



# Characteristics of Microorganisms Associated with Crude Oil Impacted Surface Water Body in Bodo/Bonny River, Nigeria

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The contamination of water is a serious environmental problem as it adversely affects the human health and the biodiversity in aquatic ecosystems. The organisms that inhabit the water space use oxygen for metabolic activities and the blocking of the water surface of the river by total petroleum hydrocarbons tend to reduce the dissolved oxygen available within the system thereby making the survival of aquatic life difficult particularly the non-hydrocarbon utilizing microorganisms. The aim of this study was to determine the microbiological characteristics of water samples from Bodo/Bonny River impacted by crude oil spill. Water and dead fish samples were collected from four stations while the fifth sample was collected from a link fish pond which served as control. Microbiological analysis of samples collected was analysed accordingly using standard analytical methods. Bacterial isolates from the sampling stations show that *Vibrio cholerae*, *Shigella* sp, *Escherichia coli*, *Vibrio* sp, *Salmonella* sp, *Bacillus* sp, *Klebsiella* sp, *Actinomycetes*, *Clostridium*, *Listeria* sp, *Acinetobacter* and *Pseudomonas* sp and fungal isolates namely, *Aspergillus niger*, *Penicillium* sp, *Aspergillus flavus*, *Rhizopus*, *Mucor* sp and *Candida* sp were identified. Percentage occurrence of

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both isolates show that *E. coli* 15%, *Vibrio* species 13%, *Pseudomonas* 12%, *Klebsiella* 12% and *Shigella* 9% while *Aspergillus niger* had 37.18%, while *Mucor* had 25.64%, *Penicillium* sp 11.38%, *Aspergillus flavus* 11.38% and *Candida* sp 10.26%. Microorganisms isolated from the river water that survived the harsh influences associated with oil spill from this study shows that most of the microorganisms could be genetically cloned as hydrocarbon utilizing organisms for cleanup of oil contaminated environments because of their existence and sustenance to mankind.

**Keywords:** Microorganisms; surface water; crude oil pollution; dead fish; bodo/bonny.

## 1. INTRODUCTION

Water is one of the most important natural resources known to mankind. Man has depended on water resources for various purposes since time immemorial as a critical component of the environment for living organisms (including plant and animals) [1,2]. The conservation and sustainable use of water resources has been considered of great importance on earth [1]. The uniqueness of water bodies often gets gradually deteriorated once it is engulfed with pollutant(s). Water pollution is one of the most principal environmental and public health problems in most water bodies [3]. Meanwhile, the surface water resources in Bodo/Bonny communities of Rivers State suffers regular pollution of its ecosystem due to increase in crude oil exploration, refining and activities of other industrial establishments operating within the coastal areas of the Niger Delta region of Nigeria. This have resulted in the wide scale contamination of most of its creeks, swamps and rivers with hydrocarbons and dispersant products. Despite community concerns about their health and the damage of their water bodies, water pollution in the Niger Delta is commonly attributed to activities related to oil exploitation; resulting in serious threat to public health and the ecosystem. Farmlands and drinkable water sources are destroyed and therefore cause a draw back in fishing activities of the coastal waters by fishermen who live on such subsistence practice as a source of livelihood. The surface water including rivers and streams have been reported to be reservoirs of a spectrum of pathogenic microorganisms, including coliforms, thermotolerant coliform bacteria, *Enterococcus faecalis*, and *Salmonella* (Fiello et al., 2014) and as such, microbiological quality evaluation of water is a critical parameter to measure health-associated risks. These microorganisms enter the water bodies through natural processes, mostly as a result of anthropogenic factors. These anthropogenic factors including agricultural activities, industrial

discharges and domestic wastes contribute as sources of water pollutants (Wilson, 2018) thereby affecting the quality of water available for plant, animal and human use [4]. The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life (Raji et al., 2015). Meeting water quality expectations for streams and rivers therefore, is required to protect drinking water sources, encourage recreational activities and to provide a good environment for fish and wildlife [5]. The importance of water resources with respect to microbiological quality is imperative to ascertain their suitability for both wild animal and human use. This study therefore examined the microbiological characteristics of water samples from Bodo/Bonny River impacted by crude oil spill.

## 2. METHODOLOGY

### 2.1 Sampling Location

Samples for this study were collected from four different location of surface water in Bodo/Bonny River in Gokana Local Government Area of Rivers State. Control samples were taken from link fish pond located away from the location where there was no record of crude oil pollution within the river environment. The sampling stations were chosen based on an experimental scheme design following ecological settings and human activities in the area. Bodo Creek is characterized by low tidal energy current, making its swamps and canals exceptional breeding grounds for a vast variety of fish and shellfish. It also provides an excellent habitat for periwinkles (*Tympanotonus fuscatus*; *Tympanotonus fuscatus varradula*; *Pachymelania aurita*; *Pachymelania fusca*) [6]. The original diversity of shellfish found in the Creek included bloody cockle (*Senilia senillis*), oyster (*Crassostrea gasar*), swimming crab (*Callinectis amnicola*), razor clam (*Tagelus adansonii*), land crab (*Cardisoma amatum*), and mangrove purple hairy crab (*Goniopsis pelli*) [6]. The Bodo/Bonny River

meet several socio-economic needs including aquaculture, fishing, sand dredging and drainage of the various towns and villages bordering it. Fig 1 is a map showing sampling points in the river.

## 2.2 Sample Collection

Samples used for this study are surface water and fishes (*Pseudotolithus elongatus*) from the river and Link fish pond as control. Dead floating fishes were collected from the seashore during the sampling period (Plate 1). Sampling was done between in the mornings between 10 am-12 noon each day for a period of 12 months covering both wet and dry seasons, at an interval

of once a month. Sample were collected in duplicates from each location, monthly. Collection of the samples was done in the hours when tide in the river was at its peak in duplicates.

