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Effect of storage duration on the microbiological and heavy metal levels in sachet water samples consumed within Enugu metropolis

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Abstract
Studies were carried out to determine the microbiological and heavy metal levels in sachet water samples stored at varied durations using standard biochemical and analytical procedures and, instrumentation.
Cadmium had a mean range of 0.02 – 0.03 µg/g over the stored duration (0 -14 days) of the samples, copper had a mean range of 0.63 -0.67 µg/g over the stored duration of the samples and zinc, had a mean range of 1.34 – 1.36 µg/g over the stored duration of the samples. Increased storage duration of the samples did not significantly alter the metal levels.
The mean range of cell count for *Staphylococcus aureus* and *Escherichia coli* over the storage duration of the samples were 43 -21500 and 31 – 2410 Cfu/g respectively. Increase in the storage duration of the water samples significantly increased the number of viable cell counts of the isolated organisms.

Keywords: Pathogenic organisms, storage duration, sachet water and heavy metals

Introduction
Water is life and its importance in the everyday life man cannot be over emphasized [2, 19]. Stated that the quality, odour, colour and its physicochemical characteristics determine its acceptability, sustainability and applicability at homes, offices and industries. The quality of any given water meant for human use is of utmost importance to the environmentalists and health regulators, because of its propensity to store and transport pollutants. The United Nations inferred that access to clean and drinkable water is a basic human right and one of the indices of a good living [26]. Therefore, the sources of the water consumed by the people to a great extent determine its quality and level of organic and inorganic pollutants. According to [2, 4, 8, 19], the extent of contamination of water with organic and inorganic materials is a function of the rainfall pattern, depth of water table from the source of contamination, soil properties as well as the geology and hydrology of the area. Contaminants when present in water distorts its quality and essentially imparts negatively on the health of the consumers and the domestic and economic activities carried out with it. Contaminants such as heavy metals, which are mainly the results of anthropogenic activities within an environment have been an issue of serious health concern to environmentalists and health regulatory authorities in recent times [2, 3, 5, 6, 14, 16, 19, 24, 25, 10, 13, 23]. Stated that the major cause of heavy metal contamination of any environment is the indiscriminate discharge of solid waste materials, untreated effluents and inorganic fertilizer application, which consequently alters the natural concentration of the environment. Cadmium, lead, arsenic and mercury are harmful heavy metals at low concentrations and even, copper, zinc and nickel required by the body in trace amounts, exert toxicity to man at high doses [4, 7, 8, 10, 13, 15, 16, 17, 18].
Heavy metal contamination of drinking water sources has been linked to deficiencies of some essential mineral elements, which as a consequence, adversely alter the immune defense system, psychological faculties and increases the incidence of gastro-intestinal diseases [3, 4, 6, 17, 18, 20, 21]. In the same vein [19, 29], stated that a water body contaminated with pathogenic organisms are the root causes of about 80% of water related diseases and 3.1% mortality in developing areas such as Sub-Saharan Africa. Water is contaminated with micro-organisms such as bacteria, virus, fungi and protozoa, and these micro-organisms have been linked in many health problems affecting people of all ages in many developing Nations. According to [9, 19], the potentiality of water to convey and transmit pathogenic bacteria to people is well documented.

The sources of the contamination of water bodies with pathogenic organisms vary from effluent discharge, organic manure application and leachates from sewers and sanitary pits [19].

Packaging of water in sachets and cans are common ways of making water available to people in almost all villages and cities in Nigeria today. The handling, source and treatment giving to water before package into sachets or cans for consumption, to a great extent determines the organic and inorganic contaminant levels of the water overtime and also, its drinkability. The packaged water, especially sachet water because of its low price, accessibility and low treatment procedure and sophistication required in its production and packaging, are vended in the nooks and crannies of many Nigerian cities, including Enugu metropolis.

Most times, the sachet water vendors store this water for as long as possible, probably due to low sales and unfortunately, unmindful of the health implications it portends. This act and the health concerns of possible contamination of sachet water with organic and inorganic materials necessitated this research.

Materials and Methods

Sample collection and Preparation

The purchase of different brands of sachet water samples consumed by the people was done at the sites of manufacture within the metropolis. The water samples were stored for 0 day, I day, 7 days and 14 days and were after proper labelling, serially taken to the laboratory for analysis.

