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Assessment of atmospheric pollution by heavy metals using transplanted lichen *Pseudevernia furfuracea* (L.) Zopf in Niğde Province, Türkiye

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Biomonitoring,
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Niğde,
Türkiye

Abstract: Air pollution has been proven to affect essential metabolic processes in algae and fungus, making lichens susceptible for over 140 years. Air contaminants such heavy metals, polycyclic aromatic hydrocarbons (PAHs), and sulfur dioxide (SO_2) are collected in lichen thallus to measure air pollution levels. Samples of *Pseudevernia furfuracea* (L.) Zopf were collected from a untouched forest zone (Yapraklı-Çankırı) and transplanted for two three-month periods in 6 stations near the polluted Niğde provincial center in Türkiye. This research aimed (i) to analyze the amounts of Cu, Cd, Ni, Pb, Mn, and Zn with help of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (ii) to compute the levels of chl-a and chl-b (iii) to create a pollution map of the city. According to analysis results for *P. furfuracea* means of heavy metals; in first period 0.36µg g^{-1},0,032µg g^{-1},0,44µg g^{-1}, 0,70µg g^{-1},1,94µg g^{-1},0,2 µg g^{-1}; in second period 0,58µgg^{-1},0,033µg g^{-1},0,36µg g^{-1} ,0,92µg g^{-1} ,1,98µg g^{-1} ,0,65µg g^{-1} for Cu, Cd, Ni, Pb, Mn, Zn. Whereas means of control stations are 0,26 µg g^{-1}, 0,028 µg g^{-1},0,23µg g^{-1},0,52µg g^{-1},1,90µgg^{-1}, 0,16µgg^{-1} in 1st period; 0,36 µg g^{-1},0,027µg g^{-1},0,29 µg g^{-1},0,56µg g^{-1},1,96µg g^{-1}, 0,58 µg g^{-1} in 2nd period for for Cu, Cd, Ni, Pb, Mn, Zn. The factors that contribute to high heavy metal levels are as follows: (i) traffic density, (ii) industrial activities, (iii) urban heating activities. *P. furfuracea* was an effective bioaccumulator organism for biomonitoring studies despite the survey's short length.

Transplante Liken *Pseudevernia furfuracea* (L.) Zopf Kullanılarak Niğde İlinde (Türkiye) Atmosferik Ağır Metal Kirliliğinin Değerlendirilmesi

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

Özet: Hava kirliliğinin alg ve mantarlardaki temel metabolik süreçleri etkilediği ve likenleri 140 yılı aşkın süredir duyarlı hale getirdiği kanıtlanmıştır. Ağır metaller, polisiklik aromatik hidrokarbonlar (PAH'lar) ve sülfür dioksit (SO_2) gibi hava kirleticileri, hava kirliliği seviyelerini ölçmek için liken tallusларında toplanır. *Pseudevernia furfuracea* (L.) Zopf örnekleri ormanlık bölgeden (Yapraklı-Çankırı) toplanmış ve üç aylık iki dönemde Niğde (Türkiye) il merkezine yakın 6 istasyona nakledilmiştir. Bu araştırmanın amacı (i) İndüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) kullanarak Cu, Cd, Ni, Pb, Mn ve Zn konsantrasyonlarını analiz etmek (ii) klorofil a ve b seviyelerini hesaplamak (iii) şehrin kirlilik haritasını oluşturmaktır. *P. furfuracea* 'nın analiz sonuçlarına göre ağır metal ortalamaları Cu, Cd, Ni, Pb, Mn, Zn için; ilk periyotta 0.36µg g^{-1},0,032µg g^{-1},0,44µg g^{-1},0,70µg g^{-1},1,94µg g^{-1},0,2 µg g^{-1} ikinci periyotta 0,58µg g^{-1},0,033µg g^{-1},0,36µg g^{-1},0,92µg g^{-1},1,98µg g^{-1},0,65µg g^{-1} olmuştur. Kontrol istasyonlarının Cu, Cd, Ni, Pb, Mn, Zn ortalamaları ise 1. periyotta 0,26 µgg^{-1},0,028 µg g^{-1}, 0,23µg g^{-1},0,52µg g^{-1},1,90µg g^{-1},0,16µg g^{-1}'dir; 2. periyotta 0,36 µg g^{-1},0,027µg g^{-1},0,29 µg g^{-1}, 0,56µg g^{-1},1,96µg g^{-1},0,58 µg g^{-1} bulunmuştur. Yüksek ağır metal seviyelerine sebep olan faktörler şöyledir: (i) trafik yoğunluğu, (ii) endüstriyel faaliyetler, (iii) kentsel ısınma faaliyetleri. *P. furfuracea*, araştırmanın kısa sürmesine rağmen ileri biyoizleme çalışmaları için etkili bir biyoakümülatör organizma olmuştur.

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1. Introduction

The utilization of lichens for the purpose of detecting and monitoring the chemical compositions of the atmosphere has been considered very important for a considerable period of time. This is due to the fact that their prolonged life cycle enables the acquisition of long-term information from the ecosystem [1]. The application of cryptogams, particularly lichens and mosses, for biomonitoring has been a popular method in recent years. It has proven to be efficient in identifying the origins and distribution patterns of many persistent airborne pollutants, such as heavy metals, PAHs, dioxins, and furans [2,3,4,5,6,7,8,9]. Contrary to plants, lichens and mosses do not possess a root system. Instead, they depend on gas interchanges, sorption of nutrients, and absorption of contaminants to take place all over their outer layer. [4,10,11]. However, the practicality of using cryptogams as bioindicators of PM contamination is often limited in cases when there are numerous human activities in a small area or when land use is diverse [12]. The utilization of nitrogen, carbon, sulfur, and heavy metal isotope patterns might be advantageous in this regard. Mass spectrometry analysis, an exceptionally efficient and accurate technique, requires just minute amounts of sample [13,14,15,16,17,18]. Nevertheless, the analyses may incur significant expenses, making them potentially unsuitable for large sample collections [9].

Lichens often inhabit many terrestrial environments, however their occurrence in metropolitan settings is frequently influenced by human activities. Passive biomonitoring is rendered useless in such cases and is substituted by active biomonitoring. A technique entails the placement of lichen-filled bags inside a contaminated environment to quantify the extent of contaminants affecting the specimen. [19,20,21,22,23,24]. The features of the pre-exposure lichen material are crucial in the bag method operation. Due to the material's frequent acquisition in untouched locations far from known sources of pollution, most of its components are anticipated to have a minimal amount of contamination. If there is a lack of naturally occurring lichens or their growth is restricted, It is still possible to conduct lichen biomonitoring by bringing live specimens from a different area into the target area. Due to insufficient lichen growth in the research area, it was necessary to use a transplanting method for conducting air monitoring. The use of lichen transplants has numerous advantages, including the ability to utilize various experimental methodologies and the capability to measure levels of elements at the beginning of the monitoring study and during the exposure period [25].

