DEVELOPING PLANT TOLERANCE INDICATOR TO AIR POLLUTION, CASE STUDY IN KRAKATAU INDUSTRIAL ESTATE CILEGON CITY, INDONESIA

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ABSTRACT
Plant tolerance against air pollutants from industrial estate can be assessed based on the change of physiological parameters calculated according to APTI (Air Pollution Tolerance Index by Singh). However, based on previous research, APTI formulation was less accurate, the results obtained between macroscopic and physiological observations are not always sync. Additional physiological parameters, i.e. total carbohydrates as main product of photosynthesis process was need to be examined. Therefore, purpose of this study were to examine the physiological parameters that indicate the level of tolerance of plants sensitivity to air pollution and to analyze the level of tolerance of tree species to air pollution in industrial estate. The method used in this research were survey method, along with macroscopic parameters (leaf area, leaf number, and leaf hue), microscopic parameters (stomatal density, leaf and palisade thickness) and physiological parameters (ascorbic acid content, total chlorophyll, leaf pH, water content, and total carbohydrate) observation of tree species which exposed to pollution and non-pollution (control). The results of this research showed that total carbohydrate as an additional parameter affected the level of tolerance by 49.2% and thus modified the APTI formulation and changed the classification range of plants tolerance. Results showed that the tolerant plants were Polyalthia longifolia Sonn., Polyalthia fragrans (Dalzell) Hook. f. & Thomson, Erythrina crista-galli L., and Casuarina junghuhniana Miq.; moderate tolerance were Hibiscus tiliaceus L., Samanea saman (Jacq.)Merr. and Acacia auriculiformis A. Cunn. ex Benth; and intolerant were Leucaena leucocephala (Lam.) de Wit, Pterocarpus indicus Wild., and Swietenia mahagoni L.

Keywords: Air Pollution Tolerance Index, modified APTI, industrial plants, relative growth rate, total carbohydrate.

INTRODUCTION

Cilegon City is one of the industrial cities in Indonesia and became an industrial estate in West Banten areas. The potential of environmental degradation was occured in the industrial estate of Cilegon, as observed from the indication of an inappropriate land convertion, the decreasing of open green space, as well as the decreasing of the environment quality as a result of potensial industrial waste pollution that exceeded the quality standard in the industrial estates in Cilegon City. According to Rahmat (2014) air condition in Ciwandan Subdistrict of Cilegon city that located directly to Krakatau Industrial Estate Cilegon cause the increasing number of people suffering the Under Respiratory Infection (URI). This matter indicated that the poor air quality in industrial estates was caused by the air pollution.

Green open space was an alternative to diminish air pollution. Besides the effectivenes of reducing pollution, plants tolerance to air pollutant was one the important factors to determined trees type as a green space element. If the plants was tollerant to air pollution then its function as an agent to diminish air pollution can work well by also keep maintaining the optimum growth and development of the plants. Plants tolerance level to pollution as a result of parameter alteration, commonly measured and correlated with plants response level (Bora and Joshi, 2014). Plants response to stress was divided into three levels, i.e growth, physiology, and molecular (Oguntimehin et al., 2010). Therefore the species tolerance level of some trees to air pollution has to be examined macroscopically, microscopically, and physiologically.

Air Pollution Tolerance Index (APTI) was used by landscape expert as one of the selection criterias to determined the type of plants that were tollerance to air pollution (Liu and Ding, 2007 Rai, 2013). However, this APTI method had a weakness, which was the unsynchronized result beetwen macroscopic and physiological observations (Sulitijorini et al., 2008). It means, the formula was not fully compatible with some types of plants. Therefore, additional parameter that affected tree's growth in physiological observation, i.e total carbohydrate content in plants, needs to be investigated. Total carbohydrate content needs to be investigated because this was the main product of photosyntetic process. With the addition of total carbohydrate parameter in this physiological observation, a more accurate tolerance level would be obtainaed as a reference to determined the type of plants that are tolerant to air pollution.

Therefore, purpose of this study were to examine the physiological parameters that indicate the level of tolerance of plants sensitivity to air pollution and to analyze the level of tolerance of tree species to air pollution in industrial estate.

MATERIALS AND METHODS

The experiment was conducted in industrial estate of PT Krakatau Industrial Estate Cilegon (KIEC), Cilegon city, Banten, Indonesia. Geographycally located at 2°30'-20°05' EL and 27°15'-50°15 ' SL. The research was carried out from April to June 2015. The research was done by several stages:

1. Survey

Field survey was done to specified the location, species of trees, and location of specimen. Sampling was carried out in two locations, which were in polluted area and non-polluted area (control). Pollution-exposed sample was carried from the areas that had highest pollution level, and as comparison, the non-pollution sample was takenfrom low pollution level areas which were resident districs, hotel districs, and golf area.

