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Short Communication: The effects of SO_2 and NO_2 fumigation on the chlorophyll of *Parmotrema perlatum* from Mt. Lawu, Indonesia

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Abstract. *Roziaty E, Sutarno, Suntoro, Sugiyarto. 2023. Short Communication: The effects of SO_2 and NO_2 fumigation on the chlorophyll of* Parmotrema perlatum *from Mt. Lawu, Indonesia. Biodiversitas 24: 2630-2637.* Lichens are symbiotic organisms composed of algae and fungi. Lichens have long been recognized as bioindicators of environmental health. One of the lichen parts that will be affected by pollution is chlorophyll. The lichen thallus was fumigated with the gases from motor vehicle emissions. The lichens under study were *Parmotrema perlatum* (Huds.) M. Choisy, found in Cemoro Sewu Forest of Mt. Lawu, Magetan District, East Java Province, Indonesia. The categorization of vehicle emission exposure levels includes Level 0 (no fumigation, control group), Level 1 (1.5 hours), Level 2 (3 hours), Level 3 (4.5 hours), Level 4 (6 hours), Level 5 (7.5 hours) and Level 6 (9 hours). The fumigated thallus had already been examined with spectrophotometry. Sulfuric dioxide (SO_2) was measured at 324 nm and 328 nm, whereas nitrogen dioxide and chlorophyll were measured at 645 nm and 663 nm. Each test was replicated three times (R_1, R_2, and R_3). Level 6 had the highest NO_2 and SO_2 content. The highest NO_2 content on thallus lichens was 4.00 at Level 6, while the lowest was 2.14 at Level 0 (Control). The highest SO_2 content was 1.856 at Level 6, whereas the lowest was 0.231 at Level 0 (Control). The highest chlorophyll content of *Parmotrema* was found at Level 0, while the lowest was identified at Level 6 (0.245 µg ml^{-1}). The highest content of chlorophyll b was 0.659 (Level 4), while the lowest was Level 6 (0.413 µg ml^{-1}). The thallus *Parmotrema* responded positively to NO_2 and SO_2 exposure. Correlation tests between the four components, specifically fumigation, NO_2, SO_2, and chlorophyll, show a positive correlation between pollutants and chlorophyll.

Keywords: Chlorophyll, lichens, NO_2, *Parmotrema*, SO_2

INTRODUCTION

Lichen is a symbiosis between algae and fungi (Gupta et al. 2016), a group of non-vascular organisms called symbionts (Shukla et al. 2014). As symbionts, lichens are composed of two parts: a photobiont (algae) and a mycobiont (fungi). The algae do the photosynthetic process, while the fungi contribute to the shape of the body, termed thallus. The thallus attaches to the substrate such as rocks, concrete, hard soil, and trees where it lives. (Parizadeh and Garampalli 2017). Lichen has been long recognized as a bioindicator of air quality (Jayalal et al. 2017) and climate (Khastini et al. 2019) as well as medicine and dye (Kusmoro et al. 2018). Since the 1900s, common studies in Europe have focused on the distribution of the type of lichen on trees, one of the Family Parmeliaceae, Genus *Parmotrema* (Stapper and John 2015). *Parmotrema* is a commonly found in the mountains, where pine trees with rough trunks dominate at temperatures ranging from 21 to 25 ^oC and humidity levels ranging from 50 to 60 % (Malaspina et al. 2020). Climate conditions and air quality affect lichen (Susilawati and Kasiamdari, 2021).

In the Cemoro Sewu Forest, East Java, Indonesia Parmeliaceae lichens are commonly found. The]mp most dominant species is *Parmotrema perlatum* (Huds.) M Choisy (Figure 1). *Parmotrema* has chlorophyll and lives in the highlands between 1,400 m and 2,700 m asl (Mansournia et al. 2012). Taxonomically, *P. perlatum* replaces *P. chinense* (Osbeck) Hale and Ahti (Naeth et al. 2016). Observations of lichen epiphyte as a bioindicator of air pollution are related to motor vehicle emissions (Coffey and Fahrig 2012). Bioindicators typically use to the extent of the population in their communities (Abas 2021). Bioindicators help indicate an environmental condition (van der Wat and Forbes 2015), (Sett and Kundu 2016). Lichen Parmeliaceae are similar to *Dirinaria*, and *Canoparmelia* is a tolerant bioindicator (Sudirman et al. 2015). Lichen can be used to predict the time of exposure to toxins (Kaasalainen et al. 2012). Another lichen is *Flavoparmelia caperrata* in Montecatini Terme, Italy can showed Pb metal concentrations and NO_2 content in their thallus (Paoli et al. 2015). Most species of *Parmelia* and *Lecanora*, as well as the genera of *Dirinaria* and *Canoparmelia*, are tolerant to air pollution (Mayer et al. 2013).

