Journal of Advances in Microbiology

14(2): 1-8, 2019; Article no.JAMB.44978
ISSN: 2456-7116

Effect of Charcoal Filters Integrated with *Moringa oleifera* Seed Extracts on Microbial Population in Water from Unprotected Sources of Kapseret Division, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2019/44978
Editor(s):
(1) Dr. Ashabil Aygan, Professor, Department of Biological Science, Kahramanmaras Sutcu Imam University (KSU), Turkey.
Reviewers:
(1) Idakwoji Precious Adejoh, Kogi State University, Nigeria.
(2) John Bassey Edet, University of Strathclyde, United Kingdom.
(3) P. Saravanakumari, Rathnavel Subramaniam College of Arts and Science, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/44978

Short Research Article

Received 12 October 2018
Accepted 24 December 2018
Published 17 January 2019

ABSTRACT

This study was carried out in Kapseret Division of Uasin Gishu County. Random sampling was used to identify 5 different rivers in the study area from which water samples were collected and analyzed using standard methods. A water extract from the seeds of *M. oleifera* was applied to the treatment sequence of coagulation–flocculation–sedimentation, followed by charcoal filtration. Each of the collected water samples were analyzed at the Eldoret Water and Sanitation Company. Data analysis was computed using SPSS. Analysis of variance test was conducted to assess whether statistically significant ($p < 0.05$) variations existed among the treatments given to assess their effectiveness in water treatment. In this study, the integration of *M. oleifera* seed suspension with charcoal filter showed a lot of potential in terms of water treatment with respect to bacteriological quality. Total coliforms were significantly reduced by 92.36% while fecal coliforms were significantly reduced by 99.23% with a p-value of 0.003 in a combined treatment of *Moringa oleifera* and charcoal filter. The integrated treatment also reduced BOD of river water by 50.66%. The *M. oleifera* integrated

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charcoal filter system if carefully studied and implemented could clarify all types of turbid and wastewater. It is also expected that a 100% disinfection rate, faster flow rates and shorter residence time with little clogging and backwashing of filter may be the potentials of this hybrid system.

Keywords: Moringa oleifera; disinfectant; charcoal filter; water; integration.

1. INTRODUCTION

Potable water is an essential component or need for a healthy living. Safe water, adequate sanitation and proper nutrition are essential health needs to be met in the developing and the developed nations [1,2,3]. However, over one billion people have no access to safe drinking water globally [4], while 2.6 billion people lack adequate sanitation leading to deaths of 1.8 million people every year from water related diarrheal diseases [5]. Among this population it has been reported that 90% of children under the age of five years, are mainly from developing countries.

Water from unprotected sources is usually turbid and contaminated with microorganisms that cause many diseases. Water-borne diseases are one of the main problems in developing countries [6]. Serving the world with adequate safe drinking water and sanitation is an important prerequisite to hygienic safety, prosperity and political stability [4]. The conventional method of water purification using aluminum sulphate (alum) and calcium hypochlorite exerts pressure on nations' over-burdened financial resources since they are imported thereby making treated water very expensive in most developing countries and beyond the reach of most rural folks. The use of alternative, non-conventional, relatively cheap, sustainable and readily available water purification methods could be the most suitable intervention for developing countries.

In many developing countries, chemical coagulants, such as aluminium sulphate and synthetic poly-electrolytes are usually unavailable [7]. Moringa tree seeds, when crushed into powder, can be used as a water-soluble extract resulting in an effective natural clarification agent for highly turbid and untreated pathogenic surface water [8]. Besides improving water drinkability, this technique reduces water turbidity (cloudiness) resulting in water being both aesthetically as well as microbiologically more acceptable for human consumption [9]. The application of this low cost *Moringa oleifera* seeds is recommended for eco-friendly, nontoxic, simplified water treatment for rural and peri-urban people living in extreme poverty.

Charcoal filters have been used for several hundred years and are considered one of the oldest means of water purification [10]. Historians have shown evidence that carbon filtration may have been used in ancient Egyptian cultures for medical purposes and as a purifying agent [11]. The first recorded use of a charcoal filter to purify potable water on a large scale occurred in 19th century England [11]. Currently, carbon filters are used in individual homes as point-of-use water filters, groundwater remediation, landfill leachate, industrial wastewater and, occasionally, in municipal water treatment facilities.

1.1 Broad Objective

The main objective of the study was to evaluate the effectiveness of using *Moringa oleifera* seed powder as a coagulant and wattle stem charcoal as filter material in purification of stream water from unprotected sources in Kapseret.

