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Research Article

Water Purification of a Calabar Municipal Stream Water with Seeds Paste of *Moringa oleifera* (Lam)

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Abstract

The water purification potential of the seeds paste of *Moringa oleifera* (Lam) was investigated. Stream water sample from a Calabar South stream was collected and analyzed for total bacterial count on nutrient agar (NA) and total coliform count on Eosin-methylene blue Agar (EMB). The stream water which is the sole source of drinking water for the locales in this area, was seen to inhabit 14 different microorganisms including *Escherichia coli, Enterobacter aerogenes, Salmonella typhi, Salmonella paratyphi (B), Salmonella typhimirium, Proteus vulgaris, Streptococcus fecalis and Pseudomonas aeruginosa*. Others were *Shigella dysenteriae, Klebsiella Oxytoca, Clostridium perfringens, Bacillus subtilis. Streptobacillus Spp*. and *Bacillus megaterium*. Both bacterial and coliform load count of the water sample, were calculated to be 5.71 x 10^3 and 3.42 x 10^3 respectively. However, the inclusion of 5%, 10% and 20% seeds paste of *M. Oleifera* reduced these values significantly ($P < 0.05$) to 4.23 x 10^3, 3.14 x 10^3 and 1.93 x 10^3 respectively for bacterial count and 2.86 x 10^3, 1.64 x 10^3 and 1.04 x 10^3 for coliform count. It was also observed that apart from improving the microbiological and aesthetic quality of the water sample by coagulation, *M. oleifera* paste directly inhibited the growth of the microorganisms. It is therefore recommended that the use of seed paste of *Moringa oleifera* in our rural households as an organic coagulant be encouraged.

Keywords: M.*oleifera*; Water Purification; Bacterial Count; Coliform Count; Seeds Paste

Introduction

Pure and clean water intake is crucial to the survival of human beings. In Nigeria today, the problem of water is really not that of availability but portability. Despite several government efforts to clean, purify and provide safer sources of water, dangerous contaminants continue to be present in our drinking water [1]. It is a well known dictum that one can survive without food but not without water. This places water at the pinnacle of man's needs for survival. The importance of having clean and safe drinking water cannot be over-emphasized. In Nigeria, the most common sterilization method employed is chlorination. Apart from the long term health issues these chemicals present, they are also very expensive and require expensive maintenance procedures to keep the plants running [2]. Thus, in some parts of the country, like Afokang community in Calabar South Local Government Area, portable drinking water is an unattainable feat and locales still depend on stream water for their daily water needs.

Before water is accepted for human consumption, it must undergo and comply with certain methods of purification, which are designed to ensure that the water is palatable and safe for drinking. These methods are chemical (chlorination), biological (using plant like *Moringa oleifera*), physical (thermal treatments) methods and UV irradiation; others are bag and cartridge filtration etc. It is common practice to boil drinking water before drinking, but exactly how pure is boiled water especially as boiling does not leave any residual disinfectant in the water?

The standards for drinking water quality are typically set by governments or by international bodies. These standards set minimum and maximum acceptable concentrations of contaminants in the water. It is not possible to tell whether water is of an appropriate quality by just physical examination. Both Chemical and microbiological analyses are the best ways to obtain the information necessary for deciding on the appropriate method of water purification.

The world Health Organization [3], has recommended continuous surveillance of water supplies, which should involve keeping a careful watch at all times, from public health point of view, over safety and sustainability of water supplies. This is to be achieved through sanitary inspection and periodic water analysis. While sanitary inspection identifies potential risk factors of contamination and source of pollution, quality analysis confirms whether the water supply is faecally contaminated or not.

It therefore becomes necessary to explore safe and inexpensive biotechnological tools that can be employed as a treatment regime for both our self- generated water sources, one of which is the borehole water and natural sources of water that mankind will always depend on. Hence the choice of *Moringa oleifera* seeds, as the best biotechnological agent for this study. *Moringa oleifera* also kown as 'drumstick' [4], a very common and well known plant here in Nigeria, has been reported as a biologically active disinfecting agent that is known to compete favorably with its chemical counterparts [3]. As a biotechnological tool, *Moringa oleifera* has been shown to be most effective as a primary coagulant for water treatment when its seeds are crushed and added to the water [5]. According to Hutchinson and Dalziel [6], *Moringa oleifera* Lam, is a deciduous plant native of India, but introduced into the tropics generally.

