Original Research Article

Human Health Through Ingestion of Freshwater Fish (*Labeocoubie* and *Hydrocynusvittatus*) from River Benue, Nigeria

ABSTRACT

Alkylphenols (APs) and Alkylphenols ethoxylates (APEs), are ubiquitous estrogenic chemicals, widely distributed in fish due to their persistence and lipophilicity. This study determined levels of APs and APEs, and the associated human health risk in two fish species, 16 *Labeocoubie*(African carp*)* and 16*Hydrocynusvittatus*(African Tiger fish) sampledquarterly from River Benue, central Nigeria between September 2019 and June 2020. Compounds were identified and quantified using Gas Chromatography with Mass Spectrometric detection (GC-MS). Levels of twenty APs ($\sum_{20}$APs) in *Labeo cubie* ranged from ND-16.0 ngg^{-1} wet wt, with total and maximum values of 94.5 ngg^{-1} wet wt, and 16.0 ngg^{-1} wet wt respectively. Residues of four APEs ($\sum_{4}$APEs) ranged from ND-3.1 ngg^{-1} wet wt, total of 5.0 ngg^{-1} wet wt, and maximum of 3.1ngg^{-1} wet wt respectively. In *Hydrocynus vitatus,* $\sum_{20}$APs ranged from ND-27.8 ngg^{-1} wet wt, total and maximum of 205 and 27.8 ngg^{-1} wet wt, respectively. Estimated risk from ingestion of compounds in *Labeo cubie* and *Hydrocynus vitatus* by adult and children indicate no immediate potential harm as the hazard quotient (HQ) value was less than one (HQ < 1). However, children could suffer higher body burdens of these pollutants than adults if they are fed with these fish. This study provides baseline data on levels and estimated human health risk from ingestion of these two species of fish contaminated by APs and APEs to regulatory agents and future researchers.

Keywords: Alkylphenols, Alkylphenol Ethoxylates, Fish, River Benue, Central Nigeria

1. INTRODUCTION

Alkylphenols (APs) are phenolic compounds obtained when one or more hydrogen atoms of the phenol are substituted by alkyl group, while ethoxylation of APs result into the nonionic surfactant known as Alkylphenol ethoxylates (APEs) [1].APs and APEs are ubiquitous in the environment owing to their wide use in agriculture, household products and industry since 1950s [1].These groups of chemicalshave been described as pollutants ofemergingconcern, and are estrogenic because their human and wildlife exposurecan cause adverse effects even atmicro or nano concentrations [2].

Due to poor waste disposal practices in most developing countries such as Nigeria, wastes containing chemicals of various kinds are either discharged directly into surrounding water bodies or heaped on illegal waste dump sites in cities [3], constituting potential source for pollutants such as AP and APEs. About 60 % of the total global APs and APEs manufactured per yearenter the world aquatic environment [4], where the pollutants degrade into various mono-, di-, tri-, etc. metabolites which could cause varying degrees of health effects to wildlife and humans [5,29,30].

Nigeria, the largest consumer of fish in Africa produces about 635,399 tons of fish annually, and imports about 560,000 tons in order to meet her local demand for fish [6].Fish is widely consumed in Nigeria principally because of its high nutritive value [7]. As such, fish could constitute a major route of Nigerians exposure to pollutants such as APs and APEs.

To the best of our knowledge, this research is pilot, aimed at generating baseline data on the human health risk assessment through ingestion of APs and APEs in fish from River Benue by adult and children.

2. MATERIALS AND METHODS

2.1 The Study Area

River Benue is the second largest river in Nigeria. Itscourse originates from the fringes of the Cameroon mountains in the Republic of Cameroon, enters Nigeria

through Adamawa highlands, passes through Taraba plains, reachesAbinsi town, then the city of Makurdi before it empties its content intothe River Niger at the confluence at Lokoja in Central Nigeria [8]. Abinsi, a fishing community is situated about l27 km east of the Makurdi Metropolitan area, also known for its fishing. These study areasexperience rainy season (April to October) and dry season (November to March), and have mostly unconventional waste management practices and weak regulations. Generally, waste of various compositions is mostly dumped illegally in openareas, community-designated dumpsitesorare discharged directly into River Benue, constituting potential source for many pollutants including APs and APEs [3, 9].

Four sampling sites (one at Abinsi town, and three at Makurdi) were purposively selected along River Benue as they act as potential flash points for pollutants (Fig. 1). Site 1 (Abinsi: 7^{o} 76.060'N, 8^{o} 72.610'E), receives effluents from town drains, fishing, laundry activities, sand dredging, boat station, riverbank commerce, and serves as domestic source of water. Site 2 (Mikap: 7^{o} 43.649' N, 8^{o} 35.302' E, has upstream from it Pure Biotech Company Nigeria Limited, makers of bioethanol and Benue Brewery Nigeria Ltd). The site is prone to effluents from the industries upstream and has vibrant fishing, sand dredging and farming activities. Site 3 (WurukumAbattoir: 7^{o} 44.076' N, 8^{o} 32.840' E)receives effluence from a major abattoir, city and rice mill drains, runoff from illegal sites for open-air incineration of old vehicle tires. Here too there is a lot of fishing and sand dredging, laundry, irrigation activities serves as asource of water for rice processing and domestic use. Site 4 (Wadata: 7^{o} 44.789'N, 8^{o} 30.624'E). Illegal waste dump sites, runoff from Wadataopen market, laundry, fishing, sand dredging, source of water for domestic and commerce, boat station, and riverbank commerce are the human activities impacting on this site. Direct waste discharge into the river is a common characteristic of all the sites.

