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Comparison of Microbial and Heavy Metal Contents in Soils and Roots under Mangrove Forest Stands with Different Levels of Pollution in the Niger River Delta, Nigeria

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Abstract: Oil and gas exploration in the Niger Delta, has resulted to increased heavy metal contamination. It is thus postulated that increased heavy metal will lead to decreased microbial content in soil and root. To test this hypothesis ten replicates each of soil and root samples were collected from already established sites. Samples were sent to the laboratory for analyses of heavy metals (spectrophotometric method), total hydrocarbon (colorimetric method) and microbial (Sabouraud Destrose Agar) contents. The result indicates that bacterial population outnumbered fungal population in soils and roots. Similarly, there were significant differences in both microbial and heavy metal contents between highly and lowly polluted plots (p = 0.0001) and between polluted and non-polluted locations (p = 0.0001). Heavy metal has negative linear relationship with microbial content. As heavy metal increases the microbial activities reduces with implication on litter decomposition and nutrient cycling, which may eventually affect mangrove growth and development.

Keywords: Microbial Content, Hydrocarbon Pollution, Heavy Metal, Mangroves, Niger Delta

Introduction

Mangroves are habitat for microscopic and macroscopic organisms such as bacteria and fungi, which inhabit water, soil and plant tissues. Bacteria have high ubiquity and control the chemical environment of the mangrove forest (Ferreira *et al*., 2007a; 2007b). They are primary decomposers in anoxic mangrove sediments (Peter and Sivasothi, 2002). They sequester nutrients in nutrient poor mangrove soils (Twilley, 1988) and are involved in nitrogen cycling (Whigam *et al*., 2009) and litter decomposition (Mishra *et al*., 2012). Some bacteria are pathogenic agents and reside in the guts of mangrove-based organisms (Harris, 1993). Others live on mangrove surfaces as epiphytes e.g., pathogenic *vibrio* that lives on mangrove pneumatophores (Abhaykumar and Dube, 1991).

Fungi are also involved in nutrient cycling (Hyde and Lee, 1995) and have more affinity for dead (high presence of amino acids) than fresh (high presence of tannins) leaves (Ravikumar and Kathiresan, 1993). Fungi reside in roots (Nair *et al*., 1991; Treseder and Cross, 2006) and sediments (Soares *et al*., 1997) and some are harmful to mangroves (Tattar *et al*., 1994).

The close proximity of mangroves to urban centers makes them recipients of heavy metal contamination (Mackey *et al*., 1992; Lacerda *et al*., 1993). Although, mangroves are regarded as poor indicators of trace metals (Kathiresan and Bingham, 2001), large amounts of heavy metals are found in mangrove soils while few are found in plant tissues (Silva *et al*., 1990). Even in low concentration heavy metals are poisonous (Goyer, 2001) because of bioaccumulation (MacFarlane and Burchett, 2000). Plants that grow in contaminated sediments absorb some amount of heavy metals.

Increasing levels of heavy metals have harmful effects on plants and microbes. Heavy metals inhibit plant growth and photosynthetic activity. Since heavy metal cannot be degraded biologically they accumulate in plant tissues from soils and cause long-term damaging effects on the plant. This is especially true in mangrove soil, which tends to accumulate heavy metals effectively due to their small particle size (Forstner, 1989; Clark *et al*., 1998; Lewis and Mc Conchie, 1994). The concentration of heavy metals in mangrove is proportional to the levels of metals in the weakly bound sediment fraction. Mangrove mud possesses intrinsic physical and chemical properties that enable them to accumulate materials

discharged to the near shore marine environment (Harbison, 1986). Although, mangrove ecosystems can act as sink for heavy metals, they can also become source of pollution to plants and soils. According to Koppe (1981), lead accumulations in localized areas of pollution sources probably have little direct effect on plants. Mangrove roots often act as a barrier and retain most of the heavy metals. This action reduce the translocation of heavy metals to other parts of the plant. Very small amount of heavy metals are found in leaf tissues as most absorbed heavy metals are accumulated in the stem and roots (Yim and Tam, 1999).

In this study, it was predicted that heavy metal levels will be higher in polluted sites while microbial content will be lower because of the negative effect of pollutants on microbes. Following this prediction the question posed was will heavy metal and microbial content have positive or negative relationship? To address this question, we outlined the following specific objectives: (1) To determine microbial content at different levels of pollution and location; (2) to determine the heavy metal content at different levels of pollution and location; and (3) to determine the relationship between heavy metal and microbial content.

