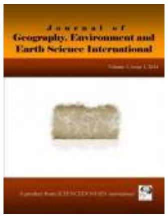

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Seasonal Variation in the Physico-chemical and Microbial Characterization of Sediment and Water Samples from Selected Areas in Ondo Coastal Region, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AS and EO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AS managed the literature searches, analyses of the study performed the physicochemical analysis. Authors AS, AC and EO managed the experimental process. Author AC identified the species of bacteria and fungi. All authors read and approved the final manuscript.

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ABSTRACT

Water and sediment samples were collected along river Ilaje in the Ondo coastal area of Nigeria during the dry and wet season. Water analysis was carried out by using standard methods for the examination of water and waste water, water and sediment samples were digested and analyzed for heavy metals using atomic absorption spectrophotometer. Pour plate technique was employed for the enumeration of bacteria and fungi using nutrient agar and potato dextrose agar respectively, the cultured media was supplemented with 2% oil (petrol, diesel and kerosene) as the only source of carbon and energy. After incubation, biochemical test and morphological features were carried out to identify the microorganisms. The result shows that the physicochemical properties of water

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varies with the seasons total hardness (620-2,424 mg/l), calcium hardness (130-1,478 mg/l), Alkalinity (120-144 mg/l), phosphate (2.21-2.78 mg/l), nitrate (0.90-22.50 mg/l), sulphate (228-395 mg/l), chloride (1990-3532 mg/l), the concentration of metals in water samples varies with the season having the lowest in Cd (0.01 mg/l) and the highest in Cr (0.66 mg/l). The concentration of metals varies in season with a spatial distribution in the top and bottom sediment samples. Oil tolerating fungi and bacteria were detected from the sampling sites with variation in the dry and wet seasons, *E. coli* was only detected in the water samples during dry season only. The presence of pathogens and water properties exceeding the WHO standard renders the water unfit for drinking and domestic use.

Keywords: Water analysis; microorganisms; heavy metals; sediment; biochemical test; morphological features.

1. INTRODUCTION

Assessment of water quality is not only for suitability for human consumption but also in relation to its agricultural, industrial, recreational, commercial uses and its ability to sustain aquatic life [1]. Sediment is the loose sand, clay, silt and other soil particles that is deposited at the bottom of body of water or accumulated at other depositional sites. Sediments can emanate from the erosion of bedrocks and soil or from the decomposition of plants and animals. Information on the study of sediment profile provides a better view on the impact of human activities on the ecosystem revealing the ultimate ability of sediment to acts as sink for heavy metals [2]. Human population growth rate has brought about an increase of water supply, irrigation, fish production and recreation offered by the aquatic system and this has put enormous pressure and stress on the quality of the aquatic environment. This highlights the significance of the need to preserve our freshwater resources. However, the amount of freshwater available for mankind is on the decrease [3]. Traditionally, the management of aquatic resources in coastal waters focused primarily on water quality. However, the importance of sediments in determining the fate and effects of a wide variety of contaminants has become more apparent in recent years. In addition to providing a habitat for many organisms, sediments are important because many toxic substances found only in trace amounts in water may accumulate to elevated levels in sediment. As such, sediments serve both as reservoirs and as potential sources of contaminants to the water column. Therefore, sediment quality data provide essential information for evaluating ambient environmental quality conditions in aquatic ecosystems. Metals are found naturally throughout the earth, in rocks, soils and sediments trapped in stable form. Toxic anthropogenic imputes results in contamination of the soil. The chemical behavior of these contaminants are controlled by soil composition, soil properties such as pH and a number of processes, such as metal cation release from contamination source, cation exchange and specific adsorption onto surfaces of minerals [4,5]. Identification of microorganisms as bioindicators for crude oil pollution and natural remediation as well as petroleum hydrocarbon levels in water and sediments has reported that microorganisms are capable of using organic substances, natural or synthetic, as sources of nutrients and energy hence, exhibiting remarkable range of degradative capabilities [6,7].

Varied concentration of crude oil and wasted engine oil has an impact on the population of fungi in soil and phytoplankton respectively [8,9]. Crude oil concentration in the range of 6.0 to 50 mg/L evoked toxicity on four Nigerian indigenous phytoplankton species, this is a potential danger to phytoplankton species biodiversity conservation [10]. The aim of the present study is to study the seasonal variation in the physiochemical and microbial characterization of water and sediment samples from Ondo coastal area, Nigeria and to access the water quality in terms of drinking water standard.

