This study has demonstrated for the first time that storing contaminated drinking water in copper containers inactivates diarrhoeagenic bacteria and rotavirus, substantiating the ancient claim in Ayurvedic texts that water stored in copper vessels can promote health. This study was the first to demonstrate that *Vibrio cholerae*, SF, ETEC, EPEC, SPT (Sudha *et al.*, 2009, 2012) and Rotavirus (Sudha *et al.*, 2011) in water could not be recovered on culture media after storage in copper pots.

Microbially contaminated water contributes to 88% of diarrhoeal deaths and about 1.5 million children under the age of five die every year due to diarrhoea (Walker *et al.*, 2012). Childhood diarrhoea creates economic burden for affected households. In India, direct and indirect cost per diarrhoeal case was INR 409 (Patel *et al.*, 2013). Providing drinking water free from microbial contamination is one of the key measures to prevent diarrhoea (Walker *et al.*, 2012).

Although community water supplies providing safe drinking water after disinfection, they may be prone to contamination during transport or handing (Gopal *et al.*, 2009). Approximately 72.2% of rural populations prefer to consume untreated water rather than from ‘treated’ source owing to various reasons including taste (in the case of chlorine) or cost (Banda *et al.*, 2013).
2007). Many studies have demonstrated that PoU water purification methods significantly improve the microbial quality of water, reduces diarrhoea by 30-40 %, and is highly cost-effective. It is also among the most effective of water, sanitation and health Interventions, that can be rapidly deployed and taken up by vulnerable populations (Fewtrell et.al., 2005; Clasen et.al., 2006; 2007). Based on these studies, WHO has concluded that household treatments provide the health benefits of safe water much faster than it will take to design, install and deliver piped community water supplies (WHO, 2007). However, many of the existing PoU water purifications systems are either expensive, are inaccessible in rural areas, need fuel or electricity and need regular maintenance (Mintz et.al., 2001).

Many simple methods are recommended in Ayurveda for enhancing the quality of drinking water. For example, storing drinking water in copper vessels overnight was believed to impart health benefits (Sharma, 2004). This has been a traditional Indian practice. However, this has not been scientifically studied. This study was undertaken to understand the 'science' behind the traditional practice and also to apply the knowledge in order to provide a low-cost household intervention for microbi ally safe drinking water. Although an indigenous tradition of India, use of copper pots are not very common today owing to several reasons including cost and easy availability of plastic and stainless steel containers.
Storing contaminated water in copper pot inactivated bacteria and rotavirus

Tap water and distilled water contaminated with 500 – 1000 CFU/mL of diarrhoea causing bacteria such as *Vibrio cholerae*, *Shigella flexneri*, *Enterotoxigenic and Enteropathogenic Escherichia coli*, *Salmonella enterica Typhi* and *Paratyphi* could not be recovered after storage in copper pots (test) for 16 h, indicating total loss of viability. It was observed that the bacteria in the test samples did not get resuscitated even after enrichment and plating on selective media. This suggested that they lost culturability on nonselective and selective media. None of the bacteria could be recovered from water when a combination of bacteria was inoculated and stored in copper pots for 16 h at RT, whereas, bacteria inoculated into tap water and distilled water and stored in glass bottles (Control), showed the presence and growth of bacteria indicating its viability. Continuous storage of water in the copper pots for five days still retained its antibacterial activity. Copper pots were also able to handle recontamination with VC upto $10^5$ CFU/mL. Storage of water in copper pots for 16 h did not alter the physicochemical parameters of water such as pH, total dissolved solids, alkalinity, hardness, presence of sulphates, chlorides, indicating that drinking water stored in copper pot is amenable for public use.

