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Antibacterial Performance Evaluation of Silver-Coated River Sand for Water Decontamination Application

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ABSTRACT

The presence of microbes in drinking water is a serious health hazard demanding immediate attention. Silver is known for centuries for its highly effective antimicrobial properties against a variety of microorganisms. Sand is a natural filter media that is widely used in water purification systems for the removal of dirt and suspended matter from water. Hence the development of additional antimicrobial features in commonly used filter material i.e. sand by coating with silver is an alternative technology for providing a safe drinking-water free from microbes. The present study was performed to develop a cost-effective material with antimicrobial properties by coating locally available river sand with silver. The coated material was subsequently used for its antimicrobial performance by using standard methods. To perform the tests *E.coli* was isolated from wastewater by using standard microbiological protocols. Thereafter, a biochemical test and antibiotic sensitivity assay were performed. Synthesised silver-coated sand was tested for its antibacterial activity against *E.coli* through the agar well diffusion method. The results showed a zone of clearance ≥40 mm with 700 mg of synthesised sample. To further determine the efficacy of developed material against *E.coli* load in artificially contaminated water, experiments were conducted by passing contaminated water through the material stuffed inside a hollow tube filter. A colony count reduction of 86.67 per cent was observed on passing 1000 ml of $3x10^3$ CFU/ml contaminated water through the filter. The present study suggests that additional functionality of microbial reduction can be introduced in the sand through the silver coating. The developed material can be effectively used for the removal of fecal coliforms (*E.coli*) present in water bodies at an effective cost in addition to the removal of traditional impurities like dirt and suspended materials.

Keywords: Antimicrobial; The zone of clearance; Silver-coated river sand; Colony count

1. INTRODUCTION

Water is fundamental to life, hence a safe and sufficient stock must be accessible to all for their well-being. But in many developing nations, access to both clean water and sanitation is not up to the prescribed standards resulting in waterborne disease outbreaks[1-2]. Intense microbial diarrheal sicknesses are a significant general medical issue in developing nations. Due to unhealthy sanitary practices, followed by common folk the highest microbial dangers are related to ingestion of water that is contaminated with human or animal dung. It has been reported that more than 1.5 million children die every year from diarrheal maladies[3]. Kids under five, principally in Asian and African nations, are the most influenced by microbial infections transmitted through water.[4-5] As per WHO, the mortality of water-related infections surpasses 5 million individuals each year. Among these, more than half are microbial intestinal contaminations with cholera at the top. By and large terms, wastewater releases in freshwaters and coastal seawaters are the significant sources of fecal microorganisms, including pathogens.[6] Even among the developed nations, in the USA, every year 560,000 individuals experience the ill effects of extreme waterborne diseases, and 7.1 million experience the ill effects of mild to direct contaminations, bringing about assessed 12,000 passing per year.[7]

In urban and rural areas, the bacteriological nature of freshwater sources is often compromised by the discharge of untreated local wastewaters.[8] The bacteriological quality of such waters is hardly resolved by utilizing any select pointers, for example, fecal coliforms, E. coli, and fecal Streptococci.[9] Drinking water if drawn from such sources, generally contains various pathogenic microorganisms and it turns out difficult to treat and deliver clean drinking water as the pathogens vary depending on the source of the contamination.[10]

The Jia-Bharali river basin is one of the most evolved regions in the north Brahmaputra plain of India.[11] The area has broad tea-estates and paddy fields. The northern part is comprised of reserve forests and scantily populated timberland towns. The area possesses large amounts of biodiversity with evergreen and deciduous trees of numerous kinds. In the ongoing years, deterioration in the quality of practically all water bodies has been observed because of intensive horticulture, urbanisation, and starting small industries in the

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basin. In the prior publications water quality of the basin was reported taking just normal estimations of the parameters as measurable investigation in both the seasons which lacked elaborate interpretation of water biology.[12-14] The sand from the river basin can be utilised for water refinement techniques with low capita investment through appropriate modifications.

Also, the antimicrobial properties of silver have been known to different cultures all around the world for many centuries. The Phonecians stored water and other liquids in silver coated bottles to discourage contamination by microbes. Silver dollars used to be put into milk bottles to keep milk fresh, and water tanks of ships and airplanes that are "silvered" can render water potable for months In 1884 it became a common practice to administer drops of aqueous silver nitrate to newborn's eyes to prevent the transmission of Neisseria gonorrhea from infected mothers to children during childbirth[15-17]. In 1893, the antibacterial effectiveness of various metals was noted and this property was named the oligodynamic effect. It was later found that out of all the metals with antimicrobial properties, silver has the most effective antibacterial action with the least toxicity to animal cells. Silver became commonly used in medical treatments, such as those of wounded soldiers in World War I, to prevent microbial growth.

