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MICROBIAL AND OTHER RELATED CHANGES IN A NIGER DELTA RIVER SEDIMENT RECEIVING INDUSTRIAL EFFLUENTS.

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ABSTRACT
The microbial and other related changes of Okpoka- Woji River serving as a sink for effluents of industries located in its vicinity within the TransAmadi Industrial area were investigated. Sediment samples were collected from six sampling stations located along the channel for the assessment of total heterotrophic and hydrocarbon-utilizing bacteria, total heterotrophic and hydrocarbon-utilizing fungi, pH, electrical conductivity, redox potential, total organic carbon, oil and grease and total petroleum hydrocarbon. The mean values for the total heterotrophic and hydrocarbon- utilizing bacteria ranged from 3.4 x 10^5 to 7.84 x 10^5cfu/g and 1.58 x 10^5 to 4.57 x 10^5cfu/g respectively. The mean values of the total heterotrophic and hydrocarbon – utilizing fungal counts ranged from 1.98 x 10^4 to 8.83 x 10^4cfu/g and 5.08 x 10^3 to 9.93 x 10^3cfu/g respectively. The pH mean values ranged from 4.87 to 6.17 while the redox potential mean values ranged from 57.70 to 125.7mV. The electrical conductivity and total organic carbon mean values ranged from 138.02 to 3113µS/cm and 3.58 to 6.32% respectively. The mean values for the oil and grease and total petroleum hydrocarbon ranged from 3791.72 to 21,537.30mg/Kg and 3037.60 to 17,461.63mg/Kg respectively. The bacterial genera isolated from the samples included *Nocardia*, *Pseudomonas*. *Klebsiella*, *Lactobacillus*, *Flavobacterium*, *Escherichia*, *Bacillus*, *Micrococcus*, *Proteus*, *Citrobacter* and *Staphylococcus*. The fungal isolates included *Rhizopus*, *Aspergillus*, *Fusarium*, *Mucor and Candida*. The study showed that the river is being polluted by the activities of the industries operating in the area.

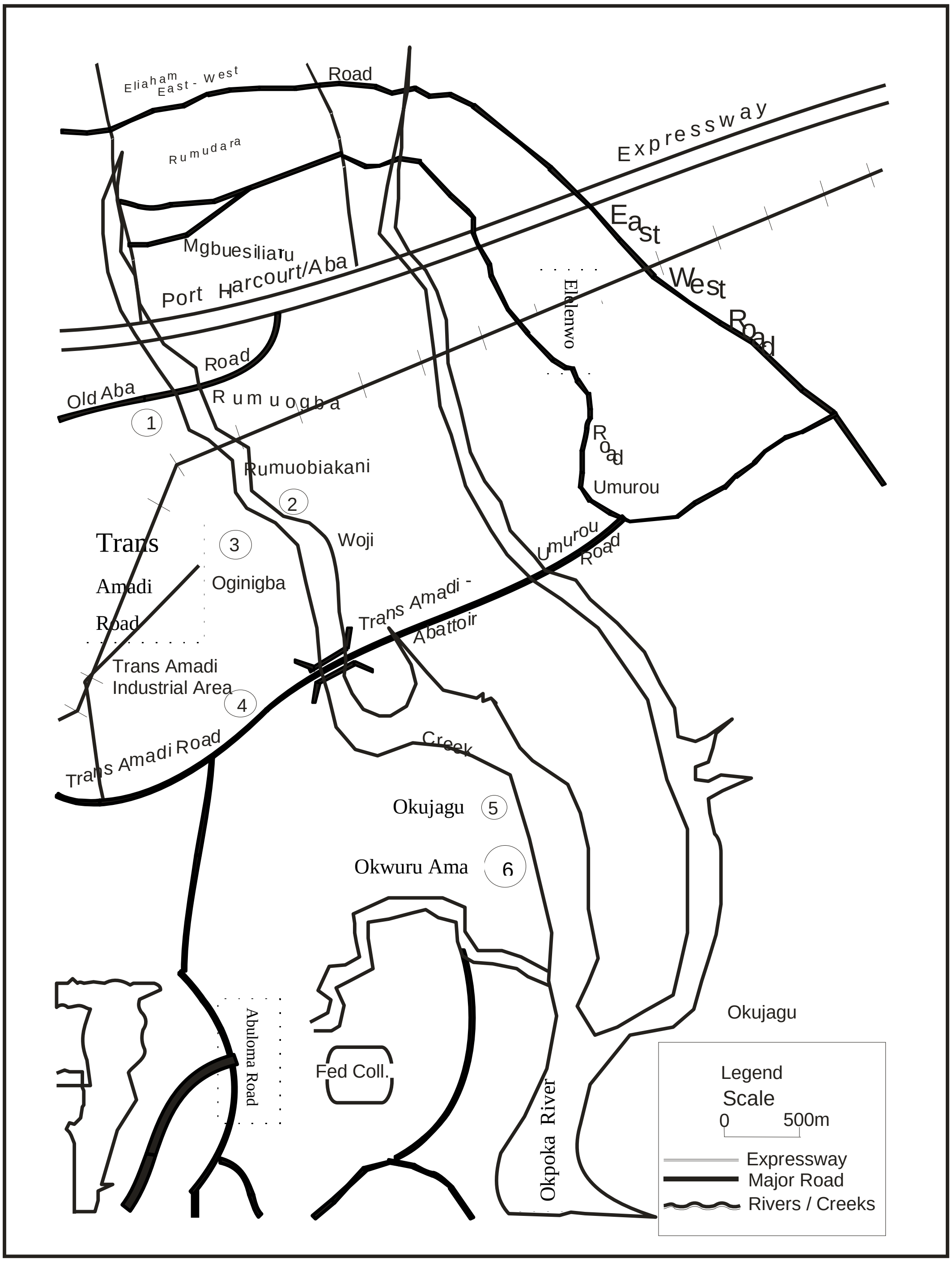
KEYWORDS: Microbial, other related changes, sediment, industrial effluents, Niger Delta River