Location of sample tress was determined by the distribution of pollutant. The highest pollution-exposed trees based on KIEC (2014) was chosen as the sample tree species. Furthermore, the chosen sample tree was a tree with a height of ≥3 m above ground level and leaves position were on the outer canopy to make sure that the leaves taken were exposed to pollution. On every sample tree, the branch was chosen randomly, and leaves taken were on ± 10-15 cm from the tip of the branch. Samples were taken from 10 different trees species which were *Acacia auriculiformis* A. Cunn. ex Benth, *Pterocarpus indicus* Wild., *Casuarina junghuhniana* Miq., *Erythrina crista-galli* L., *Polyalthia fragrans* (Dalzell) Hook. f. & Thomson, *Polyalthia longifolia* Sonn., *Leucaena leucocephala* (Lam.) de Wit, *Swietenia mahagoni* (L.) Jacq, *Samanea saman* (Jacq.) Merr., and *Hibiscus tiliaceus* L. The macroscopic, microscopic, and physiological observation was done to every trees species with three replications on every sample and parameter.

2. Data collection

a. Macroscopic observation. The observation was measuring the growth of each tree species in 2 months, from April to June 2015. Parameters observed was leaf area, leaf number, and leaf hue.

- Leaf area was determined by copied the leaf sample, except for coniferous trees, measurements was not done. Further measurement was done to the same leaf sample that was added with the newly formed leaves. Measurement was done by the tranparent plotting paper photography method (Nugraha AN, 2013). Leaf surface was affixed to a board, plotting paper was set above the leaf and then photographed. Leaf area was determined based on the number of boxes in the leaf pattern, with calculation as follow:

$$Leaf\ area = n\ x\ Lk$$

Annotation :
n = number of boxes		Lk = wide of each box

Measurement of box wide (Lk) used had an accuracy of 1 cm^2 (box size 1cm x 1 cm). Boxes that cover up the leaf pattern included to the calculation if it were covered the leaf > 0.5 of size reference, or equal to 0.5 cm^2.

- Parameter of leaf number was observed in every whole sample tree at each observation time. Leaves calculated was the leaves unfolded.

- The quality of hue rendition assesed from leaf hue that accordant to hues in Munsell Color Chart for Marketing and Merchandising with various score levels and notations (Table 1). Leaf hue measurement was not compared beetwen species because the hue standard used to them was different. Comparison done beetwen polluted-exposed and control leaves in every species.

Tabel 1. Score, color, and notation

Hue score	Leaf hue	Notation
1	Yellow	2.5 Y L1
2	Green yellow	2.5 GY DL4
3	Light green	5 GY DL4
4	Green	2.5 G DL1
5	Dark green	2.5 G DL 2
6	Very dark green	2.5 G DK1

b. Microscopic observation. Microscopic observations consist of specimen preparation with paradermal incision to observed air pollution effect to stomatal density and transversal incision to observed leaf and palisade thickness as well as the damage on the leaf tissue (Udayana, 2004). Paradermal incision was made by whole mount method (Sass, 1951) while transversal incision was made by frozen mikrotom method.

c. Physiological observation. Physiological observationof the leaf were included the analysis of ascorbic acid content, total chlorophyll, leaf pH, relative water content, and total carbohydrate content. Sampling of fresh leaves was done in the morning beetwen 07.00 to 08.00 am (Salama et al., 2011). Leaf samples were taken from the elevation of 1-2 m from ground level and then taken to the laboratory in the plastic wrap, and stored in the ice box for further analysis (Rai, 2013). Ascorbic acid content was determined by the modification method developed by Reiss (1993). Total leaf chlorophyll (mg g^{-1}) determined by visible light spectrophotometry method (Arnon, 1949). Acidity (pH) of leaves extract was measured by pH meter (Singh and Rao, 1983). Relative water content, that showed the water content percentage in the leaf, was determined by ovenmethod (Sen and Bhandari, 1978). Measurement of total carbohydrate content was determined by phenol-sulphuric acid method (Masukoet al., 2005).

d. Atmospheric (air) sampling. Measurement of ambient air consentration comprises the NO_2, NH_3, H_2S, and SO_2 gasses, dust, ozone, and hydrocarbon. Measurement was carried out in two spots, which were in the pollution exposed area in the industrial district and non-pollution area (control) in the resident districs in the morning for two hourson June 6th 2015. The instruments used was impinger for gasses parameters (NO_2, NH_3, H_2S, SO_2, ozone, and hydrocarbon), and high volume sampler (HVS) for dust/ particle parameter. Method and measurement principle for all those 7 parameters was set by PP RI No. 41 Year 1999, wich were Griess-saltzman method for NO_2, Indofenol method for NH_3, Pararosanilin method for H_2S, Metilen Blue method for Hidrogen Sulfida, Gravimetri method for dust, Neutral Buffer Potasium (NBKI) method for Ozone, and Khromatography Gas method for Hydrocarbon.

