Low cost and sustainable surface water purification methods using *Moringa* seeds and scallop powder followed by bio-sand filtration

Sharmin Zaman, Anowara Begum, K. S. Rabbani and Latiful Bari

ABSTRACT

A simple and inexpensive water purification method was developed using natural coagulant (*Moringa* seed powder) and antibacterial agents (scallop powder) followed by bio-sand filtration. In this study, surface water from different sources (e.g. pond, canal, lake and river) treated with combined *Moringa* seed powder, and scallop powder (MOSP) at a ratio of 2:1 for 10 minutes showed a clear water layer at the top and a sediment layer at the bottom. The clear water was then passed through an eight-fold sari cloth and/or natural bio-sand filtration and the resulting water was found drinkable. The microbiological and physico-chemical quality of this water showed non-significant differences compared to the US EPA drinking water quality parameters. Moreover, no cytotoxic effect of MOSP with BHK-21 and HeLa cells, nor any bacterial endotoxins were observed, indicating use of MOSP does not pose any adverse health effect. Furthermore, the quality of 6 months stored water at room temperature was found acceptable, indicating the usefulness of this technology for drinking water purposes. On the other hand, the sediment or sludge produced in this process could be used in agricultural fields after proper composting. Therefore, this simple, inexpensive and ecofriendly method could be used in drinking water scarcity areas of the world.

Key words | inexpensive water purification, *Moringa* seed powder, scallop powder, surface water

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INTRODUCTION

Safe drinking water is a fundamental need for healthy human life and water has been shown to be linked to the development of all areas including health, nature, urbanization, industrialization, energy production, food security, and equality, etc. (Colwell *et al.* 2003). In all these areas, not only water but also its cleanliness, purity, safety and quality are required to ensure proper development. According to the United Nations, to meet daily necessities such as drinking, cooking, and personal hygiene, approximately 20–40 L of clean water is required by each individual. In today's world, scarcity of safe water is a burning issue; especially in developing countries where nearly 1.0 billion people lack fresh water for daily uses (Aho & Lagasi 2012).

Approximately 71% of the earth's surface is covered with water and more than 97% of the earth's water is salty sea water, while 2% is stored in glaciers, ice caps, and snowy mountain ranges (Yongabi 2010) and thus less than 1.0% of the earth's water is surface water, of lakes, rivers, streams, ponds (0.022%) and groundwater (0.397%) available for humans for their daily needs (Yongabi 2010). However, all this accessible surface water is not necessarily safe for drinking, and depending on the location and sources, clean water is exposed to a variety of contaminants, many arising from human and animal wastes, debris from homes, chemicals from industries, fertilizers and pesticides from agricultural lands, environmental pollutants and so on (Ngwenya 2006). In addition, insanitary practices of people has greatly contributed to the deterioration of the quality of surface water sources. On the other hand, groundwater is the most important source of water supply in

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Bangladesh. Groundwater is clear, colorless with little or no suspended solids and has a relatively constant temperature. Groundwater is also free from disease-producing microorganisms which are normally present in large numbers in surface waters. However, groundwater for drinking purposes has become a problem because of: (1) the presence of arsenic; (2) excessive dissolved iron; (3) salinity in the shallow aquifers in the coastal areas (Sawai *et al.* 2013); (4) lowering of groundwater level; and (5) rock/stony layers in hilly areas. Among these problems arsenic in groundwater has become a great concern for the water supply in Bangladesh. According to a study by the World Bank's Water and Sanitation Programme (2014), about 28 million Bangladeshis, or just over 20% of the population, are living in harsh conditions for receiving clean water, and the safe, clean water supply situation will worsen in the coming decades to meet the needs of a predicted 200 million people by 2020 (Silvia *et al.* 2011).

Depending on the water sources, numerous water purification methods were developed to disinfect surface water to make clean water. For decades, the commonly used methods for treating water are reverse osmosis, ion-exchange, ultraviolet (UV)-irradiation, coagulation with aluminum sulfate (alum) and iron (II) chloride etc. The purpose of all these treatments is to inactivate microorganisms and to remove organic and inorganic particles from the water before consumption (Yongabi 2010). However, recent studies claim that these methods are becoming un-ecological, expensive to run, manage and maintain, and most importantly, unhealthy. Excessive use of coagulant chemicals has dreadful consequences for health being linked with cancer, neurological disorders, etc. (Amagloh & Benang 2009; Yongabi *et al.* 2011; Aho & Lagasi 2012; Mangale *et al.* 2012). On the other hand, several filtration methods have also been used, depending on the pore sizes, carbon block filters, porous ceramic containing colloidal silver, reactive membranes, fibre or cloth filters, to get clean water (WHO 2011).

To circumvent the use of chemical agents to purify water, scientists have started focusing on natural products which can be used to treat water (Amagloh & Benang 2009). Varieties of plants and their different parts were screened for coagulation and antimicrobial activity (Mangale *et al.* 2012). *Moringa oleifera* (drumstick) was found to be a useful plant and numbers of studies have been carried out to establish the coagulation activity of the seed of this plant (Ali *et al.* 2009; Choubey *et al.* 2012; Zaman *et al.* 2013; Kalikawe *et al.* 2015). Heated scallop shell powder has been permitted to be used as a food additive in many countries. Many study reports demonstrated that heated scallop shell powder treatment successfully decreased bacterial count in shredded cabbage, lettuce, frankfurters, and destroyed *Bacillus subtilis* spores, and inactivated or removed many foodborne pathogens including *Escherichia coli*, *Salmonella* spp., *Listeria* spp. and *Staphylococcus aureus* from the surfaces of wide ranges of fruits and vegetables (Sawai *et al.* 2001, 2003; Kim *et al.* 2006; Kubo *et al.* 2013).

