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Original Article

In-vitro inactivation of *Escherichia coli* of surface water using metals

Sharmin Zaman Emon[1], Anowara Begum[2], Md. Latiful Bari[1] and K Siddique-e Rabbani[3]

[1]Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh, [2]Department of Microbiology, University of Dhaka, Dhaka, Bangladesh, [3]Department of Biomedical Physics and Technology, University of Dhaka, Dhaka, Bangladesh

Technology for providing clean drinking water to remote areas of low-resource nations remains a significant challenge for human life The study aimed to develop a simple technology for rural households that might be adopted to utilize the bactericidal properties of metals. Three thick metal sheets made of copper, zinc, and brass (an alloy of copper and zinc) were used in this study work. These metallic sheets were placed in three plastic (polythene) containers with base areas 11cm x 7cm each so that the metallic sheets covered the entire base areas of the respective containers. Fifty ml, one hundred ml, and four hundred ml of contaminated water from a public pond were added to each container, covered with lids, and shaken/left undisturbed at room temperature. The microbial analysis of Total Aerobic Bacteria (TAB), Total Coliform Bacteria (TCC), and *E. coli* was done every 24 hours up to twenty-eight days of storage at room temperature. *E. coli* is considered an indicator of diarrhoeal pathogens. The initial bacterial counts were TAB: 4.22 log CFU/ml, TCC: 3.15 log CFU/ml, and *E.coli*: 3.13 log CFU/ml, respectively. TAB count did not reduce significantly for any of the metals used in this study. Total coliform counts decreased to almost half the original for all three metals in the first 24 hours but remained almost the same afterward. However, *E.coli* was inactivated entirely after treatment with copper within 24h and remained constant afterward. On the other hand, brass and zinc reduced *E. coli* by almost half in the first 24 hours but remained almost constant throughout the rest of the measured period. The findings mentioned above, a simple copper sheet might help inactivate diarrheal bacteria and provide safe drinking water within 24 hours. As a result, this may lead to the development of an easy technique to provide clean drinking water in remote areas of low-resource nations like Bangladesh. It is crucial to determine whether the level of copper in the water is within the safe range, as regular usage of higher doses might result in copper poisoning. A future study will attempt to optimize the relationship between the water volume to the copper sheet's exposed surface area and the treatment time.

Keywords: **Copper, brass, zinc metal sheet, microbial count, bactericidal effect.**

Introduction:

Water is a necessary resource for survival and good health of human. Water scarcity is an issue everywhere, but it is particularly acute in developing nations where rural populations depend on water from ponds, rivers, and streams for domestic use. Around 1.2 billion people of the world's population, are thought to reside in regions with physical water shortages, and another 1.6 billion people, or nearly one-fourth of the population, have a clean water deficit. Microbiologically contaminated drinking water can transmit diseases such as diarrhoea, cholera, dysentery, typhoid and polio and is estimated to cause 485 000 diarrhoeal deaths each year (WHO, 2022). Pollution from the flow of sewage and industrial waste into rivers is the main factor contributing to the lack of clean water. Heavy metals and hazardous compounds are present because industrial discharge. Both humans and animals may have major health issues as a result of exposure to certain metals. The largest source of water-borne infections in humans caused by pathogenic bacteria, coliforms which act as indicators, due to the sewage discharge into ponds and rivers (Pandey *et al*., 2014). That can be found in the environment and in warm-blooded animals' and humans' feces (Jain *et al.*, 2005; Lin *et al, 2013*). *Escherichia coli* is the most prevalent bacteria that is found in significant quantities in the intestines of humans and animals (Mitsuoka ,2014). Recently, a lot of work has gone into figuring out how to make water potable by getting rid of harmful microorganisms. So, researchers have been looking into ways to develop quick and inexpensive water disinfection techniques for usage in areas where fresh water resources are nonexistent and the available water has high levels of microbes to get clean water (Noyce *et al.,* 2006; 2007). Such systems should be independent, built and maintained by local specialists, and powered without an external source of electricity. To offer protection against infections, they should be able to entirely eradicate microorganisms.

Historical evidence suggests that Greeks and Romans used copper daily, and currency minted from this metal was widely circulated. In India, copper is the first choice in religious rituals for storing holy water and other purposes like cooking (Nordqvist et al., 2012). There is no exaggeration in saying that all Indian households would possess at least one copper vessel or a glass.

