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Microbiological and chemical risk assessment of sachet drinking water sold in selected locations in Enugu State, Nigeria

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ABSTRACT

Sachet water is widely consumed in Nigeria due to its affordability and availability. However, its safety can be compromised during production, distribution, storage and handling. This study assessed the microbiological and chemical quality of sachet drinking water obtained from selected locations in Enugu State, Nigeria. Samples were collected from two locations (manufacturing site and motor park vendor). Microorganisms were isolated using standard microbiological methods and identified using phenotypic and molecular characterization. Heavy metals were analyzed using an Agilent FS240AA atomic absorption spectrophotometer, and phthalates assessed by gas chromatography with flame ionization detection. No bacterial count was detected in the manufacturing site sample, whereas the motor park sample recorded a total heterotrophic bacterial count of 1.5×10^4 CFU/mL. *Staphylococcus aureus* which had 89.20% pairwise identity with *Staphylococcus aureus* strain IF4SW-P1 was isolated from the motor park sample. Heavy metals detected were lead (0.072–0.082 mg/L), arsenic (0.105–0.122 mg/L), and cadmium (0.037–0.052 mg/L), chromium (0.030–0.042 mg/L). The phthalates esters detected from the manufacturing site and motor park samples were Diisoundecyl phthalate, Di (2-propylheptyl) phthalate, Butyl benzyl phthalate, Diisotridecyl phthalate, Diundecyl phthalate and Diisodecyl phthalate. Di (2-ethylhexyl) phthalate and Diisoheptyl phthalate were only detected in the motor park sample. The findings highlights that although the sachet water was free from detectable bacterial contamination at the production site, contamination may occur during distribution or handling. Therefore, improved hygienic practices, proper handling, packaging and storage are recommended for consumer safety.

Keywords: Sachet water, Microbiological quality, Heavy metals, Phthalates, pH, *Staphylococcus aureus*

INTRODUCTION

Access to safe drinking water remains an important public health concern, particularly in developing countries where public water supply systems are often unreliable. In Nigeria, sachet water has become a common source of drinking water because it's affordable and convenient for consumers (Aroh *et al.,* 2013).

The production and distribution of sachet water in Nigeria are regulated by the National Agency for Food and Drug Administration and Control (NAFDAC). However, the quality of sachet water may still be affected by contamination during production, packaging, transportation, storage, and sale. Poor hygiene and environmental conditions at any of these stages can increase the possibility of contamination.

Microbial contamination of drinking water is of particular concern because contaminated water can serve as a means of transmitting diseases such as diarrhoea, cholera, typhoid fever, and dysentery. Several bacteria including *Staphylococcus* sp., *Enterobacter* sp., *Proteus* sp., *Klebsiella* sp., and *Escherichia coli* have been isolated from the sachet drinking water samples sold in Oye-Ekiti Local Government Area (2) (Adesemoye *et al.,* 2024). The detection of indicator organisms, particularly *Escherichia coli*, may suggest faecal contamination and inadequate sanitary conditions (Halage *et al.*, 2015).

Apart from microbiological quality, the chemical composition of drinking water is also important to consumer safety. Heavy metals such as lead, cadmium, arsenic, chromium, and nickel can enter water through different environmental and industrial sources. Long-term exposure to some of these metals has been associated with adverse health effects, including neurological and kidney damage and, in some cases, carcinogenic effects (Rahman and Singh, 2019). Also chemicals such as phthalates may enter into the water from plastic packaging into drinking water. These phthalates have been associated with endocrine-related, reproductive, and developmental effects (Net *et al.*, 2015).

Several conditions practiced during transportation and storage can expose the packaged water to heat, dust, and other environmental contaminants which can affect the quality of the sachet water.

Despite the widespread use of sachet water in Agbani, Enugu State, there is limited information on the quality of the same brand of sachet water obtained from various locations. Therefore, this study assessed the microbiological and chemical (including pH, heavy metals, and phthalates) quality of sachet drinking water of the same brand collected from the manufacturing site and a motor park in Agbani, Enugu State, Nigeria.

