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ECOLOGICAL ASSESSMENT OF AIR POLLUTION TOLERANCE INDICES (APTI) OF ARBOREAL EPIPHYTES UNDER AMBIENT ENVIRONMENTAL CONDITION: A WINDOW FOR SELECTION OF BIOINDICATOR AS BIOMONITORING AGENT

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Abstract
The screening of plant species is of environmental significance hence their sensitivity as bioindicator as well as their tolerance as biomonitoring agent in air pollution mitigation have been established. The present research was aimed at evaluating the potential response of arboreal epiphytes as bioindicator under ambient condition, with the objective of identifying species that can serve as biomonitoring baseline agent for impact prediction and judgment of air pollution. Classical conventional methods were used to evaluate the susceptibility level of the epiphytes under ambient environmental condition using four established physiobiochemical parameters: leaf extract pH, relative water content, ascorbic acid, and chlorophyll to extrapolate Air Pollution Tolerance Index (APTI) for 8 species. Result revealed three categories of APTI responses: APTI 1-10 = sensitivity; APTI 10.01 – 13 = intermediate and APTI 13.01-16 above = tolerance. The order of responses in sensitivity: Platycerum bifurcatum>Oleandra distenta>Nephrolepis bisserata; intermediate: Nephrolepis undulata>Nephrolepis pumicicola and tolerance: Platycerum stagelephantotis > marattia fraxinea> Platycerum grande were recorded. The order of response indicated P. stagelephantotis with higher APTI (15.19) reflecting higher tolerance level and least APTI (6.46) for N. bisserata indicating sensitivity under ambient condition as baseline against air pollution. Therefore by their level potential responses at the ambient level can be recommended for phytosequestration / mitigation hence the APTI sensitive and tolerant species can serve as bioindicator and biomonitor respectively. Such performance might be very useful in the selection of appropriate epiphytic species that can enhance the expected performance of canopy formation of greenbelt in a changing environmental condition.

Key Words: *APTI, Chlorophyll, Ascorbic acid, pH, Relative water content*

Introduction

Ecological assessment (EA) is an empirical monitoring tool used to evaluate current and changing conditions of ecological resources. Such empirical tool under surrounding ambient condition or pre and post contaminated condition can help evaluate environmental hazards posed by changes in conditions preceding any remediation approach. Ecological indicators involve many abiotic and biotic agents, reflecting the pluralistic

components of ecosystems. Hence they represent key information about structure, function, and composition of ecological system such indicators very much often are used in assessing condition of environment, provide early warning signals and diagnose causes of environmental problem or change (Dale and Beyeler, 2001). Abiotic indicators, gives information on the risks or threats from stressors to ecosystems (Rapport, 1989). Comparatively they are correlated with sources of pollutants and disturbances but may not reflect ecological end points in themselves, while biotic indicators do reflect end point of pollution and may be used to differentiate "healthy" from "sick" ecosystems (Suter, 1990). Although bioindicator has been expressed as aggregate of all sources of biotic and abiotic reactions to ecological changes; there are yet diverse living organisms utilized to screen the health of natural ecosystem as a means of assessing either positive or negative environmental health and biogeographic changes taking place in the environment, and their subsequent effects on human society (Khatri and Tyagi 2015; Trishala *et al*., 2016). Bioindicators serve as gauges of natural change, involving taxa utilized to show the impacts of natural environmental changes of positive or negative trend due to the presence of pollutants which can affect the biodiversity of the environment (Gerhardt 2002; Holt and Miller 2010). By the level of resistance and resilience of bioindicators to their tolerance as sink of ecological variability they can be christened “biomonitoring agent”.

Environmental monitoring entails using bioindicators to harness one or more variables of measurements in monitoring and observation of environmental status. Such mechanism assesses the quality of environment under the risk of pollution as well as control under ambient condition as basis for prediction and judgment in environmental impact assessments. This information can then be used to study environmental trends and to quantify the current state of the environment. Though pollution can be by anthropogenic and natural forces based on the origin; and air, land and water pollution based on the affected environment, environmental pollution is inseparable from unsustainable anthropogenic activities, causing substantial ecological problems associated with non-living and living (plant and animals) things and other vulnerable ecological systems with subsequent interference to legitimate use of the environment (Khan, 2004). Air pollution is a major and severe problem facing the world today due to industrialization and sources of transportation and vehicular emissions. This can directly or indirectly affects plants via leaves and soil acidification respectively.

Plants are integral basis for all ecosystems, most likely to be affected by air borne pollution and identified as the most potential receptors of impacts under ambient air as well as polluted conditions. Ambient air refers to the quality of air conditions of surrounding outdoors. The ambient environment of an area may be contaminated with several pollutants and the plants growing in such area would be exposed to several of such pollutants under different conditions of ambient and polluted air. The presence or absence of some specific plants or other vegetation provides ample information about environmental health. Plant species react to ecological changes in responds to their disappearance, composition and structural changes, and air quality and climatic

changes due to increase in the level of pollutants of sulfur (SO_2) and nitrogen (NO_2), particulate matter (PAM), heavy metals, benzene, CO, lead, Ozone (O_3) and PAH (Gerhardt, 2002; Holt and Miller 2010; Khatri and Tyagi, 2015).

In biomonitoring, the strategy and effort is environment specific to individual study of plants in the environment. Epiphytes are part members of vascular and non-vascular ferns and fern allies of pteridophytes that grow on the surface of other plants for support thus can be referred to as arboreal epiphytes. They are known for the following attributes: without specific roots for at least a stage in the soil (Kromer and Gradstein, 2003), characterized by high diversity based on the green belt canopy which gives them access to atmospheric water and minerals; more so are seen as sensitive indicators of climate change and environmental disturbance (Kromer *et al*., 2016), important component of global plant diversity constituting ten percent of vascular plant species of the world (Nieder *et al*., 2001), constitute a large proportion of photosynthetically active material (Hofstede *et al*., 2001), contribute to abiotic processes such as water fluxes and nutrient cycling (Gentry, 1991; Holscher *et al*., 2004) and contribute to species richness and processes and interactions in life cycle of a forest (Cummings *et al*., 2006).

