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Air Quality Bioindicator Using the Population of Epiphytic Macrolichens in Bogor City, West Java

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Studies about lichens and pollution in South-East Asia are restricted because the lichens are poorly known. A research project about air quality bioindicator using epiphytic macrolichens in Bogor City was conducted from March 2012 until July 2013. Purposive sampling method was applied in 3 plots: plot 1 was in the centre of Bogor Botanical Garden (BBG) that far from busy roads, plot 2 was in a part of BBG adjacent to main and busy roads, and plot 3 was along busy roads and near a factory. In each plot, macrolichens were observed in 8 canary trees using 2 mini quadrats (32 x 20 cm^2). The population conditions of epiphytic macrolichens were analyzed and to be used as bioindicator of air quality. Seven genera of macrolichens were found: *Coccocarpia*, *Leptogium*, *Canoparmelia*, *Parmotrema*, *Dirinaria*, *Physcia*, and *Pyxine*. Plot 1 was having *Coccocarpia* and *Leptogium* that were not found in other plots and therefore they can be used as sensitive bioindicators, none of *Canoparmelia* and *Pyxine*, a few and infrequent *Dirinaria* but with larger average coverage (AC = 6.15 cm^2), and *Physcia* was found abundantly (sensitive bioindicator). Conversely, plot 2 and 3 were having none of *Coccocarpia* and *Leptogium*, few or many *Canoparmelia* and *Pyxine* and therefore appeared to be tolerant, many and frequent *Dirinaria* but with smaller AC (plot 2 = 2.85 cm^2, plot 3 = 1.16 cm^2), and few or none of *Physcia* was found. Being found in all plots, *Dirinaria* showed a clear pattern of increasing thallus number and decreasing AC from plot 1 to plot 3, so it can be used as tolerant bioindicator. Conversely, *Parmotrema* had unclear pattern of population condition. Similar researches on different trees are necessary to explore more details regarding epiphytic macrolichens population condition.

Keywords: bioindicator, Bogor City, canary trees, macrolichens, population analysis

INTRODUCTION

Lichens are symbiotic organisms consisting of a fungus (the mycobiont), and one or more algae (the photobiont). Lichens are known to be sensitive to environmental changes, for example air pollution. This feature is related with their ability to accumulate airborne particles. Unlike flowering plants, lichens mostly have no special organs for water absorption (*poikilohydric* condition) and have no protective waxy outer cuticle, and therefore have little control over their water content. It explain the sensitivities of lichens to atmospheric pollutants, since they absorbs any particulate matter and gases from environment passively, including pollutants (Bates 2002).

For over forty years, research about lichens and air pollution has been done especially in northern hemisphere countries. Nowadays, lichens are widely used as economical and long-term biomonitoring tools in many countries. Thousands of publications about lichens and air pollution research are available (Bates 2002). In South East Asia, one of the countries with significant progress in lichen research is Thailand. The country has, for example, research conducted by Saipunkaew *et al.* (2005, 2006). Meanwhile, Indonesia is lagging behind, with only few studies that focused on lichens, especially for lichen secondary metabolites (Kusumaningrum *et al.* 2011). Vietnam has made better progress, where many species have been reported or described recently (Jayalal *et al.* 2013). Air quality monitoring more often uses macrolichens, which are lichens with foliose and fruticose life form, as they are easier to analyze.

Bogor City was chosen for sampling because the city has a good vegetation cover, and therefore a more humid microclimate. Humidity is also favored by the elevation of several areas in Bogor City, for example Cimanggu and Baranangsiang, which are more than 200 meters above sea level (Effendy 2007). Because of that, the lichens still can grow healthily. In addition, the existence of Bogor Botanical Garden (BBG) as an open space contributes to the absorption of the heat of the city, and certainly is a good habitat for the lichens. In Singapore, the botanical garden is

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also the most important habitat for lichens (Sipman 2009). However, as the population and development of Bogor City increase, air pollution increases at the same time. Sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) are among the air pollutants that are dispersed in the city. They may be derived from fumes and aerosols from domestic heatings, exhaust from road transport, and long-distance transport of emission (Zahradníková 2010).

