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NOTES and INFORMATION

LEAF MORPHO-ANATOMICAL RESPONSES OF *Mangifera indica* L. AND *Ficus benjamina* L. TO AIR POLLUTION IN SELECTED AREAS OF CEBU CITY, PHILIPPINES

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ABSTRACT – This study aims to determine leaf morphological and anatomical alterations of *Mangifera indica* L. and *Ficus benjamina* L. induced by air pollutants in a polluted area (Jones Avenue) and a non-polluted area (Bgy. Malubog) at Cebu City. Free hand sections were done to observe changes in leaf structure. Leaf sections were observed using a stereomicroscope and a light microscope. For anatomical analysis, the following parameters were obtained: length of the palisade cells (HPC), length of the upper epidermis cells (HEP), width of upper epidermis cells (WEP), length of lower epidermis cells (HELP), and width of lower epidermis cells (WLEP). Results showed that only *M. indica* sourced from the polluted area had observable lesions, burst cuticle and distorted stomatal complex. On the other hand, *F. benjamina* has no observable changes in leaf morpho-anatomy. Lesions are circular areas of raised tissue surrounding stomata indicating that open stomata were the entry points for phytotoxic gases. There were no significant differences in the length and width of the upper epidermis, length and width of the lower epidermis, and in the length of the palisade cells in the leaves of *M. indica* and *F. benjamina* between the polluted and non-polluted areas. *M. indica* was more sensitive to elevated concentrations of NO_2 and SO_2 in the atmosphere than *F. benjamina*.

Keywords: leaf structure, phytotoxic gases, stomata

INTRODUCTION

Air pollution is a major problem in many developing countries. Major cause of the increasing rate of pollutant concentrations are the growing population of human and vehicles, as well as industrial industries (The World Bank Group 2002). Kato et al. (1991) stated that the rates of pollution in the cities of developing countries are higher than in developed countries. Cebu City is the capital of Cebu province and it constitutes the core of Metro Cebu. It covers 330 square kilometers and is well known for its strong economic performance since the late 1980's (Utemadi 2000). Since it serves as the center of economic and business activities in Visayas and Mindanao areas there was a high influx of people originating from rural areas (Sajor 2001; Rodolfo 2008). The increase in population led to environmental change due to the subsequent requirements and outputs of housing, businesses, and road usage (de Sherbinin et al.

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2007). Changes in the ambient environment are caused by the presence of air pollutants in the area. In a study conducted by Sinogaya et al. (2016), the measured NO_2 concentration at the center (Jones Ave.) of Cebu City recorded an average of 73 ppb which exceeded the standard value set by Philippine Clean Air Act of 1999. However, SO_2 concentration is 47 ppb which is way below the standard set by the same written resolution.

Two of the key pollutants are SO_2 and NO_2 because they have hostile effect on human health and vegetation. Plants are one of the organisms which are sensitive to pollutants in the air. They can be utilized as biological indicators for the extent of air pollution. Various experiments have been studied showing the interaction of plants and these pollutants. Various studies have focused on morphological, physiological, and histochemical effect of these pollutants. Early greenhouse studies confirmed that regular exposure of these gases resulted in the following effects: degradation of epicuticular and epistomatal waxes on *Pinus halepensis* exposed of H_2S gases (Bartorimo et al. 2012), leaf physiognomy modification (Bacon et al. 2013) and stomatal density and index alteration (Haworth et al. 2012). These pollutants enter plants through the stomata, a minute epidermal pores mostly situated under-surface of plant leaves. When stomata open to allow exchange of carbon dioxide, oxygen and water vapor with the atmosphere, pollutants enter by diffusion due to the lower concentration gradient of these elements inside the plant. Sulphur dioxide slows down the ability of stomata to close, damaging stomatal control (McAinsh et al. 2002). At high concentrations, acidic SO_2 denature membrane-associated proteins embedded in the phospholipid bilayer of the plasma membrane that are essential for osmotic regulation (Heath 1980). This membrane is associated with calcium ions which are important messengers in signal transduction response to stimuli. Hydrogen acid, such as sulphuric acid, can displaced calcium ions in the plasma membrane impairing physiological responses of plants to environmental stresses. In addition, wax structures on the cuticle reflect and scatter photosynthetically active and UV radiation (Gausman et al. 1975). Any phytotoxic gas that degrades plant cuticle induces increased absorbance of light, thus imposing photo-oxidative damage in leaves (Shepherd and Wynne Griffiths 2006). In *Populus tremuloides*, ozone pollution leads to reduction in cuticle wax. (Mankovska et al. 1998). Degradation of wax crystal structure in *Picea abies* was recorded to be caused by nitrogen oxide and aerosol black carbon from traffic pollution (Viskari et al. 2000).

