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Uptake of polycyclic aromatic hydrocarbans by the fingerlinks of *Oreochromis niloticus* (Linneaus, 1757) from the dispersed phase of bonny light crude oil

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Abstract. In many crude oil spill sites, dispersants are widely applied during clean-up operations without adequate consideration of their environmental effects. This is despite the fact that the water accommodated fractions of these mixtures contain toxic components. This study investigated the uptake of polycyclic aromatic hydrocarbons (PAHs) from the water accommodated fractions (WAF) of a mixture of a dispersant, Goldcrew and Bonny Light crude oil using a tilapian fish, *Oreochromis niloticus*. The aim was to determine the critical body residue (CBR) of the PAHs in the fish exposed to the dispersed, dispersant and crude oil in water ($DCO_{WAF\text{-}PAH}$) and undispersed, crude oil in water only ($CO_{WAF\text{-}PAH}$) regimes of the mixtures in the WAF. The control experiment had water only. The concentrations for both regimes of exposure were at sub-lethal levels of 0.2, 0.4, 0.8 and 1.6ml/L for fifty days using renewal static bioassay. The types and total concentrations of PAHs were analyzed for in the crude oil, fish feed administered, pre-exposed fish sample, dispersant and test water before exposure. The sixteen known carcinogenic PAHs were found in the crude oil (1916.4ppm), eleven in the fish feed (0.57ppm), three in the pre-exposed fish (0.007ppm), and none in the dispersant and test medium (water). The concentration of the PAHs in the fish from the $DCO_{WAF\text{-}PAH}$ ranged from 3.6128-7.9744ppm while that of the $CO_{WAF\text{-}PAH}$ ranged from 3.4114-3.9693ppm. The concentrations of individual PAHs recovered in the fish showed that napthalene had the highest CRB level of 6.7780ppm and 3.2610ppm, anthracene was 0.6590ppm and not detected (N.D) while acenaphthene had the lowest CBR of 0.00001ppm and below the limit of quantification (<LOQ) for the $DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$ exposure phases respectively. The results showed the order of the CBR level as NAP>FL>BaF>BaP>BaA>FLU>PHE in the $CO_{WAF\text{-}PAH}$ irrespective of exposure concentration, while for the $DCO_{WAF\text{-}PAH,}$ it was NAP>FLU>BaF>BaP>B(ghi)P>BaA>BkF>PHE. The results demonstrated that the presence of the dispersant, Goldcrew increased the critical body residue of the PAHs irrespective of the concentration in the fish samples. This was especially so for the low molecular weight PAHs (<200). Thus, the application of dispersants for crude oil spills need to be done with utmost care particularly in fishing grounds.

Keywords: Polycyclic aromatic hydrocarbons, dispersant, water.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of fused-ring hydrocarbons of low-to-high molecular weights held together by strong, covalent bonds. PAHs can be classified as PBTs-persistent, bioaccumulative and toxic chemical compounds of great concern (Traas and VanLeeuwen, 2007). PAH-contaminated environments are often characterized by

mixtures derived from petrogenic (e.g. oil spills) and pyrogenic (e.g. vehicular, industrial, domestic emissions) sources (National Research Council, 2003).

PAHs are of interest because of their presence in crude oil; several of which are known to be carcinogenic, teratogenic, and immunotoxic. PAHs and their transformed products are among the most hazardous constituents of crude oil and have provided a more conspicuous view of the impacts of PAH-pollution on aquatic resources which are of long-term effects (Short *et al.,* 2004). The frequency and volume of oil spills have resulted in measures taken to reduce the spread and deleterious effects of the oil, one of which is the use of oil spill dispersants (OSD) amongst which is Goldcrew SW. OSDs reduce the adhesion between the oil and water, oil and sediments, and other surfaces by creating small droplets that move into the water column facilitating dispersion, quicker weathering and biodegradation thus, reducing the resident time of the oil within the water. OSDs are composed of surfactants, solvents and stabilizing agents and are applied as quickly as possible after a spill has occurred since there is limited 'window of opportunity' for their use due to the changing properties of oil occasioned by weathering (National Research Council, 2003).

Their use may increase the functional water solubility of PAHs resulting in increased availability and altered interactions between oil/dispersants and biological membranes (Couillard et al., 2005).This study therefore, determined the uptake (as critical body residue-CBR) of PAHs by fingerlings of *Oreochromis niloticus* from the water accommodated fractions (WAF) of the mixture of Goldcrew dispersant and Bonny Light crude oil.

Materials and Methods

Three hundred fingerlings of *Oreochromis niloticus were* procured from the African Regional Aquaculture Centre (ARAC) Aluu, Rivers State, Nigeria and kept in holding plastic tanks (36×39×53cm of 25L) for fourteen days to acclimate in the laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt using tap water. They were maintained with daily change of water to allow for stabilization before commencement of the study and fed twice daily with 'Coppen' feed (0.8-1.2mm) pellet size ad libitum at 5% body weight.