The present research was limited to only epiphytic macrolichens on canary trees (*Canarium* spp.). Canary trees are dominant, iconic, and also old trees in Bogor. The aim of the research was to investigate the possibility of using lichens for air quality bioindicator in Bogor City, by looking at its population pattern in three different plots.

MATERIALS AND METHODS

Methods and Sampling. This research was conducted from March 2012 until July 2013, using exploratory descriptive methods with survey technique. The sampling technique was purposive. Plots were determined based on air quality measurements from: several areas in Bogor City obtained from a dissertation by Santosa (2005), Bogor City Environmental Department (Badan Pengelolaan Lingkungan Hidup/BPLH) in 2012, and PT Goodyear Indonesia in September 2012. Tree location data from the Department of Sanitation and Garden (Dinas Kebersihan dan Pertamanan) of Bogor City was also determined. Three plots were selected. Plot 1 in the centre of Bogor Botanical Garden (BBG) was assumed as control area because it was located far from the traffic circulation. The criteria for a control or 'clean' area is that it features air free from anthropogenic influences and sources of air pollution, or at least close to that since it is not possible nowadays to guarantee that an area is totally free from pollution (Kularatne & de Freitas 2012). Plot 2 was still inside the BBG, but located on the edge of BBG, adjacent to Otto Iskandardinata Street with intense traffic circulation. Plot 3 was along Ahmad Yani and Pemuda Street with intense traffic and also located near PT Goodyear Indonesia factory. SO_2, NO_2, and carbon monoxide (CO) were pollutants that can be dispersed in plot 3 (PT Goodyear Indonesia 2012).

In each plot, we chose canary trees that had a girth above 60 cm (Saipunkaew *et al.* 2006). Since the tree species develops buttress roots, straight tree criteria could not be used. With eight trees on each plot, the total number of sampled trees used in this research was 24 canary trees. Lichens were sampled using a 32 x 20 cm^2 plastic quadrat, located on the main stem or a buttress side that were covered by many lichens. Two quadrates were used on each tree, placed on different sides of the tree. Every macrolichen found inside the quadrates was observed and counted. Lichen covers were measured by drawing the circumference of the whole thalli that were included in the quadrates, on a piece of transparent plastic. Sample pouches made from paper were used to collect samples of the macrolichens for further study. Samples were taken as little as possible, in view of the very slow growth rate of lichens.

Identification and Cover Measurement. Because there is a lack of lichen identification keys in tropic area, all macrolichen samples were identified only until genus level using stereomicroscope and light microscope. Observed characters were matched with Sipman (2003) and Divakar and Upreti (2005) identification keys. The samples were documented under the microscope using a digital camera. Confusing genera were identified by spot test reactions, with 10% potassium hydroxide (KOH) and "Bayclin" bleach solution.

In order to measure the coverage, each thallus that had been drawn was cut off and then weighed in an analytic scale. Weighing results were converted into centimeter square. This method is similar to the one that Mickle (1977) had done in Ohio, but using aluminum foil.

Data Analysis. Thallus numbers (TN), coverage (C), and average coverage (AC) were calculated. Many ecological parameters taken from literature with modifications (Opdyke *et al.* 2011) were also calculated: (i) Density (D: total thallus number of genus A/total quadrat area in the plot); (ii) Relative density (RD: density of genus A/total density of all genus found x 100%); (iii) Dominance (Do: total coverage of genus A/total quadrat area in the plot); (iv) Relative dominance (RDo: dominance of genus A/total dominance of all genus found x 100%); (v) Frequency 1 (F1: total thallus number of genus A/total thallus number of all genera found in the plot); (vi) Frequency 2 (F2: number of trees with genus A encountered/total tree number in the plot); (vii) Relative frequency 1 (RF1: frequency 1 of genus A/total frequency of all genus found x 100%); (viii) Relative frequency 2 (RF2: frequency 2 of genus A/total frequency of all genus found x 100%); (ix) Important Value Index 1 (IVI 1: relative density + relative dominance + relative frequency 1 of genus A); and (x) Important Value Index 2 (IVI 2: relative density + relative dominance + relative frequency 2 of genus A) .

