Case study

Microbiological Characteristics and Heavy Metal Pollution of Crude Oil Contaminated Water Bodies in Port Harcourt Metropolis, Nigeria

ABSTRACT

Crude oil exploration has been beneficial to our economy but detrimental to our environment with the artisanal refineries further compounding the challenge. This research was aimed at investigating the microbiology and heavy metal pollution of three crude oil polluted rivers in Rivers State, Nigeria and effects on living organisms inhabiting that environment. This study was carried out in three locations in South-South Nigeria (Eagle Island, Iwofe and Chokocho rivers). A total of 64 water samples (upstream and downstream points) were collected using appropriate containers and sterile polyethene bags for 6 crab samples for a period of three months. Water and crab samples were analyzed for heavy metals using Atomic Absorption Spectrophotometric method while microbiological analysis involved isolation and enumeration of microbial populations of the water and crab samples as well as characterization and identification of the isolates using standard methods Results showed Total Heterotrophic bacteria (THB) ranged from 6.0×10^6 cfu/ml to 9.0×10^8cfu/ml for the downstream locations and 1.7×10^6cfu/ml to 3.5×10^7cfu/ml for the upstream locations. Total Heterotrophic Fungi (THF) ranged from 2.0×10^4cfu/ml to 1.1×10^5cfu/ml for downstream locations and 0.1×10^4cfu/ml to 4.0×10^4cfu/ml for upstream locations, Hydrocarbon Utilizing Bacteria (HUB) ranged from 0.8×10^3cfu/ml to 4.0×10^3cfu/ml in downstream locations and 2.0×10^3cfu/ml to 7.4×10^3cfu/ml in upstream locations, Hydrocarbon Utilizing Fungi (HUF) ranged from 1.0×10^3cfu/ml to 6.0×10^3cfu/ml for downstream locations and 5.0×10^2cfu/ml to 8.0×10^3cfu/ml for upstream locations. The bacteria identified biochemically included *Serratia* sp., *Enterobacter* sp. and *Salmonella* sp. for the crab samples and *Bacillus* sp. was dominant in the water samples (*Bacillus subtilis*, *Bacillus cereus*, *Bacillus carboniphilus*). The heavy metals (Fe, Ni, Zn, Cd, Cu, Mn, Cr and Pb) were below the DPR permissible limit but are likely to increase since the activities leading to pollution are still ongoing. The crab as a filter feeder, had higher concentration of heavy metals and microbial population and the location with the highest crude oil pollution (14.5mg/l) had the lowest THB (6.0×10^6cfu/ml) as physicochemical parameters like the amount of Dissolved Oxygen had been altered.

1.0 Introduction

Pollution of the aquatic ecosystems is now a known and ever increasing problem due to the increased use of chemicals that end up as pollutants in the aquatic environment (Adati, 2012).

The extraction, refining, transport and use of crude oil and its products have caused the introduction of pollutants into the aquatic resources in the environment; this has become a source of concern for the environment and the organisms that inhabit it. Heavy metals such as Ni, Pb, Fe, Mn, Mg, Zn, Hg, Cu and Cd have been associated with oil refinery activities including oil spillage and though they are naturally occurring, their high level as pollutants in water is

traceable to anthropogenic activities particularly industrialization and mobility threatening aquatic organisms since exposure even at low levels cause bioaccumulation. Areas with mineralized rocks will usually have elevated metal levels as the trace metal content of river water is normally controlled by the abundance of metals in the rocks of the river's catchment area (Ubiogoro and Adeyemo, 2017).

The measurement of pollutants in terms of chemical concentration has provided some information that has helped in pollution studies. The use of chemical monitoring though useful in providing information on concentration and presence of chemical pollutants has left a hole with regards to concrete information on the effect of these pollutants on living organisms (both macro and micro) leading to the evolution of terms like biomonitoring, biomarkers, bioindicators, bioaccumulation, bioconcentration and biomagnification. A biomarker is any indicator of a stress agent that is somehow affecting an organism's ability to grow, reproduce, survive and adapt (or in other words, to live) in a given environment. This indicator may be alterations in molecular and biochemical processes, cellular structures and functions, tissue organization or mass and length ratios of individual organs or the whole body (Parente and Hauser-Davis, 2013).

