Antimicrobial Activity of Micro Sized Copper Particles On Water Borne Bacterial Pathogens

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ABSTRACT:- Water is the source and basis of all living things. The contamination of water is the major concern in the developing countries. In conventional wastewater treatment systems, chlorine have been used to disinfect the final effluent as it has several detrimental effects, replacement of chlorine is obviously necessary. Traditionally copper vessels are being used to treat and preserve the drinking water for house hold purposes. Therefore In this investigation attempts were made to study the possibility of employing copper micro particles to disinfect various water borne bacterial species. The result revealed that copper micro particles showed maximum inhibitory effect on E.*coli, Salmonella* and minimum on *Shigella* at the incubation time of 6hrs and the above. This finding indicated that copper containing antimicrobial properties and it can be used in the treatment of water borne pathogens.

Key note: waste water, copper, selective media like EMB agar, S.S.agar and MPN broth.

1 INTRODUCTION

Water is the vital component of all living organisms. However, various microorganisms present in water can cause diseases in humans, leading to infectious, toxigenic and parasitic processes (1). The water is the primary source to cause the diseases like diarrhea, typhoid and various diseases to the human being. According to the World Health Organization, an estimated 4.1% of the total global burden of disease is contributed by diarrhea illness: around 88% of that burden is due to unsafe water supply, sanitation and hygiene, with children in developing countries being the most common victims(2). Providing safe drinking-water to the majority of the world's population, especially to those in developing countries, is still a major problem. Approximately a billion people lack access to safe drinking water (3). The major aetiological agents that account for over a million diarrhoeal deaths per year, particularly in developing countries, are enterotoxigenic *Escherichia coli* (ETEC), rotavirus, *Vibrio cholera*, and species of *Shigella*, which are spread through contaminated water and food or from person to person (4). In India, many states still have outbreaks of cholera. During 1996-2007, at least 222,038 individuals were affected by cholera (5). The chlorine, fluorine and other chemicals has been used in the water treatment. But it causes some side effect to the human kind. So instead of that we can use copper to treat the waste water. The ancient Ayurveda recommend the use of copper metal for water purification (6) and, traditionally, Indian homes stored drinking water in copper and silver pots. Now–a-day this practice has been replaced by the use of steel and plastic containers, as copper and silver have become expensive.

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Copper can help preserve the purity of drinking water. The confirmed antimicrobial effects of copper can inhibit water-borne microorganisms, such as viruses, bacteria, infectious parasites or algae. These microorganisms can create a variety of health risks to humans, including Legionnaire's Disease, deadly *E. coli* infections (7). The antimicrobial effect of copper and copper alloys on pathogens such as *Escherichia coli*,(8,9) *Mycobacterium tuberculosis*,(10) methicillin-resistant *Staphylococcus aureus* (MRSA),(11) influenza A virus(12)and *Salmonella typhi* and *Vibrio cholera(13)* has been reported. From this study, we report the micro copper particles used to incubate with waste water which contains all type of water borne pathogens. The micro copper particles had more active against the *E.coli*, *Salmonella* and *Shigella* at the incubation time intervals of 6hrs and above. The result shows that copper has antimicrobial activity and that can be used to treat the waste water treatment and to be used to design the advance water purifier.

2 Materials and methods

2.1 Collection of Sample

The fresh pond water samples were collected in the one liter plastic container from Valankulam pond at Coimbatore town, Tamil Nadu, India, the month of February and March. We have chosen this water though the pond was fully contaminated with mankind action and natural calamity.

2.2 Preparation of Copper particles

The copper rod was made it into the micro copper particles in the size range from 20µm-100µm by using the ball milling method in Bharathiar University at Coimbatore, Tamil nadu, India. The copper particles choose in the rage of 50 µm-100 µm, cleaned and washed with sterile distilled water.

2.3 Microbiological analysis

2.3a Enumeration of bacterial growth

The copper particles was taken in ratio of 5gm: 1000ml of waste water and incubated for 2hrs, 4hrs, 6hrs, 8hrs and 10hrs time intervals at room temperature (27±2 ◦C). Duplicates were maintained each time. Aliquots amount of water were withdrawn after the incubation period of each 2hrs time intervals as well as before incubation with copper. The bacterial count was determined by plating on EMB agar

for *E. coli,* S.S agar for *S. typhi* and *Shigella* (HiMedia Laboratories Pvt. Ltd. Mumbai, India). The serial dilutions were carried out by plating on nutrient agar for accurate enumeration of bacteria. The coliform also determine by using the MPN test by using the standard method.

2.4 Detection of pH and copper content in the water

The pH and copper content of the copper and waste water mixture were analyzed before and after incubation using a pH meter (DI 707; Digisun Electronics, Hyderabad, India) and the colorimetric quantification of copper using the kit relies on the royal blue colour complex [Cu (cuprizone)2 (NH3)2]2+ that is produced on the addition of the reagents provided in the kit by using the procedures of the Bureau of Indian Standards(14).

