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RESEARCH PAPER

Biochemical and Physiochemical Assessment of Air Pollution Tolerance Index of Selected Plant Species at Ikpoba Okha Gas Flaring Site, Edo State, Nigeria

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ABSTRACT
The Air Pollution Tolerance index (APTI) of six plants located within Ikpoba Okha gas flaring site in Oredo Local Government Area of Edo State, Nigeria during wet and dry seasons were assessed. Plant samples for this research work were randomly collected from the vicinity of the flaring site. Six (6) sample of each plant was used for laboratory analysis. The plant parameters assessed include relative water content (RWC), the ascorbic acid content (AAC), total leaf chlorophyll (TLC) and pH extract of the leaves and were used to compute the Air pollution tolerance indices (APTI). Based on the analyzed result, the RWC in *Drypetes leonensis*, *Ficus exasperata* Vahl, *Chromolaena odorata* (Linn) and *Gmelina arborea* Roxb. ex Smith species in dry season were higher than those in wet season. *Icacina tricantha* showed a relatively high level of acidity when compared to others. *A. boonei* De Wild has the highest ascorbic acid content in the leaves in both seasons. The highest level of chlorophyll contents was recorded in the dry season with *Drypetes leonensis* having the highest, followed by *Icacina trichantha*. There was no statistically significant difference in pH and total chlorophyll contents between samples collected in wet and dry season; however, there were significant difference observed in ascorbic acid and RWC in both seasons. APTI in wet and dry season showed a statistically significant difference. This study recommends planting of tolerant species that can acts as bio-indicators especially in gas flaring stations in Nigeria.
Keywords: Chlorophyll - ascorbic acid-pH-tolerance- bio-indicators

INTRODUCTION

Air pollution is the introduction to the environment of substances caused mainly by human activities into the environment causing an alteration or deviation from the normal quality of ambient air. Over the years, there has been a continuous growth in human population, road transportation, vehicular traffic and industries whose activities increases the concentration of gaseous and particulate pollutants (Joshi, et.al., 2009).Air pollution is a serious environmental problem causing degradation of the environment and increasing concentrations of gases and introducing varying particulates matter into the atmosphere (Panda and Aggarawal, 2018). According to Kumari and Deswal, 2017, plants exhibits a good array of symptoms once exposed to pollutants throughout chemical process, respiration, catalyst reactions, membrane disruption, stomata behaviour and ultimately death.

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Air pollution tolerance index (APTI) is the natural quality of plants to face problems of air pollution stress. It is an inherent quality of plants to encounter air pollution stress which is presently of prime concern particularly in urban areas of the world. According to Govindaraju et al. 2012, ‘‘APTI index is effective in evaluating the effect of pollutants only on biochemical parameters, but in order to combat air pollution using green belt development, some socioeconomic and biological characteristics are considered to develop the anticipated performance index (API)’’. APTI assesses the tolerance level of plants as regards air pollution by considering the total chlorophyll, ascorbic acid (AAC), leaf extract pH and the relative water contents of such tree. It has been applied in studies like green belt development (Shannigrahi et al., 2004; Kour and Raina, 2014), traffic noise reduction (Pathak et al., 2011),urban and industrial parks (Prabhat, et al.,2013), pollution mitigation along roadsides and around industries(Iqbal, et al.,2015; Bignal,et al.,2008) and gas flare station(Tanee and Albert, 2013)

Trees do act as air pollution sinks which is a function of their tolerance level, hence some of these trees could serve as biological indicators of air pollution. Air pollutants can directly affect plants via leaves or indirectly via soil acidification. It has also been reported that when exposed to air pollutants, most plant experience physiological changes before exhibiting visible damage to leaves (Wagh et al., 2006). Plants are basic integral for all ecosystems and also most likely to be affected by airborne pollution which are identified as the organisms with most potential to receive impacts from ambient air pollution. The effects of air pollution are most often apparent on the leaves which are usually the most abundant and most obvious primary receptors of large number of air pollutants.

