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Assesing the Impact of Air Pollution on Lichen (*Dirinaria picta* (sw.) Schaer: Morphological Characteristics, Magnetic Grain Analysis, and Elemental Composition

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Abstract

Dirinaria picta is a lichen species known for its tolerance to air pollution and its ability to accumulate pollutants in its thallus, making it a suitable bioindicator in urban areas. This study aims to assess the response of lichens to air pollution by examining their morphological characteristics, analyzing the presence of magnetic grains, and identifying the origin of elements in their tissues. Samples were collected from three locations in Bandung area which represent polluted areas, namely Juanda Street (JD) and Kebon Kawung (KK), and Padalarang Street (PD) and one location which represent as a control area with low pollution levels, namely Curug Cimahi (CC). Lichen microstructure scanning was performed using a Scanning Electron Microscope (SEM), and the presence of elements was detected through Energy Dispersive X-ray Spectroscopy (EDS). The SEM analysis revealed that samples from areas with higher air pollution levels exhibited obvious damage to their thallus, characterized by a more porous structure and the presence of cuboidal particles. Conversely, samples from control area showed a more compact structure. The EDS observations identified 14 elements present in the lichen tissues, ranked in descending order of abundance: C>Ca>O>Pb>Cu>Mn>Zn>Fe>Cr>Al>Cl>Se>Cd>K. The composition and quantity of these elements in lichen tissues were influenced by the level of air pollution generated by the number of vehicles passing through the area. The more vehicles, the higher the level of air pollution, resulting in greater stress for the D. picta population.

Keywords: Bioindicator, lichen, *Dirinaria picta,* elements, microstructure, Bandung

1. Introduction

Lichens are often used as bioindicators organisms due to their sensitivity to air pollution. They are composite organisms consisting of a symbiotic association between fungi and algae or cyanobacteria. Lichens have sensitivity due to the changes in the environment such as water, temperature, and nutrient (Weber *et al.,* 2016). When exposed to air pollution, lichens can exhibit visible changes in their growth, color, or morphology. By studying these changes, the level and types of pollutants present in the environment can be assessed. Lichen biomonitoring programs involve collecting lichen samples from different locations and analyzing them to determine the extent of air pollution (Sett and Kundu, 2016).

Using lichen as bioindicator and biomonitor has several advantages. Lichens are widespread and can be found in various habitats, including urban, rural, and remote areas. The ability of lichen to accumulate pollutants over time makes it able to provide a historical record of pollution levels in the area. Additionally, using lichens as bioindicator or biomonitor is cost-effective and non-destructive survey, making them suitable for long-term monitoring studies (Giordani, 2007; Sett and Kundu 2016; Kondo *et al.,* 2017; and Benitez *et al.,* 2018).

Air quality can have a significant impact on plant diversity in an ecosystem, particularly in areas near industrial zones or heavily urbanized regions. Air pollution, especially from industrial activities and vehicle emissions, introduces various harmful substances into the atmosphere (Omar and AlKhalil, 2019, Aldgini *et al.*, 2019). Studies conducted in cities across Indonesia, including Jakarta, Semarang, Pekan Baru, Kendari, and Medan, have provided valuable insights into the relationship between lichens and air pollution (Kondo *et al.,* 2017).

The production of chemical elements, including heavy metals, is often associated with the burning of fossil fuels, primarily from vehicles and industrial activities (Nobel *et al*., 2003). The combustion of fossil fuels releases pollutants into the atmosphere, which can then be deposited onto lichens and other surfaces. Lichens, due to their ability to absorb and accumulate substances from the environment, can serve as indicators of the presence and levels of these harmful elements in the surrounding area (Kim et al., 2007; Aslan et al., 2010; Pirintsos et al., 2011; Polienik *et al*., 2008). By analyzing the elemental composition of lichens and identifying heavy metal elements like Cd, Cu, Pb, the extent of pollution and the potential risks they pose to both the environment and human health can be predicted (Bold *et al*., 1987; Al-

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Weher, 2008; Omar and AlKhalil, 2019; Alsholaili and Bani-Hasan, 2018). This information can be valuable for monitoring and mitigating the impacts of anthropogenic activities on ecosystems. Analyzing the presence and distribution of these non-metallic elements, including carbon, oxygen, and selenium, can provide insights into the sources and transport mechanisms of pollutants in the environment (Al-Weher, 2008; Omar and AlKhalil, 2019).

