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Comparative study on Pb absorption ability of five shade plant species in industrial estate and urban forests of Bekasi, Indonesia

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Abstract. *Intan DZ, Yuniati R, Lestari R. 2023. Comparative study on Pb absorption ability of five shade plant species in industrial estate and urban forests of Bekasi, Indonesia. Biodiversitas 24: 1289-1294.* The expansion of the transportation and industrial sectors is detrimental to the environment. Lead is one of the most typical contaminants they produce (Pb). As biological agents, shade plants lower the air's Pb contamination. This study observed leaf Pb absorption from five species of shade plants such as *Terminalia catappa* L. (Sea almond tree); *Swietenia macrophylla* King. (mahogany); *Samanea saman* (Jacq.) Merr. (raintree); *Mangifera indica* L. (mango); and *Nephelium lappaceum* L. (*rambutan*). This research was conducted at two different sites: Patriot urban forest (Bekasi City) and MM2100 Industrial Estate (West Cikarang Subdistrict, Bekasi District) of West Java Province, Indonesia. The objectives of this study were to analyze the ability of five plant species to absorb Pb and the correlation between Pb absorption with peroxidase enzyme activity and chlorophyll content. The Pb absorption was analyzed by Atomic Absorption Spectroscopy (AAS), while the peroxidase enzyme activity and chlorophyll content were analyzed using a spectrophotometer. The results showed that Pb absorption by plant species in research sites was not significantly different (sig.>0.05), meaning that different shade plant species and research sites did not significantly affect Pb absorption. Sea almond tree had the highest average Pb absorption (7.24 µg/g), followed by mahogany (5.44 µg/g), mango (3.4 µg/g), raintree (3.17 µg/g) and *rambutan* (2.33 µg/g). The correlation and regression analysis showed that Pb absorption had no significant effect on chlorophyll content (0.173>0.05), and the direction of its correlation was negative (-0.317). It means that the chlorophyll content does not affect lead uptake. The correlation and regression analysis showed that Pb absorption significantly affected peroxidase enzyme activity (0.028<0.05), and the direction of its correlation was positive (0.490). It means that Pb absorption did not significantly affect chlorophyll content but significantly affected peroxidase enzyme activity.

Keywords: Air pollution, chlorophyll, lead, Pb, peroxidase enzyme, shade plant

INTRODUCTION

The increase in development in various sectors, such as industry, transportation, and urban life, has both positive and negative impacts. One of the negative impacts caused by the increasing city development is the presence of air contamination (Asati et al. 2016; Ukaogo et al. 2020). Heavy metals in the air act as toxic contaminants that harm health and the environment (Lakherwal 2014; Asati et al. 2016). Some of the known heavy metals are Cadmium (Cd), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Chromium (Cr), Copper (Cu), Iron (Fe), and Lead (Pb) (Asati et al. 2016).

Lead (Pb) is a heavy metal element commonly found in the environment compared to other types of heavy metals due to its nature which has a high level of distribution and availability (Jaishankar et al. 2014). Lead (Pb) in high concentrations can cause structural and physiological disorders in plants (Jaishankar et al. 2014). The main target of Pb in plants is organelles or tissues related to photosynthesis activities, such as chlorophyll. On the other hand, Pb contamination can cause oxidative stress by producing Reactive Oxygen Species (ROS) that cause physiological disorders to plant death (Jaishankar et al. 2014; Ali et al. 2015). The accumulation of ROS is a source of oxidative damage to organism cells. The production of ROS in plants puts plants under stress conditions and causes biochemical and physiological changes in plants (Pandey et al. 2017). Therefore, ROS presence would trigger the formation of antioxidants as a plant defense against pollutant stress. Many forms of antioxidants are found in the mechanism against pollutants, one of which is forming the peroxidase enzyme. Therefore, Pb exposure will increase the activity of the peroxidase enzyme as an antioxidant as their resistance to environmental stresses. Furthermore, the peroxidase enzyme activity can indicate how plants fight heavy metal contamination (Nadgorska-Socha et al. 2013).