P. furfuracea is a kind of macrolichen that grows on acidic bark in a wide area. It is fruticose, meaning it has a shrub-like growth form, and it prefers moderate moisture levels and bright light. This lichen has been extensively used in numerous biomonitoring studies, primarily employing the transplant method [39, 58,59,60,61]. This method involves collecting thalli of suitable species from distant locations and exposing them in specific areas to analyze the deposition of trace elements and organic compounds. The lichen is chosen for its easy accessibility, high resistance to harmful gaseous contaminants and climatic stressors, and its capacity to accumulate and store heavy metals [8,26,27].

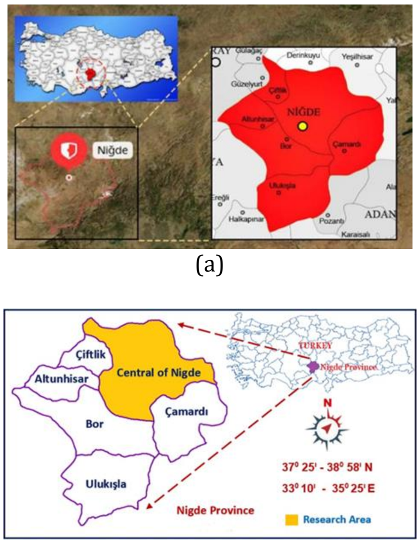
P. furfuracea has been widely used in past transplanting trials. Due to the inadequate colonization of *P. furfuracea* in the Niğde urban zone, the bag technique was employed during the transplantation operation to assess urban air pollution at selected sites. The bags utilized in this method were constructed from a nylon mesh containing water-rinsed lichens. The risk of contamination through root absorption was eliminated as the lichens contained in the bags were not connected to the soil. The "bag technique" lacks a precise definition regarding the quantity of lichen material, length of exposure, relationship to airborne depositions, and way of the absorption (either passively through trapping atmospheric particles and the cation exchange capacity of external binding points, or actively through biochemical processes in the plasma membrane and cytoplasm). However, it offers the benefit of accumulating data that is collected over the whole period of exposure [8,28]. Any lichen is uniquely adapted to the substrate it grows on, hence *P. furfuracea* has established a suitable environment on *P. sylvestris*, which will impact the accumulation of heavy metals. Furthermore, *P. furfuracea* has the ability to collect heavy metals in various substrates, as seen in Table 1 and 3. We transferred samples of *P. furfuracea* thalli, together with the tree branches (*Pinus sylvestris* L.) they were growing on, from a distant location (Yapraklı-Büyük plateau region) situated at an elevation approximately 1700 m above sea level, to 6 designated places within our study area. Yapraklı is a district of Çankırı and is a clean forested area away from the city and anthropogenic pollution factors. Two lichen bags were affixed to tree twigs or poles at each transplanting site using plastic wires. The lichens were placed at a height of around 3 meters above the soil in order to deter destruction and reduce any natural influence on their capacity to absorb elements.

The primary goal of this research was to determine Zn, Cd, Pb, Ni, Mn, Cu accumulation in thallus of *P. furfuracea* that was transplanted in the central area of Niğde. Due to variations in pollution parameters, geographical position, and climatic conditions in all regions, the city of Niğde in the Central Anatolia area of Türkiye was selected for biomonitoring atmospheric heavy metal accumulation. This location is characterized by high levels of atmospheric element enrichment due to residential areas, traffic, and industrial activities. We conducted a detailed analysis of the quantity of heavy metals present at each station and investigated the root cause. Additionally, we generated a pollution map using data on heavy metal levels, and identified the effect of heavy metal accumulation on changes in chlorophyll content.

2. Material and Method

2.1. Study area

The research area is situated in the southeastern part of the Central Anatolia Region of Türkiye and inside the Cappadocia Region. Its coordinates are between 37º 25' and 38º 58' N, and 33º 10' and 35º 25' E, as shown in Figure 1. At a height of 1,229 meters above sea level, the area is situated. The land area of this place measures around 2,223 square kilometers, and it is inhabited by a population exceeding 99.308 individuals and has number of registered vehicles in city 41,698 as of 2002 [56,57]. An excel program was used to generate a temperature-rainfall table using the temperature-rainfall data obtained from the State Statistics Institute up to 2002. Niğde experiences the characteristic continental climate of Central Anatolia, characterized by a predominance of closed weather conditions. The summers are characterized by high temperatures and lack of precipitation, while the winters are marked by low temperatures and abundant snowfall. Winter is characterized by snowfall, whereas rainfall is typically noted in spring.



(a)

(b)

Figure 1. Niğde Province (a) from [29] (b) from [30]

