Research Article
Volume 14:06, 2023

Chemical Sciences Journal

ISSN: 2150-3494 **Open Access**

Development of a Sustainable Green Chemistry Moringa-sand-charcoal Point-of-use Water Treatment System

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Abstract

The quality of water, whether it is used for non-potable purposes such as cooking, cleaning and other domestic activities, and more so when used as drinking water, is essential. The current methods of water treatment may either be costly or the chemicals used may have health problems associated with them. Curiosity-driven research about the water treatment properties of Moringa seed proteins has led to the development of a water treatment system with Moringa seed powder and eliminates the use of expensive chemicals, with their associated health and environmental effects. The water treatment system with three main water treatment compartments, i.e. a settling or coagulation/flocculation tank, a sand filter and a charcoal filter. The system was used to test the removal of turbidity and microbial pollution indicators from water. Preliminary tests show a reduction of turbidity and microbial pollution indicators by at least 96%. The preliminary results have demonstrated the potential and effectiveness of the Moringa seed powder in wastewater treatment. The next stage of the technology development will be based on applied cross-disciplinary research evolving from the lessons learned from the previous prototypes into a single unit as opposed to having separate standalone compartments**.**

Keywords: Biosorbent • Coagulation • Charcoal • Flocculation • *Moringa oleifera* • Sand • Water treatment

Introduction

Water is used for several purposes and the level of purity is very crucial since it has a direct effect on health if it is for human consumption. Water treatment is a serious challenge, particularly for developing countries. The first stage in most procedures for drinking water treatment is the coagulation and flocculation of particulate impurities. Commonly used coagulants in water treatment are aluminium salts, iron salts, polyaluminium chloride, polyferric chloride and synthetic organic polymers. These materials have cost implications and health and environmental safety concerns as their main disadvantages. The use of aluminum salts, for instance, is associated with Alzheimer's disease and can cause excessive sludge creating disposal problems production from water treatment [1]. The high cost of chemicals is beyond rural populations in developing countries and lack of clean water results in a number of preventable diseases and deaths. As a way of treating water, disinfection results in the formation of by-products, which may have cytotoxic and carcinogenic effects on humans [2]. The cost of chemical coagulants, flocculants and disinfectants thus puts pressure on the developing countries' over-burdened financial resources since they are imported thereby making treated water very expensive and beyond the reach of people living in rural areas. It is not surprising that the people in rural areas resort to sources such as dams, dugouts, streams, rivers, and lakes.

Water from these sources is usually turbid and contaminated with microorganisms that cause many diseases including guinea worm and bilharzia. Boiling water is an energy-intensive alternative, which can be economically and environmentally unsustainable. Therefore, it is desirable to develop other cost-effective, sustainable and more environmentally acceptable coagulants and flocculants. There is now increased interest in the use of natural materials such as *Moringa oleifera* (MO) seeds. The use of the MO seeds for domestic household water treatment was used first by rural women to reduce water turbidity in the countries along the Nile River, especially Sudan [3]. The MO has naturalized and is widely cultivated in the tropics including southern Africa and hence it can be a low-cost alternative to expensive water treatment chemicals which are often beyond the means of rural folks [4]. The tree grows quite well in dry and hot (tropic) climate regions. It can grow in a wide range of soils especially sandy loamy soils and easy to grow from seed and cuttings. The tree is fast growing as it can reach 5 m in 3-4 months if watered (although it doesn't really need to be watered for it to grow). All these attributes make the MO ideal in these current times of climate change and variability. Several studies reported in literature involving the use of MO seeds for water treatment have used either the powder as a biosorbent or crude aqueous extract [5-12]. In the latter case, seeds are deshelled, kernels ground into powder, dispersed in water and filtered. The main barrier to using Moringa seeds crude extract for producing potable water is that the seeds release other water-soluble proteins and Dissolved Organic Matter (DOM). The presence of this DOM supports the re-growth of pathogens in treated water, preventing its storage and later use, i.e. water treated with crude MO extract should not be stored for more than 24 h and so it's not suitable for large supply systems where the hydraulic residence time is very high. The possible solution to this shortcoming is to either remove by adsorption the organic material from crude seeds extract or extract the active coagulant/flocculant protein in pure or semi pure form, thus reducing the total amount of organic material added. To address this problem, we have been using the proteins extracted from Moringa seeds in "semi pure" form in order to understand how and why they work. This particular motivation of the studies was important in understanding first the mechanism by which a novel, natural coagulant/flocculant is effective in water purification.

