

# Distribution and Bioaccumulation of Nitro-, Oxy-, and Hydroxy-Derivatives of PAHs in the White Nile: Insights into Oil Production-Induced Contamination and Health Implications

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## Abstract

The environmental impact of oil production activities in sub-Saharan African countries remains understudied, particularly regarding their potential to release toxic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and their derivatives into ecosystems. This study investigated the concentrations and distribution of nitro-, oxy- and hydroxy-derivatives of PAHs in fish species and sediments from the White Nile near Melut oil fields in South Sudan. Analytes were extracted from fish and sediment samples using accelerated solvent extraction and ultrasonic agitation, respectively, with hydroxy-PAHs derivatized by using BSTFA+TMCS, before analysis with Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). Levels of PAH derivatives in sediments (61.8-757, 10.7-182, and 0.34-124 ng g<sup>-1</sup> dw for nitro-, oxy-, and hydroxy-PAHs, respectively) showed no significant variations amongst sampling locations. In fish, mean levels of the pollutants (748-955, 77.8-242, and 95.7-291 ng g<sup>-1</sup> ww, for nitro-, oxy-, and hydroxy-PAHs, respectively) were significantly higher in *Lates niloticus* compared to *Oreochromis niloticus* and *Clarias gareepinus*.

In all samples, low molecular weight compounds (2-3 rings) were more abundant than high molecular compounds (contributed 77%, and 81% to the  $\Sigma$ PAH derivatives in fish and sediments, respectively). Diagnostic ratios suggested that the PAH derivatives were primarily petrogenic, but health risk assessments suggested minimal health risks to humans through the consumption of fish from the White Nile. Biota-sediment accumulation factors (BSAF) values in fish revealed higher bioaccumulation of lower molecular weight PAH derivatives compared to high molecular weight derivatives. The findings underscore the need for follow-up studies within the river system and its catchments to fully understand the environmental and health implications of oil production activities in the region.

**Keywords:** Nitro-PAHs; hydroxyl PAHs; BSAFs; Health risks, White Nile

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) have been recognized as contaminants in aquatic environments due to their mutagenic and carcinogenic effects in fish and other vertebrates [1]. Derivatives of PAHs, including oxygenated-PAHs (OPAHs), hydroxy-PAHs, (OH-PAHs) and nitro-PAHs (NPAHs), are also classified among major environmental pollutants. The extra functional groups on these compounds influence their behaviours and toxic effects. For example, PAH derivatives usually have lower vapour pressures [2], and a higher tendency to remain in the solid phase compared to their parent compounds. In addition, although PAH derivatives occur in lower concentrations compared to their parent PA, some derivatives of PAHs tend to be more toxic than parent compounds [3, 4, 5].

In aquatic environments, derivatives of PAHs can be introduced through both primary and secondary sources [6]. Primary sources include the combustion of carbon-containing compounds, [7] while secondary sources include transformation processes such as photochemical, photolysis, thermochemical reactions and biological oxidation of their parent PAHs. The compounds can also exist as components of crude oil and refined petroleum products [8]. Other sources of the pollutants include wastewater treatment plants, run-off, and atmospheric deposition. Once in the environment, PAH derivatives tend to accumulate in the sediments through adsorption [9]; the extent of adsorption is influenced by the sediment organic content and how these compounds interact with some dissolved organic matter. PAH derivatives can also remobilize in the water column and become bioavailable to aquatic organisms, such as fish, potentially posing health risks to humans through diet.

The toxic effects of PAH derivatives on humans and other living organisms include carcinogenicity and mutagenicity [10]. In fact, PAH derivatives have also been classified as priority pollutants by the International Agency for Research on Cancer [11]. Despite the potential threats posed by PAH derivatives in the environment, a survey of the existing literature shows that little is known about the occurrence of these pollutants in aquatic environments, especially in sub-Saharan Africa where existing studies focused on parent PAHs [12]. This paucity of data underscores the need for monitoring these emerging pollutants within the ecosystem, such as the White Nile system.

The White Nile is a section of the longest river in the world (the Nile River), running from Lake Victoria in Uganda, through Lakes Albert, to Khartoum in Sudan. The White Nile spans areas of extensive agricultural activities and crude oil drilling activities in Uganda and South Sudan. In particular, crude oil production activities could cause environmental damage by releasing pollutants such as PAH derivatives, toxic elements, salts, and radioactive substances into the river through surface run-off and atmospheric deposition [12]. As such water systems near oil production sites, such as the Melut oil fields, are potential contamination points that could pose risks to human health, aquatic organisms and livestock. In the region near Melut, cases of still-birth, abnormal sicknesses in animals, and deformed newborns raise concerns about possible links to environmental contamination from the ongoing drilling activities. However, further investigation is needed to establish a direct correlation between these effects, emerging pollutants, and oil drilling activities.

The present study aimed to investigate the occurrence, sources, bioaccumulation potential, and health risks posed by PAH derivatives in sediments and fish from White Nile near Melut fields. The specific objectives were to; (1) determine the concentrations of nitro-, oxy-, and hydroxy-PAHs in sediments and the muscles of fish species (*Oreochromis niloticus*, *Lates niloticus* and *Clarias gariepinus*) from the White Nile, (2) estimate the carcinogenic risks of the PAH derivatives in humans, (3) identify the possible sources of the pollutants in sediment samples and, (4) evaluate the bioaccumulation potential of the PAH derivatives in fish samples relative to the sediment samples.

## Materials and Methods

### Study Area

This study was conducted in a section of the White Nile located in the vicinity of Melut oil field (Figure 1). Melut is located in the upper Nile state of South Sudan (10.440 oN, 32.202 oE). The area was chosen because of the ongoing oil drilling and the petrochemical industries. It is possible that the effluents containing PAH derivatives could be reaching the White Nile via runoffs. Three sampling sites A, B and C were selected; the locations were spaced 2 km from each other. Sampling sites A and B were close to oil fields whereas site C is close to the Melut wastewater treatment plant.

