Asian Journal of Biological Sciences 7 (5): 208-216, 2014
ISSN 1996-3351 / DOI: 10.3923/ajbs.2014.208.216

Bacterial Population of *Clarias gariepinus* (Burchell 1822) Exposed to an Oilfield Wastewater in Rivers State, Nigeria

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ABSTRACT

The constituents and bacterial population of an oilfield wastewater and tissues of *Clarias gariepinus* exposed to sub-lethal concentrations of the oilfield wastewater were investigated. Some physicochemical parameters, total heterotrophic and petroleum degrading bacterial counts of the wastewater and tissues were determined using standard methods. The mean values of physicochemical parameters obtained were: Temperature 25.93±6.7°C, pH 7.73±0.31, turbidity 40.33±1.53 NTU, salinity 6584±137 mg L^{-1}, conductivity 15200±1058.68 µS cm^{-1}, Total Dissolved Solids (TDS) 8436.33±501.68 mg L^{-1}, Total Suspended Solids (TSS) 4.67±0.58 mg L^{-1}, chloride 4033.37±208.17 mg L^{-1}, alkalinity 1296.33±2168 mg L^{-1}, Dissolved Oxygen (DO) 1.83±0.38 mg L^{-1}, Biochemical Oxygen Demand (BOD) 1.3±0.7 mg L^{-1} and Total hydrocarbon (THC) 40.54±50 mg L^{-1}. The values for TDS, salinity, conductivity and alkalinity were greater than FEPA limits while BOD and DO values were lower. Values of other constituents were within the acceptable limits of FEPA. Mean total heterotrophic counts and petroleum degraders in the oilfield wastewater were 1.59±0.57x10^{8} CFU mL^{-1} and 42.1±17.4%, respectively. Bacterial counts for the tissues of *Clarias* ranged from 0.04±0.01×10^{6} to 0.29±0.001×10^{6} CFU g^{-1} for the skin, 2.49±0.35×10^{6} to 4.15±0.13×10^{6} CFU g^{-1} for the gills and 1.22±0.00×10^{6} to 3.15±0.24×10^{6} CFU g^{-1} for the intestine. The gills had higher bacterial counts. The bacteria isolated included *Alcaligenes*, *Bacillus* spp., *Chromobacterium*, *Enterobacter* spp., *Escherichia coli*, *Klebsiella*, *Micrococcus*, *Proteus*, *Pseudomonas fluorescens*, *Serratia* spp. and *Staphylococcus aureus*. The highest occurring bacteria were *Staphylococcus aureus* (80%), followed by *E. coli* (55%) and *Enterobacter* spp. (50%) while the least was *Chromobacterium* (5%) that occurred only in the oilfield wastewater. All bacteria except *E. coli* and *Chromobacterium* were isolated from the wastewater and *C. gariepinus*, respectively. The high levels of physicochemical constituents and very low DO of the oilfield wastewater is a hazard to fish if discharged into water bodies. The presence of potential pathogens such as *Bacillus*, *E. coli* and *Staphylococcus* among others can lead to bacterial diseases of fish, economic loss and public health hazards. The proper treatment of oilfield wastewater prior to discharge into the recipient water body is advocated as to reduce ecotoxicological problems.

Key words: Oilfield wastewater, toxicity, *Clarias gariepinus*, bacteria

INTRODUCTION

Oilfield wastewater also known as produced water is an effluent produced alongside with oil and gas during drilling. At the surface, the oilfield wastewater is separated from the hydrocarbons,