Several studies have revealed the use and significant role of plant species in monitoring and maintaining the ecological balance of environment. This include the potency for impingement, absorption and accumulation and initial acceptors of air pollutants for clean air inboth ambient and polluted condition (Escobedo *et al*., 2008; Liu and Ding, 2008; Joshi and Swami, 2009), filter for dust and sink of air pollutants to check rising pollution level (Prajapati and Tripathi, 2008), evaluation of the impact of air pollution on plants as well as potential sensitivity and tolerance using chlorophyll content (Flowers *et al*., 2007); ascorbic acid content (Hoque *et al*., 2007); leaf pH (Klumpp *et al*.,2000) and relative water content (Rao, 2006); leaf or stomatal conductance, membrane permeability, peroxidase activity (Farooq and Beg, 1980, William and Christopher, 1986, Tripathi *et al*., 1991) and also the ability of plant species to detoxify the air as well as indicator of the possibility of synergistic action of pollutants (Liu *et al*., 2007; Jayashnee 2012; Lakshmi *et al*., 2008). All these imply a very close relationship between nature and plant species, and if any altered condition occurs in the atmosphere, it directly affects the physiological and biochemical constituents of plant. Ordinarily, tolerance of plants to air pollution can be measured by simple symptoms such as visible injury on the plants, but it can be correctly evaluated via tolerance index of plants to air pollutants.

To screen plants for their sensitivity / tolerance level to ambient condition and air pollutants, large number of aforementioned physiobiochemical plants parameter are been used. However, it has been observed that separate parameters gave conflicting result for a particular plant (Han *et al*., 1995). Air pollution tolerance index is based on four among the aforementioned parameters to identify tolerance levels of plant species. Air pollution tolerance index (APTI) indicates the potential of vegetation to encounter air pollution. It is used to choose tolerant species and helps in monitoring plant tolerance towards air pollution. So assessment of plants on the basis of their level of tolerance to air pollution is

essential based on biochemical parameter generally employed for recognising the tolerance level of plants. Plants species are classified into three categories of responses of APTI values with varying degree of sensitivity and tolerance to air pollution stress. These include: sensitivity (APTI 0.01 – 10); intermediate (APTI 10 – 16); & tolerant (APTI 17 ≥) (Agarwal *et al*., 1991), very sensitive (APTI <1); sensitive (APTI 1-16); intermediate (APTI 17-29); tolerant (APTI 30-100) (Lakshmi *et al.,* 2008; Enete *et al*., 2013; Lohe *et al.,* 2015), sensitivity (APTI 1-5); intermediate (APTI 5-10) and tolerant (10-15) (Dileswar *et al*., 2015), sensitivity (APTI 1-8.11); intermediate (APTI 8.12-8.14) and tolerant (APTI 8.15>) (Obidiegwu, 2019). The APTI determination provides a reliable method for screening sensitive / tolerant plants under field condition of contaminated air by variety of pollutant.

The recognition and classification of plants into tolerant and sensitive groups is essential because the sensitive plants can be used as an indicator and the tolerant as a biomonitoring sink for pollutants. Though planting of trees and shrubs forms one of the best way to mitigate air pollution however, plant selection criteria should not only be limited to robustness of species habit & life form, leaf dimension & surface area and canopy formation of space, but should also give priority epiphytic species on such trees/shrubs hence this may help improve air quality by enhancing the canopy potency for air pollution mitigation. It is important that epiphytes are considered in research, planning and implementation of ecological restoration to ensure that outdoor air quality at both ambient and polluted conditions are fully restored. Therefore, the aim of this research was to evaluate the APTI response potential of arboreal epiphytes as bioindicators by sensitivity under ambient environmental condition; with the view to identify species that can serve as biomonitoring agent of aerial pollution by tolerance level. The study is significant hence expected to provide a better understanding on the potential of epiphytes as bioindicators by their sensitivity and as biomonitors based on their tolerance on the health status of environment (the rate at which the environment is polluted) even in the presence of minute quantities of pollutant under ambient condition. It shall also provide data information that can be a baseline for impact prediction and judgment in environmental assessment of any envisaged imminent air pollution. The results obtained shall widen the knowledge of sensitivity and tolerance of some epiphytes to air pollution. It will provide additional information that would form the basis for further research, development, utilization and perpetuation of the ecological importance of epiphytic ferns. This will assist horticulturists, landscapers and environmental scientists in the selection of air pollution tolerance epiphytes that can be allowed to coexist & inhabit trees / shrubs planted in air pollution prone areas.

Materials and Methods

Study Area

Rivers state is one of the thirty-six States in Nigeria located between longitudes 6°23 E and 7°36′ E and latitude 4°18′ N and 5°45′ N of the equator (Fig.1). The State bounded by Imo River and Akwa-Ibom in the east, Bayelsa State in the west, Imo and Abia States in the North and to the south by Atlantic Ocean (Edwin-Wosu *et al.,* 2013). It vegetation belt consist of the mangrove forest, fresh

water raffia palm and tropical rain forest vegetation systems. The climatic condition of the area as at the time of study had variable rainfall with maximum range between 2,500 to 3,500 mm annually and relative humidity under the influence of latitudinal and seasonal variation, comparative uniformity due to proximity to the Atlantic Ocean (Kuruk, 2004). Mean maximum monthly temperature ranges from 28 to 33°C and minimum monthly temperature of 17°C and 24°C. The soil by gradient underlain section usually consist of sandy silt or sandy loam and impervious pan of clayey component often drained and leached by both fresh and salty water as a result of heavy rainfall thus making it alkaline or salty and sometimes acidic in nature (Egwuogu *et al.,* 2016). The State consists of twenty-three (23) local government areas including Obio/Akpor study location, which is located between latitude 4°45′0″ N and 5°0′0″ N and longitude 6°45′0″ E and 7°15′0″ E (Fig. 2) housing localities, town and suburbs, including Choba Study site (Fig. 3).