A number of studies, including the ones mentioned above, have identified that seeds from MO can be used to clarify water (i.e. seeds possess effective coagulation/flocculation properties) and that the active ingredient for this purpose is seed proteins with molecular weights in the range 6-16 kDa [13-15].

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Received: 13 October, 2023; Manuscript No. CSJ-23-116740; **Editor Assigned:** 16 October, 2023; Pre QC No. P-116740; **Reviewed:** 27 October, 2023; QC No. Q-116740; **Revised:** 01 November, 2023, Manuscript No. R-116740; **Published:** 08 November, 2023, DOI: 10.37421/2150-3494.2023.14.375

However, the nature and properties of the coagulant and flocculant component seem to differ. The conflicting reports clearly suggested that the coagulation/flocculation mechanism was not well understood. For instance, different authors attributed it to the existence of proteins and non-protein flocculating agents [16]. Extensive research, therefore, was needed to identify and characterise the whole range of proteins from MO seeds that possess the coagulation/flocculation property. As mentioned earlier, previous studies have focused on the properties and mechanism of the crude protein extract at interfaces. Recent neutron reflectivity, X-ray and biophysical studies by Moulin M, et al. [17] have provided detailed characterization and properties of individual proteins from the Moringa seeds that give new insights relating to the active compounds involved. Extensive mass-spectrometry and adsorption studies of the protein fractions clarified that specific components may bind differently to various materials confirming previous reports that proteins extracted from the seeds of MO contain several distinct proteins to account for observed high adsorption in a form of multilayer to solid surfaces [17,18]. Furthermore, it has been shown that different sequences of MO seed proteins with differences in only a few amino acid residues can show significantly different adsorption properties [17].

In our studies, the Moringa seed proteins extraction and purification was done as described by Kwaambwa HM and Maikokera R [19,20] following the method of Ndabigengesere A and Narasiah KS [14]. The procedure involves extraction with petroleum ether to remove oil, extraction of the protein with water, precipitation of protein with ammonium sulphate, filtration, dissolving the precipitate in water, dialysis to remove excess ammonium sulphate, adsorption through carboxymethyl cellulose column, and elution with 1 M NaCl, dialysis and finally freeze-drying. In the work by Moulin M, et al. [17], the resulting protein was further separated into different fractions. Curiosity-driven research about the water treatment properties of seeds led to the understanding of the mechanism of water treatment by Moringa seeds protein (Figure 1). However, these methods of extraction, purification and separation are tedious and time-consuming and hence making it unattractive for rural community use. Hence the motivation to develop processes that do not require the supervision of trained technical personnel, particularly in rural areas in developing countries. These reasons are some of the powerful drivers to investigate the exploitation of non-traditional technology in water purification being reported here. Investigations are aimed at using crude seeds that is a mixture of proteins that can be used in the new technology. The improvement and optimization of the system for practical conditions are discussed or suggested. The ultimate aim of the research, therefore, is to develop a water treatment system with Moringa seed powder aimed at eliminating expensive chemicals, with their associated health and environmental effects. The system will use the seeds powder of the Moringa instead of the purified protein seed extracts. The results presented here are preliminary results from the exploratory experiments with system being developed (Figure 1).

Material and Methods

Moringa Water Treatment System (MWTS) prototypes 1 and 2

Construction of the first prototype (Prototype 1) shown in Figure 2 was a collaboration between the Faculty of Health and Applied Sciences and the Faculty of Engineering at the Namibia University of Science and Technology. The system is divided into 4 tanks. Tank 1 contains the influent (dirty water). Water is then transferred to tank 2 by opening a tap. The powder or extract of Moringa seeds is added to the water in tank 2. Tank 2 is then stirred to allow the seeds or extract to interact with the impurities. After stirring for approximately 20-30 minutes, the water is transferred from tank 2 to tank 3, which is also referred to as the settling or coagulation/flocculation tank. The aggregates are allowed to sediment in tank 3 and the top layer of clean water is now ready to be transferred to the final tank (tank 4) containing the effluent or clean processed water.