INTRODUCTION

The deleterious effects of pollutants on the environment have led to increased awareness and vigilance against any possible contamination and damage to our environment. In recent years in Nigeria, there has been a remarkable population growth, urbanization and increase in industrial activities. These have brought about a huge increase in the quantity of discharges and wide diversification in the types and nature of pollutants that reach the river waters and sea through the estuaries. The pollutants have undesirable effects on the assemblages of microbial communities. Some workers have studied these wide scale contamination and their effects on different biota (Okpokwasili and Odokuma, 1990, 1994; Okpokwasili and Nnubia, 1995; Isirimah *et al*., 1987; Kinako and Zuofa, 1991).

Although industrialization is inevitable, various devastating ecological and human disasters, which have continuously occurred over the last three decades or so, implicate industries as major contributors to environmental degradation and pollution problems of various magnitudes, industrial wastes, most of which can be detrimental to human health. These include heavy metals such as lead, cadmium and mercury and toxic organic chemicals such as pesticides, polychlorinated biphenyls (PCBs), dioxin, polyaromatic hydrocarbons (PAHs), petrochemicals and phenolic compounds. For instance, there was a case of the Minimata disease in Japan in the 50s caused by mercury poisoning of consumer of fish from Minimata Bay Japan, which had surface waters for drinking, washing, fishing and swimming (Manahan, 2001). Industries also need water of acceptable quality for its processes (FEPA, 1991; Eze and Okpokwasili, 2008).

In view of the economic importance and inherent scientific interests in the sustainable use of the estuaries, several ecological studies on the Niger Delta estuary become imperative. Okpoka-Woji River estuary is one of the largest in the Niger Delta wetlands and harbours several industries with their accompanying urbanization. But the spite of deterioration of this ecosystem and its resultant effects on the water and sediment qualities and microbial communities is a major source of concern. Unfortunately, no in depth study of the influence of water and sediment qualities on the microbial communities in the estuary has been conducted especially on the poly-haline sector.



Source: Street Guide of Port Harcourt by SPDC 1986

Fig.1: Map of Woji Creek showing the sampling stations

The cases of industrial accidents and deliberate discharges of effluents experienced in the Okpoka –Woji River have caused an alteration on the inter-tidal microbial community structure which apart from occupying a significant position in the food chain were of tremendous economic and importance (Ekweozor *et al*., 1989). Treated and untreated effluents are discharged into the upper reaches of the river, which emanate from various industries along its course.

