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Ecoanatomical Affiliation of Halophyte in Hydrocarbon Polluted Sites in Parts of Bodo Coastal Creeks, Rivers State, Nigeria

Mojuetan, T.[1]; Tanee, F.B.G.[2]; Edwin-Wosu, N.L[3*]
Department of Plant Science and Biotechnology.
Faculty of Science, University of Port Harcourt

Corresponding Author: Edwin-Wosu, N.L[3*]

Abstract:- The increasing demand for energy supply and revenue has led to an ever increasing oil exploration and exploitation in parts of the Niger Delta, Nigeria. This has also led to an increase in hydrocarbon pollution leading to the destruction of the plant and animal ecosystems in parts of Rivers State. This study was aimed at investigating the impact of hydrocarbon pollution on the stem and root anatomy of a special group of plants- "Halophytes" present in polluted and unpolluted areas of Bodo Creek, Gokana in Ogoni land Rivers State. The result reveal diverse structural aberrations and challenges involving the species inability to pick up stains thereby limiting the clarity of focus, conversion of air/intercellular spaces into vacuoles filled with oil, rearrangement of the vascular bundles, thickening of the sclerenchyma cells, damage of vital parts of stem and root tissues as well as shrinkage and detachment of cortex from the epidermis leading to the reduction of the air compartments. All these are the anatomical distortion associated with the impact on hydrocarbon pollution on the internal structures of these halophytes when compared with the structures of sections in the controlled unpolluted site.

Keywords:- *Halophytes, Anatomy, Hydrocarbon Pollution, Paspalum Vaginatum, Rhynchospora Corymbosa, Fimbrystylis Littoralis.*

I. INTRODUCTION

The enigma of environmental pollution by extensive array of chemical pollutants and contaminants including hydrocarbons is remarkable globally based on their toxicity, threat and effect on biodiversity and potential risk to human health [1, 2]. In the Niger Delta region of Nigeria, crude oil exploration and production have been likened as a double-edged sword in the region [3]. This can be exemplified in the value chain of refined and unrefined product as a major source of revenue generation and energy for the states and nation at large [4] and concomitant release of same hydrocarbon derivatives into the diverse ecosystem (aquatic, air and the land) by legal and illegal machineries with associated number of threats to humans and biodiversity [5, 6, 7], and consequent decline of ecological resources in the region [8, 9, 10, 11, 12].

Hence it cannot be overemphasized that unsustainable oil exploration activities have rendered the Niger Delta region a derelict hotspot, it has now become one of the five most severely petroleum damaged ecosystems in the world with hazardous impacts on biodiversity (plants inclusive) which ranges from defoliation to the extinction of certain species, and death of the entire vegetation as well as damages and destruction of internal plant tissues and organs [6, 13].

Some plants groups are known to withstand and strive in stressful environments; an example of such group is the "Halophytes". Halophytes are group of plants which have the ability to grow, develop and reproduce their kind in highly saline environments [14]. Some of these highly saline environments include salt dunes, coastal marshes, saline creeks and wetland etc. These plants are salt tolerant and are able to withstand and thrive in salt water stress environment through different mechanisms and adaptations [15]. Most plants are unable to withstand such salinity increase in their environments which could lead to their decrease in soil water absorption, sodium ion absorption and consequent buildup of toxicity in plants thereby affecting their metabolism, increasing plant cells plasmolysis and invariably death.

The composition of hydrocarbon, its presence in soils and the extent or degree of pollution alters the biophysical characteristics of plants. Plants exposure to hydrocarbon pollution due to their immobile state has revealed consistent effects and damage to their physiology, anatomy and growth [16]. However, due to the ability of halophytes to grow in these saline environments that are naturally toxic for other plants, this has informed the need to investigate the impact of hydrocarbon pollution stress on their internal structures in light of their resilience to such condition. This study was aimed at examining the impact of hydrocarbon induced changes in the anatomical structures of selected halophyte species with the objective of evaluating the pollutant effect on the stem and root anatomy of three halophyte species. Furthermore, it will be significant in adding to the botany knowledge repertoire of the internal differences of these selected halophytes obtained from both hydrocarbon polluted and unpolluted environment.

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II. MATERIALS AND METHODS

A. Description of Study Area, Location and Site

Rivers state is located between longitudes 6º23'E and $7^0$6E and latitudes $4^0$18'N and $5^0$45'N of the equator (Fig. 1). It is one of the states in the Southern parts of Nigeria and geographically, it is bounded to the East, West, North and South by Imo and Akwa-ibom states, Bayelsa state, Imo and Abia states, and the Atlantic Ocean respectively [17, 18]. The state is known to be characterized by varying vegetation types (mangrove forest, coastal barrier islands, fresh water swamp and the tropical rainforest) [18, 19]. Rivers state is highly distinguished by its tropical hot monsoon climate which is attributed to its latitudinal locus resulting in a consistent annual warm temperature spanning 23.00 to 42.60°C and relative humidity within 65% to 96.80%, heavy rainfall of about 2000mm to 2500mm with an average elevation of 20m to 30m above sea levels characterizing the area as a lowland [18, 20, 21, 22]. The soil types in this area include the sandy silt, sandy loam or clayey underlain by a section of impervious pan often leached by an alkaline (salty) and seldomly an acidic rainfall [18]. The State consists of twenty-three local council areas including Gokana study location (Fig. 2) which houses the Bodo city study site.