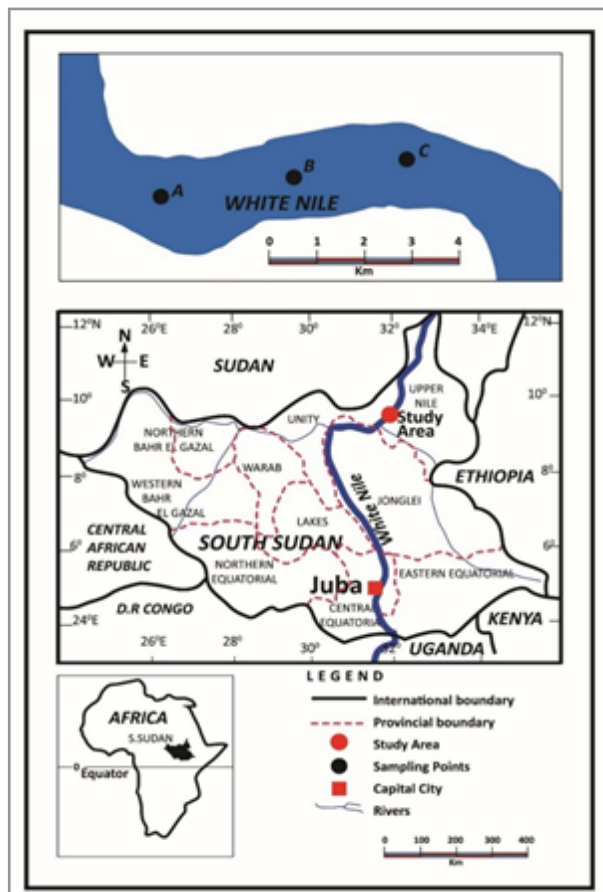


Figure 1: Map showing the study area

### Sampling

Samples were collected between May and August 2022. A total of 30 surficial sediment samples (10-20 cm depth) were collected using a sediment corer. Within each of the sampling sites, ten (10) sediment samples were collected at a distance of approximately 200 m from each other. Similarly, a total of 45 fish samples, comprising 15 samples of each of the three different fish species (*Oreochromis niloticus*, *Lates niloticus* and *Clarias gariepinus*) were collected from the same sampling locations as the sediments. These fish species are the most abundant in the White Nile and are consumed as a dominant source of protein by the local community. The lengths of collected fish samples ranged from 20 to 80 cm while the weights varied from 0.25 to 1 kg. Edible muscle (20 g) was sliced from each fish, wrapped in aluminium foil and labelled. The fish and sediment samples were then transferred into zip-lock polyethylene bags, transferred to cooling boxes packed with dry ice, and transported to the laboratory for analysis. In the laboratory, the samples were kept in a freezer at -20°C to avoid microbial degradation before the extraction of analytes.

### Chemicals and Analytical Standards

Analytical grade dichloromethane (DCM), ethyl acetate, methyl tert-butyl ether (MTBE), methanol and n-hexane were used for extraction. These reagents were purchased from Sigma-Aldrich. Reference standards were procured for PAH derivatives, including 15 NPAH derivatives [1-Nitronaphtalene (1-NNAP), 2-Nitronaphtalene (2-NNAP), 2-Nitrobiphenyl (2-NBP), 5-Ni-

troacenaphthene (5-NACE), 2-Nitrofluorene (2-NF), 9-Nitrofluoracene (9-NPHE), 9-Nitroanthracene (9-NANT), 3-Nitrofluoranthene (3-NPYR), 3-Nitrophenanthrene (3-NFA), 1-Nitropyrene (1-NPYR), 7-Nitrobenz(a)anthracene (7-NBAA), 6-Nitrochrysene (6-NC), 6-Nitrobenz(a)pyrene (6-NBaP), 1,3-Dinitropyrene (1,3-DNP), 2,7-Dinitrofluorene (2,7-DNF)], five OPAH derivatives [Naphthalene-1-aldehyde (NAA), 9-Flourenone(FN), Carbazole (CZ), Acridine (ACr), Quinoline (Qui)], and three OHPAHs derivatives [2-Hydroxybiphenyl (2-OHBP), 1-Hydroxypyrene (1-OHPHE), 6-Hydroxyphenanthrene (6-OHPHE)]. These standards were procured from Accu Standard, USA. Stock and working standards were prepared by serial dilution of the reference standards using DCM as a solvent.

### Analytical Procedure on the Samples

#### Extraction of Sediments

Before extraction, sediment samples were freeze-dried, homogenized, and then sieved using 1 mm mesh. Extraction was then done as described by. Briefly, a portion of the sediment sample (5 g) was weighed and transferred into a 50 mL polypropylene centrifuge tube. DCM (10 mL) was added to the content in the tube and the mixture vortexed for 5 min. The tube and its contents were then sonicated in a high-performance ultrasonic bath for 25 min. The tube was intermittently inverted and shaken to continually re-suspend the samples. The extract was then centrifuged at 3000 rpm for 10 min, and the supernatant was decanted into a previously DCM-rinsed polypropylene tube before clean-up.

## Extraction of Fish

The muscle (5 g) was chopped from the fish samples using a knife. The fish muscle was then ground with anhydrous sodium sulphate (10 g). A mixture of 0.2 M sodium hydroxide in MTBE (2 mL) was added to the grounded mixture, and then vortexed for 5 min before extraction. Extraction was then achieved using an Accelerated solvent extractor (ASE 350) with DCM: n-hexane (1:1 v/v) as the extraction solvent [13]. The volume of extract was reduced to 2 mL using a rotary evaporator before clean-up.

## Clean-up of Sediment and Fish Extracts

The extracts were each cleaned using Oasis® HLB cartridges (6 cc/20 mg; Waters Corporation, Milford, MA, USA) mounted on a Waters extraction manifold (Waters Corporation, Milford, MA, USA), following a method established by [14]. Each cartridge was pre-conditioned with n-hexane (5 mL) followed by 5 mL of a mixture of n-hexane: DCM (1:1 v/v) and then DCM (5 mL). Sample extracts were loaded onto a cartridge, washed with n-hexane (5 mL), and then eluted with 5 mL a mixture of n-hexane: DCM (1:1 v/v), followed by DCM (5 mL), and finally a mixture of DCM: methanol (1:1 v/v, 10 mL). The eluate was evaporated to dryness using a stream of nitrogen and reconstituted into DCM (2 mL). Each reconstituted extract was divided into two fractions. The first fraction was used for the GC-MS/MS analysis of OPAHs and NPAHs. The second fraction was used for the determination of OHPAHs concentrations after derivatization.

## Derivatisation of Hydroxy-PAHs

The derivatisation process was conducted following a method suggested by [15]. Briefly, N, O-bis (trimethylsilyl) trifluoroacetamide/ trimethyl-chlorosilane (BSTFA/TMCS) (10 µL) was added into the GC vials. The vials were quickly screwed with the cap and then placed in an oven at 60 °C for 30 min before GC-MS/MS analysis.

