

# Alterations in the Air Pollution Tolerance Indices and Foliar Micro-Structures of Five Medicinal Plants: Implications of Oil-Spill Pollution of Agricultural Farmland in Edugberi Community, Rivers State, Nigeria

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## Research Article

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## Abstract

The Nigerian costal environment has witnessed the continuous input of petroleum hydrocarbons and associated pollutants as a result of different phases of petroleum activities. These activities have elicited a devastating impact on terrestrial and aquatic biota, which constitute the people's major source of livelihood. Air pollution tolerance indices (APT<sub>I</sub>) and foliar photomicrograph were used in evaluating impact of oil spill on five medicinal plants (*Costus afer*, *Centrosoma pubescens*, *Chromolaena odoranta*, *Psidium guajava* and *Musa sapientum*) within an oil spill area in Edugberi community of Rivers State, Nigeria. The APT<sub>I</sub> of each plant was computed from four biochemical parameters namely: pH of the leaf extract, relative water content, ascorbic acid content and total chlorophyll content using standard methods. The results obtained showed that the mean values of APT<sub>I</sub> ranged from 7.98(0.78) in *C. afer* to 9.29(0.89) in *M. sapientum*

for test site, while it ranged between 7.44(0.23) in *P. guajava* and 10.69(0.22) in *M. sapientum* for control site. The foliar photomicrographs analysis revealed alterations in foliar micro-structures in leaf samples from test site as compared to control samples. The study showed that oil spillage had marked negative impact on biochemical, physiological as well as anatomical configuration of selected medicinal plants which may affect their phyto-constituents and pharmacological profiles.

**Keywords:** Oil-Spill; Air Pollution Tolerance Index; Foliar Photomicrograph; Environmental Pollution; Phytopotency; Medicinal Plants

## Introduction

Environmental pollution has been recognized as a major global problem with serious consequences for the sustainability of the ecosystems as well as the quality of life and economic performance of human communities. In Nigeria, the problem has been forced most drastically on the public consciousness by the massive impact of industrial pollution in our industrial centers and by the many “accident” and devastation brought by the activities associated with the various phases of the petrochemical industry from exploration and production to processing and transportation [1]. The Coastal area of the Niger Delta is well known for such activities. Against the backdrop of illegal oil bunkering by militants, oil spills occur quite frequently and are a major environmental challenge. The farmland in the Edugberi community of Rivers State has recently witnessed continuous input of petroleum hydrocarbons originating from oil spill with negative implication on the quality of terrestrial and aquatic biota.

The sensitivity and response of plants to air pollutant varies among species [2,3]. The climatic condition, the physiochemical properties of air pollutants and their residence time in the atmosphere have impact on surrounding plants. The plant species which are more sensitive act as bio-indicator of air pollutants whereas, tolerant species may be indicated in phytoremediation of air polluted environment. Plants act as air pollutant sink but better performance comes from the pollution tolerant species. Plantation of tolerant species may have a marked effect on varied aspect of the quality of the urban environment and the cleanliness of life in a city. Plant species tolerant to air pollution may accumulate the harmful substance that may affect human health, if such plants are used for medical or food purposes [4].

The quality of medicinal plants for use as phytotherapeutics is often affected by alteration of their

biochemical status. This alteration may be in the form of oxidative stress or changes in plant natural resilient properties against environmental pollution. World Health Organization [5] recommends that medicinal plants which form the raw materials for the finished product may be checked for the presence of pollutants. This is because after collection and transformation into final dosage form, the pollutants resident in these plants may find their way into human body with eventual physiological changes.

Air pollution tolerance index (APTI) and foliar photomicrograph are important tools used in the assessment of plant biochemical and physiological status under stressful conditions such as pollution and draught. Ordinarily, tolerance of plant to air pollution can be measured by simple symptoms such as visible injury on the plant but it can be correctly evaluated by calculating the tolerance index of plant to air pollutants and by establishing foliar microscopic changes. Hence, computation of APTI and foliar photomicrograph analysis are useful components of vegetation studies in the environmental impact assessment, auditing and evaluation of any anthropogenic activity [1]. The usefulness of evaluating APTI for the determination of tolerance as well as sensitivity of plant species in different sites were followed by several authors [6-8]. However, study within oil spill area with respect to medicinal plants has not been carried out and that necessitates this research work.

This study investigated the effects of oil spill on some medicinal plants (*Cosotus afer*, *Centrosema pubescens*, *Chromoleana odoranta*, *Psidium guajava*, and *Musa sapientum*) from oil spilled farmland in Edugberi community of Rivers State, Nigeria. The biochemical parameters (ascorbic acid content, pH, total chlorophyll content and relative water content) were used to compute the air pollution tolerance indices (APTI) of the plants,

while foliar photomicrograph was used as index of oil spill-induced micro-structural changes in the leaves.

