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Bioaccumulation of Heavy Metals and Hydrocarbons in *Hemichromis Fasciatus* Exposed to Surface Water in Borrow Pits Located Within Onshore Oil Exploration and Production Area

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Abstract

A field bioaccumulation study was carried out. Juvenile *hemichromis fasciatus* in net cages were exposed to contaminated surface water in borrow pits located within oil exploration and production (E & P) installations within the Niger Delta region of Nigeria during wet and dry seasons.

Percentage mortality ranged between 2% and 3.8%. Assuming a first order kinetics and steady state at day 14; toxicokinetic variables were obtained for xenobiotics assessed. The up-take rate constant *k*, ranged from 38.47 $\times 10^{-2}$ d^{-1} (pit B, wet season) to 40.82 $\times 10^{-2}$ d^{-1} (pit A, dry season) for TPH, while PAHs ranged between 22.40 $\times 10^{-2}$ d^{-1} (pit B, wet season) and 24.98 $\times 10^{-2}$ d^{-1} (pit A, dry season) for both pits and seasons.

Amongst the water borne metals *k* for *hemichromis fasciatus* ranged from 4.66×10^{-2} d^{-1} to 47.66 $\times 10^{-2}$ d^{-1} and 2.70 $\times 10^{-2}$ d^{-1} to 33.90×10^{-2} d^{-1} in pits A and B respectively during both seasons. The order of uptake was Fe <Pb< Cr < Ba < Cd < Zn < Cu < Ni, while As (arsenic) recorded a zero uptake.

Calculated BCF in both pits and seasons ranged from 2.832 LKg^{-1} to 4.844 LKg^{-1} for TPH and 2.636 LKg^{-1} to 8.0 LKg^{-1} for PAH, while for metals - Ba, Cd, Cr, Cu, Fe , Ni, Pb and Zn, values ranged between 0.211 LKg^{-1} to 71.727 LKg^{-1}. *Hemichromis fasciatus* exhibited greater uptake of analytes in the dry season and the amount of heavy metals accumulated were all below the provisional maximum tolerable daily intake (PMTDI).

Keywords: bioaccumulation, *hemischromis fasciatus,* hydrocarbons, heavy metals, Toxicokinetics

1. Introduction

Studies on the impact of heavy metals and petroleum hydrocarbon in aquatic ecosystems comprising of rivers, streams, lakes, aquatic organisms (fish, shrimp, mollusc etc.) and sediments have become a major global environmental issues. In nature, aquatic organisms are constantly exposed to pollutants such as metals and hydrocarbons due to natural geochemical processes (e.g., weathering of rocks, and leaching) and anthropogenic activities resulting from increase in urbanization, industrialization, agricultural practices, oil exploration and production (E&P) activities as characterized in the Niger Delta region of Nigeria (Ajayi & Osibanjo, 1981; Biney *et al.,*1994; Alloway & Ayres, 1997; Noegrohati, 2006; Godwin *et al*., 2011;). Often such increases in anthropogenic activities, usually leads to accelerated releases of chemical pollutants into the aquatic environment which poses serious and severe threat to aquatic life and man due to their proved toxicity, persistence, bioaccumulation and biomagnification in the food chain (Tulas *et al.,* 1989; Papagiannis *et al.,* 2004; Martinez-Lopez *et al*., 2005).

Heavy metals (e.g., Fe, Ni, Cd, Cu, Cr, Ba, etc) and many organics like hydrocarbons and pesticides have been reported in various concentrations in many rivers and creeks in the Niger Delta region of Nigeria (Anyakora *et al*., 2005; Olowoyo *et al*., 2010; Godwin *et al*., 2011; Nwabueze, 2011) and these pollutants have a tendency to accumulate in biota (Landrum *et al.,* 2003; Anyakora & Coker, 2007) and undergo food chain magnification (James *et al.,*1998). It is therefore imperative to monitor the concentration of these pollutants in the environment

and to analyze bioaccumulation process in order to assess the possible impact on human health and risk which man faces in such an environment (Kotze *et al*., 1999).

The use of biological accumulator species in monitoring and assessing the level of contaminants and pollution of our aquatic environment is a major thrust towards knowing the degree to which the various components of our aquatic ecosystem is impacted. Accumulator species such as mollusc and some bentho-pelagic organisms are sedimentary dwellers and have capacity to bioaccumulate relatively large amounts of certain pollutants, even from much diluted solutions without obvious noxious effects. The bioaccumulation of pollutants in organisms is the result of previous uptake from its environment in the past as well as the recent pollution level of the environment in which the organism lives, while the pollutant concentrations in the water only indicate the situation at the time of sampling (Karadede *et al*., 2004). Chemical pollutants are known to have adverse effects on aquatic environments. A negligible increase in the concentration of chemical pollutants could lead to a drastic effect on the aquatic life. Also, chemicals, which would have been harmless on their own, may become toxic by interacting in the general milieu of contaminated water.

Bioaccumulation starts with the uptake of chemical pollutants across biological membrane and could be investigated via laboratory and field study. Many laboratories bioaccumulation studies have been reported by researchers (Wang & Rainbow, 2006; Noegrohati, 2006; Martin *et al*., 2007; Kamunde, 2009). However, field bioaccumulative studies, gives a real situation approach, whereby aquatic organisms are exposed to retinue of inorganic and organic compounds that interplays within the natural environment. Oikari, 2006; Crane *et al.*,2007 reported that *in-situ* caging of fish to determine the effects of exposure to contaminants at impacted sites has many advantages over traditional in-lab testing. In a field study using net cages, Goksoyr *et al.,* 1996, reported the accumulation of PAHs in juvenile Flounder (*Platichthysflesus* L.) and Atlantic cod (*Gadusmorhua* L.). The use of caging methodology seems to be a promising way to approach eco-toxicologically relevant problems, such as bioavailability of contaminants, biomarker responses in the field, and dose-response relationships, also under mixed contaminant situations.

The environment is continuously loaded with foreign organic chemicals (xenobiotics) and inorganic compounds released by urban communities and industries (R. van der Oost *et al.,* 2003). The aquatic ecosystem is therefore continuously and seriously threatened by these substances - as it is the ultimate sink for these contaminants by either due to direct discharges or to hydrologic and atmospheric processes (Stegeman & Hahn, 1994).

More so, these effects become more pronounced in non-flowing, receiving water bodies such as lakes, borrow-pits and discharge pits. Some of these man-made pits or lakes are totally submerge during the rainy season especially when rivers around these pits over-flow their banks to cover such pits. Aquatic organisms (like fish) tend to migrate in and out of such receiving pits. In some cases communities have also fished in such pits. Some of the chemical pollutants that could have lasting effects on the natural pits are nutrients, oil and grease, refractory chemical species such as heavy metals, PAHs, etc.

In the Niger Delta region of Nigeria, where onshore operations involving petroleum exploration and production (E & P) activities are carried out within communities such occurrences are prevalence. Physical and chemical characterisations of impacted environmental components in the Niger Delta environment (and indeed in the African continent) are in most cases on air, soil, sediment and surface water, leaving out the biota. To ascertain the chemical characteristics of these environmental components (surface water, sediments, soil, biota and atmosphere) interacting in any ecosystem, the levels of pollution indicators should be evaluated. The chemical characteristics are analysed to indicate the concentration of pollution indicator parameter, selected for evaluation.

