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Impact of Polluted Soil on Herbivory of Leaves and Pneumatophore Growth of Black Mangroves (*Laguncularia racemosa*) at Eagle Island, River State, Nigeria

Peace K. Ohia [a], Aroloye O. Numbere [a*], Tambeke N. Gbarakoro [a] and Ibiene W. Dick-Abbey [a]

[a] Department of Animal and Environmental Biology, University of Port Harcourt, P.M.B. 5323, Choba, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mangroves are ecosystem along the shoreline of Nigerian coast and area of active oil exploration in Nigeria. This study is on the impact of pollution on herbivory and pneumatophore growth in black mangrove at Eagle Island. Leave herbivory, pneumatophore growth, THC and heavy metal concentration in soil, root and leave were determined. The sample site was divided into: plot A (high muddiness), plot B (low muddiness), plot C (slight muddiness) and Control (little or no pollution). Random sampling was used in obtaining leaves, soil and pneumatophore from each plot. Pneumatophore height was taken with meter rule and the weight with weighing balance. The leaf

**Corresponding author: E-mail: aroloyen@yahoo.com; aroloye.numbere@uniport.edu.ng;*

image was taken using a digital handy scanner and was uploaded in a software called Image J to measure the area consumed. EPA Method 418 was used to test for Total Hydrocarbon Content (THC). Heavy metals (Cadmium, Lead, Zinc) were determined using AAS- Atomic Absorption Spectrometric method. Results revealed that control plot had highest leaf consumption (2.200±0.33cm^2) compared to other plots. The heavy metals and THC concentrations in different plant parts (leaves and roots) revealed that THC was high in leaves (250.88±95.33mg/kg), while heavy metals were high in root The pneumatophores were taller and heavier in Plot A compared to Plots B and C. This study shows that pollution affects herbivory and pneumatophores growth in mangroves forest. Mitigation measures should be taken to prevent these pollutants.

Keywords: Herbivory; hydrocarbon pollution; mangrove; heavy metals; insects.

1. INTRODUCTION

“Mangroves are salt-tolerant plants that can thrive in marine and estuarine ecosystems” [1]. They are amphibious plants due to their ability to grow at the interface of terrestrial and aquatic ecosystems. Mangroves are land former through anchoring roots to hold sediment [2], which changes into muddy soil, which later hardens and turns to land [3]. Mangroves can endure the troublesome. Due to their unique root system, which is used for respiration [4], turbulent and salty coastal terrain, and heavy metal excretion [5], as well as salts [6]. Because it transports oxygen from the atmosphere into and out of the plant, the adventitious root of the mangrove is referred to as the "breathing root” [7]. “The breathing root fills in as the lungs of the mangrove trees” [8]. This explains why they can endure when lowered in an oceanic climate or affected by flowing flows. According to Spencer et al. [9], the mangrove coastal vegetation has evolved life-history adaptations to the difficulties of mobile establishment caused by current dynamics and the influence of ocean waves in high salinity (0-90 degrees/thousand) aqueous, anoxic sediments. Regardless, mangroves give beachfront security by lessening wave level and energy, going about as a characteristic boundary to approaching waves and diminishing disintegration [10].

“The root of the black mangroves (*Avicennia germinans*), which are vertical finger-like protrusions that stick out from the forest floor beneath the trees, is the pneumatophore” [11]. To promote atmospheric gaseous exchange, the pneumatophore emerges from the soil and grows into the atmosphere [12]. For roots that are submerged, air oxygen works in conjunction with underwater oxygen. These numerous pneumatophores create a radial circumference across the forest canopy at the foot of black mangrove trees [13]. According to Munir et al. [6], pneumatophores are biologically significant for controlling salinity exchange between the plant and the sea environment. They serve as a shutdown mechanism that stops the plant from absorbing too much salt solution, allowing the mangrove to withstand high sodium chloride concentrations and avoid osmotic cell collapse caused by too many salt crystals.

Additionally, the pneumatophores act as an erosion break [14], a conduit pipe for the delivery of water and nutritious minerals to the plant, and a means of excreting salt crystals and other toxic waste products from the plant [15]. “One of the mangrove's organs, the pneumatophore, allows it to thrive as an aquatic plant” [9]. “In addition, pneumatophores serve additional crucial roles in the mangrove ecosystem by supplying soil nutrients through the decay of their bodies” [14]. “When their litter attracts bacteria that carry out the degradation of organic materials, they increase the soil nutrients” [15]. The specific objectives of the study were to (i) Assess the herbivory on leaves, (ii) determine the Total Hydrocarbon Content (THC) and heavy metal concentration in soil, root, and leaves, (iii) compare the growth rate of pneumatophore, (iv) to compare the THC and heavy metal concentration between herbivory in the plot.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was conducted in a polluted mangrove forest in Eagle Island Port Harcourt (N04˚47.53; E006˚58.59). Eagle Island is bounded north by Rivers State University (Fig. 1), Diobu by the East, and Iwofe River by the south. The area is surrounded by swampy soil that is chocolate brown and borders a river course that is used for boat transportation. The soil is slightly alkaline, with a pH of 7.5. The temperature of the soil is 26.1±0.01 °C, the salinity is 1.16ppt, and the

TDS is 360×10 ppm. The area has two seasons, the wet and dry seasons. The dry season occurs from November to March, while the wet season is between March to October each year [15]. Several years ago, this area was covered by a luxuriant mangrove forest with some scattered Nypa palm trees.

Nevertheless, part of the forest is gone because of pollution and has also been abandoned. The area is in an early stage of succession by young mangrove seedlings with other non-mangrove plants such as grasses and aquatic weeds. The study site was randomly picked; plot A is a muddy area with stagnant water. Plenty of leaf drops on the soil, which helps to attract insects and micro-organisms for decomposition. In plot B, the area is a moist environment with little leave litter. Plot C is a dry area with little water, hence scanty vegetation. Control is an area with little or no pollution, a sandy area that is usually covered with water during high tides, hence no vegetation.