Phytotoxic gases cause different types of plant injuries. Physiological damage caused by SO_2 includes down-regulation of photosynthesis (Haworth et al. 2012). When phytotoxic gases damaged the anatomy of plant it further damaged water regulation as epidermal and neighbour cells supporting the guard cell collapse, thus leaving the stomata into permanent open (Neighbour et al. 1988). Once damage occurred to the plant cuticle, the protective barrier between plant interior and exterior is breached (Bartiromo 2012). However, morphological damaged caused by phytotoxic gases uptake also depend on plant type. Kim et al. (1997) showed that low consumption of SO_2 by gymnosperm led to epicuticular damaged, while *Gingko biloba* required very high level of SO_2 uptake to damage its cuticle.

Currently, no study has been conducted to evaluate the responses of plants in air polluted areas in Metro Cebu. Therefore, it is important to assess and provide data regarding the morpho-anatomical responses of plants as affected by air pollution. The main objective of the study is to determine the morpho-anatomical responses of selected plant species in polluted and non-polluted areas in Cebu City. The study was conducted from February to May 2016.

MATERIALS AND METHODS

Sampling Site

The sample vegetal material was obtained from two different sites in Metro Cebu. Location of the two sites as well as it coordinates were determined and recorded (Table 1). The first site is an urban area and was assigned as the air polluted site based on the recorded high mean concentration of 73 ppb for NO_2 and 47 ppb for SO_2. Site 1 is located downtown of the city at Jones Avenue. The second site, Bgy. Malubog, is a remote area which was assigned as the non-air polluted site based on the recorded low value of 8 ppb for NO_2 and 27 ppb for SO_2 concentrations.

Table 1. Selected sampling sites assigned as air polluted site and non-air polluted site.

Sites	Location	Coordinates	Mean NO_2 Concentration (ppb)	Mean SO_2 Concentration (ppb)
Site 1: Air polluted site	Cebu City downtown, Jones Avenue	10° 18' 0" N 123° 53' 49.2" E	73	47
Site 2: Non-Air polluted site	Brgy. Malubog	10° 22' 53.118" N 123° 52.5' 5.484" E	8	27

Plant Species Selection

Plant species selection was based on direct observation regarding their dominance and occurrence in both sites. *Mangifera indica* L. and *Ficus benjamina* L. were selected and are both members of the angiosperm group. Both plants were found to be common in the selected study sites.

Sample Preparation

Four trees from each site were selected. Four leaves were randomly collected from the central part of each plant species which will be utilized for epidermal observation. The abaxial side was polished with nitrocellulose dissolved in ethyl acetate and was then cut to 0.5 x 0.5 cm. The films formed from nitrocellulose were removed and placed on glass slides. These were then observed through a stereomicroscope and a light microscope. For anatomical analysis, the leaves were fixed in a solution of formalin/ethanol/acetic acid/water (FEA; 8:135:10:44 mL) and conserved in 70% ethyl alcohol.

Anatomical characters

A free-hand section technique was employed to obtain cross-sections of the leaves. The cross sections were examined under a light microscope. Anatomical characteristics of the leaves - such as the length of the palisade cells (HPC), length of the upper epidermis cells (HEP), width of upper epidermis cells (WEP), length of lower epidermis cells (HELP), and width of lower epidermis cells (WLEP) were measured using a microscope measurement tools software by Fiji app. Thirty measurements were made for every anatomical character.

