

Influence of Seasonal Variation on the Microbiological and Physicochemical Parameters of Imo River Estuary of the Niger Delta Mangrove Ecosystem

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Abstract

Surface and sub-surface water samples collected from Imo river estuary were analyzed for the seasonal variation in microbiological and physicochemical parameters. The results obtained indicate that there were no significant difference ($P > 0.05$) in the population of total heterotrophic bacteria (THB) in the surface water during the wet and dry season ($2.23 \times 10^6 \pm 2.23$ cfuml⁻¹ and $2.39 \times 10^6 \pm 1.63$ cfuml⁻¹) respectively, while there were significant difference ($P < 0.05$) in the population of THB in the sub-surface water during the dry and wet season ($2.27 \times 10^6 \pm 2.00$ cfuml⁻¹ and $2.13 \times 10^6 \pm 1.84$ cfuml⁻¹) respectively. The total heterotrophic fungi (THF) densities in the surface water were $1.17 \times 10^5 \pm 0.93$ cfuml⁻¹ and $1.38 \times 10^5 \pm 0.63$ cfuml⁻¹ during the wet and dry season respectively, the mean densities of $1.15 \times 10^5 \pm 0.63$ cfuml⁻¹ and $1.30 \times 10^5 \pm 0.48$ cfuml⁻¹ were observed in the sub-surface water during the wet and dry season respectively. However, there were significant difference ($P < 0.05$) in the population of total heterotrophic and crude oil utilizing microorganisms. The results further revealed that the water samples show a remarkable variation in physicochemical parameters during the wet and dry season. The temperature ranges for both the surface and sub-surface water revealed mesophilic levels. This study has revealed useful information about the periodic and seasonal variations in microbiological and physicochemical gradients that occur in Imo river estuary. Eight (8) species of bacteria and five (5) species of fungi were isolated. The bacteria species were *Flavobacterium* sp, *Micrococcus* sp, *Vibrio* sp, *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* sp. The fungi species isolated were *Cladosporium* sp, *Penicillium* sp, *Aspergillus* sp, *Monilia* sp and *Fusarium* sp.

Keywords: Microbiological, Physicochemical, Estuary, Surface, Sub-surface

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Introduction

Estuaries are amongst the most heavily populated areas throughout the world, with about 60% of the world's population living along estuaries and the coast. As a result, estuaries are suffering from degradation by many factors, including sedimentation from soil erosion from deforestation, overgrazing, and other poor farming practices, overfishing, drainage and filling of wetlands, eutrophication due to excessive nutrients from sewage and animal wastes, pollutants including heavy metals, radionuclides and hydrocarbons from sewage inputs (Eke, 2002; Ross, 1995). Microbial communities readily respond at faster rates (compared to other benthic organisms) to environmental and pollution changes. This reflects their micro environmental conditions and "communicates" this information to other biota in their vicinity and therefore plays key roles in benthic-pelagic coupling. The most important variable characteristics of estuarine water are the concentration of dissolved oxygen, salinity and sediment load. There is extreme spatial variability in salinity, with a range of near zero at the tidal limit of the tributary river(s) to 3.4‰ at the estuary mouth. At any one point the salinity will vary considerably over time and seasons, making it a harsh environment for organisms (Kaiser, 2005).

Determining the hydrological regime of a water body is an important aspect of a water quality assessment. Discharge measurements, for example, are necessary for mass flow or mass balance calculations and as inputs for water quality models (APHA, 1998). Water bodies undergo temperature variations along with normal climatic fluctuations. These variations occur seasonally and, in some water bodies, over periods of 24 hours. Most chlorine compounds occur as chloride (Cl⁻) in solution. It enters surface waters with the atmospheric deposition of oceanic aerosols, with the weathering of some sedimentary rocks (mostly rock salt deposits) and from industrial and sewage effluents, and agricultural and road run-off (Okonko *et al.*, 2008). Oxygen is essential to all forms of aquatic life, including those organisms responsible for the self-purification processes in natural waters. Variations in dissolved oxygen can occur seasonally, or even over 24 hour periods, in relation to temperature and biological activity (i.e. photosynthesis and respiration). Biological respiration, including that related to decomposition processes, reduces DO concentrations.