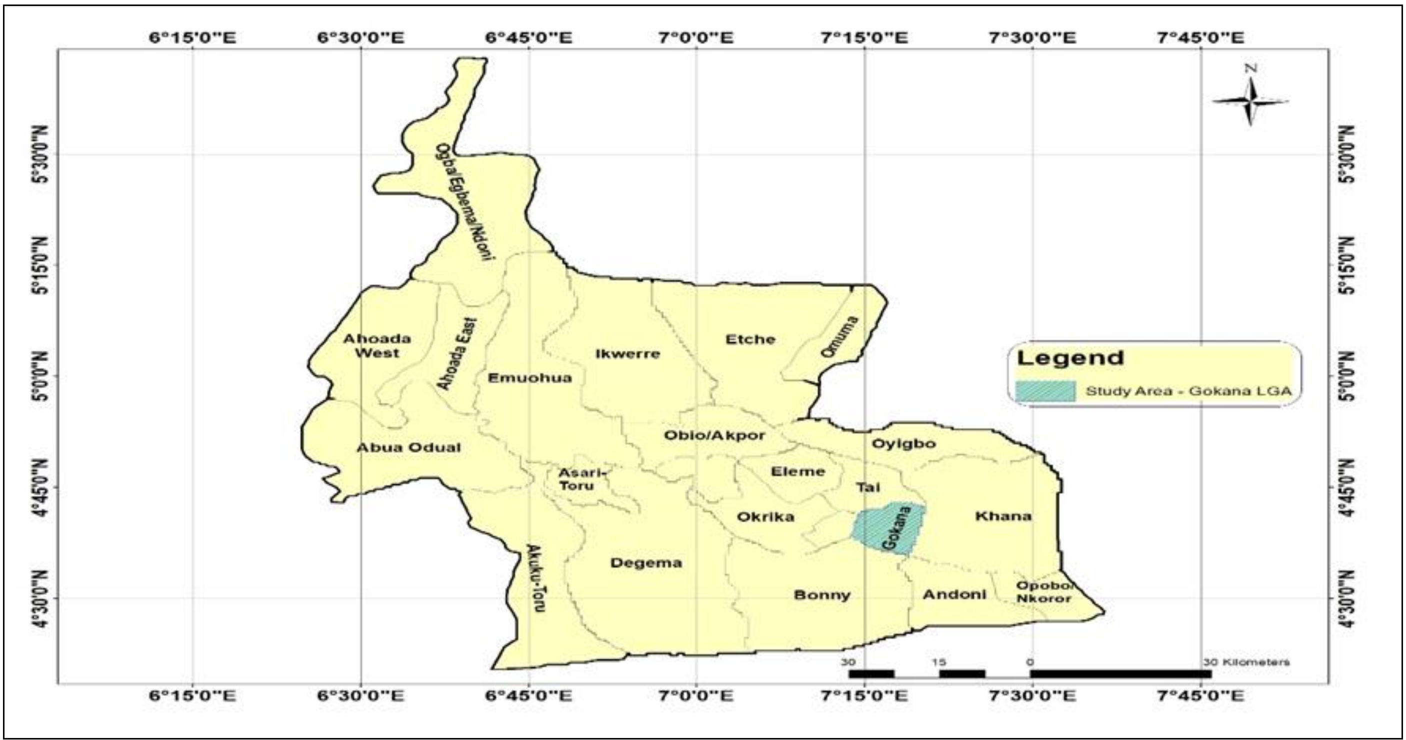

Fig 1: Rivers State Indicating Study Area – Gokana

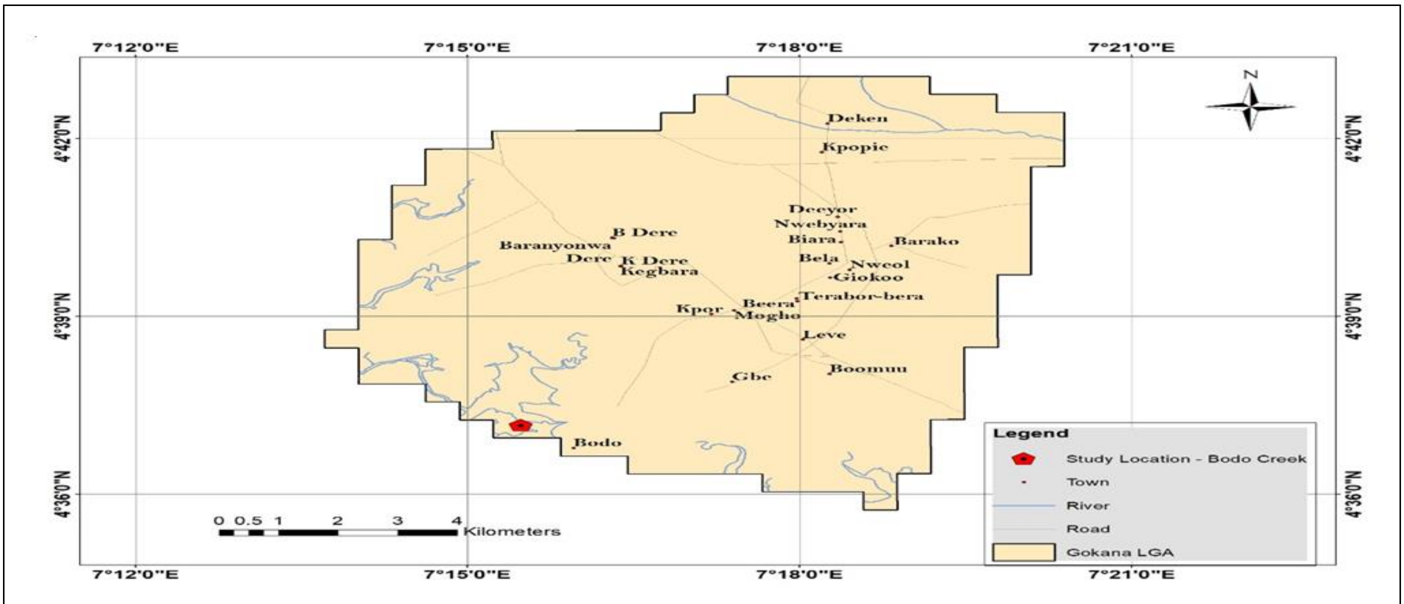

Fig 2: Gokana Indicating Study Location – Bodo Creek

The sample site situated in Bodo Creek in Bodo City study site is a littoral swampy land mass of approximately 9,230 hectares in Gokana study location. (Fig. 3). The swamp land has four channels that takes and return salt water in and out of it. Bodo creeks plays host to the sea coast, salt marshes and an expansive mangrove ecosystem which are almost totally impacted by hydrocarbon pollution [23, 24, 25]. The sample site was purposefully selected based on the presence, availability and accessibility to halophytic species found along the sea coasts, marshes and mangroves within parts of the Bodo creek with pollution scenarios.