Once antibiotics were discovered, the use of silver as a bactericidal agent decreased. However, with the discovery of antibiotics came the emergence of antibiotic-resistant strains such as CA-MRSA and HA-MRSA, the flesh-eating bacteria. Due to increasing antibiotic resistance, there has recently been a renewed interest in using silver as an antibacterial agent. As a result, different types of silver-coated/embedded materials have been developed from time to time and their microbial decontamination properties evaluated for the treatment of microbiologically contaminated water[18-23].

In the present study, sand was collected from banks of the local Jiya Bharali River. Since it was already being used by the local community as a household filter media for removal of dirt and suspended matter. Additional coating with silver was done to enhance its performance by incorporating antimicrobial property. Subsequently, the antibacterial activity of silver-coated sand was tested for its efficacy and potency against one of the major fecal coliform bacteria *E.coli* isolated from wastewater, which is widely recognised as an indicator organism of contaminated water. The material was also evaluated for reducing the bacterial load from artificially contaminated water by passing it through a hollow tube stuffed with the material. Our results demonstrate a substantial decrease in the bacterial count after passing artificially contaminated water through silver-coated sand.

2. METHODOLOGY

2.1 Materials

The sand sample was collected from the banks of the Jia Bharali river situated in the Eastern Himalayan Mountain, which originates in the Tawang district. Sterile borosil glassware was used for laboratory experiments. Doubly distilled/sterilised water was used for all experiments. Microbial experiments were carried in BSL–2 with all necessary precautions.

2.2 Preparation of Silver Coated Sand

The sand was thoroughly washed with distilled water and dried. Sieving was done by using a sieve of 35 mesh size to remove the larger particles. 500g of sieved sand with an effective size of 0.8mm was again washed thoroughly and dried in the hot air oven at 110˚C. The dried sand was sprayed with a 10 per cent silver nitrate aqueous solution and kept for drying. The silver nitrate treated sand was suspended in a 4 per cent aqueous solution of a mild reducing sugar and stirred manually in a mechanical stirrer for reduction of silver nitrate and precipitation of silver nanoparticles on the surface of the sand. The treated sand was then washed several times with distilled water and left for drying at 110˚C in the hot air oven. Energy-dispersive X-ray analysis (EDX) analysis was carried out (Carl ZEISS, EVO50) to confirm the silver coating on the surface of the sand (Fig 2).

2.3 Isolation of *E.coli* Bacteria using HiWater Test Kit (K015-1KT)

Wastewater samples were collected from a community waste treatment facility (receiving human fecal matter). *E.coli* bacteria were isolated from the human fecal matter treatment facility Tezpur, Assam (26° 39' 4.3848'' N and 92° 47' 1.7268'' E). HiWater Test Kit (K015-1KT) was used for isolation of *E.coli* species from the wastewater. MacConkey and EMB Agar plates were used for the growth of *E.coli* and their morphological characteristics (colony colour, shape, and size) were recorded.

2.4 Biochemical Characterisation of *E.coli*

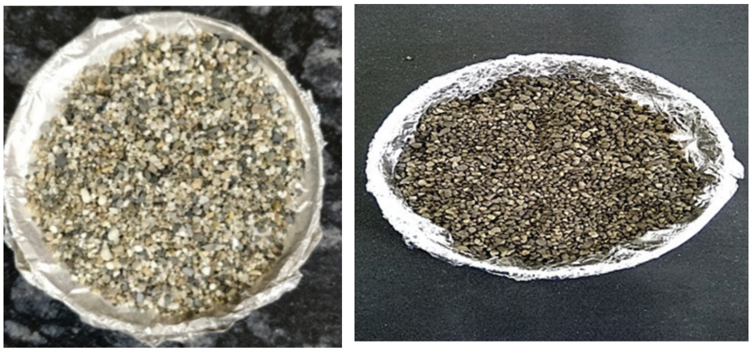
A biochemical test was performed by using the HiWater *E.coli* identification Kit (KB010). The results were observed after overnight incubation at 37˚C.