Dirinaria picta has been known as lichen species that can tolerate pollution areas of the city (Lawal *et al.*, 2023). *D. picta* is a species that may develop and grow rapidly in urban areas; therefore, *D. picta* can be regarded as inhabitant of urban areas (Abas *et al.,* 2022). A previous study in Greater Bandung showed that *D. picta* species could live in all observed locations based on the value of atmospheric purity, both in the highest and the lowest air polluted areas (*Rahmatia, 2003*; Saragih, 2022).

Based on The Central Board of the Central Bureau of Statistics for the City of Bandung for the 2020 period, Greater Bandung area is one of the 5^{th} most populous areas in Indonesia with the number of vehicles in 2018 reaching 1,811,498 with an average increase of vehicles 5% per year. The ratio of the number of vehicles to the population is 3:4 with 72% of all registered vehicles being motorbikes (The Central Bureau of Statistics for the City of Bandung, 2020).

The emissions from vehicles can include various pollutants such as particulate matter (specifically PM10, referring to particles with a diameter of 10 micrometers or less), lead, carbon monoxide (CO), hydrocarbons (HC), and nitrogen oxides (NOx) (Kim *et al.,* 2007). Monitoring the health and presence of lichens can provide insights into the extent and impacts of heavy metal pollution in ecosystems and help assess the overall health of the environment ecosystems (Urpreti *et al.*.,2015; WHO, 2016; Vannini *et al*., 2021).

This study aims to assess the response of lichens to air pollution by examining their morphological characteristics, analyzing the presence of magnetic grains, and identifying the origin of elements in their tissues. This research is focused on identifying mineral types, microstructure, and mineral content of *D. picta* samples found in Greater Bandung area.

In the investigation of lichens as bioindicators for assessing air pollution, beyond furnishing detail on the diversity and abundance of lichens in regions with differing pollution levels, it is imperative to include supplementary information pertaining to the anatomical status of lichen resulting from exposure to diverse pollutant. This additional data is crucial because alterations in anatomical structure have the potential to lead to the mortality of lichens. Studying the microstructure conditions of lichens, including their mineral and metal contents, is a valuable tool for monitoring and managing environmental pollution. It provides crucial information for understanding the impact of human activities on ecosystems and guides efforts to protect and restore the environment.

2. Material and Methods

Sampling was carried out at three locations in Bandung area heavy traffic sites, namely Juanda street (JD) (6^o52’22”S;107^o36’59”E), Kebon Kawung street (KK) (6^o54’45”S;107^o36’05”E), and Padalarang street (PD) (6^o50’38”S;107^o29’11”E), and one site with low traffic activity, i.e. Curug Cimahi as a control area (CC) (6^o47’55”S;107^o34’32”E) (Figure 1). The amount of sample taken was 10 g for each area.

Figure 1. Research Study Area in Bandung City

The four locations show differences in terms of vehicle emissions caused by differences in traffic volume and air environmental conditions. According to the categorization of air quality levels based on IAP values (Kommision Reinhaltung der Luft im VDI und DIN, 1995), the observation station with the highest to the lowest level of air pollution is KK, PD, JD, while CC showed the lowest. IAP was calculated on the preliminary study in four locations (JD, PD, KK, and CC). The results are presented in Table 1.

Table 1. IAP (Index of Atmospheric Purity) measurement results

Location	IAP	Pollution Levels	Information
Juanda Street (JD)	24.85	Level B	Highly polluted
Kebon Kawung Street (KK)	10.21	Level A	Very highly polluted
Raya Padalarang (PD)	17.70	Level B	Highly polluted
Curug Cimahi (CC)	46.65	Level E	Very low polluted