The objective of the study was to examine the microbial and other related changes of the river sediment and their effect of industrial discharges

MATERIALS AND METHODS

Study Area

The Okpoka-Woji River is situated in the coastal environment of the Niger Delta. It arises from the bifurcation to the left of the Okpoka River, which drains into Bonny River. The area has a mean water depth of 4.8m, which is tidal and gradually transits from fresh to salt water at the head. The fresh water biotope flows unidirectionally downstream from the Rumuodara swamp forest transversing Port Harcourt – Aba Express Road Bridge through Rumuogba (Mini-Okoro Police Station) where tidal effects begin, hence the beginning of the incursion of salt water (Figure 1).

Collection of Sediment Samples

Sediment samples were collected from the river at the discharge points once in a month from April, 2001 – March, 2002. The sediment samples were collected using soil grab and were put in sterile black polythene bags. All the samples were analysed immediately on reaching the laboratory.

Chemical Reagents

Chemical reagents employed in the study were of analytical grade and were products of BDH Chemicals, Pooles, England; Sigma Chemical Company, St. Louis, Missouri, USA and Hach Company Ltd. Colorado, USA. The microbiological media used were products of Oxoid and Difco Laboratories, England. They included nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification of hydrocarbon-utilizers and for pure culture; Sabouraud dextrose agar (SDA) used for the isolation of fungi. The modified mineral salt agar was used for the isolation of hydrocarbon-utilizing bacteria and fungi.

Chemical Analysis

Oil and grease and total petroleum hydrocarbon (TPH) determination

The method was adopted from ASTM (2003). The sediment samples were air dried and sieved. Ten grams of the air dried sieved samples were weighed into 60ml glass bottles and 20ml of tetrachloroethylene was poured into the glass bottles. These bottles were placed into a shaker maintained at room temperature. The system was allowed to shake for 30minutes after which they were allowed to settle. The extracts were filtered out into a 20ml glass bottle using a glass funnel stuffed with cotton wool on which anhydrous sodium sulphate was placed. Analysis of the samples was done using an infrared spectrophotometer. The TPH was determined by treating the extracts with silica gel before analyzing with the infrared spectrophotometer.

Determination of total organic carbon

The method used was adopted from ASTM (2003). One gram each of the air-dried samples was weighed out in duplicate and transferred to 250ml Erlenmeyer flask. Ten millimeters of 1N potassium dichromate solution and 20ml concentrated sulphuric acid was added and the flasks swirled until the soil and reagents were mixed. The flasks were allowed to stand on the sheet of asbestos for about 30minutes after 100mL of distilled water was added. Three drops of indicator was added and then titrated with 0.5N ferrous sulphate solution.

Enumeration of total heterotrophic bacteria and fungi

Samples of the river water were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined by plating in duplicate, using pour plate technique. Then molten nutrient agar and Sabouraud dextrose agar at 45^{o}C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi respectively. They were swirled to mix and colony counts were taken after incubating the plates at room temperature

Enumeration of hydrocarbon-utilizing bacteria and fungi

The hydrocarbon-utilizing bacteria and fungi were determined. The modified mineral salt agar of Mills *et al*. (1978) comprising per litre of distilled water NaCl, 10g; $MgSO_4.7H_2O$, 0.42g; KCl, 0.29g; K_2HPO_4, 1.25g; KH_2PO_4, 0.83g; $NaNO_3$, 0.42g; agar, 15g; pH 7.2 was used. However, for the hydrocarbon-utilizing fungi the medium supplemented with 70g of aureomycin hydrochloride in 200mL of sterile distilled water. The pH of the medium was adjusted to 5.4 with lactic acid. The hydrocarbon-utilizers were then enumerated after plating in duplicate using pour plate technique,1mL of the appropriate dilutions of the samples on Petri dishes. Then molten mineral salt agar medium and the one containing antibiotic at 45ºC were poured into the Petri dishes for the isolation of hydrocarbon-utilizing bacteria and fungi respectively. These were swirled to mix and allowed to solidify. Then filter papers (Whatman No.1) soaked with crude oil were aseptically placed on the inside of the covers of inverted Petri dishes containing the inoculated modified mineral salt medium. Enumeration of the hydrocarbon-utilizers was performed after incubation at room temperature for 7 days.

