Microbiological and physicochemical characteristics of surface water from a Local refinery site In Port Harcort, Nigeria

ABSTRACT

Water plays a vital role in maintaining human health but due to an increase in industrialization, urbanization and other anthropogenic activities, surface water pollution is on an increase. This research was carried out to investigate the microbiological and physicochemical status of a crude oil polluted surface water in Iwofe River, Rumuolemini, Port Harcourt. Samples were collected from two points (Upstream and Downstream) bi-monthly for a period of three months. The THB counts for the upstream and downstream samples were 1.7×10^8cfu/ml and 1.4×10^8 cfu/ml, respectively while the THF counts for the samples were 3.0×10^6cfu/ml and 1.4×10^6cfu/ml, respectively. Hydrocarbon utilizing bacterial (HUB) counts were higher in the upstream (1.5×10^6cfu/ml) than downstream (4.3×10^5cfu/ml) location of the Iwofe River. The Hydrocarbon utilizing fungal (HUF) count ranged from 1.0×10^5cfu/ml to 1.4×10^5cfu/ml in the upstream and downstream water samples, respectively. The results of all the microbiological parameters analyzed indicated that the total heterotrophic bacterial (THB) and Total heterotrophic fungal (THF) counts were higher in the upstream than in the downstream location. The Most Probable Number (MPN) method used for the determination of coliform counts revealed the presence of coliforms both upstream and downstream, with an MPN index of 1,600/100ml of each sample. Some of the bacterial species isolated were *Staphylococcus aureus, Escherichia coli* and *Vibrio cholerae* while the fungal isolates included *Aspergillus fumigatus, Penicillium* species and *Alternaria* species. The high level of oil contamination poses a great health threat and there is a need for remediation of the aquatic ecosystem.

Keywords: Water, pollution, bacteria and fungi.

1. INTRODUCTION

"Water is the most widely circulated and abundant substance found in nature and it is necessary for industrial, agricultural and human existence. Clean drinking water is now recognized as a fundamental right of human beings. Most of the water on earth is not used for drinking purpose because 97% is sea water and only 3% is fresh water, out of which 2% is lodged in the polar ice caps and glaciers; only 1% water is available for potable use" (WHO, 2004). "Around seven hundred and eighty million people do not have access to clean and safe water and about two million people do not have proper sanitation. As a result, around six to eight million people die each year due to water-related diseases and disasters. Therefore, water quality control is a top priority policy agenda in many parts of the world" (Ephraim, 2015).

"Water quality and suitability for use are determined by its taste, odour, colour and concentration of organic and inorganic matters" (WHO, 2004). "The healthy water ecosystem is dependent on the physicochemical and microbiological characteristics" (Ephraim, 2015). "Contaminants in the water can affect the quality and consequently, the human health. The potential sources of water contamination are geological conditions,

hydrocarbon spills, microorganisms, industrial and agricultural activities and water treatment plants" (Gundlach, 2000).

"One of the sources of water contamination, as stated earlier, is hydrocarbon spills. This arises from the use of petroleum and petroleum-based products. Petroleum refers to any mixture of hydrocarbons that can be recovered from a drill pipe. As petrochemical industries are flourishing worldwide, hydrocarbon contamination has become one of the major environmental problems faced globally" (Nwilo and Badejo, 2001). "Activities associated with petroleum exploration, development and production operations have local detrimental and significant impacts on the atmosphere, soils and sediments, surface and ground water, marine environment, biological diversity and sustainability of terrestrial ecosystems in the Niger Delta" (Nwilo and Badejo, 2001). Discharge of petroleum products into rivers have caused environmental pollution, adverse human health effects, detrimental impact on regional economy, socio-economic problems and degradation of host communities in the various oil-producing states in the Niger Delta region, especially Rivers state.

"Diseases related to contamination of drinking water constitute a major burden on human health. Interventions to improve the quality of drinking water provide significant benefits to health" (WHO, 2004). Improving access to safe drinking water can result in great benefits to health. Every effort should be made to achieve a drinking water quality as safe as practicable. This study was designed to investigate the microbiological and physicochemical properties of Iwofe River as well as to isolate and identify potential human pathogens and assess the of level of pollution.

2. MATERIAL AND METHODS

2.1 Study Area

The area under investigation (Fig. 1) forms part of Rumuolumeni, a prominent community within Port harcourt, Rivers State. It is delimited by the coordinates: longitude 6° 55' 41.8''E – 6° 55' 49.7''E of the Greenwich Meridian, and latitude 4° 48' 33.3'' N – 4° 48' 40.1'' N of the equator. The water serves as a means of transportation to neighboring towns such as Degema. An oil-refining company Delmaco which produces loads of waste effluents is an imposing edifice at the bank of Iwofe River. The bank slopes away towards the river and the area experiences two distinct seasons as common in most tropical areas; the wet season (March to October) and the dry season (November to February).

