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MICROBIAL ANALYSIS OF DRINKING WATER

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ABSTRACT

Storage container plays a major role that affects the microbial quality of drinking water as mentioned in ayurveda. **Objectives:** The present research has highlighted the effect of various storage vessels on the microbial quality of drinking water. **Methods:** Drinking water samples were collected under sterile conditions in a pre-sterilized conical flask and brought to the laboratory immediately for testing. Different vessels, including mud pot, steel container, plastic container, glass container, aluminium container, brass container, copper container and silver container, were taken and rinsed with sterile water. Most Probable Number (MPN) technique was used for analysis of total coliform and fecal coliorm bacteria in the collected water sample. **Results:** The results of microbial analysis of water stored in eight different containers, showed very clearly the decrease in coliform bacterial population by 12 hrs of storage itself and complete removal of coliform bacteria leading to microbial free safe water within 24 hrs, in water stored in mud pots, brass, copper and silver containers. However, the water stored in other remaining containers viz., steel container, plastic container, glass container and aluminium containers, show the presence of coliform bacteria even in the water stored for 24 hrs. **Conclusion:** It is concluded that household water storage containers were capable of improving quality of the microbial contaminated water.

KEYWORDS: Storage Vessels, Drinking Water, Coliforms.

INTRODUCTION

Safe and potable drinking water is essential to the well-being of community. Water related diseases continued to be one the major health problems globally (Oladipo et al. 2009; Folarin et al., 2013 and Uwah et al., 2014). Improper sanitation and fecal contamination of drinking water sources is majorly responsible for water pollution (Cabral, 2010). Safe drinking water is colourless, odourless, tasteless and free from faecal contamination. Water is considered 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. 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. Acceptable water quality occurs when there are especially no bacteria of faecal origin present that may cause human diarrhoea and other life-threatening diseases (Ezeugwunne et al., 2009).

In many parts of the developing world, drinking water is collected from unsafe surface sources outside the home and is then kept in household storage vessels. It may be contaminated at the source or during storage. 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. 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, antiinflammatory, antioxidant and anticarcinogenic activities. A simple, practical and effective method for potable drinking water in its most holistic way is drinking water used from a copper cup or a large copper vessel and silver vessel having 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 zinc and copper present in the brass boost immunity and protect against illness. Clay pots have been commonly used for a long time ago to conserve water and keep the water clean. However, plastic, steel, and glass containers are now widely used by customers to store water (Kumar and Garg, 2020).

Household water storage remains a necessity in many communities, especially in the developing countries. Water storage vessels is the main source of water contamination. The primary objective of this research is to store drinking water in a perfect storage vessel as long as possible without deterioration in its qualities. Hence, it is still necessary to investigate how each type of storage vessel affects the quality of the water stored in it. The current work examined the impact of various storage containers on the quality of water, thereby promoting human health.

MATERIALS AND METHODS

Drinking Water Sampling

Drinking water samples were collected under sterile conditions in a pre-sterilized conical flask and brought to the laboratory immediately for testing. Different vessels, including mud pot, steel container, plastic container, glass container, aluminium container, brass container, copper container and silver container, were taken and rinsed with sterile water. Each vessel (pot) having 2 litre capacity to hold the water was selected for the study. Then 200ml of the collected water sample was transferred under aseptic conditions into each vessel and stored for 24hrs at room temperature and analyses were carried out at “0” hr, 12 hr and 24 hr of storage. These vessels containing water sample were covered with their respective lid and placed in a room at a constant temperature range of 20°C–23°C to avoid any contamination and the effects of light and temperature (Salle, 1974; Jiddimani et al., 2021).

Microbial Analysis of Drinking Water

Most Probable Number (MPN) technique was used for analysis of total coliform and fecal coliorm bacteria in the collected water sample and the water stored in 8 different types of containers for different time periods. MPN method was routinely followed for the analysis of coliforms in the water (Packiyam et al., 2016).

