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EFFECT OF VEHICULAR EMMISSION ON SOME BIOCHEMICAL PARAMETERS AND STOMATA OF SELECTED TREES ALONG ZARIA-KADUNA HIGHWAY

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ABSTRACT

Biochemical and stomatal studies were conducted on selected trees along Zaria-Kaduna highway to determine their pollution tolerance level. The sites were chosen to emphasize exposure to vehicular emission pollution (VEP) and non-exposure to vehicular emission pollutants (NEP). Biochemical parameters analyzed where based on the Air Pollution Tolerance Index (APTI) which prioritizes four biochemical parameters, namely ascorbic acid, total chlorophyll, leaf extract pH and relative water content (RWC), for pollution tolerance. Ascorbic acid and RWC were higher in plants at the polluted site with *Mangifera indica* showing the highest increase of 1.2mg/g while total chlorophyll decreased most significantly in *Ficus polita* of about 4.1mg/g. Results from stomata studies reveal a significant difference ($P \leq 0.05$) in stomatal size and stomatal index for all the trees at the polluted sites. Stomata was generally found to significantly decrease in size or increase in density for plants at the polluted sites. Results computed for APTI show *M. indica* (24), *Anarcardium occidentale* (24.1), *Eucalyptus cameldulensis* (17.1) and *Senna siamea* (18.6) having the best APTI which indicate their possible tolerance to air pollution stress while *Azadirachta indica* (14.4), *Ficus polita* (13), *G. arborea* (9.4), *T. catapa* (15.2), *Parkia biglobosa* (13.4), and *Tamarindus indica* (9.9) were found to be sensitive. *M. indica* and *A. occidentale* had the best APTI indices which indicate that these plants could serve as sinks in absorbing air pollutants.

Keywords: APTI, pollution, tolerance, sensitivity

INTRODUCTION

Air pollution is a serious environmental problem due to the trans-boundary nature of the pollutants (Naeem, 2019). In most Nigerian cities, atmospheric pollution is a major problem most especially in the metropolitan areas where inefficient energy combustion has resulted in generation of high levels of confined air pollutants (Ukemenam, 2014). The rapid increase in the use of vehicles and the widespread use of motor-cycles for transporting passengers in Nigeria and other underdeveloped nations with a lack of implementation of pollution standards has added to the growing concern over vehicular pollution (Olukanni and Adebiyi, 2012; Ukemenam, 2014). These have caused a great deal of environmental problems which affect plants, animals and man. The morphology, physiology and biochemistry of plants undergoes significant changes when exposed to pollution. To achieve fine adjustments in their ability, plants conduct gaseous exchange, stomatal openings change in response to a varying environmental and internal factors (Micheal, 2018). Enormous leaf area provided by plants impinges, absorbs and accumulates air pollutants leading to reduced pollution level of the atmosphere (Escobedo *et al.*, 2008). These aspects however differ in species of plants (Costa, 2001). Use of plants as detectors of polluted air has long been established, as plants are the usually the first point of contact for air pollutants (Gharge and Menon, 2012). Plants sensitivity and their response to air pollution differs. More sensitive acts as biological signals of air pollution. APTI has been used to identify the level of tolerance of plant species based on four parameters (Singh *et al.*, 1991). APTI also have been used to classify plant species in their order of tolerance to air pollution and by landscapers to select air pollution tolerant plants (Enete *et al*, 2013). The current study aims to evaluate the biochemical and stomatal response of trees at selected sampling points and to determine their pollution tolerance status along the Zaria-Kaduna highway.

MATERIALS AND METHODS

The Zaria-Kaduna highway used to be characterized by several military checkpoints which caused long queues of vehicles to form at these specific points and this increases the load of vehicular emissions on the roads which also directly affects road side plants that remain in contact with these pollutants. Although these checkpoints have been dismantled, three of them were however identified and selected. Ten trees were selected at these points due to their reoccurrence in at least two of the sampling sites. Three replicates of fully matured leaves were sampled from the trees and transported to the Herbarium of the Department of Botany, Ahmadu Bello University for their identification. As control, leaves of the same tree species was also sampled from the Botanical garden of Ahmadu Bello University and its environs which served as control for the analysis.

