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Accumulation of Airborne Trace Elements in *Pseudevernia furfuracea* (L.) Zopf Transplanted to Yozgat Province,Türkiye

Volkan IŞIK[1], Atila YILDIZ*[2]

[1] Ankara University, Institute of Science and Technology, TR-06110, Ankara, Türkiye

[2*] Ankara University, Faculty of Science, Department of Biology, TR-06100, Tandoğan, Ankara / Türkiye

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Heavy metals,
Pseudevernia furfuracea,
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Türkiye

Abstract: Automated stations for measuring air quality consistently measure levels of airborne pollutants, but their numbers are few, they need significant maintenance expenses, and they are unable to capture the complete geographical distribution of airborne contaminants. This research involves heavy metal assessments of *Pseudevernia furfuracea* (L.) Zopf lichen specimens collected from Yapraklı-Çankırı, transplanted to five sites in Yozgat (Türkiye) and exposed to pollution for two successive three-month intervals. The main objective of our study was to analyze the levels of Cu, Cd, Ni, Pb, Mn and Zn using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), additionally to calculate the concentrations of chlorophyll a and b, as well as the ratios of Chl (a+b), Chl (a/b), and Chl (b/a), lastly to create a pollution map of the city. The analytical findings for *P. furfuracea* indicate the following mean concentrations of heavy metals in 1st period Cu-0.30µg g^{-1}, Cd-0,026µg g^{-1}, Ni-0,61µg g^{-1}, Pb-0,54µg g^{-1}, Mn-2,17µg g^{-1}, Zn-0,20µg g^{-1}; in 2nd period Cu-0,43µg g^{-1}, Cd-0,027µg g^{-1}, Ni-0,64µg g^{-1}, Pb-0,66µg g^{-1}, Mn-2,21µg g^{-1}, Zn-0,49µg g^{-1}. While means of control stations are in 1st period Cu-0,26µg g^{-1}, Cd-0,028µg g^{-1}, Ni-0,23µg g^{-1}, Pb-0,52µg g^{-1}, Mn-1,90µg g^{-1}, Zn-0,16µg g^{-1}; in 2nd period Cu-0,36µg g^{-1}, Cd-0,027µg g^{-1}, Ni-0,29µg g^{-1}, Pb-0,56µg g^{-1}, Mn-1,96µg g^{-1}, Zn-0,58µg g^{-1} in 2nd period. The variables that raise heavy metal levels include: high traffic volume, industrial operations and urban heating activities. Although the study was brief, it demonstrated that *P. furfuracea* is an effective bioaccumulator and bioindicator organism for future biomonitoring studies.

Yozgat İline (Türkiye) Taşınan *Pseudevernia furfuracea* (L.) Zopf'da Havadaki İz Elementlerin Birikimi

Anahtar Kelimeler
Biyoizleme,
Ağır metal,
Pseudevernia furfuracea,
Yozgat,
Türkiye

Öz: Hava kalitesini ölçmek için kullanılan otomatik istasyonlar havadaki kirleticilerin seviyelerini tutarlı bir şekilde ölçmektedir, ancak sayıları azdır, önemli bakım masraflarına ihtiyaç duyarlar ve havadaki kirleticilerin coğrafi dağılımını tam olarak yakalayamazlar. Bu çalışma, Yapraklı-Çankırı'dan toplanan, Yozgat'taki (Türkiye) beş alana nakledilen ve birbirini izleyen üç aylık aralıklarla kirliliğe maruz bırakılan *Pseudevernia furfuracea* (L.) Zopf liken örneklerinin ağır metal değerlendirmelerini içermektedir. Çalışmamızın temel amacı, İndüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) kullanarak Cu, Cd, Ni, Pb, Mn ve Zn seviyelerini analiz etmek, klorofil a ve b konsantrasyonlarının yanı sıra Chl (a+b), Chl (a/b) ve Chl (b/a) oranlarını hesaplamak ve son olarak şehrin kirlilik haritasını oluşturmaktır. *P. furfuracea* için analitik bulgular, 1. periyotta Cu-0.30µg g^{-1}, Cd-0,026µg g^{-1}, Ni-0,61µg g^{-1}, Pb-0,54µg g^{-1}, Mn-2,17µg g^{-1}, Zn-0,20µg g^{-1}; 2. periyotta Cu-0,43µg g^{-1}, Cd-0,027µg g^{-1}, Ni-0,64µg g^{-1}, Pb-0,66µg g^{-1}, Mn-2,21µg g^{-1}, Zn-0,49µg g^{-1} ortalama ağır metal konsantrasyonlarını göstermiştir. Kontrol istasyonlarının ortalamaları ise 1. periyotta Cu-0,26µg g^{-1}, Cd-0,028µg g^{-1}, Ni-0,23µg g^{-1}, Pb-0,52µg g^{-1}, Mn-1,90µg g^{-1}, Zn-0,16µg g^{-1}; 2. periyotta Cu-0,36µg g^{-1}, Cd-0,027µg g^{-1}, Ni-0,29µg g^{-1}, Pb-0,56µg g^{-1}, Mn-1,96µg g^{-1}, Zn-0,58µg g^{-1} olmuştur. Ağır metal seviyelerini yükselten değişkenler arasında yüksek trafik hacmi, endüstriyel faaliyetler ve kentsel ısınma faaliyetleri yer almaktadır. Araştırma süresi kısa olmasına rağmen, *P. furfuracea* gelecekteki biyoizleme çalışmaları için etkili bir biyoakümülatör ve biyoindikatör organizma olduğunu göstermiştir.