Materials and Methods

Study Area

Okrika sampling site (4°43 N and 7°05 E) is close to a major refinery that supplies petrochemical products and crude oil abroad (Fig. 1). Its mangrove forest is uniquely divided into two sections by an access road (~5 m wide) that leads from the refinery to the jetty, where crude oil is evacuated into ships. Running parallel to the road and about 1m apart are ten sets of giant nickel/steel alloy-plated crude oil pipelines (~diameter 8-10 inches) that convey crude oil and petroleum products from the refinery to the jetty head (Fig. 1). These two features created an artificial partitioning of the mangrove forest into two separate sections. For the purpose of this study, I delineated the forest into plot A (~40 away from the pipelines) and plot B (~100 m away from the pipelines). Hence plot A was assigned as the highly polluted plot because of its closeness to the pipelines and having direct impact of oil spillage. Then plot B is assigned as the lowly polluted plot because of its distance away from the pipelines and having less impact of oil spillage. Total hydrocarbon content in soil is higher than that of root for all study areas. The THC for lowly and highly polluted plot and for polluted and non-polluted locations is significantly different from each other (Table 1). This delineation is further validated by an earlier study carried out in the area which showed that total hydrocarbon content was higher in highly polluted plot than in lowly polluted plot (Numbere, 2014). Detail description of the study area is found in Numbere and Camilo (2016).

The second sampling site is Eagle Island (4°49 N and 6°58 E). Several years ago this site was exclusively a mangrove forest interconnected by rivulets and creeks. This area is now fragmented into small mangrove islands within the river system because of increased human activities such as dredging, construction of residential quarters and establishment of local wood industries. This location was delineated as the control (non-polluted area) because it has no oiling activities, but more human activities. Around the swamp there are stagnant pools of muddy water mixed with wood shavings and saw dust that came from the nearby saw mill industries. The two study areas are rain fall belt with rain occurring throughout the year except November, December and January that has little or no rains. Peak rain is in May and the average annual rainfall is 1466.0 mm. Mean soil pH range is between 6.2-6.9, mean salinity range is 21.7-26.3 ppm and mean temperature range is 27.1-30.5°C for all locations.

Sample Collection

Both locations were sampled for one year i.e., January-December, 2016 and soil and root samples taken within this period. The soil samples were collected with a hand-held soil augur (Scotts, Germany) from a depth of 5 cm below the surface under ten randomly selected and geo-referenced red mangrove trees (*Rhizophora racemosa*, Meyer) at both locations. The samples were placed in a black cellophane bag and sent in a cooler to the laboratory. Root samples were also collected with a knife from the mangrove stands at the same spot where the soil samples were collected. Because of the giant adventitious root system of the red mangroves (*R. racemosa*), which protruded from the soil, young root samples beneath the soil surface under the trees were taken. The root samples were cleaned of soil, placed in a cellophane bag and transported to the laboratory for analysis.

Laboratory Analysis

At the laboratory the following bio-pysico-chemical parameters were analyzed: Microbial content i.e., Total Heterotrophic Bacteria (THB), Total Heterotrophic Fungi (THF) and heavy metals i.e., lead (Pb), Cadmium (Cd) and Cromium (Cr) and Total Hydrocarbon Content (THC). The three heavy metals were chosen because they are often associated with crude oil pollution. The laboratory analyses of the microbial and heavy metal contents followed standard laboratory procedures (APHA, 1992). Individual bacteria and fungi species were not analyzed; only the collective microbial content using the total bacterial and fungal counts were considered. Ten replicate samples were randomly collected at highly and lowly polluted plots and another ten samples at polluted and non-polluted locations.

