Nature Environment and Pollution Technology
An International Quarterly Scientific Journal
p-ISSN: 0972-6268
e-ISSN: 2395-3454
Vol. 18 No. 1 pp. 157-162 2019

Original Research Paper Open Access

Syzigium oleina and *Wedelia trilobata* for Phytoremediation of Lead Pollution in the Atmosphere

Fida Rachmadiarti†, Tarzan Purnomo, Diana Nur Azizah and Ayudhiniar Fascavitri
Department of Biology, Faculty Mathematic and Natural Science, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia
†Corresponding author: Fida Rachmadiarti

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 30-05-2018
Accepted: 02-08-2018

Key Words:
Phytoremediation
Lead
Syzigium oleina
Wedelia trilobata

ABSTRACT

Heavy metals cause pollution in the environment, including plants. In Indonesia, *Syzigium oleina* and *Wedelia trilobata* are grown on the main roads where vehicles frequently pass through; these plants are exposed to heavy metals, such as lead (Pb). The aim of this study was to evaluate how the location of plants, the plant species, and the interaction between these two variables, affect the content of Pb metal and chlorophyll in leaves. The levels of heavy metals in plant leaves were measured by using an atomic absorption spectrophotometer, and chlorophyll content by using a spectrophotometer. Data were analysed by two-way ANOVA. The results showed that the location of plant species affected the content of Pb metal and chlorophyll in the leaves, but the interaction of the location and the type of plant only affects the chlorophyll content of leaves and not the Pb metal content.

INTRODUCTION

In general, the main cause of air pollution in cities is an increasing number of motor vehicles, including in Surabaya, Indonesia (Purwatiningsih 2015). Based on the data from Surabaya Metropolitan Police, the total length of road in Surabaya is only 2,096.69 km. However, the total number of motor vehicles reaches 3,895,061 units. This has led to changes in the air quality in Surabaya (Samsoedin et al. 2015). Motor vehicles are a moving source of contaminants so that the spread of the emitted polluting material has a wide spatial dispersion pattern (Boediningsih 2011). Motorized vehicles produce emissions, which include pollutants such as lead. The accumulative Pb is mainly derived from the imperfect combustion of gasoline additives used in motor vehicles (Sunu 2001). In addition to Pb, the exhaust of motor vehicles also contains pollutant compounds such as carbon monoxide (CO), hydrocarbons (HC), sulphur oxides (SOx), nitrogen oxides (NOx) and particulates (Kean et al. 2000).

Lead in vehicle gasoline serves to improve octane fuel efficiency and to lubricate the valve seat of cars. Pb and gasoline are burnt in the engine (EPA 2007). About 10% of Pb emissions from a motor vehicle will contaminate locations within a radius of < 100 m, whereas 5% can contaminate locations within a radius of 20 km and 35% will be carried by the atmosphere and have a potentially very wide-reaching range (EPA 2007). It is, therefore, necessary to address methods of reducing motor vehicle pollution, namely Pb emissions. One method of doing so is to use plants.

Plants that have the potential to absorb Pb include *Syzigium oleina* and *Wedelia trilobata*. Both of these plants are primarily grown on the main streets of cities, including Surabaya. Plants can be used as lead absorbers because they are able to absorb it in the air through passive absorption mechanisms. Pb is absorbed by the plant through leaf stomata and will stay in the leaf tissue and accumulate between the palisade tissue gaps (Santoso 2013). The plants that have the ability to be Pb-absorbent are those that can hold a large accumulation of Pb in their leaves, but will not show changes in leaf morphology such as in the stomata and chlorophyll (Fathia et al. 2015). Excessive Pb emissions in the air can affect the structure and physiology of plants. It may cause a decrease in growth and productivity, and can even cause death to plants. Excessive Pb absorption also causes a decrease in chlorophyll levels. The Pb entering the cytoplasm would hamper and inhibit the actions of the enzymes delta-aminolevulinic acid dehydratase (ALAD) and porphobilinogenase, which are both involved in the biogenesis of chlorophyll (Flanagan et al. 1980).

