



Microbiological and Physicochemical Analysis of Sachet Water within Awka Metropolis

Ifionu, VC^{1*}, Agu, KC², Oghonim, P.AN³, Uwanta, L.I⁴, Awari, VG⁵, Anazodo, C.A⁶, Soludo, OC⁷, Ojeah, I.K.⁸, Mbachu, IAC⁹, Okinedo, J.I¹⁰

Nnamdi Azikiwe University, Nigeria¹

Nnamdi Azikiwe University, Nigeria²

University of Delta, Nigeria³

Nnamdi Azikiwe University, Nigeria⁴

Tansian University, Nigeria⁵

Nnamdi Azikiwe University, Nigeria⁶

Nnamdi Azikiwe University, Nigeria⁷

University of Delta, Nigeria⁸

Chukwuemeka Odumegwu Ojukwu University Uli, Nigeria⁹

Southern delta University, Nigeria¹⁰

Corresponding Emil: vc.ifionu@unizik.edu.ng*

Received: 19-12-2025

Reviewed: 22-01-2026

Accepted: 20-02-2026

Abstract

Drinking water quality is one of the major significances affecting human health, lifestyle and economic well-being. Drinking water quality is solemnly dependent on the quality of source water, the treatment in water treatment plants before distributed, the water distribution system and the containers/tanks used for water storage and the household filters. This study evaluated the bacteriological, physicochemical, and heavy metal qualities of ten popular brands of sachet water commercially sold in Awka Metropolis, Anambra State, Nigeria. The aim was to assess their compliance with national and international drinking water standards and determine their potability. Standard microbiological techniques were employed to quantify microbial loads, isolate and identify bacteria, while atomic absorption spectrophotometry was used for heavy metal analysis. Physicochemical parameters were determined using standard American Public Health Association (APHA) methods. The results indicated that while the majority of the water brands were free from significant microbial contamination, two brands (FC and DS) tested positive for total coliforms, with brand FC also testing positive for faecal coliforms (*E. coli*), indicating faecal pollution. Bacterial isolates of public health concern included *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Bacillus subtilis*. Fungal counts were low but present in some samples. The physicochemical parameters (pH, turbidity, temperature, hardness, colour, and taste) for all samples were within the acceptable limits set by the WHO and NSDWQ. However, heavy metal analysis revealed alarmingly high levels of lead in one brand (FC), which exceeded the WHO permissible limit by over 12 times. The findings reveal that despite generally acceptable physicochemical qualities, some sachet water brands in Awka Metropolis

pose potential health risks due to microbiological contamination and toxic heavy metals. The study recommends stricter regulatory monitoring, improved hygiene practices among producers, and public awareness to safeguard consumer health.

Keywords: Microbiology, Physiochemical Analysis, Sachet water, Awka

Introduction

The socioeconomic status and physiological welfare of a population are inextricably linked to the integrity of its drinking water. Water functions as a sustainer of life and a pillar of a healthy environment only when it is free from harmful chemical and microbiological contaminants (Rajiv et al., 2012; Ajibade et al., 2015; Thliza et al., 2015; Hassan et al., 2016; Li & Wu, 2019). The suitability of water for human consumption depends on a continuum of factors, including the quality of the source, effectiveness of treatment processes, integrity of distribution systems, and hygiene during handling and storage (Li & Wu, 2019). Microorganisms are ubiquitous in natural and engineered water systems, and their presence may be beneficial or pathogenic depending on the environmental context and population density (Awari et al., 2023).

Globally, groundwater is a vital resource for domestic, agricultural, and industrial uses. However, its quality is strongly influenced by environmental and anthropogenic activities. Increasing incidences of groundwater contamination have raised serious public health concerns and attracted growing scientific attention (Li et al., 2017; Ilori et al., 2019; Wu et al., 2019). In urban and peri-urban settings, poor waste management practices and indiscriminate disposal of solid waste contribute significantly to groundwater pollution. In Awka, solid waste dumpsite leachates have been shown to introduce pathogenic microorganisms and heavy metals into groundwater sources, rendering them unsafe for consumption (Agu et al., 2014). Similar findings have been reported in borehole water from neighboring urban centers in southeastern Nigeria, where elevated microbial and heavy metal levels exceeded recommended safety limits (Egurefa et al., 2024).