Table 1: Mean soil and root Total Hydrocarbon Content (THC) in highly and lowly polluted plots and polluted and non-polluted locations in mangrove forest in the Niger River Delta, Nigeria

	THC (mg/l) ±SE			
Study areas	Soil	Root	F	P
Low (Site A)	234.3±1.3	208.6±1.5	168.30	0.0001*
High (Site B)	301.0±2.8	225.1±3.5	286.60	0.0001*
Polluted (Okrika)	267.7±2.0	216.9±2.7	38.01	0.001*
Non-polluted (Eagle Island)	32.5±3.80	26.4±0.60	6.40	0.02*

*Signficance

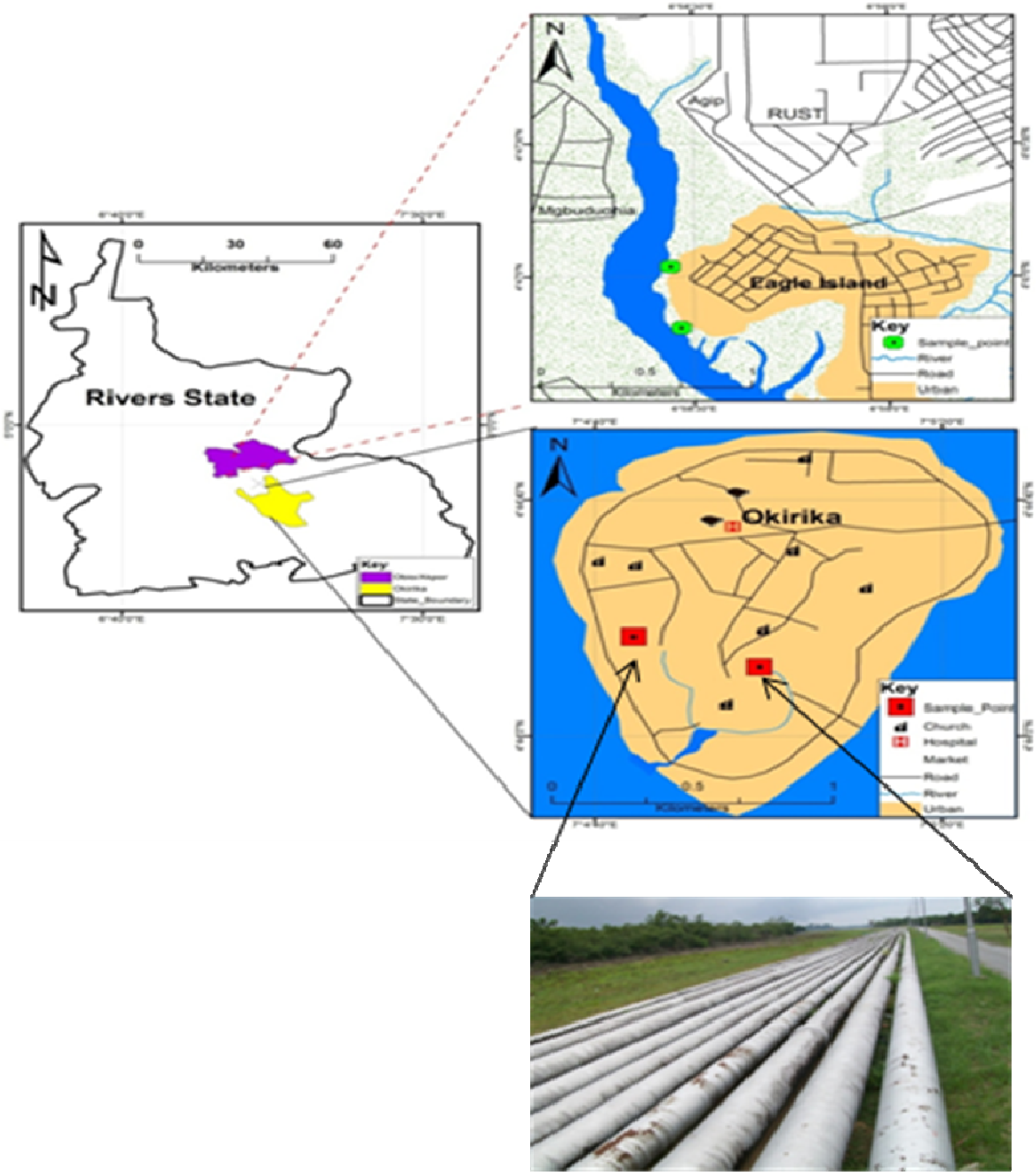

Fig. 1: Map showing study areas Okrika and Eagle Island, Niger River Delta, Nigeria

