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THE ROLE OF *DENDROBIUM* SP. AS LEAD ABSORBENT ON THE SURABAYA PROTOCOL ROADS, INDONESIA

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ABSTRACT

This study aimed to detemine the effect of lead content (Pb) on the density of stomata and lead (Pb) content on the leaves of *Dendrobium* sp. in several streets in Surabaya. Sampling was carried out at three locations with different levels of traffic density, at Campus C of Airlangga University, Walikota Mustajab Street, and Raya Darmo Street. Lead content testing was carried out using the Atomic Absorption Spectrophotometry (AAS) analysis method. The results showed that the average lead content in *Dendrobium* sp. leaves due to pollution exposure at Campus C of Airlangga University, Walikota Mustajab Street and Raya Darmo Street were 0.13 mg/kg, 0.17 mg/kg and 0.19 mg/kg respectively. The stomata density at Campus C of Airlangga University, Walikota Mustajab Street and Raya Darmo Streetwas also different. They were 30,37/mm², 37,96/mm², and 41,56/ mm²respectively. Correlation value obtained was 0.687. It meant that the relationship of lead content (Pb) in leaves to stomata density had correlation and positive effect. It showed that the the higher the lead content (Pb), the stomata density on *Dendrobium* sp. leaves would also increase.

KEY WORDS : *Dendrobium* sp., Lead, Stomata density, Atomic Absorption Spectrophotometry, Correlation

INTRODUCTION

Air pollution is a threat to every country, including Indonesia. Air pollution is the entry of living things, substances, energy and or other components into the air by human activities or natural processes so that air quality drops to a certain level which causes the air to become deficient or unable to function anymore according to its designation (Mulia, 2005). Over time, many big cities in Indonesia have the potential to be exposed with air pollution; one of them is Surabaya city. Surabaya is the second largest city in Indonesia and has a very high traffic volume.

One of the biggest contributors to air pollution is lead (Pb). Lead (Pb) in the air is released through motor vehicle emissions, which are increasing day by day. Lead (Pb) is a heavy metal that is very dangerous for living things because it is carcinogenic, decomposes over a long time and its toxicity does not change (Brass and Strauss, 1981). The concentration of lead (Pb) can be reduced by absorption by plants. The part of the plant that is able to absorb lead (Pb) is the leaves, because the particle size of lead (Pb) is smaller than the size of the stomata. The level of lead (Pb) accumulation in plants will increase with the increase of traffic density. This condition causes the plant leaf area to tend to shrink, so that the number of stomata will appear to increase along with the increase in vehicle intensity.

Basically every plant has the ability to absorb lead pollution (Pb), as well as *Dendrobium* sp. For this reason, it is necessary to carry out laboratory tests using the Atomic Absorption Spectrophotometery (AAS) method to determine the level of accumulation and lead (Pb) content in the leaves of *Dendrobium* sp. The sampling point was conducted at Campus C Airlangga University, Mayor Mustajab Street and Raya Darmo Street. The three points were chosen to represent different levels of traffic density in Surabaya City.

MATERIALS AND METHODS

Traffic Density Calculation

The number of motorized vehicles passing through

the three sampling locations was counted in the morning, afternoon and evening. The number of vehicles was counted using a hand counter for all types of motorized vehicles.

Leaves Sampling of *Dendrobium* sp

Samples of *Dendrobium*sp leaves were taken as many as 3 plants per location. Each plant wass taken as many as 3 leaves. The leaf samples that have been taken were then put in a plastic bag that has been labeled according to the sampling location. For each leaf, a sub-section of the base, middle, shoots was taken with a size of 1 x 1 cm to make preparations. Furthermore, the remaining leaves were tested for lead content (Pb) using the AAS method.

