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Analysis of the impact of Colour of Storage Container and Storage Conditions on Bacteria flora Water

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Abstract

The consequence of storage and colour of buckets on the bacteriological quality of borehole water was investigated. Water samples drawn from the borehole were collected into covered tap-fitted buckets of different colours. A set of buckets were stored outdoor while duplicates were stored indoor. The physicochemical and bacteriological parameters were analyzed. There was a gradual increase in the pH of the samples during storage. Suspended solid content, bacterial count and coliform count declined with storage. There was reduction in the total bacteria count by 94.84%; 98.72%, 91.03%; 96.15%, 92.30; 96.15 and 93.60; 97.44 from an initial 78×10^2 CFU/ml for indoor and outdoor storage in the colourless, black, green and blue buckets respectively. There was also reduction in total coliform count by 95.24%; 100.00%, 90.48%; 95.24%, 90.48%; 97.62%, 92.86%; 97.62 from an initial 42×10^2 CFU/ml for indoor and outdoor storage in the colourless, black, green and blue buckets respectively. Eleven bacteria including *Bacillus subtilis* and *Acinetobacter haemolyticus* were isolated. *Proteus vulgaris* and *Pseudomonas aeruginosa* survived till the last day of storage. Decrease in bacterial counts was prominent in the colourless and blue buckets stored outdoor making them the colour of choice for use in water storage tanks.

Keyword: Bacteria, Water, Storage, Colour, Container, Condition.

1. Introduction

Urbanization has adversely affected water quality. Water is a natural, reusable and renewable resource. It is essential to maintain life. Bacteriologically poor drinking water quality is the cause of many diseases (Odonkor and Addo, 2018; Wang *et al.*, 2020). Not much is known about the diversity of microbes and their metabolism in deep subsurface water (Purkamo *et al.*, 2018).

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Reports of diarrhoea in children and adults are not limited to rural areas but happens in urban areas too. This could be an offshoot of bacteriological pollution of drinking water supplies (Taonameso *et al.*, 2019). Water must be safe, adequately supplied and accessible to all. The constituents that may result in any significant health risk to the consumers over a lifetime of consumption must not be present (WHO, 2010). Drinking water quality management has been a key pillar of primary prevention for over one and half centuries and it continues to be the foundation for the prevention and control of water borne diseases (WHO, 2010).

The irregularity of piped water supply has however made it necessary to seek alternative water sources. This is found in rainwater and groundwater such as wells and boreholes. Groundwater sources such as wells, boreholes and springs; that are properly located produce water of very good quality (Gerald *et al.*, 1992). Rogbesan *et al.* (2002) reported heavy bacterial load in water from some boreholes in Ilorin. Contaminated water is a global public health threat placing people at risk of a host of diarrheal and other illnesses as well as chemical intoxication (Okonko *et al.*, 2009). The major risk to human health is faecal contamination of water supplies (Cheesbrough, 2006). Increase in human population pose a great pressure on provision of safe drinking water especially in developing countries (Okonko *et al.*, 2009). Providing potable water for drinking and washing is critical to reducing, diarrheal disease transmission in this setting (WHO, 2010). Unsanitary water particularly has devastating effects on young children in the developing world. Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrheal disease (Parashar *et al.*, 2003).

Olayemi *et al.* (2005) and Eniola *et al.* (2006) highlighted the importance of a few days of indoor or outdoor storage in improving the physical and microbiological quality of water. It was reported that a few days of storage of surface water would improve the physical and microbiological characteristics of the water (Olayemi *et al.*, 2005). In water treatment, storage is a preliminary step to other processes, because it reduces the bacterial

content of the raw water and also reduces the amount of total suspended matter in it, however, it cannot be relied on as sole measure of purification. Storage acts against bacteria by sedimentation which is a physical water treatment using gravity to remove suspended solids from water, microbes are usually attached to these particles.

