International Journal of Microbiology and Mycology | IJMM
pISSN: 2309-4796
http://www.innspub.net
Vol. 3, No. 1, p. 1-9, 2015
Open Access RESEARCH PAPER

Mycological flora of *Clarias gariepinus* exposed to an oilfield wastewater in Nigeria

Nedie Patience Akani*, Omokaro Obire

Department of Microbiology, University of Port Harcourt. P.M.B. 5323, Choba - Port Harcourt, Rivers State, Nigeria

Keywords *Aspergillus* spp; *Clarias gariepinus; Mucor* spp; Oilfield wastewater.

Publication date: July 07, 2015

Abstract

The Mycological flora of *Clarias gariepinus* exposed to sub-lethal concentrations of an oilfield wastewater were investigated. The concentrations included 0% (control), 10, 20, 30, 40, 50 and 60% respectively. Physico-chemistry and mycoflora of wastewater and tissues of *Clarias gariepinus* were determined using standard methods. Mean values obtained were; temperature 25.93±6.7°C, pH 7.73±0.31, turbidity 40.33±1.53 NTU, salinity 6584±137mg/l, conductivity 15200±1058.68µs/cm, total dissolved solids 8436.33±501.68mg/l, total suspended solids 4.67±0.58mg/l, alkalinity 1296.33±2168mg/l, dissolved oxygen 1.83±0.38mg/l, biochemical oxygen demand 1.3±0.7mg/l and Total hydrocarbon 40.54±50mg/l. Temperature, DO, BOD and THC were below allowable FEPA limits while all other components were higher. Mean counts of total fungi and petroleum degraders in the oilfield wastewater were $4.7\pm0.46\times10^6$ sfu/ml and 59.7±25.7% respectively. Fungal counts in the tissues of *Clarias* ranged from $0.20\pm0.00 \times 10^4$sfu/g to $3.00\pm0.00 \times 10^4$sfu/g (skin), $0.48\pm0.05 \times 10^4$sfu/g to $7.25\pm0.96 \times 10^4$sfu/g (gills), and $1.13\pm0.15 \times 10^4$sfu/g to $5.75\pm0.50 \times 10^4$sfu/g (intestine). The intestine had higher fungal counts, but the gills recorded the highest at 10% concentration. Fungi isolated included; *Aspergillus fumigatus* (46.43%), *Aspergillus niger* (100%), *Fusarium* spp. (100%), *Mucor* spp. (24.99%), *Penicillium* spp. (57.14%), *Rhizopus* spp. (32.13%) and *Saccharomyces* spp. (34.3%). All except *Saccharomyces* spp were isolated from oilfield wastewater. *Aspergillus* spp. *Penicillium* spp, *Mucor* and *Rhizopus* are considered normal flora, but can still cause infection which may result in the mortality of the fish and eventually economic loss to the aquarium fish industry. Proper treatment of oilfield wastewater prior to discharge into the recipient water body is advocated to reduce ecotoxicological problems.

* Corresponding Author: Nedie Patience Akani ✉ nedieakani@yahoo.com

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 clarifier 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; Akani and Obire, 2014).

Nigeria oilfield wastewater contains 3,000 to 9000mg/l 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. In Nigeria, information on fungal population of African catfish, *Clarias gariepinus* exposed to oilfield wastewater is scarce. However, researchers have associated fungi with smoke-dried as well as fresh *Clarias gariepinus* (Oni *et al.* 2012; Jimoh *et al.* 2014). Some of these fungi identified as *Aspergillus* has been known to cause disease outbreak in fish (Olayemi *et al*. 1990). The objective of this study therefore, was to enumerate and isolate the total saprophytic fungi associated with *Clarias gariepinus* exposed to sub-lethal concentrations of an oilfield wastewater and highlight the environmental significance of the fungi in the environment.

Materials and Methods

Twenty-eight adult *Clarias gariepinus* (mean weight 205 ± 12.89g SD; Mean length; 31.13 ± 3.82cm SD) were obtained from the African Regional Aquaculture Center (ARAC) at Aluu in Ikwerre Local government area of Rivers State. This organism 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. They were transported in 50 litres trough whose mouth was covered with a net to the Department of Applied and Environmental Biology laboratory. 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. The fish were fed twice with a 35% crude protein diet at 1% biomass daily (8.00 a.m. and 5.00p.m.). Mortality during acclimation was less than one percent.