Observation of the number of leaf stomata of *Dendrobium* sp

The preparations were observed using a microscope with a magnification of 100x and a field of view with diameter of 1 mm. Stomata count was carried out randomly, with an area of view of 0.785 mm^2. Stomata density can be calculated using the following formula according to Lestari (2006):

$$\text{Stomata density} = \frac{\text{Number of stomata}}{\text{Wide field of view}}$$

Testing the content of lead (Pb) in the leaves of *Dendrobium*sp

Sample preparation

Sample preparation was done by chopping 1-2 mm of *Dendrobium* sp leaves and then drying them at 105 ^oC. The dried leaves of *Dendrobium* sp were crushed into powder/flour and filtered using a mess with a size of 40-60. The samples were weighed as much as 10 g and put into a porcelain dish to be burned into ashes. Then the ash was burned again at temperature of 500-600 ^oC. The white ash was added with 25 ml of HCl and 5 ml of concentrated HNO3. After that the sample was added with distilled water to a volume of 100 ml and then filtered using filter paper to obtain a clear filtrate of 50 ml.

Atomic Absorption Spectrophotometry (AAS) Readings

The AAS was activated and then blank absorbance was measured 3 times. After that, the absorbance was measured for several variations of the standard solution. If the result was appropriate, then the absorbance of the sample solution was measured. After being completely burned, the absorbance value (intensity of the color of the burn) was measured so that the absorbance value was obtained using a lead (Pb) calibration curve. The data that has been obtained was stored according to the name of the sample.

Data analysis

The obtained data were analyzed using SPSS 22.0 to find out the effect or correlation of the level of lead concentration (Pb) on the number of stomata per field of view of the leaves of *Dendrobium* sp. Data was also presented in graphical form using linear regression with the formula Y = a + bX.

RESULTS AND DISCUSSION

The calculation of vehicles

Based on the results in Table 1, the lowest to highest average vehicle volume among the three locations consecutively were Campus C Airlangga University, Mayor Mustajab Street, and Raya Darmo Street. Using a lead (Pb) calibration curve, the data that has been obtained s stored according to the name of the sample.

The content of lead (Pb) in the leaves of *Dendrobium*sp

Based on the results of measurement of lead (Pb), the levels of lead (Pb) contained in the leaf samples at each location had different concentrations. This

Table 1. The calculation results of vehicles at the location of Campus C Airlangga University, Jl. Mayor Mustajab, and Jl. Raya Darmo

	Number of Vehicles			
Location	07.00-08.00 WIB	12.00-13.00 WIB	17.00-18.00 WIB	Average vehicles aday
Campus C Airlangga University	118	96	102	105
JL Walikota Mustajab	224	198	212	211
JL. Raya Darmo	367	415	442	408

was consistent with the average volume of vehicles in each location which varies. The higher the level of traffic density in an area, the higher the lead intensity (Pb) in the air. The location with the lowest to highest average lead concentration (Pb) is Campus C Universitas Airlangga with a figure of 0.13 mg / kg, Jl. Mayor Mustajab with a total average of 0.17 mg / kg, and Jl. Raya Darmo with lead content (Pb) of 0.19 mg / kg.

The levels of lead (Pb) in the leaves of *Dendrobium* sp were closely related to the intensity of motorized vehicles because otorized vehicles produce heavy metal lead (Pb). According to Siregar (2005), the maximum Pb content in various types of plants was 3.0 mg / kg. Based on this, it could be said that the lead content (Pb) at Campus C Airlangga University, Jl. Mayor Mustajab and Jl. Raya Darmowas far below the maximum limit of lead (Pb) content in leaves. This indicates that *Dendrobium* sp leaves had a poor ability to absorb Pb.

Each type of plant has a different ability to absorb Pb. *Dendrobium* sp's smooth leaf surface and narrow leaf size lower the potential for the leaves to absorb lead (Pb). The low stomata density of *Dendrobium* sp leaves is also thought to be the cause of the reduced ability of the leaves to absorb lead (Pb) in the air. According to Nurhikmah (2015), the ability to absorb lead (Pb) particles in the air is influenced by the density and size of the stomata. In addition, the ability of plants to absorb lead (Pb) is greatly influenced by the condition of the plant leaf surface. Leaves that have hair (pubescent) or leaves with a rough (wrinkled) surface have a higher ability to absorb lead (Pb), than leaves that have a smoother and flatter surface. This is supported by Sembiring's (2006) statement which states that heavy metal particles attached to different leaf surfaces will cause different concentrations. The likelihood of heavy metal particles adhering to wider and rougher leaf surfaces is seven times greater than that of smooth leaf surfaces.

