Analysis of Lead (Pb) in Leaf of Tabebuia aurea from Polluted Air

Rachmadiarti Fida[1*], *Asri* Mahanani[2], *Sahani* Kandilia Sari[3], *Nella* Yulia[4], *Nafidiastri Farah* Aisyah[5]

[1,2,3,4]Department of Biology, Faculty of Mathematic and Natural Science, Universitas Negeri Surabaya, Indonesia.
[5]Department of Biology, Faculty of Science and Technology, University of Airlangga, Indonesia.

Abstract. One of the three major heavy metals which can be harmful to plants, animals, and humans is lead (Pb). Smoke from gasoline fueled motor vehicles become the sources of these pollutants. In plants, including Tabebuia aurea can be found in the roads that are often passed by vehicles, so lead exposure is unavoidable. The purpose of this research was 1) to analyze the lead levels in T. aurea leaves, 2) to analyze the chlorophyll levels in T. aurea leaves, 3) to analyze the growth (leaf area). The Pb level in plant leaves was calculated using AAS (Atomic Absorption Spectrophotometer), chlorophyll level using spectrophotometer, growth was measured with leaf meter. Data were analyzed by descriptive and Anova. Based on the research and analysis that have been carried out can be concluded that 1) Pb metal levels 0.09 – 0.187 mg/L, 2) leaf chlorophyll levels ranges from 2.719 – 7.594 mg/L, and 3) Leaf area ranges from 186.720 – 199.288 cm2. Analysis with Anova ahows that the sampling location affected the Pb and chlorophyll content in the leaves, while the location did not affect the surface area of T. aurea leaves. The results of this research indicate that T. aurea can be used as a plant to absorb Pb pollutants in the air.

Keywords. Lead (Pb), Tabebuia aurea, Air Polluted, Anova

1 Introduction

The primary pollutants released into the atmosphere consist of Pb, Zn, Fe, and Cd[1]. One of them, namely Pb, can be harmful to plants, animals, and humans. Smoke from gasoline fueled motor vehicles become the sources of these pollutants. In Indonesia [2] research has been carried out which showed that several cities have relatively high PM 2,5 and PM 10 of Pb levels. Sampling locations in Surabaya, Jakarta and Tangerang have the higher Pb concentrations compared to other cities. From the quality standards, the Pb levels normally 2 □g/Nm3 within 24 hours [3]. Therefore efforts to ease Pb levels in the air need to be done. Several research to ease the Pb levels in the air have been carried out. One of them is using plants Syzygium oleina and Wedelia trilobata to absorb Pb [4] through a process of called phytoremediation. Phytoremediation is environmentally friendly technology in improving air pollution which is cost-effective and energy-saving. In the phytoremediation process, plant organs interact with microbes in the phyllosphere and rhizosphere to recover polluted air [5]. The previous research [6] reported that combination of microbes and leaves can ease air pollutants, such as Pseudomonas putida with the leaves of Azalea can decrease compounds of volatile organic [7], Burkholderia cepacia with the leaves of Yellow Lupine plant can reduce toluene [8], also association of Methylobacterium sp. with the leaves of Poplar plant for xenobiotic compounds reduction [9].

Leaf surfaces are acknowledged to absorb pollutants within big quantities of pollutants. Therefore, phyllospheric bacteria have a key role because they live in it. Pollutants absorbed in plants mainly create big-flowering leaf endophytes. These phyllospheric bacteria can reduce pollutants through degradation, transformation or sequestration. Furthermore, the precipitation process causes pollutants to downflow into the below soil plant then interact with the rhizosphere, roots, and soil. This is the key role of plants in easing pollution in the air, but the chlorophyll levels and growth will impact because of this ability.

One of the plants that can be used for this, namely T. aurea which is included in the Bignoniaceae family [10]. This plant has shrubs or tree habitus with a 5-50 m height and depending on different species. Morphology of leaves is complex or pairs crossed and has 3-7 leaf sheets.