* *Corresponding author: ayildiz@science.ankara.edu.tr*

1. Introduction

Lichens have been used for various purposes, including dye production, sustenance, and medicinal applications, from ancient Egyptian times up to the current era, throughout different regions of the globe [1]. Applying lichens for the detection and monitoring of airborne chemical compositions has been considered important for a lengthy period of time due to their prolonged life cycle, which enables the acquisition of long-term data from the ecosystem [2-3]. The usage of cryptogams, particularly lichens and mosses, for biomonitoring purposes has been increasingly prevalent in recent years. This strategy proves to be highly efficient in identifying both the origins and distribution patterns of many persistent airborne pollutants, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), dioxins, and furans [4-12]. In contrast to plants, lichens and mosses do not have a root system. Because of this, gas exchanges, nutrient absorption, and pollutant absorption take place throughout the entirety of the organism's surface [13-15]. However, employing cryptogams as bioindicators to assess Particulate Matter (PM) pollution may not always be practical for accurately identifying the origin of PM emissions, especially in regions with numerous human activities or diverse land use patterns [16]. The utilization of nitrogen, carbon, sulfur, and heavy metal patterns might be advantageous in this context, given that ICP-MS analysis is a highly efficient and responsive method that necessitates minimal quantities of material (for example, [17-22]). Nevertheless, the analyses may incur significant expenses, making them potentially unsuitable for large sample collections [12].

Lichens have been used to infer extensive geographical and prolonged temporal patterns of atmospheric metal deposition and non-selectively capture air particles containing trace elements inside their thalli. The elemental concentration in lichen thalli correlates with atmospheric metal levels (metal content in aerosols), which rise with increasing distance from the source [64]. Over the last 50 years, several research have conducted air quality biomonitoring using lichens. Some of these studies; detection of mercury content and elemental composition of *Protousnea magellanica* (Mont.) Krog transplants [65], airborne particulate matter captured by transplanted *Punctelia hypoleucites* (Nyl.) Krog thalli was determined to originate from mining activities [66], physiological alterations (damage to the anatomy of mycelium) in the transplanted lichen *Pyxine cocoes* (Sw.) Nyl. as a result of heavy metal accumulation [67], research revealed that the atmosphere in mountainous regions can be contaminated by certain potentially toxic elements (PTEs), primarily originating from vehicular traffic and local inhabitants, thereby validating the efficacy of the transplanted lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale as a reliable biomonitoring instrument for airborne PTEs in natural settings. [68], lichen transplants have proven effective as biomonitors of Persistent Organic Pollutants (POPs), enabling the establishment of an extensive geographical monitoring network [69], trace element accumulation efficacy of live (L) and dead (D) samples of the lichen *Pseudevernia furfuracea* (L.) Zopf was assessed by transplantation and D samples exhibited elevated levels of element bioaccumulation [70].

While lichens are a common sight in many natural terrestrial habitats, it's possible that human activity is to blame for their vanishing from cityscapes. When this occurs, passive biomonitoring stops working and active biomonitoring kicks in. One method involves exposing bags containing lichen to a polluted environment in order to ascertain the quantity of pollutants impacting the sample [5, 6, 23-29]. The features of the pre-exposure lichen material are crucial in the bag method operation. Due to the material being commonly sourced from unpolluted locations far from known sources of pollution, the majority of the components should have a minimum amount. If natural lichens are not present or are limited in their growth, lichen biomonitoring may be performed by transferring fresh non-polluted samples from a distant location into the specific region.

Pseudevernia furfuracea (L.) Zopf is has a shrub-like growth form. It prefers moderate to dry conditions and thrives in areas with ample sunlight. It is typically found in cool-temperate to mountainous regions. *P. furfuracea* mostly grows on bark that is acidic and not rich in nutrients. This lichen has been extensively used in numerous biomonitoring studies, primarily employing the transplant method. This method involves taking thalli of suitable species from distant locations and exposing them in specific locations to analyze the deposition of heavy metal elements and organic compounds. The lichen is chosen for its easy availability, strong resistance to harmful gaseous contaminants and climatic stressors, and its capacity to accumulate and store heavy metals [11, 30-31].

Prior to our research, no study on heavy metal contamination using lichens had been undertaken in Yozgat. This research aimed to analyze atmospheric accumulation of heavy metals in *P. furfuracea*'s thallus transplanted in the urban center of Yozgat and to ascertain the magnitude of fossil fuel use, vehicular emissions, and anthropogenic pollution sources. The city center is primarily affected by residential areas, traffic, and industrial operations, which are the main sources of increased atmospheric element concentrations. We conducted a detailed analysis to determine the amount of heavy metals present at each station and the factors contributing to their presence. Additionally, we created a pollution map that highlights the distribution of heavy metals. Furthermore, we analyzed the changes in the amount of chlorophyll in response to heavy metal accumulation.

