

Designing an Efficient Decentralized Water Purification/Treatment System to Treat Municipal Supply Water

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ABSTRACT

The goal is to develop a Decentralized Household Water Treatment and Storage System which provides purer, safer, accessible and affordable drinking water without removing essential minerals from water. In order to achieve awareness of sanitation and hygiene and required modifications in the lifestyle of the communities required. This should be achieved with low costs, ease of use, sustainability, low maintenance and independence of services (energy sources) or dependent on renewable sources – Solar energy as boundary conditions. Water treatment involves two steps: water purification and water disinfection. The objective of this study is to treat the municipal water supply or decentralized water supply. The study is aimed at evaluating the public preferences and expectations in the household water treatment and storage systems for drinking water and quality of community tap water through survey and sampling. The research looks at potential for eliminating microbial contamination due contaminated sources as well as recontamination possibility due to lifestyle. *Escherichia coli* and *Salmonella* are amongst the more prevalent bacterial pathogens that cause water and food borne diseases. This study is aimed to evaluate the antibacterial activity of metallic copper surfaces on these important enteropathogens. The antibacterial activity of copper surfaces was evaluated overlying them with suspensions of 20-2000CFU/100ml of *Escherichia coli* and *Salmonella*. Bacterial counts obtained after 0, 15, 30, 45, 60, 75, 90, 105 and 120mins at room temperature with copper strips were compared with those obtained in copper vessel. The results showed that when these enteropathogens were kept in contact with copper a significant antibacterial activity was noted, on the contrary when the same load of pathogen suspensions were tested over the control surfaces it was found that the bacterial counts remained unchanged or even increased with time. These results shows that metallic copper surfaces have an antibacterial activity against *Escherichia coli* and *Salmonella* and suggest its potential application as an disinfection agent in household water treatment system for safe drinking water. The target is to design a sustainable system for the removal of microbial contamination and anthropogenic source heavy metal contamination. It was concluded from the household survey that the two most important factors impacting the sustainability of the treatment system are operation and maintenance and initial cost of the system. The cost and maintenance of the system were found to be major concerns of the households. Hence, the approach for the designing of the treatment system is based on the sustainability. The treatment system shall also target to minimize the microbial recontamination due to suitable purified water storage system. The household treatment units designed with such an approach that can provide safe drinking water to small and mid-size communities like urban slums, offices, schools, apartment complexes etc. The positives and negatives of each existing HWTS device have to be kept in mind before putting together the concept. The device will sanitize water as per the Bureau of Indian Standards to the definition as safe drinking water.

1. INTRODUCTION

Global assessments show that a large proportion of the world's population does not have access to microbiologically safe sources of drinking water (WHO/UNICEF, 2011). More than 2.2 million people die every year from diseases resulting from unsanitary water conditions (Corcoran et al., 2010). Microbial quality is not only one of the parameters of safe drinking-water; it is a major problem of concern and is cause of epidemics. Disinfection plays an important role in eliminating majority of germs maintaining public health and controlling disease (Dvorak et al., 2005).

Agents particularly *Escherichia coli*, *Salmonella* species, *Vibrio cholerae* and species of *Shigella*, are one of the biggest public health risks in the developing countries (Preethi Sudha et al., 2012; McKenzie and Ray, 2007; CDC). Among them, *E. coli* and *Salmonella* lives in the digestive tract of all humans and other warm blooded animals. Its infection occurs by consumption of drinking water or food sources contaminated with the feces, or stool, of humans or animals. Health problems related to them may weaken resistance to other infections (Zhu et al., 2010). Life-threatening complications may also develop if the infection is higher.

In India, investments in community water supply and sanitation projects have increased steadily from the 1st plan to the 10th plan. However, the health benefits in terms of reduction in waterborne disease have not been commensurate (Gupta, 2012). Increase in the water demand, unwanted political interference and improper maintenance by the local authorities has intensified the deterioration of the public water supply systems (Engel et al., 2011). Due to this neglect, the quality of water flowing through the distribution network is rarely monitored for microbial contamination. Recontamination of already treated water in the water distribution system is a common problem. Lack of residual chlorination, sewage pipe networks running next to each other and other contamination source like permeation, leaching or microbiological growth inside the water pipes are the common reasons. Several investigators have isolated encapsulated bacteria from chlorinated water (Berger et al., 1992). Hence, there is an increased demand for decentralized household drinking water purification systems to eradicate these microorganisms.