2. MATERIALS AND METHODS

2.1 The Study Area

The Ondo coastal area of Nigeria is located on Latitude 5°50'N-6°09'N and on Longitude 4°45'E-5°05'E, it comprises of river Ilaje which borders with the Atlantic ocean, river Ilaje is a deltaic region which takes its origin from river Oluwa in Ondo State. The Ilaje communities depend on river Ilaje for their economic and domestic activities. The sampling sites are Abereke and Ogogoro areas in river Ilaje, Ondo state, Nigeria.

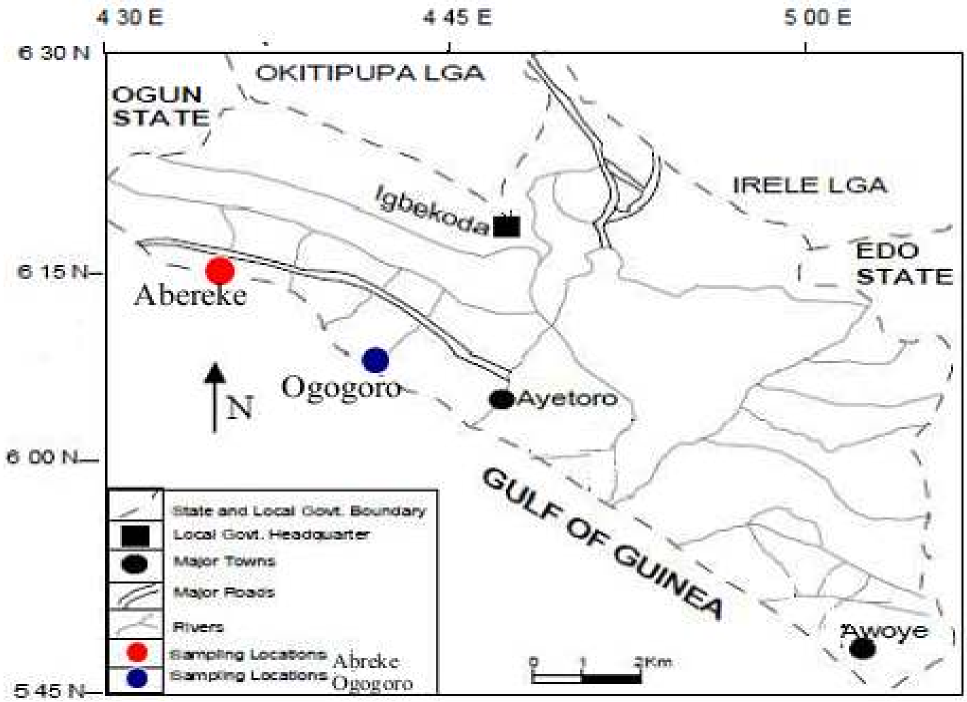

Fig. 1. Map of Ilaje community in Ondo coastal area showing the sampling sites

2.2 Sampling and Pre-treatment of Samples

Water samples were collected in glass bottles while sediment samples were collected using a soil hugger. The water and sediment samples were collected during the dry (December 2014) and rainy (May 2015) seasons, sampling points were geo-located using GPS (Global Position System) to ensure consistency. Water and microbial analysis was conducted within 24 hours of sample collection. The sediment samples were air dried for two weeks and pulverized using laboratory mortar and pestle. It was later sieved using a 2 mm mesh size sieve.

2.3 Physico-chemical Analysis

The following physicochemical parameters were analyzed; pH, temperature, turbidity, conductivity, TDS, TSS, dissolved oxygen, biochemical oxygen demand, nitrate, sulphate, phosphate, alkalinity, total hardness, calcium hardness, magnesium hardness, chloride content using standard methods for the examination of water and waste water [11]. One hundred mililiter of water samples were digested using 10 ml of HNO_3, while 2 g of air dried sediment sample was digested using 20 ml of a mixture of HNO_3, HF and $HClO_4$, (3:2:1) to near dryness. The digest of water and sediment samples was analyzed for heavy metals using an atomic absorption spectrophotometer (AAS BUCK SCIENTIFIC 210 VGP). SO_4^{2-}, PO_4^{3-} and NO_3^- were analyzed using colorimeter (DR /890). All methods of analysis were consistent with known standard methods [12-14]. All chemicals used for water analysis and elemental analysis were of analytical grade.

2.4 Microbial Analysis of Sediment and Water Samples

2.4.1 Isolation and enumeration of bacteria and fungi

Pour plate technique was employed for the enumeration of both bacteria and fungi. Potato Dextrose Agar (PDA) was used for the isolation of fungi while Nutrient Agar (NA) was used for bacteria. The medium was prepared according to the manufacturer instruction and sterilized at 121℃ for 15 min. It was supplemented with 2% (v/v), filter-sterilized oils (petrol, diesel, and kerosene) which serve as the only source of carbon [15]. The sediment (g) and water (ml) samples were serially diluted and 1ml suspension was aseptically transferred from each 10^3 dilution into sterile Petri dish and seeded with the medium. The PDA was allowed to gel and incubated at 28±2℃ for 3 days while NA plates were incubated at 35±2℃ for 24 h. A control devoid of the sample was prepared for each set of the experiments. All experiments were performed in triplicates. Colonies were counted after incubation.

