THE EFFECT OF LEAD (PB) CONTENT ON THE NUMBER OF STOMATA ON TABEBUIA AUREA LEAVES IN SURABAYA CITY

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Abstract

This study aimed to investigate the impact of lead (Pb) content on stomatal density in Tabebuia aurea leaves across various locations in Surabaya. Samples were collected from three distinct areas with different traffic densities: Campus C of Universitas Airlangga, Menur Road, and Prapen Road. Lead (Pb) content was analyzed using atomic absorption spectroscopy (AAS). The results revealed that the average lead (Pb) content absorbed by Tabebuia aurea leaves due to vehicle emissions was 1.06 mg/kg at Campus C, 1.78 mg/kg at Menur Road, and 2.35 mg/kg at Prapen Road. Stomatal density in Tabebuia aurea leaves was found to be 119.25 \pm 3.605 stomata/mm² at Campus C, 123.88 \pm 1.000 stomata/mm² at Menur Road, and 140.92 \pm 7.211 stomata/mm² at Prapen Road. These findings suggest that stomatal density in Tabebuia aurea leaves is lower on Menur Road compared to Prapen Road but higher than at Campus C. This indicates a positive correlation between stomatal density and lead (Pb) content in Tabebuia aurea leaves.

Key words: Tabebuia aurea, lead (Pb), number of stomata, stomata density, Surabaya.

Introduction

Surabaya, as the provincial capital of Indonesia, exhibits a robust socio-economic environment (Dick, 2003; Hawken & Sunindijo, 2018). Consequently, the city ranks third in Asia for air pollution, trailing only Bangkok and Jakarta. Surabaya experiences a rapid increase in motor vehicle numbers, with around 12,000 motorcycles and 3,000 cars added monthly. Annually, approximately 100,000 motorcycles and 30,000 cars enter the city (Samsoedin et al., 2015). The combustion of gasoline in these vehicles is a primary cause of air pollution (Fahruddin, 2020; Guo et al., 2023).

Lead (Pb) is recognized as a cumulative toxin, and its concentration can accumulate over time. Excessive lead (Pb) in the air can be absorbed by plants, making them effective bioindicators due to their high sensitivity to air pollution (Martuti, 2013). Plants can absorb lead (Pb) through water uptake by their roots from the soil or through their stomata on the leaves. Factors influencing the lead (Pb) content in plants include the duration of exposure to lead (Pb), the concentration of lead (Pb) in water, plant morphology and physiology, as well as the type of plant species. Accumulated lead (Pb) in significant quantities in leaves can affect the stomatal function of plants.

Tabebuia aurea plants are commonly found along the main roads of Surabaya, where motor vehicle traffic is high. Despite their prevalence, these plants frequently exhibit signs of wilting and desiccation. Given these morphological differences, there is a necessity for research on the impact of lead (Pb) content within Tabebuia aurea leaves

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along Surabaya's main thoroughfares on their stomatal density. This research is crucial due to Tabebuia aurea's role as a primary shade-providing species in Surabaya.

Research Methods

Calculation of traffic density

At the sampling location, motor vehicle counts were conducted over the span of one week. Counts were performed daily at three different times: in the morning from 07:00 to 08:00 AM, at noon from 11:00 AM to 12:00 PM, and in the afternoon from 04:00 to 05:00 PM (WIB, Western Indonesian Time). The number of vehicles passing through was tallied using a hand counter for all types of motor vehicles.

Sampling of T. aurea leaves

Leaf samples were collected from three parts to represent each location: leaves located at the lower, middle, and upper canopy of the tree. Stomatal impressions were made using the replica method, chosen for its effectiveness and efficiency. Subsequently, leaf surfaces were cut into 1x1 cm squares. The collected T. aurea leaf samples were then stored in labeled plastic bags according to their respective locations for further observation of stomatal density and measurement of lead content.

Observation of the number of stomata on T. aurea leaves

Observation of stomatal numbers on T. aurea leaves was performed using a light microscope at a magnification of 400x (40x objective lens multiplied by 10x eyepiece). Subsequently, stomatal density was calculated using the following formula as per (Mutaqin et al., 2016) :

Stomatal density = (Number of stomata) / (Area of stomatal field of view)

Testing the lead (Pb) content in T. aurea leaves

After observing and noting the stomata on the leaves, the residual leaves are cleaned. Subsequently, their lead (Pb) content is tested using the atomic absorption spectrophotometry (AAS) method.

Sample Preparation

After identifying and cleaning the leaves with observed stomata, the remaining leaves are prepared using the Dry Ashing Method. Fifty grams of leaf samples are weighed and placed into a porcelain crucible. The samples are dried in an oven at 70°C. Subsequently, the dried samples are finely cut and transferred into a beaker. Ten milliliters of nitric acid (HNO3) are added.