2.2 Experimental Design

The study collected samples randomly in an area measuring 94.78 m × 43.76 m (4152.24 m^2), which was further divided into four plots. Each plot was delineated equally using a standard tape measure at an accuracy of 0.1m. Two key areas of the plots are the seashore, which is sandy and coarse and close to the river (control), and wet soil (Plots A, B, C), which is muddy and silty and away from the river. Furthermore, the seashore site is always dry during the low tide and covered with water during the high tide, while the wet soil is constantly wet because it is supplied by river water during high tide and rainwater. Pictures of pneumatophores were also taken. The leaves were also collected from the trees in each of the plots.

2.3 Pneumatophore Growth and Development

"Pneumatophores dominate this study area because of the large population of black mangroves" [16]. "They grow in large or small populations underneath the black mangrove trees. They are made up of a soft, dark outer and a light inner coat. The outer coat is slippery and can easily be pulled away. The pneumatophores prevent the growth of other plant species at ~ 1.8 m in circumference around the black mangrove tree" [16].

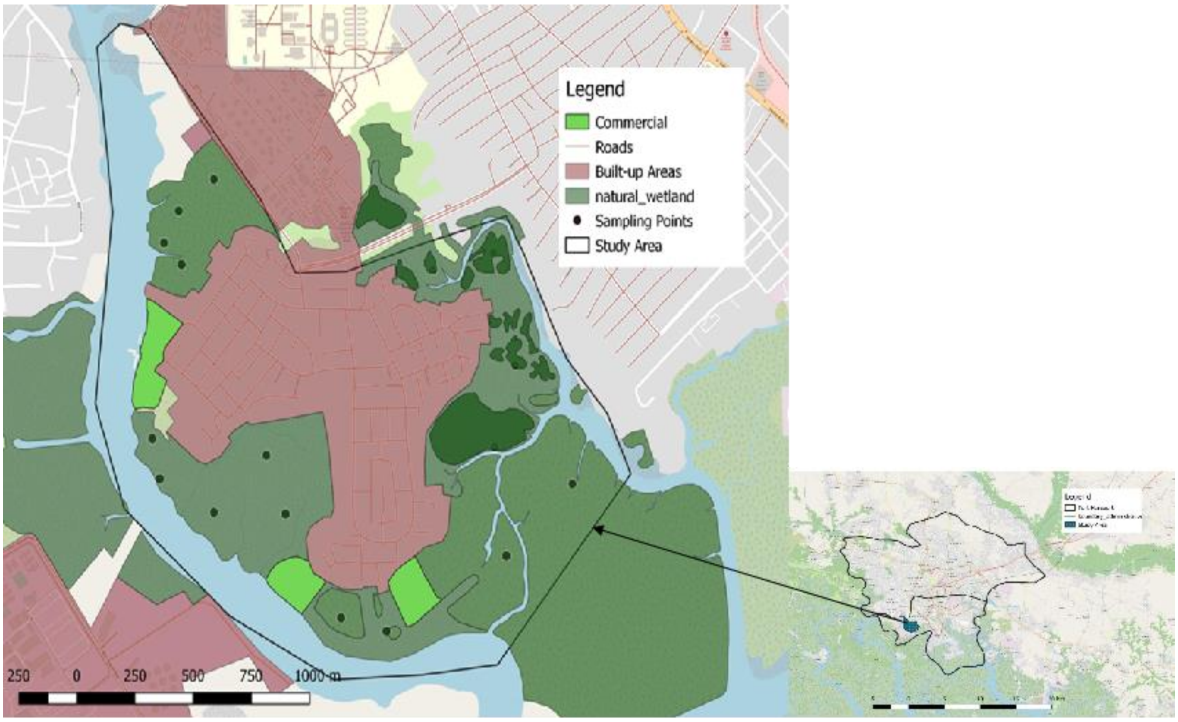

Fig. 1. Map of the study area at Eagle Island, Rivers State, Nigeria

2.4 Soil sand Pneumatophore Samples Collection

A hand-held soil augur was used to randomly collect soil samples from each plot 5cm below the soil surface within each transect (n = 10) in each plot. The samples were placed in a well-labelled polyethylene bags and sent to the laboratory for physico-chemical analysis. The seashore and stagnant pool sites were studied in detail to determine their influence on the growth and proliferation of the pneumatophore around the coastal mangrove ecosystem. The soil texture and composition were also studied. Ten pneumatophore samples from each plot were randomly pulled by hand, bagged, and sent to the laboratory for measurements of length (cm) and weight (g) at the level of accuracy of 0.1m and 0.1 g, respectively, and physico-chemically analyzed.

2.5 Herbivory Determination

The leave samples were randomly collected from trees in each plot. In each plot, the leaves were collected from the tree's high and low branches. A large number of leaves with bitten marks were collected. The total herbivory was determined by conducting an experiment in accordance with previous studies. We characterized herbivory by estimating the number of incisions or marks on leave made on the leave, which was seen as an attempt made by the herbivores to consume the leave.

2.6 Image analysis in Image J

An image impression of the leaf samples was made with a digital camera (Nikon) at a focal length of 30 cm. To confirm the validity of the images, a portable handy scan model TSN410 was used to acquire images in line with. The leaf area in pixels was converted to millimeters in image measurement software called Image J for the "pre" and "post" consumption values at a scale of 7.983 pixel mm^{-1}. The scanned leave sample was uploaded into the Image J software, after which the leave image was changed to 8 bits under the 'type' heading in the image toolbar. A freehand selection tool was used to fill the eaten area of the leave; under the 'process' toolbar, the image was converted to binary form, the leave was marked off with a rectangle, and the area of the leave calculated using the 'analyze' toolbar (Fig. 2). Also, the scaling was done using the analyze toolbar to an accuracy of 5cm. The type of herbivore bite marks made on the leaves were assessed and assigned count numbers following the example. The rate of herbivory for the exclusion experiment was calculated by computer estimation of the area of leave consumed in Image J. The leave area eaten (LAeaten) was calculated by subtracting the leave area after herbivory (LAafter) from the original leaf area before herbivory (LAbefore).