Choba is one of the communities found in Obio/Akpor. It houses the University of Port Harcourt sampled site, geo-referenced to latitude 04°54.147N and 04°54.403N and longitude 006°55.347E and 006°55.418E (Fig.4).

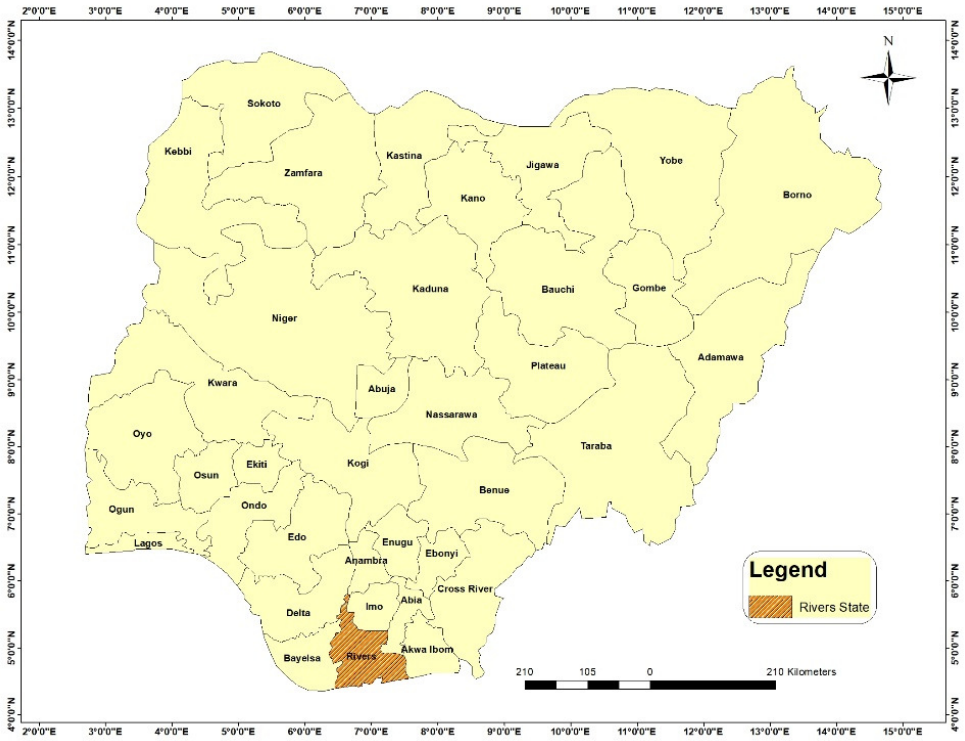

Fig. 1: Nigeria indicating Rivers State

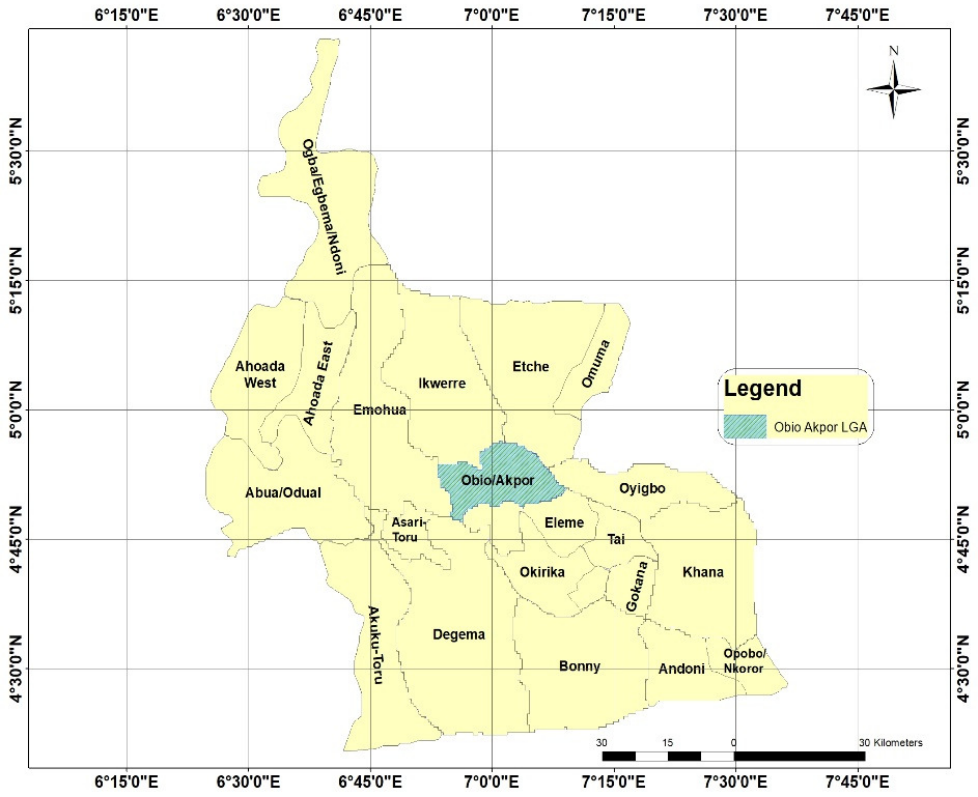

Fig. 2: Rivers State study area indicating Obio/Akpor study location

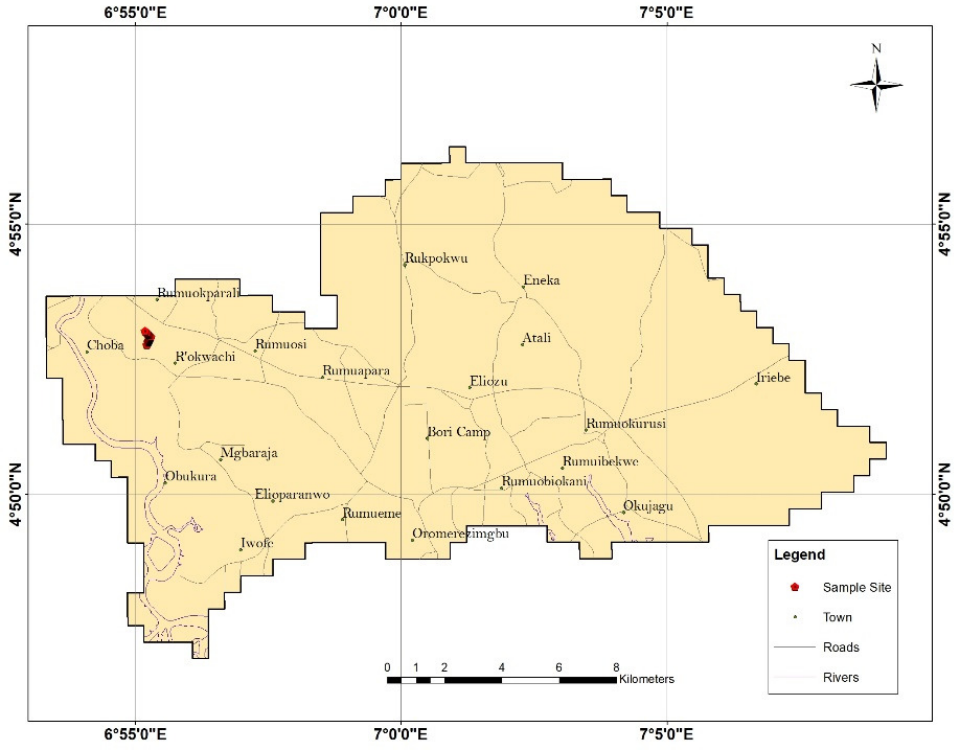

Fig. 3: Obio/Akpor study location indicating Choba study sites

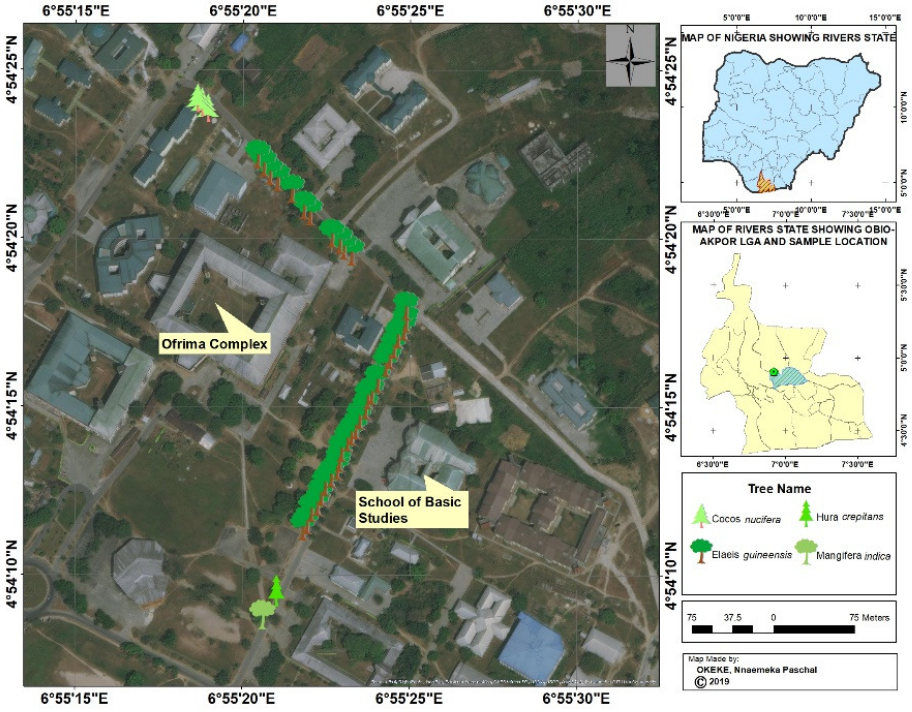

Fig. 4: Satelite imagery of the sampled greenbelt canopy formation

Experimental Field Design, Sampling Method and Procedure

An integrated sampling approach involving Rapid and Representative Epiphyte Diversity (RRED) Analysis (Shaw and Bergston, 1997) and line transect sampling (Walag and Canencia, 2016) methods were carried out in parts of the University of Port Harcourt, Abuja Campus greenbelt canopy formation under maximum ambient environmental condition. The georeferenced coordinates of the sampled site was taken using the GPS ((GPS - *Garmin Dakota 10 model*). The site with a total distance of 589.50m consisting of 42 stands of tree canopy belts was sampled. The sampled site was fragmented into 5 sub-sampled units of equal transect, with equidistance transect dimension of 117.9m consisting 5, 14, 8, 8, and 7 trees respectively.