Based on the importance of Pb involvement in the air pollution and the prediction that T. aurea can decrease Pb levels in the air, it is important to investigate the ability of this plant, so The purpose of this research

* Corresponding author: fidarachmadiart@unesa.ac.id

were 1) to analyze the lead levels in T. aurea leaves, 2) to analyze the chlorophyll levels in T. aurea leaves, 3) to analyze the growth (leaf area).

2 Material and Methods

2.1 Stage 1: Sampling of T. aurea

This research uses plants in Surabaya city and focuses on passive bio monitoring. Sample of T. aurea were carried out from 3 different roads, particularly Jl Mayjen Yono Suwoyo, Jl HR Muhammad, and Jl Hayam Wuruk. The location selection is based on traffic level and plants. The leaves of T. aurea, were carried out from 3 trees at each station. Leaves were taken from the base at the number of 3 branches for each tree. After that, the leaves will be used for some analysis.

2.2 Stage 2 : Sample Analysis

2.2.1 Analysis of Pb

Measurement of leaves Pb level used Atomic Absorption Spectrophotometry (AAS) Perkin Elmer production Medtech, Waltham, Massachusetts, United State. The first step is to weigh each sample of leaf as much 2g. Next put in the furnace within 3 hours at a temperature 800°C. Then, 2 ml of HNO3 and 10 mL or demineralized water was added to the sample. After the solution formed, next filtered the sample and used AAS to analyze it. Lead level was measured using the SNI No. 06-698945 of 2005 and calculated with below formula:

Cy' = (Cy x V/W) x 1000

Description:

Cy' = lead levels in leaves (μg/g)
Cy = measured concentration (mg/L)
V = volume of dilution (L)
W = weight of leaf dry (g)
1000 = conversion from mg to g

2.2.2 Analysis of Chlorophyll

Measurement of leaves chlorophyll level used spectrophotometers MAPADA V-1100D visible production Shanghai Mapada Instruments Co., Ltd, Shanghai, China. The first step is to weigh each sample of leaf as much 0.5 g then cut it into small pieces and macerated with 50 ml of alcohol 96% until the color of the leaf is dissolved. After the solution formed, then filtered it. The chlorophyll level was measured based on optical density using spectrophotometers at wavelengths of 665 and 649 nm.. Level of chlorophyll a, chlorophyll b, and total chlorophyll were measured using the Wintermans and de Mots formula [8]:

Chlorophyll a = 13.7 x OD 665 – 5.76 OD 649 (mg/l)
Chlorophyll b = 25.8 x OD 649 – 7.7 OD 665 (mg/l)
Total chlorophyll = 20.0 x OD 649 + 6.1 OD 665 (mg/l)

2.2.3 Leaf Area Analysis

Leaf area analysis was measured using Leaf Meter ADC AM350. The first step is to put the leaf sample into the meter area, next move the scanner while pressing the scanner button and move it from the base to tip. When the measurement was done then click the scanner button again and the result will appeare in the monitor. Furthermore, record the measurement results.

2.3 Data Analysis

Data from this research including leaves of Pb levels, leaves of chlorophyll levels, and leaf area at various locations were analyzed by Anova.

3 Results and Discussion

3.1 Result

Research has been carried on T. aurea as an absorber of Pb in the atmosphere and the results of the research are presented below.

3.1.1 Pb levels in T. aurea leaves

The results of Anova and Duncan's test of Pb levels in T. aurea leaves at various locations (Table 1) represent that location affected the Pb levels in T. aurea leaves. The Pb levels in T. aurea leaves, from high to low, were the Pb levels in T. aurea leaves on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, there was no difference in Pb levels in these two areas, followed by T. aurea leaves in Jl Hayam Wuruk.

Tabel 1. Analysis of Pb levels in T. Aurea leaves.

Plant Leaves	Average Pb Levels of Leaves on Various Street in Surabaya City (mg/L)		
	1	**2**	**3**
T. aurea	0.187 ± 0.0062B	0.186 ± 0.0130B	0.090 ± 0.0146A

Description:
1 = Sampling location on Jalan Mayjend Yono Soewoyo (with three repetition points)
2 = Sampling location on Jalan H.R Muhammad (with three repetition points)
3 = Sampling location on Jalan Hayam Wuruk (with three repetition points)
The notations A, B, dan C are the code for the sampling location.

