Scientia Africana, Vol. 15 (No. 1), June 2016. Pp 117-125
 ISSN 1118 – 1931

BIOCONCENTRATION OF WATER SOLUBLE FRACTION (WSF) OF CRUDE OIL IN *Oreochromis niloticus*.

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Received: 15-05-16
Accepted:18-06-16

ABSTRACT

Bioconcentration of water soluble fraction of Australian crude oil in 50 fingerlings of Oreochromis niloticus was conducted under laboratory conditions for 28 days. An initial acute toxicity test was carried out using different concentrations (25ml/L, 50ml/L, 75ml/L, 100ml/L and a control) of the water soluble fraction (WSF) of crude oil. The LC_{50} was 28.18 ml/L and LT_{50} were 35.5 hours, 63.1 hours, 79.4 hours and 100 hours for 100ml/L, 75ml/L, 50ml/L and 25ml/L respectively. From the LC_{50}, two concentrations (4.4ml/L and 2.2ml/L) were used to determine the bioconcentration on day 4, 7, 14, 21, and 28. The concentration of hydrocarbon in the fish tissue and test solution increased with increase in exposure time and subsequent increase in bioconcentration factor (BCF).Significant variation existed between the two concentrations of WSF of the crude oil ($F= 6.26 \geq P=0.037_{0.05}$). This implies that WSF of crude oil bioconcentrates in the tissue of aquatic organisms and may have detrimental health implications for fish consumers. Therefore, appropriate measures should be adopted for efficient effluent treatment technology and avoidance of oil spillage into the aquatic environment in the Niger Delta.

Key words: Bioconcentration, Water Soluble Fraction (WSF) of Crude Oil, LC_{50}, LT_{50}

INTRODUCTION

The bioconcentration study of water soluble fraction of Australian crude oil in *Oreochromis niloticus*, was conducted because oil, the corner stone of Nigeria's economy, has caused contamination of the environment during exploration and production (Jaja *et al*., 2015). Hydrocarbons contained in crude oil get into humans through ingestion of contaminated food and water. Other sources include biomagnification through food chain, occupational exposure or by use of hydrocarbon products. In addition, many people in some communities in Nigeria ingest crude oil directly as remedy for various conditions such as snake poisoning, convulsion, treatment of skin infection gastrointestinal disturbances and arthritis (Adesanya *et al*. 2009; Eyong *et al*. 2004). Crude oil is absorbed by living organisms through all routes of contact such as dermal, oral or respiratory tracts.

Bioconcentration refers to the absorption or uptake of a chemical from the media to concentrations in the organism's tissues that are greater than in surrounding environment (Olaifa, 2012). The degree to which a contaminant will concentrate in an organism is expressed as the bioconcentration factor (BCF), which is defined as the

concentration of a chemical in an organism's tissues divided by the exposure concentration (USEPA, 2010) and usually calculated at the steady state.

Water soluble fraction (WSF) of crude oil is described as the fraction that dissolves in the aquatic environment. This fraction comprises of toxic components such as the polycyclic aromatic hydrocarbons (PAHs), mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene and xylene (BTEX), phenols and heterocyclic compounds (Rodrigues *et al.*2010). Impurities present in crude oil include phenolic acid, napthelic acid and small quantities of most known elements except: sulphur, nitrogen, nickel, molybdenum which are present in relatively large quantities (Mason, 1966). The concentrations of WSF of hydrocarbon range from 0.008mg/l to 38.3 mg/l in Australian crude oils (Neff *et al.* 2000). The water soluble fraction (WSF) of crude oil also contains some cations (Na^+, Ca^{2+},Mg^{2+}, Fe^{2+}, Fe^{3+} and K^+); anions (Cl^-,SO_4^{2-},NO_3^- , PO_4^{2-} and HCO_3^-) and heavy metals such as lead, copper, zinc, cadmium, nickel, chromium and vanadium (Rana, 2005; Edema, 2006).