2.2 Sample Collection

Surface water samples were collected from two points of the Iwofe river, Rumuolumeni in Rivers State as shown in Table 1 below. These samples were taken bi-monthly from the upstream and downstream parts of the river for a period of three months. They were collected in sterilized ten (10) liter containers which had been properly labeled and sealed following the appropriate sampling procedures and kept in ice-boxes (Prescott, 2011). microbiology laboratory of the Rivers State University for microbiological and physiochemical analyses within three (3) hours of collection.

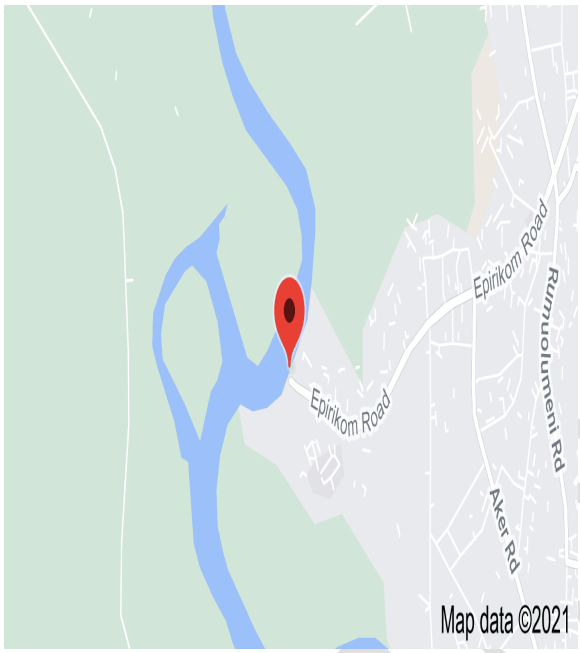

Fig 1: Map showing the sampling locations in Iwofe River, Rumuolumeni.

2.4.2 Serial Dilution

The dilution method adopted was the ten-fold serial dilution technique in which 1ml of water was added into 9ml test tubes containing sterile diluent. This was done consecutively until appropriate dilutions of 10^{2} to 10^{6} were reached.

2.4.3 Inoculation and Incubation

Inoculation of heterotrophic bacteria, total coliforms, *Salmonella, Shigella,* and *Vibrio species* was done by aseptically transferring aliquots (0.1ml) of the dilutions 10^{-6}, 10^{-3} and 10^{-2} respectively onto dried nutrient agar, macconkey agar, salmonella-shigella agar and thiosulfate citrate bile salt agar plates (containing fungosol 100 g (µ/ml) using the spread plate method and they were incubated at 35-37^{o} C for 24 hours. Inoculation of the

hydrocarbon utilizing bacteria was done by aseptically transferring aliquots (0.1 ml) of the dilution of 10^{-2} onto properly dried mineral salt agar plates using the spread plate method and incubated at 37° C for 3- 5 days (Prescott, 2011).

"Sub culturing of bacterial isolates was done to obtain pure cultures. Bacterial colonies were picked with sterile inoculating loop and streaked on freshly prepared well dried nutrient agar (NA) plates" (Prescott, 2011). "Colonial morphology such as shape, edge, color, elevation, surface, opacity and their consistency were recorded. Biochemical assay was based on Gram staining reaction, Motility, Catalase, Oxidase, Indole, Methyl red, Citrate, Voges Proskauer, Starch hydrolysis and Sugar fermentation test" (Prescott, 2011; Nollet,2000).

2.4.4 Cultural characterization of bacterial and fungal isolates

The cultural characteristics of the bacterial isolates were based on appearance on the media, shape, color, moisture, size, elevation, opacity etc. The fungal isolates were identified based on cultural characteristics such as colony growth pattern and pigmentation.

2.4.5 Morphological characterization of bacterial and fungal isolates

"Pure cultures of bacterial isolates were identified based on biochemical tests which include; gram staining, motility, catalase, oxidase, citrate utilization, indole production, methyl red test, sugar fermentations, starch hydrolysis and microscopic techniques" (Cheesebrough, 2005; Prescott, 2011). "The identification of bacterial isolates was confirmed by comparing them with Bergey's Manual of Determinative Bacteriology after microscopic examination. The fungal isolates were identified morphologically based on conidial morphology and pigmentation. The technique described by (Cheesebrough, 2005) was also adopted for the identification of the isolated fungi using cotton blue in lacto phenol 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 fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread on the slide with a 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 using ×10 and ×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 Williams and Dimbu (2005).