Atomic Absorption Spectrophotometry (AAS) Measurement

The AAS instrument is initialized, and blank absorbance is measured three times for calibration. Following this, absorbance readings are taken for various standard solution concentrations. Once validated, absorbance readings for the sample solution are recorded. After complete combustion, absorbance values (burn color intensity) are measured to determine the lead (Pb) content using a Pb calibration curve. Data collected is stored with corresponding sample names.

Data Analysis

Statistical analysis of the data is performed using SPSS 25.0 software (Ghozali, 2016). To explore the correlation or impact of lead (Pb) concentration levels on stomatal density per unit area on Tabebuia aurea leaves, Pearson correlation analysis is conducted. Results are presented graphically using linear regression with the formula Y = a + bX to visualize any relationships identified.

Results and Discussion

Motor Vehicle Counting at the Sampling Location in Surabaya City

Based on the results in Table 1, it is known that the average number of motor vehicles passing per hour at the sampling locations in Surabaya City, from lowest to highest, are at Universitas Airlangga Campus C, Jalan Raya Menur, and Jalan Raya Prapen. Traffic density is one of the causes of environmental pollution and can influence the amount of lead (Pb) in the air. To understand environmental pollution, monitoring activities can be conducted to assess the extent of urban air pollution, thereby determining priority strategies for management and control (Rinaldo, 2023).

Campus C, Menur Street, and Prapen Street.					
Number of Vehicle					
Location	07.00 -08.00 WIB	11.00 -12.00 WIB	16.00 -17.00 WIB	Average vehicle / Hour.	
Airlangga University Campus C	331	238	290	286	
Menur Street	4.905	4.141	5.250	4.780	
Prapen Street	12.515	7.428	10.385	10.110	

Table 1. Number of motor vehicles passing through the locations: Airlangga University
Campus C, Menur Street, and Prapen Street.

Lead (Pb) Content in Tabebuia aurea Leaves Based on Sampling Locations in Surabaya City

Lead (Pb) emitted from motor vehicle emissions in the air can be absorbed by plants through their leaves. Each plant has leaves with different characteristics, resulting in varying abilities to absorb lead (Pb) from the air. According to (Fathia et al., 2015), leaves with leaf hairs (trichomes) and uneven surfaces can absorb more lead (Pb) compared to plants with smooth surfaces. Tabebuia aurea plants have smooth-edged leaves (entire), leaflets resembling skin or ribs (coriaceous), covered with small scales (lepidote) on the upper and lower sides, and are glabrous.

This study tested the lead (Pb) content in Tabebuia aurea leaves using spectrophotometry atomic absorption. Accumulation of lead (Pb) content in Tabebuia aurea leaves is described in Table 2, where it is known that the lead (Pb) content ranges from lowest to highest at the following locations: Universitas Airlangga Campus C with a value of 1.06 mg/kg, Jalan Raya Menur with 1.78 mg/kg, and Jalan Raya Prapen with 2.35 mg/kg. According to Government Regulation No. 41 of 1999, the air quality standard for lead (Pb) is 2 mg/kg. The lead (Pb) content in Tabebuia aurea plants on Jalan Raya Prapen, at 2.35 mg/kg, exceeds the air quality standard threshold for lead (Pb).

University Campus C, Wenur Street, and Frapen Street			
Location	Plant	Lead Content (Pb) (mg/kg)	Average Lead Content (Pb) (mg/kg)
	1	1,08	
Airlangga University Campus C	2	0,98	$1,06 \pm 0,072$
	3	1,12	
	1	1,86	
Menur Street	2	1,52	$1,78 \pm 0,238$
	3	1,98	

 Table 2. Lead (Pb) content test results in tabebuia (T. aurea) leaves at location Airlangga

 University Campus C, Menur Street, and Prapen Street

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	1	2,18	
Prapen Street	2	2,49	$2,35 \pm 0,158$
-	3	2,39	

Stomatal density of tabebuia (T. aurea) leaves based on sampling locations in Surabaya City

Based on the observations in Table 3, it is known that the average density of stomata on Campus C, Airlangga University is $119.25 \pm 3,605 / \text{mm}^2$, Jalan Raya Menur $123.88 \pm 1,000 / \text{mm}^2$ and Jalan Raya Prapen $140.92 \pm 7,211 / \text{mm}^2$. According to (Karubuy et al., 2018) stomata density is grouped into three groups, namely $<300 / \text{mm}^2$ including the low group, $300-500 / \text{mm}^2$ including the medium group and $\geq 500 / \text{mm}^2$ including the high group. The stomata density of tabebuya (T. aurea) leaves is in the low category because it has an average stomata density of less than $300 / \text{mm}^2$.