Figure 1. *Parmotrema perlatum* (Huds.) M. Choisy (a member of foliose group) at Cemoro Sewu Forest, Magetan, East Java, Indonesia

Pollution in urban areas is typically caused by the combustion of fossil fuels (Koch et al. 2018). The pollutants, particularly gases, pass through the cuticle or wax layer on the lichen thallus (McDonald et al. 2017). In Surakarta, Central Java, research on the diversity of lichens found in urban environments, suburbs, and forests as a control yielded the following results (Roziaty et al., 2021).

Fossil fuels from vehicles produce nitrogen oxide compounds emissions that interrupt the nitrogen cycle in the atmosphere. The nitrogen dioxide (NO_2) compounds, which are currently a major pollutant on roads. One type of observed lichen is *Parmelia sulcate* Taylor (Naeth et al. 2016). Another pollutant compound found in the atmosphere caused by the combustion of fossil fuels is SO_2. It was reported that one of the effects of SO_2 in the atmosphere is a decrease in the number of lichen colony formations (colonization), while NO_2 causes significant damage to *Flavoparmelia caperata* (L.) Hale if exposed for an extended period of time (Maslać et al. 2016). Lichen can convert atmospheric nitrogen gas into nitric oxide compounds and release them into the atmosphere (Meusel et al. 2017). Because lichens are made up of algae, they contain chlorophyll, which acts as a photobiont (Muggia and Grube 2018). The presence of chlorophyll a and b and also the ratio of chlorophyll a to chlorophyll b indicate the dominance of chlorophyll in lichens (Caesar et al. 2018). Air pollution and poor environmental conditions caused by the transportation sector can interfere with lichen's physiological processes such as the photosynthetic process in algae (photobiont) (Asplund and Wardle 2017). This study aims to analyze the effect of SO_2 and NO_2 fumigation on the chlorophyll of *P. perlatum* (Huds.) M. from Mount Lawu, Cemoro Sewu, Central Java, Indonesia.

MATERIALS AND METHODS

Study area

The study was conducted from September - December 2021. The research was carried out in the Biological Education Laboratory of the Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta, Indonesia by applying the spectrophotometric method. Lichen samples were taken from Cemoro Sewu Forest, along the hiking trail of Mount Lawu, Magetan District, East Java Province, Indonesia with the coordinates of 07°39.809' S, 111°11.501' E. The exploration was applied as a preliminary stage for observation. The selected lichens, *P. perlatum*, are epiphytes. They grow along the Cemoro Sewu forest at an altitude of 1,810-2,700 m above sea level (Figure 2).

Procedures

Sample preparation and lichen exposure treatments

Parmotrema perlatum is a fruticose lichen that has been chosen as a species bioindicator (Roziaty et al. 2020). Epiphytes on Pinus merkusii bark in Cemoro Sewu Forest, pH of tree bark 5. The samples were taken in the rainy season of November 2021. The average temperature was 21 degrees Celsius, and the humidity level was 60 to 70% (Lackovičová et al. 2013). Lichens were collected from 32 different host trees. The lichen samples were then taken to the laboratory for spectrophotometric analysis of NO_2 and SO_2 (Koch et al. 2018) as well as chlorophyll content using (Adams and Gottardo 2012). In this study, smoke from diesel-fueled motor vehicles was released in 2012. The vehicles had been tested for emissions beforehand with test results below the quality standards for test vehicles based on government regulations. Lichens were previously fumigated with diesel-fueled motor vehicle emissions, modified from (Root et al. 2021).