Disinfection of surface water by boiling is an effective and better practice, because it will kill all waterborne pathogenic microorganisms. However, boiling practices are not routinely used in villages because of high costs and a limited supply of fuel and wood in rural Bangladesh. Moreover, during severe flooding, which frequently occurs in Bangladesh, there are geographical areas that experience a reduction in the quality of life to mere survival, when even the barest necessities are difficult to obtain and building fires to boil water is simply not possible. Thus, the common practice in villages in Bangladesh is to use cloth, frequently a flat, unfolded piece of an old sari, to filter water and home prepared drinks (Huq *et al.* 2010). Hence, a sari filter was used in this study.

We were looking for a simple, inexpensive and environmentally friendly technology using locally available natural coagulant and antimicrobial agents followed by bio-sand filtration to convert surface water to drinking water. Thus, in this study, *Moringa* seed powder alone and/or in combination with scallop powder was used in treating surface water and then passed through sari or natural bio-sand filtration (sand, charcoal, and gravel) to make clean and potable water at the household level.

MATERIALS AND METHODS

Sample collection

Surface water samples were collected from Shahidullah Hall, Jagannath Hall, Bangla Academy pond of Dhaka University area, Dhanmondi Lake and river water was collected from Shitalakshya River at Narayangonj, Padma River at

Manikgong, and Meghna River at Bhola District of Bangladesh. Care was taken while choosing locations and point of water collection of ponds, rivers, and lakes. Immediate vicinities of the pond corner were avoided and samples were collected from approximately 150 cm distance from the corner of the ponds. Water samples from a depth of 4.0–6.0 cm were collected in sterile polyethylene terephthalate (PET) bottles according to the standard procedures of APHA (1992) and transported to the laboratory in an insulated cool box maintaining a temperature of 6–8 °C within 4–6 hours of collection. Microbiological and physico-chemical analysis of the collected water was done on the same day. The collected water samples were either filtered through eight-fold cotton sari to reduce/eliminate protozoa and debris, or not filtered before analysis.

Treatment procedure

Moringa seed powder (1.0 g) and scallop powder (2.0 g) was added to 1,000 mL water samples and mixed vigorously using a sterile glass rod for 1 min and kept for 30 min to settle the sediment. The clear water from the upper portion was then passed through a three-step natural bio-sand filtration especially designed in a PVC pipe (Figure 1). The microbiological and physico-chemical analysis of the filtered water was carried out as per United States Environmental Protection Agency (US EPA) drinking water quality parameters (US EPA 2015). The filtered/non-filtered water samples were stored for 6 months at room temperature (26–28 °C), and periodic quality parameters were analyzed. Each experiment was performed five times and the average results were presented in tables and figures.

Sari filtration

One of the cheapest ways of removing turbidity is by filtering the turbid water with cotton clothes. These fabrics are cheap and readily available, which make them perfect materials for water treatment in rural areas. Cotton sari clothes (a traditional garment of unstitched cloth ranging from 4 to 9 m in length that is draped over the body of women) is folded over three times (eight layer) and a water sample was passed through the folded sari. Both non-filtered and filtered water were analyzed for physico-chemical and microbiological analysis.

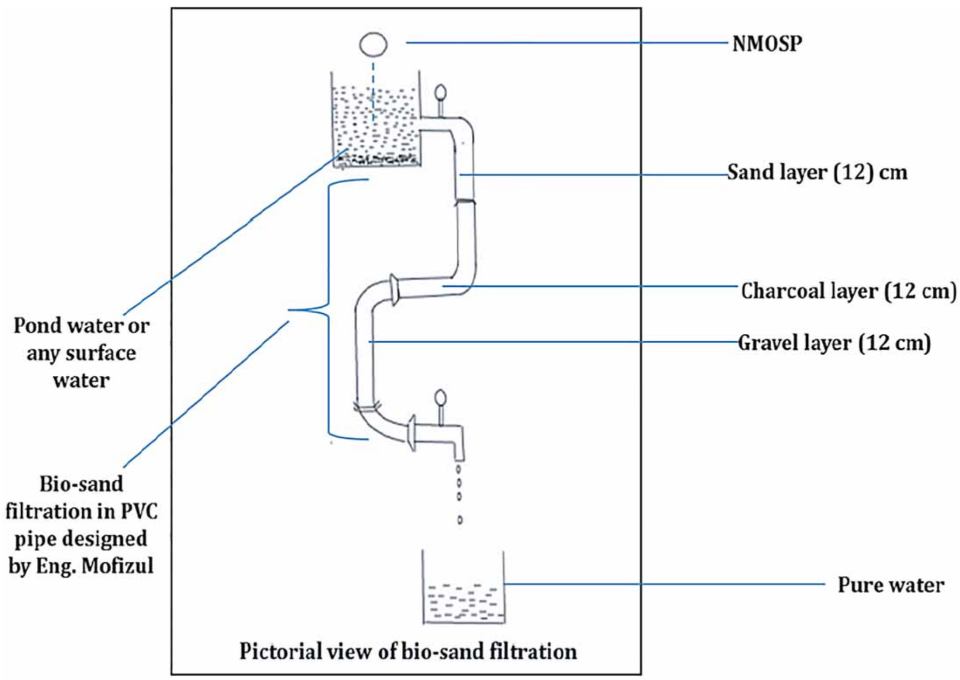

Figure 1 | Pictorial image of bio-sand filtration.

