OCTANOL/WATER PARTITION COEFFICIENT AND BIOACCUMULATION INDEX OF BONNY LIGHT CRUDE OIL IN CAT FISH *Clarias agboyiensis* IN LABORATORY-DOSED SEDIMENTS

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ABSTRACT

Octanol/water partition coefficient and bioaccumulation index of Bonny light crude oil, having a fractional percentage composition of 81.11 saturate, 7.20 aromatics, 2.48 ashphaltene and 9.21 residues, were studied in microcosm aquaria using a fresh water catfish Clarias agboyiensis. The partition coefficient (K_{ow}) of the crude oil was evaluated to be 0.74. The mean bioaccumulation values of the petroleum hydrocarbons (PHCs) in the homogenates of the whole fish, liver and kidney at intervals of 24, 72 and 120 hours were respectively 0.845 ± 0.118, 11.0 ± 0.058 and 15.0 ± 0.064 after exposing the whole fish to sediment and water, respectively containing 31 µg/g and 190 µg/l of the crude oil in the aquarium. The mean bioaccumulation values of petroleum hydrocarbons (PHCs) in the tissue homogenates of the exposed fish were higher than in the control ($p<0.05$), thus suggesting that Bonny light crude oil with K_{ow} of 0.74 could be lipophilic.

Keywords: Crude Oil, Bioaccumulation, Partition Coefficient, *Clarias agboyiensis*

INTRODUCTION

Unwholesome activities by man have created a severe imbalance in our ecosystem. In recent years, public interest in environmental pollution issues has grown that there is extensive coverage in the media. Emphasis on environmental sciences has shifted from direct toxic threat to man towards more general concern regarding pollutants impact on animals and plants, ecosystem and indeed in the whole biosphere (Peakall and Mohtadi, 1980). The society worldwide is increasingly becoming jittery over the safety and quality of the environment in which they live. There is considerable concern on the impact of oil pollution in both the terrestrial and aquatic ecosystems as a result of corrosion/rupture of oil-pipelines sabotage, accidental spills, seepage from storage tanks and tanker wash-off (Inyang, 1996). The fate and effect of crude oil and other petroleum products in the natural ecosystem have been the subject of many investigations. Obviously, the impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration and exploitation activities is a problem of environmental concern (Coon and Dieter, 1981), particularly with regards to the associated heavy metals present in the crude oil. In Nigeria, particularly in the Niger-Delta area, the discharge of crude oil into aquatic environment and its consequent pollution hazard is increasingly becoming a phenomenon of concern (Antai and Mgborno, 1993). Such episodes have created devastating socio-economic problems and health hazard to communities affected, and have been the subject of various litigation between the host community and the oil prospecting companies.

Within the framework of many Environmental Risk Assessment (ERA) procedures for chemicals, the measuring of bioaccumulation is required under certain circumstances. One main criterion for bioaccumulation potential is the n-octanol/water partition coefficient (K_{ow}), which is often used to express hydrophobicity. Chemicals that have K_{ow} values higher than 2 are usually considered liable to bioaccumulate in biota (Oliver and Charlton, 1984). Bioaccumulation studies are used to assess the rate and extent of contaminant accumulation by an organism from various media including air, water, food, soil and sediment. The rate at which a pollutant bioaccumulates in a given lower trophic level is important for assessing the hazard it poses for a higher trophic level of a food chain (USEPA, 2000). Models used to predict bioaccumulation in food webs have been reported (Sample *et al.*, 1998; 1999)

MATERIALS AND METHODS

Test Sample: The test sample for the study was Bonny light crude oil obtained from the Department of Petroleum Resources (DPR), Nigeria National Petroleum Cooperation (NNPC), Port Harcourt.

Experimental Design: *Clarias agboyiensis* caught alive in nets during the rainy season were purchased from fishers at the bank of Anambra River, at Otuocha, Anambra East Local Government Area, Anambra State in the month of May 2002. One hundred (100) healthy *Clarias agboyiensis* of average body weight of 19.33 ± 4.40g and length 15.25 ± 1.5 cm were selected and distributed into five groups with 10 catfish per group. They were allowed to acclimatize in uncontaminated aquaria in the Zoological garden, University of Nigeria, Nsukka for fourteen days prior to their exposure to crude oil-contaminated aquaria. Water in the aquarium was

changed twice weekly during the acclimatization phase. The catfish were also fed twice weekly with feed composed of 30.41% crude protein obtained from Fishery's Unit, Zoology Department, University of Nigeria, Nsukka.