3 Result and Discussion:

The collected pond water samples were tested before and after incubating with the copper particle at 2 hrs time intervals on the nutrient plate for measure the bacterial count as the colony forming unit. [Fig.1] The result was no bacterial colonies at after 10 hrs incubation time interval and no. of colonies also decreased when incubation time increased. The withdrawn water samples at the same time intervals and seen the coliforms by the MPN test in the lactose broth [Fig. 2]. The same withdrawn water sample were tested for *E.coli* on Eosin Methylene Blue Agar and for *Salmonella, Shigella* on S.S. Agar that results were shows in the fig. 3 and 4 respectively. The result found that there was no growth on EMB agar even at 6 hrs incubation time interval and low level of growth at the other 2hrs, 4hrs time intervals. On the S.S agar there was no growth of *salmonella sp.* at starting time intervals of 2hrs to 10 hrs but which contains the growth of *shigella* at less no. of colonies[Fig.3&4]. The pH and levels of copper in the tested water samples were within the permissible limits set by WHO [pH<8.5 and 1.8 mg/l of copper] the result shown in table 1 (15).

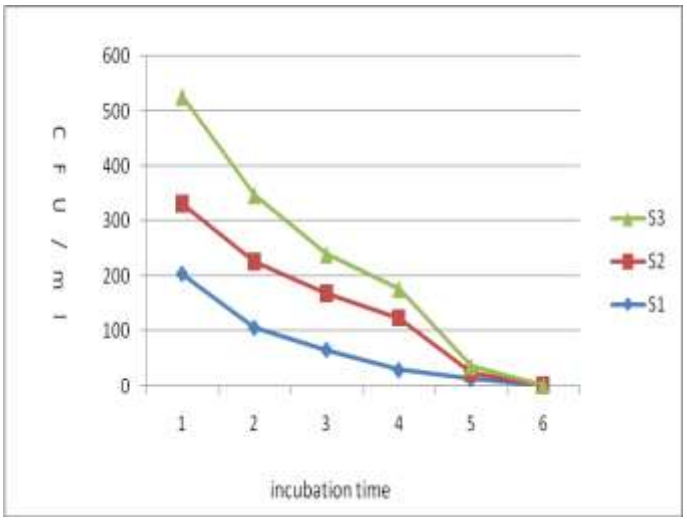

Fig. 1. Effect of incubated pond waste water inoculated with micro copper particles and before treatment of colony forming unit on nutrient plate CFU/ml

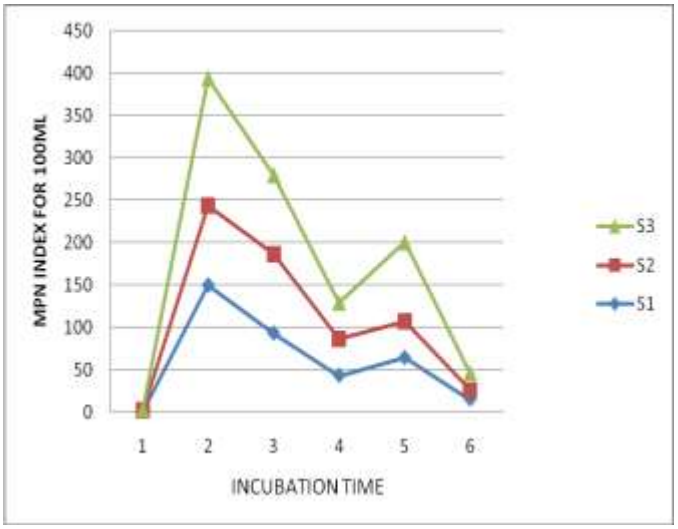

Fig. 2. Shows that result of MPN index per 100ml.

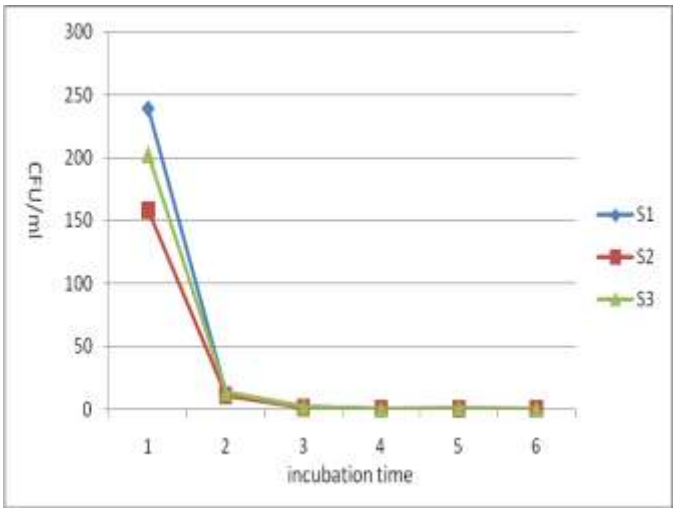

Fig. 3. Shows that the average CFU/ml of the triplet EMP agar plate.