The plants studied have ethnomedicinal importance in the community as with other tropical communities. *C. afer* is locally used in the management of a cocktail of diseases because of the reported high contents of anti-oxidants. The therapeutic potential of antioxidants (flavonoids, saponins and phenols) in controlling degenerative disease with marked oxidative damage from reactive oxygen species (ROS) or free radicals have been reported [9]. The methanol extract of the rhizome showed significant topical anti-inflammatory activity in croton aldehyde-induced mouse ear oedema [10]. In the work by Bruchhaus, et al. [11], the methanolic leaf extract showed significant cytotoxicity in the brime shrimp test. The same extract showed moderate local anesthetic activity in guinea pig skin test, and contracted the guinea pig ileum in a concentrated dependent manner. The extract exhibited antihypoglycaemic activity, and decreased the blood glucose level by 50% in streptozotocin (STZ)-induced hyperglycaemia in male rats, high doses, however, increased blood glucose level.

The fruit of *M. sapientum* is traditionally used in diarrhea (uripe), dysentery, intestinal lesion in ulcerative colitis, diabetes (uripe) in sprue, uremia, nephritis, gout, hypertension, cardiac disease. *M. sapientum* is also used in the treatment of excess menstruation with *Cana indica*. Banana leaves (ashes) are used in eczema, as cool dressings for blister and burns. Flowers are used for treating diarrhea, dysentery, and menorrhagia. The root is used as antihelminthic, blood disorders, and venereal diseases [12].

Traditionally, *P. guajava* is used to treat the various diseases conditions. The roots of the plant have been reported for their astringent property at a dose of 30-40 grams for 1 liter of 4-5 cups a day. The decoction form of the bark is used in treatment of ulcer. The leaves of the plant have been implicated in management of diarrhea, wounds, ulcer, toothache and stomach-ache and in the diabetes. The decoction of the leaves is used as gargles or sore throats, swelling of the mouth, laryngitis, external ulcer on the skin and vaginal irritations. *P. guajava* leaves poses the anti-inflammatory property and is also used in various lung problems. In addition to this, leaves are used in various bacterial infection, diarrhea, blood cleansing. Various evidence indicates that the ripe fruit of the guava has laxative action, whereas the unripe fruit decoction is used as astringent, antidiarrhoeic [13,14].

*C. odoranta* is believed to possess healing potentials for wounds and treatment of pile, sore throat ailment. A decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria. Other traditional medicinal uses include antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic. The fresh leaves extract of *C. odoranta* are traditional herbal treatment in some developing countries for burns, soft tissue wounds and skin infection. The use of the leaf extract of *C. odoranta* for sore throat and treatment of pile, burns and wound have been documented [15].

Much use has been made of *C. pubescence* for the treatment of burns among the Ibibio tribe of South-South political Zone of Nigeria. However, there is no literature report to confirm its use in ethno medicine [16].

## Materials and Methods

### Study Area

Edugberi community in Ahoada West Local Government Area of Rivers State, South-South, Nigeria was selected as study area. The area lies in the tropical rain forest with mean annual rainfall of about 2500 mm, mean annual relative humidity is 65% and mean annual temperature is 26 °C. Its geographical coordinates are 5° 51' 00" N and 6° 39' 00" E.

### Sample Collection

Fresh leaf samples were randomly collected from the test site (TS) and control site (CS). The samples were taken in quadruplet (An, Bn, Cn, Dn, En,) 100m along a transect, using a sharp kitchen knife to destalk mature leaves from the branches in morning hours. The fresh leaf samples were immediately taken to the Department of Plant Science and Biotechnology University of Nigeria, Nsukka in a perforated brown envelop for taxonomical identification and authentication. The fresh leaf weight (FM) was taken immediately upon arriving at the laboratory. Some samples were preserved in a refrigerator for other analyses while others were dried for further analysis. This protocol was observed for both samples from test and control sites.

### Analysis of Biochemical Parameters

**Relative Water Content (RWC):** The method described by Singh [17] was applied to determine and calculate relative leaf water content as follows: fresh leave sample was weighed and recorded as FM-fresh mass. It was

floated in distilled water inside a closed petri-dish, at a room temperature for 24 hrs.

At the end of the incubation period, leaf sample was wiped dry to obtain the turgid mass (TM). It was placed in a pre-heated oven at 80°C for 48 hrs. Thereafter the leaf was weighed to obtain the dry mass (DM). The relative water content was calculated using the formula:

$RWC = \frac{FW - DW}{TW - DW} \times \frac{100}{1}$ , where FW = fresh weight, DW = dry weight, TW = turgid weight.

**Leaf Extract pH:** The pH was determined by the Direct Reading Engineering Method (DREM), using digital pH meter. The leaf extract was made cold by maceration of the leaf with de-ionized water, filter through an ashless filter paper, and the filtrate was used for pH determination. The pH meter was pre-calibrated prior to its usage, using buffer solution of pH 4 and 9. The pH electrode was carefully dipped into the filtrate in a 10 ml beaker. The value on the crystal liquid display panel (CLD) of the pH meter, was taken as true pH value. The exercise was triplicated, and the average of the three readings was used.

**Ascorbic Acid:** Ascorbic acid was determined by the trimetric method after Association of Official Analytical Chemistry (AOAC) 2005. The ascorbic acid was extracted with 20 ml of distilled water, in 30 ml H<sub>2</sub>SO<sub>4</sub> and 0.5 ml, oxalic acid. 2 g of the filtrate was titrated against 0.05 mol iodine solution, using starch mucilage as indicator to end point. The ascorbic acid value was calculated from the relation:

1 ml 0.05 iodine = 0.008806 g = 8.806 mg

**Total chlorophyll content:** This was determined by the spectrophotometric method. 3 g of the leaf sample was blended then extracted with 10 ml of 80% acetone, left for 15 min, and the liquid portion was decanted, centrifuged

at 2,500 rpm for 3 min. The absorbance was measured at 645 nm and 663 nm, using spectrophotometer.