Bio-sand filtration

A bio-sand filter is a point-of-use water treatment system adapted from traditional slow fine sand filters. Bio-sand filters for this study were typically constructed using a PVC pipe with a tightly fitted lid to prevent contamination and unwanted pests from entering the filter. A 3-step bio-sand filtration was constructed as shown in Figure 1. At the top of the filter, fine sand (0.1–0.3 mm in diameter) column (12 cm), below the sand column, a layer of charcoal (0.8–1.0 mm in diameter) (12 cm) and below the charcoal column, a layer of gravel (3.0–4.0 mm in diameter) (12 cm) was placed. Each layer was separated by using plastic mesh that prevents sand particles entering into the charcoal column and charcoal particles into gravel layer. A water sample was passed through the bio-sand filter and both the non-filtered and filtered water was analyzed for physico-chemical and microbiological analysis.

Physico-chemical analysis

The physico-chemical properties including color, odor, taste, pH, turbidity, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), conductivity, major metal (Fe, Na, Ca, Mg and Zn) contents, heavy metals (As, Cd, Cr, Pb) contents were carried out according to the US EPA (2015) and BSTI (2001) standard methods.

Bacteriological analysis

The bacteriological analysis includes total aerobic bacteria, total coliform, fecal coliform and presence of *E. coli, Salmonella* sp. and *Vibrio* sp. was carried out according to the USFDA Bacteriological Analytical Methods (2001). Spread plate technique on selective and non-selective agar media was used to identify specific bacteria. Tryptic Soy Agar (TSA, Fluka, USA) for total aerobic microbial count, Hichrome TM Coliform Agar (Fluka, USA) for total coliform count, Sorbitol MacConkey Agar (Oxoid, UK) for *E. coli*, Bismuth Sulfite Agar (BD, USA) for *Salmonella* sp., and Thiosulfate–Citrate–Bile salts–Sucrose Agar for *Vibrio* sp. were used in this study. All the agar plates were incubated at 35 °C for 24–48 hours before being counted. At least five randomly picked presumptive colonies of *E. coli*, *Salmonella* sp. and *Vibrio* sp. were isolated from selective agar and confirmed using API 20E kit (bioMerieux sa, Marcy-l'Etoile, France) and biochemical test.

Challenge test

E. coli strains CARS-2 (isolated from water) were cultured at 37 °C in 5 mL of tryptic soy broth (TSB; Oxoid, UK) medium supplemented with 50 mg/mL rifampicin (TSB-Rif). Cultures were transferred to TSB-Rif using a loop at three successive 24-h intervals immediately before they were used as inocula. Cells were collected by centrifugation (3,000 × g, 10 min, 22 °C) and suspended in 9.0 mL of sterile saline water (0.85% NaCl solution). The inoculums (9.0 mL) with an initial concentration of approximately 10^7 CFU/mL were maintained at 22 ± 1 °C and added to water samples within 1 h of preparation. Plating on media containing rifampicin greatly minimized the interference of naturally occurring microorganisms and facilitated the detection of test pathogen on recovery media. The isolated colonies were confirmed by detecting the *uidA* gene, specific for *E. coli*, using a molecular method (as shown in Figure 2).

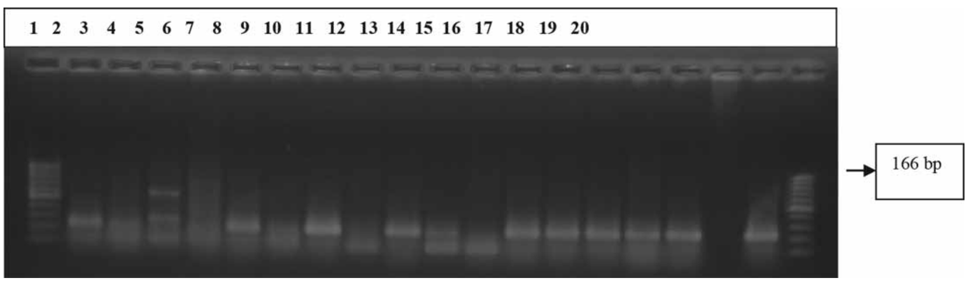

Figure 2 | Representative *E. coli* was confirmed by detecting specific uidA gene using molecular method. Lanes: 1 and 20, represent 100-bp standard DNA ladder; Lanes 2, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17 represent non-treated samples; Lanes 3, 5, 7, 9, 12 represent treated samples; Lane 18, negative control; Lane 19 positive control.

Toxicity test for *Moringa* powder and scallop powder

The cytotoxic effect of *Moringa* powder and scallop powder was carried out on BHK-21 and HeLa cells using tissue culture methods at the cell culture laboratory of the Centre for Advanced Research in Sciences, University of Dhaka. In brief, HeLa, a human cervical carcinoma cell line, and BHK-21, a baby hamster kidney fibroblast cell line, was maintained in Dulbecco's Modified Eagles medium (Sigma, UK) containing 1.0% penicillin streptomycin (1:1) and 0.2% gentamycin and 10% fetal bovine Serum. HeLa (1.4×10^4/100 μL) and BHK-21 (8×10^4/100 μL) cells were seeded onto 96-well plates and incubated at 37 °C + 5% CO_2 chamber. Cytotoxicity was examined under an inverted light microscope (Optika-XDS: 2ERGO, Italy) after 24 h of incubation. Duplicate wells were used for each sample.

Bacterial endotoxins test

The bacterial endotoxins test is an *in vitro* assay for detection and quantitation of bacterial endotoxins, a component of the cell wall of Gram negative bacteria. The endotoxins detection was carried out by limulus amebocyte lysate (LAL) test, using a lysate of amebocytes isolated from the horseshoe crab (*Limulus polyphemus*) clotting reaction. This reaction involves a coagulation cascade of sequentially activated proteases which formed clots with endotoxin. Both the non-treated and treated water samples were tested for bacterial endotoxins according to USFDA (2012) instructions.

