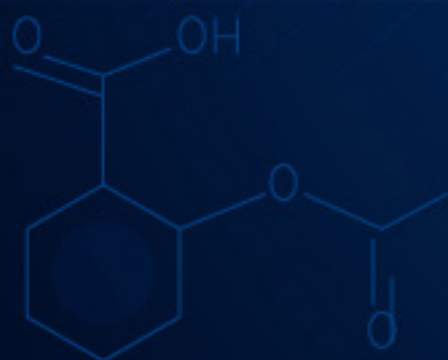




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Evaluation of the Effects of Storage Conditions and Duration on the Levels of Phthalate Esters in Brands of Plastic Bottled Water Produced in Adamawa State, Nigeria

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ABSTRACT

Phthalate acid esters (PAEs) are endocrine-disrupting plasticizers that migrate from polyethylene terephthalate (PET) bottles into drinking water, particularly under heat and ultraviolet (UV) exposure. This study quantified six PAEs; DMP, DEP, DBP, BBP, DEHP, and DNOP in two Adamawa-Nigerian bottled water brands (Gauni and Zaya) stored under refrigeration (~4°C), room temperature (~25–35°C), and direct sunlight (>40°C + UV) for four weeks. Gas chromatography–mass spectrometry (GC-MS) analysis revealed a consistent contamination gradient (refrigeration < room temperature < sunlight). Even under refrigeration, DEHP reached 0.30±0.01 mg/L in Gauni and 0.18±0.01 mg/L in Zaya, exceeding the World Health Organization (WHO) guideline of 0.008 mg/L. At room temperature, DEHP rose to 0.74±0.02 mg/L in Gauni, while DBP reached 0.38±0.01 mg/L. Under direct sunlight, extreme levels were observed. DBP peaked at 3.79±0.10 mg/L in Gauni and 1.61±0.01 mg/L in Zaya, while DEHP reached 3.66±0.02 mg/L in Zaya and 1.24±0.01 mg/L in Gauni. Control samples confirmed negligible baseline contamination (≤0.02 mg/L). DEHP and DBP were the dominant migrating compounds across all conditions. These findings highlight severe public health risks, ranking among the highest PAE levels reported globally, and underscore the urgent need for regulatory intervention, mandatory storage-condition labelling, and consumer education in Nigeria and other tropical regions.

INTRODUCTION

Polyethylene terephthalate (PET) has become the dominant material for bottled water packaging due to its durability, affordability, and recyclability (Razali *et al.*, 2021). Despite these advantages, concerns persist about the migration of phthalic acid esters (PAEs) into drinking water. PAEs are synthetic plasticizers added to enhance flexibility and strength, but because they are not covalently bonded to the polymer matrix, they remain physically dispersed and can leach into water through diffusion processes. This migration is accelerated by elevated temperature, ultraviolet (UV) radiation, and prolonged storage (Jayaweera *et al.*, 2020; Kumari & Kaur, 2020; Net *et al.*, 2015).

Daily consumption of PET-packaged water raises significant health concerns. Phthalates have been linked to malignancies, hormonal imbalances, and developmental delays (Evarist *et al.*, 2024). Epidemiological and toxicological studies implicate them in reproductive toxicity, hepatotoxicity, neurodevelopmental impairment, metabolic disorders, and carcinogenicity (Li *et al.*, 2023; Kahn *et al.*, 2020; Dutta *et al.*, 2020). DEHP, one of the most widely studied PAEs, has been prohibited in children's toys in the United States since 2009 and is classified as a Group 2B human carcinogen (United States Environmental Protection Agency, 2019). It has also been designated as a high-priority substance for risk evaluation due to its potential to cause unreasonable risks to human health and the environment (Massahi *et al.*, 2025).

Global assessments confirm the ubiquity of phthalates in bottled water. A survey of more than 300 brands across 21 countries reported dibutyl phthalate (DBP) and bis(2-ethylhexyl) phthalate (DEHP) as the most frequently detected contaminants, with detection frequencies of 67.6% and 61.7% respectively (Xu *et al.*, 2020). Similarly, Gerassimidou *et al.* (2020) found detection frequencies above 89% for DEHP and 93% for DBP in PET bottled beverages worldwide. Migration is influenced not only by storage conditions but also by factors such as pH, bottle capacity, and solar irradiance intensity (Mohammadi *et al.*, 2024). Bach *et al.* (2014) demonstrated that PET polymers undergo photochemical aging under UV exposure, thereby widening molecular diffusion pathways and accelerating phthalate release.

