COMPARITIVE MICROBIOLOGICAL WATER ANALYSIS STORED IN DIFFERENT VESSELS

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Abstract: In developing countries, a large portion of the population, suffers from health problems associated with either lack of drinking water or due to the presence of microbiological contamination in the water. To have an understanding about the drinking water quality in and around Tiruchirappalli, 4 drinking water samples were collected, out of which 3 were around Chatram bus stand and 1 from common municipal water tank near Karur bye-pass road, Trichy. It was found that the temperature of the water collected from all 4 sites were within the permissible limit as per IS: 10500. The fluctuation of pH in the samples is from 7.32 to 7.53 and the turbidity ranged from 2.31 to 2.56 NTU which was within the prescribed limit for drinking water. Among the 4 samples collected water collected from the municipal tank showed high incidence of bacteria compared to all the 3 samples. Isolated colonies were subjected for identification by macroscopy, microscopy and biochemical tests. Water samples 1 & 2 were considered as potable and water sample 3 & 4 were non-potable by MPN technique. Among all the vessels used copper was found to be effective for storing water since it exhibited antibacterial property against *E.coli, Salmonella typhi* and *Klebsiella* sp, tested.

Key Words – water quality, *E.coli, Salmonella typhi, Klebsiella* sp., MPN, copper, CFU

I. INTRODUCTION

Good drinking water quality is essential to the health and well-being of all people. Water is considered as potable when there are no levels of chemicals (e.g. heavy metals) or chemical substances that would cause harm to human health and when water does not have a bad taste or smell. The most serious water pollutants in terms of human health worldwide are pathogenic organisms. Thus drinking water must be free of these pathogens - viruses, protozoa or bacteria. Acceptable water quality occurs when there are especially no bacteria of faecal origin present that may cause human diarrhoea and other life threatening diseases (e.g. typhoid fever). To actually test water for specific harmful viruses, protozoa and bacteria is time consuming & expensive. Therefore water quality control personnel usually analyze water for the presence of coliform bacteria, any of the types that live in the colon or the intestines of humans and other animals (e.g. *E. coli*).

Coliforms are used as water quality indicators for 2 main reasons: i) Coliforms may be associated with the sources of pathogens contaminating water and their presence in drinking water may indicate a possible presence of harmful, disease causing organisms. ii) The analysis of drinking water for coliforms is relatively simple, economical and efficient. Coliforms could be easily detected by its ability to ferment lactose to produce acid and gas within 48 hrs at 35-37°C. In developing countries, people usually collect drinking water from surface sources like ponds, wells, streams, municipal pipes, stored water from tanks or storage level itself.

Water may become contaminated at any point between collections, storage, serving at homes (Rufener *et al*. 2010). The storage water for hours or even days increases the possibility of fecal contamination of otherwise good quality water inside the household (Uwah *et al.,* 2014 and Subbaraman *et al*. 2013). The Household drinking water storage containers or the point-of-use are importance for the fecal recontamination of water (Subbaraman *et al.* 2013). Higher levels of microbial contamination is associated with storage vessels having wide openings (e.g., buckets and pots), by introduction of hands, cups and dippers that can carry faecal matter, and lack of a narrow opening for dispensing water (Onigbogi and Ogunyemi, 2014). For improving and protecting the quality of water to these households, effective, affordable, functional and sustainable intervention strategies are required (CDC, 2001; Clasen *et al*. 2007).

The ancient Egyptian, Indian and Sumerian civilizations used copper, silver and gold for jewellery, cutlery and as vessels to store and drink water. These materials were not used for aesthetics alone; they have tremendous health and spiritual benefits for the human being. The Indian ayurveda describes storing water in a copper vessel overnight and drinking it in the mornings for many health benefits. Storing water in copper and silver pots finds mention in ancient texts of Ayurveda for purification of water (Sharma, 2014; Preethi Sudha *et al*. 2012; Radha and Susheela, 2015). Copper is known for its antimicrobial effect (Preethi Sudha *et al.* 2012). Sarsan, 2013 have reported that the water stored in the copper and silver vessels have antimicrobial, anti- inflammatory, antioxidant and anti carcinogenic activities.

Thus the present work was taken with the objective to study the presence of coliforms and other bacteria in drinking water sources collected in and around Chatram bus stand and from municipality tank located in Karur bye-pass road and also to analyze the effect of storage of contaminated water in different vessels like copper, brass, stainless steel and mud.