RESULTS

Epiphytic Macrolichens on Canary Trees. We found seven genera of epiphytic macrolichens in canary trees that included in four families: Coccocarpiaceae comprise of *Coccocarpia*, Collemataceae comprise of *Leptogium*, Parmeliaceae comprise of *Canoparmelia* and *Parmotrema*, and Physciaceae comprise of *Dirinaria*, *Physcia*, and *Pyxine*. Figure 1 shows the macrolichen distribution in the three plots. Plot 1 contained 5 genera (*Coccocarpia*, *Leptogium*, *Parmotrema*, *Dirinaria,* and *Physcia)*; plot 2 contained 5 genera (*Canoparmelia*, *Parmotrema*, *Dirinaria*, *Physcia*, and *Pyxine*) and plot 3 contained 4 genera (*Canoparmelia*, *Parmotrema*, *Dirinaria*, and *Pyxine*).

Macrolichens with cyanobacteria (cyanolichens), i.e. *Coccocarpia* and *Leptogium*, were only found in plot 1. *Parmotrema* and *Dirinaria* were encountered in all plots. *Physcia* occurred in plot 1 and 2 but not in plot 3. Together with *Canoparmelia*, *Pyxine* was distributed in both plot 2 and 3.

Population Patterns of All Macrolichens in Three Plots. In general for all macrolichens, plot 2 had the highest total thallus number (530 thalli) and total coverage (1323.4 cm^2) (Figure 2A). But, the average coverage of lichen thallus in plot 2 (2.5 cm^2) was smaller than in plot 1 (5.1 cm^2) (Figure 2B). In plot 1, *Physcia* had the highest important value index (IVI1 = 192%), and followed by *Leptogium* (IVI1 = 75.7%) (Figure 3). *Dirinaria* had the highest important value index in plot 2 (IVI1 = 144%), and followed by *Physcia* (IVI1 = 73.9%). In plot 3, *Dirinaria* also had the highest important value index (IVI1 = 147%), and *Pyxine* followed afterward (IVI1 = 135%).

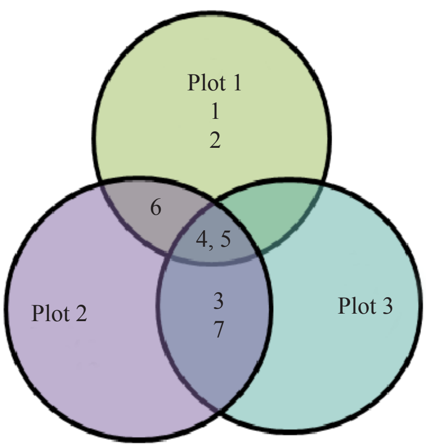

Figure 1. Macrolichens distribution in plot 1, 2, and 3. 1 = *Coccocarpia*, 2 = *Leptogium*, 3 = *Canoparmelia*, 4 = *Parmotrema*, 5 = *Dirinaria*, 6 = *Physcia*, 7 = *Pyxine*.