## 2.3 Surface Water

Surface water samples were collected using the method of Adesemoye et al. [7]. Sterile 1.5 Litre bottles were used to aseptically collect the surface water. The samples were collected at four different points (about 50m apart) in the direction of water flow while the fifth sample served as control from Link fish pond.



Plate 1. Fish (*Pseudotolithus elongatus*) Samples from the Bodo/Bonny River

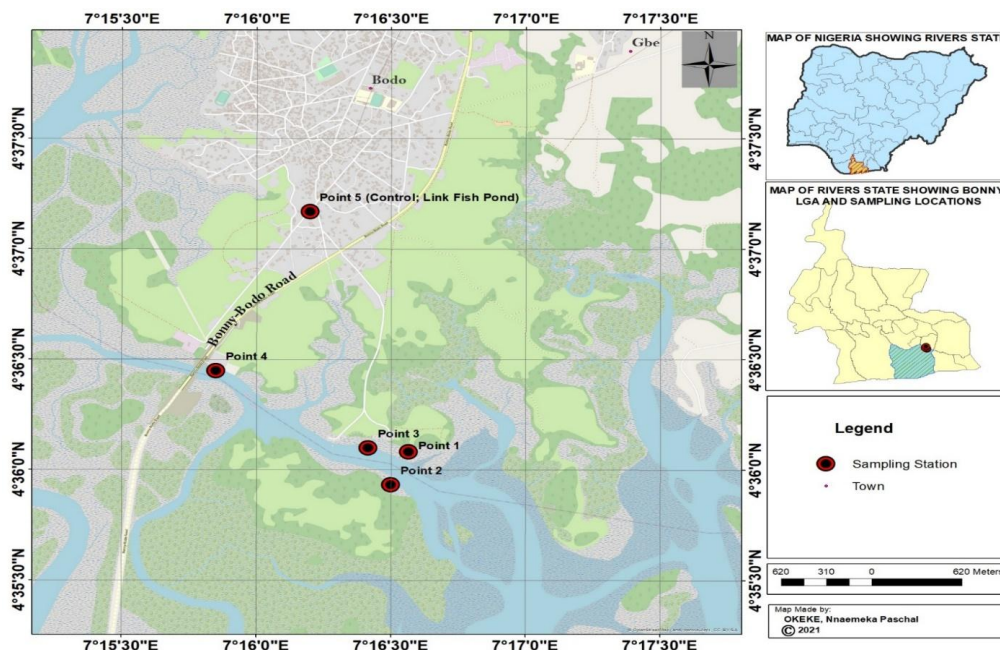


Fig. 1. Map indicating sampling stations at Bodo/Bonny River

The Link fish pond is situated on ground 100 meters away from the river but lined with mud. Actually water that percolates in the pond comes from the river water either by tide or seepage from the groundwater which could be influenced by the level of the water table. To collect the surface water, base of the sterilized sample container was held with one hand, plunged about 30 cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow [8]. About 500 ml of the sample collected from each station were pooled together to get a composite sample. After collection, the samples were placed in a cooler containing ice blocks and transported immediately to the laboratory for analysis.

## 2.4 Serial Dilution

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to a more usable concentration or countable colonies usually 30 to 300 colonies. Each dilution will reduce the concentration of bacteria by a specific amount. Thus, prior to inoculation of the samples to the respective culture medium used in this study, serial dilutions were employed using standard methods [9]. One milliliter each of the water samples was separately added to 9 ml of 0.1% peptone water diluents to give a 10-3 dilution. After thorough shaking further serial 10- fold (v/v) dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluents to a range of 10-3 dilutions. The fresh fish sample was opened up to collect the intestines and gills and then immersed in a diluent before an aliquot of it was inoculated onto appropriate media for growth of microbes inhabiting the fish sample. The smoked fish sample was macerated to homogenize the entire sample and mixed with sterile diluent before it was used for inoculation in an appropriate media

## 2.5 Inoculation, Incubation and Enumeration of Microbes in Water and Fish (*Pseudotolithus elongatus*) Samples

### 2.5.1 Total Heterotrophic Bacteria (THB)

Aliquots (0.1 ml) of various dilutions were inoculated to surface dried appropriate medium in triplicates and spreading with flamed bent glass spreader and incubated at 37<sup>o</sup> C for 24 hours. Total Heterotrophic Bacteria from the respective samples (Water, and fish) was

enumerated as described by Prescott et al. [9]. Bacterial colonies that appeared on the nutrient agar plates were counted and the mean expressed as cfu/ml for the water samples (Nrior and Odokuma, 2015). The colony forming unit per milliliter of sample was calculated using the formula below:

$$\text{CFU/ml} = \frac{\text{(number of colonies)}}{\text{(Dilution} \times \text{volume plated)}} \quad \text{(Equation 1)}$$

### 2.5.2 Total Heterotrophic Fungal Count (THF)

This was determined using the Potato Dextrose Agar (PDA) onto which 1ml of 10% lactic acid was added to suppress bacterial growth [10]. The spread plate technique as described by Prescott et al., [9] was adopted. An aliquot (0.1ml) of the appropriately serially diluted samples were inoculated in duplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at 25<sup>o</sup>C for about 5 days after which the colonies were counted and the mean of the count recorded accordingly.

### 2.5.3 Total Hydrocarbon Utilizing Bacteria (THUB)

The Vapour Phase Transfer method of Mills and Colwell (1978) was adopted to determine the population of hydrocarbon utilizing bacteria. Aliquots (0.1 ml) of the serially diluted samples were inoculated on Mineral Salt Agar media using the spread plate technique as described by Odokuma (2003). Sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar was placed aseptically in the cover of the inoculated agar plates in duplicates. The plates were incubated for 5 days at room temperature (25<sup>o</sup>C). After the incubation period, the number of colonies were counted and the mean of the colonies were determined in cfu/ml.

