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Comparative Study on Bactericidal Properties of Storage Vessels for Potable Water

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***Abstract:** Antimicrobial property of metals has been noted since the time of Hippocrates (400 BC). According to ancient Ayurvedic texts, practices such as storing Ganges water in brass or copper vessels were intended to maintain the sanitary quality of water, which points to the bactericidal activity of copper. The present study examines the antibacterial properties of different storage vessels (Copper, Bronze, Stainless Steel, Glass, Plastic). Potable water taken in these vessels was inoculated with a known volume of common pathogens and stored. Bactericidal actions of storage vessels were recorded at regular intervals. A considerable reduction in viable count was observed in storage vessels made of copper and bronze. This emphasisesthe need for research into the antibacterial properties of copper and bronze vessels, which can be utilised in the food and pharmaceutical industries.*

***Keywords:** Antimicrobial, Bactericidal, Sanitary quality, Potable water*

I. INTRODUCTION

Metals emerged as one of humanity's most pivotal inventions, profoundly shaping human culture and progress. India's metallurgy has a rich history, dating back to the Indus Valley Civilisation and earlier Vedic periods. [1] Contemporary developments in metallurgy are characterised by innovative jewellery and diverse utensils, sophisticated equipment, and more in the engineering field, yet the roots of this technology can be traced back to ancient Indian practices that laid the foundation of modern metallurgy. [2] From prehistoric copper smelting to advanced modern steelmaking, Indian metallurgy demonstrates both transformative breakthroughs and the sustained preservation of ancient techniques. Its evolution reflects not only millennia of technological growth but also the blending of traditional craftsmanship, scientific discovery, and socio-economic forces across historical periods. [3] Archaeological evidence from Harappa, Mohenjo-daro, Lothal, Rangpur, and Dholaviraconfirms smelting and finishing technologies and sophisticated practices in the working of copper, bronze, lead, gold, and silver. Later iron working marked a significant milestone in global metallurgical development. [4] Charaka Samhita, one of the remarkable books of Ayurveda, also holds bounteous references regarding the use of metals for different purposes. It includes the processing of metals such as Mercury, Gold, Silver, Lead, Zinc, Copper etc. In Ayurveda, especially in Rasashastra texts, there are references to detoxification during the manufacture of metals. Ayurvedic classics written before 8th century AD, like Charaka Samhita and Sushruta Samhita etc. contain descriptions of metals and minerals, their processing techniques and their utilization in therapeutics. [5] The Arthasastra provides insightful descriptions of pure metals such as gold, silver, copper, tin, lead, and iron, as well as two significant alloys, bronze and brass.[2] Vedic scriptures describe gold as having purifying properties. It symboliseswealth, prosperity, success, good health, richness, and femininity. Most temples house idols of worship made of gold or adorned with Gold ornaments. Silver is another auspicious metal, widely used in jewellery making, spiritual activities, and the manufacture of utensils. In Indian culture, gold is closely associated with Goddess Lakshmi and is worn as chains or as bangles. It is avoided on the lower body, so waistbands, anklets, and toe rings are typically silver. Even today, in Indian villages and tribal areas, silver signifies status and is favoured for auspicious occasions. [6] Traditional practice endorses Copper (Tambra) for water, Bronze (Kansa) for food and Brass (Pital) for cooking. Water containing copper will provide the body with a trace amount of copper sufficient to meet its daily requirement. Eating in Kansa purifies food, enhances immunity and improves brain function. Its alkaline nature reduces acidity during digestion and supports digestion. Similarly, using a Brass coffee filter reduces not only acidity due to its alkaline nature but also enhances the flavour and aroma of coffee. [7] Copper stands as the first proven solid antimicrobial metal, capable of eliminating 99% of pathogenic bacteria within two hours. [8] Copper has been a fundamental component of Indian kitchens and households for thousands of years, with a deeply rooted connection to health, spirituality, and culture. Rooted in Ayurveda and traditional practices, copper is primarily used for storing water and for cooking due to its numerous health benefits, antimicrobial properties, and its role in balancing the body's energy. Bronze is an alloy primarily composed of copper and tin.