2.4.2 Identification of bacterial isolates

The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics were observed for each bacteria colony after 24 h of growth. The appearance of the colony of each isolate on the agar media was studied and the characteristics observed include; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Biochemical characterizations were done according to the method of [16].

2.4.3 Identification of fungal isolates

The fungal colonies were sub-cultured on Potato Dextrose Agar (PDA). The isolates were identified based on their morphological and microscopic features. Two drops of cotton-blue-in-lactophenol were placed on clean glass slide and small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain on the slide. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles and observed under the microscope for vegetative and reproductive structures [17].

3. RESULTS AND DISCUSSION

The average value of the physicochemical parameters determined for the water samples are reported in Table 1 and compared with WHO standard [18-20] for the 2 sampling sites. The mean temperature value (25.2℃ -28.5℃) of river Ilaje in Ondo coastal area fell within the optimum temperature 28℃ – 30℃, within which maximal growth rate, efficient food conversion, best condition of fish and other aquatic life, resistance to disease and tolerance of toxins (metabolites and pollutants) are enhanced [21]. The mean pH value (7.40-8.00) falls within the required pH value of 6.5-8.5 for the survival of fishes and other aquatic life. The pH of an aquatic system is an important indicator of the water quality and the extent of pollution in the watershed areas. Low pH values or acidic waters are known to allow toxic elements and compounds such as heavy metals to become mobile thus producing conditions that are inimical to aquatic life [22-26]. The mean turbidity value (42.3NTU-49.6NTU) is higher than the WHO standard of 5NTU. Turbidity reflects the materials dispersed or dissolved in the water column and can indicate problems with treatment processes, particularly coagulation/ sedimentation and filtration [27]. The mean value of the electrical conductivity ranges from 520 µs/cm -5,804 µs/cm which exceed the maximum permissible WHO standard of 1,200 µs/cm, the high level of electrical conductivity of river Ilaje in Ondo coastal area indicate a brackish water because conductivity is an index of the total ionic content of water, and therefore indicates freshness or saltiness of a water sample [28,29]. The mean value of total dissolve solid (TDS) falls below 500 mg/l, which is the WHO required standard. The mean value of chloride, phosphate, nitrate and sulphate show a variation with the highest value during the dry season while the lowest value during the wet season. The observation in seasonal variation could be attributed to dilution effect which occurs as a result of increased in the volume of the water body due to heavy rain fall during the wet season. The mean value of the total hardness (620 mg/l-2,424 mg/l) and calcium hardness (130 mg/l-1,478 mg/l) of the river Ilaje in Ondo coastal area exceed the WHO maximum permissible standard of 150 mg/l. The variation in magnesium hardness is a function of total hardness and calcium hardness. The mean value for alkalinity is fairly constant at Ogogoro sampling site but varied at Abereke sampling site, at both site the mean alkalinity value exceed the WHO maximum permissible standard of 100 mg/l. The mean value of the concentration of heavy metals in water samples from the 2 sampling sites during the dry and wet season is presented in Fig. 2. The concentration levels of Zn, Fe and Cu in the water samples were lower than the WHO maximum permissible drinking water standard which makes the river Ilaje of Ondo coastal area free from Zn, Fe and Cu contamination hence no detrimental effect are expected from domestic usage in terms of Zn, Fe and Cu concentration, but the concentration levels of Cr, Cd and Pb were higher than the WHO maximum permissible drinking water standard making the river Ilaje of Ondo coastal area contaminated Cr, Cd and Pb hence, detrimental effect will be expected from domestic usage due to Cr, Cd and Pb contamination. The concentration of heavy metals in the water samples from the two sampling sites were observed to vary between the dry (December 2014) and wet (May 2015) seasons. From the two sampling site it was observed that the concentration of Pb, Cu, Cd and Fe were higher during the wet season compared to the dry season, the concentration of Cr was higher during the dry season compared to the wet season, while the concentration of Zn varies with both seasons.