Arboreal Epiphyte Assessment

The field survey on arboreal epiphyte was carried out in October, 2019 on the study site georeferenced to latitude 04°54.147N and 04°54.403N, and longitude 006°55.347E and 006°55.418E. Of the 42 epiphyte and non-epiphyte tree canopies enumerated were stands of 37 *Elaeis guineensis* L., 3 *Cocos nucifera* L., 1 *Mangifera indica* L. and 1 *Hura crepitans* L. assessed at the western location (left wing) of the green belt formation. The epiphyte sampled consisted of most prevalence and dominance species of *Oleandra distenta* Kunze, *Platycerium bifurcatum (*Cav.*)* C. Chr., *Nephrolepis bissserata (*Sw.*)* Schott, *Platycerium grande* Kunze, *Marattia fraxinea* Sm, *Nephrolepis pumicicola* F. Ballard, *Nephrolepis undulata* J.Sm and *Platycerum stagelephantotis* Schweinf. Care was taken in assessment to ensure all species undergoing investigation had isoecological conditions under ambient surrounding condition. The assessed and collected samples was by single rope tree climbing technique which helps to

determine the presence of epiphyte for maximum sampling of the microhabitats of the upper canopy community without creating injuries on the tree and with ccollected samples stored in a zipper bag for laboratory analyses.