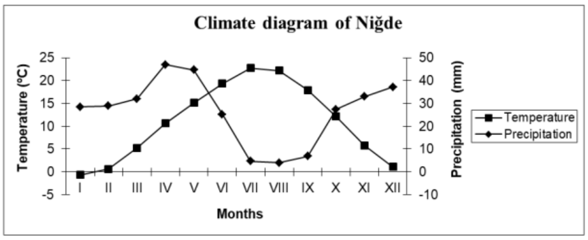


Figure 2. Climate diagram of Niğde Province **(2002)**

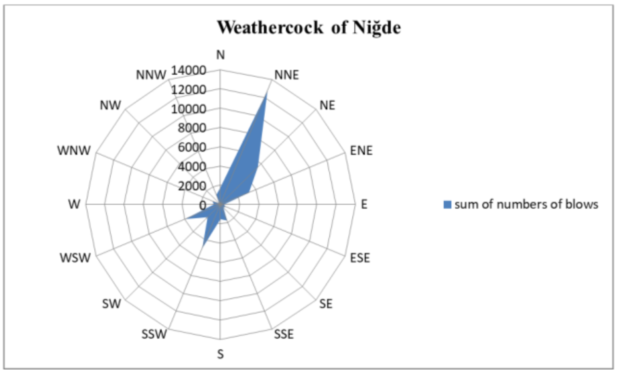


Figure 3. Weathercock of Niğde Province **(2002)**

The most industrial establishments of Niğde province; cement factory, sugar factory, flour factories, cheese-butter factory, concrete pole factory, tile-brick factories, automobile spare parts. The primary sources of possible contamination in Niğde are: (i) industrial activities, (ii) motor vehicles, (iii) heating activities, (iv) meteorological factors. According to the 2020 air quality assessment report published by the Ministry of Environment, Urbanisation and Climate Change, air pollution in Niğde shows seasonal and topographical characteristics. There is sensed and detected pollution caused by SO_2 in winter, late autumn and early spring. The aforementioned situation illustrates the origin of air pollution in Niğde (in terms of SO_2 pollution) is not a pollution caused by industry and motor vehicles, but an air pollution caused by heating and meteorological factors. Pollutants from motor vehicles also have an effect on air pollution. Traffic density, especially in the morning and evening hours, adversely affects the air. The industrial branches operating in Niğde Province vary. Air pollution caused by industry is mainly caused by wrong site selection, inappropriate fuel use and discharge of waste gases to the receiving environment without adequate technical measures. While the calcite quarries in the vicinity of the city do not directly affect residential areas, the particulate matter produced from the operations of these facilities adversely affects the air quality of the city [31].

2.2. Collection and assessment of lichen samples

P. furfuracea, which grows on other plants without harming them, was chosen as a widely applied indicator of trace elements and PAHs based on previous studies [6,7,8,26,32]. This lichen is very prevalent, easily identifiable, capable of withstanding stress, resistant to transplantation [7], and has a tendency to capture particle matter due to the formation of many finger-like vegetative propagules called "isidia" [9,33].

Table 1. Geographical positions of stations

Station no	Location	Substrate of the specimens	Elevation of see level (m)	Latitude and longitude
C1	Çankırı-Yapraklı, Yapraklı Büyük Plateau, Dikilitas area (control group)	*Pinus sylvestris*	1750m	N 40º 47' 600'' E 33º 46' 818''
C2	Çankırı-Yapraklı, Yapraklı Büyük Plateau, Dikilitas area (control group)	*Pinus sylvestris*	1750m	N 40º 47' 600'' E 33º 46' 818''
1	Niğde- Inönü Mah., Villalar, 38.Sok., No:14 garden of the house	*Populus alba*	1320m	N 37º 59' 748'' E 34º 40' 599''
2	Niğde- Süleyman Fethi Cad, Rectorate garden	*Fraxinus* sp.	1320 m	N 37º 58' 155'' E 34º 39' 906''
3	Niğde- Selçuk Mah., Old Slaughterhouse Passage, opposite the Watchman's hut, near Niğgaz	*Salix* sp.	1200 m	N 37º 57' 575'' E 34º 40' 695''
4	Niğde- Bor Cad., opposite Kardelen	*Fraxinus* sp.	1200 m	N 37º 58' 018'' E 34º 40' 427''
5	Niğde-Terminal Cad., under Orphanage, Cumhuriyet Bulvarı, Faik Şahenk Boulevard	*Fraxinus* sp.	1220 m	N 37º 58' 271'' E 34º 41' 021''
6	Niğde- Kayseri-Adana Road junction, Hub, Kayseri side, Regional traffic, Nigde exit, Intersection	*Robinia pseudoacacia*	1270 m	N 37º 57' 988'' E 34º 42' 250''

The pre-transplant samples were obtained from branches of individual *Pinus sylvestris* L. trees in the Yapraklı-Çankırı forest region at a height of 1750 meters a.s.l. These trees were located far away from any local sources of pollution in Niğde. The samples were taken at heights ranging from 1 to 5 meters above the ground (Table 1). Paper bags were used to carefully collect the specimen of lichen, which was accompanied by a twig that served as a support. It was then brought to the laboratory, where it was allowed to dry naturally at room temperature for a period of time. The exposure occurred on July 4th, 2002, within one hour of conducting fieldwork. In order to prevent damage and reduce the impact of natural variables on the lichens' ability to capture elements, two lichen bags were firmly fastened to tree branches or poles with the help of plastic cables. The bags were positioned at an elevation of around 3 meters above floor at each transplantation site. The initial **sampling from lichen thallus** was gathered on October 5, 2002, while the subsequent sampling was obtained on January 9, 2003. Following exposure, the samples were transferred to the laboratory, where the bags were unzipped and left to dehydrate at ambient temperature. Furthermore, although we initially had a total of 7 stations, the sample inexplicably vanished from one of the sites.

2.3. Lichen specimen preparation and heavy metal determinations

Following the collection of transplanted lichen specimens, they underwent twice of washing using regular and distilled water to eliminate any extraneous materials such as soil particles, sand, dust, and bark. The samples were dehydrated for a duration of 24 hours at a temperature of 80ºC using yellow paper bags. This was done to prevent any microbial decay and to get accurate reference values for dry weight. The dried samples were pulverized using a mortar to ensure uniformity and dispersion of heavy metals.

The glass, plastic, and ceramic materials were soaked in a solution of detergent and water for an extended period of time, followed by rinsing with regular water and then immersion in a 20% concentration of nitric acid overnight. Subsequently, the glassware was cleansed using double distilled water and subsequently subjected to a drying process in a 60 ºC 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 460ºC. The charred ash samples were placed into 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 capacity was expanded to 15 ml using a 1% HNO_3 solution. After centrifuging the extract at a speed of 3000 rpm for 20 minutes (equivalent to 1157 g of relative centrifugal acceleration), it was transferred to a 25 ml beaker and then diluted to a final volume of 25 ml using a 1% HNO_3 solution. The ICP-MS instrument was employed to assess the concentration of heavy metals in all samples, including the control station.

2.4. Chlorophyll measurement

5 mL DMSO was administered to the lichen thallus throughout the extraction procedure to produce chlorophyll from 20 milligrams of dried lichen material. The lichen extract-containing tubes were then held in the dark at 65 ºC for 40 minutes before

being allowed to achieve ambient temperature. Whatman no 3 filtration paper was employed to filter lichen extracts. The UV-Spectrophotometer was regulated at 750 nm. The samples' absorbance ranges were estimated at 665 and 648 nm. The calculations were done using DMSO, 100% (pure solvent) (DMSO=Dimethyl sulphoxide (for synthesis) 99% purity, Merck 8.02912). Chlorophyll content was calculated according to equations (1), (2) and (3) of [34]

$$\text{Chlorophyll-a} = 14.85A^{665} - 5.14A^{648} \quad (1)$$
$$\text{Chlorophyll-b} = 25.48A^{648} - 7.36A^{665} \quad (2)$$
$$\text{Chlorophyll (a+b)} = 7.49A^{665} + 20.34A^{648} \quad (3)$$

