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ORIGINAL RESEARCH

Levels of phthalates in two commercially important fish species from two markets in Lagos State, Nigeria

***Gabriel Olarinde MEKULEYI, Prince Emeka NDIMELE and Isa Olalekan ELEGBEDE**

[1]*Department of Fisheries, Faculty of Science, Lagos State University, Nigeria*

Correspondence
Mekuleyi Gabriel Olarinde, Department of Fisheries, Faculty of Science, Lagos State University, Nigeria.
Email: tosingabriel76@yahoo.com; gabriel.mekuleyi@lasu.edu.ng

Abstract:

Introduction: Phthalates are organic substances frequently used in many industries. In recent times, some phthalates have been demonstrated to be endocrine disruptors and detrimental to human health.

Aim: Sequel to this development, this study deem it fit to examine the levels of phthalates (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Benzyl butyl phthalate(BBP), Bis (2-ethylhexyl) phthalate(DEHP) and Di-n-octyl phthalate (DNOP)) in *Oreochromis niloticus* and *Scomber scrombrus* collected from Ojo and Agboju markets in Lagos State, Nigeria.

Materials and Methods: Fish samples were collected and analyzed using Gas chromatography-mass spectrometry (GC-MS) Analysis of Phthalate Esters' methodology while variance in the quantitative data were statistically tested using t-test analysis.

Results: The concentrations of DEP, DBP, BBP, DEHP and DNOP in µg/g for *Oreochromis niloticus* from both markets were not significantly different ($p>0.05$). However, DMP was not detected in *O. niloticus* and *Scomber scrombrus* at both markets. On the contrary, the concentrations of Dibutyl phthalate (5.43±0.06µg/g) and Bis (2-ethylhexyl) phthalate (6.78±0.06 µg/g) in *S. scombrus* from Agboju market were significantly ($p<0.05$) higher than that from Ojo market.

Conclusion: The level of all phthalates detected in *O. niloticus* (from both markets) and *S. scombrus* (from Ojo markets) were within the permissible levels of < 5 µg/g in fish. However, the level of Dibutyl phthalates and Bis (2-ethylhexyl phthalates) in *S. scombrus* from Agboju markets exceeded the permissible limit. Hence, sellers and processors of the fish species are enjoined to embrace hygienic practices to avert further contamination of the fish.

Keywords: Phthalates, contamination, fish, markets, Lagos

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Phthalates are family of chemicals used in hundreds of consumer products, such as clothing, cosmetics, building materials, household furnishings, personal care products, pharmaceuticals, nutritional supplements, medical devices, herbal remedies, dentures, automobiles, children's toys, glow sticks, modeling clay, food packaging, lubricants, waxes, cleaning materials and insecticides [1]. Phthalate esters has a common chemical structure, dialkyl or alkyl/ aryl esters of 1, 2-benzenedicarboxylic acid [2].More than three million metric tonnes of phthalates are used globally every year by different companies and due to their widespread use, populations of people, domestic animals, and wildlife regularly encounter opportunities for exposure to phthalates [3-5]**.** Studies has shown that the most commonly used phthalates in consumer products include benzylbutyl phthalate (BBP), diisononyl phthalate (DiNP), dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DIP), di-n-butyl phthalate (DBP), dicyclohexyl phthalate (DCHP), di-n-hexyl phthalate (DHP), bis (2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DOP), and diisodecyl phthalate (DiDP) [4, 6-7]. While phthalates like di(2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DiNP), di-n-octyl phthalate (DnOP) are considered high molecular weight phthalates, some such as diethyl phthalate (DEP) and dibutyl phthalate (DBP) are low molecular weight phthalates[6].

Aquatic ecosystem such as wetlands, rivers, dams, and lakes that are located within or passes through agricultural, industrial, residential and urban environments are exposed to a lot of different types of phthalate esters from various anthropogenic inputs [8]. The lipophilic nature of phthalates influences their leaching and environmental partitioning characteristics. As a result of its high octanol-water partition and low vapor pressures, most phthalates entering the water environment have extremely low volatility hence can easily migrate into various water bodies and enter aquatic organisms [4,9]. Phthalates toxicity in aquatic ecosystems has been shown to affect biota therein and is also associated with human exposure and health risks that result from human contact, ingestion of contaminated food (e.g., fish and crops irrigated with phthalates polluted water), and inhalation. However, very limited action has been implemented toward strict regulation and regulatory enforcement for these compounds in many developing countries [9].