3. Processing and data analysis

a. All of the result of macroscopic, microscopic, and physiological parameters measurement beetwen the polluted and control plants was examined by ANOVA and continued with Duncan multiple range test (α = 5%) if result showed a significant difference.

b. Comparison of plants tolerance level by RGR (Sulistijorini et al., 2008) and APTI (Singh et al.,1991).

Plants tolerance macroscopically done by observing the total increasing of leaf area (Sulistijorini et al., 2008). Relative Growth Rate (RGR) was calculated by the increasing of leaf area of each tree species with the formula:

$$RGR = \frac{\ln Ld\ (i)ta - \ln Ld\ (i)to}{t}$$

Annotation:

Ld (i)ta = leaf areatype*i*-that the end of measurement

Ld (i)to = leaf area type *i*-that the start of measurement

t = measurement interval (30 days)

Plants tollerance measured by RGR was analyzed by ANOVA, and if the result showed a significance difference then continued with Duncan test at α = 5%. After that, scoring by the Duncan test result was done (modification of Dahlan in Sulistijorini *et al.*, (2008)). Score 1 given if there was a significant difference and the average of polluted plants was lower than control. Score 2 given if the average of control and polluted plants was not significantly different; score 3 if there was a significant difference and the average of polluted plant was higher than control. Classification of plants tollerance determined by RGR score that included to tolerant was if the score= 3, moderate tolerant if the score= 2, and intolerant if score= 1.

Plants tolerance measured by APTI was obtained by calculated the ascorbic acid content, chlorophyll, pH, and relative water content based on APTI (Air Pollution Tolerance Index) formula (Singh *et al.*, 1991):

$$APTI = \frac{A(T + P) + R}{10}$$

Annotation:

A = total ascorbic acid (mg g^{-1}) T = total chlorophyll (mg g^{-1})

P = leaf pH R = leaf water content (%)

APTI value of each plant types in polluted condition was compared to the table of sensitivity criteria and plants tolerance (Singh *et al.*, 1991), that were: sensitive plant (<12), moderate (13-16), quite tolerant (17-20), and tolerant (>20).

c. Plant Carbohydrate Analysis

Additional physiological parameter that were expected to affect tollerance level was total carbohydrate content, therefore correlation analysis was done beetwen macroscopic and physiological parameters to the total carbohydrate content in the leaf.

d. Correlation analysis of macroscopic and physiological parameters

Physiological condition of plants affected the macroscopic condition, therefore the correlation analysis beetwen macroscopic and physiological parameters was done to observed the most significant relation among those parameters. This result became a reference in determining a new modification of APTI formula by using linier regresion analysis.

4. Synthesis

Data and information of result analysis was arranged by level and plants tolerance aspects, so that the list of plants that were tolerant to air pollution was obtained.

RESULT AND DISCUSSION

Air Quality

Measurement result from some of the air quality parameters showed that pollution level in polluted area was higher than control (Table 2). However, the result showed that the air quality was still under the quality standard set by PP RI No. 40/1999. Based on measurement result, pollutan particle has a high consentration in polluted area (9 µg m^{-3}) and its value was much higher than those in the control area (<0,027 µg m^{-3}). Consentration of NO_2, SO_2, ammonia, ozone, and hydrocarbon in polluted area was higher than in control area, while H_2S and CO still in a very low consentration and has a same value as in the control area.

Particle concentration was the highest because most of the factories in industrial estate did industrial activities that produced particle, that is burning, milling and raw material smelting. In addition to industrial activities, high particle concentrations were also caused by motor vehicles, especially large vehicles passing by in industrial estate. Most large vehicles passing by in the industrial estate use diesel fuel emitting particle, NO_2 and hydrocarbon pollutants. According to Fitter and Hay (1992), nitrogen oxide is a by-product of combustion arising from a combination of nitrogen and atmospheric oxygen. The main source of NO_2 was combustion, the highest burning comes from motor vehicles. The high concentration of NO_2 was also thought to be related to the high number of large vehicles passing through the measured areas and the burning results of some factories.