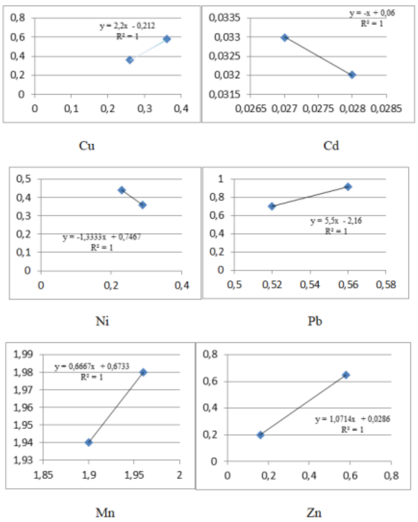
3. Results

3.1. Element contents of the lichen transplants

Clean samples obtained from the Yapraklı-Çankırı forests region and exposed near the Niğde city center revealed a significant accumulation of heavy metals. Table 2 displays the average levels of heavy metal contents in the 1st and 2nd exposure periods and in terms of these means, there is a positive correlation between each element and the control station in 1st and 2nd periods.

Table 2. Mean values of heavy metals contents in 1st and 2nd exposure periods (μg g^{-1}) and correlation of these averages between each element and the control station in the 1st and 2nd periods.

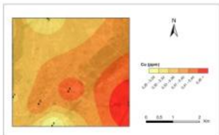
	Periods	Cu	Cd	Ni	Pb	Mn	Zn
Control stations	1	0,26	0,028	0,23	0,52	1,90	0,16
	2	0,36	0,027	0,29	0,56	1,96	0,58
Exposure stations	1	0.36	0,032	0,44	0,70	1,94	0,2
	2	0,58	0,033	0,36	0,92	1,98	0,65



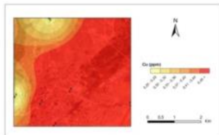
According to analysis results for *P. furfuracea* means of heavy metals; in first period 0.36 μg g^{-1}, 0,032 μg g^{-1}, 0,44 μg g^{-1}, 0,70 μg g^{-1}, 1,94 μg g^{-1}, 0,2 μg g^{-1}; in second period 0,58 μg g^{-1}, 0,033 μg g^{-1}, 0,36 μg g^{-1} ,0,92 μg g^{-1} , 1,98 μg g^{-1} , 0,65 μg g^{-1} for Cu, Cd, Ni, Pb, Mn, Zn. Whereas means of control stations are; in 1st period 0,26 μg g^{-1}, 0,028 μg g^{-1}, 0,23 μg g^{-1},0,52 μg g^{-1}, 1,90 μg g^{-1}, 0,16 μg g^{-1}; in 2nd period 0,36 μg g^{-1}, 0,027 μg g^{-1},0,29 μg g^{-1}, 0,56 μg g^{-1}, 1,96 μg g^{-1}, 0,58 μg g^{-1} for for Cu, Cd, Ni, Pb, Mn, Zn (Table 3). Figure 4 illustrates the air pollution maps created based on the heavy metal data at the exposure stations.

Table 3. Heavy metal contents of clean and exposed *P. furfuracea* lichen samples analysis for each stations (μg g^{-1})

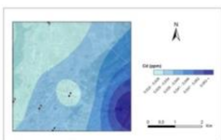
Stations	Periods	Cu	Cd	Ni	Pb	Mn	Zn
C1*	1	0.28423	0.02621	0.27508	0.51637	1.89763	0.15076
	2	0.38909	0.02757	0.28306	0.55338	1.94752	0.57671
C2*	1	0.25191	0.03153	0.20229	0.52883	1.91850	0.18884
	2	0.34413	0.02832	0.31485	0.56882	1.98790	0.58973
1	1	0,31401	0,02208	0,96226	0,63504	2,46733	0,23323
	2	0,28011	0,02564	0,59925	0,56602	2,09254	0,49185
2	1	0,32159	0,02212	0,32397	0,48804	1,66188	0,16847
	2	0,23505	0,02847	0,28212	0,48029	1,49659	0,60287
3	1	0,25080	0,03316	0,27445	0,53006	1,48448	0,16700
	2	0,43639	0,04568	0,30920	0,88345	1,96096	0,29325
4	1	0,39251	0,03098	0,56249	0,69905	2,24653	0,22314
	2	0,47377	0,03424	0,30434	0,83700	1,80414	0,90077
5	1	0,41504	0,02629	0,29624	0,88979	1,99383	0,34427
	2	0,60392	0,03162	0,31500	0,96374	1,97278	0,55663
6	1	0,47260	0,05773	0,24648	0,97326	1,82779	0,38721
	2	1,48957	0,03296	0,40016	1,80854	2,58594	1,06657



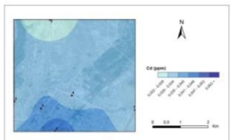
Cu - 1st period



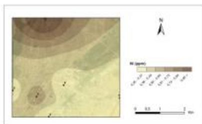
Cu -2nd period



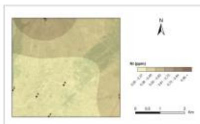
Cd - 1st period



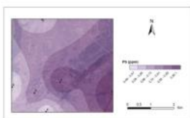
Cd -2nd period



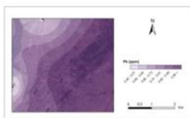
Ni - 1st period



Ni - 2nd period

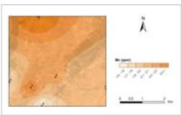


Pb- 1st period

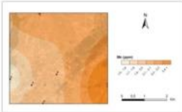


Pb - 2nd period

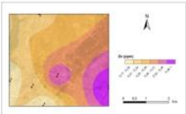
	Periods	Chl-a	Chl-b	Chl a+b
Control stations	1	6,381	1,527	7,908
	2	7,072	2,024	9,097
Exposure stations	1	3,378	0,665	4,043
	2	3,174	1,312	4,486



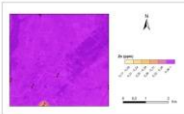
Mn- 1st period



Mn - 2nd period



Zn- 1st period



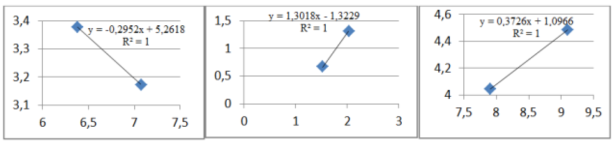
Zn-2nd period

Figure 4. Niğde pollution maps categorized by heavy metal concentrations

3.2. Chlorophyll contents

The average amount of chlorophyll of the lichen specimens transplanted to the city center of Niğde exhibited a notable decline throughout the first and second periods, as compared to the specimens collected from the forest region of Yapraklı-Çankırı. Within this particular framework, there exists a statistically significant association between pollution and a reduction in chlorophyll concentration (Table 4).