2.7 Determination of Physicochemical Parameters

Soil pH was determined with a Kelway soil tester while the soil compaction was determined with a pocket penetrometer. Soil temperature was determined with a digital dual sensor thermometer to a detection unit of ± 1˚C. The salinity of the pore water soil was determined with a salinity meter (OAKTON Salt 6 Acorn Series). The salinity meter probe was used to test standing water in dug-out holes during low tide. Total organic content (TOC) was determined using the Walkey-Black titrimetric method. The TOC was used to determine the nutrients in the soil. The TOC was determined because soil organic content influences soil texture and composition, which in turn influences mangrove growth.

2.8 Physico-Chemical Analysis

In determining the soil chemistry of the study area, the following soil physicochemical analyses were done: Total hydrocarbon (THC), Lead (Pb), cadmium (Cd), and Zinc (Zn). also, the plant sample was analyzed for the following parameters: Cd, Pb, Zn and THC using standard laboratory procedures described below.

2.9 Procedures of THC Analysis

It involved the use of a spectrophotometric method using the HACH DR 890 calorimeter (wavelength 420 nm). The samples were crushed, and 2 g of the crushed sample was weighed into a glass beaker. 20 ml of hexane was added, and with the aid of a glass rod, the mixture was homogenized by stirring. Afterward, the sample was filtered in a glass funnel packed with cotton wool, silica gel, and anhydrous sodium sulphate. After this, 10 ml of the filtered organic extract was transferred into a 10 ml sample curve and inserted into the calorimeter. The detection limit for THC is 0.01 mg/l.

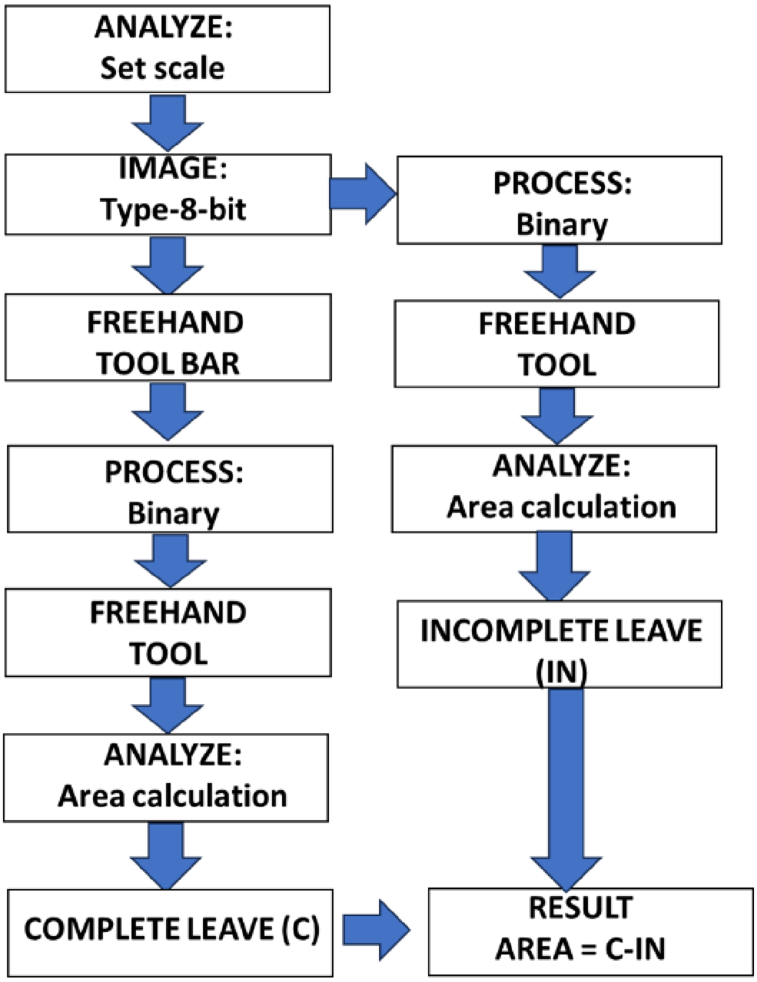

Fig. 2. Flow chart of herbivory analysis using Image J

2.10 Procedures of Heavy Metal Analysis

Heavy metals such as cadmium, chromium, lead, and zinc. These heavy metals were determined using the AAS-Atomic Absorption Spectrometry method.

2.11 Statistical Analysis

A randomized design was used in sample collection. An analysis of variance (ANOVA) was conducted since there were multiple samples per block to test whether there was any significant difference in herbivory pattern within plots and heavy metals. For the herbivory study twelve leave samples were randomly collected monthly in four plots for six months ($n=12 \times 4 \times 6 = 288$). Also, ANOVA was used to determine whether there were any significant differences between metal concentration and plots. Similarly, a post-hoc Tukey's HSD test was done to investigate pairwise mean differences between groups. Pearson's product-moment correlation was done to compare whether there was any significant difference between pneumatophore length vs. weight. All analyses were performed in the R statistical environment, 3.0.1 [17].

3. RESULTS

3.1 Amount of leave Area Consumed by Herbivores (Herbivory)

3.1.1 Complete versus incomplete

The ANOVA result indicates that there is a significant difference in the area of complete (leaf without herbivory) and incomplete leaves (leaf with herbivory) between three different sites ($F= 24.84$, $P< 0.001$, Fig. 3). Plot A has the highest area for complete and incomplete leaves, followed by plots B, Control, and Plot C.