Macroscopic Observation

Based on the result, the increasing of leaf number and leaf area was not significantly different based on location of pollution, but generally the increasing number of leaf in polluted areas was higher than in control and the increasing of leaf area in polluted area was higher than control except for *P. indicus* and *S. mahagoni* (Table 3). The inhibition of plant growth caused by air pollution only take effect on *P. indicus* and *S. mahagoni*. This suggests that both plant was sensitive to air pollution even in a very low consentration.

Observations that show higher plant growth compared to these controls were inconsistent with Kovacs (1992) assertion that air pollution causes stunted plant growth due to inhibition of photosynthetic product intake to apical cells will lead to inhibition of cell division and elongation that affect the growth of leaf surface area. The decrease in chlorophyll content leads to a decrease in photosynthesis rate so that photosynthesis results were also reduced. This was influenced by three air pollutants suspected to affect the rate of increase in the number and extent of plant leaves in polluted areas are NO_2, SO_2, and particle.

Table 2. Measurement result of some air quality parameters

Parameters	Industrial estates (polluted)	Resident district (control)	Standard quality*	Unit
Nitrogen Oxide (NO_2)	0.014	0.005	400	µg m^{-3}
Sulphur Dioxide (SO_2)	0.002	<0.001	900	µg m^{-3}
Ammonia (NH_3)	0.087	0.038	17	mg m^{-3}
Hydrogen Sulfide (H_2S)	<0.01	<0.01	14	mg m^{-3}
Ozone (O_3)	12	10	200	µg m^{-3}
Carbon Monoxide (CO)	<1000	<1000	30000	µg m^{-3}
Hydrocarbon (HC)	2.96	<0.65	160	µg m^{-3}
Particle	9	<0.027	230	µg m^{-3}

*Standard quality set by PP RI No. 41/1999

Nitrogen was absorbed root especially in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In addition to the nitrogen contained in the growing medium, two nitrogen sources in industrial estates that allegedly could be utilized by plants are NO_2 (0.014 µg/m^3) and NH_3 (0.087 mg/m^3). Higher polluted values than controls indicated that this tree species were capable of using nitrogen derived from NO_2 or NH_3 in industrial estate. According to Rogers and Aneja (1980); Farquhar et al., (1980); Castro et al. ,(2006), stems and leaves can absorb nitrogen in the form of NH_3. In addition, Nishimura et al. (1986); Rowland-Bamford and Drew (1988); Ammann et al., (1995) show that the leaves can absorb NO_2 from the air.

Table 3 Leaf number, leaf area and leaf hue of each plant types

Types of Plants	Increase of leaf number		Increase of leaf area (cm^2)		Leaf hue score	
	Polluted	Control	Polluted	Control	Control	Polluted
H. tiliaceus	2.67	1.83	36.83	21.5	3	5
S. saman	27.75	24.5	2.75	2.75	5.5	6
L. leucocephala	5	1	-	-	6	3
P. indicus	0	1	6	6.75	2.5	6
P. longifolia	2	1.75	21.75	12.5	6	3.5
P. fragrans	2.83	1.33	9.83	5.33	6	2.5
E. crysta-galli	6.67	5.5	17.83	16.5	5	6
S. mahagoni	0.5	1	6	16	5.5	6
C. junghuhniana	3.33	2.3	-	-	3	6
A. auriculiformis	4	7.5	5.75	2.83	6	5

Nitrogen is a major component necessary for vegetative growth (Marschner, 1986 and Larcher, 1995 in Sulistijorini, 2008).

The measurement of leaf hue showed that each species has a different impacts of air pollution (Table 3). Leaf hue of *of L.*

leucocephala, P. longifolia, P. fragrans, and *A. auriculiformis* species in polluted area was lower than control. Species of *L. leucocephala*, *P. longifolia*, and *P. fragrans* had a score of 2.5-3.5 (yellow green-light green-green) in polluted area. Result showed that leaf hue score of *H. tiliaceus*, *S. saman*, *P. indicus*, *E. crista-galli*, *S. mahagoni*, and *C. junghuhniana* in polluted area was higher than in control. Those species had a score of 5-6 (dark green) in polluted area.