In developing countries, rapid population growth and inadequate public water supply systems have intensified dependence on alternative drinking water sources. Sachet water has therefore become a widely consumed commodity, particularly among students and low-income populations, due to its affordability and accessibility. Despite its popularity and the misleading designation “pure water,” sachet water is often produced under suboptimal sanitary conditions. The source water—commonly boreholes and shallow aquifers—may already be microbiologically compromised, as earlier studies in Awka and its environs have shown frequent contamination of surface and groundwater by diverse bacterial populations (Agu et al., 2017). Such contamination poses a heightened health risk, especially for infants and children with underdeveloped immune systems.

This study therefore aims to evaluate the bacteriological and physicochemical properties of selected sachet water brands sold within Awka Metropolis. Specifically, the research seeks to determine microbial loads, identify bacterial isolates, and assess

Microbiological and Physicochemical Analysis of Sachet Water within Awka Metropolis

physicochemical parameters and heavy metal concentrations in order to ascertain their compliance with established drinking water standards.

Literature Review

Several studies across Nigeria have documented the poor quality of packaged drinking water. Ibrahim et al. (2015) reported significant physicochemical and bacteriological non-compliance among sachet water brands in Bauchi metropolis, with a large proportion deemed unsafe for human consumption. Within Awka, comparative studies of water samples from hostels revealed high microbial loads indicating inadequate treatment and post-production contamination (Victor-Aduloju et al., 2023). The presence of bacteria in environmental waters and effluents, including abattoir wastewater in Awka, further reflects the widespread dissemination of microorganisms capable of contaminating drinking water sources (Ezeokoli et al., 2023).

Chemical contamination also represents a major concern in drinking water safety. Heavy metals may infiltrate water sources through geogenic processes or anthropogenic activities such as waste dumping and industrial discharge. Studies have shown that although some trace metals may occur within permissible limits, others often exceed regulatory standards, constituting long-term health risks upon continuous exposure (Agu et al., 2014; Egurefa et al., 2024). In aquatic environments, diverse microbial communities—including halotolerant and environmentally persistent species—have been isolated from local rivers and salt lakes, demonstrating their adaptability and potential influence on water quality dynamics (Agu & Odibo, 2021; Agu et al., 2017).

Additionally, water quality can deteriorate during storage and distribution. Duru et al. (2017) observed that prolonged storage of sachet water led to deterioration in chemical quality, even when samples initially complied with World Health Organization standards. These findings emphasize the role of handling, storage conditions, and microbial regrowth in compromising potable water quality.

Given the documented evidence of microbiological and physicochemical non-compliance in drinking water studies (Balogun et al., 2014; Michael et al., 2015; Addo et al., 2016; Chinenye & Amos, 2017), continuous monitoring of sachet water remains essential. Considering the heavy reliance on sachet water by residents of Awka Metropolis, regular assessment of its bacteriological and physicochemical quality is necessary to ensure public health protection and regulatory compliance.

Research Method

The research was conducted within Awka Metropolis, the administrative capital of Anambra State, situated in Southeastern Nigeria. Geographically, the study area is positioned at latitude 8.2069°N and longitude 7.0678°E (Figure 1). According to the Nigeria Population Commission (2006), the metropolis supports a population of approximately 361,657 residents.