### 2.5.4 Total Hydrocarbon Utilizing Fungi (THUF)

The Vapour Phase Transfer method of Mills and Colwell (1978) was adopted to determine the population of hydrocarbon utilizing fungi. Aliquots (0.1 ml) of the serially diluted samples were inoculated on Mineral Salt Agar media added 1ml of 10% lactic acid to suppress bacterial growth [10] using the spread plate technique as described by Mills and Colwell (1978). Sterile filter paper discs soaked in filter-

sterilized crude oil which served as the only carbon source in the Mineral Salt Agar was placed aseptically in the cover of the inoculated agar plates in duplicates. The plates were incubated for 5 days at room temperature (25°C). After the incubation period, the number of colonies were counted, mean of the colonies were recorded.

### 2.5.5 Purification of isolates

After incubation, pure isolates were obtained by picking (with sterile inoculation loop) distinct culturally and morphologically different colonies from the various media plates. These were subjected to streaking on sterile nutrient agar in plates until pure distinct colonies were formed.

### 2.5.6 Identification of bacterial isolates

The discrete bacteria isolated from the fishes and water samples were characterized based on their cultural morphological which include colour, texture, shape, size, elevation, etc of the isolate while the biochemical characteristics include Gram's reaction, motility, catalase, oxidase, spore formation, indole production, methyl red, citrate utilization, voges proskauer test and sugar fermentation of the discrete bacterial isolates which were compared with the Bergey's Manual of Determinative Bacteriology for the identification of the bacterial isolate [11].

### 2.5.7 Identification of fungal isolates

The fungal isolates were identified based on morphological and microscopic characteristics such as colony growth pattern, conidial morphology and pigmentation. The technique described by Odokuma and Okpokwasili [12] was also adopted for the identification of the isolated fungi using cotton blue in lactophenol stain. This was done by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with  $\times 10$  and  $\times 40$  objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with standard scheme for identification of fungi as adopted by Okerentugba and Ezereonye [10]

## 3. RESULTS

### 3.1 Microbiology of Surface water and Link Fish Pond Water

The microbial counts for the Surface water and Link Fish Pond water from various sampling points are presented in Table 1. Microbial counts for Total Heterotrophic bacteria ranged from  $7.5 \times 10^8$  to  $9.6 \times 10^8$  cfu/ml across the stations while fungal count ranged from  $2.5 \times 10^4$  to  $3.6 \times 10^4$  cfu/ml showing that the bacterial isolates were more in number than the fungi isolates in the water samples. Hydrocarbon Utilizing Bacteria (HUB) and Fungi recorded similar values. HUB had  $4.0 \times 10^3$  cfu/ml in station 1 (BBW1) but least count in station 3 (BBW3) with  $1.9 \times 10^3$  cfu/ml while the HUF in station 1 (BBW1) had  $3.4 \times 10^3$  cfu/ml but least count in station 2 (BBW2) with  $2.1 \times 10^3$  cfu/ml.

The biochemical characteristics of bacterial isolates from the sampling stations show that the bacterial isolates were identified as *Vibrio cholerae*, *Shigella* sp, *E. coli*, *Vibrio* species, *Salmonella* sp, *Bacillus* sp, *Klebsiella* sp, *Actinomyces*, *Clostridium*, *Listeria* sp, *Acinetobacter* and *Pseudomonas* sp. Meanwhile the distribution of individual bacterial isolates from the different sampling stations are shown in Table 2 while the percentage distribution of bacterial isolates across the sampling stations indicate that *E. coli* count had the highest with 15%, *Vibrio* 13%, *Pseudomonas* 12%, *Klebsiella* 12% and *Shigella* 9% (Fig. 2). Morphological characteristics of fungal isolates are presented in Table 3. The fungal species were identified as *Aspergillus niger*, *Penicillium* sp, *Aspergillus flavus*, *Rhizopus*, *Mucor* sp and *Candida* sp while the distribution of their occurrence at different sampling stations are presented in Table 3. The percentage distribution of fungal isolates across the sampling points show that *Aspergillus niger* had 37.18%, while *Mucor* had 25.64%, *Penicillium* sp 11.38%, *Aspergillus flavus* 11.38% and the *Candida* sp had 10.26% (Fig. 3).

### 3.2 Relationship between Heavy Metal Concentrations and Microbial Counts

The XY scatter plots showing the relationship between microbial counts and each of the Heavy metals analyzed showed no correlation between the parameters and microbial counts as the points are scattered around the chart area (Figs. 4 to 9).

**Table 1. Microbial counts of surface river water and link fish pond water**

Parameter	BBW1	BBW2	BBW3	BBW4	LFPW5
Total Heterotrophic Bacteria (cfu/ml)	$9.6 \times 10^8 \pm 0.05$	$8.6 \times 10^8 \pm 0.70$	$7.5 \times 10^8 \pm 1.70$	$8.2 \times 10^8 \pm 0.05$	$2.8 \times 10^6 \pm 0.05$
Total Heterotrophic Fungi (cfu/ml)	$3.6 \times 10^4 \pm 0.70$	$3.0 \times 10^4 \pm 2.12$	$3.3 \times 10^4 \pm 1.12$	$2.5 \times 10^4 \pm 0.70$	$1.3 \times 10^3 \pm 0.70$
Hydrocarbon Utilizing Bacteria (cfu/ml)	$4.0 \times 10^3 \pm 1.41$	$2.8 \times 10^3 \pm 0.70$	$1.9 \times 10^3 \pm 0.10$	$3.0 \times 10^3 \pm 1.41$	$1.0 \times 10^2 \pm 1.41$
Hydrocarbon Utilizing Fungi (cfu/ml)	$3.4 \times 10^3 \pm 2.12$	$2.1 \times 10^3 \pm 0.05$	$2.7 \times 10^3 \pm 0.05$	$3.40 \times 10^3 \pm 2.12$	$0.5 \times 10^2 \pm 2.12$
Total Coliform (MPN/100ml)	$340 \pm 32.4$	$250 \pm 43.4$	$240 \pm 43.4$	$340 \pm 32.4$	$180 \pm 32.4$
Fecal Coliform (MPN/100ml)	$80 \pm 6.12$	$93 \pm 10.05$	$90 \pm 10.05$	$80 \pm 6.12$	$10 \pm 6.12$