Plate 1: Seeds of *M. oleifera*.

The tree is fast-growing and has been planted in many tropical countries (Africa, Asia and America). Its common name is horse-radish tree, drumstick (English), Odudu Oyibo, Zogale (Hausa), Ewe ile (Yourba), Yevutsi (Ghana), [7], The seed powder can be used as a quick and simple method for cleaning dirty water. it's been shown that this simple method of purification not only diminishes water pollution but also harmful bacteria. According to Jahn [8], there is a dual advantage to this property of Moringa:

1. It can be used as a locally- produced substitute for imported flocculants, thus reducing expenditure of foreign currency reserves by third world countries.
2. Moringa flocculant, unlike aluminum sulphate is completely biodegradable.

Using Moringa to purify water replaces chemicals such as aluminum sulphate that are dangerous to people and the environment and are expensive. Water from varying sources will need different amounts of *Moringa oleifera* powder because the impurities present might not be the same [8]. Gross [9], reports that some other plants have successfully been used to purify water. Two of such plants are *Ptreris vitatta* fern and duckweed. However, according to Doer [10], of all materials that have been used, seeds of the Moringa have been found to be one of the most effective. The world Health Organization [3], has recommended continuous surveillance of water supplies, which should involve keeping a careful watch at all times, from public health point of view, over safety and sustainability of water supplies. This is to be achieved through sanitary inspection and periodic water analysis. While sanitary inspection identifies potential risk factors of contamination and source of pollution, quality analysis confirms whether the water supply is faecally contaminated or not.

With still so much dependence on stream water as a non-portable drinking water source, it becomes imperative to investigate the bacteriological contents of the water for people still dependent on streams, wells, rivers etc. as their source of drinking water. Stream water, if properly purified, will serve for human and animal consumption where there is no alternative source of consumable water. It is based on this fact, that both heat treatment method of stream water and inclusion of *M. oleifera* seeds are being evaluated. According to Oluduro and Adewoye [11], the interaction of both physical and chemical properties of water, play a significant role in the distribution and abundance of water organisms. The availability of good quality water is an indispensable feature for preventing disease and improving quality of life.

Subsequently, Mane., *et al.* [12], stated that, drinking water treatment effort can become weighed down when water resources are heavily polluted by wastewater, micro-organism species, pathogenic viruses, bacteria, protozoa and helminthes etc. They believe, these microbes may have been present in raw municipal wastewater and will survive in the environment for longer periods. This is in line with what Apantaku., *et al*. [13] cited by Ali., *et al.* [14] states that, the greatest risk to humans from sanitary point of view is from faecal contamination of water supplies. The sanitary quality of water therefore is based on the presence and density of faecal coliform or *Escherichia coli*, followed by the untidy nature of our physical environment along with close proximity of some wells to toilet, gabbage dump and poultry house [14].

Materials and Methods

Ten (10) liters of stream water (Fig. 1), was collected from Afokang stream (Plate 2) in Calabar south, Cross River State, during the day time, when the locales usually fetch their drinking water. 10 kg Moringa seeds (Plate 1), were also sourced from Obase farms in Akamkpa local Government Area, Cross River State for this study, commercial Table water (Eva Table Water) served as the control.

Bacterial cells count was carried out using membrane filtration protocol (Hugo and Russel, 1992). Two categories of bacterial cell count was carried out viz; total count on nutrient agar (NA) and coliform count on Eosin-methylene blue Agar (EMB). 200mlUntreated stream water sample was filtered through a 0.45mm membrane filter (4-5mm diameter). The filter was removed from the holder using sterile forceps and placed on the Nutrient Agar (NA) and Eosin Methylene Blue Agar (EMB) for 24 hours incubation period, after which colonies were observed and counted according to types. Identification was carried out using standard bacteriological procedures.

The seeds of *M. oleifera* were de-hulled, air dried and milled into fine powder, using an electric milling machine (Masterchef, 1000). 100g powdered seeds were then sieved (1.8 mm mesh) and mixed with 100 ml of water to form a paste. Graded doses of 5%, 10% and 20% of the paste were prepared and added to each 1 liter of the stream water sample, stirred for 10 minutes and allowed to stand overnight and then filtered. The filtrates were then subjected to bacteriological analyses.