While historically celebrated for indigenous iron smiting and wood carving, the city has experienced rapid urbanization and a significant influx of civil servants following its designation as the state capital. This population surge has expanded the local water market, which currently hosts over sixty sachet water production facilities, though only an estimated 70% are formally certified. Climatically, Awka features a tropical environment with a six-month rainy season and a dry Harmattan period spanning November to February. The onset of the rains is typically preceded by a brief, intense dry spell characterized by daily maximum temperatures ranging between 35°C and 40°C.

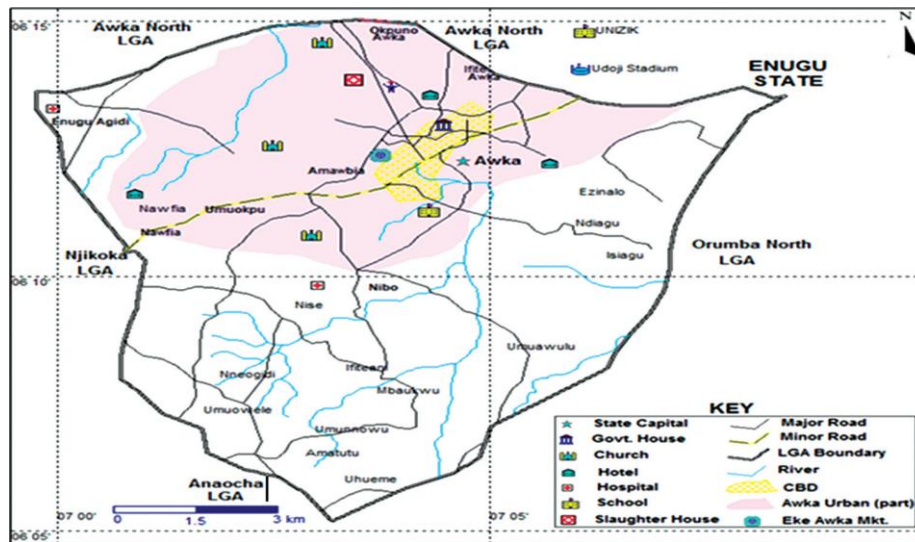


Figure 4: Map of Awka Metropolis

(Source: Department of Geography, University of Nigeria, Nsukka).

A total of twenty (20) sachet water samples, representing the ten most patronized brands within the metropolis, were procured from various retail points (two sachets per brand). Immediately after purchase, samples were coded and transported in ice-packed coolers to the Microbiology Laboratory at Nnamdi Azikiwe University to maintain a cold chain. All analyses were commenced within two hours of collection. To ensure sample integrity, the external surface of each sachet was homogenized by varying the position of the sachet, then sanitized with ethanol-soaked cotton wool. Aliquots were subsequently withdrawn using a sterile needle and syringe for the assessment of pH, turbidity, and organoleptic properties (colour, odour, and taste).

Table 1: Sampling frame

S/N	CODE
1	FC
2	AL
3	AR
4	RT
5	DS
6	BJ
7	PN
8	FN
9	CA
10	KP

Culture Media Preparation

A range of selective and differential media were utilized for microbial isolation, including Nutrient Agar (NA), Nutrient Broth (NB), MacConkey Agar (MA), Sabouraud Dextrose Agar (SDA), Eosin Methylene Blue (EMB) Agar, and Simmons Citrate Agar. All media were compounded and reconstituted in strict adherence to the manufacturers' protocols. Sterilization was achieved via autoclaving at 121 °C and 15 psi for 15 minutes.

Microbiological Analysis

Serial Dilution and Inoculation

To quantify microbial load, a 1 mL aliquot of each water sample was aseptically transferred into 9 mL of sterile distilled water and homogenized to create a stock solution. Ten-fold serial dilutions were subsequently performed using sterile distilled water as the diluent. From the appropriate dilution factor 10^{-2} , 0.1 mL aliquots were inoculated via the pour-plate method onto NA, MA, EMB, Salmonella-Shigella (SS) agar, and SDA plates.

