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Iko River estuary: Oil exploration and the microbial community shift

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Abstract

The continuous input of petroleum-based and other industrial pollutants along with heightened navigational activities in the inland and coastal waters of the Niger Delta region of Nigeria has contributed tremendously towards petroleum pollution of the aquatic environment. Standard analytical methods were employed in sample collection and analysis. The results showed the mean values of Total Heterotrophic Bacteria [THB] for tidal water 1.44±0.20 ($x10^7$), 1.42±0.62 ($x10^7$)and 1.82±0.61 ($x10^7$)for upstream, midstream and downstream respectively, while the mean values for CUB 1.06±0.12 ($x10^6$), 1.30±0.54 ($x10^6$) and 1.28±0.46 ($x10^6$) for upstream, midstream and downstream respectively. Similarly, the mean values for Total Fungi (TF) and Crude oil-Utilizing Fungi (CUF) were 1.08±0.12 ($x10^6$), 1.12±0.21 ($x10^6$), 1.18±0.20 ($x10^6$) and 8.2±0.78 ($x10^4$), 9.2±0.20 ($x10^4$), 8.8±0.26 ($x10^4$) for upstream, midstream and downstream respectively. For intertidal water, the mean values obtained for upstream, midstream and downstream were 1.24±0.82 ($x10^7$), 1.77±0.57 ($x10^7$) and 1.40±0.32 ($x10^7$) for THB, 1.08±0.92 ($x10^6$), 1.08±0.22 ($x10^6$) and1.13±0.21 ($x10^6$) for CUB, 1.00±0.60 ($x10^7$), 1.26±0.30 ($x10^6$) and 1.11±0.18 ($x10^6$) for Total fungi [TF] and 7.2±0.81 ($x10^4$), 9.6±0.4 ($x10^4$), 9.0±0.27 ($x10^4$) for CUF). While the values for benthic sediment were 1.55±0.38 ($x10^8$), 1.68±0.32 ($x10^8$), 2.24±0.34 ($x10^8$) for THB, 1.14±0.32 ($x10^7$), 1.24±0.88 ($x10^7$), 1.48±0.90 ($x10^7$) for CUB, 1.12±0.31 ($x10^7$), 1.20±0.52 ($x10^7$), 1.40±0.16 ($x10^7$) for TF and 8.2±0.12 ($x10^5$), 6.2±0.43 ($x10^5$), 1.01±0.12 ($x10^6$) for CUF. The results showed that there was no significant difference ($p > 0.05$) in the mean values of each physicochemical parameter across the different microhabitats and stations. This result revealed the massive impacts of anthropogenic gradients on the biology and physicochemistry of Iko River estuary.

Keywords: Microbiological; Physicochemistry; Crude oil-utilizing microbes; Niger Delta; Molecular analysis

1. Introduction

An estuary is a partly enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open ocean. Estuaries form a transition zone between river environments and ocean environments and are subject to both marine influences, such as tides, waves, and the influx of saline water, and riverine impacts, such as flows of fresh water and sediment [1]. The inflow of both seawater and freshwater provide high levels of nutrients in both the water column and sediment, making estuaries among the most productive natural habitats in the world [2].

The Niger Delta region of Nigeria produces more than 80% of the country's crude oil and there is presently an unprecedented increase in the upstream and downstream activities of the oil and allied companies in this area [3] Over the years, these oil companies have generated myriad of pollutants in the form of gaseous emissions, oil spills, effluents and solid waste [3] that have polluted the marine environment almost beyond sustainability. Heightened navigational

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activities in inland and coastal waters of the Niger Delta region is another anthropogenic source of petroleum pollution of the aquatic environment.

The continuous input of these petroleum-based and other industrial pollutants has resulted in an enriched microbial community capable of surviving toxic contamination as microorganisms are sensitive to fluctuations and alterations in their environment [4]. Whenever their chemical or physical environment is suddenly altered, there is a lag period during which the microbial community adapts to the new conditions [5]. This lag period is also called acclimation period and enables the microorganisms to acquire the metabolic repertoire necessary for their survival [6]. This phenomenon has been shown to occur in both terrestrial and aquatic ecosystems [7].