## Gas Chromatographic Analysis

Analysis of PAH derivatives was achieved using a gas chromatograph coupled with a triple quadrupole mass spectrometry (Agilent Technologies, USA). The GC was operated in selective ion monitoring mode. Separation was achieved with a DB-17MS capillary column (20 m length × 0.18 mm internal diameter × 0.18 µm film thickness). The oven temperature was initially set at 70°C for 1 min, then increased at a rate of 5°C / min to 230 °C, and kept at that temperature for 7 min. Helium (99.999% purity) was used as a carrier gas with a constant flow rate of 1.3 mL/min. Acquisition and processing of data was carried out using Agilent Mass Hunter quantitative software. Identification of PAH derivatives was based on a comparison of the retention times and fragmentation patterns of the peaks in the chromatograms of the sample extracts with those obtained from the external standards. Quantification was done using external calibration, with R<sup>2</sup> values all above 0.99.

Estimation of the Carcinogenic Risk of PAH Derivatives in Fish  
The carcinogenic risk of the pollutants was evaluated using the Toxic Equivalence Factors (TEFs) calculated using equation 1.  
$$\text{Carcinogenic risk} = \sum_i (C_i \times \text{TEF}_i) \times \text{URBaP} \dots\dots\dots (1)$$

Where C<sub>i</sub> and TEF<sub>i</sub> are the individual concentration of the PAH derivative and TEF of the target PAH derivative, respectively.

URBaP (unit risk) is the number of people at risk of contracting cancer from exposure to a Benzo (a) Pyrene (BaP) equivalent concentration of 1 ng g<sup>-1</sup> within a lifetime of 70 years. URBaP has a value of 1.1 × 10<sup>-6</sup> (ng g<sup>-1</sup>)-1 (OEHHA, 1994), calculated from cancer potency factor (CPF<sub>i</sub>) using the equation (2) below:  
$$\text{UR}_i = (\text{CPF}_i \times 70) / (70 \text{ Kg} \times \text{CV}) \dots\dots\dots (2)$$
  
Where CPF<sub>i</sub> is the cancer potency factor of the compound i (= 3.9 (mg (Kg-day)<sup>-1</sup>) for B[a]P, 70 Kg is the reference human body weight, and CV is the conversion factor from mg to ng (= 1 × 10<sup>6</sup>).

The calculated carcinogenic risk was compared to a screening value (SV); the threshold concentration of chemicals in edible tissues that are of potential public health concern.  
$$\text{SV} = [\text{RL}/\text{SF} \times \text{BW}] / \text{CR} \dots\dots\dots (3)$$

Where,  
RL (10<sup>-5</sup>) is the maximum acceptable risk level and defined that if a person weighing 70 Kg consumes 142.2 g of fish per day with the same concentration of carcinogen for 70 years, the risk would be less than one additional death per 100,000 persons.

SF (7.30 (µgg<sup>-1</sup>day<sup>-1</sup>)-1) is the US EPA slope factor for PAHs and their derivatives. This value is used to estimate the upper bound probability of an individual acquiring cancer due to exposure to a certain carcinogen level for 70 years.

BW (70 Kg) is the average body weight of an adult human.  
CR (142.2 (g day)<sup>-1</sup>) is the average human consumption rate of fish.

## Quality Control (QC) and Quality Assurance (QA)

QC and QA protocols included determining limits of detection (LODs) and using procedural blanks for recovery studies. LODs and LOQs were estimated as analyte concentrations corresponding to signal-to-noise ratios of 3 and 10, respectively. Samples with analyte levels ≥ LOD were considered positive and analyte levels ≥ LOQ were quantifiable. Recovery tests were conducted by spiking procedural blanks with PAH derivative standards at levels of 0.2 and 0.6 ng g<sup>-1</sup>. All the recoveries were within the acceptable range of 62 to 120% (Pulleyblank et al., 2020) and, hence, the data reported here was not corrected for recoveries.

## Identification of the Sources of PAH Derivatives

To establish whether the sources of PAH derivatives in sediments were pyrogenic (combustion-related) or petrogenic (petroleum-related), the ratio of Lower molecular PAH derivatives (ΣLPAHDs) to High molecular PAH derivatives (ΣHPAHDs) was evaluated. Normally, a ratio less than 1 indicates a pyrogenic source, whereas a ratio greater than 1 suggests a petrogenic origin of the pollutants [16].

## Determination of Total Organic Carbon (TOC) in Sediments

Total organic carbon (TOC) in the sediments was determined as described by. The sample (1 g) was weighed into a glass tube and potassium dichromate solution (5 mL) was added to it, followed by 2M sulphuric acid (7.5 mL). The mixture was heated in a water bath at 75 °C for 30 min and later allowed to cool. The digested content in the tube was transferred to a 250 mL conical flask and 0.3 mL of ferroin indicator was added to it. The mixture was then titrated with ferrous ammonium sulphate solution and the



titre value (t) recorded. TOC content was calculated according to equation 4 below.

$$\text{TOC (\%)} = (t \times 0.3 \times 0.2) / (\text{sample weight}) \dots \dots \dots (4)$$

### Determination of Lipid Content in Fish Muscles

The lipid content in fish muscles was determined as reported by [17]. The muscle (5 g) was extracted using ethyl acetate (20 mL) in an ultrasonic bath for 30 min. The extraction was repeated once. The solvent from the combined extracts were evaporated to dryness using a stream of nitrogen, and the lipid content determined gravimetrically as the residual mass of each extract after complete evaporation of the organic solvent.

### Estimating the Bioaccumulation Potential of PAH Derivatives in Fish Muscles Relative to Sediments

The bioaccumulation potential of PAH derivatives in fish was estimated using Biota-Sediment Accumulation Factors, or BSAF [18, 19]. BSAF is expressed as a ratio of the analyte's concentration in the fish's tissue normalized to lipid content in fish to the concentration of the same analyte in sediments normalized to TOC. The model assumes that: (i) equilibrium between uptake and elimination of a pollutant (ii) both the organism and its food were exposed to sediments (iii) the sediment used for estimating the pollutant concentration is the same as that to which the organism is exposed (iv) no transformation of the pollutant in the sediment, water, or organism. In the present study, BSAF were calculated using equation 5.

$$\text{BSAF} = (C_{0/f_1}) / (C_{s/f_{soc}}) \dots \dots \dots (5)$$

where;

Co is the PAH derivative's concentration (ng g<sup>-1</sup> ww) in the fish muscle

f1 is the lipid fraction of the muscles (g lipid g<sup>-1</sup> ww)

Cs is the PAH concentration (ng g<sup>-1</sup> dw) in sediment and

fsoc is the sediment organic carbon (g organic carbon g<sup>-1</sup> dw).