Bacterial cultures were incubated aerobically at 37 °C for 24–48 hours, while fungal plates were incubated at ambient temperature for 48–72 hours. Post-incubation, discrete colonies on Nutrient Agar were enumerated to determine the Total Viable Count (TVC). Distinct colonies were sub-cultured to obtain pure isolates for identification.

The colony counts were calculated using the following formula:

$$\text{TBC/TFC} = \frac{(\text{N}) \times 10}{\text{V} \times \text{D}}$$

Where TBC: Total Bacterial Count

TFC: Total Fungi Count

N: No of Colonies

V: Volume plated

D: Dilution Factor

As a confirmatory protocol (APHA, 2005), 100 mL aliquots of the samples were passed through sterile Millipore membrane filters (0.45 µm porosity) using a vacuum filtration apparatus. The membranes were aseptically transferred onto selective agar plates and incubated at 27 °C–37 °C for 24 hours. Resultant colonies were enumerated, and pure cultures were generated on fresh NA and SDA plates for further morphological and biochemical profiling (Buchanan and Gibbons, 1994; Watanabe, 2002).

Characterization of Bacterial Isolates

Bacterial isolates were identified based on colony morphology, Gram reaction, and biochemical tests including catalase, coagulase, oxidase, citrate utilization, indole, methyl red, Voges–Proskauer, motility, spore staining, and carbohydrate fermentation tests using standard protocols (Cheesbrough, 2010).

Identification of Fungal Isolates

Fungi were identified through macroscopic assessment of colony features on SDA and microscopic evaluation of vegetative and reproductive structures using the slide culture technique as described by Agu and Chidozie (2021). Identification was confirmed using standard fungal atlases (Barnett and Hunter, 1972; Larone, 2002; Klich, 2002; Samson et al., 2004).

Heavy Metal Analysis

Heavy metal concentrations were determined using a Varian AA240FS Atomic Absorption Spectrophotometer following APHA guidelines (APHA, 1995). The principle is based on the absorption of element-specific radiation by atomized metals in a flame.

Physicochemical Analysis

Physicochemical parameters were analyzed using standard methods (APHA, 1998) at Springboard Laboratories, Awka. Parameters assessed included temperature, pH, conductivity, total dissolved solids, total suspended solids, alkalinity, dissolved oxygen, biochemical oxygen demand, and chemical oxygen demand. Results were compared with NSDWQ (2007) and WHO (2011) guideline limits.

Statistical Analysis

Data analysis was performed using one-way ANOVA in SPSS version 23, with statistical significance set at $p < 0.05$.

Result

Table 2: Isolates distribution per sachet water sample

Sample	Total Coliform Count CFU/ml	Total Fungal Count CFU/ml	Total Bacteria Count CFU/ml	Total Coliform (CFU)/100 ml	Faecal Count	Salmonella typhi CFU/ml	Shigella spp CFU/ml
AR	NG	5.0 x 10 ⁰	NG	NG		NG	NG
RT	NG	4.0 x 10 ⁰	NG	NG		NG	NG
BJ	NG	NG	NG	NG		NG	NG
PN	NG	NG	NG	NG		NG	NG
FC	4.0 x 10 ⁰	5.0 x 10 ⁰	NG	1.0 x 10 ⁰		NG	NG
CA	NG	NG	5.0 x 10 ⁰	NG		NG	NG
KP	NG	1.0 x 10 ¹	NG	NG		NG	NG
AL	NG	NG	NG	NG		NG	NG
FN	NG	NG	NG	NG		NG	NG
DS	1.0 x 10 ⁰	NG	1.0 x 10 ⁰	NG		NG	NG

Key: Ng = No visible growth

Microbiological and Physiochemical Analysis of Sachet Water within Awka Metropolis