Key: BBW = Sampling Points of Bodo/Bonny River, LFPW = Link Fish Pond water

**Table 2. Distribution of bacterial isolates within the sampling stations**

S/N	Bacterial isolates	BBW1	BBW2	BBW3	BBW4	LFPW5	Total
1	<i>Vibrio sp.</i>	5.6	12.9	13.6	13.7	21.7	12.8
2	<i>Shigella sp.</i>	8.4	11.5	12.3	10.3	0.0	9.3
3	<i>E. coli</i>	17.5	20.1	15.6	14.4	8.4	15.0
4	<i>Clostridium sp.</i>	7.0	0.0	7.1	8.9	0.0	5.1
5	<i>Staphylococcus sp.</i>	8.4	7.9	8.4	6.8	9.6	8.1
6	<i>Salmonella sp.</i>	6.3	8.6	7.1	0.0	14.5	6.6
7	<i>Bacillus sp.</i>	7.7	6.5	6.5	9.6	13.3	8.3
8	<i>Klebsiella sp.</i>	9.1	12.9	11.7	10.3	15.7	11.6
9	<i>Actinomyces sp.</i>	2.8	1.4	0.0	0.7	3.6	1.5
10	<i>Serratia sp.</i>	6.3	0.0	3.9	5.5	0.0	3.5
11	<i>Listeria sp.</i>	2.1	3.6	1.3	2.7	0.0	2.1
12	<i>Pseudomonas sp.</i>	13.3	14.4	8.4	13.7	8.4	11.9
13	<i>Acinetobacter sp.</i>	5.6	3.6	3.9	0.0	4.8	3.5
<b>Total</b>		100	100	100	100	100	100

Key: BBW = Sampling Points of Bodo/Bonny River, LFPW = Link Fish Pond water

**Table 3. Morphological Characterization and identification of fungal Isolates**

Isolates	Macroscopy	Microscopy	Probable Identification
A	Cream large round	Oval budding blastoconidia	<i>Candida</i> sp
B	Black spores surrounded by cream background, brown reverse	Septate hyphae with aseptate conidiospore bearing conidia	<i>Aspergillus niger</i>
C	Fluffy white cottony, white reverse	Aseptate hyphae bearing sporangiospores	<i>Mucor</i> sp
D	Orange small round raised	Spherical budding blastoconidia	<i>Candida</i> sp
E	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	<i>Penicillium</i> sp
F	Black spores surrounded by white lawn-like growth	Aseptate conidiophores bearing conidia	<i>Aspergillus niger</i>
G	Light green lawn surrounded by white lawn-like growth	Septate hyphae with aseptate conidiospore bearing conidia	<i>Aspergillus flavus</i>
H	Fluffy white cottony, white reverse	Aseptate hyphae bearing sporangiospores	<i>Mucor</i> sp
I	Cream large round	Oval budding blastoconidia	<i>Candida</i> sp
J	Black spores surrounded by cream background, brown reverse	Septate hyphae with aseptate conidiospore bearing conidia	<i>Aspergillus niger</i>

**Table 4. Percentage frequency of occurrence of fungal isolates within the sampling stations**

Fungal isolates	BBW1		BBW2		BBW3		BBW4		LFPW5		%F-total
	Occurrence	F	Occurrence	F	Occurrence	F	Occurrence	F	Occurrence	F	
<i>Candida</i> sp	+	2	-	0	+	1	+	3	-	2	10
<i>Aspergillus niger</i>	-	8	+	6	-	5	+	4	+	6	37
<i>Mucor</i> sp	+	4	+	5	+	2	+	3	+	6	25
<i>Penicillium</i> sp	+	3	-	2	+	4	+	2	+	1	15
<i>Aspergillus flavus</i>	-	2	+	1	+	2	+	1	-	3	11
Total		19		14		14		13		18	78

Key: BBW = Sampling Points of Bodo/Bonny River, LFPW = Link Fish Pond River, %F= Frequency