2. Material and Method

2.1. Study area

Yozgat is a province (39.626°N, 35.141°E) situated in the Central Anatolia Region of Türkiye (Figure 1). The population of Yozgat province by the end of 2002 is 113.614 (current-2024, 420.699) [71]. The province has a surface area of 13.690 km^2. The economic foundation of Yozgat relies on the cultivation of crops and the rearing of livestock. The city possesses abundant subterranean resources, including highly valuable energy raw materials such as lignite, bituminous shale, and geothermal fluids. It also has metallic metals like iron, lead, zinc, and silver, as well as industrial raw materials like salt, cement and brick raw material, marble, building stone, and road material. Sarıkaya, Sorgun, Saraykent, and Yerköy Districts has abundant hot water resources [33]. Within the Yozgat Organised Industrial Zone, there are now 31 facilities operating in several industrial sub-sectors. These include facilities in the textile-garment plastic, construction, metal, health, agriculture, machinery, food and packaging, marble granite, and recycling sectors [34].

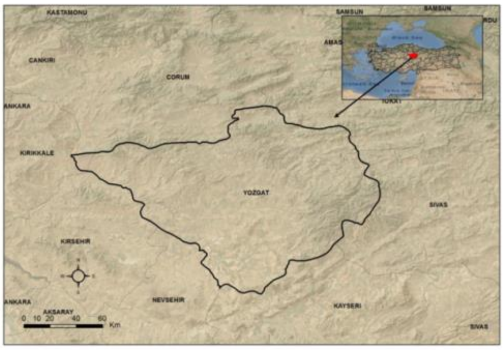


Figure 1. Yozgat province

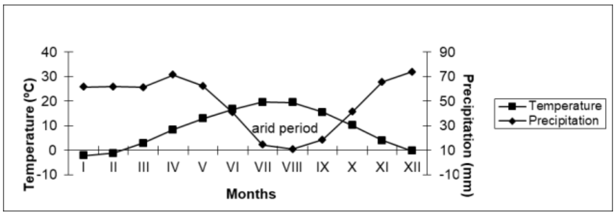


Figure 2. Climate diagram of Yozgat (2002)

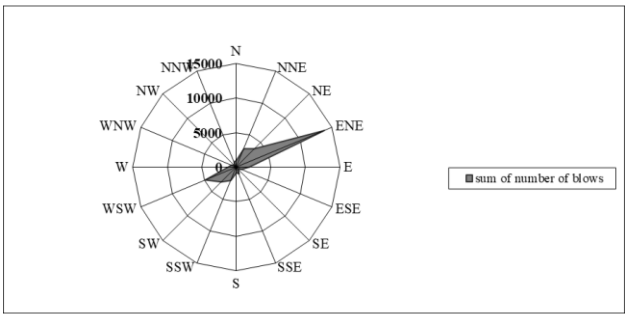


Figure 3. Wind rose diagram of Yozgat (2002)

The prevailing climate of Yozgat Province is a semi-arid continental climate (Figure 2). Due to its lack of proximity to the sea, the region has scorching and arid summers, as well as chilly and wet winters. The temperature disparities between summer and winter, as

well as between day and night, are substantial. January and February are the months with the lowest temperatures, while July and August are the months with the highest temperatures. Yozgat's geographical location results in a prevalent wind direction that is predominantly northeast, with some influence from the east (Figure 3) [35].

2.2. Bag technique

P. furfuracea has earlier been widely used in transplanting trials. Due to the inadequate colonization of *P. furfuracea* in the urban zone of Yozgat, it was necessary to apply the bag technique in the transplantation procedure to measure air pollution at chosen stations. The bags utilized in this method were constructed from a nylon mesh containing water-rinsed lichens. The risk of contamination through rhizin absorption was eliminated as the lichens contained in the bags were completely disconnected from the main tree. The "bag technique" does not have a specific definition regarding the quantity of lichen material, duration of exposure, connection to atmospheric depositions, and method of uptake (either passive through atmospheric particulate entrapment and cation exchange capacity of binding sites outside the cell, or active through biochemical activities of the cell's plasma membrane and cytoplasm).However, it offers the benefit of gathering information that is representative of the entire duration of exposure [11, 32]. We relocated thalli of the lichen *P. furfuracea* to 5 designated places in our research area. These thalli were collected along with their supporting substrate (tree twigs) from a distant location in the Yapraklı-Çankırı forest zone, which is approximately 1700 meters above sea level. To deter harm and minimize the influence of natural factors on the accumulation of elements by the lichens, two lichen bags were fastened to tree branches using plastic cables. The bags were positioned approximately 3 meters above the ground at each transplantation site.

2.3. Lichen sampling and exposure

The epiphytic lichen *P. furfuracea* was chosen as a widely used bioindicator for heavy metals and PAHs, as demonstrated by studies conducted by [10-11, 37-41]. This lichen is highly abundant, easily detectable, capable of tolerating stress, resistant to transplantation [35] and has a morphology that allows it to capture particulate materials through the growth of numerous finger-like vegetative propagules called "isidia" [12, 42]. Fresh lichen specimens were collected from branches of isolated *Pinus sylvestris* L. at a height of 1 to 5 meters above the ground in Yapraklı-Çankırı forest region, located at an elevation of 1750 m a.s.l (Figure 4 and 5). This location is far away from any local sources of pollution in Yozgat. The lichen specimen, together with a twig for support, was collected, put in paper bags, and taken to the laboratory. It was then left to dry naturally at room temperature.

Figure 4. Forested region located in the Yapraklı-Büyük Yayla district of Çankırı (Türkiye) [43].