Meitiyani (2003), states that contact with SO_2 in low concentrations over long periods causes chronic damage characterized by yellowing of leaf color due to inhibition of chlorophyll formation mechanism. However, in *A. auriculiformis* the color difference between the polluted and control areas is not much different, ie the polluted area with a score of 5 (dark green) while the control with a score of 6 (dark green). This shows that air pollution has no significant effect, didn't give serious damage to *A. auriculiformis* plants and because pollutant concentration in polluted areas is not very high.

The results showed that the color scores of polluted leaf plants were higher than controls on plant species *H. tilliaceus, S. saman, P. indicus, E. crista-galli, S. mahagoni*, and *C. junghuhniana*. The type of plant has a score of 5-6 (dark green-dark green) in the polluted area. This indicates that the leaves affected by pollutants have a darker color. This dark color indicates that plant leaves can absorb and retain dense pollutants such as particles, especially hairy leaves (Gray and Deneke, 1978). This was in accordance with ambient air conditions in industrial estate that have high concentrations of particle pollutants, resulting in darker leaf color.

Microscopic Observation

Result of the analysis of variance showed that stomatal density was not significantly different based on the location of pollution but showed a significant difference based on the species of trees. Accordingly, the stomatal density of 10 species of sample trees in this research was varied between 182.5 to 939.5 per mm^2 (Table 4). Stomatal density of *P. indicus*, *P. longifolia*, *S. mahagoni*, and *C. junghuhniana* in polluted area was higher than those in control. This plants respond to air pollutants by increasing its stomata. According to Gray and Deneke (1978), the type of plant with very high stomatal density has the potential as an agent to reduce air pollution.

Result of the analysis of variance indicated that leaf and palisade thickness were not significantly different by the location of pollution, while the species of trees only showed a significant difference on the leaf thickness. The observation result of this parameters indicated that there was a different responses on every species of trees. Leaf thickness of *H. tiliaceus*, *L. leucocephala*, *P. longifolia*, *E. crista-galli*, *C. junghuhniana*, and *A. auriculiformis* was higher in the polluted area (Table 4). Palisade thickness of all species of trees in polluted area was higher than control except for *S. saman* and *P. indicus*. Plants with higher leaf thickness and palisade thickness in polluted areas compared with controls showed plants tolerant to air pollution. In this case, *S. saman* and *P. indicus* are sensitive because both the thickness of the leaves and the palisade thickness in the polluted area are lower than the controls.

Physiological Observation

Result of analysis of variance indicated that there was a significant difference in the species of trees, but the location of pollution was not significanly different. The result of the research showed that the species of trees that had a higher ascorbic acid content in polluted area than in control was *H. tiliaceus*, *L. leucocephala*, *P. indicus*, *P. longifolia*, *C. junghuhniana*, and *A. auriculiformis*. Whereas the species of *S. saman*, *P. fragrans*, *E. crista-galli*, and *S. mahagoni* had a lower ascorbic acid content in polluted area than in control (Table 5). Plants with ascorbic acid content in polluted areas higher than control areas, they were included in tolerant plants. This was in accordance with the statement of Lima et al., 2000 and Rai et al., 2013, that plants are tolerant of air pollution have high ascorbic acid content because ascorbic acid has a function as an anti oxidant or a powerful reductor that can prevent oxidation reactions. In polluted areas, the highest permissible level of ascorbic acid is *L. leucocephala*, while the lowest is *P. fragrans*. This suggests that *L. leucocephala* has a high tolerance level for pollutants because of the high ascorbic acid content.

Analysis result showed that the chlorophyll content was not significantly different based on the species of trees, but significantly different based on location of pollution. Chlorophyll content in polluted area in almost all of the species was declining except for *P. longifolia*, *P. fragrans* and *C. junghuhniana,* while the *A. auriculiformis* was constant (Table 5). This is consistent with Carter and Knapp (2001) statement, that contaminants can induce chlorophyll reduction.

Result of the analysis of variance of this parameter indicated that the pH of leaf exctract was significantly different based on the species of trees but not significantly different based on location. Trees in polluted area has an acid pH, which was in the range of 5-7, while in control area showed a higher pH with a range of 6-7. This is due to exposure to SOx, NOx, and other acid-producing pollutants derived from emissions from industrial estates, giving a pH change to acidic leaves, low pH leaf extracts showed a good correlation to air pollution and also inhibited photosynthesis in plants (Yan-Ju and Hui, 2008; Thakar and Mishra, 2010). Species with a high pH number in polluted area was *H. tiliaceus*, *S. saman*, *L. leucocephala*, *P. indicus*, *E. crista-galli*, *S. mahagoni*, and *C. junghuhniana* (Table 5).

