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pH level, Ascorbic Acid, Proline and Soluble Sugar as Bio - indicators for Pollution

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ABSTRACT

The analysis of four biochemical parameters such as pH, ascorbic acid, proline and soluble sugar were carried out to investigate their response to pollution stress. Fifteen samples of plant leaves were collected in duplicate along the Ughelli – Patani metropolis in Ughelli North local government area of Delta State, Nigeria. The pH of the leave samples were measured after calibrating the pH meter; Ascorbic acid content (expressed in mg/g) was measured using spectrophotometric method; Proline content in the leaves of plants was determined following the protocol based on the formation of red colored formazone with ninhydrin in acidic medium which is soluble in organic solvent like toluene. Soluble sugar was determined by ion exchange chromatography. While the biochemical content of the leaf species under investigation shows a trend of a decrease of pH values; increase ascorbic acid and proline content and a reduced sugar content all from the polluted sites except for Musa paradisiaca L. and Manihot esculenta that shows alternate results and vice versa. An overview of the entire results obtained from this study reveals that different plants respond differently to air pollution with plant growing in polluted site having higher ascorbic acid content, proline values and lower pH and soluble sugar content as compared to the polluted sites and vice versa.

Keywords: Ascorbic acid, Proline, soluble sugar, pH, pollutant

INTRODUCTION

The quest for knowledge and the intuitive nature of man has undoubtedly been translated to the use of sophisticated machineries which in turn has led to the release of a more complex waste materials or substances into the environment. While these substances are toxic and pollutant to the environment, they tend to be discharged in the form of solid, liquid, or gaseous waste. The consequences of these categories of waste which could be referred to as pollutant cannot be under estimated.

General survey reveals that due to poor waste management facilities, inhabitant from the Niger Delta Region of Nigeria dispose sewage into streams, lake and in drainages which in turn, flows into rivers and other water bodies, thus resulting to water pollution. This process could cause environmental stress such as salinity, increase in the concentration of toxic metal ions, rise in water temperature among others. Also, these act, during the raining season, due to poor drainage facilities, could results to flooding and might be contaminating farmland thus leading to land pollution (Akpoghelie, *et. al.,* 2016). On the other hand, gaseous waste could be generated and if not properly handled and if by any means get into the environment could pollute the atmospheric environment.

Akpoghelie, et. al., (2016) describes air pollution as any process that leads to the change of the normal atmospheric condition by reason to alter or change the concentration of the gaseous mixtures of the atmosphere. It could also be described as the process leading to the abnormal rise, fall or the addition to the normal atmospheric constituent. Seyyed and Koochak (2013) reported that air pollution is one of the environmental stresses affecting the plant productivity (Woo, et. al., 2007). Literature reveals that pollutants could also have direct effect on plant species with the greatest effects on that part of a plant leaves that serves primarily as the plant's food - making organ in a process called photosynthesis, thus resulting to reduced productivity.

Liu and Ding (2008) observed the physiological change in the leaves of plant before physical changes appears when exposed to air pollution. These leaf injuries leads to stomata damage, thus leading to reduced photosynthetic activity and reduce growth and yield in sensitive plant species. Rai, *et. al.*, (2013) reported the visible effects of air pollution on human health and vegetation. Akpoghelie (2016) reported the detrimental effect of air pollution on nearly all phase of human lives to include health effect such as eye irritation, skin irritation, headache, sneezing, coughing, suffocation, chest pain, breathing problems among others, effects on vegetation, soil, water, manmade materials, climate and visibility (Seyyednejad, *et. al.*, 2011). When this happens, literature reveals that the human immune system led by the white blood cell automatically produced a defense against these diseases. Also, ascorbic acid (vitamin C) does not only help to prevent diseases, but also help to increase the body's resistance to infection. It also acts as an antioxidant - a nutrient that chemically binds and neutralizes the tissue - damaging effects of substances in the environment known as free radicals. Just like the human body, ascorbic acid (vitamin C) is an abundant component of plants. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth.