Determination of Microbial Content

The THB and THF were determined using similar method; the only difference was in the use of Nutrient Agar for THB, which was incubated at room temperature for 24 h. Firstly, the Sabouraud Dextrose Agar (SDA) medium was prepared and sterilized in autoclave at 121°C for 15 min, after which it was poured into a petri-dish and allowed to cool. Later 0.1% lactic acid (i.e., 10 mL) was added to the agar to inhibit bacterial growth while serial dilutions were done to further reduce the microbial load on the root and soil samples. After the serial dilution was done, 0.1 mL of the sample was taken from the last dilution factor, placed in the agar plate and cultured. The microbial content (i.e., THB and THF) was then enumerated and expressed in (cfu/g) $\times 10^5$. These laboratory procedures were done for both soils and root samples. The root samples were washed, dried in an oven at 60°C for 48 h and then crushed to extract sample for analysis.

Determination of Total Hydrocarbon and Heavy Metal Concentration

The THC was determined using colorimetric method (model: DR 890 HATCH colorimeter). The root and soil samples were oven-dried at 60°C in the drying oven (Memert U270) for 24 h to get rid of the moisture. The dried samples were crushed and 2 g weighed into a glass beaker and 2 mL of hexane added. The samples were homogenized with the use of a glass rod by stirring. After that the samples were filtered through a glass funnel packed with cotton wool and silica gel (i.e., anhydrous sodium sulphate). After the filtration, 10 mL of the filtered organic extracts were transferred into 10 mL sample covet and inserted into the colorimeter. Total hydrocarbon content was expressed in ppm which is equivalent to mg/l.

Heavy metals were determined using Atomic Absorption Spectrophotmetric (AAS) method (Model: GBC Avanta, PM). Here inorganic acid i.e., Hydrochloric acid (HCL) and nitric acid (HNO_3) were used to extract the metals from the samples before being analyzed in Atomic Absorption Spectrophotometer (AAS). The AAS was used because there is no spectral and ionization interferences. This process was used for soil and root samples. The root samples were washed, dried in an oven at 60°C for 48 h and then crushed to extract sample for analysis.

Statistical Analysis

The mean and standard error of microbial and heavy metal contents were calculated and graphed. The microbial and heavy metal data were analyzed statistically using Analysis of Variance (ANOVA) because it is a more robust tool in analyzing data with more than two treatments. The dependent variable was the microbial count while the explanatory variables were plots (high Vs. low), species (THB Vs. THF) and treatments (soil and root sample). A 3-way ANOVA was performed after logarithmic transformation to normalize the count data. Similarly a 3-way ANOVA was performed for the heavy metal concentration, where concentration was the dependent variable and the explanatory variables were treatments (soil and root), location (polluted Vs. non-polluted locations) and heavy metals (Pb, Cr and Cd). One was added to the data (i.e., Y+1) because of the zeros after which it was log transformed (Logan, 2010). To determine the relationship between heavy metal and microbial content in soil and root, an Analysis of Covariance (ANCOVA) was performed, where the categorical variable was the treatments (i.e., soil and root) and was regarded as a factor, while the heavy metal was used as a dependent variable. All the analyses were done in R statistical environment 3.0.1 (RDCT, 2016).

Results

Microbial Content

The average THB in polluted location for root is 2.0×10^5 cfu/g and for soil is 4.7×10^5 cfu/g. Also the average THB in non-polluted location for root is 2.2×10^5 cfu/g and for soil is 4.2×10^5 cfu/g. The THF in polluted location for root is 1.3×10^5 cfu/g and for soil is 3.3×10^5 cfu/g. Also the THF in non-polluted location for root is 1.5×10^5 cfu/g and for soil is 3.2×10^5 cfu/g. Soil bacteria count was more than fungal counts within plots and across locations (Fig. 2a and 2b, 3a and 3b). Similar microbial trend was observed in Okrika jetty several years ago where THB ranged from 1.9 to 3.6×10^5 cfu/g and THF ranged from 2.4 to 35×10^2 cfu/g (EIA, 2001). The range in the present study is slightly higher than root *Azotobacter* count derived by Selvam and Kathiresan (2010) for *Rhizophora* (1.3 cfu/g$\times10^5$) and *Avicennia* (1.5 cfu/g$\times10^5$) in India.

