

Quantification of Phthalates and Bisphenols in Fish Samples from Owan Market, Edo State, Nigeria

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ABSTRACT

Phthalates and bisphenols are widely used industrial chemicals known for their persistence in the environment and potential to bioaccumulate in aquatic organisms. This study aimed to quantify selected bisphenols and phthalates in two commercially important fish species—*Gymnarchus niloticus* and *Clarias gariepinus*—obtained from Owan Market, Edo State, Nigeria. A total of 30 fish samples were sampled over a five-month sampling periods (June to October 2022). The samples were homogenized and analyzed using gas chromatography–mass spectrometry (GC-MS) to detect four bisphenols (BPA, BPS, BPE, and BTMC) and five phthalates (DMP, DCHP, DEHP, DPrP, and DEP). In *G. niloticus*, phthalate concentrations ranged from not detected (ND) to 45.34 ± 0.17 µg/g, while in *C. gariepinus*, the concentrations were higher, ranging up to 97.8 ± 0.66 µg/g. Di(2-ethylhexyl) phthalate (DEHP) was the most abundant phthalate in both species while DCHP and DPrP were not detected in *G. niloticus* and *C. gariepinus* respectively. There was also significant difference in the concentration of DEHP in *C. gariepinus* compared to *G. niloticus* at $p < 0.001$. Among the bisphenols, bisphenol A (BPA) showed the highest concentrations in both fish: 584.6 ± 0.09 µg/g in *G. niloticus* and 527.5 ± 41.72 µg/g in *C. gariepinus*. There was, however, no significant difference in the concentrations of bisphenol congeners among the two fish species. The presence and elevated concentrations—particularly of BPA and DEHP—raise significant public health and environmental concerns. Routine screening of fish and other aquatic products is, therefore, essential to mitigate potential human exposure and ensure food safety.

Keywords: Plasticizers, Phthalates, Bisphenol, *Gymnarchus niloticus* and *Clarias gariepinus*, Owan market

INTRODUCTION

Phthalates and bisphenols are widely used industrial chemicals known for their endocrine-disrupting properties. Their extensive application in the manufacturing of plastics, food packaging, and a range of consumer products has raised global concerns regarding environmental contamination and human exposure (Gao et al., 2017; Rochester, 2013). Phthalate esters (PAEs), such as diethyl phthalate (DEP) and bis(2-ethylhexyl) phthalate (DEHP), are commonly found in items like cosmetics, personal care products, plastic toys, surface coatings, and food packaging materials (Savoca et al., 2018; Hahladakis et al., 2018). The primary environmental sources of phthalates stem from human activities (Wang et al., 2013). Since PAEs are not chemically bonded to polymer matrices but are instead added as plasticizers, they can easily migrate into the environment through evaporation, leaching, or abrasion (Jiménez-Skrzypek et al., 2020).

Phthalates can reach aquatic environments via both direct and indirect pathways (Sun et al., 2021). Direct sources include the discharge of industrial effluents and surface runoff from rainwater interacting with agricultural plastic films, insect repellents, and plastic debris (Huang et al., 2020). Indirectly, phthalates released into the atmosphere may later enter aquatic systems through dry deposition or rainwater leaching (Sun et al., 2021). Studies have also shown that some freshwater algae and cyanobacteria can naturally produce compounds such as monoethylhexyl phthalate (MEHP) and dibutyl phthalate (DBP), which may be released into surrounding waters (Sun et al., 2016). Because these substances are not covalently bonded to plastic matrices, they readily leach into ecosystems, making their distribution nearly as widespread as that of plastics themselves (Schmidt et al., 2018). However, the primary contributors to aquatic PAE pollution remain industrial discharges, commercial

wastewater, and plastic waste containing synthetic PAEs (Zhang et al., 2021). Due to their high n-octanol-water partition coefficients (Kow) and low vapor pressures, PAEs exhibit low volatility and are capable of migrating across diverse aquatic environments (Cao et al., 2018). These characteristics also facilitate their uptake by aquatic organisms (Zhang et al., 2021), with their hydrophobic nature enhancing their potential for bioaccumulation (Oehlmann et al., 2009). In fish, exposure primarily occurs through gill absorption from water, though dietary intake may also contribute (Barron et al., 1995).