There are many important variable characteristics of estuarine water, some of which are the concentration of dissolved oxygen, salinity and sediment load these factors are greatly impacted through anthropogenic variations [8]. There is extreme spatial variability in salinity, with a range of near zero at the tidal limit of the tributary river to 3.4% at the estuary mouth [2].

2. Material and methods

2.1. Microbiological analysis

Sediment and water samples for microbial analysis were collected aseptically, labeled and stored in ice-packed plastic coolers and transported to the laboratory where analysis within 24 - 48 hours of collection was carried out. Prior to analysis, the sediment samples were homogenized and ten 10 g of each sample was weighed out, added to 90 ml of sterile deionized water and vigorously shaken for 1 minute using a vortex shaker to dislodge the microbiota. This method disrupts the flocculent material and randomly disrupted the protists. Treated samples were allowed to settle for 10 minutes prior to withdrawal of supernatant for serial dilution. Ten-fold serial dilution of the sediment and water samples was carried out for enumeration of densities of the different microbial groups [9].

2.1.1. Estimation of microbial densities of sediment and water samples

Several methods and media were used for the enumeration of the various microbial groups. The densities of the following microbial groups were determined:

- Total heterotrophic bacteria (THB)
- Total fungi (TF)
- Crude oil-utilizing bacteria (CUB)
- Crude oil-utilizing fungi (CUF)

The basic analytical media employed in the course of this research included: nutrient agar (NA), Sabouraud Dextrose Agar (SDA), Mineral Salt Medium (MSM), thiosulfate citrate-bile salts-sucrose agar (TCBS), Salmonella-Shigella Agar (SSA) and agar-agar. The media were prepared according to manufacturer's recommendations (Difco,Biotech).The counts of total heterotrophic bacteria in sediment and water samples were determined by the pour plate techniques [10] using Nutrient Agar (NA). The NA medium was amended with nystatin (50µgml^{-1}) to prevent the growth of fungal contaminants. The total fungal count was determined by pour plate technique using Sabouraud dextrose agar (SDA) supplemented with streptomycin (50µgml^{-1}) to inhibit the growth of bacteria [11] [12]. Inoculated NA plates were incubated at 28℃ for 24 hours, while the SDA plates were incubated at room temperature for 3 days before enumeration of microbial colonies.

2.1.2. Isolation, purification and maintenance of pure microbial isolates

Distinct or representative colonies from the culture plates were selected for characterization. Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 24 hours before stocking. Similarly, distinct fungal colonies were sub-cultured repeatedly on freshly prepared Sabouraud dextrose agar plates for 72 hours before stocking. Pure isolates of the microorganisms were maintained on agar slants as stock, and preserve in the refrigerator for further use.

2.1.3. Enumeration of crude oil-utilizing microorganisms

The counts of crude oil-utilizing bacteria and fungi were enumerated by pour plate techniques [13] using vapour phase transfer technique on mineral salt medium (MSM). For the enumeration of oil-degrading bacteria, the medium was

supplemented with 30 mgl^{-1}fungizolmiconazole nitrate to prevent the growth of fungal contaminants. On the other hand, mineral salts medium supplemented with 50µgml^{-1} streptomycin to inhibit the growth of bacterial contaminants was used to ensure the enumeration of oil-degrading fungi. In both cases, the crude oil used was sterilized by Millipore filtration (0.45 µm pore size) and stored in sterile bottles. The oil agar plates were incubated at room temperature for 5 days before enumeration [14].