Nicholas and Glen, (2000) stated that vitamin C do not only plays important role in photosynthesis, but also acting in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of photosynthetic electron carriers and as a cofactor for violaxanthin de - epoxidase, an enzyme involved in xanthophyll cycle - mediated photoprotection. Just like the white blood cell, proline is a part of many protein and enzymes and has important role in plant source of energy and osmoprotectant in stress condition. It acts as free radical scavengers protecting the plant against damage due to oxidative stress. Soluble sugar in the other hand is a synthesized carbohydrate in plant. They are not only the main photosynthetic product in higher plant, but they are also the main form of carbohydrate metabolism and temporal storage. It is an important constituent and source of energy for living organism that plays important roles in carbohydrate metabolism. Soluble sugars are also very important bio-molecules involved in tolerance of water shortage during seed maturation and in storage as well (Obendorf, 1997). It has a close relationship with photosynthesis and production of starch. Ascorbic acid, proline and soluble sugar are no doubt important biochemical parameters that are not only needed by plant for growth, but the plants also need them to thrive when exposed to pollution stress.

Literature reveals that various methods such as the use of air pollution tolerance index (APTI) had been explored using the leaves of plant species to monitor air pollution in the environment [Mahecha, *et. al.,* (2013); Irerhievwie, *et. al.,* (2014)]. Hence, the ascorbic acid, proline and soluble sugar content of the leaves of fifteen plant species collected along Ughelli – Patani metropolis in Delta State, in the Niger Delta region of Nigeria observed as an experimental site by the researcher is examined and compared with the ascorbic acid, proline and the soluble sugar content of the leaves of plant species from composite sites deemed as less polluted or control site.

This study owes its significance to the facts that most cities in Delta State, Nigeria is ill planned and is characterized with poor drainage system leading to flood during heavy down pour, thus resulting to traffic. Also, improper disposal of sewage into farmland and drainage, the links of residential septic tank to canals and drainages and the combustion of bones, rubber wheel cover, bush burning among other. Hence, the aims and objectives of this study is to examine and compare the concentration of ascorbic acid, proline and soluble sugar from the leaves of fifteen plant species collected within two semi – urban cities in Delta State term as experimental and control sites, and also to attempt to establish if pollution gradient exist between all the experimental and control sites.

MATERIALS AND METHODS

Sampling

Fifteen duplicates of fully matured leaves samples of some plants species such as *Magnifera indica,*
Bambosa Bambosa, Elais guinensis, Anacadium occidentale, Psidium guajava, Citrius sinesis, Vernonia amygdalina, Musa paradisiacal L., Carica papaya L., Colocasia esculanta L., Delonix regia, Ocimum grattissimum L., Talinum triangulare J., Saccharum officinarum L., and *Manihot esculenta* were cultivated in Ughelli – Patani metropolis in Ughelli North local government area and Osubi metropolis in Okpe local government area of Delta State were randomly collected. The former is designated as an experimental site while the latter is designated as a control sites.

The plants selected for the study were those available at the experimental site. Identification of the plant species were done at the Department of plant science, Delta State University, Abraka. Replicates of fully mature leaves samples of the various plants collected were put in polyethene bags and marked with masking tape and well labeled. These were immediately taken to the laboratory for analysis.

Analysis of Samples

Biochemical parameters such as pH, ascorbic acid, proline and soluble sugar were chemically determined from the leaf extracts is as follows:

Leaf Extract pH

5g of the fresh leaves was ground to paste and then extracted using 50 cm^3 of deionize water. The leaf extract pH was then measured using a calibrated digital pH meter.

Ascorbic Acid (AA) Content Analysis

Ascorbic acid content (expressed in mg/g) was measured using the spectrophotometric method. 1g of the fresh leave extract was put in a test - tube, followed by the addition of 4ml oxalic acid - EDTA extracting solution was added; and then 1ml of orthophosphoric acid, 1ml 5%

tetraoxosulphate (vi) acid was added to this mixture. 2 ml of ammonium molybdate was added and then 3ml 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 samples was then extrapolated from a standard ascorbic acid curve.

Estimation of Proline

Proline content in the leaves of plants was determined following the method described below. The protocol is based on the formation of red colored formazone by proline with ninhydrin in acidic medium which is soluble in organic solvent like toluene. 0.5g plant leaves were homogenized in 5ml of 3% sulphosalicylic acid using pre washed mortar and pestle. The homogenate were filtered through whatman filter paper number 2.