Hydrocarbons present in crude oil are toxic to many organisms especially those at early life stages because they alter the embryonic development of fish, induce ethoxyresorufin-O-deethylase (EROD) activities in fish (Lee *et al.,* 2011). Many organisms can accumulate hydrocarbons in their bodies (Di Toro *et al.*2001, Azad, 2005, Nwabueze and Agbogidi, 2010, Rodrigues *et al*. 2010). Polycyclic aromatic hydrocarbons are toxic, persistent and carcinogenic (Bamforth and Singleton, 2005; Feijoo-Siota *et al.*, 2008).

There is incessant oil spills in oil producing region in Nigeria and the sudden input of large amounts of petroleum hydrocarbons associated with the oil spills imposes abnormal impacts on the environment unlike the naturally occurring hydrocarbons (Nwabueze and Agbogidi, 2010).

This study is aimed at investigating the bioconcentration and toxicity of water soluble fraction of crude oil in *O. niloticus*. This fish constitute an important link in the food chain of most aquatic ecosystem in the Niger Delta.

MATERIALS AND METHODS

Experimental Organisms

A total of 150 fingerlings of *Oreochromis niloticus* were collected from the African Regional Aquaculture Centre (ARAC), Aluu, in Rivers State. The fish was transported to the laboratory using plastic buckets with aeration at the early hours of the day. In the laboratory the fish length and weight were taken immediately. The weight of fingerlings was 3.5 ± 0.5 g and the length ranged from 6.0± 3.5 cm. The fishes were acclimatized for 7 days in plastic tanks containing dechlorinated tap water. The fishes were fed twice daily and water changed daily, during the acclimatization. The fishes were fed with locally produced feed purchased from African Regional Aquaculture Centre Aluu (30% protein) and feeding was stopped a day prior to exposure to the toxicant so as to reduce the amount of fecal product during the experiment.

Test Material

The crude oil used was obtained from the Port Harcourt Refining Company – a subsidiary of NNPC at Alesa, Eleme, in an air-tight 10 liters plastic container, and stored in a refrigerator in the laboratory.

Scientia Africana, Vol. 15 (No. 1), June 2016. Pp 117-125
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Water soluble fraction (WSF) of the crude oil was prepared in a ratio of one part of crude oil to 9 parts of distilled water (Anderson *et al*., 1974). The mixture was put in a conical flask (pyrex) with the mouth of the flask sealed so as to ensure that the volatile hydrocarbons do not evaporate. The flask was then placed on a magnetic stirrer which was shaken at 300r/min for 24 hours. The mixture was allowed to stand for 3 hours in order to enhance a clear interphase between the oil and the water (Reish and Oshida, 1987). The mixture was then separated using a separating funnel to ensure the extraction of a pure water fraction and the WSF. The mixture was stored in a refrigerator for further use.

Determination of lethal concentration

A total of 15 plastic aquaria were used for the experiment. Ten healthy organisms were randomly selected and placed in five concentrations 25 ml/L, 50ml/L, 75 ml/L, 100 ml/L and 0 ml/L (control) of the test solution in 3 replicates. The fingerlings were confirmed dead when they no longer responded to prodding or became motionless and showed no movement with its tail. Dead fishes were counted and removed immediately from the water.

The number of dead fishes per treatment was recorded against the time of their death and data collected were used to plot the percentage of death against concentration and time. The test assessed the lethal effect of water soluble fraction (WSF) of bonny light crude oil on *Oreochromis niloticus* at 96 hours. The median lethal concentration (LC_{50}) of WSF is the concentration required to kill half (50% mortality) of the members of the test organisms within 4 days (96 hours) and the median lethal time (LT_{50}) is the time taken to obtain 50% death or survival of the population of the test organisms. The mortality was analyzed using probit method (Finney, 1971).

Determination of the Bioconcentration

This was done in accordance to American Society for Testing Materials (ASTM) No. E 1022- 84. A total of 50 fingerlings of *Oreochromis niloticus* were used for the experiment. The $1/6^{th}$and $1/12^{th}$of the LC_{50} were used as the concentrations for the experiment which lasted for 28 days.

