International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(1): 25-28 March 2014
DOI 10.5958/j.2230-732X.7.1.004

Environmental Science

Microbiological Quality of Water Stored in Copper, Earthenware and Stainless Steel Vessels

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Paper No. 175 Received: December 28, 2013 Accepted: February 20, 2014 Published: March 03, 2014

Abstract

A study was conducted to compare the effect of storage of water in copper, earthenware and stainless steel vessels on coliform count and aerobic plate count. Water samples contaminated with animal excreta was collected from farms and aerobic plate count and coliform count was estimated. The samples were then stored in copper, earthenware and stainless steel vessels for ten days at room temperature and the counts were taken on third, sixth and tenth days. Initial mean aerobic plate count of sample was 9.18±0.38 $\log_{10}$ cfu/ml, which came down to 4.99±0.12 $\log_{10}$ cfu/ml, 5.87±0.47 $\log_{10}$ cfu/ml and 5.21±0.42 $\log_{10}$ cfu/ml respectively, in copper, earthenware and stainless steel vessels on third day of storage. By tenth day of storage of water, copper vessel could eliminate 83 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. Coliforms were completely eliminated from copper vessel by third day of storage. Whereas, earthenware vessel retained 66 percent of initial coliforms on third day of storage, and it took seven to ten days for complete removal of coliforms from earthenware vessel. Stainless steel vessel retained 23.5 per cent of initial coliforms on tenth day of storage. It could be concluded that use of copper vessels for storing drinking water in households is an effective water purification method.

Highlights

- Among copper, earthenware and stainless steel vessels, copper vessel is the best for storing water followed by earthenware and stainless steel vessels

Keywords: Aerobic plate count, coliform count, copper vessel, storage, microbiological quality

Introduction

Water is most essential for existence of life on earth. Water intended for human consumption must be safe and wholesome. Diseases related to contamination of drinking water constitute a major burden on human health. According to World Health Organization, more than 3.5 million people die each year from water, sanitation and hygiene related causes, with 99 per cent death in developing world. It is estimated that approximately 780 million (one in nine) people lack access to an improved water source (WHO, 2008). 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 (Brick *et al*., 2004). Drinking water may be contaminated at the source or during storage. Eventhough, storage of water has been recommended as a method for 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.

Traditionally, vessels made up of copper and its alloys and earthenware pots have been used in India for storing drinking water. Man exploited the antimicrobial attributes of copper long before nineteenth century, when Louis Pasteur developed the germ theory of disease (Online). Copper and its alloys have been shown to be antimicrobial and effective in eliminating both gram-negative and gram-positive bacteria, molds, fungi and viruses, which can cause various disease conditions. Research has shown that the antibacterial activity copper surfaces could be utilized in food processing operations (Faundez *et al*., 2004) and health care (Michels *et al*., 2008). Even then, in present days, majority of people store drinking water in stainless steel vessels. Keeping these facts in mind, the present study was conducted to compare the effect of storage of water in copper, earthenware and stainless steel vessels on coliform count and aerobic plate count. The findings may have significant implications in the use of vessels made of copper and its alloys for household storage of drinking water.

Materials and Methods

Water samples contaminated with animal excreta were collected from farms in sterile conical flasks and used for the study. Samples were transported in ice, reached the laboratory within ten minutes and subjected to microbiological examination immediately.

In order to estimate the microbial load per millilitre of water, the sample was agitated thoroughly and 10 millilitre was transferred to 90 millilitre sterile normal saline (diluent) so as to form one in ten dilution of the sample. Further 10 fold serial dilutions were prepared by transferring one milliliter of inoculum to nine milliliter of the diluent. Dilutions were made upto 10^{-10} and selected dilutions of each sample were used for estimation of various microbial loads per millilitre of sample. 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). Immediately after taking initial microbial counts, water samples were distributed in sterile copper and earthenware vessels of 500 ml capacity, covered with sterile paper and kept at room temperature. The stored samples were further subjected to enumeration of bacterial load on third, sixth and tenth days of storage. The study was conducted thrice by taking samples from same source at two weeks interval and mean count is expressed in $\log_{10}$ cfu/ml.

