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COMPARATIVE MICROBIOLOGICAL ANALYSIS OF WATER STORED IN DIFFERENT STORAGE VESSELS

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ABSTRACT

Water is most essential for existence of life on earth. Water is the basic necessity of all living creatures and its important use are for drinking purpose. Water intended for human consumption must be safe and free from microbes. Therefore this investigation was taken with the objective to study the effect of storage of lake water in different vessels like plastic, clay, copper and stainless steel by comparing the number of coliforms present in the water before and after storage. The findings may have significant implications in the use of vessels made of copper and its alloys for household storage of drinking water.

KEYWORDS: Lake water, physico-chemical parameters, copper vessel, aerobic plate count, coli form count.

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INTRODUCTION

Water is most essential for existence of life on earth. Water is the basic necessity of all living creatures and its important use is for drinking purpose[1]. Man can live without food for two months, but can live only three to four days without water. The crisis of water shortage is still the most important global challenge of the twenty first century. Although 75 percent of the earth surface is covered by water, fresh water has the share of only 3 percent of this amount, from which only one percent is available for different human uses[2]. Based on the reports of World Health Organization (WHO,1996) [3], nearly 85 percent out of 1.5 billion population of the world has no access to healthy and uncontaminated water and live in small communities who need safe drinking water. Water intended for human consumption must be safe and free from microbes. Diseases related to contamination of drinking water constitute a major burden on human health[4]. It is estimated that approximately 780 million people lack access to an improved water source. In India, there are additional problems due to the disruption of piped water supplies in rural and urban areas, resulting in a need to store water for drinking, food preparation and bathing purposes. Drinking water may be contaminated at the source or during storage[5]. Eventhough, storage of water has been recommended as a method water purification, contamination of treated or disinfected water can also occur during storage due to improper handling. Hence it is important from a safety point of view to maintain the quality of drinking water during storage.Water is considered as potable when there are no levels of chemicals (e.g. heavy metals or chemical substances that would cause harm to human health and when water does not have a bad taste or smell[6]. Drinking water should be pure for consumption but majority of our population in our country is not consuming the pure water. Drinking water must be free from chemical substances and microorganisms, which might be dangerous to the health of the user. Sources of water like tube well, ponds, hand pump, tanks, rivers and municipal taps are sometimes contaminated with faecal matter, which make the water unhygienic[7]. The basic problem of safe water supply and sanitation is yet to be solved. Utilization pattern of water is also not safe and healthy. Quality of water is poor, which it may be due to either inherent in sanitary water supply source or unhygienic water storage methods. The various microorganisms present in water can cause diseases in humans, leading to infectious, toxigenic and parasitic processes . The water is the primary source to cause the diseases like diarrhoea, typhoid and various diseases to human beings[8]. According to the World Health Organization, an estimated 4.1% of the total global burden of disease is contributed by diarrhoea illness: around 88% of that burden is due to unsafe water supply, sanitation and hygiene, with children in developing countries being the most common victims.The most serious water pollutants in terms of human health worldwide are pathogenic organisms. Thus, drinking water must be free of these pathogens - viruses, protozoa or bacteria. Consumable water quality occurs when there are especially no bacteria of faecal origin present that may cause human diarrhoea and other life threatening diseases (e.g. typhoid fever). To actually test water for specific harmful viruses, protozoa and bacteria is time consuming and expensive. Therefore, water quality control personnel usually analyze water for the presence of coliform bacteria, any of the types that live in the colon or the intestines of humans and other animals (e.g. *E. coli*).Coliforms are used as water quality indicators for two main reasons: i) Coliforms may be associated with the sources of pathogens contaminating water and their presence in drinking water may indicate a possible presence of harmful, disease causing organisms. ii) The analysis of drinking water for coliforms is relatively simple, economical and efficient. Coliforms could be easily detected by its ability to ferment lactose to produce acid and gas within 48 hrs at 35-37^{0}C.In many parts of the developing world, drinking water is collected from unsafe surface sources outside the home and is then held in household storage vessels. However, improving source water quality alone does not always decrease disease incidence because drinking water also becomes contaminated after collection, either during transport or storage in the home[9]. Therefore, strategies to reduce waterborne disease transmission must