Figure 1. Stomata of tabebuia (T. aurea) leaves at Airlangga University Campus C location. (magnification 400x)



Figure 2. Stomata of tabebuia (T. aurea) leaves at Menur Street location (magnification 400x)



Figure 3. Stomata of tabebuia (T. aurea) leaves at Prapen Street location (magnification 400x)

Excessive lead (Pb) content in tabebuia (T. aurea) leaves can affect leaf tissues, including stomata. The accumulation of excessive lead (Pb) due to motor vehicle

emissions can impact leaf tissues, especially stomata (Sihaloho et al., 2020). Motor vehicle emissions are capable of causing damage to plants, such as reducing stomatal numbers, damaging guard cells, increasing stomatal density, damaging leaf blades, inhibiting transformation rates, reducing leaf area, decreasing chlorophyll levels, and causing leaf death (Raharjo et al., 2015)

Table 3. Stomatal density of tabebuia (T. aurea) leaves at locations Kampus C Universitas
Airlangga, Jalan Raya Menur, and Jalan Raya Prapen

Location	Plant	Number	Stomata Density	Average Stomata
Location	1 Iunit	Stomata	(n/mm^2)	Density (n/mm ²)
Airlangga University	1	25	123	
Campus C	2	23	116	$119,25 \pm 3,605$
	3	24	118	
	1	25	125	
Menur Street	2	25	124	$123,88 \pm 1,000$
	3	25	123	
	1	28	139	
Prapen Street	2	26	129	$140,92 \pm 7,211$
-	3	31	143	

Correlation between lead (Pb) content and stomatal density in tabebuia (T. aurea) leaves

A Pearson correlation analysis using SPSS 25.0 was conducted to explore the relationship between lead (Pb) content and stomatal density in tabebuia (T. aurea) leaves (SPSS, 2015). The results, as shown in Table 4, revealed a significant correlation with a p-value of 0.030 (<0.05), indicating that lead concentration in the leaves correlates with stomatal count per field of view across multiple locations in Surabaya. The correlation coefficient for this analysis was found to be 0.716.

Correlations			
		Lead Content	Stomata
		(Pb)	Density
Lead Content(Pb)	Pearson Correlation	1	0.716^{*}
	Sig. (2-tailed)		0.030
	Ν	9	9
Stomata Density	Pearson Correlation	0.716^{*}	1
	Sig. (2-tailed)	0.030	
	Ν	9	9

Table 4. Correlation test results of lead (Pb) content on stomatal density at locations Airlangga University Campus C, Menur Street, and Prapen Street

*. Correlation is significant at the 0.05 level (2-tailed).



Figure 4. Graph showing the effect of lead (Pb) on stomatal density at locations Airlangga University, Menur Street, and Prapen Street

Figure 4 ilustrated that the relationship between lead (Pb) content in tabebuia (T. aurea) leaves has a positive effect on stomatal density. The graph shows the equation Y = 17.629x + 100.11, with a coefficient of determination (R²) of 0.5128, indicating that lead (Pb) content (X) influences stomatal density (Y) by 51.28%. This finding aligns with Sulistiana & Setijorini's research (2016), which demonstrated that an increase in stomatal number and density per mm² correlates with higher lead (Pb) absorption in puring plants. Lead absorption (positive) tends to increase stomatal density and number, with a coefficient of determination (R²) of 0.2524, contributing significantly at 25.24%. There is a positive correlation between high lead (Pb) absorption in leaves and high stomatal density and number.

High motor vehicle intensity increases airborne lead (Pb), potentially polluting the environment and affecting leaf stomatal density. According to (Santoso et al., 2012) roadside shade plants absorb lead (Pb) through stomata via passive absorption. Absorbed lead (Pb) accumulates in palisade tissue, and absorption efficiency is highly influenced by stomatal density and size.

Conclusion

The average lead (Pb) concentrations in T. aurea leaves at three different locations in Surabaya show significant differences: 1.06 mg/kg at Kampus C Universitas Airlangga, 1.78 mg/kg at Jalan Raya Menur, and 2.35 mg/kg at Jalan Raya Prapen. Stomatal densities per area in tabebuia (T. aurea) leaves vary as follows: 119.25/mm² at Kampus C Universitas Airlangga, 123.88/mm² at Jalan Raya Menur, and 140.92/mm² at Jalan Raya Prapen. Stomatal density in tabebuia plants at Kampus C Universitas Airlangga does not significantly differ from that at Jalan Raya Menur but differs significantly from that at Jalan Raya Prapen. There is a strong positive correlation of 25.24% between lead (Pb) concentration and stomatal density per area in tabebuia (T. aurea) leaves.

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