Many different disinfection technologies have been developed to get microbiologically safe water for drinking. Commonly employed disinfection methods include boiling, addition of oxidizing agent, exposure to ultraviolet radiation (UV), ozonation and recent use of silver nanoparticles in terms of their effectiveness against various microorganisms (National Drinking Water Clearing House Fact Sheet, 1996). These are expensive, and beyond the reach of common people with high operation and maintenance. The centralized drinking water treatment involves addition of strong oxidizing agent like chlorine (World Chlorine Council, 2008; Ahmad et al., 2012:132-137). This disinfectant is effective, however chlorine itself is relatively harmless but the disinfection byproducts (DBPs) like Trihalomethanes (THMs), Volatile Organic Compounds (VOCs) and Haloacetic Acid (HAAs) produced from addition of chlorine are potentially harmful to health (Clark and Boutin, 2001). Therefore great efforts are being devoted to the development of novel and effective disinfecting agents.

Sensitivity and susceptibility of microflora to some heavy metals has been known since antiquity and is in the order of $Ag > Cu > Ni > Ba > Cr > Hg > Zn > Na > Cd$ (Otitolaju and Don Pedro, 2002; Top and Ulku, 2004). In the mid 1800's, the cholera epidemic did not affect copper workers in France which shows preventive role of copper. In contrast to the low sensitivity of human tissue to copper, microorganisms are extremely susceptible to copper. The bacterial inhibitor effectiveness of copper is used in paints to render surfaces self-disinfecting and in various stages of food processing, in textile fabrics to reduce the spread of microorganisms and germs in hospital personnel thus, trimming down the chances of infection (Santo et al, 2011). Clinical uses include copper-8-quinolinolate as a fungicide against *Aspergillus* spp. (Rutala et al, 2008) and copper-silver ionization for *Legionella* disinfection (Lin et al., 1996). Cupric chloride inactivated 9 of the 13 bacteria strains that they tested by more than 5 logs within 30 minutes (Borkow and Gabbay, 2005). Also, the colonization of such harmful microbes has been shown to be 2.1 times greater with glass and 3.0 times greater with stainless steel (Health benefits of copper). It was found that water distribution systems made of copper have a greater potential for suppressing biofilm growth and for decreasing persistence of microflora in potable water than distribution systems constructed of plastic materials or galvanized steel. The 'copperized' water (Tamra Jal) also adds health benefits by treating stomach, digestive system, prevents us from gastrointestinal disorders, general cardiovascular health problems, autoimmune diseases, arthritis, Alzheimer's disease, diabetes and many other diseases (Store Drinking Water in Copper Utensils to be Healthy Naturally). Along with iron, it helps in forming the red blood cells. It prevents from ageing as it stimulates the production of collagen.

Although research studies have been done on antibacterial properties of copper, there is still effort required to use this disinfection effect in real time application in household drinking water purification systems. The exact mechanism of copper disinfection is not absolutely sure. In this study our first objective was to conduct a household survey and sampling to analyze bacteriological quality of water at the point of supply and consumer's preferences and expectations in household water treatment and storage systems for drinking water. Our second objective was to evaluate the effect of metallic copper strips and copper vessel on *E. coli* and *Salmonella* microbiologically-contaminated water in 120 minutes. By keeping fresh water in a copper vessel or with copper strip the water becomes infused with copper ions. The use of biocidal copper surface is low cost, low tech, easy maintenance and environmentally compatible technology. Since distilled water is slightly acidic (pH 6.7 $\pm$ 0.05) which might enhance copper leaching. Also the effect of copper metal in regular tap water (pH 6.5-8.5) is

demonstrated against both the important indicator bacterial strains. Moreover, the use of copper in household water purification would address health and sanitation which are of great concern. Implementation of its residual antimicrobial activity will have a major effect on the lives of the individual.

