Bacteria Free Water for Drinking by Silver Treatment
(BFWDST)

DST File No. DST/TM/WTI/2k10/22/(G) Dated 09/09/2011

Final Report

Submitted to
Department of Science and Technology
Government of India, New Delhi

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C**hapter 1**

INTRODUCTION

1.1. General:

"World Health Organization" considers that "drinking-water" should be "suitable for human consumption and for all usual domestic purposes including personal hygiene." Diverse regulatory agencies adopt similar definitions. Drinking-water should therefore be suitable for consumption, washing/showering and domestic food preparation. In human health terms, exposure to water and its constituents can occur though ingestion, contact and aerosol inhalation. Drinking-waters should be safe for lifetime use, taking account of differing sensitivities that occur across life stages, but all are not necessarily suitable for individuals suffering from certain specific immune-compromising disorders.

Drinking-waters also include those obtained from non-piped sources, such as from springs and community wells, in bottles and as ice. The control of faecal contamination in drinking-water systems and sources, where it occurs, is of primary importance. Faecal-specific indicator bacteria such as E. coli are the parameters of first importance in monitoring faecal pollution. Piped drinking-water supplies typically involve source abstraction, treatment and distribution. The latter may include ancillary devices at domestic or institutional levels, such as softeners, activated carbon treatment, vending machines, dispensers, etc.

Drinking water Standards:

Table 1.1: Bacteriological quality of drinking water [(a)] **(Indian Standard – Drinking water specification: IS 10500 -** Doc: FAD 25(2047) C**)**

Organisms	**Guidelines**
All water intended for drinking : E. coli or thermotolerant coliform bacteria [(b, c)]	Must not be detectable in any 100 ml sample.
Treated water entering the distribution system : E. coli or thermotolerent coliform Bacteria [(b)]	Must not be detectable in any 100 ml sample.
Total coliform bacteria	Must not be detectable in any 100 ml sample.
Treated water in the distribution system : E. coli or thermotolerent coliform Bacteria Total coliform bacteria [(d)]	Must not be detectable in any 100 ml sample. Must not be detectable in any 100 ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken thoughout any 12 month period.

a) Immediate investigative action must be taken if either *E.coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.

b) Although, *E.coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

c) It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for progressive improvement of water supplies

d) In the remaining five percent sample total coliform bacteria should not exceed 10/100ml.

BIS 10500: 1991

Water in Distribution System Ideally, all samples taken from the distribution system including consumers' premises, should be free from coliform organisms. In practice, this is not always attainable, and the following standard for water collected in the distribution system is therefore recommended when tested in accordance with IS 1622: 1981.

- Throughout any year, 95 percent of samples should not contain any coliform organisms in 100 ml
- No sample should contain *E. Coli* in 100ml
- No sample should contain more than 10 coliform organisms per 100 ml
- Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

ISI-IS: 2296-1982

No sample should contain *E. coli* in 100 ml; No sample should contain more than 10 coliform organisms per 100 ml; and Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

Bacteriological Standards
(http://www.indiawaterportal.org/sites/indiawaterportal.org/files/Drinking%20Water%20Quality%20Standards_BIS_0.pdf)

a) For water entering a distribution system, Coliform count in any sample of 100 ml should be zero (0).

b) For water in a distribution system –
(i) *E. Coli* count in 100 ml of any sample must be zero (0).
(ii) Coliform organisms should not be more than 10 per 100 ml in any sample.
(iii) Coliform organisms should not be present in 100 ml of any two consecutive samples or more than 5% of the samples collected for the year [5].

1.2. Water contamination:

Water polluted with microorganisms is known as the cause of several water-borne diseases including typhoid fever, diarrhea, cholera, polio and various respiratory tract diseases. Therefore, in order to assure public health, treatment of microbial contaminated water is very important. Conventional disinfection methods, such as chlorination and ozonation, effectively kill the pathogenic microorganisms; however, the formation of carcinogenic disinfection byproducts limits their potential [12].

The issue of water quality and contamination is touching alarming levels in India. There is a growing concern about increasing level of water contamination and its impact on lives of Indians, which is evident from the water audit. The biggest concern is accessibility to clean and healthy drinking water. A large section of society drinks tap water and risks the contaminants present [Times of India, Bangalore, Thursday, August 22, 2013].

Table 1.2: Diseases or pathogens that can be transmitted by water and in which the pathogens are present already in raw water [3]

Bacteria	Viruses	Protozoans
Cholera Typhoid fever Paratyphoid fever Cryptosporidiosis Salmonellosis Toxoplasmosis Shigellosis Yersiniosis Campylobacter enteritis E. coli (EHEC) Leptospirosis	Poliomyelitis Hepatitis A and E Enterovirus Rotavirus Adenovirus Norwalk like virus Coxsackievirus	Amoebiasis Giardiasis Cryptosporidiosis Toxoplasmosis

1.3. Silver susceptibility to Coliforms:

Silver has been known to be a disinfectant for about 1200 years and has been widely used during this century in the treatment of clinical diseases, including newborn eye prophylaxis, topical burn wounds, orthopedic infections, and so on. Silver serves as a potent antibacterial agent acting against an exceptionally broad spectrum of bacteria while exhibiting low toxicity to mammalian cells. Since silver therapy is of significant clinical benefit in the control of bacterial infections, various forms of new agents containing the silver ion, such as creams, solutions, electrodes, ligatures, foils, nylons, biological skin and catheters, have been developed over the past decades in medical, biological and pharmaceutical preparations. Silver is the most toxic element to microorganisms in the following sequence: Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn (Golubovich & Rabatnova 1974, Berger et al. 1976b). Silver ions are also used for a number of nonmedical purposes, such as in electrical appliances. The slow-release “nano-silver” linings of laundry machines, dishwashers, refrigerators, and toilet seats are also marketed and advertised. Silver effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules. They inhibited cell division and damaged the cell envelope and contents of bacteria. Bacterial cells increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Finally, silver ions interact with nucleic acids; they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear.

Three possible mechanisms for inhibition by silver have been proposed:

- Interference with electron transport
- Binding to the DNA
- Interaction with cell membrane [4]

1.4. Objectives of the project:

Simple and cost effective method of treating drinking water for removing bacterial contamination has been developed by the Principal Investigator and the concept needs to be evaluated and field tested for varying field conditions. Council in collaboration with Indian Institute of Science will develop a protocol for treating drinking water by using silver metal.

1. Quantification of bacterial removal in drinking water by silver metal treatment
2. Simple, cost effective and field tested products / methods for mass application and comparison with other methods
3. Documentation and publicizing of the findings

Water quality varies from source to source and place to place. The bacterial contamination of drinking water is one of the major concerns for both rural and urban population leading to health issues. The established methodology will also be compared with the domestic water purifiers in the market and also in other research laboratories. The target beneficiary is the society and public in general. As the concept is to develop a protocol for water treatment and not a product with commercial interest

A simple concept will be evolved and used both in urban and rural sectors. The product will be developed at individual household level on do-it-yourself basis. Several products may be developed using the same concept and the protocol, so developed for various applications of domestic water treatment.

C**hapter 2**

LITERATURE SURVEY

2.1. Sampling:

Grab sampling from various sources such as bore well water, open well water, pond water; tap water and rain water was carried out. The recommended maximum elapsed time between collection and analysis of sample is 8 hours. When analysis cannot begin within 8 h, maintain the sample at a temperature below 40 ^{0}C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24 h (Standard methods for the examination of water and waste water, 21st edition, 2005).

2.2. Standard methods of drinking water treatment:

Water is one of the most commonly used substances on our earth. We need water for all our activities in day-to-day life. Water supply in urban area is always short against the total demand. Surface water is inadequate to meet our demand and we have to depend on ground water. Due to rapid urbanization, infiltration of rainwater into the subsoil has decreased drastically and recharging of ground water has diminished. This scenario requires an alternative source to bridge the gap between demand and supply.

Water from Rainwater collection, open well, bore well, lakes, ponds, rivers and canals can provide water for domestic use. However, all these sources have varying degree of contaminants, rainwater being the purest and the surface water stored in an open pond may be the most contaminated. All the above sources need to be treated for potable purposes.

Rainwater collected from a surface is relatively free from salts and other dissolved impurities compared to ground water from bore well, open well or water from river. However, rainwater collected from the surface had bacteriological contamination. Fecal coliform and E coli are the common and harmful bacteria present in all the above listed sources of water.

Common methods for treating water are

- Boiling
- Chlorination
- Ozone treatment
- Ultra Violet light treatment
- Reverse osmosis
- Lime treatment
- Treatment with potassium permanganate

All these treatments are effective in large-scale water treatment and are expensive or beyond common man's reach. In addition, all the above methods need either electricity, fuel or chemicals and the water treatment vary with the process and the control mechanism.

New simple and eco-friendly methods for treating water need to be standardized and popularized for domestic and individual drinking water treatment.

2.3. Coliform bacteria

Coliform bacteria are the Gram-Negative, Non-Spore forming bacilli that ferment Lactose with the production of acid and gas. *Escherichia coli* are the most extensively studied and best understood bacterium. A large data base exists to which comparisons may be readily made. Moreover, recently *E. coli* has become a theat to human health because of publicized outbreaks of *E. coli*. Various concentrations of silver exert bacteriostatic or bactericidal action to a different degree. The group of coliform bacteria as an indicator of other pathogenic micro-organisms, specifically organisms of faecal origin, has much emphasis in all countries. This is due primarily to the fact that the coliform bacteria groups meet many of the criteria for a suitable indicator organism, and are thus a sensitive indicator of faecal pollution:

- They are abundant in faeces (normal inhabitants of intestinal tract)
- They are generally found only in polluted waters
- They are easily detected by simple laboratory tests
- Can be detected in low concentrations in water
- The number of indicator bacteria seems to be correlated with the extent of contamination [8].

It is important to remember, however, that not all coliforms emanate from human originate from other mammalian species or from other environmental sources (eg: bird droppings).When coliforms are discharged to the aquatic environment, they will tend to die at a rate which depends, amongst other things, on the temperature and turbidity of the water and the depth to which solar radiation penetrates. Therefore, it is not safe to conclude that the lack of coliforms in water means that it has not been subjected to feacal pollution.

Total Coliforms:

The Total coliform group comprises several distinct types (genera) of bacteria. These bacteria have been isolated from the feaces of humans and other warm-blooded animals. Some coliform bacteria are normal inhabitants of soil and water. In testing of coliforms, therefore tests may be run in conjunction to verify their feacel origin. However, this group of bacteria is widely used as a measure of health hazard from feacal contamination. Pathogenic bacteria and viruses causing enteric diseases in humans also originate from feacal discharges of diseased persons. Consequently, water containing coliform bacteria is identified as potentially dangerous. The Total coliform group comprises the aerobic and facultative, gram negative, non-spore forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35^0C.

Feacal Coliforms (Thermo tolerant coliforms):

The feacal coliform group of bacteria is indicative of feaces of humans and other warm blooded animals. The specific bacterium *Escherichia coli* is a part of this group. The test for feacal coliform ia at an elevated temperature, 44.5^0C, where growth of other non-feacel coliform bacteria is suppressed. Only a small percentage of non-feacal bacteria may also be identified I the feacal coliform test (<5%).

Fecal coliform is the standard EPA uses to assure the public of the safety of drinking water, reclaimed water as well as sewage sludge used as biosolids soil amendment and fertilizer. For EPA testing standards, *Escherichia coli* is the primary fecal coliform. *Escherichia coli* is the only member species for which standardized data exists."The standard test procedure showing *E. coli* growth is run at a temperature of 44.5° C. (112.1° F) for 24 hours. On the other hand, the human body will die at an internal temperature of 42.5° C. (108.5° F) and blood is said to coagulate at 42.6° C (108.68° F). The question then becomes, are the test really showing *E. coli* from the human gut? Any so called study puts public

health at risk by discussing heat inhibited thermotolerant antibiotic resistant, the high heat of the fecal coliform test inactivates many of the bacteria since the optimum growth rate for *E. coli* (coliform) is 98.6 deg F when incubated for 24 hours. At the optimum temperature *E. coli* will double every 20 minutes. As an example, if there was only one *E. coli* bacteria at the start of the test doubling every 20 minutes, in 12 hours the colony would be composed of 68 billion, 719 million, 476 thousand, and 736 hundred bacteria. That would be counted as one most probable number (MPN) colony forming unit (CFU) for the test result and the test still has 12 hours left -- but it would still be counted as one MPN. Organisms have evolved to survive at different temperatures. The fecal coliform test used for drinking water tells us nothing about the *E. coli* that grows at human body temperature or the changes due to antibiotic treatment for diseases such as Urinary tract infections that may be caused by bathing in contaminated drinking water or dental diseases caused by bacteria not shown in the test. If Total Coliform are the pathogenic entero-bacteriaceae family of enteric bacteria, including *E. coli*, found in the intestinal tract of animals and humans. E. coli 0157:H7 doesn't show up in the coliform test even when it grows at 44.5° C.

Escherichia Coli (*E. coli*):

This bacterium is a particular member of the feacal coliform group of bacteria; the presence of this organism in water indicates feacal contamination. The bacterium *E. coli* is exclusively to feacal origin. *E. coli* reside in human intestinal tracts. They are excreted in large numbers in feaces, averaging about 50 million per gram. Untreated domestic waste water generally contains 5 to 10 million coliforms per 100ml.The degree to which indicator organisms represent the presence of individual pathogens (such as salmonella) has been the subject of continuing investigation. There does seem to be a general correlation between the concentration of Feacal coliform bacteria and the occurrence of *salmonella*. When feacal coliform numbers is about 1000 per 100ml, *salmonella* occurrence is about 95% .Relationship between total coliform and individual pathogens is not so quantitative. Thus the test of total coliform is not as effective as an indicator. The Total coliform test is complicated by the presence of non-feacal bacteria. As a general rule, feacal coliform levels are about 20% of total coliform concentrations, although a wide spread exists.

Besides being the number one cause of human urinary tract infections, *E. coli* has been linked to diseases in just about every other part of the body. Pneumonia, meningitis, and traveler's diarrhea are among the many illnesses that pathogenic strains of *E. coli* can cause. Pathogenic strains of *E. coli* can cause severe cases of diarrhea in all age groups by producing a powerful endotoxin. [Central America Shigella strain Toxin] Treating *E. coli* infections with antibiotics may actually place the patient in severe shock which could possibly lead to death. This is due to the fact that more of the bacterium's toxin is released when the cell dies.

E. coli strains, tame commercial recombinant Escherichia coli Host strains, and at least 200 0: H *E. coil* strains such as 0157:H7. *E. coli* is the primary member of the coliform group with the ability to ferment lactose at 44.5 degree C (112.1°F) within 24 hours. The fermented lactose causes the test sample to change colors. Verification of *E. coli* is achieved by a color change Yellow = total coliforms, Yellow/fluorescent = *E. coli*. Twenty-four hours later you can get a most probable number of viable *E. coli* [8].

Reduction of Coliforms:

Coliform bacteria do not always show up in every sample. They can be sporadic and sometimes seasonal. If coliform bacteria are present, the source of the problem should be identified. Re-sampling from several locations within the system will be helpful. Silver is completely bound to proteins when

applied topically. The ease of formation of insoluble compounds with anions, sulfhydryl groups, and many biological materials such as enzymes, is responsible for the disinfectant activity of silver [11].

Martin noted that silver reacts with ether sulfur, as occurs in methionine. Chang reported that silver requires a long contact time for disinfection and does not enter the cell. Rather it forms reversible sulfhydryl or histidyl complexes on the cell surface and prevents the dehydrogenation process. He noted further that permissible levels of silver (50 ppb) are too low for any effect. Protein de-naturation is more difficult than oxidation of complexed sulfhydryl groups, which explains why higher residuals are needed for viruses than bacteria.

Rahn and Landry noted that silver can bind phage DNA, increasing the rate of dimerization inside the phage upon UV radiation. Grier stated that silver can complex with electron donor groups containing sulfur, oxygen or nitrogen. Reversible binding of bases occurs without aggregation or disruption of double helix. Intercalation of silver can lead to increased stability of double helix. Upon entering the cell, the molecule may dissociate where the silver binds the DNA. James reported that all silver salts are bactericidal and the metallic silver dissolves in water to an extent (10^{-5} g/l) which is toxic to *E. coli* and *Bacillus tryphoses*. It is the concentration of silver ions, not their physical nature that is responsible for disinfection capability.

Phillips and Warshowsky noted that finely divided metallic silver presents an excellent oxidative catalytic surface, which reportedly can complex proteins and nucleic acids. Chambers et al. found that the greatest kill rates occurred at pH 7.5.

Woodard explained that it is the ability of silver to absorb to surfaces which accounts for the continued germicidal effect after stopping the addition of silver to water. Muller et al. noted that no deterioration in the water quality after the addition of 100ppb of silver to water, which was then, stored 3years in polypropylene containers. During the storage period, nearly all the silver adsorbed on the walls of the container yet continued to prevent bacterial growth. It was reported that, while *salmonella* is at least as sensitive as *E. coli*, the cost and slowness of disinfection by silver may limit its use. A bactericidal effect has been reported for both silver salts (acetate, nitrate and sulfate) and electrolytically produced silver, with no significant observed difference when used in water from a public distribution system. Moroz et al. stated that a concentration of 50 to 200ppb silver kills *salmonella* and *E. coli* as well as bacteria that are highly resistant to antibiotics [11].

Since ancient times, the silver ion has been known to be effective against a broad range of microorganisms [13]. Today, silver ions are used to control bacterial growth in a variety of medical applications, including dental work, catheters, and the healing of burn wounds Silver ions are also used for a number of nonmedical purposes, such as in electrical appliances. The slow-release “nanosilver” linings of laundry machines, dishwashers, refrigerators, and toilet seats are also marketed and advertised. It is clear that we are exposed to a wide range of mostly unfamiliar uses of silver containing products intended to function as antimicrobial biocides. Therefore, it is necessary to elucidate the antimicrobial activity of the silver ion, which is widely used in these products.

2.4. Water quality analysis

Bacteriological parameters:

The standard test for the coliform group may be carried out either by the Multiple - tube fermentation technique or presence – absence procedure (though the presumptive-confirmed phases or completed test) described herein, by the membrane filter (MF) technique or by the enzymatic-substrate coliform test. Each technique is applicable within the limitations specified and with due consideration of the purpose of the examination. Production of valid result requires strict adherence to quality control procedures [1].

When multiple tubes are used in the fermentation technique, results of the examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of coliforms in the sample.

The precision of each test depends on the number of tubes used. The most satisfactory information will be obtained when the largest sample inoculums examined shows gas in some or all of the tubes and the smallest sample inoculum's shows no gas in all or a majority of the tubes. Bacterial density can be estimated from the table using the number of positive tubes in the multiple dilutions. The number of sample portion selected will be governed by the desired precision of the result. MPN tables are based on the assumption of a Poisson distribution (Random Dispersion). However, if the sample is not adequately shaken before the portions are removed or if clumping of bacterial cells occurs, MPN value will be an underestimate of the actual bacterial density.Mainly concentrating on Bacteriological parameters, Under Multiple tube fermentation technique we have following tests.

Total Coliform Test:

The test for total coliform bacteria is usually conducted using a liquid culture. Enumeration employing solid culture media is not commonly done in India. The liquid culture 'multiple tube technique' consists of 2 stages such as presumptive test and Confirmed test. The presumptive test is based on gas production during fermentation in enrichment medium which contains beef extract, peptone and lactose within 48 hours incubation at 35^{o}C. The confirmed test is used to substantiate or deny the presence of coliforms in a positive presumptive test. A small inoculum from a positive lactose broth is transferred to a tube containing brilliant green lactose bile broth. The green dye and bile salts in this broth inhibit non-coliform growth. The presence of coliform is confirmed by growth and gas production within 48 hours at 35^{o}C. The Most Probable Number (MPN) of total coliform is then calculated from the number of confirmed tubes.

Feacal Coliform Test:

Sometimes a 'completed test' may be performed to determine the feacal origin of the coliforms giving positive confirmative test. These tests involve subscribing of positive tubes on solid media and testing for further bio-chemical reactions. Elevated temperature test for the separation of organisms of coliform group into those of faecal and non-faecal origin may also be performed. In this test, transfers from all positive presumptive tubes are made to culture tubes of EC medium which contains Bile slats and sodium chloride as selective agents along with the nutrients. The inoculated tubes are incubated at $44.5 \pm 0.2\ ^{o}$C. Gas production within 24 hours is considered a positive reaction indicating coliforms of feacal origin.

Chapter 3

MATERIALS AND METHODOLOGY

3.1. Sample sources:

3.1.1. Rain water:

Rain water is the water that has fallen as rain and contains little dissolved mineral matter. Rain is a major component of the water cycle and is responsible for depositing most of the fresh water on the Earth. The most common hazard in water sources obtained from roof or surface catchments is microbial (biological and microbiological) contamination, especially enteric pathogens. Enteric pathogens are micro-organisms (bacteria, viruses, and protozoa) that cause gastrointestinal illness. These organisms are introduced into drinking water supplies by contamination with faecal material (from human or animal origin) or dead animals and insects (enHealth, 2004). The most important indicator is *E-coli*. Chemical contamination results from air pollution (industrial and traffic emissions), run-off and leaching of chemical substances (agricultural and human activities) and toxic material use; all these factors can pose a serious a health theat. However, in rural areas of developing countries, these activities are mostly absent or very small- scale (for example: fireplaces near a roof or having a chimney can cause soot to settle on the roof), and are therefore unlikely to cause significant impacts on the quality of the collected rainwater (enHealth, 2004). Physical contamination includes inorganic and organic sediments like sand, silt, clay, or plant material. Physical contamination affects the colour, odour or taste of the water, but it poses no direct health risk. Users can however object to water if its colour, odour and taste are found less attractive [10].