Table 4. Average concentrations of Chl(a), Chl(b), chl(a+b) in micrograms per milliliter (µg/ml)and correlation between exposure and control stations.

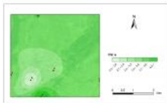


Chl-a Chl-b Chl-a+b

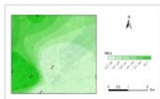
Chlorophyll contents of Chl-a, Chl-b, Chl-a+b also Chl-a/b and Chl-b/a ratios in 1st and 2nd periods are given in Table 5. Especially Chl-a, at 4th station in 1st period and at 5th station in 2nd period shows min value. Chl-b at all exposure stations in 1st period shows values that are almost close but surprisingly in 2nd period at 4th station shows max value. The chlorophyll maps of lichens exposed to pollution at the stations are presented in Figures 5 and 6.

Table 5. Chl(a), Chl(b) and Chl(a+b) contents (µg/ml) and Chl (a/b), Chl (b/a) ratios for each stations

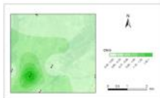
Stations	Periods	Chl-a	Chl-b	Chl a+b	Chl a/b	Chlb/a
C1*	1	7.7827	1.945	9.7277	5.0007	0.312
	2	9.252	3.013	12.265	4.5167	0.3337
C2*	1	4.9797	1.109	6.0887	5.7143	0.2017
	2	4.8937	1.036	5.9297	5.9523	0.1983
1	1	3,59	0,494	4,084	7,267	0,138
	2	4,004	0,492	4,496	8,138	0,250
2	1	3,565	0,549	4,114	6,494	0,154
	2	4,521	0,58	5,101	7,795	0,128
3	1	4,234	0,608	4,842	6,964	0,144
	2	1,898	0,596	2,494	3,185	0,314
4	1	2,291	1,295	3,586	1,769	0,565
	2	5,286	5,2	10,486	1,017	0,984
5	1	3,244	0,548	3,792	5,92	0,169
	2	0,727	0,373	1,1	1,949	0,513
6	1	3,348	0,496	3,844	6,75	0,148
	2	2,612	0,631	3,243	4,139	0,242



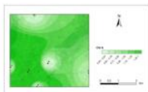
Chl(a)-1st period



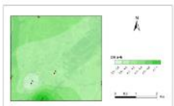
Chl(a)-2nd period



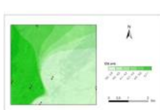
Chl(b)-1st period



Chl(b)-2nd period

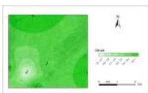


Chl(a+b)-1st period

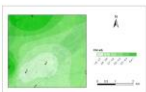


Chl(a+b)-2nd period.

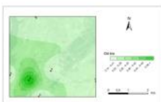
Figure 5. Niğde pollution maps based on chl(a), Chl(b), and chl(a+b) concentrations.



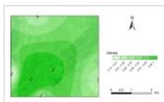
Chl(a/b)-1st period



Chl(a/b)-2nd period



Chl(b/a)-1st period



Chl(b/a)-2nd period

Figure 6. Niğde pollution maps based on chlorophyll a/b and b/a ratio

4. Discussion and Conclusion

The highest Cu contents were 0,60 μg g^{-1} at 5th station in 2nd period and lowest 0,250 μg g^{-1} at 3rd station in 1st period. When Cu contents are analysed, it is observed that there are significantly high values in the 2nd periods of the 3rd, 4th, 5th, and 6th stations. The 3rd station being close to gas station (NiğGas) and motor vehicle traffic and urban winter heating activities, and the 4th, 5th, and 6th stations being close to motor vehicle traffic and urban winter heating activities support this increase in Cu values. Similar findings for Cu had been reported by [35,36,37,38,39].

As stated in a study [40], plants from uncontaminated locations exhibit 0.01-0.3 μg g^{-1} Cd. Cd value ranges at exposure stations vary between 0.02 and 0.05. The highest Cd value was recorded at station 6 in the 1st period. Cd values at other stations did not show a significant variation compared to control stations. The fact that **6th station** is located on a high traffic route supports that Cd is associated with motor vehicle exhaust gas [41].

The maximum Ni value was found in the 1st and 2nd periods of the 1st station, as well as the 1st period of the 4th station, while the lowest Ni value was found in the 1th period of the 6th station. Seasonal winter heating activities (coal) and vehicular traffic are two variables that contribute to elevated Ni levels [42]. The authorized limit value for Ni in plants, as determined by the FAO/WHO, is 5 μg g^{-1} [43]. In this investigation, the average Ni content was 0.40 μg g^{-1}. The average Ni content observed in the current research is lower than that found in [39,44,45] investigations. The quantity is below the acceptable Ni concentration for plants.

While the 1st and 2nd periods of the 4th, 5th and 6th stations and the 2nd period of the 3rd station showed considerably higher Pb values than the control stations, the other stations showed relatively similar values. As shown from Table 2, the average Pb value of the control station in the 1st and 2nd periods was 0.52 μg g^{-1}-0.56 μg g^{-1} and the mean of the exposure stations was 0.70 μg g^{-1}-0.92 μg g^{-1}. Equivalent results were made by [46] with *Flavoparmelia caperata* (L.) Hale, by [37], [47], [48], [49] with *P. furfuracea*. The contribution of gasoline to Pb concentrations in the atmosphere is decreasing, however coal and gasoline combustion remain the primary producers of harmful chemicals in the environment [49]. The pollution of Pb is connected to the release of pollutants from vehicles and the burning of gasoline [42]. [50] examined the existence of heavy metals in a small number of samples and discovered that automobiles released significant levels of Pb and Cu. The measurements of Pb accumulation clearly reveal Pb contamination at stations 4, 5, and 6, which may be identified to motor vehicles sources.

Table 3 shows that Mn values were high in 1st and 2nd periods of 1st and 5th stations, in 1st period of 4th station and in 2nd period of 6th station. The emissions from vehicles caused considerable bioaccumulation at these stations. In contrast to the control station, fewer metrics were assessed at the 2nd and 3rd stations. 1st and 2nd period Mn average of exposure stations is 1.96 μg g^{-1}, whereas the average of the control stations is 1.93 μg g^{-1}. The mean Mn content observed is lower than the Mn content reported in [38,44,45] research.