Table 1. Mean value of the physiochemical analysis of water samples at the 2 sampling sites

Parameter	Unit	Ogogoro dry season	Ogogoro wet season	Abereke dry season	Abereke wet season	WHO standard
Temperature	℃	25.2	28.5	25.3	28.2	25-29
pH	Nil	7.50	8.00	7.40	7.9	6.5-8.5
Turbidity	NTU	45.7	49.6	42.3	48.2	<5
Electrical conductivity	µs/cm	2,169	520	5,804	1,394	<1200
Total dissolved solids	mg/L	4.22	4.53	11.53	11.53	<500
Total hardness	mg/L	1,730	620	2,424	1,797	<150
Calcium hardness	mg/L	945	130	1,478	400	-
Magnesium hardness	mg/L	785	490	946	1,397	-
Alkalinity	mg/L	120	120	150	144	<100
Chloride (Cl^-)	mg/L	2,996	1,990	3,532	2,474	<250
Phosphate (PO_4^{3-})	mg/L	2.43	2.21	2.78	2.67	<5
Nitrate (NO_3^-)	mg/L	18.4	0.90	22.5	2.43	<50
Sulphate (SO_4^{2-})	mg/L	395	228	374	365	<250
B.O.D	mg/L	0.047	0.047	0.027	0.027	0
D.0	mg/L	6.67	6.67	7.0	7.0	>7.5

Source: WHO (1996, 2004, 2011)

Table 2. WHO standard for heavy metals in drinking water

Metals in water	Zn	Fe	Cr	Cd	Cu	Pb
WHO standard	<3	<0.3	<0.05	<0.003	<2	<0.01

Source: WHO (1996, 2004, 2011

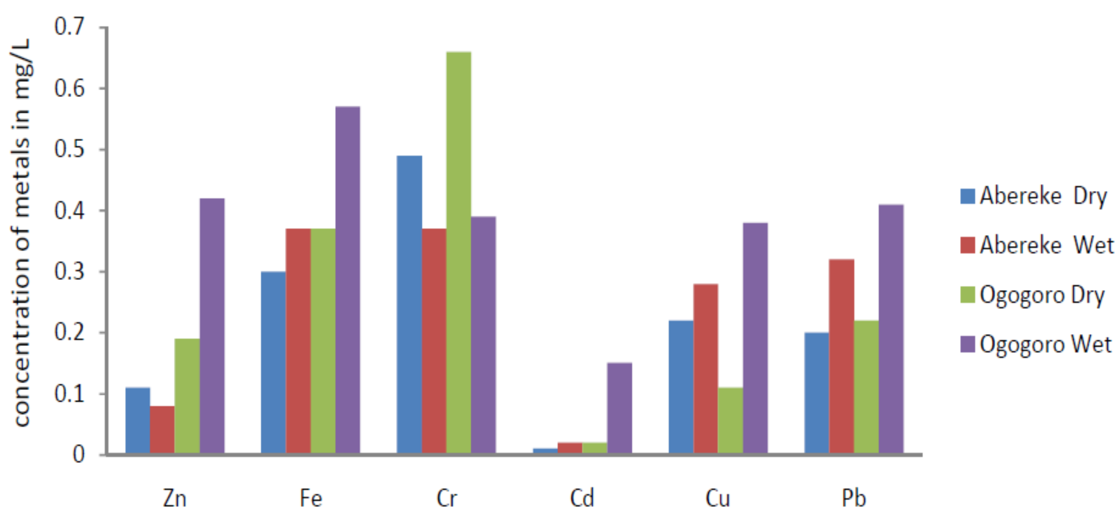

Fig. 2. A graph showing the seasonal variation of heavy metals in the water samples from the 2 sampling sites

Table 3. Morphological characteristics of fungal isolates

Isolate	Morphological characteristics
Aspergillus versicolor	Hyphae are septate and hyaline conidial heads are biseriate and loosely radiate. Vesicles are small, with metulae and phialides covering most of the vesicle
A. sydowii	The conidial heads are spread out. The stalk of the conidiophores are hyaline, vesicles are spherical. Phalides arise from the metulae.
A. terreus	Conidial heads are compact and biserate. Conidiophores are smooth and hyaline. Aleurioconida produced directly on the hyphae
A. flavus	Colonies are olive to lime green with a cream reverse. Hyphae are septate and hyaline. Conidiophores are coarsely roughened and uncoloured. Vesicles are globose, metulae are covering the entire vesicle in biserate species.
A. fumigatus	Conidia heads are long, globose to prolate. Conidiophores hyaline vesicle ovate
Mucor spp	Smooth conidiophores that are non-septate
P. chrysogenum	Septate hyaline hyphae, branched conidiophores, mutulae philades and conidia are present.
Cladosporium spp	They produce septate brown hyphae, erect and pigmented conidiophores and conidia. Conidia are elliptical to cylindrical in shape
Alternaria spp	Branched acropetal chains (blastocatenate) of multicelled conidia are produced, branched conidiophores are present.
Fusarium spp	Hyphae are septate and hyaline. Phailades are cylindrical conidiophores are medium length

