Environmental Pollution 201 (2015) 42–49

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Linking biochemical perturbations in tissues of the African catfish to the presence of polycyclic aromatic hydrocarbons in Ovia River, Niger Delta region

Blessing E. Obinaju, Carola Graf, Crispin Halsall, Francis L. Martin*

Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK

ARTICLE INFO

Article history:
Received 3 January 2015
Received in revised form
13 February 2015
Accepted 15 February 2015
Available online 9 March 2015

Keywords:
African catfish
Biospectroscopy
Environmental pollution in Nigeria
Fourier-transform infrared spectroscopy
Heterobrachus bidorsalis
Niger Delta pollution
Polycyclic aromatic hydrocarbon

ABSTRACT

Petroleum hydrocarbons including polycyclic aromatic hydrocarbons (PAHs) are a pollution issue in the Niger Delta region due to oil industry activities. PAHs were measured in the water column of the Ovia River with concentrations ranging from 0.1 to 1055.6 ng L^{-1}. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy detected alterations in tissues of the African catfish (*Heterobranchus bidorsalis*) from the region showed varying degrees of statistically significant ($P < 0.0001$, $P < 0.001$, $P < 0.05$) changes to absorption band areas and shifts in centroid positions of peaks. Alteration patterns were similar to those induced by benzo[*a*]pyrene in MCF-7 cells. These findings have potential health implications for resident local communities as *H. bidorsalis* constitutes a key nutritional source. The study provides supporting evidence for the sensitivity of infrared spectroscopy in environmental studies and supports their potential application in biomonitoring.

1. Introduction

Petroleum exploration and exploitation activities have increased the level of polycyclic aromatic hydrocarbons (PAHs) present in water and soil in the Niger Delta region. The increase in PAHs is exacerbated by the lack of the required level of regulation, which would protect the aquatic environment within the region from contamination by petroleum products or derivatives (Sojinu et al., 2010). The carcinogenicity of most PAHs, *e.g.*, benzo[*a*]pyrene (B[*a*]P) is well-established and studies have also documented their neurotoxic (Perera and Herbstman, 2011) and immunotoxic (Casale et al., 2000; N'Diaye et al., 2006) potential. PAHs, including B[*a*]P, acquire carcinogenicity or toxicity following biotransformation/activation into toxic metabolites by metabolising enzymes such as cytochrome P450s (Shimada and Fujii-Kuriyama, 2004). It is generally accepted that PAHs act through either of two mechanisms: ***1)*** "dioxin-like" toxicity mediated by activation of the aryl hydrocarbon receptor (AhR), which controls a battery of genes involved in PAH metabolism, such as cytochrome P4501A1 (CYP1A1); and, ***2)*** "nonpolar narcosis", where tissue uptake is dependent solely on hydrophobicity and toxicity is mediated through non-specific partitioning into lipid bilayers (Incardona et al., 2006). PAHs have been well-studied for their possible toxic mechanisms (Wolska et al., 2012), including photochemical-activated induced toxicity (Dong et al., 2002; Gao et al., 2005; Ibuki et al., 2002). Therefore, the increased levels of PAHs in Nigeria's delta region suggest an elevated risk of various toxic effects arising from increased exposure to PAHs in the resident population.

The dissolved phase of the water column is quite important in observing the bioavailability, eco-toxicity and endocrine disrupting impacts of all forms of environmental contaminants, especially PAHs (Zhou et al., 1996). Dissolved hydrocarbons and their degradation products bio-concentrate in organisms by passive diffusion and respiration (Berrojalbiz et al., 2011, 2009). Therefore, documenting dissolved hydrocarbon concentrations in aquatic environments is relevant to the assessment of toxicological effects, particularly for small organisms at the base of many aquatic trophic chains and might constitute the onset of PAH bio-magnification in other organisms (Guigue et al., 2014).

* Corresponding author. Centre for Biophotonics, LEC, Lancaster University, Lancaster LA1 4YQ, UK.
E-mail address: f.martin@lancaster.ac.uk (F.L. Martin).

http://dx.doi.org/10.1016/j.envpol.2015.02.031
0269-7491/

1.1. PAH contamination and monitoring in the Niger Delta

Very little is known of the existence or movement of PAHs within aquatic environments, especially within the Niger Delta region. The assessment of PAH contamination in aquatic environments of the region has mostly been conducted by assessing concentration levels of PAHs, mainly in the particle and sediment phase of the water column (Ana et al., 2009; Essien et al., 2011; Okafor and Opuene, 2007). Such information evaluating the concentrations of PAHs specifically in the dissolved phase of the water column is scarce. Sojinu et al. (2010) documents values of total PAH concentration from sampling sites with certain proximity to oil exploration locations within the Niger Delta as ranging between 23.8 and 120 ng/g in soil, and 65–331 ng/g in sediments. These values were the sum concentrations of 28 PAHs measured. Concentrations ranging from 0.1 to 28 μg/kg in sediments have also been documented (Anyakora et al., 2005).

Aquatic species, *e.g.*, mussels, molluscs and fish (Kelly and Giulio, 2000; Koehler, 2004; Nasci et al., 2002) including aquatic plants such as algae (Blasco et al., 2006; Coogan et al., 2007) are most employed in eco-toxicological studies, due to their high sensitivity to various cellular alterations (*e.g.*, oxidative damage) even at very low exposures compared to terrestrial organisms (Valavanidis et al., 2006) and perhaps due to their position in the food chain, which may affect the levels of contaminant biomagnification in organisms at higher trophic levels. Compared to studies monitoring the bioaccumulation of heavy and trace metals in sentinels, very few studies (Anyakora and Coker, 2007; Benson et al., 2008; Eduok et al., 2010) have attempted to monitor PAHs in sentinels within the Niger Delta region. Furthermore, these studies only document the concentrations of PAHs within sentinel tissues with no specific documentation of possible exposure effects, *e.g.*, DNA damage in the observed sentinels as a response to PAH exposure.