The filtrates collected were used for the estimation of proline content. 2 ml of extract were decanted into the test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added one after the other. The reaction mixtures were subjected to heating in a boiling water bath at 100°C until the brick red colour was developed. The reaction mixtures were cooled and 4 ml of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromospheres containing toluene was separated and its absorbance need at 760 nm in spectrophotometer against toluene blank. Standard curve of proline was prepared by taking 5g to 100 gml^{-1} concentrations. The proline content in samples was estimated by referring to a standard curve made from known concentration of proline.

Estimation of Soluble Sugar

Soluble sugar was estimated according to the protocol outline by bio-protocol (2014). The leaves of plant species were grind with mortar and pestle in the presence of liquid N_2 until the sample is converted to a fine powder. 100 - 300 mg leaf powder was emptied into a microfuge tube and extraction buffer 1 solution was added.

The samples were heated at 80°C for 2 hour to extract soluble sugars. The resulting solution was allowed to cool and centrifuge at 13,000 rpm in a microfuge for 10 min. The supernatant was removed into a fresh microfuge tube and then evaporated in vacuum at 45°C for 2 hour. The dried extract was re-suspended in Milli Q grade water at a ratio of water/original powdered tissue of 0.5:1 (v/w).

Clean soluble plant extracts were obtained by filtering through nylon filters. Suitable extract volumes (typically 50 µl) were made up to 200 µl with Milli Q grade water and filtered through Whatman Mini – Uniprep ™ nylon filters (0.2 µm pore size). 250 µl 21.6 mM NaOH were added to 50 µl filtered extracts and apply into a Dionex HPLC system. Injection volume of 10 µl set up. Sugars are separated by isocratic elution at 20°C with 18 mM NaOH as mobile phase at a flow of 1 ml/min [18% (v/v) 0.1 M NaOH and 82% (v/v) Milli Q grade water] through a CarboPac PA10 column and pre - column CarboPacPA10. Detection is carried out by amperometric detection with a gold electrode in nC units.

Percentage (%) of pollution gradient

The percentage of pollution gradient for biochemical parameters is as follows:

$$(\%)\ \text{Gradient} = \frac{ES - CS}{CS} \text{ X } \frac{100}{1}$$

Alternatively

$$(\%)\ \text{Gradient} = \frac{CS - ES}{ES} \text{ X } \frac{100}{1}$$

Where ES = Mean Experimental site Concentration; **CS** = Mean Control Site Concentration

RESULTS AND DISCUSSIONS

The result of the analysis of biochemical parameters such as pH, ascorbic acid, proline and soluble sugar in mg/g is as presented in Tables 1 - 2 and Figures 1 – 4. The results reveals that there is a significant relationship between the four biochemical parameters investigated in this study. This is an indication that the pressure resulting from the contamination of the earth's environment with substances that interferes with the human health, the quality of life, or the natural functioning of ecosystems - living organisms and their physical surroundings are among the factors most limiting plant productivity and survivorship. In this study, it is evidence that pH is not just the principal determinant for the response of plant leaves to pollution stress, but it is also a major determinant for the plant leaves to respond to the availability of water.

While several studies had reported the increase of leaf extract pH in the case of drought, Zamblé, *et. al*., (2015) reported that in the presence of an acidic pollutant, the concentration of the leaf extract pH is lowered and the decline is greater in sensitive than that in tolerant plant. High pH may increase the efficiency of conversion from hexose sugar to ascorbic acid, a natural antioxidant. The concentration of leaf extract pH in this study was lower in the experimental site as compared to the control sites except for *Musa paradisiacal L.* and *Manihot esculenta* which has higher concentration in experimental site as compared to the control sites. pH is a measure of the hydrogen ion concentration of a solution. Solutions with a high concentration of hydrogen ion (H^+) have a low pH and solution with low concentration of hydrogen ion (H^+) have a high pH. A shift in the pH of cell sap towards the acid side in presence of an acidic pollutant might decrease the efficiency of conversion of hexose sugar to ascorbic acid. However the reducing activity of ascorbic gives tolerance to plants against pollution. In all the species under investigation, the variation of the leaf extracts pH was not much, but a gradient was established with the ascorbic acid content. The concentration of leaf extract pH ranges from 4.60 to 6.45 in the experimental site and 4.80 to 6.90 for the less polluted site with *Delonix regia,* having the highest and lowest concentration of 6.90 and 6.45 respectively. See data from Table 1 and Figure 1.

Table 1: Mean concentration of pH, ascorbic acid, proline and soluble sugar content of the leaves of plant species collected from both experimental and control sites of Delta State, Nigeria.