Vegetation generally acts as important indicators of the overall impact of air pollution and the effect observed is a time-averaged result that is more reliable than the one obtained from direct determination of the pollutants in air over a short period. A vast majority of plants are good dust filters in checking rising urban dust pollution level (Rai *et al.*, 2010). Plants possess large leaf area for absorption and accumulation of air pollutants to reduce pollution level in the air environment with various extents for different species (Liu and Ding, 2008). The use of plants as bio-indicators of air pollution has long been established because they are the initial acceptors of air pollutants due to having scavenging property for many air pollutants (Joshi and Swami, 2009)

Air pollutants causes leaf injury, stomata damage, premature senescence, decrease photosynthetic activities, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari etal, 2006). Sensitivity and response of plants to air pollutants is variable. The plant species which are more sensitive act as biological indicators of air pollution. The response of plants to air pollution at physiological and biochemical levels can be understood by analyzing the factors determining their resistance and susceptibility.

The aim of this work is to assess air pollution tolerance indices (APTI) potentials of some selected plants growing around Ikpoba Okha gas flaring station in Edo State, Nigeria. It is expected that findings from this work will widen the knowledge of the tolerance and sensitivity of some plants to air pollution and also in identifying these trees will help probably in the selection of air pollution tolerance plants that could be planted in air pollution prone areas.

MATERIALS AND METHOD

Ikpoba Okha gas flaring station is located at Oredo Local Government Area of Edo State, Nigeria, lies at latitude 6°3’8.04” N and 5°39’51.25” E longitude. The study area lies within the coastal plain of Niger Delta which is characterized by two seasons (wet and dry). Rainfall in the study area varies, though heavy and adequate for all year-round crop cultivation. About 2279 mm of precipitation is recorded annually. The average annual temperature is 26.4°C. Relative humidity is high throughout the year and decreases slightly in the dry season. The soil in the area is brown loams and sandy loams.

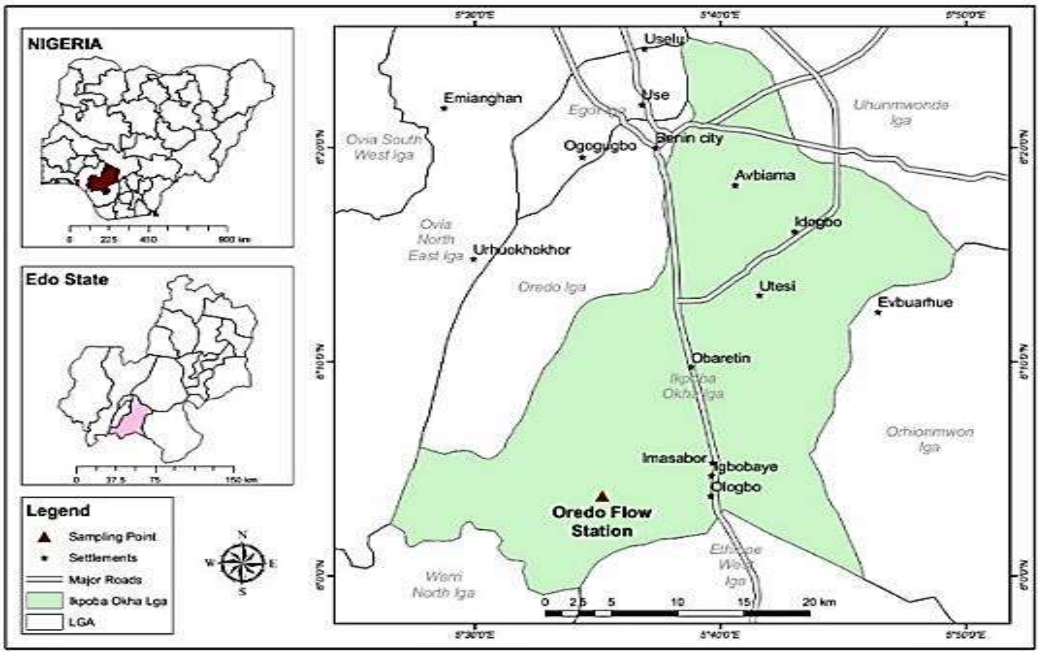

Figure 1. Study area showing sampling points

A complete randomized design method was employed in collecting leaves from the gas flaring station. The tree species selected for this work were those available within 100m of the flaring site. A total of six dominant tree species i.e. *Icacina trichantha*, *Drypetes leonensis*, *Fiscus exasperata*, *Chromolaena odorata*, *Gmelina arborea* and *Alstonia boonei* De Wild were randomly selected because of their visible presence and abundance in the gas flare sites. Leaf samples were collected in wet and dry season. The sampled leaves were put in bags and placed in liquid nitrogen container for further use. In laboratory, samples were stored at −20°C till use. Identification of the leaves was carried out at the department of Botany herbarium, Obafemi Awolowo University Ile –Ife, Nigeria; they were immediately taken to the central Science laboratory of the OAU for analysis. Composite sample of six (6) leaves for each species were used for the analysis

The following parameters were analysed: leaf relative water content (RWC), ascorbic acid content (AAC), total leaf chlorophyll (TLC) and pH of leaf extract. These were used to compute the APTI values.