There is no literature on field bioaccumulation studies within the Niger Delta region of Nigeria, considering the effect of the E & P operations on the environment. We have adopted the caging method to assess the levels of contamination of the numerous borrow pits dug around this region as a result of various infrastructural development that are on-going - this includes road construction, petroleum exploration and development operations, housing projects, etc. The aim of the study is the use of bio-indicators in assessing the level of contamination in this area and also the use of toxicokinetic variables obtained from both physico-chemical and biological properties of recipient borrow-pits and test organism – *hemichromis fasciatus* respectively, to characterize the effect and risk of onshore exploration and production (E&P) operational activities on communities situated around the Niger Delta region of Nigeria

2. Material and Methods

2.1 Reagents and Materials

Extraction solvents - dichloromethane (DCM), n-hexane, acetone, petroleum spirit and acetonitrile were all of

analytical grade and manufactured by Sigma-Aldrich (St Louis, USA) and Merck (Darmstadt, Germany). Sodium sulphate (anhydrous) and silica gel 60 extra-pure (60 – 120 mesh) for column chromatography were from BDH limited (Poole, England). Concentrated nitric acid used was of analytical grade, manufactured by Merck KGa A of Germany and BDH Limited Poole England. Distilled water used was double distilled (DD). USEPA 16 priority PAHs mixed standard (2000 µg/mL) comprising: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), Pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DaA), benzo[g,h,i]perylene (BgP) and indeno[123-cd]pyrene (IP) in dichloromethane: benzene was obtained from SUPELCO, Bellefonte, PA, USA

2.2 Instrumentation and Measurements

2.2.1 Atomic Absorption Spectrophotometer (AAS)

Heavy metal measurements were carried out using a Varian Atomic Absorption Spectrophotometer (AAS), model SpectraAA 600 with flame system inter-phased to a computer and printer.

2.2.2 Gas Chromatography – FID

The hexane reconstituted clean up extract was analysed with a Shimadzu QP – 2010 Gas Chromatography-Flame Ionization Detector (GC-FID). Chromatographic separation was carried out on a 30 m x 0.25 mm id SLB-5MS capillary column with a film thickness of 0.25 µm. The oven temperature was programmed, which was initially held at 70^{o}C for 0.2min, and was increased to 265^{o}C at a rate of 25^{o}C/min, held for 1 min and then raised to 315^{o}C at a rate of 5^{o}C /min and held for 2 min. The flow rate of the carrier gas (helium, 99.99% purity) was kept constant at 1.3 mL/min. Pulsed splitless injection mode at an injection temperature of 250^{o}C was carried out at a pressure of 30psi. Injection volume was 2µl.

All instruments were calibrated before use.

2.3 Study Areas and Experimental Procedure

2.3.1 Study Area

The study area - Ogba –Egbema Local Government Area of Rivers, lies within latitude 5^0 13'N and 5^0 22'N and longitude 6^0 33'E and 6^0 42' North West of the Niger Delta region of Nigeria (Figure 1). The main population is mostly rural communities. There are numbers of oil exploration and production (E & P) facilities and activities located around this area belonging to three major players (multinationals) in the up-stream sector of the oil industry (Figure 2). Some of the installations include oil field, gas plants, flow stations etc. Also the area is one of the prominent and highest oil/gas onshore producing areas of the Niger Delta with over 900 oil wells and about thirteen active oil fields - playing a host to three multinational oil companies. The area is criss-cross with network of pipelines carrying either oil or gas to the flow stations from the different oil wells (Avwiri & Ononugbo, 2010).

Two distinct seasons are recognized namely; the rainy and dry seasons. The vegetation is typically tropical rain-forest with fresh water swamps.

Activities of the communities inhabiting the area consist mainly of crop farming and fishing. One of the predominant fish in the study area is the genus *hemichromis (*or *Hemichromis sp.).*

Field experiment was carried out between March and April 2012 for dry season and August 2012 for wet season.

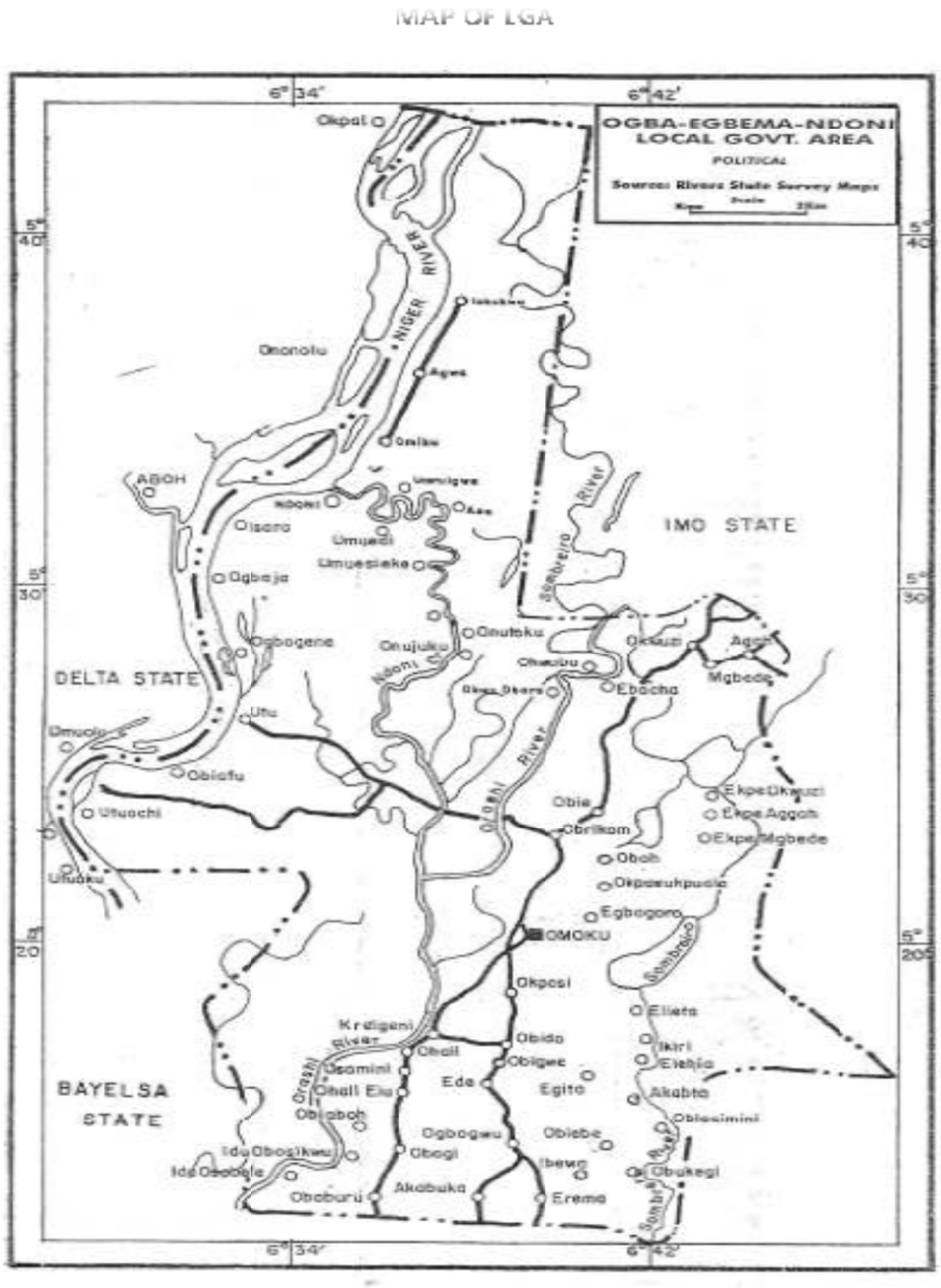

Figure 1. Map of the study area in Egba- Egbama – Ndoni Local Government Area of Rivers State