DW contaminated with Rotavirus of $2.3 \times 10^7$PFU/mL and stored in copper pots for 16 h lost its infectivity on cell lines as observed by plaque assay,
whereas 2.0 \times 10^8 \text{ PFU/mL} of rotavirus was recovered from water stored in glass bottles (control). Rotavirus causes diarrhoea in infants and are responsible for 45 \% hospitalizations in developing countries (Grimwood et al., 2009)

**The copper water (CuSN) was also observed to retain antibacterial property.**

Bacteria could not be recovered in CuSN, indicating that it was not necessary for water to be in constant touch with copper pot for exhibiting antibacterial activity. CuSN was also able to handle recontamination as VC inoculated once a day for five days continuously (\(10^5\text{CFU/mL}\)) into CuSN could not be recovered, whereas they could be recovered from controls. This indicates that, water stored in copper pots and withdrawn is able to inactivate bacteria that are introduced into water during storage and handling demonstrating its use as a household, PoU water purifier. The Effective Dose of copper wherein VC, SF, EPEC, ETEC could not be recovered from water was observed to be 200 ppb when water was stored for 16 h time. At 600 ppb copper concentration, the bacteria could not be recovered in 12 h time.

The antimicrobial activity of copper is well established (reviewed as a part of literature review). It exhibits antimicrobial activity against many pathogenic drug- resistant bacteria, viruses, fungi and protozoa (Grass et al., 2011;
Ibrahim et. al., 2011; Sunada et. al., 2012). This use of copper has been exploited and is now licensed by USEPA as an antimicrobial surface to be used in public places such as hospitals, food industries and in textiles to prevent nosocomial infections (http://www.copper.org/antimicrobial/)

**Copper in Ayurveda**

According the Ayurveda, copper produces a scrapping effect (Lekhana), heals and nourishes when administered in a smaller dose. It is astringent, sweet, bitter and sour and Mitigates Pitta and Kapha. Its Vipaka (post-digestive property) is pungent with a cold potency. It is recommended to be used in treatment of Pandu (anaemic disorders). Copper may have also play a role in physiological enhancement of water quality. Copper is also recommended to be used in conditions of Udara (ascitis), Arsa (piles), Kustha (skin disorders), cough, Svasa (dyspnoea), Kshaya (pulmonary tuberculosis), Pinasa (corryza), Amlapitta, Sopha (edema), Krimi (worm infestation) and Sula (pain). (Chunekar, 2004) Modern studies have demonstrated that copper is an essential micronutrient that is essential for the synthesis of Heamoglobin and is an essential component of many enzymes. This is the only metal that confers catalytic activity to SOD 1. Copper is a major component of catalytic centers of different redox enzymes such as metalloenzymes including superoxide dismutase. It is also essential in embryo development, mitochondrial respiration, hepatocyte and neural function, regulation of hemoglobin (Krupanidhi et. al., 2008 ). Deficeincy in copper is indicated in
anemia, neutropenia (Halfdanarson, et. al., 2008) and bone abnormalities (Haddad et al., 2008).

**Fabrication of copper device**

As copper pot is expensive (Rs. 2000/3 L), a copper device (Fig. 6.1) was fabricated and standardized for six diarrhoeagenic bacteria. After intervention with copper device of surface area 22.8 cm$^2$/L for 16 h, VC IDH 02474 could not be recovered. SF could not be recovered after intervention with copper device of 160 cm$^2$/L. None of the bacteria could be recovered on culture media even after resuscitation after intervention with copper device of surface area of 200 cm$^2$/L in 16 h time.

**Leaching of copper into water**

According to WHO, demonstration of leaching of metals within safety limits is acceptable method to demonstrate the safety of a PoU method (WHO/UNICEF, 2011). In this study, the copper content leached into tap water of pH 7.8 after storage (16 h) in copper pot and with copper device of 200 cm$^2$/L is 180 ± 14 ppb and 175 ± 28 ppb respectively. Storage of tap water in copper pots for seven days had 448 ± 0 ppb. The copper content in water after storing in copper pots or with copper device is well within the WHO limits of 2000 ppb (WHO, 2008) and BIS limits of 1500 ppb (BIS, 2009). This guideline is based on the protective effect against acute gastrointestinal
effects of copper and to provide an adequate margin of safety in populations with normal copper homeostasis. Therefore for adults with normal homeostasis, this guideline value permits consumption of 2-3 litres of water per day, use of a nutritional supplement and copper from foods without exceeding the tolerable upper intake level of 10 mg/day or eliciting an adverse gastrointestinal response.