1.2 Specific Objective

To compare the antimicrobial activity of *Moringa oleifera* seed extract integrated with wattle stem charcoal filtration alongside the independent performance of *Moringa oleifera* and charcoal filter system against microbial populations in raw water sample.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Kapseret division, Uasin Gishu County, Kenya. The region covers an area of 148.30 sq. Km. It comprises of Simat, Chepkatet and Lemook locations. It receives an average rainfall ranging between 900-1200 mm and this occurs between March and September with two distinct peaks in May and August. The dry spells begin in November and end in February while temperatures range between 8.4 and 26°C but these features are changing probably due to climate change [12]. According to the 2009 Population and Housing Census, the total population of area stood at 31,030.

The area is a peri-urban setup with an increasing population owing to outward expansion of Eldoret town and rural-urban migration. Major water sources in the area include streams, shallow wells and springs. These sources are usually unprotected and therefore exposed to pollution.

2.2 Sampling and Sample Preparation

Sampling procedures described by American Public Health Association [13] were followed. Glass sample bottles (2000 ml) were sterilized in an autoclave at 121°C for 15 minutes at 121 kPa. A sample of 1.5 litres of water was fetched from each of the five streams i.e Leberio, Malanymaina, Lemook, Nganiat and Kapbodigita in sample bottles and the bottles stoppered. To sterilize the immediate air, flaming was used at the mouth of the sample bottles to avoid possible sample contamination by bacteria in the air around the sampling locations Samples were collected from these streams in the study area since they were found to be the commonly used water sources by locals. The sampling sites were identified to represent even distribution of unprotected streams across the study area. Random sampling was used in the study. Samples collected were labeled and placed in a cooler box containing ice blocks and then transported within six hours to Eldoret Water and Sanitation (ELDOWAS) laboratories for analysis.

2.3 Preparation of *Moringa oleifera* Seed Extract

Fully matured *Moringa oleifera* seeds were collected from Marigat forest. The seeds were air-dried in direct sun for a week. The shells surrounding the seed kernels were removed using a knife and the kernels were pounded using laboratory mortar and pestle into fine powder. The powder was sieved using a strainer with a pore size of 2.0 mm to separate the coarse powder and obtain only the fine powder to achieve solubilization of active ingredients in the seed. This powder was used to prepare *M. oleifera* stock solution for water purification. The stock solution was prepared by mixing 10, 20, 30, 40, 50 and 60 g of fine seed powder in 1000 ml of distilled water and solution later filtered. The suspension was vigorously shaken for 30 min using a stirrer to promote water extraction of the coagulant proteins and this was then passed through filter paper (Whatman No. 1). The filtrate was used within an hour.

2.4 Designing an Improvised Charcoal Water Filter

Fresh wattle tree charcoal was used as it was readily available and has no known side effects. Crushed charcoal was graded from 0.5 mm to 5mm using standard sieves at the Ministry of Public Works laboratory in Eldoret. The graded charcoal sample was sterilized by boiling in water for 15 minutes before use in the filter. A 2-litre cylindrical plastic container with the lower part cut open was obtained. The smaller opening was covered with a piece of fabric that acted to prevent the charcoal from falling out or running through with the water. Approximately 500 g of crushed charcoal of varying sizes was packed into the container tightly. This was meant to create as fine a matrix as possible for the water to drip through slowly, th us trapping more sediment. The crushed charcoal was filled up to about halfway the cylinder. The filter was placed atop a sterile container to collect the filtered water.

2.5 Sample Filtration

A 500 ml sample of raw water was slowly poured into the filter and allowed to slowly percolate through. The filtrate was collected in a sterilized beaker. The raw and the filtered samples were later analysed for total coliforms, fecal coliforms and biological oxygen demand. To determine the effectiveness of combined activity of *M. oleifera* and charcoal filter, stream water was initially treated with optimum stock solution i.e. 40 g of *M. oleifera* seed powder in 1000 ml of distilled water. The treated sample was then passed through the charcoal filter in a similar procedure of filtration undertaken.

2.6 Determination of the Antimicrobial Activity of Wattle Tree Charcoal-*Moringa oleifera* Seed Filter in Water Purification

2.6.1 Estimation of total and fecal coliforms

Analysis of collected raw water samples and treated water samples to estimate the populations of total coliforms and fecal coliforms was done using the Colilert-18 test procedure. This analysis represented one aspect of water quality whose findings were used to draw inferences about the suitability of the water for use based on average microbial populations as per WHO recommendations.

One pack of Colilert reagent was added to a 100 ml room temperature water sample in a sterile water container. The container was capped and shaken until its contents dissolved. The sample/ reagent mixture was poured into a quanti tray and sealed in a quanti tray sealer. The quanti-tray 2000 of 97 wells was used. The sealed tray was incubated at 37°C for 18 hours. The results were read according to an interpretation table as described by Abida and Harikrishna [14].