MATERIALS AND METHODS

Collection of samples

The assessed sachet water samples were from the same commercial brand and were collected from different locations within Agbani in Enugu State, Nigeria. The first sample was obtained directly from the manufacturing factory and the second sample obtained from a motor park vendor.

All samples were transported to the Microbiology Laboratory, Enugu State University of Science and Technology using sterile containers and analyzed immediately.

Microbiological analysis

Total heterotrophic bacterial count

The serially diluted samples (0.1ml) were inoculated onto Nutrient Agar plates and incubated at 37°C for 24 hours. The developed colonies were counted using colony counter and expressed as colony-forming units per milliliter (CFU/mL).

Microbiological assessment of the sachet water sample

Each sample was inoculated onto MacConkey agar, Salmonella shigella agar, mannitol salt agar, sabouraud dextrose agar (SDA) and incubated at 37°C for 24 h except for SDA which was incubated at 28°C for 7 days.

Identification of the isolates

The preliminary identification and molecular profiling of the bacterial isolate was done as described by Nwachukwu and Chioke (2026). The preliminary identification of the isolate was based on colony morphology, Gram staining reaction and biochemical tests.

The molecular profiling was carried out through Genomic DNA extraction using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, USA) following the manufacturer's protocol. The DNA quality and PCR products were assessed using agarose gel electrophoresis.

The 16S rRNA gene was amplified in a 25 µL reaction containing 12.5 µL of 2× Taq Master Mix (NEB, M0270), 1 µL each of primers 27F and 1525R, 2 µL of template DNA, and 8.5 µL of nuclease-free water. The PCR products were sequenced using an Applied Biosystems Genetic Analyzer 3500 with the BigDye Terminator v3.1 kit. Sequences were edited and analyzed using Geneious Prime and compared with reference sequences for identification. Confirmed sequences were submitted to NCBI GenBank for accession numbers.

pH determination

The pH of the water samples was measured using a digital pH meter (Hanna Instruments, USA). An aliquot of each sample was transferred into a clean beaker, the electrode was then immersed in the sample and then the pH reading was recorded.

Determination of phthalates

Sample extraction and clean-up

Phthalates were extracted from the homogenized samples using a Soxhlet extraction procedure. Five grams (5 g) of each homogenized sample was mixed thoroughly with 60 g of anhydrous sodium sulphate in an agate mortar to remove moisture. The mixture was transferred into a 500 mL beaker and extracted with 300 mL of n-hexane for 24 h. The crude extract obtained was concentrated using a rotary vacuum evaporator at 40 °C to near dryness.

The concentrated residue was re-dissolved in n-hexane and subjected to column clean-up. A chromatographic column (20 cm × 8 mm internal diameter) was packed with 5.0 g of activated silica gel prepared as a slurry with 1.5% (v/m) petroleum ether. Approximately 1 mL of anhydrous sodium sulphate was placed on top of the column to absorb residual moisture. The column was pre-eluted with 15 mL of petroleum ether. The concentrated extract was then introduced into the column and allowed to pass through the sodium sulphate layer. The phthalate esters were eluted with two 10 mL portions of the extracting solvent. The eluate was collected, concentrated to dryness, and reconstituted with 2 mL of the extracting solvent prior to GC-FID analysis.

Preparation of phthalate standards

A 100 mg/L stock solution of each phthalate ester standard was prepared in isopropyl alcohol using a 5 mL volumetric flask. Lower working concentrations were prepared by appropriate dilution of the stock solutions. Aliquots (1.0 µL) of the working standards were injected into the GC-FID for calibration.

Gas chromatographic analysis

The cleaned sample extracts were analyzed using gas chromatography with flame ionization detection (GC-FID). A 1.0 µL aliquot of each reconstituted extract was injected into the GC-FID. The detected phthalates were identified by comparing their retention times with those of the corresponding standards, while their concentrations were determined from the calibration curves obtained from the standard solutions.