2. MATERIAL AND METHOD

Survey and sampling descriptive statistics

An opinion survey was conducted to comprehend community water quality and need for purification of drinking water at household level. The study area was classified to understand consumer preferences for household water treatment products in Nagpur City, India

- a. Low Income Areas - Mata Dhanteshwari Nagar Slums, Chuna bhatti Slums,
- b. Middle Income Areas - Ujawal Nagar

Local water reservoir managers were contacted to know supply timings in the study area since supply is intermittent. Mata Dhanteshwari area received the supply water from 2pm – 7pm, chunna bhatti expected from 6pm -11.30pm. Ujawal Nagar area received the supply water from 1pm –3pm. Other times people drew water from well and hand pump. At the supply level municipal water delivery points were classified in three categories: 1) Household Tap - if the tap was located above ground and on the premises of the house, 2) Well and 3) Public Stand Post-if the tap was constructed in a public location by the Board for communal use. Household selection was random. Generally, a woman was preferred to respond to a survey, as it was likely that she would be most familiar with the water collection and handling practices of the home.

Sampling was done directly from supply points in pre-sterilized 125ml polypropylene bottles. At supply points the water was allowed to run for at least 1 minute, tested for residual chlorine and then collected. The residual chlorine was measured using the visual color comparison method, by adding one drop of N, N-diethyl-paraphenylene-diamine sulfate (LTek) to a 5 ml water sample and comparing to using chloroscope. Samples were shielded from light and refrigerated. Samples were tested within one hour of reaching the laboratory and the analysis was completed within 24 hours. Thermotolerant coliform counts were determined by membrane filtration technique (MFT), with incubation at 37°C for 24 hours on selective m-FC agar plates (HIMEDIA, Mumbai).

Disinfection Studies with Metallic Copper

1. Bacterial strains

An isolate of *E.coli* strain MTCC 443 (ATCC 25922) and *Salmonella* strain were selected as test microorganism and identified by IMViC test. The culture media used for the test were Tryptone broth (HIMEDIA, Mumbai) for Indole Test, VP-MR broth (HIMEDIA, Mumbai) for Methyl Red and Voges-Proskauer Test and Citrate agar slants (HIMEDIA, Mumbai) for Citrate Test.

2. Preparation of Bacterial culture

Cultures were streaked onto selective media, EMB agar medium for *E. coli* species, Bismuth Sulphite (BS) agar medium (HIMEDIA, Mumbai) for *Salmonella* species, and were incubated at 37°C for 24-48 hours in bacteriological incubator (Bio-Technics India). After incubation, an isolated colony was picked and inoculated from each culture plate into Nutrient Broth (HIMEDIA, Mumbai) and incubated for 24 hours in bacteriological incubator at 37°C. This overnight culture was serially diluted in normal saline (NaCl, 0.85%) for inoculation. Erlenmeyer flasks (Fisher brand) of 250ml capacity were used for all batch experiments to prevent the loss of Cu²⁺ ions caused by the adsorption onto the walls of the container. They were thoroughly cleaned and containing 100ml normal saline (NaCl, 0.85%) were autoclaved (steam sterilized) each time before use.

3. Experimentation

Each culture flask containing 100ml sterilized saline solution was inoculated with 1ml of the freshly diluted bacterial suspension in order to maintain required initial bacterial concentration measured in colony-forming units (CFU)/ml. The same was enumerated by MFT on EMB agar and BS agar medium. Initial bacterial concentration was determined by plate count of the sample withdrawn at zero minute. Copper strips with 99% purity and dimensions of 4.5cm x 1 (± 0.2) cm (surface area of 4.5cm²) and weight 1.0-1.35grams were used. The strips were wiped with ethanol to remove organic matter from surface if any and air-dried. They were immediately immersed one in each experimental solution. Control experiments for both normal saline solution and culture were performed.