2.5 Antibiotic Sensitivity Assay

The antibacterial tests were done by using commercially available Hexa disc (HiMediaHexa G-Minus1 and Hexa G-Minus2: HiMedia India). i.e. Ampicillin, Amoxyclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin, Ceftazidime, Ciprofloxacin, Amikacin, Nitrofurantoin, Netillin and, Nalidixic acid. Tryptone Soya Agar (TSA) plates were prepared and 100 µl of *E.coli* bacterial culture was spread on the plates. The discs were applied using an aseptic technique and the rings were deposited at the center of the plate using sterile forceps and kept for overnight incubation at 37 ˚C (Fig. 6). Interpretation of results was done according to Clinical Laboratory Standard Institute (CLSI) guidelines as resistant, intermediate, or sensitive.

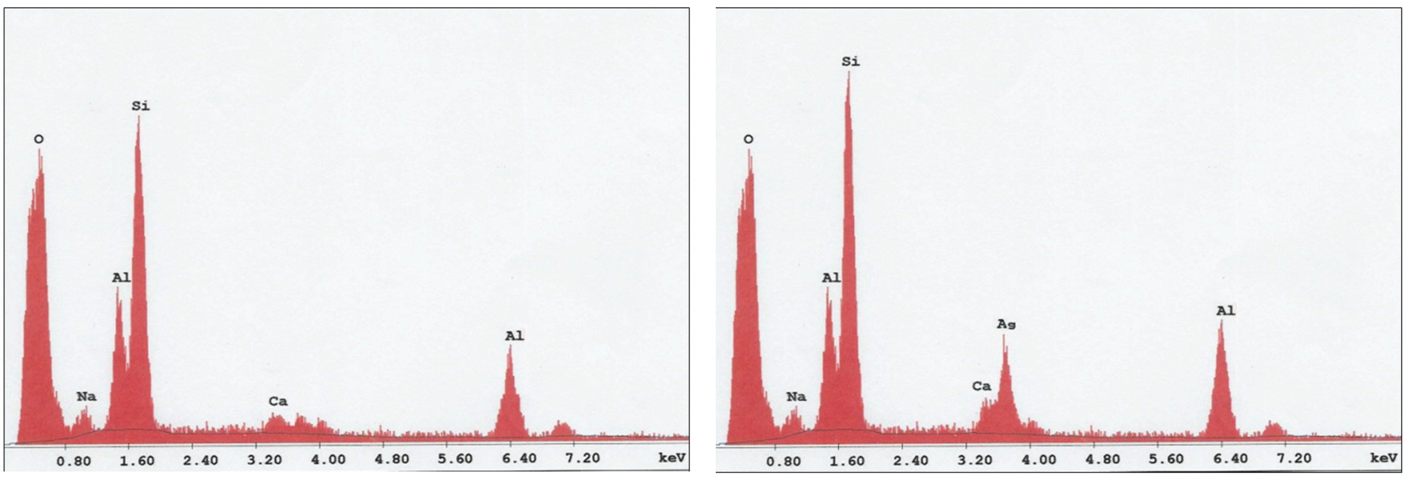
2.6 Antibacterial Activity of Test Material by Agar Well Diffusion Method

The preliminary antibacterial activity of the silver-coated sand was evaluated using the agar well diffusion assay. A colony-forming unit (CFU) of $\sim 10^6$/ml was spread on the Luria Bertani Agar (LBA) plates. Agar well (6 mm) was punched with the help of sterilised micropipette tips and loaded with 700 mg of the sample. The plate was then incubated for 20 h at 37 °C and the zone of inhibition was recorded in millimeter



(a) (b)

Figure 1. Digital images of (a) uncoated and (b) silver-coated river sand.



(a) (b)

Figure 2. EDX spectra of (a) uncoated and (b) Ag coated sand.

(mm) (Fig. 7).

2.7 Testing of Filter Loaded with Silver-Coated Sand for Antibacterial Activity

3 ml freshly prepared culture was centrifuged and the pellet was washed with sterilised distilled water. It was then resuspended with 3 ml of sterilised distilled water. 3 ml of the culture was inoculated in 1 L of sterilised tap water and mixed well. The filtrate was taken after passing it through a hollow tube filter which was half-filled with 44 g of silver-coated sand. The collected filtrate was plated on NA media and incubated at 37 ^oC for 24 h.

3. RESULTS AND DISCUSSION

3.1 Visual Inspection and EDAX Analysis

Visual observation of uncoated sand displays light-colored texture which darkened to greyish after silver coating. The colour change after the silver coating is very clearly visible to the naked eye as displayed in Fig. 1.