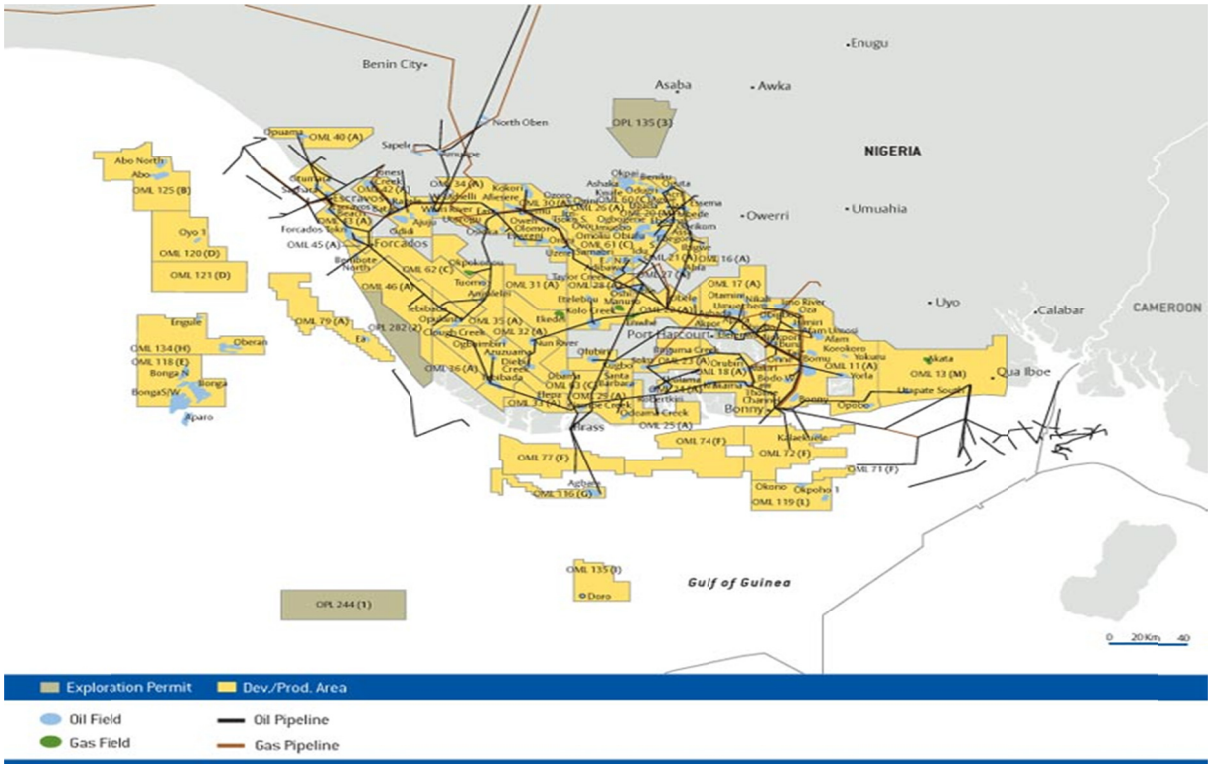

Figure 2. Map showing some onshore active oil fields around the Niger Delta Region of Nigeria

2.4 Bioaccumulation Test Procedure

The EPA (2008) and DPR (2002) bioaccumulation test procedures were adopted for *in-situ* (field) test methodology for toxic metals and hydrocarbons for biological monitoring.

Bioaccumulation test was conducted on test organism captured from a pristine environment.

Prior to experimental set up, baseline analysis of toxic metal and hydrocarbon contents in test organisms were determined to ascertain their level of 'cleanliness'.

All organisms were collected from the same pristine environment and were uniform in size (i.e., < 10 g) and age (juvenile organisms - that are post larval, actively feeding, sexually im-matured or spawning). Their lengths were standard and measurement were done from the tip of snout to end of candal peduncle of the fish and none of the test organisms had a length twice more than one another (i.e. the longest fish was not more than twice that of the shortest fish in length). All organisms were well cared for to forestall or avoid unnecessary stress during transportation and acclimatization. Harvested organisms were properly preserved before extraction of analytes (toxic metals and hydrocarbons) and the determination of these analytes were carried out in accordance with specified standard methods.

2.4.1 Test Organism

The test organism used in this study was *hemichromis fasciatus,* a benthopelagic species. It was cultured at The African Regional Aquaculture Centre, Port Harcourt, Nigeria and the specie was duly identified by a Marine Biologist as *hemichromis fasciatus*.

2.4.1.1 Collection of Test Organism and Acclimatization

Test organisms used in this test were captured using a small drag net made of rope (0.5mm thickness). They were of an average weight of 4.98 ± 1.38 g, with an average length of 2.95 ± 0.52cm. The collected organisms were carefully transported to the experimental site with minimal stress on organism and were later transferred into a transparent glass tank with a dimension of 72cm (l) x 48cm (w) x 48cm (h) containing their native water and allowed to acclimatized at a temperature range of $23^0C \pm 2^0C$ in an air condition room for 10 days. The water in the glass tank was continuously aerated during this period and organisms fed with minimal quantity of recommended fish feeds of known lipid and protein content (EPA, 2008) that does not contain any of the toxic

metals and hydrocarbon.

2.4.2 Test Media

The test media were two water bodies (borrow pits) with dimensions of approximately 72.5 m (l) x 44.2 m (b)×2.5m (d) and 82.7m (l)×45.2m (b)×1.8m (d) for pits A and B respectively. The surface water in the pits were slightly brownish, with thin films of petroleum on the surface of water in some areas, especially in pit A. The odour was characteristic of crude oil.

2.4.3 Control Site

The control experiment for this study was set up at Mgbosimiri stream at Akabuka, which is presumably a pristine environment. The major anthropogenic activities at the stream are recreational (swimming), fishing and in some cases sand mining. This stream has not witness any pollution or impact from oil exploitation or exploration exercise. Mgbosimiri stream is a fresh water environment and it is within the study area. Generally, its' vegetation and soil properties are akin to those found around the communities were these facilities are located.

2.4.4 Experimental Design

Test was conducted in the field aiming to capture the real aquatic environmental situation – vis-a-viz other variables that could interplay in the study area.

Five (5) nylon net cages with dimensions – 60cm (length) x 45cm (width) x 60 cm (height), with net space size of 3x3mm and rope thickness of 1mm were used for the experiment. They were placed at different locations in the recipient water, at least about 5 meters apart. Each cage was partially lowered into the recipient water body with weights attached to net base at four (4) ends (to restrict being drifted by current movement) from a canoe. Ten (10) acclimatized organisms were then introduced carefully into each cage with the lower part being submerged in recipient water. The cage inlet was then zipped and lowered. The top ends of the cage were tied to four (4) stakes with the aid of a rope (1.5 cm diameter) in a manner that about 5 cm – 10 cm of the top portion of the cage was not submerged.

2.4.5 Duration of Study

Test organisms were exposed to recipient water or media in the discharge pits for a duration of 14 days, with an assumption that steady-state equilibrium would have been established between test organism and test media.

2.6. Analytical Procedure

2.6.1. Determination of Hydrocarbons Concentration in Test Organism

2.6.1.1 Sample Extraction

EPA methods3540C and 8100 were adopted for sample extraction and determination of hydrocarbon (TPH and PAHs) respectively.

Harvested fish samples from study or sampling pits were air dried at room temperature (25^0C) for 10days in a well aerated room, free of hydrocarbon contaminants. A portion of the dried fish was properly grinded and homogenized into an agate mortar using a pestle. 10g dry weight (DW) of the homogenized sample was weighed into a timble and transferred into a soxhlet extractor. Hydrocarbon was extracted using 100 ml of dichloromethane: hexane mixture (3:1) for about 2 hours on a heating mantle. This was allowed to cool. The extract was then concentrated to 5ml in a rotatory evaporator and transferred quantitatively into a 10 ml beaker. The flask was rinsed further with 2 ml of hexane and combined extracts was evaporated completely with the aid of nitrogen gas.