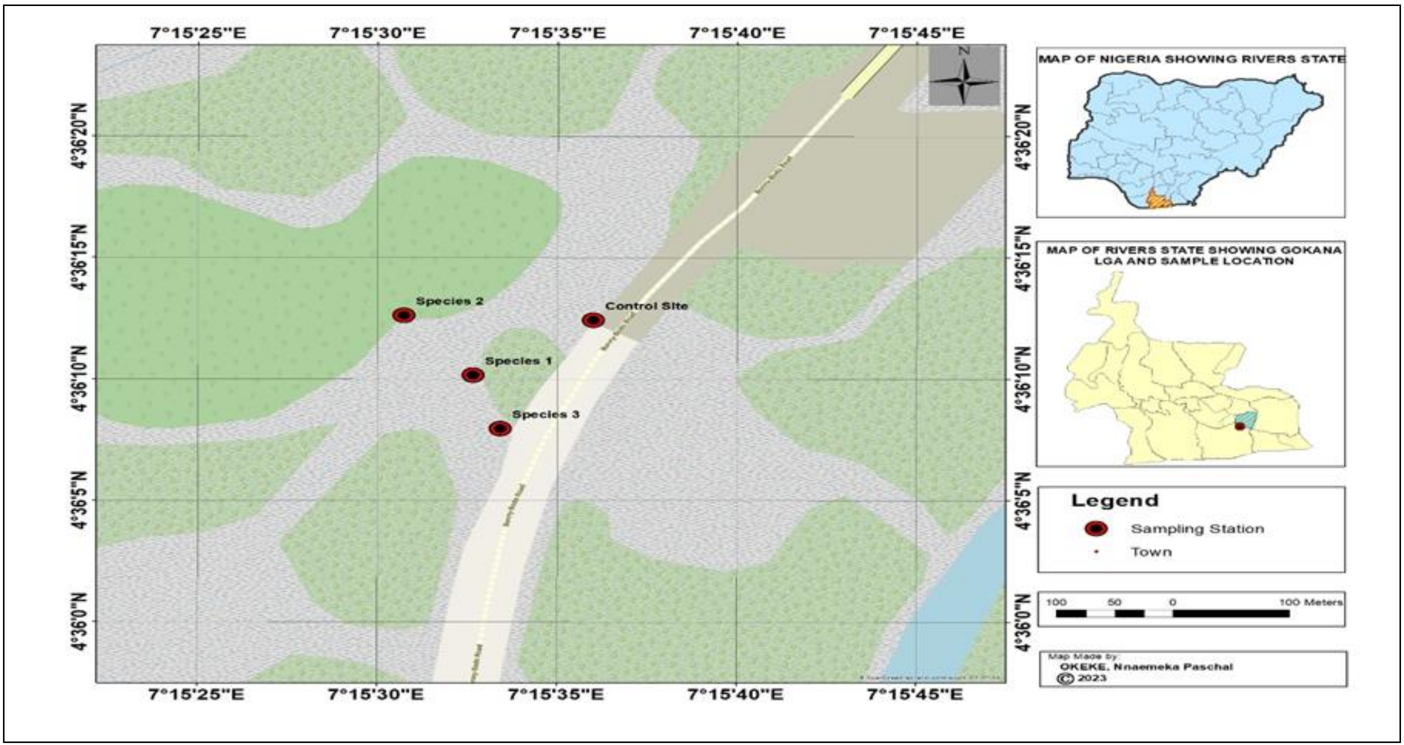

Fig 3: Study Location- (Bodo Creek) Indicating Sample Site – Bodo Bonny Bridge

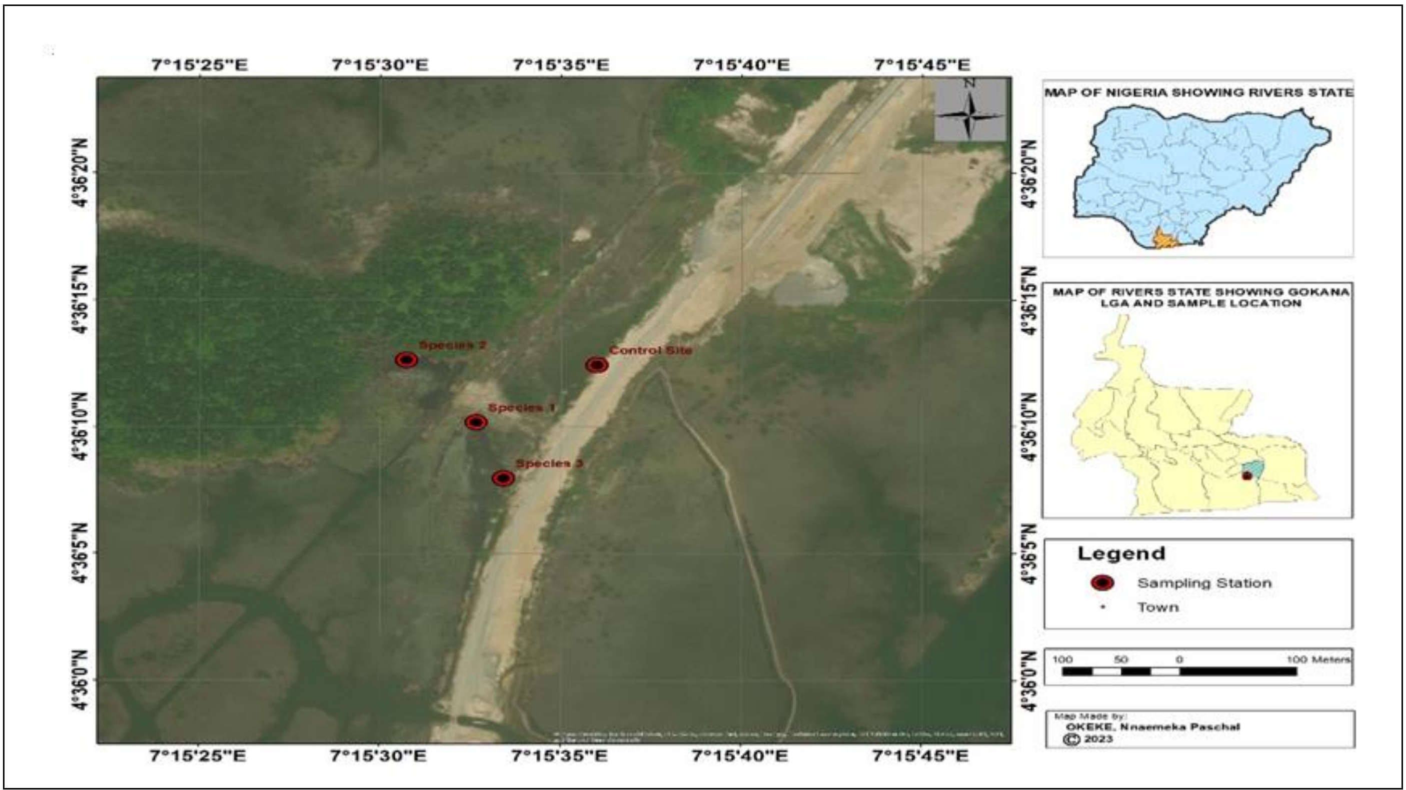

Fig 4: Satellite Imagery of Sampled Site

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B. Flora Sample Collection

A purposive selective sampling method was carried out in two seasons- dry (March) and wet (July) seasons 2021.Three halophyte species were randomly sampled from polluted site at the following georeferenced coordinates in Table I. Whole plant samples were uprooted carefully from the polluted and unpolluted soils. Each plant had their roots properly washed with distilled water to ensure total removal of soil particles attached to them. Thereafter, the plants were cut using a secateur differentiating the roots, stem and leaves which were put in fixative bottles containing F.A.A (Formalin (50%); Acetic Acid (25%); Alcohol (25%) mixture.