The test toxicant was oilfield wastewater effluent from Ebocha oilfield in Ogba/Egbema local government area of Rivers State with coordinates N05 27’ 40.45’’E006 41’ 52.14’’. The effluent (toxicant) was collected in 50l plastic containers each on three occasions. These represented different ranges of the effluent at the discharge point. Water samples for physicochemical characteristics were collected in 250ml sample bottles and their characteristics determined

according to APHA (1998). In addition water for microbiology were collected in sterile bottles and transported to the laboratory in ice cooler.

A range finding test (trial test) was carried out using the toxicant, oilfield wastewater effluent. Five concentrations (10%, 30%, 50%, 70% and 100%) 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 formula below (FAO, 1984):

$$Concentration\ of\ effluent(\%) = \frac{\mathrm{VE}}{\mathrm{VE} + \mathrm{VDW}} \mathrm{x}\ 100$$

Where, V_E = Volume of effluent

V_{DW} = Volume of dilution water

The determined volume of effluent was added to the desired quantity of dilution borehole water and stirred vigorously to disperse the effluent. One fish was exposed to each concentration of the effluent. The introduction of the toxicant was done with the aid of a measuring cylinder. The water was not changed for a period of one week. However, the fish was fed twice daily as in the acclimation period. The purpose of the test was 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).

Definitive (Sub-lethal) test

Sub-lethal concentration for the definitive test was done based on the range finding test. The concentrations were prepared by measuring 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 litres of the raw effluent and making it up to 15 litres with unchlorinated borehole water in the aquaria to make 10%, 20%, 30%, 40%, 50%, and 60% respectively.

There were six (0% (control), 10%, 20%, 30%, 40%, 50%, and 60%v/v) treatment levels with four replicates. A single fish was introduced individually into each aquarium containing the various concentration of the toxicant for a period of 28 days. Ten litres of solution was used and fish was fed as in the acclimation period. The test solution was renewed weekly after washing the aquarium.

Physicochemical Analysis of oilfield wastewater sample

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 dissolved oxygen (DO), biochemical oxygen demand (BOD),Total hydrocarbon content and heavy metal. The methods used for the analysis were as described by APHA, (1998).

Mycological examination of oilfield wastewater and tissue samples

At the end of twenty-eight (28) days, fish were killed and dissected in order to collect samples of the skin, gill and intestine tissues in sterile Petri-dishes for mycological examination.

Enumeration of heterotrophic fungi on all samples was performed using Sabouraud dextrose 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 1.0ml of oilfield wastewater to 9.0ml of sterile saline or 10g of tissue sample to 90.0ml of sterile saline to give an initial dilution of 1:10 respectively. Subsequent serial dilutions were made by adding 1.0ml of the last dilution to 9.0ml of fresh diluents. Finally, 0.1ml of appropriate dilutions were placed in duplicate on dry Sabouraud dextrose agar plates for fungi and oil agar plates (IPS,1990) for petroleum degrading fungi. The inocular was evenly spread out with a sterile glass spreader and incubated at room temperature (30±2°C) for 3days.

Enumeration of fungal counts was done using standard methods (APHA, 1998). Discrete fungal spores were randomly picked and sub-cultured onto Sabouraud dextrose agar (Oxoid).

The fungal isolates were characterized based on macroscopic and microscopic appearances, which comprised colonial morphology type of hyphae, presence of sterigma, shape and kind of spore/conidia, presence of special structure such as foot-cell, and growth on glucose. Probable identities of the fungi were determined according to Barnett *et al.* (1983).

Data Analysis: Statistical analysis was carried out on the data obtained during the study. Analysis of variance and Students Newman Kuel's test were used to test for significance and mean separation respectively. This was done using statistical package for the social sciences (SPSS) version17.0.