The severity of phthalate migration is particularly pronounced in tropical regions. Elevated concentrations have been reported in PET bottled water stored under direct sunlight in Tanzania (Evarist *et al.*, 2024), while similar findings have been documented in Iran and Saudi Arabia (Mohammadi *et al.*, 2024; Xu *et al.*, 2020). Seasonal disparities in phthalate levels have also been observed in drinking water systems, underscoring the influence of environmental conditions (Kumawat *et al.*, 2022). Despite these global findings, comprehensive research in Adamawa State, Nigeria remains limited, even though bottled water consumption is widespread and roadside retail often exposes PET bottles to intense sunlight and high ambient temperatures.

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This study therefore aimed to quantify the concentrations of six PAEs 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 two widely consumed bottled water brands (Gauni and Zaya) under refrigeration, room temperature, and direct sunlight over four weeks. The results were compared against World Health Organization (WHO, 2017) drinking water guidelines and international literature to evaluate temporal changes, brand-specific differences, and the broader public health implications of phthalate migration in tropical sub-Saharan Africa.

MATERIALS AND METHODS

Chemicals, Standards, and Reagents

High-purity standards of dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2 ethylhexyl) phthalate (DEHP), and di n octyl phthalate (DnOP) were obtained from Aldrich Chemical Co. in the United States, Scharlau Chime S.A. in Germany, and BDH Chemical Ltd. in England. Methanol, xylene, sodium hydroxide, sulphuric acid, and sodium chloride were of analytical grade. All glassware was cleaned with methanol and n hexane, then oven-dried at 120 °C for two hours to minimize background contamination before use.

Sample Collection and Pre-Treatment

Two bottled water brands, Gauni and Zaya, packaged in 75 cL PET bottles, were purchased from retail outlets in Adamawa State, Nigeria. The bottles were stored under four different conditions: refrigeration at approximately 4 °C, room temperature between 25 and 30 °C, direct sunlight exposure exceeding 35 °C with ultraviolet radiation, and a control condition under standardized laboratory storage immediately after factory production. Sub-samples were withdrawn weekly over a four-week period to monitor changes in phthalate concentrations.

Extraction of Phthalates

Phthalates were extracted using ultrasound-assisted dispersive liquid-liquid microextraction (DLLME) following the methods of Mousa *et al.* (2013) and Alshehri *et al.* (2022). Ten millilitres of each sample were transferred into a 15 mL centrifuge vial, and a mixture of 20 μ L methanol and 500 μ L xylene was rapidly injected to form a cloudy emulsion. The mixture was ultrasonicated for 20 minutes and extracted three times using the same solvent system. The organic phase was collected, combined, and transferred into gas chromatography autosampler vials for analysis.

Analysis of Phthalates (GC-MS) Method

Quantification of phthalates was performed using an Agilent 7890B gas chromatograph coupled with a 5977A mass spectrometer. Separation was achieved on an HP 5MS fused silica capillary column (30 m \times 0.25 mm, 0.25

μ m film thickness) with helium (99.999%) as the carrier gas at a flow rate of 1.0 mL/min. The oven program began at 70 °C for two minutes, ramped to 150 °C at 20 °C/min, and then increased to 280 °C at 10 °C/min with a ten-minute hold. The injector temperature was maintained at 250 °C in splitless mode with an injection volume of 1 μ L. The mass spectrometer operated in electron impact mode at 70 eV, with the ion source temperature set at 230 °C and the quadrupole at 150 °C. Data were acquired in both full-scan (m/z 50–500) and selected ion monitoring (SIM) modes. Quantification was based on a five-point external calibration curve ranging from 0.01 to 5 mg/L using authentic standards, with retention times and mass spectral confirmation ensuring accuracy (Zhou *et al.*, 2021; Mousa *et al.*, 2013; Zaki and Shoeib, 2018).

Preparation of Phthalate External Working Standards

A standard mixture containing standard solutions of dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), n-butyl benzyl phthalate (BBP), diethyl hexyl phthalate (DEHP), and Di-n-octyl phthalate (DnOP) dissolved in methanol each at a concentration of 2000 μ g mL⁻¹. A working standards solution were prepared daily by appropriate dilution of stock solution of PAEs in methanol.