The water treatment system prototype 2 (Figure 3) is similar to the first except that it has four additional compartments, i.e. sand and charcoal tanks,

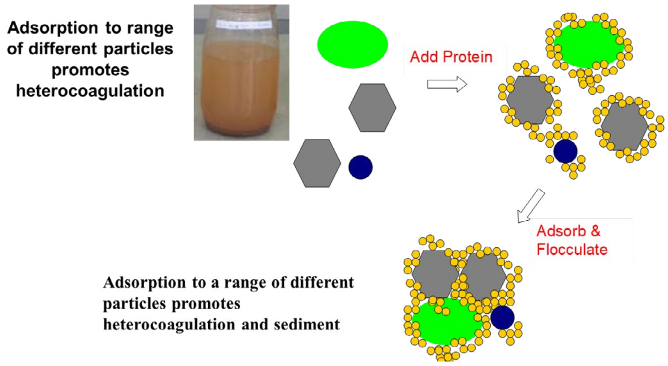

Figure 1. Mechanism of how the Moringa seeds protein works in water treatment.

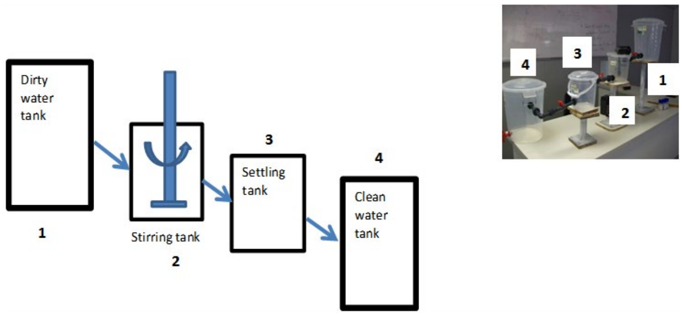

Figure 2. Schematic diagram of the Moringa water treatment system (Inset is Prototype 1 photo).

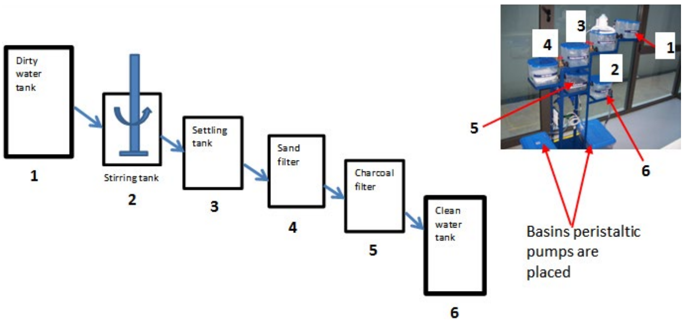

Figure 3. Schematic diagram of the Moringa-Sand-Charcoal water treatment system (Inset is Prototype 2 photo).

backwashing pump and lower raw water tank and hence the name Moringa-sand-charcoal (MSC) water treatment system. The system was fabricated by Makuva Tukeni Enterprises (Windhoek, Namibia). Water to be treated is added to the lower raw water tank or directly added to the elevated raw water tank (marked as 1 in Figure 3). The direct addition of raw water to the elevated tank could be necessary when using a large volume of water through the treatment system. The second tank is for flocculation/coagulation and is fitted with a mixer for mixing the raw water with the seed powder. The mixer has three (3) set speeds that can be selected, depending on the required speed that performs best in coagulation/flocculation. The seed powder is added to this tank manually, through the two openings on top of the tank. The flocculation/coagulation tank retention time is controlled by the two valves at the tank's outlets. They can be closed and opened manually at any time during the process. The next tank, as in the prototype 1, is for settling/sedimentation and once again the valves at the outlets of this tank make it possible to control the settling time required. After the settling time, the clarified water can be allowed to proceed to the subsequent stage by opening the two valves mentioned earlier. Desludging can be performed manually by opening the tank's lid and scooping out the flocs at the bottom of the tank. The clarified water passes through the sand filter to remove any fine suspended colloids left after flocculation/coagulation process. The water proceeds to the granulated carbon filter for further polishing. The final clean water is produced after the carbon filter. This tank too is fitted with an adjustable valve at the outlet.