Data analysis

All the trials were replicated five times. Reported data represented the mean values obtained from five individual trials, with each of these values obtained from duplicated samples. Data were subjected to analysis using the Microsoft Excel program (Redmond, Washington, DC, USA). Significant differences in plate count data were established by the least significant difference at the 5% level of significance. For other data mean ± SD values are presented in Tables 1 and 4.

RESULTS

Bacteriological quality of raw water and its effect on filtration

Microbial quality is one of the primary indicators for the safety of drinking water. Of all contaminants in drinking water, human and/or animal feces present the greatest danger to public health. The presence of waterborne diseases causing microorganisms in drinking water may result in gastrointestinal illness or diarrhea (WHO 2001). Infants, the elderly and immune-suppressed individuals are at greater risk of serious infection when exposed to these

Table 1 | Comparison of bacteriological quality of raw water (pond, river and lake) and MOSP treated water followed by filtration, with standard value

	Raw water			MOSP treatment followed by sari filtered water[a]			MOSP treatment followed by bio-sand filtration				
	Pond	River	Lake	Pond	River	Lake	Pond	River	Lake		
Bacteriological quality indicators	Average bacterial population (log CFU/ml)			No. of positive/ No. of sample tested			No. of positive/ No. of sample tested			US EPA Std for drinking water	BSTI Standard
Aerobic bacterial count	4.2	3.6	5.4	1.0[a]	1.0	1.0	1.0	1.0	1.0	TT[3]	1,000
Total coliform count	3.4	3.7	4.1	2/5	3/5	3/5	ND/5	ND/5	ND/5	NR	NR
E. coli	2.9	4.3	5.4	1/5	1/5	2/5	ND/5	ND/5	ND/5	NR	NR
E. coli O157:H7	0.8	4.7	4.4	ND/5	2/5	3/5	ND/5	ND/5	ND/5	NR	NR
Salmonella spp.	ND	3.8	4.7	ND/5	2/5	1/5	ND/5	ND/5	ND/5	NR	NR
Vibrio spp.	ND	3.6	5.4	ND/5	1/5	ND/5	ND/5	ND/5	ND/5	NR	NR

[a]Approximately 1.0 log CFU/mL present in the sample tested; Treatment technique (TT[3]) – a required process intended to reduce the level of a contaminant in drinking water; ND = below the detection limit. Detection limit: <1.0 cfu/mL; NR = not recommended.

organisms. Thus, the removal of such organisms is required from the water and many processes are available to inactivate and/or remove pathogens from drinking water. However, the minimum treatment required for water derived from a surface water source directly affected by surface water includes coagulation, sedimentation, filtration and disinfection. In this study, *Moringa* seed powder was used as a natural coagulant and scallop powder as antimicrobial agents. In addition, cotton sari clothes filtration (widely used materials in local village to reduce flocculation) and bio-sand filtration, which are cheap and easy to operate methods, were used to convert surface water to drinking water. The distribution of microorganisms and the presence of pathogens in river, pond and lake water and its effect in different treatment conditions and filtration was performed and is presented in Table 1. The bacteriological analysis results revealed that the pond river and lake water was grossly contaminated with aerobic bacteria ranging from 3.6 to 5.4 log CFU/mL; coliform 3.4–4.1 log CFU/mL), *E. coli* 2.9–5.4 log CFU/mL (Table 1). The presence of a higher number of *Salmonella* spp. ranging from 3.8 to 4.7 log CFU/mL and *Vibrio* spp. ranging from 3.6 to 5.4 was evident in river and lake water but not in the pond water. Irrespective of raw water sources, a higher presence of enteropathogenic *E. coli* O157:H7 was found, which is a strong indication of recent sewage or animal waste contamination. When all these waters were treated with MOSP followed by cotton sari filtration, a significant reduction to below the detection level of resident bacteria and no pathogenic bacteria including *E. coli* O157:H7, *Vibrio* sp. and *Salmonella* sp. was recorded with sari filtered water. Sari cloth, folded three times, provides a filter of about 20 μm mesh size, which was small enough to remove all zooplankton, most phytoplankton, all *Vibrio cholerae* attached to the plankton and other particulates larger than 20 μm. Eight layers of sari cloth was considered optimal for water filtration, since some research reports showed that eight-layer sari consistently removed >99% of the bacteria (Huo *et al.* 1996; Colwell *et al.* 2003). The scan electron micrograph image showing the comparison of a single and eight-layer of new and old cotton sari, presented in Figure 3, shows that an old cotton sari cloth has less pore size than the new cotton sari, because threads of an old cotton sari become soft and loose, reducing the pore size compared to