II. MATERIALS AND METHODS

2.1 Isolation and Identification of bacteria from drinking water samples

2.1.1 Details of study area

Tiruchirappalli (formerly Trichinopoly in English), also called Tiruchi or Trichy, is a major tier II city in the Indian state of Tamil Nadu and the administrative headquarters of Tiruchirappalli District.

Chatram bus stand is a densely populated area with schools, colleges, vendors, residents and travelers. So drinking water becomes a main issue here mainly because of 2 reasons: 1) Scarcity during summer and 2) Contamination of drinking water due to various factors.

2.1.2. Drinking water sampling points

Sterile containers of 200ml capacity were used for collecting samples. The bottles were filled leaving no air space, and then the bottle was sealed to prevent any leakage. Each container was clearly labeled. The sampling sites were in and around Chatram bus stand and drinking water from the municipal tank, near Karur bye-pass road.

2.1.3 Isolation and Identification of Bacteria

From the samples collected, bacterial strains were isolated and enumerated by culture techniques (spread plating method 0.1 ml) on nutrient and selective medium plates. The numbers of visible colonies were counted after 48hours of incubation in the order of magnitude above 10^4 CFUs cm^{-2} (CFUs -Colony Forming Units). Bacterial parameters were characterized and listed. Characterization and identification of bacteria were done based on the results of morphologically dissimilar and distinct colonies. Selected isolates were identified by macroscopy, microscopy and biochemical methods using standard techniques (Aneja, 2003). The selected isolates were purified by streak plate and preserved under refrigerated condition for further studies. Selected isolates were identified by macroscopy, microscopy and biochemical methods using standard techniques.

2.2 Potability test for the water samples by Most Probable Number (MPN) technique

Most Probable Number (MPN) technique was used for analysis of total coliform and fecal coliform bacteria in the collected water sample and the water stored in 10 different types of containers for different time periods. 3 tubes MPN method was routinely followed for the analysis of coliforms in the water. The value of MPN/100 ml was computed by referring the standard MPN table (Ravichandran *et al.*, 2016).

2.3 Effect of storage of drinking water in different containers

2.3.1 Bacterial strains

E. coli, *Salmonella typhi* and *Klebsiella sp.*, were obtained from IMTECH, Chandigarh. Cultures from the nutrient agar culture-stab were streaked onto selective media, including Eosin Methylene Blue (EMB) agar medium (HIMEDIA, Mumbai, India) for *E. coli* species, Salmonella Shigella (SS) and Mac Conkey agar medium (HIMEDIA) for *Klebsiella sp.*, were incubated at 37°C for 16-18 hours in a agar for *Salmonella typhi* bacteriological incubator (IN 18 DF, Servewell Instruments Private Limited, Bangalore, India). After incubation, a single colony was picked and inoculated into 2 ml of Luria Bertani broth (Himedia) and incubated for 16-18 hours in a bacteriological incubator at 37 °C. This overnight culture was serially diluted in normal saline (NaCl, 0.85%) for inoculation in water.

2.3.2 Vessels used for the study

Mud pot (big and small), Copper (big and small), Brass (big and small), Stainless steel (big and small) were used for the study.

Fig 2.1 Different vessels used for the study

2.3.3 Antibacterial activity of different vessels on drinking water inoculated with enteric pathogens

The volume and the holding capacity of the different vessels shown in the fig.2.1 were calculated and the vessels were autoclaved and were then filled with $1/5^{th}$ of the total capacity to allow for sufficient aeration. Suitable volumes (ml) of overnight cultures of the three different bacterial species wee inoculated into autoclaved distilled water. The cell numbers of each organism inoculated into each vessel were $1x10^6$ CFU/ml. Overnight cultures were measured using a colorimeter against a blank of nutrient broth, in which the cultures wee grown. Immediately after inoculation, the zeroth (0^{th}) OD was taken by keeping autoclaved water as a blank. Then 4ml of the water sample containing the organisms was withdrawn periodically (2 hrs and 4 hrs after inoculation into the vessels) and the samples were serially diluted in autoclaved water. From the appropriate dilutions, 50µl of the dilute culture was plated on nutrient agar plates. Autoclaved distilled water (50µl) was plated as control. The plates were incubated overnight at 37°C and about 15hrs later, the number of colonies formed were counted and the CFU/ml values were obtained using the formula:

$$\text{CFU/ml} = \frac{\text{No. of colonies counted x dilution factor}}{\text{Volume plated (ml)}}$$

A plot of CFU/ml (y-axis) vs. time (x-axis) was drawn using graph pad prism software.

