

# Effect of Crude Oil Effluent (Produce Water) on Brackish Water Fish and Microbial Growth in Aquarium Environment.

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## ABSTRACT

The toxicity of crude oil effluent (produce water) was tested on *Tilapia guineensis* and mangrove swamp microbial community (bacteria and fungi) in the aquarium environment. One hundred percent mortality occurred in the *Tilapia sp* exposed to 60% concentration of produce water within 24 hours. Mortality ranged between 50% and 80% when the same organism was exposed to 40% concentration for 96 hours. No mortality was observed below 30% concentration during 96 hours. Since total hydrocarbon levels in produce water were low with indicated value of  $\pm 2.19$  part per million (ppm), observed mortalities were attributed to the presence of toxins other than the oil. The bacterial character of the effluent was also confirmed by the marked growth inhibition at 96 hours relative to controls. Inhibition of bacterial growth resulted in competitive advantage to fungi, which show enhancement in growth relative to controls. The effect of produce water on the microbial community clearly showed the extent of ecosystem alteration associated with chronic low-level pollution from oil field produce water. There was no indication that microbial growth affected the toxicity of effluent to fish. In the light of these results, the discharge of crude oil produce water, even if it meets the oil and grease limits, must be considered with caution until comprehensive analysis of effluent characteristics and subsequent treatment has been performed.

(Keywords: crude oil, produce water, toxicity, fish, mangrove, pollution)

## INTRODUCTION

Crude oil production is usually accompanied by large amounts of oily water (produce water) which must be disposed of after separation from oil at the wellhead. Safe handling requires that the water be injected into reservoir or be thoroughly treated to reduce oil to acceptable levels before disposal into the environment. Most of the oil waters arising from oil production are discharged directly or indirectly into the environment after some level of mechanical treatment at API skimmers (Beg et al., 2001 and Helen, 2005). In most instances the water still contains oil in excess of 10 ppm (v/v). In offshore production where extracted oil is stored in underwater containers, the containers are usually filled with water, which is then displaced by the introduction of oil. When the soluble fraction of the crude oil of this water, effluents, and ballast waters are discharged near coastal shore before the taking on of new cargo, it results in contamination of coastal and inshore waters (Kuehn et al., 1995).

Considering the fact that most oil production in the Gulf of Guinea (West African Coast) is concentrated in swamp environments (Otokunefor and Obiakwu, 2005), with usually a high restricted water circulation and water exchange characteristics, discharge of oil water poses a great hazard to this very important ecological ecosystem. Since the composition of crude oil produce water may be as complex as crude oils themselves, and their impact may depend also on the receiving environment, there is need for system specific bioassay oil field produce water.

This will provide proper basis for establishment of effluent discharge limits and also show the need

for the implementation and enforcement of environmental protection legislation.

*Tilapia guineensis* is one of the widely distributed and important fish species in the Nigeria mangrove swamp ecosystem. Its use for bioassay with crude oil effluents will provide a generalized result, which could be applicable in mangrove ecosystems.

Bacteria and fungi also constitute very important members of the swamp ecosystems. They are responsible for mineralization and hence supply nutrients from organic matter back to the environment. They also provide a direct source of protein to detrital feeders. Examination of the influence of crude oil effluents on mangrove swamp microbial community will give an insight into the vulnerability of this rich ecosystem to low level chronic pollution, which has continued unabated on onshore oilfields.

## MATERIALS AND METHODS

**Collection of Organisms:** Juveniles of brackish water *Tilapia guineensis* were collected from mangrove swamp ponds of an oil producing area of South Eastern Nigeria. Fish were collected using lift nets in such a way to avoid damage or stress to the fishes. Selected fish species were immediately transferred to fish-transport containers (100 liter capacity) filled with air-cooled habitat water and fitted with aerators which provided continuous oxygenation. The organisms were transported under this condition to the laboratory for acclimation.

**Acclimation of the Organism:** Enough habitat water was transferred to the aquaria fitted with EHEIM recirculation pumps. For the acclimation of the organism, the fishes were transferred to the aquaria with hand nets. During acclimation the fishes were fed with compounded feeds (e.g. commercial feeds). Acclimation was continued for 4 weeks during which time mortality reduced to zero.

**Produce Water:** Crude oil produce water was collected from Shell Petroleum Development Oil Company Ltd Nigeria, in sealed sterile containers.