2.6.2 Clean up

The residue was re-dissolved with 5 ml of pentane and transferred into chromatography column containing silica gel (60 – 120 mesh). The total petroleum hydrocarbon (TPH) was eluted using pentane. Eluent was then concentrated for Gas Chromatography analysis to give aliphatic and PAHs profiles. All the biogenic hydrocarbons were eliminated during the cleaning exercise.

2.6.3 Determination of Heavy Metal in Test Organism

About 1.5g – 2.5g (DW) of homogenized whole fish of the test organism was transferred into a Kjeldah flash (before and after uptake phase). This was digested using 10ml of concentration of HNO_3 on a heating mantle. The digest was transferred on cooling into a 50ml volumetric flask quantitatively. Kjeldah flask was rinsed with distilled water and the volumetric flask was made to mark. Solutions were analysed for heavy metals using an

AAS. A blank solution was also prepared containing the 10ml of concentrated nitric, treated as in sample and made to 50 ml mark of volumetric flask.

2.7 Standard Calibration Graph

2.7.1 Heavy Metals

A five point calibration graph was performed for each of the heavy metal determined (Pb, Cu, Zn, Cr, As, Fe, Cd, Ni and Ba). The prepared concentrations of heavy metal standards used for the plot varied between 0.005 mg/mL and 10.00 mg/mL depending on the absorbance of the metal. Calibration curves for each metal showed good coefficient of regression (R^2) between 0.9987 and 1.0000.

2.7.2 Hydrocarbons

A five point calibration curve was performed using a mixed standard containing twenty (20) poly aromatic hydrocarbons (PAHs). The standards were co-mixed in iso-octane and each concentration contained all the pesticides being analyzed (0.063 ppm, 0.125 ppm, 0.250 ppm, 0.500 ppm and 1.00 ppm). Calibration curves for each PAHs showed good regression coefficient (R^2) between 0.9958 and 0.9999. All standards were used as external standards for the identification and quantification of corresponding PAHs present in the test organism.

3. Results and Discussion

3.1 Surface Water Characteristics

The temperatures of the surface water were 29.7 ± 2.3^{o}C and 25.4 ±1.0^{o}C in the dry and wet seasons respectively. These values are typical for the Niger Delta region (Nduka & Orisakwe, 2010; Eziekel *et al.,*2013). The pH, DO and conductivity ranged from 6.48±0.55 to 7.88±0.07, 4.63±0.42mg/L to 8.43 ± 0.31mg/L and 48.33±6.50 to 124.98±2.52 (µs/cm) respectively, while values obtained at the control were (7.25±0.24 and 6.95 ± 0.18, 5.93±0.25 mg/L, 21.28±1.28 and 18.42±0.98 (µs/cm) respectively.

3.1.1 Total Petroleum Hydrocarbon (TPH)

The total petroleum hydrocarbons mean values during the dry and wet seasons were 0.107±0.032 (mg/L) and 0.054±0.037 (mg/L) respectively in borrow pit A, while borrow pit B recorded mean values of 0.087 ± 0.014 (mg/L) and 0.045±0.024 (mg/L) respectively. The control station was 0.0100 ±0.004 (mg/L) and 0.009±0.003 (mg/L) for dry and rainy seasons respectively. These values were all below the DPR/EPA regulated limit of 10mg/L.

3.1.2 Heavy Metals in Water

The mean concentrations of heavy metals in both pits and during dry and wet seasons were Pb 0.002 – 0.014 mg/L; Cu 0.004 – 0.024 mg/L; Zn 0.011 – 0.022 mg/L; Cr 0.003 – 0.014 mg/L, As<0.001 mg/L; Fe 0.168 – 0.243 mg/L; Cd 0.002 – 0.008 mg/L, Ni 0.004 – 0.011 and Ba 0.002 – 0.014 mg/L.

Table 1. Baseline physico-chemical properties of surface water in borrow pits

PARAMETERS	Borrow Pit A		Borrow Pit B		CONTROL	
	Dry Season (Mean ± sd)	**Wet Season** (Mean ± sd)	**Dry Season** (Mean ± sd)	**Wet Season** (Mean ± sd)	**Dry Season** (Mean ± sd)	**Wet Season** (Mean ± sd)
Physcio-chemical properties						
pH	6.70 ± 0.07	7.88 ± 0.07	6.48 ± 0.55	6.97 ±0.06	7.25±0.04	6.95±0.05
Temperature (^oC)	29.8 ±1.2	25.3±0.3	30.5 ± 1.4	25.7 ± 0.2	26.3±0.6	25.4±0.4
Conductivity (µS/cm)	119.67 ± 1.53	97.04 ± 36.40	27.33 ±16.5	23.78±2.08	11.18±0.82	127±8.23
DO (mg/L)	6.53± 0.15	6.24 ± 0.42	8.13 ± 0.31	7.32 ± 0.34	4.93±0.25	6.4±0.45
TDS (mg/L)	59.40 ± 0.53	48.0 ± 17.32	14.30 ± 8.56	12.2 ± 0.72	6.88±1.23	64.8±2.54
COD (mg/L)	8.68 ± 0.59	8.33 ± 0.93	8.33 ± 0.45	6.83 ± 0.46	8.7±1.01	6.2±1.21
BOD_5 (mg/L)	5.21 ±0.36	4.99 ± 0.55	5.00 ±0.27	4.09 ± 0.28	5.22±0.98	3.41±0.29
TSS (mg/L)	14.13± 2.26	12.84± 2.27	12.37 ± 2.40	16.4 ± 4.26	3.14±0.44	16.4±1.63
Petroleum Hydrocarbon						
TPH (mg/L)	0.107±0.074	0.054±0.037	0.087±0.014	0.045±0.024	<0.001	< 0.001
PAH (mg/L)	0.009±0.003	0.004±0.002	0.011±0.004	0.003±0.001	<0.001	<0.001
Heavy Metals						
Pb (mg/L)	0.014±0.001	0.009±0.00	0.005±0.001	0.002±0.001	<0.001	<0.001
Cu (mg/L)	0.024±0.001	0.015±0.00	0.004±0.001	0.009±0.001	<0.002	<0.002
Zn (mg/L)	0.015±0.002	0.012±0.001	0.013±0.001	0.022±0.003	0.002±0.001	<0.001
Cr (mg/L)	0.014±0.005	0.007±0.002	0.003±0.00	0.002±0.001	<0.001	<0.001
As (mg/L)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fe (mg/L)	0.232±0.027	0.168±0.031	0.243±0.022	0.201±0.012	0.031	0.029
Cd (mg/L)	0.008±0.001	0.006±0.001	0.003±0.001	0.002±0.001	<0.002	<0.002
Ni (mg/L)	0.009±0.001	0.017±0.003	0.004±0.001	0.005±0.002	<0.001	<0.001
Ba (mg/L)	0.014±0.002	0.012±0.002	0.004±0.001	0.002±0.001	<0.001	<0.001

3.2 Field Bioaccumulation Study

3.2.1 Flesh Tainting and Mortality

Taint in fish is evaluated by its flavour (or taste), odour and physical appearance (organoleptic test).Flavour, odour and colour are physical properties and can be imparted to a fish as a result of its immediate surroundings and this can render the fish unmarketable. Flesh tainting of aquatic organism could be used to evaluate the quality of recipient water body.

In this study *hemichromis fasciatus* was tainted. The extent of flesh tainting was moderate in both pits and seasons.