The germicidal action of sunlight has long been recognized but the ecological implications and the potentials for practical applications have to be researched more thoroughly. UV light has been shown to effectively inactivate indicator bacteria and pathogens. The effectiveness of UV light in biological inactivation arises primarily from the fact that DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 260 nm. UV-A (wavelengths, 320 to 400 nm) causes only indirect damage to DNA, proteins and lipids through reactive oxygen intermediates. UV-B (wavelengths, 290 to 320 nm) causes both direct and indirect damage because of the strong absorption by DNA at wavelength below 320 nm (Joux *et al.*, 1999). The most common household reservoirs are plastic tanks, usually of different colours, placed outdoor. The outdoor location of the water tanks exposes them to solar radiation. Sunlight exposure is considered to be the most important cause of "natural disinfection" in surface water environments (Rijal and Fujioka, 2001). The amount of radiation available is affected by aerosol optical depth and cloud parameters (Sekiguchi *et al.*, 2003). The colour of container can impact radiative heat transfer due to varying conductivity and shading of different colours but will have basically no effect on conductive heat transfer (Penner *et al.*, 2002).

This study is an investigation of effects of the colour of containers and conditions of storage (indoor and outdoor) on the bacteriological quality of borehole water.

2. Materials and Methods

All the materials used for this project were appropriately sterilized before and after use in order to prevent contamination. The sampling site is a hand pumped borehole located adjacent the University's Senate Building within the main campus of University of Ilorin (Plate I). There were trees around the site and the immediate surroundings of the borehole were surrounded with a concrete pavement. The sampling site is mostly busy with human traffic as a road from the school's bus terminus to the academic area.

Water was obtained from the borehole into disinfected tap-fitted coloured (colourless, black, green and blue) plastic buckets as described by WHO (2005). The buckets were washed with soap, thoroughly rinsed, allowed to dry and disinfected by swabbing with cotton wool soaked with 70% alcohol. The alcohol was allowed to vaporize and the buckets were further rinsed with the water sample to be collected. The buckets were then filled to about 3cm from the top of the bucket and covered. Four buckets of different colours were moved indoor while the other set of buckets were outdoor.



Plate 1: The Sampling Site: Borehole around the UNILORIN Senate Building.



Plate II: A set of Buckets: Representing Outdoor Storage.

The experiment was carried out within the months of March and April which was during the dry season. Atmospheric conditions of the experiment were those consistent with those observed during the dry season although there were variations in the intensity of the sun, sky condition, wind and temperature. Variations in these conditions were taken by synoptic observation. The pH of the water samples was determined immediately after the sample collection. Subsequent pH readings were taken at intervals of 4 and 6 days. The readings were taken using standardized glass electrode pH meter (model 290mk 2 pH meter). (Mendham *et al.*, 2000).

Mercury bulb thermometer with a range of 0 °C – 100 °C was used for determining the temperature of the water sample. The total suspended solid was determined using the filtration method described by APHA (1995) and Sawyer *et al.* (1994) by the filtration process. Bacteriological analysis was carried out during a period of 24 days during which the samples were stored under both outdoor and indoor storage conditions. The analysis took place in intervals of 4 days for the first 12 days and in an interval of 6 days for the last 12 days. Pour plate technique was used for the analysis (Willey *et al.*,

2011); 2ml of sample from each of the storage buckets (indoor and outdoor) was collected using sterile test tubes. For each of the storage buckets one plate of nutrient agar and one plate of MacConkey agar were used for the bacteriological analysis using 0.1ml of the sample for each plate. The plates were labeled according to the colour of container and condition of storage, e.g. blue outdoor.

All samples were tested for total bacterial count by standard plate count (SPC) method with the aid of nutrient agar. All samples were tested for total coliform count by standard plate count (SPC) method with the aid of MacConkey agar. Samples were further tested for total faecal coliform count by standard plate count (SPC) method using eosin methylene blue (EMB) agar. Characterization and identification of bacterial isolates was done by determining their colonial, cellular and biochemical characteristics. The isolates were characterized and identified using standardised biochemical tests with the aid of Microbact™ ID 24E System for Identification of Enterobacteriaceae and Common Miscellaneous Gram-Negative Bacilli (MGNB). The kit was used according to the manufacturers' specifications.