Environmental contamination occurs or is more frequently encountered as complex mixtures and the behaviour of chemicals in a mixture rarely corresponds to that predicted from data on the pure compounds (Llabjani et al., 2010). Relating low environmental exposures to actual effects in organisms is often difficult and requires lab-based dose response type assays often involving sophisticated techniques and protocols requiring expensive kits. This understanding creates the need for simple, cost effective yet highly sensitive techniques and robust protocols (Baker et al., 2014) that are applicable to environmental biomonitoring in sentinels (Obinaju and Martin, 2013) and able to detect real-time contaminant exposure in these organisms, even at very low doses. Biospectroscopy, *e.g.*, infrared (IR) spectroscopy, techniques and protocols such as those documented in Baker et al. (2014), coupled with multivariate computational analysis such as principal component analysis and linear discriminant analysis (PCA-LDA) (Martin et al., 2010) have been shown to discriminate between graded tissue types (Kelly et al., 2011a) and very low-dose exposure scenarios (Llabjani et al., 2014; Pang et al., 2012). Using this technique, subtle tissue alterations have been observed in various biochemical components, *e.g.*, lipids of locally caught African catfish (*Heterobranchus bidorsalis*) within the Niger Delta region (Obinaju et al., 2014).

This study utilises attenuated total reflection Fourier-transform IR (ATR-FTIR) spectroscopy to examine alterations in various tissues of the African catfish (*H. bidorsalis*) and explain these alterations as a function of exposure to waterborne PAHs. It measures PAH concentrations in the dissolved phase of the water column at several sites along a river in the Niger Delta region, which receives chemical/hydrocarbon pollution. It aims to assess the risk of PAH exposure to the resident population along the river as the African catfish constitutes a major constituent of the resident population's diet.

2. Materials and methods

2.1. Sampling sites and sample collection

Water samples were collected at four sites (Gelegele 1, Gelegele 2, Ikoro and Nikorowa) along the Ovia River in Edo state, Nigeria (Fig. 1). The characteristics of the river and sampling sites have been previously detailed (Obinaju et al., 2014). Briefly, sampling sites were chosen to reflect distance from a known contaminant source (open gas flare site and petroleum exploration activity) at Gelegele (Fig. 1) and thus one would expect varying contaminant concentration levels (*i.e.*, decreasing with increasing distance from contaminant source) at the individual sampling sites. Clean amber glass bottles with Teflon lined lids were used to collect 2 L of water samples at approximately 30 cm below the water surface for PAH analysis. Water samples were preserved at 4 °C and processed within 48 h of collection. Water samples were pre-filtered using a filter paper (Whatman) and filtrate passed through an ENVI-18 DSK 47 mm solid-phase extraction disks (Sigma–Aldrich UK) using a vacuum filtration unit. This approach measures PAHs in the open water column rather than PAHs associated with particles and/or sediments. Each disk was preconditioned using 5 ml of methanol and 5 ml of sample prior to filtration. For each sample, a blank disk was included to account for contamination artefacts that may occur during sample transport and handling. Each disk following filtration was wrapped in thin foil and stored between 0 and 4 °C until sample extraction and clean-up. All glassware was cleaned and then baked out at 450 °C overnight prior to use.

2.2. Sample extraction, clean-up and analysis

Prior to sample analysis and detection of PAHs, each disk was spiked with a mixture of seven deuterated PAHs (10 ng μl^{-1}) to monitor losses during sample extraction/clean up. Disks were extracted with 10 ml of dichloromethane (DCM) twice and extracts reduced to 1 ml under a gentle stream of nitrogen. The final extract was cleaned on a 1 g alumina (baked at 450 °C), 2 g silica (baked at 450 °C) column with a small amount of sodium sulphate (baked at 450 °C), sequentially. Each packed column was washed with two column volumes of hexane:DCM (1:1 v/v) and target compounds eluted with 10 ml of hexane:DCM. The samples and blanks were reduced under a gentle stream of nitrogen and transferred to gas chromatography (GC) vials containing 100 μl of keeper solvent (iso-octane) containing deuterated PAH internal standards (d8-naphthalene, d-10-acenahthalene, d10-phenanthrene, d10-fluoranthene, d12-benzo[*a*]anthracene, d12-perylene) and further reduced to a final volume of 100 μl, before analysis on a gas chromatography-mass spectrometer (Thermo Trace GC Ultra/DSQ GC–MS), equipped with a Phenomenex ZB-MultiResidue-2 column (30 m × 0.25 mm × 0.2 μm).

The PAHs measured in this study were: naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnapht halene, 2,3,6-trimethylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, 1-methylphenanthrene, anthracene, fluoranthene, biphenyl, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[*c,d*]pyrene, dibenz[*a,h*]anthracene, perylene and benzo[*g,h,i*]perylene. Detected PAHs with concentrations (blank – recovery corrected) below zero were excluded from the calculated sum of all PAHs at each site. The sum of all PAHs analysed (total PAHs) is referred to as ΣPAHs hereafter.