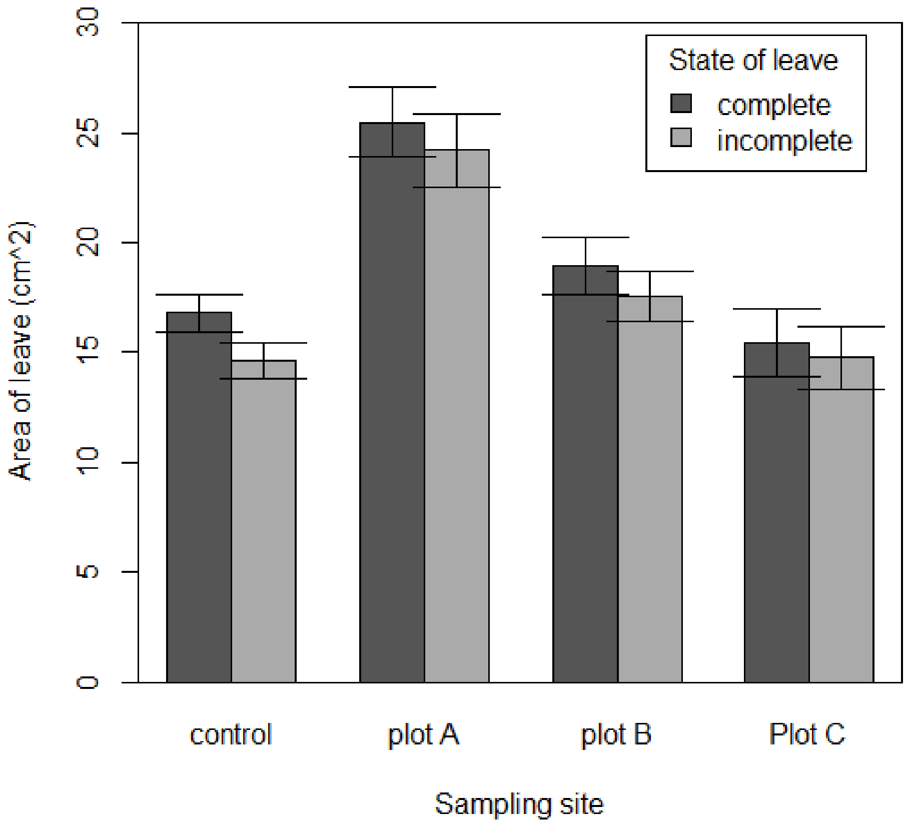

Fig. 3. Graph of complete and incomplete leaves collected from Eagle Island, Rivers State, Nigeria

3.1.2 The amount of leave area consumed from different sites

The ANOVA result reveals that there is no significant difference in the area of mangrove leaves consumed at different sites ($F_{3, 133,}$ 2.61, P>0.05, Fig. 4). However, the highest leaves were consumed in the control plot (2.200±0.33 cm^2) followed by Plots B (1.39±0.46 cm^2), A (1.26±0.33 cm^2), and C (0.65±0.24 cm^2) (Fig. 4).

3.2 Physico-Chemistry of Mangrove Plant Parts and Soil

3.2.1 The concentration of THC and heavy metals in mangrove parts and soil

The ANOVA result reveals that there is a significant difference in the concentration of THC and heavy metals (F= 3.17, P = 0.03, Fig. 5). The overall THC has the highest concentration, followed by zinc and lead. In contrast, there is no significant difference in the concentration of THC and heavy metals in soil, roots, and leaves ($F_{2\ 44}$, = 2.70, P = 0.08). However, there is a significant difference in THC between roots, soil, and leaves. Leaves have the highest concentration of THC (250.88±95.33 mg/kg) followed by root (7.21±3.68 mg/kg) and soil (0.16±0.10 mg/kg) (Fig. 5). In terms of site, there is no significant difference in the concentration of THC and heavy metals (F= 0.41, P = 0.75). The ANOVA table for all comparison is given in Table 1.

3.2.2 The concentration of THC and heavy metals in soils at different plots

The ANOVA result reveals no significant difference in the concentration of THC and heavy metals between the plots (F = 0.76, P=0.53, Fig. 6). However, the highest concentration of cadmium and lead was observed in plots B and C. In contrast, the highest concentration of THC was observed in plots in plots A and C, and the highest concentration of zinc was observed in plot A and control. Plots with the highest chemical concentration are plots B and C, while control has the most negligible chemical concentration.

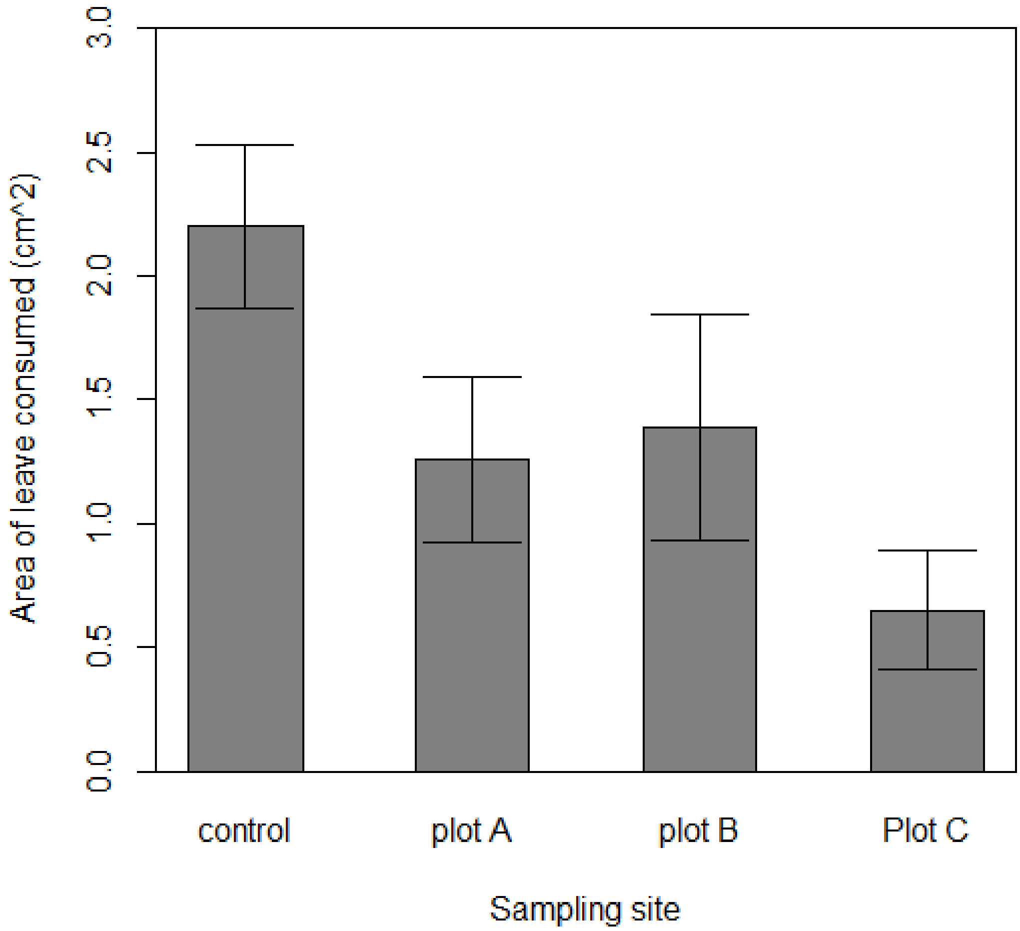

Fig. 4. The mean area of leaves consumed by herbivores at different sites in Eagle Island, Rivers State, Nigeria