Table 1: GPS Coordinates of Sampled Species

S/N	Species	Lat. (N)	Long. (E)
1	*Paspalum vaginatum*	04.60286°	007.25911°
2	*Rhynchospora corymbosa*	04.60353°	007.25852°
3	*Fimbrystylis littoralis*	04.60242°	007.25936°
	Controlled species 1,2,3	04.60345°	007.26004°

C. Sample Processing and Laboratory Analysis

The FAA preserved plant samples were transported to the laboratory within 1 – 72 hours to ensure that the cell saps were not totally plasmolyzed before viewing under the microscope. At the laboratory, they were rinsed with distilled water, followed with 30% alcohol solution twice. To kickstart the dehydration process, the specimens were put in petri dishes containing 30%, 50%, 70% and 95% alcohol for at least 5 minutes respectively before transferring to a petri dish filled with Xylene. Disposable microtome blades were used to section the roots and stem into thin sections at 10nm to be viewed under the microscope. The section slides were transferred into fresh Xylene for a minute. To obtain their respective stains; sections were transferred to petri dishes containing safranin red and methyl blue respectively and transferred to the slides. The slides were mounted on the Olympus CX31 microscope, viewed under different magnifications (x10, x40 and x100) to get the best resolution and the images were captured using the microscope's inbuilt camera.

III. RESULT

The ecoanatomical structure of the halophytes investigated in response to the hydrocarbon polluted and unpolluted conditions has been revealed as exemplified in the various species sections of the stem and root in dry and wet seasons for *Paspalum vaginatum* (Plate 1a – 1d; 2a – 2d); *Rhynchospora corymbosa* (Plates 3a – 3d; 4a – 4d) and *Fimbrystylis littoralis* (Plates 5a – 5d; 6a – 6d).

A. Paspalum vaginatum Sw.

The stem section of *P. vaginatum* in polluted and unpolluted conditions under both seasons has revealed the differences in the various parts of the stem internal structures as represented in Plates 1a-d and Table II. The differences between the stem sections of *Paspalum vaginatum* (Plates 1a – 1d) from the polluted and unpolluted sites in wet and dry seasons have been recorded. Parts of the stem tissues from the polluted sections as revealed in Plate 1a (Epidermis, Hypodermis and Sclerenchymatous tissue) dry season and Plate 1c (Pith) wet season were impacted with severe internal damages through the formation of holes in them at both the dry and wet seasons. The plant stem tissue obtained from the unpolluted sections at dry and wet season (Plates 1b and 1d) had all parts of their tissues intact devoid of any internal aberration.

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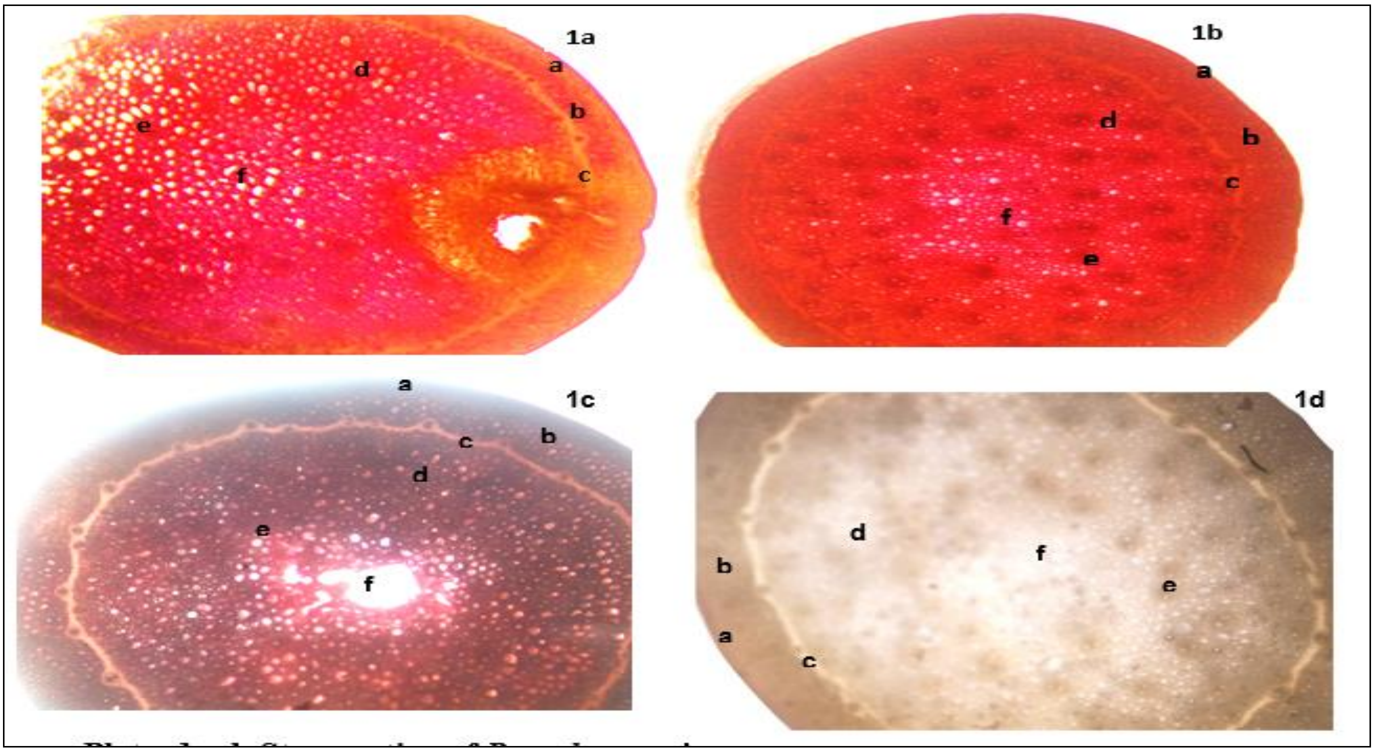

Plates 1a-d: Stem section of *Paspalum vaginatum.*
1a: Dry season polluted section; 1b: Dry season unpolluted section; 1c: Wet season polluted section; 1d; Wet season unpolluted section. (a) Epidermis; (b) Hypodermis; (c) Sclerenchymatous tissue; (d) Air spaces; (e) Vascular bundles; (f) Pith.