The measurement of DO can be used to indicate the degree of pollution by organic matter, the destruction of organic substances and the level of self-purification of the water. Its determination is also used in the measurement of biochemical oxygen demand (BOD) (Eniola, 2005; Okonko *et al.*, 2008).

Increased mineral salts in rivers may arise from several sources including release of mining wastewaters, certain industrial wastewaters and increased evaporation and evapotranspiration in the river. Ion content (principally Ca^{2+} , Na^+ , Cl^- , and SO_4^{2-}) can also be affected by other human activities such as domestic wastewater inputs, atmospheric pollution, the use of de-icing salts and fertilizer run-off (Adeniji and Mbagwu, 1990). Conductivity is sensitive to variations in dissolved solids, mostly mineral salts. The degrees to which these dissociate into ions, the amount of electrical charge on each ion, ion mobility and the temperature of the solution all have an influence on conductivity (Adeniji and Mbagwu, 1990). The nitrate ion (NO_3^-) is the common form of combined nitrogen found in natural waters. It may be biochemically reduced to nitrite (NO_2^-) by denitrification processes, usually under anaerobic conditions. The nitrite ion is rapidly oxidized to nitrate. Nitrate is an essential nutrient for aquatic plants and seasonal fluctuations can be caused by plant growth and decay (Bissett *et al.*, 2006).

Materials And Methods

Study site

The study site for this research work was Imo river estuary in the Niger Delta region of Nigeria. Imo River estuary lies between latitude $04^{\circ} 34^{\circ}52\text{N}$ and longitude $007^{\circ} 32^{\circ}59\text{E}$, with an elevation of 11 m above sea level.

Sample Collection

All samples were collected from the surface and sub-surface. The bottles were open to fill and closed below the water. All containers were rinsed at least three times with water that was to be analyzed (APHA, 1998).

Microbiological Analysis

Samples for microbial analysis were collected aseptically, labeled and stored in ice packed plastic coolers and transported to the laboratory where analysis within 24 hours of collection was carried out. Ten-fold serial dilution of the samples was carried out for enumeration of densities of the different microbial groups. 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 heterotrophic fungi (THF), crude oil utilizing bacteria (CUB), crude oil utilizing fungi (CUF)

Estimation of Densities of Heterotrophic Microorganisms

The counts of total heterotrophic bacteria were determined by the pour plate techniques (Chikere *et al.*, 2009,) using nutrient agar (NA). The NA medium was amended with nystatin ($50\mu\text{gml}^{-1}$) in order to prevent the growth of fungal contaminants. The total heterotrophic fungi count was determined by pour plate technique using Sabouroud dextrose agar (SDA) supplemented with streptomycin ($50\mu\text{gml}^{-1}$) to inhibit the growth of bacterial contaminants (Martini *et al.*, 1980; Barnett and Hunter, 1972). Inoculated NA plates were incubated at 28°C for 24 hours, while the SDA plates were incubated at room temperature for 3 days before enumeration of microbial colonies.

Enumeration of Crude oil Utilizing Microorganisms

The counts of crude oil utilizing bacteria and fungi were enumerated by pour plate techniques (Mills *et al.*, 1978; Obire *et al.*, 2008) using vapour phase transfer technique on mineral salts medium (MSM). For the enumeration of oil degrading bacteria, the medium was supplemented with $50\mu\text{gml}^{-1}$ fungizol miconazole nitrate to prevent the growth of fungal contaminants. On the other hand, mineral salts medium supplemented with $50\mu\text{gml}^{-1}$ 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 ($0.45\ \mu\text{m}$ pore size) and stored in sterile bottles. The plates were incubated at room temperature for 5 days before enumeration.

