Exploration of Parmotrema tinctorum and Leptogium sp. lichens as bioindicator of air quality in Mekarsari fruit park

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Abstract. Lichens are often used as bioindicator to monitor air quality. This study aims to use the Mekarsari Fruit Park area as a model to analyse the population of Parmotrema tinctorum and Leptogium, and considering the possibility of using them as bioindicator of air quality. The method used is descriptive exploratory with survey techniques. The observation plots were consisted of 3 zones, each of 1000 trees. The samples of each plot were identified morphologically and chemically and then the lead contents were determined using Atomic Absorption Spectrophotometry. Parmotrema tinctorum were found in all zones but Leptogium only found in zone 3. Lead contents of P. tinctorum thalli in each zone of 1, 2, and 3 were 13.34, 24.87, and 23.61 ppm with the number of total thalli were 8, 223, and 469, the thalli area of 3369.91 cm², 39259.48 cm², and 98984.97 cm² and the average thallus area (ATA) of 1.3, 13.8, and 17.2 cm². P. tinctorum tends to be tolerant lichen, because the lead content increasing of 43-46% caused increasing of 96-98% thalli number, 91-97% of thalli area, and 91-92% of ATA. Although Leptogium spp. are known as sensitive lichens, it occurred in higher lead concentration zone.

Keywords: air quality, bioindicator, lead (Pb), Leptogium, Parmotrema tinctorum

1. Introduction
Lichens is a symbiosis of mutualism between fungus and algae or cyanobacteria, or both. Lichens has been widely used, including for food, medicinal ingredients, perfume, bioindicator, and decoration. Lichen is known to be sensitive to environmental change, especially air pollution. Many researchers classify lichens based on its tolerancy to pollution. Sensitive lichens are a type of lichen that are only found in the area with good air quality or low pollution, such as Leptogium, Coccocarpia, and Lobaria pulmonaria. Intermediate lichens and tolerant lichens are types of lichen which can live in the environment with moderate until high air pollution [1, 2]. Different with plant, lichens do not have a special organ to absorb particles and protective structures such as cuticles, so that lichens could not prevent the absorption of certain air particles, including pollutants [3].

Motor vehicle fumes contribute greatly to the air pollution in big cities. The pollutants produced by motor vehicles are carbon monoxide (CO), hydrocarbons (HC), nitrogen oxide (NOₓ), sulfur oxide (SOₓ), and lead (Pb) that can cause health problems to organism [4]. Fuel burning activities in industrial activities can also donate these gases to the atmosphere. High content of sulfur dioxide...
(SO₂) in the atmosphere is the main cause of declining population and quality of lichen thalli in polluted areas. Besides that, the sensitivity to NO₂, O₃, NH₃, and heavy metals play a role in declining population and lichen diversity [5].

Many countries in the northern hemisphere use lichens as long-term biomonitoring material. Monitoring air pollution using that lichen is done by measuring the content of chlorophyll at certain levels of air pollution from time to time [6, 7, 8], or comparing changes in lichen diversity due to changes in pollutant concentration in the atmosphere [9, 10, 11]. Indonesia is a tropical country with high biodiversity, but the research about lichens and air pollution is still a little done compared to other countries. Therefore, this research is expected to increase knowledge about lichen in Indonesia.

Mekarsari Fruit Park is one of the tropical fruit biodiversity conservation centers, located in the Sub-district of Cileungsi, Bogor, West Java, Indonesia. Mekarsari Fruit Park has an area of 264 Ha, located in the lowlands at the height of 70 meter above the sea level with the rainfall of 3500-4000 mm per year. The daily average temperature in January – March is about 31-35°C with the moisture of 60-70%, and light intensity of 10000-20000 lux. Mekarsari Fruit Park is located in the middle of a residential area that is densely populated by human activities, surrounded by roads, close to a metal processing plant. Mekarsari Fruit Park has lots of vegetation which were generally quite old (around 20 years), so that is alleged to have a large populations of lichens. Were hypothesized that locations are closer to pollution sources or contain more pollutants will have fewer lichens population. Besides Mekarsari Fruit Park, the research about lichen and air quality in Bogor Botanical Garden and Cibodas Botanical Garden has been conducted [12]. So that this research is expected to be able to add information about lichen population and ecology in Indonesia. The aims of this research is to analyze air pollution effect on lichens and consider the opportunities for using lichen as the bioindicator to monitor air quality, by exploring population of Parmotrema tinctorum and Leptogium lichens in Mekarsari Fruit Park, and analyze relationship between lead content on thalli with thalli population. This preliminary result is used for further research, which can explain the relationship between lead content in thalli and lead in the atmosphere.