3.1.2 Chlorophyll levels in T. aurea leaves

The Anova result and Duncan's test of chlorophyll levels in T. aurea leaves at various locations (Table 2) represent that the location affected the chlorophyll levels in T. aurea leaves. The chlorophyll levels in T.

aurea leaves, from low to high, were the chlorophyll levels in T. aurea leaves on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, there was no difference in chlorophyll levels in these two areas, followed by T. aurea leaves in Jl Hayam Wuruk.

Tabel 2. Analysis of total chlorophyll levels in T. Aurea leaves.

Sample Type Plant Leaves	Average Chlorophyll Levels of Leaves on Various Street in Surabaya City (mg/L)		
	1	2	3
T. aurea	2.719 ± 0.855A	3.788 ± 1.846A	7.594 ± 1.767B

Description:
1 = Sampling location on Jalan Mayjend Yono Soewoyo (with three repetition points)
2 = Sampling location on Jalan H.R Muhammad (with three repetition points)
3 = Sampling location on Jalan Hayam Wuruk (with three repetition points)
The notations A, B, dan C are the code for the sampling location

3.1.3 Analysis of Leaf area in T. aurea

The results of Anova analysis of the leaves area at T. aurea at various locations (Table 3) showed that the location did not affect the leaf area of T. aurea in three street on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, and Jl Hayam Wuruk there was no difference.

Tabel 3. Analysis of Leaf Area in T. aurea.

Sample Type Plant Leaves	Average of Leaf Area on Various Street in Surabaya City (cm^2)		
	1	2	3
T. aurea	190.140 ± 528.238	199.288 ± 2.062	186.720 ± 6.055

Description:
1 = Sampling location on Jalan Mayjend Yono Soewoyo (with three repetition points)
2 = Sampling location on Jalan H.R Muhammad (with three repetition points)
3 = Sampling location on Jalan Hayam Wuruk (with three repetition points)
The notations A, B, dan C are the code for the sampling location

The results of data analysis showed that T. aurea were able to absorb Pb. The location where T. aurea grows affects the Pb levels in T. aurea leaves. The Pb levels in T. aurea leaves, from high to low, were the Pb levels in T. aurea leaves on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, there was no difference in Pb levels in these two areas, followed by T. aurea leaves in Jl Hayam Wuruk. Pb levels in these three areas have exceeded the environmental quality standards. High levels of Pb pollutants in the air can cause physiological changes that can inhibit plant growth and even death. Metal (Pb) in the air enters the leaves tissue bound by cell membranes, mitochondria, and chloroplasts. Research [11] said that metal (Pb) is one of the PM10 class pollutants with a particle size of less than 10 μm. T. aurea is a shrub-tree habitus plant with a height of up to 3 meters; has a single green leaf, oval in shape, leaf bones are fingered, the tip is tapered, and has very thick leaves and trichomes. The ability of T. aurea is considered as the most effective in absorbing Pb because the leaf morphology supports and is in line with previous research. Based on the previous research, it is stated that if plants have trichome leaves and rough surfaces they will be able to absorb pollutants more optimally than plants with smooth leaves and the surface is slippery. High levels of Pb in plant tissues can cause a decrease in leaf chlorophyll content [12]. The capability of plants to absorb Pb in the air can decrease the Pb levels in the air [13]. Physical and chemical environmental factors such as temperature, soil temperature, air humidity, soil moisture, soil pH, and light intensity affect the absorption of Pb by plants. Plants absorb Pb in the air in the way of passive absorption. Pb enters through the stomata gap, settles in the leaf tissue, and will converge between the palisade or gaps in spongy tissue.