Fluorescence to detect the presence of *Escherichia coli* was checked using a 6-Watt, 365-nm Ultra violet light lamp within 5 inches of the sample in a dark environment. This procedure ensured that the UV light was directed away from the experimenter's eyes and towards the sample. Colilert results were read after 18 hours, however if the results were ambiguous based on the initial reading, incubating up to additional four hours to allow the color and/or fluorescence to intensify was done. Only sterile, none buffered, oxidant free water for dilutions was used. Aseptic techniques were followed during analysis and good laboratory practice GLP for disposal. Sample tests were stored at 25°C away from light.

2.6.2 Measurement of dissolved oxygen

Dissolved Oxygen (DO) was measured using a SX716 Dissolved Oxygen (DO) meter. The machine calibrations were adjusted to read or display 100% active air concentration and the tip of the probe was immersed into the sample in a container and the machine allowed to stabilize before obtaining the actual level of oxygen in parts per million (ppm) which is equivalent to mg/l (APHA, 2017). To reduce errors that might affect oxygen levels during transportation from the field, the initial measurement of dissolved oxygen was done in the area where samples were collected. The Dissolved Oxygen of the treated samples was also taken in the laboratory for analysis to determine the effectiveness of each treatment process.

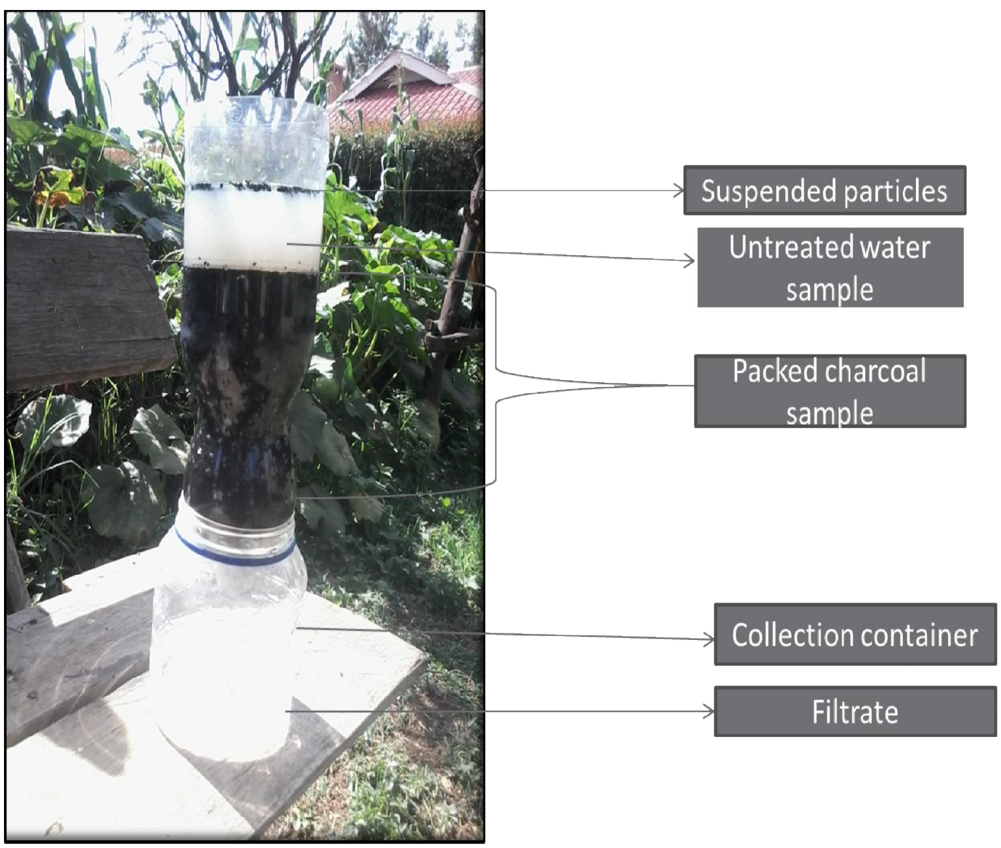

Fig. 3. An improvised charcoal filter

2.6.3 Measurements of Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) is a measure of the oxygen in the water that is required by the aerobic organisms. The biodegradation of organic materials exerts oxygen tension in the water and increases the biochemical oxygen demand (14).