We aimed to assess the lichen samples periodically throughout a one-year period; nevertheless, they were retained in the study area for a total of six months, divided into two three-month intervals, to mitigate the danger of destruction and damage in the city center. The exposure occurred on July 4th, 2002 (Figure 6) At each transplantation site, two lichen bags were securely attached to tree branches or poles using plastic cables. They were positioned approximately 3 meters above the ground. This was done to prevent damage and minimize any natural contribution to the accumulation of heavy metals by the lichens. The first collection of samples from exposure sites was obtained on October 5, 2002, while the subsequent collection was obtained on January 9, 2003 (Table 1). After being exposed, the samples were taken to the the lab, where the bags were opened and the samples were left to desiccate at ambient temperature.

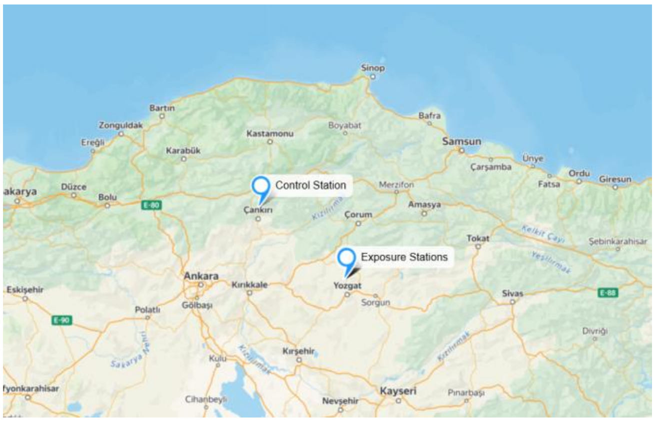


Figure 5. Control and exposure area

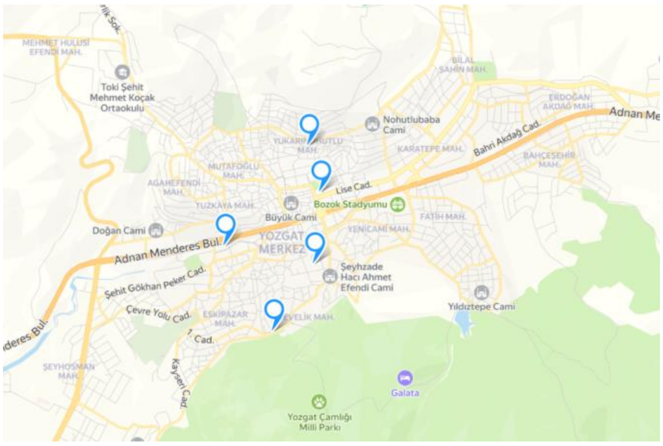


Figure 6. Exposure stations

Table 1. The sites of the exposure (Yozgat) and control (C) stations (Çankırı)

No	Location	Source of pollution	Substrate of lichen samples	Elevation of see level (m)	Latitude and longitude
C_1	Çankırı-Yapraklı, Büyük Yayla Plateau, Dikilitaş area	Clean control area	*Pinus sylvestris*	1750 m	N40º47'60'' E33º46'81''
C_2	Çankırı-Yapraklı, Büyük Yayla Plateau, Dikilitaş area	Clean control area	*Pinus sylvestris*	1750 m	N40º47'60'' E33º46'81'''
1	Meydan Park, Cumhuriyet Park, Sakarya Street	High traffic density, heating activities and residential area	*Eleagnus* sp.	1350 m	N39º49'41'' E34º48'50''
2	Yukarı Nohutlu Neighbourhood, Garden of a house on Kılıç Street	High traffic density, heating activities and residential area	*Robinia pseudoacacia*	1350 m	N39º49'59'' E34º48'52''
3	Yozgat-Sivas Street, Cumhuriyet Primary School Garden, Near Taxi Stop	High traffic density, heating activities and residential area	*Robinia pseudoacacia*	1290 m	N39º49'21'' E34º48'52''
4	Yozgat- Sivas Street, Opposite Bakgör Furniture Store, Behind Şahin Sitesi, Middle Refuge	High traffic density, heating activities and residential area	*Fraxinus* sp.	1270 m	N39º49'13'' E34º47'93''
5	Yozgat-Maternity Hospital Garden, Yozgat Çamlığı entrance	Background area	*Robinia pseudoacacia*	1330 m	N39º48'76'' E34º48'32''

2.4. Lichen specimen preparation and heavy metal determinations

After collection of transplanted lichen samples, they underwent two rounds of washing using regular and filtered water to eliminate any extraneous materials (such as soil particles, sand, dust, bark, etc.). Lichen samples were cleaned of coarse foreign material, and no more treatment was performed. No studies of sorption, desorption, or saturation capacity were conducted on the lichen samples. The samples were dehydrated for 24 hours at 80°C using yellow paper bags. The dried samples were pulverized using a mortar to ensure uniform distribution of heavy metals and protect them from microbial decay.