The culture flasks were placed at constant shaking at 55rpm in orbital shaker (Bio-Technics India) to maintain sufficient mixing along with the control for 120mins. Temperature was controlled at 27-28°C (room temperature). To find the trend of copper leaching with time, the measurements were performed at the end of every 15mins. 25ml of sample was withdrawn and plated on respective medium for the enumeration of bacteria by MFT. The sample was filtered through 0.45µm membrane filter (Millipore CAT No. HAWG04700) to separate the dissolved (free) copper ions from those attached to the cell wall. It was then collected in a polyethylene bottle for measurement of free active copper ions in the solution. Concentration of copper ions dissolved was estimated in ICP-OES (Thermo Fisher, iCAP 6300 DUO), with detection limit for Cu at 324.75 nm. The experiments also included a positive control (flask containing copper strip and saline solution, devoid of inoculum) and a negative control (flask containing inoculum and saline solution, devoid of copper strip). The negative controls indicated the microbial growth profile in the absence of copper strip. Tests and controls of the inoculated saline solution were assessed before and after incubation for pH using a pH meter (Eutech pH Tester 30) as per protocols of the American Public Health Association (APHA).

For real time study, culture was serially diluted in sterile distilled water. 500ml of tap water was filled in cylindrical copper vessel with surface area 518.1cm² and was inoculated with 500µl serially diluted culture. The vessel was kept still without shaking at room temperature. To find the trend of copper leaching in the vessel with time, the measurements were performed at the end of every 15mins. 50mL of sample was withdrawn and plated on respective medium for the enumeration of bacteria by MFT. The sample was filtered through 0.45µm membrane filter to separate the dissolved (free) copper ions. The control was a glass vessel with same inoculum. Concentration of copper ions dissolved was estimated in ICP-OES.

3. RESULTS AND DISCUSSION

Survey and Sampling

Survey was conducted within the age group of 25-70yrs in low and middle income areas. In low income areas, major drinking water source for household were private taps (35.71%), public taps (28.57%), hand pump and taps (21.42%) and well (7.14%). In middle income areas, major source for drinking water was private taps (28.57%) and well water (7.14%). According to the respondents there is no overall variation in quality of water but during monsoons and at times the water appears to be turbid and the taste changes due to chlorination. Few of the respondents were reported treating water by boiling, filtering or using chemical treatment (e.g., chlorine, alum, potash) at home during monsoon and other time of the year. However, the major concern for the respondents was microbial contamination leading to various water born diseases. Water samples were collected from the survey location and analyzed for residual chlorine and bacterial contamination. Residual chlorine was found to be less than 0.1mg/l in 24 samples and 1.5mg/l in 3 samples while 17 samples were contaminated with thermo tolerant coliforms. The WHO target for bacteriological quality of drinking water is zero *E. coli* per 100 ml, even in emergencies (WHO 2011). It was observed even with the residual chlorine in the supplied water, there was bacterial contamination at the point of use from the storage vessel. The expectations of consumers showed that they would prefer cost effective, low maintenance, greater capacity (more than 20Litres), and no electricity based drinking water purification systems for safe, pure and bacteria free drinking water.

Antibacterial activity of copper strip and copper vessel

The results for anti bacterial activity of copper using copper strips and copper vessel are presented in Figure 1 and 2. Presence of copper strips or the copper container resulted into the complete destruction of *E. coli* and *Salmonella* resembling the first order kinetic rate equation.

Figure 1(a) and (b) shows 2 log reduction of *E. coli* and *Salmonella* within 120 mins with the initial concentration ranging from 20 – 2000 CFU/100 ml. Under the test conditions 99.99% of all cells of *E. coli* and *Salmonella* were lethally damaged within 60 mins and 75 mins respectively. The growth of bacteria on selective medium was observed, where *E.coli* exhibited a typical metallic sheen colonies on EMB agar medium. The negative control with no copper strips was performed along with every experiment showed no significant reduction in the microbial colonies; the inoculated bacteria colonies either remained same or slightly decreased.

Although all the microbial contamination were removed within first 60 mins for *E. coli*, it was observed that the concentration reduction was faster in copper vessel as compared to copper strips placed in

(a)

(b)

the container with constant stirring. Studies with salmonella were conducted in copper vessel and were found to have slower destruction rate compared to *E. coli*.