Common Name	Botanical Name	Site	pH	Ascorbic Acid (mg/g)	Proline (mg/g)	Soluble Sugar (mg/g)
Mango	*Magnifera indica*	ES	5.42	1.276	1.604	2.008
		CS	5.60	1.184	1.408	2.430
Bamboo	*Bambosa bambosa*	ES	5.65	1.288	1.890	1.990
		CS	5.80	1.211	1.680	2.010
Oil Palm	*Elais guinensis*	*ES*	5.55	1.321	1.725	1.909
		CS	5.60	1.009	1.650	2.100
Cashew	*Anacadium*	ES	4.60	1.108	1.670	2.001
	accidentale	CS	4.80	1.002	1.565	2.210
Guava	*Psidium guajava*	ES	5.30	1.235	1.770	1.998
		CS	5.45	1.190	1.565	2.105
Orange	*Citrius sinesis*	ES	5.85	1.302	1.880	2.105
		CS	6.10	1.225	1.675	2.308
Bitter Leaf	*Vernonia*	ES	5.70	1.200	1.435	1.995
	amygdalina	CS	5.75	1.114	1.289	2.111
Plantain	*Musa patadisiacal L.*	ES	5.50	1.190	1.340	1.992
Leaf		CS	5.30	1.205	1.508	1.900
Paw Paw	*Carica papaya L.*	*ES*	5.55	1.300	1.600	1.992
		CS	5.95	1.198	1.440	2.101
Cocoyam	*Colocasia esculanta*	ES	5.45	1.190	1.679	1.991
	L.	CS	5.50	1.080	1.386	2.000
Flamboyant	*Delonix regia*	ES	6.45	0.990	2.009	1.650
		CS	6.90	0.890	1.788	2.190
Scent Leaf	*Ocimum*	ES	5.85	1.220	1.909	1.890
	grattissimum L.	CS	6.10	1.100	1.776	2.190
Water Leaf	*Talinum triangulare*	ES	5.60	1.390	1.887	1.799
	J.	CS	5.75	1.109	1.344	2.400
Sugar Cane	*Saccharum*	ES	5.60	1.450	1.656	1.909
	officinarum L.	CS	5.72	1.348	1.229	2.003
Cassava	*Manihot eculenta*	ES	6.15	1.120	1.690	1.890
		CS	5.90	1.329	1.880	1.800

ES = Mean Experimental site Concentration; **CS** = Mean Control Site Concentration