We selected stored rain water as sample for our experiments. We found that the silver treatment for rain water was very efficient and the treated water became potable after 8-24 h as the initial value of the sample obtained was low for MPN/100ml.

3.1.2. Open well water:

Figure 1: Open well

Open well water is underground water that is held in the soil and in pervious rocks. The contaminants in the open well may be bird droppings, leaves and other dust particles.

We selected open well water from IISc Campus and surrounding areas as sample for our experiments. We found that the silver treatment for open well water was efficient and the bacterial count (MPN/100ml) gradually decreased in the treated water up to 24 h.

3.1.3. Bore well water:

Figure 2: Bore well

Bore well water is underground water that is obtained by drilling tube wells. The contaminants differ from place to place. We selected Bore well water samples from different parts of Bangalore city such as Vijaynagar, Rajajinagar, RTNagar, Chandra Layout, Doddabommasandra, Malleshwaram. MPN Values differed from one source to another. We found that the Silver treatment was efficient in reducing bacterial count for a treatment period of 8 to 48 h.

3.2. Silver sheet immersion method:

Silver (99.9%) was tested for its purity and made in to sheet form. The method used for treating water using silver sheet of 0.1mm thickness is as follows:

- Fill 1lt of sample into a Stainless Steel container.
- Pipette out 100ml of sample though a sterilized pipette to a sterilized bottle (sample before treatment), shake well and close it with the cap.
- Close the lid of the Stainless steel container.
- Keep it in the laminar air flow chamber under aseptic conditions under room temperature until inoculation.
- Silver sheet of 31 g inserted in to the water sample (silver sheet to be washed and wiped to dry before immersing in to the water sample).
- After 2 hours of treatment, sampling is drawn as above. Repeat the process after every 2 hours.
- Repeat the sampling procedure at 24 hours.
- Subject the samples for testing using CFU or MPN method.
- Preserve the silver sheet in water until it is used for the next experiment.

Figure 3: Container with silver sheet immersed inside the water sample

3.3. Methodologies to treat water using silver sheet and to analyze water for microbial count

3.3.1. H_2S vials test

H_2S Test kit for microbiological quality of drinking water is a simple device to test the potability of drinking water in the field. This test does not need any laboratory facility [6]. It is inexpensive, reliable and convenient method of testing a sample of water in the field conditions for its potability. Water borne diseases like Cholera, Typhoid, Diarrhoea, a Jaundice are caused by consumption of polluted water. The change of color of the Test kit medium indicates its fitness for drinking or not. The conventional method of testing the microbiological quality of water test needs a microbiologist and a well equipped laboratory. It is an expensive and lengthy test. Desired result is expected only after almost 72 hours. The conventional method of testing the microbiological quality of water test needs a microbiologist and a well equipped laboratory. It is an expensive and lengthy test. However, H_2S Test Kit indicates the desired result within 18 hours and is made available at low cost and most useful in our rural areas in which test can be carried out even by an un-educated person.

Advantage of the test:

- It is field test; hence water sample can be directly collected from the tap.
- No need to remove the chlorine in chlorinated water, since the content of the bottle is instantaneously removes it.
- No need to measure the volume of water as the level of label indicates it is 20 ml.
- The test is simple, rapid and inexpensive.
- The test can be even done by uneducated person in the field.

Apparatus used: Stain less steel container, H_2S vials, silver foil (0.1mm x 23 cm x 43 cm), 1litre sterilized water bottles (PET).

Procedure:

- A stain less steel container (11litres capacity) was filled till the rim with the sample and a silver sheet (23cm x 43cm size, 136.6g) was inserted (silver sheet is washed and wiped to dry) and the container was closed with the lid.
- After 2 hours, 22ml of sample was pipette out from the container into the H_2S vial (vial shaken and kept for observation).
- Again after 4hours 22ml of sample was pipette out from the container into the H_2S vial (vial shaken and kept for observation).
- Similarly, for every 2 hours sample was pipette out from the container. Experiments were repeated at 2^{nd}, 4^{th}, 6^{th} and 8^{th} hours of the day.
- The sample in the container was kept overnight till 24 hours.

3.3.2. Heterotrophic Plate Count Method (HPC):

The Heterotrophic Plate Count (HPC), formerly known as the standard plate count, is a procedure [11] for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included the term “colony-forming units” (CFU). The final count also depends on interaction among the developing colonies; choose the greatest number of colonies within the designated incubation time. To compare data, use the same procedure and medium. Three different media are described. Selection of method out of Pour plate method, Spread plate method, Membrane filtration method is required.
The spread plate method causes no heat shock and all colonies are on the agar where they can be distinguished readily from particle bubbles. Colonies can be transferred quickly; however this method is limited by the quantity of sample or diluted sample that can be absorbed on agar; 0.1 to 0.5ml, depending upon the degree to which pre-poured plates have been dried. To use this procedure, we maintain a supply of suitable pre-dried, absorbent agar plates.
For counting and recording, Consider only plates having 30 to 300 colonies in determining the plate count. If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plates having a count nearest 300 colonies. Compute the count using the formula as given below:

CFU/ml = Colonies counted /Actual volume of sample in dish, ml

The term CFU is descriptive of the methods used; therefore report all counts as colony forming units.

If plates from all dilutions of any sample has no colonies, report the count as <1 divided by the corresponding largest sample volume used. If number of colonies per plate far exceeds 300, do not report result as “too numerous to count” (TNTC). If spreading colonies are encountered on the plate selected, count colonies on representative portions only when colonies are well distributed in spreader-free areas and the area covered by the spreader does not exceed one-half the plate area.

When spreading colonies must be counted, count each of the following types as one: a chain of colonies that appears to be caused by disintegration of bacterial clump as agar and sample were mixed; a spreader that develops as a film of growth between agar and the bottom of the Petri dish; and a colony that forms in a film of water at the edge or over the agar surface. The last two types largely develop because of an accumulation of moisture at the point from which the spreader originates. They frequently cover more than half the plate and interfere with obtaining a reliable plate count.

Count as individual colonies similar-appearing growing in close proximity but not touching, provided the distance between them is at-least equal to the diameter of the smallest colony. Count impinging colonies that differ in appearance, such as morphology or color, as individual colonies.

Equipments required are Glass bottle with cap or cotton plugs, Pipette, Petri plates, Test tubes, Glass rod, Stainless steel container and Sterile Gloves.

Preparation of Media is as follows:

- 2g of Luria Broth was put it in 100ml De-Ionized water or distilled water and mix well until it dissolves completely.
- 2g of Agar-Agar was put it in to the same bottle of 100ml DI water and mixed well.

Sterilization:

- Petri plates, test tubes in a plastic bag and pipette, glass rod were wrapped in an autoclavable plastic bag.
- By loosening the cap of media bottle, keep it in autoclave with the above mentioned equipments in a beaker.
- Autoclaving was done for at 121 ^{0}C for 15 minutes of 15 pounds.
- After autoclaving, all the equipments were kept in refrigerator and the media were kept in Laminar flow chamber.

Dilution of sample:

- Keep about 6 sterilized test tubes in an order and name them starting from 10^0 to 10^{-5}.
- Take 10ml of sample to be tested in one tube.
- Put 9ml DI water in to left out tubes.
- Take 1ml sample and put into 9ml DI water and repeat the same till the last tube.
- After completion of serial dilution, Pipette 0.1ml of diluted sample though a fresh sterilized tip.
- Now the sample is ready to inoculate.

Inoculation of sample:

- Close the door and Switch on the Ultraviolet light for 10mins before working in Laminar flow chamber.
- After switching off the UV light, switch on the blower.
- Wipe the table and hands with ethanol for maintaining aseptic conditions.
- Keep the tubes ready to inoculate and Switch on the spirit lamp to avoid any bacteria to enter the Laminar flow chamber.
- Keep the Petri plates half open and pour the hot (80 ^{0}C) Media into the plates and keep for few minutes for solidification.
- Inoculate 0.1 ml of sample on to the solidified media and spread immediately using sterilized spreader and close the lid and name them.

Incubation: Keep the Petri plates upside down in the incubator at 37 ^{0}C for 24 h for the bacterial growth.

Counting Colonies: After 24h of incubation

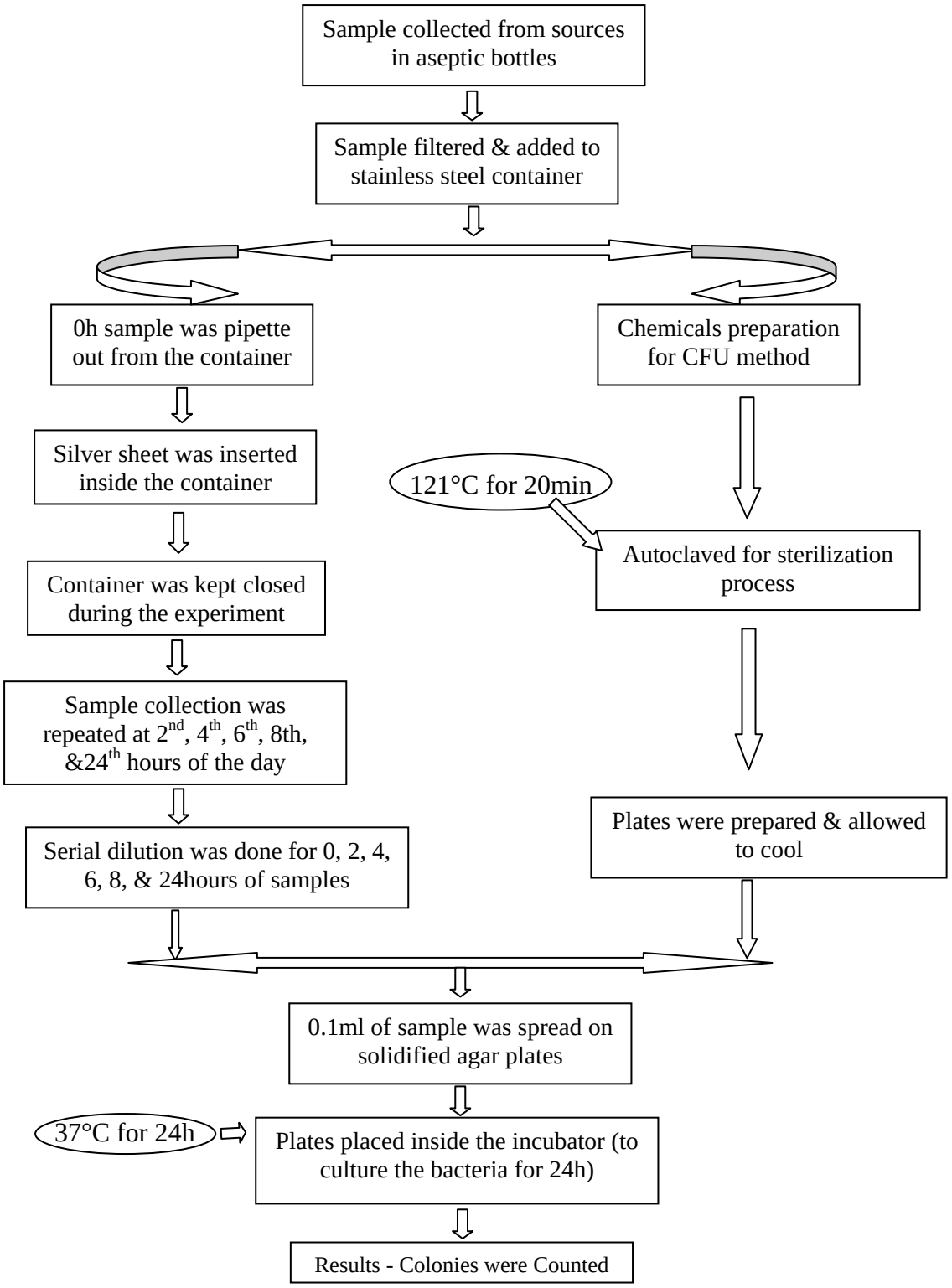

Figure 4: Flow chart and experimental details of CFU procedure

3.3.3. Multiple Tube Fermentation Technique – Most Probable Number (MPN):

This is a standard qualitative analysis of water for the determination of Most Probable Number (MPN) of Coli form Bacteria [10].

- Presumptive test
- Confirmative test
- Completed test

Materials required are Test tubes, Durham's tubes, Cotton plugs, Bunsen Burner, Inoculation Loop, Stainless steel container, Sterile Gloves, Pipettes, Distilled water, Measuring cylinders, Glass rod, Measuring Beaker, Conical Flask, Autoclavable Testube Stands, Autoclavable Covers/Bags.Equipments Required are Weighing Machine, pH meter, Autoclave, Inoculation Chamber with Laminar Air flow, Bacteriological Incubator.Chemicals required are Tryptose, Lactose, Dipotassium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium Chloride, Sodium Lauryl Sulphate, Bile salts and Sodium Hydroxide Pellets.Types of test Carried Out:

- Total Coliforms (TC)
- Feacal Coliforms (FC) / (Ecoli)

LST Broth preparation for TC presumptive test:

Tryptose	20 g
Lactose	5 g
Dipotassium Hydrogen Phosphate	2.75 g
Potassium Dihydrogen Phosphate	2.75 g
Sodium Chloride	5 g
Sodium Lauryl Sulphate	0.1 g

Mix all of the above chemicals and put it in 500ml distilled water and shake well to get double strength broth, Check the pH of the broth and it should be 6.8 ± 0.2^0C

- Take a series of 10 sterilized Test tubes for each sample and put Durham's tubes to each tube in the inverted form.
- Pour 10ml broth to all the tubes using a sterilized measuring cylinder.
- Prepare Dilution water by adding 34g of Potassium DiHydrogen phosphate in 500ml distilled water and add NaOH pellets to Maintain pH of 7.2
- Pour the dilution water of 9ml to 10ml broth in the 5 test tubes and pour 9.9ml to 5 tubes and mix well.
- Close the tubes with cotton plugs
- Keep all the tubes for sterilization in the Autoclave for 20 minutes at 121^0C.

EC Broth Preparation for FC confirmative test:

Tryptose	20 g
Lactose	5 g
Dipotassium Hydrogen Phosphate	4 g
Potassium Dihydrogen Phosphate	1.5 g
Sodium Chloride	5 g
Bile Salts	1.5 g

- Mix all of the above chemicals and put it in 1000ml distilled water and shake well to get single strength broth.
- Check the pH of the broth and it should be 6.9 $\pm$ 0.2^0C
- Take a series of 10 sterilized Test tubes for each sample and put Durham's tubes to each tube.
- Pour 10ml broth to all the tubes using a sterilized measuring cylinder.
- Keep all the tubes for sterilization in the Autoclave for 20mins at 121^0C
- Bring down all the tubes to room temperature.
- Pair each positive presumptive fermentation tube with a fermentation tube containing EC broth. Mark each EC tube to match its paired presumptive tube.
- Mark each EC tube to match its paired presumptive tube.
- Using a sterile transfer loop, transfer a portion of the liquid from each presumptive tube to its paired EC broth fermentation tube under aseptic condition.
- Incubate the tubes for 48 hs at 44.5$\pm$ 0.5^0C.

Inoculation of Sample:

- Bring down all the tubes to room temperature and put 10ml for 5tubes, 1ml for 3tubes and 0.1ml for 2tubes under aseptic conditions
- Mix well the broth and samples and keep it in incubator at 35$\pm$$0.5^0$C for 48 h
- Check for positive tubes of acid and gas formation
- Note down the Number of positive tubes in 10 ml, 1 ml, and 0.1 ml sample tubes and calculate MPN/100ml of Total Coliforms.

Aseptic Conditions:

- Working desk and Hands to be rinsed with 95% ethanol before touching any sterilized material
- Sampling to be done though a sterilized pipette
- Samples to be collected in a sterilized bottle and mix well before inoculation
- Sample Inoculation must be carried out in Laminar air flow chamber
- Flame to be used every time during inoculation and to heat the loop
- Flame sterilizes metal loops before each transfer or use individual pre-sterilized loops or wood splints for each transfer.
- All the glass wears should be washed by 1N Hydrochloric acid.

3.3.4. Reduced Flow Method

Apparatus used:

a) Inlet drum (SS) with lid and tap - 10 litres capacity.
b) Drip wire (1.5m long and 2mm pipe diameter) connected to Inlet drum.
c) Two SS containers coaxial to each other are placed one inside the other with a gap of 3 mm annular space between the walls of the container for inserting silver sheet.
d) Three different sizes of silver sheets with total weight of 156.334g,
    a. large size of silver sheet weighing117.305g
    b. small size of silver sheets weighing 15.730g and 23.296g

Experimental setup:

A total quantity of 7 litres of water was poured in to a stainless steel inlet drum of 10 litres capacity. The water sample was made to flow slowly from inlet drum though drip pipe. Then the water sample was made to flow drop by drop into the outer container at the bottom. A smaller inner container is provided with a small hole at the top for the water to enter into the inner drum. Large size of silver sheet (117.305g) placed between two containers (larger and smaller SS drums).

As the water flows into the outer larger container which has silver sheet, large surface area of the silver will come in contact with small quantity of slow moving water. Later treated water gets collected in the smaller inner container which has two silver sheets (15.730g and 23.296g) placed in circular form. Water sample is drawn from the inner drum for testing.

Grams staining method:

The Gram staining method is named after the Danish bacteriologist Hans Chistian Gram (1853 – 1938) who originally devised it in 1882 (but published in 1884), to discriminate between *pneumococci* and *Klebsiella pneumoniae* bacteria in lung tissue.
(http://www.microrao.com/micronotes/pg/Gram%20stain.pdf)

It is a differential staining method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. This reaction divides the eubacteria into two fundamental groups according to their stain ability and is one of the basic foundations on which bacterial identification are built.

Gram staining consists of four components:

- Primary stain (Crystal violet, methyl violet or Gentian violet)
- Mordant (Gram's Iodine)
- Decolourizer (ethyl alcohol, acetone or 1:1 ethanol-acetone mixture)
- Counterstain (Dilute carbol fuchsin, safranin or neutral red)

Procedure:

Prepare a thin bacterial smear on a clean glass slide and cover with a few drops of crystal violet stains. The crystal violet stain renders all the bacteria uniformly violet. After a minute of exposure to the staining solution, the slide is washed in water. The smear is treated with few drop of Gram's Iodine and allowed to act for a minute. Gram's iodine serves as a mordant. The slide is again washed in water and then decolorized in absolute ethyl alcohol or acetone. A mixture of ecetone-ethyl alcohol (1:1) can also be used for decolorization. The process of decolorization is fairly quick and should not exceed 30 seconds for thin smears. Acetone is a potent decolorizer and when used alone can decolorize the smear in 2-3 seconds. Decolorization is the most crucial part of Gram staining and errors can occur here. Prolonged decolorization can lead to over-decolorized smear and a very short decolorization period may lead to under-decolorized smear. After the smear is decolorized, it is washed in water without any delay. The smear is finally treated with few drops of counterstain such as dilute carbol fuchsin, neutral red or safranin. The slide is washed in water; excess water is removed using a blotting paper, dried in air and heat fixed before observing under microscope.

Those bacteria that hold on to primary dye-iodine complex and remain violet are called **Gram positive** and those which get decolorized and subsequently take up counter-stain (pink/red) are called **Gram negative**. Basic fuchsin (present in dilute carbol fuchsin) stains many Gram negative bacteria more intensely than safranin does, making them easier to see. Some bacteria which are poorly stained by safranin, such as *Haemophilus spp., Legionella spp*., and some anaerobic bacteria, are readily stained by basic fuchsin. In order to ascertain if the staining procedure was satisfactorily conducted, a control smear of known Gram positive organism (e.g., *Staphylococcus aureus*) and a known gram negative organism (*Escherichia coli)* must be stained simultaneously. While the fibrin in a clinical specimen may appear gram positive, the pus cells and epithelial cells are always gram negative.

Applications of Gram staining:

- Differentiation of bacteria into Gram positive and Gram negative is the first step towards classification of bacteria.
- It also the first step towards identification of bacteria in cultures.
- Observation of bacteria in clinical specimens provides a vital clue in the diagnosis of infectious diseases.
- Useful in estimation of total count of bacteria.
- Empirical choice of antibiotics can be made on the basis of Gram stain’s report.
- Choice of culture media for inoculation can be made empirically based on Gram’s stain report.