Results and Discussion

Results of physicochemical properties of raw oilfield wastewater and constituted concentrations are presented in Tables 1 and 2, respectively. All the physicochemical properties except temperature DO, BOD and THC fell above acceptable limits of the Federal Environmental Protection Agency (FEPA). Both DO and BOD levels fell below 5.0mg/l and 50mg/l, respectively which is the FEPA acceptable limit and therefore indicates that the environmental is stressed (Clerk, 1986; FEPA, 1991). THC however fell below the FEPA limits. It is therefore possible that the process of oil removal before discharge is very efficient. Otherphysicochemical properties of raw oilfield wastewater analyzed were pH 7.73±0.31, salinity 6584±137mg/l, turbidity 40.33±1.53NTU, conductivity 15200±1058.68µs/cm, total dissolved solids (TDS) 8436.33±501.68ppm, total suspended solids (TSS) 4.67±0.58mg/l and alkalinity 1296.33±2168mg/l. These high values clearly indicate that the oilfield wastewater did not undergo any treatment prior to discharge into the pit and environment. The values recorded in this study differs from that of Obire and Amusan (2003) who worked on treated oilfield wastewater and had 12800mg/l for TDS and 688mg/l for chloride respectively. FEPA acceptable limits for TDS is a maximum of 2000mg/l but this study clearly showed a very high value which may have 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.

Table 1. Characterization of raw oilfield wastewater collected at various times.

Physicochemical parameters	Number of sampling			Mean ±SD	FEPA Limits
	1st	2nd	3rd		
Temperature	25.6	26.7	25.5	25.93±0.67	35
pH	7.5	7.6	8.08	7.73±0.31	6.5 – 8.5
Salinity (ppm)	6567	6456	6730	6584.33±137.82	
Turbidity (NTU)	42	39	40	40.33±1.53	
Conductivity (µs/cm)	14000	15600	16000	15200±1058.30	400
TDS(ppm)	7864	8645	8800	8436.33±501.68	2000(max.)
TSS (ppm)	5	4	5	4.67±0.58	30
DO (ppm)	2.1	2	1.4	1.83±0.38	5
BOD (ppm)	1.8	1.6	0.5	1.3±0.7	10
Alkalinity (ppm)	46	43	3800	1296.33±2168.24	
THC (ppm)	12.13	10.26	99.24	40.54±50.84	

Table 2. Mean ± standard deviation values of physico-chemistry of constituted concentrations of oilfield wastewater used during the study.

Physicochemical properties	Concentration of oilfield wastewater (%)							Level of sig, $p \leq 0.05$
	01	02	03	04	05	06	07	
Temp.	26.17±0.29	26.07±0.4	26.5±0.5	25.0±0.0	26.83±0.29	27.33±0.29	27.0±0.0	0.001
pH	7.1±0.1	7.47±0.31	7.67±0.12	7.23±0.25	7.47±0.31	8.0±0.0	8.0±0.0	0.001
Salinity (ppm)	0.0±0.0	47.67±2.52	517.33±28.31	1500±50	2443.33±309.25	3233.33±251.66	4556±51.07	0.001
Turbidity (NTU)	1.5±0.5	5.33±0.58	13.33±4.16	16±1.0	23.33±3.06	32.0±2.0	35±0.0	0.001
Conductivity (us/cm)	183.33±28.87	3766.67±251.66	1060±121.66	2166.67±288.68	4216.67±225.46	8433.33±404.15	13666.67 ±288.68	0.001
TDS(ppm)	48.33±2.89	976.67±25.17	1620±158.75	2966.67±152.75	4783.33±256.58	6146.67±128.58	7316.67 ±275.38	0.001
TSS (ppm)	0.48±0.03	1.23±0.25	2.33±0.15	2.7±0.17	3.0±0.0	3.27±0.12	3.5±0.0	0.001
DO (ppm)	3.97±0.451	3.4±0.2	3.3±0.1	2.43±0.15	2.33±0.12	2.3±0.0	2.2±0.0	0.001
BOD (ppm)	0.82±0.03	1.02±0.03	1.18±0.06	1.52±0.06	1.58±0.02	1.64±0.0	1.73±0.02	0.001
Alkalinity. (ppm)	9.33±1.16	20.0±2.0	23.5±0.5	26.67±3.06	31.33±1.16	32±0.0	14±0.0	0.001
THC (ppm)	0.0±0.0	1.98±0.082	3.09±0.085	3.49±0.445	6.17±0.651	7.45±0.135	11.81±3.118	0.001