Colonies of the hydrocarbon-utilizing bacteria growing on the agar plates were counted, isolated, purified by streaking on nutrient agar plates and kept on nutrient agar slants as stock cultures for characterization and identification. In the case of hydrocarbon-utilizing fungi, the isolates were streaked to purify onto Sabouraud dextrose agar plates and later kept on nutrient agar slants as stock cultures for characterization and identification.

Characterization and identification of hydrocarbon-utilizing isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell micro morphology. Other tests performed included spore formation, motility, oxidase and catalase production, citrate utilization, oxidative/fermentative (O/F) utilization of glucose, indole production, methyl red-Voges Proskaur reaction, urease and coagulase production, starch hydrolysis, production of H_2S from triple sugar iron (TSI) agar and sugar fermentation. The tests were performed according to the methods described by Gerhardt *et al*. (1981), Stewart and Beswick (1977) and Cruickshank *et al*. (1980). Microbial identification was performed using the keys provided in the *Bergey's Manual of Determinative Bacteriology* (1994). Fungal isolates were examined macroscopically and microscopically using the needle mounts technique. Their identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

The statistical tools used were analysis of variance (ANOVA) and correlation analysis adapted from Agwung-Fobellah (2007).

RESULTS

The results of the changes in the values of the pH, redox potential, electrical conductivity and total organic carbon of Okpoka- Woji River sediment are shown in Figures 2a to 2d.

The mean values for pH were generally higher in the dry season months than the rainy season months. The ANOVA $P < 0.05$ showed that there significant difference in the mean values between the dry and rainy season months. The ANOVA $P < 0.05$ showed that there was significant difference in the mean values among the stations. The correlation analysis revealed that the mean values had positive influence on the total heterotrophic and hydrocarbon-utilizing fungi and negative influence on the total heterotrophic and hydrocarbon-utilizing bacteria in the rainy season months. However, the mean values had negative influence on all the microbial groups except the total heterotrophic bacteria on which it had positive influence in the dry season months.

The mean values for the redox potential were higher in the rainy season months than in the dry season months. The ANOVA $P < 0.05$ showed that there was significant difference in the mean values between the rainy and dry season months. The ANOVA $P < 0.05$ also showed that there significant difference in the mean values among the stations. The correlation analysis revealed that the mean values had positive influence on the total heterotrophic and hydrocarbon-utilizing bacteria and negative influence on the total heterotrophic and hydrocarbon-utilizing fungi in the rainy season months. The mean values had positive influence on the hydrocarbon-utilizing bacteria and total heterotrophic and hydrocarbon-utilizing fungi and negative influence on the total heterotrophic in the dry season months.

The electrical conductivity mean values were higher in the dry season months than in the rainy season months except in stations 1 and 2 where the mean values were higher in the rainy season months. The ANOVA $P < 0.05$ showed that there significant difference in the mean values between the dry and rainy season months. The ANOVA $P < 0.05$ showed that there was significant difference in the mean values among the stations with values increasing as one proceeded down the stations. The correlation analysis revealed that the mean values had negative influence on all the microbial species both in the rainy and dry season months.

The mean values for the total organic carbon were higher in the dry season months than in the rainy season months within an exception of stations 3 and 4 where the mean values were slightly above in the rainy season months than dry season months. The ANOVA $P > 0.05$ showed that there was no significant difference in the mean values between the dry and rainy season months. The ANOVA $P < 0.05$ showed that there was significant difference in the mean values among the stations. The correction analysis revealed that the mean values had positive influence on the total heterotrophic and hydrocarbon-utilizing bacteria for both the rainy and dry season months. However, the mean values had negative influence on the total heterotrophic and hydrocarbon-utilizing fungi for both the rainy and dry season months.