The acclimatized *Clarias agboyiensis* were divided into two categories A and B. Category A numbering 50 were of average body weight 16.45 ± 3.38g and length 14.3 ± 1.6 cm while category B also numbering 50 were of average body weight 22.21 ± 5.41g and length 6.2 ± 1.4 cm. *Clarias* a*gboyiensis* under the two categories A and B were each separated into five treatment groups. All the groups were introduced into the various contaminated aquaria with the exception of the control groups (uncontaminated aquaria).

Preparation of Oil-Contaminated Sediment: The method of Landrum *et al.* (2000) was used in the sediment preparation and contamination. In this method 10.0 g portions of characterized soil were spiked with 0.2, 0.4, 0.6 or 5.0g of Bonny light crude oil. The oil-spiked soil samples were mixed thoroughly after which equal volume of tap water (200 ml) was added to each portion and stirred with rod stirrer. The mixture was further shaken vigorously for 10 minutes before allowed to stand for 15 minutes. At the end of the interval, the water was decanted leaving only the sediment at the bottom of the beaker. A similar treatment was further performed on the above samples but with a standing interval of 30 minutes. The same treatment was repeated, but the duration of standing was 24 hours. The prepared sediment was transferred into plastic bowls and appropriate volume of tap water used to wash off the sediment into the plastic aquaria. More water was added to bring the final volume to 4 litres. All the aquaria were allowed to equilibrate for 4 days before introducing the acclimatized *Clarias agboyiensis*. The aquaria containing the fish were all covered with nets fastened with rubber.

Tissue Collection: Two *Clarias agboyiensis* were randomly collected from each aquarium using a plastic sieve. One of the fish was used for whole tissue assay and the other for liver and kidney assay. The sampled *Clarias agboyiensis* were sacrificed by piercing their heads with knife. The skin was wiped with tissue paper after washing in tap water. This helped to remove extra mucus secretion where oil particles could be loosely attached. The *Clarias agboyiensis* were then cut into small pieces (for whole body tissue assay) using sterile stainless steel scissors that was rinsed three successive times in hot distilled water. The second fish was dissected to expose the viscera, liver and kidney. All the tissues were homogenized and suspended in normal saline.

Determination of Petroleum Hydrocarbons (PHC) in Sediment and Water: The concentrations of petroleum hydrocarbons (PHCs) in the crude oil adsorbed to the spiked sediment were determined, using 1g portion of the sediment. The PHC was extracted with 5 ml of 1:1 mixture of chloroform/ethanol by vigorous shaking for two hours, and allowed to stand for four hours. Extraction was done twice and the optical density (OD) of the extract read at 520 nm against the extraction mixture as blank. The same approach was used to determine the concentrations of PHC that partitioned into the water phase.

Determination of Petroleum Hydrocarbons (PHC) in the Homogenates: In assaying for the total PHCs that bioaccumulated in the liver, kidney and body of the exposed fish, 1g portions of their homogenates, were mixed with 5 ml of the extraction solvent. The mixture was shaken vigorously and allowed to stand for the supernatant to be separated completely. This was repeated twice and the O.D of the supernatant read at 520 nm against the extraction mixture as the blank.

Determination of Octanol/Water Partition Coefficient (k_{ow}) of Bonny Light Crude Oil: The octanol/water partition coefficient (K_{ow}) of Bonny light crude oil was determined as described by Gobals *et al.* (2002) at 28.5^{0}C. In this method equal volume (5 ml) of water (W) and octanol (O) were equilibrated with each other for 4 hours before adding 2 ml of the crude oil (Bonny light). The mixture was shaken vigorously to effect the distribution of the crude oil to the two phases. The set-up was allowed to stand for 24 hours. When an equilibrium condition was achieved, the net volume of the two phases was determined for estimation of the partition coefficient.