be adopted that includes the conditions and practices of water collection and storage and the choice of water collection and storage containers or vessels. Improvements in source water quality generally depend on expensive, long-term, centralized projects, such as construction of wells, water treatment plants, and water distribution systems. An inexpensive strategy is storage in appropriate vessels to prevent recontamination (safe storage) or reduce the number of pathogens. The ancient Egyptian, Indian and Sumerian civilizations used copper, silver and gold for jewellery, cutlery and as vessels to store and drink water. These materials were not used for aesthetics alone; they have tremendous health and spiritual benefits for the human being[10]. The Indian Ayurveda describes storing water in a copper vessel overnight and drinking it in the mornings for many health benefits. Copper is known for its antimicrobial, anti-inflammatory, antioxidant and anticarcinogenic activities.Yogis and traditional households in India have for thousands of years (and are till today) been utilizing a simple, practical and effective method of drinking water in its most holistic way- drinking water from a copper cup or a large copper vessel where water is stored. Silver vessels were also used to give water powerful antibacterial, antifungal and antiviral properties. For centuries, storing water in brass vessels has also been said to be good for health. It is believed that the zinc and copper present in the brass boost immunity and protect against illness. Thus this project was taken with the objective to study the effect of storage of lake water in different vessels like plastic, clay, copper, and stainless steel by comparing the number of coliforms present in the water before and after storage.

MATERIALS AND METHODS

Collection of water samples

Water samples were collected from Ukkadam lake, Coimbatore, Tamilnadu. The samples were brought to the laboratory for the estimation of physico-chemical parameters and micro-biological analysis. The collected water samples were stored in different storage vessels – plastic bottle,copper bottle,clay pot and stainless steel vessel and kept for ten days. Water samples were transported to the laboratory after ten days and subjected to physico-chemical parameters and micro-biological analysis.

Physico-chemical analysis of water

The collected water samples were tested for physico-chemical properties such as water temperature (WT), transparency, pH, electrical conductivity (EC), dissolved oxygen (DO), total alkalinity (TA), free CO_2, total dissolved solid (TDS), and total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium-nitrogen (NH3-N) and total suspended solid (TSS) according to the procedures of the Bureau of Indian Standards[11].

Storage of contaminated water in different vessels and detection of coliforms

Immediately after taking initial microbial counts, water samples were distributed in different vessels of 500 ml capacity, covered with sterile paper and kept at room temperature. Different storage vessels - Copper, stainless steel vessel, plastic vessel and clay vessel were taken and rinsed with sterile water. Then 200ml of the collected water sample was transferred under aseptic conditions into each vessel and stored for 24hrs at room temperature. Water sample was tested for the presence of coliforms before and after storage in the metal vessels by using multiple tube presumptive test of coliform test.The number of positive finding of coli form group organism resulting from multiple portion decimal dilution planting was computed as the combination of positive and was recorded in terms of MPN. The MPN of variety of planting series and result was computed from statistical table.

Estimation of micro-organisms in the water

In order to estimate the microbial load per millilitre of water, dilutions were made upto 10 fold through serial dilutions. Coliform count per millilitre of sample was estimated using spread plate technique on sterile Violet Red Bile Agar (Himedia, India) plates, according to the procedure described by Kornacki and Johnson (2001).Aerobic plate count per millilitre of sample was estimated using pour plate technique, in standard Plate Count Agar (Himedia, India), according to the procedure described by Morton (2001).

RESULTS

Table 1 showed the physico-chemical parameters of water collected from lake before and after storage in different storage vessels. The physical parameters include turbidity and pH and the chemical parameters include Total dissolved solids, Total hardness, Total alkalinityand the amount of chlorides and sulphates present in the water sample. The turbidity and pH of water before storage in vessels were 1 NTU and 6.6 respectively.TDS level was 815 mg/l,Total hardness was 296 mg/l,total alkalinity was 65mg/l . All the four storage vessels showed turbidity below 1.The pH alone changed in different vessels namely pH stored in plastic bottle is 7.2, copper vessel – 8.4,clay vessel-7.5 and stainless steel vessel is 7.1.The chemical parameters in Table 1 suggested that total dissolved solids is 171 mg/L in plastic bottle, 62.9 mg/L in copper vessel, 92.0 mg/L in clay vessel and 92.0 mg/l in stainless steel vessel. If total hardness, total alkalinity and the amount of chlorides and sulphates present in the water sample stored in storage vessel are considered, storing water in copper vessel yields the best result.