According to the research method, the fumigation lasted three hours (Kuldeep and Prodyut 2015). This study develops research on the long period of time for fumigation so that fumigation is developed into 7 levels, depending on the length of exposure per hour (Cecconi et al. 2019). The levels of fumigations were categorized based on the length of exposure into: 0 (control); 1 (1.5 hours); 2 (3 hours); 3 (4.5 hours); 4 (6 hours), 5 (7.5 hours), and 6 (9 hours) as modification from Root et al. (2021). All levels were conducted three time (n=3) and indicated as R_1, R_2, and R_3 (Figure 3) (Riddell et al. 2012).

Lichens were arranged in a modified plastic box with a capacity of 108.376 cm^3 (62 cm x 46 cm x 38 cm). The sampled lichen thallus was placed on a sheet of A4 paper covered with cardboard so that it could be neatly arranged on the wall of the box. The container has a volume of 108,376 cm^3 (62 cm x 46 cm x 38 cm). The container was then fumigated. This method expands the method from Riddell et al. (2012).

Physiological analyses

Lichens were then soaked in 90 % acetone to determine the chlorophyll a and b values (Table 1). For the chlorophyll analysis, the spectrophotometer used wavelengths ranging from 645 to 663 nm (Caesar et al. 2018). The spectrophotometer was a double beam, with a wavelength range of 190-1,100 nm.

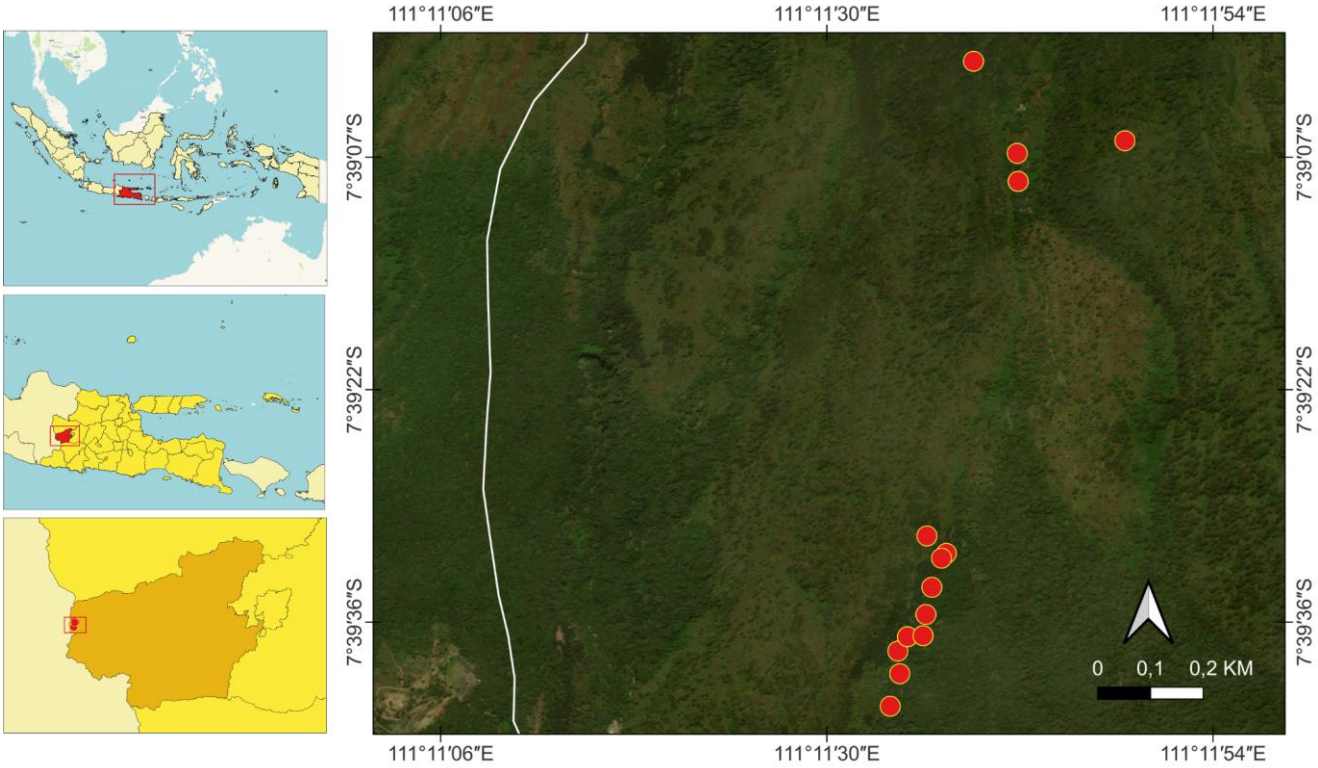

Figure 2. Map of the research location of Mount Lawu, Cemoro Sewu, Magetan, East Java, Indonesia