Heavy metal analysis

About 1 ml of each water sample was mixed with 25 ml aqua regia solution in the digestion tube. The mixture was digested at 120 °C for 3hrs. The digest was subsequently filtered into a 100ml beaker and made up to mark with de-ionized water. The sample digests were stored in labelled polyethylene containers until metal analysis. Triplicate preparation was carried out for each of the samples including the blanks as described by [1]. The heavy metals (Cd, Zn and Cu) were determined in the samples using atomic absorption spectrophotometer (AAS) (model Elico, El-194).

Microbiological/ Biochemical analysis and identification of bacterial isolates

Inoculation, gram staining, colony and morphological characterization was carried out on the samples in order to identify the physical and structural features of the bacterial isolates as described by [12, 17, 21].

Pure cultures of the isolates were subjected to various biochemical tests in order to determine the identity of the bacteria species. The result of each test was noted and the likely identity of the isolates was determined by the use of the Bergey's manual of determinative bacteriology [11]. Bacterial colonies were counted to determine the viable cell count.

Statistical analysis

The data obtained were expressed as mean ± standard deviation and subjected to one way analysis of variance (ANOVA) at 5% confidence level using IBM SPSS 22.0.

Results and Discussion

Table 1: Mean heavy metal levels in sachet water samples at varied storage time

Storage duration (Days) Metal (µg/g)	0	1	7	14	F test P value	WHO STD [27])
Cd	0.03±0.01	0.03±0.01	0.02±0.01	0.03±0.01	0.20	0.05
Cu	0.67±0.02	0.64±0.03	0.63±0.04	0.65±0.03	0.18	2.0
Zn	1.34±0.12	1.36±0.09	1.33±0.10	1.35±0.13	0.11	10.0

Cadmium

The result of Table 1 shows that the mean Cd levels in the sachet water samples stored at 0, 1, 7 and 14 days were 0.03±0.01, 0.03±0.01, 0.02±0.01 and 0.03±0.01µg/g respectively. It was observed from the result of Table 1 that the varied storage duration of the water sachet samples did not significantly affect the mean Cd levels (See Fig. 1).

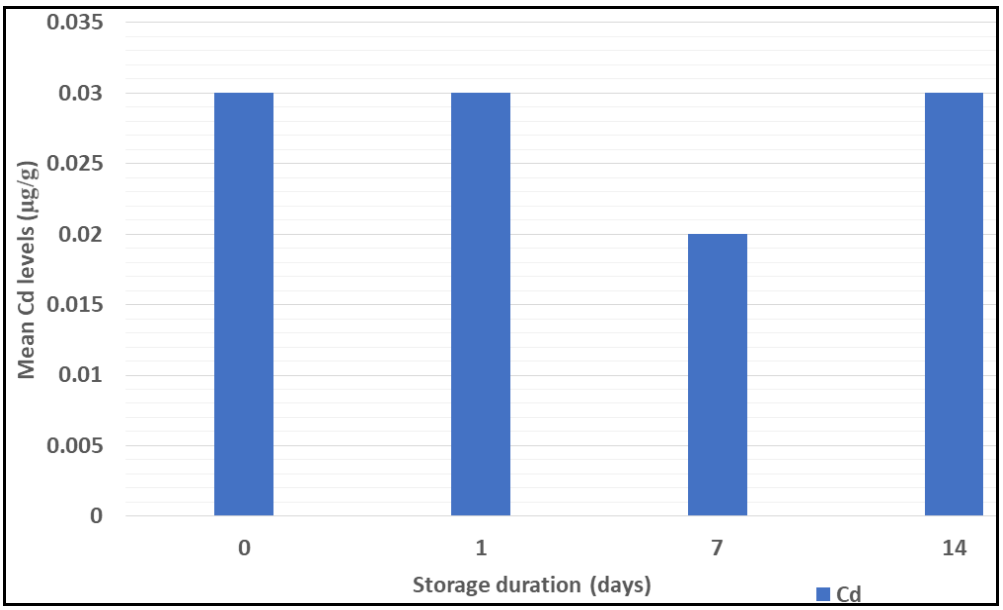

Fig 1: Bar chart representation of the mean Cd levels in the sachet water samples at varied storage durations