Nernst distribution law defines partition coefficient of a substance (X_1) between water and n-octanol phases (K_{ow}) as the ratio of equilibrium concentration in the two phases. Most frequently it is given as the logarithm to the base 10 (log K_{ow}) as shown in Equation (1).

$$K_{ow} = \frac{X_i^0 / V^0}{X_i^w / V^w} = \frac{C_i^0}{C_i^w} \quad (1)$$

Where X_i^0 is molar mass of octanol, V^0 the final volume of octanol, X_i^w the molar mass of water, V^w the final volume of water, C_i^o the molar concentration of octanol and C_i^w the molar concentration of water.

RESULTS AND DISCUSSION

The distribution of 2 ml of crude oil in the aqueous phase of octanol/water is presented in Table 1. Also the estimated concentrations of PHC in the crude oil spiked sediment and water in each aquarium are shown in Table 2. The partition coefficient (K_{ow}) of Bonny light crude oil calculated from Equation (1) was 0.743 and this tends to suggest that the crude oil will most likely not adsorb to particulate organic matter. The octanol/water partition coefficient has been shown to be the measure of a chemical's affinity for lipid portion of an organism's tissue. Since

chemicals with K_{ow} of 2-6 are said to be lipophilic for some water body (Oliver and Charlton, 1984), it shows that Bonny light crude oil is far less lipophilic in the test microcosm aquarium. The speculation that chemicals with K_{ow} values ≥ 2 are lipophilic also suggests that Bonny light crude oil is not lipophilic. But this seems not the case as shown by the results of bioaccumulation of PHCs in the liver (Tables 3) and the kidney (Table 4) of the exposed fish. The data in Tables 3, 4 and that for bioaccumulation in the body tissue (Table 5) reveal that at 72 hours the bioaccumulation values were highest in the kidney, followed by the liver whereas the whole fish body has the least PHC bioaccumulation. The reason adduced for this increase in bioaccumulation levels in the kidney and lever could be due to their involvement in the "clearance" of toxicants in the body of animals which culminates in their elimination via the kidney. Considering the role of the liver in biotransformation of xenobiotics, the reason for high PHC concentration in the former becomes more glaring. The high PHC bioaccumulation in these organs makes them to be prone to hepatic lesion or organ dysfunction (French et al, 1996). Hence both the liver and the kidney work in a concerted manner as regards detoxification and elimination of pollutants.

Table 1: Distribution of 2 ml crude oil in aqueous phase of octanol and water

Compound	Initial volume (ml)	Final volume (ml)	Molar mass of compound
N-octanol	5.0	6.8	130.2
Water	5.0	5.2	18.0
Bonny light crude oil	2.0	-	-
Total volume	12.0	12.0	-

Table 2: Concentration of PHC in each of the spiked sediment and water

Aquarium	Initial crude oil dose (g)	Final values of PHC in spiked sediment µg/g[x]	Final values of dissolved PHC in water µg/l[x]
Control	No oil	-	-
A	0.2	24	180
B	0.4	31	190
C	0.6	33	220
D	5.0	221	900

**These values were considered as the concentration of crude oil directly affecting the fish in the aquarium*

It should be stated that "purging"' of the fish after exposure to the crude oil was not carried out against the U.S. EPA protocol (U.S. EPA, 2000). This is because of the concern that tissue-bond PHC will depurate from the fish during the holding in clean water, thus under-representing the steady state in the organism (U.S. EPA, 2000). The reason for carrying out "purging" is to prevent sediment bond chemicals (PHC) in the gut of the fish from being measured as part of the body tissue burden or concentrations. Hence, Bonny light crude oil having a low K_{ow} value of 0.74 should have a low tendency to bioaccumulate in the catfish, but the result of this study suggests the contrary, in that, the bioaccumulation of PHC in the tissue homogenates of the exposed fish was high relative to the control. It has been observed that uptake rates and bioaccumulation levels of a substance within the body tissues are of particular interest because they can be related to toxicity and body burden, and these endpoint parameters can be used to predict impact (Chapman, 1997).