At the end of the incubation period, all the petri plates containing between 30 and 300 colonies were selected. Colonies on each plate were counted and compared. Number of bacteria (CFU) per millimeter of sample was calculated by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

$$\frac{\text{Number of colonies (CFUs)}}{\text{Dilution factor}} = \frac{\text{X amount of specimen plated}}{\text{No. of bacteria/ml}}$$

Photomicrographs showing isolated bacterial colonies and types were collected.

Plate 2: Collected Stream water Samples.

Results

Cultures of the collected stream water gave colonies with heavy microbial load. On Plate 3, the cultured control water (Eva table water) presents a culture with no microbial load. Microbial isolates identified from the untreated stream water (Plate 4) include *Escherichia coli, Enterobacter aerogenes, Salmonella typhi, Salmonella paratyphi, Salmonella typhimirium, Proteus vulgaris, Streptococcus fecalis, Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella oxytoca, Clostridium perfringens, Bacillus subtilis, Bacillus megaterium* and *Streptobacillus Spp*. (Table 1). The various tests carried out on the untreated stream water are also presented on table 1. These include: Motility test, Gram reaction test, catalase test, oxidase test, Indole, Methyl Red test, Citrate utilization test and Voges Proscaver test. Both bacterial and coliform load count of the water sample, before and after treatment are presented on table 2. From the Table, the bacterial load in the untreated water sample was more than the Coliform load. As the concentration of the seed paste of *M. oleifera* increased from 5% to 15%, the microbial load in the water sample decreased.

Isolates	Motility Test	Gram Reaction Test	Catalase Test	Oxidase Test	Indole Test	Methyl red Test	Citrate Utilization Test	Voges Proskaver Test
Escherichia coli	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Enterobacter aerogenes	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Salmonella typhi	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Salmonella paratyphi(B)	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Salmonella typhimirium	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Proteus vulgaris	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Streptococcus fecalis	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
Pseudomonas aeruginosa	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
Shigella dysenteriae	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Klebsiella Oxytoca	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
Clostridium perfringens	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Bacillus subtilis	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve
Bacillus megaterium	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
Streptobacillus Spp.	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve

Table 1: Microbial Isolates from Untreated Stream Water.

Source: Field Work, 2016

	Control (Table Water)	Untreated Stream Water	*Moringa oleifera* treated water		
			5%	10%	15%
Total Bacterial Count (cfu/ml)	0	$5.71x10^3$	$4.23\ x10^3$	$3.14\ x10^3$	$1.93\ x10^1$
Total Coliform Count (cfu/ml)	0	$3.42x10^3$	$2.86x10^3$	$1.64x10^3$	$1.04x10^3$

Table 2: Microbial Load Of Stream Water Before and After Treatment.

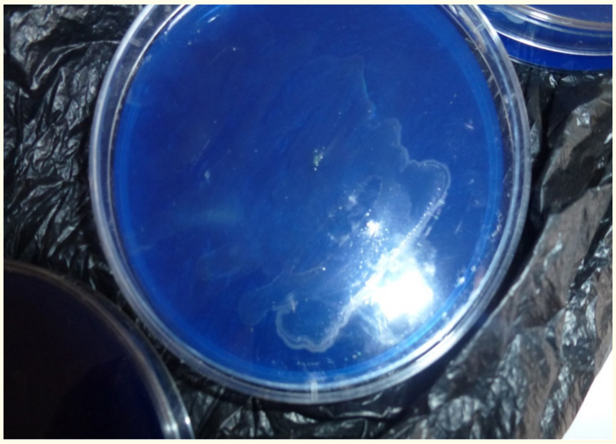

Plate 3: Cultured Table water with no microbial colonies.

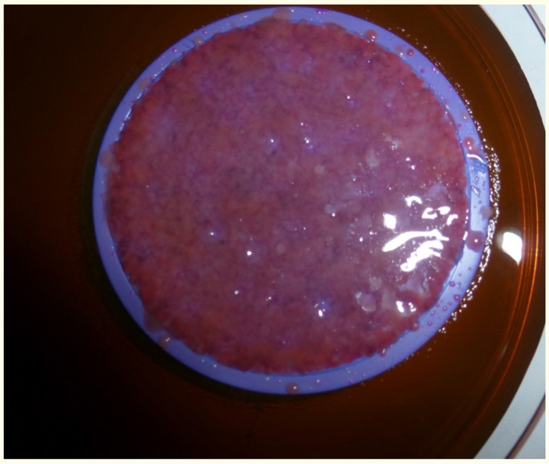

Plate 4: Cultured Untreated stream water with microbial population.