Plants also respond to the entry of Pb into tissue as an environmental stressor and undergo physiological changes as an adaptive response. The effects of Pb on plants include chlorosis, cell wall damage, and decreased chlorophyll biosynthesis. Changes in chlorophyll levels are another common physiological response (Novita et al. 2012). For example, a decrease in chlorophyll levels in *Swietenia macrophylla* leaves in conjunction with an increase of Pb levels has previously been described (Sembiring & Sulistyawati 2006).

The characteristics of plants that are effective Pb absorbers are that they have dense foliage that do not easily fall, that the leaves have a cuticle layer and are scaly, and that the leaf shape resembles a needle with a serrated leaf edge (NASA & ALCA 1989). *S. oleina* is characterized by dense foliage (single leaf) and lancet shaped leaves, with a shiny leaf surface. Conversely, *W. trilobata* herb characterized by oval-shaped leaves with a hairy leaf surface. Different plant habits affect the ability of the leaves to absorb Pb in the air. Herbs have a higher potential for heavy metal pollutants absorption than bushes and trees, as herbs are able to inhabit up more layers of ground cover (Landberg & Greger 1996).

In the context of the many problems of Pb air pollution, and with varying studies focusing on the potential of Pb absorption in different plants, it is necessary to determine both the Pb absorbing ability of individual plant species, and whether there are adverse effects of Pb on these plants, such as on leaf chlorophyll levels. The aim of this study is to evaluate how location, plant species, and the interaction between location and species affect the content of Pb metal and chlorophyll in the leaves of S. *oleina* and *W. trilobata* in Surabaya, Indonesia. Site selection was based on the fact that these plant species are widely grown at this location.

MATERIALS AND METHODS

Sample Collection

The samples of *S. oleina* and *W. trilobata* leaves were collected during the rainy season and taken from three different Surabaya town locations: Diponegoro Street, J.A. Suprapto Street and H.R. Muhammad Street. Of the three streets, Diponegoro Street is traversed by the most vehicles, about 154850 units a day, whereas J.A Suprapto Street is traversed by 99350 vehicles a day, and H.R. Muhammad Street is traversed by 19474 vehicles a day (Transportation Department of Surabaya Town, 2016). The leaves from both the plants were taken from the 7^{th} position from the bud.

Analysis of Pb Content

Sample preparation: The preparation of samples was done using destructive methods. Leaves from each sample were weighed and ± 2.0 gram samples were placed into a porcelain cup or mortar. These samples were dried in an oven at 105°C for 2 hours. The samples were then cut into small pieces and put into a glass beaker, along with 10 mL of HNO_3 65% solution. The samples were then destroyed by using a destruction flask until red NO_2 gas appeared. The sample was then cooled and 2-4 mL of $HClO_4$ 70% solution was added. The sample was reheated and cooled until evaporation and reduced volume amount. The sample was then moved into a 50 mL measuring flask and diluted with distilled water until the meniscus sat on the 50 mL mark. The sample was then ready to be analysed by Atomic Absorption Spectrophotometer (AAS).

The production of standard metal solutions: Precisely 100 ppm of lead (Pb) standard solution was made from 1000 ppm of Pb main solution, then 10 mL of it was taken by drop pipette and added into 100 mL measuring flask, then diluted with distilled water until tera mark precisely, then homogenized. A 10 ppm Pb working solution was made from the 100 ppm Pb standard solution using the same method.

The production of calibration curves: The Pb standard solution was made by diluting the 10 ppm Pb solution until the desired concentration, ranging from 0-3 ppm, was reached. Then the absorbance value of each standard solution was measured with an AAS AA-700 Perkin Elmer tool using a Pb hollow cathode lamp.

The sample measurement: The measurement of the leaf samples was done according to the method generally used in tests of Pb content, in accordance with Indonesia National Standard (SNI) number 06-698945 in 2005.