Lichen samples for elemental analysis were conveyed in falcon tubes. ICP-MS device was utilized to assess the amount of heavy metals (Cu, Cd, Ni, Pb, Mn, Zn) in all lichen samples, including the control station. The procedure for sample preparation prior to analysis by ICP-MS (Varian Liberty ICP-OES Sequential) is as follows [72].The glass, plastic, and ceramic materials were soaked in a solution of detergent and water for an entire night, followed by rinsing with regular water and then immersion in 20% nitric acid for another night. Subsequently, the glassware underwent a thorough cleaning process using double distilled water, followed by drying in a 60 ^{o}C oven for a duration of 12 hours. The standard treatments and solutions were prepared using a mixture of 65% w/w nitric acid and 35% w/w hydrochloric acid (Merck reagent). Nitric acid (HNO_3) is frequently employed for the dissolution of lichen material. A total of 1 gram of dehydrated lichen samples were incinerated in a porcelain crucible for a duration of 24 hours at a temperature of 460 degrees celsius. The charred ash samples were placed in a 100 mL beaker containing a solution of 65% 100 molar concentration nitric acid (HNO_3). The beakers were heated in a sand bath to facilitate the evaporation and precipitation of HNO_3. After the process of evaporation, the remaining fraction was transferred to a centrifuge beaker and the volume was increased to 15 ml using a 1% HNO_3 solution. After centrifugation at 3000 rpm for 20 minutes, the extract was transferred to a 25 ml beaker and then diluted to 25 ml using a 1% HNO_3 solution. It is worth noting that 3000 rpm corresponds to a relative centrifugal acceleration of 1157 g.

2.4. Chlorophyll measurement

In the extraction procedure, 5 mL of Dimethyl sulphoxide (DMSO) was applied to the lichen thallus to get chlorophyll from 20 milligrams of dried lichen material. The tubes containing the lichen extract were thereafter incubated in darkness at a temperature of 65^{o}C for a duration of 40 minutes, followed by cooling to room temperature. The lichen extracts underwent filtering using Whatman no 3 filtration paper. The UV-Spectrophotometer was adjusted to a wavelength of 750 nm. The samples were analyzed for absorbance at wavelengths of 665 and 648 nm. The calculations were conducted using DMSO with a purity of at least 99% (for synthesis), obtained from Merck (catalog number 8.02912). DMSO was used as a pure solvent at a concentration of 100%. The chlorophyll content was determined using equations (1), (2), and (3) from the study conducted by [44].

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

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

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

2.5. Pollution maps

Pollution maps were drawed using ArcGIS software and upon enlargement, the city center becomes visible.

3. Results and Discussion

3.1. Heavy metal contents of the lichen transplants

Examination of samples taken from the Yapraklı-Çankırı forest areas and transferred to a location near the city center of Yozgat showed an important accumulation of heavy metals. Figure 7 illustrates pollution maps according to heavy metal concentrations during 1st and 2nd periods. Table 2a and show the mean amounts of heavy metal concentrations throughout the first and next exposure periods. These averages indicate a positive correlation between each element and the control station at both time points (Table 2b, 2c).

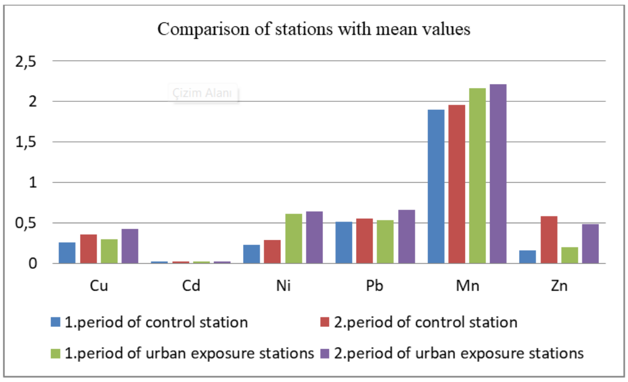
Table 2a. The average values of heavy metal levels in the 1st and 2nd exposure periods, measured in micrograms per gram (μg g^{-1}).

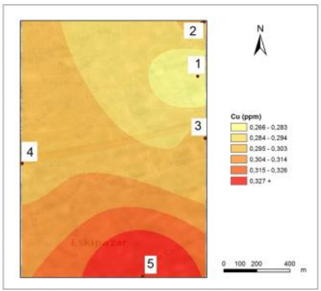
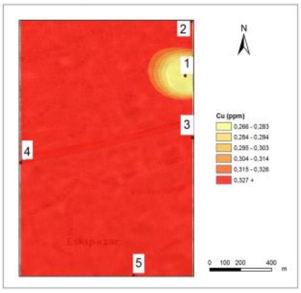
	Periods	Cu	Cd	Ni	Pb	Mn	Zn
Control stations	1	0,26	0,028	0,23	0,52	1,90	0,16
	2	0,36	0,027	0,29	0,56	1,96	0,58
Exposure stations	1	0.30	0,026	0,61	0,54	2,17	0,20
	2	0,43	0,027	0,64	0,66	2,21	0,49

Table 2b. Heavy metal contents of control and exposure stations (in μg.g^{-1})

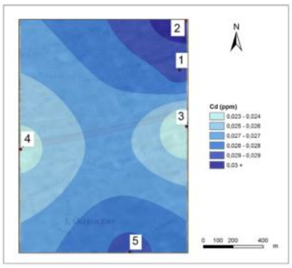
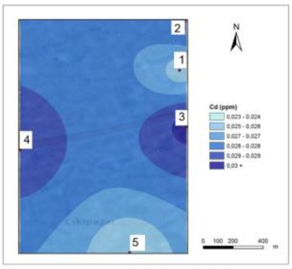
Stations	Periods	Cu	Cd	Ni	Pb	Mn	Zn
C1*	1	0.28423	0.02621	0.27508	0.51637	1.89763	0.15076
	2	0.38909	0.02757	0.28306	0.55338	1.94752	0.57671
C2*	1	0.25191	0.03153	0.20229	0.52883	1.91850	0.18884
	2	0.34413	0.02832	0.31485	0.56882	1.98790	0.58973
1	1	0,26583	0,02809	0,46892	0,53868	1,96055	0,17215
	2	0,24319	0,02523	0,26632	0,44251	0,86942	0,59502
2	1	0,29972	0,03001	0,71339	0,48454	2,25085	0,13378
	2	0,38672	0,02795	0,96429	0,57726	2,26039	0,30292
3	1	0,29616	0,02338	0,44944	0,52363	1,74723	0,12154
	2	0,51360	0,02920	0,59881	0,73851	2,84038	0,70275
4	1	0,30179	0,02428	0,49522	0,57701	2,44851	0,16671
	2	0,42328	0,02829	0,69590	0,70800	2,30566	0,40124
5	1	0,33984	0,02797	0,95382	0,60406	2,45053	0,44047
	2	0,61915	0,02496	0,69977	0,84210	2,79898	0,45785