Laboratory Analysis

The following physiobiochemical parameters of APTI were analysed: the pH of the foliar extract, relative water content (RWC), ascorbic acid content (AAC), and total chlorophyll content (TCC). These were used to extrapolate the baseline values for APTI of the species in question under surrounding ambient environmental condition.

pH Determination

The "leaf extract pH measurement" was determined electrometrically using 5g of fresh leaf sample weighed into 50ml beaker; containing 10ml distilled water, homogenised and filtered, and allowed to stand for 15min (Singh and Rao, 1983) and then read with Hanna pH meter (Model HI 9811-5N, Hanna Instrument, USA).

Relative Water Content (RWC) Determination

The methods by Liu and Ding (2008) and Gharge and Menon (2012) were adopted to extrapolate the relative water content as exemplified in the formular below. Fresh leaf samples were weighed and obtained as fresh weight (FW). The leaves then immersed in water at room temperature for incubation period of 24hrs, were wiped dry to obtain turgid weight (TW). Then were placed in an oven at 80^{o}C for 48hrs, and weighed to obtain the dry weight (DW).

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where

FW = fresh weight

DW = Dry weight

TW = Turgid weight

Ascorbic Acid Content (AAC)

The AAC was determined using the Spectrophotometric method (Abida and Harikrishina, (2010). One gram of ground fresh leaves was homogenized for 30sec in 4ml oxalic acid-ethylene-di-amine-tetra-acetic (EDTA) extracting solution containing 1ml of ortho-phosphoric acid, 1ml 5% tetraoxosulphate (vi) acid, 2ml ammonium molybdate and 3ml water. The solution was allowed to stand for 15minutes. The absorbance was read off with a spectrophotometer at 760nm. The concentration of ascorbic acid was extrapolated from a standard ascorbic acid curve.

Total Chlorophyll Content (TCC) Determination

Fresh leaves collected from the field were stored in a refrigerator at 20^{o}C to avoid degradation of coloured pigment. Wholesome fresh leaves (1g) were finely cut, thoroughlymacerated and homogenized once with a known volume (20ml) of 80% acetone and transferred by filtration to test tubes under a subdued light condition by effectively wrapping in an aluminum foil. Some quantities of the filtrate in the test tube were then transferred to a glass centrifuge tube and centrifuged with model 0406-2 centrifuge for 3 minutes at 2000 rpm. The centrifuged supernatant (0.1mill) was transferred using a sampler to a glass cuvette containing 2ml of 80% acetone. A normal 80% acetone solvent was used as blank (to normalize the spec. to 0.00) and passed through spectrophotometer model 722s. At 643nm and 663nm, the chlorophyll absorbance (wavelength) was determined and then total chlorophyll extrapolated using the formula as below: (Strickland and Parsons, 1972).

Chl a = (11.6 A663 –1.3 A643) Vx^{-1}

Chl b = (19.1 A643 – 4.7 A663) Vx^{-1}
Where a and b contents are in mgg^{-1} FW
A663 and A643 are absorbance at 663 and 643 nm.
V = vol (ml) of 80% acetone (20ml)
X = fresh weight of sample used (0.5g)
Thus total cholorophyll = (Chl a + Chl b.)

APTI (Air Pollution Tolerance Index) Determination

The Air pollution Tolerance Index (APTI) which gives an empirical value for tolerance level of plants to air pollution was extrapolated following the Singh and Rao (1983) method with the formula as:

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

A-Ascorbic acid
T-Total chlorophyll
P-Leaf extracts pH
R-Relative Water Content of the leaves

Statistical Analysis

The data were estimated using the Statistical Analysis System (SAS) PROC. NLIN procedure (2002) for the analysis of variance (PROC ANOVA) procedures. Where significant differences were observed, means were separated according to the procedures of the Duncan's New Multiple Range Test (DNMRT) using least significant difference (LSD) tests at *P<0.01* confidence level. Pearson correlation was applied to determine the relationship between the physiobiochemical parameters of the epiphytic species under ambient condition.

Result

The present study has revealed the APTI responses for four biochemical parameters (Table 1) among and within eight epiphytic ferns of green canopy under ambient environmental condition. Percentage increment in the biochemical parameters for the species under such condition are shown in Table 2. The responses of the plant species with APTI values (<1-10) less than Mean±SD (Standard deviation) indicated as sensitive plant, with those of APTI value (13.01 – 16) more than the Mean±SD as tolerant species while moderately tolerant species with value (10.01 – 13) between those of the tolerant and sensitive plant species are being recorded in Tables 3and 4.

