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Microbiological Analysis of Household Water Tanks in Egypt

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ABSTRACT

The world faces a significant challenge in meeting freshwater demands due to the limited availability of fresh and pure water. This study investigated the microbial quality of domestic water tanks in Cairo, Egypt, to assess the possible health effects of stored water. Water samples were obtained from the household tanks at Azhar University and analyzed for bacterial content using membrane filtration and serial dilution techniques. The differences observed in the microbial plate counts showed a variation across seasons and temperatures, and the total plate counts were noted in the winter and the summer water samples at 35°C and 22°C, respectively. Some samples had low microbial counts, while others had higher ones, which could imply contamination. Faecal and total coliform concentrations were relatively low and, in some cases, within the range of the WHO requirements. The study emphasizes the importance of microbial quality sampling in water samples, as some samples may pose health hazards. It suggests improved standards in cleaning water tanks to ensure safe drinking water. Proper water treatment and risk checks can help eliminate potential health risks and provide a secure water supply. Clean water is crucial for human health and sustainable development, and ensuring adequate and safe water is essential for consumption.

INTRODUCTION

Global freshwater demand cannot be satisfied despite 75% of the Earth submerging in water. Surface freshwater and subterranean water comprise only 3% of the Earth's surface, with glaciers storing an additional 2.5%. Meanwhile, what's left behind is polluted water that is unsuitable for human consumption or domestication (Naqvi *et al.*, 2015). Whether animal or plant, water is a fundamental component in cell synthesis and an integral part of every industrial and biological activity (Al-garawyi, 2019). For more than 2000 years, city residents have recognized the necessity for a safe and clean water supply (Nastić, 2021). The early Romans built an aqueduct system to transfer water from the Tiber River upstream of the city, providing a steady water supply through their enormous aqueduct system connected with the expansion as a centre of their civilization (Kulperger *et al.*, 2003). Nowadays, household water tanks are frequently used to store water for domestic purposes in numerous urban regions (Salehi, 2022).

Nevertheless, the susceptibility of these tanks to microbial contamination arises from many factors, including insufficient maintenance practices and environmental pollutants (Organization, 2004). Water contamination has emerged as a significant environmental concern since the beginning of the 21st century due to population expansion, urban development, industrial progress and pollution arising from industrial wastewater, domestication, agriculture and sewerage dumping of solid waste in streams (Badr *et al.*, 2013; DG Al-Afify & YM Aly, 2019). In contrast, rivers across the globe are subjected to substantial quantities of waste discharged by industrial and agricultural sectors (Badr *et al.*, 2013).

This has caused serious ecological challenges, as it exerts adverse effects on the general population's well-being and the aquatic ecosystem's biodiversity, generating waterborne diseases (Hunter *et al.*, 2001; Noreen *et al.*, 2022).

Waterborne diseases can be shown as illnesses that are affected by the consumption of water polluted with pathogenic microorganisms present in human or animal faeces, which may include viruses, bacteria, or protozoa (Hunter *et al.*, 2001). According to WHO, there is a significant risk of morbidity and mortality resulting from unsafe drinking water, where 200 million cases of diarrhoea and 2.1 million deaths attributed to diarrheal illness are reported annually (Organization, 2004). Approximately 20% of the global population faces the challenge of insufficient access to potable water, leading to more than 5 million deaths each year due to diseases linked to the consumption of unsafe water or inadequate sanitation (Hunter *et al.*, 2001). Infants, young children, disabled individuals, and the elderly are most likely to be affected by waterborne infections, where inadequate sanitary facilities considerably raise the risk of waterborne illnesses 25 times higher in industrial regions (de Bruin *et al.*, 2018).

Precise microbiological studies are scarce and provide information on residential water tanks in urban areas such as Cairo, Egypt (Mohamed *et al.*, 2016; Osman *et al.*, 2010). While there is some research on water quality concerns and their effects worldwide, information regarding microbial content and health risk implications tied to water tanks in homes in Cairo is scarce or lacking. Consequently, information concerning the quantity and kinds of microbial contaminants is still unknown, let

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alone efficient strategies to manage such threats.

This study is an effort to fill this gap by evaluating the microbial quality of stored water by conducting an extensive microbiological analysis of water tanks in residential buildings in Cairo-Egypt. The aim is to provide valuable information to improve water quality control and management and ultimately benefit public health. The study aims to determine these tanks' specific diseases and microbial concentrations.