**Calculation of APTI:** APTI for the selected plants was determined by method described by Sigh and Rao [18]. The formula is given as:  $APTI = \frac{[A(T+P)+R]}{10}$ , where, A= ascorbic acid content, T= Total chlorophyll content, P = pH of leaf extract, R = relative water content.

**Analysis of Foliar Photomicrograph:** The leaf samples were cleaned by washing with water. Using a camel hair brush, nail varnish was applied on 22x22 cm portion of both the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaf and left to dry for 10 mins. Subsequent coatings were applied for the second and third times and left to dry for 10 mins and 20 mins respectively. The samples were then passed through air current for 1 hr to ensure maximum dryness. Epidermal strips of the leaf samples were scraped gently with the aid of forceps and placed on a clean slide. They were stained with safranin, washed with alcohol three times and covered with a cover slip before mounting for microscope at x40 magnification and photomicrographs were taken with Zeiss light microscope with MC' 35 camera for 53 mm film at x400 magnification.

**Statistical analysis:** The data was subjected to analysis using SPSS. The values were represented as mean (SEM). Paired t-test was performed from each sample with measure from baseline value at 5% and a p value of < 0.05 was taken as indicating a statistically significant difference.

## Results

The data obtained from statistical analysis of pH of leaf extract and ascorbic acid content (AA) of selected plants species from test and control sites are presented in Table 1 below.

Plant Species	pH			Ascorbic Acid(mg/g)		
	Test	Control	P value	Test	Control	P value
	Mean(SEM)	Mean(SEM)		Mean(SEM)	Mean(SEM)	
<i>Costus afer</i>	3.80(0.27)	3.85 (0.03)	0.8588	0.88(0.003)	0.77(0.01)	< 0.0001*
<i>Centrosoma pubescens</i>	3.53(0.48)	3.58(0.03)	0.3903	1.32(0.003)	1.26(0.0)	0.0053*
<i>Chromolaena odoranta</i>	5.35(0.05)	5.55(0.03)	0.0134*	0.88(0.003)	0.79(0.02)	0.0038*
<i>Psidium guajava</i>	5.33(0.09)	5.15(0.09)	0.1002	1.32.(0.003)	1.27(0.03)	0.1202
<i>Musa sapientum</i>	5.35(0.07)	5.63(0.03)	0.0073*	0.88(0.003)	0.82(0.02)	0.0380*

**Table 1:** pH and ascorbic acid of selected plant species from test and control sites.

Values are means (SEM) for four samples,

\*Statistically significant difference in comparison with control site P < 0.05

The table shows that pH varies among the selected plant species. All the leaf samples collected from both sites exhibited a pH toward acidic side. The pH of leaf samples at the test site was relatively lower when compared to control samples. *C. pubescens* with a pH of 3.53 (0.43) and 3.58 (0.03) recorded the lowest pH for test and control respectively, while *M. sapientum* with a pH of 5.35 (0.07) and 5.63 (0.03) recorded the highest pH for test and control respectively. There was statistically significant difference ( $P < 0.05$ ) in the pH between *C. ordortranta* and *M. sapientum* collected from test and control sites.

The mean concentration of ascorbic acid content of selected plant species at the test site was relatively high

as compared to control site. *C. afer*, *C. odoranta* and *M. sapientum* had ascorbic acid content of 0.88 (0.003) at the site. However, *C. pubescens* and *P. guajava* had equal AA of 1.32 (0.003) at the test site. The ascorbic acid content varies among the leaf samples collected from control site. The statistical analysis showed that there was statistically significant difference ( $P < 0.05$ ) in the AA between *P. guajava* at the test and control site.

Table 2 shows the results obtained from statistical analysis of relative water content (RWC) and total chlorophyll content (TCh) of selected plant species from test and control site.

Plant Species	Relative Water Content (%)			Total chlorophyll (mg/g)		
	Test	Control	P value	Test	Control	P value
	Mean(SEM)	Mean(SEM)		Mean(SEM)	Mean(SEM)	
<i>Costus afer</i>	70.72(7.79)	85.77(7.41)	0.2112	6.54(0.04)	6.48(0.03)	0.2148
<i>Centrosoma pubescens</i>	65.67(8.90)	78.14(5.17)	0.2712	8.16(0.01)	7.89(0.01)	<0.0001*
<i>Chromolaena odoranta</i>	76.90(4.74)	87.32(1.68)	0.0836	5.21(0.01)	5.11(0.01)	0.0013*
<i>Psidium guajava</i>	71.23(4.80)	57.21(2.29)	0.0387*	9.59(0.01)	8.51(0.01)	<0.0001*
<i>Musa sapientum</i>	79.40(8.90)	94.18(2.42)	0.1602	9.84(0.08)	9.95(0.00)	0.2019

**Table 2:** Relative water content and total chlorophyll of selected plant species from test and control sites.

Values are means (SEM) for four samples,

\*Statistically significant difference in comparison with control site  $P < 0.05$

The table reveals that with an exception of *P. guajava*, percentage of RWC in leaf samples collected from test site was relatively low in comparison with the control samples. *C. pubescens* recorded the lowest RWC of 65.67(8.90) at test site and *P. guajava* had the lowest RWC of 57.21(2.29) at the control site. The highest RWC of 79.40(8.90) and 94.18(2.42) were recorded for *M. sapientum* collected from test and control sites respectively. There was statistically significant difference between the RWC of *P. guajava* at test and control sites ( $P < 0.05$ ).