The result under ambient condition of leaf extract pH (Table 1) has recorded the highest pH (6.57±0.36) being achieved in *Platycerum bifurcatum* with a percentage increment of 30.62%, significantly different (*P<0.01*) from the lowest pH (5.03±0.38) being recorded in *Marattia fraxinea* (Table 2), which had greater mean value pH (18.42±1.72) within species across parameters. The relative water content (RWC) as presented in Table 1 among species with the highest value (81.22±4.62) was achieved in *N. pumicicola* with significant difference (*P<0.01*) and differential increment of 58.91% while *N. bisserata* has a significantly lower RWC (51.11±12.84). Besides *N. pumicicola* had greater mean RWC value of 18.38±1.80 within species across parameters. *Marattia fraxinea* with 86% increment in ascorbic acid content among species has recorded a significantly different (*P<0.01*) higher content (1.86±0.20) beside a non-significant difference from *P. stagelephantotis* AA content, while *N. bisserata* recorded a lower ascorbic content (1.00±0.46) with significant difference (*P<0.01*) but non-significantly different from *O.distenta* AA content. *Marattia fraxinea* within species across the parameters had a higher AA mean value of 18.42±1.72. *Platycerum stagelephantotis* with over 100%

increment in total chlorophyll content (TCC) among species recorded a significantly different (*P<0.01*) higher content (9.18±0.62), though non-significantly different from *P. grande* TCC. *Nephrolepis undulata* had a significantly lower content (2.25±1.12) of TCC but non-significantly different from the content achieved in *N. pumicicola* and *N. bisserata.* Within species across parameters *P. stagelephantotis* had greater mean value of 22.24±2.71 than *Nephrolepis undulata.* The APTI content (15.19±3.11) with over 100% increment was observed in *P. stagelephantotis* with significant difference (*P<0.01*) higher than the significantly lowest APTI (6.46±4.15) achieved in *N. bisserata.* Within species across parameters *P. stagelephantotis* recorded greater means APTI of.24±2.71 than *N. bisserata.*

Discussion

By the overview of result of this present study it has been revealed based on Tables 1-4 that different species of the epiphytes under ambient environmental condition respond in different ways, by their differences of air pollution tolerant index which can denote their potential as bioindicator and biomonitor to combat air pollution and improve the ambient air quality. In this present work the plant species under ambient condition have expressed different responses to pollution tolerance capabilities depending on the physiobiochemical and other environmental factors affecting them. Hence air pollution tolerance can be affected as reported (Obidiegwu, 2019) by natural climatic conditions such as rainfall, temperature, soil type, and relative humidity; these conditions can also be influencing factor under ambient condition in this study. This justify one reason why the relationship in combining a number of physiobiochemical parameters in the extrapolation of APTI for a more reliable result to be obtained than relying on a single factor. This can be exemplified in a strong positive correlation (r = 0.68, r = 0.93, r = 0.33; *P<0.01*) of APTI with RWC, AA, and TCC respectively beside a weak negative correlation (r = - 0.26; *P<0.01*) with pH, (Table 5), thereby corroborating an earlier assertion by Liu and Ding (2008).

Though there are so many factors influencing the responses of plant species to the sensitivity and / or tolerance in polluted and / or ambient air condition. The pH of leaf extract plays an important role in deciding the tolerance level of plant against pollution. In the investigation the pH of the leaf extract had variation among the species with four species (*P. bifurcatum, N. bisserata, P. grande* and *O. distenta*) expressing a significantly higher pH level. However, all sampled species exhibited an acidic level pH under the ambient natural condition. This can imply the presence of SO_2 and NO_X in such condition which can cause a change in the pH of the leaf sap towards acidic status as similarly reported in several studies (Swami *et al*., 2004; Lohe *et al*., 2015). Plant with lower pH are more susceptible and sensitive while those with higher pH around 7 are more tolerant (Kumar and Nardini, 2013). However, in the present observation all the studied plant showed acidic pH ranging from 5.03 to 6.57 with the most significant low acidic pH found in *M. fraxinea.* On a different note studies have recorded that sensitive plants had higher leaf extract pH than tolerant species (Enete *et al*., 2013; Bakiyaraj and Ayyappan, 2014). Similar result was obtained in the present study which justifies the tolerance level of responses in

M. fraxinea and *P. stagelephantotis*, intermediate tolerance level in *N. pumicicola* and *N. undulata* and sensitivity response in *O.distenta, P. bifurcatum,* and *N. bisserata* respectively (Table 4). The changes in the pH might have influenced stomatal level of responses due to SO_2 and NO_2 under ambient condition. This corroborates the assertion that plant with high sensitivity to SO_2 and NO_2 close their stomata faster than when they are not exposed to pollutant (Larcher, 1995). This can also be a potential source of sensitivity response to air pollutant.

Water is a major essential of life hence it makes up over 80% of the protoplasmic component of every living organism including such lives as plant species in their physiobiochemical processes for existence and survival. Several studies have revealed large water content as an aid to maintaining physiological balance under stress condition such as exposure to air pollution when transpiration rates are high (Bakiyaraj and Ayyappan, 2014; Dileswar *et al*., 2015; Lohe *et al*., 2015). Relative water content (RWC) of a leaf is the water plant has in it relative to its full turgidity. Such content in plant cells is associated with protoplasmic permeability which can cause loss of water and dissolved nutrients. This was earlier reported to be one of the causes of early senescence in leaves (Enete *et al.,* 2013; Bakiyaraj and Ayyappan, 2014). Study has shown that high RWC do favour drought resistance in plants and such plants appear to be more tolerant to pollutant (Jyothi and Jaya, 2010; Enete *et al*., 2013). Similarly, a RWC range of 51.3% to 84.0% has been reported in sensitive plant species (Dileswar *et al*., 2015). Current study under ambient condition with minimal level of pollutant and other environmental related conditions have shown a RWC range of 51.11% to 60. 40% to be associated with sensitive species (*N. bisserata, P. bifurcatum* and *O. distenta*) while 62.21 to 81.22% was associated with intermediate tolerant species (*N. undulata* and *N. pumicicola*) and tolerant species (*M. fraxinea, P. stagelephantotis* and *P. grande*) (Table 4). This corroborates an observed high RWC under ambient condition in monsoon and rainy seasons (Das and Prasad, 2010; Bhattacharya *et al*., 2013). Plant with high RWC under such ambient condition of pollutant (SO_2 and NO_X) may possibly be tolerant to pollutants under polluted condition. Ascorbic acid (AA) is an important monitoring biochemical parameter and a strong reactant that activates many physiological and defense mechanism in plant species. It reducing power has been known to be directly proportional to its concentration (Raza and Murthy, 1988). By its reducing potential it plays a vital role in cell wall synthesis, defense and cell division (Conklin, 2001). Such reducing potential was earlier reported to be pH dependent (Agbaire and Esiefarienrhe, 2009), thus increases with pH increase which have been reported to enhance the efficiency of hexose sugar conversion to ascorbic acid in relation to pollution tolerance (Escobedo, 2008; Liu and Ding, 2008). Similarly, its productivity in relation to total chlorophyll based on its concentration in chloroplast has been reported (Bakiyaraj and Ayyappan, 2014). This can be exemplified in this present study wit a positive correlation ($r = 0.36$; $P<0.01$) between AA and pH, and a corresponding positive correlation ($r = 0.30$; $P<0.01$) between AA and TCC (Table 5). Increase in the concentration of ascorbic acid among plant species and