Heavy metal analysis

Heavy metal analysis was conducted using an Agilent FS240AA atomic absorption spectrophotometer (AAS) according to the method described by the American Public Health Association (APHA, 1995).

The sample was thoroughly mixed by shaking, and then 100ml of it was transferred into a glass beaker of 250ml volume. Thereafter, 5ml of conc. nitric acid was added into it and heated to boil till the volume was reduced to about 15-20ml, by adding conc. nitric acid in increments of 5ml till all the residue was completely dissolved. The mixture was cooled and was made up to 100ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. Thereafter, the sensitivity for 1% absorption was observed (Adrian, 1973).

Preparation of reference solutions and calibration

Reference standard solutions were prepared daily by appropriate dilution of individual stock metal solutions with water containing 1.5 mL of concentrated nitric acid per litre. A series of standard solutions covering the appropriate working concentration range was prepared for each metal. Calibration curves were obtained by plotting the absorbance of the standards against their corresponding concentrations. The concentrations of the metals in the samples were determined from the respective calibration curves**.**

RESULTS AND DISCUSSION

Total heterotrophic bacterial count (THBC) of the sachet water samples

The two sachet water samples showed a clear difference in their heterotrophic bacterial counts. No bacterial growth was observed in that of the manufacturing site (SW) sample. This suggests that the water was microbiologically satisfactory at the point of production and that the treatment and handling processes at the factory were effective. This findings is in line with the reports on the quality of properly treated drinking water (Halage *et al.*, 2015).

The motor park sample (SW1) recorded a THBC of 1.5 × 10^4 CFU/mL (Table 1). This suggests that the microbiological quality of the sachet water may have changed after leaving the production site. Its contamination may have occurred during transportation, storage, or handling at the point of sale. The contamination could also be from dust, frequent human contact, and exposure to the surrounding environment at the motor park. Similar increases in microbial counts have been reported in sachet water samples sold by the retailers (Edema *et al.*, 2011; Oyedeji *et al.,* 2010).

Table 1. Total heterotrophic bacterial count (THBC) of the sachet water samples

Sample code	Source	THBC(CFU/mL)
SW	Manufacturing site	0
SW1	Motor Park	1.5× 10^4

A high total heterotrophic bacterial count does not necessarily mean that the organisms present are pathogenic, but it can indicate a deterioration in water quality or conditions (Halage *et al.*, 2015). Therefore, regular quality checks along the distribution chain would help to identify possible points where contamination occurs.

Identification of the isolate from sachet water samples

The result indicates that there was no microbial growth in the manufacturing-site sample (Table 2). This indicates that the sachet water was microbiologically safe at the production point. Similar findings have been reported in packaged drinking water samples (Igbeneghu and Lamikanra, 2014).

In contrast, *Staphylococcus aureus* was recovered from the motor park sample which was closely related to *Staphylococcus aureus* strain IF4SW-P1. The 16S rRNA gene sequence showed 89.20% pairwise identity, with a bit score of 244.878 and an E-value of 1.58 × 10^{-59} (Table 3, Fig. 1&2).

The presence of this organism may indicate contamination after production, which could be through transportation, storage, or handling. *S. aureus* is commonly found on the skin, hands, and in the nasal passages of humans, and its detection in drinking water can therefore be associated with poor handling hygiene.