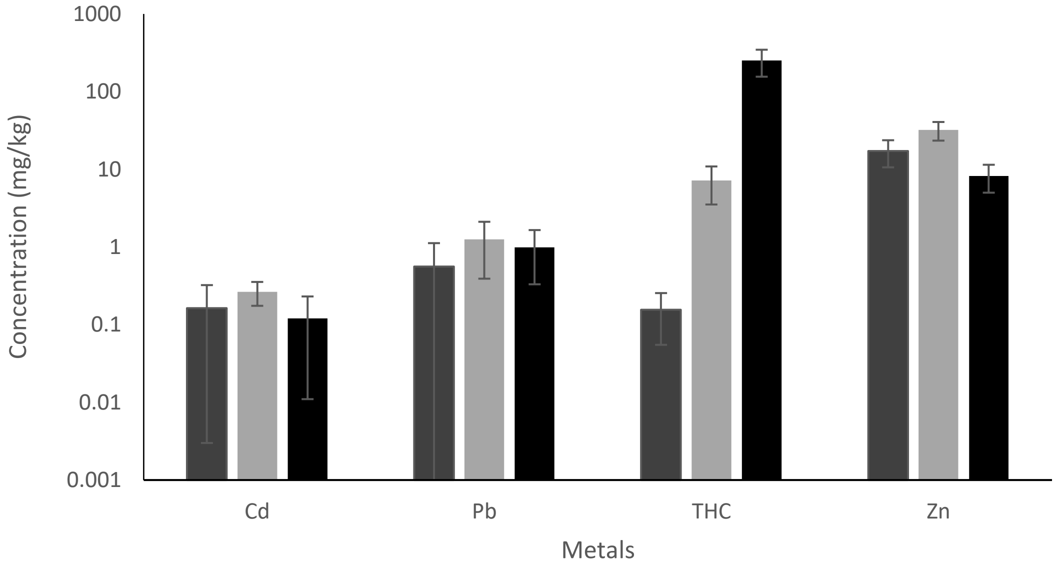

Fig. 5. The mean concentration of heavy metals in different parts of mangrove plant collected at Eagle Island, Rivers State, Nigeria

Table 1. One-way ANOVA of concentration of physico-chemical paramters, herbivory and allometry at different sites in Eagle Island, Rivers State, Nigeria

SOV	Df	SS	MS	F	P-Value
			Physico-chemistry		
Metals	3	88	111	3.17	0.03*
Plant parts	3	10	17	2.70	0.08
Sites	3	56	89	0.41	0.75
			Herbivory and allometry		
Site	3	11	123	2.61	0.08
Consumption	3	119	3980	24.84	0.001*
Height	3	95	1001	54.61	0.03*
Weight	3	95	1011	32.72	0.02*

**significant*

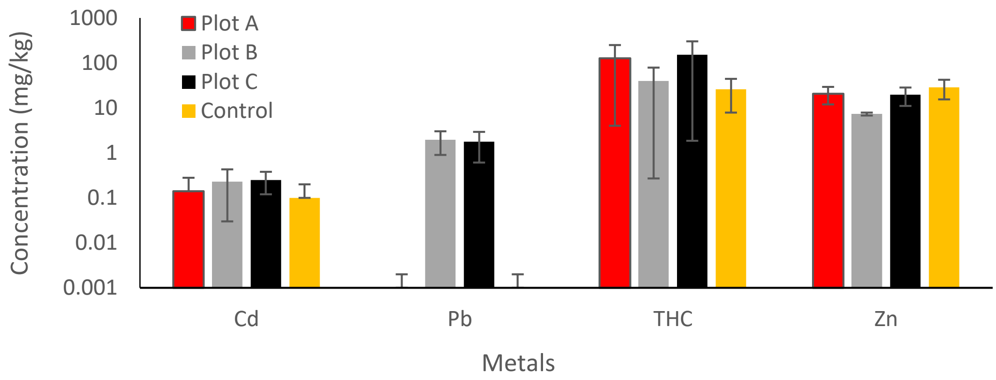

Fig. 6. Mean THC and heavy metals concentration in different plots at Eagle Island, Rivers State, Nigeria

3.3 Allometric Measurement of Pneumatophores

The ANOVA result shows that there is a significant difference in the height ($F_{3, 95,}$ = 54.61, P<0.05) and weight (F= 32.72, P<0.05) of mangrove pneumatophore between plots (Figs. 7 and 8). The plot with the longest above-ground pneumatophore is plot A (34.76±1.09 cm), while plot B has the shortest pneumatophore (17.9±1.17 cm). Similarly, the plot with the heaviest pneumatophore is plot A (7.52±0.28 g), while plot C has the lightest pneumatophore (3.10±0.21 g).

3.4 Relationship between Herbivory, soil Chemistry and Allometry

There is a positive correlation between root height and weight, which means the weight increases as the root grows taller (Fig. 9). A contributing factor to rapid growth is the presence of soil nutrient and low concentration of pollutants. There is also a relationship between the leave, root and soil metal concentration (Figs. 10 and 11).

4. DISCUSSION

Results from this study showed that polluted soil directly influenced herbivores' consumption of mangrove leaves. The study was conducted in an area that has been polluted for decades. A higher amount of leaf was consumed in plot A (i.e., high muddiness) than in plot B (medium muddiness), plot C (low muddiness), and Control (sandy). In the mean area of leaf consumed, consumption was high in Control, followed by Plot B, Plot A, and Plot C. The high consumption could result from the leaf's palatability, which influenced consumption.