When pH of TW and DW were buffered, there were changes in the leaching pattern of copper into water. DW and TW adjusted to pH 6.0 were observed to leach 1111 and 6000 ppb copper respectively whereas TW and TW adjusted to pH 8 leached 162 and 567 ppb copper respectively. However, all of them still within the stipulated limits of WHO (2000 ppb). Sharan et.al., (2010a) have also reported similar results. Copper content leached into water of pH 7 after 12 h at 35°C was 260 ppb. At pH 6 and 9, copper content leached into water was 1700 ppb and 150 ppb respectively at 35°C. Inactivation was proportional to the concentration of copper. This is suggested to be due to higher concentration of unbound ions in acidic pH (Hasman et.al., 2009). Similar results were obtained when acidic water was released from copper pipes. Water with acidic pH leached higher amounts of copper into water from delivery pipes made from copper (Boulay et. al., 2001). At higher alkaline pH of 9.1, bacteria were observed to acidify the media and leach our higher amounts of copper and thereafter copper leaching was stabilized (Lejczak et.al.,1980).
Probable target sites of copper ions on bacteria

The ‘active moiety’ that brings about loss in viability of bacteria is possibly the copper in its ionic form, since the addition of EDTA to copper water inhibited the antibacterial property of copper water by chelating the copper ions. Similar observations were made by others and is now well established that copper ions are responsible for inactivation of bacteria (Espírito Santo et al., 2008; 2011; Warnes et al., 2011a,b). Studies on bacteria exposed to copper water suggested that target sites of copper could be the cell wall, cell membrane and genomic DNA. VC exposed to copper lost their characteristic comma shape appeared oval or elongated in shape as observed under light microscope. After storage of VC in copper pots, the cell membranes were observed to be compromised as studied using a flow cytometer and stains such as propidium iodide and Thiozole. The genomic DNA many also be a site of target as genomic DNA of VC appeared as ‘smear’ on gel electrophoresis after storage in copper pots. Appearance of DNA as a “smear’ indicates that genomic DNA of the bacteria is degraded into small fragments that gives an appearance of “smear’ similar to the results obtanied by Warnes et. al., 2010 on dry copper surfaces. Genomic DNA of VC not treated with copper appeared as distinct band on the agarose gel, indicating that the DNA was intact.

Copper may inactivate bacteria by more than one mechanism (Grass et. al., 2011; Espirato Santo et. al., 2011). Studies on dry surfaces of copper have
demonstrated that copper causes structural damage and membrane damage (Molteni et. al., 2010; Espírito Santo et. al., 2012; Warnes et. al., 2012). Copper were observed to inactivate bacteria by destroying their cell wall and cell membrane (Espírito santo et. al., 2008; Hong et. al., 2012). Damage to the membrane was observed to causing leakage of potassium or glutamate through the outer membrane of bacteria, thereby disturbing the osmotic balance. Studies using copper coupons have demonstrated that copper ions were involved in binding to proteins that cause oxidative stress by generating hydrogen peroxide and damaging DNA suggesting genotoxicity as one of the mode of action of copper (Warnes et. al., 2010).

The present study on rotavirus demonstrated that the virus lost its ability to infect cells indicating its loss in pathogenicity after exposure to copper surface. Studies on coliphage MS2 have attributed the antiviral property of copper to generation of ROS and subsequent capsid protein oxidation (Nieto-Juarez et. al., 2010). Studies on H1N1 suggested that the inactivation may be due to the degradation of viral proteins (Fujimori et. al., 2012).