Table 2. Preliminary identification of the isolates

Isolate code	Colonial appearance						Biochemical tests								Presumptive identity
	Gram stain	SSA	MSA	Mac	NA	SDA	Catalase	Coagulase	Oxidase	Indole	Methyl red	Voges proskauer	Citrate	Urease	
SW	ND	NG	NG	NG	NG	NG	ND	ND	ND	ND	ND	ND	ND	ND	No detectable bacterial growth
SW1	+Rod shaped bacilli	NG	Round smooth golden yellow colonies	NG	Round golden yellow colonies	NG	+	+	-	-	+	+	+	+	*Staphylococcus aureus*

Note: ND= Not Determined, NG= No growth, SW =Manufacturing Site Sample, SW1=Motor Park Sample, SSA=Salmonella Shigella agar, Mac= MacConkey Agar, NA= Nutrient Agar, MSA= Mannitol Salt Agar

Table 3. Molecular profiling of isolated bacteria from the sachet water sample

Sample code	% pairwise identity	Accession	Bit-score	Description of related organism	E value	Hit end	Hit start
SWI	89.20%	KY218821	244.878	*Staphylococcus aureus* strain IF4SW-P1 16S	1.58EIU-59	646	246

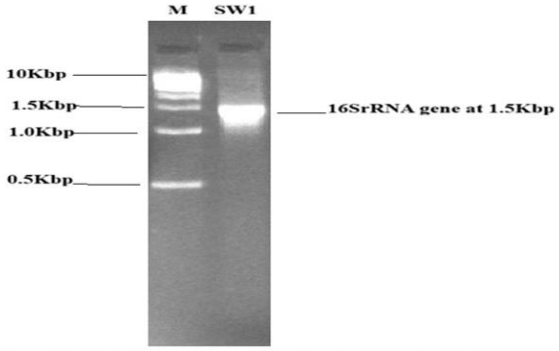


Fig. 1. The amplification of the 16S rRNA gene at 1500 bp
Key: Lane M =1Kbp DNA Ladder

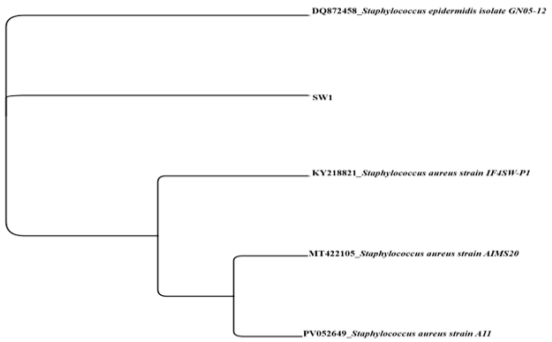


Fig. 2. Phylogenetic tree of the bacterial isolate

This result is in line with a previous study that reported the presence of *S. aureus,* along with other bacterial species, from sachet drinking water marketed in Western Nigeria (Agbasi *et al.*, 2024; Odeyemi, 2015). Their findings also showed that the microbiological quality of packaged water can change between production and the point of sale.

The present study differs from other reports in that only one bacterial species was recovered from the motor park sample, whereas other studies reported several bacterial genera, including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* spp., *Proteus* spp., and *Bacillus* spp. (Olaoye and Onilude, 2009; Odeyemi, 2015). This result also differs from the findings of Enaigbe and Imade (2021) who reported the absence of *Staphylococcus aureus* in NAFDAC-certified sachet water brands in Benin City.

pH of the assessed sachet drinking water samples

The pH values obtained for the sachet drinking water samples collected from the manufacturing site and Motor Park were 7.00 and 7.38, respectively. The manufacturing-site sample had a neutral pH, while the Motor Park sample was slightly alkaline. Both values were within the recommended range of 6.5–8.5 for drinking water, indicating that the samples had acceptable pH levels (WHO, 2017) (Fig. 3).

This result is similar with those reported for commercially available sachet drinking water in Gwagwalada, Nigeria, where pH values ranging from 6.80 to 7.43 were observed (Agbasi *et al.*, 2024). Similarly, pH values of 6.88–8.10 have been reported for sachet water samples from Ogun State, Nigeria (Taiwo *et al.*, 2023).

