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Coagulation and Antimicrobial Activities of *Moringa oleifera* Seed Storage at 3°C Temperature in Turbid Water

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ABSTRACT

Water quality and treatment is becoming of increasing concern, especially in developing nations, where water quality is poor and proper treatment is lacking. Moringa oleifera is a tropical plant whose seeds contain water-soluble substances that have coagulation activity in water. The coagulation and antimicrobial efficiency of the Moringa oleifera seed solution at different concentrations in turbid surface water (Onu-Ebonyi river) were studied and compared with alum, which is presently the most widely used industrial coagulant. The physicochemical and microbial analysis of the turbid surface water indicated that the water sample has turbidity of 28 NTU and the presence of 30×100 *MPN/ml coliform bacteria,* 286×10^2 *CFU/ml mesophilic bacteria and* 70×10^2 *CFU/ml mesophilic fungi respectively. However, microbial reduction of 70-93.3 % for coliform bacteria, 93.7-98.3 % for mesophilic bacteria and 97-100 % for mesophilic fungi was obtained following coagulation of the water sample with Moringa oleifera seed solution, as the concentration increased from 1-2 %. Also, at 1 % (1g/100ml) by weight concentration, both Moringa solution and alum coagulants gave 62.5 % and 75 % turbidity removal respectively. Moringa seed is non-toxic and environmentally friendly, and unlike alum does not significantly affect the pH and conductivity of the treated water. So, as a natural coagulant, Moringa oleifera seed may be potentially viable substitute to alum in both home and pilot water treatment especially in the rural areas of the developing countries.*

INTRODUCTION

Moringa oleifera is the most widely cultivated species of a monogeneric family, the *Moringaceae*, that is, native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. . It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands (Jed, 2005). Almost every part of the plant (leaves, flowers, seeds, roots and bark) can be used as food or with medicinal and therapeutic purposes (Anwar *et al*., 2007), specially in developing countries. *Moringa oleifera* seeds are also used as a primary coagulant in drinking water clarification and wastewater treatment due to the presence of a water-soluble cationic coagulant protein able to reduce turbidity of the water treated. Seeds are powdered and added to the water straight or after preparing crude extract (Ndabigengesere et al., 1995).

Water is one of the fundamental requirements of life and any undesired addition of chemical substances leads to its contamination and makes it unfit for human utility (Mayank et al., 2011). Many industrial and power plants use rivers, streams and lakes to dispose of waste heat and also can have a disastrous effect on life in an aquatic ecosystem (Maitera et al., 2011). The frequency of life threatening infections caused by consumption of untreated

water has increased worldwide and is becoming an important cause of mortality in developing countries (Al-Bari et al., 2006). In the recent years the use of various natural products has been widely investigated as an alternative for the currently expensive methods of water treatment. Some of the natural products can be effectively used as a low cost absorbent (Shaikh PR, Bhosle, 2011). *M. Oleifera* seed has also been found to have antibacterial activity. The ability of the *M. oleifera* coagulant to remove bacteria from water was tested in the jar test experiments with spiking of sample water with *E. coli* bacteria. The results indicated a reduction in the bacteria count similar to that of alum. However the bacteria count in the sludge reduced significantly with increased *M. oleifera* coagulant dosage unlike alum where the bacterial count in the sludge remained fairly constant with increased dosage (Broin et al., 2002). This may be an indication of bactericidal activity of *M. oleifera* although further investigation is required to verify the mechanism of action. Suarez et al. (2003) demonstrated the ability of a recombinant *M. oleifera* protein to decrease the viability of gram-negative or gram-positive bacterial cells and to mediate the aggregation of negatively charged particles in suspension, such as bacterial cells, clay or silicate microspheres.