Table 2c. Comparing average levels of heavy metal concentrations in 1st and 2nd exposure periods (μg g^{-1})

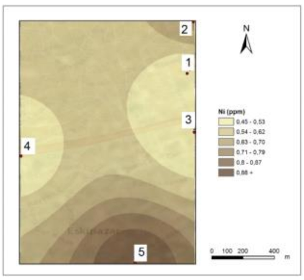
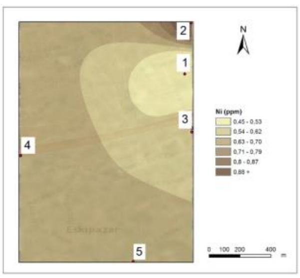


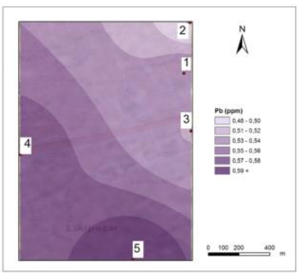
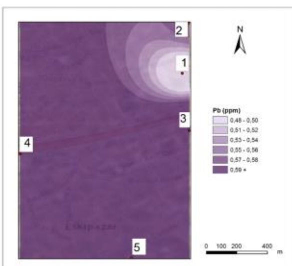
Cu, 1st period | Cu, 2nd period

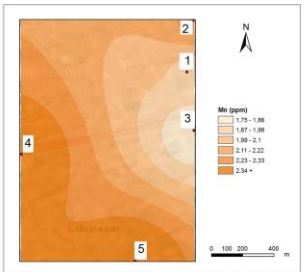
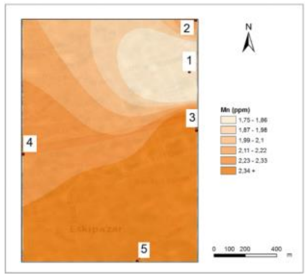
Cd, 1st period | Cd, 2nd period

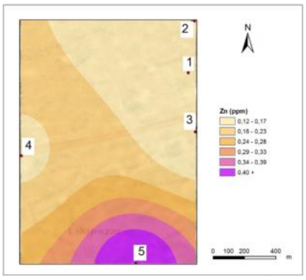
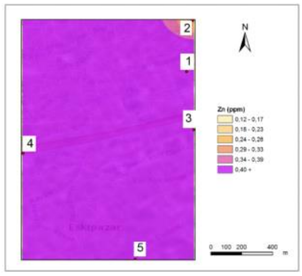
Ni, 1st period | Ni, 2nd period

Pb, 1st period | Pb, 2nd period

Mn, 1st period | Mn, 2nd period

Zn, 1st period | Zn, 2nd period

Figure 7. Maps showing the differences in heavy metal concentrations (ppm) throughout the 1st and 2nd periods (P)

3.2 Chlorophyll contents

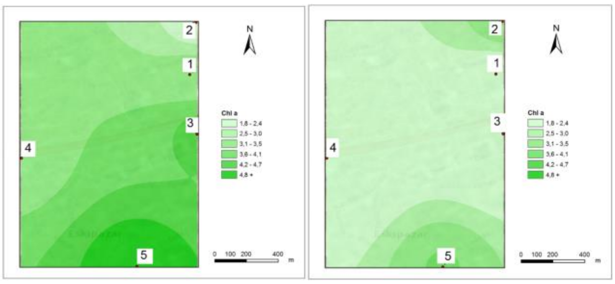
The average chlorophyll content of the lichen samples transplanted to the city center of Yozgat exhibited a notable decline throughout the first and second periods, in contrast to the samples collected from the woodland region of Yapraklı-Çankırı. Within this particular framework, there exists a statistically significant association between pollution and a reduction in chlorophyll concentration (Table 2d). Chlorophyll concentrations of chlorophyll -a, chlorophyll -b, chlorophyll -a+b, as well as the chlorophyll (a/b) and chlorophyll (b/a) ratios, during the first and second periods are provided in Table 2e. Minimum values of chlorophyll -a, particularly at the 1st, 3rd, and 4th stations during the 2nd period, and at the 2nd station during the 1st period, are observed. Chlorophyll-b levels at all exposure stations during the first period exhibit values that are almost identical. Figure 8 displays the chlorophyll maps of lichens that were subjected to pollution at the stations.