**Safety studies**

Copper content in water after the storage in copper pots or with copper device were within the limits of WHO making it safe for human consumption. The safety of copper water substantiated by studies using cell lines and small animal models. Toxicity of copper water was not observed on HepG2 cells.
and *Artemia salina* (Brine shrimp) when exposed to copper water of 1500 ppb. The taste of water stored in copper pot or copper device was observed to be unaltered as observed through a preliminary hedonicity test conducted to observe organoleptic qualities of copper water at IHST (FRLHT)

Few studies on acute copper toxicity have been conducted in human adults mainly using copper sulphate as a source of copper in drinking water. The first symptom of acute copper toxicity was observed to be mild and transient nausea. Gastrointestinal symptoms at 4 mg/L copper (delivered as copper sulfate) was observed in women and at 6 mg/L in men (Araya et. al., 2003). Adverse reactions were not observed when children 9–21 months who consumed drinking water containing 0.03–2.1mg Cu/L delivered through copper pipes (Pettersson et. al., 2003 ).

Earlier studies supported that the ingestion of high amounts of copper was responsible for Indian Childhood Cirrhosis (ICC). They correlated the use of brass and copper vessels for preparing and storing milk for children (O’Neill et. al., 1989; Tanner et. al., 1981) but recent analysis have shown this to be virtually tenable (Pandit et. al., 1996; Sriramachari, 2008). This is because stages of ICC were also encountered in cases when there was a total absence of use of copper utensils. Also, hepatic overload of copper was not observed as seen in cases of ICC. (Sriramachari, 2008). Moreover, majority of the cases of ICC were in males, suggesting it to be a genetic disease or a disease with
unknown etiology (De Romaña et al., 2011). This suggests that copper utensils may not be directly associated to ICC.

**Other similar studies**

The use of copper vessels for microbial purification drinking water was earlier demonstrated by Godbole (1971) on fecal coliforms. Tandon et al., (2005, 2007) demonstrated that isolates of *E. coli* and coliform bacteria in water stored in a brass vessel, could not be detected after 48 h. However, only after this study, other studies on the effect of storing contaminated water in copper vessels that demonstrated the contemporary relevance of using copper containers for storing drinking water has been reported (Sharan et al., 2011). The rate of inactivation of bacteria was almost similar at 15, 25 and 35°C temperatures, and pH of water from 6.0 - 9.0 (Sharan et al., 2010a). In the presence of chlorine, Na₂SO₄, NaNO₃ and NaNO₂, of amino acids, proteins, humic acid or complex organic brought about a decrease in inactivation of bacteria. (Sharan et al., 2010b). It was also demonstrated that *Salmonella* in water stored in copper vessels lost their infectivity (Sharan et al., 2011).

Electrolytically generated copper have also been extensively studied for its use in controlling *Legionella* in hospital water systems. Field trials have demonstrated its effectiveness in inhibiting the growth of the organisms and associated infections. This is especially useful in healthcare systems where
the there are presence of bacteria that are resistant to the common disinfectants (Shih et al., 2010).

**Potential of Copper device as a low-cost water purifier**

This study has demonstrated that the copper pot and copper device inactivate diarrhoea causing bacteria and rotavirus by more than 3-log, thus demonstrating its potential as a PoU water purifier. Copper device for treating one litre of water would cost INR 500 - 600/- for a life time and can be used in a regular plastic pot or any other container used to store water (Fig. 6.2). The device can be scaled up to treat different volumes of water. Its functioning is not dependent on fuel, electricity, replaceable filters, intensity of sunlight, etc. to operate or maintain it. It also reduces recontamination due to handling. It is simply a passive storage of water. This one time investment is similar to the direct and indirect cost for treating every diarrhoeal incidence (INR 409). The health benefit that can be achieved by using copper pot or device as a PoU water-purification device will far outweigh the cost, if divided over the members in a rural family, especially as it will be a one-time investment with no recurring costs. It is suitable for developing countries like India where there is frequent intermittent supply of drinking water, necessitating storage of drinking water for days. In such conditions copper device can be introduced during storage of drinking water.
Household Water Treatment Options are evaluated by WHO based on health-based target and microbiological performance. As per its evaluation criteria, copper pot or device has the potential to be grouped under “interim”. This term refers to technologies that achieve 2-log reduction for two classes of microorganisms, viz, bacteria, viruses or protozoa and have a proven impact on reducing diarrhoeal and waterborne infections an ‘protective’ or ‘interim’ HWT (WHO/UNICEF, 2011). Therefore, copper device has a tremendous potential to be promoted as one of the options for low-cost, safe, easy to use device for microbial purification of water.