The elemental composition of sand surface coated with silver was analysed by using the energy dispersive X-ray analyser. Fig. 2 (a) and Fig. 2 (b) show the EDX spectrum of uncoated and silver-coated sand respectively. The EDX spectrum of uncoated sand (2a) shows only the mineral peaks, which come from the sand constituents. The spectrum of coated sand shows an additional peak of silver which confirms the coating by silver. Thus, the presence of Ag on the surface of the sand is further confirmed by EDX.

3.2 Isolation of *E.coli* Bacteria using HiWater Test Kit

The digital image of colour change to yellow in Media A bottle after overnight incubation for 24hr at 37 ^oC indicating the presence of *E.coli.* No such colour change was seen in the bottle containing Media B. For further confirmation, a sample was withdrawn from Media A and

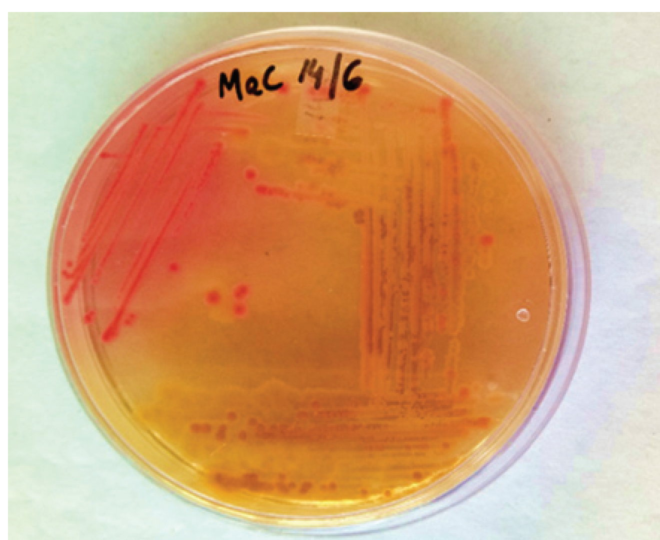


Figure 3. Growth of pink colonies on MacConkey plates showing positive results for *E.coli.*

cultured on MacConkey Agar plates and EMB Agar plates specific for *E.coli* and kept for incubation at 37 ^oC for 24 h. Rapid lactose fermenting colonies of *E.coli* on MacConkey

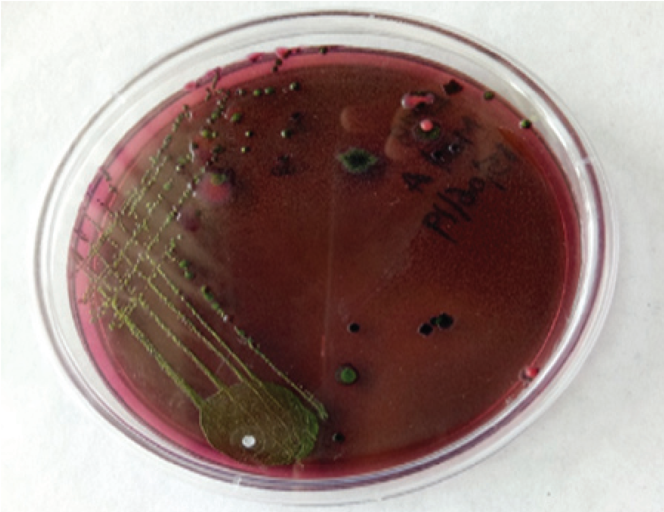


Figure 4. Green metallic sheen on EMB agar plates confirming the growth of *E.coli.*

plates (Fig. 3) indicated positive results for *E.coli*. Further EMB Agar which is selective media for *E.coli* was used for the identification and appearance of green metallic sheen (Fig. 4) on the plate confirmed the growth of *E.coli.*

Strong fermentation of lactose with high levels of acid production by the bacteria causes the colonies and confluent growth to appear bright pink to red.

Rapid fermentation of lactose and production of strong acids results in a rapid reduction in the pH of the EMB agar the critical factor in the formation of the green metallic sheen observed with E. coli.

3.3 Identification using Biochemical Studies

A biochemical test was performed using KB010 Hi E.coli Identification Kit and the results were observed after overnight incubation at 37 ˚C.