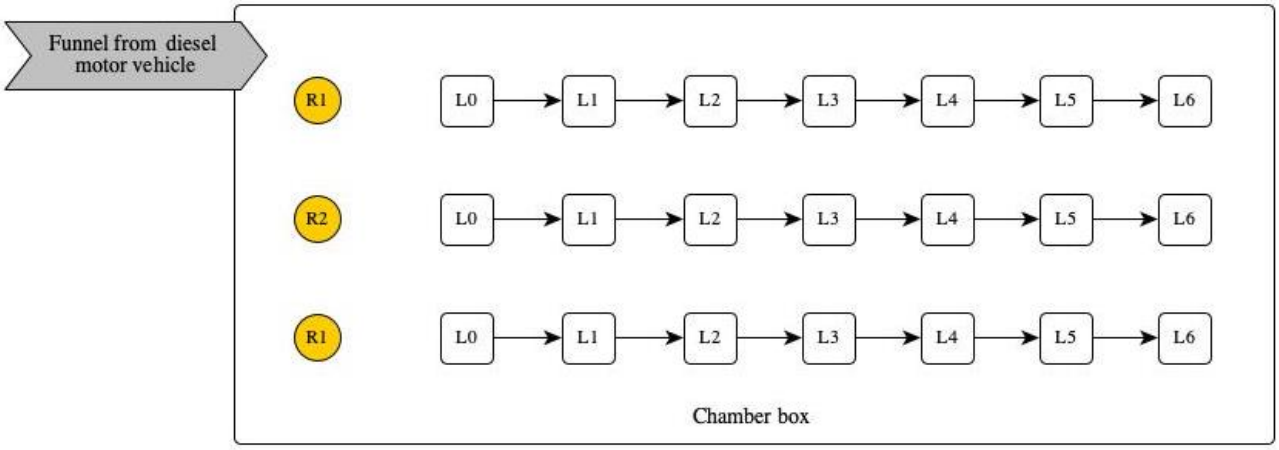

Figure 3. Diagram of experiments conducted on lichen in 6 levels for 9 hours

Table 1. Chlorophyll formulas for lichens using acetone solvent

Author	Solvent	Chlorophyll (µg mL^{-1})
Rowan (1989); Ritchie (2006)	90% acetone	Chlorophyll a, b, a + b, and the ratio of a/b
Porra et al. (1989); Pfeifhofer and Kranner (2002)	Formulae for chlorophyll concentration (µg ml^{-1}):	
	Chl a = 12.25 A^{663} - 2.85 A^{645}	
	Chl b = 20.31 A^{645} - 4.91 A^{663}	
	Chl a + b = 17.76 A^{645} - 7.34 A^{663}	
	Chl a/b (R') = *Chl a/Chl b*	

Preparation of nitrogen dioxide (NO_2) samples. The thallus lichens that were still attached to the stem at each deposition location were separated. The lichens were then washed with running water, dried using tissue paper, and then weighed 5 mg each using a digital scale. The lichens were cut into small pieces and then soaked in 10 ml of 90 % ethanol for 10 minutes. The extracts were filtered using filter paper and added ethanol again until reaching 10 ml in size. The extracts were measured with a spectrophotometer with a wavelength of 328 nm three times for each lichen species. Before the new samples (species) were measured, the cuvette was washed and rinsed using distilled water. The spectrometer was then calibrated using ethanol filled in on the cuvette. After the number showed a new 0, the chlorophyll sample measurements were repeated until completed (Lovadi et al. 2012).