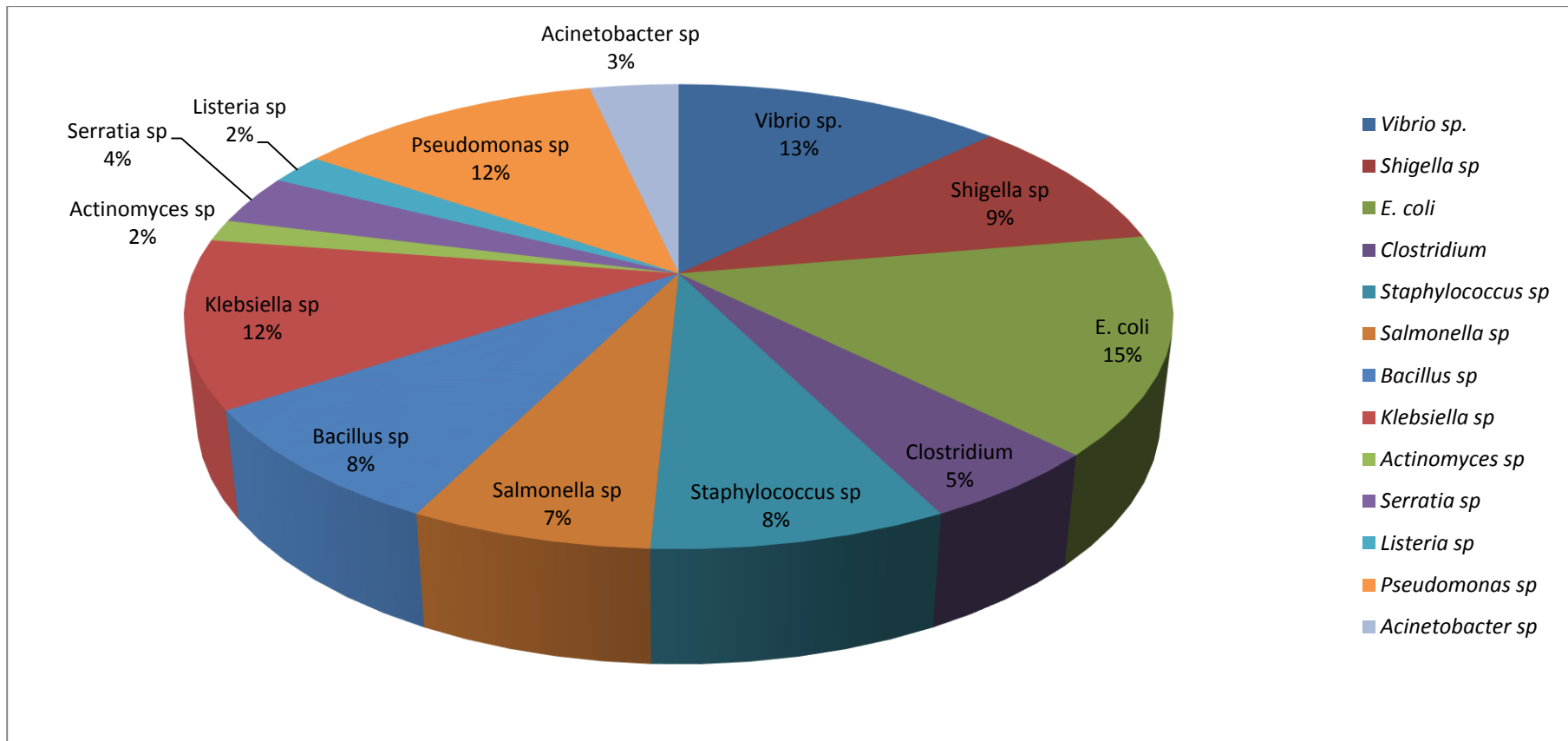
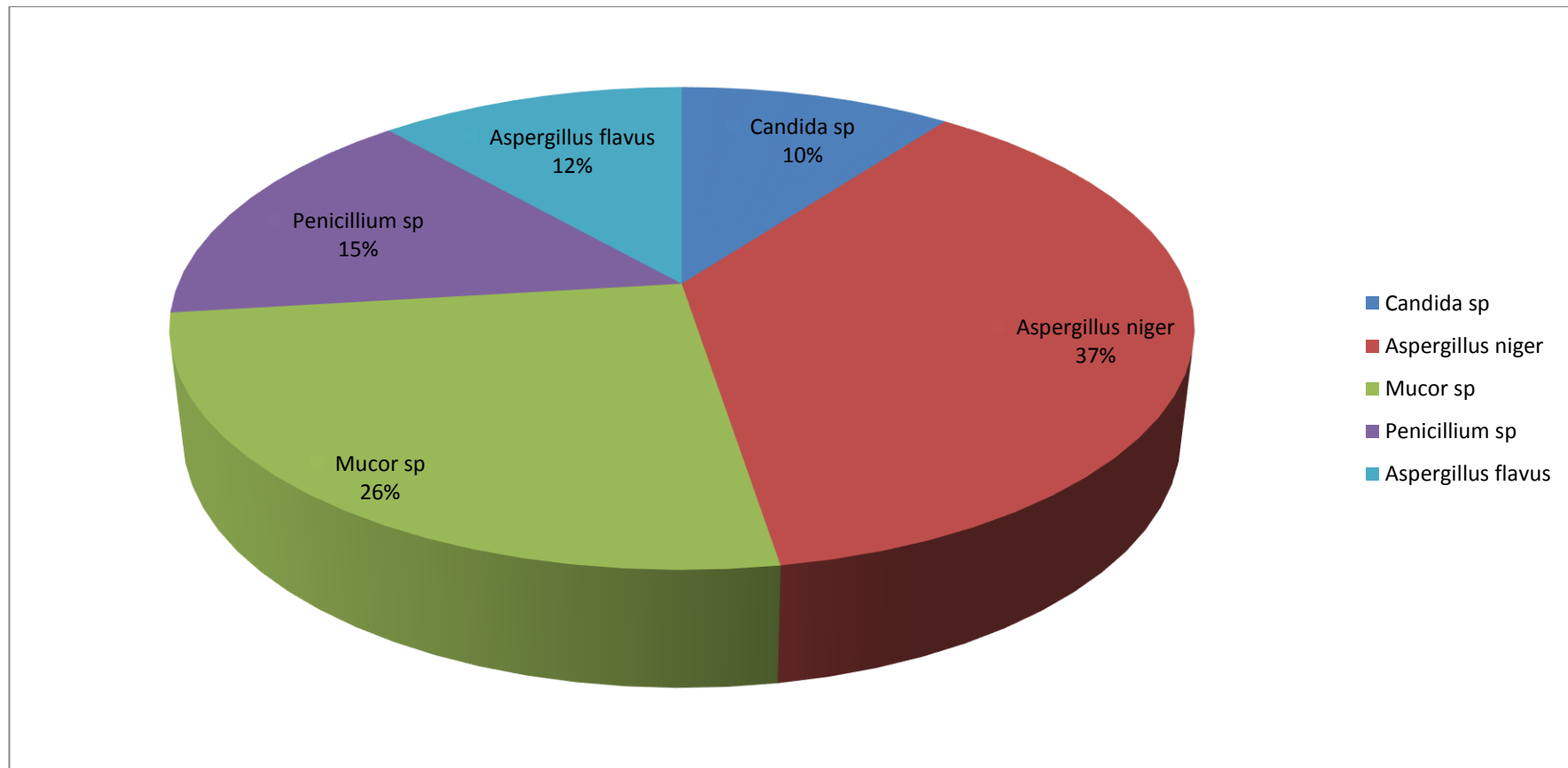