with such species considered to be tolerant to air pollutants has been recorded (Enete *et al*., 2013; Lakshmikanta Panda *et al*., 2018). In the present study diverse trend of ascorbic acid content of the epiphyte was observed in response to level of sensitivity to tolerance ranging from 1 to 1.86mg/g under ambient condition. This trend increase might be due to enhanced ascorbic acid content of all the species by the increased rate of reactive oxygen species (ROS) during photic oxidation of the absorbed SO_2 to SO_3 (sulphite) under moistened condition of high RWC as can be exemplified in a positive correlation ($r = 0.40$; $P<0.01$) between AA and RWC (Table 5).

Three species (*N. bisserata, O.distenta* and *P. bifurcatum*) being categorised as sensitive plant (Table 4) had low level range of 1 to 1.22mg/g AA (Table 1), while species (*N. pumicicola* and *N. undulata, P. grande, M. fraxinea* and *P. stagelephantotis*) categorised as intermediate tolerance to tolerance (Table 4) with high AA level had range of 1.32 to 1.86mg/g (Table 1). The high level of AA among tolerance species in present study corroborates the assertion that higher ascorbic acid content of plant species is signal of tolerance against SO_2 pollution and as well may be a defence mechanism of individual species (Varshney and Varshney, 1984; Cheng *et al*., 2007). Consequently, in the present study the higher ascorbic acid level among tolerant categories of species can suggest their tolerance as biomonitors and lower AA could supports the sensitivity nature as bioindicators toward pollutants.

Chlorophyll is one of the influential biochemical properties of plant species subject to diverse trend of environmental condition of both polluted and ambient qualities. Such influential indices of chlorophyll have been reported in several studies including: it photosynthetic potential for carbondioxide fixation, growth and biomass development (Dileswar *et al*., 2015; Enete *et al*., 2013); it importance as stress metabolites (Lakshmikanta Panda *et al*., 2018) and indicator of air pollution (Ninave *et al*., 2001). Variation in chlorophyll content of plant have been established based on species differences, age of leaf, pollution level as well as other biotic and abiotic conditions (Katiyar and Dubey, 2001; Abida and Harikrishna, 2010). Higher chlorophyll content in plant might favour tolerance to pollutants (Lakshmikanta Panda *et al*., 2018). Where as certain pollutant increase the TCC others decrease it (Joshi and Swami, 2009; Joshi *et al*., 2009; Agbaire and Esiefarienrhe, 2009; Chandawat *et al*., 2011). In the current research while lower chlorophyll (ranging from 2.25 – 2.33mg/g) was associated with intermediate tolerant species (*N. undulata* and *N. pumicicola*), higher TCC range of 5.90 to 9.18mg/g was associated with tolerant species category (Tables 1 & 4). Similarly was a decrease (2.33mg/g) and increase of 5.33 and 7.95mg/g achieved in sensitive species under ambient condition. The decrease might be due to the pollutant (SO_2 and NO_x) presence under ambient condition. Similar reduction in chlorophyll concentration might be due to increased chlorophyllase enzyme activities which often affect TCC in plants (Mandal, 2000). Also decrease due to alkaline condition created by dissolution of chemical present in dust particles, thus blocking stomatal pores for diffusion as well as possing stress in metabolism has been reported (Anthony, 2001). Similar phenomenon can be depicted in the current study under ambient condition.

Air pollution Tolerance Index (APTI) signifies the potential capability of plant species (vegetation) to combat air pollution. Plant species naturally have the innate potential via physiobiochemical processes to purify the aerial medium of the environment by scavenging or sequestering particulate matter and non particulate matter (phytosequestration) in what ever prevailing environmental condition they found themselves. It has been established that APTI plays a significant role in determining the resistivity and susceptibility of plant species against pollution levels (Dileswar *et al*., 2015). This implies that plant exhibit different pollution response capabilities depending on the species and environmental factors affecting them. This can be depicted from Table 1 that different plants respond differently to air pollutant even under ambient condition ranging from sensitive through intermediate tolerance to tolerance responses. In the present study the variation in the APTI values could be attributed to the different responses of the plant to the physiobiochemical factors (pH, RWC, AA, and TCC) which in turn are affected by variation in the air pollution level of the ambient environment. Such influencing relationship of the APTI dependence on biochemical parameters can be represented in a positive correlation ($r = 0.26$; $r = 0.68$; $r = 0.93$; $r = 0.33$; $P<0.01$) of APTI with pH, RWC, AA, and TCC respectively (Table 5).