Fifteen fingerlings were randomly distributed into the two concentrations (4.4 and 2.2 ml/L and a control). The fish were fed twice daily. The organisms and test solution were collected and analyzed for hydrocarbon. Three fingerlings were sacrificed and water collected for analysis on $4^{th,}$ 7^{th}, 14^{th}, 21^{st} and 28^{th} day. The test solution was renewed after the collection.

Collection for the control was done twice, at the beginning and at the end of the experiment i.e. the 4^{th} and 28^{th} day. Acid digestion was carried out on the water sample and fingerlings respectively and the extract was analyzed using the Atomic Absorption Spectrophotometer (AAS). The pH, temperature and dissolved oxygen were monitored regularly as recommended by APHA (1998). Results were tested for significant differences using one way ANOVA at 0.05 levels. All statistics were done with the aid of Microsoft Excel.

RESULTS

Lethal concentration

The pH and dissolved oxygen content decreased on introduction of the toxicant (WSF) in each treatment, while temperature varied between 27^0C – 30^0C (Table 1).

Table 1. Mean andStandard Deviation (S.D) of the physiochemical parameters measured.

Treatment of (WSF) in ml/L	pH ± S.D	Dissolved oxygen (mg/L) ± S.D	Temperature (^{O}C) ± S.D
Control (0)	8.00± 0.7a	7.2± 0.07a	30.2±0.03a
25	7.00± 1.2ab	6.7± 0.8a	27 ±1.0a
50	6.00±1.6bc	6.3± 0.14a	28.9±0.19a
75	5.00±2.9b	5.0 ±3.1ab	28±1.8a
100	5.50±1.3bc	4.0±2.3ab	30±0.14a
FEPA (1991)	6.0-9.0	6.0-8.0	27-38

NB: Values in each column with the same superscript are not significantly different at P >0.05

The results of mortality of *Oreochromis niloticus* fingerlings exposed to water contaminated with WSF showed that no mortality was recorded in the control; but mortality increased with increased in concentration and time of exposure (Table 2). The 96hrs LC_{50} was at 28.18ml/L concentration, and the LT_{50} were 100, 79.43, 63.10 and 35.48 hrs respectively for 25ml/L, 50ml/L, 70ml/L and 100ml/L as shown in figs 1 and 2. There were significant variations in the means of the treatments at $P \leq 0.05$,

Table 2: Probit values for the 96th hrs

Concentration of (WSF) in ml/L	Log of concentration	Probit value of % mortality
Control	0.00	0.00
25	1.40	5.00
50	1.70	5.25
75	1.90	5.52
100	2.00	8.14

Table 3: LT_{50} of values

Concentration of (WSF) in ml/L	Percentage mortality in Probit values	Log of Time
100ml/L	5.00	1.38 (24hrs)
	5.25	1. 68 (48hrs)
	5.52	1.86 (72 hrs)
	8.14	1.98 (96hrs)
75ml/L	4.48	1.38(24hrs)
	5.00	1.68 (48hrs)
	5.00	1.86 (72hrs)
	5.52	1.98 (96 hrs)
50ml/L	3.72	1.38 (24hrs)
	4.16	1.68 (48hrs)
	4.48	1.86(72hrs)
	4.48	1.98 (96hrs)
25ml/L	3.72	1.38(24hrs)
	4.16	1.68 (48hrs)
	4.16	1.86(72hrs)
	4.16	1.98 (96 hrs)