RESULTS AND DISCUSSIONS

Morphological Responses

Lesions: Raised areas of damage on leaf surfaces

Lesions were observed on *M. indica* leaf sections obtained from Site 1 (Figure 1). Lesions were observed as raised and circular areas on the leaf surface. On the other hand, these lesions were not observed in the samples from Site 2. For *F. benjamina*, there were no observed lesions on the leaf sections for both site 1 and site 2 (Figure 2). Stomata were seen on the top of each dome-shaped lesion, indicating that the structures were not just raised cuticle but raised abaxial epidermal tissue as this is where damaging may have occurred through the stomatal pore. The presence of these lesions on the leaf samples are distinct damage response. The presence of these circular areas of raised tissue surrounding the stomata indicates that the open stomata were the entry point for phytotoxic gases. However, it is unclear on what gas specifically contaminated the leaves collected. Cosgrove (2005) showed that SO_2 subsequently damaged the underlying cells of plant leaf, leading to uplifting of epidermal and possibly mesophyll tissue. Raised lesions maybe filled with liquid water, gases, vapor or swollen plant tissues. When SO_2 enters the plant leaf it produces turgor pressure which results into irreversible growth of cell walls. Loss of osmotic control within the mesophyll layer may lead to irreversible outgrowth of mesophyll tissues.

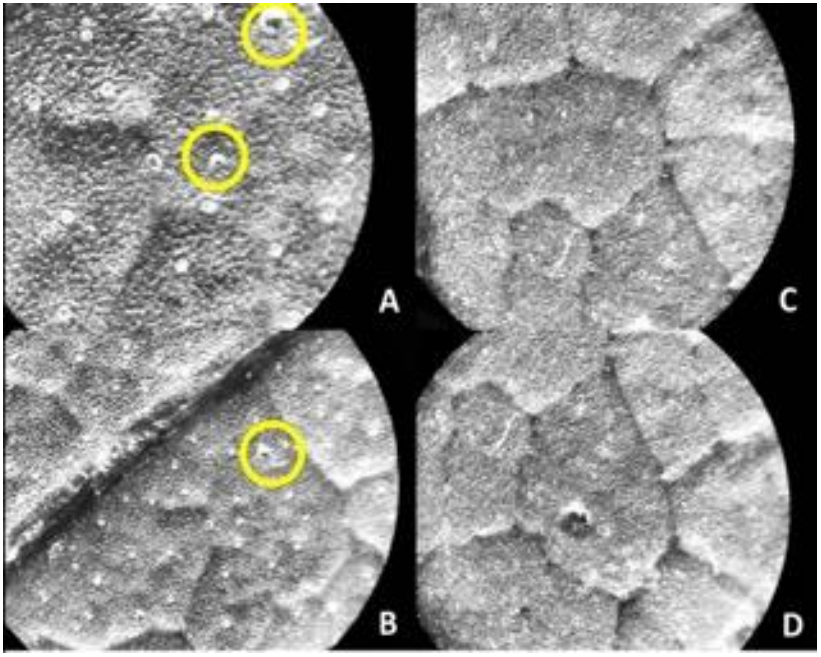

Figure 1. Abaxial leaf sections of *Mangifera indica* L. with lesions (yellow circle) obtained from polluted site (A&B) and non-polluted site (C&D) observed under LPO (100X).

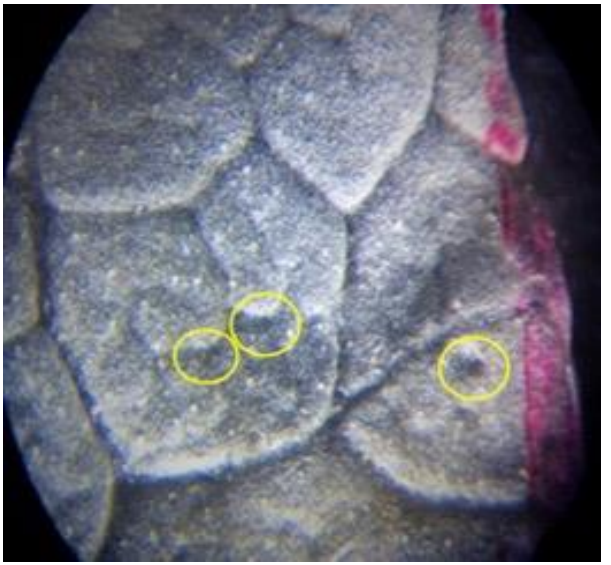

Figure 2. Abaxial leaf sections of *Ficus benjamina* L. obtained from polluted site observed under LPO (A) showing lesions (yellow circle).