treated to remove as much oil as possible and then either discharged into the surrounding aquatic environment, into a pit or injected back into the wells (Wills, 2000). Oilfield wastewater contains inorganic and organic constituents which vary from location to location and even over time in the same well (Wardley-Smith, 1979; Veil *et al*., 2004). Of importance are the oil and grease constituents of produced water and has received most attention in both onshore and offshore operations while salt content (expressed as salinity, conductivity, or TDS) is a primary constituent of concern in onshore operations (Veil *et al*., 2004). Microorganisms have also been associated with oilfield wastewater (Obire and Amusan, 2003; Wemedo *et al*., 2009, 2012; Obire *et al*., 2008) as they are known to clog equipment and pipelines (Veil *et al*., 2004). They can also form difficult-to-break emulsion and hydrogen sulphide which can cause corrosion (Veil *et al*., 2004). During treatment of wastewater, however, chemicals such as water clarifer and biocides are added to reduce microbial populations (Sloat and Ziel, 1991; Obire and Wemedo, 2002). The numerous inorganic constituents dissolved in oilfield wastewater can be potentially or actually far more hazardous than the crude oil itself (Wardley-Smith, 1979; Akani *et al*., 2009).

Nigeria oilfield wastewater contains 3,000-9000 mg L^{-1} chloride ions (Ibiebele, 1985; Oteri, 1985; Obire *et al*., 2008) and the continuous discharge of such wastewater into the surrounding aquatic environment or into a pit could cause major damages to aquatic and agricultural resources. Fish live in very intimate contact with their environment and are therefore very susceptible to microbial contamination (Wilson and Taylor, 1993). In Nigeria, information on bacterial population of African catfish, *Clarias gariepinus* exposed to oilfield wastewater is scarce. The objective of this study therefore, was to enumerate and isolate the total aerobic heterotrophic bacteria associated with *Clarias gariepinus* exposed to sub-lethal concentrations of an oilfield wastewater and highlight the environmental significance of the bacteria in the environment.

MATERIALS AND METHODS

Twenty-eight adult *C. gariepinus* (mean weight 205±12.89 g SD; mean length; 31.13±3.82 cm SD) were obtained from the African Regional Aquaculture Center (ARAC) at Aluu in Ikwerre local government area of Rivers State. This fish was chosen because of its availability all the year round, ease of maintenance in laboratory conditions and relative sensitivity (high level of tolerance) to petroleum products. The *Clarias gariepinus* were put into 50 L trough containing borehole water. The mouth of the trough was covered with a net and transported to the Department of Applied and Environmental Biology laboratory for analysis. On arrival, the fish were acclimated individually in rectangular aquaria for two weeks. The top of the aquaria were covered to control escape of fish. The water was changed daily and the aquaria washed with a piece of foam without using any form of soap or detergent. The fish were fed twice with a 35% crude protein diet at 1% biomass daily (8.00 am and 5.00 pm). Mortality during acclimation was less than one percent.

The test toxicant (oilfield wastewater) was collected from Ebocha oilfield in Ogba/Egbema Local Government Area of Rivers State with coordinates N05 27' 26.7"E006 41' 38.9". The effluent was collected in 50 L plastic containers on three occasions. These represented different ranges of the discharge at the discharge point. The oilfield wastewater samples were immediately transported to the laboratory after collection.

Preliminary investigation was conducted as to determine the range or concentrations of oilfield wastewater that exhibited sub-lethal effect on the *C. gariepinus*. Five concentrations (10, 30, 50, 70 and 100%) of the oilfield wastewater were prepared by serially diluting from each effluent sample on a volume to volume (v/v) ratio so that the percentage (%) concentration in each test solution is obtained by using the equation given as follows (FAO, 1984):

$$\text{Volume (\%)} = \frac{\text{Volume of effluent}}{V_E + V_{DW}} \times 100$$

Where:
V_E = Volume of effluent
V_{DW} = Volume of diluted water

The determined volume of effluent was added to the desired quantity of dilution (borehole) water and vigorously mixed.

The water was not changed for a period of one week. However, the fish were fed twice daily as in the acclimation period. The purpose of the preliminary investigation was also to determine the range of concentration to be used for the definitive (main) test. Concentrations that caused death within one week were omitted from the definitive test (Gurure, 1987). Each prepared concentration the oilfield wastewater then had a fish sample (*C. gariepinus*) exposed to it.