The TCh of leaf sample collected from the test site from the test site was relatively high as compared to samples from control site. The lowest TCh was seen in *C. odoranta* with concentrations of 5.21(0.01) and 5.11(0.01) at test and control sites respectively, while *M. sapientum* with concentrations of 9.84 (0.08) and 9.95 (0.00) for test and control respectively had the highest TCh. There were statistically significant difference between the TCh of *C. pubescens*, *C. odoranta* and *P. guajava* collected from test and control sites ( $P < 0.05$ ).

Plant Species	Test	Control	P value
	Mean (SEM)	Mean (SEM)	
<i>Costus afer</i>	7.98(0.78)	9.37(0.74)	0.2439
<i>Centrosoma pubescens</i>	8.11(0.89)	8.98(0.73)	0.4763
<i>Chromolaena odoranta</i>	8.62(0.48)	9.58(0.18)	0.1085
<i>Psidium guajava</i>	9.20(0.52)	7.44(0.23)	0.0215*
<i>Musa sapientum</i>	9.29(0.89)	10.69(0.22)	0.1766

**Table 3:** Air Pollution Tolerance Indices of the plants.

Values are means (SEM) for four samples

\*Statistically significant difference in comparison with control site  $P < 0.05$

The data obtained from computation of air pollution tolerance indices (APTI) of various plant species are presented in **Table 3**.

The table demonstrates a relatively decrease in the APTI of plant species collected from test site as compared to control site. *C. afer* recorded the lowest APTI of 7.98(0.78) at test site and *P.guajava* had the lowest APTI of 7.44(0.23) at the control site. The highest value of APTI

was recorded for *M. sapientum* with APTI of 9.29(0.81) and 10.69(0.22) at test and control sites respectively. The statistical analysis showed that difference in the mean values of *P. guajava* at test and control sites was statistically significant ( $P < 0.05$ ).

Foliar photomicrographic features of selected medicinal plants are presented in Table 4.

	Sample ID	Leaf Surface	Somata type		Pore size ( $\mu\text{m}$ )	
			Test	Control	Test	Control
1	<i>Costus afer</i>	Adaxial	Anisocystic	Diacytic	0.2 $\mu\text{m}$	0.1 $\mu\text{m}$
		Abaxial	Anisocystic	Diacytic	0.1 $\mu\text{m}$	0.2 $\mu\text{m}$
2	<i>Centrosoma pubescens</i>	Adaxial	Cytocytic	Anisocystic	0.2 $\mu\text{m}$	0.3 $\mu\text{m}$
		Abaxial	Cytocytic	Anisocystic	0.3 $\mu\text{m}$	0.2 $\mu\text{m}$
3	<i>Chromolaena odoranta</i>	Adaxial	Anisocystic	Actinocytic	0.2 $\mu\text{m}$	0.2 $\mu\text{m}$
		Abaxial	Anisocystic	Actinocytic	0.1 $\mu\text{m}$	0.1 $\mu\text{m}$
4	<i>Psidium guajava</i>	Adaxial	Diacytic	Cytocytic	0.2 $\mu\text{m}$	0.2-0.4 $\mu\text{m}$
		Abaxial	Diacytic	Cytocytic	0.1-0.2 $\mu\text{m}$	0.1 $\mu\text{m}$
5	<i>Musa sapientum</i>	Adaxial	Anisocystic	Cytocytic	0.1 $\mu\text{m}$	0.2 $\mu\text{m}$
		Abaxial	Anisocystic	Cytocytic	0.2 $\mu\text{m}$	0.1 $\mu\text{m}$

**Table 4:** Photomicrographic features of leaf surface, stomata type and pore size.

The foliar epidermal analysis of *C.afer* showed that the test sample expressed anisocystic stomata with pore sizes of 0.2  $\mu\text{m}$  and 0.1  $\mu\text{m}$  at the adaxial (upper) and abaxial (lower) surfaces respectively, while the control sample expressed diacytic stomata with pore sizes of 0.1  $\mu\text{m}$  and 0.2  $\mu\text{m}$  at the adaxial and abaxial surfaces respectively.

*C. pubescens* collected from test site expressed cytotocytic stomata with pore sizes of 0.2  $\mu\text{m}$  and 0.3  $\mu\text{m}$  at adaxial and abaxial surfaces respectively, whereas the control sample expressed anisocystic stomata with pore sizes of 0.3  $\mu\text{m}$  and 0.2  $\mu\text{m}$  at adaxial and abaxial surfaces respectively.

The test and control samples of *C. odoranta* demonstrated anisocystic stomata with pore sizes of 0.2  $\mu\text{m}$  and 0.1  $\mu\text{m}$  at adaxial and abaxial surfaces respectively.