2.4.6 Characterization and identification of fungal isolates

Discrete fungal isolates from the 5-7 days incubated plates were selected based on the differences in their morphologies and purified by sub-culturing on freshly prepared Sabouraud Dextrose Agar plates using spread plate technique. The sub-cultured plates were marked, labelled properly and incubated at 28 ± 2° C for 5- 7 days. Pure cultures obtained after 5-7 days of incubation were subjected to characterization and identification based on cultural and morphological characteristics such as colony growth pattern, conidial morphology, and pigmentation

2.4.7 Purification and preservation of bacterial cultures

Ten percent (10%) glycerol solution was prepared, dispensed in McCarteny bottles and autoclaved at 121°C for 15 minutes, and allowed to cool, discrete colonies were purified by repeated sub-cultures unto Nutrient Agar. Pure cultures were inoculated in duplicates then stored in nutrient agar slants kept in the refrigerator at 4°C for further tests (Amadi *et al.,* 2014).

2.5 Physicochemical analysis

Water samples were analyzed for the following parameters: Temperatures, pH, Turbidity, Salinity, Dissolved Oxygen (DO), Bio-chemical Oxygen Demand (BOD), Total suspended solids (TSS), Total dissolved solids (TDS), Electrical conductivity (EC), Phosphates and Nitrate were determined using standard laboratory methods of APHA (2004) to ascertain whether the values were within the WHO permissible limits.

2.6 Statistical Analyses

The results were statistically analyzed using one-way Analysis of Variance (ANOVA) and an unpaired t-test involving a two-tailed p- value was calculated. The results were presented as mean ± SD where necessary. Statistical significance was defined as a p-value less than 0.05 at 95% confidence interval (Aliyu, 2011).

3.0 RESULT

3.1 Microbial Analyses

The microbiological analyses indicated that the total heterotrophic bacterial count for upstream water sample was higher (1.7×10^8cfu/ml) than that of the downstream water sample (1.4×10^8cfu/ml) as shown in Table 1. The fungal count for upstream was also higher (3.0×10^6cfu/ml) than that of downstream sample (1.4×10^6cfu/ml) as shown in Table 1. Thus, the microbial load was higher in the upstream part of the river than the downstream.

The prevalence of bacterial and fungal isolates in upstream and downstream locations are presented in Tables 2 and 4 respectively. The morphological characteristics and biochemical reactions of the bacterial isolates revealed the presence of microorganisms such as *Hafnia alvei, Staphylococcus aureus, Escherichia coli* and *Brenneria species* in the surface water. Some of the fungal species isolated from the water samples were *Aspergillus species, Penicillium species* and *Fusarium species* which are known hydrocarbon utilizing organisms (Chibuike *et al.,* 2021) (Table 4). In the MPN test for detection of coliforms, Table 5 shows that all the tubes produced acid and gas in each dilution after incubation. Fifteen test tubes were used for each water sample; a dilution had 5 test tubes.

3.2 Physicochemical Analyses of Water

Results of the physicochemical analyses are presented in Table 6. The electrical conductivity (EC) values for the upstream and downstream water samples were 5450µs/cm and 5740µs/cm, respectively, while the turbidity values were quite high, ranging from 13.1 ± 19.9NTU–38.4NTU. However, parameters such as pH, temperature, dissolved oxygen (DO) and biological oxygen demand had values within WHO permissible limits for normal drinking water.

Table 2. Microbial Population of the upstream and downstream Iwofe River

Microbial population	Upstream (cfu/ml)	Downstream (cfu/ml)
Total heterotrophic bacterial count	1.7×10^{8}	1.4×10^{8}
Total heterotrophic fungal count	3.0×10^{6}	1.46×10^{6}
Total coliform count	7.9×10^{5}	7.7×10^{5}
Total Salmonella-shigella count	2.0×10^{3}	3.0×10^{3}
Total Vibrio Count	1.0×10^{3}	1.0×10^{3}
Hydrocarbon utilizing bacterial count	1.15×10^{6}	4.3×10^{5}

Hydrocarbon utilizing fungal count	1.4×10^5	1.0×10^5

Table 3: Prevalence of Bacterial Isolates in River Water

Isolate	Upstream	Downstream
1_{THB}	+	+
2_{THB}	+	-
3_{THB}	+	-
4_{THB}	+	-
5_{THB}	+	+
6_{THB}	-	+
7_{THB}	+	-
8_{THB}	+	-
9_{THB}	+	-
10_{THB}	-	+
11_{THB}	-	+
12_{THB}	-	+
13_{THB}	-	+
A_{TCC}	+	+
B_{TCC}	+	+
C_{TCC}	+	+
D_{TCC}	+	+
E_{TCC}	-	+
$S_{1\ TSSC}$	+	+
$S_{2\ TSSC}$	-	+
T_{TVC}	+	+
1_{HUB}	+	+
2_{HUB}	+	-