Earlier research revealed that species with higher index value are tolerant to air pollution, thus depicting their potential as sink to mitigate pollution, while species with low index value show less tolerance depicting their sensitivity level to air pollution (Singh and Rao, 1983; Tanee and Albert, 2013; Nwadinigwe, 2014). Similar trend of response occurred in the present study in which the eight epiphyte species had shown various levels of APTI values ranging from 1 – 10 as sensitive, 10.01 – 13 as intermediate tolerant and 13.01 – 16 as tolerant species and in the following order of response: *P. bifurcatum*>*O. distenta*>*N. bisserata* for sensitivity; *N. undulata*>*N. pumicicola* as intermediate tolerant and *P. stagelephantotis* > *M. fraxinea*> *P. grande* as tolerant (Table 4). The highest APTI value (15.19) and least APTI value (6.46) were shown by *P. stagelephantotis* and *N. bisserata* respectively. As earlier suggested based on high and low APTI values of responses (Raina and Sharma, 2006; Mingorance *et al*., 2007), the sensitivity species in the current study can be suggested as bioindicators while tolerant species are biomonitors for pollutants.

Since tolerant species serve as sink for biomonitoring, it can then be depicted as an important tool to evaluate the impact of air pollution on plants. Thus APTI can be indicated as a phytosequestration tool for selecting tolerant species for air pollution monitoring. Such APTI valuation can be window for selection via transition of species responses from sensitivity through APTI levels of environmental tolerance of species. It can therefore be recommended that selection criteria should not only be limited to higher plant with robustness of morphological and anatomical features but should be inclusive of lesser known understudied species like the epiphytic ferns. Epiphytic fern can help improve air quality through their potential sink as biomonitoring agent via transition levels of tolerance. They can also improve the phytosequestration potency of higher species by modifying the microhabitat of

green belt canopies for enhanced air quality mitigation.

Conclusion

In conclusion APTI is important hence it will guide against envisaged air pollution and maintain air quality. The study also provides useful information for selecting biomonitors via tolerance levels. Epiphytes ranked as tolerant and intermediate tolerance should be considered for their air quality usefulness, other than their attachment or support dependence on higher plant. Species ranked as sensitive can also help as bioindicator of air quality. This by implication via APTI valuation means that epiphytes has tremendous role on the life potential usefulness of higher tree canopies in air quality monitoring.

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Table 1: Air Pollution Tolerance Indices of Epiphytic Species of Green Belt Canopy under Ambient Environmental Condition

Species Indices	*Oleandra distenta*	*Platycerum bifurcatum*	*Nephrolepis bisserata*	*Platycerum grande*	*Marattia fraxinea*	*Nephrolepis pumicicola*	*Nephrolepis undulata*	*Platycerum stagelephantotis*	Mean	LSD (*P<0.01*)
pH	6.03±0.55^{c}	6.57±0.36^{a}	6.18±0.28^{b}	6.27±0.10^{b}	5.03±0.38^{d}	5.83±0.49^{e}	5.87±0.19^{e}	5.33±0.48^{f}	5.74	0.12
RWC	60.40±1.63^{a}	58.84±5.61^{b}	51.11±12.84^{c}	68.39±6.20^{d}	65.14±5.48^{e}	81.22±4.62^{f}	62.21±9.35^{g}	66.89±9.13^{h}	66.35	0.17
AA	1.12±0.71^{de}	1.22±0.51^{i}	1.00±0.46^{d}	1.65±0.35^{c}	1.86±0.20^{b}	1.32±0.23^{i}	1.67±0.24^{c}	1.85±0.22^{b}	1.58	0.15
TCC	5.53±2.55^{f}	7.95±1.86^{d}	2.33±1.58^{h}	7.72±1.50^{a}	5.90±0.55^{c}	2.33±1.28^{h}	2.25±1.12^{h}	9.18±0.62^{a}	5.33	0.14
APTI	8.15±5.24^{j}	9.22±4.53^{h}	6.46±4.15^{g}	13.67±3.31^{k}	14.18±1.97^{a}	11.83±2.38^{d}	11.96±3.62^{d}	15.19±3.11^{i}	12.44	0.15
Mean	16.25	16.76	13.42	19.54	18.42	18.38	16.37	22.24		
LSD (*P<0.01*)	0.15	0.19	0.35	0.10	0.11	0.28	0.25	0.24		

Note: values represent means ± standard deviation. Means followed by the same superscript in the same column are not significantly different at *P<0.01* and vice versa. pH = pH of the leaf extract. RWC = Relative Water Content. AA = Ascorbic Acid. TCC = Total Chlorophyll Content. APTI = Air Pollution Tolerance Index.

Table 2: Percentage change (increments) of biochemical parameters between species

Parameter	Percentage increment per species
Leaf extract pH	30.62 in *P. bifurcatum*
Relative Water Content	58.91 in *N. pumicicola*
Ascorbic Acid	86 in *M. fraxinea*
Total Chlorophyll content	100> in *P. stagelephantotis*
APTI	100> in *P. stagelephantotis*

Table 3: Responses of APTI of the epiphytic species under ambient environmental condition

Range of index value	Response
<1 – 10	Sensitivity
10.01 – 13	Intermediate
13.01 – 16	Tolerant

Table 4: Response level of plants

S/N	Epiphytic species	Responses Sensitive (APTI <1-10)	Intermediate (APTI 10.01 – 13)	Tolerant (APTI 13.01 – 16)
1	*O. distenta* Kunze	x (8.15)		
2	*P. bifurcatum(*Cav.*)* C. Chr.	x (9.22)		
3	*N. bisserata (*Sw.*)* Schott	x (6.46)		
4	*P. grande* Kunze			x (13.67)
5	*M. fraxinea* Sm			x (14.18)
6	*N. pumicicola* F. Ballard,		x (11.83)	
7	*N. undulata* J.Sm		x (11.96)	
8	*P. stagelephantotis* Schweinf.			X (15.19)

Table 5: Pearson correlation coefficient of physiobiochemical parameters among epiphytic species under ambient condition

Parameter	pH	RWC	AA	TCC	APTI
pH	1.00				
RWC	- 0.01	1.00			
AA	0.36**	0.40**	1.00		
TCC	- 0.18	- 0.04	0.30**	1.00	
APTI	0.26**	0.68**	0.93**	0.33*	1.00

** Correlation is significant at the 0.01 level.

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