Scientia Africana, Vol. 15 (No. 1), June 2016. Pp 117-125
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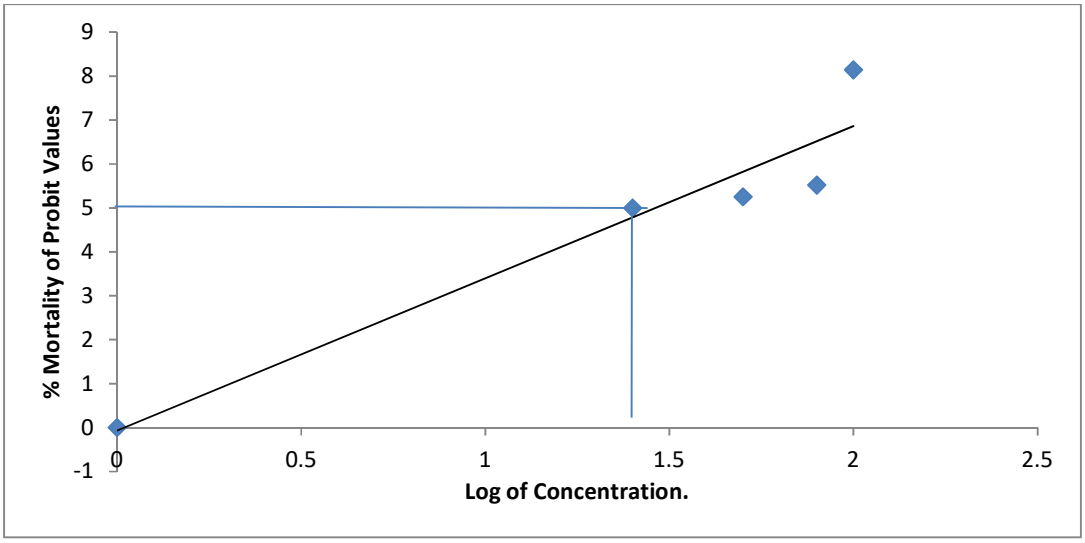

Fig 1: Probit plot of mortality against log of concentration
LC_{50} = Antilog of 1.45 = 28.18ml/L

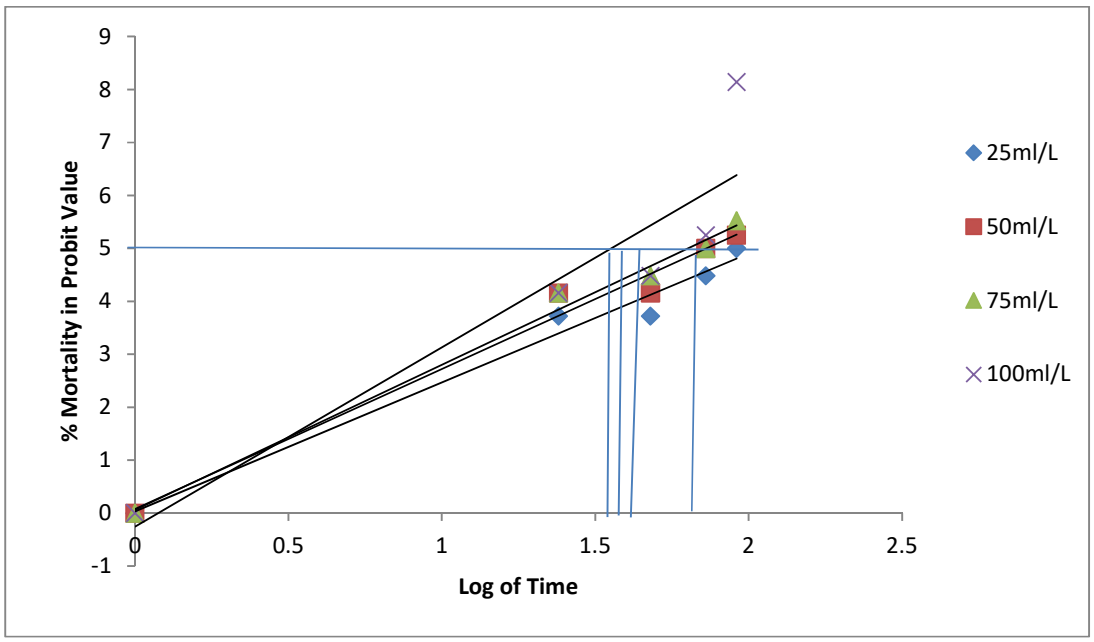

Fig. 2: Probit plot of mortality against log of time.