### Statistical Data Analysis

Statistical analysis was done using Origin Pro (version 8) software. Descriptive statistics, reporting the means, medians, ranges, and standard deviations were reported. Arithmetic means were calculated from only positive quantifiable samples. T-tests were used to determine the statistical differences between the means of individual PAH derivatives while a Student t-test was used to determine the difference between means of lower-molecular weight (with 3 aromatic rings; LPAHDs) and high-molecular-weight PAH derivatives (> 3 aromatic rings; HPAHDs) in sediments and fish. One-way ANOVA was used to establish the statistical differences among mean concentrations of different groups of PAH derivatives at different sampling sites, between fish species, and among various groups of PAH derivatives in both sediments and fish. Statistical differences were confirmed using a Tukey's HSD post-hoc test. To establish the relationships between the levels of PAH derivatives and organic carbon content for sediments or lipid content for fish samples, Pearson correlation coefficients between log-transformed PAH derivative levels and the sample parameters were calculated. In all cases, the normality of the data was checked using the Shapiro-Wilk test and statistical differences were deemed significant at p<0.05.

### Results and Discussion

#### Concentrations of PAH Derivatives in Sediment Samples

The concentrations of the 15 NPAHs, 5 OPAHs and 3 OHPAHs quantified in sediments are shown in.

Table 1. Levels of total (Σ) NPAHs, OPAHs and OHPAHs were 319.28, 54.05 and 53.07 ng g<sup>-1</sup>dw at station A, 312.68, 34.31 and 47.02 ng g<sup>-1</sup>dw at station B, and 184.21, 30.85 and 17.81 ng g<sup>-1</sup>dw at station C, respectively. These levels did not differ significantly amongst sampling locations (p>0.05, ANOVA), suggesting a similar source.

**Table 1: Mean levels (ng g<sup>-1</sup>dw) of the PAH derivatives in sediment samples**

| PAH derivatives | NR | Sediment sites |        |           |              |        |           |             |        |           |
|-----------------|----|----------------|--------|-----------|--------------|--------|-----------|-------------|--------|-----------|
|                 |    | A (N=10)       |        |           | B (N=10)     |        |           | C (N=10)    |        |           |
|                 |    | Mean ± STD     | Median | Min – Max | Mean ± STD   | Median | Min-Max   | Mean ± STD  | Median | Min-Max   |
| 1-NNAP          | 2  | 121± 154       | 56.9   | nd-437    | 96.5 ± 107   | 49.6   | nd-310    | 67.7 ± 128  | 5.44   | nd-436.7  |
| 2-NNAP          | 2  | 77.7 ± 128     | 1.1    | nd-388    | 87.7 ± 99.6  | 64.7   | nd-315    | 27 ± 57.5   | 0.42   | nd-192    |
| 2-NBP           | 2  | 15.6 ± 7.79    | 13.9   | 4.02-32.3 | 9.89 ± 4.42  | 9.86   | 1.65-19.4 | 12.1 ± 5.62 | 9.6    | 7.42-24.6 |
| 5-NACE          | 3  | 6.83 ± 3.58    | 6.09   | nd-15     | 20.0 ± 26.9  | 7.01   | nd-79.3   | 2.14 ± 3.45 | -      | nd-9.52   |
| 2-NF            | 3  | 11.3 ± 16.1    | 7.65   | nd-57.8   | 14.3 ± 8.02  | 12.9   | nd-27.8   | 10.6 ± 8.42 | 8.09   | nd-27.3   |
| 9-NPHE          | 3  | 5.03 ± 3.37    | 6.38   | nd-9.62   | 4.94 ± 3.51  | 5.3    | nd-10.6   | 4.13 ± 4.23 | 2.47   | nd-10.2   |
| 9-NANT          | 3  | 6.83 ± 6.91    | 5.25   | nd-24.3   | 6.05 ± 3.99  | 7.56   | nd-10.8   | 10.1 ± 12.1 | 6.93   | nd-44.6   |
| 3-NFA           | 4  | 7.93 ± 3.18    | 8.81   | nd-11.8   | 5.75 ± 2.63  | 6.47   | nd-8.69   | 4.77 ± 2.82 | 5.17   | nd-8.66   |
| 3-NPHE          | 3  | 3.67 ± 5.11    | 0.87   | nd-15.1   | 7.97 ± 12.7  | -      | nd-37     | 2.50 ± 4.02 | -      | nd-11.6   |
| 1-NPYR          | 3  | 15.3 ± 15.5    | 10.1   | nd-45.8   | 16.8 ± 15.2  | 12.2   | nd-54     | 14.9 ± 10.3 | 13.15  | nd-29.4   |
| 7-NBAA          | 4  | 3.93 ± 8.55    | -      | nd-28.6   | -            | -      | -         | -           | -      | -         |
| 6-NC            | 4  | 11.9 ± 7.35    | 12.9   | nd-25.9   | 12.11 ± 8.96 | 10.1   | nd-36.2   | 8.62 ± 5.57 | 7.66   | nd-17.5   |
| 6-NBaP          | 5  | 15.2 ± 9.29    | 15.4   | nd-30.7   | 19.1 ± 10.4  | 15.4   | 13.6-50   | 16.1 ± 8.04 | 15.3   | nd-35.5   |
| 1,3-DNP         | 4  | 6.75 ± 4.84    | 7.09   | nd-18.1   | 2.49 ± 1.86  | 2.09   | nd-5.19   | 3.21 ± 3.83 | 2.08   | nd-13.5   |
| 2,7-DNF         | 3  | 10.5 ± 8.59    | 10.2   | nd-29.2   | 8.81 ± 6.33  | 8.45   | nd-24.7   | 7.08 ± 5.66 | 6.8    | nd-20.1   |