Table 3: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Marginal	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Oxi	Ure	Lac	Glu	Suc	Fru	Mal	Identity	
A	Circular	Glistening	Cream	Entire	Raised	Transparent	Rod	-	+	+	-	+	+	+	-	+	+	+	+	-	+	<i>Proteus mirabilis</i>
B	Irregular	Glistening	Cream	Entire	Raised	Opaque	Rod	+	-	-	+	-	+	-	+	+	+	+	+	(-)	+	<i>Klebsiella pneumoniae</i>
C	Circular	Shiny	White	Entire	Convex	Moist	Rod	+	+	-	-	+	+	-	-	+	+	+	+	+	+	<i>Enterobacter aerogenes</i>
D	Circular	Shiny	Cream	Entire	Flat	Opaque	Rod	+	+	+	+			-	-	-	+	+	+	+	+	<i>Escherichia coli</i>
E	Circular	Shiny	Cream	Entire	Flat	Opaque	Cocci	+	-	-	-	-	-	-	-	+	+	+	-	+	+	<i>Streptococcus faecalis</i>
F	Irregular	Dry	Cream	Lobate	Flat	Opaque	Rod	+	+	+	-	-	+	+	-	-	+	+	-	+	+	<i>Bacillus subtilis</i>
G	Irregular	Shiny	green	Lobate	Flat	Opaque	Cocci	+	+	-	-	+	+	-	+	+	+	+	+	+	+	<i>Staphylococcus aureus</i>

Key:

Gram: Gram reaction

Cat: Catalase test

Mot: Motility test

Ind: Indole test

MR: Methyl-red test

VP: Voges-Proskauer test

Cit: Citrate Utilization test

Oxi : Oxidase test

Ure: Urease test **Sugar Fermentation Tests:**

Lac: Lactose Fermentation

Glu: Glucose Fermentation

Suc: Sucrose Fermentation

Fru: Fructose Fermentation

Mal: Maltose Fermentation

Table 4: Result of Heavy Metal Analysis

Samples	Lead (mg/l)	Silver (mg/l)	Mercury (mg/l)	Arsenic (mg/l)
FC	0.376 ± 0.006	0.068 ± 0.020	0.354 ± 0.004	0.014 ± 0.004
AL	0.030 ± 0.010	0.041 ± 0.010	0.084 ± 0.030	0.005 ± 0.002
AR	0.002 ± 0.001	0.031 ± 0.011	0.046 ± 0.004	0.013 ± 0.007
RT	0.009 ± 0.008	0.031 ± 0.010	0.039 ± 0.002	0.027 ± 0.007
DS	0.000 ± 0.000	0.022 ± 0.005	0.021 ± 0.010	0.022 ± 0.010
BJ	0.031 ± 0.001	0.022 ± 0.010	0.016 ± 0.003	0.011 ± 0.010
PN	0.000 ± 0.000	0.013 ± 0.003	0.002 ± 0.002	0.016 ± 0.006
FN	0.000 ± 0.000	0.017 ± 0.002	0.014 ± 0.004	0.008 ± 0.001
CA	0.000 ± 0.000	0.013 ± 0.003	0.013 ± 0.003	0.031 ± 0.010
KP	0.002 ± 0.001	0.010 ± 0.005	0.002 ± 0.002	0.016 ± 0.001
P Value	0.000	0.000	0.000	0.003