In many developing countries, access to clean and safe water is a major problem. According to the UN, 1.1 billion people still do not have access to an adequate supply of drinking water and these people are among the worlds poorest. Due to limited clean and safe water source, surface water either from rivers or rain fed ponds has become one of the main sources of water supply. This water is vulnerable to various forms of pollution generated from different sources mainly households, agriculture and industries (Abaliwano et al., 2008). Hence the continous treatment of waste water is more suitable and ideal (Deviram et al., 2011).

The coagulant activity of *Moringa oleifera* seeds is widely known and applied in water treatment at household level in rural areas of developing countries (Jahn, 1988). Coagulant recovery from waterworks sludge for re-use, though not a new concept remains a key option towards the reduction of chemical usage in the water industry (Abdullahi and Musa, 2011). However there are constraints encountered in the use of chemical coagulants (e.g. alum), such as scarcity of foreign currency for importation and inadequate supply of chemicals. Although aluminum is the most commonly used coagulant in the developing countries, studies have linked it to the development of neurological diseases (e.g. pre-senile dementia or Alzheimer's disease) due to the presence of aluminum ions in the drinking water (Jekel, 1991). Subsequently, large non-biodegradable sludge volumes are produced comprising of residual aluminum sulphate requires treatment facilities to prevent further contamination into the environment. Hence as a result of this consequence mentioned above, there is a need to develop alternative, cost effective and also environmentally friendly coagulants.

MATERIALS AND METHODS

Collection of water sample: The water sample used for this study was aseptically collected from Onuebonyi river using the method given by Cheesbrough, 2000 for river water sample collection.

Collection and Identification of *M. oleifera* seeds: Seeds of *M. oleifera* used in this study were collected from a single tree located at Km 6, Enugu-Abakaliki Express way, Mgbabo Village, Abakaliki, Ebonyi State. It was identified and authenticated by Prof. S.S.C. Onyekwelu, taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State.

Preparation of *M. oleifera* seed powder: The *Moringa* seeds were de-shelled and dried at ambient temperatures (23 to 25°C) for a period of five days before milling. The white kernels were milled into a fine powder using with the aid of a Starlite blender (Model SL-999) and was sieved through a small mesh to get the fine powder. The powder were collected into a sterile bottle with cap and stored in the refrigerator at 3 ^{0}C for seven days.

Preparation of *M. oleifera* seed solution and water treatment: Different concentrations of *Moringa* seed solutions were made by dissolving 1 g, 1.5 g, and 2 g of the *Moringa* seed powder weighed on a triple beam balance into a 100 mls of distilled water each contained in a conical flask to obtain 1 %, 1.5 % and 2 % concentration of the solution respectively (Schwarz, 2000). The solution was shaken properly for 1 minute to extract and activate the coagulant and antimicrobial proteins in the seed powder. It is important to not that 5 *Moringa* dried seeds make up 1 g of the seed powder. Each of the concentrations was poured into one liter of the raw water contained in a beaker (2 liter capacity) and the water stirred for 60 seconds and then slowly for 2 munities. The treated water was then allowed to stand undisturbed for 6 hours. After which 100 mls was collected from the top of the water and subjected to post-treatment analysis (Suleiman and Evison, 1994; Folkard et al., 1999 and Doerr, 2005).

Preparation of alum coagulant and water treatment: The alum used for this study was obtained from the local market popularly known as Meat market located within Abakaliki metropolis. The 1 % solution of alum was made by adding 1 g of alum in 100 ml distilled water and shake for 60 seconds. The alum was totally soluble in the water. The solution was then added into 1 litre of the raw water sample and the treatment procedure was as described above with *Moringa* solution.

Microbial analysis of the water sample: The microbial analysis was performed to determine the microbiological quality of the water sample. These tests which include the total viable counts, i.e., the total mesophilic bacteria count and total mesophilic fungal count, and the estimation of the most probable number (MPN) of faecal coliform bacteria were conducted prior to treatment and after the incorporation of the *Moringa oleifera* seed solution into the water sample.