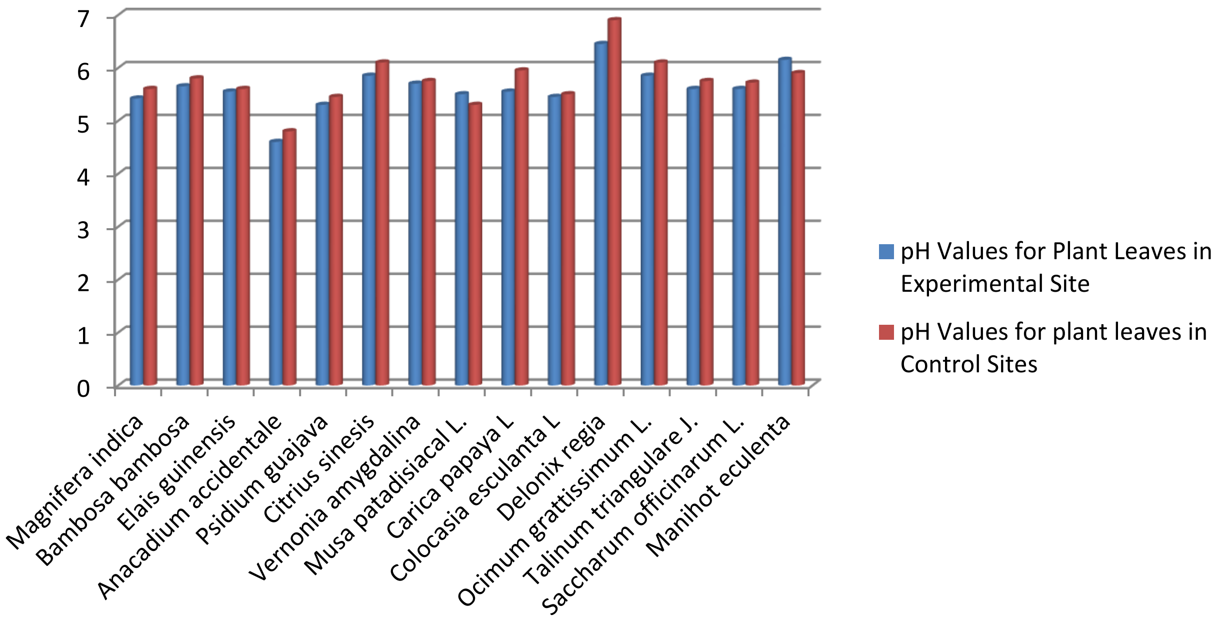

Figure 1: Chart Showing pH Value for Plant Leaves in Experimental and Control Sites

Data from Table 1 and Figure 2 reveals that the ascorbic acid content increases at a lower pH. Ascorbic acid is a metabolite that is regarded as a natural detoxicant that exist in sufficient quantity and may prevent the damaging effect of air pollutants in plant tissues and high amount of this substance favors pollution tolerance in plants. Level of this acid declines on pollutant exposure. Ascorbic acid content accumulates in plant leaves when subjected to environmental stress such as air pollution – they tend to accumulate in polluted site than in less polluted site (Table 1, Figure 2).

The increase in the level of ascorbic acid reported in this study may be due to the defense mechanism of the respective plant and also to the growth of plants (University of Exeter, 2007). Data's from table 1 and figure 2 further reveals the increase of ascorbic acid in the experimental site regarded as the more polluted site as compared to the less polluted sites. The ascorbic acid content for all leaves of the plant species under investigation range from 0.89 mg/g to 1.329 mg/g for the control site and 0.99 mg/g to 1.390 mg/g in the experimental sites.

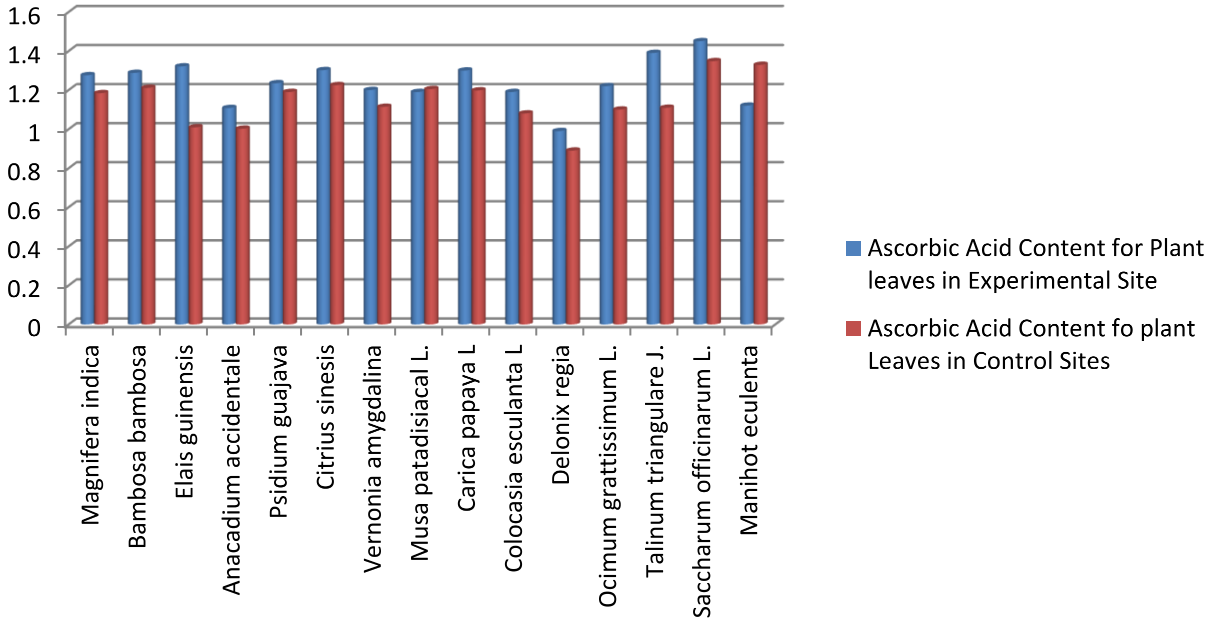

Figure 2: Chart Showing Ascorbic Acid Content for Plant Leaves in Control and Experimental Site