MATERIALS AND METHODS

The water samples were collected from a single storage tank used by one of the students from Azhar University located in Cairo Governorate City. They were numbered A1, A2, A2a, A3, B1, B2, Ba, B2b, and B3. All samples were collected separately in sterile tubes and were kept at a temperature of 4°C before further testing.

Sample Collection and Preparation

The membrane filtering technique was used to isolate bacterial analyses following the methods outlined by Clark (1980) and Hoadley (1981) as well as the standard procedure as outlined in the American Public Health Association protocols (APHA) (APHA, 2017; Clark, 1980; Hoadley, 1981). The water samples were not stored longer than 24 hours after collection to prevent changes in microbial growth patterns. The temperature was kept at or below 10 °C, preventing freezing during transport. The samples were also collected for chemical analysis using clean, sterile borosilicate plastic bottles with wide openings.

A 0.1 ml sodium thiosulfate solution ($\text{Na}_2\text{S}_2\text{O}_3$) with a concentration of 3% was employed as an extraction and purification agent. This solution was added to a 120 ml container, effectively neutralizing residual chlorine levels of up to 5 mg/L. The substance effectively counteracts any remaining halogen and prevents the ongoing bactericidal activity during transportation. The $\text{Na}_2\text{S}_2\text{O}_3$ solutions were prepared according to Table 1.

Table 1: Equivalents of sodium thiosulfate

$\text{Na}_2\text{S}_2\text{O}_3$ Concentration	Weight of Compound
3% anhydrous	3 g/ 100 ml
3% pentahydrate	4.6 g/ 100 ml
10% anhydrous	10 g / 100 ml
10% pentahydrate	15.21/ 100 ml

Serial Dilution

This study used a serial dilution technique, as described by (Niemi, 2002). Each sample was made by carefully adding approximately 10cc distilled water into eight glass bottles and autoclaving them at a control temperature of 121°C for 30-60 min. After each water sample was taken from the tanks, it was diluted in the glass bottles (1) with sterile distilled water at a ratio of 1:10. The procedure was continued until 10-4 dilutions were reached; for 1:100 dilutions, 1 cc of sample from a bottle (1) was added to bottle (2).

The next step was to run the samples through a sterile, gridded membrane filter paper with a pore diameter of 0.45 mm and a length of 47 mm. The samples were shaken vigorously at low speed for 7 to 15 seconds using a mechanical shaker to mix the sample dilutions uniformly. A 50-9 mm diameter agar petri dish was aseptically put on the filter paper using flame-sterilized forceps. Each plate was marked with the proper data before the investigation, including the sample points, dilution, date, and other relevant information. Each sample volume or dilution tested for compliance was tested using membrane filtering procedures with a minimum of two duplicate plates. Replicas were suggested for non-compliance testing. The prepared plates were turned upside down and placed in a sealed container or plastic bag at 2° to 8° C for two weeks. The agar plates were kept at a consistent humidity level during incubation to prevent them from drying out by more than 15%.

Optimum Colony Density Count

The standard protocols of the APHA (2017) have been adhered to get the most suitable colony density count, which was obtained to be 20 to 200 per filter (APHA, 2017). After the membrane filtering, the resulting colonies were counted under a stereoscopic microscope and adjusted to a 10 to 15-fold magnification power. The petri dish containing the colonies was rotated to an angle of 45 degrees on the microscope stage, and a light source was passed across the colonies' plane. To determine the average count of such colonies per square, 10 squares were counted, and the count of the colonies varying from three to ten was noted. Five squares were assessed in the 10-20 count per square to identify the number of squares whose counts fell in that count range. The number of colonies, the average count per square, was multiplied by 100 and thus divided by the sample volume to ascertain the number of colonies per millilitre. If there was more than one colony in each square, the resulting figure was read as greater than 2000 divided by its volume, which was rounded to give the mean colony counts, referred to as the Colony Forming Unit, or CFU. Spreaders were only estimated in round figures where two or more geographically separated colonies were joined together.

Total Bacterial Count

In compliance with the recommended methods outlined by the American Public Health Association, 1 ml of each potable water sample was pipetted and cultured on R2A agar and spread plate agar to determine the overall bacterial loads (APHA, 2017).

Faecal Coliform Counts

The faecal coliform counts were ascertained using the membrane filter technique on an improved lactose medium based on the said protocol (Guillemin *et al.*, 1991).