Fish concentrate (bioaccumulate) lipophilic contaminants mainly by exchange across the gills (depending on the gill ventilation rate of the animal). However, Table 4 illustrates the extent to which *Clarias agboyiensis* could bioaccumulate PHC in the liver. Although dietary uptake of the same contaminant is negligible in fresh water fish because of their natural low feeding rates, poor adsorption efficiency and rapid elimination rates of the contaminants (Niimi and Dookhram, 1989; Randall *et al.*, 1998), the bioaccumulation levels of Bonny light crude oil in the various tissue homogenates of the *Clarias agboyiensis* exposed to the crude oil were significantly high ($P \leq 0.05$) relative to the parallel controls. This suggests that the exposed fish are prone to the toxic injury of the test crude oil sample.

The fact that PHC could bioaccumulate in the various tissues of the fish as shown in the study in spite of the "poor" lipophilicity of the crude oil sends a warning signal to the higher trophic levels that may depend on fish for food, especially in areas prone to crude oil spillage, considering the inherent toxicity of crude oil and the fact that its body burden builds up with time. Consequently, incessant consumption of "sea foods" exposed to crude oil pollution could lead to disease conditions caused by the carcinogenic, mutagenic or even teratogenic properties of crude oil and its derivatives. These disease conditions may lead to death as terminal results.

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Table 3: Bioaccumulation of petroleum hydrocarbon in fish liver homogenate after 24 hourly interval of exposure in crude oil- contaminated aquaria

Treatment group	Crude oil concentration		Concentration of PHC in homogenates (mg/g)		
	Sediment (μg/g)	Water (μg/l)	T_1 = 24 hrs	T_2 = 72 hrs	T_3 = 120 hrs
Control	No oil	No oil	0.00	0.00	0.00
A	24	180	9.0 ± 0.119**	10.0 ± 0.190**	9.0 ± 0.099**
B	31	190	9.0 ± 0.091**	11.0 ± 0.058**	8.0 ± 0.058**
C	33	220	9.0 ± 0.110**	14.0 ± 0.100**	7.0 ± 0.101**
D	221	900	8.0 ± 120**	11.0 ± 0.020**	ND*

*** Results are significantly different (P ≤ 0.05) from each other; thus showing effect of concentration and time of exposure.* Not determined because the fish died before this time*

Table 4: Bioaccumulation of petroleum hydrocarbon in fish kidney homogenate after 24 hourly interval of exposure in crude oil- contaminated aquaria

Treatment group	Crude oil concentration		Concentration of PHC in homogenates (mg/g)		
	Sediment μg/g	Water μg/L	T_1 = 24 hrs	T_2 = 72 hrs	T_3 = 120 hrs
Control	No oil	No oil	0.00	0.00	0.00
A	24	180	10.0 ± 0.068**	20.0 ± 0.071	14.0 ± 0.081**
B	31	190	7.0 ± 0.040**	15.0 ± 0.064**	19.0 ± 0.046**
C	33	220	9.0 ± 0.085**	39.0 ± 0.122	17.0 ± 0.091**
D	221	900	10.0 ± 0.113**	29.0 ± 0.122**	ND*

*** Results are significantly different (P ≤ 0.05) from each other; thus showing effect of concentration and time of exposure. * Not determined because the fish died before this time*

Table 5: Bioaccumulation of petroleum hydrocarbon in whole fish homogenate after 24 hourly interval of exposure in crude oil – contaminated aquaria

Treatment group	Crude oil concentrations		Concentration of PHC in homogenates (mg/g)		
	Sediment μg/g	Water (μg/l)	T_1 = 24 hr	T_2 = 72 hr	T_3 =120 hr
Control	No-oil	No-oil	0.00	0.00	0.00
A	24	180	0.2160 ± 0.106**	0.9193 ± 0.023**	0.8173 ± 0.050**
B	31	190	0.3630 ± 0.046**	0.8453 ± 0.118^{xx}	0.9897 ± 0.100**
C	33	220	0.8949 ± 0.076**	1.0670 ± 0.113**	1.0900 ± 0.100**
D	221	900	1.0787 ± 0.110**	1.1250 ± 0.044**	ND*

*** Results are significantly different (P≤ 0.05) from each other; thus showing effect of concentration and time of exposure. *Not determined because the fish died before this time*

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