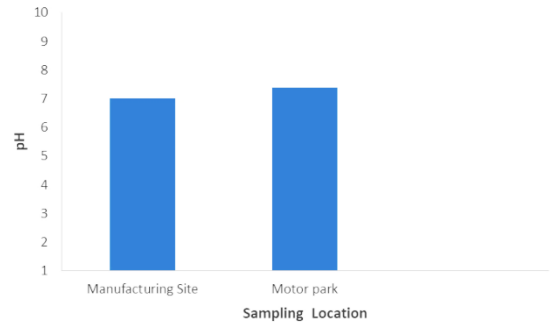


Fig. 3. pH of sachet drinking water samples from different locations

The slightly higher pH recorded in the Motor Park sample may be related to differences in storage and handling after production. Variations in the physicochemical properties of sachet water obtained from different locations have been reported (Mohammed *et al.,* 2023).

The present findings, however, differ from those reported in some sachet water samples from Kano State, where a pH value of 6.39 was observed (Mohammed *et al.*, 2023).

Determination of heavy metals in sachet water samples

The presences of heavy metals were detected from both samples but the concentrations varied between the two sampling points. Lead recorded 0.072 mg/L and 0.082 mg/L in Samples A and B, respectively. Both values were above the WHO guideline value of 0.010 mg/L. Lead is a toxic metal and prolonged exposure to high levels may cause neurological and developmental problems, kidney damage, and cardiovascular effects (Tchounwou *et al.*, 2012). The present of lead in drinking water may be from contaminated water sources, environmental pollution, industrial activities, or the leaching of metals from pipes and storage materials.

Arsenic recorded 0.105 mg/L in Sample A and 0.122 mg/L in Sample B (Table 4). These values were more than the WHO guideline value of 0.010 mg/L. Arsenic observed in drinking water is of great concern because long-term exposure has been linked with skin lesions, cardiovascular and neurological effects, and certain types of cancer.

Table 4. Heavy metal concentrations in sachet water samples

Heavy metal	Sample A (mg/L)	Sample B (mg/L)	WHO limit(mg/L)	Interpretation
Lead (Pb)	0.072	0.082	0.010	Above limit
Arsenic (As)	0.105	0.122	0.010	Above limit
Cadmium (Cd)	0.037	0.052	0.003	Above limit
Chromium (Cr)	0.030	0.042	0.050	Within limit

Cadmium recorded 0.037 mg/L and 0.052 mg/L in Samples A and B, respectively. These values were also higher than the WHO guideline value of 0.003 mg/L. Cadmium is a toxic metal that can accumulate in the body following prolonged exposure. Continuous intake of water containing high levels of cadmium has been associated with kidney damage, skeletal effects, and reproductive toxicity (Jaishankar *et al.,* 2014). The presence of cadmium in the samples may be related to environmental contamination, industrial activities, waste disposal, or contamination of the water source.

Unlike the other metals, chromium was detected at 0.030 mg/L in Sample A and 0.042 mg/L in Sample B, which were below the WHO guideline value of 0.050 mg/L. This suggests that chromium was not a major chemical concern in the samples analyzed. However, continued monitoring is still necessary because excessive exposure to some forms of chromium can have adverse health effects.

The results of the present study are in agreement with other reports in which relatively high levels of lead and cadmium were detected in some sachet water samples, with reported concentrations ranging from 0.002 to 0.036 mg/L (Orisakwe *et al.*, 2006; Rahman and Singh, 2019). In contrast, Makwe and Ukah (2025) reported absence of heavy metals in

investigated 15 sachet water samples in Gwagwalada, Abuja. These differences may be related to variations in water sources, production processes, environmental conditions, and handling or storage practices at the different locations.

Determination of phthalates in sachet water samples

Sachet water sample A

Chromatographic analysis of Sachet Water Sample A collected from the manufacturing site showed the presence of several phthalate compounds. Phthalates are commonly used as plasticizers in the production of flexible plastics and other packaging materials. Their detection in the sample may be related to the migration of these compounds from the packaging material into the water during processing, storage, or transportation.