RELATIVE WATER CONTENT (RWC) DETERMINATION

RWC was determined following the method described by Agbaire and Esiefarienrhe (2009).

DETERMINATION OF LEAF EXTRACT pH

This was carried out as described by Singh *et al.*, (1991). 5 g of the fresh leaves was homogenized in 10 ml distilled water. This was then filtered and the pH of leaf extract was then determined after calibrating pH meter with buffer solution of pH 4 and pH 9.

ASCORBIC ACID CONTENT DETERMINATION

This was determined using the procedure as described by Bajaj and Kaur (1985). 4ml oxalic acid – EDTA extracting solution was added to 1g of sample. Then 1ml of orthophosphoric acid, 1ml 5% H_2SO_4, 2ml of ammonium molybdate and then 3ml of water was added to this mixture then allowed to stand for 15 minutes. The absorbance at 760nm was then measured using spectrophotometer. The concentration of ascorbic acid in the sample was then extrapolated from a standard ascorbic acid curve.

TOTAL CHLOROPHYLL CONTENT (TCH)

This was done as described by Bojović and Stojanović (1981). 0.5 g of leaf samples was measured on a weighing balance. The measured material was then homogenized using a pestle and mortar with the addition of 10 ml of 80 % acetone. A primary acetone extract containing all chloroplast pigments was obtained in this way. The extract was then centrifuged at 2500 rpm for 5 min. Since the concentration of pigments was too great for reading on a spectrophotometer, the obtained extract was diluted by adding 9 ml of 80% acetone per ml of extract. The extract produced in this way was subjected to reading on a spectrophotometer (Spectrumlab 752S). Chlorophyll was calculated using the formula

$$C_a = 11.75\ A_{662} - 2.350\ A_{645}$$

$$C_b = 18.61\ A_{645} - 3.960\ A_{662}$$

Total chlorophyll = $C_a + C_b$, where C_a = Chlorophyll A, C_b = chlorophyll B

DETERMINATION OF STOMATAL LENGTH, WIDTH AND INDEX

This was done as described by Ogaya *et al*., (2011).

DETERMINATION OF AIR POLLUTION TOLERANCE INDEX (APTI)

Air pollution tolerance index was determined following the method of Singh *et al*., (1991).

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

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

STATISTICAL ANALYSIS

Descriptive statistics was used to obtain the mean values and standard error of means for the chlorophyll, carotenoid, leaf extract pH, RWC and ascorbic acid at 95% confidence interval. Students t-test was used to compare the means of the stomata length, width and index between the control and polluted site at $p \leq 0.05$.

RESULTS AND DISCUSSION

Figure 1A-D gives the mean chlorophyll for TCH, AA, leaf extract pH and RWC obtained for the trees.