|         |        |             |      |          |             |      |           |              |      |           |
|---------|--------|-------------|------|----------|-------------|------|-----------|--------------|------|-----------|
| ΣNPAHs  |        | 319 ± 189   | 257  | 116-621  | 313 ± 186   | 306  | 88.4-757  | 184 ± 158    | 120  | 61.8-602  |
| NAA     | 2      | 10.8 ± 7.51 | 9.83 | nd-24    | 7.29 ± 5.43 | 6.04 | nd-21.5   | 6.69 ± 4.16  | 6.34 | nd-16.4   |
| FN      | 3      | 2.57 ± 2.51 | 2.27 | nd-8.34  | 1.85 ± 1.15 | 1.99 | nd-3.32   | 1.73 ± 1.06  | 1.95 | nd-3.54   |
| CZ      | 3      | 15.4 ± 11.6 | 14.6 | nd-37.4  | 8.59 ± 7.78 | 7.72 | nd-26.5   | 8.77 ± 6.29  | 8.68 | nd-21.9   |
| ACr     | 3      | 16.8 ± 24.3 | 10.1 | nd-87.8  | 10.5 ± 7.44 | 10.9 | nd-28.1   | 11.2 ± 12.6  | 8.3  | nd-46.5   |
| Qui     | 2      | 8.48 ± 8.56 | 4.58 | nd-24.9  | 6.07 ± 10.2 | -    | nd-28.6   | 2.44 ± 5.13  | -    | nd-15.8   |
| ΣOPAHs  |        | 54.1 ± 47.1 | 41.3 | 15.5-182 | 34.3 ± 18.5 | 29.8 | 16.2-78.7 | 30.9 ± 21.8  | 22.9 | 10.7-87.4 |
| 2-OHBP  | 2      | 15.8 ± 10.2 | 12.3 | nd-41.4  | 10.0 ± 11.0 | 7.2  | nd-40.4   | 0.45 ± 0.88  | -    | nd-3.10   |
| 1-OHPYR | 4      | 20.2 ± 32.1 | 3.52 | nd-101   | 25.8 ± 34.4 | 3.77 | nd-97.8   | 8.17 ± 10.8  | 2.88 | nd-28.7   |
| 6-OHPHE | 3      | 17.2 ± 13.2 | 15   | nd-44.9  | 11.2 ± 8.41 | 9.2  | 1.94-32.2 | 9.19 ± 7.65  | 6.81 | nd-27.7   |
| ΣOHPAHs |        | 53.1 ± 35.2 | 33.4 | 15.9-114 | 47.0 ± 42.1 | 21.4 | 15.1-124  | 17.8 ± 19.4  | 9.85 | 0.34-59.5 |
| LPAHDs  | NR ≤ 3 | 361 ± 426   | 187  | 11.3-437 | 328 ± 339   | 221  | 5.29-309  | 198.8 ± 277  | 85.4 | 9.25-437  |
| HPAHDs  | NR ≥ 4 | 65.8 ± 65.3 | 47.3 | 2.64-101 | 766 ± 58.2  | 38.1 | 16-97.8   | 41.2 ± 31.1  | 33.4 | 0.83-35.5 |
| % TOC   |        | 0.93 ± 0.01 |      |          | 0.66 ± 0.27 |      |           | 3.09 ± 0.476 |      |           |

NR= number of rings, nd=non-detectable (below the limit of detection), N= number of sediment samples, levels reported as mean ± standard deviation

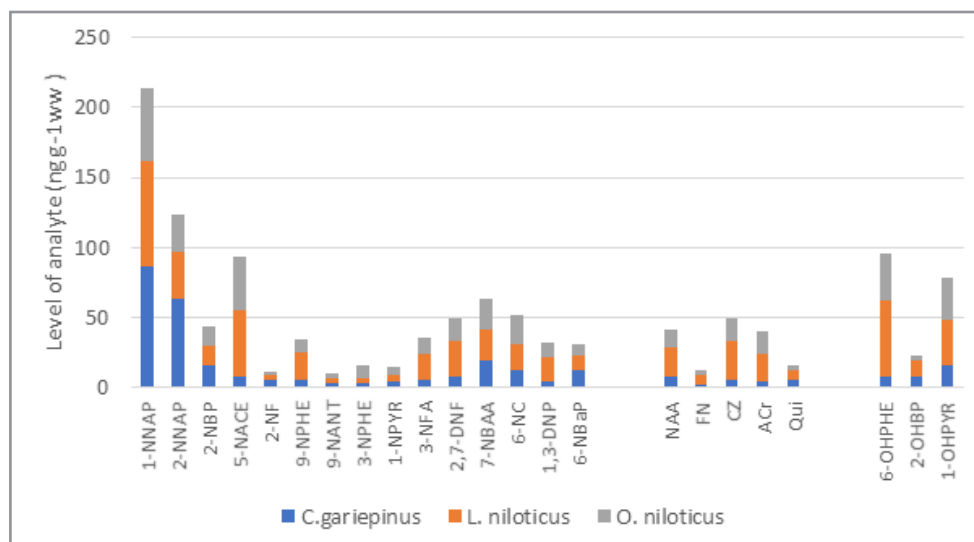
Overall, at the studied sites, low molecular weight PAH derivatives (LPAHDs) accounted for 83.8 % of total PAH derivatives. The predominance of LPAHDs over HPAHDs in the sediments could be related to combustion-related sources, such as vehicular emissions and petroleum residues. Besides, PAHs of lower molecular weight are easier to degrade into their corresponding derivatives compared to PAHs of high molecular weight.

In terms of specific analytes, 1-nitronaphthalene was the most abundant with 33% of the ΣLPAHDs and 1-hydroxypyrene was the most abundant analyte among HPAHDs (contributing 27% to ΣHPAHDs). Among nitro-PAH derivatives, 1-nitronaphthalene was the most common analyte, contributing 35% to total nitro-PAH derivatives, on average, followed by 2-nitronaphthalene (24%), while 7-nitrobenzo (a)anthracene was the least abundant pollutant (0.46%). For oxy-PAHs, acridine and 1-hydroxizine were the most dominant, contributing 28% and 59%, respectively. The similarity in the distribution patterns of PAH derivatives among different sites was indicative of a common source of pollution [20]. The mean difference between LPAHD and HPAHDs at site A was statistically significantly different ( $p < 0.05$ , student t-test). At sites B and C, the mean differences between LPAHD and HPAHD did not differ significantly ( $p > 0.05$ , student t-test). There was a significant variation in the mean difference of the sediments across the three sampling sites A, B and C ( $p < 0.05$ , one-way ANOVA). Tukey's (post-hoc) test revealed that the mean differences of the levels in sediments at sites A and B were statistically similar compared to that of site C. There was a significant variation in the mean levels of the three different groups of PAH derivative compounds (NPAHs, OPAHs and OHPAHs) in sediment ( $p < 0.05$ , ANOVA).