Butyl benzyl phthalate (BBP) was the most abundant detected, with a concentration of 74.23 µg/L and a retention time of 2.202 minutes. Others detected were diundecyl phthalate (11.56 µg/L), diisodecyl phthalate (5.33 µg/L), diisotridecyl phthalate (1.30 µg/L), di(2-propylheptyl) phthalate (0.94 µg/L), and diisoundecyl phthalate (0.11 µg/L) (Fig. 4).

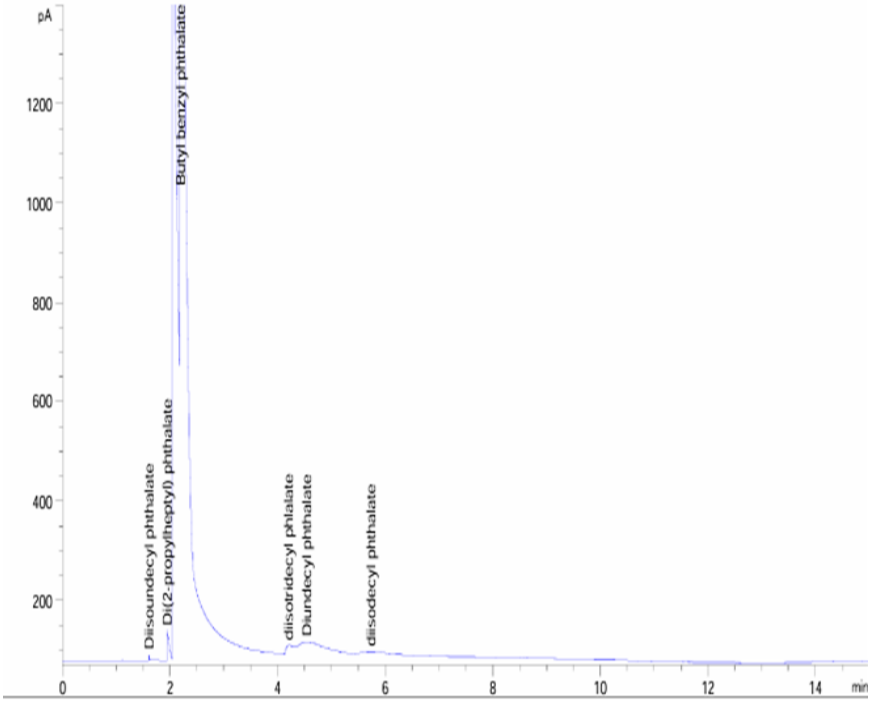


Fig. 4. Chromatograph of the phthalates in sachet water samples A (Manufacturing site)

Diisoundecyl phthalate=1.14621e-1 ug/l; Di (2-propylheptyl) phthalate=9.35887e-1 ug/l; Butyl benzyl phthalate=74.22614 ug/l; Diisotridecyl phlalate=1.29782 ug/l; Diundecyl phthalate=11.56409 ug/l; Diisodecyl phthalate=5.32997 ug/l.

Di(2-ethylhexyl) phthalate (DEHP) and diisoheptyl phthalate were not detected. This may indicate that they were absent from the sample or present at concentrations below the detection limit of the analytical method. The detection of several phthalate compounds in the sample collected directly from the manufacturing site suggests that contact with plastic packaging may contribute to the presence of these compounds in sachet water. Phthalate migration from plastic materials can be influenced by factors such as storage duration and temperature. Considering the widespread consumption of sachet water, the presence of these compounds highlights the need for continued monitoring of phthalates in packaged drinking water.