Result of fungal population revealed that mean fungal population of the raw oilfield wastewater was $4.7\pm0.46x10^4$ sfu/ml, while the mean percentage petroleum degrading fungi was 59.7±25.7% (Table 3). The high value in fungal population may be due to the high turbidity which contains nutrient substances that could support the growth of microorganisms. This finding does not however, agree with that of Wemedo *et al.* (2012) who recorded lower values of 0.3 to 8.8 x 10^1sfu/ml. The difference may be attributed to peculiarity of the well in terms of age and chemical treatment applied during separation process. The high occurrence of petroleum degrading fungi could also be attributed to the inefficient separation process hence a proliferation of these group of organisms. This finding was recorded by Atlas, (1981) and Akani *et al. (*2008). According to them petroleum degrading microorganism abound in the environment and will increase in the presence of hydrocarbons.

Table 3. Fungal populations (mean±SD) of raw oilfield wastewater sampled during the studyperiod.

Saprophytic fungi	Number of sampling			Mean±SD
	1st	2nd	3rd	
TSF(x10^6sfu/ml)	4.6	4.3	5.2	4.7±0.46
PDF(x10^6sfu/ml)	2.4	3.8	2	2,73±0.95
PDF (%)	52.17	88.37	38.46	59.7±25.7

*Legend:TSF = Total Saprophytic Fungi.

PDF =Petroleum Degrading Fungi.

Table 4. Mean fungal counts of the constituted concentrations of the oilfield wastewater effluents.

Microbial types	Concentration of oilfield wastewater (%)							Level of sig, $p \leq 0.05$
	Control (0)	10	20	30	40	50	60	
TSF (x10^4sfu/ml)	1.00 ± 0.00^a	2.83 ± 0.29^c	2.83 ± 0.29^c	2.10 ± 0.36^b	1.07 ± 0.12^a	1.03 ± 0.06^a	2.77 ± 0.25^c	0.001
PDF (%)	0.00 ± 0.00^a	3.78 ± 0.39^b	3.67 ± 0.33^b	5.84 ± 1.25^c	11.39 ± 1.99^d	13.61 ± 1.63^e	4.13 ± 0.59^{bc}	0.001

Legend: Same as in Table 3.

*Means with the same superscript in the rows are not significantly different (P≥0.05).

Fungi have been associated with different species of fish. Fungal populations of the different tissues at the constituted concentration revealed a difference (P≤0.05) at all the concentration in the tissues tested (Table 5). Mean value of total saprophytic fungi and petroleum degraders in the oilfield wastewater was 4.7±0.46x10^6 sfu/ml and 59.7±25.7%, respectively. Fungal counts in the tissues of *C. gariepinus* ranged from 0.20±0.00 x 10^4sfu/g to 3.00±0.00 x 10^4sfu/g for the skin, 0.48±0.05 x 10^4sfu/g to 7.25±0.96 x 10^4sfu/g for the gills, and 1.13±0.15 x 10^4sfu/g to 5.75±0.50 x 10^4sfu/g for the intestine. Generally, the intestine had higher fungal counts even though the highest was recorded in the gills at 10% concentration.

Table 5. Variation of mean – standard deviation of Total saprophytic fungal (x10^4sfu/g) counts of the different tissues at the various concentrations of the oilfield wastewater.

Tissue	Concentration of oilfield wastewater (%)							Level ofsig,p≤0.05
	Control (0)	10	20	30	40	50	60	
Skin	1.50±0.58^{ab}	0.20±0.00^{a}	0.65±0.40^{a}	0.20±0.00^{a}	1.15±1.90^{a}	1.60±1.63^{ab}	3.00±0.00^{c}	0.008
Gills	0.475±0.05^{a}	7.25±0.96^{b}	3.40±0.94^{c}	1.10±0.12^{a}	1.15±0.10^{a}	1.78±0.05^{a}	0.60±0.00^{a}	0.001
Intestine	1.13±0.15^{a}	4.25±1.89^{cd}	3.25±0.50^{bc}	2.25±0.50^{ab}	3.50±0.58^{bcd}	5.75±0.50^{e}	4.75±0.50^{de}	0.001

*Means with the same superscript in the rows are not significantly different at P≥0.05.