The result indicates that there was a significant difference in bacteria and fungi contents ($F_{1, 176} = 76.9$, $p = 0.0001$) for lowly and highly polluted plots. Similarly, there was also a significant difference in the microbial content in soil and root ($F_{1,176} = 361.8$, $p = 0.0001$), but no significant difference across plots ($F_{1, 176} = 0.7$, $p = 0.4$). There were significant differences in bacterial and fungal counts ($F_{1,116} = 111.1$, $p = 0.0001$) at both locations ($F_{1,176} = 1.3$, $p = 0.0001$) and between root and soil samples ($F_{1,176} = 454.0$, $p = 0.0001$). Lowly polluted plots had more soil microbial content than highly polluted plots, but for the root the reverse is the case as highly polluted plot had more microbial content than lowly polluted plot (Fig. 3a). The microbial content in non-polluted location is slightly higher than the content in polluted location (Fig. 2b and 3b). Similarly, the root microbial contents for polluted and non-polluted locations are not too different from each other (Fig. 3a and 3b).

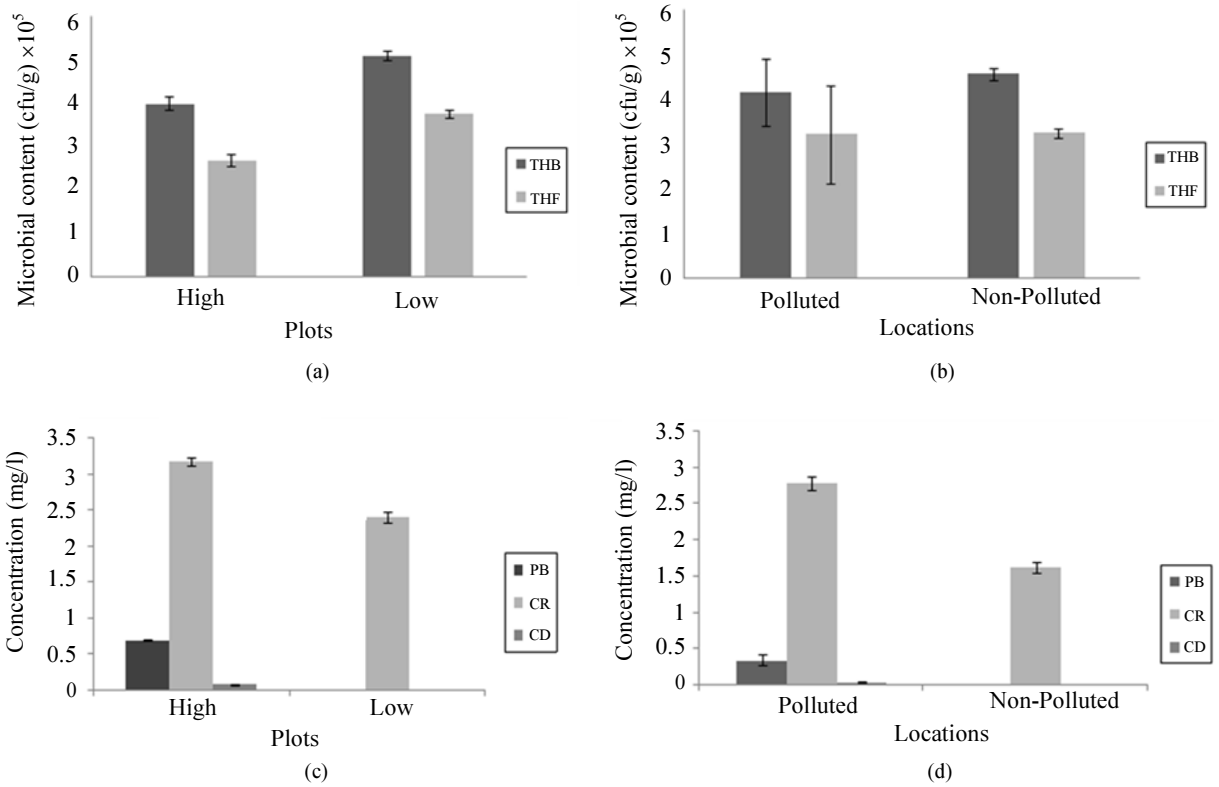

Fig. 2: Graphs of microbial and heavy metal concentrations in mangrove soil: (a) Microbial content in highly and lowly polluted plots (b) Polluted and non-polluted locations; and heavy metal concentrations in (c) Highly and lowly polluted plots; (d) Polluted and non-polluted locations in the Niger Delta, Nigeria. THB = Total Heterotrophic Bacteria and THF = Total Heterotrophic Fungi. Vertical lines show ±1 standard error of the mean