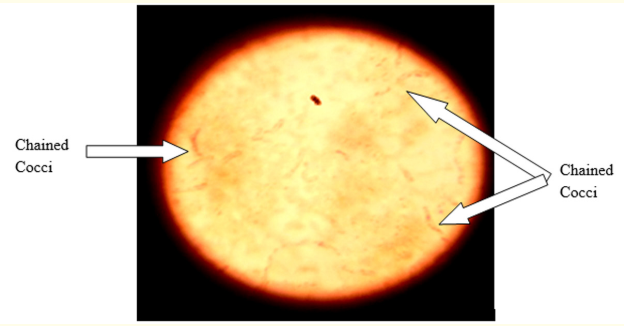

Plate 5: Chained Cocci isolated from untreated water sample.

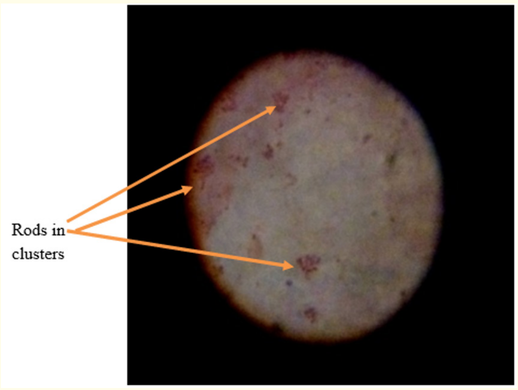

Plate 6: Gram +ve rods in clusters from 15% *M. oleifera* seeds treated water sample.

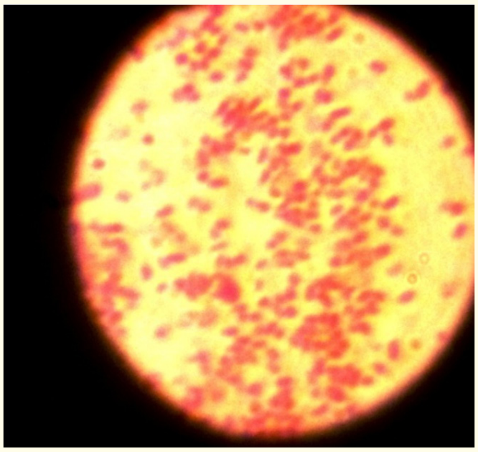

Plate 7: Gram +ve large rods from 10% *M. Oleifera* treated water sample.

Discussion and Conclusion

Using natural materials to clarify water is a technique that has been practiced for centuries and of all the materials that have been used, seeds of the Moringa have been found to be one of the most effective. According to Kansal and Kumari, [5] *Moringa oleifera* seeds treat water on two levels, acting both as a coagulant and an antimicrobial agent. It is generally accepted that Moringa works as a coagulant due to positively charged, water-soluble proteins, which bind with negatively charged particles (silt, clay, bacteria, toxins, etc) allowing the resulting "flocs" to settle to the bottom or be removed by filtration. The low molecular weight of *M. oleifera* cationic proteins (MOCP) extracted from the seeds is very useful and is used in water purification, because of its potent antimicrobial and coagulant properties [15]. Our findings support research reports by Gassenschmidt., *et al*. [16] and Choy., *et al*. [17] that the flocculant capacity, determined in glass powder suspension, is comparable to that of a cationic polymer on polyacrylamide basis. Flocculation activity, according to them, may be explained by the patch charge mechanism due to low molecular weight and high charge density. The control (Eva Table water) had no microbial load (0cfu/ml) (Plate 3) while the untreated stream water recorded 5.71 x 10^3cfu/ml for total bacterial count and 3.42 x 10^3cfu/ml for total coliform count (Table 2). This suggests the presence of pathogens in the water samples and this data thus indicate how unsafe the untreated stream water is for human consumption as it could cause gastrointestinal diseases. Identification of gram positive bacteria (Plate 5, 6 and 7) using different tests ranging from Motility test to Voges Proskaver test showed different microbial isolates from the untreated stream water. After treatment with *M. oleifera*, the water sample showed a mean count of 3.10 x 10^3±1.15 for total bacterial load and 1.85 x 10^3±0 for coliform load per ml of stream water (Table 3). This supports the report by Gross (2014) that the process of flocculation removes about 90 -99% of bacteria which are normally attached to the solid particles. This result affirms WHO [18] recommendation that raw water without treatment is not safe for drinking.