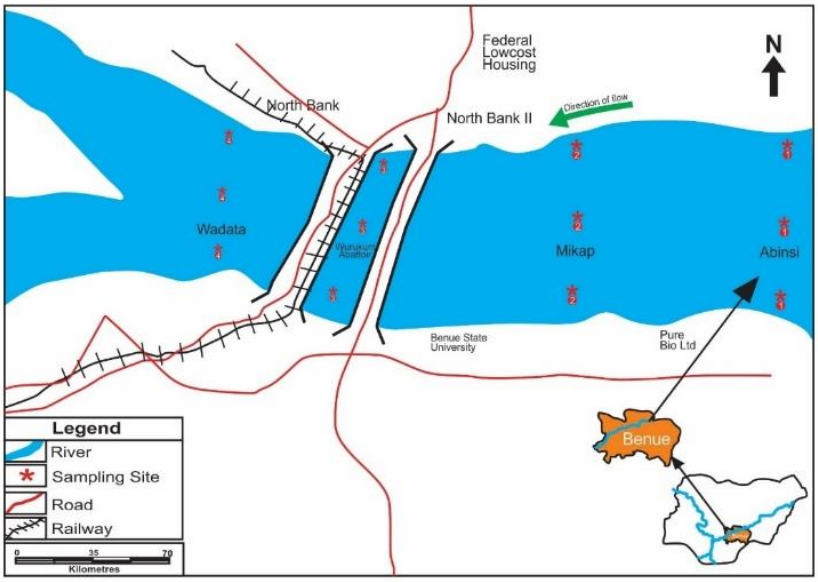

Fig. 1: Map of River Benue showing sampling sites in the study areas

2.2 Sample Collection

Samples were collected according to Chokwe et al.[10] with slight modification. In brief, with the assistance of artisanal fishermen, samples of one bottom feeder, *Labeo cubie,* Rupell 1832, or African Carp, and one predator fish, *Hydrocynusvitatus,* Castelnau 1861, or Tiger fish were caught using nets in order to minimize skin damage; these were subsequently immobilized by the percussive stunning method [11], and wrapped individually in heavy-duty aluminium foil, labelled properly and placed in a chest packed with ice, and transported to the laboratories of Joseph SarwuanTarka University, Makurdi and kept frozen pending the time for sample preparation. The fish species were purposively selected as they are the commonly consumed species by the local population. A total of 32 fishes, 16 each of *Labeo cubie* and *Hydrocynus vitatus* were sampled quarterly between September 2019 and June 2020.

2.3Sample Preparation, Extraction, Cleanup and Derivatization

The frozen fish were thawed enough toremove the foil wrapper and rinsed with tap water, then deionized water to remove any adhering debris, internal organs removed within 48 hours, and muscle tissue filleted, then homogenized on electric blender, and the homogenate stored frozen at -20 °C until the time of extraction.

The sample extraction procedure was a modification from Chokwe et al. [10]. Briefly, 5 g of the homogenized fish tissues were weighed and ground with anhydrous sodium sulfate (1:4 w/w) to uniform paste using mortar and pestle, then extracted with 300 mL of hexane/acetone (4:1v/v) mixture in 500 mL round bottom flask at 55 ^{o}C for 12 hours, and extracts concentrated to 20 mL by solvent recovery. The resulting 20 mL extracts were placed in 250 mL separatory funnel, and 10mL of concentrated sulfuric acid added to remove lipids, and the mixture mechanically shaken for 3 minutes, then 7 mL acetone was added and shaken for 5 minutes for enhanced lipids removal and separation of layers. Subsequently, the acid layer was separated first followed by the hexane/acetone layer. The acid layerwas washed with 25 mL hexane for further extraction of compounds. The hexane/acetone layer and recovered solvent were combined and washed with 20 mL of 40% (v/v) sulfuric acid to further remove residual lipids, and organic layer collected, and reduced to incipient dryness at 30 ^{o}C.

The residue was re-constituted with 2.5 mL methanol, diluted to 250 mLwith MilliQ water, acidified to pH 3 with acetic acid, then subjected to solid phase extraction (SPE) process as follows.

The SPE column was plugged at the bottom with glass wool and 0.5 g of C_{18} reversed phase silica gel placed on top of the wool, conditioned with 6 mL of 30% methanol in dichloromethane, followed by 6 mL dichloromethane, then solution extracted under gentle suction at 10 mL^{-1}. The column was dried under gentle suction for 15 minutes, and compounds eluted with 6 mL of dichloromethane/hexane (4:1v/v) mixture. Theeluate was subsequently concentrated to dryness under a gentle stream of nitrogen, then subjected to derivatization.

Reconstitution of APs and APEs residues was carried out using 0.1 mL hexane in a Pyrex tube, 40 µL of 0.1 M triethylamine (TEA) and 4 µL heptafluorobutyric anhydride (HFBA) added, and vortexed for 1 min, and the contents gently heated to 50°C for 30 minutes using GC to ensure derivatization of compounds. Contents were cooled, quenched with 0.3 mL of 5% aqueous solution of potassium carbonate (K_2CO_3), the

organic phase drawn off. The aqueous phase was washed twice with 0.5 mL of hexane for compound recovery, and the organic phase extracts were concentrated to 100 µL. Chrysene was added as internal standards, volume made up to 200 µL, and 1 µL of the mixture injected into GC-MS.