III. RESULTS

4 drinking water samples were collected, out of which 3 were in and around Chatram bus stand and 1 from the municipal tank, near Karur bye-pass road. The physical parameters such as temperature

and pH of the water sample were analyzed. It was found that the temperature of the water collected from all 4 sites were within the permissible limit as per IS: 10500 during winter (Fig. 3.1).

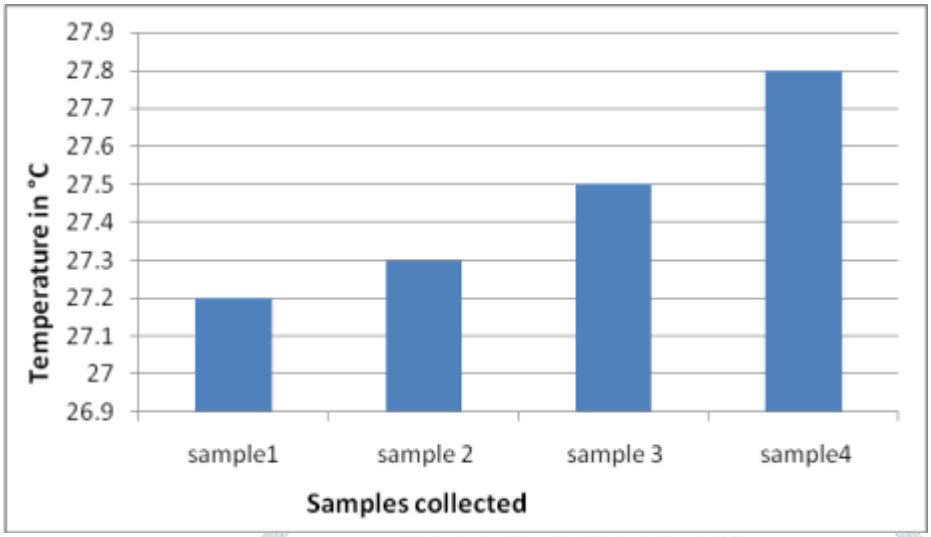

Fig.3.1. Average Temperature of drinking water samples collected

The pH is a measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. It has no direct adverse affect on health, however, a low value, below 4.0 will produce sour taste and higher value above 8.5 shows alkaline taste. A pH range of 6.5 – 8.5 is normally acceptable as per guidelines suggested by ISI. In the present study, the fluctuation of pH in the samples is from 7.32 to 7.53 (Fig. 3.2).

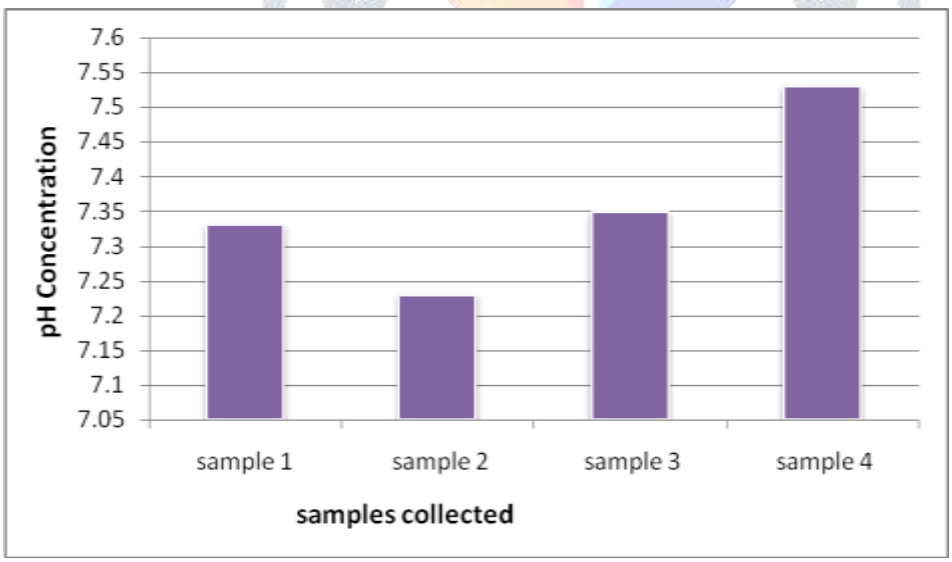

Fig.3.2. Average pH of the water samples collected

Measurement of Turbidity reflects the transparency in water. It is caused by the substances present in water in suspension. In natural water, it is caused by clay, silt, organic matter and other microscopic organisms. It ranged from 2.31 to 2.56 NTU. However the prescribed limit of Turbidity for drinking water is 5 NTU (IS: 10500). Turbidity was found within the permissible limit in all the water samples (Fig. 3.3).