This therefore substantiates the fact that heavy metals are non- dissoluble, non-biodegradable but bioaccumulates in biological systems over a long period of time as described by [6, 9, 10, 16, 22, 23]. The mean Cd levels of 0.04±0.01µg/g obtained in sachet water samples in Aba metropolis as reported by [9] agreed with the mean values reported for Cd in the studied sachet water samples [19]. Reported a slightly higher mean value of 0.05±0.01µg/g for Cd in well water samples consumed in Akwuke community of Enugu State, than what was obtained for the metal in the investigated sachet water samples. The mean Cd levels in the studied sachet water samples at varied storage duration were within the recommended permissible limits. Cadmium is a known toxic metal, even at low concentrations and the debilitating effects of it on man is well documented and, this include anemia, renal failure, bone impairment, lung infection, neuropathy and lung cancer [3, 5, 6, 7, 13, 18, 21, 24].

Copper

Copper is a trace element required in minute amounts for body's enzymatic and biochemical function.

The result of Table 1 shows that the mean Cu levels in the sachet water samples stored at 0, 1, 7 and 14 days were 0.67±0.02, 0.64±0.03, 0.63±0.04 and 0.65±0.03 µg/g respectively. As was previously observed for Cd in the samples, the varied storage duration of the sachet water samples did not significantly influence the mean Cd levels (See Fig.2).

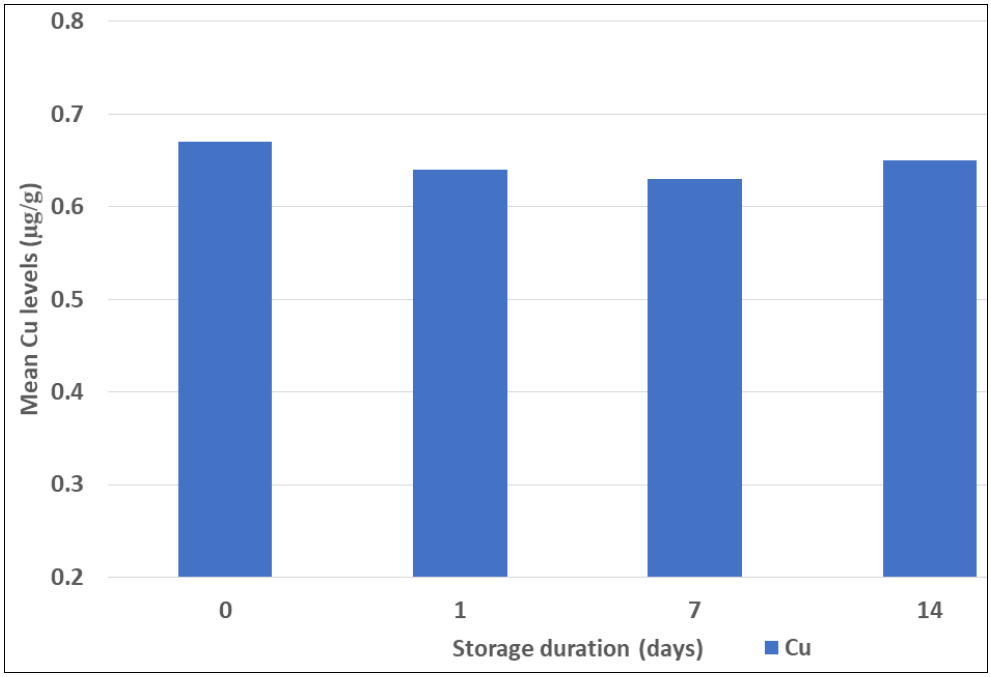

Fig 2: Bar chart representation of the mean Cu levels in the sachet water samples at varied storage durations

The result of Table 1 indicates that the mean Cu levels in the water samples were within the recommended permissible limits. The mean Cu levels of 0.62±0.04µg/g in sachet water samples consumed in Aba metropolis as reported by [9] was not significantly different from the mean Cu levels in the stored sachet water samples, despite the varied geography and hydrology of the two environments. [19] reported a higher mean Cu levels of 1.71±0.21µg/g in the well water samples consumed in Akwuke community of Enugu State, than this study got as mean values for the metal [5, 8, 10, 16, 18, 22]. Stated that although copper is essentially required by the body in trace amounts for optimal function, however, at high concentrations over a consistent level of exposure, results in excessive retention in the liver and invariably, damage of this vital organ of the body.