Both the sand and charcoal filters are fitted with the backwashing pump by opening the valve to the filter one wishes to backwash while the valve to the other filter you do not intend to backwash is closed. The system has two peristaltic pumps (Waterfall 2 Core Flow 1000 Pump Cable, 220 V, 1000 L/h maximum flow rate) such that one is for the lower raw water tank and the other for the backwashing basin. In order to stay cool, the pumps are always submerged in water when they are running. Water movement throughout the system is by gravity, except from the main two (lower) tanks. All water tanks are made of clear and transparent plastic for visibility during experimentation or demonstration. The model is mounted on a wheeled trolley for easy movement either indoor or when it has to be moved from one point another, say within a building.

Preparation of moringa seed powder

Dry Moringa seeds were deshelled by hand and the kernels were washed with copious amounts of double distilled water to remove any adhering dirt before drying them in the oven at 65℃ for 24 hours. It is worth mentioning that the use of doubly distilled was done for research but in practice other cleaned water would be used (from a previous purified batch) of available. The seed kernels were ground to a fine powder using either a mortar and pestle or kitchen blender. Since the oil valuable, it was extracted by mixing the seeds powder (75-80 g) in 40-60℃ petroleum ether (200 mL) for 30 minutes using a magnetic stirrer. Since the use of petroleum ether is quite expensive, oil could be extracted by pressing and this might even be the preferred method if the oil is to be used for cooking or personal care products although there may be slightly more oil left in the seed powder. The powder was then obtained by suction filtration and dried at room temperature in open air on a bench for 24 hours. The material was further processed into finer powder using a mortar and pestle. Before use, the defatted and undefatted powder was sieved through mesh sieves to obtain a homogeneous biomass (150-200 μm). No other chemicals or physical treatments were used prior to the water treatment experiments.

Wastewater samples, treatment and water quality measurements

The samples of water came from three sampling points at the Gammams Wastewater Care Primary recycling plant of the City of Windhoek, Namibia, and labelled as GWCW, GW and SW. To determine the dosage for use in the treatment system, 0.1 g, 0.3 g, 0.5 g and 1 g was added to 3 L of each wastewater in 5 L beakers. After stirring for 30 minutes using a magnetic stirrer, the samples were left to stand for 1 hour. The turbidity of the supernatants were measured using a turbidimeter (Hach 2100P). 0.3 g, 0.5 g and 1 g were determined to be the optimum dosages in turbidity removal for SW, GW and GWCW, respectively, and were used for experiments to test the MSC water treatment system. To 3 L of wastewater in the MSC system, the optimum dosage of Moringa seed powder prepared was added. After addition of each amount of seed powder, the wastewater was stirred for 2 minutes at 150 rpm (rapid stirring) followed by slow speed of 20 rpm for 20 - 30 minutes. For all data reported here, 1 hour was allowed for the sedimentation. At the dosages of the Moringa seed powder examined, no change in solution pH was observed with the treated water whereas the conductivity decreased to less than 200 μS/cm as shown in Table 1. The turbidity of the wastewater before treatment and after sedimentation (sedimentation aliquot) and filtration was measured using the turbidimeter (Hach 2100P). The turbidity measurements were done in triplicate and the average values are reported here.

The microbiological quality of untreated and treated wastewater was evaluated by the use of pollution indicators, *viz*. total coliforms, faecal coliforms, *Escherichia coli* (*E. coli*) and faecal streptococci in the water environment. The methods to evaluate these pollution indicators are described below.

Estimation of total and faecal coliforms in different samples

The membrane filtration procedure as described by Fattouh FA and Al-Kahtani MT [21] was used for the enumeration of coliforms. Briefly, suitable dilutions of water samples were filtered through Millipore membranes (0.45 μm and 0.7 μm) for Total (TC) and Faecal (FC) coliforms. Each membrane was then placed on absorption pads soaked with m-ENDO MF broth (Difco Laboratories, USA) for the enumeration of TC and incubated at 37 ℃ for 24 hours. Typical dark colonies with sheen were counted as TC. To enumerate FC, filters were placed on absorbent pads saturated with m-FC broth (Difco Laboratories, USA) supplemented with 1% (w/v) rosalic acid and incubated at 44.0 ± 0.5 ℃ for 24 hours. All typical blue colonies were counted. TC and FC were estimated as colony-forming units/ 100 mL (cfu/100 mL).

Table 1. Turbidity removal by MSC (Prototype 2) system.