Three leaves of different weights were used to estimate the RWC of leaves. Fresh Weight (FW) was obtained by weighing the fresh leaves, Turgid Weight (TW) of the leaves were obtained by immersing the leaves in water overnight, blotted dry and then weighed to get the turgid weight. Dry Weight (DW) was obtained from drying the leaves overnight in an oven at 70°C and reweighed. Relative water content (% RWC) of the leaf was determined using the method of Liu and Ding (2008) as follows:

$$\mathrm{RWC\%} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100 \quad \#\mathrm{Eqn1}$$

Where FW is the fresh weight, DW is the dry weight of turgid leaves while TW is the turgid weight after immersion in water overnight.

Using the method of Aasawari et al. (2017), 5g of blended fresh leaves was homogenized in 10ml distilled water and the extract filtered; pH of the leaf extract was determined after calibrating pH meter with buffer solution of pH 4, 7 and 9.

10.0g of fresh leaves was blended and then extracted with 10ml of acetone and left for 15 minutes for thorough extraction. The liquid portion was decanted into centrifuge tube and centrifuged at 4,000rpm for 2 minutes. The supernatant was then collected and diluted with acetone. The absorbance of the extracts were taken at 645nm and 663nm using spectrophotometer. Calculations were done using the formula below.

$$\text{Chlorophyll a} + \text{Chlorophyll b} = \text{Total chlorophyll} \ \#\text{Eqn. 2}$$

$$\text{Chlorophyll (mg/g)} = 20.2\ (D_{645}) + 8.02\ (D_{663}),\ D_{645} = \text{absorbance at 645nm}$$

$$D_{663} = \text{absorbance at 663nm}$$

$$\text{Total chlorophyll} = \frac{0.1\ \text{CT x (Leaf Dry Weight}}{\text{Leaf Fresh Weight}} \ \#\text{Eqn. 3}$$

0.5g each of the plant was weighed using Mettler Toledo weighing balance. 5ml Oxalic-EDTA was added to each of the samples and then left for 24hrs. 2.5ml of the standard ascorbic acid was pipetted into 25ml volumetric flask, 2.5ml of oxalic EDTA was then added to each of the samples followed by addition of 2ml of ammonium molybdate (5%).In each of the samples, 1ml of 5% H_2SO_4 was added and made up to the mark in 25ml volumetric flask with distilled water, the same was repeated for the samples and read at 760nm wavelength using UV spectrophotometer.

The method of Sing et al. (1991) was used in calculating APTI, i.e

$$APTI = \text{A(T + P) + R}\frac{}{10} \ \#\text{Eqn. 4}$$

Where;

A = ascorbic acid content (mg/g);
T = total chlorophyll content (mg/g):
P = pH of leaf extract;
R= relative leaf water content (%).

RESULTS AND DISCUSSION

The relative water content (RWC) (%) of a leaf depicts its full turgidity. It is linked with protoplasmic permeability in cells. High water content within plant body serves as an indicator of drought resistance in plants and helps to maintain its physiological balance under stress conditions such as exposure to air pollution when the transpiration rates are usually high. As shown in table1, the relative leaf water content of *Drypetes leonensis* (40.65), *Ficus exasperata* (47.7*), Chromolaena odorata* (49.93) and *Gmelina arborea (48.05)* tree species in the dry season were higher than those in the wet season. A possible explanation could be that those tree species have developed adaptive features for water storage which helps in maintaining their physiological balance against pollution stress. A similar study was carried out by Kashish and Bhardwaj (2017) who in their work showed that seasons has a good significant influence on leaf relative water content of plants. In their work to determine APTI, maximum relative water content of 80.10 per cent in plant leaves was recorded during wet season followed by winter (64.43 per cent) and minimum of 55.45 per cent in summer season. Jyothi and Jaya (2010) in their work observed the highest relative water content of water in plants in monsoon season followed by winter and wet season. Tanee et al., (2014) also showed that plants at polluted site absorbed more water than those in unpolluted sites, which could be an adaptive physiological mechanism of plants to withstand the effect of pollution in its environment; their study concluded that during rainy season *D. sissoo* growing at 0-100 m distance contained relative water content to the tune of 86.05 per cent which was significantly higher as compared to other treatment combinations.