### **Physicochemical Analysis of Produce Water:**

**-Solubility** was assessed by observing whether the crude oil produce water was miscible or not with deionized water.

**-Volatility** was determined as the difference of produce water sample before and after exposure in Petri dish under laboratory conditions for a period of 24 hours.

**-Conductivity** was measured directly using conductivity meter (Model WTWLF 90).

**-pH** was measured with a pH meter (Model WTW pH 90).

**-Dissolved oxygen (DO)** was measured with dual DO/Temperature meter (Model Oxyguard Handy MK II).

**-Biochemical Oxygen Demand (BOD<sub>5</sub>)** was determined as difference in DO before and after incubation of sample at 20°C for 5 days.

**-Chemical Oxygen Demand (COD)** was determined by the 4-hours permanganate test.

**-Total Suspended Solid (TSS), Turbidity, Color, and Ammonium-Nitrogen** were determined using 3000 direct reading spectrophotometer.

**-Total Hydrocarbon** was measured spectrophotometrically at 370 nm wavelength using HACH DR 3000 Spectrophotometer.

**-Habitat Water (Dilution Water)** for bioassay was prepared from pure habitat water by filtration through 0.45 µm membrane filter.

### **Physicochemical Analysis of Habitat Water:**

This was performed on habitat water collected from acclimation aquaria after filtration as reported above. Nitrate was determined spectrophotometrically by the diazotization method after cadmium reduction using HACH DR 3000 Spectrophotometer. Phosphate was determined by the phosphomolybdenum method using HACH DR 3000 Spectrophotometer.

**Microbiology Analysis:** Water samples to analyze for bacteria and fungi were collected from the flasks using sterile pipette. Water was analyzed on a daily basis. Any water sample collected was first diluted up to dilution factor 10<sup>-5</sup> (i.e. dilution that will give visible colonies between 30-200 cfu/ml). Then 0.1 ml of the dilution was

plated out in duplicate using the spread plate method. Media used to cultivate organisms were Plate Count Agar and Yeast Extract Agar for the enumeration of bacteria and fungi, respectively. All Plate Count Agar were incubated at 37 °C for 48 hours and Yeast extract Agar plates were incubated at room temperature for 72 hours.

### **Bioassay:**

**-Selection of Test Concentration:** The test concentrations were selected from preliminary range finding test with *Tilapia guinensis*. During the range finding test, 80% test concentration (i.e. 80 ml of produce water + 20 ml of habitat water) produced 100% mortality while 30% test concentration gave 0% mortality within 96 hours. For the experiment, 40% and 60% test concentrations were selected. The control consisted of pure filtered water.

**-Toxicity Test with *Tilapia guinensis*:** Test Concentrations and controls were prepared in duplicate using 1 liter Erlenmeyer flasks. Then 10 juvenile *Tilapia sp* were transferred to each flask which was then subjected to gentle aeration supplied by air pump connected to fritted glass ends through Teflon tubing attached to each set (comprised of 3 assay media) of bioassay containers. Two concentrations of test mixture (produce water) were made. A control was included in the bioassay (the flasks were covered with porous polyfoams to reduce external influences). The following parameters DO, pH, and conductivity were monitored at the beginning and after 24 hours duration.

Fish mortality was monitored hourly during the first 12 hours and then daily over a period of 96 hours. Owing to the presence of suspended solids in produce water and its low DO content, it

was filtered through a 0.45 µm membrane filter under vacuum. This process led to a significant increase in DO concentration of the effluent before being used for preparation of test concentrations.

### **RESULTS**

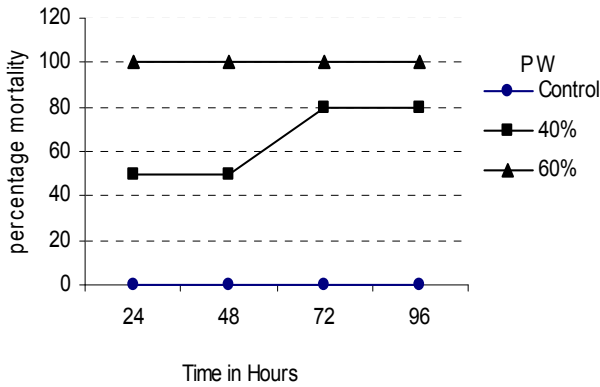
The results of the physicochemical analysis of produce and habitat water are given in Table 1. Table 2 presents the results of DO, pH, and conductivity measurements during the initial 24 hours toxicity test. The results in Figure 1 indicate that 100% mortality was recorded in *Tilapia sp* exposed to 60% test concentration within 24 hours but at 40% test dose, mortality ranged between 50% and 80% during 96 hours.