Zinc

Zinc as well as copper are mineral elements, whose deficiency in the body can disrupt important biochemical activities and are therefore required in trace amounts for the body's well-being [4, 8, 10, 18].

The result of Table 1 shows that the mean Zn levels in the sachet water samples stored at 0, 1, 7 and 14 days were 1.34±0.12, 1.36±0.09, 1.33±0.10 and 1.35±0.13 µg/g respectively. The mean Zn levels in the sachet water samples at the varied storage durations was not statistically significant (See Fig. 3).

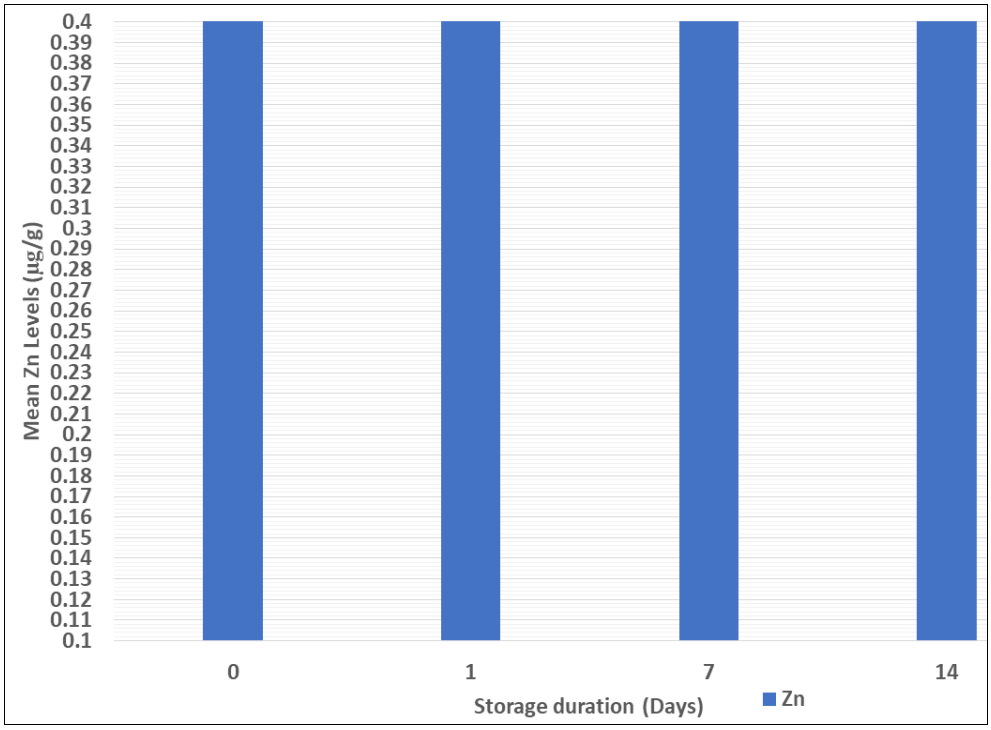

Fig 3: Bar chart representation of the mean Zn levels in the sachet water samples at varied storage durations

The water samples had mean Zn levels at the recommended permissible limits as established by [27, 19] reported a higher mean Zn value of 7.03± 0.29 µg/g in the well water samples consumed in Akwuke community of Enugu State, than it was obtained for the metal in the studied sachet water samples. Just as stated earlier, zinc is not essentially toxic, however, excessive exposure to it could result in vomiting, abdominal pain, lethargy and brain apoptosis [4, 7, 9, 19, 22, 23, 24].