There was mortality of the test organism in all the discharge pits. The percentage mortality in the pits ranged between 2 and 3.8 %, with zero mortality in the control (Figure 3). The highest percentage mortality was observed in pit A during dry season.

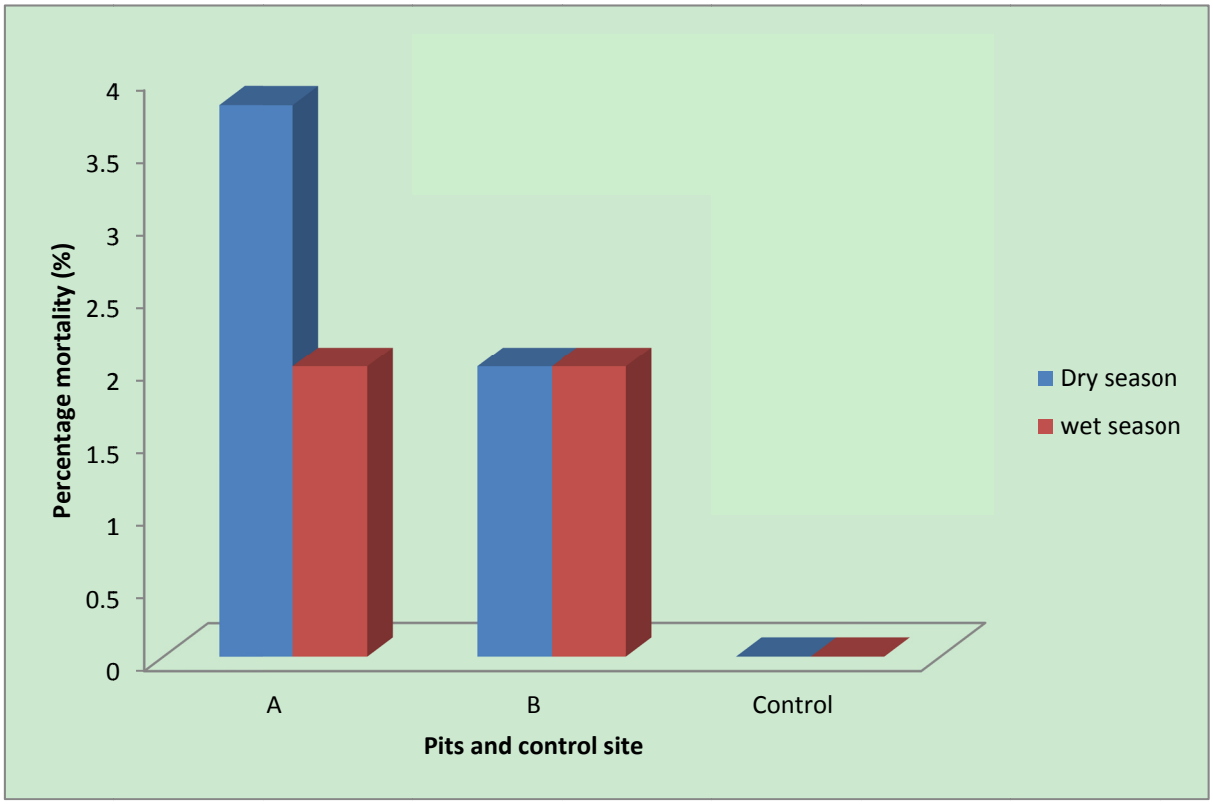

Figure 3. Percentage mortality of test organism in pits and control site

3.3 Bioaccumulation of TPH, PAHs and Heavy Metals

The results of bioaccumulation test of *hemichromis fasciatus* for pits A and B and control station for dry and wet seasons are presented in Figure 4. Values obtained are in wet weights (WW).

3.3.1 Hydrocarbon (TPH and PAH)

The TPH content in *hemichromis fasciatus* ranged between 0.218 ± 0.052 mg/kg and 0.303 ±0.013 mg/kg in both pits and seasons (Figure 4). The highest bioaccumulation was observed in pit A, during the dry season, while the least was in the wet season in pit B. The mean concentrations for the control location were 0.009 ± 0.003 mg/kg and 0.010 ±0.004 mg/kg for wet and dry seasons respectively.

The mean concentration of TPH in the pits for both seasons showed significant increase when compared to values obtained for the control samples. This suggested that there was bioaccumulation of TPH in *hemichromics fasciatus* in pits.

It is pertinent to note that the level of TPH determined in whole fish samples is higher than the recorded for the surface water samples in the pits. *Hemichromis fasciatus* is benthopelagic in nature and can easily contact the sedimentary environment because of its bottom dwelling character. Also, concentrations were relatively higher in the dry season, when compared to the wet season. Davies *et al*., 2006 reported that many aquatic organisms have the ability to accumulate and bio-magnify contaminants in the environment. Polycyclic aromatic hydrocarbons (PAHs) mean concentration in pit A were 0.033 ±0.013 mg/kg and 0.032 ± 0.018 mg/kg during the dry and wet seasons respectively, while pit B recorded an accumulation of 0.029±0.026 mg/kg and 0.023±0.009 mg/kg for dry season and wet seasons respectively.

Animal studies have shown that TPH have some toxicological effect on the blood, immune system, liver, spleen, kidneys, lungs, reproductive system and even developing foetus (USEPA 2000).

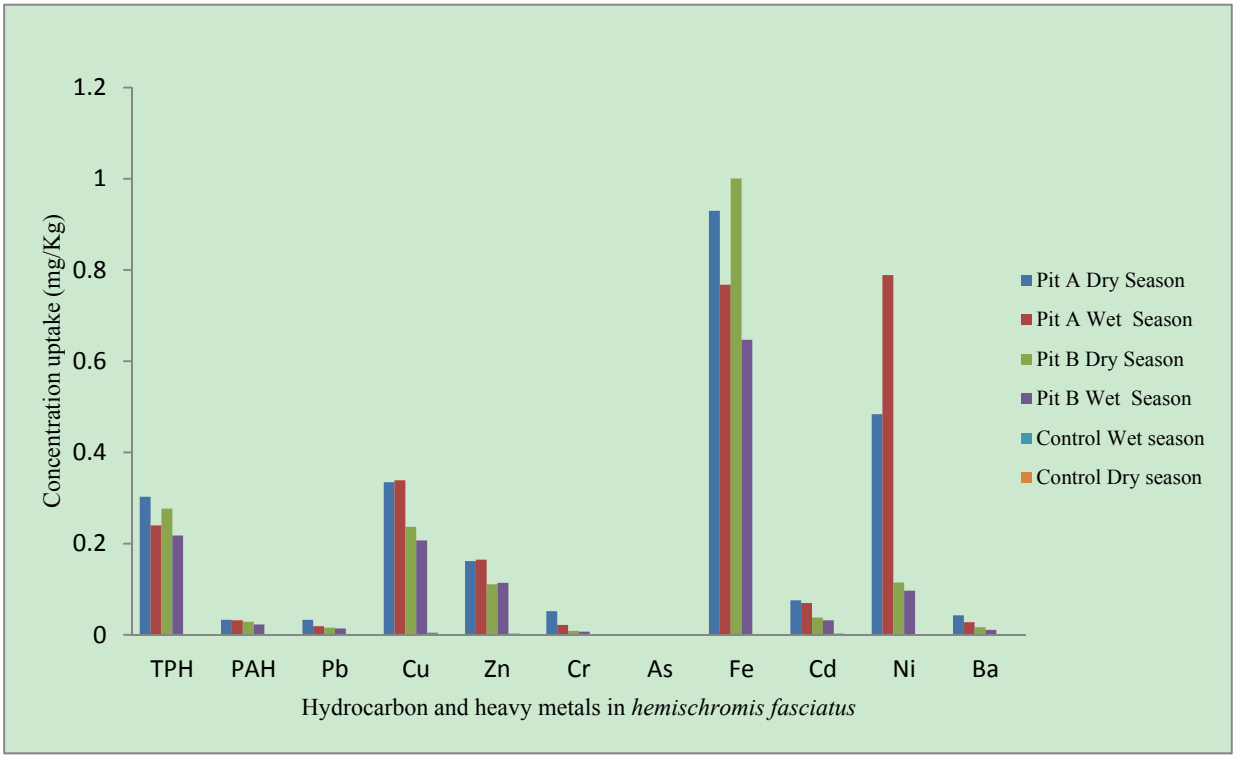

Figure 4. Bioconcentration of hydrocarbons and heavy metals in *hemischromis fasciatus* over 14 days exposure

3.3.2 Heavy Metals

3.3.2.1 Lead (Pb)

The concentration of Pb in *hemichromis fascistus* for pits A and B ranged from 0.004±0.002 mg/kg to 0.033 ± 0.014 mg/kg during both seasons. The highest value was obtained in the season in pit A during the dry season.