The Bonny Light crude oil and Goldcrew (SW) were procured from an oil company in I.5L air tight plastic bottles and stored at 28^0C for preparation of the test solution. The water accommodated fractions (WAF) of the mixture of Bonny Light crude oil and Goldcrew (SW) dispersant ($DCO_{WAF\text{-}PAH}$) and that without Goldcrew ($CO_{WAF\text{-}PAH}$) were prepared under laboratory conditions as described by Reish and Oshida (1986); Khan and Payne,(2005). The application ratio of crude oil to Goldcrew (SW) was determined to be 1:30, with a 1:30 dilution ratio of dispersant to distilled water. Range finding tests were done to determine the threshold concentrations (Lelei, 2007).

A static renewal bioassay of the two test exposures ($DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$) was conducted for fifty days with four concentrations each and three replicates with a factor of

0.2 that gave 0.2, 0.4, 0.8 and 1.6ml/L made up to 12L with 10 fish per tank. There was 12hr light and dark regime respectively during exposure with a 48hr renewal of test media and mixtures to prevent sequestration (aging) by which chemicals tend to become less available with time for uptake by organisms for partitioning into the aqueous phase.

The fish samples and feed were oven dried and prepared to determine PAHs levels using Hewitt Packard 5890 Series II gas chromatography equipped with Flame Ionization Detector (GC-FID).

Results and Discussion

The data showed that the sixteen carcinogenic PAHs were present in the crude oil, eleven were in the fish feed and three in the pre-exposed fish in varying concentrations (Table 1). In the crude oil, B(b)F had the highest concentration of 1711.829ppm or 89.34% of the total concentration of PAHs while B(ghi)P had the lowest concentration of 0.39457ppm at 0.014% of the total PAHs present in the Bonny Light crude oil. The order of the PAHs in the crude oil was B(b)F>IP>ACT>FL>ANT>FLU>PYR>ACE>PHE>B(a)A>B(k)F>B(a)P>CHY>D(ah)A>NAP>B(ghi)P. NAP had the highest value of 0.27024ppm (47.20%) and PYR the lowest with 0.00001ppm (0.002%) of the total PAHs in the Coppen fish feed. The order of the PAHs in the feed was NAP>FLU>ACT>B(k)F>ACE>B(a)A>PHE>FL>IP>ANT>PYR. The pre-exposed fish samples also showed that NAP had the highest concentration of 0.00589ppm at 80.25% and ANT the lowest concentration of 0.00064ppm at 8.72%. The order of the PAHs was NAP>PHE>ANT.

The critical body residue (CRB), the lethal body burden, the internal response or whole body concentration of a chemical in an aquatic organism which is a constant is the bioavailable concentration. It depends on the fugacity of the chemical, and is the concentration of the chemical vis-à-vis the storage capacity of the test organism. The CBR in the fish is relevant for the assessment of the risk of the PAHs. The CBR (Table 2) expressed as the total concentration of the PAHs showed that values from the concentrations of the $DCO_{WAF\text{-}PAH}$ were higher (3.6128-7.9744ppm) than those from the $CO_{WAF\text{-}PAH}$ (3.4114-3.9693ppm). Similarly, the PAHs in the medium/water (0.0577-0.6306ppm) were lower than in the fish.

Table 1: Concentrations (ppm) and Percentages (%) of Individual PAHs present in Bonny Light crude oil, Coppen feed and Pre-exposed fish samples

Individual PAHs	Abbreviation	Bonny Light Crude oil	%PAH present	Coppen feed	% PAH present	Fish Sample	% PAH Present
Acenaphthene	ACE	1.40945	0.074	0.00820	1.433	-	-
Acenaphthylene	ACT	5.29376	0.276	0.03145	5.493	-	-
Anthracene	ANT	3.97793	0.208	0.00002	0.004	0.00064	8.719
Benzo(a) anthracene	B(a)A	0.89904	0.047	0.00470	0.821	-	-
Benzo(a) pyrene	B(a)P	0.39457	0.021	-	-	-	-
Benzo(b) fluoranthene	B(b)F	1711.82872	89.326	-	-	-	-
Benzo(g,h,i) perylene	B(ghi)P	0.27392	0.014	-	-	-	-
Benzo(k) fluoranthene	B(k)F	0.54669	0.029	0.01087	1.898	-	-
Chrysene	CHY	0.35757	0.019	-	-	-	-
Dibenz(a,h) anthracene	D(ah)A	0.33503	0.018	-	-	-	-
Fluoranthene	FLU	2.31912	0.121	0.25849	45.143	-	-
Fluorene	FL	4.64420	0.242	0.00008	0.014	-	-
Ineno(1,2,3-cd) pyrene	IP	180.64988	9.427	0.00003	0.005	-	-
Naphthalene	NAP	0.31152	0.016	0.27024	47.195	0.00589	80.245
Phenanthrene	PHE	1.27761	0.067	0.00051	0.089	0.00079	10.763
Pyrene	PYR	1.87589	0.098	0.00001	0.002	-	-
ΣPAHs		**1916.39488**	**100.0**	**0.5726**	**100.0**	**0.00734**	**100.0**