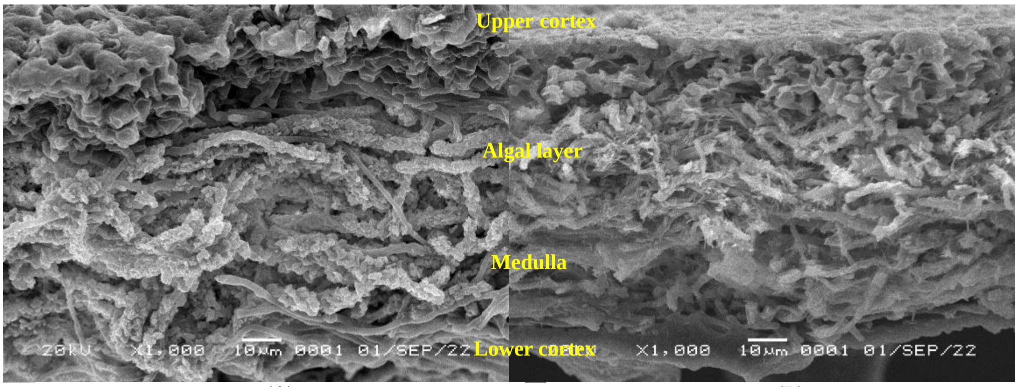
Characterization test of lichen samples from each study location using Scanning Electron Microscopy (SEM) accompanied by Electron Dispersive Spectroscopy (EDS) was carried out to observe how the shape of the topographic structure or lichen surface, structural defects, shape, and size of material granules, as well as an elemental composition and impurities in the lichen sample. The analysis of the structure and composition of the lichen was carried out in the laboratory of the Geological Survey Center Laboratory, Bandung. The sample was fixed using formalin then dehydrated and embedded in paraffin. The sample was then sliced using a microtome with a final sample thickness of 3μm, then the incision was placed on an object glass. Furthermore, the incision was deparaffinized by washing it with xylene at 37^oC for 2 hours. After that, the samples were washed using 100% ethanol at room temperature for 1 hour. The samples were then left overnight at room temperature to dry (Saragih, 2022).

Before being observed by SEM, the sample was glued to carbon tape that had been placed in a cuvette and then coated with a conductive layer, namely aluminum using an ion sputtering machine for 1 minute. The cuvettes were then placed in the specimen holder and inserted into the scan tool. Observations to scan the topography and morphology of the sample were carried out with an accelerating voltage of 20 kV with a magnification of 2000x (determination of the elemental composition of individual particles), 1000x and 5000x magnification (lichen surface), and 1000x magnification (lichen cross-section) (Conti *et al.,* 2001).

3. Results and Discussion

3.1. Micrograph Cross-Sectional

A comparison of the results of cross-sectional micrograph analysis of the *D. picta* samples that were obtained from KK (very highly polluted) and CC (very low polluted) are shown in figure 2 (A and B). Sample of *D. picta* that was collected from KK location had cubes resembling particles with uneven and irregular sizes, while sample of *D. picta* that was collected from CC location showed less number of those particles. Lichen that live on high levels of pollution absorb more particles than lichen that live on low level of pollution, and this particle was shown uneven and irregular sizes, which could disrupt metabolic processes in lichen tissue and lead to the death of the tissue (Taufikurahman *et al.,* 2010).



(A) (B)

Figure 2. Micrograph cross-sectional SEM images of *D. picta* (1000x magnification) from: (A) KK location (very high polluted area); (B) CC location (very low polluted area).

The tissue in figure 2A (on area with high pollution) appears more sparse compared to figure 2B (area with low pollution). The presence of pollutants causes gaps in the algal tissue and medulla. The damage, porous structure, and accumulation of particles seen in lichens from polluted areas are a consequence of the pollutants emitted by vehicles, industries/factories, and other human activities, while lichens from unpolluted areas maintain a healthier and more intact surface structure (Kim *et al.,* 2007; Wintermans *et al.,* 1965).

3.2. Optical Micrography

Optical micrography results of an examination of the surface microstructure of the *D. picta* thallus at 1000x magnification with a resolution of 10µm and a voltage of 20 kV can be seen in Figure 3 (A-D). It can be observed that lichens from areas with higher levels of air pollution exhibit visible damage to their thallus. Air pollutants can lead to a porous structure in the lichen and an accumulation of particles that form cubes on the surface of the thallus. In contrast, lichen samples from very low polluted area typically have a more compact and denser structure due to the lower levels of air pollution, and there is no significant accumulation of particles observed on the surface of the lichen's thallus (Taufikurahman *et al.,* 2010). The accumulation of pollutants, especially heavy metals, in the lichen thallus can lead to a range of physiological and structural changes in these organisms (Tripp *et al.,* 2016). This disparity in surface structure between lichens from polluted and unpolluted areas serves as a notable indicator of the impact of air pollution on lichens.