Both *E. coli* and *Salmonella* cultures inoculated separately with copper strips and in copper vessel were not recovered on the specific growth medium over a time interval. This indicated that the bacteria in the test samples was completely killed or had lost their culturability on the medium.

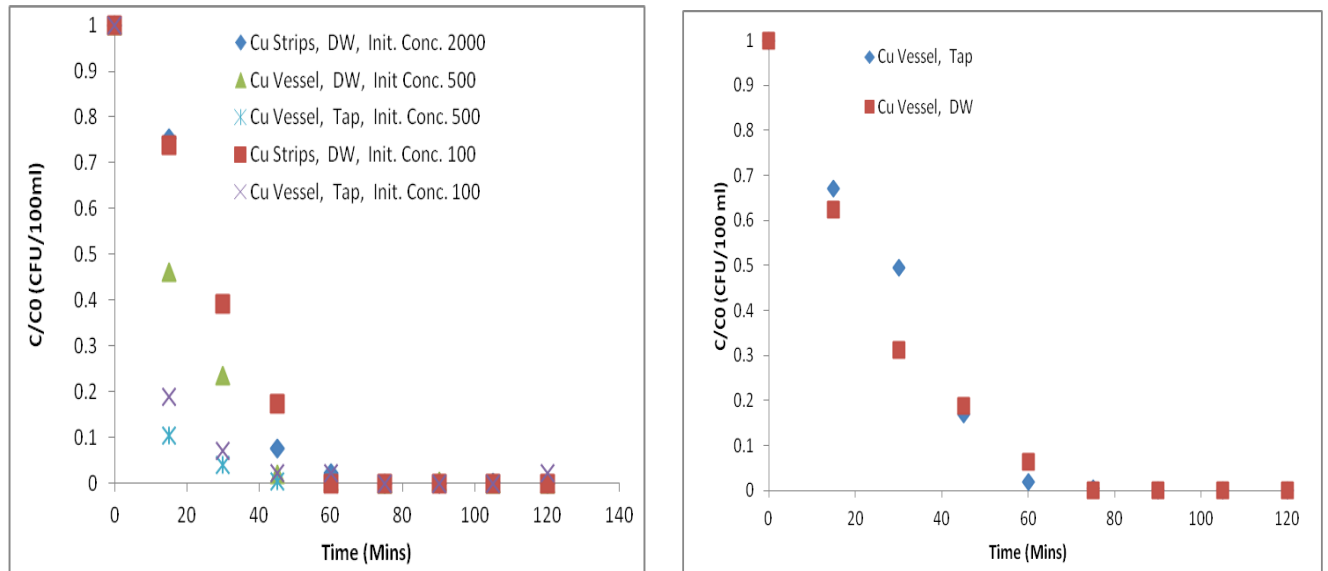


Figure 1: (a) Antibacterial activity of copper strip with *E. coli*; (b) Antibacterial of copper vessel with *S. enterica*

Further, when only bacteria (*E. coli* and *Salmonella*) were inoculated without copper in batch system, a normal bacterial growth curve was observed. The population of cells initially adjusted to the new medium (lag phase) until they started dividing regularly by the process of binary fission (exponential phase) at regular interval of time under favorable conditions. When their growth became limited, the cells stop dividing (stationary phase), eventually they show loss of viability since the nutrients were not renewed (death phase). Different bacteria has different growth curve. The growth rates depend on standard nutritional conditions (culture medium, temperature, pH, etc.). In these experiments the cultures used were *E. coli* and *Salmonella*. The negative control (flask containing inoculum and saline solution, devoid of copper strip) showed a steady increase and decrease in number of bacteria (*E. coli* and *Salmonella*) in 120mins inoculated at similar rates figure 2. This might be because the bacterial growth in culture was asynchronous and cell age was completely random.