Physicochemical Analysis

Many physicochemical parameters such as temperature, DO, BOD, TDS, EC and pH were determined in situ using their different meters (Udo *et al*, 2009; Ubalua and Ezeronye 2005; APHA, 1985, 1998).

Determination of Chloride

The chloride content of the samples was determined titrimetrically by the silver-nitrate method (APHA, 1985, 1998).

Determination of Salinity

Percentage salinity of the samples was determined by the method described by Miroslav and Vladimir (1999)

Nitrate and Phosphate Content Determination

Nitrate and phosphate content was determined using the method of APHA (1985, 1998; Radojevic and Bashkin, 1999).

Determination of COD

COD was determined by the method described by AOAC (1990).

Total Hydrocarbons Content (THC)

The method used for determination of total hydrocarbon content (THC) follows a standard procedure (AOAC, 1990; APHA, 1998; Law and Klungsoyr, 2000; Radojevic and Bashkin, 1999).

Statistical Analysis

Data collected were subjected to 2x3x6 factorial experiment in a Completely Randomized Design (CRD), significant means were separated using Least Significant Difference (LSD) test at 5% probability level.

Results

Total Heterotrophic Microorganisms

The results show that there were no significant difference ($P > 0.05$) in THB of the surface water during the wet and dry season ($2.23 \times 10^6 \pm 2.23$ and $2.39 \times 10^6 \pm 1.63$) respectively, while significant difference ($P < 0.05$) were observed in the sub-surface water ($2.27 \times 10^6 \pm 2.00$ and $2.13 \times 10^6 \pm 1.84$) respectively as shown in Table 1. The total heterotrophic fungi (THF) density (Table 1) revealed that in the surface water, the mean densities observed were $1.17 \times 10^5 \pm 0.93$ cfuml⁻¹ and $1.38 \times 10^5 \pm 0.63$ cfuml⁻¹ during the wet and dry season respectively, the mean densities of $1.15 \times 10^5 \pm 0.63$ cfuml⁻¹ and $1.30 \times 10^5 \pm 0.48$ cfuml⁻¹ were observed in sub-surface water during the wet and dry season respectively

Crude Oil Utilizing Microorganisms

The results show that there were significant difference ($P < 0.05$) in population of crude oil utilizers with respect to season (Table 1). The mean densities of crude oil utilizing bacteria observed were $1.22 \times 10^5 \pm 1.20$ cfuml⁻¹ and $1.32 \times 10^5 \pm 1.05$ cfuml⁻¹ in surface water during the wet and dry season respectively, in the sub-surface water, the mean densities observed were $1.18 \times 10^5 \pm 1.06$ cfuml⁻¹ and $1.32 \times 10^5 \pm 0.98$ cfuml⁻¹ during the wet and dry season respectively. The mean densities of crude oil utilizing fungi (CUF) observed for the surface water were $7.2 \times 10^3 \pm 0.23$ cfuml⁻¹ and $8.9 \times 10^3 \pm 0.63$ cfuml⁻¹ during the wet and dry season respectively. In the sub-surface water, the mean densities were $7.4 \times 10^3 \pm 0.78$ cfuml⁻¹ and $8.8 \times 10^3 \pm 0.84$ cfuml⁻¹ during the wet and dry season respectively.

Physicochemical Characteristics of the Water Samples

Temperature and pH Levels

The result of the physicochemical investigation showed that there were no significant difference ($P > 0.05$) in temperature range throughout the study period. The results show that the pH levels in both microhabitats were slightly acidic throughout the study period. The mean fluctuations in physicochemical parameters and total hydrocarbon content during wet and dry season are shown in Figure 1 – 3.