Therefore, the necessity to investigate levels of phthalates in fish especially those sold in our market (which is the major contact for fish purchase by consumers) cannot be over- emphasized. On this note, two commercially important and highly demanded fish (*Scomber scombrus* and *Oreochromis niloticus*) were selected for phthalates assessment to determine if their persistent consumption are safe for the consumers.

2. MATERIAL AND METHODS

2.1 Study Area

Two sampling stations (Agboju and Ojo markets) were selected for this study. Agboju fish market is on latitude 6.4612786^{o}N and longitude 3.2861868^{o}E while the Ojo fish market is on latitude and longitude 6.4696065^{o}N and 3.2072879^{o}E respectively as shown in Figure 1 below.

Agboju market (Site 1) is a very popular market located at the second gate of Festac town in Lagos State. It's a multipurpose market with different segments of traders including foodstuff segments, fashion accessories, electronics, and other things while Ojo market (Site 2) is a very popular market in the Iyana Iba area along Lagos State University (LASU) road. This market is also divided into different segments of multipurpose trades but it's mostly big on different kinds of food stuffs.

2.2 Collection and Identification of Fish Samples

A total of eight samples of fish were collected from the two sampling markets. Specifically, two (2) samples each of *Oreochromis niloticus* (Tilapia fish) and *Scomber scrombrus* (Titus fish) were collected from each sampling market and were identified by Fisheries experts in Lagos State University, Ojo. Sample of specimens of *O. niloticus* and *S. scrombrus* collected from the markets are presented in Plates 1 and 2 respectively.

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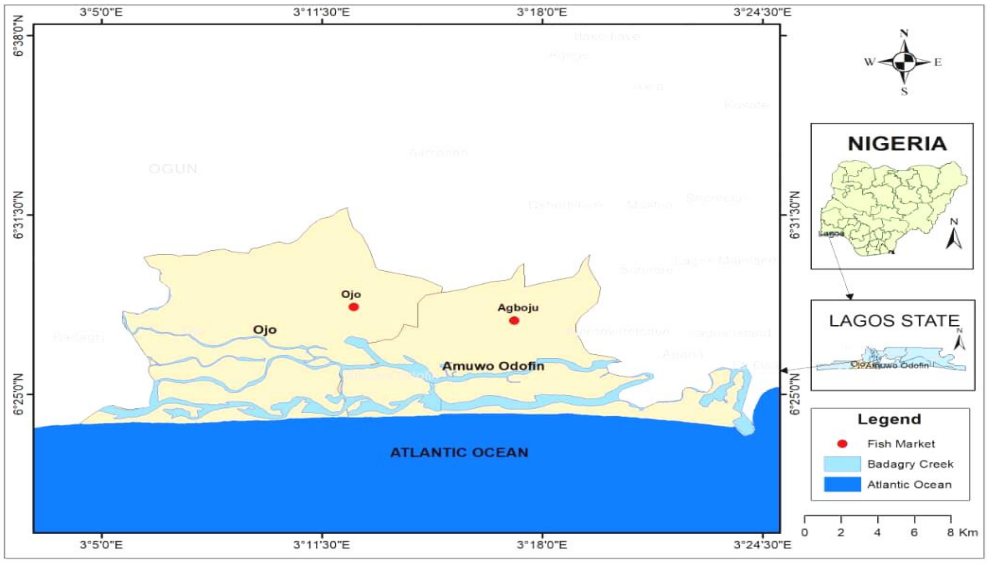

Figure 1: A map showing the location of the selected fish markets (Agboju and Ojo) in Lagos State, Nigeria

Plate 1: Sample of *O. niloticus* from Agboju market

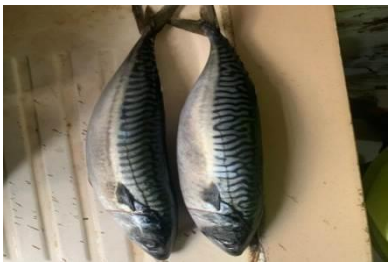

Plate 2: Sample of S. *scombrus* from Ojo market

2.3 Chemical Analysis

2.3.1 Gas chromatography-mass spectrometry (GC-MS) **Analysis of Phthalate Esters**

At the laboratory, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of m/z 69, 219, 502 and other instrument optimal and sensitivity conditions. Determination of the levels of Pthalate Esters in the fish sample was carried out using GC-MS by operating MSD in selective ion monitoring (SIM) and Scan mode to ensure low level detection of the target constituents.