The Zn level of lichen specimens proved to be related to road traffic. Based on research, the primary sources of Zn pollution include petroleum, fuel oils, composting, and metal alloys. The elevated levels of zinc in areas such as industry, urban roads, urban sites, urban parks, and shanty settlements indicate the influence of high traffic intesity and the wearing of car tires [37,51]. At exposure sitations, highest Zn value was measured in the 2nd period of the 6th station (1,06 μg g^{-1}), 4th sations, which were related to traffic and the lowest value was measured in the 1st period of the 2nd and 3rd stations (0,16 μg g^{-1}). Similar results were found by [52] and [47].

Photosynthetic pigment levels may be readily quantified and are commonly employed to evaluate the impact of metal toxicity on plants and lichens. Heavy metals are believed to impact enzymes responsible for regulating chlorophyll production, hence inhibiting the productivity of chlorophyll pigments. The study [53] revealed that Zn decreased photosynthetic pigments by replacement of Fe, which is essential for the synthesis of chlorophyll. At 4th and 6th stations, this situation was observed for Zn. Based on reference [54], the presence of Cu has resulted in a decrease in chlorophyll levels. At the 5th and 6th stations, this situation was observed for Cu. The degradation of chlorophyll in plant cells is induced by the buildup of heavy metals. In a study [63], the concentrations of Cu at 100 and 200μmol·L^{-1} had no significant impact on the levels of Chl a, Chl b, Chl a + b, and Chl a/b. However, when the concentrations were raised to 400 and 800μmol·L^{-1}, there was a substantial drop in the levels of Chl a, Chl b, and Chl a + b. Zn caused a substantial drop in the levels of chlorophyll a (Chl a), total chlorophyll (Chl a + b), and the ratio of Chl a to Chl b in the treated plants. However, this effect was seen only at a dosage of 800μmol·L^{-1}. Zinc had no significant impact on the content of chlorophyll b (Chl b).

Heavy metal presence owing to heating and vehicle traffic (especially at 3rd,4th,5th,6th stations) is related with reduced chlorophyll content in lichen thallus, as seen in Table 5. The chl(a+b) decline map confirmed that the photosynthetic pigments had totally degenerated, providing evidence for this outcome. The chl(a/b) maps indicated that chl(a) was more adversely afflicted by air pollution compared to chlorophyll b. Furthermore, the pollution resulted in a reduction in photosynthetic pigments, as anticipated. The accuracy of the finding was further confirmed by the examination of the chlorophyll b/a maps. The variations in the amounts and ratios of chlorophyll a and b might potentially be caused by environmental stressors, such as pollution. The level of chlorophyll in lichen thallus is often correlated with environmental adversity, exhibiting higher chlorophyll content in stressful settings compared to non-stressful situations [55]. Furthermore, [55] observed that the positioning of the station did not impact the amount 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, the amount of light, and the lichen organism itself.

From chemical point of view, heavy metal contents of lichen tallus exposed to pollution for 6 months at 3-month intervals are expected to increase. The findings of the present investigation suggest that the accumulation of metals in lichens is significantly affected by the location of the station and the duration of exposure. This survey found that heavy metal concentrations of *P. furfuracea* in Niğde was higher than at the control station and suggests that *P. furfuracea* is a species that effectively collects heavy metals in lichen-bags that are exposed in urban areas.

Before the start of our biomonitoring research, we underline the importance of how the amount of elements in tallus of lichen samples with low elemental content, taken from natural flora away from pollution factors, may react to heavy metals after transplantation to polluted areas. This will assist us in improving the quality of the bioaccumulation data, allowing us to enhance the accuracy of interpretations of our findings in future studies.

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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.

References

[1] Augusto, S., Máguas, C., & Branquinho, C. 2013. Guidelines for biomonitoring persistent organic pollutants (POPs), using lichens and aquatic mosses – A review. *Environmental Pollution*, *180*, 330–338.

[2] Augusto, S., Sierra, J., Nadal, M., Schuhmacher, M. 2015). Tracking polycyclic aromatic hydrocarbons in lichens: It's all about the algae. Environ Pollut. 207:441-5.

[3] Bargagli, R., Mikhailova, I. 2002. Accumulation of Inorganic Contaminants. In: Nimis, P.L., Scheidegger, C., Wolseley, P.A. (eds) Monitoring with Lichens — Monitoring Lichens. NATO Science Series, vol 7. Springer, Dordrecht.

[4] Lucadamo, L., Anna, C., Gallo, L. 2017. Local wind monitoring matched with lichen Pseudevernia furfuracea (L.) Zopf transplantation technique to assess the environmental impact of a biomass power plant," *Turkish Journal of Botany*: Vol. 41: No. 2, Article 4.

[5] Nascimbene, J., Tretiach, M., Corana, F., Lo, Schiavo F., Kodnik, D., Dainese, M., Mannucci, B. 2014. Patterns of traffic polycyclic aromatic hydrocarbon pollution in mountain areas can be revealed by lichen biomonitoring: a case study in the dolomites (Eastern Italian Alps). Sci. Total Environ. 475:90–96.

[6] Tretiach, M., Adamo, P., Bargagli, R., Baruffo, L., Carletti, L., Crisafulli, P., Giordano, S., Modenesi, P., Orlando, S., Pittao, E. 2007. Lichen and moss bags asmonitoring devices in urban areas. Part I: influence of exposure on sample vitality. Environ. Pollut. 146:380–391.

[7] Tretiach, M., Candotto, Carniel. F., Loppi, S., Carniel, A., Bortolussi, A., Mazzilis, D., Del Bianco, C. 2011. Lichen transplants as a suitable tool to identify mercury pollution from waste incinerators: a case study from NE Italy. Environ Monit Assess. 175(1-4):589-600.

[8] Kodnik, D., Winkler, A., Candotto, Carniel F., Tretiach, M. 2017. Biomagnetic monitoring and element content of lichen transplants in a mixed land use area of NE Italy. Sci Total Environ. 1;595:858-867.

[9] Bargagli, R. 1998. Trace Elements in Terrestrial Plants. An ecophysiological approach to

biomonitoring and biorecovery. Springer, Berlin, 324 p.

[10] Garty, J. 2000. Environment and elemental content of lichens. In: Trace Metals in the Environment. Chapter 8. Volume 4, 2000, Pages 245-276.

[11] Capozzi, F., Giordano, S., Di Palma A., Spagnuolo, V., De Nicola, F., Adamo, P. 2016. Biomonitoring of atmospheric pollution by moss bags: Discriminating urban-rural structure in a fragmented landscape. Chemosphere. 2016 Apr;149:211-8.