Proline is amino acid. They are biomolecule that is thought to play a cardinal role as an osmoregulatory solute in plant subjected to hyperosmotic stress primarily drought and salinity. Shamsul *et. al.*,(2012) reported that the accumulation of large quantity of proline is an adaptive response to various biotic and abiotic stresses. Higher concentration of proline is an indication that the plants are exposed to higher stress. In response of the plant to stress, plant accumulate large quantity of different type of compatible solutes. Compatible solutes are low molecular weight, highly soluble organic compound that are usually not toxic at high molecular concentration. These solutes provide protection to plant from stress by contributing to cellular osmotic adjustment.

Ashton and Verma, (1993) stated that the hyperosmotic stress caused by drought and salinity are the most important factor limiting plant growth and productivity. Data's from Table 1 and Figure 3 reveals higher accumulation of proline by the leaves of the plant species under investigation except for *Musa patadisiacal L.* and *Manihot eculenta*. The increase proline accumulation in the leaves of plant species in this study may be due to the decrease in proline degradation thereby increasing proline synthesis and the hydrolysis of proline. Data's from Table 1 and Figure 1, 2 and 3 shows that when the plant species exposed to environmental stress, the pH of the cell sap of the leaves of the plant decreases, resulting to the accumulation of ascorbic acid : a strong antioxidant, and the accumulation of proline.

Several pollution studies reveals that the deleterious effect of pollutant are caused by the production of reactive oxygen species in plant which cause peroxidative destruction of cellular constituent. Nathalie and Christian, (2008) reported the confirmation of the benefit of proline accumulation during stress by the transgenic approach, but they however reported that a consensus was not achieved on the exact role of proline accumulation. This was because classical gain or loss function strategies could not bring clearer answer probably because proline accumulations also display the essential of protein component. Several factors could however alter the results of this finding. For instance, the determination being tolerance or sensitive for one tested plant species could be changed during different seasons (Liu and Ding, 2008). *Delonix regia* show the highest concentration of proline with 2.009 mg/g while *Saccharum officinarum L.* show the lowest proline concentration with the value of 1.229 mg/g.

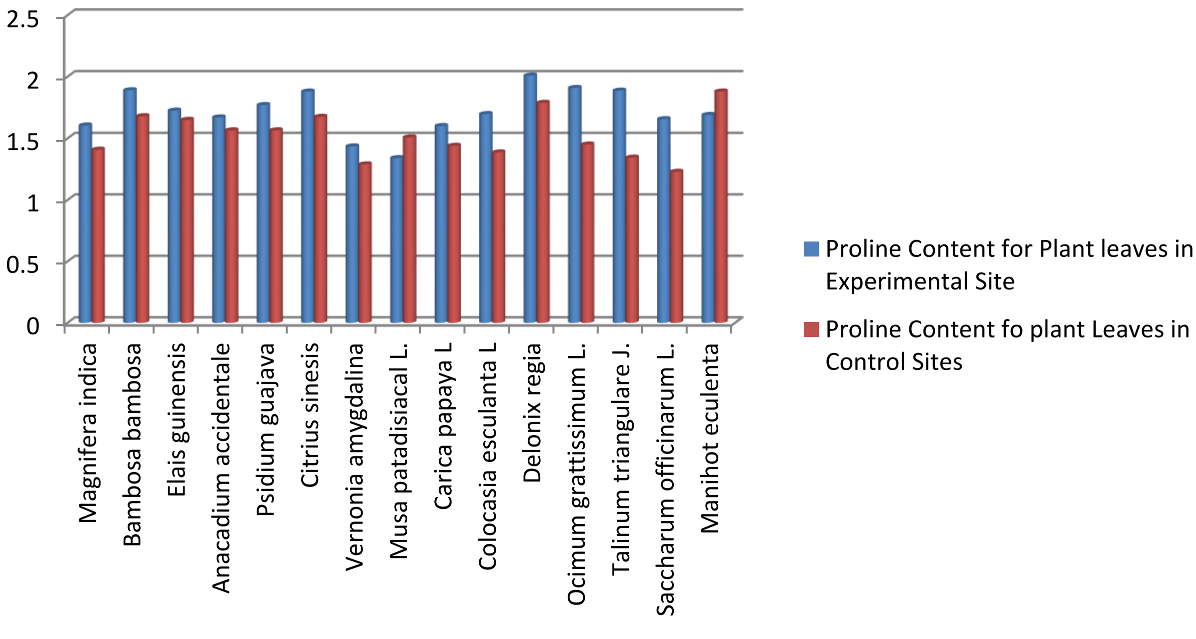

Figure 3: Chart of Showing Proline Content for Plant Leaves in Experimental and Control Sites