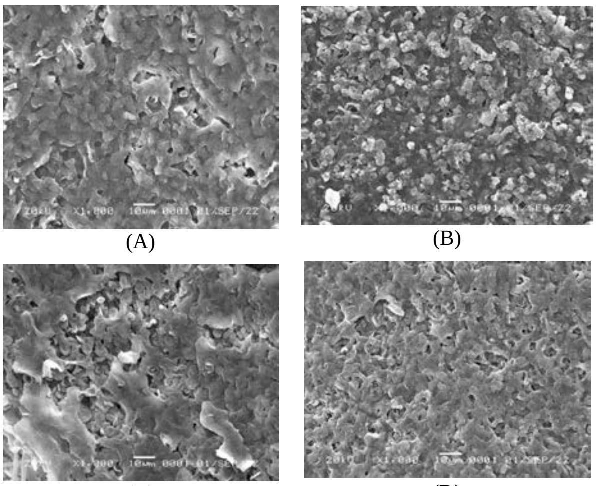


Figure 3. SEM image micrograph surface of the *D. picta* sample (1000x magnification). Sample were obtained from: (B) JD (Highly polluted area); (A) KK (highest polluted area); (C) PD (Highly polluted area); (D) CC (lowest polluted area)

3.3. Topographical Structure

The differences in the topographical structure and defects in the surface structure of the lichen samples from each study location can be seen in SEM-EDS with a magnification of 5000x with a resolution of 5µm and a voltage of 20 kV (Figure 4). The results of surface and elemental composition observations showed that lichens in highly polluted areas appear to have many cube-shaped particles which is magnetic grains, whereas at lower pollution levels, the number of cube-shaped particles is less. Meanwhile, in areas with very low pollution, no cubic particles were seen.

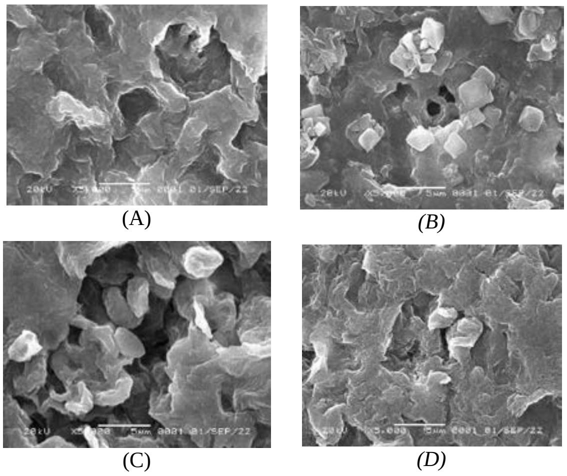


Figure 4. SEM image micrograph of the surface and elemental composition of the talus of the *D. picta* sample (5000x magnification) from: (A) JD (Highly polluted area); (B) KK (Very highly polluted area); (C) PD (Highly polluted area); (D) CC (Very low polluted area)

Magnetic grains in the sample which have been suggested to come from two different sources, namely pedogenic and anthropogenic. In the samples obtained from KK (very highly polluted) and PD (highly polluted area), there are a few magnetic grains that have an octahedral or angular shape. These grains show signs of damage at the corners, indicating that they might originate from pedogenic sources, such as natural magnetic minerals found in the environment (Olomukoro and Azubuike, 2009). On the other hand, the dominant spherical grains are believed to come from anthropogenic sources from polluted area. These grains are typically associated with ash or dust found on the sides of roads, as well as pollutants emitted from the burning of fossil fuels by vehicles (Ribeiro *et al.,* 2016; Richardson, 1993).

3.4. Elemental Composition

The results of the analysis of elemental composition in the lichen samples indicated the presence of several elements present in the thallus. Several metallic and non-metallic elements were detected in all samples including C, Ca, O, Pb, Cu, Mn, Zn, Fe, Cr, Al, Cl, Se, Cd, and K (Table 2). The average EDS spectrum of *D. picta* shows the percentage by weight of elements (%) found in the lichen thallus from highest to lowest to be C>Ca>O>Pb>Cu>Mn>Zn>Fe>Cr> Al>Cl>Se>Cd>K (Figure 5).