3. Result and Discussion

Table 1 shows the atmospheric conditions during the storage period of storage. The table shows that atmospheric temperature was least at 31°C on the 8th day and peaked on the 12th day at 36°C. Figure 1 shows the variation in the mean pH during storage. There was a steady increase in the mean pH during the period of storage from the initial pH of 6.6 which was the lowest pH and occurred on day 0 in all buckets to the highest pH of 9.2 which occurred in the colourless outdoor bucket on day 24. Figure 2 shows the variation in the mean temperature during storage. Temperature was lowest on day 4 and 12 with 26.3°C in the blue indoor bucket and was highest at 35.0°C in the black outdoor bucket on day 24. Figure 3 shows the variation in the mean total suspended solid content during storage. Steady decrease in the mean total suspended solid content was observed. The

initial total suspended solid content of 0.0057 g/ml (Figure 3) was the highest total suspended solid content and it occurred on day 0 in all the experimental buckets. The lowest total suspended solid content was 0.0005 g/ml and occurred on day 24 in the colourless bucket placed outdoor.

Shown in figure 4 is the variation in the mean total bacteria count during storage. There was a steady decrease in the mean total bacteria count during the period of storage from the initial and highest count of 78×10^2 CFU/ml occurring in all storage buckets on day 0 to the lowest count of 1×10^2 CFU/ml in the colourless outdoor bucket on day 24. Figure 5 shows the variation in the mean total coliform count during storage. There was a steady decrease in the mean total coliform count during the period of storage from the initial and highest count of 42×10^2 CFU/ml occurring in all storage buckets on day 0 to the lowest count of 0×10^2 CFU/ml in the colourless outdoor bucket on day 24.

Table 1: Atmospheric Conditions during Storage Period.

Time of Storage (Days)	Atmospheric Conditions			
	Temperature	Sky Condition	Wind Condition	Solar Intensity
0	35°C	Clear	Mild	High
4	36°C	Clear	Gentle	High
8	31°C	Mild	Calm	Low
12	35°C	Mild	Mild	Mild
18	34°C	Clear	Gentle	High
24	33°C	Clear	Gentle	High

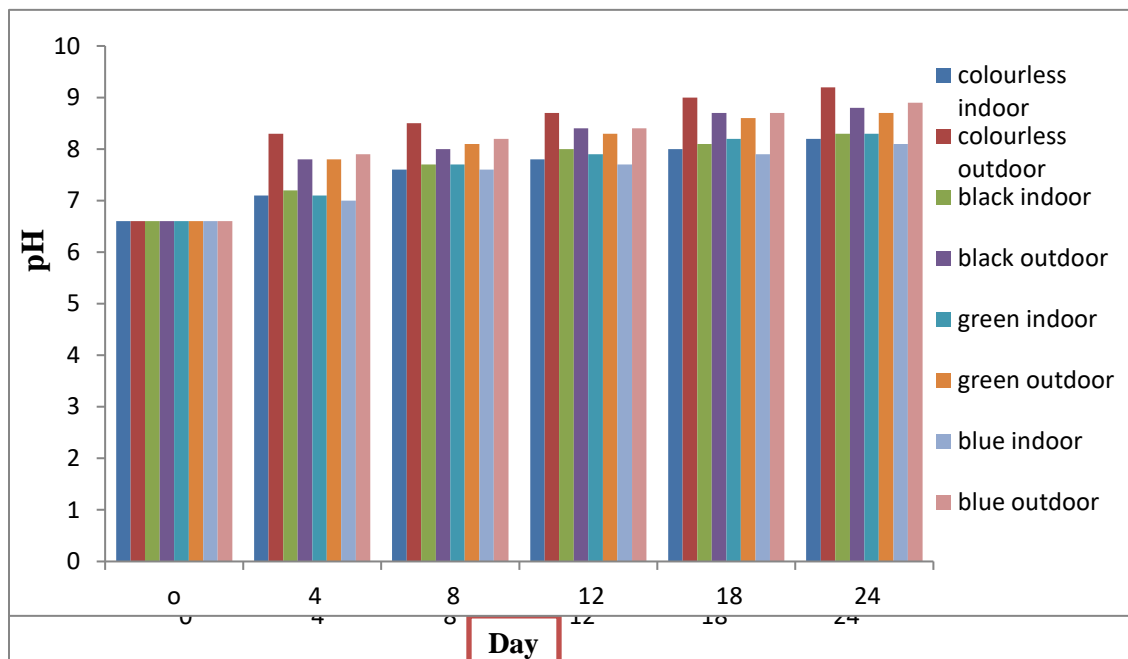


Figure 1: Variation in the Mean pH during Storage.