Stomata density in *Dendrobium* sp

Based on the results of the stomatal density

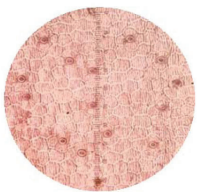

Fig. 1. Leaf stomata of *Dendrobium* sp at Campus C location

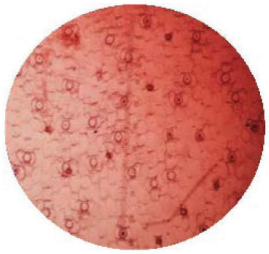

Fig. 2. Leaf stomata of *Dendrobium* sp at Walikota Mustajab Street

calculation, it showed that the average number of stomata density at the location of Campus C, Airlangga University was 30.37 /mm^2, on Jl. Mayor Mustajabwas 37.96 / mm^2, and on Jl. Raya Darmo was 41.56 /mm^2. The number of stomata can be classified into: few (1-50), many (101-200), very many (201->300) and infinite (301->700) (Haryanti, 2010). Based on this, the stomata density in the leaves of *Dendrobium* sp was classified as small category because it had an average number of stomata per field of view of less than 50.

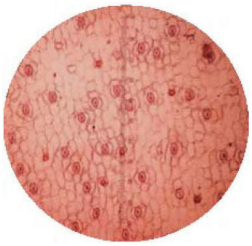

Fig. 3. Leaf stomata of *Dendrobium* sp at the location of Raya Darmo Street

The variation in the number of stomata density at the three locations was caused by the different lead (Pb) content in each leaf sample of *Dendrobium* sp. This is related to the different levels of traffic density at each research location. This is in line with the research of Azmat (2009), which states that the level of lead accumulation in the leaves of each type of plant varies depend on the location that is used as the research location, whether it is seen from the sampling location, and the level of motorized vehicle density. Mulia (2005) stated that the higher the number of stomata density, the higher the potential to absorb heavy metals or particles in the air. According to Gunarno (2014), the number of stomata in polluted areas is higher than in non-polluted areas. This is a form of physiological adaptation due to air pollution.

Stomata at each location showed a difference in density and different conditions. At the location of Jl.

Mayor Mustajab (Fig. 2.) and Jl. Raya Darmo (Fig. 3). It is clear that there are many black spots on the stomata and damage to the surrounding epidermal cells. This was due to the high levels of Pb pollution in both locations. The indication of stomata that is damaged due to the influence of pollutants, including Pb, will show a narrowing of the stomata gaps with a black color (Yanuar and Widyastutik, 2011).

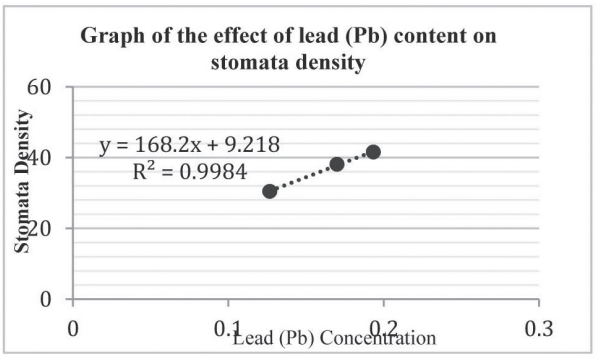

Fig. 4. Graph of the effect of lead (Pb) content on stomata density at the location of Campus C Airlangga University, Jl. Mayor Mustajab and Jl. Raya Darmo

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Effect of lead (Pb) content on stomata density in *Dendrobium* sp

Based on the correlation graph presented in Figure 4, it showed that the relationship between lead (Pb) levels in *Dendrobium* sp leaves had a positive effect on stomata density. From the graph, it obtained the formula Y = 168.2X + 9.218 and the coefficient of determination (R2) was 0.9984, which meant that the variable Pb (X) level affected the stomatal density variable (Y) by 99.84%, while the rest was influenced by factors - other variable factors.