Preparation of sulfur dioxide (SO_2) samples. The thallus lichens were separated from the stem and then washed with running water and dried with tissue paper. Each sample of thallus lichen was weighed 5 mg using a digital scale. The lichens were cut into small pieces and then soaked in 10 ml EDTA solution 0.001M for 10 minutes. The extracts were sieved using filter paper and added again with EDTA solution 0.001 M until reaching 10 ml in size. The extracts were measured with a spectrophotometer with a wavelength of 423 nm and repeated three times. The cuvette was first rinsed with distilled water and the spectrometer was calibrated with a 0.0001 M EDTA solution filled in the cuvette. After the number showed a new 0, the chlorophyll sample measurements were repeated until completed (Hamutoğlu et al. 2020).

Preparation for the analysis of chlorophyll samples in thallus lichens. The tallus lichens were separated from the stem, rinsed with running water, dried with tissue paper, and then weighed 5 mg each with a digital scale. The lichens were cut into small pieces and then soaked in 10 ml of 90% acetone (Zrnzević et al. 2017) for 10 minutes. The extracts were filtered with filter paper and then added again with ethanol until reaching a size of 10 ml. The extracts were then measured with a spectrophotometer at a wavelength of 645 nm and 663 nm and repeated 3 times for each sample (Tabel 1).

Before the new samples/species were measured, the cuvette was cleaned using distilled water, and the spectrometer was calibrated using ethanol put into the cuvette. After the value was 0, the chlorophyll sample measurement was repeated until completed.

RESULTS AND DISCUSSION

Nitrogen dioxide and sulfur dioxide in the thallus

The nitrogen dioxide gas content in the samples of *P. perlatum* is presented in Table 2. The lowest NO_2 content was found in the control group (level 0). After smoke exposure treatment was given, the lowest NO_2 content was 2.673 mg m^{-3} while the highest content was 4.000 mg m^{-3}. Exposure to smoke for three hours (Level 2) produced 3.324 mg $m^{-3.}$ In plants, the critical SO_2 value is 10-30 µg m^{-3} (0.01-0.03 mg m^{-3}). The high SO_2 concentration disrupts the airflow between the cell layers in thallus lichens. This causes water deficiency (dehydration) in the thallus as well as other active physiological disorders, such as impaired respiratory processes (Araújo et al. 2015). The critical level of NO_2 in a lichen is 30 µg m^{-3} (Atator et al. 2021).

Parmotrema perlatum will show a change in the thallus color, which is getting darker at the apex and rolling because it is thinning, the thallus shrinks, and the very end will disappear. These symptoms also appear in the lichen foliose group, particularly the Parmeliaceae family (Araújo et al. 2015). These morphological changes are linked to the process of pollutant absorption by the surface of thallus lichens (Naeth et al. 2016).

Sulfur dioxide (SO_2) causes disruptions in the movement of plasma within cells. This will affect the plasmolysis process. Cells containing a dissolved homogeneous mass in this compartment will cause discoloration and damage to the chlorophyll found in the thallus. This need disrupts the process of photosynthesis. SO_2 has both direct and indirect effects on lichens. The direct effect takes the form of chronic damage to the energy transfer system, which will inhibit the results of the photochemical process to CO_2 in the respiration process. SO_2 exposure will have an effect on chloroplasts. The acute effect of SO_2 is to directly attack the plasma of the cell. Lichens exposed to SO_2 for 24 hours at 5 ppm will cause chlorophyll cells in algae to bleach due to permanent plasmolysis that appears in thallus cells and then appears as brown spots on chloroplasts. When lichens are exposed to SO_2 in humid environments, the spots will multiply (Araújo et al. 2015).

The highest SO_2 content was found in the Level 6 group, which experienced the longest fumigation process for thallus lichens, while the lowest content was identified in the control and Level 1 groups (Figure 4). SO_2 is a bleaching agent for plants and can reduce pigment absorption, causing the pH value in thallus lichen cells to increase. Because of the high concentration of SO_2 in the thallus, the range of chlorophyll absorption to the waves is shorter, and the absorption value of chlorophyll decreases, which affects the photosynthesis process. SO_2 causes a disorder, known as the toxicity mechanism, in which algae cells bleach and convert chlorophyll to pheophytin. Along with increased SO_2 absorption comes an increase in pH in the thallus. This lichen condition explains why the rupture of chlorophyll produces strong acids in sulfuric acid in their bodies. Sulfuric acid is formed when oxygen in the atmosphere binds to sulfur oxide compounds (sulfuric acid). The chemical oxidation of chlorophyll induces quinone ferricyanide and other iron salts, resulting in chlorophyll bleaching in the blue and red bands (Pareek et al. 2018).