Bioconcentration of the Samples

The hydrocarbon concentrations in the fish tissue and water sample showed a continuous increase in concentration. The BCF was lowest at the 4th day and highest at the 28th day. The control showed little hydrocarbon concentration in the fish tissue and water sample and had a BCF of 1.0.

Table 4: Hydrocarbon content for the sublethal concentrations (2.2ml/l and 4.4ml/L)

Days	Conc. in Fish Tissue		Conc. in Water		BCF		Conc. in fish	Conc. in water	BCF
	2.2ml/L	4.4ml/L	2.2ml/L	4.4ml/L	2.2ml/L	4.4ml/L	Control	Control	
4th	$2.8x10^3$	4.34×10^3	$3.20X10^3$	$5.00x10^3$	0.88	0.87	1.50×10^3	1.52×10^3	1.0
7th	$3.00x10^3$	$4.60x10^3$	$3.30X10^3$	$5.10x10^3$	0.91	0.90			
14th	$3.15x10^3$	5.00×10^3	$3.40X10^3$	5.12×10^3	0.93	0.98			
21st	$3.50x10^3$	$5.20x10^3$	$3.50X10^3$	$5.20x10^3$	1.00	1.00			
28th	$3.70x10^3$	$5.30x10^3$	$3.65x10^3$	5.25×10^3	1.04	1.01	1.80×10^3	1.81×10^3	1.0

DISCUSSION

A 96-hour bioassay was carried out using the WSF of a Nigerian Light crude oil. The observations of the physicochemical characteristics of the water were similar to earlier studies (Nwabueze and Agbogidi, 2010, Rodrigues *et al*, 2010) who reported decreased dissolved oxygen content with increasing hydrocarbon content of water. All the recorded dissolved oxygen concentrations (4 – 6.7mg/L) and temperature (27 – 30°C) were however adequate to support fish and within range for tropical fish.

In the present study, percentage mortalities were concentration-dependent. Similar results were presented by Ogundiran *et al.* (2010) when investigating toxicological impacts of detergent effluent in fingerlings of African catfish *Clarias gariepinus*: Calta *et al.* (2004) when studying acute toxicity of the synthetic pryrethroid deltamethrinon young minnow cap, *Cyprinus carpio*, and Ubong *et al*., (2015) in their study of toxic effect of Crude Oil on Hatchery Reared *Oreochromis niloticus* Fingerlings. The 96 h LC_{50} is known to vary for toxicants (Ayotunde *et al.* 2010) and from one concentration to another (Cagauan *et al.*, 2004). The 96 h LC_{50} was 28.18 mg/L which is comparable to 20ml/L recorded by Ubong *et al*. (2015).

The concentration of the WSF of crude oil (chemical) moved at first into the fish more rapidly than it was stored, degraded and depurated. With constant exposure, its concentration inside the organisms gradually increased until the concentration of the chemical inside the organism reached an equilibrium or a steady state with the concentration outside the organism (in water), this was observed at the 21st day, and the amount inside the organisms remains the same as the amount leaving the organisms. Similarly, the rate of elimination and depuration did not affect the results gotten due to the exposure of the fish in clean water which lasted for only 24 hours. This confirms the report of Lelei and Sikoki (2014). Also Bioaccumulation varies between individual organisms as well as between species. Thus it can be concluded that the bioconcentration of crude oil has a serious consequences on aquatic life.

Water soluble fraction of crude oil is toxic and has adverse effect on *Oreochromis niloticus* at low concentrations. In view of the toxicity effect, the recommended level of this toxicant in aquatic environment should not exceed 10% of their 96 h LC_{50}. As a result of high percent mortalities of *Oreochromis niloticus* when exposed to the water soluble fraction of crude oil, appropriate measures should be adopted for efficient effluent treatment technology and avoidance of oil spillage into the aquatic environment in the Niger Delta.

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