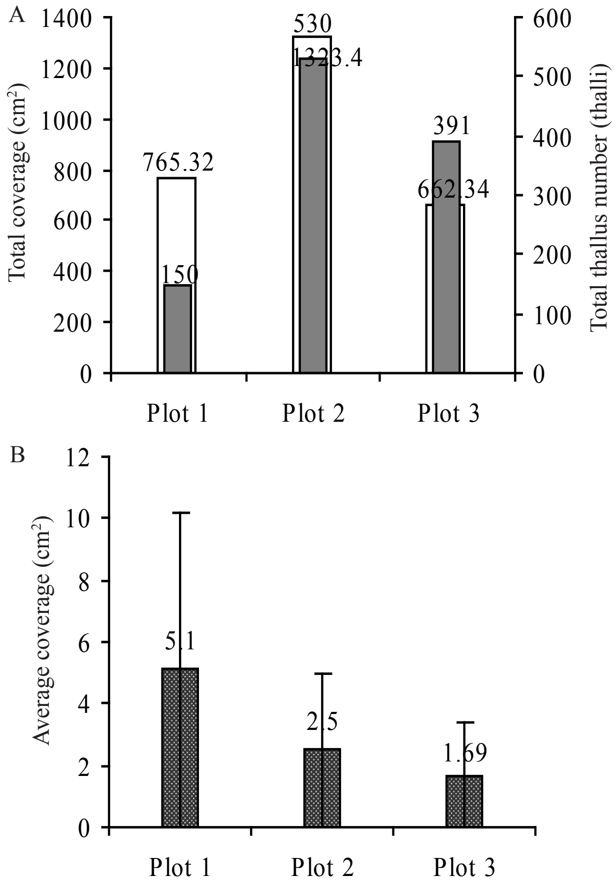

Figure 2. Diagram of total thallus number (TN) and total coverage (C) in (A), and average coverage (AC) in (B) of all epiphytic macrolichens found on canary trees in plot 1, 2, and 3. □ Total coverage, ■ total thallus number, ▩ average coverage.

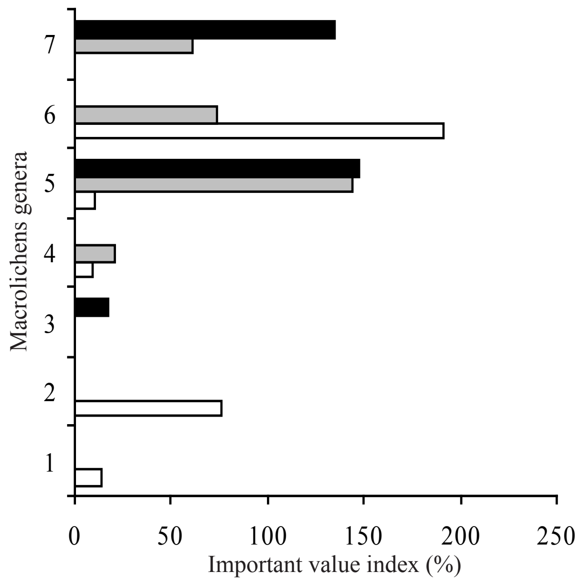

Figure 3. Comparison of important value index (IVI) of all macrolichens genera in three plots. IVI1 = Important Value Index with the frequency based on thallus number. Macrolichens genera: 1 = *Coccocarpia*, 2 = *Leptogium*, 3 = *Canoparmelia*, 4 = *Parmotrema*, 5 = *Dirinaria*, 6 = *Physcia*, 7 = *Pyxine*. □ Plot 1, ▩ Plot 2, ■ Plot 3.

Macrolichens of Bioindicator in All Plots. From this study, a population condition of epiphytic macrolichens was analyzed and to be used as indicator of air quality. Plot 1 with location far from intense traffic circulation and with assumption of having better air quality, were having some conditions: (i) having *Coccocarpia* and *Leptogium* that were not found in other plots (Figure 1); (ii) none of *Canoparmelia* and *Pyxine* were found (Figure 4); (iii) a few *Dirinaria* was found, but with larger average coverage (TN = 5 thalli, AC = 6.15 cm^2, Figure 5), and also infrequent (FR1 = 3.33%, FR2 = 10%); and (iv) *Physcia* was found abundantly (RD =

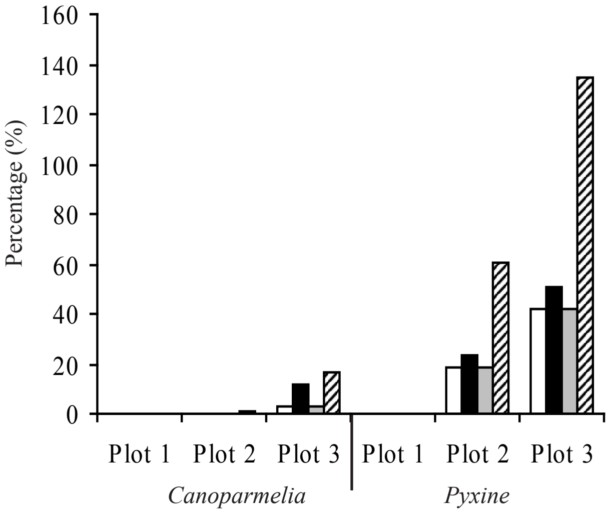

Figure 4. Diagram of relative density (RD), relative dominance (RDo), relative frequency 1 (RF1), and important value index 1 (IVI1) of *Canoparmelia* and *Pyxine* in plot 1, 2, and 3. □ Relative density (RD), ■ Relative frequency1 (RF1), ■ Relative dominance (RDo), ▨ Important value index1 (IVI1).