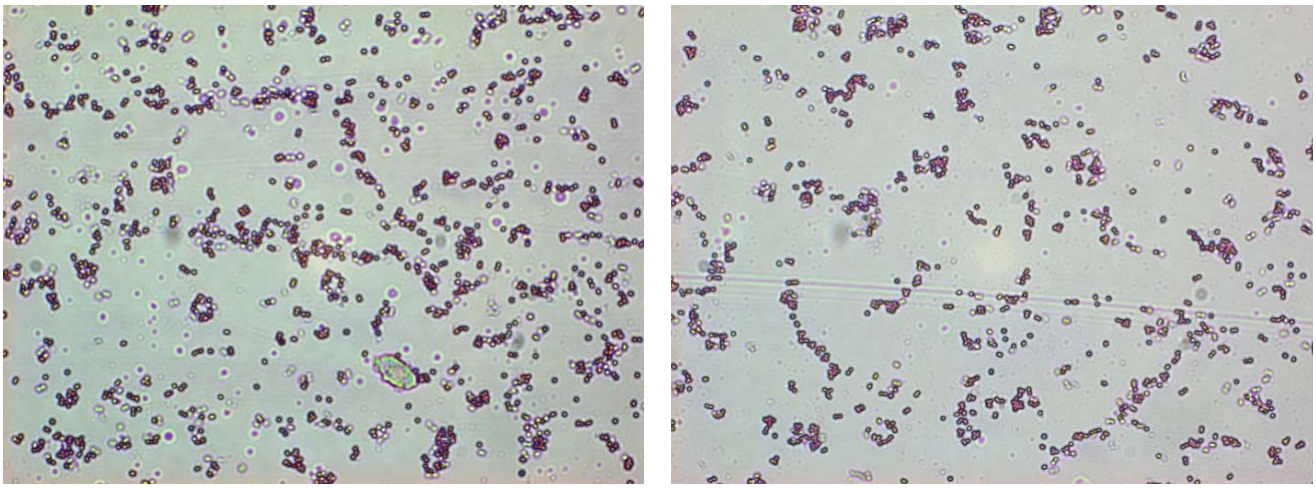

Figure 5: Gram-negative bacteria are red or pink color - indication observed in the water sample

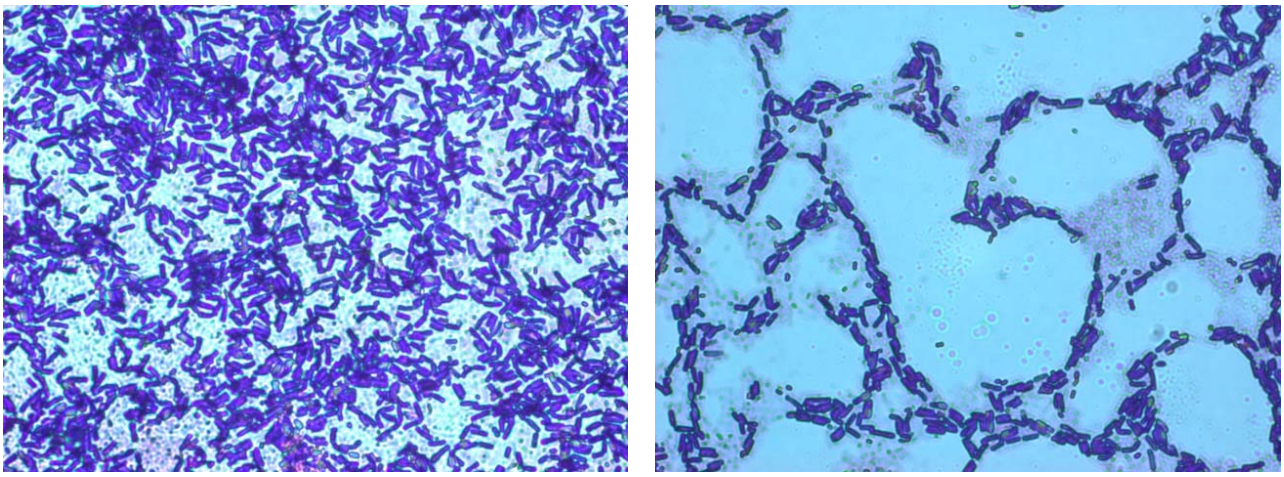

Figure 6: Gram-positive bacteria are purple color - indication observed in the water sample

Microscope used – Compound light microscope

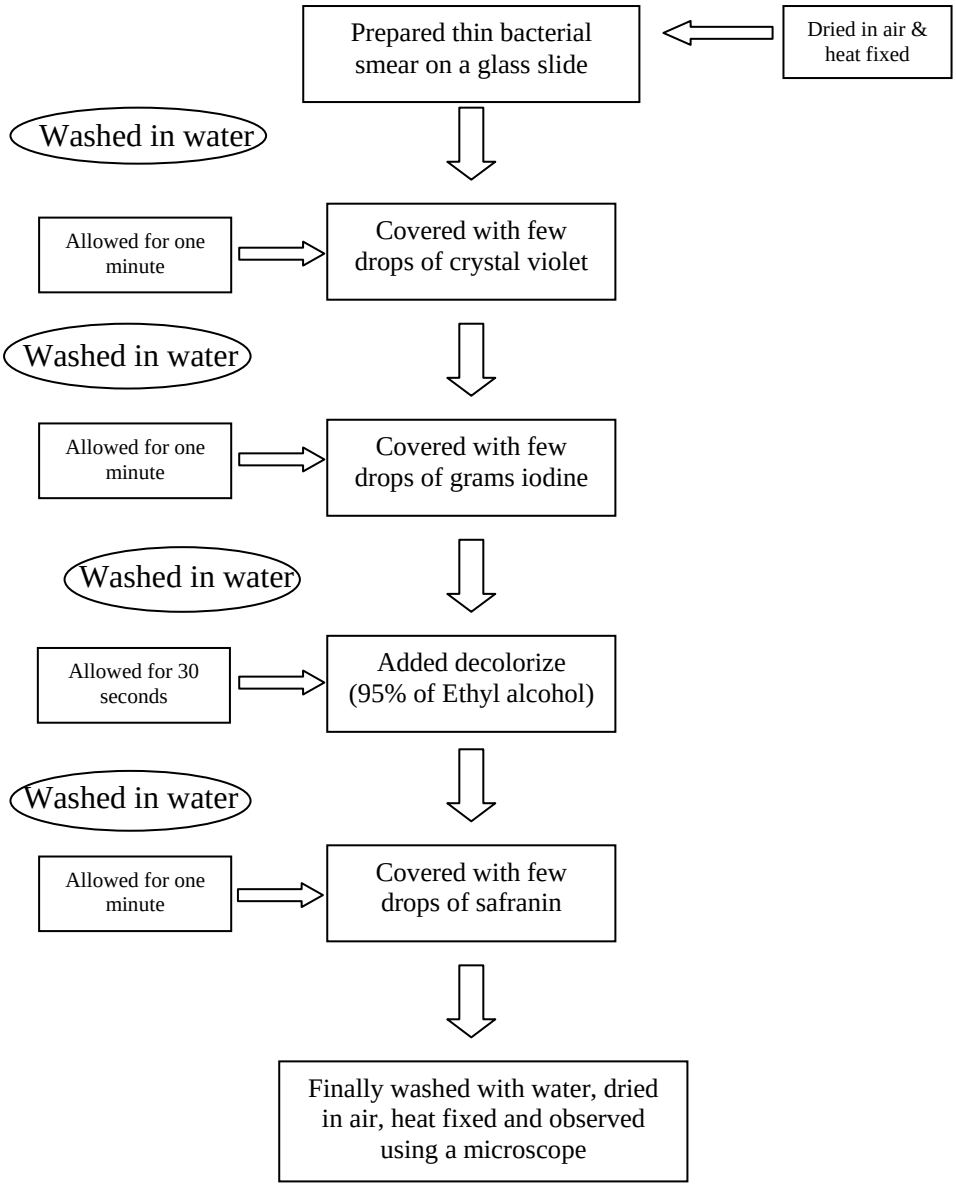

Figure 7: Flow chart on grams staining procedure

Chapter 4

EXPERIMENTS AND RESULTS

4.1. Analysis of water quality by silver treatment

- Quantity of water:

  Water samples of 1, 2, 4 and 11litres were collected to examine the efficiency of silver treatment for different types of bacteria. Samples were drawn from different sources such as bore well water, rain water, pond water and open well water. Experiments were conducted in Three methods (H_2S vials, CFU, and MPN methods) to identify the presence of coliform bacteria.

- Quantity of silver sheet:

  Silver (99.9%) was tested for its purity and made in to sheet form. Following are the different sizes and weight of silver sheets used in the treatment:

  - Sheet 1 – 23cm x 43cm, 136.600 gram (g)
  - Sheet 2 – 22cm x 48cm, 156.507 g
  - Sheet 3 – 3cm x 32cm, 14.004 g
  - Sheet 4 – 5cm x 21cm, 15.730 g
  - Sheet 5 – 9.9cm x 21cm, 23.146 g
  - Sheet 6 – 10cm x 21cm, 23.295 g
  - Sheet 7 – 15cm x 21cm, 31.345 g
  - Sheet 8 –117.305 g
  - 287.759 g (total weight of all silver sheets)

- Container sizes:

  The stainless steel containers were selected for the standard silver sheet immersion test method. Following are the different capacity of the containers (Figures 8 to 10).

Figure 8: 11 litres capacity SS drums – 24.5cm(D) x 23.5cm(H)

Figure 9: 1 litre capacity SS drum – 15.5cm(D) x 18cm(H)

Figure 10: 1litre capacity jar

- Methods of water sampling:

  Samples were collected from a sterile Schott test bottle under aseptic conditions.

  The different methods of sampling:

  - Pipette
  - Pouring
  - Direct insertion of Schott bottle inside the sample container

  Samples of about 100ml were taken out from SS container every time after specified duration of treatment for the test.
- Duration of treatment:
  - A raw sample without any treatment was collected as one sample (0hours).
  - After every two hours, sample was taken.
  - In the later stages of experiment, 0, 8, 24 hours became the standard sampling duration.
  - Control - a sample kept in the SS container without any treatment with lid closed. Silver sheet was not immersed in this sample. The samples from control container were taken at the same time as that of the treated water.
  - For more accuracy of results, 24, 32 and 48 hours of treated samples were also taken for few samples.
- Stirring and mixing:

  Samples in SS container were mixed by stirring several specified number of rotations using sterile glass rod before pipette the sample.

  For few samples mixing was tried with following:

  - Every 2 hours sample mixed for 5 rounds clock wise and 5 rounds anti clock wise, rotated with sterilized glass rod.
  - Every 1 hour sample mixed for 10 rounds clock wise and 10 rounds anti clock wise, rotated with sterilized glass rod.
  - Sample collected 0 h, 8 h, 24 h and 24C h were mixed for 5 rounds clock wise and 5 rounds anti clock wise, rotated with sterilized glass rod.
  - In the later stages an electronic blender was used for stirring the sample to achieve uniform mixing for reduced flow method

4.1.1. H_2S Vials

Experiments were conducted using H_2S vials, Bore well water sample collected from Vijaynagar was tested using H_2S vials procedure as mentioned in chapter 3 (3.3.1). Silver sheet 1 was inserted in 11liters of water sample (before inserting silver sheet, 0 h sample was drawn) and the sample collection was repeated (every time 22ml of sample was collected) at 2^{nd}, 4^{th}, 6^{th}, and 8^{th} hours of treatment. The sample was transferred to the H_2S vials. After 24 hours the H_2S vials were examined. The vials in the solution was black were deemed to have nonpotable water. Conversely, the water was deemed to be potable if the solution remained collarless. The experimental results are tabulated below.

Table 3: Experimental results of H_2S vials test

SAMPLE – Bore well water, Vijayanagar		
Treatment Time in hours	**Sample color in vial / Coliform Count**	**Result**
0	TC – 110 MPN/100ml, FC – 70 MPN/100ml	Contaminated, Non Potable
2	Black	Contaminated, Non Potable
4	Colorless	Non Contaminated
6	Black	Contaminated, Non Potable
8	Colorless	Non Contaminated
24	Colorless, TC – 10 MPN/100ml	Non Contaminated, Potable

Figure 11: Change in color of H_2S vials, color less vials indicate non-contaminant water black color indicate contaminated water

Figure 12: Sample kept in stain less steel container with silver sheet

4.1.2. Heterotrophic Plate Count method:

Bore well water was used for Plate Count Method of water analysis.
Silver sheets were washed with a Modi-Care solution.
Sterile pipettes were used for sampling.
Water was stirred before collecting sample using a sterile glass rod.
Raw & treated samples were given for Physico-chemical & bacteriological analysis at
1. Mines & Geology Department and
2. Essen & Co. Lab.
DI - Water was taken as one sample for comparison.
Sheet 4 was taken in a 1lt SS cointainer.
SS container was filled with the sample till the rim and silver sheet was inserted (silver sheet was washed and wiped to dry before inserting in to water). The container was closed with the lid.

Figure 13: Media Preparation for CFU method

The details of test procedure are given below:

- In this method samples from different sources (Rainwater and Borewell water) were collected.
- 11L of water sample was taken in a stainless steel container to which silver sheet-1 (as mentioned above 4.1) was inserted.
- From which 100 mL of raw water sample was pipetted out into a sterilized Schoot duran bottles before inserting the silver sheet.
- After 2, 4, 6, 8 and 24h of treatment the water sample was pipetted to a sterilized schoot duran bottle and placed it inside the laminar air flow chamber.
- Procedure used: (as per the standard methods) here 0.1 mL of sample from raw water sample and 2, 4, 6, 8 and 24h treated water sample was also pipetted out and spread on a solidified agar plates inside the laminar flow chamber aseptically maintained.
- Agar plates were placed inside the incubator invertedly for 24h at a temperature of 37°C.
- Similar procedure was followed using Rainwater sample.

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Figure 14: Auto clave for sterilization of equipments, De-ionised water and media

Figure 15: Laminar air flow chamber for keeping the samples aseptically

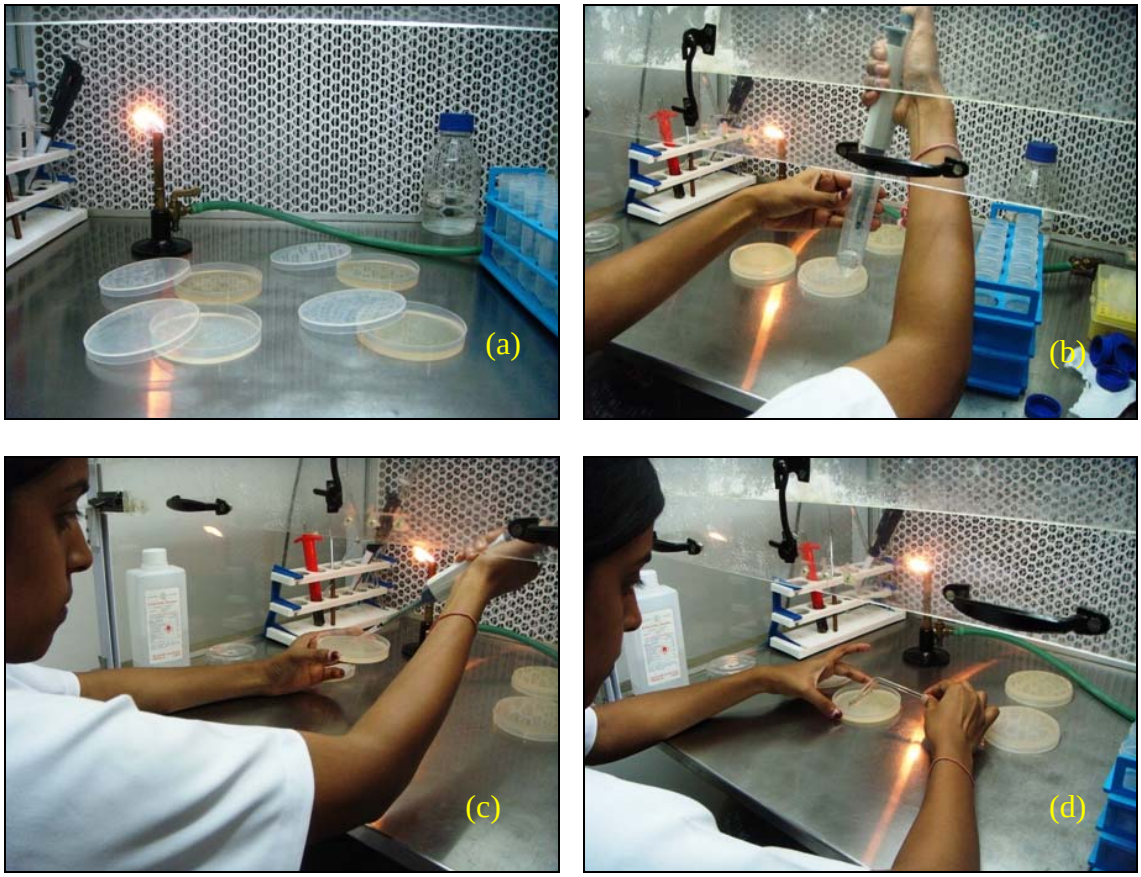


Figure 16: **(a) Agar media plates kept for cooling aseptically inside the laminar air flow chamber**
(b) Diluted water sample for inoculation - 0.1mL
(c) Water sample of 0.1mL being added to agar media
(d) Water sample being spread on agar media plates (aseptically maintained)

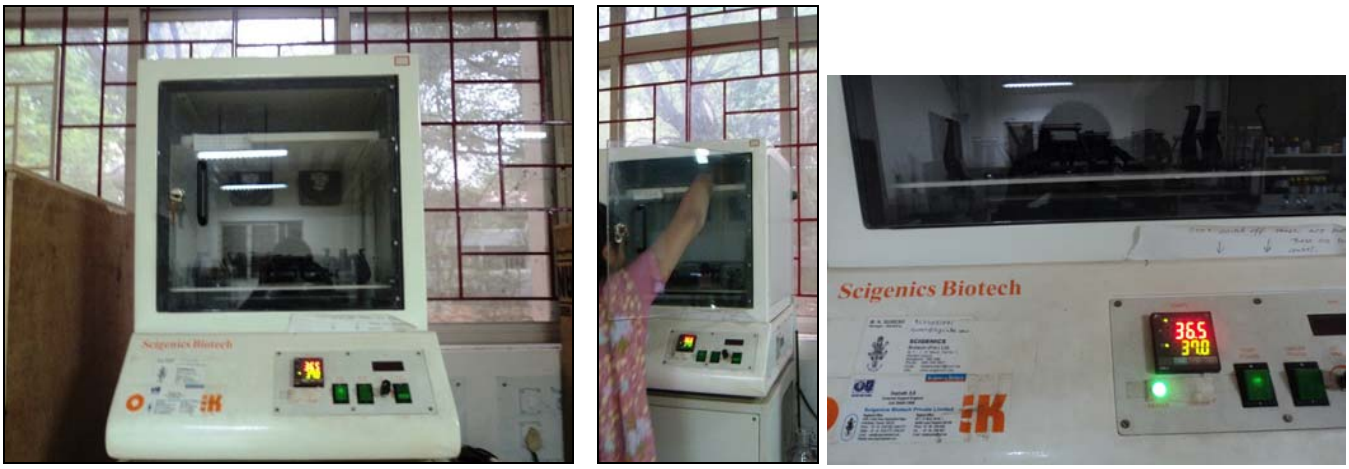


Figure 17: Inoculated samples are kept for incubation for 24 hours at 37°C

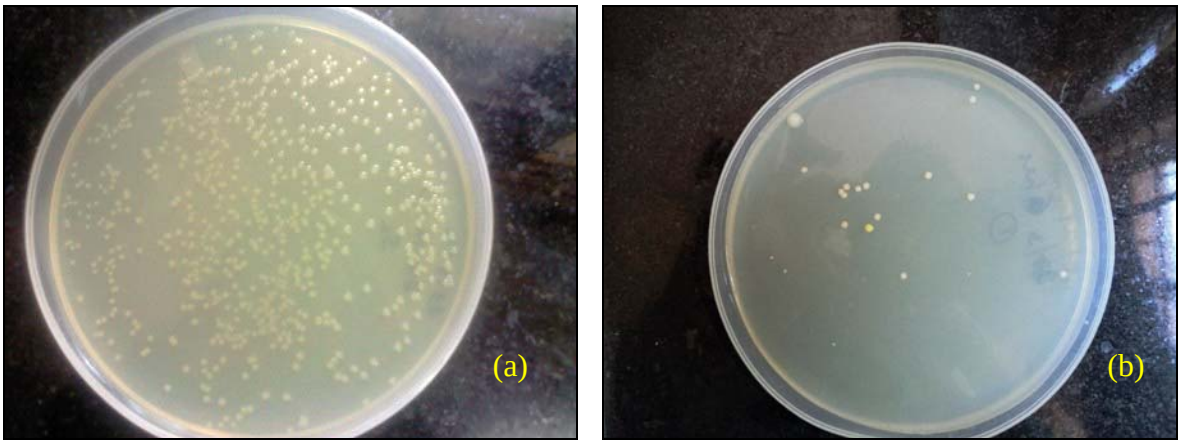


Figure 18: Bacteria growth observed after 24 h of incubation
(a) Growth of too many bacterial colonies in raw water
(b) Reduced bacterial colonies after treating water with silver sheet for 24h

Table 4.1.2: Experimental results of HPC (CFU) method

Sl.No	Sample			CFU / 100ml						
	Source	Quantity	Silver size	0 h	2 h	4 h	6 h	8 h	10 h	24 h
1	Bore well	11 lts	23cm x 43cm	-	26	20	125	80	-	-
2	Bore well	11 lts	23cm x 43cm	28	20	14	8	6	-	127
3	Bore well	11 lts	23cm x 43cm	5	10	14	18	-	-	TNTC
4	Bore well	11 lts	23cm x 43cm	TNTC	7	3	0	-	-	-
		11 lts	22 cm x 48cm	TNTC	4	0	0	-	-	-
5	Bore well	11 lts	23cm x 43cm	4	5	3	2	0	-	0
		11 lts	22 cm x 48cm	4	10	8	6	3	-	1
6	Bore well	11 lts	23cm x 43cm	22	22	15	12	9	-	-
		11 lts	22 cm x 48cm	4	5	3	2	11	-	-
7	Bore well	11 lts	23cm x 43cm	90	_	3	0	-	-	0
		11 lts	22 cm x 48cm	90	_	9	5	-	-	4
8	Bore well	11 lts	23cm x 43cm	16	9	2	2	2	0	1
		11 lts	22 cm x 48cm	16	86	59	15	3	1	1
9	Bore well	11 lts	23cm x 43cm	49	32	13	9	0	0	-
		11 lts	22 cm x 48cm	49	40	27	18	3	3	-
10	Bore well	11 lts	23cm x 43cm	76	56	67	29	5	0	-
		11 lts	22 cm x 48cm	76	51	55	31	7	0	53
11	Bore well	11 lts	23cm x 43cm	63	38	28	3	0	0	-
		11 lts	22 cm x 48cm	63	28	43	4	0	1	-
12	Bore well	11 lts	23cm x 43cm	12	12	0	0	0	0	-
		11 lts	22 cm x 48cm	12	14	0	0	0	0	-
13	Bore well	11 lts	23cm x 43cm	72	0	0	0	0	0	-
		11 lts	22 cm x 48cm	72	2	0	0	0	0	-
14	Bore well	11 lts	23cm x 43cm	15	18	11	26	26	12	18
		11 lts	22 cm x 48cm	33	28	25	43	32	30	28
15	Bore well	11 lts	23cm x 43cm	1	1	0	-	-	0	-
		11 lts	22 cm x 48cm	1	0	0	2	12	0	-
16	Bore well	11 lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		11 lts	22 cm x 48cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
17	Bore well	11 lts	23cm x 43cm	1	15	42	_	23	6	40
		11 lts	22 cm x 48cm	1	TNTC	7	_	30	11	35
18	Bore well	11 lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		11 lts	22 cm x 48cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
19	Bore well	11 lts	23cm x 43cm	TNTC	5	20	14	5	6	_
		11 lts	22 cm x 48cm	TNTC	_	6	22	7	5	42
20	Bore well	11 lts	23cm x 43cm	_	41	23	25	27	25	25
		11 lts	22 cm x 48cm		63	_	20	25	17	22
21	Bore well	11lts	23cm x 43cm	15	17	_	0	0	-	10
		11lts	22 cm x 48cm	15	14	_	0	0	-	7
22	Bore well	11lts	23cm x 43cm	11	20	22	18	20	21	-
		11lts	22 cm x 48cm	16	18	14	23	21	18	-