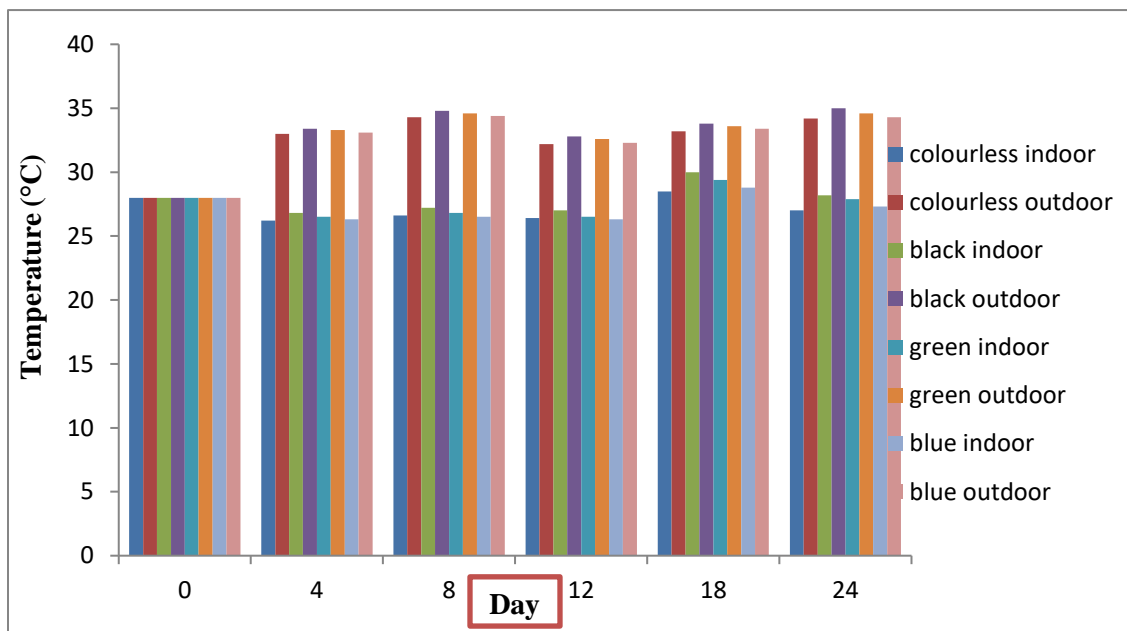


Figure 2: Variation in the Mean Temperature during Storage.

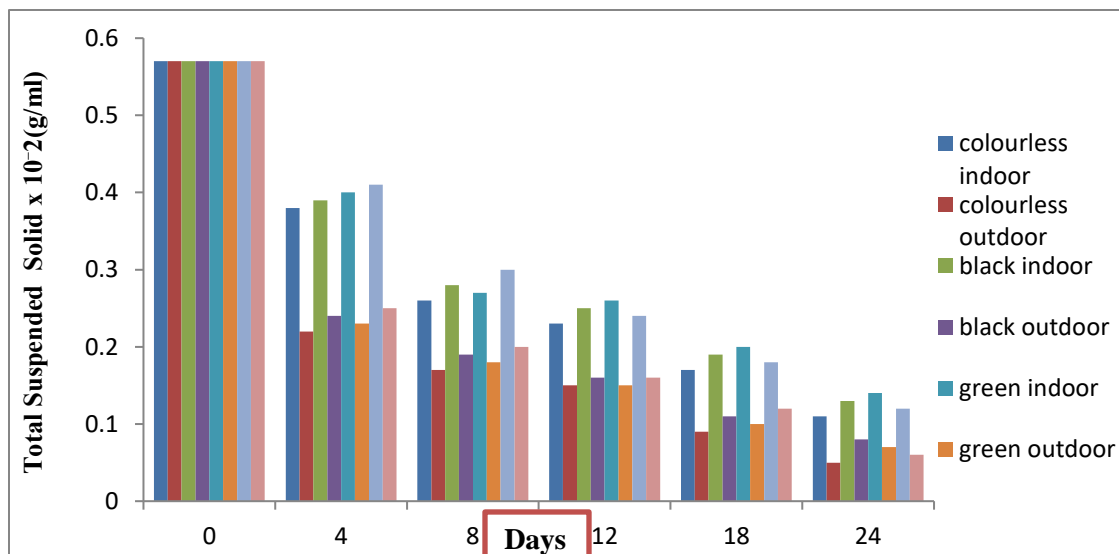


Figure 3: Variation in the Mean Total Suspended Solid Content during Storage.