The concentration levels of the heavy metals vary both seasonally and in spatial distribution in terms of the top and bottom sediment deposit of river Ilaje in the Ondo coastal area of Nigeria. The concentration of most metals including Zn, Cr, Cd, Cu and Pb were higher during the wet season than the dry season for both the top and bottom sediments in the two sampling sites. Except for Fe, whose concentration was lower in the wet season compared to the dry season at the two sampling site. From the result obtained in Fig. 3, the Ondo coastal area is highly enriched with Fe and Cr. Fe concentration level was high in both top and bottom sediment deposit for both seasons from the two sampling sites, but Cr concentration level was higher in the bottom sediment compared to the top sediment in both seasons from the two sampling sites. As a result of this high concentration of Fe and Cr, leaching of Fe and Cr into the body is very possible.

3.1 Fungal Population in Sediment

The result showing the load of oil tolerating fungal in two different seasons (dry and wet season) of two different locations, when grown on Potato Dextrose Agar (PDA) supplemented with kerosene, diesel and petrol are presented in Fig. 4. The population of oil-tolerating fungi that grew on kerosene, diesel, and petrol during dry season ranged between 8.67±0.33 and 19.33±0.33 sfu/g (Abereke sediment), and 1.33±0.33 and 2.00±0.00 sfu/g (Ogogoro sediment) respectively. Fungal load during wet season ranged between 4.33±0.33 and 10.67±0.33 (Abereke sediment), and 1.67±0.33 and 2.67±0.33 (Ogogoro sediment). The fungal population obtained from the wet season sediment samples contained fewer fungal than the dry season sediment samples in Abereke, while there was no significant different in the fungal count of Ogogoro sediment samples for both dry and wet season.

3.2 Fungal Population in Water

The lowest fungal population from oil tolerating water that survived on diesel and petrol during the dry season was 1.00±0.00 sfu/ml (Abereke). The highest fungal populations observed were 3.00±0.00 sfu/ml (petrol), 2.00±0.00 sfu/ml (Diesel), in Ogogoro (Fig. 5).

3.3 Bacterial Population in Sediment

The population of bacterial obtained from oil tolerating bacterial in two different seasons (dry and wet season) when grown on nutrient agar (NA) supplemented with kerosene, diesel and petrol are presented in Fig. 6. During the dry season, the count ranged between 12.67±0.67 and 28.33±0.33 cfu/g (Abereke sediment) and 9.33±0.33 and 29.33±0.33 cfu/g (Ogogoro sediment) respectively. Bacterial load during wet season ranged between 3.00±0.00 cfu/g and 28.33±0.3 cfu/g (Abereke sediment) and 2.67±0.33 cfu/g and 4.67±0.33 sfu/g (Ogogoro sediment) respectively. Bacterial count obtained from dry season is significantly higher than those obtained for wet season in the examined locations.

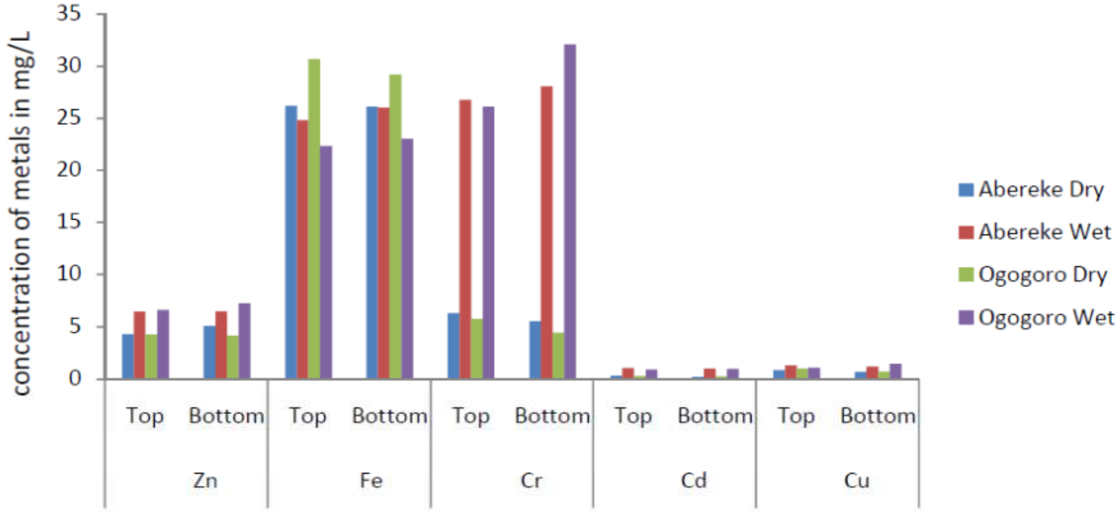

Fig. 3. Showing the seasonal variation in the spatial distribution of metals in the sediment (top=0-15 cm, bottom= 15-30 cm) samples from the 2 sampling sites