In comparison with established Sediment Quality Guidelines (SQGs), the levels of PAH derivatives quantified in the present study were within acceptable ranges, suggesting little to no probable effects on organisms resulting from exposure to PAH derivatives. The results of the present study (nd-437.3 ng g<sup>-1</sup> dw for NPAHs, nd-87.8 ng g<sup>-1</sup> dw for OPAHs and nd-100.9 ng g<sup>-1</sup> dw for OHPAHs) were also compared to other studies. These levels of PAH derivatives were lower than those reported in the Niger River, West Africa (533.6-2022.8 ng g<sup>-1</sup> dw for 8NPAHs; However, they were higher than those reported in Jiuxiang river, China (0.07-45.4 ng g<sup>-1</sup> dw for 14 NPAHs, 0.46-11.1 ng g<sup>-1</sup> dw for 50PAHs; (0.28-176 ng g<sup>-1</sup> dw for 12 NPAHs) [21]. Our results were also higher than those reported in Gotska Sandon island, Sweden (1.91- 2.65 ng g<sup>-1</sup> dw for 7 NPAHs; Brorstrom-Lunden et al, 2010), in river Elbe basin, Czech Republic (5.02-18.9 ng g<sup>-1</sup> dw for 9NPAHs; The differences in PAH derivative levels in various studied sites may be due to differences in pollution sources.

#### Concentrations of PAH Derivatives in Fish Samples

Total (Σ)15 Nitro-PAHs, (Σ)5 Oxy-PAHs and (Σ)3 Hydroxy-PAHs were 764.2, 77.8, and 95.7 ng g<sup>-1</sup> ww, respectively in *C. gariepinus*; 954.7, 242.3, and 291.3 ng g<sup>-1</sup> ww, respectively, in *L. niloticus*; and 747.8, 159.2, and 204.1 ng g<sup>-1</sup> ww, respectively, in *O. niloticus* (Figure 2) Like in sediments, 1-Nitronaphthalene was the most abundant analyte in all fish samples (contributed 26% to ΣNitro-PAHs). Naphthalene-1-aldehyde at 26% and 6-hydroxyphenanthrene at 70% had the highest concentrations among OPAHs and OHPAHs respectively, in all the three fish species in this study.



**Figure 2:** Levels of NPAHs, OPAHs and OHPAHs in three fish species from the White Nile (all sites A, B, and C).

In the present study, *L. niloticus* is found to have higher concentrations of PAH derivatives compared to other fish species (Tables S2-S4). This trend can be explained by the versatile feeding behaviours of *L. niloticus* that are characterised by a generalist and opportunistic omnivorous [22]. The diet composition of *L. niloticus* also varies within a wide range of temporal and spatial conditions of the environment [23], making the fish species susceptible to feeding on more contamination sources.

In fish, the mean levels of LPAHDs and HPAHDs were statistically different in both *L. niloticus* and *C. gariepinus*, ( $p < 0.05$ , student t-test), but statistically similar for *O. niloticus*, ( $p > 0.05$ , student t-test). There was a significant variation in the mean difference in the concentration of PAH derivative across the three different fish species, ( $p < 0.05$ , one-way ANOVA). Tukey's (post-hoc) test revealed that the mean difference of the PAH derivatives in *L. niloticus* and *C. gariepinus* was statistically similar compared to that of *O. niloticus*. The three different groups PAH derivative compounds (NPAHs, OPAHs and OHPAHs) showed a significant variation in the three different fish species *L. niloticus*, *O. niloticus* and *C. gariepinus*, ( $p < 0.05$ , one-way ANOVA). Tukey's (post-hoc) test revealed that the mean differences were statistically different across all the three different groups of compounds (NPAHs, OPAHs and OHPAHs) in all the three fish species *C. gariepinus*, *L. niloticus* and *O. niloticus*.

Like in sediments, LPAHDs were more abundant than HPAHDs (accounted for 77% of the PAH derivatives in all three fish species). This pattern could be attributed to the higher aqueous solubilities and lower octanol-water partition coefficients of LPAHDs which enhances their higher water-gill transfer efficiencies compared to HPAHDs (Nwaichi and Ntorgbo, 2016). In addition, compared to LPAHDs, HPAHDs are also more rapidly metabolized by fish due to their high enzyme affinity [24].

In comparison to other studies, PAH derivative levels in fish of this study were lower than those reported in Ghana, Africa (28-1534 ng g<sup>-1</sup> ww for 15 OPAHs), The OHPAH levels reported were within the range (nd-239 ng g<sup>-1</sup> ww) reported for 15 OHPAHs in *L. Microdon*, *L. Argentimaculatus* and *S. Guttatus* from Iran (Cappello, 2018). However, the levels in the present study were higher than those reported for 9 OPAHs in Mackerel fish species from Cheech Republic, (0.04-21.2 ng g<sup>-1</sup> ww; and for 13 NPAHs, 2 OPAHs and 4 OHPAHs (nd-187.7 ng g<sup>-1</sup> ww) in Salmon, Herring, Mackerel and Halibut fish species from Denmark, Europe [25].

#### Health Risks Associated with Human Consumption of fish PAH Derivatives in Fish

Only the published TEF values of selected NPAHs (Table S1) were used to evaluate the carcinogenic risk of PAH derivatives in this study because there are no published TEF values for OPAHs and OHPAHs. The total carcinogenic risk of PAH derivatives was  $1.8 \times 10^{-3}$  ng g<sup>-1</sup> ww with *O. niloticus* associated with the highest risk of  $7.82 \times 10^{-4}$  ng g<sup>-1</sup> ww. The carcinogenic risk values in the present study were lower than the screening values, implying that the consumption of fish from White Nile is safe for human health as a result of exposure to NPAHs, OPAHs and OHPAHs.

#### Source Apportionment of PAH Derivatives in Sediments

- As discussed above, the ratio of lower molecular PAH derivatives ( $\sum$ LPAHDs) to high molecular PAH derivatives ( $\sum$ HPAHDs) can be used to identify the source of PAH derivatives. As shown in Table 2,
- most of these ratios were  $>1$  at all sites, suggesting a predominantly petrogenic origin of the PAH derivatives.
- Only two sampling points showed a ratio of  $<1$ .