The level of Pb in test organism before the test was <0.001mg/kg. These slight increase in the test organism indicated minimal bioaccumulation. The low Pb bioaccumulation values recorded for *hemichromis fasciatus* tend to agree with the findings of Wong *et al*. (1978) and Eisler (1988b) - that aquatic organisms such as fish can bioconcentrate Pb from water, but does not bioacumulate and tends to decrease with increasing trophic levels in freshwater habitat. The mean dietary exposure of Pb to children (1 – 4 years) and adult are 0.03 – 9.0 µg/kg/day and 0.02 – 3.0 µg/kg/day respectively.

3.3.2.2 Copper (Cu)

The concentration of Cu in test organism (*hemichromis fasciatus*) during the dry season were 0.335±0.048 mg/kg (pit A) and 0.237±0.039 mg/kg (pit B), while the wet season recorded 0.339 ±0.047 mg/kg (pit A) and 0.207 ± 0.016 mg/kg (pit B). The highest value was recorded in pit A during the wet season and may have resulted from run-off/wash-off due to E&P operations. The mean concentrations of Cu in ‘‘clean’’ test organism was 0.003±0.001mg/kg (baseline concentration), thus giving a net up-takeof 0.333 mg/kg (dry season) and 0.337 mg/kg (wet season) in pit A and 0.235 mg/kg (dry season) and 0.205 mg/kg (wet season) for pit B, while the control recorded 0.005±0.002 mg/kg and <0.002 mg/kg for dry and wet seasons respectively. These increases in the test environments compared to values obtained in the control depicted bioconcentration of Cu by the test organism. Copper has been reported to bioaccumulate in many different organs in fish and mollusks (Owen 1981).

The provisional maximum tolerable daily intake (PMTDI) of Cu is 0.5 mg/kg body weight (FAO/WHO, 2011). For an average body weight of 60 kg, the maximum daily intake for an adult human is 3.0mg Cu. To exceed this toxicological limit over 8.85kg of *hemichromic fasciatus* has to be consumed per day.

3.3.2.3 Zinc (Zn)

The concentration of Zn in the test organism was 0.162 ± 0.028 mg/kg (pit A, dry season), 0.165 ± 0.029 mg/kg

(pit A, wet season), 0.111 ±0.005 mg/kg (pit B, dry season) and 0.214 ± 0.011 mg/kg (pit B, wet season). The net bioconcentration of Zn in *hemichromis fasciatus* for both pits ranged between 0.108mg/kg (pit B, dry season) and 0.211mg/kg (pit B, wet season) for both seasons.Values obtained in the wet season for both pits were relatively higher than in the dry season. The high concentration in the test organism in the wet season in pit B is due to the concentration of the Zn in the surface water in th pit B, this may be due to run-off from farms and overflow from the river around the farmland and pit B. The uptake of Zn in this study agrees with the report of Murugan *et al* (2008) that zinc can be accumulated via the gills or the digestive track, however, the role of water as source of zinc uptake is not fully elucidated. The provisional maximum tolerable daily intake (PMTDI) for Zn ranges from 0.3 to 1.0 mg/kg body weight (FAO/WHO, 2011). The uptake concentrations in *hemichromis fasciatus* does not pose any toxicology threat with respect to maximum limit stipulated.

3.3.2.4 Arsenic (As)

Arsenic (As) concentration in test organism for both pits in dry and wet seasons were <0.001mg/kg. The same concentration was recorded for test organism at the control. The level of As in both pits were less than 0.001mg/l.

3.3.2.5 Iron (Fe)

The level of Fe uptake by *hemichromis fasciatus* (test organism) harvested from both pits ranged between 0.384 mg/kg and 0.737 mg/kg for both seasons, while the mean concentrations ranged from 0.647±0.152mg/kg (pit B, wet season) to 1.001±0.209 mg/kg (pit A, dry season) for both seasons in the test organism, with the highest mean value recorded in pit A in the dry season. These bioconcentration values recorded in pits A and B, were relatively and significantly higher than the concentration of Fe in the control test organism (0.236 mg/kg and 0.242 mg/kg for dry and wet seasons respectively), this showed that pits had higher levels of Fe, indication anthropological activities around pits. The baseline concentration of Fe in test organism before commencement of test was 0.263 mg/kg and when this is compared to levels in test organism, significant decrease was observed. This significant decrease connoted depuration or loss of Fe from *hemichromis fasciatus* (test organism) in the control environment. Iron is an essential trace metal required by all forms of life, especially in the synthesis of haem protein and many enzyme systems. The FAO/WHO average daily intake for male and female are 17 mg/day and 9 -12 mg/day respectively.

3.3.2.6 Chromium (Cr)

The bioconcentration of Cr in *hemichromis fasciatus* during both seasons ranged between 0.007±0.001mg/kg and 0.052±0.004 mg/kg in both pits. The highest mean concentration was recorded at pit A in the dry season. Uptake concentrations in the dry season were higher than for the wet season.

3.3.2.7 Cadmium (Cd)

The mean concentration uptake of Cd in test organism *(Hemichromis fasciatus*) for dry and wet seasons was 0.076 ± 0.008 mg/kg (pit A, dry season), 0.070 ± 0.005 mg/kg (pit A, wet season), 0.038 ± 0.008 mg/kg (pit B, dry season) and 0.032 ± 0.007 mg/kg (pit B, wet season). Cadmium is bioaccumulated at all trophic levels, especially in the livers and kidneys of fish (Sindayigaya, *et al*. 1994; Sadiq 1992). The provisional tolerable monthly intake (PTMI) for Cd is 0.025mg/kg body weight (ie 1.5 mg/adult of an average body weight of 60 kg) (FAO/WHO 2010). Cadmium levels in test organism were significantly below the PTMI stipulated by the JECFA of FAO/WHO.

3.3.2.8 Nickel (Ni)

The concentration of Ni in the test organism (*hemichromis fasciatus*) in the pits A&B ranged between 0.097 ± 0.007mg/kg and 0.789 ± 0.0039 mg/kg for both seasons. The highest uptake was observed in the wet season in pit A, while the least mean concentration was obtained in pit B during the wet season.

There were significant changes in the concentration ranges obtained in this study during uptake, when compared to base level concentration of Ni in *hemichromis fasicatus* (<0.001mg/kg) and levels determined from the control site. This indicated bioaccumulation. Javid*et al.,* (2000) reported the bioaccumulation of Ni in three species of fish - *Catlacatla, Labeorohita* and *Cirrhinamrigala* , with bioaccumulation concentration of 10.12 ±1.27 mg/Kg, 14.41 ±2.24 mg/Kg and 13.46 ±3.39 mg/Kg respectively, on exposure to 0.05 mg/L of Ni for 96 hours. These values were significantly higher when compared to values obtained in this study for *hemichromicfasciatus*.