2.0 Materials and Methods

2.1 Area of Study

This study was undertaken in three coastal waterfronts in Rivers State; Iwofe waterside ($4^0$48'46.551"N, $6^0$56'12.0906"E), Eagle Island/Nkpor waterside ($4^0$47'47.302"N, $6^0$58'24.5496"E) and Chokocho waterside ($4^0$59'53.75688" N, $7^0$3'39.93084"E) all in Rivers State, Nigeria (Fig. 1). These three watersides are located in the South-South geopolitical zone of Nigeria where crude oil exploration takes place. The three locations have been implicated for crude oil spills as a result of the activities of artisanal refineries. The Iwofe location sees more of these activities as pipes were seen at low tide that channelled crude to the nearby environment for refining. The Eagle Island location also had spills as the oil sheen could be seen covering the water at high tide. The Chokocho location was a fresh spill but not very close to residential areas like the other two locations.

These three waterside locations were selected due to the fact that they are sites known for various activities including bunkering/local refining of crude oil, transportation of petroleum products, sand dredging, indiscriminate dumping of waste and other anthropogenic activities that pollute the surface water as well as the seafood obtained from such ecosystems.

2.2 Sample Collection

2.2.1 Collection of Water Samples

The collection of water samples was carried out using the appropriate sampling containers for various analyses: microbiology, physicochemistry, heavy metals concentration and Total Petroleum Hydrocarbon (TPH). Water samples were collected at 2 points; Upstream (Towards the source of the river) and Downstream (Towards the shore); Iwofe (IW1 and IW2), Eagle Island (EI1 and EI2) and Chokocho (CH1 and CH2). The water samples were put in icepacks and conveyed to the Post Graduate Microbiology Research Laboratory of the Department of Microbiology, Rivers State University, Port Harcourt for analyses.

2.2.2 Collection of *Callinectes* sp. Samples

The crab samples were collected at low tide in the morning on each sampling day when the water had receded and they were asceptically picked at the river shore along the sampling stations and kept in sterile black polythene bags and conveyed to the laboratory the same day. They were preserved in the freezer and analyses were carried out subsequently. A total of seventy(70) samples: 64 water samples from the three locations and 6 crab samples from the brackish locations (Iwofe and Eagle Island) as crabs were not present in the fresh water habitat in Chokocho.

Fig 1: Map showing the Three River Locations Sampled in this Study

2.3 Microbiological Analysis

2.3.1 Serial Dilution of Water and Crab Samples

Dilution of water samples adopted the ten-fold serial dilution technique as 1ml of water sample was transferred to 9ml of earlier prepared diluent(normal saline) in test tubes. This was done

consecutively from tube to tube until appropriate dilutions were reached with dilution factor from10-1to 10-6.

The dilution method adopted was the ten-fold technique in which 10g of the homogenized crab tissue was soaked in hundred millilitres of diluent(normal saline) to produce a stock solution from which one millilitre was taken to carry out serial dilution from 10-1to 10-6.

2.3.2 Inoculation and Incubation of Cultures

Isolation and enumeration of THB and HUB was done by aseptically transferring an aliquot (0.1ml) of the dilution of 10^{-5} and 10^{-6} for THB and 10^{-1} and 10^{-2} for HUB onto properly dried NA and MSA (containing fungosol 0.25g/ml serving as antifungal agent to prevent fungal contamination) in duplicates using spread plates method. The inoculated plates were incubated at 35-37^{0}C for 24 to 48 hours and 37^{0}C for 5-7 days respectively (Udotong *et al*., 2015).

Isolation and enumeration of fungi and HUF was also done by aseptically transferring an aliquot (0.1ml) of the dilution of 10^{-2} and 10^{-3} for both THF and HUF onto SDA plates and MSA plates (containing chloramphenicol to suppress bacterial growth) in duplicates using spread plates method. The inoculated plates were incubated at 28 ± 2^{0}C for 5-7 days.

For the crab sample, dilutions were plated on the selective media (EMB, TCBS and SSA) to isolate and enumerate *E. coli*, *Vibrio* sp. and *Salmonella-Shigella* sp.