The root sections of *P. vaginatum* under polluted and unpolluted conditions in both seasons have revealed differences in the various parts of the root internal structures as represented in Table III. The root sections under polluted condition in both dry and wet seasons (Plate 2a & 2c) revealed shrinkage of the cortex (Plate 2a) and a detachment of some part of the cortex from the epidermis of the root section (Plate 2c).

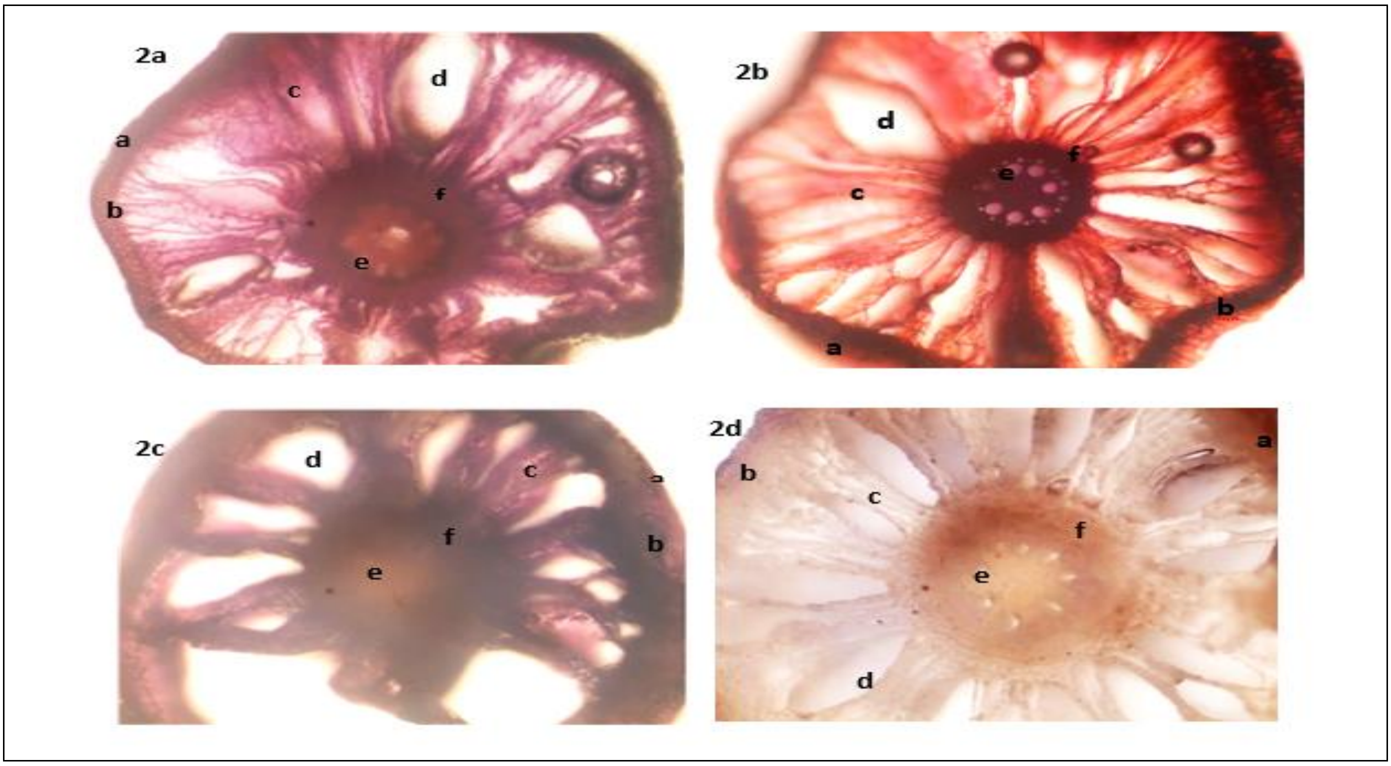

Plate2 (2a - 2d): Root sections of *Paspalum vaginatum*
2a – Dry season polluted section; 2b - Dry season unpolluted section; 2c – Wet season polluted section;
2d - Wet season unpolluted section Piliferous layer, b- Epidermis, c- cortex, d- air chambers, e- vascular bundles, f- pericycle

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B. Rhynchospora corymbosa L

The stem section of *R. corymbosa* on polluted and unpolluted conditions in both seasons have revealed differences in the various parts of the stem internal structures as represented in Table 2. In Plate 3 the epidermal tissue (epidermis) (Plate 3a) is detached from the other part of the stem tissue and a separation of the ground tissues (lacking cohesion) is seen in *R. corymbosa* stem tissues (plate 3c). Furthermore, the vascular bundles were shrunken and not robust (Plate 3a) and not visible (Plate 3c) unlike the stem sections obtained from the unpolluted site at both dry and wet seasons that were robust and visible (Plate 3b & 3d).