Total Coliform Counts

The total coliform membrane filter technique was used to

obtain the colony-forming units (CFU) from the sampled drinking water, following the method described (Gautam & Adhikari, 2018). Briefly, 10 ml of pH-adjusted dilution water was pipette into the funnel with 100 ml of filtered sample, then washed in with 25 – 50 ml of buffer. At other times, the probable density of bacteria, the level of turbidity, and legal provisions were used to show the number of samples for collection. An optimum sample volume was estimated to yield 20 to 80 coliform colonies and 200 colonies of all types on a membrane-filter surface. One membrane filter was placed in each dish incubated for 22 to 24 hours at 35.5°C. The following equation calculated the density of the coliforms:

$$\text{(Total coliforms, No./100 mL)} = \frac{\text{coliform colonies counted} \times 100}{\text{sample filtered (ml)}} = \text{No.CFU/100 mL} \quad (1)$$

Membrane(s) with adequate colonies and 200 colony forming units (CFU) or less on each membrane were chosen. When no coliform colonies are found in drinking water samples, the total coliform colonies should be reported as 1 CFU/100 ml or total *Escherichia coli* (*E. coli*) absent per 100 ml sample.

Statistical Analysis

Every data set underwent statistical analysis in compliance with the protocol defined by the (Institute, 1985) computer software, and means were contrasted using the technique as per (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSIONS

The results of this study's microbiological analysis are displayed in Table 2, which represents the total count of microbiological plates observed within household water tanks during the winter and summer seasons at two different temperatures. It can be observed that the plate count for all samples in A1 and B1 was less than 1 CFU except sample A1, observed at 22°C in summer (2 CFU). For A2, the highest count of 5 CFU was observed at 22°C in summer, while at 35°C, it was less than 1 CFU. In winter, it was observed to be as low as 350×10^{-3} and 260×10^{-3} CFU at 35°C and 22°C respectively. The plate count observed for sample A2a was 15×10^{-3} CFU at 35°C and 18×10^{-3} CFU at 22°C for summer. Whereas 4.20×10^{-2} colonies were observed at 35°C and 8.9×10^{-2} colonies were seen at 22°C in winter.

Table 2: Total number of microbiological plates observed in household water tanks

Sample ID	Winter		Summer	
	35°C	22°C	35°C	22°C
Total plate count (CFU/100ml)				
A1	<1	<1	<1	2
A2	350×10^{-3}	260×10^{-3}	<1	5
A2a	15×10^{-3}	18×10^{-3}	4.20×10^{-2}	8.9×10^{-2}
A3	48	1.5×10^{-2}	<1	11×10^{-1}
B1	<1	<1	<1	<1
B2	1	7	350×10^{-3}	310×10^{-3}
B2a	2.30×10^{-2}	2×10^{-2}	4×10^{-3}	13×10^{-3}
B3	<1	<1	3×10^{-1}	16×10^{-1}

The highest colonies of 48 CFU were observed to be found in winter in sample A3 at 35°C, while in summer, there were fewer than 1 at the same temperature and at 22°C, 1.5×10^{-2} and 11×10^{-1} CFUs were observed in winter and summer respectively. Colonies of the bacteria were 1 and 7 CFU/100mL of B2 in winter at 22°C and 35°C respectively, whereas 350×10^{-3} and 310×10^{-3} CFU/100mL values were reported in winter at 22°C and 35°C. For B2a, 2.30×10^{-2} at 22°C and 2×10^{-2} at 35°C were seen in winter, while 4×10^{-3} at 22°C and 13×10^{-3} at 35°C were grown in summer. Both the samples of B3 in winter showed <1 CFU/100ml, while in summer, the CFU/100mL value of the water samples observed in winter was 3×10^{-1} at 35°C and 16×10^{-1} at 22°C. Moreover, Table 3 in this study showed the total *E. coli* or coliform count of summer and winter samples was

found to be in the range of 1 CFU/100 mL in almost all samples, except for the winter water sample B2a, which had 4.5×10^{-1} CFU/100 ml. If no coliform colonies are found in drinking water samples, the total coliform colonies should be reported as 1 CFU/100 ml or total coliform bacteria missing per 100 mL sample. Faecal coliform densities were estimated in terms of CFU per 100 mL, taking into account the density arrived at from the sample volumes yielding MFC within the range of 20-60 thermotolerant coliform colonies, as shown in Table 4. This colony density range is more restrictive than the 20 to 80 total coliform ranges because the total count on MFC is normally bigger due to larger colony size. The outcome displayed all samples as 1. The WHO has approved these results as acceptable (Organization, 2004).