Wastewater Type	GWCW	GW	SW
Dosage (g/L)	1.0	0.5	0.3
pH raw water	7.5	7.8	7.5
pH after treatment	7.5	7.4	7.7
Conductivity of raw water (S/cm)	1310	1270	1310
Conductivity of after treatment (S/cm)	197	175	186
Turbidity of raw water (NTU)	352	250	225
Turbidity of supernatant in the settling tank (NTU)	6	2	8
Turbidity of treated water after filtration (NTU)	0.2	0.4	1
Turbidity removal in settling tank (%)	98.3	99.2	96.4
Post filtration turbidity removal (%)	99.9	99.8	99.6

The counts of faecal streptococci before and after treatments were evaluated using the method as described by Beltrán-Heredia J and Sánchez-Martín J [9]. The aliquots for faecal streptococcus evaluation were sequentially diluted in sterile 0.85% saline solution (NaCl) under laminar flow. Subsequently, the dilutions were plated on Petri dishes containing Tryptic Soy Agar (TSA), which were incubated for 24 hours at 36-37℃. After the incubation time, bacterial growth counts were performed in cfu/100 mL. Total coliform and *E. coli* in water samples were determined using the Colilert systems that can simultaneously detect and enumerate both organisms using the method described by An YJ, et al. [22]. The density units are expressed with Most Probable Number (MPN) per 100 mL. Reverse Osmosis (RO) purified water was used as a negative control and RO water with one drop of a log phase culture of *E. coli* ATTC strain #15597 was used as a positive control. The limit of quantification for this method is 1 MPN/100 mL. Total coliform and *E. coli* in sediment samples were enumerated by spreading suitable dilutions of the sediments on Eosin Methylene Blue (EMB) plates that select for Gram negative bacteria. An aliquot of sediment (the equivalent of 1.5 g dry wt.) was placed in a 40-mL glass vial and 15-mL of sterile RO water was then added to the vial. The vials were closed and tumbled on a low-profile roller (Stovall, Life Science, Inc.) at 8 rpm for 1 hour to suspend the sediment; this is a 1:10 dilution. Further, 10-fold dilutions were made to 100:1 to 1000:1. 0.5 mL aliquots of these dilutions were placed on the surface of EMB plates in duplicate and the aliquot spread over the surface of the agar with a sterile L-shaped glass rod. The inoculated EMB plates were incubated for 48 hours at 36℃ and the number of colonies was counted on plates by utilizing a colony counter. The number of colonies with a green-metallic sheen were counted and recorded as the cfu/100 mL of *E. coli* per gram soil of dry weight and the total number of colonies present recorded as the cfu per g of dry weight. When the number of cfu on duplicate plates varied by greater than 30%, the dilution was replated (Figure 2,3).

Results and Discussion

Table 1 shows the measured turbidity values before and after treatment through the MSC system. The dosages indicated are the optimum amounts for the different types of wastewater after sedimentation and filtration through sand and charcoal. At the dosages examined, no change in solution pH and conductivity was observed with the treated water. The turbidity removals percentages after sedimentation in the settling were 98.3% for GWCW, 99.2% for GW and 96.8% for SW. By allowing the water from the settling tank to pass through sand and charcoal filters, the turbidity removal percentages for GWCW, GW and SW changed to 99.9%, 99.8% and 99.5%, respectively. The results demonstrate the effectiveness of the Moringa seed powder in removing turbidity of the wastewater from three sampling points. In terms of microorganism population, a high decrease was obtained as shown in Figures 4-6 for all the pollution indicators studied, *viz.* total coliforms, faecal coliforms, *E. coli* and faecal *streptococcus*. For instance, the coagulation/flocculation process achieves up to 85% total coliforms removal, which increases to 97% following the filtration process whereas faecal coliforms, *E. coli* and faecal *streptococcus*, 100% removal was generally observed after filtration for the wastewater sample types studied (Table 2). The results demonstrate that Moringa seed powder alone is effective enough in reducing in microorganism populations with coagulation/flocculation and sedimentation process, but filtration further improves the quality of treated water, as it is quite effective in removing remaining pathogens. This is in agreement with findings of Beltrán-Heredia, J and Sánchez-Martín, J [9].