**Table 1:** Physicochemical Characteristics of Habitats and Crude Oil produce Water used for the Toxicity Test.

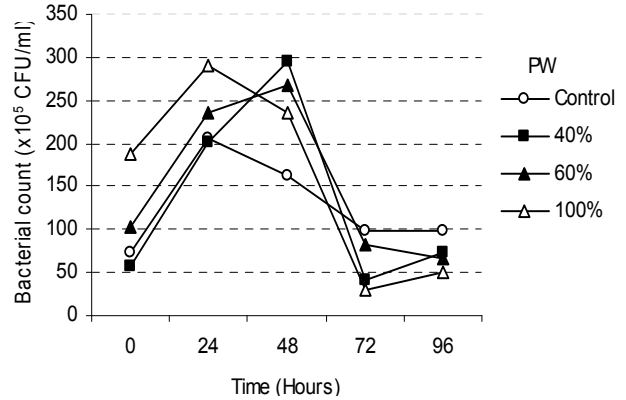
Parameters	Habitat water	Produce water
Solubility (g/hr)	-	Miscible
Volatility	-	0.006
Conductivity (µs/cm)	26.44	16.15
pH	7.43	7.48
DO (mg/l)	5.9	0.8
BOD5 (mg/l)	4.1	0.4
TSS (mg/l)	-	16
COD (mg/l)	-	10.8
Turbidity (Formazin Turbidity Unit)	-	18
Color (Platinum Cobalt Unit)	-	158
Ammonium (mg/l)	9.00	0.76
Nitrate (mg/l)	1.15	-

**Table 2:** Physicochemical Characteristics of Test and Control during the first 12 hours (hr) and 24 hours of Test.

Parameters	Produce water (PW) -Test concentrations						Habitat water (Control)	
	PW 100%		PW 60%		PW 40%		12 hr	24 hr
	12 hr	24 hr	12 hr	24 hr	12 hr	24 hr		
pH	8.05	-	7.9	-	7.79	-	7.43	-
Dissolved Oxygen (mg/l)	6.7	5.6	5.3	5.5	6.0	5	5.9	5.6
Conductivity (µs/cm)	17.10	-	17.85	-	18.53	-	26.44	-



**Figure 1:** Percentage Mortality of Fish.



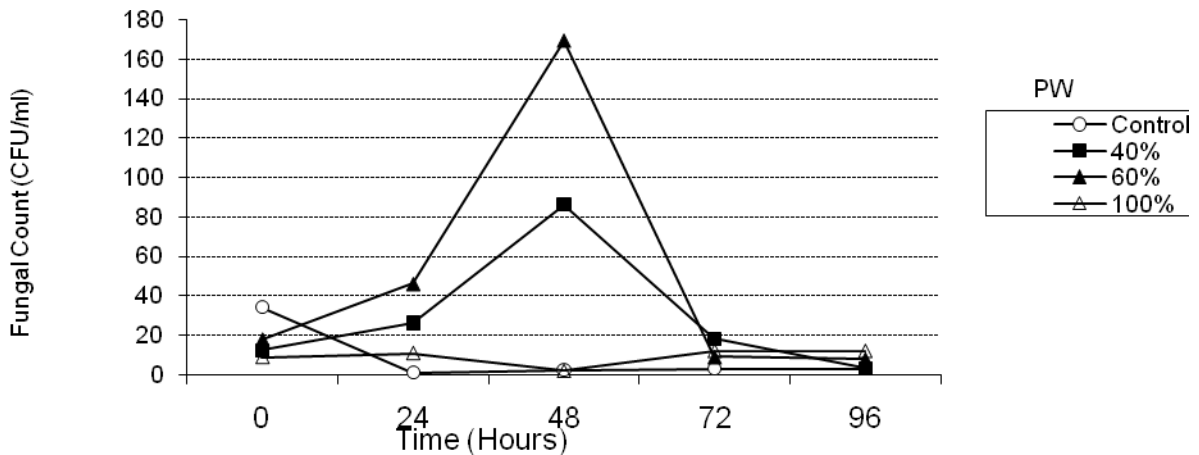
**Figure 2:** Growth Curve of Bacteria Exposed to Different Concentrations of Produce Water (PW).