2.4Instrumental Analysis

The derivatized extracts were analyzed using an Agilent 6890 GC equipped with 5975 mass selective detector (MSD) comprising of capillary column: Restek RTx1614, film thickness of 0.10 µm, 15 m x 0.25 mm I.D. and fitted to an Agilent autosampler A673. Using Helium as carrier gas, the GC/MS conditions were: linear velocity of 40 cm/s, 150 °C ion source, maximum injector temperature of 280 °C, and 300°C transfer line temperature. The temperature programme was: a starting temperature of 50 °C, heated to 120 °C by a temperature ramp of 7.5 °C/min, then 275 °C by a temperature ramp of 15 °C/min, and finally heated to 300 °C (held for 2 min) by a temperature ramp of 25 °C/min.

2.5Quality Assurance and Statistical Analysis

Fish were caught using nets to minimize skin damage, and fish spines carefully sheared to minimize punctures during the aluminium foil packaging. Samples were transported in chest packed with ice to avoid decomposition. Edible parts (muscle tissue) were filleted within 48 hours to avoid contamination by ruptured internal organs. Monitoring chromatographic conditions to check variations during analysis, then running blanks at interval of five samples, and duplicate sample analysis were the other quality assurance measures adopted. All data were analyzed using Microsoft Windows Excel 2019 version.

2.6Human Health Risk Estimates

In this study, the risk assessment tool recommended by USEPA 1989 [12], was adopted to estimate the non-carcinogenic hazard quotient (HQ) due to adult and children exposure through ingestion of fish contaminated by NP, NPE, OP, OPE and 4t-BP as follows:

$$HQ = ADD/RfD \quad (1)$$

$$ADD = Ci \times IR/BW \quad (2)$$

Where, ADD is the average daily intake of a chemical (μgkg^{-1} BWday^{-1}) and RfD is the daily intake reference dose (μgkg^{-1} BWday^{-1}). The tolerable daily intake (TDI) for NP was 5000 ngkg^{-1} BWday^{-1}, and 13000 ngkg^{-1} BWday^{-1} for NPE as proposed by the Danish Institute of Safety and Toxicology were adopted as their RfD [13]. The United States Environmental Protection Agency (USEPA) sets a RfD for phenol at 300 000 ngkg^{-1} BWday^{-1}[14], and this RfD value was employed for estimating the HQ of OP, OPE and 4t-BP during this study, since their RfD have not yet been reported in literature. C_i is the concentration of APs and/or APEs in fish (μgkg^{-1} wet wt). Ingestion rate (IR) is the amount of fish Ingested per day (kg person^{-1}day^{-1}) and was calculated based on per capital consumption of 0.021 kg adult^{-1}day^{-1} for adult Nigerians, while one-third IR of the adult is consumed by children (i.e. 0.007 kg child^{-1}day^{-1}). BW is the body weight (kg) of adult (70 kg), and children (16 kg) respectively. The above parameters were obtained from [15], [16],[17] and [12]. An HQ > 1 indicates that there may be concern for potential human health effects, and vice versa.

3. RESULTS AND DISCUSSION

3.1 Levels of APs and APEs in *Labeo cubie* from River Benue

Sum of twenty APs($\sum_{20}$APs) detected in *Labeo cubie* samples from River Benue as obtained from Table 1, ranged from 14.6-28.0 ngg^{-1} wet wt, with total and maximum values of 94.5 ngg^{-1} wet wt, and 16.0 ngg^{-1} wet wtrespectively. Meanwhile, the range, total and maximum levels of sum of nine short chain APs ($\sum_{9}$SCAPs) were ND-4.16, 11.4 and 4.16 ngg^{-1} wet wt, respectively. Similarly, sum of eleven long chain APs documented were in range of ND-12.3 ngg^{-1} wet wt, total and maximum of 83.1 ngg^{-1} wet wt, and 16.00 ngg^{-1} wet wt, respectively. Sum of four APEs ($\sum_{4}$APEs) were observed to range from ND-3.12 ngg^{-1} wet wt, total of 5.01 ngg^{-1} wet wt, and maximum of 3.12ngg^{-1} wet wtrespectively (Table 1). $\sum_{11}$LCAPs contributed

about 88 % abundance of total mean concentration over about 12 % abundance donated by $\sum_9$SCAPs(Table 1). Concentrations of $\sum_9$SCAPs were highest in site 2 followed by site 3, then site 4, but below the limit of detection of the instrument used for this work in site 1, while sum of three ethyl phenol ($\sum_3$EP) was most abundant metabolites(8 %) among the short chain APs detected, but levels of sum of three methyl phenol ($\sum_3$MP) and sum of three propyl phenol ($\sum_3$PP) were less than limit of detection of our analytical instrument (Table 1). Again, site 3 recorded the highest level of sum of eleven long chain APs ($\sum_{11}$LCAPs) followed by site 4, then sites 1 and 2, respectively. 2-octyl phenol (2-OP) was most abundant (63 %) among the LCAPs detected in *Labeo cubie*, followed by 4-octylphenol (4-OP) with 50%, then, 4-nonylphenol (4-NP) (19%), 4-hexylphenol (4-HxP) and 4-heptylphenol (13% each), 4-butylphenol (4-BP) and 2-hexylphenol (2-HxP) (6% each), while 4-tert butylphenol (4t-BP), 2-pentylphenol (2-PnP), 4-pentylphenol (4-PnP), 4-tert octylphenol (4t-OP) recorded the detection frequency of zero percent (0%) respectively (Table 1)

Exception of site 1, concentrations of $\sum_4$APEs in tissues of *Labeo cubie* obtained in all other sites were below the limit of detection of the analytical instrument, and NPE1 was the dominant homologue among these group of compounds. Generally, the long chain AP homologues were most frequently detected in the *Labeo cubie* tissues, while the SCAPs homologues were least detected (Table 1). Variation in the sources of these pollutants at the studied sites might account for the differences in concentrations of these pollutants at the studied sites. Although fish are mobile, therefore, the effect of site-specific anthropogenic activities on their tissue accumulation of pollutants might be less effective [18], nevertheless, this factor becomes effective when considering the broader habitat of the fish. The impact of environmental concentration of APs and APEs on the body burden of the chemicals in fish was previously shared by [12].