Bisphenols, including Bisphenol A (BPA), B, C, S, F, and AF, are phenolic compounds extensively used in producing plastic containers, epoxy resins, food and drink cans, water pipes, electronics, thermal paper, kitchenware, toys, and dental materials (Rochester, 2013; Andujar et al., 2019). BPA, in particular, serves as a key monomer in the production of polycarbonate plastics and epoxy resins and is known to leach into the environment and foodstuffs through depolymerization (Pisciottano et al., 2020; Lim et al., 2018). Given their widespread use and prolonged release from plastic materials, bisphenols are frequently detected in human biological samples—even in the absence of known direct exposure (Volkel et al., 2005; Owczarek et al., 2018). These compounds are known to disrupt endocrine function and impair reproductive health (Chen et al., 2016; Sidorkiewicz, 2018). Even low levels of chronic exposure can suppress luteinizing hormone, follicle-stimulating hormone, and prolactin, producing both estrogenic and anti-androgenic effects that can negatively impact spermatogenesis (Owczarek et al., 2018; Ullah et al., 2018; Shi et al., 2017). BPA and related endocrine-disrupting chemicals (EDCs) can also be absorbed by aquatic organisms through ingestion, dermal contact, gill uptake, and trophic transfer (Baluch and Hashmi, 2019).

Furthermore, as freshwater habitats are critical to aquatic life, the continuous introduction of pollutants into these ecosystems can disrupt ecological balance and lead to the bioaccumulation of contaminants in fish through ingestion, skin absorption, and respiration via the gills (Brodin et al., 2014). In Nigeria, fish constitute a vital component of the human diet and can serve as bioindicators of aquatic pollution. This study aims to quantify the concentrations of selected phthalates and bisphenols in fish samples collected from a major food market in Edo State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Thirty (30) fresh fish samples; *Gymnarchus niloticus* and *Clarias gariepinus* were procured monthly for five consecutive months June-October, 2022 from Owan Market in Edo State, Nigeria. The market is a major commercial hub known for its vibrant trade in fresh and preserved food items, including fish sourced from various aquatic environments.

Reagents and Standards

All chemicals used were of analytical reagent grade. Solvents were further purified via distillation. Phthalate ester standards, including dimethyl phthalate (DMP), diethyl phthalate (DEP), and bis(2-ethylhexyl) phthalate (DEHP), were obtained from Chem Services (West Chester, PA, USA) and Sigma-Aldrich (Milwaukee, WI, USA), with purities exceeding 99%. The internal standard, *n*-butyl benzoate (99% purity), was purchased from Fisher Scientific. Derivatizing agents such as trimethylchlorosilane (TMCS), N-methyl-N-trimethylsilyltrifluoroacetamide with 1% TMCS, and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, PI-48915) were obtained from Pierce Chemical Company.

Analytical standards of bisphenols with purities exceeding 98% were obtained from Sigma-Aldrich (USA). The internal standard used for quantification was d_{16} -bisphenol A (BPA- d_{16} ; 98 atom% D). Stock solutions of both the standards and the internal standard were prepared in HPLC-grade methanol at a concentration of 2000 $\mu\text{g/L}$.

Sample Preparation

Samples were homogenized with C18-bonded silica sorbent using a mortar and pestle. Each sample was spiked with 1 μL of a 5 mg/100 mL standard mixture of DMP, DEP, DCHP, DPrP and DEHP. Clean-up was performed

using pre-conditioned Florisil columns. Common solid-phase clean-up materials such as 3% deactivated Florisil (Giam, 1975), alumina, and 5% deactivated silica gel (Tanaka et al., 1984) were also referenced.

The spiked C18-sample mixture was loaded onto Florisil columns and eluted at a flow rate of 1 mL/min. A total of 15 mL of CH₃OH/CH₂Cl₂ mixtures in varying ratios (10:90 to 90:10 v/v) were used for elution. The eluents were evaporated under nitrogen vacuum. Once dried, 1 µL of 1.0 mg/L *n*-butyl benzoate in methanol was added as the internal standard.

The extraction of bisphenols from fish muscle and liver tissues was adapted from the method described by Cunha et al. (2012), with modifications to optimize recovery and matrix compatibility. Fish tissue samples were accurately weighed into pre-cleaned glass vials. Each sample was fortified with 80 µL of the internal standard solution (BPA-d₁₆ at 250 ng/mL) and extracted using 5 mL of MeCN for muscle samples. The extraction was carried out overnight at approximately 21 °C using a platform shaker set to 420 rpm (D-72379 Hechingen, Edmund Bühler GmbH, Germany).