2.3.3 Maintenance of Pure Cultures

Discrete colonies of bacterial and fungal isolates were purified by several sub-cultures unto appropriate agar media (SDA and NA). Pure bacterial cultures were inoculated in duplicates and then stored in pre sterilized NA slants and kept in the refrigerator at 4^{0}C for further test (Amadi *et al.*, 2014, Uzorigwe and Okpokwasili, 2012). The pure colonies were then identified through colonial and cellular morphology as well as molecular identification.

2.3.4 Characterization and identification of Bacterial isolates

a. Cultural/Morphological Characterization

The cultural characteristics of the discrete bacterial isolates were based on appearance on the media that includes shape, color, moisture, size, elevation, opacity etc.

b. Cellular/Biochemical Characterization

Pure cultures of bacterial isolates were broadly divided into Gram-positive and Gram-negative bacteria using Gram staining and microscopy. Further identification waa based on biochemical tests which included catalase, oxidase, citrate utilization, indole production, Voges Proskauer and methyl red test as well as sugar fermentations (Cheesebrough, 2006; Prescott *et al.*, 2011). The identification of bacterial isolates was confirmed by comparing them with recommendation in Bergey's manual of determinative bacteriology and using the ABIS system.

2.3.5 Characterization and Identification of Fungal Isolates

Discrete colonies from the 5-7 days incubated plates were selected based on the differences in their colonial characteristics and purified by sub-culturing on freshly prepared SDA plates. The sub-cultured plates were marked, labelled properly and incubated at 28 ± 2^0C for 5-7 days. Pure cultures obtained after 5-7 days of incubation were subjected to characterization and identification using macroscopy and microscopy.

2.4 Heavy metal concentration

Heavy metals concentration of the water and crab samples were determined using the Atomic Absorption Spectrophotometric Method. The dried samples of crab were put in a cleaned dried mortar separately and were grounded to fine particles and then sieve using a sieve of particle size 0.02 mm. 0.5 g each of samples were measured into clean dried beaker (100 mL), 5 mL of aqua regia HCl and HNO3 (3:1) was then added to the sample for digestion. The samples were allowed to be evenly distributed in the acid by stirring with a glass rod and then the beaker was place on the heater. The digested sample was filtered into a graduating cylinder and the filtrate was made up to 50 mL using distilled water. UNICAM AA 919 atomic absorption spectro□photometer model was used to analyse the concentration (µg/g) of heavy metals in the six different samples of crab (Mebom *et al*, 2021).

Fig. 2: 

UNICAM AA 919 atomic absorption spectro□photometer

3.0 Results

3.1 Microbial Population

3.1.1 Microbial Population of Water Samples

Results of the microbial population of the water samples (upstream and downstream) for the three locations are presented in Table 1 and 2 respectively. Each table shows THB, HUB, HUF, Fungi and Total Coliform count (TCC). The highest values recorded are boldly represented.

Table 1: Microbial Population of the Upstream Locations

Parameters (cfu/ml)					
Locations	**THB**	**FC**	**HUB**	**HUF**	**TCC**
Iwofe	**1.7×10^9**	2.0×10^4	2.6×10^3	1.1×10^3	3.5×10^4
Iwofe	**2.0×10^9**	2.0×10^4	2.0×10^3	1.9×10^3	6.1×10^4
Eagle Island	7.0×10^6	2.0×10^4	**7.4×10^3**	5.0×10^2	**6.4×10^4**
Eagle Island	2.0×10^6	1.0×10^4	**5.2×10^3**	1.0×10^3	**5.8×10^4**
Chokocho	3.5×10^7	**4.0×10^4**	2.1×10^3	**4.0×10^3**	$8{,}0 \times 10^3$
Chokocho	1.2×10^7	**3.0×10^4**	1.8×10^3	**8.0×10^3**	1.1×10^4

Table 2: Microbial Population of the Downstream Locations

Parameters (cfu/ml)					
Locations	**THB**	**FC**	**HUB**	**HUF**	**TCC**
Iwofe	**6.3×10^8**	**4.0×10^4**	1.9×10^3	1.1×10^3	2.1×10^4
Iwofe	**9.0×10^8**	**11.0×10^4**	2.6×10^3	2.0×10^3	6.6×10^4
Eagle Island	3.0×10^7	5.0×10^4	**2.1×10^3**	2.6×10^3	**7.9×10^4**
Eagle Island	8.0×10^7	2.6×10^4	**4.0×10^3**	1.0×10^3	**6.1×10^4**
Chokocho	6.0×10^6	2.0×10^4	1.6×10^3	**3.0×10^3**	5.0×10^4
Chokocho	1.2×10^7	3.0×10^4	0.8×10^3	**6.0×10^3**	3.0×10^4