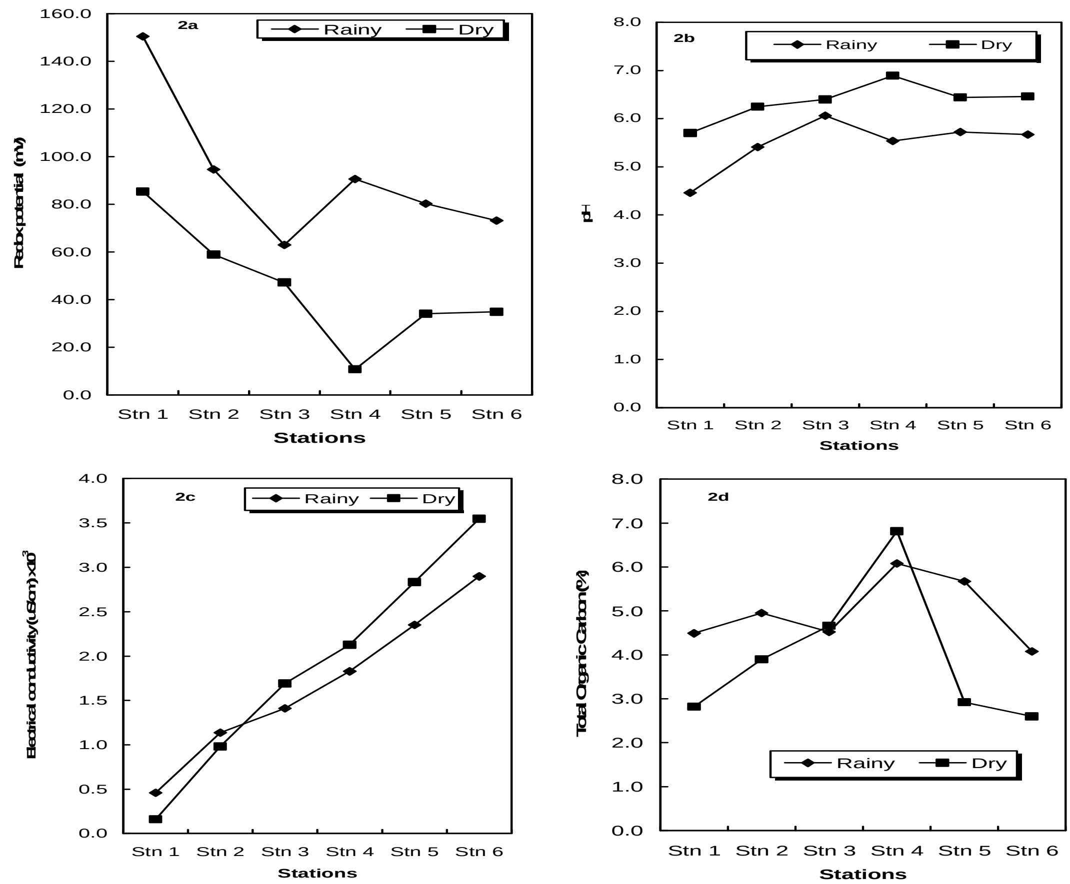


Fig. 2: Changes in the monthly mean values of redox potential, pH, electrical conductivity and total organic carbon (TOC) levels of Okpoka-Woji River sediment across the stations.

The changes for oil and grease and total petroleum hydrocarbon (TPH) are shown in Figures 3a and 3b.

The mean values for oil and grease and TPH were in the rainy season months than in the dry season months. The ANOVA P > 0.05 for oil and grease and TPH respectively showed that there was no significant difference in the mean values between the dry and rainy season months. The ANOVA P < 0.05.for oil and grease and TPH showed that there was significant difference in the mean values among the stations. The statistical analysis for TPH showed that there was no significant difference in the mean values among the stations. The correlation analysis revealed that oil and grease and TPH mean values had positive influence on the total heterotrophic and hydrocarbon-utilizing fungi and negative influence on the total heterotrophic and hydrocarbon-utilizing bacteria in the rainy season months. However, they had positive influence on all the microbial groups in the dry season months.