It is important to note, that while the Moringa seed powder is able to remove most impurities, the water at this stage, is not yet safe for human consumption. Filtration through sand and charcoal improves the quality of the water even more. Further analysis is underway to determine the chemical and microbial quality of the water to meet drinking water standards. A barrier to using Moringa seeds powder for producing potable water is that the seeds

Table 2. Percentage reduction of microorganisms at different stages of the MSC system.

Microorganism	% Reduction					
	After Sedimentation Stage			After Filtration Stage		
	GWCW	GW	SW	GWCW	GW	SW
Total coliforms	89 ± 4	88 ± 3	85 ± 5	96 ± 4	96 ± 2	97 ± 2
Faecal coliforms	96 ± 5	87 ± 4	88 ± 2	100	98 ± 2	99 ± 1
Faecal *streptococcus*	78 ± 7	82 ± 5	80 ± 6	100	97 ± 3	100
E. coli	83 ± 3	95 ± 3	94 ± 3	98 ± 1	100	100

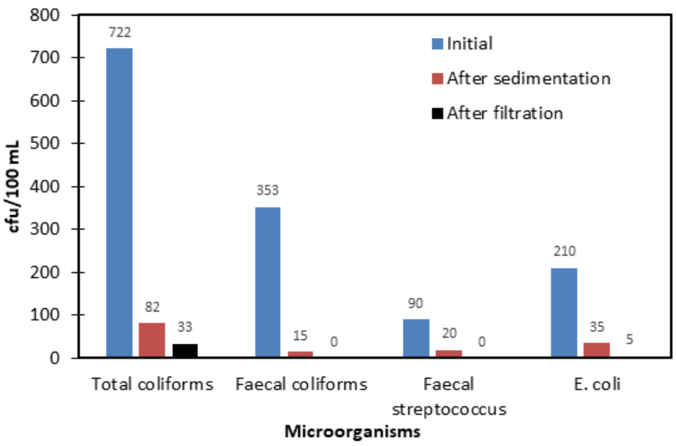

Figure 4. MSC (Prototype 2) system microorganisms removal (3 L of GWCW treated with 1 g of *M. oleifera* seed powder).

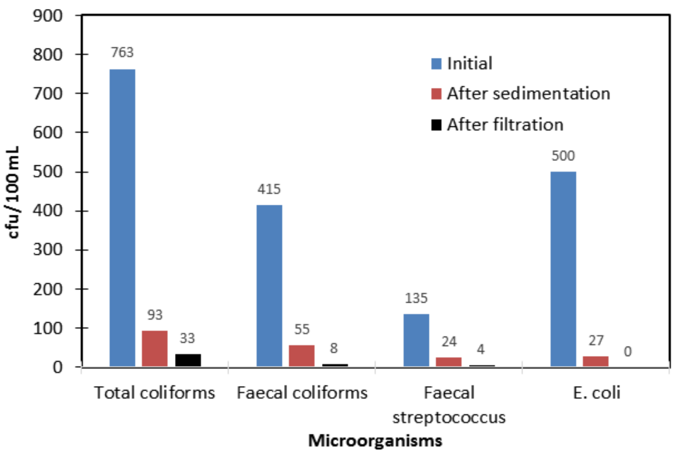

Figure 5. MSC (Prototype 2) system microorganisms removal (3 L of GW treated with 0.5 g of *M. oleifera* seed powder).

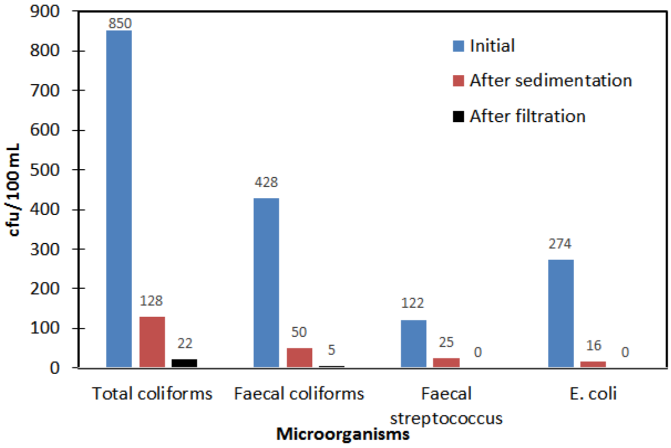

Figure 6. MSC (Prototype 2) system microorganisms removal (3 L of SW treated with 0.3 g of *M. oleifera* seed powder).