The results of the microbial count are presented in Figures 2 and 3. At the beginning of the test, bacterial density increased with concentration of effluent while an opposite trend was observed at the end of the 96 hours. Although there was significant growth enhancement relative to control at 48 hours, bacterial growth was markedly inhibited at 96 hours, relative to controls as presented in Figure 3.

Marked growth enhancement was observed at 40% and at 60% test doses at 48 hours relative to control and 100% produce water. In general growth characteristics in test solutions were markedly different from those in controls.

In contrast to bacteria, fungal density in the control was significantly higher than in the test solution at the start of the experiment (Figure 3).

At 96 hours fungal density increased with increase in concentration of produce water. There was no direct relationship between microbial growth and fish mortality during the test.



**Figure 3:** Growth Curves of Fungi Exposed to Different Concentrations of Produce Water (PW).

## DISCUSSION

From the results obtained, it could be seen that fish survived in the polluted water until the 5<sup>th</sup> day at a concentration of 40% whereas all the fish died within 24 hours exposure at a concentration of 60%. No death was recorded in the control. This shows that, at low levels, the pollution damaging or killing effect is less than at chronic levels. Low chronic levels of pollution usually creates much stress on aquatic life. If this is compared to the situation in an open estuary polluted with oil, research has shown that oil travels upstream and downstream after release and still more oils are washed into smaller creeks near the spill site. Oil slicks are spread into small quantities to various points near the spill site. At the end one finds that within few weeks the oil has disappeared, with the reason being that natural dispersal of oil is higher in the open sea than in the stagnant pool (aquarium environment in the laboratory).

A study carried out by Onwumere and Aladimeji (1990) in Nigeria on fish mortality *Oreochromis niloticus* revealed a great reduction at the oiled site than the un-oiled site. It suggests that the spillage had a considerable effect on the macro fauna at the spill site. Although fish have the ability to induce detoxifying enzymes, these enzymes do not confer resistance to acute exposures to oil (Jewel et al., 2002). In fact it is thought that the metabolic intermediates of these enzymatic reactions could be more toxic than the unmetabolized aromatic components (Jewel et al., 2002). More so damages are done much earlier before the enzymes are induced prior to exposure to high doses of water-soluble fractions of the oil.

In our study more than 30% acute lethal concentrations of produce water was used. The observed high mortalities at 40% and 60% test concentrations indicate that severe sub-lethal effects may already occur at much lower concentrations. Looking further, it would be seen that within 24 hours of exposure of fish to 60% concentrations, fatal damage was inflicted on all fish. This shows that immediately after pollution, damage to the animals could occur during the first few hours of exposure. Therefore the fish will not have enough time to induce any enzyme to detoxify pollutants and stay alive.

A study on the toxicity of different Nigerian crude oils on brackish water species (*Tilapia guineensis*,

*Aplocheilichthys spilauचना*) and on fresh water species (*Pariocephalous spp*, and *Serotherodon spp*) revealed that *Tilapia guineensis* was the most affected recording 80% mortality whereas the fresh water species are resistant to damage within 24 hours of test period. This result was attributed to degree of solubility of pollutant observed in both environments.

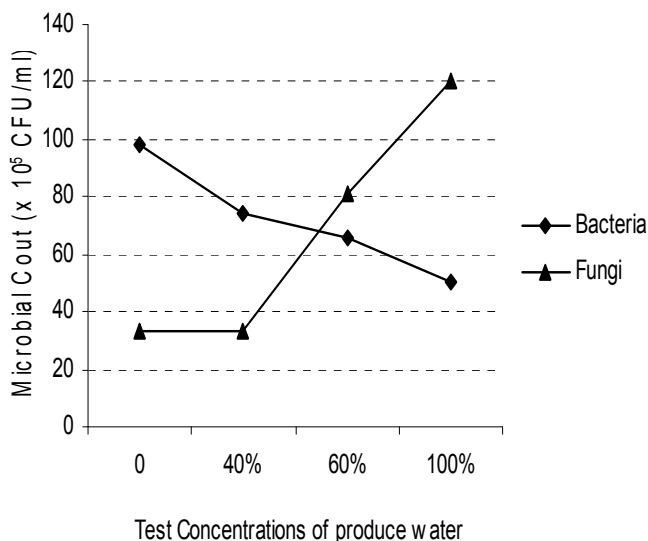
Water soluble fractions of crude oil have higher solubility in brackish water or distilled water than in fresh water, though the fraction of the oil which results to higher solubility is not known. It is however known that several factors influence the solubility of hydrocarbons in sea water including salinity, water temperature. The presence of certain types of dissolved organic compounds can enhance the solubility of hydrocarbons. Poorly soluble hydrocarbons can also associate with these relatively soluble materials and become distributed in sea water in true solution or in finely dispersed micelles (Beg et al., 2003).