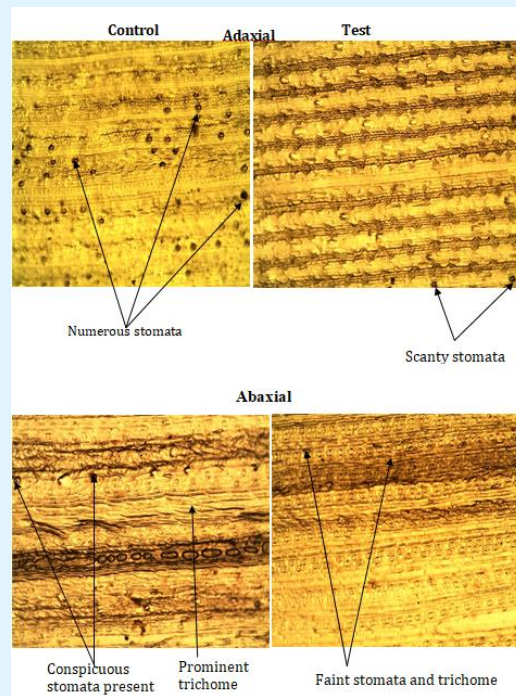
The foliar epidermal traits of *P. guajava* collected from test site revealed diacytic stomata with pore sizes of 0.1-0.2  $\mu\text{m}$  and 0.2  $\mu\text{m}$  at adaxial and abaxial surfaces respectively, in contrast to control sample that revealed

cytotocytic stomata with pore sizes of 0.1  $\mu\text{m}$  and 0.2-0.4  $\mu\text{m}$  at adaxial and abaxial surfaces respectively.

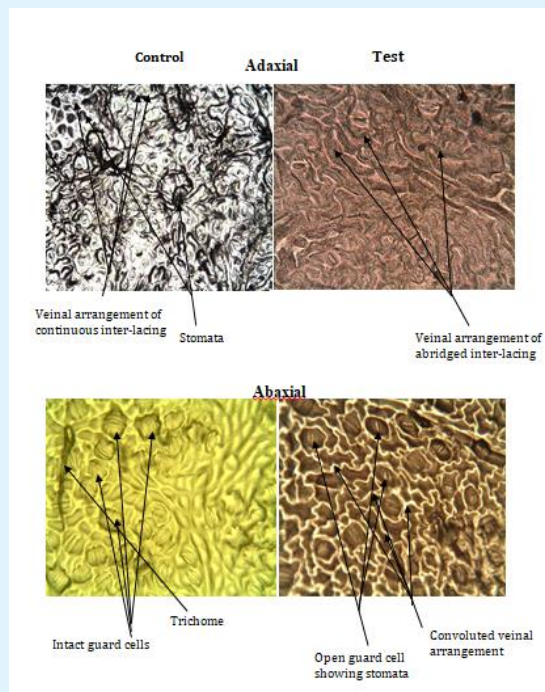
*M.sapientum* revealed anisocystic stomata with pore sizes of 0.1  $\mu\text{m}$  and 0.2  $\mu\text{m}$  at adaxial and abaxial surfaces respectively, contrarily, the control sample expressed cytotocytic stomata with pore sizes of 0.2  $\mu\text{m}$  and 0.1  $\mu\text{m}$  at adaxial and abaxial surfaces respectively.

Figures 1-5 represent comparative analysis of foliar photomicrographs of leaf samples collected from control and test sites. Figure 1 represents foliar photomicrographs of *C. afer*.

The adaxial surface of control sample showed a well distributed and numerous stomata, whereas test sample showed an abraded foliar structure with fuzzy thick lines and scanty stomata. In the abaxial photomicrograph, a conspicuous stomata and prominent trichome were observed in the control sample, whereas indistinct stomata and trichome were seen in test sample. Figure 2 represents foliar photomicrographs of *C. pubescens*.



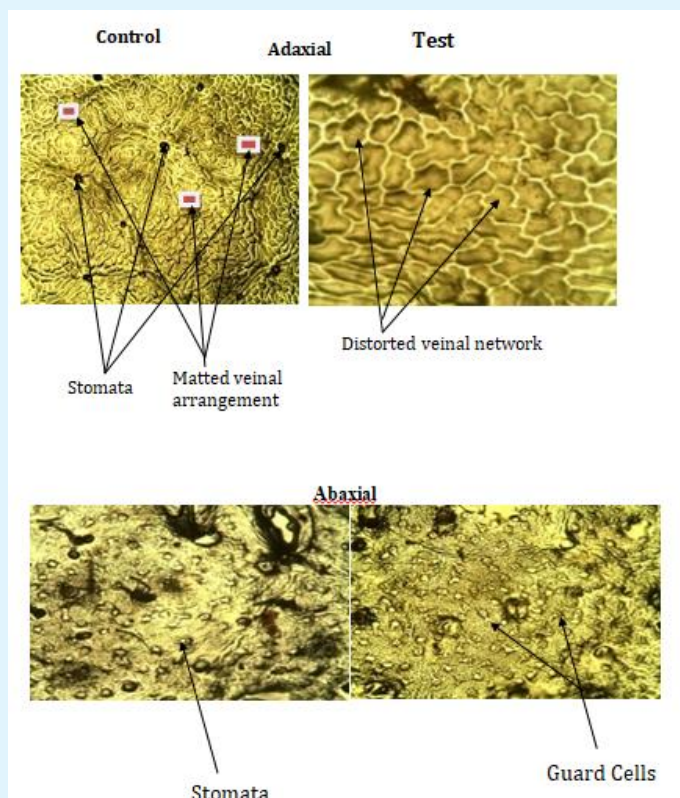
**Figure 1:** Foliar photomicrographs of *Costus afer* showing the alterations in the test sample across the adaxial and abaxial surfaces as compared to control sample.