Quality Control and Quality Assurance Procedures

Several quality control precautions were taken to reduce the phthalates cross-contamination that may occur throughout the steps of analysis. Control samples of each selected unpackaged plastic bottle water were analysed. The quality assurance procedures were determined by analysing three replicates of ultrapure Milli Q water blanks spiked with PAEs; the precision of the analysis was determined by estimating relative standard deviation and percent recoveries of the phthalates.

Statistical Analysis

Triplicate determinations were carried out and the results were expressed as the mean \pm SD. Significant differences in PAE concentrations among storage conditions (refrigeration, room temperature, sunlight) and between brands (Gauni and Zaya) were evaluated by one-way analysis of variance (ANOVA), with storage condition and brand as fixed factors. All statistical calculations were performed using IBM SPSS Statistics version 26.0.

RESULTS AND DISCUSSION

The concentrations of six PAEs detected in Gauni and Zaya bottled water are showed in Table 1. Tables 2–4 present weekly progression data per storage condition. Control samples confirmed negligible baseline contamination (\leq 0.02 mg/L or N.D), confirming contamination is storage-driven rather than a manufacturing failure consistent with global.

Table 1: Mean \pm SD concentrations (mg/L) of phthalate esters in Gauni and Zaya bottled water under three storage conditions after four weeks

SN	Phthalate Compound	Gauni Fridge	Gauni Room	Gauni Sun	Gauni Control	Zaya Fridge	Zaya Room	Zaya Sun	Zaya Control
1	Dimethyl phthalate (DMP)	0.00 \pm 0.00	0.00 \pm 0.00	0.08 \pm 0.00	N.D	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.00 \pm 0.00
2	Diethyl phthalate (DEP)	0.01 \pm 0.00	0.01 \pm 0.00	0.15 \pm 0.02	N.D	0.00 \pm 0.00	0.00 \pm 0.00	0.03 \pm 0.00	0.00 \pm 0.00
3	Dibutyl phthalate (DBP)	0.02 \pm 0.00	0.38 \pm 0.01	3.79 \pm 0.10	0.00 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	1.61 \pm 0.10	0.00 \pm 0.00
4	Benzyl butyl phthalate (BBP)	0.00 \pm 0.00	0.00 \pm 0.00	0.05 \pm 0.01	0.00 \pm 0.00	0.02 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.00 \pm 0.00
5	Bis(2-ethylhexyl) phthalate (DEHP)	0.30 \pm 0.00	0.74 \pm 0.00	1.24 \pm 0.01	0.02 \pm 0.00	0.18 \pm 0.00	0.22 \pm 0.01	3.66 \pm 0.02	0.02 \pm 0.00
6	Di-n-octyl phthalate (DNOP)	0.00 \pm 0.00	0.01 \pm 0.00	0.06 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.00 \pm 0.00

Concentrations (Mg/L) Of Phthalate Esters in Gauni and Zaya Bottled Water Under Three Storage Conditions After Four Weeks

Table 1 shows a consistent gradient of phthalate contamination across both brands, with concentrations lowest under refrigeration, higher at room temperature, and highest under sunlight exposure. DEHP and DBP were the dominant compounds detected in all conditions. Under sunlight, Gauni exhibited higher DBP concentrations (3.79 \pm 0.10 mg/L compared to 1.61 \pm 0.01 mg/L in Zaya), whereas Zaya showed elevated DEHP levels (3.66 \pm 0.02 mg/L compared to 1.24 \pm 0.01 mg/L

in Gauni). These variations likely reflect differences in polymer grade or plasticizer composition. Xu *et al.*, (2020) reported DBP and DEHP as the most frequently detected phthalates in bottled water across 21 countries, with detection rates of 67.6% and 61.7% respectively. Similarly, Gerassimidou *et al.*, (2022) observed detection frequencies of 93.3% for DBP and 89.6% for DEHP in PET bottled beverages worldwide. Xu *et al.*, (2020) demonstrated a significant correlation between PET bottle wall thickness and phthalate content ($r = .71$, $p = .05$), suggesting that material properties may contribute to the observed brand specific differences.