Table 5: Result of Physicochemical Analysis of Water samples

Samples	pH	Turbidity (NTU)	Temperature (°C)	Hardness (mg/L)	Colour	Taste
FC	6.82 ± 0.010	0.8 ± 0.200	22.40 ± 0.300	68 ± 5.000	Colourless	Unobjectionable
AL	6.50 ± 0.500	0.40 ± 0.050	20.80 ± 0.100	76 ± 2.000	Colourless	Unobjectionable
AR	7.12 ± 0.020	0.60 ± 0.040	22.20 ± 0.200	44 ± 4.000	Colourless	Unobjectionable
RT	7.40 ± 0.300	0.80 ± 0.150	24.20 ± 0.050	90 ± 2.000	Colourless	Unobjectionable
DS	6.58 ± 0.040	0.30 ± 0.100	20.60 ± 0.400	72 ± 1.000	Colourless	Unobjectionable
BJ	6.92 ± 0.010	0.60 ± 0.100	20.40 ± 0.150	78 ± 6.000	Colourless	Unobjectionable
PN	6.8 ± 0.600	0.90 ± 0.300	19.80 ± 0.400	84 ± 3.000	Colourless	Unobjectionable
FN	7.5 ± 0.400	1.05 ± 0.050	18.20 ± 0.100	96 ± 3.000	Colourless	Unobjectionable
CA	7.15 ± 0.050	0.03 ± 0.010	20.50 ± 0.300	74 ± 4.000	Colourless	Unobjectionable
KP	6.90 ± 0.150	0.70 ± 0.200	22.80 ± 0.100	38 ± 3.000	Colourless	Unobjectionable
P Value	0.009	0.000	0.000	0.000		

Discussion

This study evaluated the microbiological quality, heavy metal content, and physicochemical parameters of ten popular sachet water brands commercially available in Awka Metropolis, Anambra State, Nigeria. The findings reveal significant concerns regarding the safety and compliance of some of these products with established national and international drinking water standards.

The microbiological analysis indicated a relatively low level of visible microbial contamination in most samples. As shown in Table 2, the majority of the samples (BJ, PN, AL, FN) showed no growth for total coliform, total bacteria, total faecal coliform, *Salmonella typhi*,

Microbiological and Physiochemical Analysis of Sachet Water within Awka Metropolis

and *Shigella* spp. This is a positive indicator and suggests that some producers adhere to good manufacturing practices. However, the presence of microbial counts in certain brands is a cause for public health concern.

Notably, samples FC and DS tested positive for total coliforms, with FC also testing positive for faecal coliforms (1.0×10^9 CFU/100ml). The presence of faecal coliforms, specifically *Escherichia coli*, is a definitive indicator of recent faecal contamination and the potential presence of enteric pathogens (WHO, 2011). This finding is consistent with several other studies in Nigeria. For instance, a study in Lagos State also reported faecal coliform contamination in sachet water, linking it to poor source water quality and inadequate treatment (Oluwafemi and Ojo, 2017). Similarly, Oparaocha *et al.* (2022) in a broader review of water quality in Southeastern Nigeria, highlighted that breaches in hygiene during production and packaging are primary routes for post-treatment contamination.

The identification of specific bacterial isolates (Table 3) further elucidates the potential health risks. The isolation of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter aerogenes*—all members of the *Enterobacteriaceae* family—points to faecal or environmental contamination. These organisms are opportunistic pathogens capable of causing gastrointestinal infections, urinary tract infections, and pneumonia, particularly in immunocompromised individuals (Cheesbrough, 2010). The presence of *Staphylococcus aureus* is particularly alarming, as it is often associated with human skin and nasal flora, indicating poor personal hygiene of handlers during the packaging process. Its ability to produce heat-stable enterotoxins can lead to food poisoning (Ateba and Maribeng, 2019). The recovery of *Bacillus subtilis*, a spore-forming bacterium, suggests possible soil contamination or the survival of spores despite treatment processes.

The fungal contamination observed in samples AR, RT, FC, and KP, though at low counts, is another indicator of inadequate sanitation or post-production contamination. Fungi can affect the organoleptic properties of water and certain species can produce mycotoxins (Samson *et al.*, 2004). The findings align with a study by Nweze *et al.* (2018) in Enugu, which also reported fungal contamination in packaged water, attributing it to the lack of proper filtration and sanitization of production equipment.