Table 1 shows results of the biochemical characterisation of isolated bacteria. It was observed that the test showed positive results for catalase, indole production, methyl red, glucuronidase, nitrate reduction, and production of acid from glucose, lactose and sucrose, and sorbitol. However, the test was negative for Voges-Proskauer reaction, urease production, and citrate utilisation. Hence from the above results as well as literature reports[24] it might be *E.coli*.

Table1. Biochemical characterization of isolated bacteria

Test	Inference
Methyl red	+
Voges Prokauer’s	_
Citrate utilization	_
Indole	+
Glucuronidase	+
Nitrate reduct-ion	+
Urease	_
Lysine utilization	+
Lactose	+
Glucose	+
Sucrose	+
Sorbitol	+
Catalase	+

3.4 Antibacterial Activity of *E.coli*

The antibacterial tests were done using HiMedia Hexa G-Minus1 and Hexa G-Minus 2 for 12 antibiotics i.e. Ampicillin, Amoxyclav, Cefotaxime, Co-Trimoxazole, Gentamicin, Tobramycin, Ceftazidime, Ciprofloxacin, Amikacin, Nitrofurantoin, Netillin, Nalidixic acid. The results of these antibacterial activities against *E.coli* are shown in Table 2 and Fig. 5.

Table 2. Results of antibacterial activity against *E.coli*

Antibacterial activities	Result
Antibiotics	Inference
Ampicillin	Resistant
Amoxyclav	Resistant
Cefotaxime	Intermediate
Co-Trimoxazole	Resistant
Gentamicin	Intermediate
Tobramycin	Resistant
Ceftazidime	Intermediate
Ciprofloxacin	Intermediate
Amikacin	Sensitive
Nitrofurantoin	Sensitive
Netillin	Sensitive
Nalidixic acid	Resistant

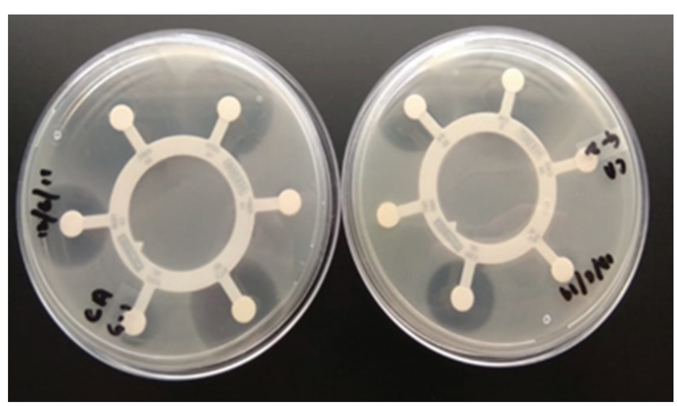


Figure 5. Antibiotic susceptibility test of *E.coli.*

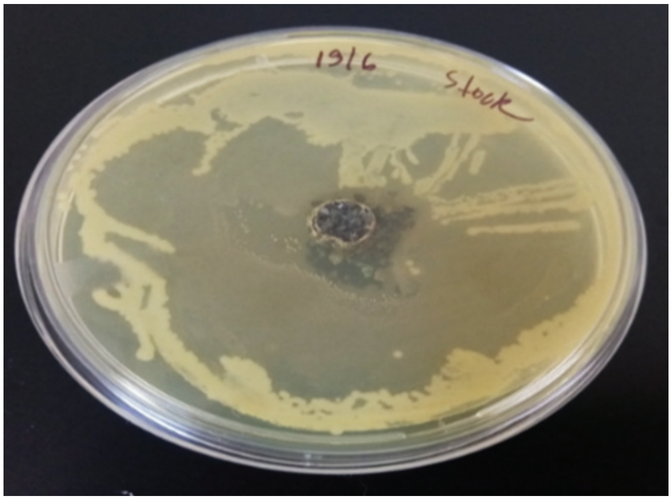


Figure 6. Antibacterial activity test showing zone of inhibition on the plate.

3.5 Antibacterial Activity of Test Material against *E.coli*

To evaluate the antibacterial activity of silver-coated sand, an agar well diffusion assay was done with 700 mg of the synthesised material. The plate was incubated overnight at 37 °C and the zone of inhibition was measured to be >40 mm as displayed in Fig. 6.