Blistered and burst cuticle

Circular holes were observed in the cuticle of *M. indica* obtained from site 1 which may indicate that the bursting of cuticle due to elevated concentration of phytotoxic gases. There were no observable holes in the cuticle of the leaf sample from site 2. Furthermore, there were no evidences of blistered and burst cuticle on *F. benjamina* both from polluted and non-polluted area. These blisters may be originally a raised area of cuticle which eventually burst open, collapsing from the stress. Air pollutants cause damage to leaf cuticle and affect stomatal conductance (Saxena and Kulshrestha 2016).

Distortion of stomatal complexes

Only *M. indica* samples exhibited distorted stomata in both sites 1 and site 2. It is unclear whether the epidermal cells alone collapsed or whether the underlying mesophyll cells were also damaged. The stomatal complex also collapsed for *M. indica* samples obtained from site 2 (Figure 3). The stomata of the samples obtained from site 1 and 2 were irregular in shape and seemed to have burst wide open. On the other hand, no evidence in distortion of stomatal complex is present from both polluted and non-polluted area for *F. benjamina* (Figure 4). In a study conducted by Ashden et al. (2003), stomatal conductance was reduced in plants that were exposed to exhaust gas pollution as compared to plants exposed to clean air.

Exposure to different concentrations of SO2 gas has been reported to induce both stomatal opening and closing depending on the concentration of gas (McAinsh et al. 2002). Mansfield (1998) suggested that increases in stomatal conductance occur when SO_2 damages the epidermal cells surrounding guard cells, removing structural resistance to the guard cells and preventing guard cell closure. But, when the guard cells themselves are damaged by SO_2, they lose turgor and the stomatal pore

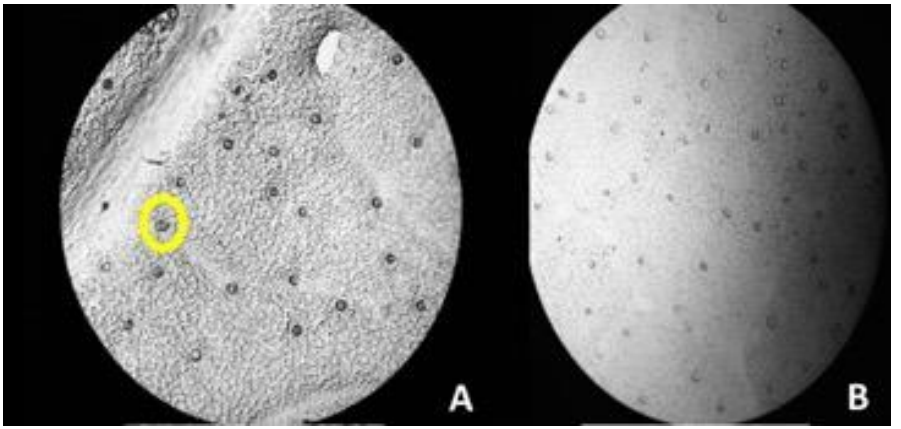

Figure 3. Leaf morphology of *Mangifera indica* obtained from the polluted area observed under LPO (A) showing stomatal distortion or irregular stomatal shape (yellow circle) and from the non-polluted area observed under LPO (B).