Key: THB (Total Heterotrophic Bacteria), FC (Fungal Count), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi), TCC (Total Coliform Count)*Means with same superscript across the column shows no significant difference ($p \geq 0.05$)

3.1.2 Microbial Population of Crab Samples

Results of the microbial population of the crab samples in two locations are presented in Table 3. The total heterotrophic bacterial count was higher in Eagle Island ($2.7x10^6$cfu/g) and lower in Iwofe ($5.0x10^5$cfu/g). There was significant difference ($p \geq 0.05$) in the total heterotrophic bacterial (THB) counts in the two locations. The coliform count (CC) was higher in Eagle Island ($2.9x10^4$cfu/g) and lower in Eagle Island ($1.7x\ 10^4$cfu/g). There was no significant difference ($p \leq 0.05$) in the CC in the crab samples. The *E. coli* count (EC) was lower ($1.1x10^3$cfu/g) in Eagle Island and higher ($1.2x10^3$cfu/g) for Iwofe. There was no significant difference ($p \leq 0.05$) in the EC of crab samples. The *Salmonella-Shigella* count (SSC) was higher ($6.5x10^3$cfu/g) in Iwofe and lower ($1.3x10^3$cfu/g) in Eagle Island. There was significant difference ($p \geq 0.05$) in the SSC in the crab samples. The *Vibrio* count (VC) was lower in Eagle Island (2.5×10^2cfu/g) and higher (3.3×10^2cfu/g) in Eagle Island. There was no significant difference ($p \geq 0.05$) between the two locations.

Table 3. Microbial Populations of the Crab Samples

Microbiological Parameters (cfu/g)	Locations	
	Eagle Island	**Iwofe**
THB	**$2.7\ x10^6$**	$1.0\ x10^5$

TCC	**2.9 x10^4**	1.7 x10^4
EC x10^{3}Cfu/g	1.1 x10^3	**1.2 x10^3**
SSC x10^{3}Cfu/g	1.2 x10^3	**6.5 x10^3**
VC x10^{2}Cfu/g	**3.3 x10^2**	2.5 x10^3

Key: THB (Total Heterotrophic Bacteria (THB), Total Coliform Count (TCC), *E.coli* Count (EC), *Salmonella-Shigella* Count (SSC), *Vibrio* Count (VC)

*Means with same superscript across the column shows no significant difference ($p \geq 0.05$)

3.2 Heavy Metal Content of the Samples

Results of the heavy metal analyses of the three upstream locations are presented in Table 4 and for downstream in Table 5. Highest values are boldly represented and alphabet superscripts show significant difference.

In summary, the heavy metal content of each heavy metal did not vary significantly across the three locations sampled except for Fe content in Chokocho. Figure 2 is the comparism chart for the Total Petroleum Hydrocarbon Content for upstream and downstream locations.

Table 4: Heavy Metals Concentrations of Upstream Locations

Heavy Metals (mg/l)	Locations		
	Chokocho	**Eagle Island**	**Iwofe**
Cd	0.03±0.01^a	0.0±0.0007^a	**0.05±0.01^a**
Fe	**4.85±6.03^a**	0.28±0.19^a	0.18±0.08^a
Cr	0.003±0.00^a	**0.07±0.08^a**	0.003±0.00^a

Cu	**0.03±0.03[a]**	0.02±0.02[a]	0.01±0.01[a]
Ni	0.16±0.08[a]	0.16±0.01[a]	**0.25±0.17[a]**
Mn	**0.27±0.07[a]**	0.03±0.01[a]	0.01±0.01[a]
Zn	0.14±0.08[a]	**0.14±0.15[a]**	0.06±0.003[a]
Pb	0.23±0.12[a]	**0.95±0.63[a]**	0.56±0.25[a]