***Corresponding author:**
Sharmin Zaman Emon, Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh.
Email: sharmin_micro@du.ac.bd, ksrabbani@gmail.com

Due to growing awareness of traditional cooking and eating methods, copper-bottomed utensils made a comeback into our kitchens in recent years. Interestingly, our bodies, too, possess a small amount of copper in the form of a trace mineral at around 1.4 to 2.1mg, and the deficiency of copper can lead to various chronic conditions (Ensoy *et al.,* 2015)

In addition, copper has significant antimicrobial properties, making it ideal for eliminating disease-causing bacteria, fungi, and viruses (Konieczny *et al.,* 2012). When water gets stored for up to 8 hours in a copper bottle or a vessel, it undergoes an oligodynamic effect, which means it gains the ability to kill several infection-causing microbes and fungi besides maintaining the natural pH levels (Parihar *et al.,* 2020).

Based on studies that certain copper alloys can kill 99.9% disease causing bacteria within just 2 hours of regular cleaning (Grass *et al.,* 2011). The United States Environmental Protection Agency (EPA) approved the usage of copper coated door knobs, bedrails, handrails, sinks, faucets, toilet hardware, etc in hospitals to arrest and reduce the transfer of contagious diseases like cold, cough, influenza and contamination (Michels *et al.,* 2008)

Besides killing highly resistant bacteria, fungus and virus copper water is a treasure trove of medicinal properties that can play a pivotal role in maintaining the overall health (Rosenberg et al., 2018). Some research works showed that, copper can prevent cancer, stimulates thyroid function, Boost hemoglobin count, regulates hypertension, fights infections, soothes joints and strengthens bones, maintains heart health, helps lose weight, improves brain function, heals wounds, delays aging and etc (Weaver *et al.,* 2008). Copper has also beneficial effect in environment like leaching, nuclear waste handling etc.

From this point of view, this research team inititated a research work using the metals like copper, zinc and their alloy brass.The germicidal function of metals, especially heavy metals, is thought to be a result of the oligodynamic effect, in which metal and metal compounds have the capacity to alter and ultimately kill off bacteria in a distinctive manner when introduced into the interior of bacterial cells (Sudhaa *et al.,* 2009). Among the metals, copper and silver are the most investigated metals for their oligodynamic effects. Silver ions denature proteins in Gram-positive and Gram-negative bacterial cells by attaching to reactive groups and rendering them inactive (Yamanaka *et al.,* 2005; Shrestha *et al.,* 2009). In disinfection systems that contain both copper and silver, copper ions permeate the cell wall, opening a pathway for silver ions to permeate into the microorganisms. The binding of the silver ions to the cell's proteins, respiratory enzymes, DNA, and RNA causes the cell's entire life support system to become immobilized (Lenntech, 2010).According to a recent study on copper surfaces showed that hydroxyl radicals in the solution are responsible for microbes fatal action (Oyanedel *et al,* 2008; Santo *et al.,* 2011.

This method is typically employed in large-scale, commercial water disinfection systems where metal ions are produced chemically or electrolytically, and it has been found to be extremely successful (Varkey, 2010). The fact that electrolytic systems require a power source in order to function is a drawback (Mehtar *et al.,* 2008). Chemical ion production is not preferred either since the by-products must be removed by an extra filter system. The current water issue in rural parts of developing nations may be resolved by a straightforward point-of-use (POU) water treatment system that makes advantage of metals' antibacterial properties (Lin *et al*., 2013). A significant disease-causing pathogen frequently found in contaminated water in rivers and ponds is the coliform bacterium, particularly the *E. coli* strain O157:H7. Processes used to disinfect water must get rid of these microbes (Gerba, 2019).

At first, the research work initiated with brass filing for destruction of *E.coli*, because brass is the alloy of copper and zinc metal. The copper in brass makes brass germicidal. Depending on type and concentration of pathogens present, brass kills microorganisms within a few minutes to hours of contact. Antimicrobial effect of copper and copper alloy on *E. coli*, *Mycobacterium tuberculosis*, methicillin resistant *Staphylococcus aureus*, influenza A virus, *Salmonella typhi* and *Vibrio cholera* have been reported in different research works (Sudhaa *et al.,* 2009; Dhanalakshmi *et al.,* 2013).

This study sought to determine the efficacy of three different metals to eliminate coliform bacteria especially *E.coli* by just submerging them for varying lengths of time in order to determine if the findings could provide any useful information to get the potable water in rural areas.