Sulfur dioxide has an impact on both vascular and lichen plants, with lichens being more sensitive to pollutants than vascular plants. Non-vascular plants have a mechanism that allows them to absorb all of the compounds present in the atmosphere across their entire body; therefore, lichens will indirectly accumulate pollutants in the atmosphere in their habitat. When exposed to smoke for more than 5 hours, stomata on the surface of the *P. perlatum* tissue open (Podaril and Colbert 2016).

Table 2. The nitrogen dioxide and sulfur dioxide content by three repetitions in the fumigation process to *P. perlatum* R1, R2, and R3 indicate repetition no 1, 2, and 3 respectively. Level 0 (control); level 1 (1.5 hours); level 2 (3 hours); level 3 (4.5 hours); level 4 (6 hours), level 5 (7.5 hours), and level 6 (9 hours)

	Nitrogen dioxide (NO_2) mg m^{-3}			Sulfur dioxide (SO_2) mg m^{-3}		
Exposure level	**(R_1)**	**(R_2)**	**(R_3)**	**(R_1)**	**(R_2)**	**(R_3)**
Level 0	0.00	0.00	0.00	0.00	0.00	0.00
Level 1	2.28	2.78	2.87	0.260	0.26	0.23
Level 2	2.51	2.81	2.91	0.281	0.28	0.23
Level 3	2.71	3.07	3.13	0.369	0.37	0.35
Level 4	2.98	3.21	3.46	0.478	0.48	0.32
Level 5	3.00	3.67	3.89	0.523	0.52	0.06
Level 6	3.50	3.98	4.00	0.558	0.56	0.59

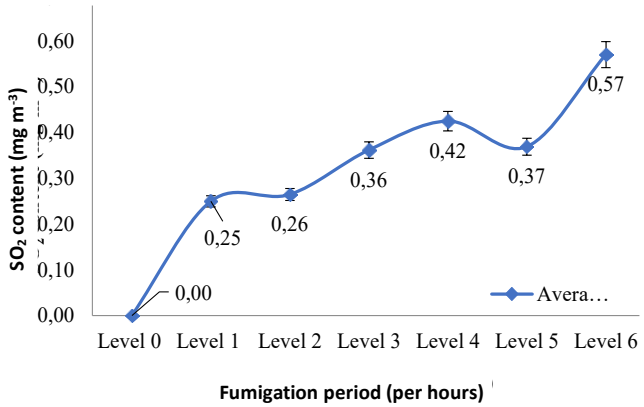

Figure 4. Content of Sulphur dioxide (SO_2) in *Parmotrema perlatum* (Huds.) M. Choisy

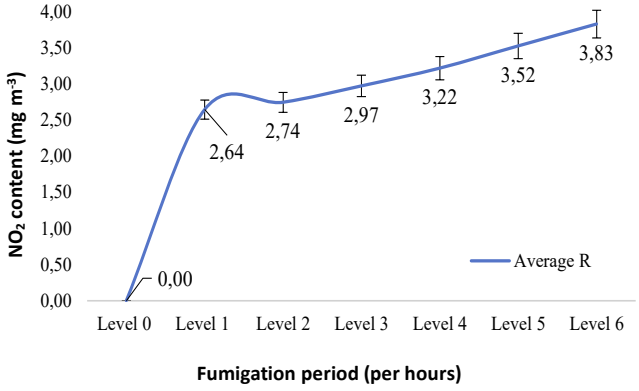

Figure 5. Nitrogen dioxide (NO_2) in thallus *P. perlatum* fumigated in various levels of exposure (Level 0-6)

The activities of urban communities have a significant effect on the climate in the urban environment. Lichen fumigation produces pigments that are mostly found in algae because photobionts degrade quickly. Furthermore, this exposure causes chlorophyll to bleach. *Parmotrema perlatum* are lichens that are sensitive to SO_2. Sulfur dioxide fumigation has an indirect effect on lichen by lowering the pH on the surface of the lichen substrating stems to grow. The average pH encountered is 5, with a peak of pH 3.5 shortly after fumigation. When the pH is very low, SO_2 dissolved in the rain is harmful to lichens, threatening the lichen propagules that will appear, causing only a few propagules to survive in very acidic habitat conditions (Tripp et al. 2016).