2. Materials and methods
The research was conducted from December 2018 until May 2019. The method used was descriptive exploratory with survey technique. Three observation zones were used, each of them had a certain distance from the source of pollution. Zone 1 was assumed to be an area with high levels of air pollution because the edges was close to Jonggol-Cileungsi Highway (0-650 m from the highway), that has a high frequency of vehicle activities. Zone 2 was assumed to be an area far from the highway, but the edges were surrounded by residential area and road with lower frequency of vehicle activities. Zone 3 was located far from the highway but is located ±250 m from metal processing plant, and the edges were surrounded with other roads which has lower vehicle activity frequency (figure 1). The data of lichen population taken was Parmotrema tinctorum and Leptogium. The number of trees observed in each zone were 1000 trees. The samples taken were lichen which grows on tree trunks and branches, starting from 0 until the height of 2 meters, as an observation plots. Tree surface area or observation plot were not exactly cylindrical, it will be difficult to calculate the tree surface area surely. The largest total thalli area in a tree was regarded as observation plot area, which is 0.089 m². Each zone consists of 1000 trees, so the area of observation plots per zone was 89 m².

Identify the types of lichens morphologically was done by using stereomicroscope. Observed lichens characters matched with key identification by Sipman (2003) and Ellix (1994) [13, 14]. Chemical identification was done by using spot test reagent reaction using potassium hydroxide (KOH) 10% and sodium hypochlorite (NaClO) 5.25% (“Bayclin”) on the cortex and medulla thalli. Identification with UV light used wavelength of 302 and 365 nm in the cortex and medulla thalli. The measurement of lead (Pb) content in Parmotrema tinctorum thalli uses Atomic Absorption Spectrophotometry (AAS) at Terpadu IPB Baranangsiang Laboratory.

Population or the area of thalli were measured by drawing the circumference of lichen thalli using transparent plastic. This method are similar to Mickle 1970, but uses aluminium foil. The area of
thalli was calculated by cutting transparent plastics, the plastics were then weighed by using analytic scale. The result in weight (g) were converted to area (cm$^2$), 0.008 g is equal to 1 cm$^2$. Besides using analytic scale, thalli area measurements was also done by Image J application. Plastics of thalli tracing results were photographed, then the photos were put in Image J. The area of thalli used was the average of calculation result by using analytic scale and Image J. The parameters of population were the frequency of lichen (F), distribution of tree species covered with lichens, total thalli number (TTN), total thalli area (TTA), average thallus area (ATA), and thalli area per m$^2$ observation plot. Thalli area per m$^2$ observation plot is calculated with the following formula. The observation plot used was 89 m$^2$.

\[
\text{Thalli area per m}^2 \text{ observation plot} = \frac{1 \text{ m}^2 \times \text{ total thalli area in n zone (cm}^2\)}{\text{Observation plot (m}^2\)}
\]

![Figure 1. Observation zone map at Mekarsari Fruit Park](image)

3. Results and discussion

The result shows that Parmotrema tincorum was found in all zones, meanwhile Leptogium sp. was only found in zone 3. Besides P. tincorum, Parmotrema sancti-angelii was also found in Mekarsari Fruit Park (MFP) which grew abundantly in all zones. The highest frequency of P. tincorum was found in zone 3, which were found in 72 trees. The lowest frequency of P. tincorum was found at zone 1, which was only found in 3 trees, while in zone 2 it was found in 58 trees. P. tincorum in zone 1 was found at a distance of 650 meter from the highway, meanwhile at a distance of 0 until 649 meter from the highway, has not yet been found. Leptogium sp. was found in zone 3, and was only found in 1 tree, with total thalli area was 353.93 cm$^2$.