Analysis result showed that both location and species of trees did not gave significant difference to water content parameter. The average value of relative water content in polluted area was lower than in control (Table 5). In polluted area, the highest water content was found in *S. saman* and the lowest was in *P. fragrans*. In control area, the highest was in *P. indicus* while the lowest was in *S. saman*. Higher water content in industrial estates responds to the normal functioning of biological processes in plants (Meerabai et al., 2012), which occur in *S. saman, S. mahagoni*, and *C. junghuhniana*.

Table 4 Stomatal density, leaf thickness, and palisade thickness of each plant types

Types of Plants	Stomatal density		Leaf thickness		Palisade thickness	
	Control	Polluted	Control	Polluted	Control	Polluted
H. tiliaceus	575.2	504.6	139.7	188.6	46.7	60.7
S. saman	836.6	664.9	188	126.8	95.6	54.4
L. leucocephala	237.2	186.9	145.2	180	42.8	67.9
P. indicus	182.5	311.1	166.2	133.6	65.9	37.4
P. longifolia	315.9	489.8	119.8	156.7	21.6	44.3
P. fragrans	407.7	358.2	167.3	158.7	47	60.2
E. crysta-galli	454.9	424.5	267.1	290.2	70.7	92.9
S. mahagoni	542.7	743.2	157.5	152.5	33.1	43.5
C. junghuhniana	864.5	939.5	656.2	725.9	71.6	95.9
A. auriculiformis	466.2	387	152.5	189.6	40	46.7

Table 5 Ascorbic acid content, Chlorophyll, pH, and water content of each plant types

Types of Plant	Ascorbic acid (mg g^{-1})		Chlorophyll (mg g^{-1})		pH		Water content (%)	
	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control
H. tiliaceus	12.62	6.40	2.80	10.70	7.2	6.9	65.48	65.66
S. saman	8.08	10.51	6.15	11.10	6.8	6.7	67.36	58.64
L. leucocephala	15.54	15.50	4.60	6.40	6.9	6.5	66.54	68.96
P. indicus	8.30	3.37	8.90	14.05	6.8	6.2	66.21	77.82
P. longifolia	7.54	2.31	7.65	6.35	5.8	6.2	57.28	59.84
P. fragrans	4.33	6.65	3.50	7.00	5.8	6.1	53.89	59.84
E. crysta-galli	5.21	10.94	6.30	15.05	6.8	7.3	66.64	68.75
S. mahagoni	10.25	12.70	9.15	14.35	6.5	6	61.53	60.5
C. junghuhniana	6.54	6.32	4.35	2.35	6.3	6.2	61.63	59.67
A. auriculiformis	13.61	16.01	3.70	3.70	5.4	6.4	61.04	66.53

Comparison of Plants Tolerance Level Between RGR and APTI

Based on the calculation result, tolerance level obtained based on APTI and RGR value was quite significantly different (Table 6). Table 5 showed that the most significant result of tolerance level difference was in *P. fragrans* and *S. mahagoni* species. Species *P. fragrans* included to tolerant plants based on RGR but come under a sensitive type based on APTI. On the contrary, *S. mahagoni* was included to intolerant based on RGR but included to tolerant type based on APTI. Determination of tolerant plants based on APTI (Singh *et al*. 1991) was really influenced by ascorbic acid parameter. Based on APTI, if the content of ascorbic acid was high then the plants would considered as tolerant type, like *L. leucocephala* species, and contrarily if ascorbic acid content was low, plants would be included to sensitive type, for example *P. fragrans* species. APTI formulation showed that the alteration of ascorbic acid gave the best effect rather than other parameters like total chlorophyll, leaf exctract pH, and leaf water content.

Analysis of Total Carbohydrate Content

Analysis of correlation between carbohydrate content with macroscopic and physiological parameters was done to discovered the effect of carbohydrate level to those parameters. The result indicated that the correlation of total carbohydrate to other parameters was insignificant ($p>0.05$). However, the highest correlation was with RGR (correlation = 0.492). Analysis result of linier regression also showed a positive correlation between RGR and total carbohydrate content. The equation obtained was $y = 0.078x + 0.81$ with $R^2 = 0.492$, which means that the escalation of RGR number by about 49.2% was affected by the total carbohydrate content of the leaf, and the rest was affected by other parameters.

Based on the calculation result, total carbohydrate content as an additional parameter had a highest correlation number compared to other parameters, that equal to 0.429 with $p= 0.053$ (credibility level approaching 95%). It indicated that carbohydrate gave a big impact to the tolerance level of plants. In consequences, APTI formulation put forward by Singh et al. (1991) needs to be modified because of the unsynchronicity between the RGR number with the quality of every physiological parameters in it. Furthermore, based on the result of correlation analysis, total carbohydrate content as a physiological parameter had a big role toward RGR.