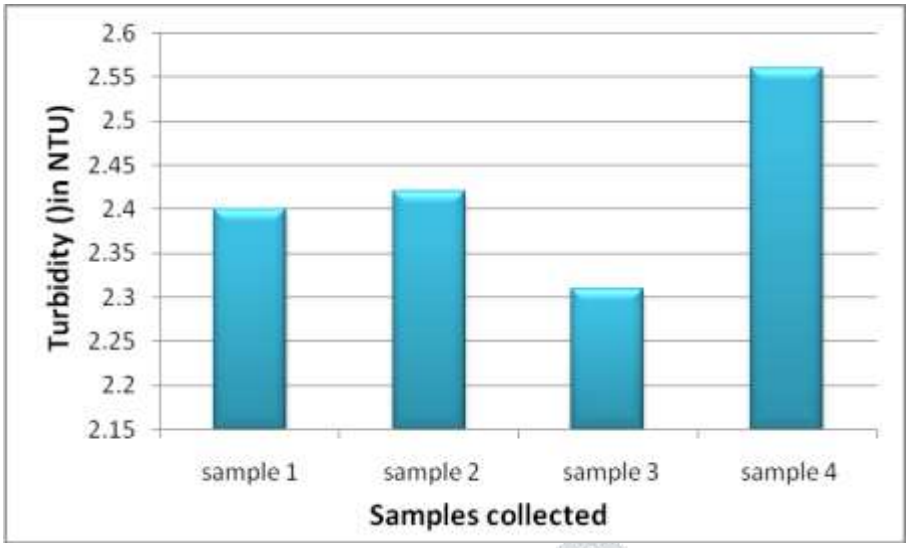

Fig.3.3. Turbidity of the water samples collected

Water samples collected from different sites were subjected for bacteriological analyzes. Among the 4 samples collected water collected from the municipal tank showed high incidence of bacteria compared to all the 3 samples (Table 3.1).

S. No	Sampling site	Cultivable bacteria obtained
1.	Sample 1	12
2.	Sample 2	9
3.	Sample 3	10
4.	Water from the municipal tank	23

Table. 3.1 Details of the samples collected and bacteria isolated

Potability of the collected water samples were analyzed by MPN technique. In sample 3 and 4, a positive result of presumptive test was observed with the production of acid and gas bubble in Durhams tube whereas sample 1 and 2 showed a negative result. Sample 3 and 4 were further preceded for comfirmed and completed test.

Production of gas in the brilliant green lactose bile broth tubes after 14 hrs of incubation indicates a positive confirmed test for coliform bacteria in sample 3 and 4. Production of gas from lactose confirms the presence of gram negative, non-spore forming rods in the completed test indicating the faecal contamination of water. Thus the water sample 3 and 4 are not considered as non-potable (Table 3.2).

S. No	Sampling site	Potability of water

1.	Sample 1	potable
2.	Sample 2	potable
3.	Sample 3	Non-potable
4.	Water from the municipal tank	Non-potable

Table. 3.2Potability of the samples collected by MPN technique

The efficacy of copper (big), brass (big) and pot (big) against *E.coli* was more efficient compared to the small vessels used (Fig 3.4). At the 2h point, CFU/ml for *E.coli* was 0 (0 colonies were obtained) for these vessels alone. These three vessels are large and therefore, oxygen dissolution in these vessels would be much greater.

$1x10^6$ CFU/ml *E.coli* cells were inoculated into each of the vessels. At first, the total capacity (volume) of the vessel was measured.