2.2. PCR reaction for characterization and identification of isolates

The PCR reaction was performed on the extracted DNA samples using universal degenerate primers 27F.1 Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3' that amplifies the entire 16s variable region at annealing temperature of 58.Similarly, universal degenerate primers were used to amplify ribosomal internal transcribed spacer (ITS). The primers sequences were ITS1: 5´TCC GTA GGT GAA CCT TGC GG 3 and ITS4 5´TCC TCC GCT TAT TGA TAT GC 3´.Each PCR reaction contained 5µl of 10 × Taq buffer, 2mMMgCl 2,1.5 U Super-Therm DNA Polymerase (Southern Cross), 0.25mMdNTP's, 0.1µM of each primer, 1µl of extracted DNA and Nuclease Free Water (NFW) up to the final reaction volume of 50µl. Few microliters of the samples were run on a 1% agarose gel at 90V for 30min in order to verify amplification to check presence of amplification band corresponding to expected band size1.

DNA sequencing was performed by Sanger (dideoxy) sequencing technique to determine the nucleotide sequence of the specific microorganism isolated using automated PCR cycle-Sanger Sequencer™ ABI3500XL DNA Analyzers from Applied Biosystems. Sequencing was then done with the ABI V3.1 Big dye kit according to manufacturer's instructions. The cleaned products were then injected on the analyzers with a 50cm array, using POP7. Sequencing data were obtained and read on a Geospiza FINCH TV software version 1.4.0, Chromatogram viewer that can display DNA sequence traces on Windows.

2.3. Physicochemical analysis

Physicochemical analysis of the sediment and water samples were determined using standard analytical methods [15] [16]. Many fast changing parameters were analyzed *in situ*.

3. Results and discussion

The results presented in Tables 1 represent the mean values of total heterotrophic bacteria (THB), crude oil-utilizing bacteria (CUB), total fungi (TF), and crude oil-utilizing fungi (CUF) respectively. The results showed that anthropogenic variations influence greatly the microbial proliferation as significantly ($p<0.05$) higher microbial levels were observed across all microhabitats (tidal water, intertidal water and benthic sediment) as well as stations (upstream – Okoro, midstream – Kampa and downstream – Emeroke).

It was observed that the sediment samples produced significantly ($p<0.05$) higher THB counts than tidal and intertidal water samples. Similar trends were observed for CUB, TF and CUF respectively. There was no significant difference ($p>0.05$) between the mean values of upstream, midstream and downstream. In all microhabitats and stations, the densities of crude oil-utilizing microorganisms were significantly ($p<0.05$) low compared to total heterotrophic counts. The total fungal counts were significantly ($p<0.05$) low compared to total heterotrophic bacteria counts.

The mean values of THB for tidal water 1.44±0.20 ($x10^7$), 1.42±0.62 ($x10^7$)and 1.82±0.61 ($x10^7$)for upstream, midstream and downstream respectively, while the mean values for CUB 1.06±0.12 ($x10^6$), 1.30±0.54 ($x10^6$) and 1.28±0.46 ($x10^6$)for upstream, midstream and downstream respectively. Similarly, the mean values for TF and CUF were 1.08±0.12 ($x10^6$), 1.12±0.21 ($x10^6$), 1.18±0.20 ($x10^6$) and 8.2±0.78 ($x10^4$), 9.2±0.20 ($x10^4$), 8.8±0.26 ($x10^4$) for upstream, midstream and downstream respectively. For intertidal water, the mean values obtained for upstream, midstream and downstream were 1.24±0.82 ($x10^7$), 1.77±0.57 ($x10^7$) and 1.40±0.32 ($x10^7$) for THB, 1.08±0.92 ($x10^6$), 1.08±0.22 ($x10^6$) and1.13±0.21 ($x10^6$) for CUB, 1.00±0.60 ($x10^7$), 1.26±0.30 ($x10^6$) and 1.11±0.18 ($x10^6$) for TF and 7.2±0.81 ($x10^4$), 9.6±0.4 ($x10^4$), 9.0±0.27 ($x10^4$) for CUF). While the values for benthic sediment were 1.55±0.38 ($x10^8$), 1.68±0.32 ($x10^8$), 2.24±0.34 ($x10^8$) for THB, 1.14±0.32 ($x10^7$), 1.24±0.88 ($x10^7$), 1.48±0.90 ($x10^7$) for CUB, 1.12±0.31 ($x10^7$), 1.20±0.52 ($x10^7$), 1.40±0.16 ($x10^7$) for TF and 8.2±0.12 ($x10^5$), 6.2±0.43 ($x10^5$), 1.01±0.12 ($x10^6$) for CUF.