Table 2d. Average concentrations of Chl-a, Chl-b, and Chl (a+b) (µg/ml)

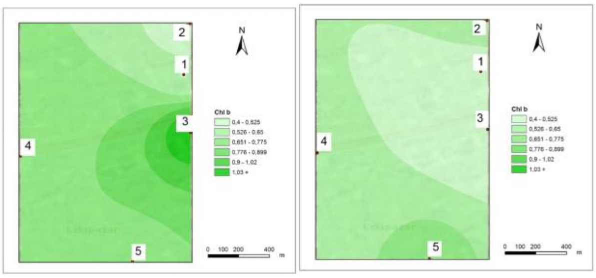
	Periods	Chl-a	Chl-b	Chl (a+b)	Chl (a/b)	Chl (b/a)
Control stations	1	6,381	1,527	7,908	5,357	0,256
	2	7,072	2,024	9,097	5,234	0,265
Exposure stations	1	3,564	0,710	4,275	5,195	0,205
	2	2,168	0,539	2,708	3,846	0,350

Table 2e- Chl -a, Chl -b and Chl (a+b) contents (µg/ml) and Chl (a/b), Chl (b/a) ratio

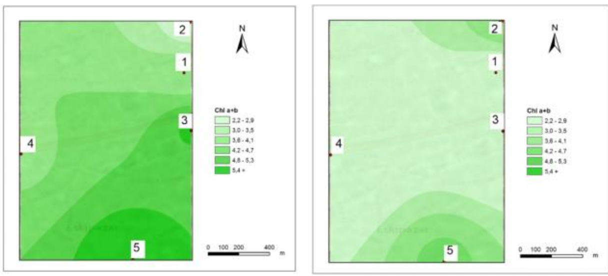
Stations	Periods	Chl-a	Chl-b	Chl (a+b)	Chl (a/b)	Chl (b/a)
C1*	1	7.782	1.945	9.727	5.000	0.312
	2	9.252	3.013	12.26	4.516	0.333
C2*	1	4.979	1.109	6.088	5.714	0.201
	2	4.893	1.036	5.929	5.952	0.198
1	1	3,249	0,523	3,772	6,212	0,161
	2	1,875	0,447	2,322	4,195	0,238
2	1	1,831	0,4	2,231	4,578	0,218
	2	3,549	0,626	4,175	5,669	0,176
3	1	4,299	1,149	5,448	3,742	0,272
	2	1,14	0,375	1,515	3,04	0,329
4	1	3,203	0,752	3,955	4,259	0,235
	2	0,666	0,541	1,207	1,231	0,812
5	1	5,24	0,729	5,969	7,188	0,139
	2	3,613	0,709	4,322	5,096	0,196



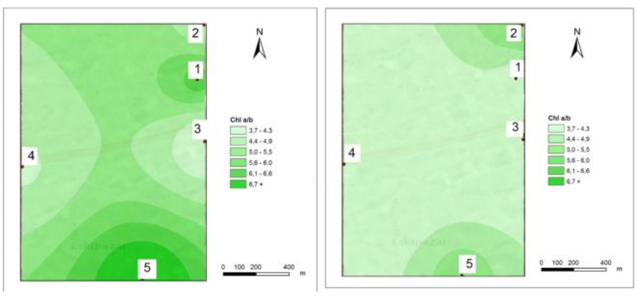
Chl-a, 1st period Chl-a, 2nd period



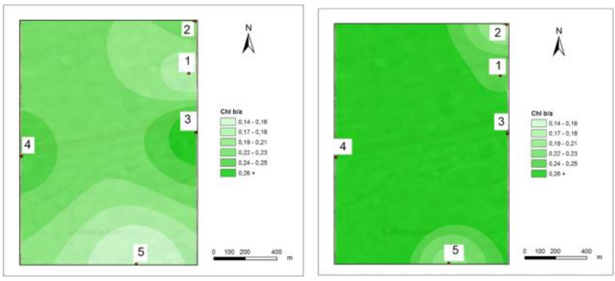
Chl-b, 1st period Chl-b, 2nd period



Chl (a+b), 1st period Chl (a+b), 2nd period



Chl (a/b), 1st period Chl (a/b), 2nd period



Chl (b/a), 1st period Chl (b/a), 2nd period

Figure 8. Maps of Yozgat according to chlorophyll-a, chlorophyll-b and chlorophyll (a+b) contents and chlorophyll (a/b) and chlorophyll (b/a) ratio

The highest Copper (Cu) concentration was 0.619 µg g^{-1} at the 5th station during the 2nd period, whereas the lowest value was 0.243 µg g^{-1} at the 1st station over the same time. Upon analysis of Cu contents, it is evident that there are notably elevated levels in the 2nd periods of 3rd, 4th, and 5th stations. The proximity of the 3rd, 4th, and 5th stations to heavy motor vehicle traffic and urban winter heating operations contributes to the elevated levels of Cu values. [39, 45-48] all showed similar findings for Cu.

According to [49], plants from unpolluted areas show a concentration of 0.01-0.3 µg g^{-1} of Cadmium (Cd). The Cd values at exposure stations range from 0.023 to 0.03 µg g^{-1}. The maximum concentration of Cd was observed at 2nd station during 1st period, with a value of 0.03 µg g^{-1}. The Cd results at other locations exhibited no substantial deviation in comparison to the control stations.

Highest Nickel (Ni) value was observed during the 2nd period at 2nd, 3rd,4th and 5th stations, as well as during 2nd period at 5th station. Conversely, the lowest Ni value was recorded during 2nd period at 1st station. Two factors that lead to increased levels of nickel (Ni) include seasonal winter heating activities using coal and automobile traffic [50]. The authorized limit value for nickel in plants, as determined by the Food and Agriculture Organization/World Health Organization (FAO/WHO), is 5 µg g^{-1} [51]. The mean Ni concentration observed in this research was 0.62 µg g^{-1}. The mean Ni concentration discovered in the present study is lower than that reported in the investigations conducted by [39, 52-53]. This quantity falls below the acceptable Ni concentration for plants.