P. tincorum population in MFP was distributed on durian trees (Durio zibethinus), mango (Mangifera indica), rambutan (Nephelium lappaceum), jackfruit (Artocarpus heterophyllus), nona (Annona squamosa) and coconut (Cocos nucifera) tree, meanwhile Leptogium sp. was only found in rambutan tree. P. tincorum population in zones 2 and 3 were concentrated growing on durian trees with the highest total thalli area. Besides durian trees, many P. tincorum also grew on mango trees in zone 3 (figure 2). It seems durian trees (Malvaceae) and mango trees (Anacardiaceae) were suitable substrate for P. tincorum growth. Before this study it was reported that P. tincorum was also found in family Podocarpaceae, Myrtaceae, and Araucariaceae in Cibodas Botanical Garden and walnut trees (Canarium sp.) in Bogor Botanical Garden [12, 15]. This indicates that P. tincorum had a broadly host distribution or substrate. Besides tree types, the tree age which was quite old causing abundant population of P. tincorum in MFP. The average age of trees in MFP was 20 years old. Old tree age gives the opportunity for lichen to grow, because lichen has a slow growth, so that it takes years to
colonize a substrate. The growth and colonization of lichen is affected by vegetation type, host age, and substrate condition [16].

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Distribution of Parmotrema tinctorum on different tree species in MFP.}
\end{figure}

*P. tinctorum* has the highest total thalli number (TTN) and total thalli area (TTA) in zone 3, which was 469 thalli with 98984.97 cm² total area, meanwhile zone 1 has the smallest TTN and TTA, which was 8 thalli with 3369.91 cm² total area (figure 2). Zone 1 had smallest average thalli area (ATA), while zone 3 had a largest ATA. It means that, zone 1 had smallest population, while zone 3 had a largest population of *P. tinctorum* in MFP. Based on thalli area per m² observation plot, zone 1 also has the smallest thalli area /m², and zone 3 has the largest thalli area/ m². Outline, zone 1 had the least *P. tinctorum* population and it was not found in the area near the road area.

Zone 3 has a higher total thalli area than zone 2, it was cause by zone 3 has more open canopy than zone 2. Study in India forest, *Quercus semecarpifolia* trees in open canopy forest had maximum lichens cover (70%), meanwhile closed canopy forest only had 40% lichens cover [16]. Open canopy causes more sunlight received by lichen thalli. Sunlight is needed for photosynthesis process done by photobiont. *Usnea rubescens* had growth faster at a higher light intensity (5000 lux) compared to a lower light intensity (500 lux) [17].

The highest lead content was found in *P. tinctorum* thalli in zone 2, which was 24.87 ppm, meanwhile the least was found in zone 1 which was 13.34 ppm. (figure 3). The lead content in the zone 2 was not much different from zone 3 (23.61 ppm), which was the difference of 1.2 ppm. The result showed nonconformity with initial assumption, which was the first zone with highest lead content, because the location was adjacent to the highway. The lower concentration of lead in zone 1 was caused by *P. tinctorum* thalli samples taken from a distance of 650 m from the highway, so that the concentration of lead atmosphere had declined as it goes away from the source of pollution. The high lead content of thalli in zone 2 and 3, there were suspected by other lead source, such as metal processing plant which was located 250 m from zone 3, and other roads surrounded zones 2 and 3.