Total mesophilic bacteria count: 10-fold serial dilution of the water sample was made before plating using the methods given by Amadi and Ayogu (2005). Using a sterile syringe 9 mls each of the diluents (sterile water) was placed into 10 different test-tubes arranged in a rack. The water sample was then shaked to mix and 1 ml was taken using sterile 5 ml syringe and then added into the first test tube in the rack and shaken properly to mix. 1 ml of the water was taken from the first test tube and delivered into the second test tube and mixed. This process was repeated for the 10-test tubes. 0.1 ml aliquot of each dilution 1 to 5 test tubes was then plated on an already solidified nutrient agar. The water sample was spread evenly on the surface of the agar using sterile swab stick, after which the inoculated media was allowed to dry and then incubated at 37 0C for 24 hours (Amadi and Ayogu, 2005). After the incubation period, number of colony growths on the agar were counted and recorded.

Total mesophilic fungal counts: 1o-fold serial dilution of the water was made using the method already described above. Then 0.1 ml aliquot from 1-5 dilution plated on each plat containing already solidified Sabauroud dextrose agar medium. The water was spread evenly on the surface of the plate using a sterile swab stick and the plate allowed to dry and then incubated at 25 ^{0}C for 48 hours. The number of colonies growth on the plates was counted after the specified period of incubation.

Most probable number (MPN): The water sample was thoroughly mixed by inverting the bottle several times. The cap was then removed and 50 mls of water was added to the bottle containing 50 mls of MarConkey broth (double strength), using a 10 mls syringe, 10 mls of water as added to each of the five bottles containing 10 mls of MarConkey broth (double strength). Also, 1 ml of the water was added into each of the 5 bottles containing 5 mls of MarConkey broth (single strength). For the treated water sample, 50 mls of water was added to the bottle containing 50 mls of MarConkey broth (double strenght0 and 10 mls of water placed into each of the 5 bottles containing 10 mls of MarConkey broth (double strength). The inoculated broths were then incubated at 44 ^{0}C for 24 hours with the bottles loosely caped. After the incubation period, the results were read and recorded using Cheesbrough (2000) standards.

Physicochemical analysis of the water sample

The water sample physicochemical parameters were determined prior and after treatment with *M. Oleifera* seed solution using specific methods. The parameters determined were:

Determination of turbidity: The turbidity of the water sample was determined using both Nephelometric machine (Gallenkamp, England) and Digital photocolorimeter (312 E, India). The machine was switched on and then calibrated with distilled water. 5 mls of the water sample was poured into a cuvette holder with the vertical line on the cuvette aligning with the horizontal mark on the instrument. The value of the turbidity was then read on the crystal liquid display (CLD) as soon as the ready signal was seen on the screen.

Determination of salinity, total dissolved solids (TDS) and conductivity: These parameters were determined using a mulitmeter analyzer (HACH, Cloverland) that has a software application that can inter-change to read different parameters when the 'mode' button is pressed. The instrument was switched on and calibrated with distilled water. Then 5 mls of sample to be determined were poured into a test tube, the sensor (electrode) of the instrument was now inserted into the test tube and the mode button pressed for the reading of each parameter. The values for each of the parameters were read from the crystal liquid display (CLD) as soon as the instrument indicates ready signal.

Determination of pH: This was done using pH meter (Suntex TS-2, Taiwan). The instrument was calibrated with distilled water. 5 mls of the water sample was measured into a clean test tube and the electrode of the instrument inserted into the water and the start button pressed, the reading was taken as displayed directly on the crystal liquid display panel of the instrument.

Determination of total suspended solids (TSS): This was determined by weighing a filter paper on weighing beam balance, the water sample was then filtered using the filter paper. The wet paper was then dried in the hot air oven at 103 to 105 ^{0}C, after which the filter paper was removed and reweighed. The increase in weight was read and recorded as the total suspended solids in the water sample.