Fig. 2. Percentage occurrence of bacterial isolates across the sampling stations





**Fig. 3. Percentage occurrence of the fungal isolates in the five sampling stations**

**Table 5. Microbial counts of Fish samples from Bodo/Bonny and Link fish Pond River**

Parameter cfu/g	Fresh fish			Link fish pond fresh fish			Smoked fish	Link fish pond smoked fish
	Gill	Intestine	Muscle	Gill	Intestine	Muscle		
Total Heterotrophic Bacteria	1.68x10 <sup>7</sup>	1.80 x 10 <sup>7</sup>	1.28x 10 <sup>7</sup>	4.2 x 10 <sup>7</sup>	1.75x 10 <sup>7</sup>	1.08x10 <sup>7</sup>	9.6 x 10 <sup>6</sup>	1.8X 10 <sup>6</sup>
Total Heterotrophic Fungi	5.0 x 10 <sup>3</sup>	5.8 x 10 <sup>3</sup>	4.7 x 10 <sup>3</sup>	2.6 x 10 <sup>2</sup>	2.9 x 10 <sup>2</sup>	4.0 x 10 <sup>2</sup>	6.6x 10 <sup>3</sup>	1.4 X 10 <sup>2</sup>
Hydrocarbon Utilizing Bacteria	2.6 x 10 <sup>2</sup>	3.7 x 10 <sup>2</sup>	3.4 x 10 <sup>2</sup>	8.0 x 10	5.0 x 10 <sup>3</sup>	6 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	<0.001
Hydrocarbon Utilizing Fungi	1.4 x 10 <sup>2</sup>	2.0x 10 <sup>2</sup>	1.8 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	4 .0 x 10 <sup>2</sup>	1.4x 10 <sup>2</sup>	<0.001

The linear regression equation of the line of best fit is presented with the equation  $y=mx+b$ , with  $y$  being the microbial counts (dependent variable), while  $x$  is the value of the Heavy metal concentrations (independent variable),  $m$  the slope (representing the value of  $y$  when  $x$  is increased by a unit and  $b$  intercept of the line graph (the value of  $y$  when  $x=0$ ). The value for  $R^2$  is the coefficient of determination which expresses the variation in  $y$  that can be

explained by a variation in  $x$ . The  $R^2$  value obtained shows that the Heavy metal concentrations contributed very little to the microbial counts obtained in the samples. Figs. 4 and 6 shows a positive relationship between the two variables indicating an increase in concentration of Cd and Cu to a corresponding increase in the Total Heterotrophic Bacterial Count.

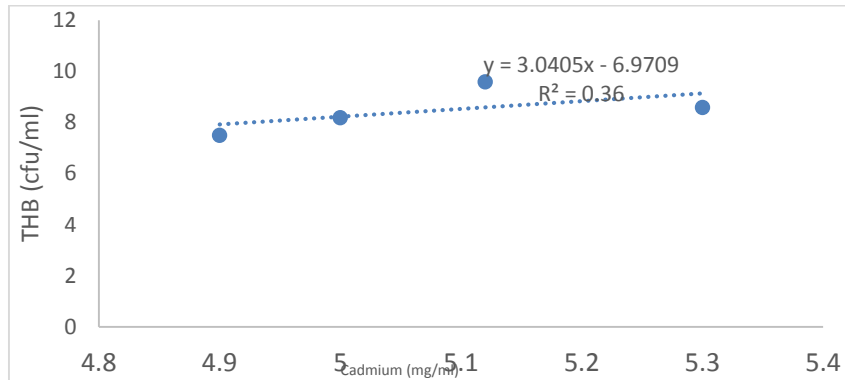


Fig. 4. XY scatter plot showing relationship between microbial counts and cadmium