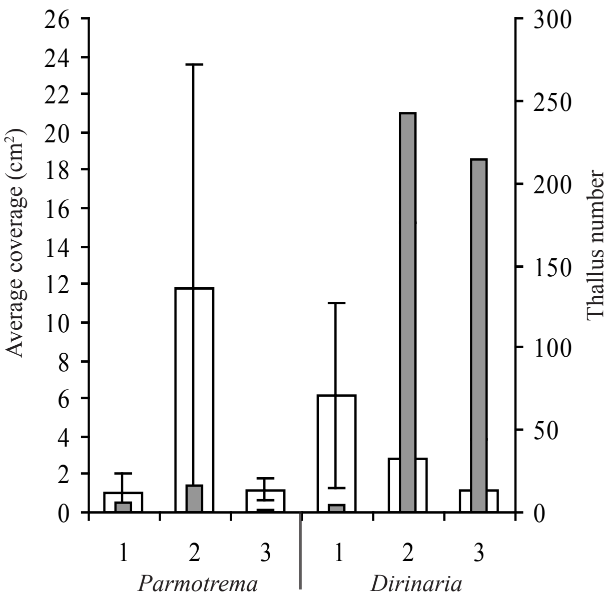

Figure 5. Diagram of thallus number (TN) and average coverage (AC) of *Parmotrema* and *Dirinaria* in plot 1, 2, and 3. □ Average coverage, ■ Thallus number.

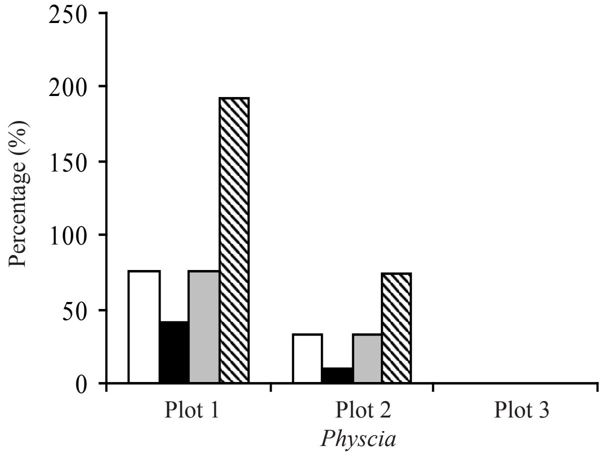

Figure 6. Diagram of relative density (RD), relative dominance (RDo), relative frequency 1 (RF1), and important value index 1 (IVI1) of *Physcia* in plot 1, 2, and 3. □ Relative density (RD), ■ Relative frequency1 (RF1), ■ Relative dominance (RDo), ▨ Important value index1 (IVI1).

75.58%, RDo = 40.8%, Figure 6). Conversely, plot 2 and 3 with location near intense traffic circulation and with assumption of having polluted air quality, were having some conditions: (i) none of *Coccocarpia* and *Leptogium* were found (Figure 1); (ii) few or many *Canoparmelia* (RD plot 2 = 0.377%, RD plot 3 = 2.558%) and *Pyxine* (RD plot 2 = 18.49%, RD plot 3 = 42.2%) were found (Figure 4); (iii) many *Dirinaria* was found, but with smaller average coverage as in plot 2 (TN = 243 thalli, AC = 2.85 cm^2) and plot 3 (TN = 214 thalli, AC = 1.16 cm^2) (Figure 5), and also frequent (RF1 plot 2 = 45.85%, RF1 plot 3 = 54.73%); and (iv) few or none *Physcia* was found (RD plot 2 = 32.26%, RD plot 3 = 0) (Figure 6).

Being found in all plots, *Parmotrema* and *Dirinaria* were showed different population pattern (Figure 5). *Parmotrema* showed unclear population pattern. Thallus number and average coverage of *Parmotrema* were the highest in plot 2 (TN = 16, AC = 11.84 cm^2), but equally smaller in both plot 1 (TN = 6, AC = 1.002 cm^2) and plot 3 (TN 2, AC = 2.42 cm^2). *Dirinaria* showed a clear pattern of increasing TN from plot 1 to plot 2 and plot 3, and decreasing AC from plot 1 to plot 2 and plot 3.