In the current study initial DO values were recorded in the field and the same samples incubated at 20°C for 5 days in dark bottles. This was done in order to avoid some processes like photosynthesis and respiration that could have released or consumed oxygen hence affecting its concentration. Final DO was recorded at the end of 5 days. Biological Oxygen Demand after the 5^{th} day was determined in the formula given below:

> BOD_5= Final DO-Initial (12). Similar procedure was done for *Moringa oleifera* treated samples and charcoal filtered samples in the laboratory.

2.7 Data Processing and Analysis

Analysis of variance (ANOVA) was conducted to assess whether significant ($p < 0.05$) variations existed among the treatments given to assess their effectiveness as water coagulants. Analysis of data was computed using GenStat Discovery Edition III, 2008.

3. RESULTS

Based on the objectives of the study, the findings of the study were as follows;

3.1 Effects of *Moringa oleifera* and Charcoal Filter on Assessment of Microbiological Parameters

In this study, the microbiological parameters under investigation were total coliforms, fecal coliforms and biological oxygen demand. Summaries of the findings for these parameters are shown in tables.

The interactions between *Moringa oleifera* and charcoal filter had significant ($p \leq 0.05$) effects on all microbiological parameters.

3.2 Total Coliforms

The results of total coliforms in the sample water were as shown in Table 2. There were significant differences in total coliforms count ($p<0.05$) among the different treatments of the sample water in the area of study. *Moringa oleifera* reduced the total coliforms by approximately 33%. Filtration over charcoal reduced the population significantly by a further 33%. A combination of *M. oleifera* and charcoal filtration reduced the total coliform population by 92%. This reduction was significantly different from either using charcoal or *M. oleifera* singly (Table 2).

3.3 Fecal Coliforms

The results of fecal coliforms (Table 2), shows that there were significant differences in fecal coliforms count ($p<0.05$) among the different treatments. *Moringa oleifera* reduced the population by 21%. Charcoal filtration further reduced the population by a significant 82%. A combination of the two treatments reduced the population by approximately 99%. This reduction was significantly different from either using charcoal or *M. oleifera* singly (Table 2).

3.4 Biological Oxygen Demand (BOD)

The BOD levels were found to be significantly different ($p< 0.05$) among the different treatments (Table 2). BOD reduction by *Moringa oleifera* was 20%. Filtration over charcoal reduced the BOD concentration by a further 12%. A combination of the two treatments reduced the BOD concentration by 51%.

4. DISCUSSION

4.1 Effects of *Moringa oleifera* and Charcoal Filter on Microbiological Parameters

Moringa oleifera reduced the total coliforms by approximately 33%. Filtration over charcoal reduced the population significantly by a further 33%. A combination of *M. oleifera* and charcoal filtration reduced the total coliform population by 92%. This reduction was significantly different from either using charcoal or *M. oleifera* singly.

Processing the water by coagulation using *M. oleifera* as natural coagulant showed that the treatment with *M. oleifera* provided additional advantage of reduced total coliforms.

Moringa oleifera seeds can be applied to treat water on two levels, acting both as a coagulant and an antimicrobial agent [15]. It is generally accepted that *Moringa* plant works as a coagulant that leads to the formation of “flocs” that settle at the bottom of water [15]. The antimicrobial aspects of *Moringa* plant continue to be investigated [16]. While there are on-going research work being conducted on the nature and characteristics of these components, it is accepted that treatments with *Moringa* solutions remove 90-99.9% of the impurities in water [17]. A viable alternative to the chemical coagulants is natural coagulant [18]. *Moringa* seed pods are allowed to dry naturally on the tree prior to harvesting. The mature seeds are readily removed from the pods, easily shelled and then may be crushed and sieved using traditional techniques such as those employed for the production of maize flour [19]. The crushed seeds’ powder, when mixed with water, yields a solution [12]. To treat surface water, the equivalent weight of seed powder required to make up a crude extract solution is dependent upon the turbidity [9].

Moringa oleifera derived coagulants offers several advantages over conventional coagulants such as aluminium sulphate [12]. This includes its activity being maintained over a wide range of influent pH values i.e. no pH correction is required. Natural alkalinity of the raw water also remains unchanged following coagulation i.e. no addition of alkalinity is required. Sludge production is also greatly reduced and is essentially organic in nature with no aluminium residuals sludge volumes are reduced by a factor of up to 5 [20].

