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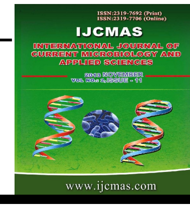

Original Research Article

Effect of storage of water in different metal vessels on coliforms

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A B S T R A C T

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Good drinking water quality is essential to the health and well-being of all people. The most serious water pollutants in terms of human health worldwide are pathogenic organisms. Acceptable water quality occurs when there are especially no bacteria of faecal origin i.e coliforms. The pond water was collected and stored overnight at room temperature in vessels made of copper, silver, brass, aluminium and stainless steel. Coliform counts of the water were taken before and after storage. The results showed that the no: of coliforms reduced on storing water in all the metal vessels used. But there was a significant reduction in the no: of coliforms in water stored in brass and copper vessels. The no: of coliforms have significantly reduced from >1800 to 43 in the water sample stored in brass (an alloy consisting mainly of copper) vessel while from >1800 to 75 in the water sample stored in copper vessel. Thus it was concluded that copper is the most effective metal in killing the coliforms.

Introduction

Good drinking water quality is essential to the health and well-being of all people. 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. 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 (e.g. typhoid fever).

To actually test water for specific harmful viruses, protozoa and bacteria is time consuming & 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 2 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. Drinking water 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. Therefore strategies to reduce waterborne disease transmission must safeguard against both events. Key factors in the provision of safe household water (decreased microbial contamination) include 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. 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 contaminated water in different metal vessels like copper, silver, aluminium, brass and stainless steel by comparing the number of coliforms present in the water before and after storage.

Materials and Methods

A uniform water sample from the centre of pond was collected under sterile conditions in a pre sterilized conical flask and brought to the lab immediately for testing. Different metal vessels - Copper, silver, brass, aluminium and stainless steel vessels 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 (Salle,1974). Lactose broth of single strength and double strength were used for the test. The single strength lactose broth medium was prepared by dissolving 3 gms of beef extract, 5 gms of Peptone, 5 gms of Lactose dissolved in 1000 ml distilled water and required volumes were dispensed into culture tubes containing an inverted Durham tube and sterilized in an autoclave at 115^0C for 10 minutes.

In the multiple tube fermentation method, a series of tubes containing lactose broth medium was inoculated with test portions of water sample. After a specified incubation time at a given temperature, each tube showing gas formation is regarded as "presumptive positive" since the gas indicates the possible presence of coliforms. Three rows (F1, F2, F3) of 5 tubes each in a test tube rack were arranged. The tubes in the first row (F1) contained 5ml of double strength lactose broth medium while the tubes in F2 &F3 rows contained 5ml of single strength medium. 10ml of water sample was added to each of the 5 tubes in row F1, and 1ml into each of the 5 tubes in row F2, and 0.1ml into each of the 5 tubes in row F3. The tubes were gently shaken to mix the contents and then incubated at 37^0C for 24hrs and looked for growth and gas production. The tubes which showed gas production as indicated by bubble formation in the inverted Durham's tube in the test tube were regarded as positive and those without gas formation as negative. The most probable number (MPN) of bacteria present were then estimated from the no: of tubes inoculated and the no: of positive tubes obtained in the test using specially devised statistical tables. This technique is known as the MPN method. There are different MPN methods which can be employed for testing the coliforms in different samples. Usually 3 tube MPN is used for testing most foods, 5 tube MPN for water, shellfish and shellfish harvest water testing and 10 tube MPN for testing bottled water or samples that are not expected to be highly contaminated. In the present test, 5 tube MPN method was used.

Results and Discussion

The pond water was collected and stored overnight at room temperature in vessels made of copper, silver, brass, aluminium and stainless steel. 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 most probable no: of bacteria present were then estimated by scoring the no: of positive tubes in each row and referring to the standard MPN table (Aneja, 2003). The results are tabulated (Table 1,2,3,4,5,6).

The results showed that the no: of coliforms reduced in water stored in all the metal vessels used. But there was a significant reduction in the no of coliforms in water stored in brass and copper vessels. The no of coliforms have significantly reduced from >1800 to 43 in the water sample stored in brass vessel while from >1800 to 75 in the water sample stored in copper vessel. Brass is an alloy consisting mainly of copper (over 50%) and zinc, to which smaller amounts of other elements may be added. Thus it can be inferred that copper metal is the most effective metal in killing the coliforms.