The microbial species identified from the molecular analysis were: *Pseudomonas putida, Bacillus aquimaris, Lysinbacillus macroides, Rhodococcus equi, Achromobacter xylosoxidans, Enterobacter cloacae, Marinobacter hydrocarbonoclastius, Penicillium citrinum, Aspergillus japonicas* and *Tinctoporellus epiphiltinus.*

Table 1 Microbial analysis of tidal, intertidal and benthic water samples

Sampling locations /Parameters	Upstream (Okoro)	Midstream (Kampa)	Downstream (Emeroke)
Tidal Water (TW) (cfu/ml)			
THB	$^{b}1.44\pm0.20(x10^{7})$	$^{a}1.42\pm0.62(x10^{7})$	$^{a}1.82\pm0.61(x10^{7})$
CUB	$^{a}1.06\pm0.12\ (x10^{6})$	$^{b}1.30\pm0.54(x10^{6})$	$^{b}1.28\pm0.46(x10^{6})$
TF	$^{a}1.08\pm0.12\ (x10^{6})$	$^{a}1.12\pm0.21(x10^{6})$	$^{a}1.18\pm0.20(x10^{6})$
CUF	$^{a}8.2\pm0.78(x10^{4})$	$^{a}9.2\pm0.20\ (x10^{4})$	$^{a}8.8\pm0.26(x10^{4})$
Intertidal water (ITW) (cfu/ml)			
THB	$^{a}1.24\pm0.82(x10^{6})$	$^{b}1.77\pm0.57(x10^{7})$	$^{c}1.42\pm0.32\ (x10^{7})$
CUB	$^{a}1.08\pm0.92(x10^{6})$	$^{a}1.08\pm0.92\ (x10^{6})$	$^{a}1.13\pm0.21\ (x10^{6})$
TF	$^{a}1.00\pm0.60(x10^{6})$	$^{b}1.26\pm0.30(x10^{6})$	$^{c}1.11\pm0.18\ (x10^{6})$
CUF	$^{a}7.2\pm0.81(x10^{4})$	$^{a}9.6\pm0.4(x10^{4})$	$^{a}9.0\pm0.27(x10^{4})$
Benthic sediment (BSD) (cfu/g)			
THB	$^{a}1.55\pm0.38\ (x10^{8})$	$^{a}1.68\pm0.32\ (x10^{8})$	$^{b}2.24\pm0.34\ (x10^{8})$
CUB	$^{a}1.14\pm0.32\ (x10^{7})$	$^{a}1.24\pm0.88\ (x10^{7})$	$^{b}1.48\pm0.90\ (x10^{7})$
TF	$^{a}1.12\pm0.31\ (x10^{7})$	$^{a}1.20\pm0.52\ (x10^{7})$	$^{a}1.40\pm0.16\ (x10^{7})$
CUF	$^{b}8.2\pm0.12\ (x10^{5})$	$^{a}6.2\pm0.43\ (x10^{5})$	$^{a}1.01\pm0.12\ (x10^{6})$

Similar superscripts represent significant mean ± SD across the rows for each of the parameters for tidal water, Intertidal water and benthic sediment.

Table 2 represents the results of the physicochemical analysis of the tidal and intertidal water samples while Tables 3 represents the results of the physicochemical analysis of benthic sediment samples respectively.

