	Kırşehir- Industrial Area, Kılıçözü Industry Site, Opposite of OK Akü	*Salix* sp.	1060 m	N 39º 07' 22" E 34º 11' 28"
2	Kırşehir-Terme hot spring entrance	*Thuja orientalis*	990 m	N 39º 08' 16" E 34º 09' 19"
3	Kırşehir- High School Intersection, Yavuz Sultan Selim Boulevard, Centre	*Ailanthus* sp.	990 m	N 39º 08' 86" E 34º 09' 84"
4	Kırşehir- Military Service Branch intersection, Mehmet Ali Yapıcı Avenue, Yavuz Sultan Selim Boulevard, Gar den of the Military Service	*Populus alba*	980 m	N 39º 09' 28" E 34º 09' 55"

Fig. 4. Forest area in Yapraklı-Büyükyayla district of Çankırı province. (from https://www.dogakolik.com/cankiri/yaprakli-buyuk-yayla/)

to polluted air to remove any substances that were present. The samples were left to dry in paper bags for 24 hours at 80°C both for drying and to prevent any microbial effect. To guarantee homogenous distribution of heavy metals in totally dried samples, crushing was carried out with the help of a mortar. Experimental equipments (glass, porcelain, plastic) were left in detergent water overnight, then washed with tap water and left in 20% nitric acid solution overnight. After these processes, the samples were washed twice with distilled water and dried at 60°C. For all standard treatments and solutions, twice distilled water and 65% w/w nitric acid and 35% w/w HCl in aqua regia were used. In order to accelerate the disintegration processes in the lichen material, the commonly used HNO_3 solution was used to dissolve it (Halıcı et al., 2005). Process steps continued as follows;

(a) After 24 hours of burning in a porcelain crucible at 460°C, 1 g dried lichen samples were transferred in a 100 mL beaker and 10 mL of 65% HNO_3 solution was added, (b) the beakers were heated in a sand bath to evaporate the excess HNO_3 solution. Just before evaporation of HNO_3 solution, the beakers were removed from the sand bath and allowed to cool at room temperature, (c) after evaporation and cooling, the remaining portion of the solution was placed in centrifuge tubes for centrifugation and the volume of the samples was adjusted to 15 mL with 1% HNO_3 and centrifuged at 3000 rpm (3000 rpm= 1157 g (relative centrifugal acceleration)) for 20 min, (d) following centrifugation, the upper component (supernatant) was moved to a 25 mL volumetric flask and diluted to 25 mL with 1% HNO_3. ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) was used to detect concentrations of heavy metals (Halıcı et al., 2005).

Physiological parameters (Chl_a, Chl_b, Chl_{a+b}, $Chl_{a/b}$, $Chl_{b/a}$)
Amount of chlorophyll

As a first step for measuring the amount of chlorophyll, dried 20 mg samples of *Pseudevernia furfuracea* lichen were extracted with 99% pure DMSO (for synthesis, Merck 8.02912). Then 5 mL DMSO was added to the lichen thallus. Test tubes containing DMSO and lichen material were incubated in the dark at 65°C for 40 minutes before cooling to room temperature. The lichen extracts were filtered through Whatman no 3 filter paper. The UV-Spectrophotometer was calibrated with DMSO at 750 nm. The absorbance of the lichen extracts was adjusted at 665 and 648 nm and the values were read. Chlorophyll extractions and following calculations were done according to Barnes et al. (1992).

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

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

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

RESULTS AND DISCUSSION

The chlorophyll-a and chlorophyll-b contents of *Pseudevernia furfuracea* samples hung at 4 stations in Kırşehir and 2 stations in Çankırı (control station) and the results of heavy metals (Cu, Cd, Ni, Pb, Mn, Zn) analyses measured by ICP-MS are shown in Table 2.

It was determined that heavy metals accumulated in lichen samples collected twice in 3 month periods exposed to polluted air in the city centre of Kırşehir province and a decrease in chlorophyll content was detected due to pollution stress.