Methodology:

- **Sample collection:** The surface water samples were collected from Shahidullah Hall, Jagannath Hall and Bangla Academy pond water of Dhaka University area, as a preliminary study sites for this research work. The water samples were collected in pre-sterilized glass bottles or PET bottles from relatively fresh flow and from a depth of 4-6 cm. Standard procedures were followed for sampling (Clesceri, 1998). After collection, water samples were transported to the laboratory in an insulated foam box with ice to maintain a temperature ranging from 4-6 °C and microbiological tests and treatment process were performed immediately after sampling.
- **Use of Brass granules:** one **(**1.0g) of brass filing was poured into a test tube. Water sample of a measured volume (5/50/100 ml) strained using the cloth filter was added to the test tube and then shaken gently. Then the solution was left for 30 minutes. After that the treated water was separated from brass filing and microbiological analysis of treated water was done (Figure 1).
- **Use of brass, copper and zinc sheet/plates:** After that, the research work has been expanded to two constituents of brass: copper and zinc, and three sheets of these metals were used for the treatment (Figure 2). Brass, copper and zinc metallic sheets of 11cm×7cm sized were placed in three plastic

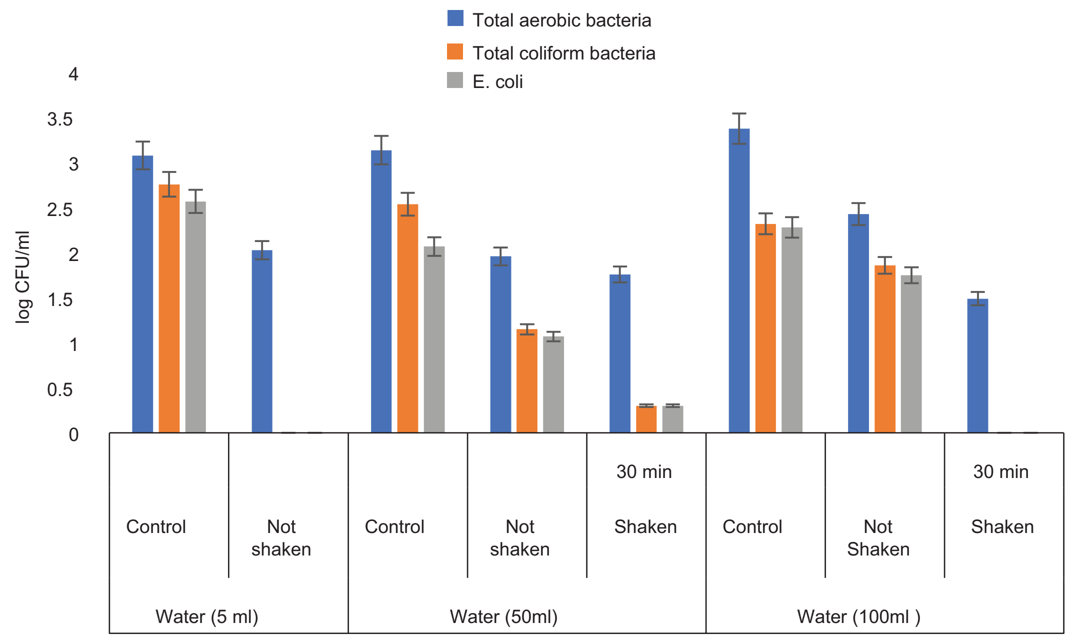

Fig. 1: *Average result of three pond water after treating with brass filings.*

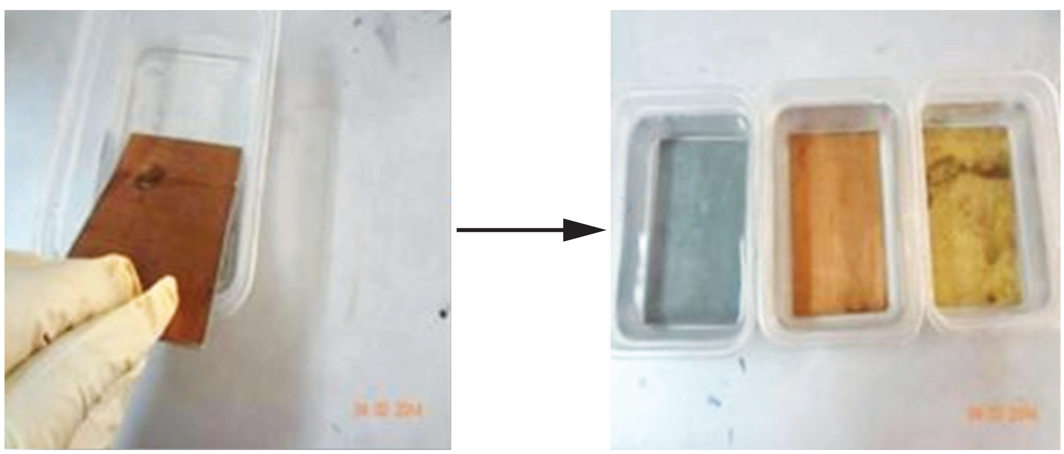

Fig. 2: *Experiment with brass, copper and zinc sheet/plates*

containers of fitting size. 50/100/400 ml of filtered water was added to each container. All the samples were shaken by hand, or using a shaker machine (50 rpm) later, continuously for 30 minutes after which the samples were subjected to microbiological analysis. In the comparative study of copper, brass and zinc in figure 2, for 50 ml of water, which only created a thin layer over the 11cm x 7 cm plates and shaken for 30 minutes. Then the containers were left for different period i.e. 24, 48 hours. Microbiological analysis of treated water was done in an attempt whether the water could be drinkable.

Effectiveness of copper inactivation in the long term

Second step experiments revealed that, copper was the best in inactivation of *E. coli*. So, next step was evaluation of the effectiveness of copper plate in inactivation of diarrhoeal pathogens in the long term. The methodology is copper plate of 11cm×7 cm were placed in two plastic containers of fitting size. 400ml of filtered water was added to each container. One container was left in a cool and dry place at room temperature with copper plate for 24 hours (microbiological study was done after 24 hours), while another one was used for periodic microbiological study of 1, 2, 4 and 24 hours of treatment with copper plates and the samples were stored at room temperature for 28 days.

- **Inoculation study**