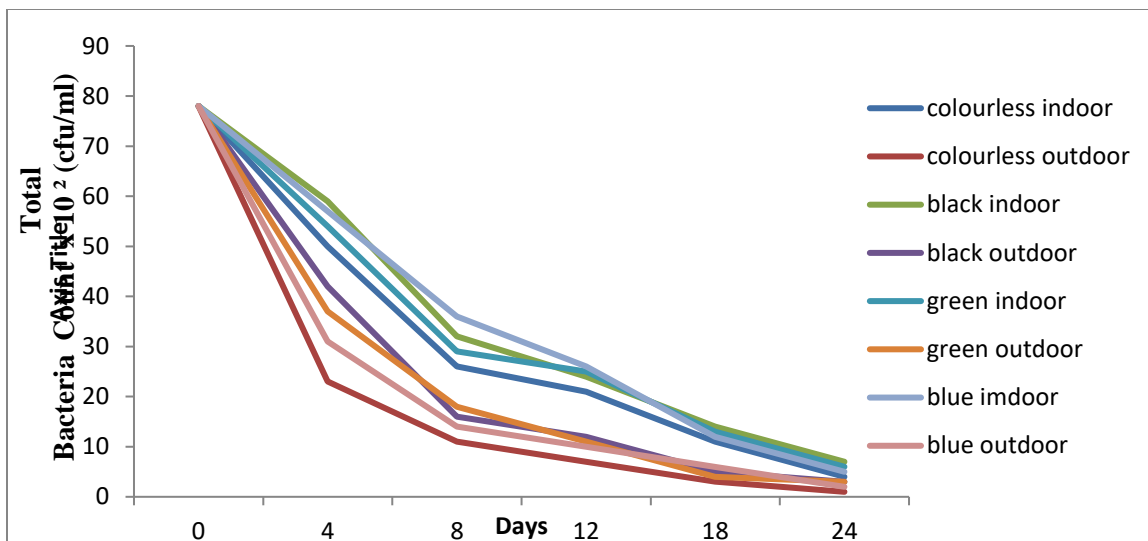


Figure 4: Variation in the Mean Total Bacteria Count during Storage.

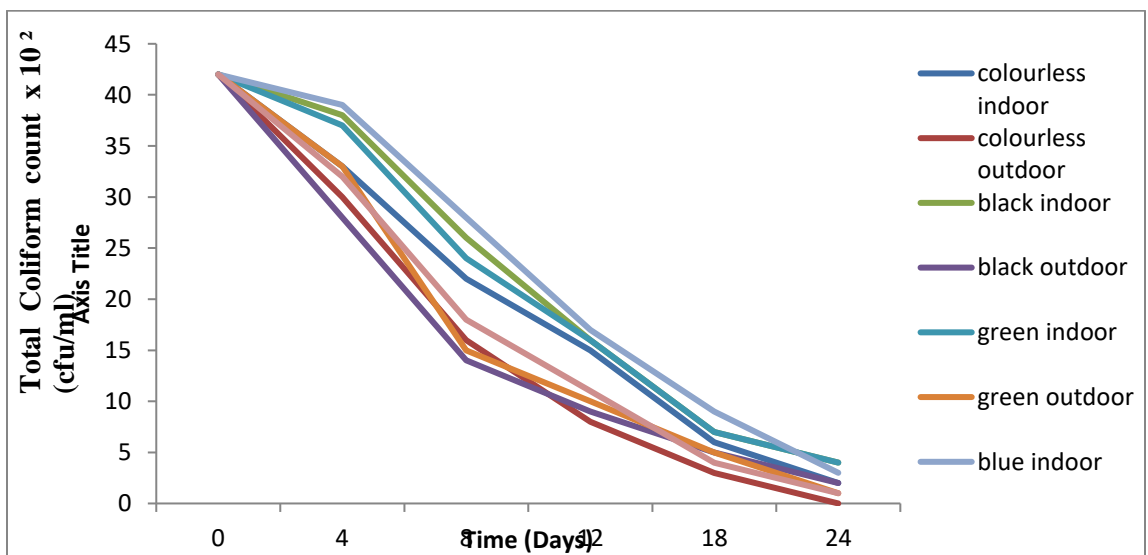


Figure 5: Variation in the Mean Total Coliform Count during Storage.