The TTA, TTN, and ATA of *P. tinctorum* showed an association with lead content on thalli. The smallest lead thalli content had the smallest population (zone 1), while an increase in lead thalli content caused an increase in population (zones 2 and 3). The increase of lead content of 43-46%, caused a TTA increase of 91-97%, an increasing of TTN by 96-98%, and an increasing of ATA by 91-92% (figure 4). It was thought that the high lead content of thalli did not cause extensive decline the population by shrinkage of the size of thalli. High lead thalli content might be considered to stimulate thallus growth, but this statement is still to be confirmed by population pattern in other location. Based on a study with copper (Cu), there were lichens whose population decreases with increasing heavy metal content such as *Hypogymnia physoides*, but there was also a population of lichens whose numbers were not affected by heavy metal content such as *Tuckermanopsis specincola*. [18]. In contrast *P. tinctorum* is sensitive to SO₂, its existence is threatened. It was not even found in urban areas with
average annual SO$_2$ concentration more than 20 ppb [8, 19]. A high content of SO$_2$ caused disintegration of cortex, inhibit lobule formation, and lower of secondary metabolites [8].

The presence of air pollution is not the only one cause of differences in lichens population. The growth and colonization of lichen is also affected by terrain variation, vegetation type, human disorder, light intensity, humidity, host age, and substrate condition [16]. The abundant population of \textit{P. tinctorum} in zone 2 and 3 was assumed by the presence of host trees. Many \textit{P. tinctorum} were found growing in durian and mango trees. Zone 2 and 3 has more durian trees compared to zone 1, it causes \textit{P. tinctorum} population was higher in zone 2 and 3. It had been discussed earlier that \textit{P. tinctorum} had extensive host tree distribution, so that it is very possible to grow in trees besides durian and mango trees. The further research is needed to be done.

Lichens that grow in polluted areas and can accumulate heavy metals (Cu, Zn, Pb, Cd, Mn) in large quantities are called metal tolerance lichens. Resistant lichens can accumulate a lot of heavy metals but the chlorophyll contents were slightly affected or not affected by the presence of heavy metals [20, 21]. \textit{Hypocenomyce scalaris} (320 ppm Pb), \textit{Lepraria elobata} (718 ppm Pb), and \textit{Furcata cladonia} (331 ppm Pb) were metal-tolerant lichens and the photosynthetic pigment showed only a slight change due to high concentrations of Zn or Pb [22, 23]. Every lichen has a different level of tolerance. \textit{Hypogymnia physoides} is also categorized as tolerant lichen but had a lead accumulation limit of up to 58 ppm of lead [24]. In contrast to sensitive lichens, exposure of 0.01 ppm lead in sensitive lichens caused chlorophyll degradation [25]. The accumulation of lead metal in sensitive lichens of \textit{Lobaria pulmonaria} had been reported as much as 9.76 ppm.

Lead accumulation on \textit{P. tinctorum} is lower when compared to other metal tolerant lichens, for example on \textit{Hypocenomyce scalaris}, \textit{Lepraria elobata}, and \textit{Cladonia furcate}. An increase in lead content does not decrease the population of \textit{P. tinctorum}, whereas thalli with high lead content actually had a larger population. Based on these results, \textit{P. tinctorum} could be categorized as lichen that tends to be tolerant of lead. This result also was supported by a study of \textit{P. tinctorum} in Cibodas Botanical Garden with the thalli area per m$^2$ observation plot was 22.86 cm$^2$ per m$^2$ [15] which was very small when compared with \textit{P. tinctorum} population in MFP (figure 2). Cibodas Botanical Garden is located far from pollution source, and assumed has a better air quality than MFP. Besides calculating the population, to determine the sensitivity of \textit{P. tinctorum} to lead, a study of lead effects on physiological thallus is also needed with the calculation of chlorophyll content on photobiont.