The physicochemical parameters analyzed for tidal and intertidal water samples were; temperature (℃), pH, electrical conductivity (µS/cm), dissolved oxygen (DO) (mg/l), biological oxygen demand (BOD) (mg/l), total dissolved solids (TDS) (mg/l), salinity (ppt), turbidity (NTU), total hardness, phosphate (mg/l), calcium (mg/l), magnesium (mg/l), sodium (mg/l), potassium (mg/l), acidity (mg/l), sulphate (mg/l), nitrate (mg/l), nitrite (mg/l), chloride (mg/l), ammonium (mg/l) and Total Hydrocarbon Content (THC) (mg/l). The results showed that there was no significant difference ($p> 0.05$) in the mean values of each parameter across the different microhabitats as shown below.

However, the physicochemical parameters of the benthic sediment analyzed were; pH, electric conductivity (µS/cm), total dissolved solids (TDS), Available phosphorus (mg/l), total nitrogen (mg/l), exchangeable bases as well as total organic carbon (TOC) (%), sulphide (mg/l), exchangeable acidity, effective cation exchange capacity (ECEC), base saturation, particle size distribution (PSD) (%): Sand, Silt, Clay and total hydrocarbon content (mg/kg). From the results obtained, there was no significant difference ($p> 0.05$) in the mean values of the parameters as shown below.

The complex microbial diversities and dynamics in contaminated ecosystems such as Iko River estuary offer a resounding opportunity for environmental sustainability function and strategies for bioremediation as revealed in this study. The results obtained showed that the great the microbial proliferation observed may be as a result of high nutrient availability and concentrations during the period of study. Similar reports were recorded by [10] [14].

It was observed that the sediment samples produced significantly ($p<0.05$) higher THB counts than tidal and intertidal water samples. Similar trends were observed for CUB, TF and CUF respectively. There was no significant difference ($p>0.05$) between the mean values of upstream, midstream and downstream respectively. In all microhabitats and stations, the densities of crude oil-utilizing microorganisms were significantly ($p<0.05$) low compared to total heterotrophic counts. The total fungal counts were significantly ($p<0.05$) low compared to total heterotrophic bacteria counts.

Generally, the high level of microbial proliferation in the estuary was due mainly to the continuous input of petroleum-based and other industrial pollutants resulting in an enriched microbial community capable of surviving toxic contamination. Microorganisms are sensitive to fluctuations and alterations in their environment. Whenever their chemical or physical environment is suddenly altered, there is a lag period during which the microbial community adapts to the new conditions [2].