RESULTS

The microbial assessment of the raw water sample before treatment indicated that the water sample contains 286×10^2 CFU/ml of mesophilic bacteria, 30×10^2 MPN/ml of coliform bacteria and 70×10^2 CFU/ml of mesophilic fungi (Table 1). Also, the physicochemical analysis showed that the water sample has turbidity of 28 NTU, total suspended solids of 0.8 gml, pH of 6.06, conductivity of 106.2 µS as indicated in Table 2.

Table 1: Microbial analysis of the water sample before treatment with *Moringa oleifera seed solution*

Microbial counts	Number of colonies
Total mesophilc bacteria	286×10^2 CFU/ml
Total mesophilic fungi	70×10^2 CFU/ml
Total coliform	30×10^2 MPN/ml

CFU = Colony forming unit and MPN = Most probable number

Table 2: Physicochemical analysis of the water sample before treatment with *Moringa oleifera*

Physicochemical parameters	Value
TDS (Total dissolved solids)	50.0 ppm
TSS (Total suspended solids)	0.8 g/ml
Conductivity	106.2 µS
Salinity	0.00
pH	6.06
Temperature	28.4 ^{0}C
Turbidity	28 NTU

PPM = Part per million, g = gram, µS = Microsiemens, ^{0}C = Degree Celsius and NTU = Nephelometric turbidity units.

The results obtained for the microbial analysis of the water samples following treatment with *Moringa oleifera* seed solution at different concentrations are presented in Table 4. The data indicate that the microbial load in the water sample reduced drastically as the solution of the *Moringa* solution increased from 1 to 25 %. Also, the results obtained fro the microbial analysis of the water treatment with alum solution contained in Table 5. The results suggest that the alum do not have significant effect on microbial concentration in water. The results obtained for the physicochemical analysis of the water sample after treatment with 1 % (1 g /100ml) by weight concentration of the *Moringa* and alum solutions indicated that the turbidity reduced to 10.5 NTU and 7 NTU respectively, but no significant changes seen on the pH, conductivity and salinity for the water sample treated with *Moringa* solution (Table 3).

Table 3: Physicochemical analysis of the water sample after treatment with *Moringa oleifera* seed solution and alum as a control

	Coagulants		Physicochemical Parameters				
	TDS (ppm)	TSS (g/ml)	Conductivity (µS)	Salinity	Temperature (^{0}C)	pH	Turbidity (NTU)
M. oleifera seed solution	48.7	0.15	104.9	0.0	28.4	5.65	10.5
Alum solution	302.0	638.0	638	0.3	28.4	3.62	7.0

Table 4: Microbial assessment of the raw water sample after treatment with *Moringa oleifera* seed solution at different concentration

Concentration of *M. Oleifera* solution (%)	Total mesophilic bacteria count (CFU/ml)	Total mesophilic fungi count (CFU/ml)	Total coliform bacteria count (MPN/ml
1.0	18×10^2	2×10^2	9×10^2
1.5	10×10^2	No growth	6×10^2
2.0	5×10^2	No growth	2×10^2

Table 5: Microbial assessment of the raw water sample after treatment with 1 % by weight concentration of alum concentration a control

Microbial count	Number of colonies
Total mesophilic bacteria (CFU/ml)	192×10^2
Total mesophilic fungi (CU/ml)	57×10^2
Total coliform bacteria	18×10^2

DISCUSSION

The finding from this study showed that the active agents in the *Moringa oleifera* seeds solution are water soluble materials as seen from their coagulation and antimicrobial activities coagulation of the raw turbid water. Thus one can easily recover turbid water with low microbial concentration consumption. These active agents have been reported to possess antimicrobial activities, they include: 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocy-anate (Abrams et al., 1993), 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate (Abuye et al., 1999), niazimicin (Akhtar and Ahmad, 1995), pterygospermin (Anderson and Bell, 1986), benzyl isothiocyanate (Anwar and Bhanger, 2003), and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Asres, 1995).