Agilent 8860A gas chromatograph coupled to 5977C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies) was used. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). For the carrier gas, Helium was used at constant flow of 1.2 mL/min at an initial nominal pressure of 10.26psi and average velocity of 40.00 cm/sec. One (1) µL of the samples were injected in splitless mode at an injection temperature of 250°C. Purge flow to spilt vent was 30.0 mL/min at 0.35 min with a total flow of 31.24 mL/min; and thereafter gas saver mode was switched off. The oven was initially programmed at 100°C (1 min), and then ramped at 20°C/min to 280°C (7 min) while the run time was 12 min with a 3 min solvent delay.

The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 300°C.

Acquisition of ion was via Scan mode (scanning from m/z 50 to 500 amu at 2.0s/scan rate) and selective ion mode (SIM) [10]

2.3.2 Calibration Procedure

Pthalate Esters standard, 2000ppm (Catalog Number: M-606) containing 6 Phthalate components was purchased from AccuStandard. Three (4) point serial dilution calibration standards (0.3, 0.6,1.5, 3.00ppm) was prepared from the stock and used to calibrate the GC-MS. After calibration, the samples were analyzed and corresponding Pthalate Esters concentration obtained[10].

2.3.3 Extraction Procedure

A 5g of properly homogenized samples were weighed into beakers and mixed with 10ml n-Hexane: Acetone (1:1). The beakers were then placed into an ultrasonic bath and sonicated for 20 mins. The mixture was allowed to settle and solvent layer was decanted and concentrated down to 2ml using a rotary evaporator [11].

2.3.4 Cleanup Procedure

Granular silica gel (Mesh Size 60-200A) was activated by heating at 130^{o}C for 16hrs and stored in a desiccator. A glass column was packed with 5g of silica gel and 1g of Anhydrous Na_2S0_4 was added, 20ml n-Hexane was added to the column and eluted into a beaker. Thereafter, 2ml sample extract was added to the top of the column quantitatively. While another 10ml of n-Hexane was added to the column and eluted to waste. Before the column head dried out,10ml (1+1) Dichloromethane + Hexane was added and the eluent was collected. Then, the eluent was concentrated to 2ml using a rotary evaporator and analyzed [11].

2.4 Statistical Analysis

Data obtained were computed using Statistical Package for Social Sciences (SPSS version 22) and analyzed with t-test analysis at significant level of $p < 0.05$..

3. RESULTS

3.1 Identification and Quantitation of the Phthalates Compounds

From the fish samples examined for this study, some compounds identified as phthalate components in the samples include Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Benzyl butyl phthalate(BBP), Bis (2-ethylhexyl) phthalate(DEHP) and Di-n-octyl phthalate (DNOP) (Table 1). As shown in Table 2, Dimethyl phthalate was not detected in *Oreochromis niloticus* in both markets (Agboju and Ojo markets). The concentrations of Diethyl phthalate, Dibutyl phthalate, Benzyl butyl phthalate, Bis (2-ethylhexyl) phthalate and Di-n-octyl phthalate in *O. niloticus* (µg/g) from both markets were not significantly different ($p>0.05$).

Table 3 shows the levels of phthalate compounds recorded in *Scomber scombrus* (Mackerel/Titus) from Ojo and Agboju markets. While no Dimethyl phthalate was detected in Mackerel from Ojo markets, other phthalate components were obtained in *S. scombrus* collected from the two markets. However, only the concentrations of Dibutyl phthalate (5.43±0.06 µg/g) and Bis (2-ethylhexyl) phthalate (6.78±0.06 µg/g) in *S. scombrus* from Agboju market were significantly ($p<0.05$) higher than that from Ojo market.