  In addition, Tryptic soy agar (TSA; Oxoid) and Sorbitol MacConkey (SMAC) agar supplemented with 50 µg/ml rifampicin was used as non-selective and selective medium, respectively, for the recovery of *E. coli* and their presence in storage study for 28 days.

- **Statistical analysis**

  All trials were replicated three times. Reported plate counts determined on all agar media were converted to Log CFU/ml and numbers represent the mean values obtained from three individual trials, with each of these values being obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA.). Significant differences in plate count data were established by the least-significant difference at the 5% level of significance.

Results and Discussion

Brass filling granules in 5ml, 50 ml and 100 ml of water shows that, for 1 g brass granules in 5 ml water without shaking, total coliform and *E. coli* counts were inactivated after 30 minutes of treatment. However, with 50 ml and 100ml of water, inactivation was only possible with shaking for 30 minutes. The samples without shaking showed significant reduction in counts but cannot be considered safe for drinking from a microbiological point of view (Figure 1).

In the comparative study of copper, brass and zinc in figure 2, for 50 ml of water, which only created a thin layer over the 11cm x 7 cm plates and shaken for 30 minutes. All the three metals used (50 ml water) in this study were seen equally effective in reducing *E. coli* population to below detectable range. However, the brass was found reduced the coliform counts to non-detectable level, but larger water volume (100ml) was found not effective under similar experimental conditions. The brass and zinc used in 100 ml study was not found effective in reducing coliform bacteria, in contrast, copper showed higher effectivity in reducing *E. coli*. In case of 400 ml, in which the water was left undisturbed in the boxes with the respective metallic plates at the bottom for periods of 48 hours. Small samples of water were collected for microbiological analysis at 24 and 48 hours (figure 3). Copper appears to be effective in inactivating *E. coli* successfully while brass and zinc could not, although the counts decreased for all, compared to the control water samples.

Comparing the result of copper, brass and zinc metallic sheets, it seems that, copper was the best in killing the indicator bacteria, *E. coli*. So, the research work has been proceeding to disinfection of pond water using copper plates and storage of treated water samples. Pond was used for periodic microbiological study of 4 and 24 hours of treatment, and copper plate was removed after 4 hours of treatment. Treated water was stored at room temperature (26-28 °C) without the copper plate for 28 days and periodic count was performed with 7 days intervals. In this experiment, all the resident bacteria along with coliform and *E. coli* were found to increase after storage at room temperature (figure 4).