[12] Batts, J.E., Calder, L.J., Batts, B.D. 2004. Utilizing stable isotope abundances of lichens to monitor environmental change. Chem. Geol., 204 (2004), pp. 345-368.

[13] Cloquet, C., Muynck, D.D., Signoret, J.,Vanhaecke, F.2009. Urban/periurban aerosol survey by determination of the concentration and isotopic composition of Pb collected by transplanted lichen *Hypogymnia physodes* Environ. Sci. Technol., 43 (2009), pp. 623-629,

[14] Purvis, O.W., Chimonides, P.J., Jones, G.C., Mikhailova, I.N., Spiro, B., Weiss, D.J., Williamson, B.J. 2004. Lichen biomonitoring near Karabash smelter town, ural mountains, Russia, one of the most polluted areas in the world. Proc Biol Sci. 2004 Feb 7;271(1536):221-6.

[15] Spiro B, Weiss DJ, Purvis OW, Mikhailova I, Williamson BJ, Coles BJ, Udachin V. 2004. Lead isotopes in lichen transplants around a Cu smelter in Russia determined by MC-ICP-MS reveal transient records of multiple sources. Environ Sci Technol. 2004 Dec 15;38(24):6522-8.

[16] Wadleigh MA. 2003. Lichens and atmospheric sulphur: what stable isotopes reveal. Environ Pollut. 2003;126(3):345-51.

[17] Wiseman, R., Wadleigh, M. 2002. Lichen response to changes in atmospheric sulphur: isotopic evidence. Environmental Pollution 116, 235–241.

[18] Boonpeng, C., Sangiamdee, D., Noikrad, S., Boonpragob, K. 2023. Assessing seasonal concentrations of airborne potentially toxic elements in tropical mountain areas in thailand using the transplanted lichen Parmotrema Tinctorum (Despr. ex Nyl.) Hale. Forests. 2023; 14(3):611.

[19] Loppi, S., Ravera, S., Paoli, L. 2019. Coping with uncertainty in the assessment of atmospheric pollution with lichen transplants, Environmental Forensics, 20:3, 228-233.

[20] Godinho, R.M., Freitas, M.C., Wolterbeek, H.T. 2004. Assessment of lichen vitality during a transplantation experiment to a polluted site. J Atmos Chem 49, 355–361 (2004).

[21] Carreras, H.A., Pignata, M.L. 2001. Comparison among air pollutants, meteorological conditions and some chemical parameters in the transplanted lichen Usnea amblyoclada. Environ Pollut. 2001;111(1):45-52.

[22] Carreras, H.A., Pignata, M.L. 2002. Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. Environ Pollut. 2002;117(1):77-87.

[23] Abas, A., Aiyub, K., Awang, A. 2022. Biomonitoring potentially toxic elements (PTEs) using lichen transplant Usnea misaminensis: A Case Study from Malaysia. Sustainability. 2022; 14(12):7254.

[24] Gallo, L., Corapi, A., Loppi, S., Lucio, L. 2014. Element concentrations in the lichen Pseudevernia furfuracea (L.) Zopf transplanted around a cement factory (S Italy), Ecological indicators. 2014, Vol 46, pp 566-574, 9 p ; ref : 3/4 p.

[25] Incerti, G., Cecconi, E., Capozzi, F., Adamo, P., Bargagli, R., Benesperi, R., Carniel, F.C., Cristofolini, F., Giordano, S., Puntillo, D., Spagnuolo, V., Tretiach ,M. 2017. Infraspecific variability in baseline element composition of the epiphytic lichen Pseudevernia furfuracea in remote areas: implications for biomonitoring of air pollution. Environ Sci Pollut Res Int. 2017 Mar;24(9):8004-8016.

[26] Paola, M., Paolo, G., Paolo, M., Luisa, A.M., MAGI,E., Francesco, S. 2014. Bioaccumulation capacity of two chemical varieties of the lichen Pseudevernia furfuracea, Ecological indicators. 2014, Vol 45, pp 605-610, 6 p ; ref : 3/4 p.

[27] Brodo, I.M. 1961. Transplant experiments with corticolous lichens using a new technique. - Ecology 42(4): 838-841.

[28] Niğde ili ve ilçeleri haritası https://sehirsorgula.com/nigde-ilceleri/ (Erişim Tarihi: 12/12/2023)

[29] Bağdatlı, M. C. & Öztekin, M. E. 2021. Determination of Land Use Capabilities by GIS Analysis in Nigde Province, Turkey . Eurasian Journal of Agricultural Research , Volume 5 Issue 2 , 121-129 .Retrievedfrom

https://dergipark.org.tr/en/pub/ejar/issue/66343/986937.

[30] Air Quality Bulletin. 2020. Turkey Ministry of Environment, Urbanisation and Climate Change, (2020). Air Quality Bulletin, Annual.

[31] Kodnik, D., Candotto, Carniel F., Licen, S., Tolloi, A., Barbieri, P., Tretiach,M. 2015. Seasonal variations of PAHs content and distribution patterns in a mixed land use area: a case study in NE Italy with the transplanted lichen Pseudevernia furfuracea. Atmos. Environ. 113, 255–263.

[32] Tretiach, M., Crisafulli, P., Pittao, E., Rinino, S., Roccotiello, E.,Modenesi, P. 2005. Isidia ontogeny and its effects on the CO_2 gas exchanges of the epiphytic lichen Pseudevernia furfuracea (L.) Zopf. Lichenologist 37, 445–462.

[33] Barnes, J, D., Balaguer, L., Manrique, E., Elvira, S., Davison, A,W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environ Exp Bot, 1992, vol.32, pp. 83–100.

[34] Giordano, S., Adamo, P., Spagnuolo ,V., Tretiach, M., Bargagli, R. 2013. Accumulation of airborne trace elements in mosses, lichens and synthetic materials exposed at urban monitoring stations: towards a harmonisation of the moss-bag technique. Chemosphere. 2013 Jan;90(2):292-9.

[35] Onder, S., Dursun, S. 2006. Air borne heavy metal pollution of Cedrus libani (A. Rich) in the city centre of Konya (Turkey). Atmospheric Environment, 2006, vol. 40, pp.1122-1133.

[36] Aksoy, A., Leblebici, Z., Halici, M.G. 2010. Biomonitoring of heavy metal pollution using lichen (Pseudevernia furfuracea (L.) Zopf Exposed in bags in a semi-arid region,Turkey. M. Ashraf et al. (eds.), Plant Adaptation and Phytoremediation, DOI 10.1007/978-90-481-9370-7_3, C Springer Science+Business Media B.V. 2010.