Based on the results of the preliminary investigation, the following concentrations (10, 20, 30, 40, 50 and 60% v/v) of oilfield wastewater were prepared as the sub-lethal concentrations that were used for the study, including a control.

There were six [0.00 (control), 10, 20, 30, 40, 50 and 60% v/v] treatment levels each with four replicates. A fish was then introduced into each of these concentrations contained in an aquarium and incubated for a period of 28 days at room temperature (30±2°C). Fifteen litres of each prepared concentration was used and fish was fed as in the acclimation period. The test solution was renewed weekly after washing the aquarium to get fresh toxicant as the old one would have deteriorated with time.

At the end of 28 days, fish were killed and dissected in order to collect samples of the skin, gill and intestine tissues in sterile petri-dishes for bacteriology.

Physicochemical and bacteriological analysis of oilfield wastewater and tissue samples: The following physicochemical properties of the various oilfield wastewater samples collected at a weekly interval were analyzed; they include temperature, pH, salinity, turbidity, electrical conductivity, Total Dissolved Soilds (TDS), Total Suspended Solids (TSS), chloride, alkalinity total hydrocarbon content and heavy metal.

Temperature of the oilfield wastewater was measured at the sampling point immediately after collection using mercury in glass thermometer graduated in centigrade (°C). The thermometer was first allowed to equilibrate in air before lowering the sensitive (i.e., bulb) end into the water sample. The thermometer remained immersed for 5 min to stabilize and the thermometric readings were then recorded.

Measurement of pH was done using a pH meter (model No. 7 corning). The electrode was first dipped into distilled water in beaker to stabilize the reading at zero. After which, the electrode is removed and immediately put into the sample and left to remain for a few minutes to stabilize before taking the reading. The electrode was flushed with distilled water before and after taking the pH measurement.

Salinity of produced water was determined by the method described in Sterling (1999); the titre in milliltre is directly equal numerically to the salinity of the sample, to within about 0.3%.

The turbidity of the samples was measured using absorptometric method described by APHA (1998). The absorbance of the sample was read with a direct reading spectrophotometer 2000 (HACH model) at 450 nm.

The electrical conductivity of the sample was determined using a Jenway 4020 conductivity meter; the electrode was immersed into the sample, allowing the meter to stabilize and results recorded.

Determination of the total dissolved solids was done by using ASTMD 1888-78 methods. A dry evaporating crucible was weighed and its initial weight (W_1) recorded. A specific volume of water sample was added into the crucible and put into water bath until evaporation was complete. Crucible was then placed in an oven at 105°C for about 30 min then transferred into a desiccator to cool. The crucible was weighed again and final weight (W_2) recorded. The total dissolved solid was estimated by calculating the difference between W_1 and W_2 (APHA, 1998).

Determination of total suspended solids was determined by filtering through a previously dried, weighed filter paper, a known volume of the wastewater sample (APHA, 1998).

The determination of chloride was performed using the silver nitrate method as described by APHA (1998).

Total alkalinity was measured titrimetrically using methyl red indicator. Titration was continued until an orange color appeared and the mean titre value calculated (APHA, 1998).

Dissolved Oxygen (DO) was determined based on the Modified Oxygen Depletion (MOD) method known as Winkler's method described by APHA (1998); the results were recorded as BOD (mg LG^{1}).

Toluene extraction method was used (Odu *et al*., 1985). The extract was read directly at 420 nm using Spectronic 20. Hydrocarbon concentrations were calculated by multiplying with appropriate dilution factor and the results expressed as parts per million (ppm).