The result was matched the research of Seyyednejad et al. (2011) which found that plants with a high sugar content was a species that were tolerant toward air pollution due to the polymer of

Table 6 Comparison of plants tolerance level based on RGR and APTI

Type of Plants	RGR		Score	Tolerance level	APTI score	Tolerance level
	Polluted	Control	**	(RGR)		(APTI)
H. tiliaceus	1.228 b*	0.717 c	3	Tolerant	19.16	Quite tolerant
S. saman	0.094 a	0.092 a	2	Medium	17.20	Quite tolerant
L. leucocephala	***	***	***	***	24.53	Tolerant
P. indicus	0.200 a	0.225 ab	1	Intolerant	19.65	Quite tolerant
P. longifolia	0.725 a	0.417 ab	3	Tolerant	15.86	Moderate
P. fragrans	0.328 a	0.178 ab	3	Tolerant	9.42	Sensitive
E. crysta-galli	0.594 a	0.550 bc	3	Tolerant	13.48	Moderate
S. mahagoni	0.200 a	0.533 abc	1	Intolerant	22.19	Tolerant
C. junghuhniana	***	***	***	***	13.12	Moderate
A. auriculiformis	0.192 a	0.094 a	2	Medium	18.48	Quite tolerant

* Values in each column with the same letter arenot significantly different according to Duncan Multiple Range Test at α=5%

** Based on Dahlan modification (1995)

*** RGR measurement was not done due to technical problems in measuring the increasing of leaf area

fructose that could help the tolerant species under the unfavorable condition. For example, the ryegrass plant was undergo the increasing of fructans content when exposed to a momentarily air pollution in Sao Paolo (Brazil) (Moretto et al., 2009). Sandrinet al. (2013) reported that the enhancement of fructans particularly in the root of *Lollium multiflorum* L. at noon of spring and fall season, consist when the temperature was high, the light intensity was low, and the concentration of pollutant was at the highest level. In those condition, photosynthesis process was well stimulated so that it accumulated a higher concentration of sugar. The fructans (polyfructosylsucrose) itself was a major carbohydrate storage in the plants. The synthesis of fructans in the leaf was correlated with the synthesis of sucrose and translocation along with the sucrose metabolism in the stem and root. Fructans was instrumental to the plant growth under an unfavorable condition, such as in the condition of polluted air, because it has a role as an absorbent of reactive oxygen or indirectly stimulated the mecanism of antioxydative defense.

Analysis of Correlation Between Macroscopic and Physiological Parameters

The analysis of correlation was done to macroscopic parameter that consist of the increasing of leaf number, leaf hue, leaf thickness, RGR 1 (RGR number of the first one month interval observation), RGR 2 (RGR number of the second one month interval observation), and RGR (RGR number of the two months interval observation), as well as the physiological parameter that consist of total carhodyrate, ascorbic acid, chlorophyll, pH, and water content. The result of correlation analysis showed the most significant correlation occured between RGR 1 and the total carbohydrate, with the correlation value of 0.717 (categorized as strong correlation) with p= 0.045. However, the correlation between RGR 1 and other parameters tend to be low, with the ascorbic acid, chlorophyll, and pH included to low correlation category, and water content included to a very low correlation category.

Correlation analysis result between RGR 1 and physiological parameter showed that ascorbic acid, as the main parameter in determining tolerance level in plants, evidently had a low correlation with RGR 1 (correlation= 0.287). This result was in accordance with Sulistijorini et al. (2008) which reported that the tolerance based on RGR was not affected by the ascorbic acid of leaf, as in *Lagerstroemia speciosa* L. (included to tolerant type) that had a lowest ascorbic acid content compared to other seven species. Chlorophyll parameter (correlation= 0.390) also did not brough any significant difference to RGR 1. This result was in a contrary with Carter and Knapp (1991) which found that pollutants could induced chlorophyll reduction, as well as Carter and Knapp (1991) which reported that the presence of air pollution could induce necrosis and chlorosis that involved the mecanism of chlorophyll damage. Pandey and Agrawal (1994) proved that plants in urban areas that relatively polluted had a lower chlorophyll content and significantly different to those in uncontaminated areas. Leaf water content as an element in APTI formula also could not able to describe therole of water content in the defense mecanism from the exposured of pollutants. As seen on the correlation analysis result, water content only had correlation level by -0,046. The increasing of water content in polluted plants was allegedly because of water uptake from the ground and the transpiration of plants was in a equilibrium state. This presumption was supported by Taiz and Zeiger (2002) which stated that water availability on the leaf was affected by ability of root absorption and plants transpiration. The pH parameter also insignificantly correlated with RGR 1 (correlation= 0.293). In accordance to previous calculation, total carbohydrate as the additional parameter had a highest correlation number compared to other parameters, with the value of 0.717 and p=0.045 (credibility level 95%).