According to Numbere and Camilo [18], the palatability of mangrove leaves increases the herbivory rate of mangrove leaves. Environmental factors such as rain, temperature, and salinity could also play a vital role in the high consumption of leaves in the study area; this finding is in agreement with the findings of Silva and Maia [19], who stated that the salinity of the environment was a determining factor for herbivory since the highest values of leaf herbivory in mangrove species occurred during high salinity.

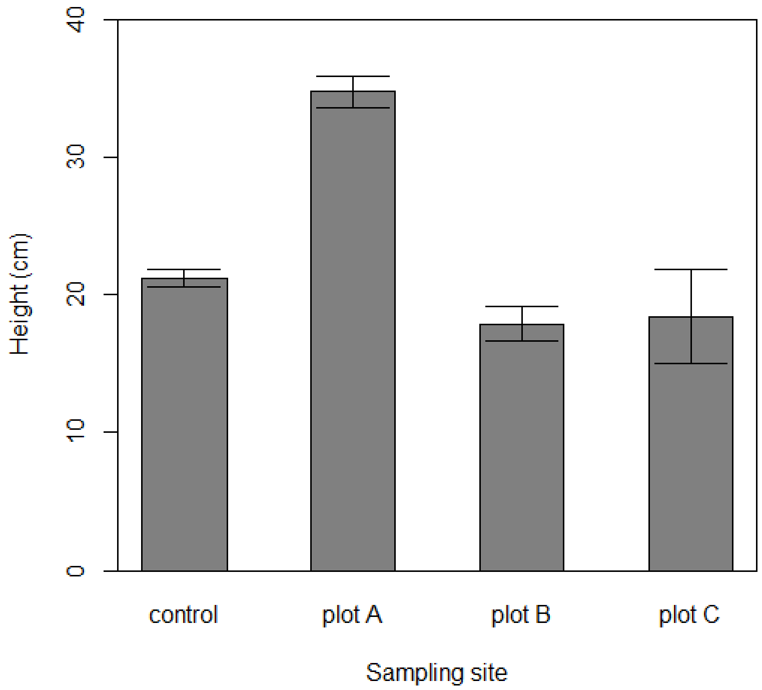

Fig. 7. Mean height of pneumatophore collected from different plots at Eagle Island, Rivers State, Nigeria

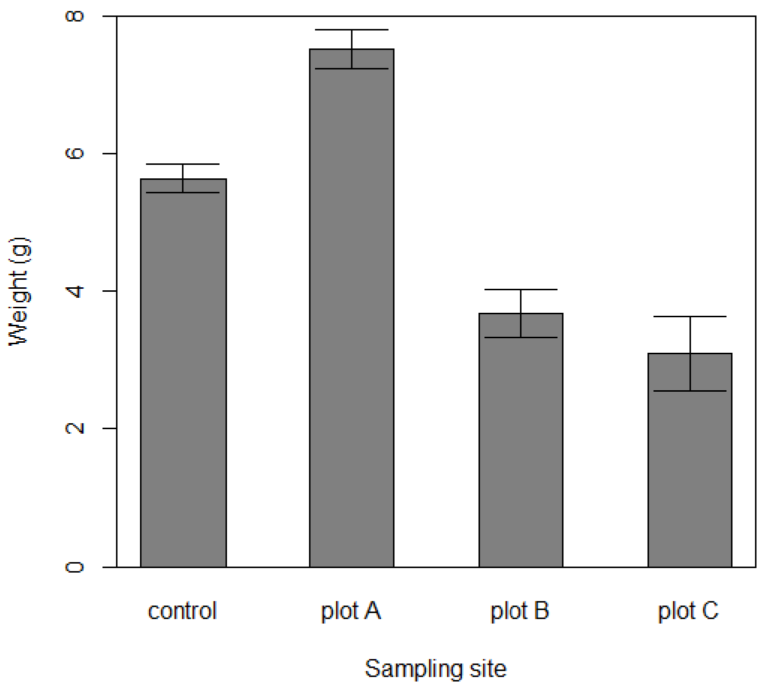

Fig. 8. The mean weight of pneumatophore was collected from different plots at Eagle Island, Rivers State, Nigeria