Table 2 Physicochemistry of the tidal and intertidal water samples

Parameter / Location	Upstream (Okoro)		Midstream (Kampa)		Downstream (Emeroke)	
	TW	ITW	TW	ITW	TW	ITW
Temp (℃)	^{a}23.2±1.45	^{a}23.1±1.14	^{a}23.4±1.31	^{a}23.3±1.23	^{a}23.4±1.36	^{a}23.3±1.57
pH	^{a}6.71±0.51	^{a}6.74±0.57	^{a}6.96±0.53	^{a}6.88±0.58	^{a}6.62±0.52	^{a}6.72±0.60
DO(mg/l)	^{a}10.50±0.71	^{a}10.24±0.76	^{b}5.76±0.44	^{b}5.11±0.51	^{b}7.81±0.61	^{a}8.22±0.71
BOD(mg/l)	^{b}12.6±0.91	^{a}3.82±0.22	^{a}4.88±0.31	^{a}4.72±0.34	^{a}4.91±0.41	^{a}5.31±0.34
TDS (mg/l)	^{a}1242±6.64	^{a}1280±6.43	^{a}1248±6.74	^{a}1371±6.60	^{a}1210±6.43	^{b}2187±6.49
Salinity (ppt)	^{a}1168±5.01	^{a}1325±5.21	^{a}1244±5.23	^{a}1314±5.31	^{b}3192±5.47	^{b}4288±5.10
Turbidity (NTU)	^{a}12.3±0.91	^{a}12.5±0.93	^{a}12.9±0.94	^{a}13.1±0.95	^{a}14.6±0.91	^{b}16.10±0.97
Total Hardness	^{a}112.1±3.94	^{a}112.7±4.87	^{b}106.8±3.14	^{a}114.4±2.14	^{a}115.2±1.98	^{a}115.7±2.14
Phosphate (mg/l)	^{a}10.22±0.45	^{a}9.44±0.41	^{a}8.61±0.39	^{a}8.81±0.42	^{a}7.42±0.47	^{a}9.96±0.42
Ca (mg/l)	^{a}88.7±3.42	^{a}84.2±2.98	^{a}80.1±3.11	^{a}82.8±3.49	^{a}85.4±2.99	^{a}82.7±3.22
Mg (mg/l)	^{a}22.5±1.01	^{a}20.7±1.21	^{a}23.1±1.04	^{a}23.0±1.09	^{a}24.8±1.12	^{b}31.9±1.31
Na (mg/l)	^{a}542.7±3.46	^{a}528.2±3.74	^{a}531.1±3.91	^{a}521.8±3.44	^{a}511.2±3.85	^{b}516.7±3.14
K (mg/l)	^{a}176.3±1.52	^{a}164.7±1.53	^{a}169.4±1.47	^{a}171.3±1.47	^{a}172.7±1.60	^{a}177.4±1.60
Acidity (mg/l)	^{a}2.61±0.02	^{a}2.49±0.01	^{a}2.55±0.01	^{a}2.54±0.01	^{a}2.61±0.02	^{a}2.60±0.03
Sulphate (mg/l)	^{a}128.8±0.97	^{a}129.9±1.21	^{a}126.3±1.34	^{a}129.4±1.41	^{a}131.2±1.12	^{a}140.7±1.28
Nitrate (mg/l)	^{a}21.0±0.86	^{a}21.7±1.01	^{a}21.4±1.03	^{a}21.4±1.21	^{a}22.1±1.37	^{a}22.0±1.42
Nitrite (mg/l)	^{a}0.27±0.01	^{a}0.24±0.02	^{a}0.29±0.02	^{a}0.26±0.02	^{a}0.27±0.02	^{a}0.26±0.02
Chloride (mg/l)	^{a}484.1±3.10	^{a}479.7±2.58	^{a}432.4±2.61	^{a}412.9±2.91	^{a}484.1±2.98	^{a}456.5±2.63
NH_4(mg/l)	^{b}0.16±0.01	^{a}0.27±0.01	^{b}0.16±0.01	^{b}0.17±0.01	^{a}0.20±0.01	^{a}0.24±0.01
THC (mg/l)	^{a}36.7±0.42	^{a}32.1±0.34	^{a}38.2±0.31	^{a}40.0±0.29	^{a}39.4±0.31	^{a}39.4±0.33

Similar superscripts across the columns represent significant ANOVA ($p < 0.01$) for each of the parameters and as well significant Student t-test ($p<0.01$) for each sampling stations for TW and ITW.KEY: TW = Tidal water, ITW = Intertidal water