Fish mortality in produce water may be attributed to other toxic components (Helen, 2005). The relatively high COD (10.8 mg/l) compared to BOD<sub>5</sub> (0.8 mg/l) of produce water may be an indication as to the presence of bacterial components in the effluent water. Such components may also be directly toxic to fish.

Total hydrocarbon content of produce water was quite low (2.19 ppm) and well below regulatory/standard limits (10 ppm). The high mortality of *T. guineensis* at 60% and 40% test concentration, may thus be attributed to other factors or components other than oil; this observation has been reported by Helen (2005). The influence of microbial growth on mortality of *T. guineensis* is excluded since no direct relationship was found between microbial growth and fish mortality. Significant modifications and alterations were observed in microbial growth characteristics under the influence of produce water (Figures 2-4).

The bactericidal character of the influent is indicated by the marked growth inhibition of bacteria at 96 hours relative to controls (Figure 4). The initial enhancement of growth during the exponential phase may be attributed to dilution of concentration of toxic components and presence of nutrients from habitat water. Similar enhancement of growth in fungal community is attributed to same factors. The increase of fungal growth with increase in contaminant at 96 hours

(Figure 4) may be attributed to reduce competition for substrates and nutrients by bacteria.



**Figure 4:** Microbial Count after 96 hour Exposure to Produce Water (PW).

Studies on the effect of pollution on marine microbial growth shows that both inhibition of sensitive species and enhancement of petroleum degrading species may occur (Helen, 2005).

According to Otokunefor and Obiaku (2005), changes in microbial community structure may become permanent in areas exposed to chronic oil pollution and eventually result in complete alteration of structure of bacterial and fungal populations with serious consequences.

## CONCLUSIONS

In view of the results obtained in our study it could be said that in environments with characteristic poor circulation and exchange of water, lethal concentrations of crude oil produce water may be readily attained at the commonly encountered waste water discharge rates in Nigeria. Crude oil produce water, even where it meets the recommended oil and grease limit, may contain other toxic components as indicated in this study.

The solubility of water soluble fractions of crude oil effluents is higher in brackish water and sea water. Hence the environment is not safe should

any spill occur whether small or chronic because within 24 hours, damage can be done to aquatic life and subsequent defoliation of mangrove trees should it occur near mangrove swamp. Swamps especially mangrove swamps represent important nursery and breeding grounds for a wide range of fish and shellfish species, discharge of such water poses a direct danger to fisheries production. The toxicity to bacteria is an indication of the extent of vulnerability of this important ecosystem to chronic low-level pollution. The contrasting trends exhibited by bacteria and fungi at 96 hours exposure indicate the extent of ecosystem modification and alteration, which could occur under condition of produce water pollution in mangrove ecosystems.

We could recommend that treatment of produce water should be carried out beyond the mechanical separation of oil. Rigorous screening of waste water for all possible toxins must be considered before issuing of discharge permits. In no case should any waste be discharged into the environment without proper toxicity testing involving all possible trophic levels in the receiving environment. The re-injection of effluent into reservoirs should also be considered as an alternative. Hence, extreme care should therefore be taken in the oil industry to avoid oil spillage, since most offshore wells, operations and transport distribution facilities are at sea. It must be noted that at this point good management strategies together with active check on operations reduce the risk of pollution in our waters hence should be imbibed

It is necessary to add that, in the laboratory, experiments are carried out in static conditions in aquaria, and as such, no tides disperse pollutants and the surface area used is quite small, hence data obtained should not be a total and dependent model to the situation outside because the natural environment is a multivariable system and is quite complex. Therefore, caution should be exercised in the extrapolation of such data to a spill situation in the open sea.

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