Electrical conductivity (EC)

In the overlying and sub-surface water, the mean values of electrical conductivity recorded were $554.5 \pm 2.94 \mu\text{scm}^{-1}$ and $586.5 \pm 2.45 \mu\text{scm}^{-1}$ during the wet and dry season respectively (Table 2). The results further revealed that the surface water had a significantly ($P < 0.05$) higher conductivity level than the sub-surface water.

Total Dissolved Solids (TDS)

The mean values of total dissolved solids (TDS) recorded for the overlying and sub-surface water were $290 \pm 2.06 \text{mg l}^{-1}$ and $162.25 \pm 2.21 \text{mg l}^{-1}$ during the wet and dry season respectively (Table 2). The result obtained revealed a significantly ($P < 0.05$) higher values of TDS during the wet season.

Dissolved Oxygen (DO)

The results show that there were no significant differences ($P > 0.05$) in DO during the wet and dry season, though the levels observed during the dry season were slightly higher than those observed during the wet season. The mean values recorded were of $5.35 \pm 0.13 \text{mg l}^{-1}$ and $5.48 \pm 0.09 \text{mg l}^{-1}$ during the wet and dry season respectively for surface and sub-surface water.

Biological Oxygen Demand (BOD)

The mean biological oxygen demand was $2.57 \pm 0.10 \text{mg l}^{-1}$ in surface and sub-surface water during the wet season and $2.70 \pm 0.09 \text{mg l}^{-1}$ during the dry season.

Salinity

In the surface and sub-surface water, the mean salinity level was 5.54 ± 0.24 ppt during the wet season and 5.69 ± 0.18 ppt during the dry season. The salinity levels observed during the dry season were significantly ($P < 0.05$) higher than the levels observed during the wet season (5.69 ± 0.18 and 5.54 ± 0.24) respectively.

Nutritive salts (Ammonium, Phosphate, Sulphate, Nitrate and Chloride)

The result presented in Table 2 shows that the mean concentrations of nutritive salts varied with season and microhabitats. The mean concentrations of Cl^- , SO_4^{2-} , P^{2+} , and NH_4^+ during the dry season were significantly ($P < 0.05$) higher than the mean concentrations observed during the wet season, while the mean N^{2+} concentration observed during the wet season was significantly ($P < 0.05$) higher than the mean concentration observed during the dry season.

Chemical Oxygen Demand (COD)

The result shows that during the wet and dry season, the mean concentrations of COD in surface and sub-surface water were $32.27 \pm 1.32 \text{ mg l}^{-1}$ and $32.91 \pm 0.98 \text{ mg l}^{-1}$ respectively.

Table 1: Influence of season and source of sample on the microbial population of the water samples

	Wet season		Dry season		LSD
	SW (cfuml ⁻¹)	SSW (cfuml ⁻¹)	SW (cfuml ⁻¹)	SSW (cfuml ⁻¹)	
THB	$2.23^c \times 10^6 \pm 2.23$	$2.13^d \times 10^6 \pm 1.84$	$2.39^c \times 10^6 \pm 1.63$	$2.27^c \times 10^6 \pm 2.00$	1.46
CUB	$1.22^a \times 10^5 \pm 1.20$	$1.18^b \times 10^5 \pm 1.06$	$1.32^b \times 10^6 \pm 1.05$	$1.32^b \times 10^5 \pm 0.98$	8.5
THF	$1.17^c \times 10^5 \pm 0.93$	$1.15^c \times 10^5 \pm 0.63$	$1.38^c \times 10^3 \pm 0.63$	$1.30^c \times 10^3 \pm 0.48$	1.52
CUF	$7.2^c \times 10^3 \pm 0.23$	$7.4^c \times 10^3 \pm 0.78$	$8.9^c \times 10^3 \pm 0.63$	$8.8^c \times 10^3 \pm 0.84$	1.05

Means with the same superscript along the horizontal array represent no significant difference ($P > 0.05$).

KEY: SW = surface water, SSW = sub-surface water, THB = total heterotrophic bacteria,

THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit.