Spiked/ inoculation study was done whether copper plate has the disinfection effect on rifampicin resistant *E. coli* (figure 5), here, rifampicin was used due to its broad spectrum activity on both intracellular and extracellular activity (Brunton et al., 2018). Like environmental samples, marker bacteria also resuscitated on storage. Inoculation study also resembles with the environmental study (figure 3). Explanation of this result is that, copper injured the bacteria for some period and at that time bacteria were in non

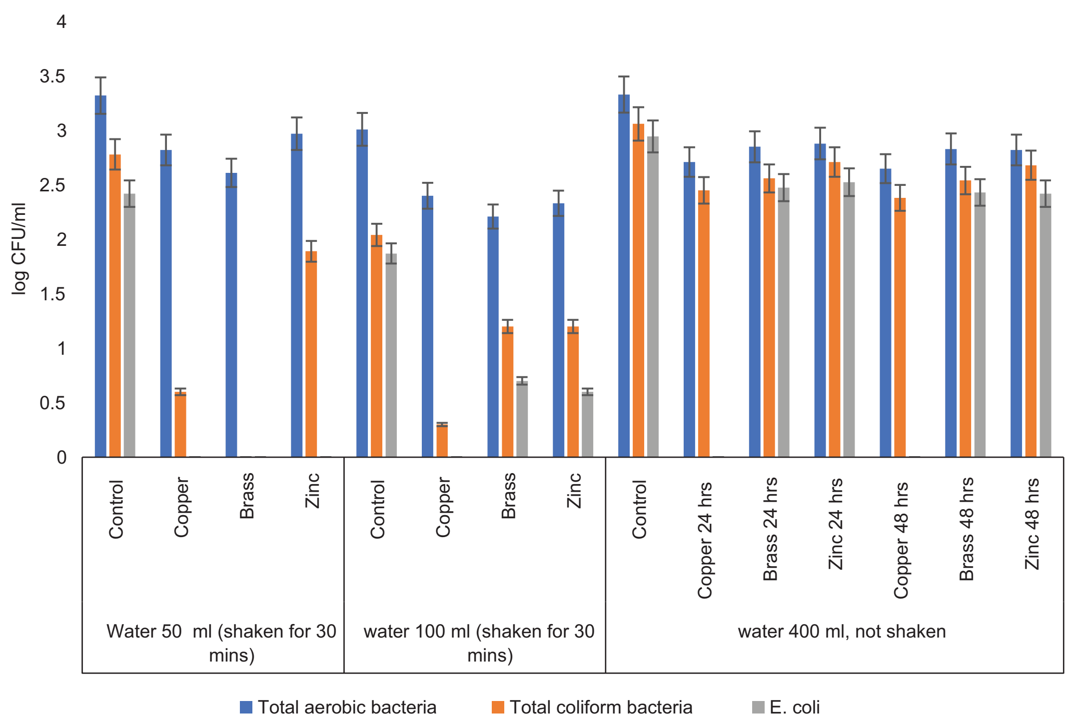

Fig. 3: *Average result of three pond water after treating with copper, brass and zinc metallic sheets in different experiment condition*