After extraction, 6 mL of ultrapure water was added to each vial and manually shaken for 1 minute. Then, 1.5 g of MgSO₄ and 0.25 g of NaCl were added, followed by another 1-minute shaking. Samples were centrifuged at room temperature for 5 minutes at 1690×g to facilitate phase separation. The supernatant was collected and subjected to dispersive liquid–liquid microextraction (DLLME) for derivatization and concentration.

In the DLLME step, 0.85 mL of the supernatant was mixed with 85 µL of T4CE and 125 µL of acetic anhydride. This mixture was then transferred to a 4 mL vial containing 3 mL of ultrapure water adjusted to pH ≥ 10 with 5% potassium carbonate (K₂CO₃). After vortexing for 1 minute, samples were centrifuged at 1690×g for 3 minutes. Fifty microliters of the resulting lower organic phase was carefully collected and subjected to GC-MS analysis (Varian 3800/4000). All sample preparations and analyses were performed in duplicate.

Statistical Analysis

Data obtained were computed using Graphpad Prism 5.0 version. T-test analysis was carried out to determine the significance level at $p < 0.05$.

RESULTS

The average concentrations of phthalates and bisphenols in the analyzed fish samples are summarized in Tables 1 and 2. The phthalate concentration ranged from not detected (ND) to 45.34±0.17 and 97.8±0.66 in *G. niloticus* and *C. gariepinus* respectively with DEHP having the highest value (Table 1). The concentrations of DMP and DEP were significantly different in *G. niloticus* when compared with *C. gariepinus* at $p < 0.001$ while DEHP was significantly higher in the latter species.

The highest concentration was recorded for BPA in both fish samples as 584.6±0.09 and 527.5±41.72 respectively (Table 2). There was no significant difference in the mean concentration of bisphenol congeners among the two fish species.

Table 1. Average Concentration of Phthalates in *G. niloticus* sold in Owan Market

Congeners (µg/g)	<i>G. niloticus</i>	<i>C. gariepinus</i>
DMP	8.68±0.06***	4.14±0.05
DCHP	ND	45.08±0.11
DEHP	38.5±1.71	97.8±0.66***
DPrP	45.07±0.06	ND

DEP	45.34±0.17***	8.12±0.13
ΣPAEs	137.59	155.14

Mean±SD with *** along the same row are significantly different at $p<0.001$.

Table 2. Average Concentration of Bisphenol in *G. niloticus* sold in Owan Market

Congeners (µg/g)	<i>G. niloticus</i>	<i>C. gariepinus</i>
BPA	584.6±0.09	572.5±41.72
BPS	41.16±1.39	41.21±1.45
BPE	47.09±9.53	42.59±0.66
BTMC	192.5±10.54	207.6±6.24
ΣBPs	865.35	863.90

Mean±SD along the same row are not significantly different at $p>0.05$.

DISCUSSION

Concentrations of PAEs in Fish Samples

In *G. niloticus*, the concentrations of DMP, DEHP, DPrP, and DEP were recorded as 8.667 ± 0.06 , 38.5 ± 1.71 , 45.07 ± 0.06 , and 45.34 ± 0.17 µg/g, respectively, with DCHP not detected. In contrast, *C. gariepinus* exhibited higher levels of DEHP (97.8 ± 0.66 µg/g) and DCHP (45.08 ± 0.11 µg/g), while DMP (4.135 ± 0.05 µg/g) and DEP (8.12 ± 0.13 µg/g) were present in lower concentrations. DPrP was not detected in *C. gariepinus*. Among the analyzed phthalate esters (PAEs), DEHP exhibited the highest concentration in *C. gariepinus*, while DMP was consistently the lowest across both species.