Bronze, traditionally known as *Kansa* or *Pital* in India, is highly valued for making utensils due to its durability, excellent heat conductivity, and significant Ayurvedic health benefits, such as improving digestion and retaining food nutrients. [9] Bronze ornaments such as rings and bracelets benefit from the antimicrobial properties, making them suitable for everyday wear without the risk of harbouring harmful bacteria. Historically, various civilisations recognised the health benefits of copper and bronze and used these metals to make tools, utensils, and ornaments, thereby reducing the risk of disease. [5]

Stainless steel, composed mainly of iron, carbon and chromium, is renowned for its resistance to corrosion and staining making it a common material in health care, food processing. Stainless steel is ubiquitous in Indian kitchens due to its durability, nonreactivity with acidic spices, and hygienic properties. It also offers a long-lasting, low-maintenance, and cost-effective approach for daily use. Stainless steel is characterised by an addition of chromium of at least 10-15% of the total composition. Stainless steel is one of the most common materials used in healthcare environments. However, the lack of antibacterial activity has limited their practical use. Antibacterial stainless steel surfaces with different copper contents have been prepared by plasma surface alloying technology (PSAT).

Plastics are synthetic or semi-synthetic materials primarily composed of long polymer chains that confer flexibility and durability. Common types of plastics include polyethene (PE), polypropylene (PP), polyethene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC). These materials are known for their resistance to degradation, which poses significant environmental challenges as they persist in the environment for hundreds of years. However, the efficiency of microbial degradation varies widely depending on the type of plastics and environmental conditions. Plastic utensils leach toxic byproducts when they come into contact with heat and release microplastics into food. [10] Glass has made a significant contribution to human civilisation, reaching modern times. Important glassy materials have a history of 5000 years. Glass and glass ceramic materials have a wide range of use and are essential in modern life. Glass is commonly used in important areas of human life like food and beverage, glassware, medicine, etc. [10] Despite advances in modern utensils, unidentified reasons underlying in the storing utility, cooking advantage and health benefits of traditional vessels need further study. This paper investigates vessel effects on inoculum survival and quantifies post-storage bacterial counts.

II. METHODOLOGY

A. Collection of Vessels and Sterilisation Protocol

Experimental vessels composed of Copper, Bronze, Plastic, Stainless Steel, and Borosilicate Glass were procured from local vendors in Thrissur, Kerala. To eliminate indigenous microbial flora, vessels were subjected to differential sterilisation based on material properties. Thermo-tolerant Vessels (Steel and Glass)were cleaned with non-ionic detergent, rinsed with distilled water, and sterilised via autoclaving.Copper, Bronze and Plastic vessel surfaces were chemically disinfected with 70% (v/v) ethanol. Following solvent evaporation, the vessels were placed in a Class II Biosafety Cabinet and exposed to UV-C radiation for 20 minutes to ensure complete surface sterility.

B. Evaluating the impact of vessels on known inoculum

The antimicrobial efficacy of the vessels was challenged using four distinct bacterial isolates: *Bacillus sp.*, *Proteus sp.*, *Staphylococcus sp.*, and *Escherichia coli.* cultures were grown in Nutrient broth for 18–24 hours at 37°C.Cell suspensions were adjusted using sterile 0.85% (w/v) NaCl to match the 0.5 McFarland turbidity standard, approximately equivalent to 1.5 X 10^8 colony-forming units per milliliter (CFU/mL).

A volume of 50 mL of sterile distilled water was aseptically transferred into each test vessel. Each vessel was then spiked with a predetermined volume of the standardized bacterial inoculum to achieve a uniform initial microbial load.Vessels were sealed with sterile lids to prevent secondary contamination and maintained at an ambient temperature of 28 ± 2 for a total duration of 72 hours.[12]

C. Determination of Bacterial Count in Stored Water

Survival kinetics were monitored at periodic intervals of 24, 48, and 72 hours. 1 mL aliquots were aseptically withdrawn from each vessel after gentle agitation to ensure a homogenous distribution of cells.Samples were subject to ten-fold serial dilutions in sterile distilled up to a factor of 10^{-4}. 100 µLof the 10^{-4}dilution was plated onto Nutrient Agar (NA) using the spread-plate technique with a sterile L-shaped spreader. Plates were incubated at 37°C for 24 hours. The bacterial density was expressed as CFU/mL using the formula

CFU/mL = (Number of colonies × Dilution factor/Volume plated (mL)[12]

III. RESULT AND DISCUSSION

Comparative Efficacy of Vessel Materials

The survival kinetics of the four bacterial isolates (*Bacillus sp., E. coli, Staphylococcus sp.,* and *Proteus sp.*) varied significantly across the test materials. As indicated in Tables 1–4, copper-based alloys (Copper and Bronze) demonstrated the most robust antimicrobial activity.