3_{HUB}	**+**	**+**

Key: + =Present; - = Absent; THB=Total heterotrophic bacteria; TCC=Total Coliform Count; TSSC=Total Salmonella-Shigella Count; TVC=Total Vibrio Count; HUB = Hydrocarbon Utilizing Bacteria

Table 4: Prevalence of Fungal Isolates in River Water

Isolate	Upstream	Downstream
1_{THF}	**+**	**+**
2_{THF}	**+**	**+**
3_{THF}	**+**	**+**
4_{THF}	**+**	**-**
5_{THF}	**+**	**+**
6_{THF}	**-**	**+**
7_{THF}	**+**	**+**
1_{HUF}	**-**	**+**
2_{HUF}	**+**	**-**
3_{HUF}	**+**	**+**
5_{HUF}	**+**	**+**
6_{HUF}	**+**	**+**

Key: + =Present; - = Absent; THF=Total heterotrophic Fungi; HUF= Hydrocarbon Utilizing Fungi

Table 5: MPN results for water samples

	Dilution			
Sample	**10ml**	**1ml**	**0.1ml**	**MPN Index/100ml**
Upstream	5 + (A+G)	5 + (A+G)	5 + (A+G)	1600
Downstream	5 + (A+ G)	5 + (A+G)	5 + (A+G)	1600

KEY: 5 + (A+G) indicates that all the test tubes in each dilution produced acid and gas.

Table 6: Physicochemical Analyses of River Water from Upstream and Downstream

Parameters	Upstream	Downstream	USEPA	WHO
pH	**6.43**	**6.51**	**6.5-8.5**	**6.5-8.5**
Temperature (^{0}C)	**30.7**	**30.4**	**40.0**	**20-30**
Turbidity (NTU)	**19.9**	**38.4**	**5.1**	**5.0**
Electrical Conductivity (µs/cm)	**5450**	**5740**	**400**	**600**
Salinity %/ppt	**2.8**	**3.0**	**NA**	**3.0**
Dissolved Oxygen (mg/L)	**4.1**	**0**	**NA**	**14**
Biochemical Oxygen Demand (mg/L)	**0.9**	**70.0**	**10**	**40**

4.0 DISCUSSION

4.1 Microbiological Analyses of the Surface Water

The diversity of microbial groups observed upstream and downstream in this study may have been favoured by the interplay of the various ecological factors and anthropogenic activities. Such plethora of microorganisms have also been reported by other researchers (Amadi *et al*., 2020; Chibuike *et al*., 2021; Woke and Umesi, 2018). Results of the microbiological analyses indicated that the microbial load was higher in the upstream part of the river than the downstream (Table 1). The higher microbial count upstream could be attributed to anthropogenic activities, nutrient and aeration that enhance microbial growth. The lower microbial count observed downstream (Table 1) could be attributed to anaerobic conditions and nutrient depletion that inhibit

microbial growth. Most of the microorganisms identified in this study such as *Escherichia coli, Vibrio diazotrophicus, Staphylococcus* and *Pseudomonas species* (Table 4) are of public health importance since they are associated with different types of diseases ranging from food poisoning, boils, skin infections and Urinary tract infection (Prescott *et al*., 2011). Their presence in this river may be linked to open defecation, soil erosion, discharge of industrial and domestic wastes, bathing/washing and recreational activities, thus resulting in proliferation of waterborne pathogens as earlier reported in different rivers (Javed *et al*., 2014). Other microorganisms such as *Anabaena* and *Nostoc* species have been successfully used in the remediation of oil-polluted aquatic ecosystems (Williams and Youngtor, 2017). Among the isolates were *Penicillium* and *Aspergillus species* which have been said to have hydrocarbon utilizing abilities (Chibuike *et al.,* 2021).

The high presence of coliforms is a clear indication of fecal contamination of the water. In cases where it is desirable to determine whether fecal contamination may have occurred, *E. coli* is the most widely used indicator of such, the presence of which implies a risk that other enteric pathogens may be present in the food or water (Prescott, 2011). The presence of a local refinery site in proximity to the surface water could have enhanced the growth of Hydrocarbon Utilizing Bacteria and Hydrocarbon Utilizing Fungi observed in this study and microbial contamination possibly reflects the indiscriminate human defecation and poor waste disposal system prevalent in the area (Adekunle, 2009).