It is necessary to lessen Pb contamination in the air since lead has a negative impact on both living beings and the environment. Plants have the ability to catch and absorb polluting particles so that they can lower the concentration of Pb in the air (Shahid et al. 2012). Building greenways near the source of lead pollutants is one of the efforts to absorb pollutants and airborne dust, especially that contain Pb (Agustin and Hamidah 2019; Fida et al. 2021). The shading plant is widely used as a component of greenways. Reduced environmental pollution, such as heavy metal contamination brought on by human activity from transportation or industrial activities, is the goal of having shade plants near the source of pollution (Agustin and Hamidah 2019).

Therefore, the objectives of this study were to; (i) examine the Pb absorption capacities of various plant species; (ii) examine the Pb contamination at various sites; (iii) examine the plant species with the highest Pb absorption capacities; (iv) examine the research site with the highest Pb contamination; (v) examine the relationship between Pb uptake and enzyme activity; and (vi) examine the relationship between Pb uptake and chlorophyll content. This study used five types of shade plants as research objects: *Terminalia catappa* L. (Sea almond tree), *Swietenia macrophylla* King. (mahogany), *Samanea saman* (Jacq.) Merr. (raintree), *Mangifera indica* L. (mango) and *Nephelium lappaceum* L. (*rambutan*) from two locations, namely Patriot urban forest (Bekasi City) and MM2100 Industrial Estate (West Cikarang Sub-district, Bekasi District) of West Java Province, Indonesia.

MATERIAL AND METHODS

Sample collection

Leaf collection

The samples used in this study were shade plant leaves from five different species, i.e., *Terminalia catappa* L. (Sea almond tree), *Swietenia macrophylla* King. (mahogany), *Samanea saman* (Jacq.) Merr. (raintree), *Mangifera indica* L. (mango), and *Nephelium lappaceum* L. Each leaves sample from five different species was taken in three replications. This study was conducted at MM2100 Industrial Estate located in West Cikarang, Bekasi District and Patriot urban forest of Bekasi City of West Java Province, Indonesia (Figure 1).

The sampling technique used in this study was purposive sampling, by selecting subjects based on the criteria set by the researcher. The criteria for the samples taken were: a minimum plant height of two meters to limit the plants not being too young; the leaves taken were healthy without pests, the leaves were dark green on the branch closest to the source of contamination, and the plants had leaves in large numbers so that when taking the leaves does not cause the plant to die. In addition, heavy metal uptake by shade plants, chlorophyll content, and peroxidase enzyme activity were selected as the parameters.

Sample preparation

The leaf sample was weighed as much as 3-4 grams, placed in a porcelain dish, and furnaced at 400°C for 2 hours. First, the sample was cooled, and then 10 mL of HNO_3 and aqua regia were added. The sample was then heated until the volume was reduced to 5 mL and cooled. After the cooling process, the sample was filtered using filter paper and put into a 25 mL volumetric flask. Next, Atomic Absorption Spectrometry (AAS) was carried out to determine the Pb content in the samples at the Department of Chemistry Laboratory, Universitas Indonesia, Depok.

Parameter measurement

Measurement of leaf Pb content

Sample measurement obtained in the lab is calculated following SNI number 06-698945 of 2005.

Leaf Pb content measurement:

$$Cy' = (Cy \times \frac{v}{w}) \times 1000$$

Where:

Cy' : Pb content in leaves (µg/g)
Cy : Measured Pb concentration in AAS (mg/L)
V : dilution volume (L)
W : dry leaf weight (g)
1000 : mg to µg conversion

Measurement of chlorophyll content

The leaf samples were cleaned using distilled water; the midrib was removed. Next, leaf samples were cut, weighed as much as 0.5 g, and ground with a mortar. Next, 20 mL of 80% acetone was added until the chlorophyll dissolved. Next, the crushed leaf sample was filtered into a measuring flask, and then 80% acetone was added to 50 mL. Finally, a spectrophotometer measured the sample solution for absorbance read at 645 and 663 nm. The content of chlorophyll a, chlorophyll b, and total chlorophyll were calculated based on Arnon's (1949) equation:

Chlorophyll a (µg/mL) : 12,7 (A_{663}) - 2,69 (A_{645})
Chlorophyll b (µg/mL) : 22,9 (A_{645}) - 4,68 (A_{663})
Total chlorophyll (µg/mL): 20,2 (A_{645}) + 8,01 (A_{663})

Measurement of peroxidase enzyme activity

Leaf samples were ground using a mortar with the addition of 0.01 M phosphate buffer solution (pH 6.0) in a ratio of 1:4 (1 g leaf sample was added 4 mL of phosphate buffer). Then the samples were filtered and centrifuged for 30 minutes at 5,000 rpm at a temperature of 4°C. The supernatant was used as an enzyme preparation.

Pyrogallol solution was prepared as a reagent by mixing 10 mL of 0.5 M pyrogallol added with 0.066 M phosphate buffer pH 6.0 and diluted with water until a volume of 100 mL. The peroxidase enzyme activity was observed by mixing 0.2 mL of enzyme preparation. Next, added to a reagent solution containing 5 mL of 0.5 M pyrogallol solution and 0.5 mL of 1% H_2O_2 in a cuvette. The mixture of enzyme preparations and reagents was homogenized for 5-10 seconds, and the absorbance was measured at a wavelength (λ) of 420 nm. The absorbance value was calculated every 30 seconds for 3 minutes.

Data analysis

The Pb contents data from five different shade plant species at two different locations were statistically tested using ANOVA with Factorial CRD design using the SPSS program to see the significance of the results. In addition, the correlation between leaf Pb content data on peroxidation enzyme activity and chlorophyll content data was analyzed by regression correlation analysis method in the SPSS program.

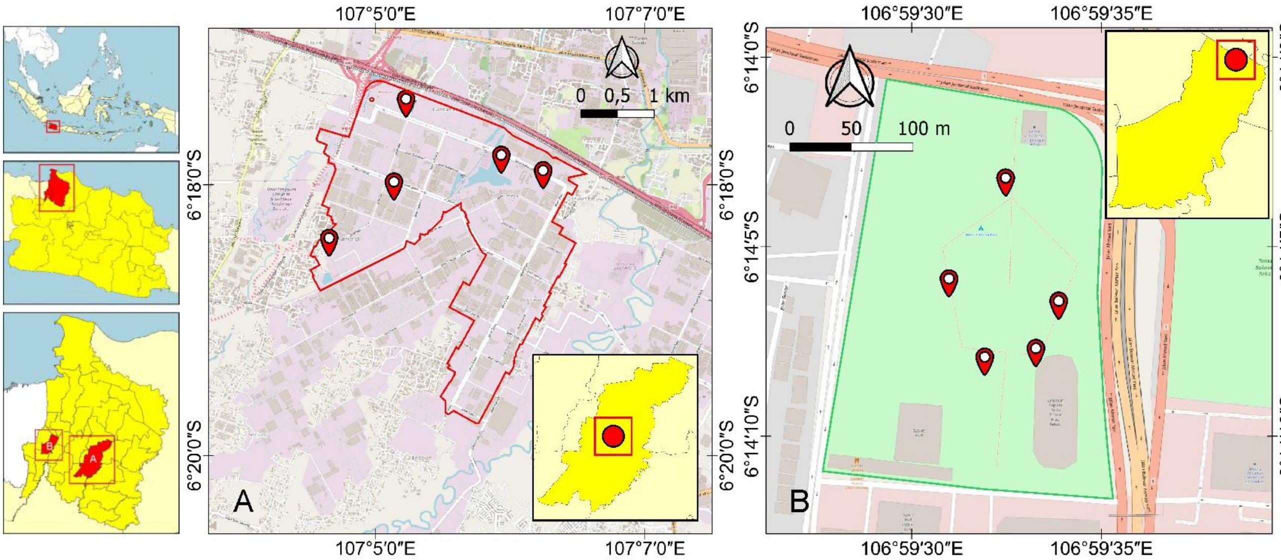

Figure 1. A. MM2100 industrial area location map, West Cikarang Sub-district, Bekasi District; B. Patriot urban forest area map, Bekasi City, Indonesia