Fig. 1. Various vessels (Copper, Bronze, Plastic, Stainless Steel, and Glass) used for the comparative evaluation of bacterial survival in stored water.

Copper and Bronzeachieved the most rapid decline in viable counts. for *E. coli*, complete inhibition was achieved within 48 hours (Table 2). This demonstrates the potent "contact killing" ability of copper-based surfaces. Conversely, plastic vessels consistently harboured the highest microbial loads, particularly for *Bacillus sp.* (64 X 10^4 CFU/mL) and *Proteus sp.* (72 X 10^4 CFU/mL) at the 72-hour mark. This confirms that synthetic polymers provide a conducive environment for bacterial persistence, likely due to their inert nature and potential for biofilm initiation.

E. coli was the most susceptible to the oligodynamic effect of copper and bronze. The total elimination of viable cells by 48 hours suggests that the Gram-negative cell envelope of *E. coli* is highly vulnerable to the cupric ions (Cu^{2+}) released from the vessel walls, which induce rapid membrane peroxidation and cytoplasmic leakage. In contrast, *Proteus sp.* exhibited marked resilience. Even on copper and bronze surfaces, viable colonies persisted at 72 hours (Table 4). This resistance may be attributed to the swarming motility and robust outer membrane structures characteristic of *Proteus*, which may partially buffer the organism against oxidative stress.Gram-positive organisms such as (*Staphylococcus* sp. and *Bacillus*sp) showed intermediate survival. The thicker peptidoglycan layer in *Bacillus* and *Staphylococcus* likely provides a temporary mechanical barrier against ion penetration.

Bronze which is alloy of copper and tin showed superior performance. While copper is the primary antimicrobial agent, the alloying with tin in bronze may optimize the rate of ion release or surface oxidation. The mechanism of action includes the disruption of the cellular homeostasis by displacing essential metals from their binding sites in proteins, inducing the production of Reactive Oxygen Species (ROS) and by causing irreversible damage to genomic DNA.

Inert materials like Glass and Stainless Steel lack these active biochemical pathways and thus cannot reduce a pre-existing inoculum, as evidenced by the relatively stable viable counts across 72 hours in Tables 1 and 4.

The data suggests that traditional storage vessels (Bronze and Copper) serve as "active" antimicrobial systems. In areas where waterborne pathogens remain a concern, the transition from plastic storage to copper-based alloys could provide a sustainable, chemical-free method for enhancing point-of-use water safety.

Table 1. Comparative survival (CFU/mL) of select bacterial isolates (*Bacillus* sp., *E. coli*, *Staphylococcus* sp., and *Proteus* sp.) in water stored in copper, bronze, plastic, stainless steel, and glass vessels over 72 hours.

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	Viable count (CFU/ml)											
Vessels	*Bacillus*			*E coli*			*Staphylococcus sp.*			*Proteus sp.*		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Copper	80×10^4	1×10^4	-	1×10^4	-	-	2×10^4	5×10^4	2×10^4	50×10^4	9×10^4	2×10^4
Bronze	4×10^4	41×10^4	2×10^4	1×10^4	-	-	1×10^4	1×10^4	5×10^4	1×10^4	19×10^4	1×10^4
Plastic	115×10^4	63×10^4	64×10^4	234×10^4	57×10^4	55×10^4	3×10^4	119×10^4	10×10^4	95×10^4	49×10^4	72×10^4
Steel	88×10^4	35×10^4	33×10^4	158×10^4	40×10^4	45×10^4	81×10^4	35×10^4	11×10^4	40×10^4	50×10^4	17×10^4
Glass	47×10^4	61×10^4	44×10^4	138×10^4	80×10^4	72×10^4	32×10^4	17×10^4	16×10^4	64×10^4	57×10^4	18×10^4