Sl.No	Sample			CFU / 100ml						
	Source	Quantity	Silver size	0 h	2 h	4 h	6 h	8 h	10 h	24 h
23	Bore well	11lts	23cm x 43cm	1	63	0	0	_	-	23
24	Bore well	11lts	22 cm x 48cm	1	1	0	0	0	-	13,16
25	Bore well	11lts	23cm x 43cm	21,30	24	44	48	66	-	0
26	Bore well	11lts	23cm x 43cm	3	27	_	_	0	-	-
27	Bore well	11lts	23cm x 43cm	_	_	_	0	0	-	-
28	Bore well	11lts	22 cm x 48cm	1	1	1	0	0	-	3
29	Bore well	11lts	23cm x 43cm	60	30	26	20	16	-	TNTC
30	Bore well	11lts	23cm x 43cm	60	45	27	25	58	-	TNTC
31	Bore well	11lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
32	Rain water	11lts	23cm x 43cm	37	9	4	23	40	-	-
		11lts	22 cm x 48cm	70	_	_	_	55	-	-
33	Rain water	11lts	23cm x 43cm	48	TNTC	50	70	TNTC	-	-
		11lts	22 cm x 48cm	56	_	_	_	TNTC	-	-
34	Rain water	11lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
		11lts	22 cm x 48cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
35	Rain water	11lts	23cm x 43cm	40,42	38,40	32,35	35,40	60,52	-	21
		11lts	22 cm x 48cm	40,42	_	_	30	25	-	21
36	Rain water	11lts	23cm x 43cm	TNTC	132,138	1,2	28,20	172,125	-	-
		11lts	22 cm x 48cm	TNTC	_	_	_	37,33	-	-
37	Rain water	11lts	23cm x 43cm	11,9	3	3	6	7	-	-
38	Bore well	11lts	22 cm x 48cm	67,51	42,32	35,31	65,53	120,156	-	-
39	Bore well	11lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	-	-
40	Bore well	11lts	23cm x 43cm	43,36	10,4	4,2	2	2	25,28	-
41	Bore well	11lts	23cm x 43cm	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	-
42	Bore well	11lts	23cm x 43cm	10	42	88	TNTC	TNTC	TNTC	-
43	Pure culture	1lt	5cm x 21cm	4	30	27	45	_	-	20
44	Pure culture	1lt	5cm x 21cm	125	110	100	100	96	-	-
45	Bore well	1lt	5cm x 21cm	_	52	50	50	50	-	-
46	Bore well	1lt	5cm x 21cm	2	3	5	1	2	-	-
		2lts	10cm x 21cm	2	2	2	1	5	-	-
47	Bore well	1lt	5cm x 21cm	1	TNTC	72	63	TNTC	-	-
		2lts	10cm x 21cm	TNTC	TNTC	TNTC	TNTC	TNTC	-	-
48	Bore well	1lt	5cm x 21cm	42	TNTC	TNTC	TNTC	TNTC	-	-
		2lts	10cm x 21cm	42	TNTC	TNTC	TNTC	TNTC	-	-

4.1.3. Multiple Tube Fermentation Technique (MPN Method):

In this method samples (source-Bore well) were collected from Vijaynagar, 1litre of sample was taken in a SS container and silver sheet 4 (as mentioned above 4.1) was inserted. Presumptive test was conducted for 0h, 2h, 4h, 6h, and 8h of treatment. Here 10 tubes were used out of which 5 tubes for 10mL, 3 tubes for 1mL and 2 tubes for 0.1mL. This experiment was continued for about 20 days, the values obtained were not consistent (because of some contamination problem and also incubator could not reach constant temperature of 35°C). Hence, small changes were made in this experimental set up;

Change 1: here we used 11 lts of rain water sample, silver sheet 1 (as mentioned above 4.1) was immersed inside the sample, Same procedure was followed with different volume of samples and number of tubes (5 tubes for 10mL, 5 tubes for 1mL, and 5 tubes for 0.1mL).

Change 2: For 1lt of rain water sample, Sheet 7 (as mentioned above 4.1) was immersed, experiments were continued using sheet 4 (as mentioned above 4.1), Here we got some consistent values and thus we continued the same procedure and same experimental set up for about 30 tests.

Change 3: In the later stage, the bore well water (Magdi road) was used in the experiment for accurate results. Same procedure was followed for number of tests.

Change 4: Later for every one hour before sampling, sample was mixed (by manual stirring) for 30 secs clock wise and anti clock wise with sterilized glass rod.

Change 5: Samples collected for every 2 hours were stirred for 5 times in clock wise and 10 times anti clock wise direction.

Change 6: The samples were collected at 0, 8, 24 hours and 24C (Control - without silver sheet) test was conducted for the sample after 24 hours.

Change 7: Tests were conducted for different water samples from different sources from different areas in Bangalore city, such as bore well water from Vijayanagar, rain water from Vijayanagar, pond water from Vijayanagar, bore well water from Nandini lay out, bore well water from Rajajinagar, bore well water from Magadi road, bore well water from Malleswarum, open well water from Malleswarum, and open well water from Indian Institute of Science Campus. Here above mentioned MPN test experimental procedure was followed, In which changes with respect to the size of silver sheet and quantity of water samples was carried out to get more accurate values.

The standard procedure of MTFT was followed as mentioned in chapter 3 (3.3.3) and the results are tabulated.

Figure 19: Known quantity of water sample inoculated in sterilized test tubes and kept aseptically inside the laminar air flow chamber

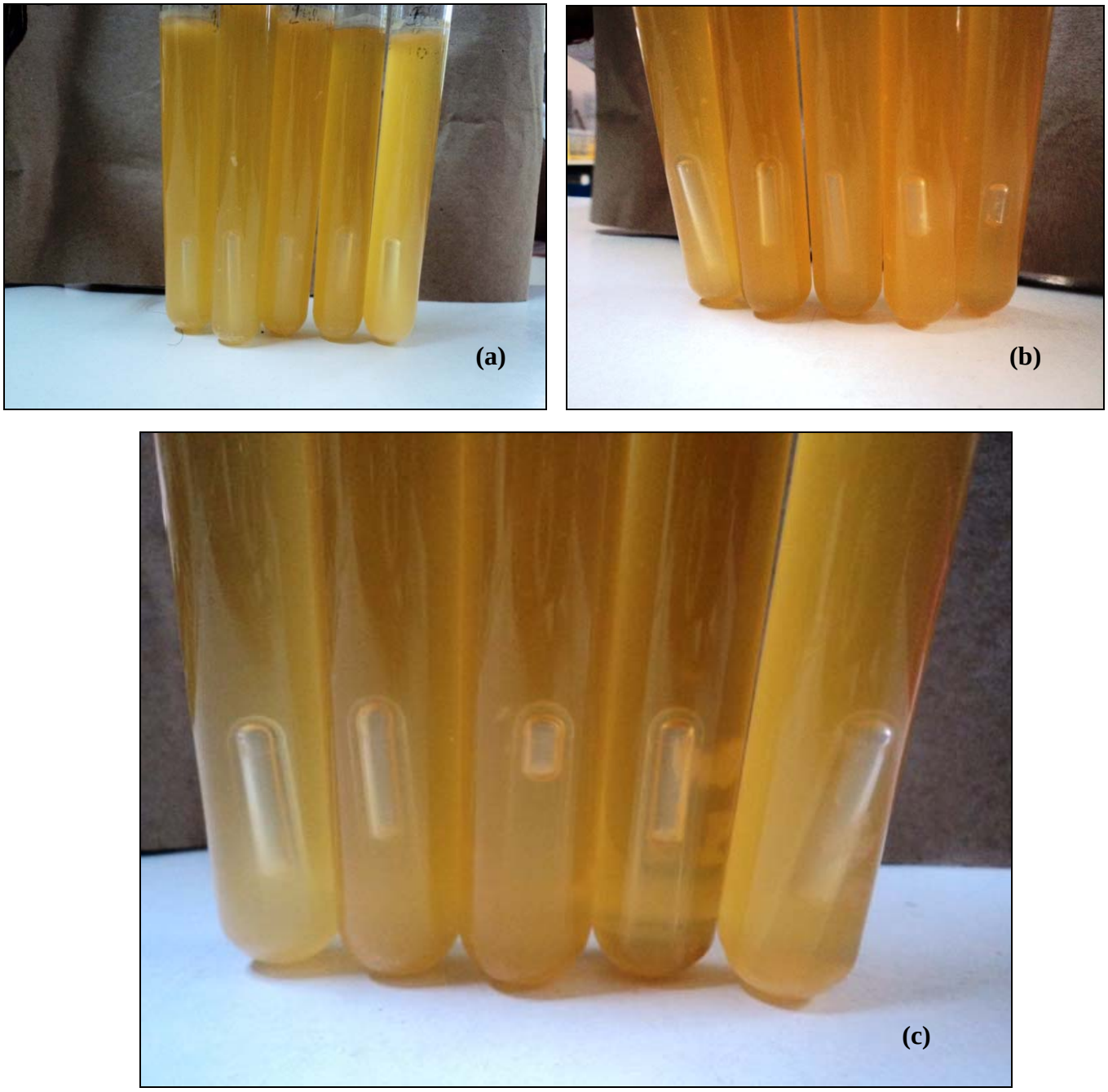


Figure 20: (a) Test tubes observed - all 5 positive tubes – raw water 10mL sample
(b) Test tubes observed - 4 positive tubes – raw water 1mL sample
(c) Test tubes observed - 4 positive tubes – raw water 0.1mL sample
Positive 5 – 4 – 4 tubes indicate 350MPN/100mL

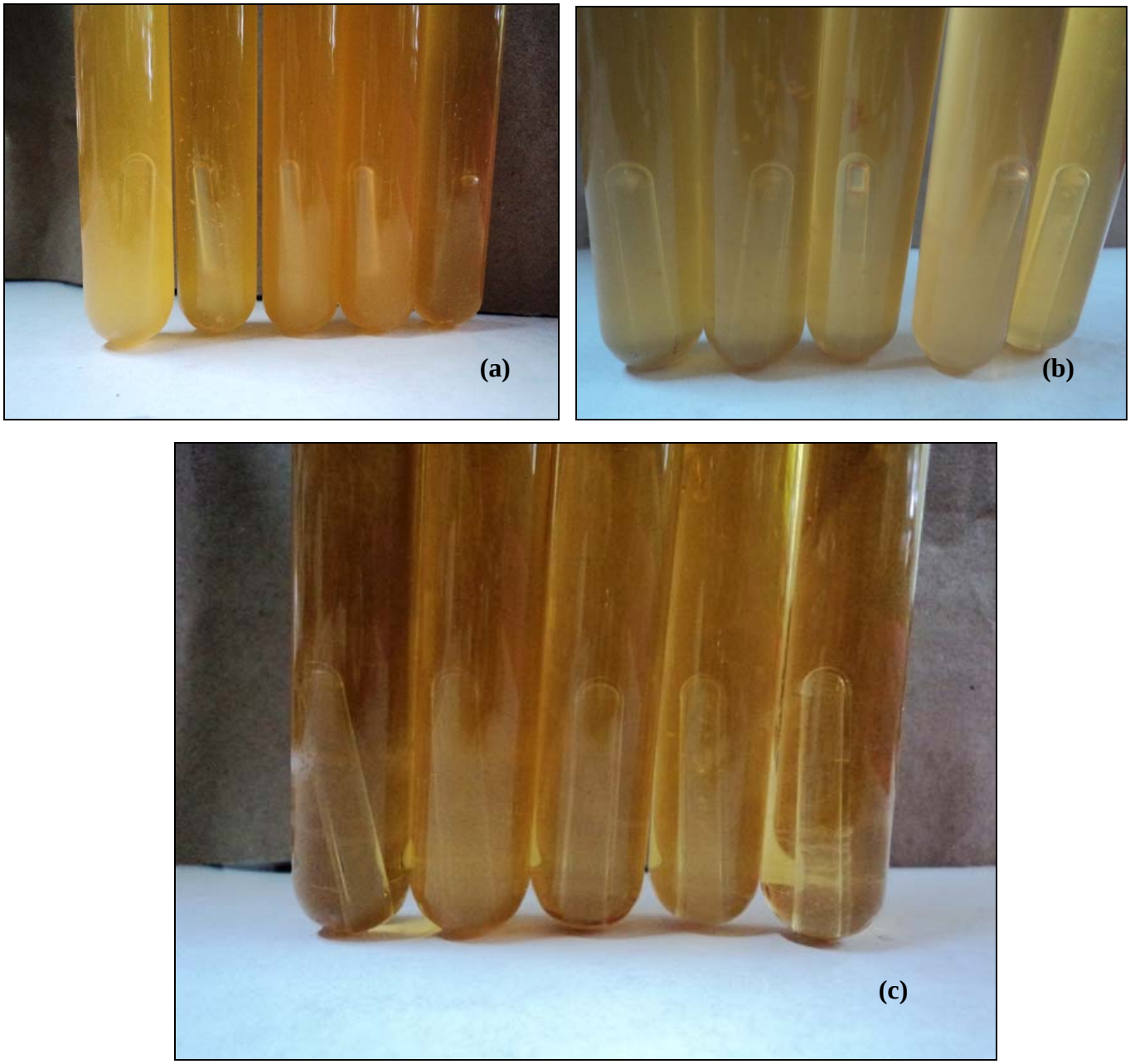


Figure 21: (a) Test tubes observed - 3 positive tubes –water treated for 24 h 10mL sample
(b) Test tubes observed - all negative tubes – water treated for 24 h 1mL sample
(c) Test tubes observed - all negative tubes – water treated for 24 h 0.1mL sample
Positive 3 – & 0 – 0 tubes indicate 9MPN/100mL - water potable

Table 4.1.3: Experimental results of MTFT (MPN) method

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
1	Bore well	1lt	5cm x 21cm	460	240	240	240	210		-			
2	Bore well	1lt	5cm x 21cm	<3	<3	<3	<3	_		-			
3	Rain water	1lt	5cm x 21cm	9.1	<3	3	7.3	11		-			
4	Rain water	1lt	5cm x 21cm	9.1	3.6	7.3	<3	<3		-			
5	Rain water	1lt	5cm x 21cm	93	39	9.1	9.1	9.1		-			
6	Rain water	1lt	5cm x 21cm	<3	<3	<3	<3	<3		-			
7a	Rain water	1lt	5cm x 21cm	9.1	3.6	<3	<3	<3		15			
7b	Rain water	1lt	5cm x 21cm	7.3	_	_	_	<3		-			
7c	Rain water	1lt	5cm x 21cm	3.6	<3	<3	<3	<3		-			
8	Rain water	1lt	5cm x 21cm	9.2	9.2	6.6	6.9	3.6		-			
9	Rain water	1lt	5cm x 21cm	2	30	23	8	4		-			
10	Rain water	1lt	5cm x 21cm	2	23	7	13	8		-			
11a	Rain water	1lt	5cm x 21cm	140	_	30	_	23		-			
11b	Rain water	1lt	5cm x 21cm	140	90	90	90	70		-			
12a	Rain water	1lt	5cm x 21cm	80	110	50	50	110		-			
12b	Rain water	1lt	5cm x 21cm	80	_	80	_	23		-			
13a	Rain water	1lt	5cm x 21cm	30	22	30	23	23		-			
13b	Rain water	1lt	5cm x 21cm	23	23	23	23	13		-			
14a	Rain water	1lt	5cm x 21cm	2	4	2	2	2		-			
14b	Rain water	1lt	5cm x 21cm	2	<2	<2	<2	<2		-			
15a	Rain water	1lt	5cm x 21cm	110	80	70	50	30		-			
15b	Rain water	1lt	5cm x 21cm	110	80	70	50	30		-			
15c	Rain water	1lt	5cm x 21cm	110	80	50	33	49		-			
16a	Rain water	1lt	5cm x 21cm	140	140	140	110	30		-			
16b	Rain water	1lt	5cm x 21cm	140	90	90	90	70		-			

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
17	Rain water	1lt	5cm x 21cm	50	13	17	17	17		-			
18	Rain water	1 lt	10cm x 21cm	23	13	8	4	2		-			
19a	Rain water	1lt	10cm x 21cm	4	13	2	2	4		-			
19b	Rain water	1lt	10cm x 21cm	4	2	<2	<2	<2		-			
20	Rain water	1lt	10cm x 21cm	8	_	<2	<2	2		-			
21a	Rain water	1lt	10cm x 21cm	80	40	30	12	6		-			
21b	Rain water	1lt	10cm x 21cm	80	_	50	_	30		-			
21c	Rain water	1lt	10cm x 21cm	50	_	30	13	4		-			
21d	Rain water	1lt	10cm x 21cm	50	_	23	_	4		-			
22a	Rain water	1lt	10cm x 21cm	13	_	8	_	4		-			
22b	Rain water	1lt	10cm x 21cm	8	_	8	_	2		-			
23a	Rain water	1lt	10cm x 21cm	170	_	140	_	140		-			
23b	Rain water	1lt	10cm x 21cm	1600	_	900	_	90		-			
23c	Rain water	1lt	10cm x 21cm	110	_	_	_	110		-			
23d	Rain water	1lt	10cm x 21cm	23	_	_	_	13		-			
24a	Rain water	1lt	10cm x 21cm	30	_	13	_	8		-			
24b	Rain water	1lt	10cm x 21cm	13	_	4	_	2		-			
25a	Rain water	1lt	10cm x 21cm	>1600	_	_	_	1600		-			
25b	Rain water	1lt	10cm x 21cm	>1600	_	>1600	_	>1600		-			
26a	Rain water	1lt	10cm x 21cm	>1600	_	>1600	_	>1600		-			
26b	Rain water	1lt	10cm x 21cm	>1600	-	-	-	383		-			
27	Rain water	1lt	10cm x 21cm	84	-	-	-	60		-			
28	Bore well	1lt	10cm x 21cm	2	-	4	-	2		-			
29a	Rain water	1lt	10cm x 21cm	50	-	30	-	23		-			
29b	Rain water	1lt	10cm x 21cm	23	-	17	-	8		0			

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
30b	Rain water	1lt	10cm x 21cm	240	-	-	-	240		-			
31a	Rain water	1lt	10cm x 21cm	4	-	4		2		-			
31b	Rain water	1lt	10cm x 21cm	4	-	-	-	2		-			
32	Bore well-N L	1lt	10cm x 21cm	540	-	-	-	49		-			
33	Pond Water	1lt	10cm x 21cm	60	-	11	-	4		-			
34	Bore well-N L	1lt	10cm x 21cm	>1600	-	>1600	-	280		-			
35	Bore Well-R N	1lt	10cm x 21cm	350	-	-	-	220		79			
36	Bore Well-R N	1lt	10cm x 21cm	170	-	80	-	50		4			
37	Bore Well-R N	1lt	10cm x 21cm	17	-	13	-	2		0			
38	Bore Well-R N	1lt	10cm x 21cm	94	-	-	-	17		4			
39	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	280					>1600
40	Bore Well-R N	1lt	10cm x 21cm	130	-	-	-	46		13			
41	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	280		8			>1600
42	Bore Well-R N	1lt	10cm x 21cm	140	-	-	-	46		4			
43	Bore Well-R N	1lt	10cm x 21cm	30	-	-	-	13		2			
44	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	30		8			
45a	Bore Well-R N	1lt	10cm x 21cm	46	-	-	-	23		2			
45b	Bore Well-R N	1lt	10cm x 21cm	130	-	-	-	23		2			
–	Bore Well-R N, NL, VN, Pond water, Rain water	–	10cm x 21cm	70,94,4 ,110,23	-	-	-	-		-			-
46	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	920		220			>1600
47	Bore Well-R N	1lt	10cm x 21cm	110	-	-	-			30			110
48	IISc kaveri water	1lt	10cm x 21cm	0	-	-	-	0		0			0
49	Bore Well-R N + Pond water	1lt (1:1)	10cm x 21cm	240	-	-	-	30		13			23
50a	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	>1600		>1600			>1600