The calculation of the Pb content of the leaves was obtained using the following formula:

$$Cy' = \left(Cy \times \frac{V}{W} \right) \times 1000$$

Where,
Cy' = Pb content that was absorbed in the leaves (µg/g)
Cy = Pb content that was measured by AAS (ppm or mg/L)
V = Volume of dilution solution (L)
W = Leaf biomass as dried leaf weight (g)

Analysis of Chlorophyll Content

About 1 gram sample was ground by mortar and pestle and made into an extract by mixing the leaf smear with 95% alcohol solution by as much as 100 mL, then filtering this solution with filter paper until the obtained filtrate volume was approximately 100 mL. The chlorophyll content contained in the leaf filtrate was measured using a spectrophotometer at wavelengths of 649 and 665 nm. Then the absorbance values of chlorophyll *a*, *b*, and the total chlorophyll content of the solution were calculated using the formula of Wintermasn and de Mots as follows.

Chlorophyll $a = 13.7 \times$ OD 665 - 5.76 OD 649 (mg/L)

Chlorophyll $b = 25.8 \times$ OD 649 - 7.7 OD 665 (mg/L)

Chlorophyll total = $20.0 \times$ OD 649 + 6.1 OD 665 (mg/L)

Data Analysis

ANOVA analyses were carried out to assess the impact of location, plant species, and the interaction between location and species, on both the Pb and chlorophyll content of the leaves.

RESULTS

Table 1 gives the results of ANOVA on the influence of plant species, location, and the interaction between plant species and location, on Pb content. ANOVA results showed that the location and species of plants affect Pb content ($p < 0.05$), but the interaction of location and plant species did not affect the Pb content ($p > 0.05$).

The Pb content of *S. oleina* and *W. trilobata* leaves that were exposed to pollutants from motor vehicle emissions is presented in Table 2. The results of the Duncan test showed that Pb content in both the plants was highest in plants grown at station 1, second highest at station 2, and lowest at station 3. The Pb content of *W. trilobata*, in general, was higher than *S. oleina* plants.

Table 2 showed the ANOVA results investigating the influence of location and plant species as well as the interaction of location and plant species on Pb content.

Table 1: ANOVA result of location and type of plant *S. oleina* and *W. trilobata* with Pb content in the leaves.

Source	Dependent Variable	Sign
Location	Pb content	0.05
Plant	Pb bontent	0.00
Location*Plant	Pb content	0.92

Table 2: The average and standard deviation of Pb content in *W. trilobata* and *S. oleina* leaves at various locations.

Type of Plants	Station	Lead content (Pb) in the leaves (ppm)
W. trilobata	St. 1	0.288 ± 0.040bB
	St. 2	0.273 ± 0.016abB
	St. 3	0.258 ± 0.026aB
S. oleina	St. 1	0.186 ± 0.005bA
	St. 2	0.165 ± 0.010abA
	St. 3	0.146 ± 0.011aA

Notes: a, b: location; A, B: species of plant
St. 1 Diponegoro Street, St. 2 J.A Suprapto Street and St. 3 H.R Muhammad Street.

These results show that the location and species of plants, as well as the interaction between location and the plant species, affect the Pb content ($p < 0.05$).

The chlorophyll content of *S. oleina* and *W. trilobata* leaves that were exposed to pollutants from motor vehicle emissions in the form of Pb is presented in Tables 3 and 4. The results of the Duncan test showed that chlorophyll content in both the plants was highest in plants grown at station 1, second highest at station 2, and lowest at station 3. In general, the chlorophyll content of *S. oleina* was higher than in *W. trilobata*.

DISCUSSION

In this research, *S. oleina* and *W. trilobata* grown on Diponegoro Street, J.A. Suprapto Street, and H.R. Muhamad Street in Surabaya city displayed differing levels of Pb absorption as well as different chlorophyll contents. There were no measurements of Pb content in the ambient air in this study, but the Pb content in Surabaya was assumed to be 2.664 $\mu g/m^3$ (Siregar 2005). Based on the National Ambient Air Quality Standards (NAAQS), the standard of Pb content in the air was equal to 0.15 $\mu g/m^3$ while the Pb content in Surabaya for particular matter ($PM_{2.5\text{-}10}$) was 0.27 $\mu g/m^3$. However, the quality standard established by the Indonesian Government Regulation 41 in 1999 for Pb was 2 $\mu g/m^3$ (24 hours) or 1 $\mu g/m^3$ (12 hours), so the quality of Pb in air particulate in Surabaya from October 2012 to February 2014 has not exceeded this established quality standard. So, it can be concluded that Pb content in $PM_{2.5}$ and $PM_{2.5\text{-}10}$ categories has exceeded the quality standard of NAAQS from the United States Environmental Protection Agency (USEPA), but has not exceeded the quality standard of the Government Regulation 41 in 1999.