RESULTS AND DISCUSSION

Pb absorption

The data of Pb uptake were analyzed using the Kruskal-Wallis and Mann-Whitney tests to determine the influence of the factors tested. The Kruskal-Wallis test was used to observe the effect of shade plant types on Pb uptake, and the Mann-Whitney test was used to observe the effect of the research site on Pb uptake. Kruskal-Wallis analysis results showed that the plant type did not show a significant effect (significance value of 0.131>0.05) on Pb uptake. Similar results were also observed in Yuniantara and Kuntjoro's (2021) study, which reported that the Pb absorption ability of several plants did not show significantly different results. The ability of the shade plants tested only gave a slight difference in the value of Pb uptake.

Although statistically not significantly different, each plant species has a varying Pb absorption ability. The results of the average Pb absorption are shown in (Table 1). The average value of Pb absorption was ordered from the highest to lowest: Sea almond tree>mahogany>mango> raintree>*rambutan*.

The results of previous studies reported that the Sea almond tree has a good adsorption capacity due to its wide leaves. Leaf size and shape affected the amount of Pb uptake in plants. The broad leaves of the Sea almond tree make it easier for airborne contaminants to be trapped and absorbed by plants (Istiaroh et al. 2014). Mahogany plants also show high Pb uptake because, in addition to having wide leaves, they have many branches and twigs, thereby increasing the ability to absorb Pb. Mango, raintree, and *rambutan* have a lower ability to absorb Pb than Sea almond trees and mahogany. Mango is reported to be able to absorb Pb contamination in the moderate category, with an absorption amount of around 6.33 ppm (Maulida 2016). Raintrees generally have a maximum height of about 15-25 m. It is easily recognizable by its wide, domed umbrella-shaped canopy structure. Raintree is commonly planted for shade plants, wood, and many are found at the roadside (Kabir et al. 2011; Sowjanya et al. 2014). The canopy structure allows for better pollutant absorption. Raintree also has a rough leaf structure with fine hairs on its surface (Krisnandika et al. 2019), but the small surface area of the leaves makes the raintree's ability to absorb Pb still below that of other plants.

The Mann-Whitney test results showed that the research site did not have a significant effect (significance value of 0.364>0.05) on the uptake of Pb. In their investigation, Uka et al. (2021) also discovered that the content of heavy metals in the leaves was not significantly affected by different geographical regions. According to Das et al. (2018), the industrialization process and emissions from transportation-related activities both played a major role in the contamination of the air with lead. Based on the results of statistical analysis on two research sites did not show the significance of the Pb absorption value. Still, the data on the average absorption of Pb by five types of plants in the two locations showed a difference in the average Pb uptake (Table 1). Based on the results of this study, the average value of Pb absorption in Patriot urban forests is 4.43 μg/g. Chealsiyana et al. (2021) stated that the Patriot urban forest of Bekasi City is located in the city center, opposite the Major's Office and the Bekasi City Patriot Stadium, and side by side with national and provincial highways. According to Das et al. (2018), Pb derived from vehicle emission contributes significantly to Pb levels in the air as the increasing number of motor vehicle users causes recirculation of the previous Pb that has not disappeared and remains in the environment. In addition to these things, the location of Patriot urban forests has higher humidity compared to industrial areas. According to Istiaroh et al. (2018), humid air causes contamination to settle more easily because polluting particles will bind to the water in the air and allow a higher Pb weight so that it will be easier to settle to the leaves surface.

Based on Indonesian Government regulation (Peraturan Pemerintah No. 41 Tahun 1999), the maximum Pb content analyzed using the AAS method is 2 μg/m^3. The average value of Pb uptake in MM2100 industrial areas, West Cikarang, is 4.20 μg/g. It shows that lead concentration has exceeded the contamination threshold. Das et al. (2018) stated that industrial activities contribute to high Pb environmental contamination. The metallurgical, paint, smelting, mining, and combustion industries of industrial residues release a lot of Pb into the environment. Das et al. (2018) also reported that due to coal burning, industrial activities, and transportation that have continued to increase to date causing, the concentration of air lead in Asia measured in the past decade had increased many times compared to other regions.