Table 2: Biochemical characteristics of bacterial isolates from the water samples at varied storage durations

Cultural characteristics	Morphology	Gram staining	Glucose	Indole	Coagulate	Catalase	Citate	Most probable identity
Yellowish orange and slimy	Cocci in pairs	+	+	-	-	+	+	*S. aureus*
Red coloured with a smooth serrated edge	Rods straight	-	+	+	NA	+	+	*E. coli*

Table 3: Mean bacterial count (Cfu/g) from the samples at varied storage durations

Duration (Days) Bacterial isolates (Cfu/g)	0	1	7	14	F test P value	WHO STD ([26]
S. aureus	43.10±8.41	106.09±14.22	1470.83±23.07	21500.81±27.13	0.01	$< 10^2$
E. coli	31.16±12.07	77.63±10.18	114.17±19.11	2410.87±35.33	0.01	$< 10^2$

The result of Table 2 shows that the two bacteria (*S. aureus* and *E. coli*) were identified and isolated from the water samples at the investigated storage durations. Despite that the water samples were packaged in sachets and assumed to have undergone some treatment compared to water harvested from fresh water habitats, pathogenic bacteria such as *S. aureus* and *E. coli* were noticeably present in the samples at the very point of production (0 day). Result of Table 2 further buttresses the fact that most water vendors do not source water from environments with little or no contamination with microorganisms and, after getting the water do not subject it to necessary heat treatment at least, before packaging for sale. These unscrupulous water vendors are solely driven to make quick returns from the business at the expense of the consumers' health. The result of Table 2 totally agreed with the findings of [19], who isolated some pathogenic bacteria from well water samples consumed by people in Akwuke community of Enugu State.

The result of Table 3 shows that the mean counts for *S. aureus* in the water samples at 0, 1, 7 and 14 days storage durations were 43.10±8.41, 106.09±14.22, 1470.83±23.07 and 21500.81±27.13 Cfu/g respectively. Also, from the result of Table 3, the mean counts for *E. coli* in the water samples at 0, 1, 7 and 14 days storage durations were 31.16±12.07, 77.63±10.18, 114.17±19.11 and 2410.87 ±35.33 Cfu/g respectively. The result of Table 3 indicates that the two isolated pathogens (*S. aureus* and *E. coli*) festered and multiplied in the sachet water samples as the storage duration extended from 0 to the 14day (See Figs.4 and 5).

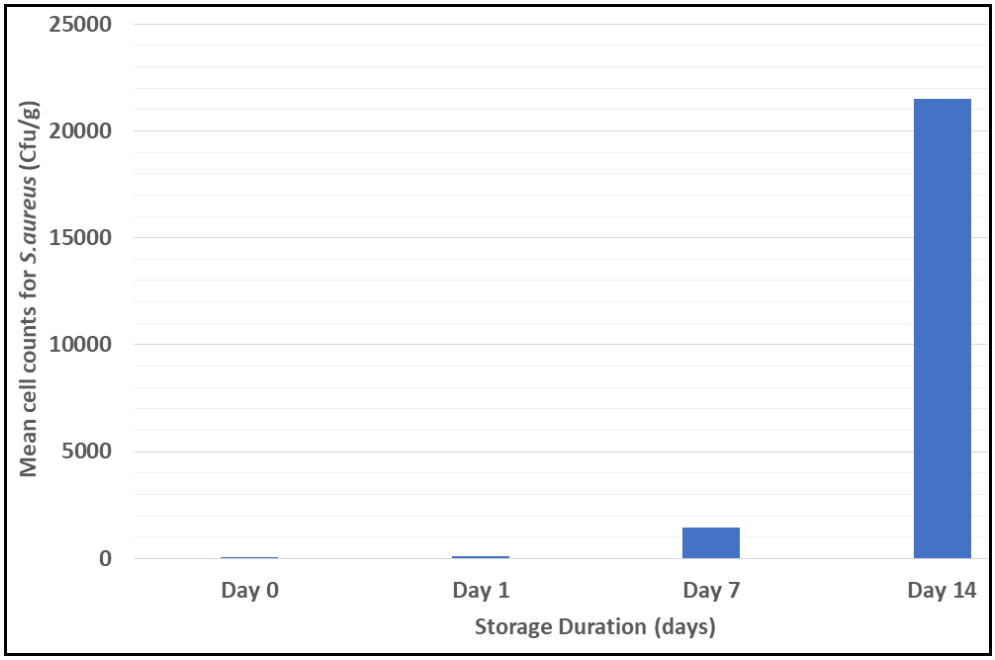

Fig 4: Bar chart representation of the mean cell counts for *S. aureus* in the water samples at varied storage durations