Table 1: Identification and Quantitation of the phthalate compounds

Compounds	Abbreviation	Chemical type	Quantitation ions (m/z)
Dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	163
Diethyl phthalate	DEP	$C_{24}H_{38}O_4$	149
Dibutyl phthalate	DBP	$C_{24}H_{38}O_4$	149
Benzyl butyl phthalate	BBP	$C_{24}H_{38}O_4$	149
Bis (2-ethylhexyl) phthalate	DEHP	$C_{24}H_{38}O_4$	149
Di-n-octyl phthalate	DNOP	$C_{10}H_{10}O_4$	149

Table 2: Concentrations of Phthalate compounds detected in *O.niloticus* from the markets

Phthalate compounds (µg/g)	Agboju Market	Ojo Market
Dimethyl phthalate	**ND**	**ND**
Diethyl phthalate	0.03±0.01^{a}	0.03±0.02^{a}

Dibutyl phthalate	0.26±0.01[a]	0.03±0.01[a]
Benzyl butyl phthalate	0.10±0.01[a]	0.02±0.01[a]
Bis (2-ethylhexyl) phthalate	0.02±0.00[a]	0.07±0.02[a]
Di-n-octyl phthalate	0.05±0.01[a]	0.03±0.01[a]

Mean with the same superscript on the row are not significantly different (p >0.05)
ND = not detected

Table 3: Concentrations of Phthalate compounds detected in *S. scombrus* from the markets

Phthalate compounds (µg/g)	Agboju Market	Ojo Market
Dimethyl phthalate	0.04±0.01	**ND**
Diethyl phthalate	0.49±0.02[a]	0.15±0.04[a]
Dibutyl phthalate	5.43±0.06[a]	1.57±0.06[b]
Benzyl butyl phthalate	0.10±0.01[a]	0.02±0.01[a]
Bis (2-ethylhexyl) phthalate	0.02±0.00[a]	0.07±0.02[a]
Di-n-octyl phthalate	0.05±0.01[a]	0.03±0.01[a]

Mean with the same superscript on the row are not significantly different (p >0.05)
ND = not detected

4. DISCUSSION

Fish play a significant role in human nutrition especially in Nigeria where most people consume fish as a source of protein. In aquatic ecosystem, fishes are reliable bio-indicators of pollutants as they occupy higher trophic levels. In this study, the concentrations of Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Benzyl butyl phthalate (BBP), Bis (2-ethylhexyl) phthalate(DEHP) and Di-n-octyl phthalate (DNOP) in µg/g for *Oreochromis niloticus* (Tilapia) from Ojo and Agboju markets were not significantly different from each other. In line with this study, Salaudeen *et al*.[12] had reported the presence of DMP, DEP, DBP, BBP, DEHP, and DOP of with DBP and DEHP being the most abundant phthalate esters in the wastewater treatment plants of Amathole Municipality in Eastern Cape province of South Africa.

Dimethyl phthalates (DMP) was not detected in *O.niloticus* from Ojo and Agboju markets. Similarly, it was not detected in *Scomber scombrus* (Mackerel) at Ojo market but present in Mackerel from Agboju market. The differences in the observation of DMP content in *S. scombrus* at Ojo market and that of Agboju market might suggest that the fishes were caught from different aquatic stations or due to variance in handling and hygiene of fish sellers across the sampled markets. Furthermore, absence of DMP in *O. niloticus* could suggest that the fish might be adjudged safer than *S. scombrus.* The reason is that DMP has been reported to be an endocrine disrupting chemical (EDC) which promotes chromosomal injuries in human leucocytes, thereby causing abnormalities in reproductive system and interference with the development of animals and humans [13]. However, acceptable daily intake values of DMP have not been calculated due to lack of comprehensive studies pertaining to particular organ systems or exposure duration [14].

In the foregoing, absence of DMP in *O. niloticus* and *S. scombrus* from both markets and Ojo market respectively could suggest presence of adequate microbial biodegradation of the compound in the habitats where the fish resides naturally. Documented literatures have shown that marine microalgal and some microbial species possess the capability to degrade phthalates esters [15-16]. For instance, *Bacillus mojavensis* [17] and *Paracoccus kondratievae* [18] have been reported to have high efficiency for the degradation of phthalates esters. However, it has been opined that microbial degradation is generally affected by external environmental factors and typical lack of specific degrading bacteria in the aquatic ecosystem, which in turn makes phthalate esters degradation difficult under natural conditions, hence their long half-life, that range from a few years to several hundred years [4].