Table 2: The CBR as total concentration of PAHs analyzed in the fish samples (ppm) from the concentrations of $DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$

Exposure concentration (ml/L)	Fingerlings		Test medium (water)	
	A	B	A	B
0.2	7.9744	3.4114	0.0958	0.0819
0.4	4.2212	3.8567	0.1552	0.1552
0.6	3.6128	3.6718	0.1400	0.1400
1.6	4.9218	3.9693	0.1020	0.1020

Key: A: Concentrations of $DCO_{WAF\text{-}PAH.}$ B: Concentrations of $CO_{WAF\text{-}PAH.}$

Generally, the fish samples from the $DCO_{WAF\text{-}PAH}$ had higher CBR of the PAHs when compared with fish from the $CO_{WAF\text{-}PAH}$ at the same concentration. Conversely, the CBR of the individual PAHs varied irrespective of the exposure concentration (Figures 1 & 2).This was obvious in ACT, ANT, B(a)A, CHY and D(ah) A. Data for the individual PAHs showed that NAP had the highest CRB levels of 6.7780ppm and 3.2610ppm. The values for ANT were 0.6590ppm and not detected (ND) while ACE had the lowest CBR of 0.00001ppm and below the limit of quantification (<LOQ) for the $DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$ respectively. The order of the CBR levels was NAP>FL>BaF>BaP> BaA>FLU>PHE in the $CO_{WAF\text{-}PAH}$ irrespective of the exposure concentration while for the $DCO_{WAF\text{-}PAH,}$ it was NAP>FLU>BaF>BaP>B(ghi)P>BaA>BkF>PHE.

The data revealed that the Bonny Light crude oil, Coppen fish feed and pre-exposed fish all contained PAHs but the bulk (16) of individual PAHs were recovered in the crude oil. The fish feed contained ten out of the sixteen PAHs while the pre-exposed fish contained NAP, PHE and ANT. It is likely that the fish accumulated these PAHs from the environment from which they were procured or from maternal transfer. The level of the PAHs in the test organisms post-exposure can thus be attributed to the mixtures as well as the feed administered. This is in agreement with the findings Oterhals and Nygård (2008) and Nácher-Mester et al., (2010).

The concentrations of the PAHs in the water column were lower than those in the fish (with the $CO_{WAF\text{-}PAH}$ having the lower values) possibly due to reduced hydrophilicity and high lipophilicity. This is related to their molecular weights, partition coefficient, solubility and abilty to bioconcentrate in fish tissues (Froehner *et al.,* 2011). These CBR values were higher than the 0.03ppm level of concern for B(a)P in finfish (Anyakora *et al*., 2008).

The higher PAH levels in the $DCO_{WAF\text{-}PAH}$ than the $CO_{WAF\text{-}PAH}$ may have been triggered by the presence of Goldcrew attesting to its 'additive' quality to the crude oil. This increased the bioavailability of the PAHs for uptake by the fish in line with findings by Couillard et al. (2005) that used a different dispersant. Among the individual PAHs, our results showed that CBR was influenced differently by the treatments and not necessarily by the concentrations. The $DCO_{WAF\text{-}PAH}$ affected the CBR level of the PAHs of low molecular weight (LMW) and high molecular weight (HMW) attributed to the presence of the dispersant. On the other hand, $CO_{WAF\text{-}PAH}$ affected the CBR of HMW PAHs as previously reported (Froehner *et al.,* 2011).

