

Development of the Biochemical Parameters of Selected Plants Exposed with Ozone Gas

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Abstract

Air pollution tolerance index (APTI) is used by landscapers to select plant species tolerant to air pollution. To develop the usefulness of plants as bioindicators requires an appropriate selection of plant species, which is of utmost importance in specific situations. In order to evaluate the tolerance level of plants to air pollutants, four parameters, namely ascorbic acid, chlorophyll, relative water content, and leaf-extract pH, were determined and computed together in a formulation signifying the air pollution tolerance index (APTI) of plants. The study examined the APTI of seven selected species which were fumigated with 60 ppb ozone gas (O₃) for 10 days. The results showed that the APTI of *Palaquium formosanum* exhibited the most significant decrease after fumigation with O₃. Based on the nature of their tolerance, the experimental plants could be arranged in tolerance order as *Palaquium formosanum* > *Aglaia formosana* > *Cerbera manghas* > *Nageia nagi* > *Millettia pinnata* > *Terminalia catappa* > *Tournefortia argentea*.

Keywords: Biochemical; Plant; Ozone gas; Air pollution tolerance index

Introduction

Air pollution is the human introduction into the atmosphere of chemicals, particulate matter, or biological materials that cause harm or discomfort to humans or other living organism, or damage the environment. Air pollutants can directly affect plants via leaves or indirectly via soil acidification [1]. Plants provide an enormous amount of leaf area for impingement, absorption and accumulation of air pollutants and thus reduce the pollutant levels in the air [2], with a varied extent of mitigation for different species [3]. Plants also can green the environment, clean the air and adjust the climate. In both city and industrial areas, it is crucial to improve the air quality with long term planting and maintenance efforts, so plants should therefore readily adapt to the surrounding environment, even in a polluted environment [4,5]. Different plant species vary considerably in their tolerance to air pollutants [6]. The identification and categorization of plants into tolerant groups is important because the former can serve as indicators and the latter as sinks for the abatement of air pollution. It has been reported that when exposed to air pollutants, most plant experience physiological changes before exhibiting visible damage to leaves. Previous studies also showed the impact of air pollution on ascorbic acid content [7], chlorophyll content [8], leaf extract pH [9] and relative water content [10]. These separate parameters gave conflicting results for same species. However, the air pollution tolerance index (APTI) based on all parameters has been used for identifying tolerance levels of plants species [11-15].

Ozone gas (O₃) can harm the human respiratory system, and it can also impact plant health. The cells membranes of plant leaves are damaged under conditions of high concentrations O₃ in the environment. Cells die, and then symptoms appear on the leaves. When a plant comes into contact with O₃, the common symptoms are leaf rolling and drooping, as well as dark brown and ashen spots, among other visible symptoms. In order to achieve better air quality in industrial settings and urban environments, planted trees should have high tolerance toward pollutants. In this research, the O₃ concentrations of the experiment were set at 60 ppb. This research assess the tolerance of common green trees to O₃ in order to use this as an indicator of air quality, and fumigation equipment was utilized for this purpose. The results lead to a better understanding of plant tolerance to O₃.

Material and Methods

Experiment setup

Leaves of seven kinds of Taiwan protophyte plants were collected for this experiment, including *Aglaia formosana* (A. f.), *Cerbera manghas* (C. m.), *Millettia pinnata* (M. p.), *Nageia nagi* (N. n.), *Palaquium formosanum* (P. f.), *Tournefortia argentea* (T. a.), and *Terminalia catappa* (T. c.). The tested plants were put in a fumigation chamber for this experiment. The chamber was transparent and was placed in natural sunlight. An air conditioner was placed in the chamber to control the temperature between 25 to 30°C during the fumigation experiment. A fan was used on the inside ceiling of the chamber to mix the O₃ with the inner air in order to achieve a uniform O₃ concentration environment in the chamber. An O₃ gas manufacture machine (OW-K1/A-O, Taiwan) supplied the O₃ gas into the chamber using a calibrated flow meter to control the fumigation volume of the O₃. An O₃ gas detector (Photometric O₃ Analyzer-model-400a, USA) measured the O₃ concentration in the chamber, and as the concentration changed, it was adjusted to keep the gas concentration constant in the chamber during the experiment. The O₃ concentration in the sample was calculated as following equation [16],

$$C_{O_3} = -\frac{10^9}{a \times L} \times \frac{T}{T_0} \times \frac{P_0}{P} \times \ln \frac{I}{I_0}, \quad (1)$$

where

C_{O_3} = O₃ concentration

a = Absorption efficiency

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L = Light wave length

I_0 = Incidence ultraviolet light strength

I = Outlet ultraviolet light strength

T_0 = Temperature of standard gas temperature (273 K)

P_0 = Pressure of standard gas (29.92 in-Hg)

T = Temperature of sample

P = Pressure of sample

4.2 Air pollution tolerance index

During present investigation ascorbic acid content, leaf extract pH, total chlorophyll content and relative water content was taken together in the form of mathematical expression to obtain an empirical value, signifying the air pollution tolerance index (APTI) [17].

$$APTI = \frac{A \times (T + P) + R}{10}, \quad (2)$$

where

A = Ascorbic acid content (mg g⁻¹ fresh wt.)