The high fungal load in the intestine may be attributed to the fact that fungi seemed to thrive in extreme environments in the intestine. Microorganisms are abundant in the environment where fish live so it becomes difficult for the fishes to avoid them; hence during the feeding process they get acclimatized in the intestine which forms a favourable environment for its growth making the intestine have higher counts than the surrounding wastewater (Olafsen, 1990; Sakata, 1990) Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations (Vaupotic *et al.* 2008) of which the intestine of this freshwater fish *C. gariepinus* is no exception. Although the counts in the intestine did not follow any particular trend the fungal load seemed to be higher in the test concentrations than the control. 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 reduction in the population of fungi on the skin may be due to the presence of the mucilaginous substances that may have prevented fungi from attaching to it (Tsutsui *et al.* 2011). During the feeding process, also, fungi get trapped in the gills during ingestion of food hence increasing the fungal load in the gills.

Result showed that a total of 6 fungal genera were identified in the oilfield wastewater, skin, gills and intestine, respectively (Fig. 1) The fungi isolated and frequencies included; *Aspergillus fumigatus* (46.43%), *Aspergillus niger* (100%), *Fusarium* spp. (100%), *Mucor* spp. (24.99%), *Penicillium* spp. (57.14%), *Rhizopus* spp. (32.13%) and *Saccharomyces* spp. (34.3%) All fungi except *Saccharomyces* spp. were isolated from oilfield wastewater.

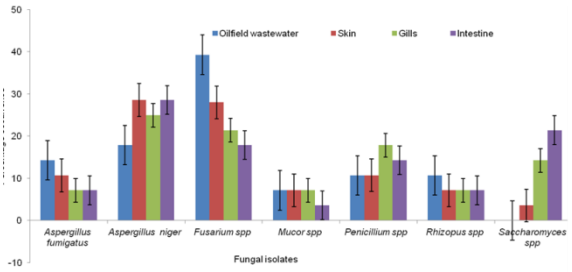

Fig. 1. Comparing occurrence of fungal isolates in oilfield wastewater sample and the various tissues analyzed.

These saprophytic fungi have at various times being isolated from either fresh or smoked *C. gariepinus*. Abolude *et al.* (2013) carried out a survey on fresh water fungi associated with eggs and brood stock of *C. gariepinus* in fish hatchery farm. A total of 6 fungal genera were isolated. Their isolates which include *Mucor* sp, *Aspergillus flavus*, *Aspergillus niger*, *Acreomonium* sp. Yeast sp. *Penicillium* sp. *Saprolegnia* sp. and *Trichophyton* sp.; with *Penicillium* sp having the highest frequency of 23%. They had similarities with the present study. However, this study recorded 100% occurrence for *Aspergillus niger* and *Fusarium* spp. Other workers have also recorded similar results while working on fresh *C. gariepinus* (Oni *et al.,* 2012; Jimoh *et al.,* 2014) Among the commonest different fungi found to be associated with smoked fish samples in Nigeria are *Aspergillus flavus*, *A. terens*, *A. fumigatus*, *A. niger*, *Mucor,* sp., *Cladosporium* sp., *Penicillium* sp., *Candida tropicalis* and *Fusarium monilliformis* (Adebayo-Tayo *et al*. 2008; Fafioye *et al*. 2002; 2013). Although pathogenecity is not our focus in this study the presence of *Aspergillus* spp. *Penicillium* spp*, Mucor* and *Rhizopus* which are considered normal flora can still cause infection which may result in the mortality of the fish. Infection does not always result in disease but environmental stress such as is posed by the oilfield wastewater may upset the balance between potential pathogens and their host (Igba *et al.* 2012). Under such conditions the chances of infection increases. Fungal infections of many fishes abound (Eli and Abowei, 2011). *Aspergillus* and *Penicillium* associated with smoked *C. gariepinus* with percent occurrence of 37.7 and 25 respectively probably were toxigenic as tested by Edema and Agbon (2010). *Aspergillus* has also been associated with disease outbreak in fish (Olayemi *et al*. 1990)

Conclusion

This study has showed that the oilfield wastewater did not have much adverse effect on the fungal flora of *Clarias gariepinus* but the presence of some potential pathogens which may be a normal flora of the fish or be introduced from the wastewater could pose a threat to the fish and reduce its performance, hence oilfield wastewater should be treated to remove all potential pathogens andtoxic chemical substances so as to conform to FEPA acceptable limits before being discharged into the environment.