The mean bacterial counts for *E. coli* at 7 and 14 days storage durations exceeded the recommended permissible limits as established by [26]. Also, the mean bacterial counts for *S. aureus* at 1, 7 and 14 days storage durations exceeded its permissible limits. The isolated organisms from the water samples are pathogenic organisms that have been implicated in many food and water borne ailments and most importantly, finds their way into food and water from unhygienic environments, faecal contamination and poor storage, packaging and handling of food and water [10, 17, 19, 20, 21].

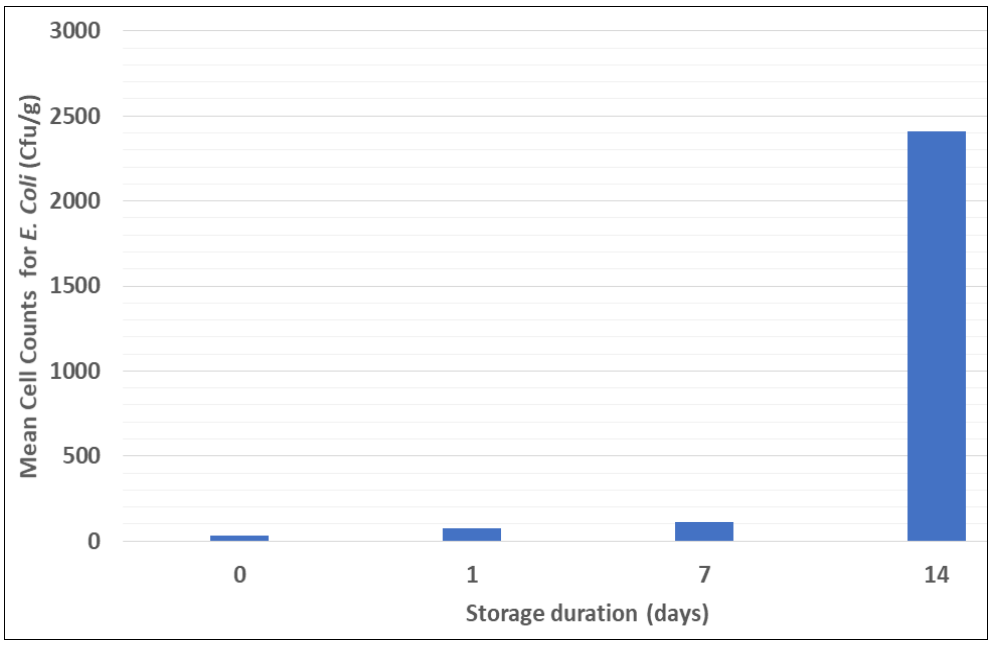

Fig 5: Bar chart representation of the mean cell counts for *E. coli* in the water samples at varied storage durations

[19], reported a higher mean count of 3750± 600 and 2535±129 Cfu/g for *S. aureus* and *E. coli* respectively in the well water samples consumed in Akwuke community of Enugu State, than what this study reported as mean cell counts for the isolated pathogenic bacteria in the sachet water samples.

Conclusion

The three investigated heavy metals (Cd, Cu and Zn) were present in the sachet water samples at the studied storage durations. The mean levels of the metals were at non-toxic levels in the samples at the investigated storage durations. Increased storage duration of the water samples did not significantly affect the metal levels. *Staphylococcus aureus* and *Echerichia coli* were isolated from the water samples and increase in the storage duration of the samples significantly increased the mean cell counts for the two isolated organisms. The mean cell counts for the isolated organisms in the water samples exceeded their permissible limits. Water meant for human consumption should be

sourced from healthy environments and undergo at least a strong heat treatment to eliminate the possible contamination and festering of water with microorganisms that endanger the health of the people.

The production, packaging and storage of water meant for human consumption should follow best practices and this can be enforced by authorized health regulatory authorities.

Conflicts of Interest

The authors' of this research bears no conflict of interest in carrying out this research and its publication.

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