Table 2: Weekly progression of phthalate ester concentrations (mg/L) in Gauni and Zaya under refrigeration

Phthalate Compound	Brand	Week 1	Week 2	Week 3	Week 4	WHO (mg/L)
Dimethyl phthalate (DMP)	Gauni	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	-
	Zaya	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	
Diethyl phthalate (DEP)	Gauni	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	-
	Zaya	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	
Dibutyl phthalate (DBP)	Gauni	0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.3
	Zaya	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	
Bis(2-ethylhexyl) phthalate (DEHP)	Gauni	0.08 \pm 0.00	0.15 \pm 0.02	0.23 \pm 0.01	0.30 \pm 0.01	0.008
	Zaya	0.05 \pm 0.00	0.09 \pm 0.01	0.14 \pm 0.01	0.18 \pm 0.02	

BBP and DNOP were below the MDL throughout refrigeration storage

Refrigeration Storage

Phthalate concentrations were minimal under refrigeration relative to room temperature and sunlight. DEHP levels under refrigerator storage surpassed the World Health Organization (WHO) recommendation of 0.008 mg/L. In Gauni, DEHP grew progressively from 0.08 \pm 0.00 mg/L in week 1 to 0.30 \pm 0.01 mg/L by week 4, whereas Zaya rose from 0.05 \pm 0.00 to 0.18 \pm 0.01 mg/L throughout the same timeframe (Table 2). DBP levels were negligible, reaching a maximum of 0.02 \pm 0.00 mg/L in Gauni and 0.01 \pm 0.00

mg/L in Zaya. The dominance of DEHP relative to lower molecular weight PAEs under refrigeration is observed, although DEHP is more resistant to migration due to its hydrophobicity and higher partitioning coefficient (Net *et al.*, 2015; Gerassimidou *et al.*, 2022). Notably, although refrigeration represented the lowest-risk storage condition, DEHP concentrations in both brands exceeded the WHO guideline by more than twenty-fold, demonstrating that cold storage alone does not ensure compliance with international drinking water safety standards.

Table 3: Weekly progression of phthalate ester concentrations (mg/L) in Gauni and Zaya under room temperature storage

Phthalate Compound	Brand	Week 1	Week 2	Week 3	Week 4	WHO (mg/L)
Dimethyl phthalate (DMP)	Gauni	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
	Zaya	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
Diethyl phthalate (DEP)	Gauni	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00	-
	Zaya	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
Dibutyl phthalate (DBP)	Gauni	0.10±0.00	0.19±0.02	0.29±0.01	0.38±0.01	0.3
	Zaya	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00	
Bis(2-ethylhexyl) phthalate (DEHP)	Gauni	0.19±0.01	0.37±0.10	0.56±0.01	0.74±0.02	0.008
	Zaya	0.06±0.01	0.11±0.01	0.17±0.00	0.22±0.01	

BBP and DNOP were below the MDL throughout refrigeration storage

Room Temperature Storage

At ambient room temperature, PAE concentrations grew considerably. DEHP increased from 0.19±0.01 mg/L (week 1) to 0.74±0.02 mg/L (week 4) in Gauni and from 0.06±0.01 to 0.22±0.01 mg/L in Zaya. DBP exhibited a particularly notable growth in Gauni from 0.10±0.00 to 0.38±0.01 mg/L, while staying comparatively low in Zaya (0.01±0.00 mg/L at week 4). Gauni's week-4

room temperature value of 0.74±0.02 mg/L is much higher than DEHP in PET bottled water averaged 0.006 mg/L at ambient circumstances in Saudi Arabia (Xu *et al.*, 2020). The substantial inter-brand difference Gauni DBP approximately 38-fold higher than Zaya most likely reflects differences in PET polymer grade or bottle wall thickness (Xu *et al.*, 2020).