Presumptive Test

The water samples were analyzed for the presence of coliforms in lauryl tryptose broth (g/L: Lactose, 20; Sodium chloride, 5; Dipotassium phosphate, 2.75; Mono Potassium phosphate, 2.75; Sodium Lauryl Sulphate, 0.10; pH at 25^{0}C 6.8 ± 0.2) by using presumptive test specific for coliforms (Salle, 1974). Ten mL of either single strength or double strength Lauryl Tryptose Broth were made in test tubes containing an inverted Durham’s tube and sterilized at 121^{0}C, for 15 min. The broth was aseptically inoculated with 0.1 ml of drinking water to 10 mL to double strength and 1ml, 10ml of bore well water to single strength Lauryl Tryptose broth and incubated at 35°C. The results were recorded after 24 hrs. The formation of gas indicated by a bubble in Durham’s tube were recorded as positive indicating the presence of coliforms and those without bubble were regarded as negative for coliforms.

Confirmatory Test

Confirmatory test for the positive water samples were done in brilliant green 2% bile broth (g/L: peptic digest of animal tissue, 10; Lactose, 10; Ox gall, 20; Brilliant green, 0.0133; pH (at 25°C) 7.8 ± 0.2). One loop full of the inoculums from positive tubes of Lauryl Tryptose broth was inoculated into the sterile brilliant green 2% bile broth tubes and incubated at 37°C for 24hrs. After the incubation period, the tubes were examined for the gas production. The numbers of positive tubes were counted and the value of MPN/100 mL as computed by referring the standard MPN table (Aneja, 2003).

Completed Test

The test is aimed for the identification of coliforms through various biochemical means. Streak one loop full of the positive confirmed culture on sterile EMB agar plate and incubated at 37°C for 24 hrs. Nucleated colonies with or without metallic sheen colonies were marked as typical colonies and transfer to sterile Lauryl Tryptose Broth and nutrient agar slants. Observe gas production on LT broth. Gas production on LT broth indicates completed test.

RESULTS AND DISCUSSION

The results of microbial analysis of water stored in eight different containers, showed very clearly the decrease in coliform bacterial population by 12 hrs of storage itself and complete removal of coliform bacteria leading to microbial free safe water within 24 hrs, in water stored in mud pots, brass, copper and silver containers. However, the water stored in other remaining containers viz., steel container, plastic container, glass container and aluminium containers, show the presence of coliform bacteria even in the water stored for 24 hrs (Table 1). The results clearly show that household water storage containers were capable of improving quality of the microbial contaminated water. The quality improvements of water by storing them in different containers were already reported and our results are in accordance to those results (Thompson et al., 2003; Clasen et al., 2007).

Mud pot gradually increased the cooling effects to the stored water and also reduced the microbial population because of the temperature decrease below 30°C, which is not suitable for *E. coli* growth and it needs 30-35°C in aerobic condition (Tandon et al., 2005).

It can be inferred from the published literatures that copper metal is the most effective metal in killing the coliforms (Shrestha et al. 2009; Delgado et al., 2011; Sarsan 2013; Samanovic et al., 2012; Radha and Susheela, 2015). The reduction in population of total bacteria as the day of storage increased in similar to the observation by Payment et al. (1985) and Eniola et al., (2007). Many researches indicate that the copper and brass is a low cost microbial safety drinking water storage container (Mehta et al., 2004; Faundez et al., 2004; Brick et al., 2004; Sudha et al., 2009, 2012 and Sharan et al., 2010). Brass is an alloy consisting mainly of copper (over 50%) and zinc with smaller amounts of other elements (Tandon et al., 2005).