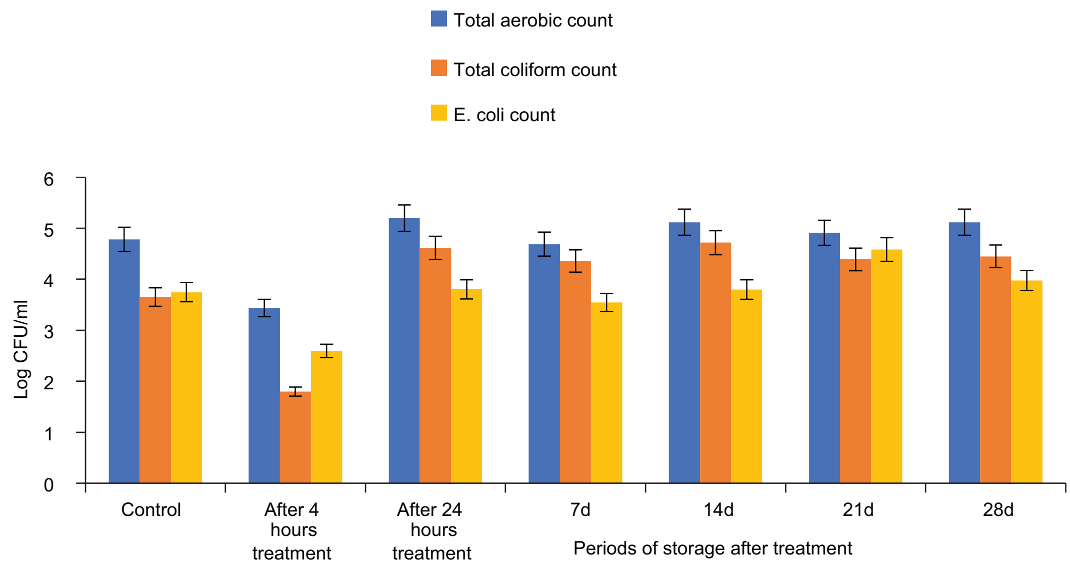

Fig. 4: *Effect of copper plate on total aerobic, total coliform, E. coli count on pond water*

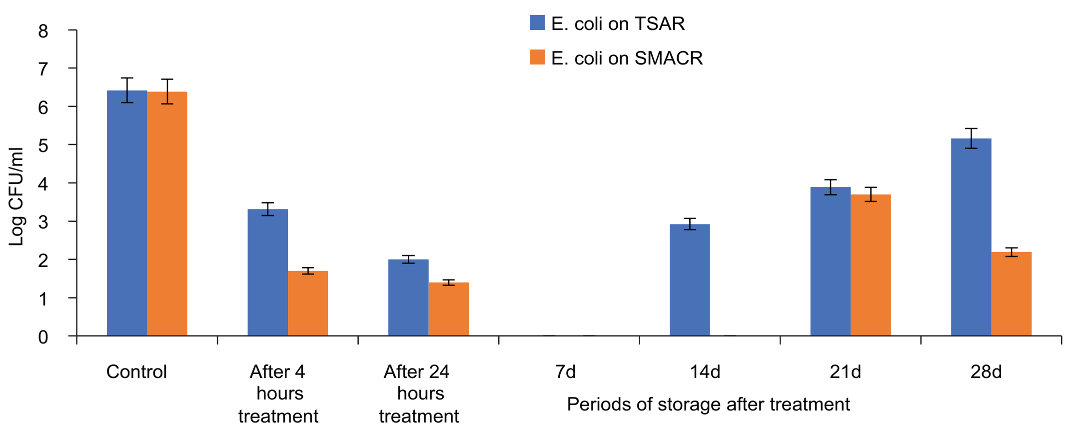

Fig. 5: *Effect of copper plates on rifampicin resistant E. coli count (TSAR and SMACR) of pond water.*

culturable stage but during the storage, they recovered and came to culturable state.

Conclusion

The above study suggests that coliform and *E. coli* were possibly injured and therefore inactivated temporarily due to the effect of copper, but resuscitation was observed after storage. This suggests that copper does not destroy bacteria, rather it inactivates them. However, this study was not performed for brass and zinc and therefore, nothing may be said about the effect of these metals at this stage. Several studies have suggested that, after incubation of water with copper, concentration of copper in water was increased over the permissible limit of World Health Organization (2.0 mg/l). Higher concentration of copper has been reported to cause diarrhoeal diseases, nausea and other symptoms (Dhanalakshmi *et al*., 2013; Pizarro *et al.,* 1999). So; further studies will be needed to find out the relationship of the volume of water sample exposed to metal sheet surface and experiment time duration to get a potable water to the rural areas people.

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References

1. Brunton, L. L., Lazo, J. S., & Parker, K. L. (2018). The pharmacological basis of therapeutics. *Goodman, Gilmans, editors, 18 Edition.*. https://accessmedicine. mhmedical.com/ book.aspx? bookID=2189 (Accessed on June 2022).
2. Clesceri, L. S. (1998). Standard methods for examination of water and wastewater. *American Public Health Association*, *9*.
3. Dhanalakshmi. T., and Rajendran, S. (2013). Antimicrobial Activity of Micro Sized Copper Particles On Water Borne Bacterial Pathogens. *International Journal of Scientific and Technology Research*, 2(1), 115-118.

4. Ensoy, C., Rakhmawati, T. W., Faes, C., & Aerts, M. (2015). Final evidence report as part of preparatory work for the setting of Dietary Reference Values for magnesium, copper and phosphorus. *EFSA Supporting Publications*, *12*(10), 691E.