Table 4: Weekly progression of phthalate ester concentrations (mg/L) in Gauni and Zaya under direct sunlight exposure (>40°C + UV radiation)

Phthalate Compound	Brand	Week 1	Week 2	Week 3	Week 4	WHO (mg/L)
Dimethyl phthalate (DMP)	Gauni	0.02±0.00	0.04±0.00	0.06±0.00	0.08±0.00	-
	Zaya	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00	
Diethyl phthalate (DEP)	Gauni	0.04±0.01	0.08±0.00	0.12±0.00	0.15±0.01	-
	Zaya	0.01±0.00	0.02±0.00	0.02±0.00	0.03±0.00	
Dibutyl phthalate (DBP)	Gauni	0.95±0.02	1.90±0.10	2.85±0.10	3.79±0.10	0.3
	Zaya	0.40±0.00	0.80±0.01	1.20±0.02	1.61±0.10	
Bis(2-ethylhexyl) phthalate (DEHP)	Gauni	0.31±0.00	0.62±0.01	0.93±0.00	1.24±0.01	0.008
	Zaya	0.91±0.01	1.83±0.10	2.74±0.01	3.66±0.02	

All six target PAEs were detectable under sunlight conditions in at least one brand by week 4

Sunlight Storage

Table 4 showed the results under direct sunlight. Direct sunlight showed by far the most extreme PAE concentrations. DBP in Gauni escalated from 0.95±0.02 mg/L (week 1) to 3.79±0.10 mg/L (week 4) which was far above the 0.3 mg/L of WHO guideline while Zaya's DBP reached 1.61±0.10 mg/L. DEHP was markedly higher in Zaya (3.66±0.02 mg/L) than in Gauni (1.24±0.01 mg/L). DMP, DEP, BBP, and DNOP negligible under refrigeration also became detectable under sunlight. Similar result was obtained by other companies. Diethyl phthalate (DEP) and Dibutyl phthalate (DBP) were not detected in any samples analysed by Evarist *et al.*, (2024). Net *et al.*, (2015) reported that phthalates are ubiquitous in various environmental matrices and that their release from plastics is enhanced by photodegradation and thermal degradation, which progressively widen molecular diffusion pathways. The escalation observed

values in (Gauni DBP 0.95±0.02 mg/L/week; Zaya DEHP 0.91±0.01 mg/L/week) demonstrated that repeated heat-UV exposure cycles cumulatively degrade the PET polymer matrix (Jayaweera *et al.*, 2020). High temperature (sunlight), and the duration of storage are the primary drivers of phthalate transfer from plastic and paper containers into water (Mohammadi *et al.*, 2024).

Health Implications

The levels measured under direct sunshine and room temperature storage constitute a severe public health concern in the analysed samples in Adamawa State, Nigeria. Phthalates are recognized endocrine-disrupting chemicals (EDCs) capable of interfering with hormonal signalling pathways in humans and wildlife (Li *et al.*, 2023; Kahn *et al.*, 2020). Low molecular weight PAEs such as DMP, DEP, and DBP mimic natural hormones and have been implicated in reproductive impairment,

neurodevelopmental delays, and metabolic dysfunction (Balalian *et al.*, 2019; Dutta *et al.*, 2020). High molecular weight PAEs such as DEHP and BBP are classified as probable carcinogens and are associated with hepatotoxicity and reproductive developmental anomalies (Gerassimidou *et al.*, 2024).

Chronic ingestion of water containing DEHP at $0.74 \pm 0.02 - 3.66 \pm 0.02$ mg/L (from the analysed samples) represents exposures hundreds of times beyond the WHO recommendation. Even refrigeration-stored samples revealed DEHP values 22–37 times the WHO guideline, refuting the premise that cold storage promotes safety (Wowkonowicz, 2023). Urgent regulatory interventions required include NAFDAC-mandated PAE maximum residue levels linked with WHO; mandatory storage condition labelling and shelf-life limits; prohibiting outside sunlight display of PET bottled water; and concentrated consumer education initiatives.

CONCLUSION

This study shows that storage conditions play a critical role in the migration of phthalates from PET bottled water. Contamination was consistently lowest under refrigeration, higher at room temperature, and most extreme under direct sunlight. The findings confirm that phthalate migration is storage-driven and intensified by tropical climates, where heat and ultraviolet radiation accelerate polymer degradation. The results highlight serious public health risks, as consumers are exposed to levels far above international safety recommendations. Importantly, the study demonstrates that even refrigeration does not guarantee safe limits, challenging assumptions about storage practices. Urgent regulatory action is needed to address this issue. Measures such as setting maximum residue limits, enforcing proper labelling, prohibiting outdoor sunlight display, and educating consumers are essential to reduce exposure.

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