The number of elements found in highly polluted areas (KK) was 12 elements, while in less polluted areas (PD and JD) 11 elements. In contrast, area with very low pollution (CC), only 8 elements were found. C levels at all locations were the highest in lichen tissue compared to other elements (more than ten times), with the lowest C value (54.57%) found in highly polluted areas and the highest C value (63.82%) in areas with lower pollution. Compared to polluted area, percentage of Pb levels in low polluted area was very low (2.07%), while oxygen (13.26%) and Ca levels (12.56%) showed the highest level. In polluted areas, the accumulation of polluting elements causes oxygen and Ca levels in lichen tissue to decrease.

Carbon and oxygen are non-metallic elements that can be found in both solid and gaseous forms. In the air, oxygen constitutes approximately 20.95% of the composition of dry air. Carbon can be present in the form of aerosols or small solid objects that are suspended in the air. Carbon aerosols can originate from various sources, including combustion processes, industrial emissions, vehicle exhaust, and natural sources such as wildfires. These aerosols can contain carbonaceous particles, which contribute to air pollution and can be deposited on surfaces such as lichen thalli (WHO 2016).

Table 2. Elemental composition in *D. picta* samples obtain from EDS analysis

Element	Mass (%)			
	KK (Very highly polluted area)	PD (Highly polluted area)	JD (Highly polluted area)	CC (Very low polluted area)
C	54.57	59.29	63.82	61.43
O	3.85	7.23	4.48	13.26
Al	1.52	0.57	3.02	0.42
Cl	1.92	-	0.41	-
K	1.05	-	1.21	-
Ca	8.05	7.47	6.93	12.56
Cr	7.02	-	-	-
Mn	4.17	5.14	4.36	-
Fe	2.38	2.19	1.3	3.41
Cu	6.16	5.97	6.18	3.61
Zn	2.09	4.05	2.77	3.24
Pb	8.27	7.5	6.73	2.07
Se	-	0.35	-	-
Cd	-	0.24	-	-
Total	100	100	100	100

The analysis of elemental composition in lichens could provide information on sources and types of contamination in the research area. The elemental composition and mass percentages found in lichens are influenced by both biological processes carried out by the lichens themselves and anthropogenic inputs from human activities (WHO, 2016). Road traffic activity, in particular, can contribute to the contamination of lichens by increasing the emission of soil particles that are absorbed into the lichen thallus. These particles can contain various elements, including heavy metals such as Cd, Cu, and Pb (Cite). These heavy metal elements are known to be harmful to human health (Urpeti *et al.,* 2015; Kim *et al.,* 2007**;** Ezemonye and Tongo, 2009).

In addition, the non-metallic element found in the samples is selenium (Se). Se element can be naturally present in soil and rocks, and it can become airborne through processes such as wind erosion or human activities like mining and agricultural practices (Omar and AlKhalil, 2019; Ezemonye and Tongo, 2009)**.** The presence of Se in lichen samples suggests that it may have originated from the deposition of soil particles onto the lichen thallus (Aslan *et al.,* 2010; Vannini *et al.,* 2021).

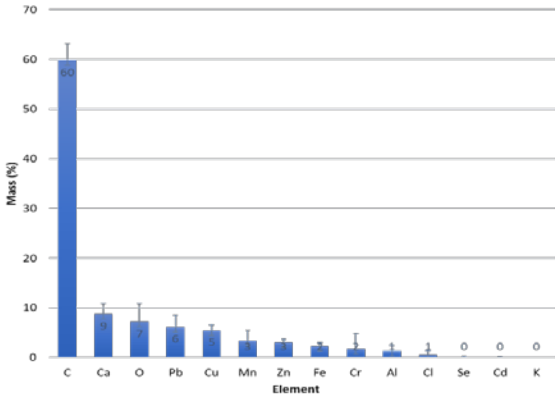


Figure 5. Element composition and the average elemental mass in samples of lichen D.*picta*;Bar showed standar deviation

The accumulation of particles in the algal layer and medulla of *D. picta* is influenced by pollution from vehicles and daily weather conditions. When the lichen is hydrated, it can absorb nutrients and contaminants directly from the air through its surface (Varela *et al.,* 2018; Tripp *et al.,* 2016; Urpeti *et al.,* 2015). These substances are then concentrated and transformed into a slow-release form within the lichen's structure. This process allows the lichen to efficiently extract and store nutrients while also retaining pollutants present in the surrounding environment (Pirintsos *et al.,* 2011; Vannini *et al.,* 2021).