Correlation of Pb uptake and chlorophyll content

Leaf chlorophyll content ranged from 8.23 to 29.99 mg/g (Table 2). Moreover, chlorophyll a & chlorophyll b were measured. The measurement of chlorophyll a and b is one of the important indicators to determine the effects of air pollution and heavy metal stress on plants (Petrovic and Krivakopic 2020). It can be observed that the average chlorophyll of all plants in the MM2100 Industrial Estate is lower than that in the Patriot urban forest. The highest chlorophyll content was in the mango plant in the Patriot urban forest, and the lowest chlorophyll content was in the mahogany plant in the industrial area. The low chlorophyll content is because chlorophyll is damaged due to the degradation by Pb. According to Hou et al. (2017); Hou et al. (2018), lead contamination can destroy the light-harvesting pigment and damage the reaction center pigments.

High exposure to Pb can cause changes in chlorophyll content. Lead may decrease the chlorophyll content dan performance (Li and Zhang 2015; Hou et al. 2018). Singh et al. (2012) confirmed that the leaves have enzymes known as α-amino levulinate dehydrogenase enzymes for chlorophyll biosynthesis. The presence of lead strongly inhibits the action of the enzyme. Changes in chlorophyll content occur due to the contamination of heavy metals such as Pb, which will damage the structure of chloroplasts. Lead can damage the structure of chloroplasts, especially in the grana structure. Kovacs (1992) stated that the structure of chloroplasts is strongly influenced by nutrients such as Mg and Fe. The influx of excess heavy metals in plants will reduce Mg intake, causing changes in the volume and number of chloroplasts. The element Mg is an element that is included in the type of macronutrients that are the constituents of chlorophyll molecules.

The regression analysis results showed that Pb uptake and chlorophyll content had a linear relationship pattern with the equation $Y= -0.4994x+17.668$ with $R^2= 0.1003$. That means 10% of the chlorophyll content is affected by Pb uptake, and 90% is influenced by other factors (Figure 2). The correlation analysis results showed insignificant results of 0.173>0.05, meaning that the Pb absorption relationship had no significant effect on chlorophyll levels. Pearson correlation value shows the number -0.317. That means the relationship between Pb uptake and chlorophyll content has a negative direction with a weak correlation level. The higher the Pb uptake, the lower the chlorophyll content in plants, and vice versa. Olivares (2003) found a connection between changes in leaf chlorophyll content and heavy metal correlations. As the amount of chlorophyll in the leaves declines, the amount of heavy metals in the environment will rise.

Correlation of Pb uptake and peroxidase enzyme activity

Peroxidase enzyme activity in leaves ranged from 0.12-2.91 mg/L (Table 3). The highest peroxidase enzyme activity was in mahogany plants located in MM2100 Industrial Estate areas. Increased activity of peroxidase enzymes in plants is a defense mechanism against environmental contamination (Nadgorska-Socha et al. 2013).

Table 1. Average Pb uptake from five different shade plants in two different research sites

Plant	Research site		Average (μg/g)
	MM2100 Industrial Estate (μg/g)	Patriot urban forest (μg/g)	
Sea almond tree	4.27	10.20	7.24
Mahogany	7.21	3.66	5.44
Mango	4.82	1.98	3.40
Raintree	3.15	3.19	3.17
Rambutan	1.54	3.11	2.33

Table 2. Average leaves chlorophyll content from five different shade plants in two different research sites (mg/g)

Plant	Peroxidase enzyme activity	
	MM2100 Industrial Estate (mg/L)	Patriot urban forest (mg/L)
Sea almond tree	2.71	2.62
Mahogany	2.91	1.54
Mango	2.14	0.12
Raintree	0.84	0.64
Rambutan	0.64	0.29

Table 3. Average leaves chlorophyll content from five different shade plants in two different research sites

Plant	Peroxidase enzyme activity	
	MM2100 Industrial Estate (mg/L)	Patriot urban forest (mg/L)
Sea almond tree	2.71	2.62
Mahogany	2.91	1.54
Mango	2.14	0.12
Raintree	0.84	0.64
Rambutan	0.64	0.29