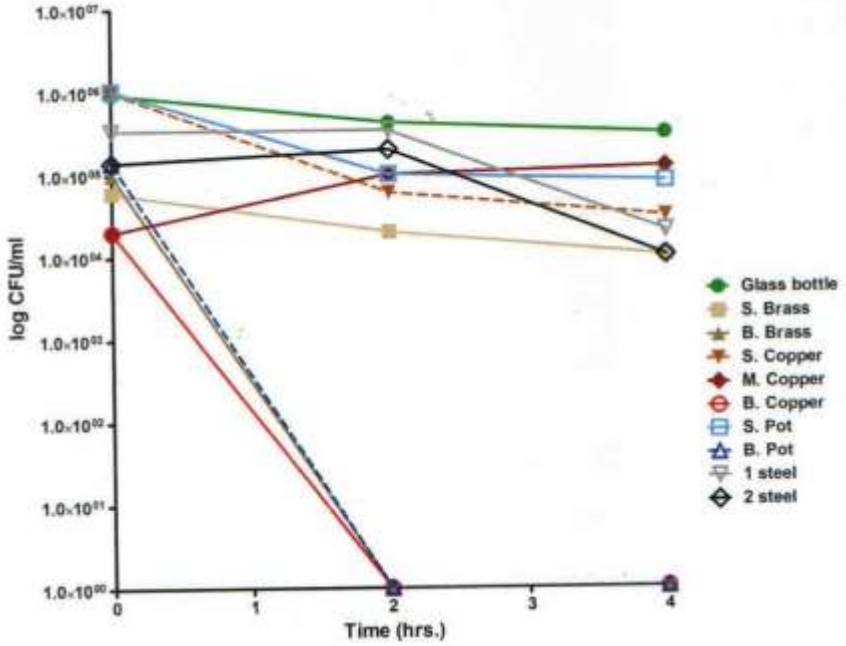

Fig. 3.4 CFU/ml plot of *E.coli*-vessels vs. time

In the Fig. 3.5, once again, B. Copper, B. brass and B. pot vessels showed the highest efficacy against *Salmonella typhi*. At the 2h point, CFU/ml for *S.typhi* was less (0 colonies were obtained) for these vessels alone. The slopes for the other vessels are not that sleep, and hence, the killing effect is lesser. Copper, iron and other transition metals which exude from the vessels may get into the water and cause the destruction of microbial cells. Again the three vessels which showed the best effects are large and therefore, oxygen dissolution in these vessels would be much greater.

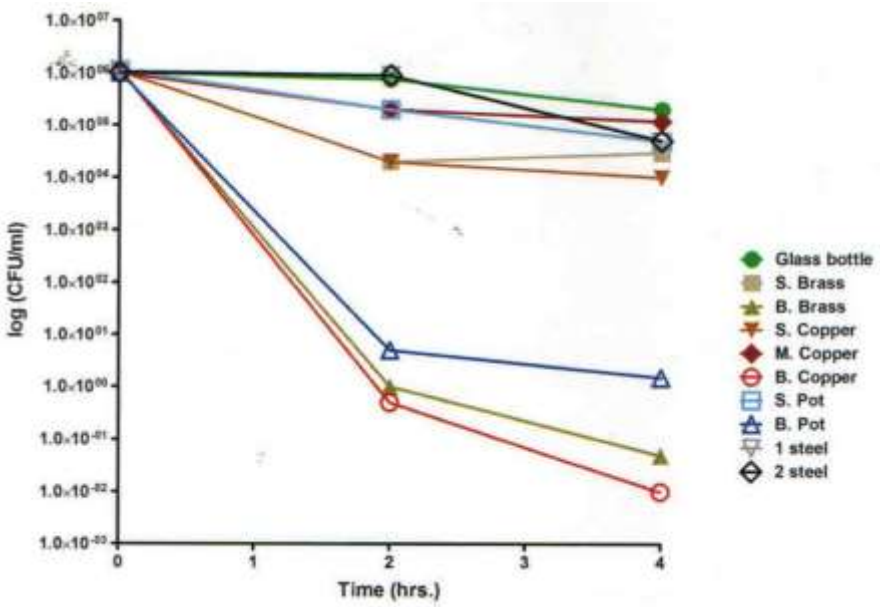

Fig. 3.5 CFU/ml plot of *Salmonella typhi*-vessels vs. time

In the Fig. 3.6, as seen for the previous two organisms, B. Copper, B. brass and B. pot vessels showed the highest efficacy against *K.pneumoniae*. At the 2h point, CFU/ml for *K.pneumoniae* was much lower for these vessels alone. Among the three, B. Copper vessel had the greatest bactericidal effect. The larger the mouth of the vessel, the greater is the propensity for increase in oxygen dissolution (dissolved oxygen concentration).