5. Gerba, C. P., & Pepper, I. L. (2019). Microbial contaminants. *Environmental and pollution science*, 191-217.

6. Grass, G., Rensing, C., & Solioz, M. (2011). Metallic copper as an antimicrobial surface. *Applied and environmental microbiology*, *77*(5), 1541-1547.

7. Jain, P., and Pradeep, T. (2005). Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnology and Bioengineering*, 90(1), 59–63.

8. Konieczny, J., and Rdzawski, Z. (2012). Antibacterial properties of copper and its alloys. *World Academy of Materials and Manufacturing Engineering*, 56(2), 53-60.

9. Lenntech (2010). http://www.lenntech.com/processes/disinfection/chemical/disinfectants-copper-silverionization.htm.

10. Lin, S., Huang, R., Cheng, Y., Liu, J., Lau, B. L. T., and Wiesner, M. R. (2013). Silver nanoparticle-alginate composite beads for point-of-use drinking water disinfection. *Water Research*, 47, 3959–3965.

11. Mehtar, S., Wild, I., and Todorov, S. D. (2008). The antimicrobial activity of copper alloys against nosocomial pathogens and *Mycobacterium tuberculosis* isolated from healthcare facilities in the Western Cape: an in-vitro study. *Journal of Hospital Infection*, 68, 45-51.

12. Michels, H., Moran, W., & Michel, J. (2008). Antimicrobial properties of copper alloy surfaces, with a focus on hospital-acquired infections. *International Journal of Metalcasting*, *2*(3), 47-56.

13. Mitsuoka, T. (2014). Establishment of intestinal bacteriology. *Bioscience of microbiota, food and health*.

14. Nordqvist, K., Herva, V. P., Ikäheimo, J., & Lahelma, A. (2012). Early copper use in Neolithic north-eastern Europe: an overview. *Estonian Journal of Archaeology*, *16*(1), 3.

15. Noyce, J. O., Michels, H., and Keevil, C. W. (2006). Potential use of copper surfaces to reduce survival of methicillin resistant *Staphylococcus aureus* in the healthcare environment. *Journal of Hospital Infection*, 63, 289-297.

16. Noyce, J .O., Michels, H., and Keevil, C. W. (2007). Inactivation of influenza A virus on copper versus stainless steel surfaces. *Applied and Environmental Microbiology*, 73, 2748-2750

17. Oyanedel, C., and Smith, V. J. (2008). Sustainable colloidal-silver impregnated ceramic filter for point of-use water treatment. *Environmental Science and Technology*, 42(3), 927–933.

18. Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *Amb Express*, *4*(1), 1-16.

19. Parihar, K., Sankhla, M. S., Kumar, R., & Singh, A. (2020). Assessment of copper and iron concentration in water of Yamuna River, Delhi, India. *Letters in Applied NanoBioScience*, *10*, 2251-2257.

20. Rosenberg, M., Vija, H., Kahru, A., Keevil, C. W., & Ivask, A. (2018). Rapid in situ assessment of Cu-ion mediated effects and antibacterial efficacy of copper surfaces. *Scientific reports*, *8*(1), 1-12.

21. Santo, C. E., Lam, E. W., Elowsky, C. G., Quaranta, D., Domaille, D. W., Chang, C. J., & Grass, G. (2011). Bacterial killing by dry metallic copper surfaces. *Applied and environmental microbiology*, *77*(3), 794-802.

22. Shrestha, R., Joshi, D. R., Gopali, J., & Piya, S. (2009). Oligodynamic action of silver, copper and brass on enteric bacteria isolated from water of Kathmandu Valley. *Nepal Journal of Science and Technology*, *10*, 189-193.

23. Sudhaa, V. B. P., Singha, K. O., Prasad, S. R., and Venkatasubramaniana, P. (2009). Killing of enteric bacteria in drinking water, by a copper device for use in the home: laboratory evidence. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103, 819-822.

24. Varkey, A. J. (2010). Antibacterial properties of some metals and alloys in combating coliforms in contaminated water. *Scientific Research and Essays*, *5*(24), 3834-3839.

25. Weaver, L., Michels, H. T., & Keevil, C. W. (2008). Survival of Clostridium difficile on copper and steel: futuristic options for hospital hygiene. *Journal of Hospital Infection*, *68*(2), 145-151.

26. Yamanaka, M., Hara, K., & Kudo, J. (2005). Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Applied and environmental microbiology*, *71*(11), 7589-7593.

27. WHO, 2022. https://www.who.int/news-room/fact-sheets/detail/drinking-water.