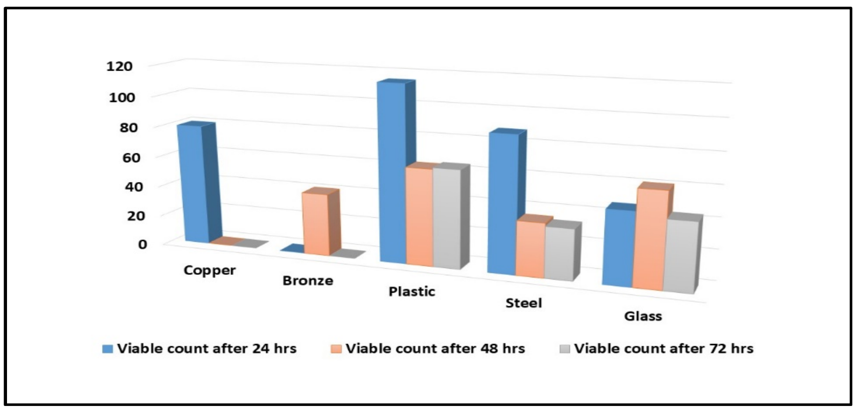

Fig. 2. Comparative survival (CFU/mL) of *Bacillus* sp. in water stored in copper, bronze, plastic, stainless steel, and glass vessels over 72 hours.

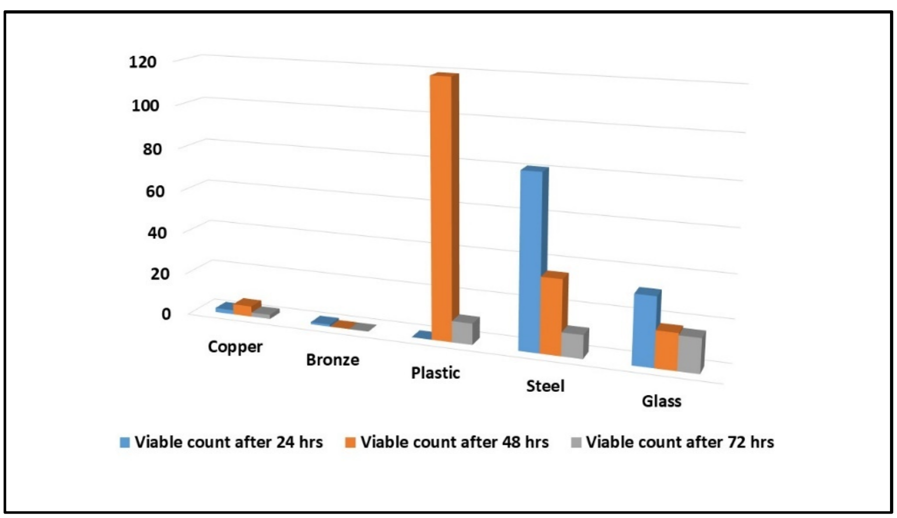

Fig. 3 Comparative survival (CFU/mL) of *Staphylococcus* sp., in water stored in copper, bronze, plastic, stainless steel, and glass vessels over 72 hours.

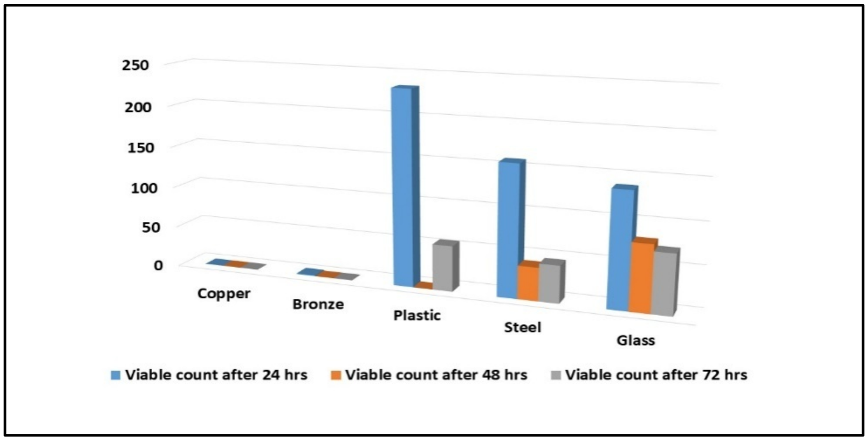

Fig. 4. Comparative survival (CFU/mL) of *E. coli* in water stored in copper, bronze, plastic, stainless steel, and glass vessels over 72 hours.