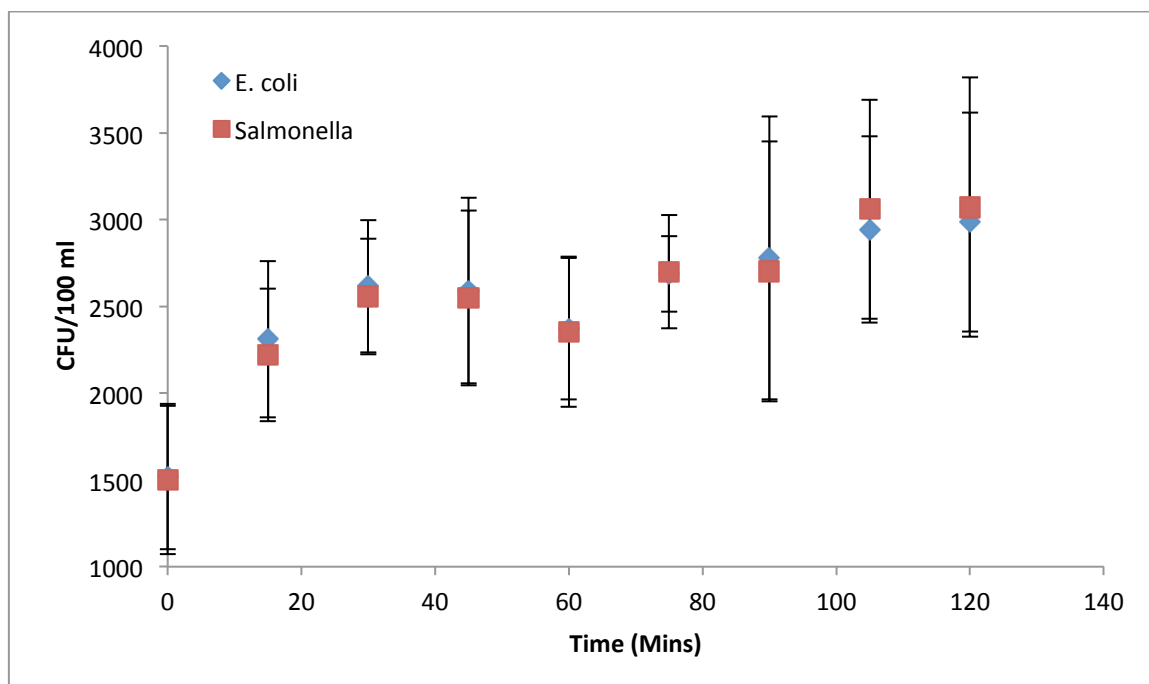


Figure 2: *E.coli* and *Salmonella* culture in normal saline solution (0.85%) without copper strip (Negative control) Eradication of microorganisms within the experimental setup with copper occurred at an early stage due to copper toxicity. This might be due to displacement of essential metal by copper from their native binding sites, through ligand interactions mechanism, changes in the conformational structure of nucleic acids and proteins in cells or redox cycling damaging lipids, proteins, DNA and other biomolecules (Borkow and Gabbay, 2005; Warnes and Keevil, 2011). The exact mechanism behind bactericidal effect of copper is not clear. The overall charge of bacterial cells at biological pH-values is negative due to the excess of carboxylic groups present in the lipoproteins at the bacterial surface, which, upon dissociation, makes the cell surface negative (Stoimenov et al., 2002). The opposite charges of bacteria and copper ions are thought to cause adhesion and bioactivity due to electrostatic forces. Generally exposure of most microorganisms to high concentrations of this trace element results in damage to cellular components. At low concentration copper acted as a micronutrient for bacteria (Raffi et al., 2010). Copper in cuprous state Cu^{2+} has a specific affinity for DNA. It can bind at two binding sites competing with hydrogen bonding present within the DNA molecule. Thus, the helical structure happens to be disordered by cross-linking within and between strands causing multiple damage to the nucleic acids, denaturing DNA (Marzano et al., 2009). Copper also readily catalyzes reactions that result in the production of hydroxyl radicals through the Fenton and Haber-Weiss reactions (Santo, 2011). It was also found in a study of the biochemistry of copper that the biocidal effects of copper are achieved mainly by the inactivation of 3 SH-groups per copper ion present in glutathione due to interaction of Cu^{2+} with SH- moieties present in the cell membrane and within cells (Borkow and Gabbay, 2005).