3.3.2.9 Barium (Ba)

The concentration and net uptake levels of Ba in the test organism (*hemichromis fasciatus*) in the dry and wet season were 0.043 mg/kg (pit A, dry season), 0.028 mg/kg (pit A, wet season), 0.017 mg/kg (pit B, dry season,)

and 0.011 mg/kg (pit B, wet season).

The concentration of Ba in *hemichromis fasciatus* for the control was <0.001mg/kg for wet and dry seasons, this was relatively and significantly lower to values obtained in the test organisms from both pits, thus indicating bioaccumulation. Previous studies have shown that Ba does not bioaccumulate easily and concentrations in higher species rarely exceed 10 mg/kg (Moore 1991).

3.4 Bioaccumulation Factor and Rate Constant

3.4.1 Uptake Rate Constant

The uptake rate constants for each of the heavy metals and hydrocarbons (TPH and PAHs) were calculated, assuming first-order kinetics.

$$\mathbf{C_t = C_{equil}(1 - e^{-kt})}$$

Where C_t and C_{equil} are concentrations at time t (d) and steady state respectively, k is the rate constant (d^{-1}).

The uptake rate constants for TPH, PAHs and heavy metals in *hemichromis fasciatus* in this study were obtained applying the field kinetic model and also assuming a steady-state after 14 days of exposure.

The up-take rate constants are presented on Table 2 for TPH, PAHs and the heavy metals.

The up-take rate constant *k*, of TPH ranged from 38.47 $\times 10^{-2}$ d^{-1} (pit B, wet season) to 40.82 $\times 10^{-2}$ d^{-1} (pit A, dry season) for both pits and seasons, while PAHs ranged between 22.40 $\times 10^{-2}$ d^{-1} (pit B, wet season) and 24.98 $\times 10^{-2}$ d^{-1} (pit A, dry season).

Uptake of TPH and PAHs seemed slightly higher in the dry season than wet season in both pits. The higher concentrations of both organics in the dry season may have resulted to higher uptake rate in the dry season and the order of bioaccumulation rate was TPH>PAHs in both pits and seasons. In aquatic system, uptake and elimination of contaminants ceases when a steady-state or equilibrium is reached between test organism and surrounding media.

Amongst the metals, the uptake rate constants for water borne metals by *hemichromis fasciatus* ranged from 4.66$\times 10^{-2}$ d^{-1} to 47.66 $\times 10^{-2}$ d^{-1} and 2.70 $\times 10^{-2}$ d^{-1} to 33.90$\times 10^{-2}$ d^{-1} in pits A and B respectively during both seasons. The highest and least uptake rates were recorded in both pits for Ni and Fe respectively for both seasons. The order of rate of uptake constant was Fe <Pb< Cr < Ba < Cd < Zn < Cu < Ni, while As (arsenic) recorded a zero uptake. The detection limit of instrument was <0.001 mg/l.

Table 2. Concentration bioaccumulation (up-take), Bioaccumulation Factor and rate constant of TPH, PAH and heavy metals in *Hemichromics fasciatus* after 14 days exposure to surface water in borrow pits

	Concentration Up-take (mg/Kg)(ww)				**Rate Constant (k)(d^{-1})**				**Bioaccumulation Factor (BCF) (Lkg^{-1})**			
	Borrow Pit A		Borrow Pit B		Borrow Pit A		Borrow Pit B		Borrow Pit A		Borrow Pit B	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
Petroleum Hydrocarbon												
TPH	0.303	0.240	0.277	0.218	40.82×10^{-2}	39.15×10^{-2}	40.18×10^{-2}	38.47×10^{-2}	2.832	4.444	3.184	4.844
PAH	0.033	0.032	0.029	0.023	24.98×10^{-2}	24.76×10^{-2}	24.06×10^{-2}	22.40×10^{-2}	3.667	8	2.636	7.667
Heavy Metals												
Pb	0.033	0.019	0.011	0.004	28.87×10^{-2}	21.04×10^{-2}	12.80×10^{-2}	9.90×10^{-2}	2.357	0.211	2.2	2
Cu	0.333	0.337	0.235	0.205	33.65×10^{-2}	33.73×10^{-2}	31.55×10^{-2}	30.18×10^{-2}	13.878	22.467	16.786	22.778
Zn	0.159	0.162	0.108	0.211	31.26×10^{-2}	31.40×10^{-2}	33.28×10^{-2}	28.69×10^{-2}	10.60	13.5	8.308	9.591
Cr	0.052	0.022	0.009	0.007	28.23×10^{-2}	22.08×10^{-2}	15.70×10^{-2}	13.90×10^{-2}	3.714	3.143	3	3.5
As	<0.001	<0.001	<0.001	<0.001	-	-	-	-	-	-	-	-
Fe	0.667	0.504	0.737	0.384	6.65×10^{-2}	4.66×10^{-2}	7.37×10^{-2}	2.70×10^{-2}	2.875	3.006	3.037	1.911
Cd	0.076	0.07	0.038	0.032	30.94×10^{-2}	30.35×10^{-2}	25.99×10^{-2}	24.76×10^{-2}	9.5	11.667	12.667	16
Ni	0.484	0.789	0.115	0.097	44.17×10^{-2}	47.66×10^{-2}	33.90×10^{-2}	32.68×10^{-2}	53.778	71.727	28.750	19.4
Ba	0.043	0.028	0.017	0.011	26.87×10^{-2}	23.81×10^{-2}	20.24×10^{-2}	17.13×10^{-2}	3.071	2.333	4.25	5.5

At steady-state, Fe had the lowest uptake rate constants for both seasons and pits; rate constants were 6.65×10^{-2} d^{-1} (pit A, dry season), 4.66×10^{-2} d^{-1} (pit A, wet season), 7.37×10^{-2} d^{-1} (pit B, dry season) and 2.70×10^{-2} d^{-1}(pit B, wet season). This relatively low rate of uptake constants for Fe by *hemichromis fasciatus*is was likely due to its initial high concentration in test organism (0.263 mg/kgFe) and as well as in the surface water of the pits (0.201 – 0.243 mg/kgFe), this situation may have resulted to a gradual attainment of equilibrium between the test organism and the surrounding medium (surface water in pits), thereby leading to slow uptake rate for Fe over a period of 14 days.

3.4.2 Bioaccumulation Factor

The degree to which bioaccumulation occurs is normally expressed as bioaccumulation or bioconcentration factor (BAF/BCF). In an aquatic environment, BAF/BCF quantitatively describes bioaccumulation in fish or aquatic organism and it is defined as the field observed ratio of the concentration of a given chemical in biota to the concentration in corresponding water (Mackay & Fraser, 2000) or as the dimensionless ratio of wet-weight contaminant concentration in fish or aquatic organism (C_F), to the corresponding contaminant concentration in the water (C_W) . It also describes the equilibrium reached between uptake and depuration of contaminant by fish and it is the ratio of respective rate constants for those processes.

$$\mathbf{BCF = C_F \times C_W^{-1}}$$

The BCF calculated in this study is based on a steady state system at day 14.

The BCF values (dry and wet seasons) for hydrocarbons and heavy metals are presented in Table 3.