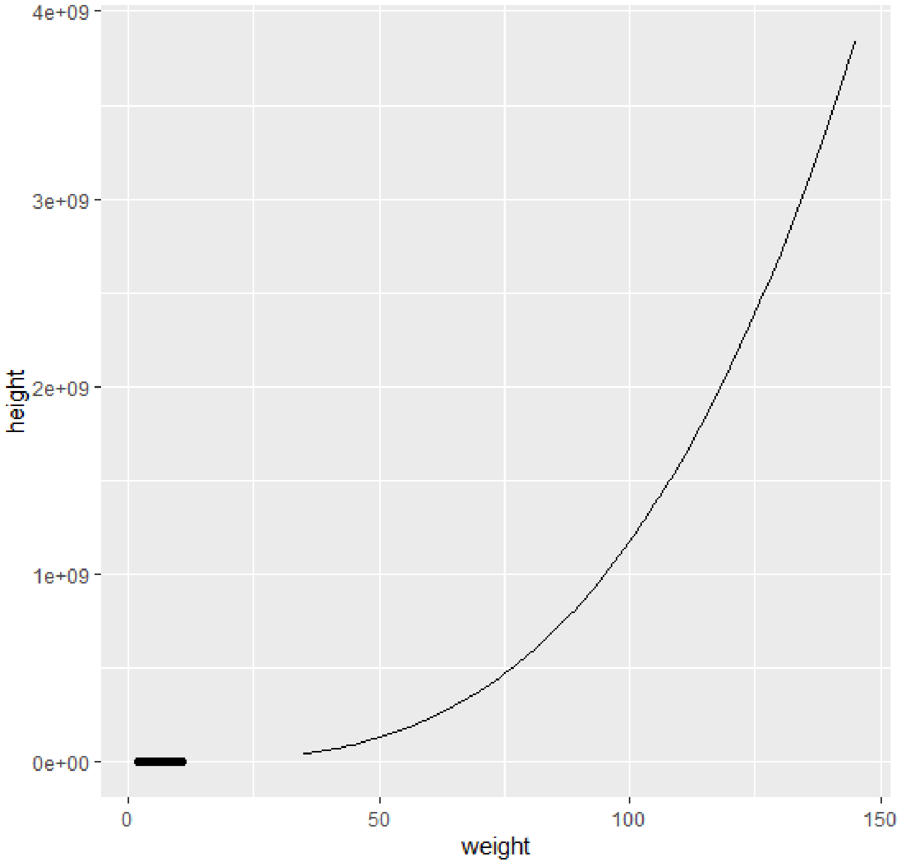

Fig. 9. Correlation between mangrove pneumatophore weight and height

The low consumption in plots B, plot C, and Control is likely to result from the presence of chemicals in the leaf, which is in line with the findings of Tong et al. [20], who revealed that the presence of chemicals in the leaf can prevent herbivore feeding. This aligns with the hypothesis that pollution will prevent the herbivory of mangrove leaves. The herbivory on the leaf is an indicator that shows that a mangrove forest with adequate environmental factors such as humidity, temperature, and salinity and the absence of pollutants will increase the herbivory rate on the leaf.

“Mangroves are habitat-specific and grow only in swampy soils. This is because most mangrove species apart from *A. aureum* (mangrove fern) cannot grow well in sandy soil because it has low salinity and conductivity. A mangrove swamp is one of the largest carbon sequesters in the world because of its air purification ability and high productive capability” [21, 18]. Swampy soils have higher heavy metal loads because of their exposure to oil spillages from oiling activities onshore and offshore. Pollution of the shorelines destroys swampy soils by reducing salinity and destroying microbes within the soil.

A known characteristic of mangrove swamps is their ability to decompose [22], making them a biodiversity hot spot [23]. But when human activities such as deforestation, sand mining, and urbanization degrade the soil, they find it challenging to carry out their function as host to numerous soil-dwelling organisms. There was no significant difference in THC concentration between heavy metals in the soils. This means total hydrocarbon contents percolate into the sub-surface quickly after decomposition to contaminate the groundwater aquifer. The result of no significance of heavy metals and THC across soil gradients shows that soil pollutants from polluted sites can migrate and spread outwardly or circumferentially to contaminate neighboring soils [24]. This can be harmful to organisms around the sites, such as the fiddler crabs (*Uca tangeri*), west African red mangrove crab (*Goniopsis pelii*), mud skipper, and tilapia species, which are captured and eaten by the local people. “Changes in heavy metals and nutrients can also influence the distribution of mangroves and other plant species in a wetland area. Mangroves play an environmental role by acting as a biofilter of heavy metals" (Kangkuso *et al*., 2017). High concentration of Zn can be ascribed to increased land runoffs and influx of metal-rich water in the soil, giving rise to elevated metal levels.

"Pneumatophore is vital in circulating oxygen and as a breathing apparatus in mangrove forests. Pneumatophore helps in carbon sequestration by absorbing atmospheric carbon dioxide through the root system. This study shows that soil chemical and microbial composition influences the growth of pneumatophores" [25] "Areas with silt and muddy soils have taller and more abundant pneumatophores than areas with sandy soil" [26]. In this study, the result showed that plot A has the highest mean weight and mean height of pneumatophore; this could be because of a stagnant pool in the plot. The stagnant pool, being a more stable environment, supports more pneumatophores, which grow taller and transmit more oxygen into the mangrove environment and surrounding sediment. Although the rich supply of pneumatophores attracts spawning organisms [27], it also serves as a trap for plastic pollutants [28] that contaminate organisms [29]. The stagnant pool has the largest population of pneumatophores because the standing water acts as a trap for organisms, which die and decompose to increase the total organic content of the soil. In addition, when the sun heats the pond, chemical reactions do occur (i.e., hydration and hydrolysis), which erodes the subsoil, leading to an increase in the water's acidity and heavy metal concentration.