The Lead (Pb) results in 1st and 2nd period of 5th station, as well as 2nd period of 3rd and 4th stations, exhibited significantly higher levels compared to the control stations. Conversely, the remaining stations had roughly similar values. According to the data shown in Table 3a, the average lead (Pb) concentration at control stations during the 1st and 2nd periods ranged from 0.52 µg g^{-1} to 0.56 µg g^{-1}, whereas the average concentration at the exposure stations ranged from 0.54 µg g^{-1} to 0.66 µg g^{-1}.

[54] achieved similar outcomes using *Flavoparmelia caperata* (L.) Hale, whereas [47, 55-56] obtained same findings using *P. furfuracea*. The atmospheric impact of gasoline on Pb levels is decreasing, although coal and gasoline burning remain the main producers of harmful substances in the environment [57]. The contamination of this substance is linked to the discharge of automobiles and the burning of gasoline [50]. [58] examined a small number of samples and discovered that cars released significant levels of lead (Pb) and copper (Cu) into the environment. The observations of Pb accumulation unambiguously indicate the presence of Pb contamination at stations 3rd, 4th, and 5th, which may be attributed to emissions from motor vehicles.

According to Table 3b, the Manganese (Mn) values were elevated during 1st and 2nd periods of the 2nd, 4th, and 5th stations, as well as during 2nd period of 3rd station. The pollutants generated by cars resulted in significant bioaccumulation at these sites. Compared to the control station, lower values were recorded at 1st station during 2nd period and at 3rd station during 1st period. The average exposure to Mn in the stations during the 1st and 2nd periods is 2.19 µg g^{-1}, while the average in the control stations is 1.93 µg g^{-1}. The mean Mn content detected is lower than the Mn content reported in the studies conducted by [39, 52-53].

Zinc (Zn) concentration in lichen samples was found to be correlated with road traffic. Based on research, the primary sources of Zn pollution include fuels, fossil fuels, fertilizers, and metal alloys. Elevated zinc concentrations are caused by high traffic volume and tire wear in industry, urban highways, urban locations, parks, and shanty communities [47,59]. The largest concentration of Zn was seen during the second time of exposure at 3rd and 1st stations, which are associated with traffic. Conversely, the lowest concentration was detected during 1st period of exposure at the 2nd and 3rd stations. [55, 60] had comparable findings.

Quantifying photosynthetic pigment levels is a straightforward method commonly employed to evaluate the impact of metal stress on plants and lichens. Heavy metals are believed to impact enzymes responsible for regulating chlorophyll biosynthesis, hence inhibiting the production of chlorophyll pigments. [61] posits that Zn replaces Fe, a crucial element for the formation of chlorophyll, thereby reducing photosynthetic pigments. According to [62] the presence of Cu has resulted in a decrease in chlorophyll levels. Heavy metal buildup in plant cells leads to the degradation of chlorophyll.

The elevated levels of heavy metals resulting from heating and vehicle traffic, particularly at the 2nd, 3rd, 4th, and 5th stations, are associated with a decrease in chlorophyll content in the lichen thallus, as seen in Table 3e. The chlorophyll a+b degradation map confirmed that the photosynthetic pigments had completely degenerated, providing evidence for this outcome. The chlorophyll a/b maps indicated that chlorophyll a was more adversely affected by air pollution compared to chlorophyll b. Additionally, the presence of pollution resulted in a reduction in photosynthetic pigments, as anticipated. The accuracy of the findings was additionally confirmed by the use of chlorophyll b/a maps. The variations in the amounts and ratios of chlorophyll a and b might potentially be linked to environmental stress, such as pollution. The level of chlorophyll in lichen thalli is often correlated with environmental stress, exhibiting higher chlorophyll content under stressful conditions compared to non-stressful ones [63]. Furthermore, [63] observed that the geographical placement of the station did not have any impact on the quantity of chlorophyll present in the lichen thalli. Further inquiry is necessary to establish the correlation between these alterations and factors such as contaminants, climatic conditions, seasons, light intensity, and the lichen organism itself.

4. Conclusions

Chemically, heavy metal levels in the lichen thallus are expected to increase after being exposed to pollution at 3-month intervals for 6 months. The study's results indicate that the accumulated amount of heavy metals in lichen thallus is notably influenced by the station's location and the length of exposure. The research found higher amounts of heavy metals in *P. furfuracea* in Yozgat than in the control station. This indicates that *P. furfuracea* is a species that effectively collects heavy metals in lichen-bags when exposed to urban settings.

Prior to commencing our biomonitoring study, we emphasize the significance of investigating how the quantity of elements in the thallus of lichen samples with low elemental content, obtained from unpolluted natural vegetation, may respond to heavy metals following transplanting to contaminated regions. By utilizing this, we will enhance the caliber of the bioaccumulation data, enabling us to produce more accurate analyses of our discoveries in subsequent investigations.

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Declaration of Ethical Code

In the present research, we ensure compliance with all the regulations outlined in the "Higher Education Institutions

Scientific Research and Publication Ethics Directive" and confirm that none of the actions listed under the section "Actions Against Scientific Research and Publication Ethics" are performed.

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