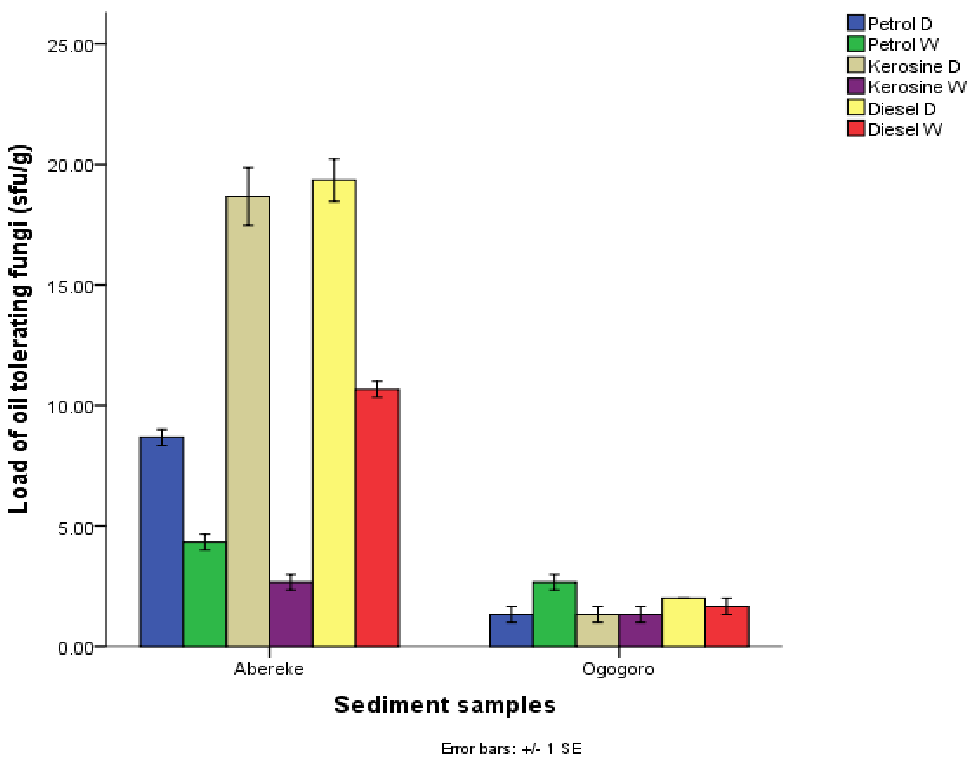

Fig. 4. Seasonal variation in the population of oil tolerating fungi present in sediment samples *(D= dry, W = wet)*

Table 4. Biochemical characteristics of isolates

Tests	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Colonial shape	**Large flat greenish colony with irregular eges and typical metallic luster**	**Circular bright yellow colonies with convex entire margin**	**Colonies are large, margin is undulated, with circular form and flat elevation**	**Small, non glistening yellowish colonies**	**Circular tiny colonies**	**Colonies with greenish metallic sheen on EMB**
Gram rxn	-	+	+	+	+	-
Shape	Rod	Cocci	Rod	Cocci	Cocci	Rod
Motility	+	-	+	-	-	+
Spore formation	-	-	+	-	-	-
Citrate	-	+	-	-	-	-
Urease	-	-	-	-	-	-
Catalase	+	+	+	+	-	-
Coagulase	-	-	-	+	-	-
Oxidase	+	+	-	-	-	-
Methyl red	-	-	-	+	-	+
Indole	-	-	-	-	-	+
Glucose	+	+	+	+	+	-
Lactose	-	-	+	+	-	+
Probable organisms	*Pseudomonas aeruginosa*	*Micrococcus* spp	*Bacillus* spp	*Staphylococcus aureus*	*Streptococcus* spp	*E. coli*

Microorganisms isolated and identified were Aspergillus versicolor, A. sydowii, A. terreus, A. flavus, A. fumigatus, Mucor spp, Penicillum chrysogenum, Cladosporium spp, Alternaria spp, Fusarium spp, Pseudomonas aeruginosa, Micrococcus spp, Bacillus spp, Staphylococcus aureus, Streptococcus spp, and Escherichia coli