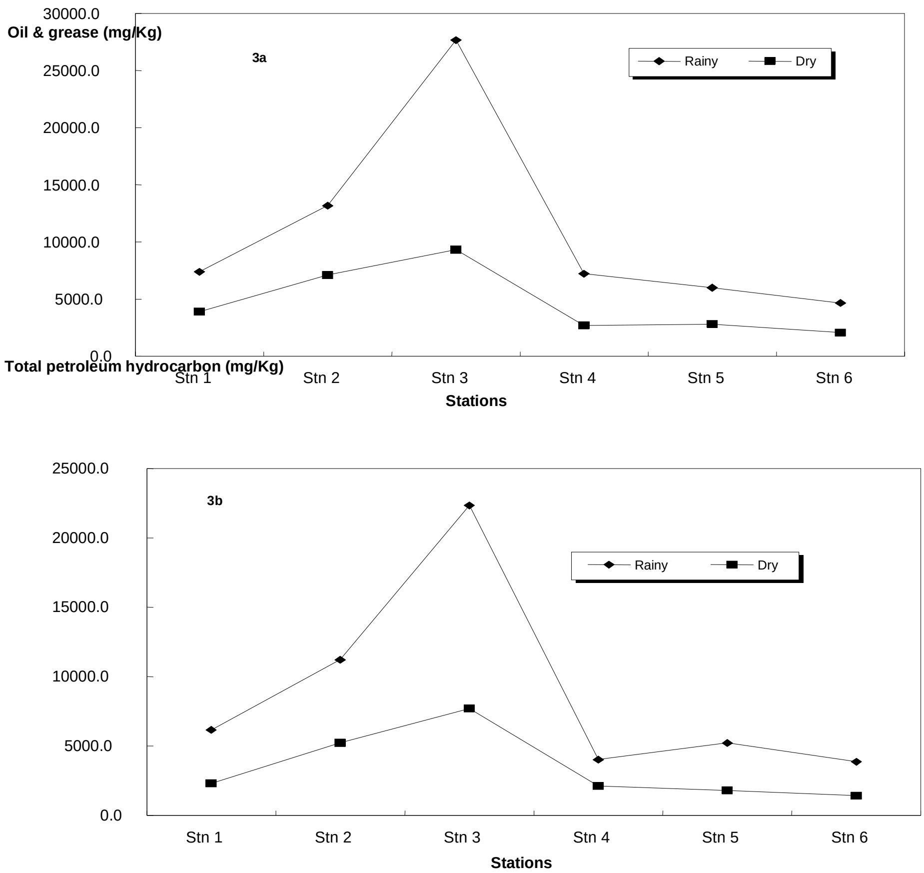


Fig. 3: Changes in the monthly mean values of oil & grease and total petroleum hydrocarbon levels of Okpoka-Woji River sediment across the stations.

The mean values of total heterotrophic bacteria, hydrocarbon-utilizing bacteria, total heterotrophic fungi and hydrocarbon-utilizing fungi are shown in Figures 4a-4c and 5.

The mean values for the total heterotrophic bacteria were higher in the dry season months than in the rainy season months. The ANOVA P > 0.05 showed that there was no significant difference in the mean values between the dry season months and the rainy season months. The ANOVA P < 0.05 showed that there was significant difference in the mean values among the stations with the values increasing from station 1-4 and decreasing from stations 5 and 6.

The mean values for the hydrocarbon-utilizing bacteria were higher in the dry season months than in the rainy season months. The ANOVA P > 0.05 showed that there was no significant difference in the mean values between the dry and rainy season months. However, the ANOVA P < 0.05 showed that there was significant in the mean values among the stations with the values fluctuating downstream.

The mean values for the total heterotrophic fungi were higher in the rainy season months than in the dry season months for stations 3-6 while the mean values were higher in the dry season months for stations 1 and 2. The ANOVA P > 0.05 showed that there was no significant difference in the mean values between the dry and rainy season months as well as among the stations.

The mean values for the hydrocarbon-utilizing fungi were higher in the rainy season months than in the dry season months for stations 3-6 while the mean values were higher in the dry season months for stations 1 and 2. The ANOVA P > 0.05 showed that there was no significant difference in the mean values between the dry and rainy season. However, the ANOVA P < 0.05 showed that there was significant difference in the mean values among the stations.