The results of data analysis of the chlorophyll levels in T. aurea leaves at various locations showed that the location affected the chlorophyll levels in T. aurea leaves. The chlorophyll levels in T. aurea leaves, from low to high, were the chlorophyll levels in T. aurea leaves on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, there was no difference in chloropyll levels in these two areas, followed by chlorophyll levels of T. aurea leaves in Jl Hayam Wuruk. The results of the chlorophyll levels analysis are in line with Pb levels. T. aurea on Jl Mayjen Yono Suwono and Jl H.R Muhammad, where Pb levels in T. aurea are relatively high compared to Pb levels in T. aurea leaves on Jl Hayam Wuruk followed by low chlorophyll levels in these two areas compared to chloropyll levels on Jl Hayam Wuruk. This shows that the levels of Pb in tissue affect the levels of chlorophyll in the leaves. The test results are in line with the research of [14] and [4] which states that the increase in leaf chlorophyll levels is directly proportional to the decrease in pollution by Pb, so that leaf chlorophyll levels can be an identification of the resistance of a plant to air pollution by Pb. The mechanism of plants dealing with toxic environments is by way of amelioration with removing ions from the circulation site using several pathways, then tolerant in the cytoplasm. The chloroplast structure formation is affected by Mg and Fe mineral nutrients. The Pb entry in plants will influence the number and volume of leaf chloroplast [15]. The longer the leaves on plants are exposed to vehicle exhaust emissions, the higher the Pb absorbed by the leaves will cause disruption of the photosynthesis process. Heavy metals will inhibit enzymes involved in the dark reaction photosynthesis process [16]. High levels of Pb in leaves can cause stomata size to shrink and the number to decrease due to accumulation of Pb in leaves [17]. The test results are different from research that says plants that have the potential as pollutant adsorbent can accumulate lead in large

quantities without causing a decrease in chlorophyll in the leaves and without poisoning in plants [18].

The results of Anova analysis of T. aurea leaf area at various locations (Table 3) showed that the location did not affect the leaf area. The leaf area of T. aurea in three street on Jl Mayjen Yono Suwoyo, Jl H.R Muhammad, and Jl Hayam Wuruk had no difference. The results of measuring the leaf area of T. aurea became an indication that this plant is resistant to Pb, this is shown by the leaf area in the three areas there is no difference. This means that in areas exposed to low and high Pb, it has no effect on the leaf area. This condition indicates that the decrease in chlorophyll in the leaves can still be tolerated so that the plant continues to grow and shows no change in leaf area. In contrast, the research of [19] also said that the reduction in chlorophyll levels also affected the leaf area. Low chlorophyll levels cause the photosynthesis process to be less effective, so that the leaf area will also decrease due to the inhibition of the process of division and elongation of leaf apical cells [20].

Conclusion for the research that the sampling location affected the Pb and chlorophyll levels in the leaves, while the location did not affect the leaf area of T. aurea leaves. The results of this research indicate that T. aurea can be used as a plant to absorb Pb pollutants in the air.

Acknowledgement

The acknowledgments Thank you to Universitas Negeri Surabaya for funding this research through Unesa PNBP 2022 funds.

References

[1]. Archibald, AT, Folberth, G., Wade, DC, Scott, D. A World Avoided: Impacts of Changes in Anthropogenic Emissions On The Burden And Effects Of Air Pollutants In Europe And North America. farads. Discuss (2017).

[2]. JPNN. (2020). Air Pollution: 3 Cities with the Highest Lead Levels, Oh Surabaya. https://www.jpnn.com/news/pollusi-air-3-kotawith-highest-lead-level-oh-surabaya?page=2. Retrieved April **2** (2021).

[3]. Decree of the Minister of the Environment Number: 554/MENLH/10/1997 concerning the Air Pollutant Standard Index (ISPU). Jakarta.

[4]. Rachmadiarti, F., Purnomo, T., Azizah, DN, Fascavitri, A. Syzigium oleina and Wedelia trilobata for Phytoremediation of Lead Pollution In The Atmosphere. Nature Environment and Pollution Technology, **18(1)**:157-162 (2019).

[5]. Lee, BXY, Hadibarata, T. & Yuniarto, A. Phytoremediation Mechanisms in Air Pollution Control: a Review. Water Air Soil Pollut **231**, 437 (2020). E3S Web of Conferences **328**, 0 (2021) ICST 2021 8003 https://doi.org/10.1051/e3sconf/202132808003 3

[6]. Weyens, N., Thijs, S., Popek, R., Witters, N., Przybysz, A., Espenshade, J., Gawronska, H., Vangersveld, J., Gawronski, SW, The Role Of Plant– Microbe Interactions And Their Exploitation For Phytoremediation Of Air Pollutants. int. J. Mol. Sci., **16**:25576-25604 (2015).