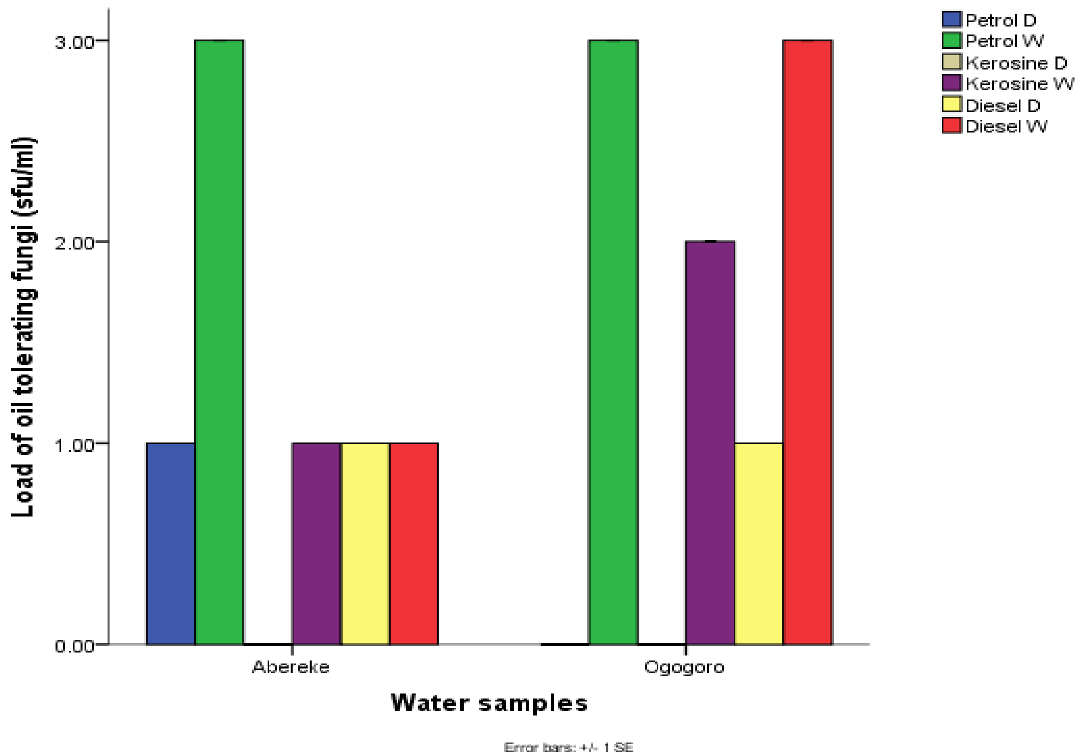

Fig. 5. Seasonal variation in the population of oil tolerating fungi present in water samples
(D= dry, W = wet)

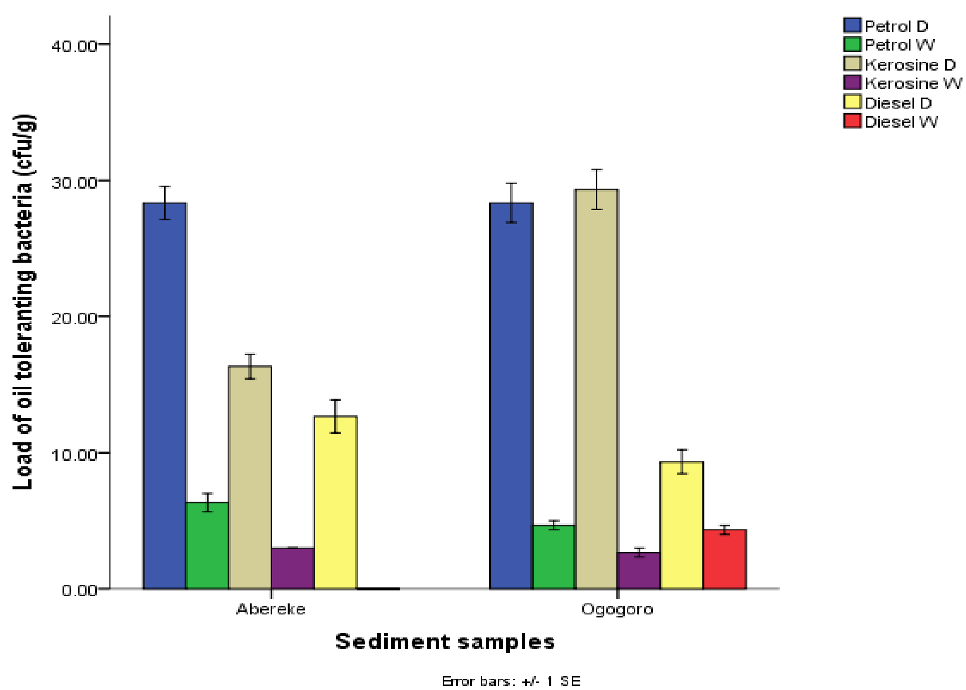

Fig. 6. Seasonal variation in the population of oil tolerating bacterial present in sediment samples
(D= dry, W = wet)