5.2 Physicochemical Analyses of the Surface Water

The mean pH values recorded were near neutral (6.43 and 6.51 respectively) and varied at sampled points (upstream and downstream) (Table 6). Such variations in pH at very small distances have been reportedly linked to atmospheric and anthropogenic emissions such as defaecation, disposal of domestic wastes buffering and dilution effects of heavy rains (Amadi *et al*., 2020). This acidic-alkaline pH of the river water may not only relief physiological stress but best suited to support aquatic life, and corroborates earlier reports by (Parmar *et al.,* 2016). Basically, pH of water is determined by the amount of dissolved carbon dioxide (CO2) which forms carbonic acid in water. It is an integral parameter that plays a vital role in evaluating the quality of drinking water. Decrease or increase in pH values of water below or above the WHO permissible limits can result in serious complications such as vomiting, cholera, diarrhea, kidney and liver diseases, stomach cramps and nausea after its consumption (Adekunle, 2009). Furthermore, leakage of acidic water into the river can directly harm the aquatic animals by lowering the sodium and oxygen activities within their systems. An important

problem associated with acidic nature of surface water is that this water favors the mobility of non-biodegradable and hazardous trace elements within them (Ephraim and Ajayi, 2015).

EC is frequently used as a water quality parameter, especially in coastal areas and it is also an indicator of salinity level which makes it very useful in studying sea water intrusion (Rusdyi, 2018). Mean EC values suggest addition of dissolved solids from atmospheric and anthropogenic emissions as well as erosion activities which corroborate reports of other workers on related water bodies (Amadi *et al.,* 2020; Omonona, 2019). EC in water is due to ionization of dissolved inorganic solids and becomes a measure of total dissolved solids. It is used as a primary index to select the suitability of water for all purposes (Sivakumar, 2011). The Electrical Conductivity and Turbidity of the samples were higher than the WHO permissible limit of 400min-1000max (Table 6). The EC values of upstream and downstream water samples ranged from 5450-5740 µs/cm, beyond the WHO permissible limit for normal drinking water with the higher value obtained from the downstream water sample. The EC levels were possibly influenced by geology and soil constituents (Akpan, 2015).

Turbidity, on the other hand, is considered an important water quality parameter because of the properties it has on drinking water. The higher the mineral content, the more the total suspended solids present in the water. The higher the turbidity level, the more likely taste and odour problems may arise in the water. The turbidity values were quite high, ranging from 13.1 ± 19.9-38.4 NTU (Table 6). Thus, the water could be a source of water-borne diseases to people that consume it raw without treatment. Typical sources of increased turbidity in the area were suspended and colloidal matters such as clay, silt, and organic matter (Akpan, 2015).

There was a significant difference ($p \leq 0.05$) in the DO and BOD of the two locations sampled. The upstream water sample recorded a DO of 4.1 mg/l and a BOD of 0.9 mg/l of oxygen while the downstream water sample recorded a BOD of 70.0 mg/l. Depletion of DO in the downstream could suppress respiration, cause death of fish, depress feeding or affect embryonic development (Clark, 2001) which could lead to reproductive failure and changes in the composition, abundance and diversity of species in the community. The mean dissolved oxygen concentration measured during the analysis was 2.9 ± 2.05 mg/l and it remained well below the minimum level required to support good fish production throughout the year. The DO values were below the 14 mg/l limit set by the world health organization (WHO, 2004). The observed low DO values possibly reflects early indication of undesirable conditions in the physical, chemical and biochemical factors within the water bodies. BOD values for the investigated waters ranged from 0-9 to 70.0 mg/L, with a mean value of 35.45 mg/L. A very low BOD of 0.9 mg/L was observed in the upstream water sample and conversely, the

downstream water sample had a high BOD value (70.9mg/L). Ephraim and Ajayi (2015) interpreted low BOD values as an indication of limited levels of organic matter decomposition requiring oxygen from the water.

5.3 Conclusion

Iwofe river is contaminated with bacteria including enteropathogens such as the enteropathogenic *Escherichia coli (*EPEC) and some mycotic fungi. This is attributed to the indiscriminate, uncontrolled discharge of untreated domestic sewage into the river. The presence of coliform bacteria in any water body renders the water unfit for human consumption. Therefore, good management measures should be employed to ensure that the river regains its fitness for the support of aquatic life and other domestic uses. The inhabitants of this area should be enlightened through public awareness programs on the effects of polluted water on their health and the health of their children. Maintaining adequate flow is critical to protecting water quality and preserving aquatic habitat.

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