Table 2: Succession of Bacteria in Water Samples During Storage.

Legend: += Present. - = Absent. 0, 4, 8, 12, 18 and 24 = days of storage.

Bacteria Isolates	Occurrence of Bacteria Isolates																																															
	White Buckets						Black Buckets						Green Buckets						Blue Buckets																													
	Indoor			Outdoor			Indoor			Outdoor			Indoor			outdoor			indoor			outdoor																										
	0	4	8	12	18	24	0	4	8	12	18	24	0	4	8	12	18	24	0	4	8	12	18	24	0	4	8	12	18	24	0	4	8	12	18	24	0	4	8	12	18	24						
<i>Proteus vulgaris</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						
<i>Bacillus subtilis</i>	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-
<i>Micrococcus luteus</i>	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-
<i>Citrobacter freundii</i>	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	-	-	-
<i>Klebsiella pneumonia</i>	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
<i>Enterobacter aerogenes</i>	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
<i>Pseudomonas stutzeri</i>	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-
<i>Acinetobacter haemolyticus</i>	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
<i>Alcaligenes faecalis type ii</i>	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-
<i>Pseudomonas fluorescens</i>	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-

4. Conclusion

The clear nature of the atmosphere and high solar intensity during the storage period suggests that more radiation is likely to reach the earth. The effect of radiation of water had been reported previously. High relative humidity and cloud cover was reported to have resulted in high water temperature (Penner *et al.*, 2002; Takemura *et al.*, 2002). This agrees with our findings where there was significant temperature gradient between indoor and outdoor conditions. (Figure 2). The zero total faecal coliform count shows that the borehole water is free of fecal contamination, contrary to the finding by Rogbesan *et al.* (2002) that water from this borehole contained coliforms exceeding the WHO (2005) standard required of untreated drinking water. This is suggestive of an improvement in the handling of the borehole and its catchment.

The presence of *Citrobacter freundii*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Alcaligenes faecalis* type ii in the water however shows it is not fit for consumption. *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Alcaligenes faecalis* type ii and *Acinetobacter haemolyticus* are opportunistic pathogens. Members of the genus *Proteus*, *Citrobacter* and *Enterobacter* are common causes of bladder, kidney and other body infections (Stanier *et al.*, 1987). *Pseudomonas aeruginosa* was associated with cystic fibrosis and was isolated from 524/7904 (6.6%) waters examined in a study by Caskey *et al.* (2018). The WHO (1996) standard requires that water intended for drinking should be free of pathogens and bacterial indicative of faecal contamination. Although opportunistic pathogens are known to be naturally present in the environment and not formally regarded as pathogens, they are able to cause disease in people with impaired local or general defense mechanism such as the elderly or very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy or those with AIDS (WHO, 1996). The variation in the temperatures of the samples under storage ranged from 28 °C-35 °C. This temperature range falls within the optimum growth for mesophytes which include the majority of bacterial and have their temperature growth within the range of 20°C and 45°C. There was a 78% decrease in the average total suspended solid of samples (figure 3) under indoor storage and 88.60% average decrease for samples under outdoor storage. The progressive reduction in suspended solid content is similar to the findings of Olayemi *et al.* (2005) and Eniola *et al.* (2006). It has

been attributed to gravitational pull, which causes suspended materials to settle out of the water over time. It is likely that if the time of storage is increased, all suspended materials present in the water could settle out under gravitational pull. This is probably the basis for the influence of storage on bacteriological quality of water as observed by Maggy *et al.* (2003).