Surface released copper ions from copper strips and copper vessel

Copper is a transition metal that is stable in its metallic state and forms cuprous (Cu^{1+}) and cupric (Cu^{2+}) cations. The copper ions released from copper surfaces contributed in killing of test organisms. As the assays were carried out, the appearance of the copper surfaces changed, the metallic surface of new copper strips turned dark brown and corroded. In addition, it was realized that the bacterial suspensions were acquiring a pale blue color, indicative of the release of Cu ions.

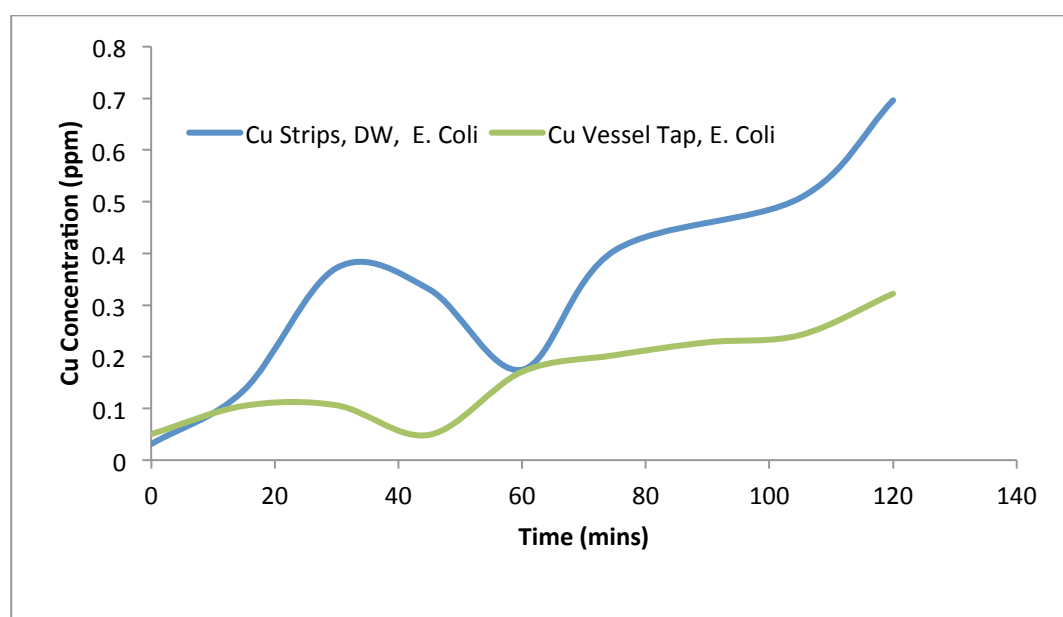


Figure 3: Concentration of dissolved Copper in Water

In order to understand the rapid and efficient inhibitory effect of copper the residual copper concentration was analyzed from the water. Figure 3 shows the buildup of the copper ion in the solution with respect to time in copper vessel as well as glass container with copper strips. In copper strip experiments the copper leaching was more as comparative to the copper vessel experiments over the time. This might be due to constant shaking condition with copper strips. The maximum concentration of free copper ions in solution with copper strips were 0.736 ppm and in copper vessel the maximum copper leaching was 0.322ppm for *E.coli* at the end of 120 minutes. For *Salmonella* species the maximum copper ion concentration obtained was 0.250ppm in copper vessel. The dip in the concentration of copper at 60 minutes and 40 minutes in copper strip experiments and Copper vessel experiments respectively coincides with the complete removal of the *E. coli*. The buildup of the

total copper in the solution can be then divided into three zones. Zone 1 indicates the steady increase in the copper concentration; Zone 2 indicates the reduction in the copper ion concentration and zone 3 indicates the increase in the copper ion concentration. During zone 1 leaching of copper from strips or container from the surface increases the dissolved copper concentration. Within zone 2 the rate of absorption of copper ions by microorganisms is higher than as compared to the rate of leaching of copper from the surface. This decrease continues until all the microorganisms are inactive or destroyed. With complete absence of microbial culture in the water there is again steady increase in the copper concentration attributed to leaching from the surface with a similar rate as in zone 1.