DISCUSSION

The finding of the cyanolichens *Coccocarpia* and *Leptogium* only in plot 1 indicates that plot 1 still has a good air quality, since cyanolichens are sensitive to acidification from air pollution (Cameron & Richardson 2006). This data was supported with a dendrogram of location cluster which analyzed by MEGA 5.05 with UPGMA method (unpublished data). The dendrogram showed a location cluster of

Coccocarpia and *Leptogium* that was far apart from other location clusters (cluster of *Canoparmelia* and *Pyxine*, cluster of *Physcia*, and cluster of *Parmotrema* and *Dirinaria*) with about 36% of location cluster distance. To compare, cluster of *Canoparmelia* and *Pyxine* was closer separated from the last two location clusters with about 22% location cluster distance.

Being located in the centre of BBG, plot 1 has more closed canopied trees than plot 2. Habitats like in plot 1 are more suitable for bryophytes, ferns, orchids, and other epiphytic angiosperms to grow. In this kind of habitat, lichens only have little space to colonize (Kumar 2009). In Oregon and Washington, cyanolichens representing only 24% of the total species diversity, accounted for a disproportionate share of rare species, being especially vulnerable to extirpation in air pollution affected areas (Geiser & Neitlich 2007). In Mediterranean forests, cyanolichens were related with low management intensity, high shrub cover and areas with steeper slopes (Aragón *et al.* 2010). *Leptogium* which was the second important in plot 1 is known from Europe as particularly sensitive to air contamination. This lichen is decreased in Singapore and it might be an indication for air quality changes (Sipman 2009). Therefore, the existence of the botanical garden in Bogor City is very important, especially as a habitat for lichens, as was also observed in Singapore (Sipman 2010).

Plot 2 is located at the edge of BBG, and has a closer access to air pollution from main streets in Bogor City, in particular Otto Iskandardinata Street. Despite of that, descriptively plot 2 is more suitable as a lichen habitat because it provides a higher light intensity. In a temperate forest in India, *Quercus semecarpifolia* trees in open canopied forest exhibit maximum lichen cover (70%) while close canopied forest has only 40% (Kumar 2009). Nevertheless, the highest value of total thallus number and total coverage of all macrolichens in plot 2 did not show that they grow as healthy as in plot 1, because the average coverage of each thallus is smaller than in plot 1. Effects of pollutant might be related to this (Bates 2002). BBG does not have air quality measurement data and BPLH did not measure pollutant concentration around Otto Iskandardinata Street, so we took into account pollutant concentration from the closest source which were surrounding BTM – Ir. H. Juanda Street circle that about 500 m distance from plot 2 (SO_2 = 34.5 µg/m^3, NO_2 = 48.12 µg/m^3) and at Pangrango 2 Hotel crossroad that about 1.5 km distance from plot 2 (SO_2 = 22.04 µg/m^3, NO_2 = 30.13 µg/m^3).

Parmotrema and *Dirinaria* appeared to be tolerant lichens because of their survival in all plots, but they showed different ecological patterns between the plots. In this study, populations of *Dirinaria* were conspicuous in plot 2 and 3 that are adjacent to main and busy roads. But, the *Dirinaria* thallus average coverage was shrinking from plot 1 to 3 (Figure 5), which might be an air pollution effect (Bates 2002). As a member of Physciaceae, *Dirinaria* is often observed in urbanized areas (Sipman 2009). *Dirinaria picta* and *D. applanata* can be found both in rural and urban areas in lowland area (Saipunkaew *et al.* 2006). *Dirinaria* also can still be found in Jenderal Sudirman Street – Pekanbaru (highly traffic density) (Nursal *et al.* 2005). Conversely, there were no consecutive changing pattern of thallus number and average coverage of *Parmotrema* from plot 1 to plot 3 (increase or decrease), so that this genus cannot be used as an indicator. Identification until species level probably generate better result, considering that in plot 1 and plot 2 we found *P. tinctorum*, which is stated to be sensitive to air pollution in Japan (Ohmura *et al.* 2009).