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
50b	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	>1600		>1600			>1600
51	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-			280			>1600
52	Bore Well-R N	1lt	10cm x 21cm	>1600	-	-	-	1600		280			920
53	Bore Well-R N + Pond water	1lt(1:1)	10cm x 21cm	280	-	-	-	240		110			240
54	Bore Well-R N + Pond water	2lts(500 ml+1500 ml)	10cm x 21cm	170	-	-	-	79		49			130
55	Bore Well-R N + Pond water	4lts (500ml+3 500ml)	10cm x 21cm	240	-	-	-	30		23			130
56	Bore Well-R N + Pond water	4lts (2000ml+ 1750ml+ 250ml)	10cm x 21cm	17	-	-	-	7		4	7		7
57a	Pond Water + Bore Well-R N	2lts (500ml + 1500ml)	10cm x 21cm	70	-	-	-	13		>1600	4	240	70
57b	Pond Water + Bore Well-R N	2lts (500ml + 1500ml)	10cm x 21cm	30	-	-	-	23		2			49
57c	Bore Well-R N	1lt	10cm x 21cm	49	-	-	-	13		<2			49
58	Pond water, IISc	1lt	10cm x 21cm	79	-	-	-	49		26	23	<2	130
59	Bore Well-R N	1lt	10cm x 21cm	30	-	-	-	23		<2	2	_	30
60	Bore Well-R N	1lt	10cm x 21cm	79	-	-	-	13		8			46
61	Borewell-Magadi Road	1lt	10cm x 21cm	>1600	-	-	-	1600		220	170	140	1600
62a	Borewell-Magadi Road	1lt	10cm x 21cm	920	-	-	-	540		49	33		350
62b	Borewell-Magadi Road(Tank)	1lt	10cm x 21cm	540	-	-	-	79		23	13		350
63a	Borewell-Magadi Road(Direct)	1lt	10cm x 21cm	23	-	-	-	13		8			23
63b	Borewell-Magadi Road(Direct)	1lt	10cm x 21cm	2	-	-	-	2		2			

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
64	Borewell-Magadi Road(Tank)	1lt	10cm x 21cm	540	-	-	-	170		140	70	49	
_	Bore well (Tank) RT nagar	1lt	_	1200									
65	RT nagar (T W)	1lt	10cm x 21cm	170				49		0	0	0	
66a	RT nagar (T W)	1lt	10cm x 21cm	170				79		33	0	0	
66 b	RT nagar (T W)	1lt	10cm x 21cm	33				13		0			27
67	Magadi Road (T W)	1lt	10cm x 21cm	170				110		8	5		
68	Magadi Road (T W)	1lt	10cm x 21cm	>1600				>1600		350	240	23	>1600
69	Shankar Mut (T W)	1lt	10cm x 21cm	8				0		0	0		
70 a	Magadi Road (T W)	1lt	10cm x 21cm	>1600				23		5	5	0	
70 b	Magadi Road (T W)	1lt	10cm x 21cm	13				2					
71	Magadi Road (T W)	1lt	10cm x 21cm	920				540		79	33	13	
72	Magadi Road (T W)	1lt	10cm x 21cm	>1600				>1600		>1600			
73	Well Water,17th cross, Malleswaram	1lt	10cm x 21cm	>1600				>1600		>1600	>1601		
74	Well Water,4th main 15th cross, S1	1lt	10cm x 21cm	>1600				>1600		1600			
75	IISc well water	1lt	10cm x 21cm	280				180		79	13		49
76	IISc well water	1lt	10cm x 21cm	110				-		79	8	0	110
77	IISc well water	1lt	10cm x 21cm	920				540					
78	IISc well water	1lt	10cm x 21cm	>1600				>1600		>1600	>1600	140	>1600
79	IISc well water	1lt	10cm x 21cm	350				240		79	2		
80	IISc well water	1lt	10cm x 21cm	>1600					180	22			
81	IISc well water	1lt	10cm x 21cm	>1600				280		0	0	0	
82	IISc well water	1lt	10cm x 21cm	170				79		23	5		

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
83	IISc well water	1lt	10cm x 21cm	920					23	5			240
84	IISc well water	1lt	10cm x 21cm	350				240		79	32	13	22
85	IISc well water	1lt	10cm x 21cm	1600				350		240	240		
86	IISc well water	1lt	10cm x 21cm	920					540	350			
87	IISc well water	1lt	10cm x 21cm	>1600				920		540			920
88	IISc well water	1lt	10cm x 21cm	1600				920		920			
89	IISc well water	1lt	10cm x 21cm	>1600				>1600		>1600			
90	IISc well water	1lt	10cm x 21cm	1600				920		350			
91	IISc well water	1lt	10cm x 21cm	170				110		33			350
92	IISc well water	1lt	10cm x 21cm	170				130		49			
93	IISc well water	1lt	10cm x 21cm	140				130		79			130
94	IISc well water	1lt	10cm x 21cm	>1600				280		23			
95	IISc well water	1lt	10cm x 21cm	>1600				>1600		>1600			1600
96	IISc well water	1lt	10cm x 21cm	920				540		350			350
97	IISc well water	1lt	10cm x 21cm	>1600				1600		70			540
98	IISc well water	1lt	10cm x 21cm	540					23	8			170
99	IISc well water	1lt	10cm x 21cm	920				540		280			540
100	IISc well water	1lt	10cm x 21cm	540				140		33			110
101	IISc well water	1lt	10cm x 21cm	220				79		23			220
102	IISc well water	1lt	10cm x 21cm	920				280		280			920
103	IISc well water	1lt	10cm x 21cm	1600				350		34			920
104	IISc well water	1lt	10cm x 21cm	>1600				920		240			920
105	IISc well water	1lt	10cm x 21cm	1600					280	140			180
106	IISc well water	1lt	10cm x 21cm	280				170		130			920
107	IISc well water	1lt	10cm x 21cm	1600				49		13			920

Sample No.	Source	Quantity	Silver size	MPN/100 ml									Control
				0 h	2 h	4 h	6 h	8 h	16 h	24 h	32 h	48 h	24 h
108	IISc well water	1lt	10cm x 21cm	540				130		79			350
109	IISc well water	1lt	10cm x 21cm	280					170	49			220
110	IISc well water	1lt	10cm x 21cm	170				49		23			110
111	IISc well water	1lt	10cm x 21cm	170				130		130			540
112	IISc well water	1lt	10cm x 21cm	540				350		70			130
113	IISc well water	1lt	10cm x 21cm	540				140		17			49
114	IISc well water	1lt	10cm x 21cm	180				13		4			
115	IISc well water	1lt	10cm x 21cm	220				49		30			
116	IISc well water	1lt	10cm x 21cm	540				350		49			920
117	IISc well water	1lt	10cm x 21cm	540				240		70			110
118	IISc well water	1lt	10cm x 21cm	920				-		49			
119	IISc well water	1lt	10cm x 21cm	540				220		170			350
120	IISc well water	1lt	10cm x 21cm	>1600				>1600		1600			1600
121	IISc well water	1lt	10cm x 21cm	1600				1600		540			920
122	IISc well water	1lt	10cm x 21cm	920				240		130			540
123	IISc well water	1lt	10cm x 21cm	1600				920		170			920
124	IISc well water	1lt	10cm x 21cm	140				49					
125	IISc well water	1lt	10cm x 21cm	1600						920			920
126	IISc well water	10lt	10cm x 21cm	1600				220		17			
127	IISc well water	10lt	10cm x 21cm	1600				300		30			50

4.1.4. Reduced flow method:

The open well water was taken as the sample to this method. The quantity of water sample was 7 liters. The experimental procedure was followed as mentioned in 3.3.4 of chapter 3 and the results are tabulated below:

Table 4.1.4: Experimental results of Reduced flow method

		MPN /100mL					Control
Sample NO.	**Silver Weight**	**0 h**	**8 h**	**16 h**	**24 h**	**48 h**	**24 h**
1	131.32	>1600	1600				920
2	131.32	1600		110			180
3	131.32	280	350				920
4	131.32	1600	240				920
5	131.32	540	79				350
6	131.32	280		13			220
7	131.32	1600	540			170	110
8	131.32	170			130		
9	156.455	170	130			23	540
10	156.455	540		180			130
11	156.455	540	350		13		49
12	156.455	220	30		13		
13	156.455						
14	156.455	540			79		920
15	156.455	540			130		110
16	156.455	920			23		
17	156.455	540			130		350
18	156.455	>1600			1600		1600
19	156.455	1600			540		920
20	156.455	920			79		540
21	156.455	1600			130		920

4.2. Residual effect of Silver in water treatment

IS 10500 standards: Maximum acceptable limit of Silver as Ag in drinking water - 0.1mg/l

The World Health Organization's (WHO) guidelines for drinking water quality indicate that there are no adequate data with which to derive a health-based value for silver in drinking water. These guidelines state that, "where silver salts are used to maintain the bacteriological quality of drinking-water, levels of silver up to 0.1 mg/litre can be tolerated without risk to health". Silver is regulated by US Environmental Protection Agency (EPA) National Secondary Drinking Water Regulations.

The WHO does not describe any health effects associated with the ingestion of silver other than a condition called Argyria, which causes discolouration of the skin and hair. This is considered a cosmetic effect and does not impair body function.

During the experiment of silver treatment of water for drinking, silver sheets were immersed in water. The silver ions will be released in to water to reduce the bacterial activity.

Equipment and method used for testing:
Equipment: Induction Coupled Plasma – Atomic Emission Spectrometer (ICP – AES)
Method: Water treated by dipping silver sheet was tested for residual silver and values are as follows:

Sl. No.	Treatment Time in hours	Silver in water (mg/l)
1	4	0.0027
2	6	0.0031
3	24	0.0065

The above values show very low level of silver in treated water which is far below the acceptable limit as per WHO and IS acceptable standards and has no health effects on humans.

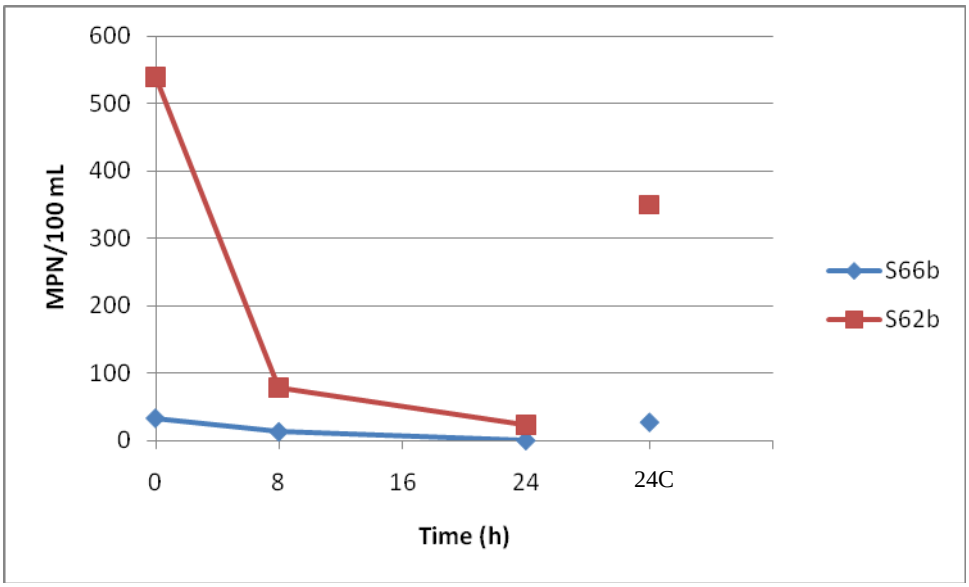
Purity of Silver sheet used in the project:

Silver sheet tested for purity at Mahalakshmi Refinery, C T Street, Bangalore 560 002 and the results showed the silver purity of 99.99%.

4.3. Graphical Representation

Table 4.3.1: Results and Graphs on Domestic tank water

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
66b	Domestic Tank water	31.325	33	13	0	27
62b	Domestic Tank water	31.325	540	79	23	350



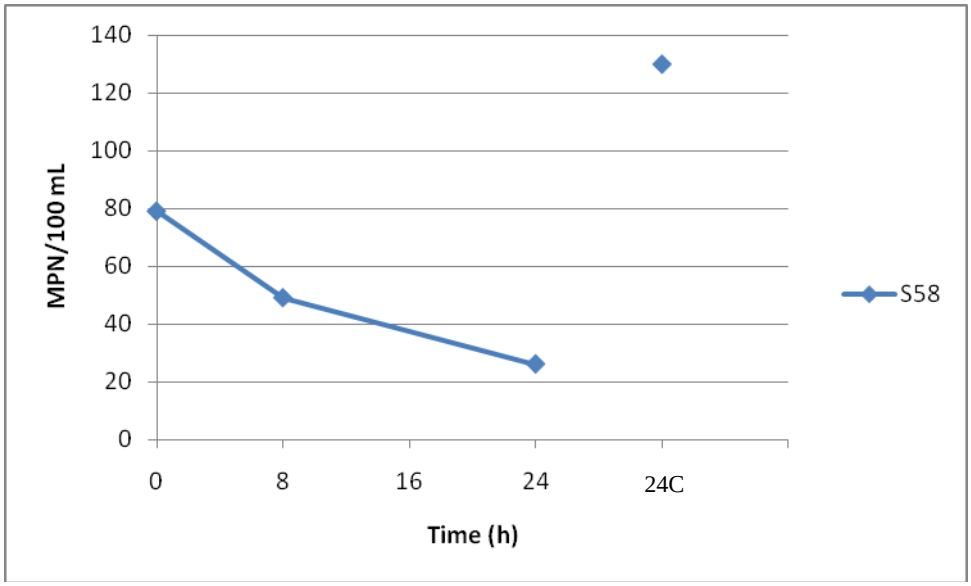
Graph 1: Graph depicting variations in MPN values for different samples at 0h, 8h, 24h and 24hC (C-Control), Source: Domestic tank water

For S66b - Sample at 0h of treatment MPN value is 33/100 mL, after 8h of treatment MPN value is 13/100 mL, further after 24h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 27 MPN/100 mL.

For S62b - Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 79/100 mL, further after 24h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

Table 4.3.2: Results and graphs on Pond water

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
58	Pond water	31.325	79	49	26	130

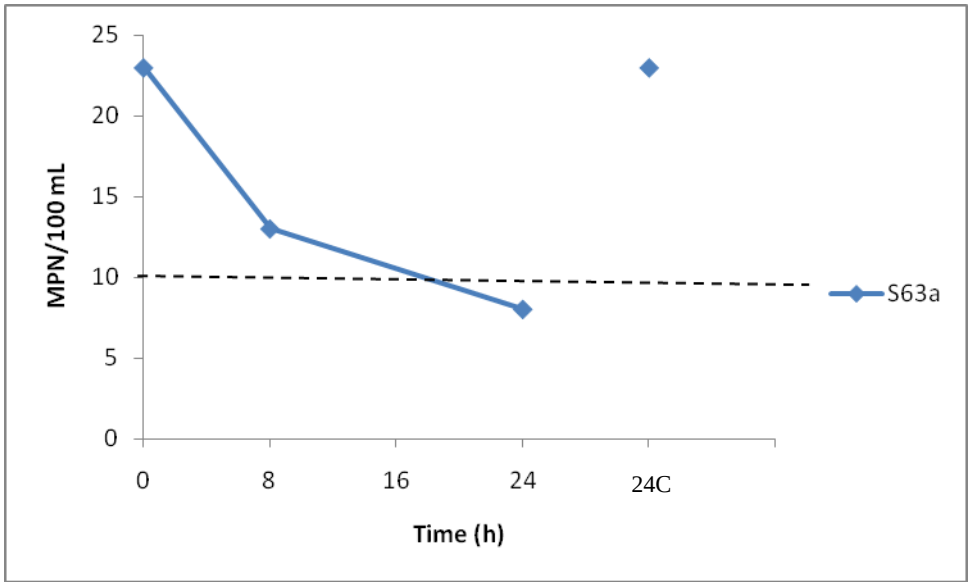


Graph 2: Graph depicting variations in MPN values for different samples at 0h, 8h, 24h and 24hC control respectively. Source: Pond water

For S58 - Sample at 0h of treatment MPN value is 79/100 mL, after 8h of treatment MPN value is 49/100 mL, further after 24h of treatment MPN value is 26/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

Table 4.3.3: Results and graphs on Bore well water collected directly

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
63a	Direct water (Bore well)	31.325	23	13	8	23

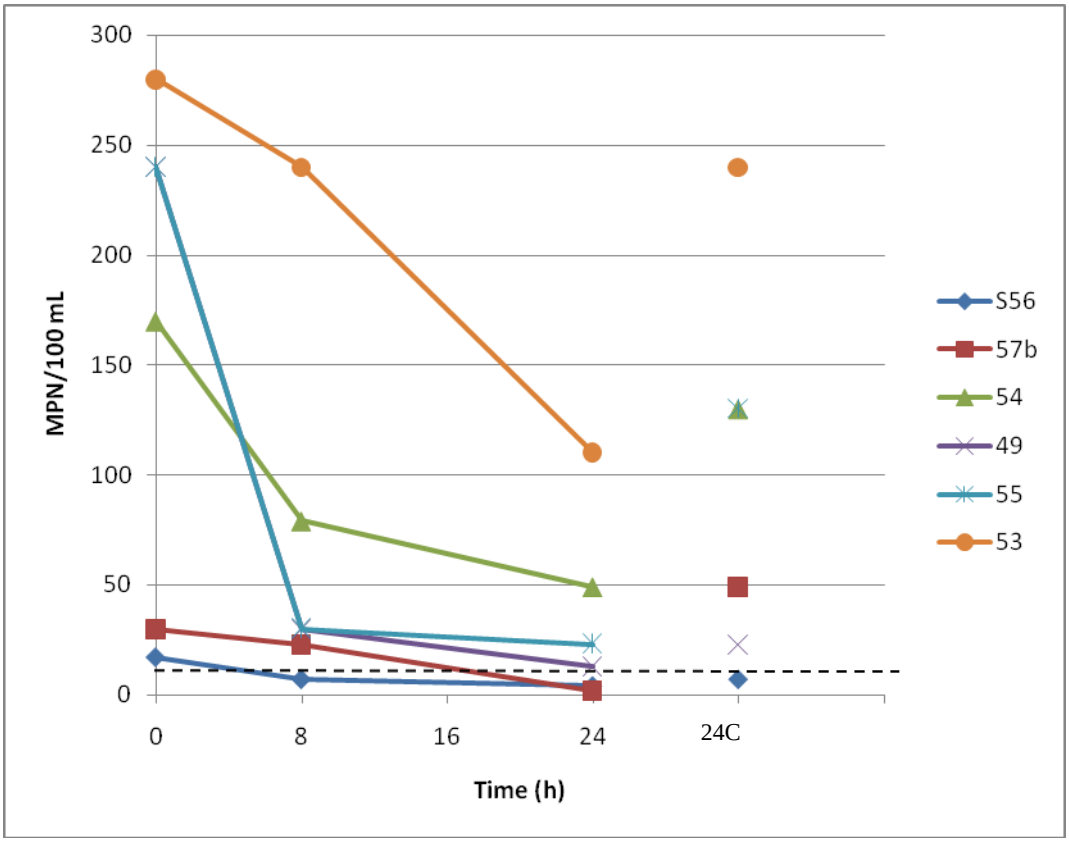


Graph 3: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Bore well water collected directly, (---------): indicates values which are portable.

For S63a: Sample at 0h of treatment MPN value is 23/100 mL, after 8h of treatment MPN value is 13/100 mL, further after 24h of treatment MPN value is 8/100 mL, Where as for 24hC (without silver sheet) is 23 MPN/100 mL.

Table 4.3.4: Results and Graphs on Water collected from multiple sources

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
56	Multiple source	31.325	17	7	4	7
57b	Multiple source	31.325	30	23	2	49
54	Multiple source	31.325	170	79	49	130
49	Multiple source	31.325	240	30	13	23
55	Multiple source	31.325	240	30	23	130
53	Multiple source	31.325	280	240	110	240



Graph 4: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Water collected from multiple sources, S1, S2, S3, S4, S5, and S6: Sample numbers, (---------): Indicates values which are portable.

For S56: Sample at 0h of treatment MPN value is 17/100 mL, after 8h of treatment MPN value is 7/100 mL, further after 24h of treatment MPN value is 4/100 mL, Where as for 24hC (without silver sheet) is 7 MPN/100 mL.

For S57b: Sample at 0h of treatment MPN value is 30/100 mL, after 8h of treatment MPN value is 23/100 mL, further after 24h of treatment MPN value is 2/100 mL, Where as for 24hC (without silver sheet) is 49 MPN/100 mL.

For S54: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 79/100 mL, further after 24h of treatment MPN value is 49/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

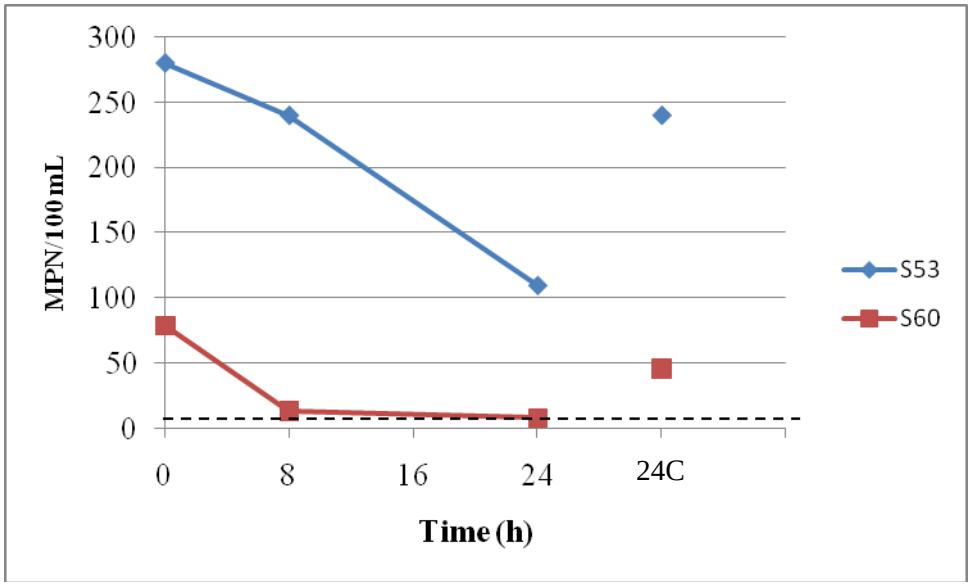
For S49: Sample at 0h of treatment MPN value is 240/100 mL, after 8h of treatment MPN value is 30/100 mL, further after 24h of treatment MPN value is 13/100 mL, Where as for 24hC (without silver sheet) is 23 MPN/100 mL.