**Table 2: Diagnostic ratios of PAH derivatives in sediments**

| Site | Samples | $\Sigma$ LMWPAHDs | $\Sigma$ HMWPAHs | $\Sigma$ LMWPAHDs/ $\Sigma$ HMWPAHs |
|------|---------|-------------------|------------------|-------------------------------------|
| A    | SA1     | 702               | 106.94           | 6.56                                |
|      | SA2     | 270               | 9.84             | 27.39                               |
|      | SA3     | 207               | 34.81            | 5.95                                |
|      | SA4     | 94.7              | 74.91            | 1.26                                |
|      | SA5     | 493               | 10.80            | 45.62                               |
|      | SA6     | 623               | 56.30            | 11.06                               |
|      | SA7     | 174               | 44.82            | 3.88                                |
|      | SA8     | 396               | 147.93           | 2.69                                |
|      | SA9     | 128               | 124.48           | 1.03                                |
|      | SA10    | 428               | 129.12           | 3.32                                |
| B    | SB1     | 390               | 46.39            | 8.39                                |
|      | SB2     | 320               | 24.54            | 13.05                               |
|      | SB3     | 91.4              | 123.72           | 0.74                                |
|      | SB4     | 346               | 49.33            | 7.01                                |
|      | SB5     | 777               | 133.49           | 5.82                                |
|      | SB6     | 447               | 43.58            | 10.25                               |
|      | SB7     | 77.5              | 67.18            | 1.15                                |
|      | SB8     | 282               | 196.19           | 1.44                                |
|      | SB9     | 278               | 65.91            | 4.21                                |
|      | SB10    | 130               | 45.12            | 2.87                                |
| C    | SC1     | 119               | 88.82            | 1.34                                |
|      | SC2     | 361               | 40.48            | 8.91                                |
|      | SC3     | 115               | 24.23            | 4.73                                |
|      | SC4     | 60.4              | 30.33            | 1.99                                |
|      | SC5     | 77.7              | 61.69            | 1.26                                |
|      | SC6     | 609               | 25.18            | 24.19                               |
|      | SC7     | 172               | 32.47            | 5.29                                |
|      | SC8     | 51.4              | 54.04            | 0.95                                |
|      | SC9     | 212               | 115.88           | 1.83                                |
|      | SC10    | 82.1              | 46.26            | 1.77                                |

LMWPAHDs- Lower molecular weight PAHs; HMWPAHDs – High molecular weight PAHs

### Correlation of PAH Derivatives Concentrations with TOC in Sediment Samples and Lipid in Fish Samples

Generally, the PAH derivative levels in sediments did not show a significant correlation with TOC ( $p > 0.05$ ) at sites A, B and C, except for 2-OHBP at site A and 1-NNAP at site B. The lack of significant correlation suggested that, apart from TOC, other factors such as the intensity of input from the primary sources of the location rather than sedimentary characteristics may have influenced the sediment burdens with respect to the pollutants. A high mobility, degradation potential, and formation of these compounds in the sediments could also explain the lack of correlation observed [26].

Similarly, no significant correlation ( $p > 0.05$ ) was observed between PAH derivative concentrations in the fish species with the lipid content, except 5-NACE in *L. niloticus* ( $p = 0.039$ ), and 3-NFA in *C. gariepinus* ( $p = 0.038$ ). This could suggest that levels of PAH derivatives in fish muscle may not have reached chemical equilibrium, and that the lipid content was not a key factor for the

PAH derivative's accumulation in the fish tissues. A similar study of 15 oxygenated PAHs in muscle and gut +gill tissues of demersal fishes (*Drapane Africana*, *Cynoglossus senegalensis* and *Pomadasys peroteti*) from three locations along the coast of the Gulf of Guinea (Ghana) also reported no significant correlations between the pollutant levels and lipid content [27]. The findings suggested that additional factors such as age and feeding habits were more important than lipid content in explaining the observed differences between the concentrations of the PAH derivatives in fish.

### Estimation of Accumulation of PAH Derivatives in Fish Relative to Sediments

Table 3 shows the BSAFs for the PAH derivatives in the three fish species (*C. gariepinus* L. *niloticus* and *O. niloticus*) at sampling sites A, B and C. BSAF values for the PAH derivatives in *C. gariepinus* ranged from 0.03 g OC g-lipid to 1116 g OC g-lipid, 2.0 to 1674 g OC g-lipid in *L. niloticus*, and 9.39 to 2138 g OC g-lipid in *O. niloticus*.