Table 5: Heavy Metals Concentrations of Downstream Locations

Heavy Metals (mg/l)	Locations		
	Chokocho	**Eagle Island**	**Iwofe**
Cd	0.02±0.004 [a]	0.02±0.02[a]	**0.04±0.01[a]**
Fe	**2.16±0.48[b]**	0.21±0.14[a]	0.14±0.05[a]
Cr	0.003±0.000[a]	**0.032±0.041[a]**	0.011±0.012[a]
Cu	0.001±0.00[a]	**0.009±0.011[a]**	0.008±0.009[a]
Ni	0.12±0.04[a]	**0.18±0.11[a]**	0.15±0.05[a]
Mn	0.02±0.00[a]	0.02±0.00[a]	0.02±0.00[a]
Zn	**0.06±0.03[a]**	0.03±0.01[a]	0.02±0.02[a]
Pb	0.28±0.26[a]	0.49±0.36[a]	**0.55±0.29[a]**

Key: Cd (Cadmium), Fe (Iron), Cr (Chromium), Cu (Copper), Ni (Nickel), Mn (Manganese), Zn (Zinc), Pb (Lead)

*Means with same superscript across the column shows no significant difference ($p \geq 0.05$)

Fig. 3: Total Petroleum Hydrocarbon for Upstream and Downstream Water Samples in each location

Results of the heavy metal analyses of crab sample for two locations (Iwofe and Eagle Island) were presented in Table 6.

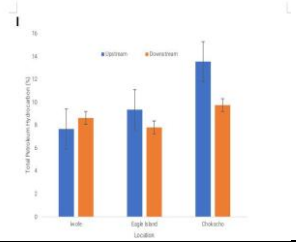

Table 6: Heavy metal concentration in Crab samples

Heavy Metals (mg/kg)	Locations	
	Eagle Island	**Iwofe**
Fe	416.65±154.22[a]	1409.45±281.49[a]
Pb	115.65±30.33[a]	95.05±20.86[a]
Cd	11.55±2.62[a]	12.80±3.39[a]
Cr	9.50±1.27[a]	19.05±4.59[a]
Cu	114.00±23.48[a]	69.30±22.49[a]

Mn	101.90±23.76[a]	300.70±28.51[b]
Ni	45.40±8.34[a]	42.00±13.15[a]
Zn	227.00±76.37[a]	216.00±50.91[a]

Key: Cd (Cadmium), Fe (Iron), Cr (Chromium), Cu (Copper), Ni (Nickel), Mn (Manganese), Zn (Zinc), Pb (Lead)

*Means with same superscript across the column shows no significant difference ($p \geq 0.05$)

Heavy metal concentrations varied across locations except Mn which was the same across all points in all locations.

4.0 Discussion

4.1 Microbial Population

This study revealed as expected that total heterotrophic bacteria had the highest occurrence at both upstream and downstream points along the rivers. There was significant difference between the total heterotrophic bacterial counts of the three rivers. The high occurrence of total heterotrophic bacteria in Iwofe River can be attributed to the nature of the environment, the age of the spill, the total petroleum hydrocarbon content and the high amounts of utilizable organic matter present in the river. Iwofe River is used as a site for indiscriminate dumping of refuse, including household waste and sewage thus enriching the soil with various forms of utilizable organic matter.

Eagle Island also had the highest fungal count in the downstream point followed by Iwofe and then Chokocho. There was no significant difference ($p \leq 0.05$) in the fungal counts. The high occurrence of fungi mostly *Penicillium* sp. the Eagle Island River can be attributed to the nature of the environment and the pH of the river. *Penicillium* sp. is commonly found in temperate regions growing on wood and decaying vegetative matter and Eagle Island riverbank is mostly occupied by carpenters who carry out their wood work and carving of boats in the water front. *Penicillium* sp. has an optimum pH between 7.0 and 9.0 and the river in Eagle Island has a pH range of 8.2-8.5 (Dhakar and Pandey, 2013).