These interspecies differences in PAE concentrations may reflect variations in ecological behavior, metabolic capacities, and differential exposure routes (Adeniyi et al., 2011; Huang et al., 2008). The notably higher DEHP levels in *C. gariepinus* may be attributed to its benthic feeding habits, which increase contact with sediment-bound pollutants. This observation aligns with prior findings indicating elevated DEHP levels in bottom-feeding fish (Adeniyi et al., 2011; Huang et al., 2008). For instance, Sioen et al. (2012) reported an average DEHP concentration of 86 µg/g in fish tissue, while Cheng et al. (2013) found DEHP levels between 0.87 and 9.47 µg/g in fish from Hong Kong markets—significantly lower than those found in the current study. Similarly, data from Taiwan also highlight DEHP as the most prevalent PAE in aquatic species (Huang et al., 2008). Generally, the bioavailability of PAEs in fish appears to be strongly influenced by both species-specific ecological traits and the physicochemical properties of the compounds (Zhang et al., 2021).

Concentrations of Bisphenols in Fish Samples

Bisphenols, such as BPA, BPF, and BPS, are commonly incorporated into plastics as additives to enhance material properties (Beltifa et al., 2017; Net et al., 2015; Rochester and Bolden, 2015). Their presence in aquatic species is largely due to environmental release from degrading plastics in water bodies (Fikarová et al., 2019). In the current study, BPA and BTMC were detected at the highest levels, whereas BPS showed the lowest concentrations across both fish species.

Specifically, BPA levels were slightly higher in *G. niloticus* (584.6 ± 0.09 µg/g) than in *C. gariepinus* (572.5 ± 41.72 µg/g), although the difference was marginal. BPS concentrations were nearly identical in both species: 41.16 ± 1.39 µg/g in *G. niloticus* and 41.21 ± 1.45 µg/g in *C. gariepinus*. Similarly, BP was slightly more

concentrated in *G. niloticus* ($47.09 \pm 9.53 \mu\text{g/g}$) compared to *C. gariepinus* ($42.59 \pm 0.66 \mu\text{g/g}$). Conversely, BTMC was more abundant in *C. gariepinus* ($207.6 \pm 6.24 \mu\text{g/g}$) than in *G. niloticus* ($192.5 \pm 10.54 \mu\text{g/g}$). The BPA concentrations observed in this study are considerably higher than those reported in earlier studies (Barboza et al., 2020; Rios-Fuster et al., 2022), possibly due to differences in environmental exposure at the fishing sites.

BPS, often considered a safer alternative to BPA, was also detected in both species. However, emerging evidence suggests that BPS may possess comparable endocrine-disrupting potential (Rochester and Bolden, 2015). The observed variations in bisphenol levels between species may be influenced by factors such as feeding behavior, habitat preference, and metabolic processing (Adeniyi et al., 2011; Sala et al., 2022; Zhang et al., 2021).

Notably, the concentrations of BPA and DEHP detected in this study far exceed the safety thresholds established by international regulatory bodies such as the European Food Safety Authority (EFSA, 2015) and the U.S. Environmental Protection Agency (US EPA, 2020). BPA, a widely used monomer in polycarbonate plastic production, was found at particularly high levels, indicating widespread environmental contamination. Likewise, the presence of DEHP—a commonly used plasticizer in PVC products—at elevated levels raises significant concern. The detection of substantial amounts of BTMC and DCHP further suggests continued use of legacy plasticizers despite international regulatory efforts to phase them out.

These differences in contaminant levels may reflect disparities in environmental persistence, pollutant input sources, and compound-specific bioaccumulation tendencies (Net et al., 2015). The findings of this study are consistent with other regional reports that document elevated levels of plasticizers in fish and other aquatic food sources (Mekuleyi et al., 2024; Ajayi and Osibanjo, 2021; Kiralan, 2020). Given the known links between phthalate and bisphenol exposure and adverse effects on reproductive, endocrine, and neurological health (Rubin, 2011; Meeker et al., 2009), the levels detected in this study raise serious public health concerns, particularly in communities that rely heavily on fish as a dietary staple.

CONCLUSION

This study reveals concentration levels of bisphenols and phthalates in fish sold at Owan Market, Edo State, Nigeria. The elevated concentrations, particularly of BPA and DEHP, highlight the urgent need for comprehensive environmental monitoring, policy enforcement, and consumer education. Regulatory authorities must strengthen efforts to limit the use of hazardous plasticizers and implement routine screening of food items to safeguard public health.

DECLARATIONS

Disclosure statement: The author declared that there is no conflicting interest.

Availability of data and materials: All data sets, on which the conclusions of the manuscript rely, are present in the results section in the manuscript.

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