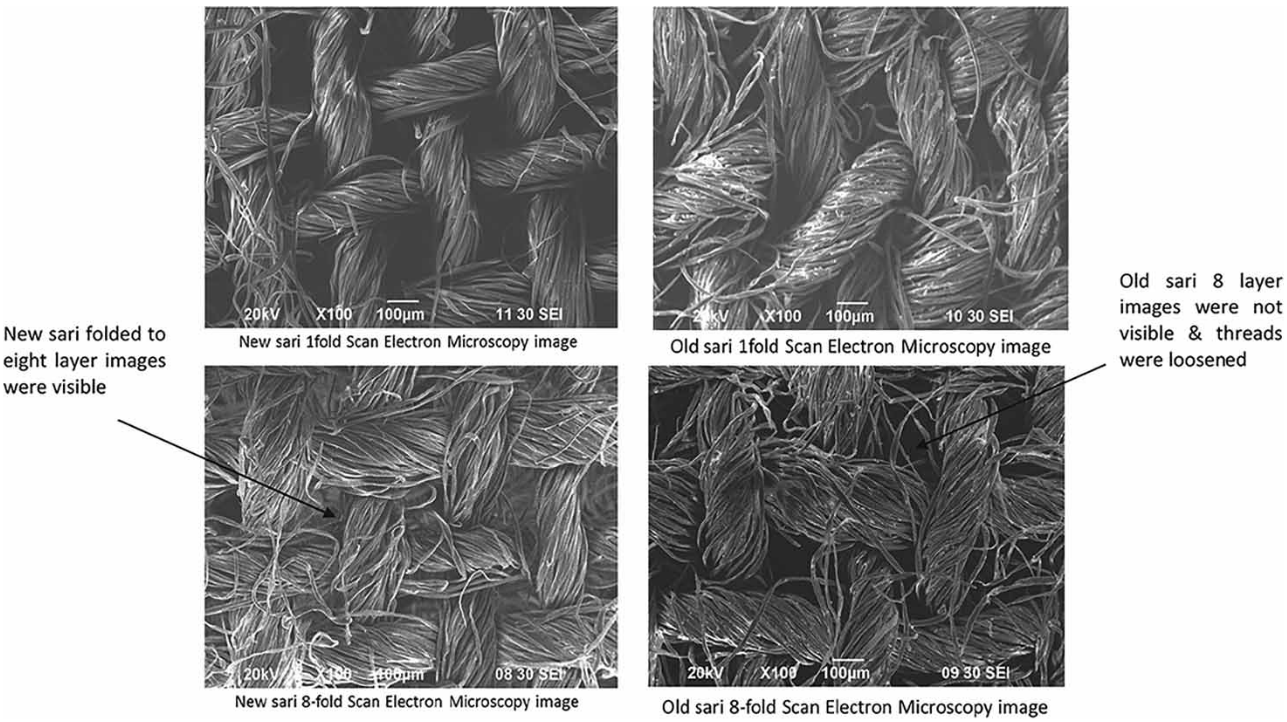

Figure 3 | Comparison of electron micrographs of one- and eight-layer new and old cotton sari.

a new cotton sari whose pores are visible in Figure 3 but were not visible in the old or used sari.

However, a rare presence of pathogen was also evident and this finding suggests that complete elimination of pathogen is not possible with these treatment methods (Table 1). In contrast, when the same water samples were treated with MOSP followed by bio-sand filtration, no pathogenic bacteria was detected throughout the study. Similar experimental results were obtained in a challenge test (data not shown). This finding suggests that bio-sand filtration techniques are better than cotton sari and could be useful in eliminating bacterial contaminants in water.

Physico-chemical quality of raw water and its effect on filtration

Furthermore, in order to treat river/lake or pond water for drinking purposes, removal of high turbidity, bad taste and odor, and appearance are some of the main challenges and must be removed before being used for drinking purposes. One of the cheapest ways of removing turbidity is by filtering the turbid water with cotton sari clothes. These fabrics are cheap and readily available, which make them perfect materials for water treatment in rural areas. The turbidity of river/lake/pond water and sari filtered and/or bio-sand filtered water is presented in Table 2.

Turbidity is the measure of relative clarity of a liquid and the measurement of turbidity is a key test of water quality. In drinking water, the higher turbidity level may lead to gastrointestinal disease (Mann *et al.* 2007). Moreover, high turbidity in water is a possible source of microbial contamination and it also reduces the efficiency of the water purification systems (WHO 2011). The suspended solids interfere with water disinfection because the particles act as shields for the virus and bacteria. Similarly, suspended solids can protect bacteria from UV sterilization of water. Irrespective of water sources, higher turbidity ranging from 54.0 to 59.0 NTU was observed in raw water samples. Sari filtration alone can reduce the turbidity to 38.0–42.0 NTU or 30.0% and MOSP treatment further reduced the turbidity to 50% and MOSP treatment followed by cotton sari filtration was able to reduce it to approximately 70%. Goyal (2015) has shown that on passing a water sample through a cotton cloth folded over three times (eight layers), the turbidity of that water sample was reduced by 48.23%. This finding is also similar to this study of turbidity reduction using cotton sari cloths. In contrast, bio-sand filtration alone was able to reduce the turbidity by up to 80% and MOSP treatment followed by bio-sand filtration was able to reduce the turbidity by up to 99.9% and hence would be applicable for water filtration as the best filtration method compared to cotton sari filtration. According to the World Health Organization, the turbidity of drinking water should ideally be less than 1.0 NTU. Water with turbidity more than 1.0 NTU may be safe for drinking, but the visible cloudiness has a negative impact on consumer acceptability.

Irrespective of treatment and filtration, the other physico-chemical quality parameters including TDS, conductivity, BOD, COD, metal and heavy metal content was found within the recommended level except for pH. The pH of cotton sari filtered water was 9.9; this might be due to the addition of 0.01% scallop powder (Table 3). On the other hand, bio-sand filtration showed acceptable pH levels (7.3–7.6) over sari filtered water (9.8–9.9). When MOSP was

Table 2 | Comparison of turbidity value of raw water and MOSP treated water followed by filtration

Treatment conditions	Turbidity value (NTU)[a]	US EPA Std turbidity value (NTU) for drinking water	BSTI Standard turbidity value (NTU) for drinking water
Control (non-treated)	54.0–59.0	0.5–1.00	5.0
Sari filtration (eight layer)	38.0–42.0		
Sari filtration followed by MOSP treatment	30.0–35.0		
MOSP treated water	26.0–30.0		
MOSP treated followed by Sari filtration	20.0–25.0		
Bio-sand filtration only	10.9–14.4		
MOSP treatment followed by bio-sand filtration	0.9–4.1		

[a]Average value range of raw water from pond, river and lake.