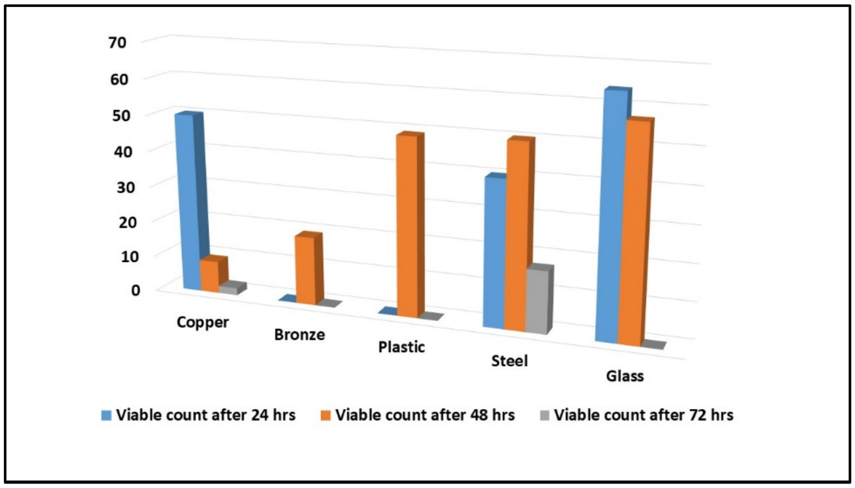

Fig. 5. Comparative survival (CFU/mL) of *Proteus* sp. in water stored in copper, bronze, plastic, stainless steel, and glass vessels over 72 hours.

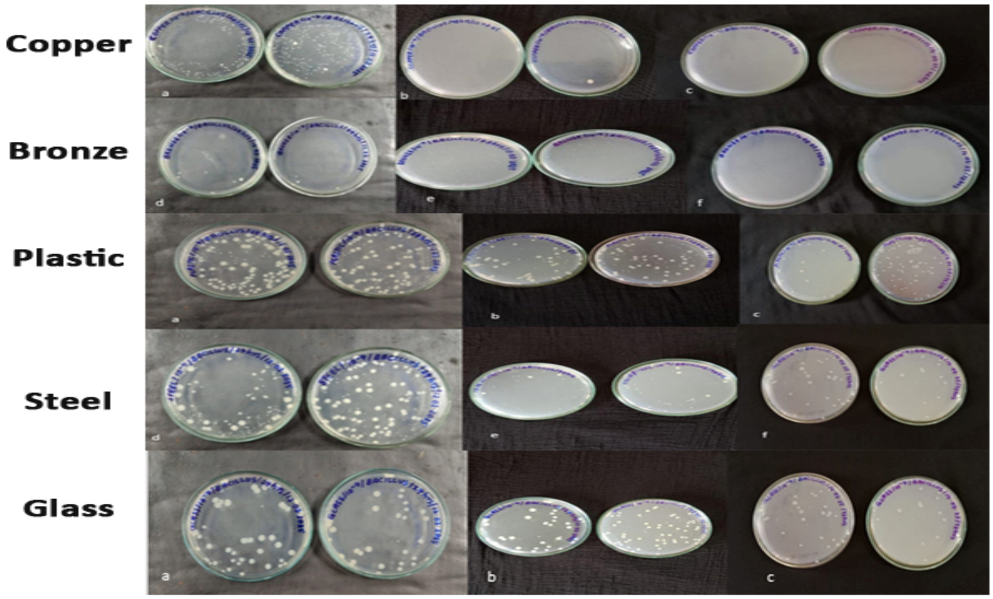

Fig. 6. Viability of *Bacillus sp.* in various vessels (a - 24 hrs, b - 48 hrs, c - 72 hrs)

IV. CONCLUSION

The present study provides a strong scientific basis for the antimicrobial superiority of traditional metallurgical vessels over modern storage materials. The results of comparative analysis of survival kinetics reveal that copper-based alloys, specifically bronze and copper, function as active antimicrobial systems rather than passive containers made of steel or glass or plastic.Traditional vessels demonstrated significant bactericidal activity, with bronze exhibiting particularly potent antimicrobial effects. Plastic and stainless-steel surfaces harboured high bacterial loads throughout the 72-hour observation period, which might be due to the chemical inertness of synthetic polymers and the lack of bioactive ion release in steel.

This research suggests that the adoption of traditional copper and bronze vessels could serve as a sustainable, eco-friendly, and cost-effective intervention for water sanitation. By utilising the intrinsic oligodynamic properties of these alloys, significant reductions in waterborne pathogen transmission can be achieved without needing chemical additives.

Future investigations should be done toelucidate the specific molecular mechanisms and oxidative stress pathways induced by bronze alloys. Furthermore, there is significant potential for translating this traditional knowledge into contemporary applications within the food processing, pharmaceutical packaging, and clinical surface engineering sectors to combat the rising challenge of surface-mediated microbial transmission.

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