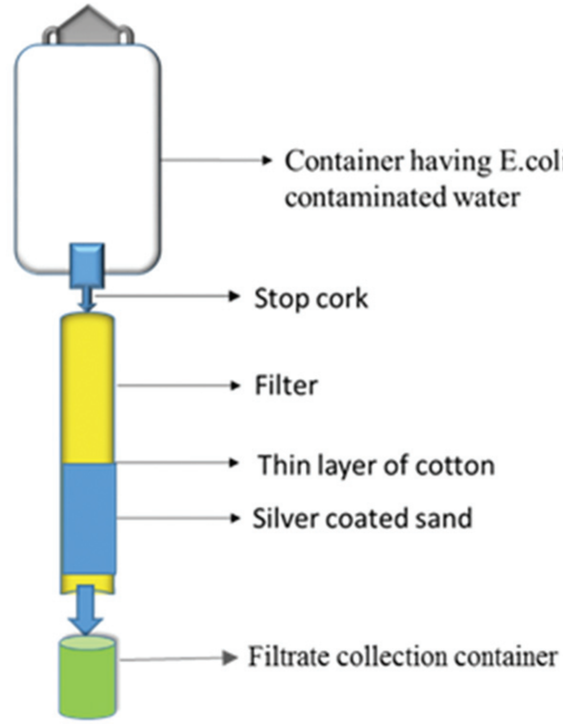


Figure 7. Experimental set up of the filter.

3.6 Testing of the Filter

Table 3. Comparison of results with literature

Base Material	Coating/ Modification	Targeted Microbes	Reference	Percent Removed (%)
Polyurethane (Thermoplastic Polyurethane-TPU and Waterborne Polyurethane-WPU)	Silver nanoparticles, Titanium dioxide, and Chitosan	*E. coli, Staphylococcus aureus*	25	E.coli- 96.5 S.aureus – 93.7
Luffa Cylindrica (Oyong fiber)	Coated with silver nanoparticles	Removal of *coliform* bacteria	26	2.3 LRV
Luffa Cylindrica fibers (LCF)	Coated with Chitosan, Silver particles	*Staphylococcus aureus, E.coli*	27	S.aureus- 62 E.coli- 74
Cotton (Ct)	Iron, Zinc, Silver oxide nanoparticles (FeCt),(ZnCt),(AgCt)	Reduction of bacterial count from Rawal lake wastewater	28	90
Nano-silica	Silver nanoparticles (AgNPs)	*E.coli, Pseudomonas aeruginosa*	29	80
Silver	Titanium dioxide (nAg/TiO_2)	Photocatalytic inactivation of *E.coli , B.subtilis*	30	bacterial inactivation
Activated carbon	Green silver nanoparticles	*Bacillus subtilis , E.coli , Candida albicans*	31	B.subtilis-85 E.coli- 76
Activated carbon	Silver nanoparticles	*E.coli*	32	100 in 5 mins
Activated charcoal	Silver nanoparticles	*P.aeuruginosa , E.coli, B.subtilis, S.aureus*	33	82, 97, 90, 95 respectively
Locally available river sand	Silver	*E.coli*	Current work	86.67

A hollow cylindrical stainless steel pipe with a porous cap on one end was used to test the antibacterial activity of silver-coated sand. The experimental setup is shown in Fig. 7. A fine layer of sterilised cotton was placed at the outlet having pores and then 44 g of silver-coated sand was added which half fills the volume of the tube filter. The *E.coli* contaminated water was passed through the filter and the filtrate was plated on Nutrient agar plates. The stock solution showed 3000 CFU/ml and the filtrate showed 400 CFU/ml. There is a decrease of 86.67 per cent.

Similar results have been found in prior publications where silver was coated on a variety of substrates and studied for removal of microorganisms including *E.coli.* The results are shown in Table 3.

4. CONCLUSION

In the present study, the silver-coated sand was used for the removal of microbial contamination from water. The prepared material is quite cost-effective as the base material is locally available in all areas including the remote regions of North-Eastern parts. From the results, it can be concluded that silver-coated sand can be used effectively for the removal of microbes as silver is very effective in removing bacteria due to its positive ion charge which acts on the microbial cell wall and causes its degradation. Besides the biochemical test was done to confirm the identification of isolated bacteria and it was found to be *E.coli.* Furthermore, the zone of inhibition assay showed around 40 mm zone of inhibition against *E.coli.* 86.67 per cent reduction in the bacterial count was observed when a stock solution of 3000 CFU/ml was passed through filter that the silver-coated sand may be a alternative option to achieve satisfactory bactericidal performance with a relatively low material cost and consumption rate. Hence this material can be efficiently used in small-scale or household purpose water filters.

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She has contributed to the overall execution, writing, and editing of the paper.

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