The NO_2 content in the thallus *P. perlatum* is detailed in Figure 5. The lowest content was found in the control group and the group with Level 1 fumigation. The inhibited value was 2.14 mg m^{-3} while the highest NO_2 content was 4.00 mg m^{-3}. NO_2 and SO_2 are chemical compounds that are produced when fossil fuels are burned. These gases are released into the atmosphere and accumulate in vegetation in urban areas. There is a positional correlation between NO_2 and SO_2 levels in the atmosphere (Atator et al. 2021). In Europe, atmospheric disturbances caused by increases in pollutant pollutants NO_2 and SO_2 are reported to be highly critical (Jayalal et al. 2017). Due to soil acidification and cation deficiency, the deposition of these compounds causes changes in nutrient compounds in the roots of the spruce tree and interferes with spruce growth and development. The photo-oxidation of chlorophyll in ethanol or acetone solution causes pH to decrease, which is similar to the chemical oxidation process of chlorophyll by SO_2.

Chlorophyll a and b in *Parmotrema perlatum*

The control group has the highest chlorophyll concentration. As with other plants, chlorophyll a is generally more dominant than chlorophyll b. The value of chlorophyll a in the treatment of vehicle smoke exposure of Level 3 (4.5 hours) remains high, reaching 1.45 µg ml^{-1}.

At Level 6, exposure to 9 hours of vehicle smoke reduces both chlorophylls a and b. This dissertation describes a change in the morphological appearance of the thallus end, which is black brown, and rolled. Chlorophyll a + b is a combination of chlorophyll content found in both vascular and non-vascular plants. The highest total chlorophyll (chlorophyll a + b) content of *P. perlatum* is in the control group (Level 0), followed by the stacking at Levels 3 and 1. The chlorophyll a/b ratio is the highest among all chlorophylls. Table 3 presents that chlorophyll a dominates chlorophyll b in thallus cells. Level 2 fumigation produces a chlorophyll value of 3.554 µg mL after three hours of exposure. Table 3 shows the different reactions of chlorophyll a and b in thallus lichen treated by fumigation and exposure to vehicle emissions. The exposure of lichens to smoke during the fumigation process reduces their photosynthetic capacity.

Table 3. The concentration of chlorophyll a and b to different periods of fumigation and exposure to motor vehicle fumes. All experiments were conducted with triplicate (n=3). A663= wavelength (nm) 663; A645=wavelength (nm) 645; chl a, chl b and chl a + b : chlorophyll a, chlorophyll b and chlorophyll a + b

Fumigation	Wavelengths on a spectrophotometer (nm)		The concentration of chlorophyll (µg ml^{-1})		
	A^{663}	A^{645}	chl a	chl b	chl a + b
Level 0	0.117	0.054	1.29	0.36	1.65
Level 1	0.090	0.047	0.68	0.35	1.03
Level 2	0.078	0.031	0.61	0.27	0.88
Level 3	0.103	0.044	0.55	0.26	0.81
Level 4	0.092	0.045	0.52	0.22	0.74
Level 5	0.064	0.035	0.48	0.17	0.65
Level 6	0.056	0.027	0.43	0.15	0.57

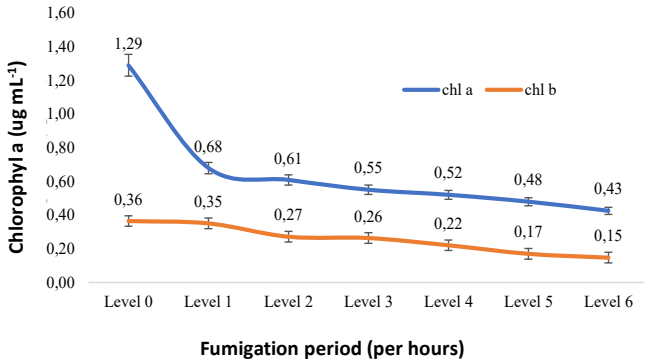

Figure 6. The chlorophyll a and b content decreased along with the increasing exposure of fumigation level (Level 1 to 6) to the thallus of *P. perlatum*