Heavy Metal Concentration

The mean soil concentrations for Pb (0.34 mg L^{-1}), Cr (2.80 mg L^{-1}) and Cd (0.04 mg L^{-1}) in the non-polluted site were lower than the mean soil concentration for Pb (1.35 mg L^{-1}) Cr (3.9 mg L^{-1}) and Cd (0.08 mg L^{-1}) in the polluted site. Then for the non-polluted site the root concentration is Pb (0.001 mg L^{-1}), Cr (1.23 mg L^{-1}) and Cd (0.001 mg L^{-1}) while for the polluted site the values are Pb (0.001 mg L^{-1}), Cr (2.43 mg L^{-1}) and Cd (0.001 mg L^{-1}). At the Okrika jetty previous studies had indicated the values of Cd for soil at all sampling points were < 0.02 mg L^{-1}, Cr ranged from 17 to 35 mg L^{-1} while Pb ranged from 8.0 to 101 mg L^{-1} (EIA, 2001). There was a significant difference in the concentration of heavy metals ($F_{2, 115}$ = 404.9, p = 0.0001) in lowly and highly polluted plots.

There was also significant difference between root and soil samples ($F_{1, 115}$ = 70.5, P = 0.0001) and across plots ($F_{1,176}$ = 144.7, p = 0.0001). Similarly, there was also a significant difference in heavy metal concentration in root ($F_{1,165}$ =3.8, P = 0.0001) and soil ($F_{1, 165}$ = 15.4, p = 0.0001) and between polluted and non polluted locations ($F_{1,176}$ = 9.1, P = 0.0001). Chromium (Cr) has the highest concentration in soil and root samples in lowly and highly polluted plots and in polluted and non-polluted locations. Lead (Pb) has the second highest concentration followed by Cadmium (Fig. 3c and 3d). However, the concentration of lead (Pb) and Cadmium (Cd) were negligible in mangrove roots within plots and across locations.

Relationship between Heavy Metal and Microbial Content in Soil and Root

Microbial content in soil and root has no interaction ($F_{1, 176}$ = 0.6, P = 0.44, Fig. 4). Also the slope for heavy metal and microbial content for soil and root are not similar. The regression line (Fig. 4) indicates that soil has higher intercept (a = 3.15) than root (a = 2.08), which means the concentration of heavy metals and microbial contents were higher in soil than in root. There is a negative linear relationship between heavy metal and microbial content in both root and soil. This means as the heavy metal increases the microbial content drops indicating that the relationship is inverse. The regression equation for heavy metal and microbial content is given below:

$$Y = 2.0769 - 0.5134 * MIC \quad (1)$$

$$X = 3.1522 - 0.7463 * MIC \quad (2)$$

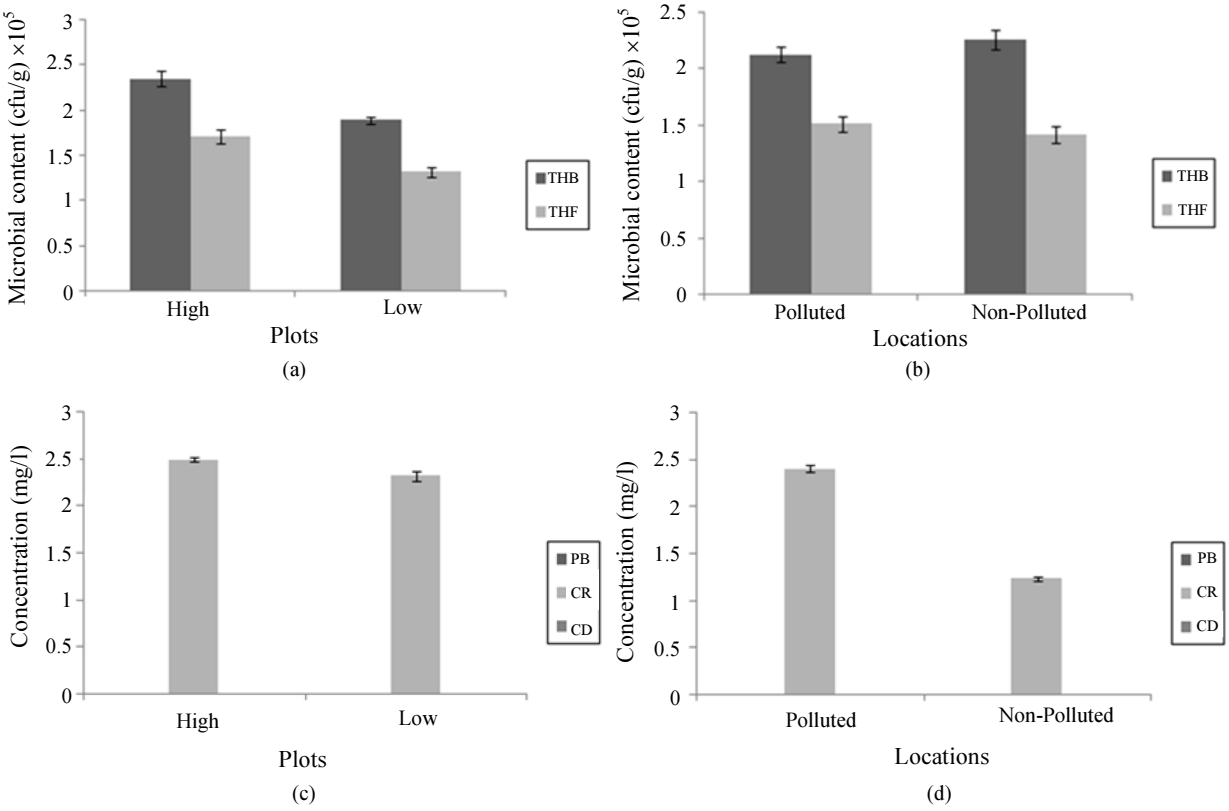

Fig. 3: Graphs of microbial and heavy metal concentrations in mangrove root: (a) Microbial content in highly and lowly polluted plots (b) Polluted and non-polluted locations; and heavy metal concentrations in (c) Highly and lowly polluted plots; (d) Polluted and non-polluted locations in the Niger Delta, Nigeria. THB = Total Heterotrophic Bacteria and THF = Total Heterotrophic Fungi. Vertical lines show ±1 standard error of the mean

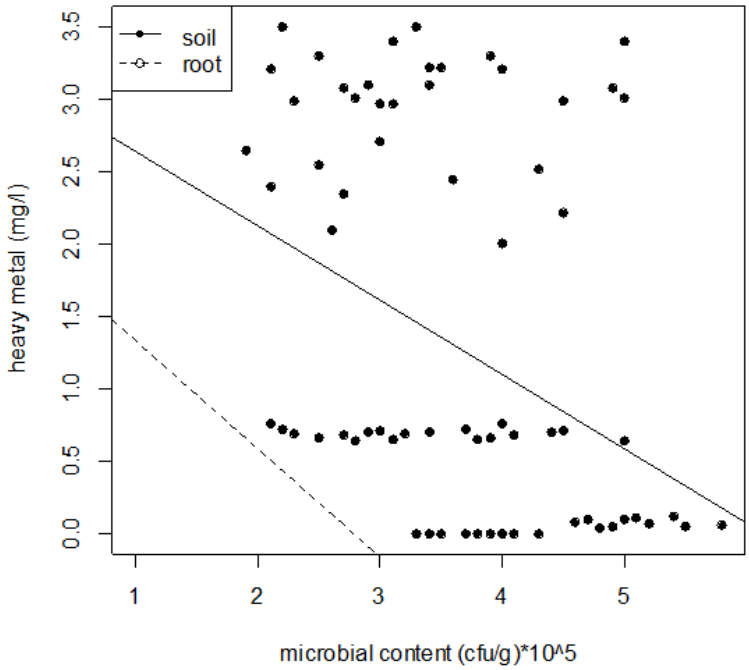

Fig. 4: Correlation between heavy metal and microbial content in soil and root in mangrove forest (*R. racemosa*) in the Niger River Delta, Nigeria. It indicates a negative relationship, as heavy metal increases the microbial content decreases