release other water-soluble proteins and organic matter. This increases the concentration of Dissolved Organic Matter (DOM) in the water [23]. The presence of this DOM can support the re-growth of pathogens in treated water, preventing its storage and later use. In this setup, extra MO protein from the water is adsorbed and immobilized onto sand granules. A rinsing step follows to remove the excess organic matter, thereby preventing later growth of bacteria in the purified water. The protein remains adsorbed onto the sand after the functionalisation treatment [11-26]. The ability of the antimicrobial functionalized sand to clarify turbidity and kill bacteria, as MO protein does in bulk solution, is maintained [24-27].

Charcoal, also known as activated carbon, is one of the common adsorbents used in water treatment. Charcoal is a well-known adsorbent that is used to remove impurities such as organic-chemical substances including materials giving rise to odor, colorants and pharmaceuticals from wastewater. In addition, it also decreases the residual Chemical Oxygen Demand (COD) of wastewater [28]. Hence, integrating charcoal into a water treatment system comprising of *M. oleifera* and a sand filter generates highly improved results for both physical, chemical and biological quality of the purified water.

Conclusion and Future Design Plan

The preliminary results have demonstrated the potential and effectiveness of the Moringa seed powder in wastewater treatment. The future studies will therefore establish the effectiveness of the three components (*M. oleifera*, sand and charcoal) on improving the physicochemical and biological quality of wastewater as an integrated system. The third prototype of a water treatment system with Moringa seed powder, sand and charcoal filters is being been radically redesigned and improved by adopting modular, user centric, crowd-design methodologies. The result is an affordable, simple, resilient water purification system, built in Africa with local materials, that delivers clean water where it is needed the most. It eliminates expensive chemicals, with their associated health and environmental effects. Additionally, the system eradicates the presence of organic material in the effluent securing water storage without bacterial re-growth. The new system will not use power but solely depend on gravity, and the stirring/settling tank, sand filter and charcoal filter will all be merged into one unit as opposed to having separate standalone compartments (prototypes 1 and 2). The objective of this research is to create a point-of-use continuous-flow water purification device that couples the available filtration capabilities of sand and charcoal with the antimicrobial and flocculating properties of the cationic protein found in a Moringa seed. The envisaged water treatment system will be fully sustainable, economical and easy-to-use by the rural people. To transfer the technology to the communities where it is needed most, the project addresses key issues that make the technology sustainable. Some of them include: 1) the system can be used as a locally produced substitute for imported, expensive water treatment chemicals (such as alum and chlorine which can lead to toxic by-products), thus reducing expenditure for poor populations; 2) Moringa filtration materials are 100%

biodegradable; 3) use of naturally dried seeds eliminates the need to boil water, thereby reducing the energy expenditure involved in finding and burning fuels; and, 4) MSC filters are renewable, degrade naturally and non-toxic. The total amount of treated per hour will be determined after the optimisation tests. The sludge characteristics depend on both the original feedwater quality and the type of the unit operation from which the sludge is discharged and hence determine the processing method before discharge or managment strategy.

Author Contributions

Conceptualization - HMK and ARR; Data Collection - MNN, MAE, NS, and MKM; Future work - NS, MAE, HMK, EN and MKM; Writing original draft- HMK; Review and editing – HMK, MKM, ARR, EN and NS.

Funding

The work was supported by the Swedish Research Council (VR/SIDA) Research Links grant with reference 348-2011-7241 and in part by National Commission on Research Science and Technology (NCRST) and National Research Foundation (NRF) under the Namibia - South Africa Research Partnership Bilateral Agreement programme.

Acknowledgement

We acknowledge the support of the National Institute of Standards and Technology (NIST), US Department of Commerce, and Institut Laue Langevin (ILL), Grenoble, France, in providing the neutron research facilities used in some of the experiments.

Conflict of Interest

The authors declare that no conflict of interest exists.

Data Availability

The authors declare that the relevant data supporting the findings of this study are available in this paper.

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How to cite this article: Kwaambwa, H. M, Manoria N. Niingo, Marta A. Elvin and Natalia Shakela, et al. “Development of a Sustainable Green Chemistry Moringa-sand-charcoal Point-of-use Water Treatment System.” *Chem Sci J* 14 (2023): 375.