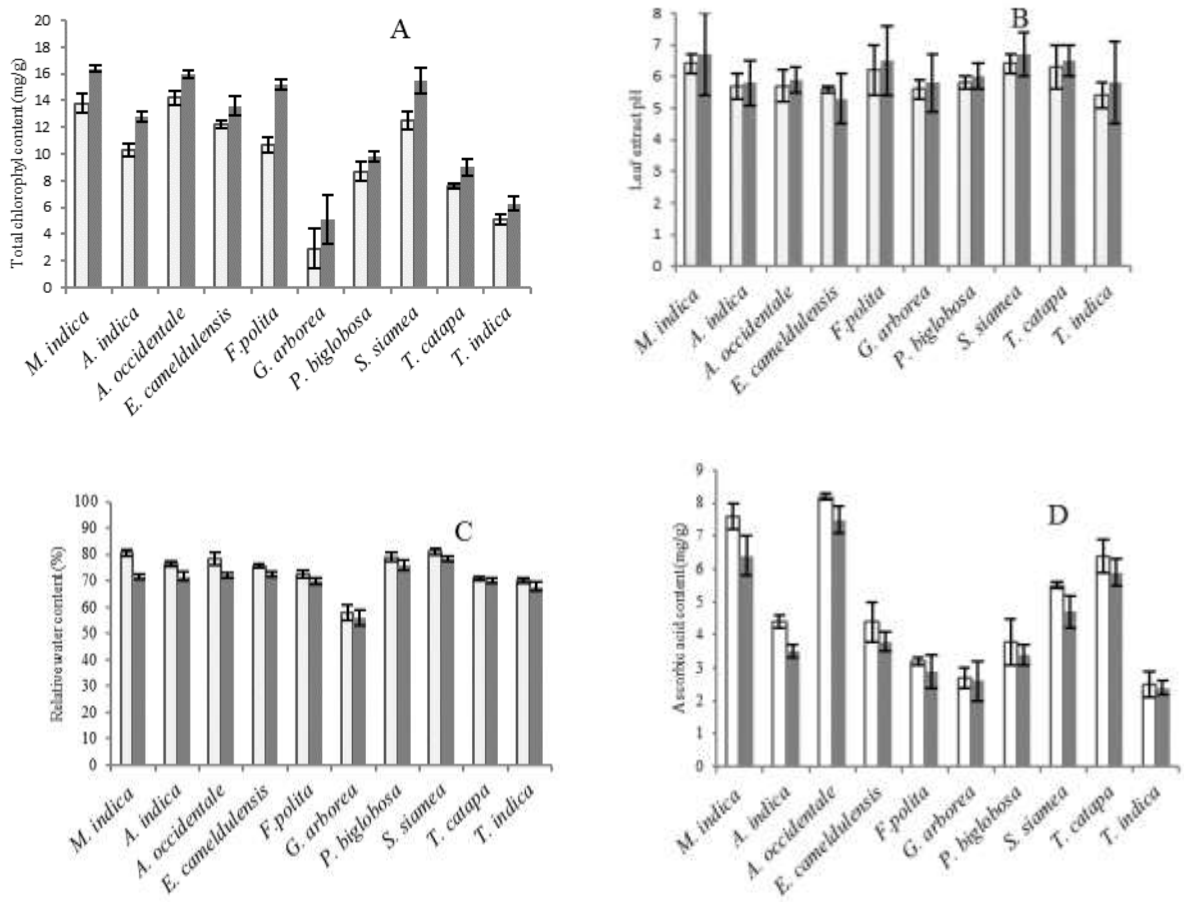

Figure 1 showing the mean values for total chlorophyll, leaf pH, RWC and ascorbic acid along the highway which were used in the computation of APTI of tree species. Parameters were determined as described in materials and methods. ○ = polluted sites ● = control site

The highest amount of ascorbic acid at the polluted site was recorded for *A. occidentale* (8.2mg/g) while *Tamarindus indica* recorded the lowest (2.4mg/g). The order in the concentration of ascorbic acid was found to be *A. occidentale* > *M. indica* > *T. catapa*>*S. siamea*> *E. cameldulensis*> *A. indica* > *P. biglobosa*> *F. polita*> *T. indica* >*G. arborea.* When compared to the control, a general increase in the concentration of ascorbic was observed with *M. indica* having the highest increase while *T. indica* recorded the lowest concentration increase. Total chlorophyll was highest in *A. occidentale* (14.1mg/g) while *Gmelina arborea* recorded the lowest at the polluted site (2.1mg/g). The control site had a much higher chlorophyll content for all of the trees with *M. indica* having the highest concentration (16.1 mg/g) while *G. arborea* had the lowest (4.2 mg/g). The highest decrease in chlorophyll content occurred in *F. polita* as the leaves of this loss about 4.2mg/g of its chlorophyll content at the polluted site while *P. biglobosa* recorded the lowest reduction in its chlorophyll of 1.2mg/g. The pH value was observed to range from 6.2 in *F. polita* which recorded the highest value to 5 in *Azadirachta indica* which recorded the lowest. None of the trees however had values above 6.3 for plants at the control site, however, *E. cameldulensis* recorded the lowest with a value of 5.4. Relative water content was highest in *S. siamea* (82%) while *G. arborea* recorded the lowest (57%) at the polluted site. The highest decrease in relative water content was observed in *M. indica* which had a reduction of about 9.8% at the control site as compared to the polluted site. *A. occidentale* had a reduction in water content of 7.1%. The rest of the tree species had less than 5% reductions in their relative water content value.