Based on the results of the Pearson correlation test in Figure 5. a significance value of 0.041 (<0.05) was obtained, which means that there was a correlation between variables. The correlation results also showed a positive relationship, this indicated that the higher the lead (Pb) content, the stomata density in *Dendrobium* sp leaves would also increase. While the correlation value of this test was 0.687. If the correlation value is in the range> 0.5 - 0.75, it meant that the correlation was included in a strong correlation (Jonathan, 2009). Based on this, the relationship between lead (Pb) levels in leaves and stomatal density was classified as a strong correlation.

Table 2. Test results for the lead content of *Dendrobium* sp leaves at the location of Campus C Airlangga University, Mayor Mustajab Street, and Raya Darmo Street.

Location	Plant	Lead concentration (mg/kg)	Lead concentration Average (mg/kg)
Campus C Airlangga University,	1	0.12	0.13 ± 0.02
	2	0.15	
	3	0.11	
Jl. WalikotaMustajab	1	0.19	0.17 ± 0.02
	2	0.15	
	3	0.17	
Jl. Raya Darmo	1	0.19	0.19 ± 0.02
	2	0.22	
	3	0.17	

Table 3. The results of the stomata density calculation results of *Dendrobium*sp

Location	Plant	Stomata density (/mm^2)	Stomata density average (/mm^2)
Campus C Airlangga University,	1	34	30.37±4.559
	2	29	
	3	29	
Jl. WalikotaMustajab	1	36	37.96±3.818
	2	37	
	3	42	
Jl. Raya Darmo	1	40	41.56±3.776
	2	41	
	3	43	

Table 4. The results of the correlation test of the effect of lead (Pb) content on stomata density at the location of Campus C Airlangga University, Jl. Mayor Mustajab and Jl. Raya Darmo

Correlations		Lead (Pb) concentration	Stomata density
Lead (Pb) concentration	Pearson Correlation	1	.687*
	Sig. (2-tailed)		.041
	N	9	9
Kerapatan stomata	Pearson Correlation	.687*	1
	Sig. (2-tailed)	.041	
	N	9	9

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

The same thing happened in Farli's (2019) study of *Dracaena marginata* tricolor leaves, which showed a strong influence between Pb levels in leaves on the number of stomata, as well as a positive relationship, namely the higher the Pb lead levels in the leaves, the more stomata number. In addition, the angsana leaf study conducted by Yudha*et al*. (2013) also showed a positive correlation between Pb accumulation and leaf area and stomatal density per mm2.

According to Sukarsono (1998), lead (Pb) exposure affects stomatal density, this is a form of physiological adaptation. Lead (Pb) which has attached to the stomata will accumulate. If lead (Pb) is found in large quantities, it will damage the stomata cells. Lead (Pb) as a whole can potentially contaminate plants. Symptoms due to heavy metal contamination are chlorosis, necrosis of the tips and sides of the leaves and early late blight. Factors that influence Pb levels in plants are the length of time the plant is in contact with Pb, Pb levels in the soil, plant morphology and physiology, plant age. Factors that affect the area such as the number of cover crops and the types of plants around the plant. The absorption of Pb through the leaves occurs because the Pb particles in the air fall and settle on the leaf surface. Pb absorption in leaves occurs because the Pb particles in the air enter the leaves through a passive absorption process. The entry of Pb particles into the leaf tissue is strongly influenced by the size and number of stomata. The larger the size and the greater the number of stomata, the greater the absorption of Pb into the leaves (Rachmawati, 2005).

CONCLUSION

Based on this research, we conclude that :

1. The average lead content (Pb) in the leaves of *Dendrobium* sp due to pollution exposure at the Campus C of Airlangga University, Mayor Mustajab Street and Raya Mustajab Street were 0.13 mg/kg, 0.17 mg/kg, and 0.19 mg/kg respectively.
2. The lowest to highest stomatal densities were in the location of Campus C Airlangga University, Mayor Mustajab Street, and Raya Darmo Streer. This result showed that the higher the lead (Pb) content in the protocol road, the stomata density in the leaves of *Dendrobium* sp will also increase.

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