The data obtained from this microbial analysis of the raw water before treatment with *Moringa* seed solution (Table 1) showed that the total mesophilic aerobic bacteria and the total mesophilic fungi concentrations are as high as 286×10^2 CFU/ml and 70×10^2 CFU/ml respectively. Also, the total feacal coliform bacteria were found to be 30×100 MPN/ml, suggesting the presence of pathogens in the raw water. These data thus indicate how unsafe the raw water is for human consumption and other domestic uses as it could cause gastrointestinal diseases. The use of the water for bathing, washing of hands, face and legs could expose one to skin and cutaneous diseases, especially, the immune-compromised individuals. The presence of mesophilic fungi in the water supports this.

However treatment of the water with *Moringa oleifera* seed solutions at different concentrations led to drastic reduction in the microbial counts in the water (Table 4). At 1 % (1g/100ml) concentration of the *Moringa* solution, the total mesophilic bacteria counts, total mesophilic fungal count and total coliform bacteria count were reduced to 18×10^2 CFU/ml, 2×10^2 CFU/ml and 9×10^2 MPN/ml respectively. At 1.5 % (1.5 g/100ml) *Moringa* concentration, the total mesophilic bacteria counts and total coliform bacteria count were reduced to 10×10^2 CFU/ml and 6×10^2 MPN/ml respectively, while the mesophilic fungal showed no growth. And at 2% concentration of the *Moringa* solution, the total mesophilic bacteria counts and total coliform bacteria count were reduced to 5×10^2 CFU/ml and 2×10^2 MPN/ml, while he mesophilic fungal showed no growth. The production of antibiotic metabolites, such as carboxylic acid (Thomasshow and Weller, 1988) and 2, 4 – diacetyl phloroglucinol (Vincent et al., 1991) may also be involved in the elimination of fungal pathogens. Some researchers demonstrated that cell wall degrading enzymes and chitinases could be involved in antagonism towards phyto-pathogenic fungi (Budi et al., 2000). Therefore, it can be suggested that may be these metabolites had an antagonistic activity in our results. The results from this work also indicated that the *Moringa* solution showed antimicrobial efficiency of 70 - 93.3 % for the coliform bacteria, 93.7 - 98.3 % for mesophilic bacteria concentration and 97-100 % for fungi count as the concentration of the *Moringa* solution is increased from 1-2 %, indicating that the inhibition of the microbial growth by *Moringa* solution increase as the concentration of the *Moringa* solution increase. In other words, our extracts worked in doze dependent manner, as the concentration of the extract increased the activity also increased. In the same vein, Ordonez et al. (2006) reported that this is due to susceptibility of the species towards concentration of the extracts, after which this extract damage that species which is not tolerable for it. Similarly, Raheela et al. (2008) showed the antimicrobial activity of *M. oleifera* is dependent on dose of the extracts, as the concentration of the extracts decreased the activity also decreased, indeed different minimum inhibitory concentrations (MIC) values were observed against different microbial species. Also the result obtained for the bacteria count after treatment

were in line with the reports of Madsen et al., 1987 and Babu and Chaudhuri, 2005. Also, the result in Table 4 for total coliform bacteria are in agreement with the reports of Babu and Chaudhuri, 2005 and fall into the range given by Cheesbrough, 2000 for WHO standards for drinking water.