Results and Discussion

Initial mean aerobic plate count of water sample was 9.18±0.38 $\log_{10}$ cfu/ml, which was reduced on storage in all the three vessels. The comparative assessment of mean aerobic plate count of water stored in copper, earthenware and stainless steel vessels is shown table 1.

Table1: Mean aerobic plate count of water stored in copper and earthenware and stainless steel vessels

Days of storage	Mean Aerobic Plate Count ($\log_{10}$ cfu/ml)		
	Copper vessel	Earthenware vessel	Stainless steel vessel
3	4.99±0.12	5.87±0.47	5.21±0.42
6	3.46±0.35	4.41±0.96	3.99±0.10
10	1.57±0.79[a]	2.15±0.82[b]	2.83±0.22[b]

Mean values in a row bearing different superscripts differ significantly

Initial mean coliform count of water sample was 5.24±0.45 $\log_{10}$ cfu/ml, which was decreased on storage in all the vessels. The comparative assessment of mean coliform count of water stored in copper, earthenware and stainless steel vessels is shown table 2.

Table 2: Mean coliform count of water stored in copper and earthenware and stainless steel vessels

Days of storage	Mean Coliform Count ($\log_{10}$ cfu/ml)		
	Copper vessel	Earthenware vessel	Stainless steel vessel
3	Nil[a]	3.46±0.26[b]	3.95±0.45[b]
6	Nil[a]	1.98±0.23[b]	2.45±0.43[b]
10	Nil[a]	Nil[a]	1.23±0.78[b]

Mean values in a row bearing different superscripts differ significantly

Statistical analysis of data (One-way Analysis of Variance, using SPSS package version 20) revealed significant difference ($p<0.05$) between copper, earthenware and stainless steel vessels in their ability to inactivate bacteria. Initial mean aerobic plate count of sample was 9.18±0.38 $\log_{10}$ cfu/ml, which came down to 4.99±0.12 $\log_{10}$ cfu/ml, 5.87±0.47 $\log_{10}$ cfu/ml and 5.21±0.42 $\log_{10}$ cfu/ml respectively, in copper, earthenware and stainless steel vessels on third day of storage. By tenth day of storage of water, copper vessel could eliminate 83 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. Coliforms were completely eliminated from copper vessel by third day of storage. Whereas, earthenware vessel retained 66 percent of initial coliforms on third day of storage, and it took seven to ten days for complete removal of coliforms from earthenware vessel. Stainless steel vessel retained 23.5 per cent of initial coliforms on tenth day of storage.

Mehta *et al.*, (2004) 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 *et al.*, (2004) showed that copper sheets have an inhibitory effect on enteropathogens such as *Salmonella enterica* and *Campylobacter jejuni*. Sharan *et al.*, (2011) 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 h. Brick *et al*., (2004) 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) 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) 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 (WHO guidelines, 2006 value 2 mg Cu/l).

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 2600 and 2200 B.C. It is still used in many households for storing water. Copper is considered an essential mineral for our body. Ayurveda recommends storing water overnight in a copper jug and drinking the same in the morning keeps good health.

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 (Sani, *et al*., 2001), and have a toxic effect on most microorganisms. The mechanism of action of copper on bacteria is not completely understood (Sudha *et al.*, 2012). Some studies conclude that the copper ions brought about complete killing of bacteria by membrane damage (Santo *et al*., 2011). 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.

Conclusion

From the present study, it is clear that copper vessel is superior over earthenware and stainless steel vessels in eliminating coliforms and other aerobic bacteria. Copper has the potential to provide microbiologically safe water and storage of water in copper vessels can be considered as a cost-effective, point-of-use water purification method. Therefore use of copper vessels to store drinking water in households is recommended.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, for providing facilities to carry out the research.

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PRINT ISSN.: 0974-1712 ONLINE ISSN.: 2230-732X

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