The results from this study also showed that microbial counts were higher downstream than in the upstream which is in accordance with works done by Udotong *et al*. (2008). This could be

attributed to the higher availability of nutrients from dumping of sewage and other biological materials into the river, Iwofe had the highest total heterotrophic bacterial count followed by Eagle Island, then Chokocho. Only five genera of bacteria were identified in this study from the three rivers of which *Bacillus* sp. was the most occurring. The organisms isolated and identified from the water samples in this study include *Bacillus carboniphilus, Bacillus subtilis, Bacillus cereus, Paenibacillus pectinilyticus, Paenibacillus massiliensis, Brochothrix thermosphacta, Salmonella* sp. and *Escherichia coli* while from the crab samples, *Staphylococcus aureus, Enterobacter hormaechei, Enterobacter cancerugens, Serratia liquefaciens* and *Salmonella bongori/enterica*. The isolates from water samples have significant ability to utilize crude oil as a sole source of carbon and energy, and the occurrence of these organisms have been reported by different researchers as crude oil degraders (Chikere and Okpokwasili, 2004; Olukunle and Boboye, 2013).

The high occurrence of fungi in Eagle Island River can be attributed to the age of the spill, total petroleum hydrocarbon content and the pH of the river. Odokuma and Dickson (2003) reported that total petroleum hydrocarbon content reduces with aging of a polluted site. Iwofe River has the oldest spill of the three rivers hence, the least total petroleum hydrocarbon content which is in agreement with works by Odokuma and Dickson (2003). Iwofe River also had a pH of (7.6), *Penicillium sp.* was the most occurring fungi and had a pH range between 7.0 and 7.5 observed in Iwofe river which showed that Eagle Island River had the most suitable pH for the proliferation of *Penicillium sp.* (Udotong *et al.*, 2008).

Hydrocarbon-degrading microorganisms are ubiquitously distributed in crude oil polluted river environments. According to Odokuma and Dickson (2003), populations of hydrocarbon degraders normally constitute less than 1% of the total microbial communities, but when oil pollutants are present in an environment, the hydrocarbon-degrading populations increase, typically to 10% of the community. This study revealed that not all the members of the heterotrophic population could utilize the crude oil and petroleum products spilled in the studied rivers, hence a decrease in the count of hydrocarbon utilizing organisms (Udotong *et al.*, 2008). The high occurrence of hydrocarbon utilizing bacteria in Eagle Island River can be attributed to the age of the spill, the total petroleum hydrocarbon content and the physicochemical characteristics of the river. It has been observed that the older the crude oil spill, the higher the

hydrocarbon utilizing bacterial counts and the less the total petroleum hydrocarbon content (Odokuma and Dickson, 2003). The high hydrocarbon utilizing bacterial count can also be attributed to the utilizable organic matter present in the crude oil, which is in accordance with Eze *et al*., (2014). .

The high occurrence of fungi in the Iwofe River can be attributed to its acidity, the age of the spill, the petroleum hydrocarbon content of the soil and the organic matter present in the river.

The high occurrence of coliform in the Iwofe River can be attributed to the high organic matter present in the river from anthropogenic activities.

The high occurrence of THB in the Iwofe crab sample can be attributed to the high organic matter present in the river while TCC which was highest in Eagle Island can be attributed to the high organic matter present in the river. The *E. coli* count in the crab sample was highest in Iwofe and its high occurrence in the Iwofe sample can be attributed to the high organic matter present in the river especially fecal materials. The *Vibrio* count in the crab sample was highest in Eagle Island and this could be due to the high organic matter present in the river especially fecal materials. The *Salmonella-Shigella* count in the crab sample was highest in Iwofe. The high occurrence in the Iwofe crab sample can be attributed to the high organic matter present in the river especially fecal materials.

4.2 Heavy Metals Analyses

Results of heavy metal analysis showed variations in their concentrations of the three sampled rivers. The concentrations of Fe recorded in the Chokocho River were below the safe limit reported by FEPA (1991). However, the concentration of Fe recorded in the Eagle Island and Chokocho Rivers studied were above the permissible limit set by FEPA (1991). The high amounts of Iron (Fe) in the Eagle Island and Chokocho rivers could be attributed to indiscriminate dumping of sewages, municipal and industrial wastes into the study river. Lead (Pb) in high amounts is very toxic to humans.. The concentration of Pb recorded in Chokocho and Eagle Island rivers were above the permissible limit set by FEPA (1991) but were below the safe limit reported by WHO (1996). However, the concentrations of Pb recorded in Iwofe were above the permissible limit set by FEPA (1991; 1996).