The pH (Figure 1) of the samples under storage ranged from an average initial 6.6 to a final average of 8.23 for indoor storage and 8.90 for outdoor storage. This is little above the range that would favour bacterial proliferation (Atlas, 1995). The observed increases in pH during storage could be due to the activities of the resident flora and or their death, which results in the release of inorganic substances such as ammonia (Rogbesan *et al.*, 2002). The observation of the pH samples of the borehole water which tilted towards alkaline during storage but still falls within a tolerable range for bacterial growth, the pH value of water is a very important indication of its quality (El Ghandour *et al.*, 1985). The decline in bacterial population over time is in concord with the report of Payment *et al.* (1985). Decline in the bacterial population can be attributed to death of the resident bacteria during the storage period due to depletion of nutrients (Olayemi *et al.*, 2005). These could be due to the removal of aggregated particle-associated resident microbes through sedimentation by gravity (WHO, 2006_a). Plain sedimentation has been approved as a simple pre-treatment of household water which improves the microbial quality of water (WHO, 2006_b). Sedimentation of suspended material in the water due to gravitational force could also contribute to the decline in bacterial populations (Salle, 1973; Eniola *et al.*, 2006). It is possible in such a situation that the organisms remain in the biofilm produced. The survival of *Proteus vulgaris* and *Pseudomonas aeruginosa* after 24 days of storage portends some dangers to consumers of the water due to their pathogenic nature (Stanier, *et al.*, 1987).

The rate of reduction in the population of bacteria and coliform was less in the samples under indoor storage compared to the population in the water samples under outdoor storage during the period of the experiment. This is attributable to direct radiation from sunlight on the buckets outside. Among those outdoor, penetration by radiations would be more pronounced in the transparent bucket. There was also an observable significant difference effect of radiation by exposure to sunlight on the bacteriological quality of the borehole water samples. This is discovered by notable difference between the numbers of organisms that survived relative to days of storage when the water samples were stored indoor and from those that survived relative to days of storage when the water samples were stored under outdoor condition. There was a reduction in the total bacteria count (Figure 4) by 94.84%; 98.72%,

91.03%; 96.15%, 92.30; 96.15 and 93.60; 97.44 from an initial 78×10^2 CFU/ml in the colourless, black, green and blue buckets stored indoor and outdoor respectively. There was also a reduction in total coliform count (Figure 5) by 95.24%; 100.00%, 90.48%; 95.24%, 90.48%; 97.62%, 92.86%; 97.62 from an initial 42×10^2 CFU/ml in the colourless, black, green and blue buckets stored indoor and outdoor respectively.

Eleven bacterial species including *Bacillus subtilis*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis type ii*, *Pseudomonas fluorescens*, *Pseudomonas stutzeri* and *Acinetobacter haemolyticus* were isolated; two of them: *Proteus vulgaris* s *Pseudomonas aeruginosa* survived till the last day of storage. Sunlight exposure is considered to be the most important cause of "natural disinfection" in surface water environments. Although, visible light is the most conspicuous and important aspect of our environment in terms of its positive involvement in photosynthesis, if present in sufficient intensity, it can damage or kill microbial cells. Exposure to near-UV radiation that reaches the earth surface from the sun can also harm microorganisms. Their destructive effect on DNA is the most important cause of death (Prescott *et al.*, 2005). The Ultraviolet B (UV-B) portion of the solar spectrum is the most bactericidal, causing direct (photo-biological) DNA damage; radiation intensity and depth of water are important factors to be considered. There is a relationship between exposure to heat and radiation. Effects of sunlight was crucial in disinfecting water by solar units. Direct radiation of sunlight works in synergy with solar heating of water for disinfection (Rijal and Fujioka, 2001).

There was also a more prominent reduction in the bacterial and coliform load in the white and blue buckets stored outdoor compared to the black and green buckets indoor. This could be attributed to the high intensity of light rays allowed into these buckets due to no shading in the white bucket and the light shading in the blue bucket.

This study buttress that storage is valuable as a preliminary accessory stage of treatment but it cannot be relied on as a sole measure of purification. The colour of the bucket and fluctuation in light and radiation are also important. There was a little difference in effect of the different colour of buckets.

In conclusion, storage was found to be desirable as it brought about improvement in the quality of the water sample, reducing the types and number of bacteria in water. Outdoor storage of water in light coloured container was found to be more desirable than indoor

storage. It is therefore recommended that borehole water should be stored outdoors in light coloured container for about 18 days.

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