For S55: Sample at 0h of treatment MPN value is 240/100 mL, after 8h of treatment MPN value is 30/100 mL, further after 24h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

For S53: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 240/100 mL, further after 24h of treatment MPN value is 110/100 mL, Where as for 24hC (without silver sheet) is 240 MPN/100 mL.

Table 4.3.5: Results and graphs on Bore well water

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
53	Bore well water	31.328	280	240	110	240
60	Bore well water	31.328	79	13	8	46



Graph 5: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Bore well water, S1, S2: Sample numbers, (---------): Indicates values which are portable.

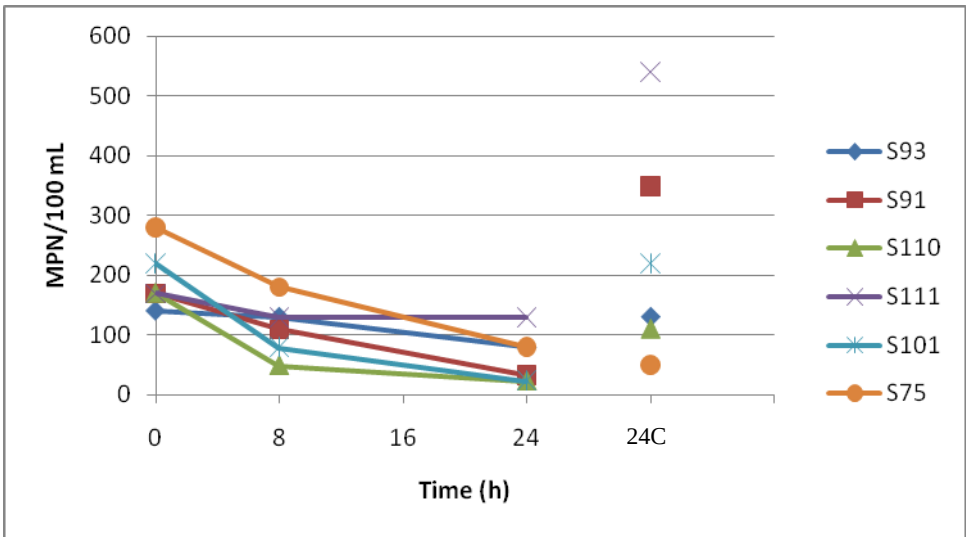
For S53: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 240/100 mL, further after 24h of treatment MPN value is 110/100 mL, Where as for 24hC (without silver sheet) is 240 MPN/100 mL.

For S60: Sample at 0h of treatment MPN value is 79/100 mL, after 8h of treatment MPN value is 13/100 mL, further after 24h of treatment MPN value is 8/100 mL, Where as for 24hC (without silver sheet) is 46 MPN/100 mL.

Table 4.3.6: Results and graphs on Open well water

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
93	Open well water	31.325	140	130	79	130
91	Open well water	31.325	170	113	33	350
110	Open well water	31.325	170	49	23	110
111	Open well water	31.325	170	130	130	540
101	Open well water	31.325	220	79	23	220
75	Open well water	31.325	280	180	79	49
106	Open well water	31.325	280	170	130	920
109	Open well water	31.325	280	170	49	220
84	Open well water	31.325	350	240	79	22
100	Open well water	31.325	540	140	33	110
108	Open well water	31.325	540	130	79	350
112	Open well water	31.325	540	350	70	130
113	Open well water	31.325	540	140	17	49
116	Open well water	31.325	540	350	49	920
117	Open well water	31.325	540	240	70	110
96	Open well water	31.325	920	540	350	350
99	Open well water	31.325	920	540	280	540
102	Open well water	31.325	920	280	280	920
122	Open well water	31.325	920	240	130	540

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL			Control (without silver sheet) MPN/100mL
			0h	8h	24h	24hC
103	Open well water	31.325	1600	350	34	920
107	Open well water	31.325	1600	49	13	920
121	Open well water	31.325	1600	1600	540	920
123	Open well water	31.325	1600	920	170	920



Graph 6: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Well water, S1, S2, S3…: Sample numbers.

For S93: Sample at 0h of treatment MPN value is 140/100 mL, after 8h of treatment MPN value is 130/100 mL, further after 24h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

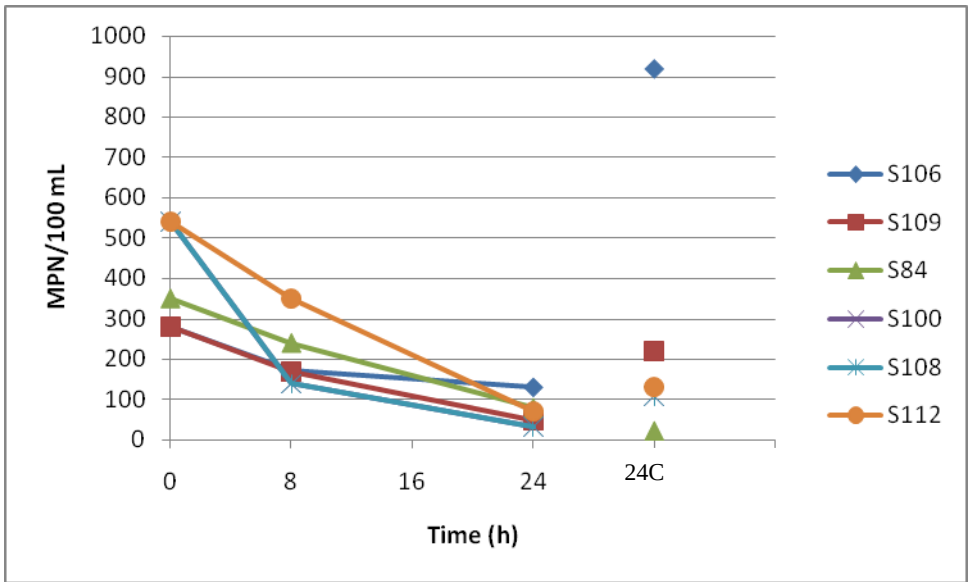
For S91: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 113/100 mL, further after 24h of treatment MPN value is 33/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S110: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 49/100 mL, further after 24h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S111:Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 49/100 mL, further after 24h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S101: Sample at 0h of treatment MPN value is 220/100 mL, after 8h of treatment MPN value is 79/100 mL, further after 24h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 49 MPN/100 mL.

For S75: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 180/100 mL, further after 24h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 49 MPN/100 mL.



Graph 7: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Well water, S1, S2, S3…: Sample numbers.

For S106: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 170/100 mL, further after 24h of treatment MPN value is 130/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

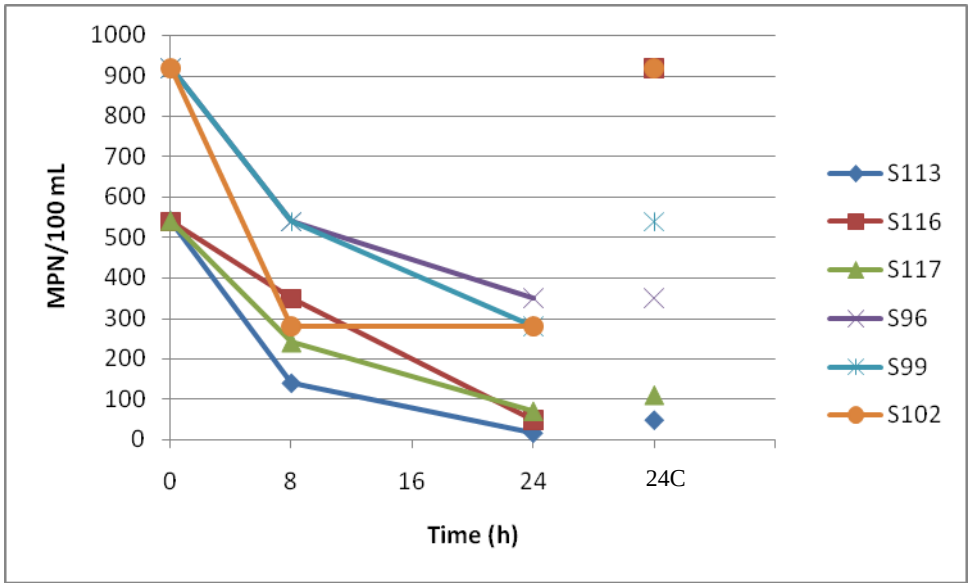
For S109: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 170/100 mL, further after 24h of treatment MPN value is 49/100 mL, Where as for 24hC (without silver sheet) is 220 MPN/100 mL.

For S84: Sample at 0h of treatment MPN value is 350/100 mL, after 8h of treatment MPN value is 240/100 mL, further after 24h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 22 MPN/100 mL.

For S100: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 140/100 mL, further after 24h of treatment MPN value is 33/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S108: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 130/100 mL, further after 24h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S112: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 350/100 mL, further after 24h of treatment MPN value is 70/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.



Graph 8: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Well water, S1, S2, S3…: Sample numbers.

For S113: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 140/100 mL, further after 24h of treatment MPN value is 17/100 mL, Where as for 24hC (without silver sheet) is 49 MPN/100 mL.

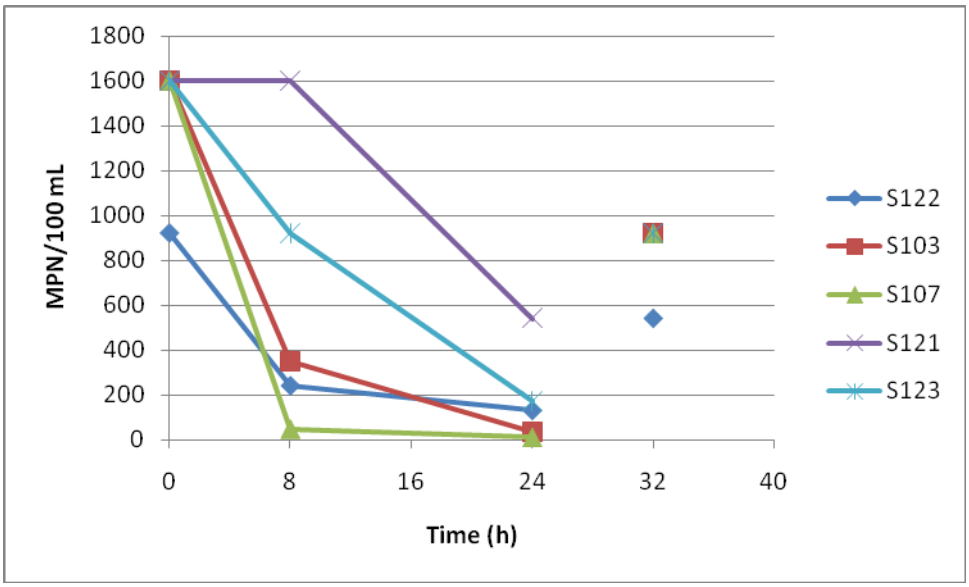
For 116: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 350/100 mL, further after 24h of treatment MPN value is 49/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S117: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 240/100 mL, further after 24h of treatment MPN value is 70/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S96: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 540/100 mL, further after 24h of treatment MPN value is 350/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S99: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 540/100 mL, further after 24h of treatment MPN value is 280/100 mL, Where as for 24hC (without silver sheet) is 540 MPN/100 mL.

For S102: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 280/100 mL, further after 24h of treatment MPN value is 280/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.



Graph 9: Graph depicting the variations in MPN values for different samples at 0h, 8h, 24h and 24hC respectively. Source: Well water, S1, S2, S3…: Sample numbers.

For S122: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 240/100 mL, further after 24h of treatment MPN value is 130/100 mL, Where as for 24hC (without silver sheet) is 540 MPN/100 mL.

For S103: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 350/100 mL, further after 24h of treatment MPN value is 34/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

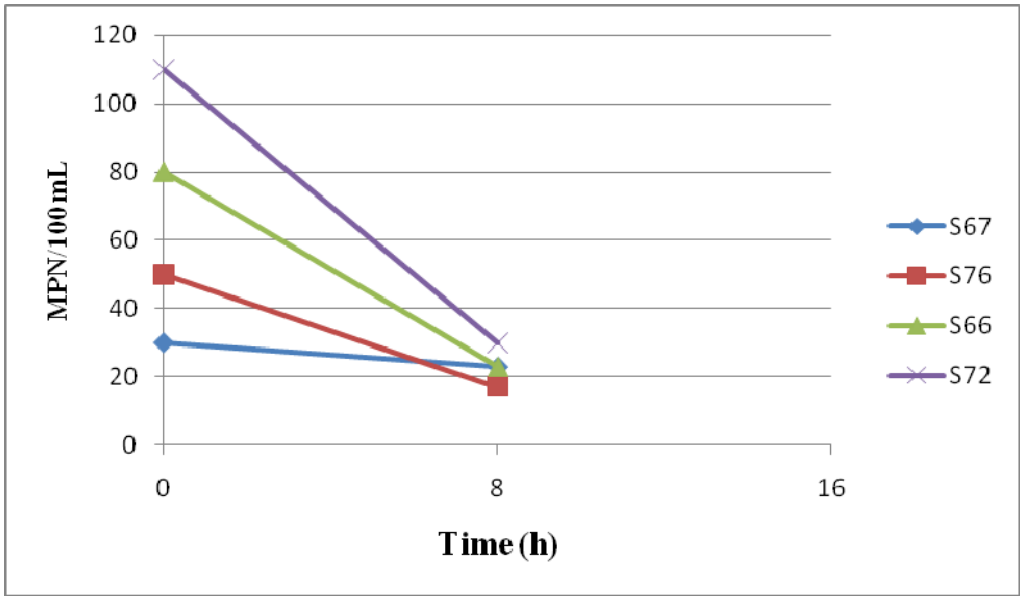
For S107: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 49/100 mL, further after 24h of treatment MPN value is 13/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S121: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 1600/100 mL, further after 24h of treatment MPN value is 540/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S123: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 920/100 mL, further after 24h of treatment MPN value is 170/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

Table 4.3.7: Results and graphs on Rain water

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL	
			0h	8h
67	Rain water	15.730	30	23
76	Rain water	15.730	50	17
66	Rain water	15.730	80	23
72	Rain water	15.730	110	30
73	Rain water	15.730	110	49
63	Rain water	15.730	140	23
64	Rain water	15.730	140	70
74	Rain water	15.730	140	30
102	Rain water	31.328	4	2
86	Rain water	31.328	8	2
85	Rain water	31.328	13	4
92	Rain water	31.328	13	2
77	Rain water	31.328	23	2
90	Rain water	31.328	23	13
99	Rain water	31.328	23	8
91	Rain water	31.328	30	8
84	Rain water	31.328	50	4
98	Rain water	31.328	50	23
81	Rain water	31.328	80	6
97	Rain water	31.328	84	60
87	Rain water	31.328	170	140
88	Rain water	31.328	1600	90



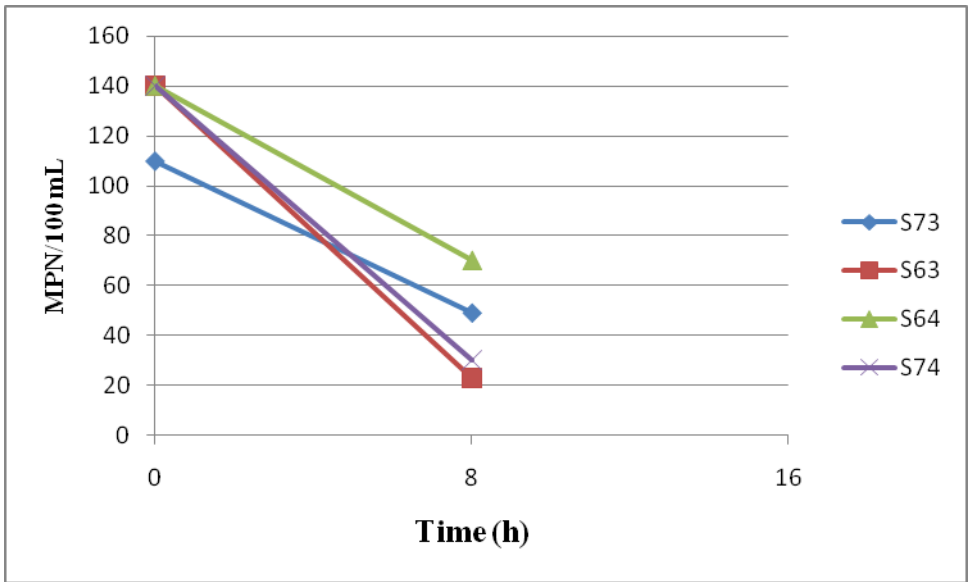
Graph 10: Graph depicting the variations in MPN values for different samples at 0h and 8h respectively. Source: Rain water, S1, S2, S3….: Sample numbers.

For S67: Sample at 0h of treatment MPN value is 30/100 mL, after 8h of treatment MPN value is 23/100 mL.

For S76: Sample at 0h of treatment MPN value is 50/100 mL, after 8h of treatment MPN value is 17/100 mL.

For S66: Sample at 0h of treatment MPN value is 80/100 mL, after 8h of treatment MPN value is 23/100 mL.

For S72: Sample at 0h of treatment MPN value is 110/100 mL, after 8h of treatment MPN value is 30/100 mL.



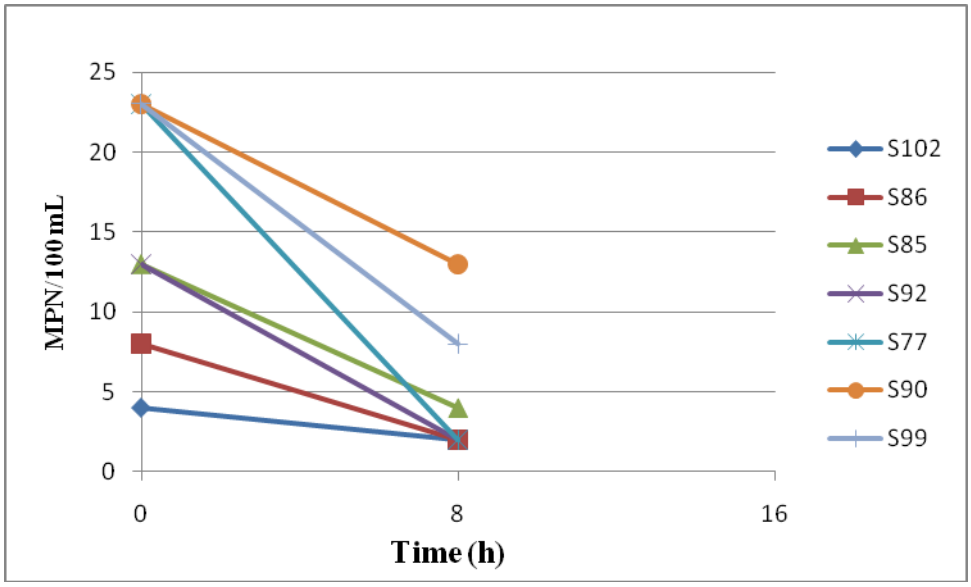
Graph 11: Graph depicting the variations in MPN values for different samples at 0h and 8h respectively. Source: Rain water, S1, S2, S3….: Sample numbers.

For S73: Sample at 0h of treatment MPN value is 30/100 mL, after 8h of treatment MPN value is 49/100 mL.

For S63: Sample at 0h of treatment MPN value is 140/100 mL, after 8h of treatment MPN value is 23/100 mL.

For S64: Sample at 0h of treatment MPN value is 140/100 mL, after 8h of treatment MPN value is 70/100 mL.

For S74: Sample at 0h of treatment MPN value is 140/100 mL, after 8h of treatment MPN value is 30/100 mL.



Graph 12: Graph depicting the variations in MPN values for different samples at 0h and 8h respectively. Source: Rain water, S1, S2, S3….: Sample numbers.

For S102: Sample at 0h of treatment MPN value is 4/100 mL, after 8h of treatment MPN value is 2/100 mL.

For S86: Sample at 0h of treatment MPN value is 8/100 mL, after 8h of treatment MPN value is 2/100 mL.

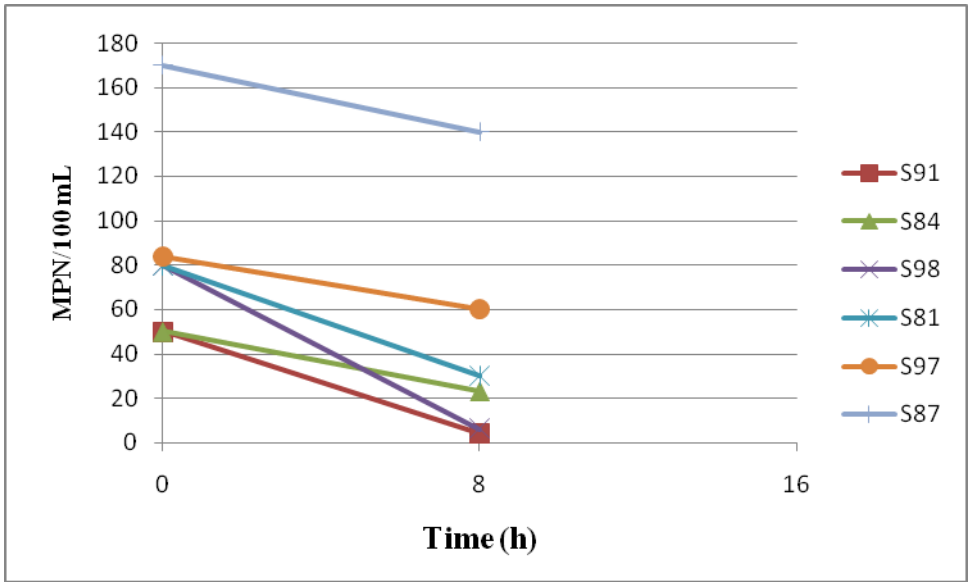
For S85: Sample at 0h of treatment MPN value is 13/100 mL, after 8h of treatment MPN value is 4/100 mL.