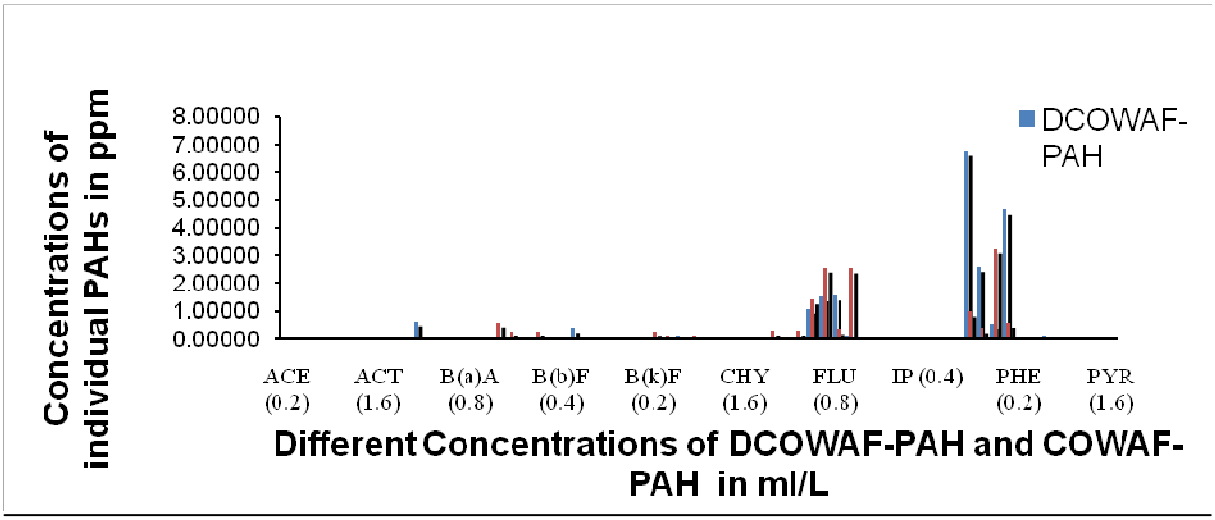

Figure 1: Concentration of Individual PAHs in the tissues of fish exposed to different concentrations of $DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$.

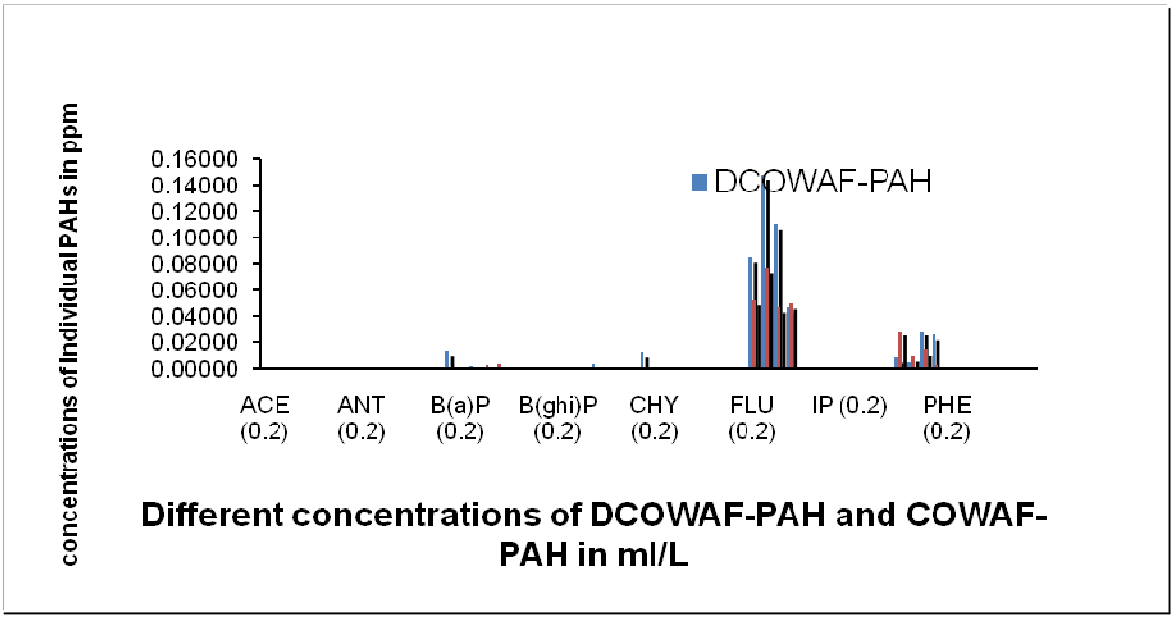

Figure 2: Concentration of Individual PAHs in the test medium for fish exposed to different concentrations of $DCO_{WAF\text{-}PAH}$ and $CO_{WAF\text{-}PAH}$.

NAP had the highest CBR levels in the various concentrations of the two exposure regimes pre-and post-exposure while CHY had the lowest values due to its insolubility, followed by ANT, as earlier reported (Perugini *et al*., 2007). However, in this study, the higher level of NAP is attributable to the fish feed administered.

Conclusion

PAH levels of 0.03ppm in finfish generally illicits concern but from this study, NAP had 6.78ppm as the CBR level in fish exposed intermittently to 0.2ml/L concentration of the dispersant and crude oil. This makes it worrisome for 'spill situations' where the levels are in thousands of litres. The use of Goldcrew SW in 'spill situations' should therefore be considered only under situations where other clean-up methods are not feasible. The use of mechanical clean-up approaches should be the preferred approach since they are less hazardous.

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