Both C*anoparmelia* and *Pyxine* were found in plot 2 and 3, but not found in plot 1. One of *Canoparmelia* species that visually identified as *Canoparmelia texana* was found during the research (Divakar 2013, personal communication). This species had been known to have high tolerancy of air pollution (Fuga *et al*. 2008; Barbosa *et al*. 2010). *C. texana* was studied in Brazil as an indicator of natural radionuclide element and rare earth element (REEs) (Leonardo *et al*. 2014), and also as indicator of pollutant that originated from lead and tin industry (Leonardo *et al*. 2011). One of *Pyxine* species that also found in this research was visually identified as *Pyxine cocoes* (Divakar 2013, personal communication). This species was also had been known to have high tolerancy of air pollution (Rout *et al*. 2010; Danesh *et al*. 2013; Shukla *et al*. 2014). *P. cocoes* was used in India to determine air quality in an area, by analyzing its chlorophyll content inside the thallus (Rout *et al*. 2010). Frequency of *P. cocoes* was increasing with the increasing of transportation activity, and its thallus was able to accumulate heavy metal up to 97% (Shukla *et al*. 2014). In Philippine, *P. cocoes* was dominant in the city that closer to intense transportation circulation, and hard to find in area that scarcely passed by vehicles.

In this research, *Canoparmelia* and *Pyxine* had the higher relative density, relative dominance, and relative frequency in plot 3 than in plot 2 (Figure 4), but we can not predict that plot 3 has higher level of pollution than plot 2 because the lack of accurate air quality measurement. Plot 3 located directly beside main streets, while some trees at plot 2 were still inside BBG and indirectly correlated with main streets. Relating the air quality, the data measurement of PT Goodyear Indonesia (2012) found that the sulfur dioxide (SO_2) content surrounding plot 3 was 55 µg/m^3 and NO_2 content was 11 µg/m^3 (September 2012). However, plot 3 had higher temperature (30-33 ^{o}C) and lower humidity (60-74%) compared to plot 2 (temperature 27-31 ^{o}C, humidity 71-93%). From the secondary data (PT Goodyear Indonesia 2012), the SO_2 level contained in plot 2 and 3 was far below the quality standards for humans (SO_2 = 900 µg/m^3, NO_2 = 400 µg/m^3). Current major cities in Korea exhibit similarly low levels of SO_2, and therefore SO_2 is assumed not to act as a pollutant restricting lichen distribution as in year 1980. Instead, NO_2 content that had been increasing since 20 years ago, recently appears to affect species composition and richness, because many nitrophilic or tolerant species were found (Ahn *et al*. 2011).

This research also described *Physcia* as a moderately sensitive lichens. The relative density, relative dominance, and relative frequency of *Physcia* were higher in plot 1 compared to plot 2 (Figure 6). The absence of *Physcia* in plot 3 cannot be explained, considering that this genus is a member of Physciaceae family that abundantly found in the cities. Identification until species level probably generate clearer population pattern, because species of *Physcia* show different sensitivity to air pollution. In Serbia, *P. tenella* is sensitive lichen, while *P. adscendens* is tolerant (Stamenković *et al.* 2010).

According to Yazici and Aslan (2006), an area that still having many foliose lichens is provide favorable conditions for lichen growth. Futhermore, we should observe other area in Bogor city to conclude the above argument. More detailed results from this kind of research can be expected, when the lichens are identified until species level, which requires additional lichenological expertises that currently lacking in Indonesia. To support results from this research, more studies in other city trees is needed to observe the distribution patterns of macrolichens. Through this research, *Coccocarpia*, *Leptogium*, and *Physcia* were appeared to be sensitive bioindicators. Meanwhile, *Dirinaria*, *Canoparmelia*, and *Pyxine* were showed up as tolerant bioindicators. Although *Parmotrema* was found in all plots, its population condition had unclear pattern, so that it cannot be used as a bioindicator. Transplantation of indicator lichens in three plots can be conducted to ensure whether those lichens are sensitive or tolerant bioindicator. For example, *Coccocarpia*, *Leptogium*, and *Physcia* will be transplanted from plot 1 (control area) to plot 2 or plot 3, as being done by Picotto *et al*. (2011). These macrolichens have similar habitus and easier to collect and transplanted.

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