Chlorophyll is an index of productivity in plant and decreases under pollution stress (Gbem *et al.,* 2016). In the present study, a decline in total chlorophyll was observed for trees at the polluted site for all trees. This lower value recorded for the polluted site could be attributed to the presence of gaseous pollutants like SO_2 in greater concentration due to increased vehicular movement which enters through the stomata of the leaves causing a degradation of chlorophyll into phaeophytin by loss of magnesium ions. Heavy metals produced from vehicle exhausts could also may have caused the reduction in photosynthetic pigment as they interfere with pigment metabolism which could result to chloroplast number reduction, structural injuries, pigment synthesis reduction and their increased destruction. The result obtained from this study is similar to that obtained by Bhavika *et al* (2017). In her study, the concentration of concentration of cholorophyll was observed to be directly proportional to the distance of plants from the highway i.e. the further plants were away from the road side the higher the concentration of their chlorophyll contents. Mohammed *et al.,* (2015) also observed a reduction in chlorophyll at three different locations within Maiduguri.

Leaf extract pH plays a significant role in regulating gaseous pollutants sensitivity of plants. The polluted site recorded lower values when compared to control for all the trees. This reduction in pH at polluted site could be as a result of the presence of SO_2 or NO_2 which must have entered the stomata, got dissolved in cell sap to produce acid like sulphuric or nitric acid. Any plant species which demonstrate faster and greater acidification of the cell sap, are liable to be more affected since pH change will disrupt the entire metabolism of the plants. The result obtained is similar to that of Ogbonna *et al.,* (2015) who in his study around Ishiagu Lead-Zinc Mining Area, South Eastern Nigeria, observed pH values from the of 6.90 to as low as 3.80 for plants at the polluted site. Sabri *et al.,* (2019) also observed a decrease in pH of plants as the vehicular load increases.

Protoplasmic permeability of cells determines RWC. This could cause loss of water and dissolved nutrient, resulting in early senescence of leaves. Retaining more water in the leaves is advantageous to plants as it helps maintain physiological balance under stress condition of air pollution (Tripathi *et al.*, 2015). In this study, higher water content was observed for trees at the polluted sites when compared with same species at control site. Results for these agree with the findings of Agbaire and Esiefarienrhe (2009); Enete *et al.,* (2013), who found higher relative water content at the experimental site than the control site, thus indicating an adaptation by these plant species to withstand pollution.

All of the trees showed increase in ascorbic acid at the polluted site when compared with control. Ascorbic acid is an antioxidant, which protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis, and a range of pollutants (Gheorghe and Ion, 2017). Reactive oxygen species (ROS) are produced in plants when exposed to toxicants such as air pollutants and plants respond by building up ROS and reducing the amount of water that escapes through the leaves. The increased ascorbic acid reported maybe due to the defence mechanism of the respective plants. Similarly, a study conducted at a gas plant by Agbaire and Esiefarienrhe (2009) showed an increased level of ascorbic acid in the leaves of plants exposed to the gas plant emissions. Bamidele *et al.* (2016) observed a similar pattern of increase in ascorbic acid content in his study on dumpsites air pollution in medicinal plants. Medicinal plants growing around the dump sites showed an increase in ascorbic contents of about 20.39% as compared to those at the control site.