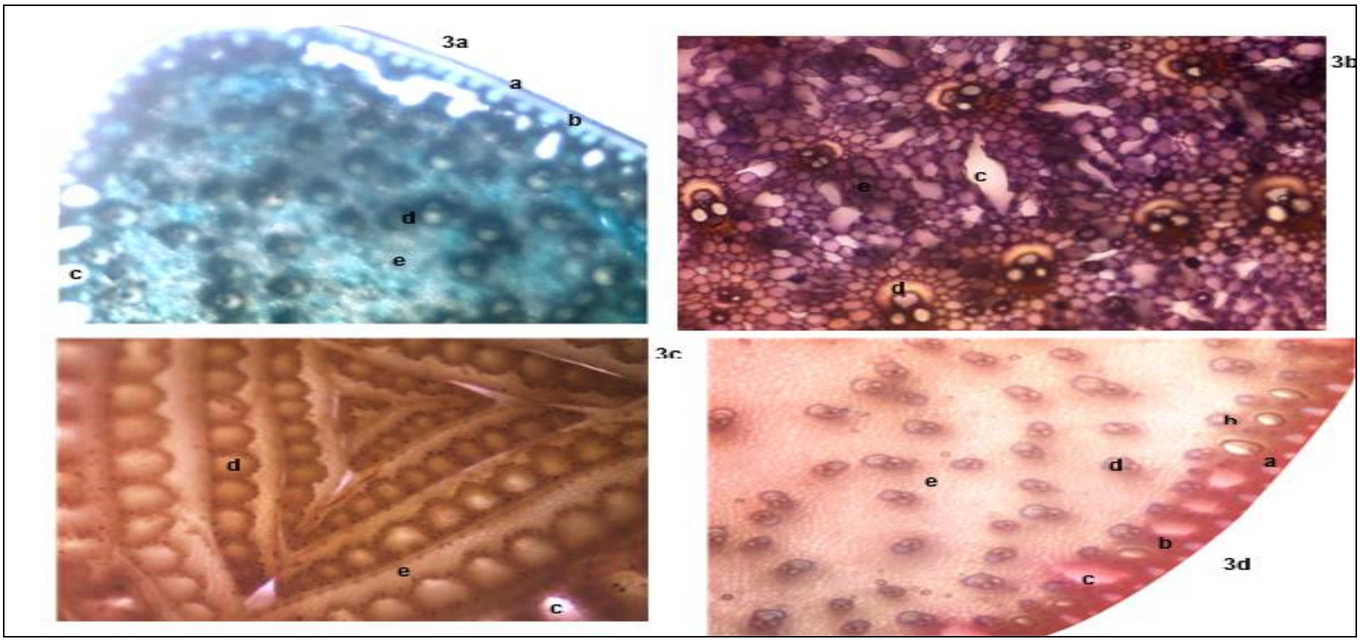

Plate3 (3a - 3d): Stem sections of *Rhynchospora corymbosa*
3a – Dry season polluted section; 3b - Dry season unpolluted section; 3c – Wet season polluted section; d - Wet season unpolluted section. a-Epidermis, b -Hypodermis, c- air spaces/compartment, d – vascular bundles, e – ground tissue

The root section of *R. corymbosa* under polluted and unpolluted conditions in both seasons has revealed their differences in the various parts of the root internal structures as represented in Table 2. The cortex had shrinkage and detachment from the epidermis of these root section (Plate 4a & 4c) in both season.

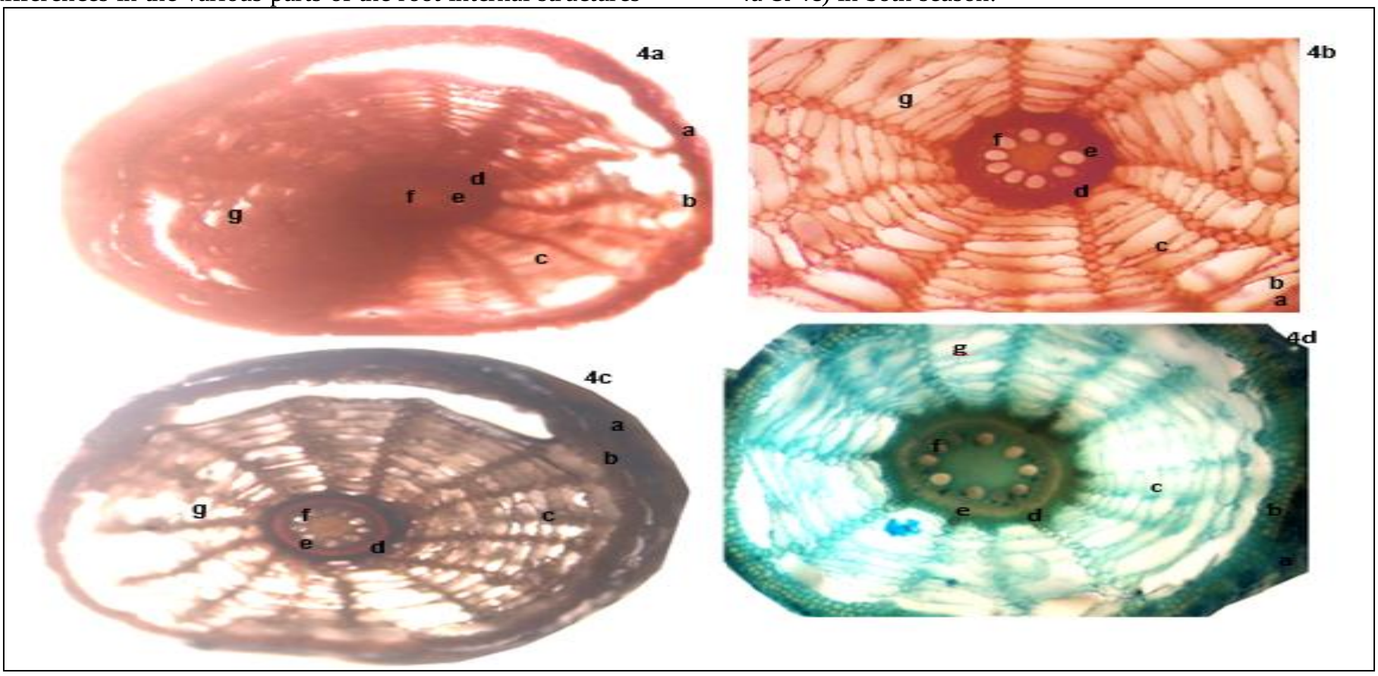

Plate 4 (4a - 4d): Root sections of *Rhynchospora corymbosa*
4a – Dry season polluted section; 4b - Dry season unpolluted section; 4c – Wet season polluted section; 4d - Wet season unpolluted section, a – Epidermis; b – Hypodermis; c – Cortex; d – pericycle; e– Endodermis; f – Vascular bundle; g – Aerenchyma/Airspaces;

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C. Fimbristylis littoralis Gaundich

The stem section of *F. littoralis* of polluted and unpolluted conditions in both seasons has revealed differences in the various parts of the stem internal structures as represented in Table 2. The air compartment/aerenchyma as shown in plate 5b and 5d are converted into vacuoles filled with oil films (plate 5a & 5c). Also, there is a thickening of the sclerenchyma cells (plate 5a & 5c) which is absent in the sections under unpolluted conditions.