Enumeration and isolation of heterotrophic bacteria: Enumeration and isolation of Total Heterotrophic Bacteria (THB) on all samples was performed using nutrient agar. The method used was the ten-fold serial dilution method of Harrigan and McCance (1990). Decimal dilutions of the samples were made by adding 1.0 mL of wastewater of sample to 9.0 mL of sterile saline or 10 g of tissue sample to 90 mL of sterile saline to give an initial dilution of 1:10, respectively. Subsequent serial dilutions were made by adding 1.0 mL of the last dilution to 9.0 mL of fresh diluents. Finally, 0.1 mL of appropriate dilutions ($10G^{2}$ and $10G^{3}$) were inoculated onto sterile solidified agar in Petridish and evenly spread out with a sterile glass spreader. Cultures were prepared in duplicates on dry media and incubated at room temperature (30±2°C). Mineral salts medium was used for the isolation and enumeration of Petroleum-Degrading Bacteria (PDB) as described by IPS (1990).

Cultures for heterotrophic bacteria, were incubated for 24 h, after which plates which developed colonies between 30 and 300 colonies (APHA, 1995) were counted and recorded. Cultures for petroleum degraders were incubated for 7 days at room temperature (30±2°C).

Colonial morphology of discrete bacterial colonies were described and aseptically sub-cultured onto Nutrient agar (Oxoid).

Identification of bacterial isolates: Identification of bacterial isolates was based on methods of Buchanan and Gibbons (1974), Cowan (1974) and Cruickshank *et al*. (1975). These tests included Gram reaction, motility, catalase, oxidation/fermentation, hydrogen sulphide production, coagulase, oxidase, indole production, Methyl-Red Voges-Proskauer (MR-VP) reactions, starch hydrolysis, nitrate reduction and fermentation of the following carbohydrates, glucose, lactose, mannitol, arabinose.

Statistical analysis: Statistical analysis was carried out on the data obtained during the study. Analysis of variance and Student Newman Keul's (S-N-K) test were used to test for significance and means separation, respectively. This was done using a computer-based programme-SPSS version 17.

RESULTS

Results of physico-chemical properties of the raw wastewater effluent are presented in Table 1. Bacterial populations of the oilfield wastewater are presented in Table 2. Bacterial population and trend in the various tissues are presented in Table 3 and Fig. 1, respectively. All the physicochemical properties except temperature (25.93±6.7°C), DO (1.83±0.38 mg L^{-1}), BOD (1.3±0.7 mg L^{-1}) and THC (40.54±50.87 mg L^{-1}) fell above acceptable limits of FEPA. Other physicochemical properties of raw oilfield wastewater analyzed were pH 7.73±0.31,

Table 1: Characterization of raw oilfield wastewater collected at various times on a weekly basis

	Sampling				
Physicochemical parameters	1st	2nd	3rd	Mean±SD	FEPA limits
Temperature (°C)	25.6	26.7	25.5	25.93±0.67000	35
pH	7.5	7.6	8.08	7.73±0.31000	6-9
Salinity (ppm)	6567	6456	6730	6584.33±137.820	
Turbidity (NTU)	42	39	40	40.33±1.53000	5,82
Conductivity (µS cm^{-1})	14000	15600	16000	15200.00±1058.30	400
TDS (ppm)	7864	8645	8800	8436.33±501.680	2000 (max.)
TSS (ppm)	5	4	5	4.67±0.58000	30
Chloride (ppm)	3800	4100	4200	4033.33±208.170	250 (2000)
Alkalinity (ppm)	46	43	3800	1296.33±2168.24	
DO (ppm)	2.1	2	1.4	1.83±0.38000	5
BOD (ppm)	1.8	1.6	0.5	1.30±0.70000	50
THC (ppm)	12.13	10.26	99.24	40.54±50.8400	48

Table 2: Bacterial population (Mean±SD) of oilfield wastewater sampled during the study period

Sampling	THB ($\times 10^8$ CFU mL^{-1})	PDB ($\times 10^7$ CFU mL^{-1})	PDB (%)
1st	1.09	6.5	59.60
2nd	1.48	6.2	41.89
3rd	2.22	5.5	24.77
Mean±SD	1.59±0.57	6.07±0.51	42.10±17.4