Table 2: Influence of season and source of sample on the physicochemical properties of the water samples

	Wet season		Dry season		LSD
	SW	SSW	SW	SSW	
pH	6.65 ^a ±0.08	6.72 ^a ±0.04	6.7 ^a ±0.1	6.77 ^a ±0.09	0.65
EC (μscm^{-1})	315.0 ^a ±1.06	333.0 ^a ±0.98	252.0 ^a ±0.24	266.5 ^a ±0.46	61.65
TDS (mg l^{-1})	168.5 ^a ±0.98	163.53 ^a ±1.40	127 ^b ±1.36	3.71 ^a ±0.86	6.44
DO (mg l^{-1})	5.5 ^a ±0.09	5.63 ^a ±0.08	5.5 ^a ±0.04	5.56 ^a ±0.10	0.42
BOD (mg l^{-1})	2.95 ^a ±0.05	3.06 ^a ±0.10	2.22 ^b ±0.01	2.28 ^b ±0.08	0.28
Salinity (ppt)	3.41 ^b ±0.23	3.92 ^a ±0.14	2.48 ^d ±0.10	3.04 ^c ±0.09	0.13
Chloride (mg l^{-1})	288.42 ^d ±1.04	303.38 ^b ±1.08	292.27 ^c ±0.94	307.91 ^a ±1.62	3.26
Nitrate (mg l^{-1})	16.05 ^a ±0.11	8.52 ^b ±0.09	16.31 ^a ±0.14	9.27 ^b ±0.08	2.18
Sulphate (mg l^{-1})	4.77 ^a ±0.18	7.29 ^b ±0.11	4.81 ^a ±0.09	7.52 ^b ±0.1	0.73
Phosphate (mg l^{-1})	4.82 ^b ±0.08	5.07 ^a ±0.04	0.02 ^c ±0.01	0.10 ^c ±0.02	0.22
Ammonium (mg l^{-1})	0.03 ^d ±0.01	2.97 ^c ±0.04	39.5 ^b ±0.06	8.15 ^a ±0.05	0.25
Acidity (mg l^{-1})	240.0 ^b ±1.32	262 ^a ±1.04	75.25 ^c ±0.98	90.25 ^c ±1.00	16.73
Alkalinity (mg l^{-1})	67.0 ^c ±0.21	82 ^b ±0.14	77 ^b ±0.09	92.0 ^a ±0.18	7.99
COD (mg l^{-1})	21.5 ^a ±0.26	21.90 ^a ±0.18	21.27 ^a ±0.20	22.12 ^a ±0.10	2.84

Means with the same superscript along the horizontal array represent no significant difference ($P > 0.05$).

KEY: SW = surface water, SSW = sub-surface water, LSD = least significant difference

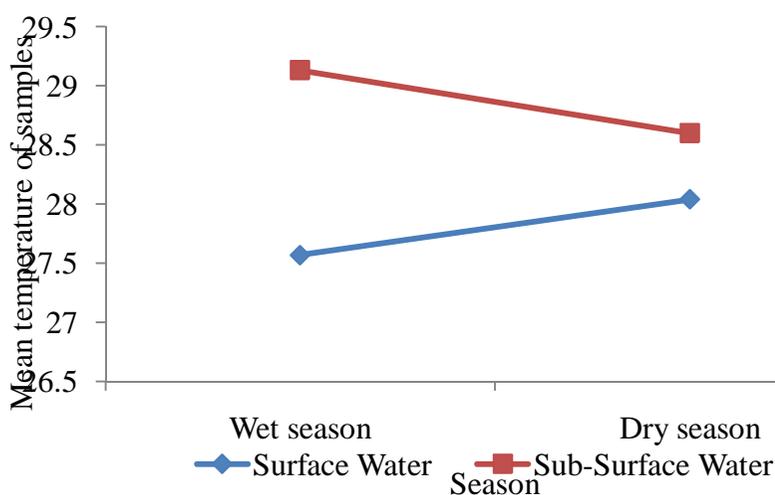


Figure 1: Mean temperature ($^{\circ}\text{C}$) levels in surface and sub-surface water during the wet and dry season