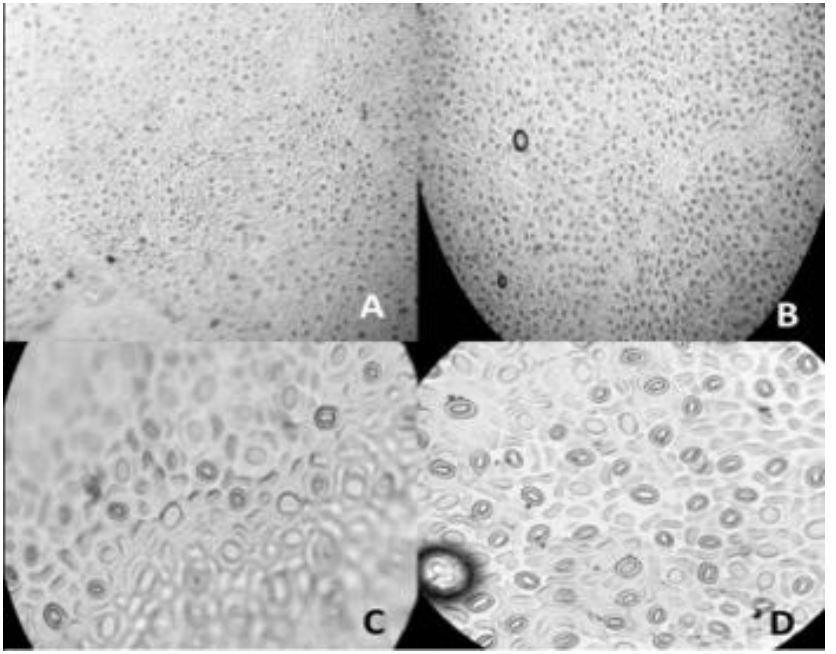

Figure 4. Stomatal complexes of *Ficus benjamina* obtained from polluted site observed under LPO (A) & HPO (B) and from the non-polluted site observed under LPO (C) & HPO (D) showing no distinct differences.

closes. In this study, all stomata on *M. indica* had collapsed in area of elevated air pollutants. In contrast, stomata in *F. benjamina* do not appear to have collapsed and closed (Figure 2) indicating a major difference in phytotoxic damage uptake between the two species. Additionally, in *M. indica*, the interveinal tissue collapsed, indicating that possibly the mesophyll and epidermal cells were damaged, and the stomatal complex was also damaged.

Anatomical Responses

There were no significant differences in the length and width of the upper epidermis, length and width of the lower epidermis, and in the length of the palisade cells in the leaves of *M. indica* between the polluted and non-polluted areas (Figure 5).

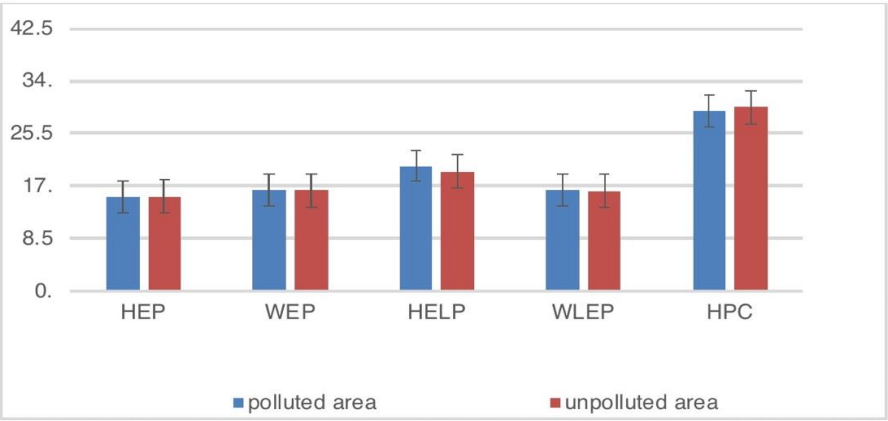

Figure 5. Comparison on the anatomical parameters of leaves from *M. indica* (HPC = length of the palisade cells, HEP = length of the upper epidermis cells, WEP = width of upper epidermis cells, HELP = length of lower epidermis cells, and WLEP = width of lower epidermis cells).

There were no significant differences in the length and width of the upper epidermis, length and width of the lower epidermis and in the length of the palisade cells in the leaves of *F. benjamina* in both the polluted and non-polluted areas (Figure 6).