From results obtained, reduction in microbial load with inclusion of *Moringa oleifera* seed solution was concentration- dependent. As the concentration increased from 5% to 15%, the microbial load decreased. The reduction observed in bacterial population of raw stream water treated with Moringa seed paste can be attributed to the antibacterial properties of its bioactive ingredient. Seed lectin, flavonoids and phenolics have been implicated in studies by Govardhan Singh., *et al*. [19], Ferreira., *et al*. [20] and Onsare and Arora [21].

Untreated Water Sample	Moringa oleifera Treated Water Sample		
	5%	10%	15%
Escherichia coli	*Escherichia coli*	-	-
Enterobacter aerogenes	*Enterobacter aerogenes*	-	-
Salmonella typhi	-	-	-
Salmonella paratyphi	-	-	-
Salmonella typhimirium	-	-	-
Proteus vulgaris	-	-	-
Streptococcus fecalis	*Streptococcus fecalis*	*Streptococcus fecalis*	-
Bacillus subtilis	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*
Bacillus megaterium	*Bacillus megaterium*	*Bacillus megaterium*	*Bacillus megaterium*
Streptobacillus spp.	*Streptobacillus spp.*	*Streptobacillus spp.*	*Streptobacillus spp.*
Shigella dysenteriae	*Shigella dysenteriae*	*Shigella dysenteriae*	-
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	-
Klebsiella oxytoca	*Klebsiella oxytoca*	*Klebsiella oxytoca*	*Klebsiella oxytoca*
Clostridium perfringenes	*Clostridium perfringenes*	*Clostridium perfringenes*	*Clostridium perfringenes*

Table 3: Microbial load of both untreated and treated water sample.

Thus, a revival of interest in natural coagulants has emerged because of the high-cost factor, health-related issues, and the environmental impacts associated with synthetic coagulants [17]. Also, Folkard and Sutherland [22] reported that *M. oleifera* seed does not have toxic effect in drinking water. Studies carried out to determine the potential risks associated with the use of the seeds in water treatment, suggest that the seeds do not have any acute or chronic effects on human, particularly at low doses required for water treatment [22].

The microorganisms isolated from the untreated water sample have been implicated in gastroenteritis infections [23]. Some of these are *E. coli, Salmonella* spp. and *Shigella* Spp. It is on record that recombinant proteins in the seeds paste of *M. oleifera* remove microorganisms by coagulation as well as act directly as growth inhibitors of the microorganisms [16]. Treatment with 15% *M. oleifera* removed the microbes reducing their numbers from 14-spp. to 5 spp. Including *Bacillus subtilis, Bacillus megaterium, Streptobacillus spp., Klebsiella oxytoca* and *Clostridium perfringenes*. Most of the microbes removed in our investigations were also recorded by Bukar., *et al*. [24], Emmanuel., *et al*. [25] and Ferreira., *et al* [20].

According to recent reports, a new study compares protein from the seeds of different varieties of Moringa trees that are grown in different countries. It also highlights estimates of the optimum amount of seed extract that should be used to minimise residues in treated water [17]. It's also on record that there is a broad interest in new, sustainable methods for water treatment. A research group has already presented results to government agencies and public bodies, particularly in Namibia and Botswana. There are now discussions on best use of Moringa seeds, both to substitute conventional materials in large water treatment plants and in small scale units [16].

Using Moringa to purify water replaces chemicals such as aluminum sulphate that are dangerous to people and the environment and are expensive. Water from varying sources will need different amounts of *Moringa oleifera* powder because the impurities present might not be the same (Jahn, 1989). The seed paste of *M. oleifera* has proven to be effective in reducing the microbial load and purifying stream water. At larger doses, it is expected to perform even better.

It is recommended that this biological method of water purification be adopted in water treatment plants at both small and large scale. However, the water will still have to be filtered to have a completely safe product. If this is put in place, then large Moringa farmlands will spring up providing farming jobs and a source of income for locales, improving economies, adding carbon value to the environment and providing a purified water source for consumption.

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