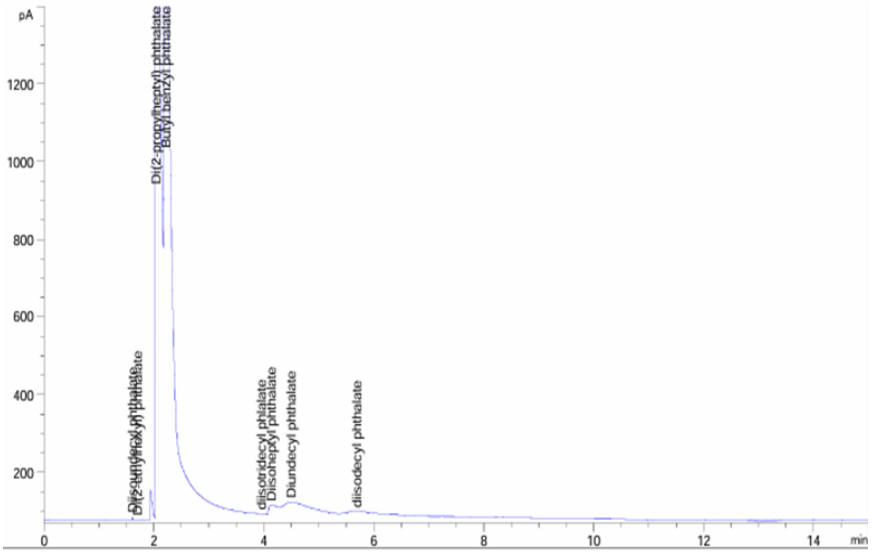


Fig. 5. Chromatograph of the Phthalates in Sachet Water Samples B (Motor Park)
Diisoundecyl phthalate=8.35831e-2 ug/l; Di (2-ethylhexyl) phthalate =1.53777e-1ug/l; Di (2-propylheptyl) phthalate =78.98231 ug/l; Butyl benzyl phthalate=71.59454 ug/l; Diisotridecyl phlalate=6.18471e-2 ug/l; Diisoheptyl phthalate=1.34234e-1 ug/l; Diundecyl phthalate=15.24215 ug/l; Diisodecyl phthalate=9.60787 ug/l

Sachet water sample B

The chromatographic analysis of Sachet Water Sample B collected from the motor park showed the presence of several phthalate ester compounds at different concentrations. Compared with Sample A obtained from the manufacturing site, Sample B contained higher concentrations of a number of the detected compounds. This difference may be related to conditions after production, including transportation, storage, handling, and exposure to the surrounding environment.

Butyl benzyl phthalate (BBP) was detected at 71.59 µg/L, while di(2-propylheptyl) phthalate had the highest concentration of 78.98 µg/L. Other compounds detected were diundecyl phthalate (15.24 µg/L), diisodecyl phthalate (9.61 µg/L), diisoheptyl phthalate (0.13 µg/L), di(2-ethylhexyl) phthalate (0.15 µg/L), diisoundecyl phthalate (0.08 µg/L), and diisotridecyl phthalate (0.06 µg/L) (Fig. 5).

The detection of several phthalate compounds in the same sample is of interest because these substances are commonly used as plasticizers in different plastic materials, including materials used for packaging (Naïja *et al.*, 2026).

The higher concentrations observed in Sample B compared with Sample A suggest that conditions occurring between production and the point of sale may have contributed to the observed differences. Exposure of packaged water to heat, prolonged storage, or contact with contaminated surfaces during transportation and handling could potentially influence the migration or presence of these compounds (Benjamin *et al.*, 2017).

Although the concentration detected in this study was low, the presence of phthalates in drinking water deserves attention.

CONCLUSION

The findings indicate that sachet water quality deteriorates after production, posing potential health risks to consumers and highlighting the need for stricter hygiene and regulatory control. This study has important implications for achieving the United Nations Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being) and SDG 6 (Clean Water and Sanitation). It provides scientific evidence supporting improved water quality monitoring and safer drinking water practices by reducing waterborne diseases and exposure to hazardous contaminants.

RECOMMENDATION(S)

Future studies should involve a larger number of sachet water samples from different brands and locations to provide more information on their microbiological and chemical quality. Sampling at different stages of production, transportation, distribution and storage would help identify possible sources of contamination.

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