For S92: Sample at 0h of treatment MPN value is 13/100 mL, after 8h of treatment MPN value is 2/100 mL.

For S77: Sample at 0h of treatment MPN value is 23/100 mL, after 8h of treatment MPN value is 2/100 mL.

For S90: Sample at 0h of treatment MPN value is 23/100 mL, after 8h of treatment MPN value is 13/100 mL.

For S99: Sample at 0h of treatment MPN value is 23/100 mL, after 8h of treatment MPN value is 8/100 mL.



Graph 13: Graph depicting the variations in MPN values for different samples at 0h and 8h respectively. Source: Rain water, S1, S2, S3….: Sample numbers.

For S91: First sample at 0h of treatment MPN value is 30/100 mL, after 8h of treatment MPN value is 8/100 mL.

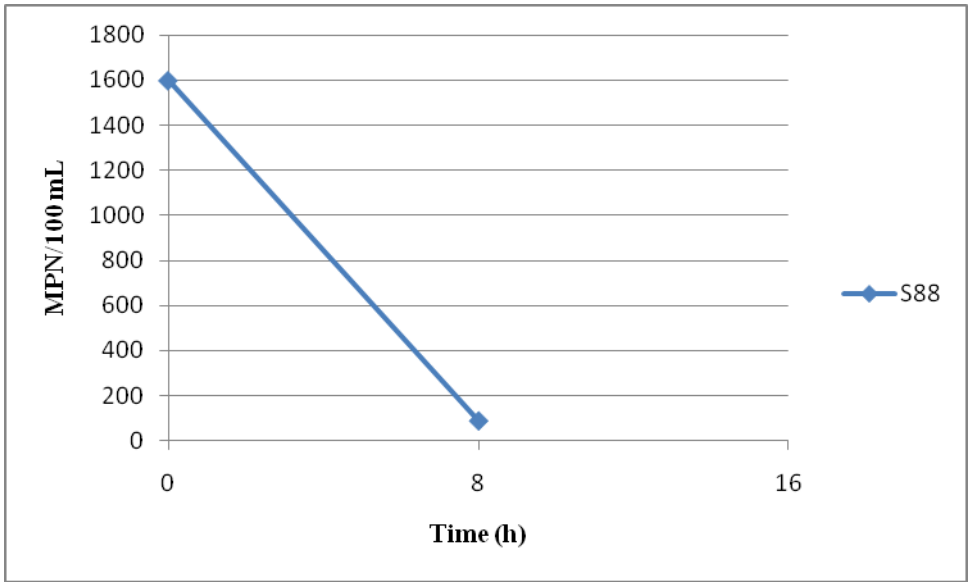
For S84: Sample at 0h of treatment MPN value is 50/100 mL, after 8h of treatment MPN value is 4/100 mL.

For S98: Sample at 0h of treatment MPN value is 50/100 mL, after 8h of treatment MPN value is 23/100 mL.

For S81: Sample at 0h of treatment MPN value is 80/100 mL, after 8h of treatment MPN value is 6/100 mL.

For S97: Sample at 0h of treatment MPN value is 84/100 mL, after 8h of treatment MPN value is 60/100 mL.

For S87: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 140/100 mL

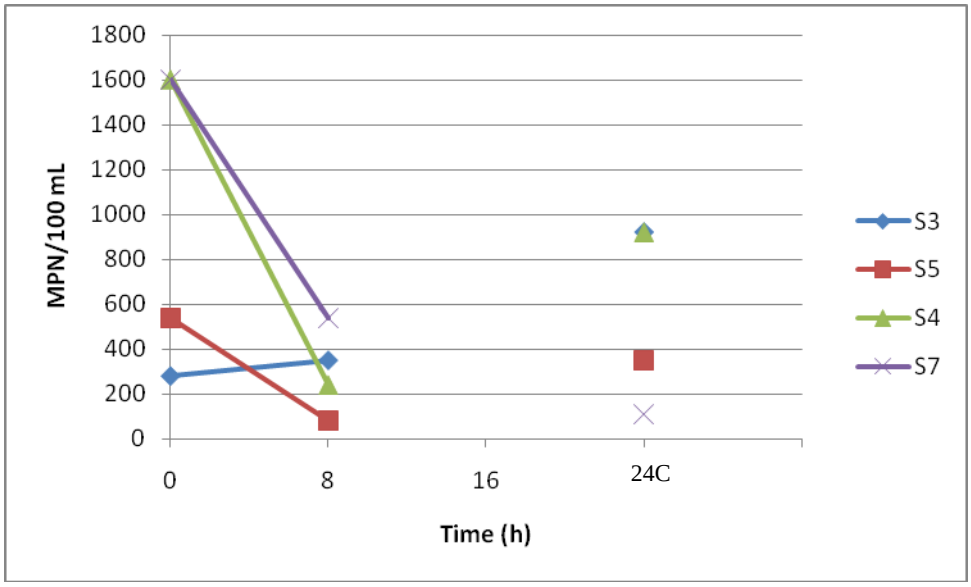


Graph 14: Graph depicting the variations in MPN values for different samples at 0h and 8h respectively. Source: Rain water, S1, S2, S3….: Sample numbers.

For S88: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 90/100 mL.

Table 4.3.8a: Results and graphs on Reduced flow method

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL		Control (without silver sheet) MPN/100mL
			0h	8h	24h C
3	Open well water	131.32	280	350	920
5	Open well water	131.32	540	79	350
4	Open well water	131.32	1600	240	920
7	Open well water	131.32	1600	540	110
9	Open well water	156.455	170	130	540
11	Open well water	156.455	540	350	49



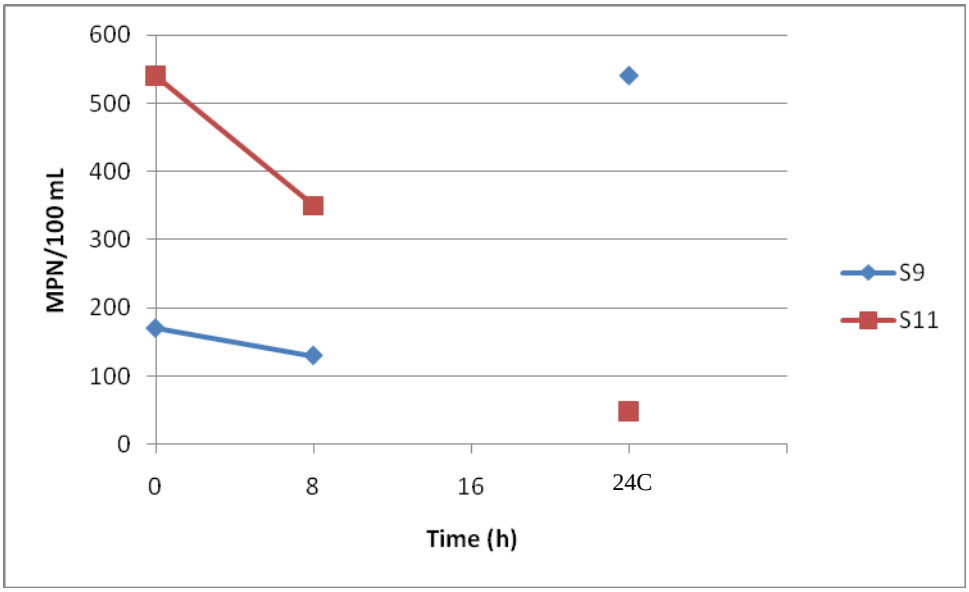
Graph 15a(1): Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC (C-control), Source: Open well water, S1, S2, S3….: Sample numbers.

For S3: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 350/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S5: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S4: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 240/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S7: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 540/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.



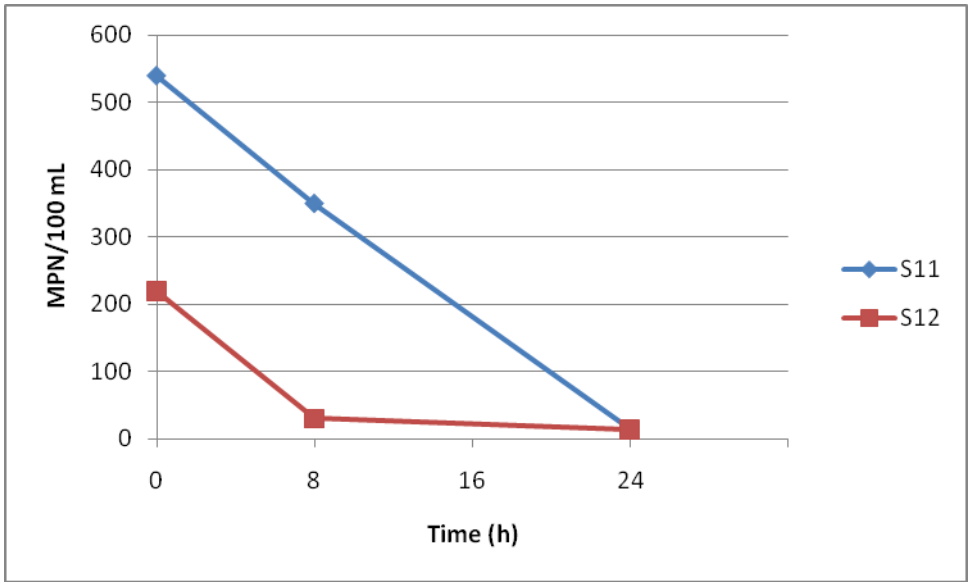
Graph 15a(2): Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC (C-control), Source: Open well water, S1, S2, S3….: Sample numbers.

For S9: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 130/100 mL, Where as for 24hC (without silver sheet) is 540 MPN/100 mL.

For S11: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 350/100 mL, Where as for 24hC (without silver sheet) is 49 MPN/100 mL.

Table 4.3.8b: Results and graphs on Reduced flow method

Sample No.	Water sources	Weight of silver sheet in Grams	Results on MPN/100mL		
			0h	8h	24h
11	Open well water	28	540	350	13
12	Open well water	156.455	220	30	13



Graph 15b: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24h respectively, Source: Open well water, S1, S2: Sample numbers.

For S11: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 350/100 mL, further after 24h of treatment MPN value is 13/100 mL.

For S12: Sample at 0h of treatment MPN value is 220/100 mL, after 8h of treatment MPN value is 30/100 mL, further after 24h of treatment MPN value is 13/100 mL.

4.4. Performance studies of water purifiers bought from market:

We selected three different types of water purifiers to study their performance and to compare our treatment method.

4.4.1. Kent Gold (UF membrane)

Figure 21: Kent Gold (UF membrane) used for comparison with our experimental method

Technical Specifications:

Model No. – 1046
Mounting – table top
Dimensions – 600L*355W*326H (mm) (When assembled)
Bottom tank storage capacity – 13litres
Upper storage capacity – 7litres
Membrane type – hollow fibre hydrophilic UF membrane
Filters used – sediment, silver carbon, SS screen
Expected life of UF membrane – 4000litres
Filtration capacity – 0.13litre/min

Salient features:

a) Ideal for water from most sources (preferably low TDS water)

b) Use of hallow fibre UF membrane removes bacteria (certified by Toray, Japan) as well as cyst (certified by NSF international). Cyst is the deadly pathogens that cannot be removed by most non – electric storage water purifiers.

c) No use of chemicals such as chlorine, bromine or iodine for purification. There by, provides healthier and testier drinking water.

d) Use of nano silver carbon for better disinfection of water

e) Very high storage capacity of 20litres (13litres in bottom tank and 7litres in the top tank), which makes purified water available on demand.

f) Food grade, plastic construction for trouble free maintains and long life.

Filtration process:

This particular filter works on the basis of 3 steps of filtration

a) Removal of suspended particles

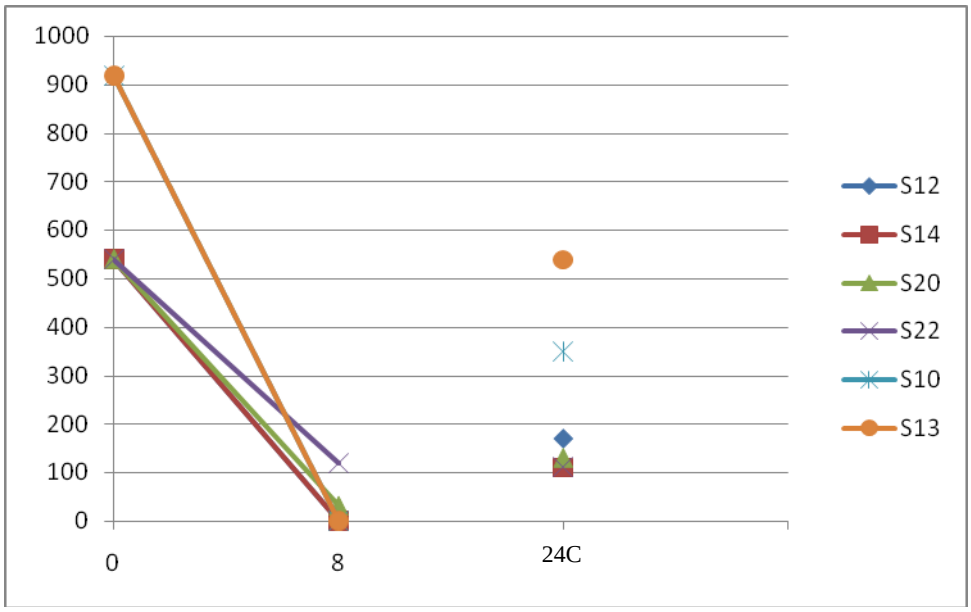
b) Removal of organic or chemical impurities

c) Removal of disinfection

Water from more sources (preferably low TDS water) is filled from the top tank which passes though sediment filter which removes the suspended particles.
Removal of organic / chemical impurities such as chlorine, bad taste and odor is done by passing though the silver impregnated carbon granules.
Water from the top tank flows down to the bottom tank though Ultra Filtration membrane, in this process the bacteria and cyst have been removed and thus purified water collects in the bottom tank, from where it can be dispensed for drinking.

Table 4.4.1: Results and graphs on Purifiers (Kent)

Sample No.	Water sources	Results on MPN/100mL		Control (without silver sheet) MPN/100mL
		0h	8h	24h C
12	Open well water	540	0	170
14	Open well water	540	0	110
20	Open well water	540	31	130
22	Open well water	540	119	110
10	Open well water	920	0	350
13	Open well water	920	0	540
9	Open well water	1600	0	1600
25	Open well water	1600	0	920



Graph 16: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC respectively, Source: Open well water, S1, S2: Sample numbers.

For 12: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 170 MPN/100 mL.

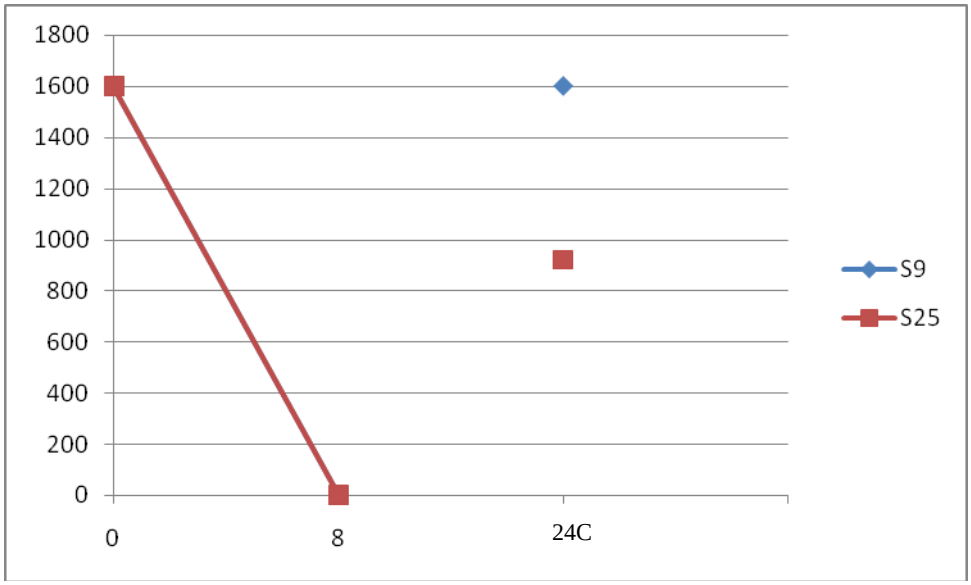
For S14: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S20: Sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 31/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

For S22: Fifteenth sample at 0h of treatment MPN value is 540/100 mL, after 8h of treatment MPN value is 119/100 mL, Where as for 24hC (without silver sheet) is 110 MPN/100 mL.

For S10: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S13: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 540 MPN/100 mL.



Graph 17: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC respectively, Source: Open well water, S1, S2: Sample numbers.

For S9: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 1600 MPN/100 mL.

For S25: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

4.4.2. Aqua Sure from Aqua guard

Figure 22: Aqua Sure from Aqua guard used for comparison with our experimental method

Technical Specifications:

Capacity of purifiers - 20 litres
Dimensions - 560h*230d*335w (mm)
Net weight - 2.710Kg
Top container of water capacity - 9 litres
Bottom container of water capacity - 11 litres
Purification capacity - 750 litres
100% chemical free - kitanu magnet
Material of construction - food grade, non toxic and engineering plastics

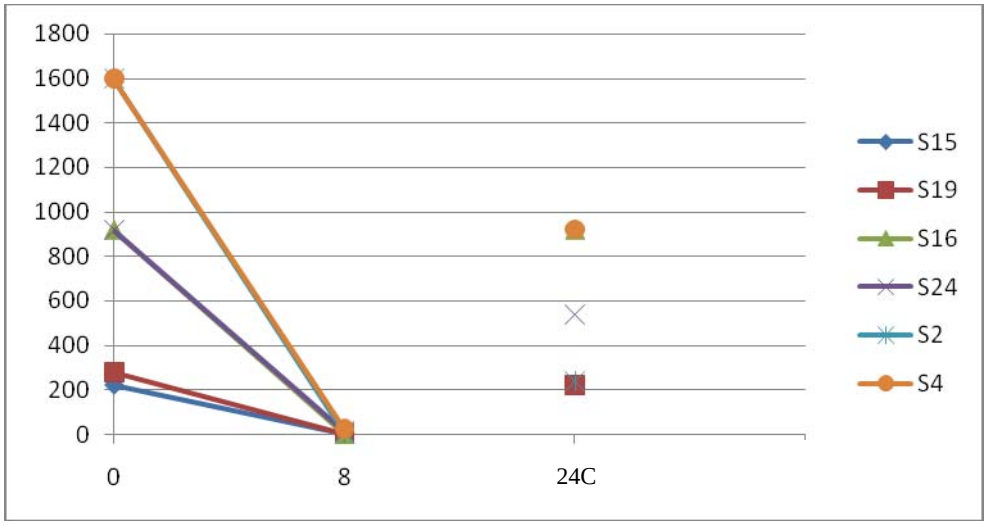
Tested by Aqua Diagnostic Water Research and Technology Centre. It conforms to Indian standard IS: 10500, 1991 drinking water specification.

Mechanism:-

(a) Special fine micro fiber filter mesh = removes all visible dirt and impurities (particulate filter).
(b) Sediment filter = highly advanced, panted design, microfiber mesh with high surface area = removes invisible impurities and provide very high water clarity.
(c) Kitanu magnet with '+'ve change technology = heart of purification system = two stages of purification compressed into one resulting complex "nano fibers" that attract and pull out harmful diseases causing bacteria and virus from water.
(d) Natural shutoff = activates once it nears its useable life.
Replacement of cartridge - after every 750 litres (3 months for 8 litres to 10 litres per day)

Table 4.4.2: Results and graphs on Purifiers (Aqua sure)

Sample No.	Water sources	Results on MPN/100mL		Control (without silver sheet) MPN/100mL
		0h	8h	24h C
15	Open well water	220	5	220
19	Open well water	280	2	220
16	Open well water	920	0	920
24	Open well water	920	13	540
2	Open well water	1600	9	241
4	Open well water	1600	23	920
17	Open well water	1600	2	920
25	Open well water	1600	13	920



Graph 18: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC respectively, Source: Open well water, S1, S2: Sample numbers.

For S15: Sample at 0h of treatment MPN value is 220/100 mL, after 8h of treatment MPN value is 5/100 mL, Where as for 24hC (without silver sheet) is 220 MPN/100 mL.