The absorption of lead (Pb) on *W. trilobata* and *S. oleina* plants: This study found that the Pb metal content of plant leaves grown in areas through which motor vehicles pass were affected by the number of vehicles that pass. *W. trilobata* and *S. oleina* leaves grown on Diponegoro Street, which, of the three streets is traversed by the most vehicles, had a higher Pb content than leaves grown on the other two streets. This indicated that the location of growth has an effect on the Pb content of leaves, both in *W. trilobata* and *S. oleina* (Samsoedin et al. 2015).

This research also found that the species of the plants affects the Pb content in leaves. The Pb content of *W. trilobata* and *S. oleina* ranged from 0.146-0.288 µg/g. The Pb content of both the plants is still within the normal range, as the accepted Pb content for many plant species range from 1.0-3.5 µg/g (Siregar 2005). However, of the two plants, *W. trilobata* had higher Pb levels than *S. oleina*. The high

Table 3: ANOVA result of location and type of plant *Wedelia trilobata* and *Oleina syzygium* with chlorophyll content in the leaves.

Source	Dependent Variable	Sign
Location	Chlorophyl content	0.00
Plant	Pb bontent	0.00
Location*Plant	Pb content	0.03

Table 4: The average and standard deviation of chlorophyll content in *S. oleina* and *W. trilobata* leaves at various locations.

Type of Plants	Station	Chlorophyll content in leaves (mg/L)
W. trilobata	St. 1	15.563 ± 1.886bA
	St. 2	12.593± 0.357abA
	St. 3	13.200± 3.346aA
S. oleina	St. 1	23.186 ± 0.804bB
	St. 2	16.403 ± 1.092abB
	St. 3	19.766 ± 4.570abB

Notes: a,b: location; A,B: species of plant
St. 1 Diponegoro Street, St. 2 J.A Suprapto Street and St. 3 H.R Muhammad Street.

absorption of Pb by *W. trilobata* leaves is associated with its herbaceous plant habit, as opposed to the shrubby habit of *S. oleina*. The height of a plant and the position of its leaves has an effect on its ability to absorb Pb. A lower plant height is able to absorb more Pb (Istiharoh et al. 2014). Another study stated that Pb is part of PM_{10}, particulate matter sized ≤ 10 µm, which is optimally distributed to shrub habit plants (Muzayanah et al. 2016). The level of pollutant distribution varies between altitudes from the soil surface upwards, whilst the number and distribution of plant leaves vary between canopy and stem so that higher floating pollutants can be absorbed by trees, while those floating at lower altitudes or those that fall are absorbed by ground cover plants (Inayah et al. 2010).

In addition, some studies have reported that furry leaves and rough surfaces will absorb more dust pollutants than leaves with slippery surfaces. Based on this, *W. trilobata* has a rough and wrinkled leaf surface that was effective in the absorption of Pb from the air. This was in line with the research that stated that feathered or wrinkled leaf surfaces have a high ability to absorb Pb compared with slippery and flat leaf surfaces (Nurhikmah et al. 2015). Thus, *S. oleina* plants have a low ability to absorb the Pb in the air compared with *W. trilobata*, as *S. oleina* has flat leaves, and a slippery surface in terms of leaf texture; *S. oleina* has leaves that are less effective in absorbing lead from the air. Furthermore, with the research by Gothberg (2008) that growing plants in areas with high air pollution may disturb the growth of plants susceptible to particulate Pb absorption. The lead is deposited on the surface of the plants and can cause a decrease in the chlorophyll content of leaves (Gothberg 2008). The effectiveness of a plant's ability to absorb Pb, to a certain extent, will decrease with increasing Pb content (Kurnia 2004). Another factor that affects the mechanism of Pb absorption by plants is the opening of the leaf stomata. A Pb particle is roughly less than 2 µm in diameter, so plants with large stomata and/or large numbers of stomata can absorb more Pb into their leaf tissue during photosynthetic gas exchange than plants with small and/or few stomata. Besides these factors, the process of Pb absorption by the leaves is also influenced by temperature, light and moisture.