Moreover, alum coagulant gave no significant reduction in the microbial load in the water after treatment as the bacteria count only reduced to 192×10^2 CFU/ml, fungi count reduced to 57×10^2 CFU/ml and had no effect on the coliform bacteria as the whole MPN showed positive for the presence of coliform bacteria (Table 5). This indicates that only very few of the microbial load settled with the flocs formed by the alum coagulant at the bottom of the beaker after treatment. However, alum produces large sludge volumes (James et al., 1982), reacts with natural alkalinity present in the water, leading to pH reduction (Ndabigengesere and Narasiah, 1998), and demonstrates low coagulation efficiency in cold waters (Haaroff and Cleasby, 1988). In addition, alum has raised a number of concerns including: Ecotoxicological impacts when introduced into the environment as post-treatment sludge, impacts on human health as a result of consumption in finish water, and the cost of importing these chemicals for developing communities (Ndabigengesere et al., 1995). Furthermore, optimal implementation of alum requires technical skill and training (WHO, 2007). For these reasons, there is a need to design and develop appropriate water treatment technologies for developing communities. One component of this may be alternative coagulants that are less expensive, inherently benign, renewable, locally available, and readily implementable.

The turbidity of the raw water before treatment was 28 NTU (Table2) but after coagulation with *Moringa* solution (1 %), the turbidity reduced to 10.5 NTU whereas that of alum coagulant (1%) reduced the initial turbidity to 7 NTU (Table 3). This result do not correspond to the 1.5 NTU residual turbidity reported by Babu and Chaudhuri, 2005, this is probably due to some ecological factors and the fact that the species of the *Moringa* used in this work may be different from the species used in this work as it is known that different species of *Moringa oleifera* exists and do not have the same coagulation efficiency (Jahn, 1988). Also, the treated water was not filtered before the turbidity measurements were made. However, *Moringa* solution showed 62.5 % turbidity removal which is in agreement with the reports of Katayon et al., 2004 for low turbid water having initial turbidity level less than 50 NTU.

It is also observed during the study that the flocs formed by the *Moringa* seed coagulant proteins were tiny and light thus settled so slowly where as the flocs formed by the alum coagulant were large enough and sedimentation rate was higher and faster.

The result presented in the Table 2 and 3 further indicate that the pH and conductivity of the water sample was not significantly affected following coagulation with *Moringa* solution compared to alum coagulant which adversely affects the pH and conductivity of the water sample. This result is in agreement with the report of Ndabigengesere et al., 1995. Therefore, this offers a significant advantage to the alum coagulant as little or no further addition of chemical may be required to correct pH of the finished water. Also, the slight decrease in pH following treatment with *Moringa* seed solution may be due to hydrogen ions of the weak acidity of *Moringaoleifera* solution, which balanced the hydroxide ions in the raw water treatment with alum (Table 3) indicates dissolution of metals in the water since metallic ions are highly conductive in aqueous environment especially water.

Moreover, *Moringa oleifera* solution did not affect the salinity of the water while the salinity increased significantly after coagulation with alum. This can be explained by the fact that alum coagulant contains trace amount of sodium chloride salt and also dissolution of potassium and sulphate ions from the alum in the water help to increase the salinity of the water which is a measure of salt and ionic content of water. The total suspended solids in the water sample drastically reduced following the treatment with alum and *Moringa* seed solution.

Generally, the analysis has shown the efficacy and effectiveness of *Moringa oleifera* seed solution in the treatment of raw turbid water as it helps to improve the microbiological and aesthetic quality of water sample. Although the water can still be filtered after coagulation, for further purification as the water coagulated with *Moringa* solution does not guarantee that the water is 100 % free of pathogenic germs. In addition, the seeds of this plant material are non-toxic as the 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 requires for water treatment (Folkard et al., 1999).

CONCLUSION

This study has successfully revealed that seed extracts of *M. oleifera* seed powder solution possesses antimicrobial properties against mesophilic bacteria, mesophilic fungi and coliform bacteria. These extracts could be promising natural antimicrobial agents and coagulant with potential applications in controlling bacteria that cause water borne diseases and reduces the number of suspended particles in raw water drastically. While water coagulation with alum are usually very acidic and thus dangerous for human consumption as it is liable to harming the gastrointestinal tract. *M. oleifera* can be cultivated very cheaply at the household level or in small communal nurseries which is to be encouraged among the rural populations.

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