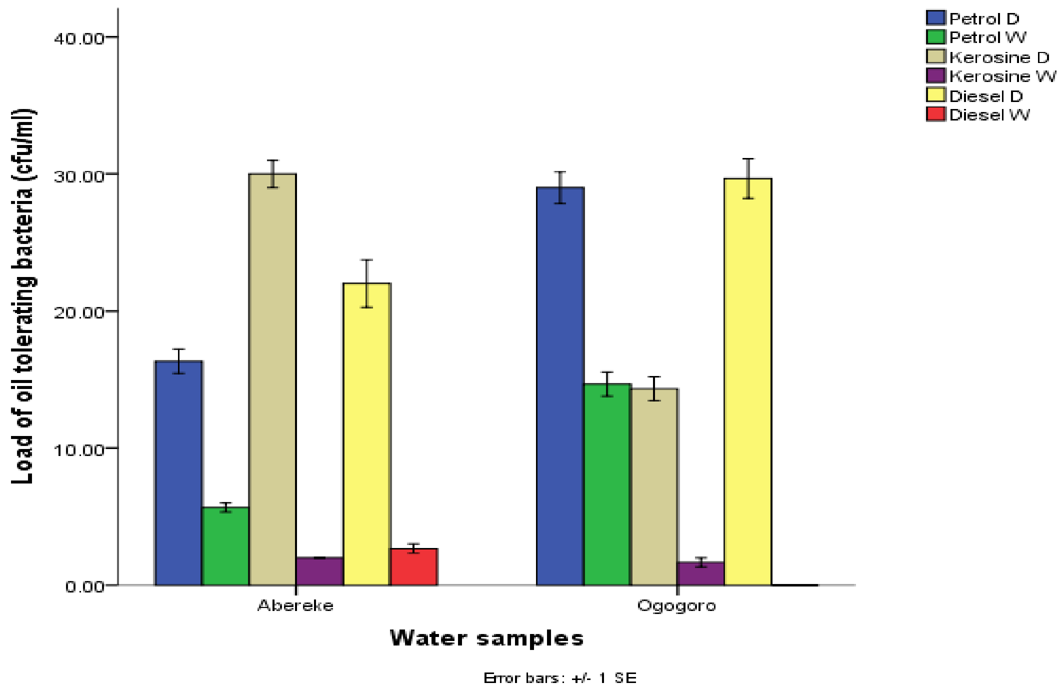

Fig. 7. Seasonal variation in the population of oil tolerating bacterial in the water samples
(D= dry, W = wet)

3.4 Bacterial Population in Water

The bacterial isolated from water samples during dry season when grown on kerosene, diesel, and petrol had the populations of 14.33±0.33 (Ogogoro) and 30.00±0.00 cfu/ml (Abereke); 22.0±0.00 (Abereke) and 29.67±0.67 cfu/ml (Ogogoro); 16.33±0.33 (Abereke) and 29.00±0.00 cfu/ml (Ogogoro) respectively. Bacterial load for wet season when grown on kerosene, diesel and petrol were 2.00±0.00 (Abereke) and 1.67±0.33 cfu/ml (Ogogoro); 2.67±0.67 cfu/ml (Abereke); 5.67±0.33 (Abereke) and 14.67±0.33 cfu/ml (Ogogoro) respectively. Relatively the bacterial grow better in the dry season than wet season. However, bacterial also grow more on petrol and diesel than kerosene.

The tolerating capabilities on different oils revealed that the microorganisms isolated from the sediment and water samples were able to tolerate oil. The load increased during the incubation periods, indicating that they were able to tolerate and utilize the oils for their growth and development. All the organisms maximally utilized all the hydrocarbon substrates as the sole source of carbon and energy. More oil tolerating bacteria were recorded in the dry season than wet season. It can be deduced that these microorganisms require hydrocarbon as their sole source of energy. The result of this finding agrees with the report of Seasonal dynamics of microbial population and physicochemical characteristics of a water body receiving industrial pollutants in Port Harcourt [30], which reported higher concentration of bacteria in the dry season when seasonal dynamics of microbial population of a water body was examined.

4. CONCLUSION

The parameters obtained from the water analysis were high with most of the parameters exceeding the WHO standard for drinking water, the heavy metals concentration in the water samples were higher than the WHO standard rendering the water body unfit for drinking purposes. The heavy metals concentration in the top and bottom sediment varies with the dry and wet seasons were high in some of the metals which could lead to leaching into the water body. The presence of pathogens render the water unfit for drinking purposes also this study shows that oil tolerating microorganisms could act as a bioindicators for crude oil pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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