Many of the currently available water purification systems are expensive and beyond the reach of the rural population in countries such as India. The available inexpensive methods for household purification of water have shortcomings. Candle filters (with diatomaceous earth) require regular cleaning and replacement, which are tasks usually ignored by users (Mintz et al., 2001). The use of regular, household, solar heaters for disinfection is in danger of leaching harmful chemicals from the plastic bottles and also prone to re-contamination during handling; moreover the functioning of the heaters on a cloudy day is not dependable.

**Challenges for copper pot/device as a HWT method**

1. The copper device does not address the chemical or physical contamination of water. It cannot be compared with other existing water
filters or comprehensive purification system. It has been observed to inactivate diarrhoeagenic bacteria and Rotavirus. In case of turbidity, water can be filtered before using the copper device. However, it is demonstrated that immediate risk to human health is due to water contaminated with enteric microorganisms of faecal origin, although chemical impurities in water are a public health concern (Mintz et al., 2001).

2. The purity of copper that is used to make the device or container is an important criterion. The device or pot that was used in this study was 98-99% pure. It did not have other toxic metals such as lead or arsenic. Copper containing impurities such as lead or any other metals may also leach into water making it unsafe. Strict quality control checks need to be performed when fabricating and implementing in large scale or for regular use.

3. The time taken by copper pot or device for inactivating the microorganisms in water is a limitation. However, it has been observed that about 15% of households treat water by simply straining it through a cloth or letting it stand and settle, that are unlikely to render water microbiologically safe. This suggests that a substantial number of households are committing time and effort to treat their water (Rosa et al., 2010). Comparatively, using copper device is not laborious as it involves only placing the device in the containers after cleaning.
4. Development of resistance in bacteria may also be of concern. However, one study was conducted to demonstrate that copper surface may reduce the horizontal gene transfer that contributes to development of resistance in bacteria. *Escherichia coli*, virulent clone ST131 harboring extended-spectrum-β-lactamase *bla*CTX-M-15, and *Klebsiella pneumoniae* harbouring metallo-β-lactamase *bla*NDM-1 as donor cells were used. Sodium azide-resistant *E. coli* J53 were used as recipients. Horizontal gene transfer of *bla* occurred on stainless steel surface but not on copper surface, although the transconjugants exhibited the same resistance profiles of the donors. The authors concluded that copper surface may reduce the incidence of horizontal gene transfer among bacteria if they are exposed to it and thereby bringing about reduction in development of resistance. Moreover, it was also argued that on exposure to copper surfaces, degradation of DNA occurs within 5-7 minutes reducing the possibility of resistance (Warnes et al., 2012).

5. Field trials need to be conducted to demonstrate the performance of copper pot and copper device on different types of water under real-life conditions in the dynamics of the target households in developing countries to fully understand the limitations. Although the copper content in water is within the permissible safety limits of WHO, it is recommended that safety studies on animals be done before conducting field trials.
6. Scaling up and implementation may also pose a challenge. Studies have shown that even though the International Network, to promote Household Water Treatment and Safe storage, hosted by WHO and other initiatives to scale up household interventions have been a part of international development efforts since 2003, the desired results have not yet been achieved in terms of its use. This is due to the constraints on distribution, user acceptance, effective use of products, price-economics, training-methods, sustainability, inadequate maintenance, monitoring and evaluation (Brown et. al., 2012). Therefore any intervention that aims to significantly reduce diarrhoeal disease in India should also include a focus on sanitation and hygiene practices (Clasen et. al., 2004, 2005). Hence, a thorough study of knowledge, attitudes, behaviour and practices related to water, water treatment, hygiene and sanitation, together with socio-cultural aspects of the communities or households needs to be undertaken before conducting field trials (Lantagne et. al., 2007). In many cases, connecting the importance of protecting water quality to the cultural or religious significance has made a difference in increasing the use of a technology and are more likely to be sustained (Palaniappan et. al., 2010; USAID, 2010)