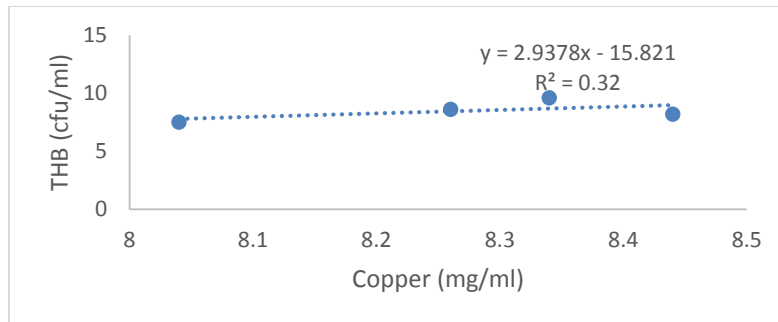


Fig. 5. XY scatter plot showing relationship between microbial counts and copper

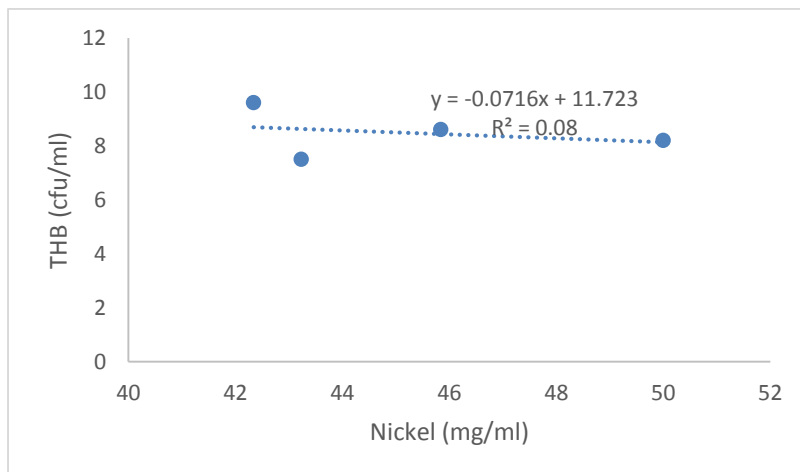


Fig. 6. XY scatter plot showing relationship between microbial counts and nickel

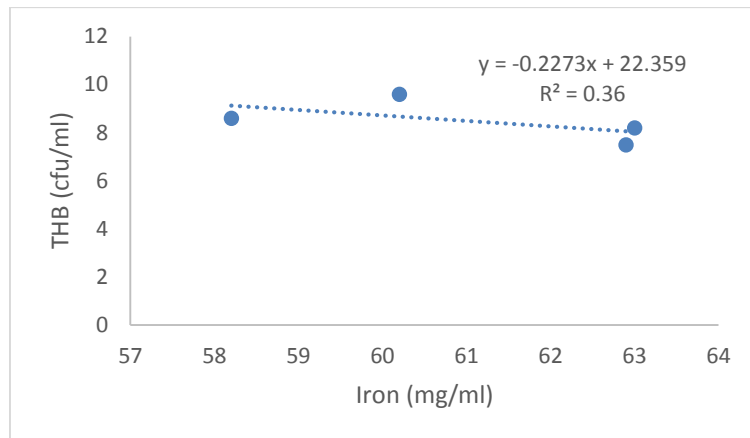


Fig. 7. XY scatter plot showing relationship between microbial counts and iron

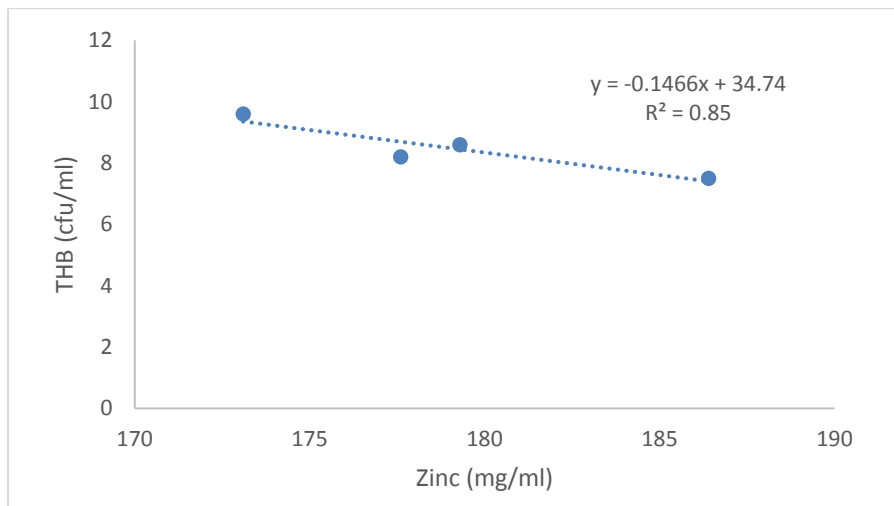


Fig. 8. XY scatter plot showing relationship between microbial counts and zinc

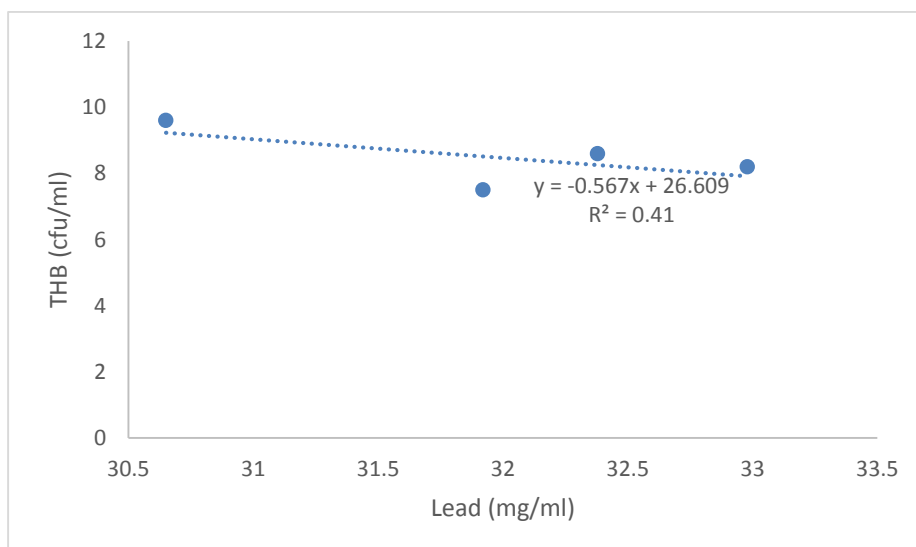


Fig. 9. XY scatter plot showing relationship between microbial counts and lead

Figs. 7 to 9 showed a negative relationship between both variables; an increase in concentration of Iron, Nickel, Zinc and Lead caused a decrease in microbial load of the water samples from the various sampling stations. The high  $R^2$  value for Zn indicates that its concentration contributed largely to the microbial load of the sampling stations while the lower values recorded for the other metals indicate that their concentrations did not largely affect the microbial load. Other factors such as temperature and time of sample collection may have also affected the microbial load of the samples.