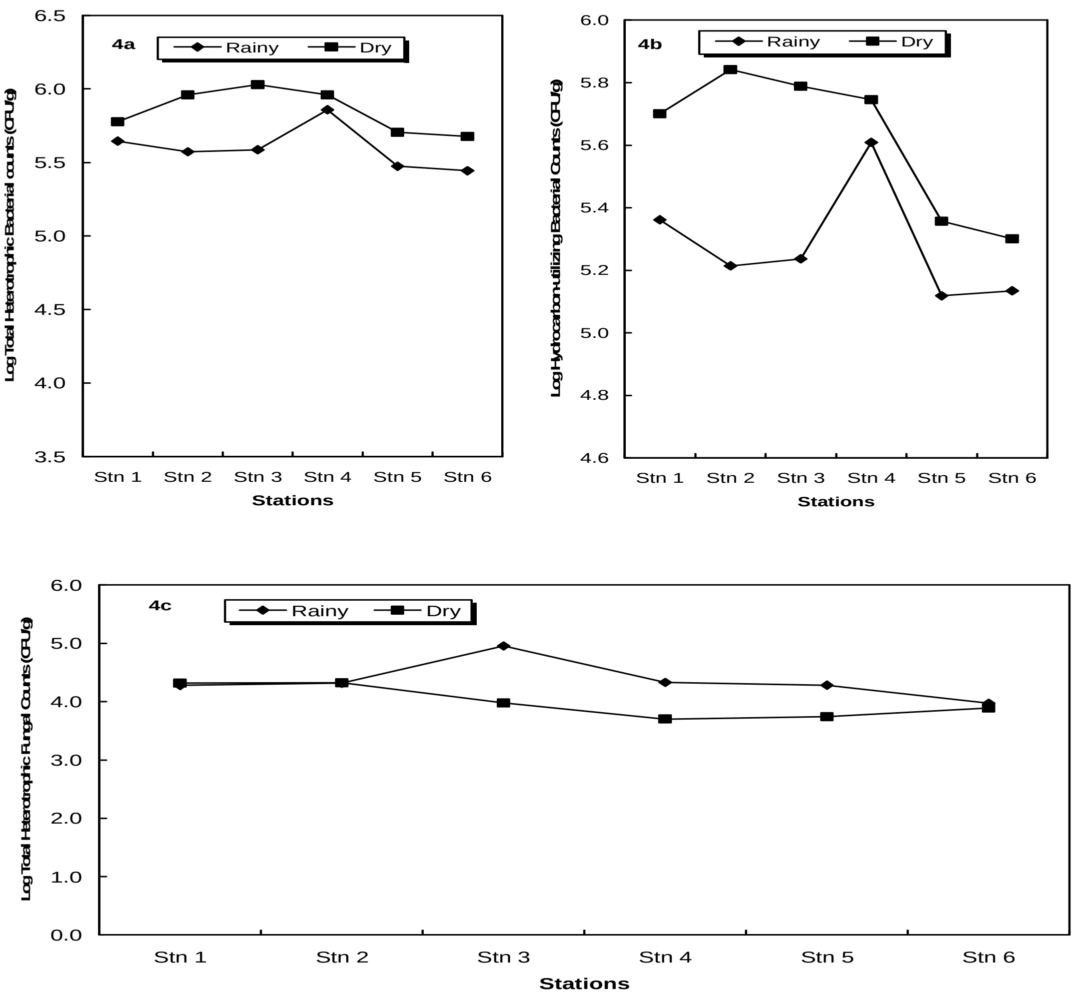


Fig. 4: Changes in the monthly mean values of total heterotrophic bacterial, hydrocarbon-utilizing bacterial Counts and Total Heterotrophic Fungal counts of Okpoka-Woji River sediment across the stations.