Table 1: Concentrations of APs and APEs (ngg^{-1} wet wt) in *Labeo cubie* from River Benue

	Site 1			Site 2		Site 3		Site 4	
Analyte	Sum	Max	Sum	Max	Sum	Max	Sum	Max	FD
2-MP	ND	ND	ND	ND	ND	ND	ND	ND	0
3-MP	ND	ND	ND	ND	ND	ND	ND	ND	0
4-MP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_3$MP	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	0
2-EP	ND	ND	2.82	2.82	ND	ND	ND	ND	6
3-EP	ND	ND	ND	ND	4.16	4.16	1.14	1.14	13
4-EP	ND	ND	3.30	3.3	ND	ND	ND	ND	6
$\sum_3$EP	**ND**	**ND**	**6.12**	**5.12**	**4.16**	**4.16**	**1.14**	**1.14**	8
2-PP	ND	ND	ND	ND	ND	ND	ND	ND	0
3-PP	ND	ND	ND	ND	ND	ND	ND	ND	0
4-PP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_3$PP	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	0
$\sum_9$SCAPs	**ND**	**ND**	**6.12**	**6.12**	**4.16**	**4.16**	**1.14**	**1.14**	3
4-BP	ND	ND	0.73	0.73	ND	ND	ND	ND	6
4t-BP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum$2BP	**ND**	**ND**	**0.73**	**0.73**	**ND**	**ND**	**ND**	**ND**	3
2-PnP	ND	ND	ND	ND	ND	ND	ND	ND	0
4-PnP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_2$PnP	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	0
2-HxP	3.84	3.84	ND	ND	ND	ND	ND	ND	6
4-HxP	ND	ND	2.89	2.89	3.13	3.13	ND	ND	13
$\sum_2$HxP	**3.84**	**3.84**	**2.89**	**2.89**	**3.13**	**3.13**	**ND**	**ND**	6
4-HP	ND	ND	ND	ND	1.13	1.13	3.13	3.13	13
2-OP	9.71	3.82	2.37	2.37	9.29	4.5	7.71	3.62	63
4-OP	ND	ND	2.89	2.89	15.7	6.19	16.0	5.93	50
4t-OP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_3$OP	**9.71**	**9.71**	**5.26**	**2.89**	**25.2**	**15.7**	**23.7**	**16.0**	38
4-NP	1.09	1.09	3.37	2.7	ND	ND	ND	ND	19
$\sum_{11}$LCAPs	**14.6**	**9.71**	**12.3**	**3.37**	**29.3**	**15.7**	**26.9**	**16.0**	15
$\sum_{20}$APs	**14.6**	**9.71**	**18.4**	**3.37**	**33.5**	**15.7**	**28.0**	**16.0**	**10**
OPE1	0.27	0.27	ND	ND	1.61	1.61	ND	ND	13
OPE2	ND	ND	ND	ND	ND	ND	ND	ND	0

$\sum_2$OPE	**0.27**	**0.27**	**ND**	**ND**	**1.62**	**1.61**	**ND**	**ND**	6
NPE1	3.12	3.12	ND	ND	ND	ND	ND	ND	6
NPE2	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_2$NPE	**3.12**	**3.12**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	3
$\sum_4$APEs	**3.39**	**3.12**	**ND**	**ND**	**1.62**	**1.62**	**ND**	**ND**	5

$\sum_3$MP = 2-MP + 3-MP + 4-MP. $\sum_3$PP = 2-PP + 3-PP + 4-PP. $\sum_9$SCAPs = $\sum_3$MP + $\sum_3$EP + $\sum_3$PP. $\sum_2$BP = 4-BP + 4t-BP. $\sum_2$PnP = 2-PnP + 4-PnP. $\sum_2$HxP = 2-HxP + 4-HxP. $\sum_3$OPs = 2-OP + 4-OP+ 4t-OP. $\sum_{11}$LCAP = $\sum_2$BP + $\sum_2$PnP + 4-HP + $\sum_2$HxP + $\sum_3$OPs + 4-NP. $\sum$20APs = $\sum_9$SCAPs +$\sum_{11}$LCAP + 4-HP + 4.NP. $\sum_2$OPEs = OPE1 + OPE2. $\sum_2$NPEs + NEP1 + NPE2. ND = Not Detected

When compared with similar other studies, levels of sum of the compounds recorded in *Labeo cubie*from River Benue were in range with the reports of [19] and [20], but lower than results reported by [10], and [21], respectively. The sum of mono-, and di-nonylphenol ethoxylate ($\sum_2$NPE) obtained from *Labeo cubie* were dominant over that of mono-, and di-octylphenol ethoxylate ($\sum_2$OPE), which is consistent with the reports of [10] and [22].

Distributionof compounds in *Labeo cubie* per chain length per site was also considered (Fig. 2). Accumulation of $\sum_{11}$LCAPs in *Labeo cubie* was consistently highest in all the sites followed by $\sum_4$APEs in Abinsi, then $\sum_9$SCAPs in Abattoir and Wadata, respectively, which suggestsvariationin degradation rates of APEs into APs, andlipophilicity of these pollutants. The impact of degradation on the tissue accumulation of APs and APEs was previously reported in literature [21].