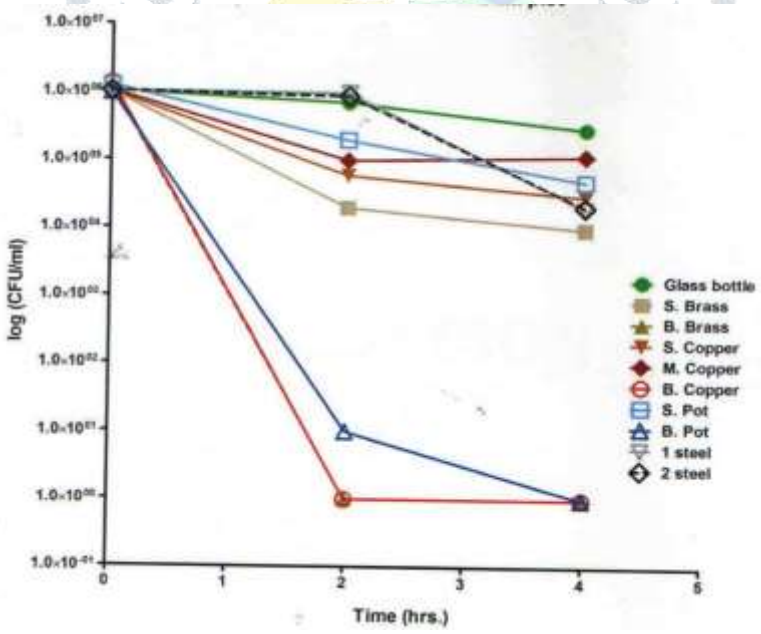

Fig. 3.4 CFU/ml plot of *K.pneumoniae*-vessels vs. time

IV. DISCUSSION

Water is essential to life and one of the most important of all natural resources known on Earth. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health (UNICEF, 2008). It is necessary that the quality of drinking

water should be checked at regular time intervals, because due to use of contaminated drinking water, human population suffers from varied of water borne diseases (Basavaraja, *et al.*, 2011). There is no single or simple measurement for water quality.

Determining water quality has been adopted as one of the main criteria for the establishment of water use, because these standards seek the safety of the consumer population. This is due to the fact that water quality is not necessarily a state of purity, but is configured by its chemical, physiological and biological. In developing countries, a large portion of the population, suffers from health problems associated with either lack of drinking water or due to the presence of microbiological contamination in the water (Saravanakumar, 2011).

E.coli is the best coliform indicator of fecal contamination from human and animal wastes. *E.coli*'s presence is more representative of faecal pollution because it is present in higher numbers in faecal material and generally not elsewhere in the environment. The Enterococci are a group of bacteria that have been most often suggested as alternatives of coliform. *Enterococcus* are formed by the splitting of *Streptococcus faecalis* and *Streptococcus faecium* (Premlata and Vikal, 2009).

Generally, for water examination purposes enterococci can be regarded as indicators of fecal pollution. *C. perfringens* is the only reliable indicator of faecal contamination and is being proposed for use in establishing satisfying water quality standards. *C. perfringens* spores were identified as the best indicator of faecal pollution and were the only indicator group significantly correlated to any of the pathogen groups in the water column (*Giardia* sp. and *Aeromonas* sp.) (Gerbaand Smith, 2005).

The drinking water quality in and around Chatram bus stand were collected and analyzed. The physico-chemical parameters directly related to the safety of the drinking water to human consumption. The physio-chemical water quality parameters provide important information about the health of a water body. These parameters are used to find out the quality of water for drinking purpose. All the 3 physio-chemical parameters such as pH, temperature and turbidity were under permisable limit (WHO, 2008).

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. Childhood diarrhoea creates economic burden for affected households. In India, direct and indirect cost per diarrhoeal case was INR 409. Providing drinking water free from microbial contamination is one of the key measures to prevent diarrhoea (Duru Majestyet.al., 2013).

The present study revealed the presence of different bacteria from all the samples collected. Bacterial colonies were high in number from the drinking water sample collected from municipal tank compared to the other 3 samples.

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). 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. 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. Deficiency in copper is indicated in anemia, neutropenia and bone abnormalities (Hundakova *et.al*., 2013). In the present study copper vessel controlled the growth of *E.coli*, *Klebsiella pneumonia* and *Salmonella typhi* compared to other vessels used.

V. CONCLUSION

Contact killing is very rapid and cells are not dividing on copper surfaces which indicates that copper possess good antibacterial activity. Further study in the mechanism of antibacterial activity of copper and its nanoparticles against disease causing microbes and with molecular docking and simulation studies (combined with drug target identification in microbes), new antimicrobial compounds of copper compounds may be obtained.

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