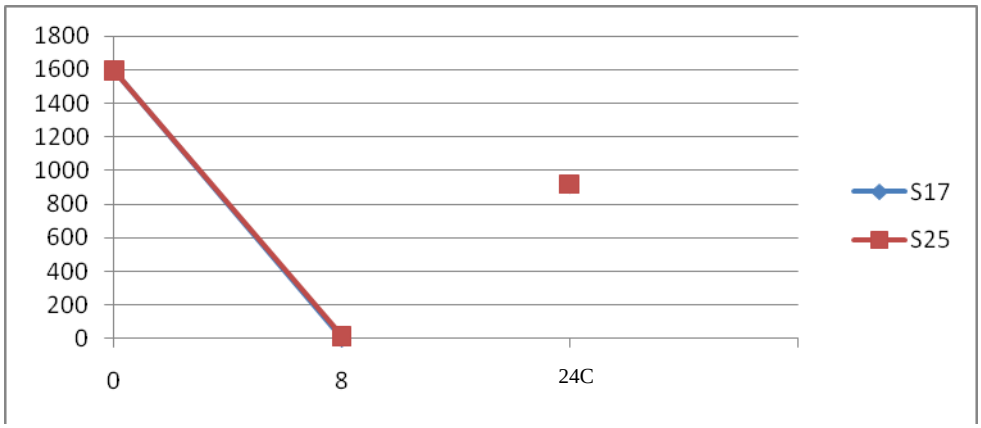
For S19: Sample at 0h of treatment MPN value is 280/100 mL, after 8h of treatment MPN value is 2/100 mL, Where as for 24hC (without silver sheet) is 220 MPN/100 mL.

For S16: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S24: Sample at 0h of treatment MPN value is 920/100 mL, after 8h of treatment MPN value is 13/100 mL, Where as for 24hC (without silver sheet) is 540 MPN/100 mL.

For S2: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 9/100 mL, Where as for 24hC (without silver sheet) is 241 MPN/100 mL.

For S4: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 23/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.



Graph 19: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC respectively, Source: Open well water, S1, S2: Sample numbers.

For S17: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value s 13/100 mL, where as for 24hC (without silver sheet) is 920 MPN/100 mL.

For S25: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 13/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

4.4.3 Pure it:

Figure 23: Pure it used for comparison with our set up of experimental method

Technical Specifications:

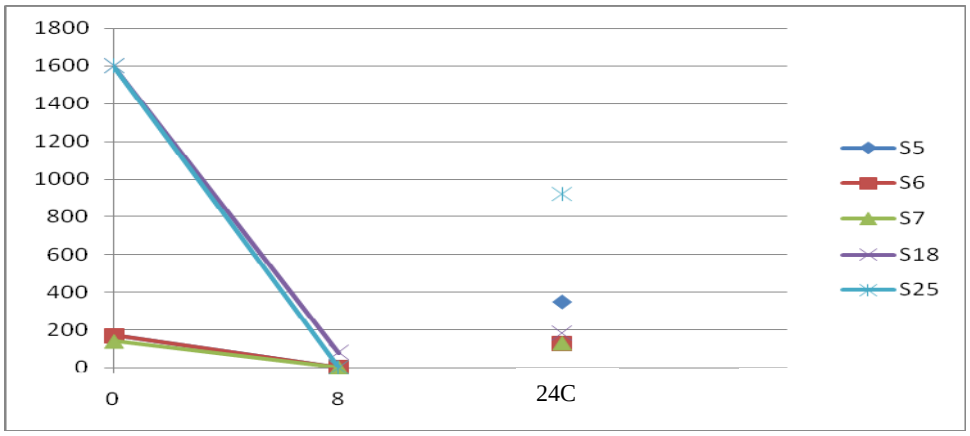
Capacity of purifier - 14 litres
Dimension - 49h*25.3d*24.7w in cm
Net weight - 2.4 Kg
Water storage capacity - 5 litres
Material of construction - food safe, non toxic and engineering plastics
Purification capacity - The germ kill kit has been designed to typically give 1250 liters of water at a temperature to 25° C, in moderate humidity conditions.
Germ kill performance standard - meets stringent international criteria of the Environmental Protection Agency (EPA), USA, for harmful viruses and bacteria removal.

Filtration process:

In this filter, as the water fed to it passes though the micro fibre mesh where the physical/visible contaminants, sediments have been removed and then with the help of activated carbon and the germkill kit processor the invisible, harmful bacteria, viruses and pesticides have been removed, this is the main filtration unit it also has an indicator to know the condition of the germkill kit. Though the batching chamber water flows though the polisher which helps in the removal of chlorine and other contaminants to make water clear, odourless and natural tasting. Then the clean water is collected in the cleansing collection chamber, from that the clean water is dispensed for drinking.

Table 4.4.3: Results and graphs on Purifiers (Pure it)

Sample No.	Water sources	Results on MPN/100mL		Control (without silver sheet) MPN/100mL
		0h	8h	24h C
5	Open well water	170	0	350
6	Open well water	170	2	130
7	Open well water	140	0	130
18	Open well water	1600	79	180
25	Open well water	1600	0	920



Graph 20: Graph depicting the variations in MPN values for different samples at 0h, 8h and 24hC respectively, Source: Open well water, S1, S2: Sample numbers.

For S5: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 350 MPN/100 mL.

For S6: Sample at 0h of treatment MPN value is 170/100 mL, after 8h of treatment MPN value is 2/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

For S7: Sample at 0h of treatment MPN value is 140/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 130 MPN/100 mL.

For S18: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 79/100 mL, Where as for 24hC (without silver sheet) is 180 MPN/100 mL.

For S25: Sample at 0h of treatment MPN value is 1600/100 mL, after 8h of treatment MPN value is 0/100 mL, Where as for 24hC (without silver sheet) is 920 MPN/100 mL.

Table 4.4: Comparison between silver treatment and other three brands of water purifiers available in the market

Detail of water purification for four types of process:

Sl.No	Purifier Name	Silver sheet	Kent Gold - B	Pure it - A	Aqua Sure - C
1	Source of sample	Open well, Bore well, Rain water	Water with low TDS value	Chlorinated water	Chlorinated water
2	Container Capacity	1lt	20 lts	14 lts	20 lts
3	Material of construction	Stainless Steel and Tupper wear	Food grade plastic	Food grade, Non toxic Engineering plastics	Food grade, Non toxic Engineering plastics
4	Filter Element	99.9% pure silver sheet metal	Sediment and silver impregnated carbon granules, UF membrane	Microfibre mesh filter, activated carbon, germ kill kit	Particulate filter which is a Fine Micromesh, Sediment filter, Kitanu Magnet with Positive charge Technology
5	Treatment Time	8-24 hours	8 hours	3 hours	3 hours
6	Bacteria removal efficiency	Bacteria free water	Removes chlorine, bad taste and odour, most of the bacteria and 99.99% cysts	Removes harmful virus and bacteria, Pesticides, chlorine	Removes Particulate matter like visible dirt and impurities,
7	Life of purifier	Life time	Upto 4000 lts	Upto 1250 lts	5 years
8	Total sample purification capacity	1lt of water = 30g of silver	4000 lts	1250 lts	750 lts
9	Advantage	Eco friendly, Chemical free, Self fabrication, Portable, Treats without change in colour, odour, taste	Chemical free	as safe as boiled water without the hassles of boiling	100% chemical free, Attracts Bacteria, Virus and Cysts, Drugs and pesticide removal, crystal clear Sweet tasting water

Sl.No	Purifier Name	Silver sheet	Kent Gold	Pure it	Aqua Sure
10	Disadvantage	Takes longer time to treat water	Microbiologically unsafe water cannot be used	Microbiologically unsafe water cannot be used	Slime growth may occur at the bottom of the container
11	Treatment efficiency	1600 MPN/100mL to 13 MPN/100mL	1600 MPN/ 100mL to NIL	1600 MPN/100mL to 79 MPN/100mL	1600 MPN/100mL to 2 MPN/100mL
12	Cost	Rs 1500/-	Rs 2550/-	Rs 2800/-	Rs 2390/-
13	Certified by	Mines and Geology Department, GOK	NSF International, Toray and Japan	EPA, USA	IS: 10500, 1991 Drinking water specification

Test results of water purifiers:

Sample No.	Raw sample	Silver sheet method	Brand - A	Brand - B	Brand - C
	MPN/100ml	Results at 8 hours of treatment MPN/100ml			
87	>1600	920			0
88	1600	920			9
90	1600	920			23
91	170	110	0		
92	170	130	2		
93	140	130	0		
94	>1600	280	94		
96	920	540		0	
98	540	8 (after 24h)		0	
99	920	540		0	
100	540	140		0	
101	220	79			5
102	920	280			0
103	1600	350			2
105	1600	140 (after 24h)	79		
109	280	170			2
112	540	350		31	
114	180	13	0		
117	540	240		119	
122	920	240			13
125	1600	920 (after 24h)	0	0	13
125 - Test at Mines and Geology	1600	110 (after 24h)	0	23	23

- As per the above table the MPN values for 87th sample at 0hours of treatment is >1600 MPN /100 mL, after 8hours of treatment using Silver sheet as treatment media the MPN value is 920 MPN/100 mL. In comparison with the commercially available purifiers (C) after 8 hours of treatment for 8liters of water sample MPN value is found to be 0 MPN/100 mL.
- For 90th sample the MPN values at 0 hours of treatment are found to be 1600 MPN/100 mL after 8 hours of treatment using silver sheet as treatment media the MPN value is 920 MPN/100 mL. In comparison with the commercially available purifiers (C) after 8 hours of treatment for 32liters of water sample MPN value is found to be 23 MPN/100 mL.
- For 91st Sample the MPN values at 0 hours of treatment are found to be 170 MPN/100 mL after 8 hours of treatment using silver sheet as treatment media the MPN value is 110 MPN/100 mL. In comparison with the commercially available purifiers (A) after 8 hours of treatment for 20 liters of water sample MPN value is found to be 0 MPN/100 mL.
- For 94th Sample the MPN values at 0 hours of treatment is found to be >1600 MPN/100 mL after 8 hours of treatment using silver sheet as treatment media the MPN value is 280/100 mL. In comparison with the commercially available purifiers (A) after 8 hours of treatment for 36 liters of water sample MPN value is found to be 94 MPN/100 mL.
- For 112th Sample the MPN values at 0 hours of treatment are found to be 540 MPN/100 mL after 8 hours of treatment using silver sheet as treatment media the MPN value is 350/100 mL. In comparison with the commercially available purifiers (B) after 8 hours of treatment for 30 liters of water sample MPN value is found to be 0 MPN/100 mL.
- For 117th Sample the MPN values at 0 hours of treatment are found to be 540 MPN/100 mL after 8 hours of treatment using silver sheet as treatment media the MPN value is 240/100 mL. In comparison with the commercially available purifiers (B) after 8 hours of treatment for 80 liters of water sample MPN value is found to be 119 MPN/100 mL.
- For 125th Sample the MPN values at 0 hours of treatment are found to be 1600 MPN/100 mL after 24 hours of treatment using silver sheet as treatment media, the MPN value is 110/100 mL. In comparison, the commercially available purifiers (A) - MPN value of 0/100 mL (B) - MPN value of 23/100 mL (C) MPN value of 23/100 mL.

4.5. Verification of results with other laboratories:

To authenticate our laboratory performance, we gave samples to the following two laboratories.

4.5.1. Mines and Geology Department:

Test results of water samples tested at Department of Mines and Geology, Kanija Bhavan, Race Course Road, Government of Karnataka.

Table 4.5.1: Water sample test results from Mines and Geology Department

SL.NO	DATE	SAMPLE	TOTAL COLIFORMS (MPN/100mL)	FEACAL COLIFORMS (MPN/100mL)
1	30/10/2010	Treated water	NIL	NIL
2	30/10/2010	Rain water	350	280
3	30/10/2010	OHT water	900	900
4	30/10/2010	Sump	900	900
5	30/10/2010	Bore well water	500	500
6	20/1/2011	Washing machine water	350	240
7	20/1/2011	Pond water	900	900
8	20/1/2011	Fish Pond water	900	500
9	20/1/2011	Well Water	300	300
10	4/11/2010	Drinking water	NIL	NIL
11	4/11/2010	Rain water	5.1	3.6
12	23/7/2011	Silver treated water	NIL	NIL
13	23/7/2011	Rain water	500	110
14	23/7/2011	Bore well water	230	230
15	28/11/2011	Silver treated water	23	16.1
16	28/11/2011	Rain water	230	170
17	28/11/2011	Bore well water	700	700
18	23/12/2011	Silver treated water	110	70
19	23/12/2011	Rain water	130	110
20	16/1/2012	Bore well water	110	70
21	21/1/2012	Silver treated water	NIL	NIL
22	21/1/2012	Silver treated water	23	23
23	21/1/2012	Silver treated water	NIL	NIL
24	4/5/2012	Silver treated water	700	700
25	4/5/2012	Untreated water	900	900
26	17/5/2012	Silver treated water	70	30

4.5.2. Essen & Co:

Test results from Essen & Co., private testing and analysis laboratory, 8th Main Road, Malleshwaram, Bangalore.

Table 4.5.2: Water sample test results from Essen & Co.

SL.NO	DATE	SAMPLE	TOTAL COLIFORMS (MPN/100mL)	FEACAL COLIFORMS (MPN/100mL)	*E.coli*
1	1/6/2012	Untreated Rainwater	541	Absent	Absent
		6hs Treated water	46	Absent	Absent
2	1/6/2012	Drinking water	NIL	Absent	Absent
3	28/6/2012	Untreated Borewell water	21	Absent	Absent
		2hs Treated water	9	Absent	Absent
		4hs Treated water	5	Absent	Absent
		6hs Treated water	NIL	Absent	Absent
		8hs Treated water	NIL	Absent	Absent
4	1/7/2012	Untreated water	148	Absent	Absent
		1lt - 8hs Treated water	94	Absent	Absent
		2lts - 8hs Treated water	6	Absent	Absent
5	9/10/2012	Untreated water	345	Absent	Absent
		Treated drinking water	221	Absent	Absent
6	27/11/2012	Untreated water	94	Present	Present
		8hs Treated water	46	Present	Present

CONCLUSIONS

- Dipping Silver sheet inside water for few hours will decrease bacterial count.
- Water purification for bacterial contamination using silver sheet has been established through experiments. The test results have been verified through water sample analysis after and before treating bacteriological contaminated water with silver sheet at:
    - Indian Institute of Science
    - Mines and Geology Department, Government of Karnataka
    - Ms Essen & Co Labs, Bangalore
- Immersion of silver sheet inside drinking water will not leave residual silver in the treated water (Silver treated water is not harmful to human health).
- Water samples from different locations of Bangalore were treated with silver sheet and the bacterial count reduced considerably with time and size of silver sheet. Samples were collected from following sources:
    - Rainwater
    - Open well water
    - Bore well water
    - Tap water
    - Open pond water
- A total of 220 water quality tests using MPN method and 50 water quality tests using CFU method from 172 water samples for bacterial contamination have been conducted in the laboratory of Indian Institute of Science:
    - Rainwater samples 50
    - Open well samples 61
    - Borewell water samples 46
    - Tap water 11
    - Open Pond water 4
- Bacterial count of over 1600 MPN/100ml was recorded from water samples collected for treatment.
- Water sample (sample no.70a) collected from one of the residence in Magadi Road, Bangalore (tap water from multiple sources = city supply + borewell) had bacterial contamination of over 1600 MPN/100ml and when treated with silver sheet for 8 hours, the bacterial count reduced to 23 MPN/100ml and the water was potable in 24 hours of treatment and the bacterial count reduced to 5 MPN/100ml. The 48 hours treatment recorded Zero bacterial activity.
- Water samples treated for 8 hours by dipping silver sheet had the effect of removing bacterial contamination as follows – total samples 224:
    - Bacteria removal up to 90% was achieved in 12 samples
    - Bacteria removal up to 75% was achieved in 36 samples
    - Bacteria removal up to 50% was achieved in 71 samples
    - Bacteria removal up to 25% was achieved in 97 samples
    - Bacteria removal of less than 25% was observed in 8 samples
- Comparison of water treatment by dipping silver sheet against three commercially available water purifiers

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ANNEXURES

Annexure I

Abbreviations:

H – Himedia
M – Merck
H_2S – Hydrogen Sulphide
HPC – Heterotrophic Plate Count
CFU – Colony Forming Unit
MPN – Most Probable Number
MTFT – Multiple Tube Fermentation Technique
IS – Indian Standard
BIS – Bureau of Indian Standard
CPCB – Central Pollution Control Board
EPA - Environmental Protection Agency
UF - Ultra Filtration
NSF - National Science foundation
GOK - Government of Karnataka

Annexure II

Silver sheet size and weight:

- Sheet 1 – 23cm x 43cm, 136.600 g
- Sheet 2 – 22cm x 48cm, 156.507 g

Above sheets were cut to the following sizes for experiments:

- Sheet 3 – 3cm x 32cm, 14.004 g
- Sheet 4 – 5cm x 21cm, 15.730 g
- Sheet 5 – 9.9cm x 21cm, 23.146 g
- Sheet 6 – 10cm x 21cm, 23.295 g
- Sheet 7 – 15cm x 21cm, 31.345 g
- Sheet 8 – 19.5cm x 43cm, 117.305 g

Annexure III

Details of Chemicals used in different methods of water testing:

- **Chemicals used in CFU method**

1) Luria broth from Himedia – 500g
2) Agar Agar type – 1 from Himedia – 500g

- **Stains used in gram staining method**

1) Grams crystal violet staining solution
2) Grams iodine stating solution
3) Grams safranine staining solution

- **Chemicals used in MPN method**

1) Lactose monohydrate – RM565 – 500g – (H)
2) Sodium chloride / GR – 500g – (M)
3) Di - potassium hydrogen phosphate / GR – 500g – (M)
4) Potassium di hydrogen phosphate / GR – 500g – (M)
5) Tryptose – RM030 – 500g – (H)
6) Hydrochloric acid CR / SD's – 500mL
7) Ethanol – AR – 99.9 %

Source of Chemicals:

M/S Mahajan chemicals
32, 1st floor, 2nd cross, sirur park road junction,
Malleshwaram, Bangalore – 560 003
Go down: No. 19, 4th main road, D.D.T.T. Ltd., industrial suburb, 2nd stage,
yeshwanthpur, Bangalore – 560 022
Tel: 41686930, 41223063
Fax: 23465459

M/S Lab supplies India pvt. Ltd
(Formerly laboratory supplies Co. Estd. 1925)
14, 21st cross, cubbonpet, Bangalore – 560 002
Tel: 22212256, 22485257
Fax: 22292848

M/S Lab Needs
No. 49, 7th main road, opp: lakshminarasimha temple,
3rd block, thyagaraja nagar, Bangalore – 560 028
Tel Fax: 26772200, Mob: 9886401900

Annexure IV

MPN table: MPN Index and 95% confidence limits for various combinations of positive results for Methods 9221B and 9221E: Multiple-Tube Fermentation Technique for Fecal Coliforms (EC Medium) and Standard Total Coliform Fermentation Technique where five tubes per dilution are used (10 mL, 1.0 mL and 0.1 mL sample portions).

Combination of positive tubes	MPN/100 mL	95% Confidence Limit		Combination of positive tubes	MPN/100 mL Lower	95% Confidence Limit	
		Lower	Upper			Lower	Upper
0-0-0	<2	1.0	10	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	14	4-3-0	27	12	67
0-2-0	4			4-3-1	33	15	77
				4-4-0	34	16	80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1	50	20	150
				5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	4	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1300
4-1-0	17	7.0	46	5-5-2	500	200	2000
4-1-1	21	9.0	55	5-5-3	900	300	2900
4-1-2	26	12	63	5-5-4	1600	600	5300
				5-5-5	>1600		

Annexure V

Equipment Details

Autoclave:

PSM vertical type
Temperature – Digital display,
Pressure set – 140^0C, 15pounds
Heater – on/off
Temperature up to 125^0C

Incubator:

Bacteriological type
30tubes capacity
Length = 28cm. Width = 13.5cm
Temperature up to 50^0C (0.3+ or - variation)
Digital display, 230v

PH meter:

Calibrate the instrument every time during PH check
Order of the calibrating standard solutions is 4, 10, and 7
Digital, PH tutor

Weighing machine:

Digital display, DC – 10v, 0.3A
Electronic Balance d=0.001g, Max=310g (AFCOSET)

Laminar Air Flow Chamber:

Horizontal airflow chamber
Motor ¼ HP, 1440rpm, HEPA: 3"*2"

Hot Air Oven:

Non digital display
Temperature – 140^0C

Acknowledgement

The financial assistance was provided by the Department of Science & Technology, Government of India New Delhi to implement this project "*Bacteria Free Water for Drinking by Silver Treatment (BFWDST)"*.

I sincerely thank Secretary KSCST Prof M.S. Mohan Kumar and Executive Secretary Dr M. Prithviraj for their continued support and guidance, from conceptualization of the project to successful implementation, and constant encouragement provided at various levels for successful completion of the project.

My special thanks are to Dr. Sanjay Bajpai, Dr. Neelima Alam and Mr. Vineet Saini, DST GOI for their encouragement and support.

This Project has the unique distinction of having Karnataka State Council for Science & Technology and Chemical Engineering Department of Indian Institute of Science, Bangalore collaborating for research project on water purification. Chemical Engineering Department of Indian Institute of Science played the key role in providing the infrastructure for the research activities and also providing technical guidance. The tireless team of Project associates Tejaswini K, Sowmya Shree S.D, Bhargavi M.P, Prajanya G.P and Navya Shree R. carried out various aspects of the project at different period of the project duration. My sincere thanks are due to them.

This programme is the result of collective efforts of KSCST, RWH Team at KSCST, Chemical Engineering Department of IISc and many institutions and individuals who had the sprit to respect water conservation and treating water using simple methods. I acknowledge the commitment and cooperation of each and every one who made this project a success.

I acknowledge the guidance provided by Ms Shashirekha, Former Chief Chemist, Mines and Geology Department, Government of Karnataka.

My earnest thanks to KSCST for providing this opportunity and to all my colleagues at KSCST for their support in implementing this Project.

Project Co Investigator Prof. K. Kesava Rao was an inspiration and a bank of information who relentlessly guided the project team for the successful implementation. I thank him for the partnership.

A. R. Shivakumar
Principal Investigator