THB: Total heterotrophic bacteria, PDB: Petroleum degrading bacteria

Table 3: Variation of Mean±Standard deviation of total heterotrophic bacterial counts ($\times 10^6$ CFU g^{-1}) of the different tissues at various concentrations of the oilfield wastewater

	Concentration of Oww (%)							
Tissue	0	10	20	30	40	50	60	Level of significance
Skin	0.04±0.01^{a}	0.05±0.01^{a}	0.16±0.04^{b}	0.19±0.01^{c}	0.24±0.01^{d}	0.28±0.01^{e}	0.29±0.001^{e}	0.00
Gills	2.44±0.35^{a}	2.91±0.09^{b}	3.44±0.15^{c}	3.68±0.11^{cd}	3.89±0.08^{de}	4.15±0.13^{e}	3.50±0.35^{c}	0.00
Intestine	1.44±0.46^{a}	2.83±0.04^{b}	3.07±0.05^{bc}	2.91±0.02^{bc}	2.91±0.01^{bc}	3.15±0.24^{c}	1.22±0.01^{a}	0.00

*Means with the same superscript, in the row are not significantly different at p$0.05

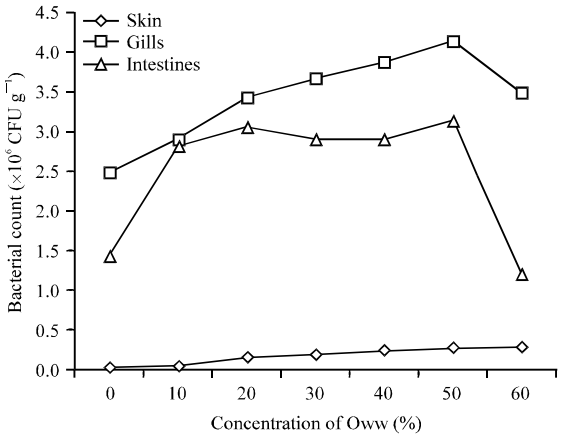

Fig. 1: Trend of bacterial load from skin to intestine of *C. gariepinus* exposed to an oilfile wastewater

salinity 6584±137 mg L^{-1}, turbidity 40.33±1.53 NTU, conductivity 15200±1058.68 µS cm^{-1}, Total Dissolved Solids (TDS) 8436.33±501.68 ppm, Total Suspended Solids (TSS) 4.67±0.58 mg L^{-1}, chloride 4033.37±208.17 mg L^{-1} and alkalinity 1296.33±2168 mg L^{-1}.

Mean bacterial population of the raw oilfield wastewater was $1.59\pm0.57\times10^{8}$ CFU mL^{-1} while the mean percentage petroleum degrading bacteria was 42.1±17.4%.

Total Heterotrophic Bacterial (THB) counts of the different tissues at the constituted concentration showed a significant difference (p#0.05) at the different concentration for each tissue tested. However, it was observed that the mean bacterial population on the skin ($0.18\pm0.1\times10^{6}$ CFU g^{-1}) was fewer than the gill ($3.44\pm0.57\times10^{6}$ CFU g^{-1}); while the intestine ($2.50\pm0.81\times10^{6}$ CFU g^{-1}) had fewer bacterial loads than the gills (Fig. 1).

Bacterial load on the skin ranged between 0.04±0.01 at the control (0%) and $0.29\pm0.001\times10^{6}$ CFU g^{-1} at 60%. In this case, the bacterial load increased with increasing concentration of the toxicant; bacterial load in the gills ranged between 2.49±0.35 at the control (0%) and $4.15\pm0.13\times10^{6}$ CFU g^{-1} at (50%). it also followed the same trend as in the skin but dropped slightly at 60% to $3.5\pm0.35\times10^{6}$ CFU g^{-1}. The bacterial load in the intestine, however, ranged between 1.22±0.01 at 60% and $3.15\pm0.24\times10^{6}$ CFU g^{-1} at 50%; the counts in the intestine were fewer at the control (0%) and the highest (60%) concentration of the toxicant.