Studies have also shown that cells of the epidermis and mesophyll layer have decreased in size and structure in plant species which were exposed to polluted areas than those in non-polluted areas. Those that were exposed to air pollution had thinner palisade mesophyll walls and were directly exposed to the environment than those which were in remote areas (Gostin 2009). However, in this study there had been no significant decrease in the size of the epidermis or the mesophyll layer of plants from polluted and non-polluted areas.

The palisade mesophyll layer of the plants in both areas had almost equal sizes, though those in the polluted area exhibited paler cells. The Spongy mesophyll layer was not measured, but it was observed that there were differences in the organization of the cells. There was a looser arrangement of the spongy mesophyll cells in the polluted area as compared to the non-polluted area.

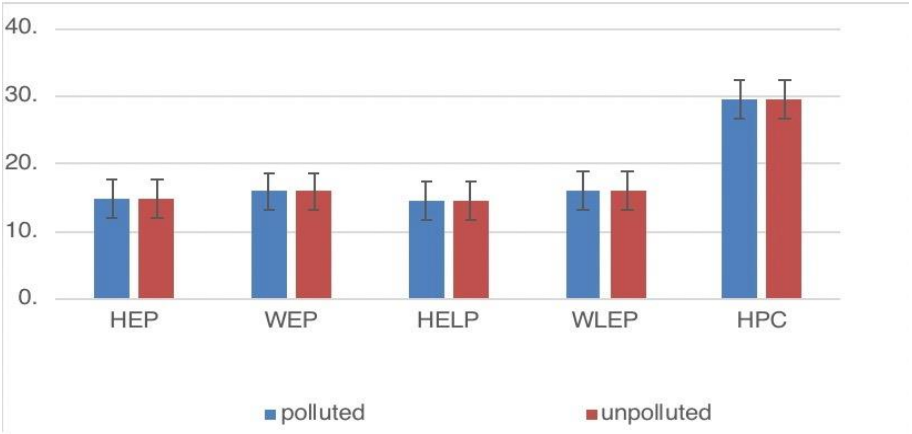

Figure 6. Comparison on the anatomical parameters on *F. benjamina* (HPC = length of the palisade cells, HEP = length of the upper epidermis cells, WEP = width of upper epidermis cells, HELP = length of lower epidermis cells, and WLEP = width of lower epidermis cells).

CONCLUSIONS AND RECOMMENDATIONS

Elevated concentrations of NO2 and SO2 may have resulted to leaf damage to *M. indica*. Data showed that *M. indica* leaf sections obtained from a polluted area had raised areas of tissue (lesions) and blistered or burst cuticle. On the other hand, *F. benjamina* obtained from the polluted and non-polluted site had no observable changes in the leaf structure. There were no significant differences in the length and width of the upper epidermal cells, length and width of the lower epidermal cells, and in the length of the palisade cells in the leaves of *M. indica* and *F. benjamina* between the polluted and non-polluted areas. Between the two plant species, *Mangifera indica* was more sensitive to elevated concentrations of NO_2 and SO_2 present in the atmosphere.

STATEMENT OF AUTHORSHIP

The first author is part of the study since the inception up to the writing of the publication. She gave numerous inputs with regard to the objectives, methodology, and the flow of the discussion. As the main author, she finalized the writing of this paper for publication. The second and third author gathered and recorded data, and consolidated reference materials.

REFERENCES

Ashden, T.W., Ashmore, M., Bell, J.N.B., Bignal, K., Binnie, J., Cape, J.N., Caporn, S.J.M., Carroll, J., Davison, A., Hadfield, P., Honour, S., Lawton, K., Moore, S., Power, S., and Shields, C. 2003. Impacts of Vehicle Emissions on Vegetation. Transactions on the Built Environment, 64: 316-317.

Bacon, K., Belcher., Haworth, M., and J.C., M. 2013. Increased atmospheric sulfur dioxide detected from changes in leaf physiognomy across the Triassic-Jurassic boundary interval of East Greenland. *PLoS ONE* 8(4). E606014. DOI. https://doi.org/10.1371/journal.pone.0060614.