It had been reported previously that cyanolichens (cyanobacteria photobionts) was more sensitive to heavy metal compared to phycolichens (algae photobionts) [26]. \textit{Leptogium} is a cyanolichen, but found in zone 3 with higher thalli lead content of \textit{P. tinctorum}. This Lichen population was declining in Singapore that it was indicated a sign of changing air quality [20]. The presence of \textit{Leptogium} in a location could be a sign of good air quality. The population of \textit{Leptogium} sp. which was found in zone 3 was also very little, this might be a sign of starting changes in air quality due to pollution. Research in the Bogor Botanical Gardens showed that \textit{Leptogium} sp. was only found in areas far from pollution sources (controls) observed in walnut trees, and not found in walnut trees in areas adjacent to pollution sources [12]. The research of \textit{Peltigera canina} showed that cyanolichen could grow in polluted areas. \textit{Peltigera canina} had tolerance mechanism by accumulating lead extracellularly, so that the effect of lead to photobiont could be minimized. Mycobiont of \textit{P. canina} contained more chitin so that Pb could be tied and accumulated extracellularly. \textit{Leptogium} sp. was also assumed to have the same mechanism with \textit{Peltigera canina}, so that it could grow eventhough in the environment with lead which was relatively higher [20]. Therefore, the existence of \textit{Leptogium} is needed to be further study and the research on \textit{Leptogium} is in progress.

Lichens has slow growth, the lead content in the thallus is an accumulation many years. The high lead content of the thallus has the possibility of high lead in the atmosphere, because the lead content in the thallus comes from the atmosphere. Lead measurements on thallus are thought to be more reliable for measuring air quality, because measurements of lead in the atmosphere are fluctuating over the time because many factors such as wind direction and speed. Based on lead content that zone 1 had lower air pollutant levels than zones 2 and 3, because zone 1 had the smallest lead content on the
thallus. Studies on lichen *Xanthoparmelia xanthofarinosa* showed a positive relationship between lead on thallus and lead in the atmosphere with a correlation coefficient of 0.657 [21].

**Figure 3.** Comparison of total thalli area (TTA) with Pb thalli content (a), total thalli number (TTN) with Pb thalli content (b), average thallus area (ATA) with Pb thalli content (c), and comparison of thalli area per m² in three zones (d). Different letter indicate significantly different (p<0.05).

**Figure 4.** Comparison of Pb thalli content, thalli number, thalli area, and average thallus area in three zones.
Unlike plants, the source of lead on lichens comes from the atmosphere, while the source of lead on plants comes from the atmosphere and soil. Plants tend to absorb less lead from the atmosphere compared to lichens because they have a cuticle layer on the leaf surface as a protective structure from exposure to pollutants in the atmosphere [27]. The lead content in roadside plants could reach 50 ppm [28]. A study showed that in the same atmospheric conditions, tree bark (6.3 ppm) accumulated less lead than lichen thallus (17 ppm) [29]. On the other hand, similar to lichens, there are also plants that can accumulate lead at high concentrations, such as the leaves of the angsana tree (326.24 ppm) and leaves of mahogany trees (80.75 ppm) [30]. Therefore, measurement of lead content on trees, especially durian trees is needed to be done in future studies, so that it become a comparison between lead accumulation in lichen thalli and tree organs.

Lichens are not suitable to be used as certain pollutant indicator [31]. Certain lichen species may be affected with certain pollutants presence in the air, but not affected by other pollutant types. That statement was supported by the research of Cladonia convoluta and Cladonia furcata in relationship of chlorophyll contents and heavy metals of Cu, Pb, and Zn. C. convoluta and C. furcata did not show the changes of significant chlorophyll contents as the changes of Pb concentration. However, the content of chlorophyll changes as the changes of Cu and Zn concentration in the atmosphere. That matter shows that C. convolute and C. furcata could be used as bioindicator of Cu and Zn changes, but could not be used as the indicator for Pb [6].

4. Conclusion

Parmotrema tinctorum was abundant at Mekarsari Fruit Park. P. tinctorum population was most found in durian and mango trees. Leptogium sp. was only found in rambutan trees in the zone 3. The concentration of Pb in thalli showed the relationship with the population. The increase of Pb content caused the increase of thalli area, thalli number, and average thalli area. So that P. tinctorum could be categorized as a lichen that tends to be tolerant of lead. Leptogium sp. is known as a sensitive lichen, but was only found in zone 3. The presence of Leptogium sp. was assumed to be affected by other factors which were not analysed yet. The analysis of chlorophyll contents on P. tinctorum, lead contents on trees and on Leptogium sp. is needed to be done in the further study in order to determine the sensitivity of P. tinctorum and Leptogium sp. clearly.

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