**Figure 2:** Foliar photomicrographs of *Centosoma pubescens* showing alterations in the test sample across the adaxial and abaxial surfaces as compared to contol sample.

The adaxial surface of control sample demonstrated well defined stomata and venial arrangement of continuous inter-pacing, while the test sample showed veinal arrangement of abridged inter-lacing. The abaxial photomicrograph of control sample showed well

distributed intact guard cells and defined trichome, but the test sample revealed distorted guard cells and convoluted veinal arrangement. Figure 3 represents foliar photomicrographs of *C. odoranta*.



**Figure 3:** Foliar photomicrographs of *Chromolaena odoranta* showing alterations in the test sample across the adaxial and abaxial surfaces as compared to control sample.

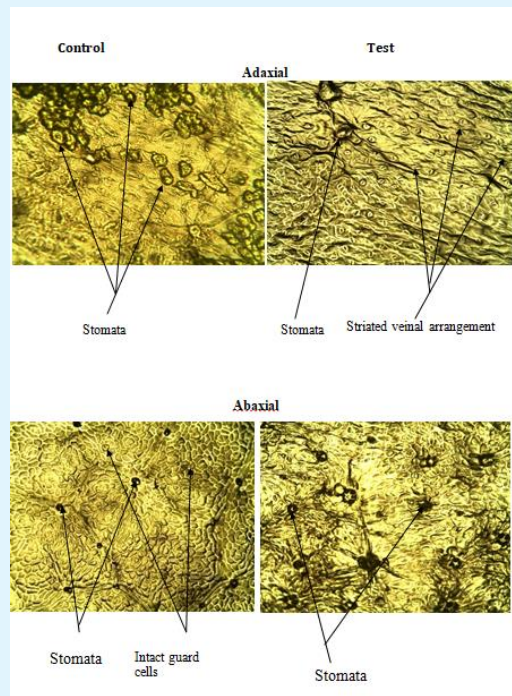
The abaxial surface of control sample revealed evenly distributed stomata and matted veinal arrangement, however, the test samples showed marked distorted veinal network.

The abaxial surface of the control showed preserved micro-architecture, whereas the test sample demonstrated bleached-like internal structure, abrasion

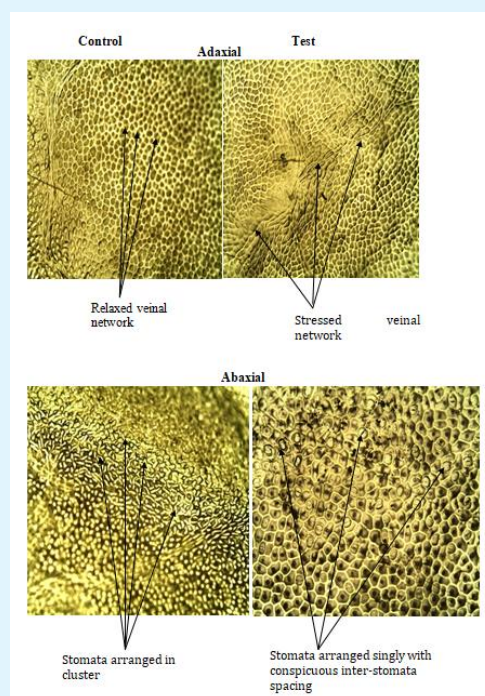
and necrosis. Figure 4 represents photomicrographs of *P. guajava*.

The adaxial surface of control sample revealed presence of numerous stomata, while test sample revealed scanty, slightly risen stomata and striated and often discontinued veinal arrangement. The abaxial photomicrograph of control sample showed evenly distributed stomata with intact guard cells, while the test sample showed degenerated stomata architecture.





**Figure 4:** Foliar Photomicrographs of *Psidium guajava* showing alterations in the test sample across the adaxial and abaxial surfaces as compared to control sample



**Figure 5:** Foliar photomicrographs of *Musa sapientum* showing alterations in the test sample across the adaxial and abaxial surfaces as compared to control sample.

Figure 5 represents photomicrographs of *M. sapientum*.

The adaxial surface of control sample revealed a relaxed veinal network, but the test sample showed stressed veinal network. The abaxial photomicrograph of control sample showed densely packed stomata, contrarily, test sample revealed sparsely arranged stomata with conspicuous inter stomata spacing.

## Discussion

The plants being constantly exposed to the environment absorb, accumulate and integrate pollutants impinging on their foliar surfaces given to the fact that they are relatively static in nature. Consequently they show visible or subtle changes depending on their sensitivity level [6]. In this study, the air pollution tolerance indices (APTI) and foliar photomicrographs were used in evaluating impact of oil spill in five medicinal plants (*C. afer*, *C. pubescens*, *C. odoranta*, *P. guajava* and *M. sapientum*) within oil spilled farmland.