Table 1 shows results for stomatal studies of trees. Results reveal *E. cameldulensis, G. arborea, T. catapa* and *T. indica* to be amphistomatic while *M. indica, A.indica, A. occidentale, F. polita, P. biglobosa* and *S. siamea* were hypostomatic. Except for *G. arborea* and *T. catapa*, all the trees showed significant diffrence ($p \leq 0.05$) in the stomatal index for both control and polluted sites. Statistically significant difference was also observed by Alves *et al*.(2011) in *Tradescantia* which he attributed to probably pollution effects, suggesting that this anatomical parameter is a potential indicator of pollution. Analysis of results recorded for stomatal length do not reveal significant difference in the stomatal length of trees except for *G. arborea* and *T. catapa*. Similarly, both *G. arborea* and *T. catapa* showed significant differences in stomatal width except for the adaxial surface of *T. catapa.* Stomata is a pore found on the epidermis of leaves and generally aids the exchange of gases. In controlling the absorption of pollutants, plants may tend to modify the frequency and size of their stomata. The current study revealed an increase in stomatal index for most of the plants studied while a decrease in size was also found to occur. Reduction in the stomata under environmental stress is not uncommon as the decrease could be an avoidance mechanism against the inhibitory effect of pollutant on important physiological activities such as photosynthesis (Verma *et al.*, 2006). The result obtained from this study is similar to that of Shri and Haritha, (2019) who also observed structural changes in the stomata of plants exposed to air pollutants. A reduction of more than 42.5 % was observed by him for plants at the polluted site as compared to plants at the control location. The study conducted by Gostin (2009) also revealed a similar pattern of reduction in the size and density of stomata.

Table 1; Stomatal length, stomatal width and stomatal index of treees

		Stomatal length (µm)			Stomatal width (µm)			Stomatal index (%)		
		Polluted	Control	p- value	Polluted	Control	p- value	Polluted	Control	p- value
M. indica	Abaxial	13.8±3.82	16.2±1.21	0.06	12.7±3.41	15±4.01	0.12	18.2±2.77	25.1±1.84	0.0007
	Adaxial	-	-	-	-	-	-	-	-	-
A. indica	Abaxial	24.2±1.32	26.1±3.33	0.19	11.5±2.8	9.9±0.37	0.13	17.6±0.85	22.2±1.63	0.005
	Adaxial	-	-	-	-	-	-	-	-	-
A. occidentale	Abaxial	23.0±2.01	19.6±1.91	0.05	16.2±2.07	15±2.07	0.26	18.1±1.12	23±1.78	0.006
	Adaxial	-	-	-	-	-	-	-	-	-
E.cameldulensis	Abaxial	21.9±2.01	19.6±1.71	0.12	19.6±1.60	19.6±1.60	1	17.3±0.58	22.7±0.81	0.0003
	Adaxial	20.4±1.76	19.3±2.30	0.24	19.6±0.6	19.6±1.05	1	9.2±0.37	8.6±0.25	0.3
F. polita	Abaxial	19.6±1.91	18.5±1.54	0.25	12.7±0.26	11.5±1.23	0.26	18.6±1.22	24.8±0.56	0.0007
	Adaxial	-	-	-	-	-	-	-	-	-
G. arborea	Abaxial	19.6±2.19	23.3±2.19	0.05	16.2±2.08	10±2.12	0.0009	25.8±0.68	26.8±0.78	0.22
	Adaxial	19±0.97	22.8±3.92	0.04	17.3±0.56	9.74±3.64	0.0002	11.9±4.57	10.78±3.6	0.59
P. biglobosa	Abaxial	21.9±0.19	20.7±0.14	0.19	15±2.08	11.5±1.96	0.05	17.7±1.11	24.9±1.98	0.003
	Adaxial	-	-	-	-	-	-	-	-	-
S. siamea	Abaxial	26.4±1.96	24.2±0.62	0.06	19.6±3.20	17.4±2.05	0.06	17.9±0.85	16.4±0.45	0.02
	Adaxial	-	-	-	-	-	-	-	-	-
T. catapa	Abaxial	29.9±1.89	22.8±3.96	0.03	18.5±2.76	15±2.07	0.05	7.6±2.74	10.7±2.01	0.09
	Adaxial	26.8±2.89	22.8±5.62	0.02	18.7±4.21	15.3±3.44	0.09	4.2±5.63	6.18±3.87	0.07
T. indica	Abaxial	19.6±0.92	17.4±1.35	0.06	9.9±0.87	8.9±1.74	0.06	19.9±1.6	24.7±0.32	0.004
	Adaxial	17.14±5.3	17.4±5.91	0.8	9.4±1.9	8.9±0.9	0.6	7.3±3.6	6.8±2.5	0.7