**Table 3: BSAFs (g OC g<sup>-1</sup> lipid) values for PAH derivatives in the fish species**

| Analyte |             | C.<br>gariepinus |              |             | L.niloticus |              |             | O. niloticus |              |
|---------|-------------|------------------|--------------|-------------|-------------|--------------|-------------|--------------|--------------|
|         | SITE A      | SITE B           | SITE C       | SITE A      | SITE B      | SITE C       | SITE A      | SITE B       | SITE C       |
|         | BSAF        |                  |              | BSAF        |             |              | BSAF        |              |              |
| 1-NNAP  | 5.7 ± 4.90  | 45.2 ± 62.5      | 18.9 ± 23.5  | 110 ± 154   | 40.8 ± 2.7  | 320          | 23.9 ± 13.0 | 15.6 ± 19.3  | 14.6         |
| 2-NNAP  | 50.1 ± 31.7 | 10.1 ± 0.20      | 1116         | 622 ± 845   | 3.4 ± 1.5   | 7.5          | 285 ± 487   | 19.2 ± 25.7  | 1979 ± 2766  |
| 2-NBP   | 39.7 ± 22.4 | 76.6 ± 92.5      | 283.2 ± 109  | 16.3 ± 9.30 | 12.5 ± 8.5  | 294 ± 118    | 40.4 ± 28.3 | 123 ± 100    | 488 ± 431    |
| 5-NACE  | -           | 26.8             | -            | 82.8 ± 13.5 | 7.1 ± 2.6   |              | 157 ± 63.9  | 21.9 ± 20.1  |              |
| 2-NF    | -           | 28.9 ± 21.1      | 80 ± 72.9    |             | 6.9         | 46.9         |             | 24.9 ± 0.7   | 53.6 ± 22.6  |
| 9-NPHE  | 17.3        | 11.7 ± 8.60      | 35.6 ± 5.60  | 32.6        | 30.1 ± 6.8  | 340 ± 261    | 103.4       | 98 ± 58.1    | 173 ± 17.9   |
| 9-NANT  | -           | 325              | -            | -           | 63.2        | 295.1 ± 31.8 | -           | 181.1        | 260          |
| 3-NFA   | 5.7 ± 2.1   | 204              | 19.6         | 67.4 ± 67.4 | 27.6 ± 11.2 | 1674 ± 2300  | 46.8 ± 37.9 | 84.5 ± 51.1  | 102 ± 56     |
| 3-NPHE  | 28.2 ± 12.9 | 1.32             | 76.9         | 15.2 ± 10.8 | 2.6         | 124 ± 98.5   | 48.2        | -            | 421 ± 589    |
| 1-NPYR  | -           | 24.7 ± 17.5      | 90.7 ± 34.2  | 5.3         | 3.6         | 191.9 ± 142  | -           | 103 ± 143    | 142 ± 92.1   |
| 7-NBAA  | 31.2        | -                | -            | -           | -           | 150          | 350         | -            | -            |
| 6-NC    | 16.4 ± 7.90 | 57.3 ± 30.8      | 113.1 ± 56.8 | 19.7        | 16.8 ± 1.9  | 272 ± 139    | 75.8        | 116 ± 23.1   | 245 ± 31.5   |
| 1,3-DNP | -           | -                | -            | 47.1 ± 22.5 | 60.5 ± 47   | 638          | -           | 481 ± 619    | 686          |
| 6-NBaP  | 17.6        | 130              | 110 ± 63.2   | 8.5         | 6.3         | -            | 18.7        | 48.7         | 133.5        |
| 2,7-DNF | -           | 51.2 ± 45.7      | 37.2 ± 2.90  | 16.8        | 31.7 ± 12.9 | 386 ± 84.1   | 55          | 102 ± 98.2   | 272 ± 85.1   |
| NAA     | 8.9 ± 1.10  | 135.4 ± 210      | 50.9 ± 12.5  | 24.3 ± 12.1 | 28.5 ± 11.4 | 572 ± 401    | 36.8 ± 19.3 | 94.5 ± 60.3  | 242 ± 63.9   |
| FN      | -           | 2.6 ± 1.7        | 12.4         | 11.4        | 9.1 ± 0.9   | 199 ± 158    | 37.9        | 28.4 ± 13.9  | 117.2 ± 39.8 |
| CZ      | 11.4 ± 6.40 | 0.03             | 28.5         | 20.7 ± 7.90 | 30.8 ± 21.5 | 316 ± 32.8   | 32.7 ± 14.4 | 92.4 ± 68.2  | 232 ± 87.9   |
| ACr     | 14.4 ± 6.10 | -                | 91.8 ± 48.3  | 46.5 ± 29.8 | 16.6        | 392 ± 362    | 102 ± 50.1  | 60.9 ± 49    | 383 ± 180    |
| Qui     | 35.8 ± 3.40 | 8.9              | 79.8 ± 25    | 11.6 ± 1.90 | 2           | 127 ± 107    | 31.3        | -            | 45.6         |
| 2-OHBP  | 113 ± 184   | 27.4             | 290          | 128         | 32.2 ± 20.4 | -            | 27.6        | -            | -            |
| 1-OHPYR | 13.9 ± 5.40 | 0.09             | 71.7 ± 62.4  | 19.7 ± 20.9 | 6.3         | 397 ± 233    | 16.6        | 9.4          | 1832 ± 1791  |
| 6-OHPHE | 4.9 ± 0.3   | 4.2              | 30           | 181 ± 262   | 46.4 ± 17.8 | 1223 ± 1029  | 44.1 ± 26.8 | 162 ± 102    | 1429 ± 1706  |

BSAFs are reported as mean ± standard deviation

BSAFs in fish varied significantly. *O. niloticus* exhibited higher bio-accumulation levels (mean BSAFs: 223 g OC g<sup>-1</sup>lipid) compared to *L. niloticus* (165) and *C. gariepinus* (73.5). This difference could be attributed to feeding behaviours, reproductive status, metabolism, trophic level and biomagnification capacity of each fish species (Yu et al., 2019). The lower bio-accumulation levels of PAH derivatives in *C. gariepinus* could be attributed to its low ranking in trophic level compared to *O. niloticus* and *O. niloticus* [28]. The higher BSAF values in *O. niloticus* could be due to its inshore-dwelling, omnivorous feeding behaviours compared to the pelagic, piscivorous behaviours for *L. niloticus* [29].

Noteworthy, sampling site C had significantly higher BSAF values (mean: 342.54 g OC g<sup>-1</sup>lipid) compared to A (60.32) and B (58.69). Site C is close to the Melut water treatment plant whose effluents may have contributed to high levels of PAH derivatives in the fish species. Generally, LPAHDs had higher BSAF values compared to HPAHDs. This trend could be attributed to the high aqueous solubility of LPAHDs which increases their bioaccumulation through direct water-gill and skin transfers in addition to dietary uptake. On the other hand, the lower BSAF values in HPAHDs could be attributed to their strong adsorption to sediments containing organic matter. HPAHDs are generally less water soluble which lowers their bioavailability and bioaccumulation in the fish's tissues [30].

### Relationship Between BSAF Values for PAH Derivatives in Fish and Log Kow Values

Both positive and negative linear relationships between BSAF values of PAH derivatives and their respective Log Kow values were established in all the fish species studied (Figures S1-S3). The positive relationship could be attributed to PAH derivative congeners with larger Log Kow that tend to be more sorbed into the sediment organic matter than those with lower Log Kow [31]. It may also be attributed to PAH derivative congeners with larger Log Kow being less readily transported to the river system due to their sorption to soils, leading to higher emission of PAH derivatives with higher Log Kow before observing their concentrations in river sediments. The negative linear relation could be attributed to the higher gill transfer efficiencies for bioaccumulation and biotransformation in fish for PAH derivatives with lower Log Kow values [32, 33]. Therefore, PAH derivatives of lower Log Kow bioaccumulate more in fish compared to those with larger Log Kow values.

### Conclusions

This study has investigated 23 PAH derivatives (including NPAHs, OPAHs and OHPAHs) in fish and sediments from the upper Nile States of White Nile near Melut oil fields, South Sudan. The concentration levels of PAH derivatives did not differ significantly in sediments from different sampling sites, indicat-

ing a common source of pollution. However, the different groups of PAH derivatives showed a significant variation in the three different fish species *L. niloticus*, *O. niloticus* and *C. gariepinus*. Low molecular weight compounds were more abundant in both sediments and fish samples [34-45]. The total carcinogenic risk value is lower than the screening value and the maximum limit set by the European Union for fish, implying minimal risks to humans from the consumption of fish from White Nile with respect to PAH derivatives. Diagnostic ratios revealed the derivatives are mainly of petrogenic origin, such as oil production activities and the petrochemical industries near the study area [45-65]. BSAF values showed that LPAHDs accumulate more than HPAHDs. A study on other environmental matrices like water to better understand the extent of pollution by PAH derivatives in the White Nile is recommended. Follow-up studies with a wider range of sampling locations within the Nile River system and its catchment and an extended list of analytes are recommended. Such studies will improve our understanding of the magnitude and spatial distribution of PAHs, as well as their fate, bioaccumulation, and probable effects in the White Nile environment [66-77].

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