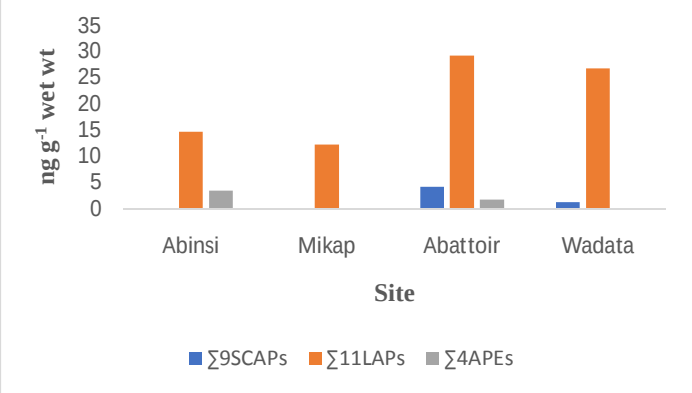

Fig.2:Accumulation of $\sum_9$SCAPs, $\sum_{11}$LCAPs and $\sum_4$APEs in *Labeo cubie*from River Benueacross the sites

Accumulation of compounds in *Labeo cubie* (Fig. 3) was predominant in the 3rd quarter (March) in all the sites.APs and APEs are lipophilic, as such, their bioavailability might be highest in March, the month of the year in Nigeria when fishes are gorged with fats [18].Fish are mobile, this may account for the observed irregular or diffused pattern of accumulation of the chemicals in *Labeo cubie* across the sites (Fig. 3).

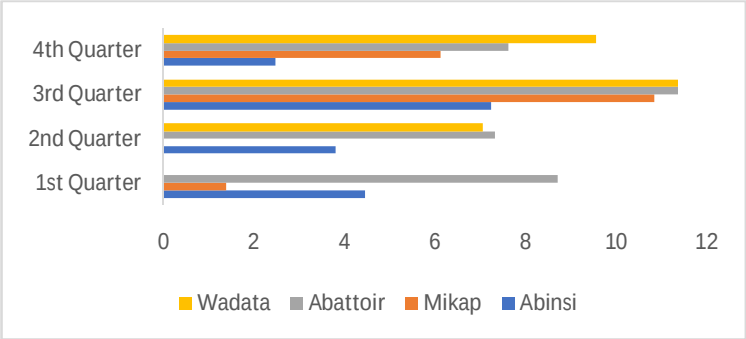

Fig. 3:Quarterly variation of theconcentrations of APs, APEs and metabolites taken togetherin *Labeo cubie* from River Benueacross sites

3.2 Levels of APs and APEs in*Hydrocynus vitatus* from River Benue

The levels of $\sum_{20}$APs detected in *Hydrocynus vitatus* (Table 2) ranged from 40.31-68.60 ngg^{-1} wet wt, with total and maximum of 205.32 and 68.60 ngg^{-1} wet wt respectively. Similarly, $\sum_{9}$SCAPs ranged from 0.61-35.27 ngg^{-1} wet wt, total and maximum concentrations of 65.81 ngg^{-1} wet wt and 35.27 ngg^{-1} wet wt, and $\sum_{3}$MP were most frequently detected (10%) homologue among the short chain APs, while concentrations of $\sum_{3}$EP were below the limit of detection of the instrument used for this study.

Table 2: Concentrations of APs and APEs (ngg^{-1} wet wt) in *Hydrocynusvitatus* from River Benue

	Site 1		Site 2		Site 3		Site 4		
Analyte	Sum	Max	Sum	Max	Sum	Max	Sum	Max	FD
2-MP	ND	ND	1.35	1.35	4.25	4.25	ND	ND	13
3-MP	ND	ND	20.52	20.52	3.80	3.80	ND	ND	13
4-MP	ND	ND	ND	ND	ND	ND	25.04	25.04	6
$\sum_{3}$MP	**ND**	**ND**	**21.87**	**20.52**	**8.06**	**4.25**	**25.04**	**25.04**	10