P = Leaf extract pH

T = Total chlorophyll (mg g⁻¹ dry wt.)

R = Percent relative water content of leaf (%).

Ascorbic acid content was measured using spectrophotometric method. 1 g of the fresh foliage was put in a test-tube, 4 mL oxalic acid - EDTA extracting solution was added, then 1 mL of orthophosphoric acid and then 1 mL 5% tetraoxosulphate (VI) acid added to this mixture, 2 mL of ammonium molybdate was added and then 3 mL of water. The solution was then allowed to stand for 15 minutes. After which the absorbance at 760 nm was measured with a spectrophotometer. The concentration of ascorbic acid in the sample was then extrapolated from a standard ascorbic acid curve.

Fresh leaf (0.5 g) sample was homogenized in 10 ml deionized water. This was then filtered and the pH of leaf extracted determined after calibrating pH meter with buffer solution of pH 4 and pH 9.

Total chlorophyll content in the leaves, 3 g of fresh leaves were blended and then extracted with 10 mL of 80% acetone and left for 15 minutes for thorough extraction. The liquid portion was decanted into another test-tube and centrifuged at 2,500 rpm for 3 minutes. The supernatant liquid was then collected and the absorbance taken using a spectrophotometer at 645 nm and 665 nm.

Relative leaf water content the fresh leaves were weighed and then immersed in water over night, blotted dry and then weighed to get the turgid weight. The leaves were dried overnight in an oven at 55°C and reweighed to obtain the dry weight.

Results and Discussion

The highest leaf ascorbic acid content of tested trees was recorded in *Palaquium formosanum* (6.67 mg/g FW), and the lowest was recorded in *Nageia nagi* (1.16 mg/g FW) before fumigation (Table 1). After 60 ppb O₃ fumigation for 10 days, most of the tested trees' ascorbic acid was lower than before fumigation except *Aglaia formosana*, *Nageia nagi* and *Terminalia catappa*. The *Palaquium formosanum* showed the greatest drop with the reducing rate 56%. But the ascorbic acid value of *Palaquium formosanum* also was the highest of all tested trees after

fumigation. The ascorbic acid of *Nageia nagi* displayed a 62% increase, which was the most increasing rate than other plants.

A range from 38.6 mg/g of *Tournefortia argentea* to 67.4 mg/g of *Nageia nagi* was observed in this experiment for chlorophyll content before fumigation (Table 2). After fumigation with 60 ppb O₃ for 10 days continually, the chlorophyll content did not show significant variance, the *Tournefortia argentea* and *Millettia pinnata* had a decrease of 1 - 2% after O₃ gas fumigation, the other plants showed increase with a range 1 - 9%.

The leaf extract pH was recorded from 5.21 of *Terminalia catappa* to 6.47 of *Aglaia formosana* before fumigation. After 60 ppb O₃ fumigation for 10 days, the pH of the *Tournefortia argentea*, *Millettia pinnata* and *Palaquium formosanum* increased by 3%, and the other trees showed a lower pH than before fumigation ranging from 3% (*Cerbera manghas*) to 8% (*Terminalia catappa*) (Table 3).

The results of the study showed the leaf relative water content to vary from 83% for *Cerbera manghas* to 94% for *Tournefortia argentea* before fumigation. After fumigation, the leaf relative water content of the test trees ranged from 86% to 93%. The variance under the O₃ environment of tested plants was -3% for *Millettia pinnata* to +8% for *Aglaia formosana* (Table 4).

The APTI is an index indicating the tolerance of plants to air

Species	Before (mg/g)	After (mg/g)	Relative (%) ^a
<i>A. f.</i>	1.82	2.28	125
<i>C. m.</i>	2.53	1.74	69
<i>M. p.</i>	1.79	1.45	81
<i>N. n.</i>	1.16	1.87	162
<i>P. f.</i>	6.67	2.89	43
<i>T. a.</i>	1.56	1.29	83
<i>T. c.</i>	1.77	1.79	101

^aRelative rate(%) = After/Before fumigation

Table 1: Leaf ascorbic acid content of experiment plants before and after fumigation with 60 ppb O₃ gas for 10 days.