Limitations/gaps in the current study

1. The study does not evaluate the ability of copper to inactivate a waterborne protozoan. However, preliminary studies conducted at NICED,
on trophozoites of *Giardia lamblia* demonstrated its ability to significantly inactivate the trophozoites. This has not been reported in this study as trophozoites are not present in the atmosphere and are sensitive to any subtle change in the environment (Adam, 1980). Water borne protozoan such as *Cryptosporidium* or *Giardia* that are significant are present as oocysts or cysts in the atmosphere and excysts as trophozoites only in the stomach (Adam, 1980). Facility for collection of large amounts of cysts and testing them is very limited. Moreover protozoan cysts can be easily removed using a filter due to their size. The popular disinfectant chlorine also does not inactivate cysts but still is used regularly as a water disinfectant. Also, *Cryptosporidium* is known to be significant in causing diarrhoea in immune compromised people and the elderly whereas diarrhoeagenic bacteria and rotavirus are known to cause diarrhoea and gastrointestinal infection largely in children, out of which rotavirus is responsible for 45% of diarrhoea disease in children (Parashar *et al.*, 2009)

2. The study demonstrates that bacteria in water stored in copper pots could not be recovered on culture medium even after resuscitation. However, we still need to confirm whether they have transformed into the viable but non culturable bacteria (VBNC) state. VBNC bacteria are defined “as bacteria that are living but unable to grow or divide when inoculated into routinely used bacteriological culture media (Senoh *et al.*, 2012). Recent studies have shown that VBNC states of many diarrhoeagenic bacteria including *Vibrio cholerae* non-O1 and non-O139, can be converted to
culturable state by co-culturing with eukaryotic cells such as Caco-2, T84, HeLa, Intestine 407, HT-29, and CHO cells (Senoh et al., 2010; 2012). Studies need to be carried out using this technique to check if the bacteria have entered the VBNC state after treatment with copper pot and copper device.

3. Copper device has not been standardized for inactivation of rotavirus although it has been established using a copper pot. The device also needs to be tested for its rigour in different types of water and conditions.

**Conclusion**

Traditional knowledge method of using copper containers for storing drinking water offers a contemporary PoU solution to microbial water purification. As copper pot is expensive, a low-cost copper device was fabricated, standardized and demonstrated that can be used instead of copper pot. The amount of copper leached into water is about 200 ppb which is well within the safety limits of WHO (2000 ppb). Copper device is inexpensive, is easy to use, requires no fuel or electricity and does not need any maintenance and replacements, making it ideal for situation prevailing in developing countries. Field trials also have to be conducted to assess its performance in the field as well as its social acceptance. Nonetheless, copper pot and copper device have the potential to be one of the simplest PoU household interventions that can bring about a significant reduction in mortality and morbidity rates in diarrhoea due to contaminated water especially in children in developing
countries. However, it does not address the chemical and other contamination of water.

In this study only one aspect of health benefit, (in terms of copper pots being able to inactivate diarrhoea causing bacteria) of storing drinking water in copper pots was demonstrated as a first attempt to understand the 'science' involved in traditional knowledge. However, there may be physiological benefits such as in improving immunity, treatment of anemia, its influence of gut microflora etc that needs to be explored. Transdisciplinarity studies need to be undertaken across various disciplines to including Ayurveda, local health recommendations, folk medicine and bioscience etc., that could guide us to discover other new benefits of traditional recommendations and contribute to well-being.

Figure 6.1: Prototype of a Copper device  

Figure 6.2: Storing drinking water with copper device