The mechanism of Pb absorption in *S. oleina* and *W. trilobata* leaves is through passive absorption. The Pb particles enter into the leaf through the stomatal gap and settle in the leaf tissue, accumulating between the palisade tissue gap or the spongy tissue. Because Pb particles are insoluble in water, Pb in the tissue is trapped in the cavity between the cells around the stomata. The contaminated gas enters the leaf tissue through the holes of stomata located on the upper epidermal layer. Through these holes, the pollutant dissolve in the surface water of the leaf cells (Santoso 2013). This Pb will accumulate inside the palisade tissue (Fitter & Hay 1991).

The chlorophyll content in *W. trilobata* and *S. oleina* leaves: Other research has found that location, plant species, or interaction of location and plant species affect the chlorophyll content of plant leaves. Plants that grow in areas where many motor vehicles pass through modify the chlorophyll content of their leaves. The higher number of passing vehicles lower the chlorophyll content of the leaves. The chlorophyll content in the leaves of *W. trilobata* and *S. oleina* on Diponegoro Street was lower than on JA Suprapto and HR Muhammad Street. This indicates that location affects the chlorophyll content of the leaves, both in *W. trilobata* and *S. oleina*.

W. trilobata has a higher Pb content and a lower chlorophyll content compared with *S. oleina*. *S. oleina* has a lower Pb content and a higher chlorophyll content than *W. trilobata*.

The chlorophyll content in both *S. oleina* and *W. trilobata* was still within the normal range and did not indicate a physiological response due to Pb exposure. This was supported by research that finds that plants with Pb absorbing capabilities tend to have a high accumulation of Pb in leaves but do not show any changes in leaves structure and physiology, such as in the stomata and in chlorophyll levels (Fathia et al. 2015).

S. oleina and *W. trilobata* leaves with exposure to Pb emissions on some streets in Surabaya town did not decrease the chlorophyll content of leaves, and chloroplast structure was not damaged. The formation of chloroplast structure is strongly influenced by mineral nutrients such as Mg and Fe. The entry of Pb in plants will reduce the intake of Mg and Fe, which can change the amount and volume of chloroplasts (Olivares 2003). From this description, it was evident that the accumulation of Pb in the leaves did not cause the inhibition of Mg and Fe minerals intake in the plants researched in this study. The plants did not change their absorption of mineral nutrients due to the influence of Pb and working of enzymes that were necessary for chlorophyll formation were not inhibited. These enzymes included porphobilinogen deaminase, protochlorophyllide, and a delta-aminolevulinic acid dehydratase. In terms of mechanisms that plants may use to deal with toxic concentrations, one method is through amelioration by moving ions from the circulation place with some way or become tolerant in the cytoplasm (Olivares 2003). The Pb particles that entered into the leaves will pass through the stomata, the size and number of which greatly affecting the entry of lead particles (Gunarno 2014). Plants act as absorbers because they are capable of absorbing Pb in the air through passive absorption mechanisms. Pb is absorbed by plants through leaf stomata located in the upper epidermal layer and settles in leaf tissue, accumulating between the palisade tissue gap or the spongy tissue (Santoso 2013).

The ability of plants to absorb Pb metal is also influenced by the leaf area. *W. trilobata* has wider leaves that are able to absorb more metals than *S. oleina*. This is in line with the study which concluded that *Codiaeum variegatum*, which has wide leaves, has the ability to absorb Pb in greater quantities than the leaves of *Hymenocallis speciosa* and *Cerbera manghas* leaves, which have an oval leaf shape (Sari et al. 2016). A wide leaf shape is more effective in absorbing pollutants compared to leaves with small surface areas (Dewi & Hapsari 2012).

CONCLUSION

The results showed that the location of a plant species affected the content of Pb metal and chlorophyll in leaves, but the interaction between the location and the species of plants only affects the chlorophyll content and not the Pb metal content of leaves.