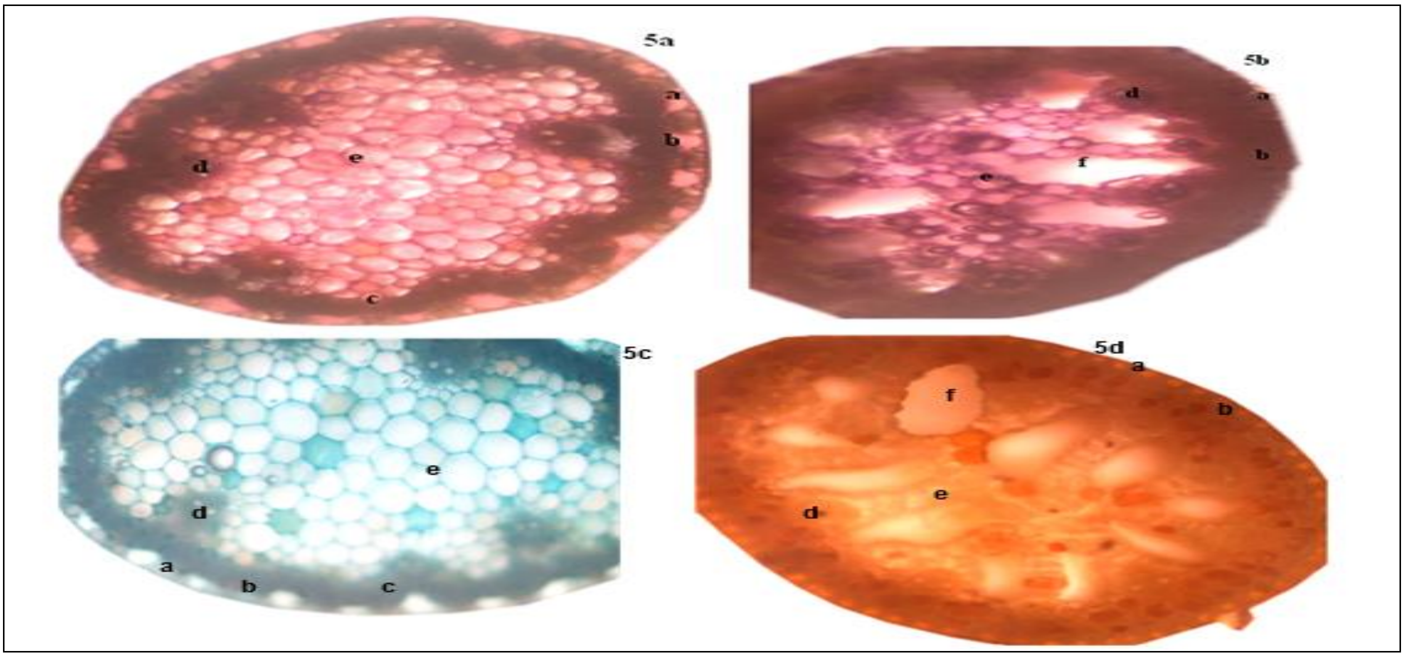

Plate 5 (5a - 5d): Stem sections of *Fimbrystylis Littoralis*

5a – Dry season polluted section; 5b - Dry season unpolluted section; 5c – Wet season polluted section; 5d - Wet season unpolluted section

a-Epidermis, b -Hypodermis, c- sclerenchymatous tissue, d – vascular bundles, e – vacuoles, f - aerenchyma

The root section of *F. littoralis* of polluted and unpolluted conditions in both seasons has revealed differences in the various parts of the root internal structures as represented in Table 3. the impact of hydrocarbon pollution revealed in the shrinkage and detachment of the cortex from the epidermis of the root section as seen in Plate 6a & 6c as well as their inability to pick up stains.

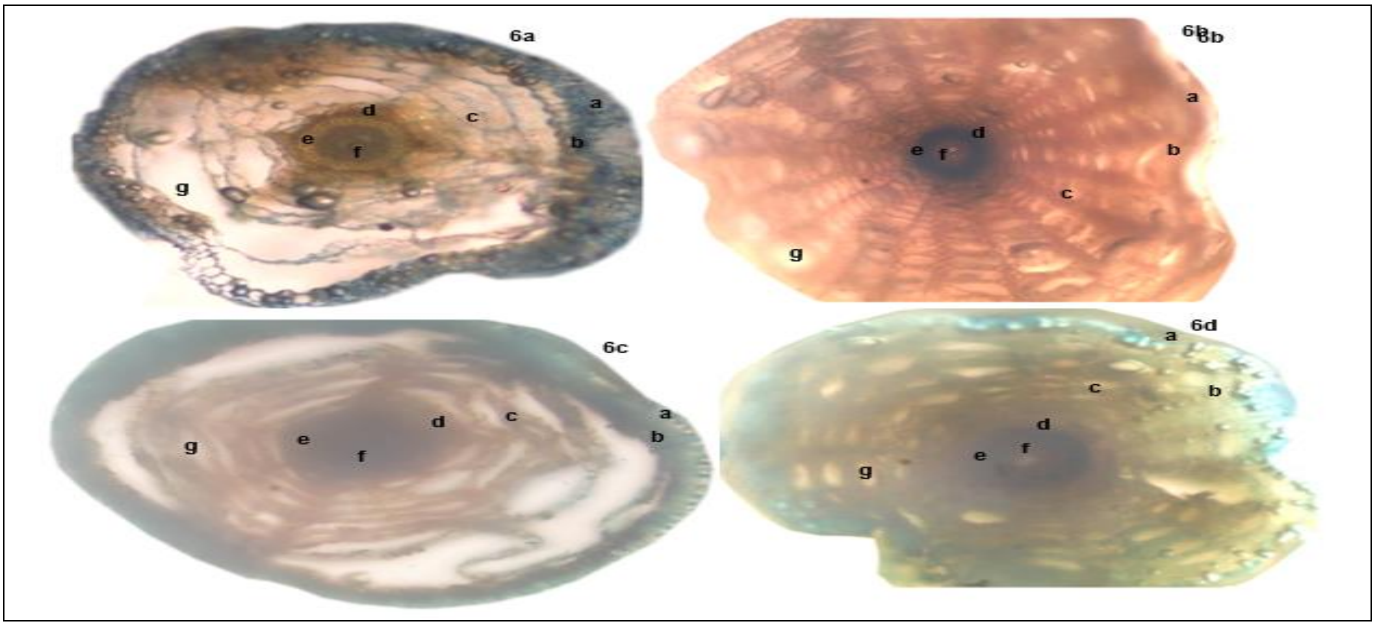

Plate 6 (6a - 6d): Root Sections of *Fimbrystylis Littoralis*

6a – Dry season polluted section; 6b - Dry season unpolluted section; 6c – Wet season polluted section; 6d - Wet season unpolluted section

a – Epidermis; b– Hypodermis c – Cortex; d – pericycle; e – Endodermis; f – Vascular bundle; g – Aerenchyma/Airspaces;