Table 3 | Comparison of physico-chemical characteristics of raw water and MOSP treated water followed by various filtrations with standard value

Physico-chemical parameter	Raw water samples	MOSP treatment followed by bio-sand filtered water	MOSP treatment followed by sari filtered water	US EPA Standard for drinking water	BSTI Standard
Color	Gray to yellowish	15 (color unit)	15 (color unit)	15 (color unit)	5 (Haxen unit)
Odor	Odorous	Odorless	Odorless	3 threshold odor number	Unobjectionable
Taste	Fishy-sour smelly	Agreeable	Agreeable	Agreeable	Agreeable
pH	7.62–8.01	7.37–7.89	9.91–9.94	6.5–8.5	6.4–7.4
TDS	259–374	232–241	131–155	500	500
Conductivity μs/cm	750–780	620–640	260–380	NS	NS
BOD (mg/L)	2.1–2.5	2.71–2.76	2.79–2.80	5.0	5.0
COD (mg/L)	2.214–3.195	2.27–2.31	2.84–2.91	40	40
Iron (mg/L)	0.00	0.00	0.00	0.3	0.30
Manganese (mg/L)	0.00	0.038–0.039	0.019–0.021	0.05	0.50
Zinc (mg/L)	0.00	0.08–0.10	0.12–0.15	5.00	3.00
Lead (mg/L)	0.0003–0.0004	0.0004	0.0003–0.004	0.015	0.01
Arsenic (mg/L)	0.002–0.003	0.002	0.002	0.01	0.05
Cadmium (mg/L)	0.00	0.00	0.00	0.005	0.003

NS = no standard set yet.

added to the water the resulting pH was recorded as 9.8–9.9. This water passes through sand, charcoal and gravel and comes into contact with organic matter, and the presence of charcoal may absorb some color and stone might add some minerals to the water and consequently, the pH was reduced to neutral. In contrast, the sari filter does not have these materials and thereby retains the higher pH. When sari filtered water was treated with MOSP followed by second filtration with bio-sand filtration, the pH was found to be within acceptable limits. All these experiments demonstrated that bio-sand filtration is a better choice than the sari filter in converting surface water to clean water.

In addition, the aesthetic characteristics including bad color, taste and odor of water do not pose any health concerns to the people. However, clean water with a good taste and odor is always more acceptable to the consumer. The dead pathogens and dirt particles impart typically unpleasing taste, color and odor to the water and removing these tastes and odorants from the water will improve its aesthetic qualities considerably. The sand, charcoal and gravels used in bio-sand filtration reduced bad odor and taste and improved the appearance of the water. They also filtered out the microbial contaminants.

To see whether *Moringa* seed powder and scallop powder used in this study possess any toxicity, a toxicity test was carried out using BHK-21 or HeLa cells and the results showed no toxic effect on BHK-21 or HeLa cells (Figure 4), which indicates the material's safety and does not pose any health effect. In contrast, chlorine, a frequently used disinfectant, has a carcinogenic impact on human health, thus the use of non-toxic, natural antimicrobial and coagulant could be introduced instead of chlorine compound for water disinfection.

Moreover, clean water produced by the above mentioned method may contain bacterial endotoxins, which could be released during cell division and lysis of cells by the action of scallop powder, thus a bacterial endotoxin test was carried out and the results showed negative results (no gel formation after addition of LAL reagent and incubation at 37 °C for 1 h on a heat block machine) (Figure 5). This study also supports the use of *Moringa* seed powder and scallop powder to produce clean water.

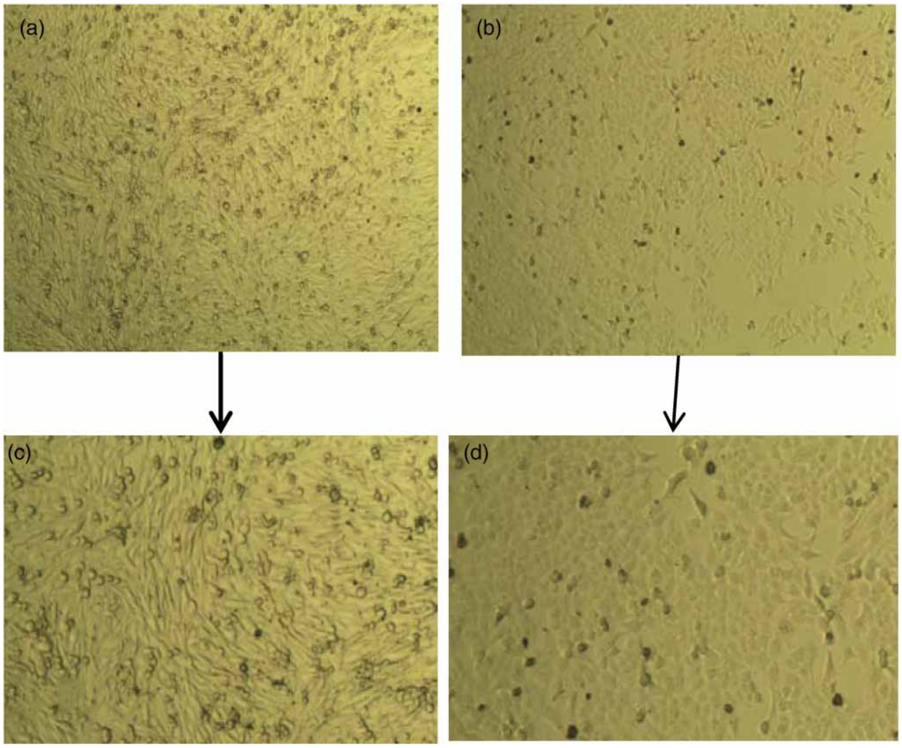

Figure 4 | Cytotoxic effect of *Moringa* and scallop powder on BHK-21 and HeLa cell. (a) Control of BHK, (b) Control HeLa, (c) After *Moringa* and SP treatment, (d) After *Moringa* and SP treatment Hela cell.