With proper mixing, the moving particles enlarged and formed flocs that fall to the bottom of the vessel due to gravity. This confirms the effectiveness of *Moringa oleifera* as coagulant for the purification of dirty water. Furthermore, the decrease in total coliform number was also affected by alkaline condition generated by *Moringa oleifera*. Most microorganisms grow well at pH 6.0-8.0, but some of them can grow well at pH 3 (acidophiles) and at pH 10.5 (alkaliphiles). Coliform bacteria are facultative anaerobic microorganisms that can grow in aerobic environments and in fermentation condition that produces lactic acid. Therefore these bacteria can still grow at low pH environment, coliform bacteria can still grow, but they cannot survive alkaline pH 14. Additions of *Moringa* as coagulant affect the increase in pH which in turn stops bacteria from growing.

Bacterial species *S. faecalis* and P*. aerugenosa* which were cultured in water, stop growing back after *M. oleifera* seeds were added [21]. When the seeds of *M. oleifera* are crushed and dissolved into the water, protein produces a positive charge that acts like a magnet and attracts dominant negatively charged particles such as clay, silk, and other toxic particles. This is in accordance with the invention that the flocculation process removes about 90-99% of bacteria that are usually attached to solid particles, so the bacteria will be aggregated together to form flocs and can be removed from the water [21]. The control treatment had the highest counts of coliform. This affirms earlier stated recommendation above that raw water without treatment is not safe for drinking.

It was observed that the BOD of raw water was very high. This was due to the presence of high amount of decomposable organic matter in the water samples. Generally, use of *M. oleifera* jointly with charcoal filters had significant effects on nutrients and BOD. BOD reduction by *Moringa oleifera* was 20%. Filtration over charcoal reduced the BOD concentration by a further 12%. A combination of the two treatments reduced the BOD concentration by 51%. This was probably due to the fact that the phosphates and nitrates were filtered out mechanically by adsorption and retention in the charcoal filter.

The porosity and large surface area of charcoal provides a multitude of reactive sites for the attachment of dissolved compounds. These reactive sites can bind non-problematic dissolved organic compounds as well as targeted hazardous contaminants. Background dissolved organic matter, present in all natural waters, can occupy sites on charcoal surfaces and thereby exclude contaminants of concern. This is called “fouling.” Fouling in charcoal filters is mitigated by upstream unit processes – in our case, the *Moringa* seed treatment – that act to remove a substantial portion of background dissolved organic matter from the source water before it encounters the charcoal. The principle is to achieve a high level of treatment prior to the charcoal filter, in order to “save the carbon” for removal of targeted problematic dissolved compounds that make it through the previous treatment steps.

The charcoal filter in this case functions as a post-coagulation adsorber. The charcoal filter is placed after the *Moringa* seed treatment in order

Table 1. Analysis of variance (ANOVA) summary on the effect of treatment on percentage reduction of biological parameters (TC, FC and BOD) in sampled water

Source of variation	Total coliforms		Fecal coliforms		BOD	
Treatment	F-value	P-value	F-value	P-value	F-value	P-value
	23.38	0.000**	60.996	0.000**	29.402	0.000**

Table 2. Mean (±) percent reductions of microbiological parameters using *Moringa oleifera*, charcoal filter and *Moringa oleifera* and charcoal filter combined in water treatment

Treatment	Total coliforms	Fecal coliforms	BOD
A(*M. oleifera*)	32.56±15.88a	21.37±16.94a	19.95±9.36a
B(Charcoal)	66.05±10.68b	82.44±11.19b	31.51±4.76b
C(Combined)	92.36±14.48c	99.23±0.84c	50.66±3.52c

Means followed by different letters within a column are significantly different at $p<0.05$

to target specific components of background organic matter (for example, compounds that cause undesirable tastes, odors, or appearance) or synthetic organic compounds (SOCs) such as pesticides, pharmaceuticals, fuel compounds, etc., that are not well removed by the preceding unit processes.

The two most important factors affecting the efficiency of charcoal filtration are the amount of charcoal in the unit and the amount of time the contaminant spends in contact with it. The more the charcoal used the better. Similarly, the lower the flow rate of the water, the more time that the contaminants will be in contact with the charcoal, and the more absorption that will take place. Particle size also affects removal rates. The effective lifetime of the charcoal filter media depends upon the quality of the charcoal, as well as the characteristics of the source water and efficacy of upstream treatment steps.

5. CONCLUSIONS

Based on this study, the following conclusions were made;

i) *Moringa oleifera* seed powder demonstrated the presence of coagulating properties in water treatment.
ii) There was enhanced improvement in water quality when *Moringa oleifera* seed extracts were used in combination with charcoal filter against the test microorganism.

6. RECOMMENDATIONS

i) The *Moringa oleifera* seed extracts can be used in the formulation of a chemical coagulant in water treatment only after scientific validation of their safety.
ii) There is need to further elucidate phytochemical components present in the *Moringa oleifera* seed extracts which might be responsible for the antimicrobial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/44978