BCF values calculated for *Hemichromics fasciatus* in both pits and seasons ranged from 2.832 LKg^{-1} to 4.844 LKg^{-1} for TPH and 2.636LKg^{-1} to 8.0 LKg^{-1} for PAH. These values are significantly below the OEHHA recommended BCF defaults of 583 for PAH in fish.Several studies have reported BCFs in fish for benzo[a]pyrene as PAH. Species such as bluegill sunfish have produced BCF values ranging between 224 and 490 (Spacie *et al.*, 1983, McCarthy and Jimenez, 1985) for PAH. Balk *et al*., 1984, reported BCF ranging from 50 to 80000 in internal organs, with the greatest bioconcentration occurring in the gallbladder and bile on exposing Northern pike fish for 10 or 21 days to benzo[a]pyrene in water.

Amongst the metals, BCF values for Pb, Cd and Cr ranged from 0.211 - 2.357 LKg^{-1}, 9.50 – 12.667$LKg^{-1}$and 3.0 – 3.714 LKg^{-1} respectively in both pits and seasons. From previous study, Taylor (1983), reported that vertebrate

fish species that were exposed to Cd have shown a BCF of <20. While Fish BCFs ranging from 5.1 to 300 have been reported for lead (SCAQMD., 1988). The arithmetic mean of these values (155) is recommended as the default BCF for lead (OEHHA, 2000). Also, BCF values ranging from 22 to 200 have been reported for Cd in brook trout fish (US EPA., 1985 and Atchison *et al*., 1977), while for Cr, BCF values ranging from 1-3.4 have been reported (OEHHA, 2000). The OEHHA recommended default BCF for Cd and Cr are 366 and 2 respectively. Other BCF values in this study ranged as follows; Cu, 13.878 – 22.778LKg^{-1}; Zn 8.308 – 13.50 LKg^{-1}, Fe 1.911 – 3.037 LKg^{-1}, Ni 19.4 – 71.727 LKg^{-1} and Ba 2.333 – 5.5LKg^{-1}. In the bioaccumulation studies of some heavy metals in *oreochromis nilotycus* BCF ranges of 38 – 56, 179 – 199 and 21 – 24 were reported by Noegrohati (2006) for Cu, Zn and Cd respectively. These values are comparable with values obtained in this study for *hemichromics fasciatus.*

It has been reported that chemical substances having a BCF or BAF >1000 are characterized by a tendency to accumulate in organisms (Moss and Boethling, 1999), however, in this study, the highest BCF value was 71.727 LKg^{-1} for Ni (pit A and wet season), this was about 14-fold below the stipulated value of 1000.

3.5 Seasonal Variation

As shown in Table 1, concentrations of analytes were generally higher in the dry season than the wet season in both pits, except for Ni (pit A) and Zn (pit B), however these changes were considered not very significant or drastic. Relatively, lower concentrations in the wet season may have been due to dilutions from rainfall, especially for the metal analytes that are waterborne in nature. The increases recorded for the wet season may have resulted from ruff-off and washout from some the oil exploration and production facilities and farmlands where fertilizers were applied within this location. Besides, there is the possibility of an overflow from the stream close to the pit A, as often witnessed at periods when rainfall is high. However, these seasonal changes in concentrations of analytes were distinctly expressed in the level of Ni uptake in *hemichromics fasciatus* (Figure 4) during the rainy season. There were no sharp changes in the up-take rate constants between the seasons in each pit (Table 2), as changes were considered insignificant.

Finally, there were strong positive correlations between dry and wet seasons (Figure 5). BCF values obtained for the xenobiotics showed strong seasonal correlation between dry and wet seasons for both pits and between pits. Correlation coefficient ranged between 0.6743– 0.9945 ($p < 0.005$).

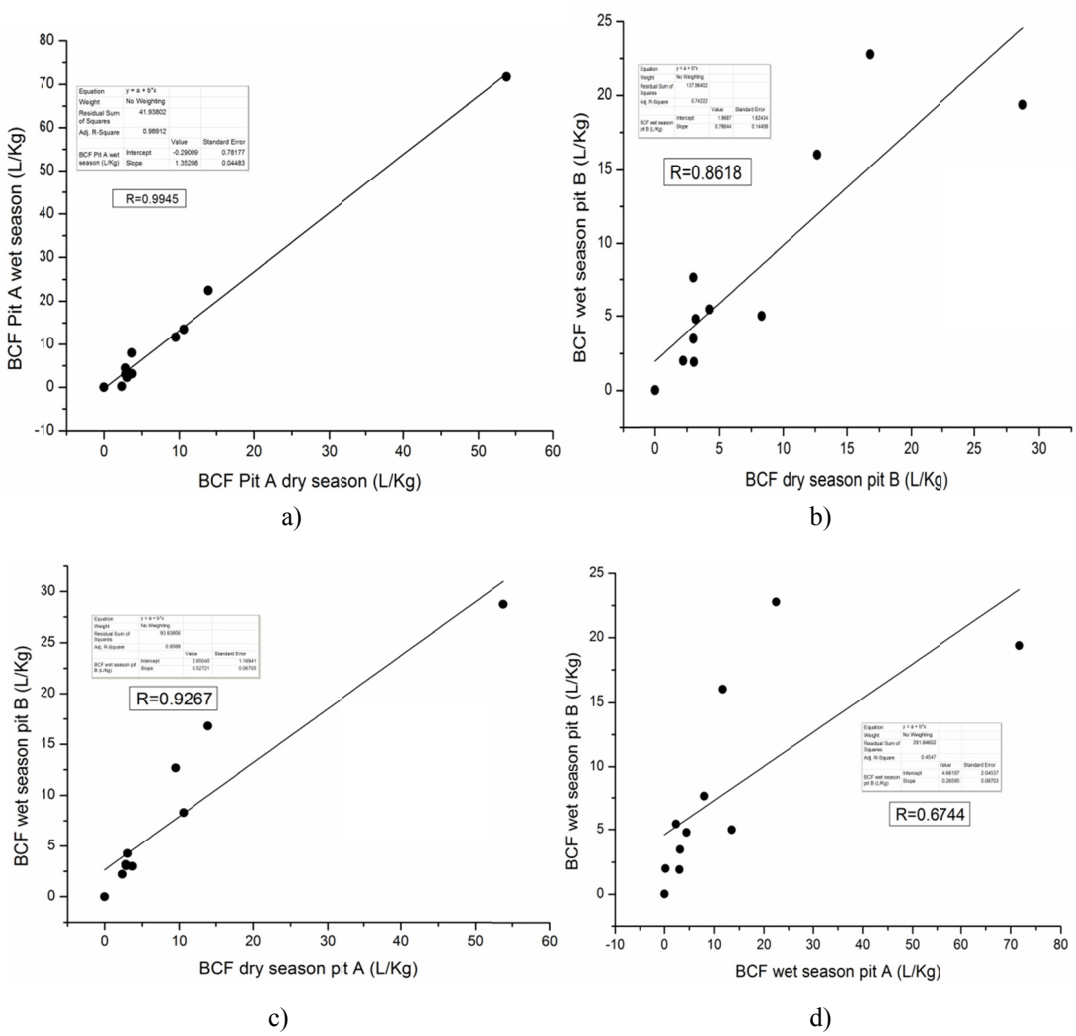

Figure 5. (a) Seasonal correlation between BCF dry and wet seasons for pit A (b) Seasonal correlation between BCF dry and wet seasons for pit B (c) Correlation between pits A and B for dry season (d) Correlation between BCF pits A and B for wet season

4. Conclusion

The results presented from this study clearly demonstrated that the borrow pits around the Niger Delta region are contaminated and aquatic organism in such water bodies are also contaminated. *Hemichromics fasciatus* exhibited greater uptake of analytes in the dry season than the wet season and the amount of heavy metals accumulated were all below the provisional maximum tolerable daily intake (PMTDI) as stipulated by the JECFA of FAO/WHO. The toxicokinetic parameters obtained for both pits and season in this study does not pose any toxicological threat to man if fish from such pits are consumed.

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