Table 1
Physico-chemical parameters of water collected from lake before and after storage in different storage vessels

Parameters	Before storage	After Storage for 10 days			
		Plastic bottle	Clay vessel	Stainless steel vessel	Copper vessel
A. PHYSICAL PARAMETERS					
Turbidity	1 NTU	0.6 NTU	0.6 NTU	0.6 NTU	0.6 NTU
pH at 25.5^0 C	6.68	7.2	7.5	7.1	8.4
B. CHEMICAL PARAMETERS					
Total dissolved solids	815 mg/L	171 mg/L	92.0 mg/L	92.0 mg/L	62.9 mg/L
Total hardness	296 mg/L	14.1 mg/L	18.98 mg/L	15.98 mg/L	17.18 mg/L
Total alkalinity	65 mg/L	250 mg/L	27.0 mg/L	27.0 mg/L	35 mg/L
Chloride as Cl	75.2mg/L	364.95 mg/L	38.55mg/L	38.51mg/L	15.26mg/L
Sulphates as SO_4	80.5mg/L	72.04 mg/L	7.67 mg/L	7.67 mg/L	9.61mg/L

The succession of bacteria in water samples during storage for 10 days was shown in Table 2. It was observed that the occurrence of bacterial isolate is less in copper vessel when compared to the other storage vessels. The decreasing succession of bacterial isolates after storage in different vessels was noted in the order: copper vessel > clay vessel > stainless steel vessel > plastic bottle.

Table 2
Succession of Bacteria in water samples during storage for 10 days

Type of storage vessel	Occurrence of Bacterial Isolate
Before storage	+
After Storage	
Plastic bottle	+++
Copper vessel	-
Clay vessel	-
Stainless steel vessel	+

Key: +: Present; -: Absent

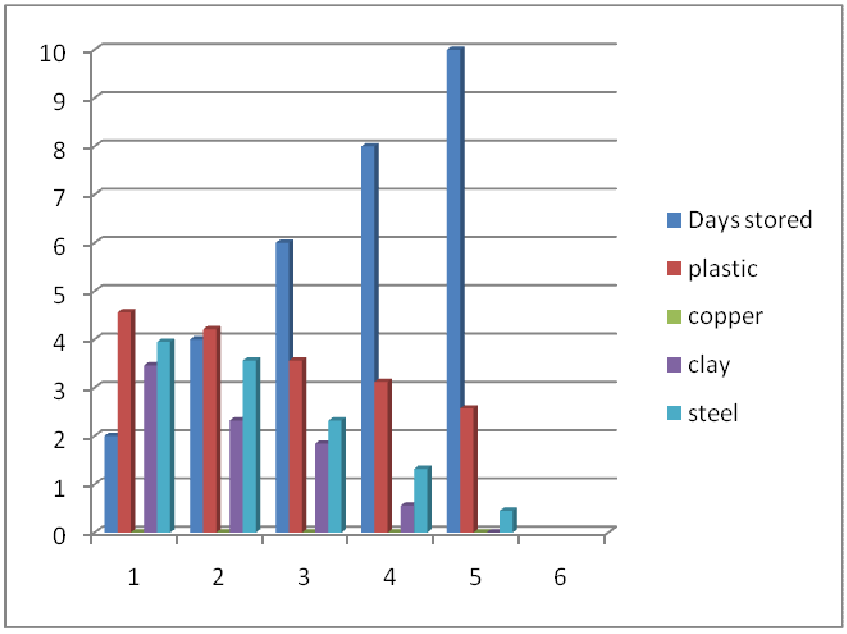
Table 3 represented the mean aerobic plate count of water stored in different storage vessels.It was found that after 10 days of storing water in storage vessels, the bacterial count decreases in all the storage vessels. But the mean aerobic plate count was negligible in copper vessel and maximum in plastic bottle.

Table 3
Mean aerobic plate count of water stored in different storage vessels

Days of storage	Mean Aerobic Plate Count (log_{10} cfu/ml)			
	Plastic bottle	Copper vessel	Clay vessel	Stainless steel vessel
2	5.98±0.11	4.32±0.11	5.21±0.45	5.11±0.40
4	4.96±0.35	3.97±0.54	4.97±0.31	4.83±0.76
6	4.32±0.21	3.53±0.33	4.66±0.75	4.00±0.10
8	3.64±0.81	2.63 ±0.56	3.61±0.80	3.54 ± 0.74
10	3.074±0.81	1.34 ±0.56	2.54±0.80	2.85±0.74

The mean coliform count of water stored in different storage vessels was depicted in Figure 1. After 10 days of storage, the coliform count was decreased in all the storage vessels. After two days of storage in copper vessel, it was found that there was absence of coliform bacteria in copper vessels. After 10 days of storage, there was absence of coliform bacteria in clay vessls also.