[7]. De Kempeneer, L., Sercu, B., Vanbrabant, W., Van Langenhove, H., And Verstraete, W. Bioaugmentation of The Phyllosphere For The Removal Of Toluene From Indoor Air. app. Microbiol. Biotechnol, **64**:284–288 (2004).

[8]. Barac, T., Taghavi, S., Borremans, B., P, Ovoost, A., Oeyen, L., Colpaert, JV, Et Al. Engineered Endophytic Bacteria Improve Phytoremediation of Water-Soluble, Volatile, Organic Pollutants. Nat. Biotechnol, **22**:583–588, (2004).

[9]. Van, AB, Yoon, JM, Schnoor, JL Biodegradation of Nitro-Substituted Explosives 2,4,6-Trinitrotoluene, Hexahydro-1,3,5-Trinitro1,3,5-Triazine, And Octahydro-1, 3,5,7-Tetranitro1,3,5-Tetrazocine by A Phytosymbiotic Methylobacterium Sp. Associated with Poplar Tissues (Populus Deltoides × Nigra DN34). app. environment. Microbiol., **70**:508–517 (2004).

[10]. Singapore Government Agency Website. 2022. Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S.Moore. NParks | Tabebuia aurea

[11]. Muzayanah, Ariffin, Sudarto and Yanuwiadi, B. Effects of the green space proportion with cumulative concentration of particulate matter 10 (PM10) in Surabaya-Indonesia. International Journal of ChemTech Research, **9(4)**: 431-436 (2016).

[12]. Gothberg, A. Metal fate and sensitivity in the aquatic tropical vegetable Ipomoea aquatica. Department of Applied Environmental Science, Doctoral Thesis, Stockholm University (2008).

[13]. Kurnia, R. Laporan Kegiatan Kursus Identifikasi Dampak Lingkungan. Yogyakarta: GEGAMA (2004).

[14]. Sari, F.R., Purnomo. T. and Rachmadiarti, F. Ability of betel plant (Epipremnum aureum) as lead (Pb) absorbent in the air. Lentera Bio., **5(3)**: 117-124 (2016).

[15]. Olivares, E. The effect of lead on phytochemistry of Tithonia diversifolia exposed to roadside automotive pollution or grown in pots of Pb-suplemented soil. Brazilian Journal Plant Physiology, **15(3)**: 149-158 (2003).

[16]. Harianto, V and Pohan, S. D. Respon Pertumbuhan dan Fisiologis Tanaman Sawi (Brassica rapa var. Parachinensis) yang dipapar Timbal (Pb). Jurnal Biosains **4(3)**: 154-160 (2018).

[17]. Xiao Shu & LiYan Yin & QuanFa Zhang & Wei Bo Wang. 2011. Effect of Pb toxicity on leaf

growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of Jatropha curcas L. Environ Sci Pollut Res DOI 10.1007/s11356-011-0625-y.

[18]. Fathia, L.A.N., Baskara, M. and Sitawati, S. The Analysis of plants capability at Median road in absorbing heavy metals Pb. Journal of Plant Production, **3(7)**: 528-534 (2015).

[19]. Satolom, A. W., N. Y. Kandowangko, dan A. S. Katili. 2013. Analisis Kadar Klorofil, Indeks Stomata dan Luas Daun Tumbuhan Mahoni (Swietenia macrophylla King.) pada Beberapa Jalan di Gorontalo. http://kim.ung.ac.id/index.php/KIMFMIPA/article/view/3700.

[20]. Sembiring, E. and Sulistyawati, E. Pb absorption and its effect on leaf condition of Swietenia macrophylla King. Paper presented at the National Seminar on Environmental Research at Higher Education 2006, at Bandung Institute of Technology Campus, 17-18 July 2006 (2006).