Table 3: Household water tanks' microbiological total coliform content

Sample ID	Winter	Summer
	Total coliform (CFU/100mL)	
A1	<1	<1

A2	<1	<1
A2a	<1	1
A3	<1	<1
B1	<1	<1
B2	<1	<1
B2a	4.5×10 ⁻¹	1
B3	<1	<1

Table 4: Household water tanks’ microbiological Faecal coliform levels

Sample ID	Winter	Summer
	Faecal coliform	
A1	<1	<1
A2	<1	<1
A2a	<1	<1
A3	<1	<1
B1	<1	<1
B2	<1	<1

DISCUSSION

The measurable value of CFU is a crucial statistic in microbiology that provides insights into the number of viable microorganisms inside a certain specimen, like bacteria, yeast, or mould. It is used in this inquiry (Cundell, 2015). This concept is considered to be crucial, especially in aspects such as water quality analysis and microbiology (Angnunavuri *et al.*, 2022; Yeboah *et al.*, 2022). Seasonal fluctuations might be attributed to differences in water source and treatment used over the period. Other differences in sample collection could also explain changes in total microbial plate count, as shown in Table 2. One of the main factors of bacterial formation within the home water storage tanks is the temperature fluctuations of 22–35°C. The research focuses on the fact that bacterial activity increases in relevance with water temperature, which may result in the variation of water quality based on global change (Jeon *et al.*, 2019).

From the samples obtained from the research, most of them had a total and faecal coliform of less than 1, implying that the amount of bacteria in the water was very little; hence, the water was clean. According to Adzitey, Sumaila, & Saba (2015), clean water containing *E. coli* can lead to undetectable coliform bacteria, supporting our findings in the study (Adzitey *et al.*, 2015). In this context, total coliforms are a robust group of bacteria that can be used in the evaluation of water quality and potential faecal contamination (Byappanahalli *et al.*, 2012; Mabvouna Biguioh *et al.*, 2020; McLellan & Eren, 2014; Noble *et al.*, 2003; Ramteke *et al.*, 1992). This information is particularly useful regarding the microbiological quality of water and how it can be utilized. The research underscores the importance of identifying *E. coli*, a rod-shaped, gram-negative facultative anaerobe bacterial species belonging to the Enterobacteriaceae family that can cause lethal diseases (Jang *et al.*, 2017; Torres, 2010). Total coliforms do not have any pathogenic potentialities;

however, they express definite threats regarding contaminating risks. Thus, there is a need to perform other tests to identify the presence of certain pathogenic bacteria, including *E. coli*, which may negatively impact health (McLellan & Eren, 2014). To minimize the spread of *E. coli*, it is necessary to detect and avoid water pollution by specializing in wash practices. To prevent the outbreak of waterborne diseases such as hazardous *E. coli* and other diseases that cause bacteria, it is crucial to have adequate and safe water supply and inadequate water sanitation facilities (Jang *et al.*, 2017). The findings show measures to lower the microbial load on water and discuss the risk of using water from taps or tanks, which may pose a health risk to families that do not have filtering systems.

Based on this study, it can also be deduced that CFU is an effective quantitative parameter in microbiology that can be used to determine the contents of water sources in terms of microbes. These changes are presumed to be due to fluctuations in plate number as a function of the passage of time, seasonal changes and water treatment. The study also recommends further research to determine the extent of pathogenic bacteria coverage like *E. coli* and the importance of monitoring total coliform as a water quality index. Studies show that water purification systems and sanitary measures are essential to prevent harm from contaminated water. Microbial and water quality analyses are a lot more helpful in obtaining results.

CONCLUSION

The study on microbial content in domestic water tanks in Cairo, Egypt, has provided valuable insights into the presence and dangers of consumable stored water. The results suggest high water quality and low levels of faecal coliform in most samples. However, further research is needed to manage potential health risks associated with potable water consumption. Protecting the population

from waterborne diseases requires strong technical examination and sound water treatment and supply maintenance procedures. Ensuring the public's right to pure, clean water is crucial for individual and community health preservation and long-term environmental-friendly development, ensuring safe drinking water for all.

LIMITATION

The study's findings are limited due to the limited sampling of water samples from storage tanks of only one student at Azhar University, making it impossible to extend the results to other households or areas in Cairo.

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