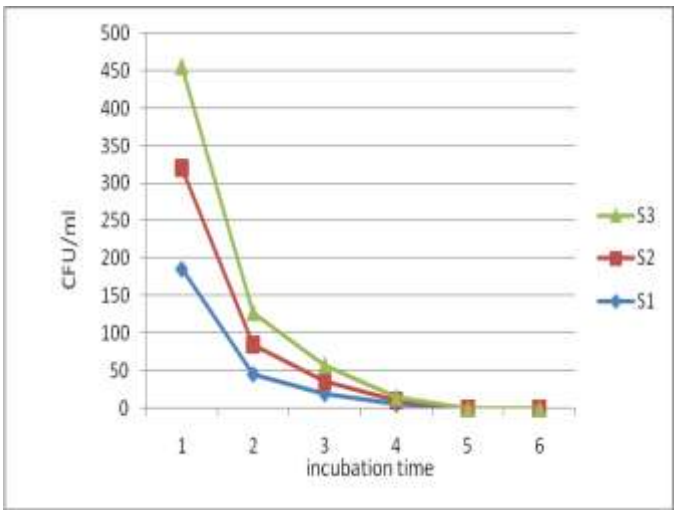

Fig. 4 shows that the average CFU/ml of triplet S.S agar plate: only observed the transparent colony of *Shigella.*

The table 1 shows that PH and copper contents of water before and after incubation with copper particle

Parameters	Permissible limit BIS/WHO	Before incubation	After incubation
pH	8.5-9.0	7.92±0.8	7.82±0.7
Copper content(mg/l)	2	1.2±0.5	2.4±0.2

The major public health problems in many developing countries are the enteric fever and cholera by the global initiatives in water and sanitation [16,17], prior most due to lack of adequate sanitation facilities and safe drinking water, alongside issues related to personal hygiene [18]. The contamination occurs during the transport and /or storage at the household level [19]. In the experiments described here we were not recover bacteria such as E. *coli, S. typhi* and *shigella*. The same results were found by other scientists also [12, 20]. As we known an ancient time we have used the copper pot for storage of drinking water and many experiment proved the copper has antimicrobial activity mainly on the water borne pathogens [12.13, 20] but in this study we have chosen the micro copper particle for water treatment process and seen the copper micro particle also have more active on water borne pathogens. Many studies they have been found that the copper devises such as copper coil copper rod and copper pot used to treat the waste water [6, 12]. Another study was reported that none of the test pathogens was recovered from drinking-water stored in copper pots even after enrichment culture [21]. The permissible limits of the leached copper 2 mg Cu/L is safe that shown in the current WHO guideline [22, 23], and the levels leached in the study were 2.4±0.2 of the permissible limits somewhat and increased only the 0.4±0.2 levels. We observed that the no growth of E.*coli*, *salmonella typhi* and limited growth of shigella in the water treated with copper. Since the result shown that the copper more actively act on the water borne pathogens and the same result were reported by many experiments [12, 20, 21, 22, and 24]. The studies concluded that the copper ions brought about complete killing of bacteria by membrane damage [24].But still we do not get clear mechanism of action of copper on bacteria. In the present study the micro copper particles are used to treat the waste water and we found that the copper micro particles are active against the water borne pathogens. Ancient time the use of copper pots in Indian households is common and is, therefore, likely to be socially accepted by the people. Its functioning is not requiring the fuel, electricity, replaceable filters, intensity of sunlight, etc. to operate or maintain it; it is simply a passive storage of water. This takes into account the conditions prevailing in rural villages and the urban slums of developing countries. The health benefit that can be achieved by using copper pot as a PoU water-purification device will far outweigh the cost of the pot, if divided over the members in a rural family, especially as it will be a one-time investment with no recurring costs. However, it is important to challenge its use under real-life conditions in the dynamics of the target households in developing countries to fully understand the limitations [21]. So the present study found that the instead of using the copper pot, rod and other devises can be use the micro copper particles also have the effect on water borne pathogens.

4 Conclusions

Water disinfectant is very much essential to reduce/or kill the water borne pathogens. Mainly in the developing countries such as India, as we are using the copper pot traditionally to store the drinking water and which is more active against the water borne pathogens. This present investigation also proved that the copper is the most active disinfectant on the water borne pathogens and we can be used to treat the waste water and this can be applicable in the water purifier system.

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