Acknowledgement

We wish to acknowledge the African Regional Aquacultural Center (ARAC) at Aluu in Port Harcourt for providing the fish, *C. gariepinus* (Burchell 1822)*;* the Department of Petroleum Resources (DPR) in Port Harcourt for the oilfield wastewater samples and the Rivers State University of Science and Technology for the use of their laboratory.

References

Abolude DS, Opabunmi OO, Davies OA. 2013. Freshwater fungi associated with eggs and broodstock of African catfish (*C. gariepinus* Burchell 18220 in fish hatchery farms, Zaria, Kaduna State, Nigeria. Journal of Research in Environmental Science and Technology **2(7),** 131 – 135.

Adebayo-Tayo B, Onilude AA, Patrick UG. 2008. Mycoflora of smoke-dried fishes sold in Uyo, Eastern Nigeria. World Journal of Agricultural Sciences **4(3),** 346-350.

Akani NP, Amadi EN, Braide SA. 2009. Long-term effects of spilled crude oil on a sandy loam soil in Ejama-Ebubu in Rivers State, Nigeria. Nigerian Journal of Microbiology **23(1)**, 1935 – 1944.

Akani NP, Braide SA, Amadi EN. 2008. Physicochemical and Microbiological Assessment of Oil-impacted Sediment in Ejama-Ebubu in Rivers State - a Case Study. Nigerian Journal of Microbiology **22(1),** 1725- 1731.

Akani NP, Obire O. 2014. Bacterial population of *Clarias gariepinus* (Burchell 1822) exposed to an oilfield wastewater in Rivers State of Nigeria. Asian Journal of Biological Sciences **7(5),** 208 - 216.

American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater. 20th edition, American Public Health Association, American Water Works Association and Water Environment Federation. USA. ISBN 0-877553-235-7, ISSN 55-1979.

Atlas RM. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological Reviews **45,** 180 – 209.

Barnett JA, Payne RW, Yarrow D. 1983**.** Yeast characteristics and identification. Cambridge University Press, Great Britain.

Clerk RB. 1986. Marine Pollution. Clarandan Press, Oxford 120 -131.

Edema MO, Agbon AO. 2010. Significance of fungi associated with smoke-cured *Ethamlosa fimbrata* and *C. gariepinus*. Journal of Food Processing and Preservation **34**, 353 – 363.

Eli AC, Abowei JF. 2011. A review of some fungal infection in African fish *Saprolegniasis,* Dermal mycoses; Branchiomces-infections, systemic mycoses and Dermocystidium. Asian Journal of Medical Sciences **3(5),** 198-205

Fafioye O, Fafioye O. 2013. Microbial identification of smoke-dried fish (*Clarias gariepinus*) from some local markets in Ibadan metropolis. Wudpecker Journal of Agricultural Research **2(11),** 294-298.

Fafioye OO, Efunloye MO, Osho A. 2002. Studies on the fungal infestation of five traditionally smoke-dried freshwater fish in Ago-Iwoye, Nigeria. Mycopathologia **154**, 177-179.

FAO. 1984. Meeting on the toxicity and bioaccumulation of selected substances in marine organisms. FAO Fisheries Report, No 334, Rovinj, Yugoslavia 5-9 Nov.FIR/R334.

FEPA. 1991. Guidelines and Standards for Environmental Pollution Control in Nigeria. Federal Environmental Protection Agency. Abuja. Nigeria Pp54 - 58.

Gurure RM. 1987. Influence of two organochloride pesticides, thiodan and lindance on survival of fingerlings of *O. niloticus* and *T. zilli.* African Regional Aquaculture Centre Working Paper ARAC/WP,6/87.

Harrigan WF, McCance ME. 1990. *Laboratory Methods in Food and Dairy Microbiology*.Academic Press, London.

Ibiebele DD. 1986. Oilfield water treatment utilizing adapted bacteria and aquatic macrophytes. In: Proceedings of international Seminar on Petroleum Industry and the Nigerian Environment, Kaduna Pp. 101 – 107.