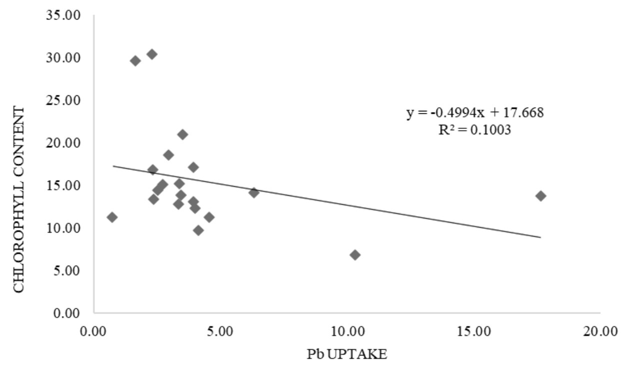

Figure 2. Correlation of Pb uptake and chlorophyll content

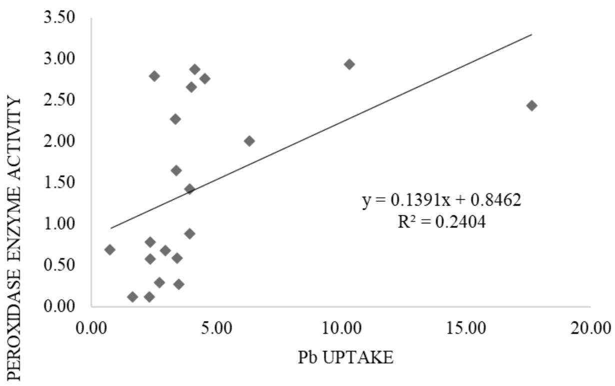

Figure 3. Correlation of Pb uptake and peroxidase enzyme activity

While Kasperczyk et al. (2015) stated, Pb toxicity could be associated with oxidative stress that increases ROS-forming concentration by reducing cellular antioxidant capacity. Sharma et al. (2016) reveal that the results of studies that several researchers have carried out reported that antioxidant enzymes could reduce ROS degree of danger to protect plants from heavy metal toxicity. Then according to Nadgorska-Socha et al. (2013), the peroxidase enzyme is one of the enzymes that has a function of resistance to heavy metals. Therefore, the activity of antioxidant enzymes in plants will increase when the level of heavy metal contamination has exceeded the plant's tolerance limit.

The regression results showed that Pb uptake and the peroxidase enzyme activity had a linear relationship with the equation Y= 0.1391X+0.8462 with R^2= 0.2404. That means 24% of enzyme activity is influenced by Pb uptake, and 76% is influenced by other factors (Figure 3). The correlation analysis results showed a significance of 0.028<0.05, meaning that the Pb uptake relationship had a significant effect on the activity of the peroxidase enzyme. Pearson correlation value shows the number 0.490. That means the relationship between Pb uptake and peroxidase enzyme activity has a positive direction with a moderate correlation. That means the higher the Pb uptake, the higher the peroxidase enzyme activity in plants, or vice versa. That follows the results of research by Sharma and Dubey (2005), which reported that the increase in peroxidase enzyme activity was directly proportional to the amount of Pb absorbed by the plant. The higher accumulation of Pb in the plant caused cell damage that triggered the formation of free radicals, and cells responded by increasing the peroxidase enzyme activity.

In conclusion, the results of this study indicate that different plant species and research sites did not significantly affect Pb uptake. Because the two research sites are both close to lead pollutant sources, the MM2100 Industrial Estate with industrial activities and the Patriot urban forest with vehicle activities, on the other hand, different species tested only gave a slight difference in the value of Pb uptake. That indicates that each species tested can be a shade plant. However, the data showed that the Pb uptake ability of each plant was different. For example, the sea almond tree had the highest Pb uptake, and the *rambutan* had the lowest Pb uptake. Based on correlation and regression analysis, Pb uptake did not affect chlorophyll content, and their correlation direction was negative. In addition, correlation and regression analysis for Pb uptake on peroxidase enzyme activity showed significant results, and the direction of the relationship was positive.

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