Where:
Y = Root sample
X = Soil sample and *MIC* represents microbial content

Discussion

Bacterial counts in mangrove soils and roots in our study area are high and indicate high decomposition activities in polluted environment (Numbere and Camilo, 2016). Heterotrophic bacteria play significant roles in litter decomposition, nutrient cycle and food production (Odum and Heald, 1972; Agate, 1988). Bacteria dominate the mangrove swamps because they can survive in both high (i.e., fastidious bacteria) and low (i.e., non-fastidious bacteria) nutrient-rich environments. In addition, their distribution is dependent on various physicochemical parameters within the mangrove forest (Kathiresan *et al*., 1995). Roots in highly polluted plot had more microbial content because the polluted environment requires more hydrocarbon utilizing bacteria to break down crude oil. Mangrove roots are microbe-rich because they serve as nurseries and host to many aquatic creatures such as fishes, barnacles, oysters, crabs, sponges and anemones. These associations predispose them to having more microbes which attach to these aquatic organisms. Microbial presence in mangrove ecosystem is also influenced by the age of the tree (Kohlmeyer and Kohlmeyer, 1993) and the chemical content of the soil (Bano *et al*., 1997).

The chemical content of the soil vary significantly due to industrial and anthropogenic activities in the study areas. For instance, polluted plot has eight times more THC than the non-polluted plot (Table 1). Heavy metal concentration is high in highly polluted sites because of high amount of crude oil spillages (Snowden and Ekweozor, 1987). The polluted location is found around a major refinery this is the cause of its high heavy metal load. The non-polluted location had no major oiling activity, but more human activities which lead to more organic waste load. The lower amount of heavy metals in roots in lowly polluted plot and non-polluted area is due to low bioavailability of the metals in these areas. Other contributing factors are exclusion of the metal and physiological adaptations that prevent metal accumulation in mangroves (Kathiresan and Bingham, 2001).

Chromium has the highest concentration in soil and root samples followed by lead and cadmium for both polluted and non-polluted sites, which follows the profile: Cr > Pb > Cd. Soil heavy metal profile of Punta Mala Bay, Panama indicates that Lead concentration was more than Chromium and Cadmium concentrations (Defew *et al*., 2005). The concentration of the three heavy metals in our study area (i.e., Lead, Chromium and Cadmium) is above the Department of Petroleum Resources (DPR, 2002) limit. This is mainly attributed to oiling activities at the polluted site and anthropogenic activities at the non-polluted site. However, the heavy metal level in this study is not as high as it was several years ago (EIA, 2001) which may be attributed to the adoption of good environmental practices as are sult of increased environmental awareness. Nevertheless, microbial content in this study is low in areas with high heavy metal concentration. Root usually has a high heavy metals concentration (Yim and Tam, 1999) because of its direct contact with the soil as compared to other parts of the plant. This is because mangrove roots act as a barrier that prevents the uptake of heavy metals to other sensitive parts of the plant (Baker and Walker, 1990; Tam and Wong; 1997; Walsh *et al*., 1979). This is why the mangroves are still growing despite the high heavy metal level in the soil.

Disturbances might cause the mangrove soils to lose their metal binding capacity resulting in the mobilization and movement of heavy metals into the plants. This makes the mangroves soil to be a heavy metal sink rather than a heavy metal source (Lacerda, 1998). Other studies have shown that disturbances such as long drought (Clark *et al*., 1997), tidal flooding (Chiu and Chou, 1991) and changes in salinity (Spratt and Hodson, 1994) can cause mobilization of heavy metals. In the same vein, this study indicates that hydrocarbon pollution, which is a serious form of disturbance, might also cause the movement of heavy metals into mangrove parts through the root. Further studies are needed to clarify the dynamics of heavy metal movement into different mangrove parts.

Conclusion

The reduced microbial content in areas with high heavy metal concentration is an indication of contamination. Even though other researchers have reported that heavy metals have no harmful impact on mangroves (Gleason *et al*., 1979; MacFarlane *et al*., 2003) field observation have shown that mangroves absorb hydrocarbons into their root, stem leaf and seed. There should be a paradigm shift from the conduction of soil quality control test to the conduction of plant parts (i.e., root, stem, leave and seed) quality control test in mangroves. This will help to establish a threshold of pollutants for mangrove parts as obtained in soil and water. Also microbial and heavy metal concentration should be determined and compared with other mangrove species such as black (*laguncularia racemosa*) and white mangroves (*Avicennia germinans*) to detect inter-specific differences.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that there are no ethical issues involved.

Conflict of Interest

The author has no conflict of interest to declare.

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