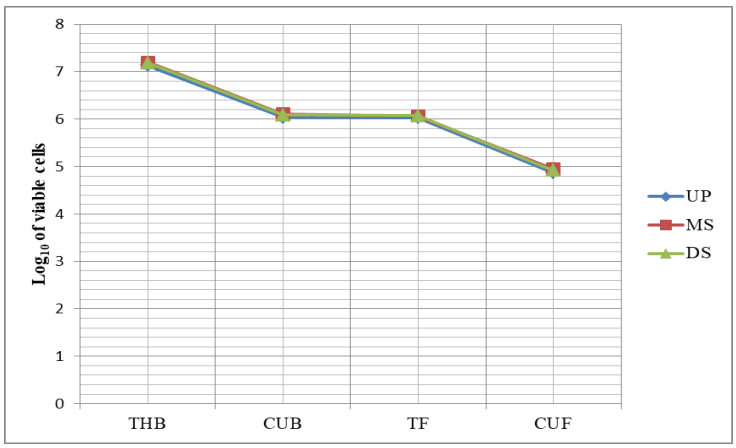
The mean values of THB for tidal water were 1.35±0.18 (x10^7), 1.59±0.64 (x10^7) and 1.56±0.52 (x10^7) for upstream, midstream and downstream respectively, while the mean values for CUB were 1.08±0.12 (x10_6), 1.28±0.58 (x10^6) and1.24±0.44 (x10^6) for upstream, midstream and downstream respectively. Similarly, the mean values for TF and CUF were 1.06±0.12 (x10^6), 1.16±0.22 (x10^6), 1.16±0.18 (x10^6) and 7.4±0.84 (x10^4), 9.0±0.20 (x10^4), 8.4±0.28 (x10^4) for upstream, midstream and downstream respectively. For intertidal water, the mean values obtained for upstream, midstream and downstream were 9.9±0.76 (x10^6), 1.79±0.52 (x10^7) and 1.42±0.32 (x10^7) for THB, 1.12±0.90 (x10^6), 1.18±0.20 (x10^6) and1.13±0.21 (x10^6) for CUB, 1.09±0.62 (x10^6), 1.24±0.30 (x10^6) and 1.11±0.18 (x10^6) for TF and 7.4±0.84 (x10^4), 9.8±0.6 (x10^4), 8.9±0.26 (x10^4) for CUF). While the values for benthic sediment were 1.68±0.40 (x10^8), 1.78±0.36 (x10^8), 2.13±0.32 (x10^8) for TH`B, 1.23±0.42 (x10^7), 1.26±0.92 (x10^7), 1.46±0.98 (x10^7) for CUB, 1.14±0.78 (x10^7), 1.26±0.62 (x10^7), 1.38±0.14 (x10^7) for TF and 8.8±0.14 (x10^5), 6.8±0.48 (x10^5), 1.02±0.14 (x10^6) for CUF. Figures 1, 2 and 3 represent the Semi-log plot of the total viable cells for tidal water, intertidal water and the benthic sediment samples respectively.

Table 3 Physicochemistry of the benthic sediment sample

Parameters/ Location	Upstream (Okoro)	Midstream (Kampa)	Downstream (Emeroke)
Ph	[a]6.71±0.20	[a]6.72±0.21	[a]6.70±0.22
TDS	[a]424±3.44	[a]477±3.43	[b]512±4.24
Avail. P (mg/l)	[a]1.14±0.01	[a]1.22±0.02	[a]1.27±0.03
Total N (mg/l)	[a]0.52±0.02	[a]0.64±0.02	[a]0.65±0.02
Ex. Bases (meq/100g)			
Ca	[a]5.11±0.57	[a]5.02±0.58	[a]4.07±0.61
Mg	[a]4.41±0.34	[a]4.68±0.41	[a]4.72±0.42
Na	[a]0.40±0.02	[a]0.38±0.02	[a]0.43±0.02
K	[a]0.34±0.02	[a]0.43±0.02	[a]0.39±0.02
TOC (%)	[a]1.41±0.10	[a]1.54±0.14	[a]1.56±0.13
Sulphide (mg/l)	[a]1.01±0.08	[a]1.15±0.07	[a]1.19±0.07
EA	[a]1.53±1.10	[a]1.47±1.20	[a]1.49±1.30
ECEC	[a]11.84±1.78	[a]11.98±1.81	[a]11.1±1.91
B. saturation	[a]84.90±2.41	[a]85.72±2.52	[a]86.6±2.74
PDS (%)			
Sand	[a]72.7±2.01	[a]73.5±2.51	[a]75.6±2.61
Silt	[a]21.2±1.97	[a]20.1±1.91	[a]19.8±1.81
Clay	[c]6.1±0.31	[b]6.4±0.27	[a]4.6±0.23
THC (mg/kg)	[a]322.6±3.23	[a]315.9±3.41	[a]321.4±3.51