Air pollution tolerance index gives an empirical value of tolerance level of plants to air pollution. The APTI analysed for each plant species at the test site (oil spilled area) and control site (non oil spill area) are presented in table 3. The study showed a relatively decrease in the APTI of selected species collected from test site as compared to control site with an exception of *P. guajava* which had an increase in the APTI of the test site as compared to the control site. Similar results were reported by Rai, et al., [19] for *P. guajava* situated in industrial site (Roukela) and non industrial site (Aizawl) of India. From this present study, it was found that the samples collected from the control site were in better condition than those collected from the test site. However, they still fell under sensitive group based on the APTI scale. Following the scale, any plant species with the value less than 1 is considered as very sensitive, plant with value from 1 to 16 is considered as sensitive, plant with value in the range of 17 to 29 is considered as intermittently tolerant and plant with value from 30 to 100 is considered as tolerant [8]. The relative decrease in the APTI of the plant species collected from the test site as compared to the species collected from control site could be due to the air pollution factor in which gaseous pollutants caused leaf injury, stomata damage, early senescence, decreased photosynthetic ability, distorted membrane permeability and condensed growth, and yield in sensitive plant species. The tolerance index for test site is given here in the decreasing order: *Musa sapientum* < *Psidium guajava* < *Chromolaena odoranta* < *Centrosema*

*pubescens* < *Costus afer* < *Centrosema pubescens* < *Costus afer*. Likewise, APTI of plants studied at the control site decrease as follows: *Musa sapientum* < *Chromolaena odoranta* < *Costus afer* < *Centrosema pubescens* < *Psidium guajava*. The study showed that there was no significant difference between the APTI of the plants collected from the both sites, but also with an exception of *P. guajava* which showed that there was significant difference between the APTI of the species collected from the test and control sites. An overview of the results obtained from the study reveals that different plants respond differently to air pollution. This variation of the APTI can be attributed to the difference in any of the four biochemical parameters which govern the computation of the index.

The results shown in Tables 1 & 2 reveal the significant role of the biochemical parameters in the determination of APTI and hence, plants' resistivity and susceptibility. It is observed that all the plant species collected from both sites exhibited a pH towards acidity. This might be due to the presence of NO<sub>x</sub>, SO<sub>x</sub>, CO<sub>x</sub>, CH<sub>x</sub>, which are components of oil spill and other acidic pollutants in the ambient air. The lower pH seen in *C. afer* and *C. pubescens* collected from both sites may be associated with high sensitivity of these species to the ambient air pollutants. This has been supported by Gostin (2009) [20], that low leaf extract pH has good correction with sensitivity to air pollution. *M. sapientum* recorded the highest pH at test and control sites and it accounted for the highest value of APTI seen in the species at the both sites.

It is also observed that the mean concentration of ascorbic acid in the leaves of plants collected from the test site was relatively higher than those of the control samples. This may be associated with increase in formation and regeneration of this metabolite by the plants due to increase in the rate of production of reactive oxygen species (ROS) under stress environmental condition. This is more pronounced in *C. pubescens* and *P. guajava* from the test site and is related to the tolerance to air pollution supporting the study of Arora, et al. [21], that ascorbic acid activates defense mechanism under stress condition. Ascorbic acid being the multiplication factor, greatly determines the APTI [3].

Measurement of relative water content (RWC) was used to study water status of the plants. The study showed that with an exception of *P. guajava*, percentage of water content in the leaf samples collected from test site is relatively low in comparison with the samples. The

maximum percentage of water content seen in *M. sapientum* at the both sites is attributed to the highest APTI of the species at the sites, whereas the minimum water content percentage observed in *P. guajava* from the control site account for its lowest APTI at the site. Therefore, RWC is a strong determinant of the APTI value. From visual inspection of the plants at both sites, the size of the leaves of various plant species varied, where the largest leaves were from the sample at the control site. And also, the leaves of the plants collected from the control site looked fresher with nice green leaf colour compared to leaves of the plants collected from the test site that had stipple and somewhat greenish-yellow colour which are susceptible features of plant exposed to air pollution.

The present study shows that the total chlorophyll content in the leaf samples collected from the test site was relatively high as compared to leaf samples collected from control samples. This is in conflict with the report of Rai, et al. [19], that the more polluted the area, the lower the total chlorophyll. *M. sapientum* recorded highest values of total chlorophyll at the test and control sites and it also

accounted for the APTI values seen in the species at both sites.

Table 5 and Figures 1-5 represent photomicrographs analysis of selected plant species at control and sites. From this present study, it was found that the adaxial foliar photomicrograph of *C. afer* collected from the control site showed well distributed and numerous diacytic stomata with pore of 0.1  $\mu\text{m}$ . The test sample showed an abraded foliar structure with fuzzy thick lines and scanty anisocytic stomata with pore of 0.2  $\mu\text{m}$ . The alteration of foliar integrity may be associated with negative consequence of spilled oil. In the abaxial foliar photomicrograph, a conspicuous stomata and prominent trichome were observed in the control sample whereas indistinct anisocytic stomata with pore of 0.1  $\mu\text{m}$  and trichome seen in the test sample were indicative of foliar disruptive micro-structures of plants exposed to polluted environment [22]. The relative decrease in the stomata pore size seen in the leaf sample collected from test site as compared to control sample could have protective value as part of avoidance mechanism. Similar report is received in studies of the foliar epidermal traits from other works [23,24].