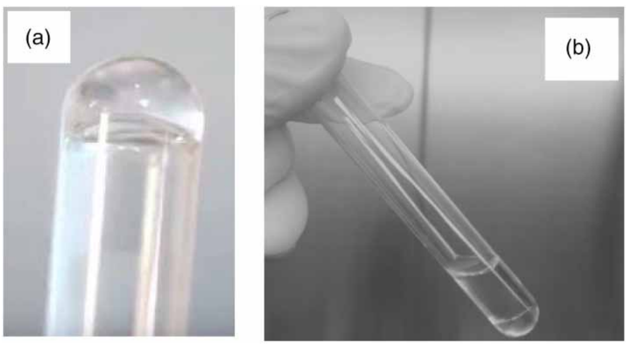

Figure 5 | (a) Represents the positive control (*E. coli* O111:B4) result showing the gel clot after adding the LAL reagent and incubation; (b) represents the negative result of the test sample showing no gel formation after addition of LAL reagent and incubation.

Household level study

A household scale filtration system was constructed as shown in Figure 6, and set up in the laboratory. MOSP treatment followed by filtration was carried out to evaluate the bacteriological and physico-chemical quality of the filtered water. Flocculation of impurities present in turbid water caused by *Moringa* seed powder is an effective approach for a reduction in turbidity. The results revealed that the turbidity values were reduced significantly from 183.7 ± 0.6 to 8.2 ± 0.1 NTU. It was also observed that most of the larger flocs settled faster with 0.01% MOSP and reduction of turbidity to 4.1 ± 0.2 NTU was observed after bio-sand filtration water. Similar findings were reported by other researchers (Yongabi *et al.* 2011; Mumuni & Elizabeth 2013). The MOSP treatment alone reduced the turbidity to 95.55% and additional reductions of 2.5% were obtained by bio-sand filtration (Table 2). Similarly, bacteriological data showed that the MOSP treatment followed by bio-sand filtration revealed a 99.94 and 99.97% reduction of total coliforms and *E. coli,* respectively (Table 1), which was statistically significant. This is an indication that MOSP treatment followed by the bio-sand filtration method had the advantage of reducing microbial load better than the single treatment (Sobsey & Stauber 2006). The combined method gave the lowest coliform count per 100 mL of the samples as compared to the MOSP treatment method. Irrespective of sources and

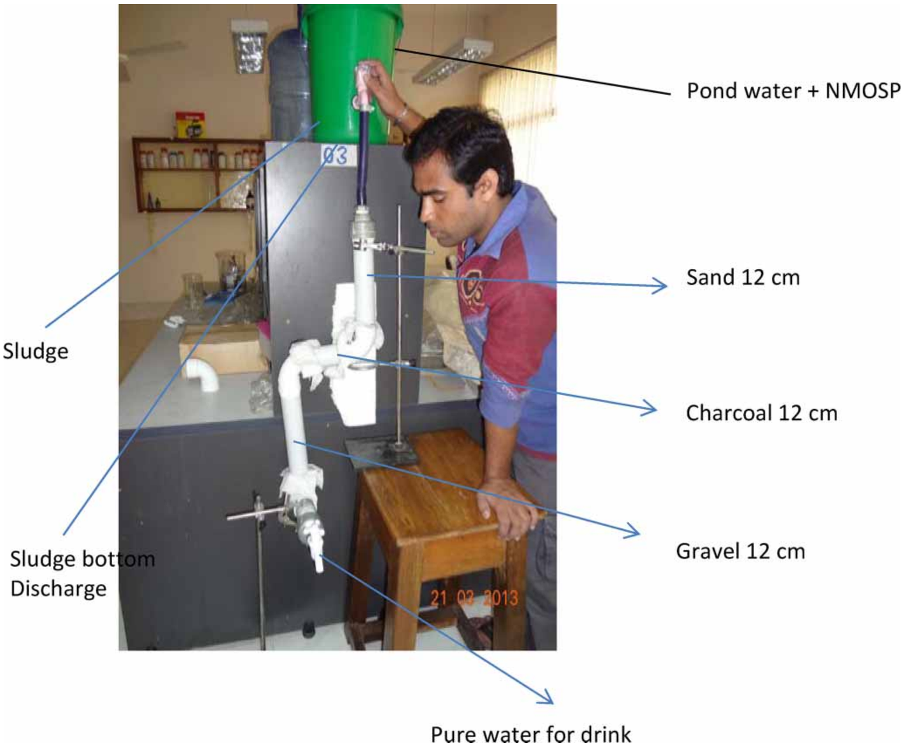

Figure 6 | Household scale treatment device.

types of water, when MOSP treatment followed by bio-sand filtration water was stored at room temperature for 6 months, no bacteria was detected throughout the study (Table 4). This finding suggested that MOSP treatment followed by bio-sand filtration could be useful at the household level.