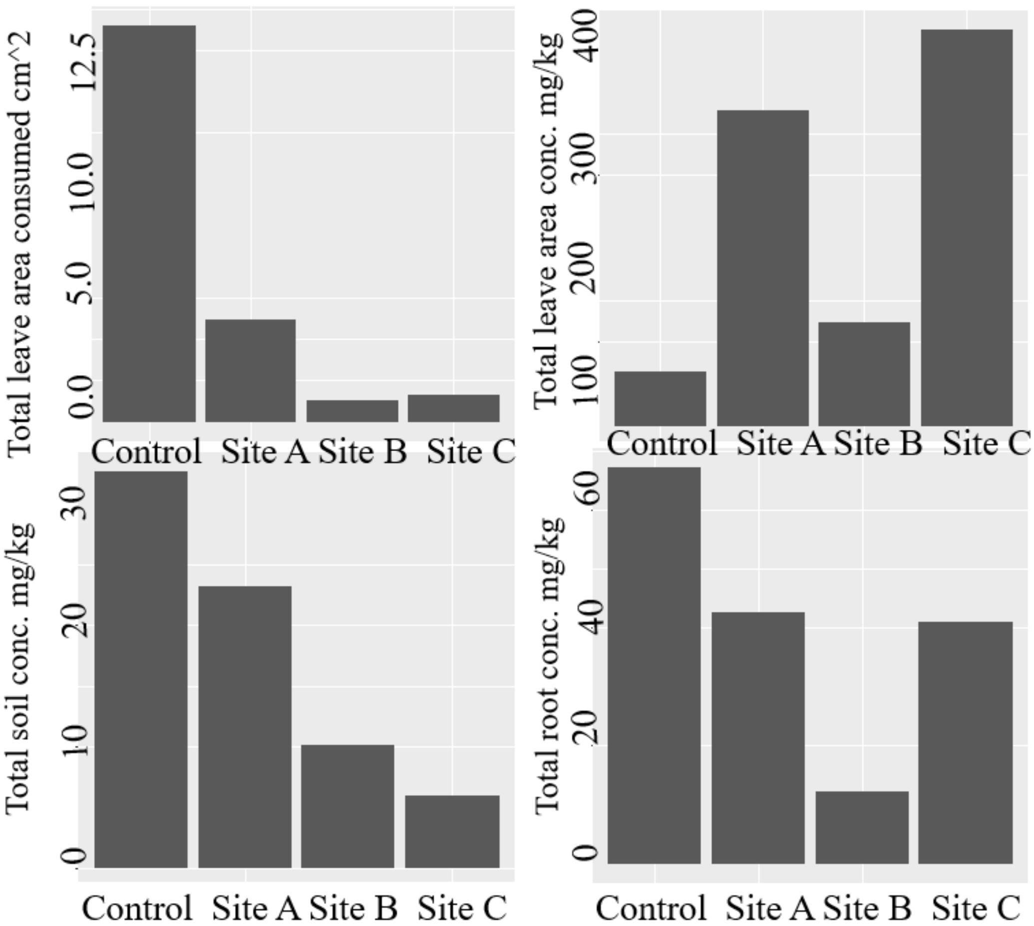

Fig. 10. Bar chart showing the relationship between the area of leaves consumed, leaves, soil, and root chemistry of mangroves at Eagle Island, Rivers State, Nigeria

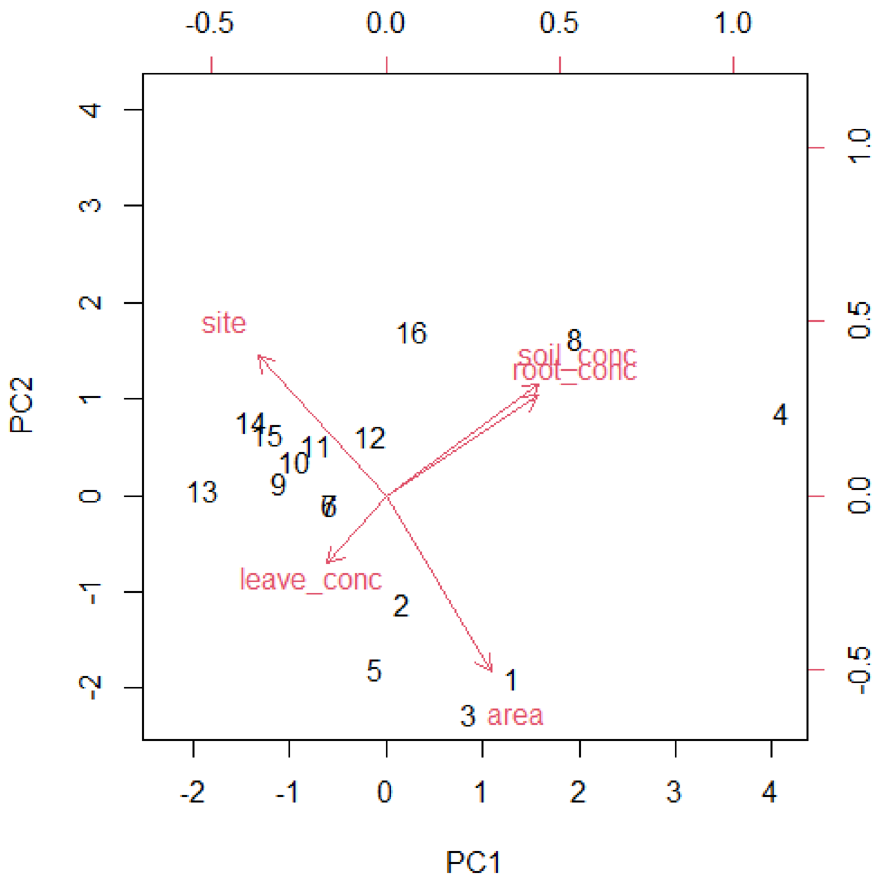

Fig. 11. PCA diagram showing the relationship of leave, soil and root concentration at different sites in Eagle Island, River State, Nigeria

The decomposed leaves form organic matter that increases the fertility of the soil, leading to the growth of more pneumatophores. Pneumatophores help the black mangroves to survive the harsh swampy environmental conditions by acting as the channel for oxygen transmission into the plants. Their presence in the mangrove forest also contributes to climate stabilization through their action of carbon sequestration. In this study, Plot B, Plot C, and Control have the lowest mean weight and mean height of pneumatophore, which may be because of the presence of sandy soil that has little organic content, mostly because of the "flushing action" of the tidal force that sweeps the surface clean of any plant and animal materials during high tide.

In the interaction effect, plot has low chemical concentrations followed by plots C and A, while control has high concentration because it is close to the shoreline and makes the first contact with pollutants brought in by tidal currents. Control has high herbivory compared to plots A, B and insect and other herbivores consumed more leaves from the control plot. But in contrast control has higher soil, root and leave chemical concentration. The leaves are free from pollutants because some leaves absorb pollutants and defoliates thereby freeing other leaves from pollutants.

Chemical concentration in leave is high in plots A and C while the chemical concentration of soil and root is high in control. Pollutants in the soil migrate through the root to the leaves, which eventually defoliates to free the tree from taking in excess chemicals. Thus, for an area to have high herbivory several factors play a role such as the site, soil and the pollution activities. Our study revealed that soil and leave pollution does not influence the amount of leave consumed (herbivory). This is because insects consume more leave area in trees growing in polluted soil. Mangroves are able to survive in polluted soil because of some defensive mechanism such as shutting down system of the root which blocks the transmission of substances from the soil through the root. There is also the accumulation of pollutants in leaves which later falls off to prevent the contamination of other plant parts.

5. CONCLUSIONS

The mangroves of the Niger Delta are one of the most productive systems in terms of biodiversity and ecosystem services worldwide. However, because of a lack of data, they are often not mentioned in many literature. This study shows that herbivory was influenced by soil pollution, which led to an increased consumption of mangrove leaves in lowly polluted plots. Since soil plays a crucial role in mangrove growth, there should be constant monitoring of soil quality to forestall drastic changes that will jeopardize the survival of the mangroves. Finally, it concludes on the importance of having prior knowledge about the sources, chemistry and potential risks of toxic heavy metals in contaminated soils to select the appropriate corrective options to conserve these productive and diverse ecosystems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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