Figure1
Mean coliform count of water stored in different storage vessels



DISCUSSION

An investigation has been conducted to determine the comparative microbiological analysis of lake water stored in different storage vessels namely plastic bottle, copper vessel, stainless steel vessel and clay vessel. It was observed that by tenth day of storage of water, copper vessel could eliminate 90 per cent of aerobic bacteria, earthenware vessel could eliminate 77 per cent of aerobic bacteria and stainless steel vessel could eliminate only 70 per cent of aerobic bacteria. Mehta *et al.,* (2004)[13] demonstrated that the MPN of coliform organism present in sewage contaminated water stored in copper vessel, was negligible within 36 hours of storage, indicating that copper has disinfecting property. Faundez (2004) showed that copper sheets have an inhibitory effect on enteropathogens such as *Salmonella enterica* and *Campylobacter jejuni*. Sharan *et al.,*

(2011)[15] confirmed that water-borne pathogens such as *Salmonella typhi,Salmonella typhimurium* and *Vibrio cholerae* are inactivated by storage in a copper water storage vessel within 24 hours. Brick *et al*., (2004)[16] reported significantly decreased contamination due to faecal coliform bacteria and *Escherichia coli* in household waters kept in brass storage vessels, both under field conditions and in the laboratory. Sudha *et al.,* (2009)[17] reported that water stored overnight in copper pots gave no counts for *E. coli*, *S. typhi* and *V. cholerae*, demonstrating inactivation of these bacteria. Sudha *et al.* (2012)[18] inoculated drinking water with 500 cfu/ml of diarrhoeagenic bacteria, including *V. cholerae*,*Shigella flexneri*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, *S. enterica* Typhi, and *S. paratyphi* and stored in copper vessels. No bacteria could be recovered after 16 hours, confirming the antibacterial property of copper. The study also demonstrated that, the level of leached copper was approximately 1/20th of the permissible limits of copper in drinking water. The use of copper in history is found to be about 10,000 years ago. In 3000 B.C., copper ores were found in the island of Cyprus. Romans named the metal as cyprium which was later known as cuprum and then copper in English. In India copper was used to sterilize drinking water. It is still used in many households for storing water. Copper is considered as an essential mineral for our body. Water stored in copperware is the boon of life[19]. Storing water in copper pots finds mention in ancient texts of Ayurveda for purification of water. Copper is a naturally hygienic metal that slows down the growth of germs such as E-coli. Our scriptures also advocate the drinking of water stored overnight termed as “Tamra Jal” and has been used for controlling ageing process. Water is energized if stored in copper vessels. Taste the water from a copper cup to experience the energized quality of water! It is said to be "blessed". When water is 'blessed', its crystalline structure is changed. When water is blessed, the water has super healing properties. Living organisms requires copper at low concentrations as cofactors for metalloproteins and enzymes, however at high concentrations, copper induces an inhibition of growth in bacteria, and have a toxic effect on most microorganisms. The mechanism of action of copper on bacteria is not completely understood. Some studies conclude that the copper ions brought about complete killing of bacteria by membrane damage. Nies *et al*., (1999) opined that the effect of copper may involve substitution of essential ions and blocking of functional groups of proteins, inactivation of enzymes, production of hydroperoxide free radicals by membrane bound copper, and alterations of membrane integrity. Copper is one of the essential metallic elements required for proper metabolic functioning of the body. However, the amount required is very less i.e. 1.2mg/day (trace amount) but our body does not synthesize copper so it needs to be supplied from dietary source. Copper is an important part of a healthy, natural environment, and is necessary to the well-being of every living thing. The best method of fulfilling the body’s need of copper is by having water stored overnight in a copper vessel. Earlier yogis would carry their “kamandalam” i.e. a copper vessel with them and used to have water from that vessel only. They believed that copper is the ideal material for charging water positively and is known for balancing the three doshas of our body i.e. kapha vata and pitta. The water stored in copper vessel was termed as “Tamra Jal” and has been used for controlling ageing process known as vaya-sthapak. This whole process of dissolving copper in water and making it more as an electrolytic is termed in scientific language as “Oligodyanamic process”. This process is known to destroy all the micro-organisms like virus and bacteria in it, hence making it fit for drinking purpose.

CONCLUSION

The present investigation proved that water stored in copper surfaces completely killed the bacteria, *E. coli*. The studies concluded that the copper ions brought about complete killing of bacteria by membrane damage. However, the mechanism of action of copper on bacteria is not completely understood. Although studies have shown the merits of copper surfaces for their use in improving public hygiene in healthcare facilities, the potential use of copper for the purification of drinking-water, especially in developing countries, has

not been widely studied. Therefore, results of our study indicate that copper holds potential to provide microbially-safe drinking-water to the rural masses in developing countries. Hence, it can be inferred that copper metal is the most effective metal in killing the coliforms. The traditional Indian practice of storing drinking water in a copper vessel overnight is the simplest way to obtain the health benefits of copper.

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