Soluble sugar is a synthesized carbohydrate in plant. They are not only the main photosynthetic product in higher plant, but they are also the main form of carbohydrate metabolism and temporal storage. Plants manufacture this organic substance during photosynthesis and break them down during respiration. Soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis. The level of soluble sugar content was a sign of the supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha et al., 2001). In drought stress, high soluble sugar content of them plays osmotic protection role to adapt plant to new condition. Obendorf, (1997) reported that soluble sugar are unique bio-molecules compound also involved in tolerance of drought stress during seed maturation and in storage as well.

Data from Table 1 and Figure 4 reveals that the concentration of the sugar content was higher with those from the less polluted sites as compared to those from the polluted sites. While the biochemical content of the leaf species under investigation shows a trend of a decrease of pH values; increase ascorbic acid and proline content and a reduced sugar content from all plant samples from the polluted sites and vice versa, *Magnifera indica* has the highest value of 2.43mg/g recorded for the less polluted sites *and Ocimum grattissimum L.* has the least value with 1.799mg/g. In this study, the concentration of soluble sugar in the leaves of plant species is an indicative of the physiological activities of the various plant species and its activities determines the sensitivity of the plant to air pollution. The reduction in starch content in polluted stations in this study could also be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration. Hence, it has been mentioned that pollutants like SO_2, NO_2 and H_2S under hardening conditions can cause more depletion of soluble sugars in the leaves of plants grown in polluted area. The reaction of sulphite with aldehydes and ketones of carbohydrates can also cause reduction in carbohydrate content (Tripathi and Gautam, 2007).

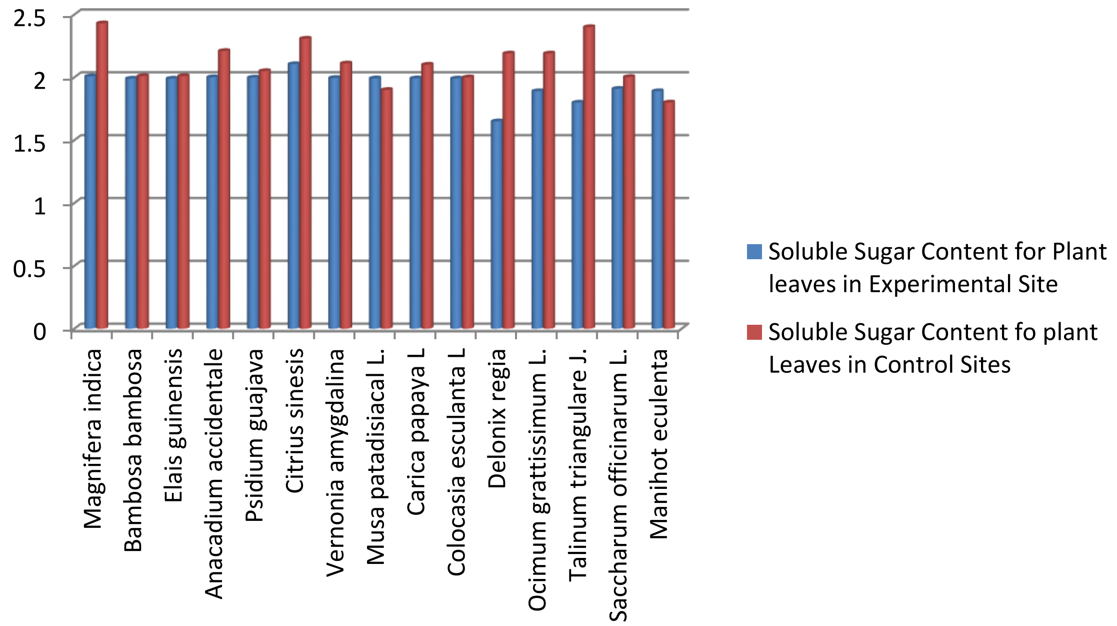

Figure 4: Chart showing Soluble Sugar Content for plant leaves in Experimental and Control Sites