Copper and its alloys (brasses, bronzes, cupronickel, copper-nickel-zinc, and others) are natural antimicrobial materials. Ancient civilizations exploited the antimicrobial properties of copper long before the concept of microbes became understood in the nineteenth century (Dollwet *et al.*, 1985). Ancient greeks

Table.1 Water sample without storage

Row no	**Vol. of water sample**	**1**	**2**	**3**	**4**	**5**	**MPN**
F1	10ml	+	+	+	+	+	5
F2	1ml	+	+	+	+	+	5
F3	0.1ml	+	+	+	+	+	5

+ : Gas production ; - : No gas production No: of coliforms present /100ml = **>1800**

Table.2 Water sample after storage in stainless steel vessel

Row no	**Vol. of water sample**	**1**	**2**	**3**	**4**	**5**	**MPN**
F1	10ml	+	+	+	+	+	5
F2	1ml	+	+	+	+	+	5
F3	0.1ml	+	+	+	+	-	4

No: of coliforms present /100ml = **1600**

Table.3 Water sample after storage in silver vessel

Row no	**Vol of water sample**	**1**	**2**	**3**	**4**	**5**	**MPN**
F1	10ml	+	+	+	+	+	5
F2	1ml	+	+	+	+	+	5
F3	0.1ml	+	+	+	+	-	4

No: of coliforms present /100ml = **1600**

Table.4 Water sample after storage in aluminium vessel

Row no:	**Vol. of water sample**	**1**	**2**	**3**	**4**	**5**	**MPN**
F1	10ml	+	+	+	+	+	5
F2	1ml	+	+	+	+	+	5
F3	0.1ml	+	+	+	+	-	4

No: of coliforms present /100ml = **1600**

Table.5 Water sample after storage in brass vessel

Row no	**Vol. of water sample**	**1**	**2**	**3**	**4**	**5**	**MPN**
F1	10ml	+	+	+	+	+	5
F2	1ml	+	-	-	-	-	1
F3	0.1ml	+	-	-	-	-	1

No: of coliforms present /100ml = **43**

Table.6 Water sample after storage in stainless steel vessel

Row no:	Vol. of water sample	1	2	3	4	5	MPN
F1	10ml	+	+	+	+	+	5
F2	1ml	+	+	+	-	-	3
F3	0.1ml	-	-	-	-	-	0

No: of coliforms present /100ml = **75**

were the first to discover the sanitizing power of copper; early American pioneers moving west across the continent put copper coins in large wooden water casks to provide them with safe drinking water on their long voyage. In addition to several copper medicinal preparations, it was also observed centuries ago that water contained in copper vessels or transported in copper conveyance systems was of better quality (i.e., no or little visible slime formation) than water contained or transported in other materials. In 1973, researchers at Battelle Columbus Laboratories conducted a comprehensive literature, technology and patent search that traced the history of understanding the “bacteriostatic and sanitizing properties of copper and copper alloy surfaces” which demonstrated that copper, in very small quantities, has the power to control a wide range of molds, fungi, algae and harmful microbes (Thurman et al., 1989).

The antimicrobial properties of copper are still under active investigation. Molecular mechanisms responsible for the antibacterial action of copper have been a subject of intensive research. The authors noted that the antimicrobial mechanisms are very complex and take place in many ways, both inside cells and in the interstitial spaces between cells (Dick et al., 1973). Currently, researchers believe that the most important antimicrobial mechanisms for copper are as follows: i) Elevated copper levels inside a cell causes oxidative stress and the generation of hydrogen peroxide. Under these conditions, copper participates in a chemical reaction causing oxidative damage to cells. ii) Excess copper causes a decline in the membrane integrity of microbes, leading to leakage of specific essential cell nutrients, such as potassium and glutamate. This leads to desiccation and subsequent cell death.iii) While copper is needed for many protein functions, in an excess situation (as on a copper alloy surface), copper binds to proteins that do not require copper for their function. This “inappropriate” binding leads to loss-of-function of the protein, and/or breakdown of the protein into nonfunctional portions.

Copper has proven to kill bacteria due to what is called the ‘oligodynamic effect.’ The oligodynamic effect was discovered in 1893 by the Swiss KW Nageli as a toxic effect of metal ions nonliving cells, algae, molds, spores, fungi, viruses, prokar yotic and eukaryotic microorganisms, even in relatively low concentrations (Nageli,1983). This antimicrobial effect is shown by ions of copper as well as mercury, silver, iron, lead, zinc, bismut gold, and aluminium. Copper is known to be far more poisonous to bacteria than others e.g. stainless steel or aluminium. When copper dissolves in water, water becomes ionic (electrolyte) as can be ascertained by its pH measurement. The production of copper ions kills the algae and bacteria. Electrically charged copper ions (Cu^{+2}) in the water search for particles of opposite polarity, such as bacteria, viruses and fungi. Positively charged copper ions form electrostatic compounds with negatively charged cell walls of microorganisms. These compounds disturb cell wall permeability and cause nutrient uptake to fail. The copper ions have the ability to pierce the protective outer membrane of a cell and disrupt the enzyme balance thereby killing microbes.

The results showed that the no: of coliforms reduced more in water stored in brass and copper vessels. As brass is an alloy consisting mainly of copper (over 50%), 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 avail of the health benefits of copper on a regular basis. The science underlying the traditional practice of storing water in copper vessel is thus an interesting and valuable method of purifying water.

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