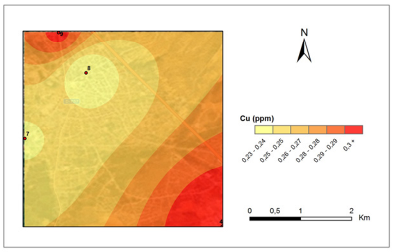
Lichens have long been known to be sensitive organisms to air pollution due to the impact of pollutants on both symbionts fundamental metabolic activities (Brodo et al., 2001). Pollution sensitive lichen species may be adversely affected in urban areas or near industrial plants, while a few hardy species with pollution resistance will survive (Riddell et al., 2008). Brodo's (1961) lichen transplantation approach (bag technique) was applied in our heavy metal biomonitoring investigation in Kırşehir province. Pollution maps according to heavy metal (Cu, Cd, Mn, Ni,

Table 2. Results of *Pseudevernia furfuracea* lichen 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 $thallus^{-1}$).

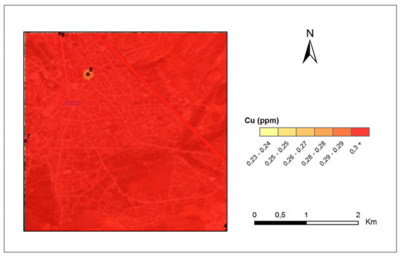
Elements	Periods	Cu	Cd	Ni	Pb	Mn	Zn	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a/b	Chlorophyll b/a
C1	1	0,28423	0,02621	0,27508	0,51637	1,89763	0,15076	7,7827	1,945	9,7277	5,0007	0,312
	2	0,38909	0,02757	0,28306	0,55338	1,94752	0,57671	9,252	3,013	12,265	4,5167	0,3337
C2	1	0,25191	0,03153	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	0,30256	0,02139	0,25920	0,46506	1,42946	0,15215	4,531	1,653	6,184	2,741	0,365
	2	0,38122	0,02883	0,36163	0,60247	1,95279	0,84667	1,531	0,228	1,759	6,715	0,149
2	1	0,24027	0,02416	0,25637	0,39846	1,88095	0,14822	5,806	0,7	6,506	8,294	0,121
	2	0,33031	0,02762	0,30009	0,58480	2,02716	0,24937	5,352	0,765	6,117	6,996	0,143
3	1	0,22969	0,02278	0,21035	0,37290	1,79213	0,17229	1,596	0,172	1,768	9,279	0,108
	2	0,28724	0,02875	0,25933	0,48198	1,89326	0,33416	1,529	0,598	2,127	2,557	0,391
4	1	0,29405	0,02581	0,24518	0,46107	1,70152	0,19297	6,031	0,91	6,941	6,627	0,151
	2	0,53776	0,02225	0,37492	0,66028	1,96426	2,441	1,211	0,295	1,506	4,105	0,244

Pb and Zn) levels are shown in Fig. 5.

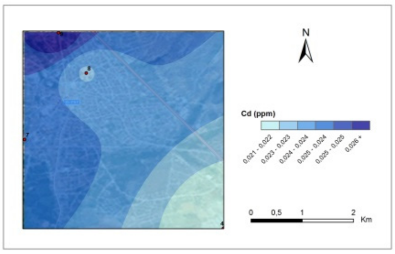
When Cu values are analysed from the data in the Table 2, an increase is observed in all 4 stations between 2 periods, especially a significant increase is observed in the 4th station. The main factors in that increase are heavy traffic and industrial activities. The Cu values at other stations are closer to the control stations. Martínez-Guijarro et al. (2021) reported that regular transportation, manufacturing, building (roofing, decoration, etc.), and electricity transit, such as conductive dyes, are all industrial sources of Cu. Cu is present in cylinder head gaskets, oil