The different environmental gradients that existed in the sample sites in this study explains why all the biochemical parameters for the leaves of plant species investigated showed positive significant percentage increase as seen in table 2. Data from table 2 reveals that the pollution gradient for ascorbic acid for the samples of the leaf extracts range from 1.26 to 30.92. It could be observed that at lower pollution gradient for ascorbic acid, the higher the pollution gradient for proline. Also, the higher the pollution gradient for ascorbic acid, the lower the pollution gradient for soluble sugar. The higher the pollution gradient for proline, the lower the pollution gradient for soluble sugar. Previous study reveals that using individual biochemical parameters to computes values that explain the effects of pollution load on our vegetation could be unreliable and could give alternative results (Irerhievwie *et. al.*, (2014). This explains the sharp deviation of pollution gradient between the individual biochemical parameters investigated in this study. Data's further reveals that the pH gradient of the leaves of plant extracts range from 0.88 to 7.21. *Vernonia amygdalina* had the lowest pH gradient of 0.88, while *Carica papaya L* had the highest pH gradient of 7.21. The pH gradient of the leaf extract appears in the order 7<3<10<14<2<13<5<1<8<15<6, 12<4<11<9.

Table 2: Percentage Response of Biochemical Parameters to Pollution Stress

Common Name	Botanical Names	pH	Ascorbic Acid	Proline	Soluble Sugar
Mango	*Magnifera indica*	3.32	7.77	13.92	21.02
Bamboo	*Bambosa bambosa*	2.65	6.36	12.50	1.01
Oil Palm	*Elais guinensis*	0.90	30.92	7.50	1.01
Cashew	*Anacadium accidentale*	4.35	10.58	1.21	10.44
Guava	*Psidium guajava*	2.83	3.78	13.10	2.60
Orange	*Citrius sinesis*	4.27	6.29	12.24	9.64
Bitter Leaf	*Vernonia amygdalina*	0.88	7.72	11.33	5.81
Plantain Leaf	*Musa patadisiacal L.*	3.77	1.26	12.54	4.84
Paw Paw	*Carica papaya L.*	7.21	8.51	11.11	5.52
Cocoyam	*Colocasia esculanta L.*	0.92	10.19	21.14	11.82
Flamboyant	*Delonix regia*	6.98	11.24	12.36	5.82
Scent Leaf	*Ocimum grattissimum L.*	4.27	10.91	7.49	21.67
Water Leaf	*Talinum triangulare J.*	2.68	25.34	40.40	19.52
Sugar Cane	*Saccharum officinarum L.*	2.14	7.57	34.74	4.92
Cassava	*Manihot eculenta*	4.24	18.66	11.24	5.00

CONCLUSION

This study has been able to establish a correlation between environmental stress and plant productivity as revealed in this study. This was achieved when plants grown in polluted environment were compared with those grown from a less polluted environment. Just like how the human body will react to disease and other materials that invades the body cells, the cell sap of the plant leaves becomes more acidic when exposed to environmental stress such as environmental pollution, which thus triggers the production of increased antioxidant such as ascorbic acid and an osmoprotectant metabolite, such as proline the study notes, thus reducing crop productivity as shown by reduced soluble sugar content. Though, *Musa paradisiaca L.* and *Manihot esculenta* produced an alternate result, the reason could be attributed to the degree of anthropogenic or industrial activities that took place, coupled with the season the samples were collected. Data's from the control site was observed to be significantly high because pollution from the plant source is spreading gradually to the control sites. The researcher desire to revisit this study with biochemical analysis on the leaves of plant species subjected to low concentration prolong exposure; high concentration prolong exposure and high concentration with limited exposure to air pollution with the analysis done in both season of the year. An overview of the entire results obtained from this study reveals that different plants respond differently to air pollution with plant growing in polluted site having higher ascorbic acid content, proline values and lower pH and soluble sugar content as compared to the polluted sites and vice versa. Hence, there is need for individuals, government, co - operate bodies and other stake holders to put all hands on deck to reduce the pollution load in the environment in order to improve crop productivity especially in this period of economic holocaust ravaging the world.

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