The result of occurrence of bacterial isolates from Oww and tissues as presented in Fig. 2 revealed that the bacteria isolated included *Alcaligenes*, *Bacillus* spp., *Chromobacterium*, *Enterobacter* spp., *Escherichia coli*. *Klebsiella*, *Micrococcus*, *Proteus*, *Pseudomonas fluorescens*, *Serratia* spp. and *Staphylococcus aureus*. All bacteria except and *Chromobacterium* were isolated from the wastewater and *C. gariepinus*, respectively. The highest occurring bacteria were *Staphylococcus aureus* (80%), followed by *E. coli* (55%) and *Enterobacter* spp. (50%) while the least was *Chromobacterium* (5%) that occurred only in the oilfield wastewater.

DISCUSSION

All the physicochemical properties except temperature DO, BOD and THC fell above acceptable limits of Federal Environmental Protection Agency (FEPA). Both DO and BOD levels fell below 5.0 and 50 mg L^{-1}, respectively which is the FEPA acceptable limit and therefore indicates that the

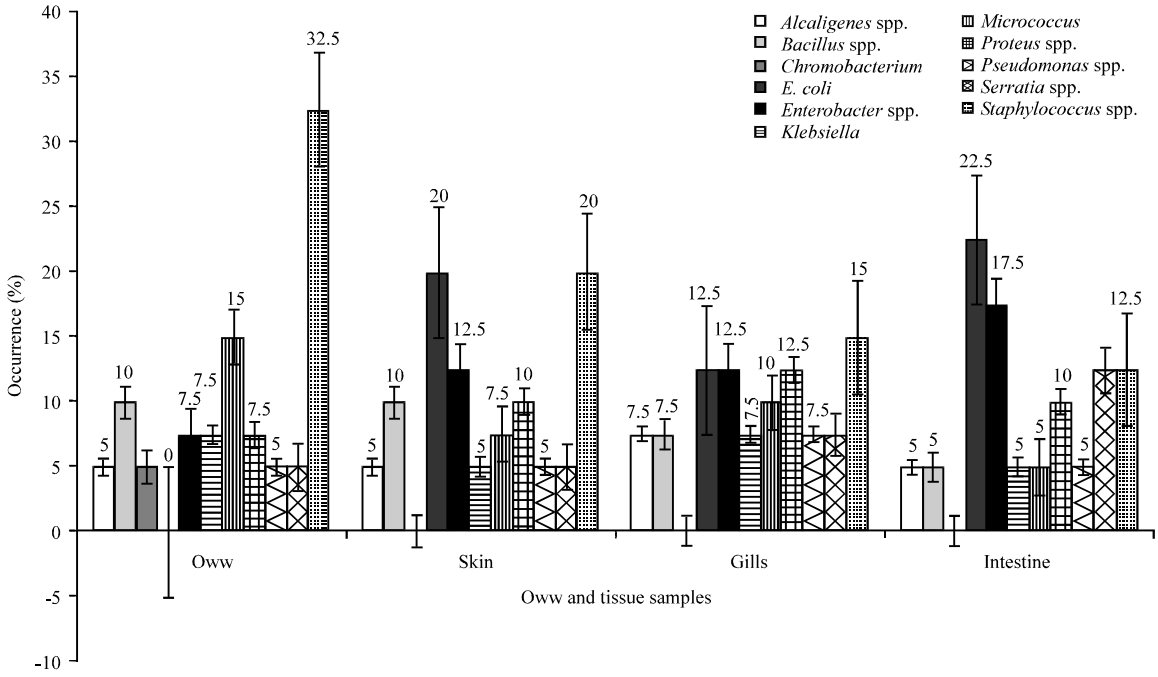

Fig. 2: Occurrences of bacterial isolates in Oww and examination of different tissue samples