The concentration of Cd recorded in the three rivers studied were above the permissible limit set by WHO (1996) but were below the safe limit reported by FEPA (1990). The high amounts of Cadmium (Cd) in the three rivers studied could be attributed to mainly indiscriminate dumping of sewages, municipal and industrial wastes into the study river.

The concentrations of Cr recorded in the three rivers studied were below the permissible limit set by FEPA (1990).

The concentrations of Cu recorded in the three rivers studied were below the permissible limit set by FEPA (1991). The concentration of Ni recorded in the three rivers studied were above the permissible limit set by FEPA (1991) but were below the safe limit reported by WHO (1996). The concentrations of Zn recorded in the three rivers studied were below the permissible limit set by FEPA (1991) and WHO (1996).

Although the results of surface water concentrations of heavy metals observed in the present study agrees with the general opinion of low level heavy metal concentrations in surface water within the study area and Niger Delta (Asonye *et al*. 2007; Chindah *et al*. 2004; Ubalua *et al*. 2007), some of the toxic metals such as Pb, Cd, Zn, Ni and Cr were higher than stipulated limits by WHO (2006) and require continuous monitoring to detect malicious increases as a result of anthropogenic input so as to avert possible public health implications of these metals on consumers of water and seafood from the study area.

For the crab sample, the heavy metal concentrations were also analyzed as indicators for the safety of those eating crabs extracted from these polluted environments. There was no significant difference in the heavy metal concentration except for Mn. The crab sample presented higher concentrations of heavy metals while in surface water; these metals exist in high levels of dissolution. In other studies, metal accumulation levels in crabs were high and this is in agreement with this study. This observation could be due to the fact that crabs are bottom feeders and are generally expected to concentrate more metals than surface feeders like prawn which is in agreement with earlier report (Okoye *et al*., 1991, Kakalu *et al*., 1987, Olowu *et al*., 2009).

5.0 Conclusion

Most of the rivers in Port Harcourt and even in Rivers State are being impacted by anthropogenic activities and being polluted. This study provides information on the microbiology and physicochemistry including nutrient content, heavy metal and total petroleum hydrocarbon

content of three coastal wetlands (Iwofe, Eagle Island and Chokocho) in Rivers State which have been polluted by oil spills due to the transportation of artisanal crude oil, and anthropogenic activities due to urbanization.

The study revealed a decrease in total heterotrophic bacterial and fungal counts (microbial population) with increasing total petroleum hydrocarbon content. The river with the highest total petroleum hydrocarbon content had the least microbial counts in general. This study also showed that microorganisms in rivers were more downstream which is by the shore than upstream towards the source of the rivers which is less impacted by anthropogenic activities. The study revealed that *Bacillus species* was the most dominant bacterial species at all points in all three river locations studied while *Penicillium* sp. and *Aspergillus* sp.were the most dominant fungal species.

Generally, the study revealed microbial counts with respect to physicochemical parameters, heavy metals and total petroleum hydrocarbon content. This information is useful in understanding microbiology of crude oil polluted aquatic environments and inference can be made on the health of the environment as well.

This study revealed that the three crude oil polluted rivers were deficient in chlorine and bromine which are micronutrients that regulates enzyme activities in plants during their growth and development. However, the river with the least Petroleum hydrocarbon content (Iwofe) had high amounts of dissolved oxygen since the oil droplets did not hinder the dissolution of oxygen with the water.

Heavy metals such as Lead (Pb), Zinc (Zn), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Manganese (Mn) and Iron (Fe) were also considered in this study. The study revealed that the rivers with the highest total petroleum hydrocarbon content had the highest concentrations of all the heavy metals except Lead (Pb). However, all the heavy metals were present in all three crude oil polluted rivers in minute or high levels which are poisonous to humans. The crab samples also showed the bioaccumulation of all the heavy metals studied.

From this study it can be inferred that the higher the level of pollution with crude oil and petroleum products, the less the microbial population found in that environment. It can also be inferred that microorganisms in rivers were less upstream than downstream. *Bacillus* sp. was the

most dominant bacteria species found in rivers polluted with crude oil while *Penicillium* sp. and *Aspergillus* sp. were the most dominant fungal species found in rivers polluted with crude oil. The study also showed an increase in heavy metal content with an increase in TPH and vice versa.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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