DISCUSSION

Access to clean water is a basic human right for everyone in the world and is a prerequisite for sanitation and health improvement. Water contamination is posing serious health problems in every developing nation. Considering the

Table 4 | Bacteriological quality of raw and treated (pond, river and lake) water stored for 6 months at ambient temperature

	Raw water stored for 6 months			MOSP treatment followed by bio-sand filtered water stored for 6 months		
	Pond	River	Lake	Pond	River	Lake
Bacteriological quality parameter	Average bacterial population (log CFU/mL)			No. of positive/No. of sample tested		
Aerobic bacterial count	4.1 ± 1.2	3.56 ± 1.3	5.38 ± 0.5	*ND/5	ND/5	ND/5
Total coliform	3.3 ± 0.9	3.68 ± 0.2	4.11 ± 0.3	ND/5	ND/5	ND/5
Escherichia coli	2.9 ± 0.5	4.30 ± 0.1	5.36 ± 0.1	ND/5	ND/5	ND/5
Escherichia coli O157:H7	0.83 ± 0.2	4.68 ± 0.1	4.44 ± 0.1	ND/5	ND/5	ND/5
Salmonella sp.	ND	3.84 ± 0.1	4.70 ± 0.1	ND/5	ND/5	ND/5
Vibrio sp.	ND	3.56 ± 0.1	5.38 ± 0.1	ND/5	ND/5	ND/5

*ND = not detected.

global statistics, around 135 million people will die from water-related diseases by 2020 if no requisite actions are taken. In order to improve the clean water situation, use of surface water from rivers is a better choice than withdrawal of excessive ground water. In Bangladesh, tube wells became a solution, but soon turned out to be a menace as they were contaminated with arsenic in many areas. Cities and towns are supplied with expensive large scale water treatment plants but this is not viable for the majority of the population in Bangladesh who live in isolated villages. A similar situation exists in most of the low resource countries of the world. Therefore, in this study, simple and inexpensive domestic scale technology for drinking water was developed for the rural population using locally available materials.

The *Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in the tropical and subtropical regions. It has an impressive range of medicinal uses with high nutritional value. The seed kernel of this plant has a considerable amount of low molecular weight and water soluble proteins. These proteins are characterized as positively charged in solution. After adding to water, the positively charged proteins primarily magnetize negatively charged particles (clay, silt bacteria, and other toxic particles). As a result, large flocs are formed by continuous agitation. Thus, the flocs are removed by settling or filtration (Aho & Lagasi 2012). The seed also serves as an antimicrobial agent as it contains a few antibiotics such as benzyl iso-thiocyanate and benzyl glucosinolate. These antimicrobial peptides either disrupt cell membrane or inhibit essential enzymes (Mangale *et al.* 2012). According to Ali *et al.* (2009), *Moringa* seed showed high coagulation activity in highly turbid water and vice versa. Water treated with *Moringa* seed showed decreased turbidity, total hardness, acidity, alkalinity and chloride concentration (Mangale *et al.* 2012). In addition, *Moringa* seed has not been found to contain any toxicity or cause any health risk to date (Mangale *et al.* 2012; Eze & Ananso 2014). This information is in agreement with the results obtained in this study.

On the other hand, scallop shell powder is known as the commercial waste product of scallop. The main component of scallop shells is calcium carbonate ($CaCO_3$), which is converted to calcium oxide (CaO) when heated (Zaman *et al.* 2012). Heated scallop shell powder possesses broad antimicrobial action against the vegetative cells of bacteria, spores, and fungi (Sawai *et al.* 1999, 2001, 2003). Scallop powder is applied to fresh vegetables and processed foods to reduce the number of viable bacterial cells. The Japan Ministry of Health and Welfare and FDA approved this heated shell powder as calcinated calcium for washing fresh fruits and vegetables and are thus used as an antimicrobial agent in this study.

Furthermore, activated carbon works via a process called adsorption, whereby pollutant molecules in the water are trapped inside the pore structure of the carbon substrate (Desilva 2000). Active charcoal carbon filters are most effective at removing chlorine, sediment, volatile organic compounds (VOCs), taste and odor from water (Taraba *et al.* 1990).

Sand filtration of drinking water has been practiced since the early 19th century and various scales of slow sand filters have been widely used to treat water at the community, and sometimes local or household, level (Droste & McJunkin 1982; Cairncross & Feachem 1983; Chowdhuri & Sattar 1990; Logsdon 1990; Palmateer *et al.* 1999). Bio-sand filters have been shown to remove 90–99% of pathogens found in water. The filter has been tested by various government, research, and health institutions, as well as by non-governmental agencies in both laboratory and field settings (Duke *et al.* 2006; Stauber *et al.* 2006; Ngai *et al.* 2007). A modified bio-sand filtration was found to be effective in removing both pathogens and heavy metal from source water (Muhammad *et al.* 1997; Ngai *et al.* 2007). On the other hand, the bio-sand filter designed in this study was appropriate for household use and contains a bed of about 12 cm of fine sand supported by charcoal and a gravel layer incorporated into a plastic pipe and a flow rate of about 0.1 m/hour. However, in the bio-sand filtration process, the accumulation of particles occurs within the top few centimetres of sand and can be cleaned manually or hydraulically by opposite direction or replaced on a regular but usually infrequent basis. Thus, additional spare PVC pipe could be provided to each household for obtaining a continuous supply of clean water.

CONCLUSIONS

Surface water treated with MOSP followed by natural bio-sand filtration was found to produce clean water. The bacteriological and physico-chemical quality parameters of this water showed non-significant differences in quality parameters recommended by EPA and BSTI. Moreover, the treated water stored for 6 months at room temperature did not deteriorate in quality, indicating the usefulness of this technology in clean water scarcity areas of the world, because the ingredients used are readily available, inexpensive, user friendly and natural. Therefore, despite many similar water purification systems available commercially, this new method would be the most simple, inexpensive and ecofriendly.

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