Copper has proven to kill bacteria due to what is called the oligodynamic effect, even in relatively low concentrations. This antimicrobial effect is shown by ions of copper as well as mercury, silver, iron, lead, zinc, bismuth gold and aluminium. Copper is known to be far more poisonous to bacteria than other metals such as stainless steel or aluminium (Sarsan, 2013). At the same time the silver has most antibacterial activity when compare to copper as evaluated by the antibacterial effects of Ag and Cu on gram-positive and gram-negative bacteria, which are resistant to nosocomial infections (Hundakova et al., 2013; Zanzen et al., 2013; Losasso et al., 2014; Paredes et al., 2014; Ben-Knaz Wakshlak et al., 2015; Dugal and Mascarenhas, 2015). When all the containers were compared, silver and copper containers were good for storage of drinking water, having antibacterial activities within 24 hrs against *E. coli* (Shrestha et al. 2009).

Studies have shown that improving the microbiological quality of household water by on-site or point-of-use treatment and safe storage in proper containers reduces diarrhoeal and other waterborne diseases in communities and households of developing and developed countries (Thompson et al., 2003). The traditional Indian practice of storing drinking water in a copper vessel overnight is the simplest way to obtain the health benefits of copper (Radha and Susheela, 2015). The antibacterial potential of copper and brass vessels against common waterborne pathogens such as *Escherichia coli*, *Enterococcus faecalis* (Tandon et al., 2005), *Salmonella* sp. and *Vibrio cholerae* (Sudha et al., 2009) has been studied. In conclusion, traditionally certain metal containers/pots/vessels were used to store drinking water in order to ensure safety. A study was conducted with the aim of evaluating the effect of metals against enteric coliforms in drinking water. Complete reduction of the tested organisms in mud pot, copper, brass and silver containers was recorded within 0 to 24 hrs of holding time. This study suggested the promotion of use of water storage containers/vessels made of oligodynamic metals such as copper and brass to control the coliform bacteria in drinking water as silver being expensive. Future studies need to elaborate the mechanism of interaction between Silver, Copper and Brass containers.

Table 1: Microbial contamination of Drinking Water Stored in Various Containers.

Name of Vessel/ Container	Volume of Water Sample (ml)	Time Duration (hours)							
		0-12 hours				13-24 hours			
		1	2	3	MPN	1	2	3	MPN
Control	10	+	+	+	1100	+	+	+	1100
	1.0	+	+	+	1100	+	+	+	1100
	0.1	+	+	+	1100	+	+	+	1100
Mud pot	10	+	+	+	1100	-	-	-	3
	1.0	+	+	+	1100	-	-	-	3
	0.1	+	+	+	1100	-	-	-	3
Steel container	10	+	+	+	1100	+	+	+	1100
	1.0	+	+	+	1100	+	+	+	1100
	0.1	+	+	+	1100	+	+	+	1100
Plastic container	10	+	+	+	1100	+	+	+	1100
	1.0	+	+	+	1100	+	+	+	1100
	0.1	+	+	+	1100	+	+	+	1100
Glass container	10	+	+	+	1100	+	+	+	1100
	1.0	+	+	+	1100	+	+	+	1100
	0.1	+	+	+	1100	+	+	+	1100
Aluminium container	10	+	+	+	1100	+	+	+	1100
	1.0	+	+	+	1100	+	+	+	1100

	0.1	+	+	+	1100	+	+	+	1100
Brass container	10	+	+	+	1100	-	-	-	3
	1.0	+	+	+	1100	-	-	-	3
	0.1	+	+	+	1100	-	-	-	3
Copper container	10	+	+	+	1100	-	-	-	3
	1.0	+	+	+	1100	-	-	-	3
	0.1	+	+	+	1100	-	-	-	3
Silver container	10	+	+	+	1100	-	-	-	3
	1.0	+	+	+	1100	-	-	-	3
	0.1	+	+	+	1100	-	-	-	3

CONCLUSION

Microbial analysis of water stored in eight different containers, showed very clearly the decrease in coliform bacterial population by 12 hrs of storage itself and complete removal of coliform bacteria leading to microbial free safe water within 24 hrs, in water stored in mud pots, brass, copper and silver containers. However, the water stored in other remaining containers viz., steel container, plastic container, glass container and aluminium containers, show the presence of coliform bacteria even in the water stored for 24 hrs. It is concluded that household water storage containers were capable of improving quality of the microbial contaminated water.

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