Table 4. Pearson Correlation and Regression (R) values between the length of fumigation with NO_2 and SO_2 and chlorophyll a + b. ns = non-significant; * P <= 5 %; ** P <= 1 %; *** P <= 0.01 - 0.05 %

Parameters	Correlation	Regression (*R*)
NO_2, SO_2	+	0.0699 ^{ns}
Fumigation, NO_2	+	0.9458 ***
Fumigation, SO_2	+	0.8606 ***
Fumigation, NO_2, Chl a + b	+	0.9458 ***
Fumigation, SO_2, Chl a + b	+	0.6082 ***

The content of chlorophyll decreases with the length of smoke pollutant exposure to the thallus *P. perlatum* of nine hours exerts morphological and physiological influences on lichens (Figure 6). Chlorophyll a + b and the chlorophyll a/b ratio constitute the mass or molar amount of chlorophyll molecules. Chlorophyll a + b is the determination between the molecules of chlorophyll a and b in the thallus *P. perlatum*. Chlorophyll a + b and the chlorophyll a/b ratio indicate consistency (Table 2) (Caesar et al. 2018). The chlorophyll content in thallus lichens is highly dependent on the environmental pressures under which the species lives. The lower the chlorophyll content, the greater the environmental pressure on the lichens.

Chlorophyll b content experienced a decrease, increased at Level 3, and then decreased again due to fumigation exposure to motor vehicle smoke (Figure 6). Measurements of SO_2 content revealed that this compound accumulates the most in chloroplasts in response to a decrease in plasma pH in plants. This will interfere with physiological mechanisms, particularly photosynthesis. Although the rate of photosynthesis remains constant, the accumulation of compounds in plasma cells will result in a decrease in the quality of chlorophyll produced by photosynthesis (Paoli et al. 2015).

Correlation between parameters in the research

The degradation of chlorophyll has been reported to be delayed as a result of SO_2 fumigation performed on lichens. This is because chlorophyll is detected to be at the stage of breakdown within 24 hours of fumigation (Table 4). The next day, the breakdown of chlorophyll will increase, so that on the fourth day, fumigation of chlorophyll is detected reduced due to component damage. The chlorophyll molecules have changed within 24 hours, as evidenced by the change in chlorophyll fluorescence parameters as the photosynthesis process begins (Sett and Kundu 2016). If left untreated for an extended period of time, this damage will kill the lichens because photobionts are extremely sensitive to chlorophyll degradation (Zhang et al. 2014).

Table 4 details three statistical parameters that show the relationship between fumigation parameters at six different levels (0-9 exposure/hour) and NO_2 and SO_2 pollutants and chlorophyll content. Positive correlations can be found for all five types of parameters. This indicates that the five parameters are related to one another. The presence of these two pollutants is indicated by the NO_2 and SO_2 parameters. These compounds are detected in motor vehicle emissions, particularly those derived from diesel fuel. The disclosed correlation is a straight line with a P value of 0.0699. The highest R-value as the ratio of F, NO_2, and Chlorophyll a + b was R = 0.9458. The correlation analysis of the research parameters obtained is greater than zero, indicating a positive correlation.

The correlation between NO_2 and SO_2 was insignificant because the NO_2 with SO_2 value was the same as (P value = 5 %), with the lowest value of 0.0699. The treatment of

fumigation correlates with all parameters, including NO_2, SO_2, and chlorophyll. A significant influence was obtained in this study on the fumigation treatment of thallus lichens exposed to pollutants. NO_2 and SO_2 have a high correlation value, as well as the correlation value between *P. perlatum* and chlorophyll a + b.

The *P. perlatum* lichens respond positively to exposure to environmental pollutants, specifically NO_2 and SO_2, which are derived from motor vehicle fumes. Pollutants and chlorophyll have a positive correlation, as evidenced by correlation tests that show values between the three components, namely fumigation, NO_2, SO_2, and chlorophyll.

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