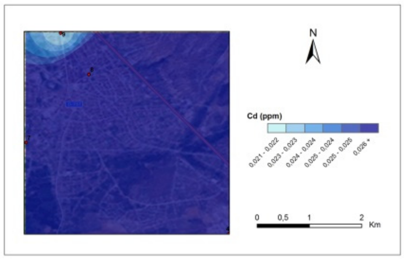
Cu, first period



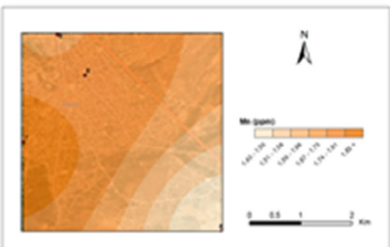
Cu, second period



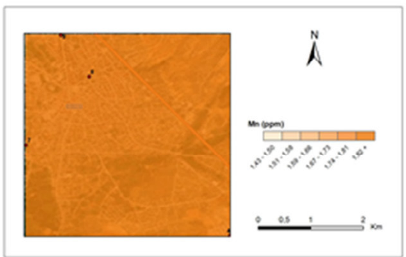
Cd, first period



Cd, second period

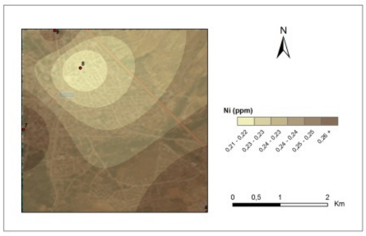


Mn, first period

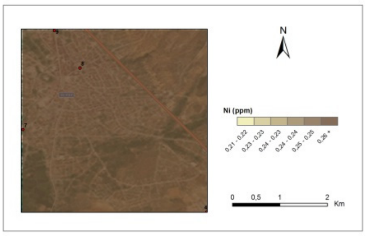


Mn, second period

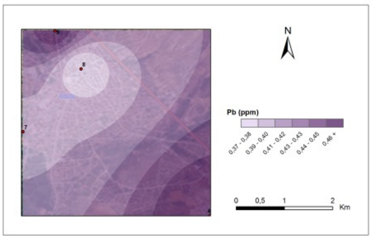
Fig. 5. Pollution maps of Kırşehir according to the heavy metals Cu, Cd, Mn, Ni, Pb and Zn.



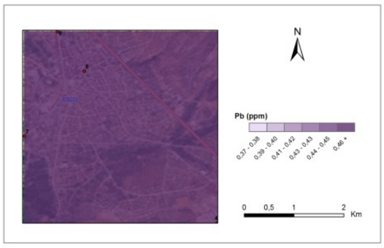
Ni, first period



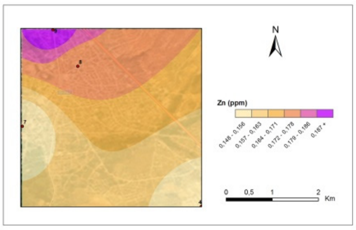
Ni, second period



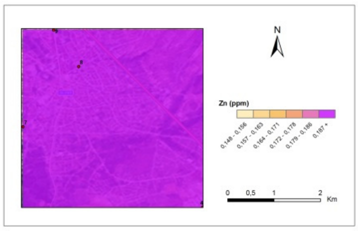
Pb, first period



Pb, second period



Zn, first period



Zn, second period

Continued Fig. 5. Pollution maps of Kırşehir according to the heavy metals Cu, Cd, Mn, Ni, Pb and Zn.

leak sumps, and brake linings and is used in the fabrication of brass alloys for vehicles. Cu mean values were found to be higher due to industrial effects in the same transplanted study with *Pseudevernia furfuracea* by Lucadamo et al. (2022) and Saib et al. (2023).

Although its low crustal abundance, Cd is commonly used in industrial processes, such as phosphate fertilizers or anticorrosive compounds in Ni-Cd battery manufacture. Other sources for Cd emissions including transportation pollution from fossil fuels, metallurgical emissions from petroleum, electroplating, and polyvinyl chloride polymers (Scerbo et al., 2002). In comparison with the control station, there is no significant difference between the Cd values of the samples of *Pseudevernia furfuracea* that were transplanted.

Many investigations have found that the vicinity of roadways increases Ni accumulation in lichens. Vehicle traffic had a significant impact on the Ni concentrations in the transplanted lichen (*Usnea misaminensis* (Vain.) Motyka) (Abas et al., 2022). Due to the traffic and industrial area, the highest Ni values were recorded in the second period of 1. and 4. stations.

Pb is emitted by traffic as a result of fossil fuel combustion, tyre and brake pad abrasion, corrosion, lubricating lubricants, and gasoline additives (Garty, 2001; Sujetoviene, 2015; Aguilera et al., 2021). The highest Pb values were obtained in the second period of 1. and 4. stations caused by traffic and industrial area.

Although the reality that it is often produced by traffic and industrial sources, the Mn value in the second period of the 2. station had been found to be the highest due to the heating of the hot spring facility.