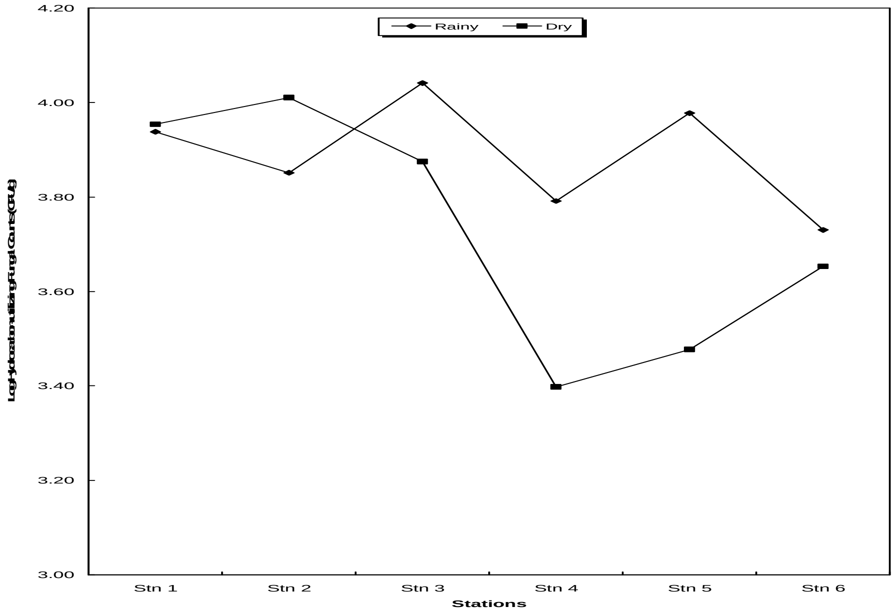


Fig. 5: Changes in the monthly mean values of Hydrocarbon-utilizing Fungal counts of Okpoka-Woji River sediment across the stations.

DISCUSSION

Sediment physicochemical characteristics cannot be viewed in isolation from the water quality. In general, natural and anthropogenic elements are transported either in dissolved or particulate phases in the water column to the sediment, which acts as a sink. Therefore, sediment can act as an effective trap for water borne elements and compounds but may also be potential sources of bioavailable elements and compounds to benthic flora and fauna. The bioavailability is dependent on the physicochemical characteristics of the sediments (Parry, 2002).

The pH mean values of the monitored sediment were within the acidic region during the study period. This may be as a result of no reclamation programme going on in the river. Ferreira *et al*. (1996) reported that the pH of sediment may be high due to reclamation programme. The complicated composition and sources of the reclamation materials may cause the pH of the sediment to be higher at the location. The pH values also increased downstream.

Some monitored redox data were negative while some were positive. The negative redox data shows that the sediment exhibits reducing conditions. Near the sampling stations, there were local point sources where an abattoir exists (Ferreira *et al*., 1996).

The values of the electrical conductivity being higher in the dry season months than rainy season months can be attributed to the fact that as rainfall dilutes the water bodies so also the effect on the sediment. The electrolytes therefore become concentrated in the dry season as a result of reduced water volume caused by evaporation. The lowest value was observed in the month of September which is the peak of rainfall (Izonfuo and Bariweni, 2001).

The total organic carbon mean values were generally higher in the rainy season months than in the dry season months. The higher values of the TOC showed how the sediment is being enriched with organic matter. The presence of organic matter shows the presence of other nutrients and these encourage the growth of microbial population (Atlas, 1992).

The oil and grease and total petroleum hydrocarbon (TPH) contents were also on the increase much greater than the concentration in the surface water. They were also high in the all the stations and this shows anthropogenic contribution of the hydrocarbon into the system. This can also be due to improper discharges of wastes from different human activities in the study area mainly from industrial oil sub-sector and municipal discharges (Kakulu and Osibanjo, 1992; Chindah *et al*., 2004).

The observed seasonal differences with higher concentration of oil and grease in wet than in dry season months may be attributed to increased water content and wave action, which may largely disturb the sediment with the concomitant resurfacing of the previously leached hydrocarbon in the sediment. The fluctuating values observed in the stations can be attributed to hydrological dynamics and the proximity to the vicinity of discharges (Chindah *et al*., 2004).

Sediments are generally a favourable habitat for the proliferation of microorganisms. Numbers of microorganisms in sediment habitat are much higher than those found in freshwater and marine habitats. A high proportion of bacteria isolated in the sediment are Gram negative. The hydrocarbon-utilizing bacteria and fungi isolated from the river sediment were *Pseudomonas*, *Klebsiella*, *Nocar*dia, *Escherichia*, *Lactobacillus*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Proteus*, *Flavobacterium*, *Citrobacter*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Mucor and Candida*. Amanchukwu (1996) also reported the same when she worked on sediment from Bonny estuary.

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