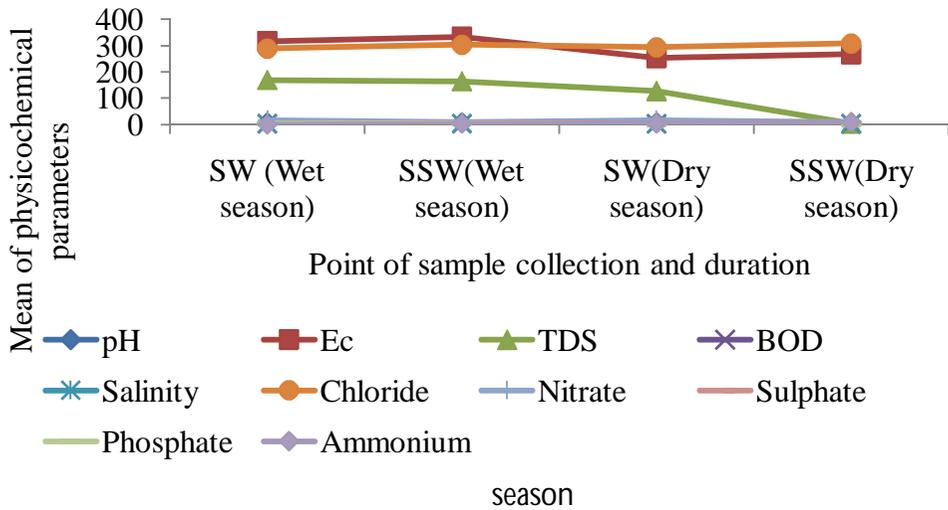


Figure 2: Mean fluctuations in the physicochemical parameters of the water samples during the wet and dry

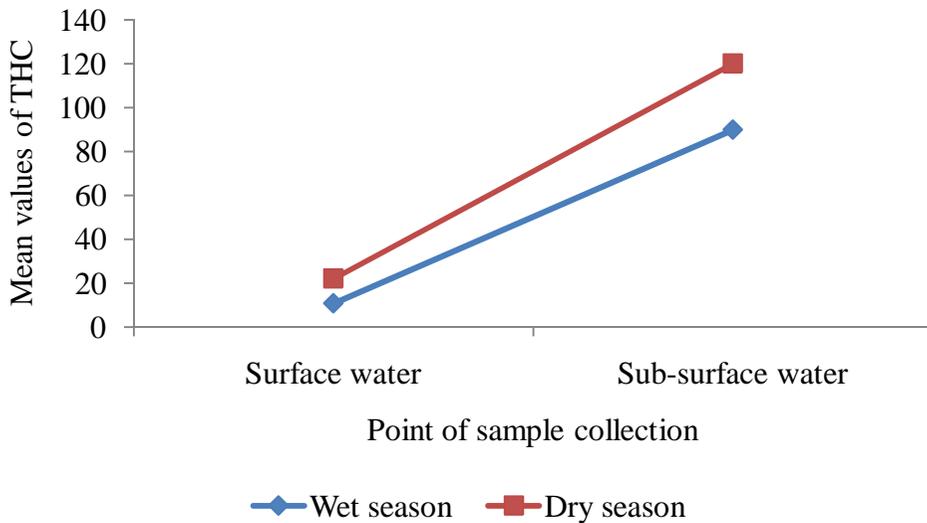


Figure 3: Mean fluctuations in the total hydrocarbon content (THC) of the surface and sub-surface water during the wet and dry season