.Table 2 gives the computed APTI for trees with *A. occidentale* having the highest of 24.2 which puts it in the intermediate range while *G. arborea* recorded the lowest with 8.1 which makes it sensitive based on the APTI classification. Along with *A. occidentale, M. indica, E. cameldulensis* and *S. siamea* which had values of 23.4, 15.4 and 18.5 were also classified as intermediate. Along with *G. arborea* Trees classified in the sensitive range includes *F. polita, P. biglobosa, A.indica, T. catapa* and *T. indica.*

Table 2; Calculated air pollution tolerance indices (APTI) for tree species along Zaria-Kaduna highway. Four parameters of total chlorophyll, relative water content, pH and ascorbic acid contents were used in the calculation as given in materials and methods section.

Tree species	APTI value	APTI classification
M. indica	23.4	Intermediate
A. indica	14.7	Sensitive
A. ccidentale	24.2	Intermediate
E. cameldulensis	15.4	Intermediate
F. polita	12.7	Sensitive
G. arborea	8.1	Sensitive
P. biglobosa	13.4	Sensitive
S. siamea	18.5	Intermediate
T. catapa	16	Sensitive
T. indica	9.3	Sensitive

APTI index is a combination of the four variables which include chlorophyll, ascorbic acid, leaf extract pH and RWC. It should be pointed out here that a single variable, even though robust may not give a complete insight into pollution tolerances of plants species. Table 2 shows the mean APTI values as calculated for plants at polluted sites. *M. indica* and *A. occidentale* have the best APTI indices which indicate that these plants would serve as sinks absorbing air pollutants, particulate matter and other emission if planted near or along highways thereby improving the air quality. The APTI value obtained for *M. indica* is much higher than that obtained by Sadia *et al*. (2019) in her study of the tree species growing around the greater Dhaka region, Bangladesh. She obtained a calculated APTI value of 10.52. Ogbonna *et al*., (2013) obtained a slightly higher computed APTI value for *A. occidentale* of 26.95 in his study around the vicinity of an automobile repair shop.

CONCLUSION

The presence of atmospheric pollutants generally affected the biochemical properties and stomata of plants selected for this study. This, however, was found to vary between plants with the most significant effect occurring in *F. polita, T. indica* and *M. indica* for total chlorophyll, leaf pH and ascorbic acid respectively. *M. indica* and *A. occidentale* had the best APTI indices indicating tolerance while *G. arborea* and *T. indica* had the lowest indicating sensitivity of these plants to air pollutants. This study has helped to understand the impact of automobile pollutants on selected trees located along the Zaria Kaduna highway and has helped bridge the gap on the information available on how the stomata of some selected trees are affected by roadside pollution. Further investigation may be needed to find out the distribution and how other tree species found along the highway are affected by gaseous pollutants.

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