2-EP	ND	ND	ND	ND	ND	ND	ND	ND	0
3-EP	ND	ND	ND	ND	ND	ND	ND	ND	0
4-EP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_3$EP	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	0
2-PP	ND	ND	ND	ND	ND	ND	4.11	4.11	6
3-PP	ND	ND	ND	ND	ND	ND	6.12	6.12	6
4-PP	0.61	0.61	ND	ND	ND	ND	ND	ND	6
$\sum_3$PP	**0.61**	**0.61**	**ND**	**ND**	**ND**	**ND**	**10.23**	**6.12**	6
$\sum_9$SCAP	**0.61**	**0.61**	**21.87**	**20.52**	**8.06**	**4.25**	**35.27**	**25.04**	6
4-BP	ND	ND	5.85	4.24	ND	ND	11.15	5.61	25
4t-BP	ND	ND	ND	ND	ND	ND	5.11	5.116	6
$\sum_2$BP	**ND**	**ND**	**5.83**	**ND**	**ND**	**ND**	**16.26**	**11.15**	16
2-PnP	ND	ND	ND	ND	4.39	4.39	ND	ND	6
4-PnP	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_2$PnP	**ND**	**ND**	**ND**	**ND**	**4.39**	**4.39**	**ND**	**ND**	3
2-HxP	ND	ND	2.11	2.11	ND	ND	0.97	0.97	13
4-HxP	ND	ND	6.80	6.80	ND	ND	ND	ND	6
$\sum_2$HxP	**ND**	**ND**	**8.91**	**6.80**	**ND**	**ND**	**0.97**	**0.97**	9
4-HP	3.28	3.28	4.87	4.87	ND	ND	ND	ND	13
2-OP	27.77	24.73	ND	ND	22.94	22.01	2.54	2.54	31
4-OP	8.22	4.26	3.36	3.36	4.92	2.44	2.48	2.48	44
4t-OP	3.90	3.90	ND	ND	ND	ND	ND	ND	6
$\sum_3$OP	**39.89**	**27.77**	**3.36**	**3.36**	**27.86**	**22.94**	**5.02**	**2.54**	29
4-NP	ND	ND	7.79	3.94	ND	ND	11.08	6.71	25
$\sum_{11}$LCAP	**43.17**	**27.77**	**30.76**	**12.35**	**32.25**	**27.77**	**33.33**	**11.15**	16
$\sum_{20}$APs	**43.78**	**27.77**	**52.63**	**21.87**	**40.31**	**27.77**	**68.60**	**25.04**	12
OPE1	ND	ND	3.06	3.06	ND	ND	ND	ND	6
OPE2	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_2$OPE	**ND**	**ND**	**3.06**	**3.06**	**ND**	**ND**	**ND**	**ND**	3
NPE1	ND	ND	ND	ND	ND	ND	ND	ND	0
NPE2	ND	ND	ND	ND	ND	ND	ND	ND	0
$\sum_2$NPE	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	0
$\sum_4$APE2	ND	ND	**3.06**	**3.06**	**ND**	**ND**	**ND**	**ND**	2

Again, 4-OP was the most frequently detected (44 %) among the LCAPs, whereas, 4-pentyl phenol (4-PnP) was least detected (0 %).$\sum_4$APEs ranged from ND-3.06 ngg^{-1}wet wt, total and maximum of 3.06 ngg^{-1} wet wt each (Table 2). Concentration of $\sum_2$NPE were below the limit of detection of our instrument, while $\sum_2$OPE were most frequently detected (3 %), and most abundant homologue recorded among the APEs in the *Hydrocynus vitatus***.** Generally, variation in the detection frequencies of compounds in the tissues of *Hydrocynus vitatus* suggests point and non-point source emissions of these pollutants around the sites studied.$\sum_9$SCAPs contributed about 37 % of the sum of mean concentration of $\sum_{20}$APs while $\sum_{11}$LCAPs homologues represented about 63 %. APs are lipophilic, and this property increases with chain length [23], this might account for the differences in percentage absorption of these chemicals to the fish tissues

In comparison with similar other literatures, [19] and [20] reported levels of APs and APEs in similar range with our study, while [10], and [12] published higher values of these pollutants. Long chain APEs can degrade into mono and di-ethoxylate metabolites which tend be more persistent, more lipophilic and bioaccumulative step-wisein the trophic [24].

Trend of chemicals accumulated by *Hydrocynus vitatus* at the various sites followed the pattern: $\sum_{11}$LCAP > $\sum_9$SCAP > $\sum_4$APE (Fig.4).Variation in degradation rate and/or fat solubility of these chemicals might be responsible for this trend [21].

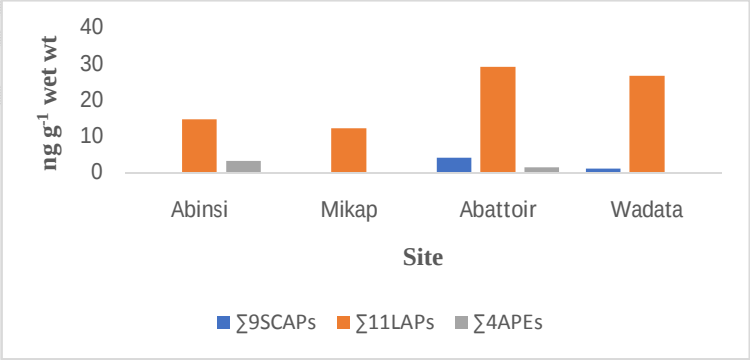

Fig. 4:Accumulation of $\sum_9$SCAPs, $\sum_{11}$LCAPs and $\sum_4$APEsin *Hydrocynus vitatus*from River Benueacross the sites

APs and APEs are lipophilic, and as such, are most bioavailable around March, the month of the year in Nigeria when fishes are rich in lipids [18]. This explains why the highest concentrations of chemicals reported *Hydrocynus vitatus* in this research were observed in the 3rd quarter (March) in all the sites (Fig. 5).

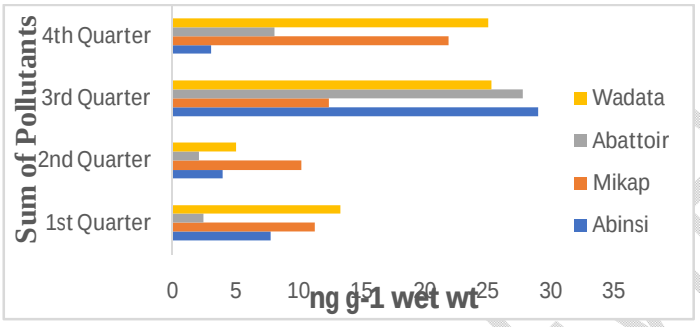

Fig. 5: Quarterly Trend of concentrations of APs, APEs and metabolites taken together in *Hydrocynus vitatus* from River Benue across sites

Accumulation of compounds across the sites followed a diffused pattern, which might be the consequence of mobility of fish which makes site-specific influence of these pollutants on fish difficult to determine.