Discussion

Estuaries form a transition zone between river environment and the open ocean and are subject to both marine influences such as tides, waves and the influx of saline water and riverine influences such as flows of fresh water and sediments (Kaiser, 2005). The results of the present study agrees with the report of Ross (1995) that estuaries are suffering degradation by many factors, including sedimentation from soil erosion, deforestation, overgrazing, overfishing, drainage and filling of wetlands, eutrophication due to excessive nutrients from sewage and animal wastes, pollutants including heavy metals, radionuclides and hydrocarbons. Water and sediment microbial community are a major component of microbial food webs, biogeochemical cycles and energy flow. Bacteria and fungi are the predominant organisms in these microhabitats. Their biodiversity is structured and determined by the temporal and spatial variability of physicochemical and biotic parameters and thus, can reflect local environmental conditions (Zhang *et al.*, 2008).

The slightly lower population of heterotrophic microorganisms during the wet season may be due to changes in biological oxygen demand, dissolved oxygen levels, temperature and salinity. The densities of crude oil utilizing microorganisms were low compared to total heterotrophic counts. The results show that there was a significant difference ($P < 0.05$) in crude oil utilizers with respect to season and microhabitats. This may have resulted from the high heterotrophic activities in the environment. In this study, eight (8) species of bacteria and five (5) species of fungi were isolated, characterized and identified. The bacteria species were *Flavobacterium* sp, *Micrococcus* sp, *Vibrio* sp, *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* sp. The fungi species isolated were *Cladosporium* sp, *Penicillium* sp, *Aspergillus* sp, *Monilia* sp and *Fusarium* sp.

Water bodies undergo temperature variations along with normal climatic fluctuations (Achudume, 2009). The temperature range of surface and sub-surface water revealed mesophilic levels. The result shows that there was no significant difference ($P > 0.05$) in temperature levels of the water samples throughout the study period, but slight vertical variations were observed. This may be attributed to the fact that water bodies exhibit vertical stratification of temperature within the water column. Temperature affects physical, chemical and biological processes in water bodies and therefore the concentration of many variables.

The results observed however, are within the 35 °C recommended limit for aquatic environment (APHA, 1995). The surface water temperature levels however exceeded the 25 °C limit recommended by WHO for surface water sources (APHA, 1995). The pH was slightly acidic throughout the study period. The values are within WHO recommended limits of 6.5 – 8.5. pH is critical to the activity and biodiversity of aquatic organisms because it influences the function of virtually all enzymes, hormones and protein which control all aspects of metabolism, growth and development. The conductivity levels observed in this study is in conformity with the report of Hogan and Ward (1998) that the conductivity of most water bodies ranges from 10 – 1000 μscm^{-1} but may exceed 1000 μscm^{-1} especially in polluted waters, or those receiving large quantities of land run-off.

Though, the BOD levels were slightly higher during the dry season than the wet season, there was no significant difference ($P > 0.05$) throughout the study period. This may be associated with the relatively stable level of pollution during the period of study. The slightly lower but insignificant ($P > 0.05$) levels of DO experienced during the wet season may be due to the level of biological respiration, including that related to decomposition processes which reduce DO concentrations (APHA, 1998). The significantly ($P < 0.05$) higher levels of salinity observed are typical of a brackish water environment with high concentration of nutritive salts. The amount of salts seems to be adequate for the growth and productivity of microorganisms. The changes in ionic contents and ionic ratio of waters are very often linked to pH changes (Barlett and Stirling, 2003).

The levels of nutritive salts observed may be attributed to the level of crude oil pollution, which has been associated with increase in nutritive salts and salinity levels of aquatic systems. It may also be attributed to the diversity of the estuarine ecosystem and the allochthonous sources of nutrients into the estuaries. The nitrate ion (NO_3^-) is the common form of combined nitrogen found in natural waters. The relatively low concentrations of total nitrogen in this study area may be ascribed to the level of crude oil pollution which can decrease the availability of nitrogen in an environment. Generally, higher concentrations of THC were observed during the dry season than the wet season in both microhabitats. This may be ascribed to increase bunkering activities and oil spillage observed in the estuary during the dry season.

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