environmental is stressed (Clerk, 1986; FEPA, 1991). Total hydrocarbon content however fell below the FEPA limits. It is therefore possible that the process of oil removal before discharge is very efficient. Other physicochemical properties of raw oilfield wastewater analyzed were pH 7.73±0.31, salinity 6584±137 mg L^{-1}, turbidity 40.33±1.53 NTU, conductivity 15200±1058.68 µS cm^{-1}, Total Dissolved Solids (TDS) 8436.33±501.68 ppm, Total Suspended Solids (TSS) 4.67±0.58 mg L^{-1}, chloride 4033.37±208.17 mg L^{-1} and alkalinity 1296.33±2168 mg L^{-1}. These high values clearly indicates that the oilfield wastewater do not undergo any treatment prior to discharge into the pit. The values recorded in this study differs from that of Obire and Amusan (2003) who worked on treated oilfield wastewater and had 12800 mg L^{-1} for TDS and 688 mg L^{-1} for chloride, respectively. The FEPA acceptable limts for TDS is a maximum of 2000 mg L^{-1} but this study clearly showed a very high value which also caused an increase in conductivity as both are a measure of solutes in a water body (Wemedo *et al*., 2012). Turbidity and TSS had values above FEPA limits hence are considered hazardous if discharged into the environment in this state.

Mean bacterial population of the raw oilfield wastewater was$1.59\pm0.57\times10^{8}$ CFU mL^{-1} while the mean percentage petroleum degrading bacteria was 42.1±17.4%. The high value in bacterial population may be due to the high turbidity which contains nutrient substances that could support the growth of microorganisms. Wemedo *et al*. (2012) recorded similar findings.

Bacterial populations of the different tissues at the constituted concentration showed a significant difference at p#0.05 at the different concentration for each tissue tested. However, it was observed that the mean bacterial population on the skin ($0.18\pm0.1\times10^{6}$ CFU g^{-1}) was fewer than the gill ($3.44\pm0.57\times10^{6}$ CFU g^{-1}); while the intestine ($2.50\pm0.81\times10^{6}$ CFU g^{-1}) had fewer bacterial loads than the gills (Fig. 1). The reduction in the population of bacteria on the skin may be due to hardness of the wastewater on the skin which caused an erosion of the integument of the skin preventing the bacteria from attaching to it. During the feeding process, also, bacteria get trapped

in the gills during ingestion of food hence increasing the bacterial load in the gills. The intestine harbored the least population at 60% concentration because of the poor feeding ability as a result of the toxicant; the fish remained above the water because of low DO and the adverse effects of the chemicals in the oilfield wastewater.

The species of heterotrophic bacteria isolated included *Pseudomonas fluorescens*, *Klebsiella*, *Bacillus* spp., *Alcaligenes*, *Serratia* spp., *Proteus*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Micrococcus* and *Escherichia coli*. Although, pathogenecity was not the focus many bacterial species encountered are no doubt potentially pathogenic to different fish species under certain conditions as reported for *Pseudomonas* and *Staphylococcus* (Varvarigos, 1997; Sowunmi *et al*., 2008), *Bacillus* spp., *E. coli* and *Staphylococcus aureus* were also implicated in fish-borne (Babu, 2000) and shrimp-borne (Raghavan, 2003) diseases of humans.

CONCLUSION

In conclusion, this study has showed that an untreated oilfield wastewater could have adverse effects on the fish *Clarias gariepinus* by reducing its performance. The fish was also found to contain some potential pathogens which may be a normal flora of the fish or be introduced from the wastewater. It is therefore, recommended that oilfield wastewater be treated to remove all potential pathogens and toxic chemical substances so as to conform to FEPA acceptable limits before being discharged into the environment.

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