3.3 Accumulation of APs, APEs and their Metabolites by *Hydrocynus vitatus* Versus *Labeo cubie* from River Benue

It could be observed from Tables 1 and 2 that LCAPs are the dominant homologues among the pollutants detected during this study, followed by SCAP, then APEs. $\sum_3$OP was observed to be most concentrated among the LCAP with the total concentration of 63.87 ngg $^{-1}$ wet wt, and detection frequency of 38 % for *Labeo cubie*, while the total concentration of 76.13 ngg^{-1} wet wt, and detection frequency of 29 % were recorded for *Hydrocynus vitatus.* 2-OP monopolized dominance withtotal concentration of 29.08 ngg^{-1} wet wt for *Labeo cubie*, and 53.25 ngg^{-1} wet wt for *Hydrocynusvitatus*. Mixed ratio of dominance was observed among the SCAP. $\sum_3$EP recorded the highest total concentration (11.42 ngg^{-1} wet wt) in *Labeo cubie*, and 3-EP toped the total concentration (5.3 ngg^{-1} wet wt), and frequency of detection (13 %)

among the ethylphenol metabolites in this species of fish. $\sum_3$MP had the highest total concentration (54.97 ngg^{-1} wet wt) and detection frequency (13 %) among the SCAPs for *Hydrocynus vitatus*, while 4-MP dominated total metabolite concentration (25.04 ngg^{-1} wet wt) of this AP homologue in this species of fish.

APEs showed inverse abundance of total metabolite concentration of pollutants among the two fish species: NPE > OPE in *Labeo cubie*, while OPE > NPE in *Hydrocynus vitatus* respectively (Tables 1 and 2), and this suggests variation in sources and/or tissue metabolism of these compounds by the two fish species. Chokwe et al. [10] reported higher abundance ratios of di-NPE and mono-NPE (23.52 ng g^{-1} versus 12 ng g^{-1}lipids) in fish, the remaining 10.6 ngg^{-1} lipids was contributed by sum of mono- and di- OPEs, and this report was consistent with [22] and [25].

Result in Fig.6 indicates that, the predatory *Hydrocynusvittus* consistently accumulated higher levels of pollutants than the bottom feeder *Labeo cubie* across sites.

This trend of accumulation is consistent with the fact that the feeding habit of fish contributes to the bioaccumulation of pollutants in its tissues[12, 10], although, fish physiology, metabolism and environmental levels of pollutants are factors to consider as well. Trend of accumulation of compounds in the two species of fish was statistically significant across sites except for abattoir, and this implies variation in of level of exposure fish to these pollutants.

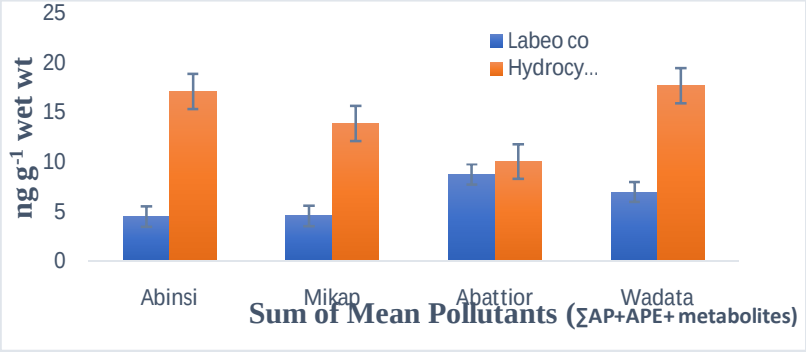

Fig.6: Comparison of the accumulation of total APs, APEs and their metabolites taken together in *Labeo cubie* and *Hydrocynus vitatus* from River Benue

3.4 Human Health Risk Assessment of APs and APEs in *Labeo cubie*and *Hydrocynus vitatus*from River Benue

The HQ for ingestion of APs and APEs in *Labeo cubie*by adults and children (Table 3) was less than one (HQ < 1), which suggests that consumption of this species of fish by the two groups posed no significant health risk. Lee *et al*. [21] reported HQ for the ingestion of NP in fish by adults and children from Tamsui river to be less than one as well.

Table 3: Hazard Quotient for Ingestion of APs and APEs (ngg^{-1} wet wt) in Fish (*Labeo cubie*) from River Benue by Adult and Children

	Abinsi		Mikap		Abattoir		Wadata		Adult		Children	
Analyte	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Sum	Mean	Sum	Mean
4t-BP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4t-OP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4-NP	3.76E-06	5.36E-06	1.17E-05	1.67E-05	NA	NA	NA	NA	1.55E-05	3.87E-06	2.21E-05	5.52E-06

***** NA = Not applicable as the contaminants were not detected

The risk estimates of exposure to 4t-BP and 4t-OP through ingestion of *Labeo cubie* by the two groups was effectively not applicable (NA), as concentrations of these chemicals in this fish tissues were less than the limit of quantitation of our instrument. Nevertheless, HQ of 4-NP across sites was found to be less than the threshold value of one for the two groups, and risk was higher for adults in Abinsi and Mikap, respectively, while children recorded higher sum and mean HQ than adults (Table 3). The body burden of 4-NP for the two groups is approximately close, which might account for variation of the inverse sum and mean risk observed.