Igbal Z, Sheikh U, Mughal R. 2012. Fungal infections in some economically important freshwater fishes. Pakistan Veterinary Journal **32(3)**, 422-426.

Institute of Pollution Studies, IPS. 1990. Ecological Impact Assessment of Ebubu-Ochani, SPDC, Institute of Pollution Studies Rivers State University of Science and Technology, Port Harcourt 236pp.

Jimoh WA, Oladele-Bukola MO, Adebayo MD, Yusuff AA, Azeez FA, Salami OO. 2014. Microbial flora of the gastro-intestinal tract of *Clarias gariepinus* caught from river Dandaru

Ibadan, Nigeria. SokotoJournal of Veterinary Sciences **12(2)**, 19-24.

Obire O, Adigwe PC, Ramesh RP, Okigbo RN. 2008. Effect of oilfield wastewater on microbiota of a freshwater in Nigeria. Journal of Pharmacy Research **1(2**), 264 – 270.

Obire O, Amusan FO. 2003. The Environmental Impact of oilfield formation water on a freshwater stream in Nigeria. Journal of Applied Sciences and Environmental Management **7,** 61 - 66.

Obire O, Wemedo SA. 2002. Seasonal effect on the bacterial and fungal population of an oilfield wastewater-polluted soil in Nigeria. Journal of Applied Sciences and Environmental Management **6**, 17 – 21.

Olayemi AB, Adebayo O, Ojo AO. 1990**.** Microbiological studies on freshwater fishes from Asa river, Iiorin, Nigerian. Journal of Aquacultre in the Tropics **5**, 133-139.

Oni TA, Olaleye VF, Omafuvbe BO. 2013. Preliminary studies on associated bacteria and fungal load of artificially cultured *Clarias gariepinus* (Burchell1822) fingerlings. Ife Journal of Science **15(1)**, 9-16.

Oteri AU. 1985. Groundwater Pollution Monitoring in Environmental Investigation. In: Proceedings of joint (FMWH and NNPC) conference on the Petroleum Industry and the Nigerian Environment, Kaduna, Nigeria pp. 257 – 264.

Sakata T. 1990. Microflora in the digestive tract of fish and shell-fish. In: *Microbiology in poecilotherms (sic) (Lesel R, editor)*. Elsevier Science, Amsterdam pp.171-251.

Sloat S, Ziel C. 1991. The use of indicator organisms to assess Public Water Safety. HACH Technical center for Applied Analytical Chemistry. Colorado U.S.A. pp1-20.

Tsutsui S, Komatsu Y, Sugiura T, Araki K, Nakamura O. 2011. A unique epidermal mucous lectin identified from catfish (*Silamis asotus);* First evidence of intelectin in fish skin slime. Journal of Biochemistry **150(5)**, 501-514.

Vaupotic T, Veranic P, Jenoe P, Plemenitas A. 2008. Mitochondrial mediation of environmental osmolytes discrimination during osmoadaptation in the extremely halotolerant black yeast, *Horiaea werneckii.*Fungal Genetics and Biology **45(6),** 994-1007.

Veil JA, Puder MG, Elcock D, Redwelk RJ (Jr.). 2004. "A white paper describing produced Water from Production of Crude Oil, Natural gas and Coal bed methane"prepared for U.S. department of Energy. National Energy Technology Laboratory. Under Contract W – 31 109-Eng-38.

Wardley-Smith J. 1979. The prevention of oil pollution. Graham and Trotman Ltd. London pp 69 – 75

Wemedo SA, Akani NP, Eke CE. 2009. Fungi in an oilfield wastewater in Nigeria. Asian Journal of Biological Sciences **2**, 54 – 57.

Wemedo SA, Obire O, Akani NP. 2012. Bacterial population of an Oilfield Wastewater in Nigeria. Asian Journal of Biological Sciences **5(10),** 46 – 51.

Wills J. 2000. A survey of offshore oilfield drilling wastes and disposal techniques to reduce the ecological impact of sea dumping: the effect of discharges of produced waters. Ekologicheaya Vahktan Sakhalina (Sakhalina Environmental Watch) 25th May, Sakhalina, London. pp 1 – 5.