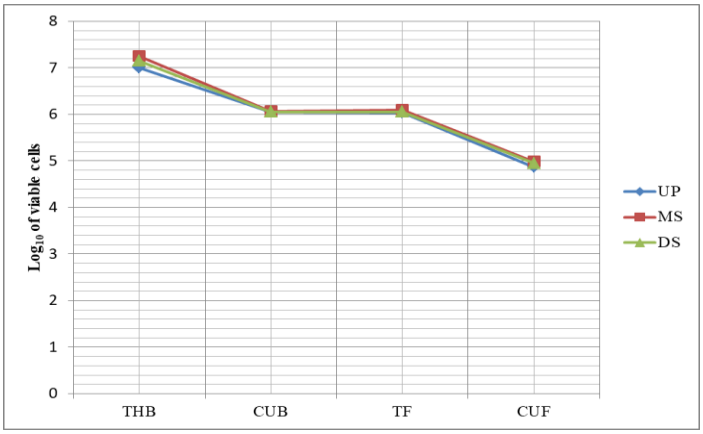
Similar superscripts across the columns represent significant ANOVA ($p < 0.01$) for each of the parameters and as well significant Student t-test ($p < 0.01$) key: PSD = Particle size distribution

The results obtained indicate that the water and sediment samples show a remarkable variation in physicochemical parameters across all microhabitats and stations revealing the tremendous anthropogenic and natural gradients in the estuary.



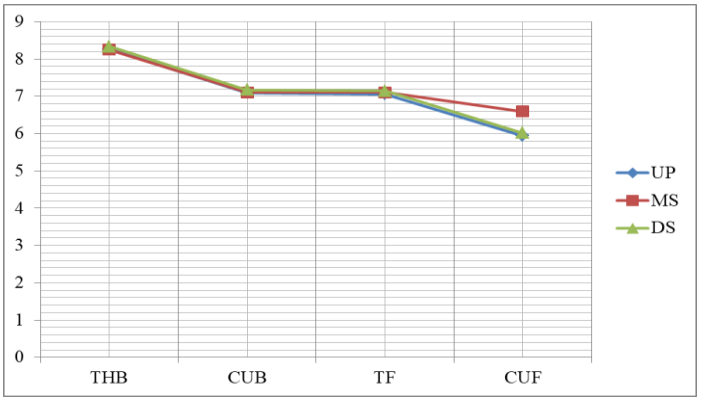
Key: THB = total heterotrophic bacteria, CUB = crude oil-utilizing bacteria, TF =total fungi, CUF = crude oil-utilizing fungi, UP = upstream, MS = midstream, DS = downstream

Figure 1 Semi-log plot of the total viable cells for tidal water sample



Key: THB = total heterotrophic bacteria, CUB = crude oil-utilizing bacteria, TF =total fungi, CUF = crude oil-utilizing fungi, UP = upstream, MS = midstream, DS = downstream

Figure 2 Semi-log plot of the viable cells for intertidal water sample



Key: THB = total heterotrophic bacteria, CUB = crude oil-utilizing bacteria, TF =total fungi, CUF = crude oil-utilizing fungi, UP = upstream, MS = midstream, DS = downstream

Figure 3 Semi-log plot of the viable cells for benthic sediment sample

4. Conclusion

In this study, marginal variations in microbiological and physicochemical characteristics were observed across all microhabitats and stations with the sediment producing higher levels of microbial counts than the tidal and intertidal water samples. The significantly high levels of hydrocarbon-utilizing microorganisms in Iko River estuary can be taken as a sensitive index of environmental exposure to hydrocarbon pollutants and other anthropogenic influences in the estuary. The benthic sediment accumulates more hydrocarbons than the tidal water and the intertidal water. The findings of this research also revealed microbial indices of pollution. From the study, all the microbial isolates exhibited hydrocarbonoclastic activity revealing their active involvement in the biodegradation and other processes in the study site. The variations observed may be use in environmental monitoring and evaluation for ecosystem studies.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no conflict of interest exists.

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Author's short biography

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