[37] Ferah, K. 2019. Rize, Trabzon ve Artvin illerindeki hava kirliliğinin Cladonia rangiformis ve Pseudevernia furfuracea türleri kullanılarak belirlenmesi. Recep Tayyip Erdoğan Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.

[38] Işık, V., Vardar, Ç., Aksoy, A., Yıldız, A. 2023. Biomonitoring of heavy metals by Pseudevernia furfuracea (L.) Zopf in Aksaray city, Turkey. EQA - International Journal of Environmental Quality, 2023, vol. 56(1), pp. 52–61.

[39] Allen , S.E 1989. Analysis of ecological materials. Blackwell Scientific Publications, Oxford.

[40] Zschau, T., Getty, Y., Ameron, A, Zambrano., Nash, T, H. 2003. Historical and current atmospheric deposition to the epilithic lichen Xanthoparmelia in Maricopa County, Arizona. Environmental Pollution, 2003, vol.125, pp.21-30.

[41] Valkoviç, V. 1983. Trace elements in coal, Vol I & II. Boca Raton, 1983, Florida CRC Press.

[42] Ozkan, A. 2017. Antakya-Cilvegözü karayolu etrafındaki tarım arazilerinde ve bitkilerdeki ağır metal kirliliği. Çukurova Üniversitesi Mühendislik Mimarlık Fakültesi Dergisi, 32, 9-18.

[43] Agnan, Y., Probst, A., Sejalon-Delmas, N. 2017. Evaluation of lichen species resistance to atmospheric metal pollution by coupling diversity and bioaccumulation approaches : a new bioindication scale for french forested areas. Open Article in Ecological Indicators, 72, 99-110.

[44] Gerdol, R., Marchesini, R., lacumin P., Brancaleoni L. 2014. Monitoring temporal trends of air pollution in an urban area using mosses and lichens as biomonitors. Chemosphere, 108, 388-395.

[45] Loppi, S., Frati, L., Paoli, L., Bigagli, V., Rossetti, C., Bruscoli, C., Carsini, A. 2004. *Flavoparmalia coperata* thalli as indicators of temporal variations of air pollution in the town of Montecatini Terme (central Italy). Sci Total Environ 326:113–122.

[46] Guidotti, M., Stella, D., Dominici, C., Blasi, G., Owczarek, M., Vitali, M., Protano, C. 2009. Monitoring of traffic-related pollution in a province of central Italy with transplanted lichen *Pseudevernia furfuracea*. Bull Environ Contam Toxicol. 2009 Dec;83(6):852-8.

[47] Sorbo, S., Aprile, G., Strumia, S., Castaldo, C. R., Leone, A., Basile. A. 2008. Trace element accumulation in *Pseudevernia furfuracea* (L.) Zopf exposed in Italy's so called Triangle of Death. Sci Total Environ 407:647–654.

[48] Sujetovien, G. 2015. Monitoring lichen as indicators of atmospheric quality. In Recent Advances in Lichenology; Upreti, D., Divakar, P.,Shukla, V., Bajpai, R., Eds.; Springer: New Delhi, India, 2015; pp. 87–118.

[49] Hölwarth M. 1982. Überwachung statischer schwermetallimmissionen mit hilfe eines

bioindikators. Stab-Reinhalt luft 1982;42:373-378.

[50] Markert, B. 1993. Plants as biomonitors: indicators for heavy metals in the terrestrial environment. VCH Publishers Ltd.

[51] Lucadamo, L., Gallo, L., Vespasiano, G., Corapi, A. 2021. The contributions of an airport and related road network to *Pseudevernia furfuracea* bioaccumulation of trace elements and polycyclic aromatic hydrocarbons. *Ecological Indicators*, *125*.

[52] Woolhouse, H.W. 1983. Toxicity and tolerance in the responses of plants to metals. In:Lange.O.L.. Nobel.P.S.; Osmond. C.B.; Zielgler. H.(Eds.).Encyclopedia of Plant Physiology. New Series. 12.246–299. ISBN:978-3-642-68155-4.

[53] Lu, C., Chau, C., & Zhang, J. 2000. Acute toxicity of excess mercury on the photosynthetic performance of cyanobacterium, *S. platensis* – assessment by chlorophyll fluorescence analysis. *Chemosphere*, *41*(1–2), 191–196.

[54] Wakefield, J, M., Bhattacharjee, J. 2012. Effect of air pollution on chlorophyll content and lichen morphology in northeastern louisiana. The American Bryological and Lichenological Society, Inc, 2012, vol. 28(4), pp. 104-114,

[55] Türkiye State Istatistical Institut. 2002. Population census report.

[56] Traffic Statistical Yearbook. 2002. Traffic education and research department.

[57] Yıldız, A., Aksoy, A., Akbulut, G., Demirezen, D., Işlek, C., Altuner, E. M., Duman, F. 2011. Correlation Between Chlorophyll Degradation and the Amount of Heavy Metals Found in Pseudevernia furfuracea in Kayseri (Turkey). *Ekoloji*, *20*(78), 82–88.

[58] Yildiz, A., Aksoy, A., Tug, G. N., Islek, C., & Demirezen, D. 2008. Biomonitoring of heavy metals by Pseudevernia furfuracea (L.) Zopf in Ankara (Turkey). *Journal of Atmospheric Chemistry*, *60*(1), 71–81.

[59] Yıldız, A., Işık, V., & Aydın, S. S. (2023, October 17). Heavy metal biomonitoring study using transplanted lichen, Pseudevernia furfuracea (L.) Zopf, in Kırıkkale, Turkey. *MOJ Ecology & Environmental Sciences*, *8*(5), 192–200.

[60] Yıldız, A., Vardar, I., Aksoy, A., & Ünal, E. (2018, February 8). Bıomonıtorıng of heavy metals deposition with Pseudevernıa furfuracea (L.) Zopf In Çorum Cıty, Turkey. *HEALTH SCIENCES QUARTERLY*, *2*(1), 9–22.

[61] Işık, V., Yıldız, A. 2024. Biomonitoring of Heavy Metals Accumulation with Pseudevernia furfuracea (L.) Zopf in Kırşehir Province, Türkiye. Pollution, 10 (1), 566-579.

[62] Chen, H., Song, L., Zhang, H., Wang, J., Wang, Y., & Zhang, H. (2022, June 17). Cu and Zn Stress affect the photosynthetic and antioxidative systems of alfalfa (Medicago sativa). *Journal of Plant Interactions*, *17*(1), 695–704.