In urban areas, Zn originates from motor vehicle traffic via the consumption of greasing lubricants (Boamponsem et al., 2017). Heavy traffic at stations 1 and 4 increased Zn concentration in *P.furfuracea* tissues significantly.

There has never been a heavy metal biomonitoring research carried out using lichens in Kırşehir province, so the data we got in 2002 are quite valuable, because there is no study that can detect the pollution in 2002. It has significant value in terms of providing data for comparison with new research. In addition, the fossil fuel used in Kırşehir province has been replaced by natural gas within 21 years. When we look at the literature, studies that we can compare in terms of heavy metals as current data in Kırşehir province are limited.

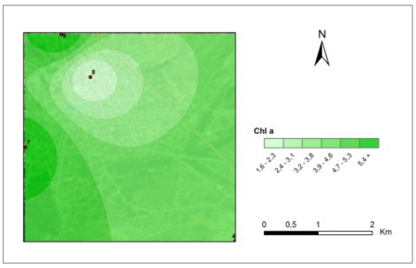
The mean concentrations heavy metals in our study were Cd :0,025 μg g^{-1}, Mn :1,83 μg g^{-1}, Zn :0,56 μg g^{-1}, Ni :0,28μg g^{-1}, Pb :0,5μg g^{-1} and Cu :0,32μg g^{-1}. Çiftçi et al. (2021) examined Ni, Pb and Cu concentrations in *Populus nigra* L. and *Cedrus libani* A. Rich. vicinity of casting factory in Kırşehir in 2021. The mean Ni, Pb and Cu values in *P. nigra* are as follows; 2,49μg g^{-1}, 0,50μg g^{-1}, 6,73μg g^{-1}. In *C. libani*, the values are as follows; 0.66 μg g^{-1}, 1,74 μg g^{-1}, 4,27 μg g^{-1}. The activities of the casting factory are effective in the high values compared to our study.

In another study conducted by Akkan et al. (2018) in dam lake sediment in Kırşehir, the mean values of Mn, Ni, Cu, Zn, and Pb were 113.324μg g^{-1}, 3.25μg g^{-1}, 0.97μg g^{-1}, 7.19μg g^{-1}, and 5.59μg g^{-1}. These values are considerably higher than our study.

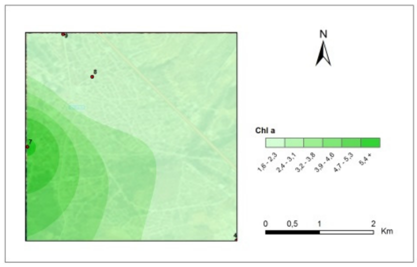
Palabıyık et al. (2022) investigated heavy metal deposition in northern pike in Kırşehir's dam lake. According to mean values of heavy metal concentration in different fish tissues varied as dry weight Cu: 0,24 μg g^{-1}, Mn: 0,35 μg g^{-1}, Zn: 2,89μg g^{-1}. So only Zn value showed high value compared to our study.

Pollution damage to lichens has been assessed using a variety of biological parameters, including respiration (Baddeley et al. 1972), photosynthesis (Showman, 1972; Puckett et al., 1973; Richardson & Puckett, 1973; Ronen et al., 1984), chlorophyll degradation (Ronen et al., 1984), and chlorophyll fluorescence (Kauppi, 1980). One of the most visible signs of lichen damage induced by air pollution is chlorophyll loss, which results in thallus bleaching or browning (Niewiadomska et al ., 1998). Gonzalez and Pignata (1994) reported a decrease in the ratio chlorophyll a, in transplanted thalli of *Punctelia subrudecta* (Nyl.) Krog, after exposure to polluted urban air in Cordoba, Argentina.