## 4. DISCUSSION

### 4.1 Microbiological Characteristics

Enumeration of the isolates in the various locations in the Bodo/Bonny river showed variations which may be linked to several anthropogenic activities which are on the rise around coastal rivers [13]. Petroleum hydrocarbons in the aquatic environments contribute basically organic complexes which may lead to the accumulation in the water and sediment columns of the water body/river, where the hydrocarbons undergo the processes of dispersion and weathering such as biodegradation, evaporation and dissolution, which results in changes that affect the toxicity level and general composition of the petroleum hydrocarbons mixtures in the system [13]. The organisms that inhabit the water space use oxygen for metabolic activities and the blocking of the water surface of the river by total petroleum hydrocarbons tend to reduce the dissolved oxygen available within the system thereby making the survival of aquatic life difficult. The water becomes unfit for use in agriculture, tourism and even domestic use due to the presence of total petroleum hydrocarbons [14]. The consumers of seafood including the fishermen are prone to health threat, due to the inhalation and body contact of oil spills and other gases associated with hydrocarbons pollution [15]. Although microorganisms are ubiquitous and are able to carry out functions such as decomposition of organic matter, factors such as temperature, pH and nutrient availability are said to critically affect their abundance in an environment [16]. In this study, microbial counts as high as  $7.5 \times 10^5$ -  $9.6 \times 10^8$ cfu/ml was recorded for Total Heterotrophic Bacterial count,  $2.5 \times 10^4$ -  $3.6 \times 10^4$  cfu/ml for Total Heterotrophic Fungi while Hydrocarbon Utilizing Bacteria had a range

of  $1.9 \times 10^3$ -  $4.0 \times 10^3$ cfu/ml and Hydrocarbon Utilizing Fungi recorded a range of  $2.1 \times 10^3$ -  $3.4 \times 10^3$  cfu/ml. Statistically there were significant differences between the microbial counts in the various locations at the surface river water samples.

Microorganisms isolated in this study included those of the genera *Vibrio cholerae*, *Shigella* sp, *E. coli*, *Salmonella* sp, *Bacillus* sp, *Klebsiella* sp, *Actinomycetes*, *Clostridium*, *Listeria* sp, *Acinetobacter* and *Pseudomonas* sp while the fungal species were identified as *Aspergillus niger*, *Penicillium* sp, *Aspergillus flavus*, *Rhizopus*, *Mucor* sp and *Candida* sp. While the microorganisms are widely distributed in nature, their abundance and diversity may be used as indicators of water quality [17]. Their availability in most surface water like rivers and streams has implications for environmental health as averred by Omonona et al [18]. The THBC and THFC were observed to be higher during the sampling and may be attributed to run-off as a result of high rainfall [19]. High surface flows during rainy seasons resulting in increase in erosion and the transport of sediment carrying bacteria into rivers are also on record [20]. The fungi species such as *Aspergillus flavus*, *Aspergillus niger* and *Fusarium* sp. observed in the study are of ecological health importance. Omonona et al. [19] also reported similar findings in their study. Furthermore, *Salmonella* / *Shigella* (enteric pathogens) and *Staphylococcus aureus*, are seen as important indicators of the health of aquatic ecosystems [21] were observed in the water samples. The coliforms *Escherichia coli* was observed to be present in the sample. ). Fecal contamination of the river water can be through non-point sources (surface runoff and soil leaching), the wildlife animals and grazing livestock faeces, and also the farmyard manure used in agricultural fields. Ajibade et al. [22] had also reported similar findings in Kainji Lake National Park in Nigeria. Adetuga et al. (2019b) and Omonona et al. [19] also reported similar findings and attributed the probable cause to more contact from animals while drinking. Coliforms generally are important among bacterial indicators that are used in water quality monitoring and assessment [23]. Their presence can be attributed to excessive nutrient run-off and / or the washing off the microbes from the land during the rainy season [24]. Similar isolates have been reported from various sources both in Nigeria and other countries (Ezeronye and Ulalua, 2005; Adesomoye et al., 2006; Adekanmbi and Falodun, 2015; Onuoha, 2018).

However, the presence of *Pseudomonas* sp in this ecology may be linked with hydrocarbon oxidation (Faria and Bharathi, 2006). Their presence in the surface river samples as natural hydrocarbon utilizing microbes can possibly be involved in the cleanup of crude oil polluted rivers. These microorganisms can basically be used in biodegradation, which may involve complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [25]. This mechanism has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. It has been reported that microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment [26].

#### 4.2 Relationship between Heavy Metal Concentrations and Microbial Counts

The coefficient of determination of the XY scatter plots showed that each of the heavy metals concentrations could influence the microbial counts obtained in the surface water samples. The presence of heavy metals in rivers can influence the degradation of the environment especially sediment, resulting in a substantial decline in biodiversity and affect public health and disrupt life support system. Other anthropogenic factors may have influenced the microbial counts recorded. Water pollution by trace metal ions is one of our most serious environmental problems. Effluents resulting from daily domestic and industrial activities may induce considerable changes [27].

#### 5. CONCLUSION

The assessment of microbial contaminants on the surface river water indicated very high microbial load of pathogenic bacteria that are of public health importance. Fungi that are known

for producing aflatoxins were also isolated from the surface water body. Apart from the fact these microorganisms will continue to proliferate and decrease the shelf life of the fish particularly when they die due to asphyxiation as we observed in this study during the sampling period, such seafood can serve as a point of conveying microbial infection to man. This is due largely to the fact that the fishes were being deprived of oxygen, as a result of the excess spill of petroleum which resulted in suffocation causing their death in the river. Isolation and identification of microorganisms from the water resources indicated an abundance of hydrocarbon utilizing and/or degrading bacteria thus indicating the possibility of crude oil exploitation activities could generate hydrocarbons being deposited in the river water. These isolates may possess the functional ability for heavy metals transformation and poly aromatic hydrocarbon degradation. Therefore, the Microorganisms isolated from the river water that survived the harsh influences associated with oil spill from this study shows that most of the microorganisms could be genetically cloned as hydrocarbon utilizing organisms for cleanup of oil contaminated environments because of their existence and sustenance to mankind.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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