The particles that accumulate in the algal layer will interfere with the photosynthesis process in the lichen because the chlorophyll in the algal layer cannot function optimally to absorb sunlight in the form of electrons, so the photosynthesis process will be disrupted due to being covered by ever-increasing particles (Aslan *et al.,* 2010; Kim *et al.,* 2007).

The specific types of contaminants and their sources may vary depending on the location and environmental conditions (Aldgini *et al.,* 2019; Al-Weher, 2008; Kim *et al.,* 2007). Vehicle emissions, which often contain pollutants such as particulate matter, nitrogen oxides, and sulfur compounds, can contribute to the accumulation of particles in lichens. Additionally, weather conditions, such as wind patterns or atmospheric deposition, can also influence the level of particle deposition on lichens (Kim *et al.,* 2007; Tripp *et al.,* 2016).

The presence of Zn is often associated with urban and industrial areas. Industrial processes, emissions from factories, and urban pollution, including vehicle exhaust, can release zinc-containing particles into the air (Omar and AlKhalil, 2019). Element Mn on the other hand, is generally linked to agricultural activities. Agricultural practices, such as the use of fertilizers or the application of pesticides, can introduce manganese into the environment (Ezemonye and Tongo, 2009). It can then be transported through air or water and subsequently deposited onto lichen thallus.

Element Fe in lichen thallus are often associated with the contribution of small soil and airborne particles, including dust, smoke, and vapor, with a diameter of fewer than 100 micrometers. These particles can be derived from various sources, including natural processes (e.g., wind erosion, volcanic emissions) and human activities (e.g., combustion of fossil fuels, industrial emissions) (Nobel *et al.,* 2003; Omar and AlKhalil, 2019). The deposition of these particles onto lichen surfaces can lead to the accumulation of Fe in lichen thalli (Aslan *et al.,* 2010; Vannini *et al.,* 2021). The accumulation of heavy metal elements such as Pb, Cu, Cd, Al, Zn, and Cr in lichens can have detrimental effects on their health and survival.

When lichens are exposed to high levels of these heavy metals, they can exhibit various symptoms of damage and stress such as chlorosis and necrosis. This discoloration occurs due to the disruption of chlorophyll production and photosynthetic processes (Cevik *et al.,* 2008; Kondo *et al.,* 2017). The accumulation of heavy metals can disrupt cellular functions and lead to the death of lichen tissues, resulting in the browning or blackening of affected areas (Benitez *et al.,* 2018; Kaffer *et al.,* 2011). The toxic effects of heavy metals can impair the lichen's metabolic processes (Muthu *et al.,* 2020), including nutrient absorption, respiration, and reproduction, leading to their decline and eventual death.

Heavy metal elements such as Pb, Cu, and Cd are harmful to human health (Giordani 2007; Rundel 1978; Robiansyah 2006). The amount of these elements in the air is affected by the volume or density of traffic, distance from highways and industrial areas, and wind direction. Other elements such as Ca and K (alkaline earth metals) are thought to accumulate in lichen due to particles in the form of dust carried by the wind, while Cl elements (halogen gases) are elements with a low pH and are acidic (Aslan *et al.,* 2010; Vannini *et al.,* 2021). The presence of Cl in a lichen is thought to occur due to the accumulation of rainwater flow and the absorption mechanism of lichen from its substrate.

The biodiversity of organisms like lichen, fungi, and those attached to plant parts like fruits and leaves refers to the physiochemical properties of the environment such as PH of the soil, temperature, and humidity (Alsohaili and Bani-Hasan, 2018). Different environmental conditions create distinct habitats, which, in turn, affect the types of organisms that can thrive in those environments (Sett & Kundu 2016)**.**

4. Conclusion

Lichens from areas with higher levels of air pollution showed obvious damage to the thallus, resulting in a more porous structure and the accumulation of cube-like particles on the surface. Lichens exposed to high levels of pollution absorbed more particles than those in areas with low pollution levels. These particles were uneven and irregular in size, which could disrupt metabolic processes in lichen tissue and potentially lead to tissue death. Meanwhile, lichen samples from very low polluted areas had a smoother and compact surface structure and showed no piles of particles. The number of elements found in highly polluted areas was higher than in less polluted areas.

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