However, wet deposition is less important than dry deposition since the pollutants present in the atmosphere may have been washed away and even from the leaf surface so much less duration of exposure but in the case of dry deposition, leaves are exposed to ambient condition for a very long time. Table 1 also shows that *Alstonia boonei in* both seasons has the highest RWC (%).

All the plant species sampled during wet season show acidic pH which may be due to the presence of oxides of Sulphur and Nitrogen in the atmosphere. Plant samples collected and analyzed showed common trends in pH values; *Icacina tricantha* showed a relatively high level of acidity when compared to others. Plants with lower pH are more susceptible, while those with pH around 7 are more tolerant (Singh and Verma, 2007; Kumar and Nandini (2013).Generally, plants with low pH value shows good correlation with sensitivity to air pollution and plants with high pH may be considered to be tolerant under polluted environment (Escobedo et al. 2008). High pH is known to boost tolerance of plants to air pollution. High pH value in *Icacina tricantha* in the study area indicates its tolerance nature. Acidic pH recorded in both wet and dry season showed that some plants are more susceptible to pollutants in comparison to other plant species. Jyothi and Jaya (2010) had similar result in their work as they recorded a higher pH of 6.48 and opined that the high pH in wet season may be due to washing effect of rains to the acidic pollutants. They also reported maximum pH during monsoon season with gradual reduction through winter and minimum in summer.

Table 1. Biochemical properties of selected tree species

Plant species	Season	RWC (%)	p^H	Total chlorophyll(mg/g)	Ascorbic acid(mg/g)	APTI
Icacina trichantha	Wet	35.11	4.75	0.21	0.66	3.84
	Dry	17.23	5.5	23.17	0.44	2.99
Drypetes leonensis	Wet	40.65	4.95	5.35	1.21	5.31
	Dry	42.09	8.6	25.22	0.64	6.38
Fiscus exasperata	Wet	37.1	6.1	4.79	2.79	6.75
	Dry	47.47	8.9	8.8	0.36	5.38
Chromolaena odorata	Wet	34.55	5.1	7.22	1.54	5.36
	Dry	49.93	6.2	11.6	0.24	5.42
Gmelina arborea	Wet	31.88	4.88	7.56	1.58	5.15
	Dry	48.05	5.6	4.08	0.61	5.4
Alstonia boonie	Wet	88.19	5.09	1.65	0.43	9.11
	Dry	65.93	5.6	4.63	3.19	9.86

Five of the plants (table 1) collected in the dry season showed a lower ascorbic acid content except Alstonia *boonei* when compared to those in wet season. The higher ascorbic acid content in the leaves of *A. boonei* in the study area compared to other plants may be due to its higher adaptive capacity to tolerate air pollution and other stresses; it could also be due to improvement in the defense mechanism of the plants which has also been reported to vary with different plants (Kashish and Bhardwaj 2017). This study is in agreement with that of Cocklin, (2001) and Aguiar et al., (2016) whose study showed that higher ascorbic acid content in plant is a sign of its tolerance against pollution.

The chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is fixed to yield carbohydrates and oxygen. It varies from species to species, age of leaf and with levels of pollution as well as with other biotic and abiotic conditions (Katiyar and Dubey, 2001). Studies has shown that air pollutants reduce chlorophyll contents (Joshi and Swami 2007; Tiwari et al., 2006) while others increase it (Tripathi and Gautam 2007; Agbaire and Esiefarienrhe, 2009). As shown in table 1, the highest level of chlorophyll contents was recorded in the dry season with *Drypetes leonensis*(25.22 mg/g) having the highest, followed by *Icacina trichantha*(23.17mg/g).This study recorded its highest chlorophyll contents during dry season, this differs from the study carried out by Katiyar and Dubey,2006 who recorded highest chlorophyll content in their study area during wet season followed by winter and minimum in summer season and also by Jyothi and Jaya (2009) who also reported high chlorophyll content in plants during wet season followed by winter and summer seasons. The high chlorophyll content recorded in dry season in table 1 may be due to the deposition of soot and dust particles from the gas flaring environment.