Sum and mean HQ for consumption of APs and APEs in *Hydrocynus vitatus* was consistent for the two groups and decreased in the sequence: 4-NP > 4t-BP > 4t-OP, with children having the highest body burden of the pollutants inAbinsi, Mikap and Wadata (Table 4).∑HQ recorded in *Hydrocynus vitatus*ranged from ND-1.70E-04 for adult and ND-2.50E-04 for children, while their total and sum of mean as obtained from Table 4 were 7.67E-05 and 8.69E-05, respectively. Children have lower body weight (16 kg), and their bodies are more sensitive to pollutants than adults (body weight = 70 kg), which explains why children were observed to have suffered morebody burden through ingestion of APs and APEs in *Hydrocynus vitatus* than adults.

Although the risk values for consumption of *Hydrocynus vitatus* contaminated by APs and APEs by the two groups suggests no immediate potential harm (HQ <1), continuous exposure to these chemicals might eventually become harmful since APs and APEs arepersistent and bioaccumulative, hence the need for routine monitoring of the chemicals in the River Benue.

Table 4: Hazard Quotient (ngg^{-1} wet wt) for Ingestion of *Hydrocynus vitatus* from River Benue by Adult and Children

	Abinsi		Mikap		Abattoir		Wadata		Adult		Children	
Analyte	Adult	Children	Adult	Children	Adult	Children	Adult	Children	Sum	Mean	Sum	Mean
4t-BP	NA	NA	NA	NA	NA	NA	1.86E-06	2.70E-06	1.86E-06	4.65E-07	2.7E-06	6.75E-07
4t-OP	1.41E-06	2.05E-06	NA	NA	NA	NA	NA	NA	1.41E-06	3.53E-07	2.05E-06	5.13E-07
4-NP	NA	NA	1.69E-04	2.46E-04	NA	NA	4.00E-06	5.84E-06	1.73E-04	4.33E-05	2.52E-04	6.30E-05
∑HQ	1.41E-06	2.05E-06	1.70E-04	2.50E-04	NA	NA	5.86E-06	8.54E-06	1.80E-04	4.41E-05	2.60E-04	6.41E-05

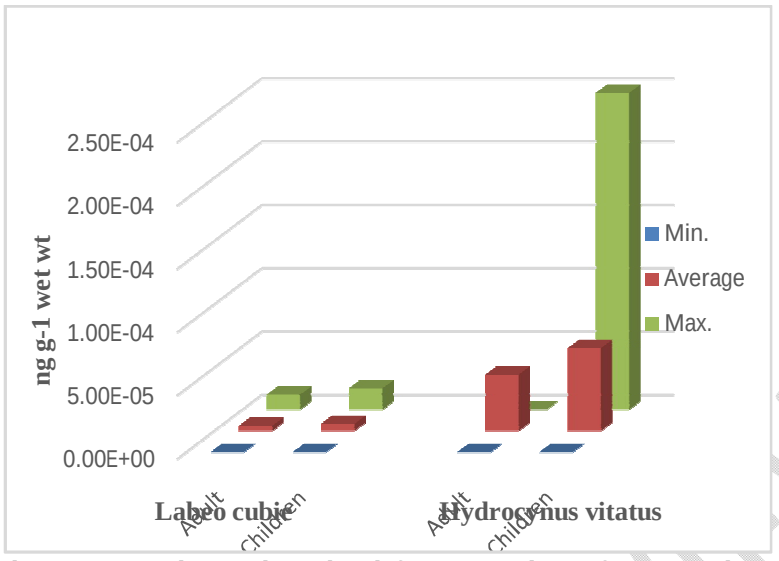

Fig. 7: Hazard Quotient (HQ) for Ingestion of APs and APEs Taken Together in *Labeo cubie* and *Hydrocynus vitatus* Fish from River Benue

Comparative trend of exposure through ingestion of the two fish species (*Labeo cubie* and *Hydrocynus vitatus*) by adult and children was equally evaluated as shown in Fig.7.Exposure to APs and APEs through ingestion of *Hydrocynus vitatus* by the two groups was consistently higher than *Labeo cubie*, except that the maximum for adultswas higher in *Labeo cubie*. It is worth to note that *Hydrocynus vitatus* is predatory, while *Labeo cubie* is bottom feeder, hence, feeding habit might account for the higher tissue levels of the pollutants recorded in *Hydrocynus vitatus*than *Labeo cubie*. Bioaccumulation of APs and APEs in fish was previously reported in literature [12,10, 26]. Nevertheless, overlapping of the maximum levels of the pollutants in adult through ingestion of pollutants in the bottom feeding *Labeo cubie*over the predatory *Hydrocynus vitatus*suggests that factors other than feeding habit might favour accumulation of the pollutants infish tissues as well.Lee et al. [12] reported that factors such as environmental concentration of chemicals (exposure level), physiology and burrowing behaviuor of fish might also favour accumulation of chemicals in fish tissues, which demands for further research in the future.

4. CONCLUSION

Baseline data on levels and human health risk estimates on exposure to APs and APEs through consumption of*Labeo cubie* and *Hydrocynus vitatus*from Rive Benue was generated fromthis study. Results indicated that the two species of fish were minimally contaminated by the studied pollutants, and as such, adults and children were not likely to be affectedby consumption of these fishes in their diets, sincetheHQ was less than one for the two groups. Nevertheless, the high levelof local consumption of fish [6], coupled with the lipophilicity of the chemicals is a concern because these chemicals can bioaccumulate and likely biomagnify in food chain, and eventually cause harm to ecology, then humans, hence, the need for routine monitoring of these compounds in the area studied. This study provides baseline data for regulatory authorities. Modification of the lipid extraction and SPE cleanup methods reported by [10] provides an opportunity for researchers working in less equipped laboratories to use our own approach.

Data Availability Data for this work will be made available on official request.

Ethical Declaration/consent Not applicable.

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