The reviewed ecotoxicity studies for phthalate esters have shown adverse effects to aquatic organisms with broad range of endpoints and at much lower concentration levels [4]. The authors also noted acute and chronic toxic effects on aquatic organisms, with symptoms such as crooked tails, necrosis, cardiac edema, lack of tactile response and death on aquatic animal embryos, while on adult organisms, phthalates exposure could lead to adverse effects on reproduction, damage to the liver, kidney, and other organs [4, 19]. He *et al*. [20], studied bioaccumulation of phthalate esters in fish in China and reported

that most phthalate esters congeners are not likely to accumulate in fish due to their bioaccumulation factors (BAF) being less than 2 except for DEP, DMP and BBP which were reported to comprise the BAFs values greater than 2.

For this study, the concentrations of Dibutyl phthalate (DBP) in *S. scombrus* from Agboju market were significantly higher than that from Ojo market. Higher level of DBP is an indication that Agboju community is more contaminated with these compounds. Mekuleyi *et al.*[21] had reported high level of some endocrine disrupting chemicals such as lead and cadmium from water and sediments of Agboju and Ajegunle water stations. *Oreochromis niloticus* and *S. scombrus* collected at Agboju and Ojo markets had almost equal levels of Diethyl phthalate (DEP) whose values were less than 1µg/g. The low level might suggest that both fishes had less affinity for the compound or they are present at low concentration in their original natural habitat. Diethyl phthalate (DEP) is an industrial chemical such as pesticides, detergents and plasticizers originated from a variety of compounds of anthropogenic used in products like insecticides, mosquito repellants, camphor substitute, plasticizer for cellulose, bathing soaps, cosmetics, pharmaceutical coatings, after shave-lotion, detergent, ester plastic film and sheets [16].
Unlike concentration of Dibutyl phthalates in *O. niloticus* from both markets that was not significantly different, *S. scombrus* from Agboju market had higher level of Dibutyl phthalates than *S. scombrus* from Ojo. The differences could be attributed to sources of catch of the fish. According to Besthseda [22], Dibutyl phthalate is used in making flexible plastics that are found in a variety of consumer products. It appears to have relatively low acute (short-term) and chronic (long-term) toxicity. However, effects of Dibutyl phthalate on humans at high levels may include irritation of the eyes, nose and throat. It may cause nausea, tearing of the eyes, vomiting, dizziness, and headache. Also, long-term exposures may cause liver and kidney damage and may also harm the developing foetus and the male testis when it's taken in at high levels [4]. Levels of Benzyl butyl phthalate, Bis (2-ethylhexyl) phthalate and Di-n-octyl phthalates in *S. scombrus and O.niloticus* that were not significantly different between Agboju and Ojo markets might suggest similar level of contamination of the fish species with those compounds. However, these chemicals have the potential to accumulate in the tissues of fishes and could cause specific effects including behavioral changes, changes in metabolic processes and endocrine disruption [6].It is mundane that endocrine system performs fundamental tasks for the life of an organism and the hormones produced by the endocrine glands have the task of controlling delicate and complex phenomena such as reproduction, growth, development, as well as the metabolism. However, Endocrine disrupters (EDCs) can imitate, compete or stop the synthesis of endogenous hormones and this translates into alterations of glands' function, alteration and reduction of reproduction with consequent low birth rates and potential loss of biodiversity [15].
All the values of phthalates recorded in *S. scombrus and O.niloticus* from Agboju and Ojo markets were lower than those reported by Ai *et al.* [23] when they investigated the distribution and bioaccumulation of dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP)) in 11 edible fish species collected from Poyang Lake, China. Also, only the level of Dibutyl phthalates and Bis (2-ethylhexyl phthalates) in *S. scombrus* from Agboju markets slightly exceeded the permissible limit of < 5µg/g in fish but are less than maximum level of 100 µg/g recommended in aged human [5].

5. CONCLUSION

It has been established in this study that the level of most phthalates detected in *Oreochromis niloticus* and *Scomber scombrus* from both sampling markets were within the permissible levels recommended in fish while all the phthalates level were still below the concentration that could be deleterious to human. Nevertheless, sellers and processors of the fish species should embrace hygienic practices to avert further contamination of the fish.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Mekuleyi Gabriel Olarinde conceived and designed the study, performed the statistical analysis and managed the literature searches. Ndimele Prince Emeka and Elegbede Isa Olalekan edited the first draft of the manuscript while all authors reviewed the final draft before submission.

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