Species	Before (mg/g)	After (mg/g)	Relative (%) ^a
<i>A. f.</i>	64.2	64.6	101
<i>C. m.</i>	48.5	51.6	106
<i>M. p.</i>	50.0	49.7	99
<i>N. n.</i>	67.4	67.2	100
<i>P. f.</i>	58.8	61.1	104
<i>T. a.</i>	38.6	38.0	98
<i>T. c.</i>	42.2	46.1	109

^aRelative rate(%) = After/Before fumigation

Table 2: Leaf total chlorophyll content of experiment plants before and after fumigation with 60 ppb O₃ gas for 10 days.

Species	Before	After	Relative (%) ^a
<i>A. f.</i>	6.47	6.12	95
<i>C. m.</i>	6.11	5.95	97
<i>M. p.</i>	6.47	6.68	103
<i>N. n.</i>	6.17	5.89	95
<i>P. f.</i>	5.62	5.81	103
<i>T. a.</i>	6.44	6.63	103
<i>T. c.</i>	5.21	4.8	92

^aRelative rate(%) = After/Before fumigation

Table 3: Leaf extract pH of experiment plants before and after fumigation with 60 ppb O₃ gas for 10 days.

Species	Before (%)	After (%)	Relative (%) ^a
<i>A. f.</i>	85	92	108
<i>C. m.</i>	83	87	105
<i>M. p.</i>	94	91	97
<i>N. n.</i>	93	93	100
<i>P. f.</i>	86	91	106
<i>T. a.</i>	94	92	98
<i>T. c.</i>	84	86	102

^aRelative rate = After/Before fumigation

Table 4: Leaf relative water content of experiment plants before and after fumigation with 60 ppb O₃ gas for 10 days.

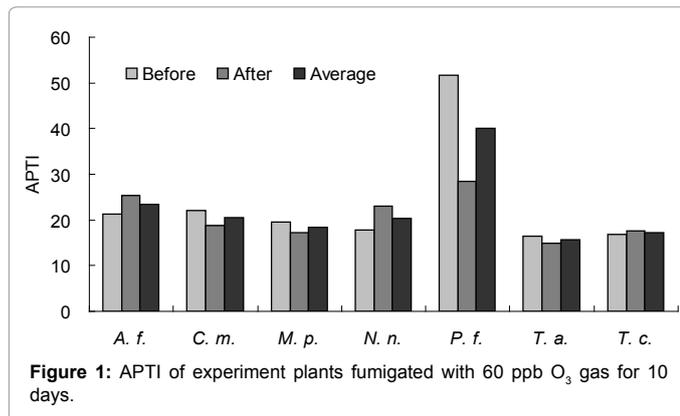


Figure 1: APTI of experiment plants fumigated with 60 ppb O₃ gas for 10 days.

pollution. Higher values indicate higher resistance to air pollutants [18,19]. The average APTI before and after fumigation showed in Figure 1. The results showed the range for *Tournefortia argentea* (16.4) to *Palaquium formosanum* (51.6) before fumigation. After fumigated with 60 ppb O₃ gas for 10 days, the *Tournefortia argentea* and *Palaquium formosanum* also had the lowest and highest APTI which were 15.0 and 28.4, respectively. The *Palaquium formosanum* showed a most decrease rate of 45% as compared to before fumigation and the *Nageia nagi* had the highest increase rate 29%. The next higher raise was *Aglaia formosana* with increase rate 18% and *Terminalia catappa* was 5% higher than before fumigation. The final tolerance order to O₃ could display as *Palaquium formosanum* > *Aglaia formosana* > *Cerbera manghas* > *Nageia nagi* > *Milletia pinnata* > *Terminalia catappa* > *Tournefortia argentea*. The observations in this study suggest that plants have the potential to serve as excellent quantitative and qualitative indices of pollution. Since biomonitoring of plants is an important tool by which to evaluate the impact of air pollution on plants, *Palaquium formosanum* can be used as biomonitors of O₃ gas pollution.

Conclusions

APTI index combined a variety of parameters can yield more reliable results as compared to an APTI only based on a single biochemical parameter. This study provided useful information by which to select tolerant species fit for landscape on sites continuously exposed to O₃ gas. In this research, a fumigation experiment was used to evaluate the air pollution tolerance index characteristics of seven kinds plants with 60 ppb O₃ gas for 10 days. The results showed *Palaquium formosanum* had the most tolerance to O₃ and *Tournefortia argentea* was the weakest among seven tested plants. The species are ranked by ability to tolerate O₃ gas, the experimental plants could be arranged in the following order: *Palaquium formosanum* >

Cerbera manghas > *Aglaia formosana* > *Milletia pinnata* > *Nageia nagi* > *Terminalia catappa* > *Tournefortia argentea*.

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