The leaching of copper in the experiments with copper strips was higher as compared to the leaching from the copper container experiments. However, the destruction of *E. coli* is faster in copper vessel as compared to copper strip experiments. This also indicates the available total copper surface area also plays a role in destruction of microorganisms along with the dissolved copper in the water. This also indicates that the microbial destruction is also taking place on the surface and not only with the dissolved copper in the water.

The contribution of both the gram negative bacteria to copper solubilization and uptake is not investigated yet. Here, the amount of copper being released from copper surfaces with *E. coli* cells strongly increased as compared to *Salmonella* species. The cell membrane phospholipid composition of the two organisms is quite similar, the only difference is the absence of one of the minor components (phosphatidylserine, phosphatidic acid, or two partially characterized lipids) and a decreased level of other membrane components (phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin) in *E. coli* (Ames, GF., 1968; Kozloff, LM et al., 1991).

Further, the decline in microbial count was observed with increase in leaching of copper ions. The uptake of the copper ions from by bacteria from solution resulted in the killing of bacteria. As the bacterial count declines the free copper ion concentration again increased giving residual disinfection. Thus, it shows bacteria exposed to metallic copper may accumulate cuprous copper ions intracellularly (Raffi et al., 2010). However, it could not be determined if this accumulation is the primary cause of lethality or a secondary result. Previous studies also show in *E. coli*, this stress causes the inactivation of hydratases, which are necessary for normal cell function. Specifically, cuprous copper ions can damage exposed Fe-S clusters in these proteins, resulting in growth defects of challenged cells (Santo et al., 2011). In our study, gram-negative *E.coli* and *Salmonella species* were both damaged after very similar exposure times. Combining copper leaching data and killing kinetics clearly demonstrated a correlation between release of copper from the copper surfaces and its antibacterial activity over the cells, with lethal consequences.

The Recommended Dietary Intake (RDI) for copper is 0.9 mg for adults (19–50 years), while the upper limit of intake for this metal is 10 mg for the same population (Faúndez et al., 2004). An excessive copper release is therefore a limiting factor to take into account. Thus the free copper ion concentration was monitored at regular intervals of experimentation. Although studies have been done on the anti-bacterial action of copper on various pathogenic micro-organisms, its application as disinfectant for the purification of drinking water in water purifiers has not been investigated. The main merit of copper metal would be decreasing the bacterial load which could survive after regular water treatment process. Studies have shown that copper surfaces completely kill bacteria mainly by membrane damage and other mechanisms. Copper act as auto-disinfectant to decrease the capacity of harmful bacteria to remain viable or to form biofilms after some hours on the surface, and may be useful to reduce cross contamination. Its functioning is fuel free, not dependent on electricity to operate or to maintain it; it is simply a passive flow and storage of water. It will be a one –time investment with no recurring cost. *Salmonella* and *E.coli* outbreaks are both rooted by the contamination of feces, but are different bacteria that pose different risks. It is important to maintain clean drinking water in your household so that our family does not consume *E.coli* or *Salmonella* from water source. Therefore the results of this study indicate that metallic copper after silver holds potential to provide microbiologically safe drinking water.

4. CONCLUSION

The use of copper vessels in Indian households is common and is, therefore, likely to be socially accepted by the people. The biocidal effect of copper as a contact surface has also been extensively investigated in a wide variety of laboratory studies and appears to have a potential application in healthcare infection prevention and control efforts. The objective of this study was to evaluate the antibacterial activity of copper metal on two highly prevalent enteropathogens, *E.coli* and *Salmonella* species. The results showed that the copper metal do have an important antibacterial activity on these two pathogens. Antimicrobial copper is effective touch surface, killing greater than 99.9% bacteria within 120 mins exposure. It is inherently antimicrobial with no chemicals added and completely

recyclable. The natural tarnishing does not impair efficacy. These findings suggest that metallic copper may have a potential application in decreasing the bacterial load in household drinking water purification system. Thus, the use of copper metal would be particularly useful to decrease the cross contamination caused by bacterial pathogens like *E.coli* and *Salmonella*. Copper hold promise as a point of use solution for microbial purification of drinking water.

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