The results of the heavy metal analysis (Table 4) present a critical aspect of this study. While silver and mercury levels were relatively low across most samples, the concentrations of lead and, to a lesser extent, arsenic in some brands are troubling. Heavy metals are often referred to as common pollutants which are widely distributed in the environment with sources mainly from the weathering of minerals and soils (O'Neil, 1993). However, the level of these metals in the environment has increased as a result of increase in human activities (Marian, 1991). All the heavy metals analyzed (Lead, Silver, Mercury and Arsenic) were detected in all the samples. However, their levels were within the acceptable limits prescribed by the WHO.

Sample FC had the most alarming heavy metal profile, with a lead concentration of 0.376 mg/L, which is over 12 times the WHO (2011) and NSDWQ (2007) maximum permissible limit of 0.01 mg/L for lead in drinking water. Lead is a cumulative toxicant that can cause severe neurological, renal, and cardiovascular damage, with children being particularly vulnerable (WHO, 2011). The high lead level in FC could be attributed to the leaching from substandard plumbing materials, industrial effluents contaminating the source water, or the use of contaminated raw materials.

The variation in heavy metal concentrations among the brands, as indicated by the significant P-values ($P < 0.05$), underscores that the source of water and the production process are not uniform. This variability has been documented in other Nigerian studies. For example, Adetunde *et al.* (2021) found elevated levels of lead and cadmium in sachet water from Northern Nigeria, linking them to industrial and anthropogenic activities. Comparatively, a global review by Uddin *et al.* (2021) on packaged water quality in developing countries identified heavy metal contamination as a recurring issue, often stemming from a lack of regulatory enforcement and inadequate water treatment infrastructure.

The physicochemical analysis (Table 5) showed that most parameters were within acceptable limits for drinking water. The pH values for all samples ranged from 6.50 to 7.50, falling within the NSDWQ (2007) range of 6.5-8.5. This indicates that the water was neither highly acidic nor alkaline, reducing the risk of corrosion or scale formation. Turbidity levels were all below 1.05 NTU, which is well within the 5 NTU standard, suggesting effective filtration in removing suspended particles. The water was consistently colourless, odourless, and had an unobjectionable taste, which are important consumer acceptability factors.

The temperature and hardness values, though showing statistical significance ($P < 0.05$), were within typical ranges for groundwater in the region and do not pose a direct health risk. The general conformity of physicochemical parameters, in contrast to the sporadic microbiological and heavy metal contamination, suggests that the primary risks in sachet water are not from basic physical and chemical properties but from specific biological and toxicological agents.

Conclusion

In conclusion, this study demonstrates that while a number of sachet water brands in Awka Metropolis meet certain physicochemical standards for drinking water, a significant proportion are compromised by microbiological and heavy metal contaminants. The isolation of pathogenic and opportunistic bacteria like *E. coli*, *K. pneumoniae*, and *S. aureus* indicates faecal and human-handling contamination, posing a direct risk to consumer health. The alarmingly high level of lead in one of the most contaminated samples (FC) highlights a severe toxicological threat that requires immediate regulatory attention.

Microbiological and Physiochemical Analysis of Sachet Water within Awka Metropolis

The findings of this study resonate with a body of evidence from across Nigeria and other developing regions, which consistently show that the sachet water industry, while providing an essential service, is plagued by inconsistent quality control. The reliance on this source for drinking water by a large segment of the population, therefore, constitutes a significant public health gamble.

Recommendation

There is need for aggressive public awareness by way of organizing seminars, putting up educative jingles in the media and the inclusion of environmental education in school curricula. It is believed that if the populace is aware that aesthetically attractive and colourless water can be unsafe for human consumption, and that all water sources need to be further treated before consumption, the incidence of water related diseases will further be reduced.

Routine checks of the quality of sachet water production in the study area are advocated for, so as to ascertain its pollution status. This situation should be communicated to inhabitants each time it is checked so that necessary precautions should be taken by the users. Both the three tiers of government, non-governmental agencies and international organizations should join hands together to ensure that adequate and safe drinking water are provided for as many people as possible. Also grants received from international donors such as World Bank assisted water projects should be maximally and efficiently utilized.

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