Generally, result from table 1 indicates that most of the plants in the study area showed higher chlorophyll level (%) in wet season compared to the dry season. The reduction in total chlorophyll contents of plants in the dry season in the study area may be due to effects of degradation of chlorophyll metabolic processes or synthesis. This is in agreement with the report that one of the effects of air pollution is the gradual disappearance of chlorophyll and concomitant leaf chlorosis which is associated with consequent decrease in photosynthetic capacity (Ninave, 2001). Chlorophyll degradation of photosynthetic pigment has been widely used as an indicator of air pollution (Joshi and Swami, 2007)

Table 2: Air Pollution Tolerance Index (APTI) of the selected plants

Plant species	Season	APTI mean value	APTI (%)	Tolerance
Icacina trichantha	Wet	35.11	10.82	Tolerance
	Dry	17.23	8.44	intermediate
Drypetes leonensis	Wet	40.65	14.96	Tolerance
	Dry	42.09	18.01	Tolerance
Fiscus exasperata	Wet	37.1	19.01	Tolerance
	Dry	47.47	15.19	Tolerance
Chromolaena odorata	Wet	34.55	15.08	Tolerance
	Dry	49.93	15.31	Tolerance
Gmelina arborea	Wet	31.88	14.5	Tolerance
	Dry	48.05	15.22	Tolerance
Alstonia boonei	Wet	88.19	25.64	Tolerance
	Dry	65.93	27.82	Tolerance

APTI values shown in tables 2 and 3 indicates that only two out of the tree species sampled during the wet season had higher APTI value while the rest had lower APTI values. In the dry season however, four tree species had a higher APTI values while two had lower APTI values.

The results of the air pollution tolerance index (APTI) determined for the six plants showed that *Alstonia boonie* has the highest APTI value, followed by *Ficus exasperata* .The plant with lowest APTI value was *Icacina trichantha* in both seasons respectively. Plant species collected during dry season gave higher APTI values. Plants with low and high APTI percentage values can serve as tolerant and sensitive plant respectively.

Table 3: Standard range of tolerance

Range of APTI	Tolerance
30-100	Tolerance
17-29	Intermediate
1 to 16	Sensitive
<1	Very sensitive

Based on seasonality (wet and dry season), paired t-tests was carried out on all the physiochemical and biochemical parameters of the plants to determine if there were any statistically significant differences between them. Results of the various paired t-tests analysed showed that there was no statistically significant difference in pH, $t(6)= -2971$, $p=0.031$ and total chlorophyll, $t(6)= -1.975$, $p=0.105$ between samples collected in both season; however there were statistical significant difference observed in ascorbic acid, $t(6)= -0.008$, $p=0.994$ and RWC, $t(6)= -0.78$, $p= 0.941$. Paired t-test for APTI in both season showed a statistically significant difference, $t(6) = 0.061$, $p=0.954$.

CONCLUSION

This study clearly showed the impact of air pollution in terms of changes that took place in various biochemical and physiochemical parameters of the studied species. It also showed how plants of various species tolerate pollution based on their APTI values. All the trees except *Drypetes leonensis* in dry season show tolerance to pollutants released in the gas flare station. Based on the APTI values obtained from the different plant species, it can be concluded that *Alstonia boonei* is the most tolerant species in both seasons (though other tree species also shown tolerance) and hence *Alstonia boonie* can be planted within the gas flaring zones of Ikpoba Okha flare station as it will act as a buffer against pollutants. The results of this study therefore suggest that plants have the potential to serve as excellent qualitative and quantitative indices of pollution since bio monitoring of plant is an important tool to evaluate the impacts of air pollution on plants.

This study provides important information for town planners, environmentalist, horticulturist, etc. when selecting plant species for landscaping and urban heat island reduction and for future planning. In conclusion, with gas flaring unabated in Nigeria, planting of tolerant species should be the first check against environmental pollution before enactment of Government laws. The present study recommends the planting of tolerant species that can acts as bio-indicators especially in gas flaring stations in Nigeria.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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