The result matched the previous estimation and calculation that total carbohydrate gave the biggest effect to RGR compared to physiological parameter in APTI formula put forward by Singh et al. (1991). The RGR 1 had the most significant correlation also caused by the sampling time to physiological analysis that were coincide with the second observation of plant growth.

Classification of plants tolerance by RGR had a strength because RGR can describe plants ability to adapt in polluted condition. Mecanism of plants adaptation to air pollutants can be measured based on the change of physiological condition. Therefore, determination of tolerance level of plants was based on the correlation between physiological parameter and RGR 1. Result of multiple linier regresion analysis obtained a new APTI formulation as follows:

$$Modified\ APTI\ = 1{,}05K - 0{,}287A - 0{,}002T - 0{,}997P + 0{,}18R$$

Annotation:

K = Total carbohydrate (%) A = Total ascorbic acid (mg g^{-1})

T = Total chlorophyll(mg g^{-1}) P = leaf pH R = leaf water content (%)

Scoring and classification of tolerance level was refers to Dahlan modification (1995). Plants tolerance level shown at Table 7 and the result indicated that tolerance level between RGR 1 and modified APTI was same, with *P. longifolia*, *P. fragrans*, *E. crista-galli*, and *C. junghuhniana* classified as tolerant species, *H. tiliaceus*, *S. saman* and *A. auriculiformis* included to moderate tolerant, along with *L. leucocephala, P. indicus*, and *S. mahagoni* included to intolerant.

Table 7 Plants tolerance based on RGR 1 and modified APTI

Type of Plants	RGR 1		Score **	Modified APTI		Score **	Tolerance level
	Polluted	Control		Polluted	Control		
H. tiliaceus	0.99 b*	0.69 b	2	6.24 ab*	8.58 ab	2	Moderate
S. saman	0.12 a	0.11 a	2	7.30 ab	6.80 ab	2	Moderate
L. leucocephala	***	***	***	4.18 ab	10.06 bc	1	Intolerant
P. indicus	0.12 a	0.28 ab	1	5.16 ab	8.82 b	1	Intolerant
P. longifolia	1.03 b	0.67 ab	3	9.48 b	9.10 bc	3	Tolerant
P. fragrans	0.24 a	0.07 ab	3	5.68 ab	4.92 a	3	Tolerant
E. crysta-galli	1.02 b	0.13 ab	3	6.53 b	10.59 bc	3	Tolerant
S. mahagoni	0.27 a	0.87 ab	1	5.94 ab	6.70 b	1	Intolerant
C. junghuhniana	***	***	***	8.82 ab	6.40 b	3	Tolerant
A. auriculiformis	0.15 a	0 a	2	2.84 a	3.80 a	2	Moderate

* Values in each column with the same letter are not significantly different according to Duncan Multiple Range Test at α=5%

** Based on Dahlan modification (1995)

*** RGR measurement was not done due to technical problems in measuring the increasing of leaf area

CONCLUSION

Comparison of plants tolerance level based on APTI and RGR showed a different results. Classification of plants tolerance based on RGR had a strength because RGR can describe adaptation ability of plants in polluted conditions. The results showed that total carbohydrate as an additional parameter of APTI affected the level of tolerance by 49.2% and also the most sensitive parameter to air pollutants rather than others physiological parameters, thus modified the APTI formulation and changed the classification of plants tolerance. Result of modified APTI if A = ascorbic acid content, T = total chlorophyll, P = leaf pH, R = water content, and K = total carbohydrate is: $Modified\ APTI = 1.05K - 0.287A - 0.002T - 0.997P + 0.18R$. Results showed that the tolerant plants were *Polyalthia longifolia*, *Polyalthia fragrans*, *Erythrina crista-galli*, and *Casuarina junghuhniana*; moderate tolerance were *Hibiscus tiliaceus*, *Samanea saman* and *Acacia auriculiformis*; and intolerant were *Leucaena leucocephala, Pterocarpus indicus*, and *Swietenia mahagoni*.

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