IV. DISCUSSION

In light of the current study sections of the various halophyte species at variance in the hydrocarbon polluted sites had diverse structural aberrations and challenges involving the species inability to pick up stains thereby limiting the clarity of focus under the microscope (Plates 2c & 4c), conversion of air/intercellular spaces into vacuoles filled with oil (Plate 4a & 4c), rearrangement of the vascular bundles (Plate 4a & 4c), thickening of the sclerenchyma cells (Plate 4a & 4c), damage of vital parts of stem and root tissues (Plates 1a, 1c, 2a, 2c, 3a, 3c, 4a, 4c, 5a, 5c, 6a, 6c) as well as shrinkage and detachment of cortex from the epidermis leading to the reduction of the air compartments (Plates 2a, 2c, 4a, 4c, 6a & 6c). All these are the anatomical distortion associated with the impact on hydrocarbon pollution on the internal structures of these halophytes when compared with the structures of sections in the controlled unpolluted site. This corroborates the assertion that crude oil hydrocarbon and contaminants can penetrate the roots of plants, thus affecting its nutrient uptake, thereby filling its intercellular spaces with oils, damage cell membranes and reduce the turgidity of plants by shrinking the cortex [16, 26]. Inability of these sections to pick up stains can be attributed to the non-polar and hydrophobic nature of the hydrocarbon and the degree of exposure these plants have with the hydrocarbon over a period of time. Also, thickening of the sclerenchyma is an adaptive strategy of some halophytes under acute stress to preserve its succulence [27] as seen in stem section of *Fimbrystylis littoralis* (Plates 5a & 5c). These three halophytes studied for their eco-anatomical affiliation in hydrocarbon polluted sites has revealed similarity as well as variation in the impacts, degrees and modifications of their internal structures which could also be attributed to their physiological adaptation, particularly in the root and stem tissues of these halophytes within saline hydrocarbon-polluted and unpolluted sites.

This research can be applied in subsequent scientific investigations as a foundation for the understanding of structural modifications and adaptations of plants, particularly in saline environment exposed to hydrocarbon pollution. It can also be applied in decision-making, ensuring that scientists and stakeholders place effective policies that control crude oil exploration and exploitation activities as well as illegal refining and vandalism within saline ecosystem. It can be applied to ascertain the level of anatomical modification and damage to plants in a polluted or stressed environment.

This result of this work can impact greatly the futuristic conservatory and environmental policies and standards. It would also act as a guide for researchers, institutes, Research and Development Unit of oil exploration organizations, Environmental regulatory bodies and the government to checkmate their activities and proffer workable solutions and/or alternatives, as a plant striving in a polluted environment can be damaged internally. By implication, the three halophyte species studied will add in easier understanding and add to existing knowledge of halophytes in saline ecosystem. The results of this study would help in revealing the impact of hydrocarbon pollution on the anatomy of halophytes.

V. CONCLUSION

The internal structures of the halophytes studied has revealed that changes in environmental conditions plays a significant role in their anatomy which also impacts on their morphology and growth over a period of time based on its resilience, degree and extent of pollution as well as the climatic season. Hydrocarbon pollution in the environment impacts negatively on plants since they are sessile organisms. However, the impact of hydrocarbon pollution is higher during the dry season than the wet season.

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Table 2: Summary of Hydrocarbon Pollution Impact on the Stem Anatomical Structures of Halophytes in Parts of Bodo Creek

S/N	Anatomical Part	Season	*Paspalum vaginatum*		*Rhynchospora corymbosa*		*Fimbrystylis Littoralis*	
			Polluted	**Control**	**Polluted**	**Control**	**Polluted**	**Control**
1	Epidermis	DRY	Partial distortion	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
2	Hypodermis	DRY	Partial distortion	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
3	Sclerenchymatous Tissues	DRY	Partial distortion	Intact	Intact	Intact	Thickening	Intact
		WET	Intact	Intact	Intact	Intact	Thickening	Intact
4	Air spaces/Air chambers/ Aerenchyma	DRY	Intact	Intact	Partial distortion	Intact	Conversion to vacuoles	Intact
		WET	Intact	Intact	Partial distortion	Intact	Conversion to vacuoles	Intact
5	Vascular bundles	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
6	Pith	DRY	Partial distortion	Intact	Intact	Intact	Not Applicable	Not Applicable
		WET	Partial distortion	Intact	Intact	Intact	Not Applicable	Not Applicable
7	Ground Tissue	DRY	Partial distortion	Intact	Partial distortion	Intact	Not Applicable	Not Applicable
		WET	Partial distortion	Intact	Partial distortion	Intact	Not Applicable	Not Applicable

Table 3: Summary of Hydrocarbon Pollution Impact on the Root Anatomical Structures of Halophytes in Parts of Bodo Creek

S/NO	Anatomical Part	Season	*Paspalum vaginatum*		*Rhynchospora corymbosa*		*Fimbrystylis littoralis*	
			Polluted	**Control**	**Polluted**	**Control**	**Polluted**	**Control**
1	Piliferous layer	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
2	Epidermis	DRY	Shrunk	Intact	Detached from cortex	Intact	Detached from cortex	Intact
		WET	Intact	Intact	Detached from cortex	Intact	Detached from cortex	Intact
3	Cortex	DRY	Partial distortion	Intact	Partial but severe distortion	Intact	Severe distortion	Intact
		WET	Severe distortion	Intact	Partial but severe distortion	Intact	Severe distortion	Intact
4	Vascular bundle	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
5	Air chamber	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
6	Pericycle	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact
7	Endodermis	DRY	Intact	Intact	Intact	Intact	Intact	Intact
		WET	Intact	Intact	Intact	Intact	Intact	Intact