Plant	Control	Test	%Difference
<i>Costus afer</i> 1	10.34	8.86	14.31
<i>Costus afer</i> 2	7.22	5.76	20.22
<i>Costus afer</i> 3	9.55	8.08	15.39
<i>Costus afer</i> 4	10.38	9.23	11.08
<i>Controsma pubescens</i> 1	8.92	8.97	-0.56
<i>Controsma pubescens</i> 2	9.74	6.72	31
<i>Controsma pubescens</i> 3	6.96	6.56	5.75
<i>Controsma pubescens</i> 4	10.31	10.19	1.16
<i>Chromolaena odoranta</i> 1	9.24	7.26	21.43
<i>Chromolaena odoranta</i> 2	10.01	8.99	10.19
<i>Chromolaena odoranta</i> 3	9.7	8.77	9.59
<i>Chromolaena odoranta</i> 4	9.35	9.46	-1.18
<i>Psidium guajava</i> 1	7.63	10	-34.6
<i>Psidium guajava</i> 2	6.75	7.68	-13.78
<i>Psidium guajava</i> 3	7.74	9.4	-21.45
<i>Psidium guajava</i> 4	7.64	9.73	27.36
<i>Musa sapientum</i> 1	11.02	9.69	12.07
<i>Musa sapientum</i> 2	11.12	10.66	4.14
<i>Musa sapientum</i> 3	10.32	10.11	2.03
<i>Musa sapientum</i> 4	10.3	6.68	35.16

**Table 5:** Comparison of Air Pollution Tolerance Index of Control and Test Plants.

The adaxial foliar photomicrograph of *C. pubescens* collected from the control site (Figure 2) revealed well

defined anisocystic stomata with pore of 0.3  $\mu\text{m}$  and veinal arrangement of continuous interlacing which are

established features of healthy plants. In the test sample, these features were altered and they suggest environmental stress [22]. The abaxial foliar photomicrograph of control sample showed well distributed intact guard cells and defined trichome. Contrarily, the test sample showed distorted guard cells and convoluted venial arrangement. These aberrations demonstrated marked alterations in foliar surface architecture of *C. pubescens* at the test site due to impact of oil spillage.

In figure 4, the control sample of *C. odoranta* showed evenly distributed aniscystic stomata with pore of 0.1  $\mu\text{m}$  and 0.2  $\mu\text{m}$  for the adaxial and abxial surfaces respectively. The adaxial surface also showed matted veinal arrangement. These foliar epidermal traits are of advantages, make the leaf to achieve high photosynthetic rate in full sunlight. The above traits were contrasted in the test sample, although the same pore sizes were recorded for both surfaces. The adaxial surface revealed that destruction in veinal network brought about separation of upper foliar surface into several compartments. Bleached-like internal structure, abrasion and necrosis were remarkable features in the abaxial. The foliar alterations may be due to the presence of NO<sub>x</sub> and other acidic pollutants in the ambient air. The foliar epidermal analysis was in conformity with the finding of Otuu [1] which evaluated foliar photomicrography of five medicinal plants at gas flaring site.

In figure 5, the foliar epidermal analysis of *P. guajava* collected from control site revealed numerous cytoytic stomata with pore of 0.1  $\mu\text{m}$  and 0.2- 0.4  $\mu\text{m}$  for adaxial and abaxial surfaces respectively. In contrast, test sample revealed scanty and slightly risen diacytic stomata with pore of 0.1-0.2  $\mu\text{m}$  and 0.2  $\mu\text{m}$  for adaxials and abaxial surfaces respectively. It was observed that the test sample had striated and often discontinued veinal arrangement. This observation was in tandem with the report of Gostin (2009) [20], that pollution stress altered the structure of the leaves in the industrial areas. The modification of the frequency and sizes of stomata as a response to the environmental stress is an important manner of controlling the absorption of pollutants by plants [24].

In *M. sapientum*, distinctions were observed in the control adaxial foliar photomicrograph (figure 5). The stomata were cytoytic with pore of 0.2  $\mu\text{m}$ , and relaxed veinal network were revealed. But scanty aniscystic stomata with pore of 0.1  $\mu\text{m}$ , and distorted veinal network were observed in the test adaxial photomicrograph. These features could be an evidence of oil spill-induced foliar

disruption. The abaxial surface of control sample had densely packed cytoytic stomata with pore of 0.1  $\mu\text{m}$ . Contrary, this prominent feature was lacking in the test sample whose aniscystic stomata with pore of 0.2  $\mu\text{m}$  were sparsely arranged and inter-stomata spacing were conspicuous. These foliar deformities were indications of polluted environment.

## Conclusion

The study shows that oil spillage had marked devastating impact on biochemical, physiological as well as anatomical configuration of selected medicinal plants. Although sensitively varies among species. *M. sapientum* with relative tolerance potential may be recommended for green belt plantation in the area with frequent incident of oil spillage for air pollution abatement. It is advised that members of Edegbiri community should avoid the use of plants within oil spill area for food and ethno-medicinal purposes.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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