Bartiromo, A.B. 2012. Influence of volcanic gases on the epidermis if *Pinus halepensis* Mill. In Campi Flegrei, Southern Italy: A possible tool for detecting volcanism in preent and past floras. *J.* Volcano Geotherm Res., 233-234: 1-17.

de Sherbinin, A., Carr, D., Cassels, S., and Jiang, L. 2007. Population and Environment. Annu Rev Environ Resour. 32: 345-373.

Gausman, H.W., Rodriguea, R.R., and Escobar, D.R. 1975. Ultraviolet radiation reflectance, transmittance, and absorptance by plant leaf epidermises. Agron. J. 67: 720-724.

Gostin, I.N. 2009. Air Pollution Effects on the Leaf structure of Some Fabaceae species. No. Bot. Hort. Agrobot. Cluj, 37(2): 57-63.

Haworth, M., Elliot-Kingston, C., Raschi, A., Marandola, D., and McElwain, J. 2012. Stomatal index response of Agrostis canina to carbon dioxide and Sulphur dioxide: implications for paleo-[CO2] using stomatal proxy. New Physiology. 188: 845-855.

Heath, R. 1980. Initial events in injury to plants by air pollutants. Annual Revision Plant Physiology 31: 395-431.

Kato, N., Ogawa, Y., Koike, Y., Sakamoto, S., and Environment, R.G. 1991. Analysis of the structure and energy consumption and the dynamics of emissions of species related to the global change in Asia. 4th Policy-oriented Research Group, National Institute of Science and Technology Policy, Tokyo: NISTEP Report no. 21.

Kim, Y., Lee, J., and Chung, G. 1997. Tolerance and susceptibility of Ginkgo to air pollution. *Ginkgo biloba*, A Global Treasure: From Biology to Medicine. Springer-Verlag, Tokyo: 233-242.

Mankavoska, B., Percy, K., and Karnosky, D. 1998. Impact of ambient tropospheric O_3, CO_2 and particulates on the epicuticular waxes of aspen clones differing in O3 tolerance Ekologia, 18: 200-210.

McAinish, M., Evans, N., Montgometry, L., and North, K. 2002. Calcium signaling in stomatal responses to pollutants. New Phytology, 153: 441-447.

Neighbor, E., Cottam, D., and Mansfield, T. 1988. Effects of Sulphur dioxide and nitrogen dioxide on the control of water loss by birch (*Betula* spp.). New Phytology, 108: 149-157.

Philippine Clean Air Act of 1999.

Rodolfo, W. 2008. Cebu Boom Continues. A report on the Business Mirror. Feb 2008.

Sajor, E. 2001. The real estate boom on the 1990s and land use allocation for socialized housing in Metro Cebu, Philippines. UN Urban Management Program (UMP) – Asia Occassional Paper No. 52: 1-33.

Saxena, P and Kulshrestha, U. 2016. Biochemical Effects of Air Pollutants on Plants in Plant Responses to Air Pollution. Spriger-Science. Pp. 60.

Shepherd, T. and Wynne Griffiths, D. 2006. The effects of stress on plant cuticular waxes. New Phytol., 171: 469-499.

Sinogaya, J., Galapate, R., Rosa, E.D., and Oppus, Z. 2016. Assessment of air quality as initiative for climate change mitigation in Metro Cebu, Philippines. Natural Sciences and Mathematics Division. Unpublished. Online. https://www.researchgate.net/publication/255609531_ASSESSMENT_OF_AIR_QUALITY_AS_INITIATIVE_FOR_CLIMATE_CHANGE_MITIGATION_IN_METRO_CEBU_PHILIPPINES.

The World Bank Group. 2002. The Philippines Environment Monitor. 2002. Online. <http://documents.worldbank.org/curated/en/381861468294104928/pdf/253950REPLACEM1at0are0in0imagebank1.pdf>

Utemadi, F.U. 2000. Civil Society Participation in City Governance in Cebu City. Environment & Urbanization, 12(1): 57-72.

Viskari, E., Holopainen, T., and Karenlampi, L. 2000. Responses of Spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions – II. Ultrastructural changes and stomatal behavior. Environ Pollut. 107: 99-107.