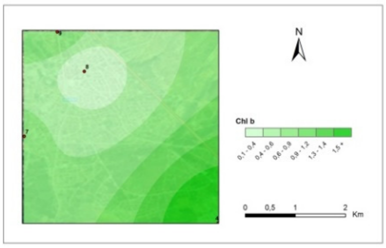
In lichens, decreasing parameters such as chlorophyll a, chlorophyll b, and chlorophyll a+b concentrations, as well as chlorophyll a/b ratios, are used to assess the photobiont's exposure to environmental stress. Chlorophyll-a is the chlorophyll type that is most impacted by pollution agents such as heavy metal accumulation, and it is the first to be eliminated. If the pollution is sufficiently strong, chlorophyll-b is destroyed. In photosynthesis, the ratio of chlorophyll b to a (Chlorophyll b/a) indicates destruction. Because of the minor variations in chlorophyll b levels, chlorophyll b/a changes rely on chlorophyll a, and a reduction in chlorophyll a results in an increase in chlorophyll b/a. Photosynthetic deterioration begins at low chlorophyll-a stations (Yıldız et al., 2008). Looking at the low chlorophyll-a values in Table 2, we can see that photosynthetic deterioration has begun at stations 1, 3, and 4. And also the changes of chlorophyll-a and chlorophyll-b in the first and second periods are shown in figure 6.



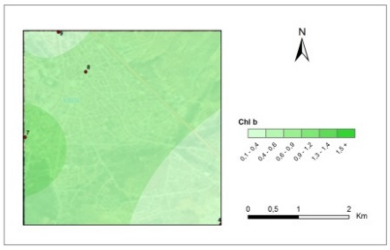
Chl-a, first period



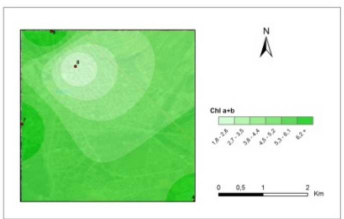
Chl-a, second period



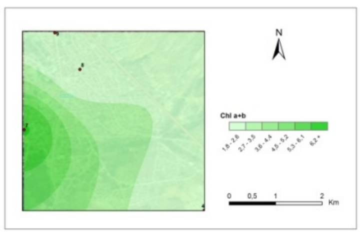
Chl-b, first period



Chl-b, second period

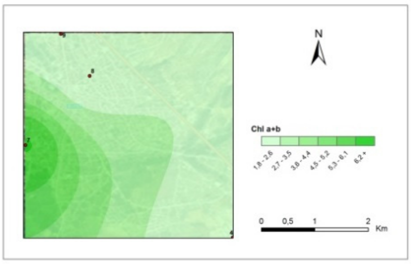


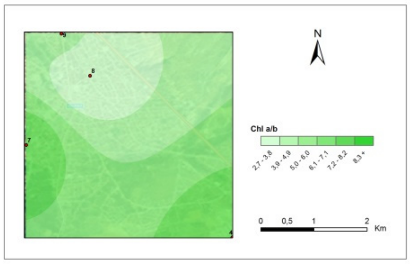
Chl-a+b, first period



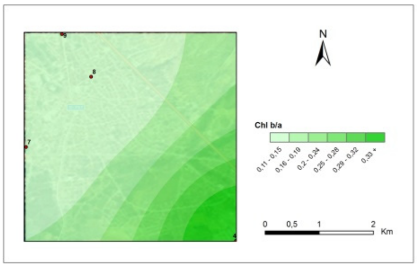
Chl-a+b, second period

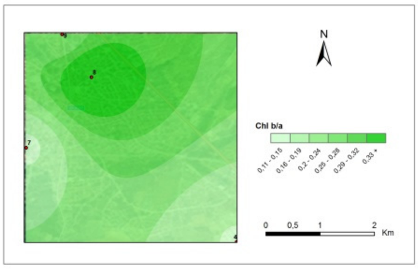
Fig. 6. Pollution maps of Kırşehir according to Chlorophyll a and b degradation.





Chl-a/b, first period Chl-a/b, second period





Chl-b/a, first period Chl-b/a, second period

Continued Fig. 6. Pollution maps of Kırşehir according to Chlorophyll a and b degradation.

CONCLUSIONS

This research was undertaken to investigate the use of lichen transplanting techniques (bag technique) to monitor heavy metal deposition in the atmosphere and to develop a pollution map for Kırşehir city, Türkiye. In this study, we found that *Pseudevernia furfuracea* accumulated Cu, Cd, Ni, Pb, Mn, Zn heavy metals in its tissues due to traffic and industrial activities. The decrease in the amount of chlorophyll and the decrease in photosynthesis due to pollution were as we predicted. The work carried out strengthens that *Pseudevernia furfuracea* is an excellent bioindicator lichen species for urban environment biomonitoring investigations.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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