ACKNOWLEDGMENTS

We would like to thank Dr. Sunu Kuntjoro and Dr. Yuliani for reviewing the article.

REFERENCES

Boediningsih, W. 2011. The impact of traffic density on air pollution in Surabaya City. Jurnal Fakultas Hukum, 20(20): 119-138.

Dewi, Y. S. and Hapsari, I. 2012. The effectiveness study of curing leaf (*Codiaeum variegatum*) and mother in-laws tounge (*Sansevieria trispasciata*) in absorbing lead in ambient air. Satya Negara Indonesia Scientific Journal, 5(2): 1-7

EPA 2007. Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. https://www.epa.gov/sites/production/files/2015-08/documents/method_200-7_rev_4-4_1994.pdf.

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

Ferdhiani, S.L. and Proklamasiningsih, E. 2016. Peroxidase enzyme activity and chlorophyll content at Angsana leaves. Biosfera, 32(2): 126-133

Fitter, A.H. and Hay, R.K.M. 1991. Physiology of Plant Environment. Yogyakarta: Gadjah Mada University Press.

Flanagan, J. T., Wade, K. J., Currie, A. and Curtis, D. J. 1980. The deposition of leads and zinc from traffic pollution on two road-side scrubs. Environmental Pollution, 1: 71-78.

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

Gunarno 2014. Influence of air pollution on leaf area and number of stomata leaves of *Rhoeo discolor*. Jakarta, University of Indonesia Press.

Inayah, S. N., Las, T. and Yunita, E. 2010. Pb content in Angsana leaf (*Pterocarpus indicus*) and mini elephant grass (*Axonopus* sp.) in Tangerang city protocol street. Valence Chemistry Journal, 2(1): 340-346.

Istiharoh, P.D., Martuti, N.K.T. and Bodijanto, F.P.M.H. 2014. Lead (Pb) content test by leaves based on tree location and crown position: Case study of green path Acacia mangium Jogorawi toll road. Media Conservation, 16(3): 101-107.

Kean, A., Harley, R.A., Littlejohn, D. and Gery, K. 2000. On-road measurement of ammonia and other motor vehicle exhaust emissions. Env. Sci. Techno. L., 34: 3535-353

Kurnia, Rahma 2004. Laporan Kegiatan Kursus Identifikasi Dampak Lingkungan. Yogyakarta: GEGAMA.

Landberg, T. and Greger, M. 1996. Differences in uptake and tolerance to heavy metals in Salix from polluted and unpolluted areas. Applied Geochemistry, 11: 175-180.

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

NASA and ALCA 1989. Interior landscape plants for indoor air pollution abatement. National Aeronautics and Space Administration John C. Stennis Space Center Science and Technology Laboratory Stennis Space Center, MS 39529-6000.

Novita, Yuliani and Purnomo, T. 2012. Lead metal (Pb) absorption and *Elodea canadensis* chlorophyll content on liquid waste of pulp and paper factory. Journal of Lentera Bio., 1(1): 1-8.

Nurhikmah, A., Syamsidar, H. S. and Ramadani, K. 2015. Bogenvil biosorption (*Bougainvillea spectabilis* Wild) against lead emissions (Pb) on motor vehicles. Journal of Al Chemistry, 3(2): 42-51

Olivares, E. 2003. 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.

Purwatiningsih, S. 2015. Review of city development concepts: Compact city. Urban Planning Courses RP 141314. (https://www.academia.edu/12525336/ReviewCompactCity).

Samsoedin, I., Susidharmawan, I. W., Pratiwi and Wahyono, D. 2015. The Role of Trees in Maintaining Urban Air Quality. Forda Press, Bogor.

Santoso, S.N. 2013. Use of plants as air pollution reduction. http://digilib.its.ac.id/public/ITS-Undergraduate-16616-Paper-pdf.pdf.

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

Sembiring, E. and Sulistyawati, E. 2006. 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.

Siregar, E.B.M. 2005. Air pollution, plant response, and its effect on humans. Faculty of Agriculture, Forestry Study Program, University of North Sumatra, Medan.

Sunu, P. 2001. Protecting The Environment by Applying ISO 14001. Jakarta: PT. Gramedia Widiasarana, Indonesia.