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Use of moringa oleifera seed extracts as alternative natural material for water purification

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Abstract

This research paper investigated the effectiveness of the use of different Moringa oleifera coagulants for the removal of turbidity, bacteria, and natural organic matter (NOM) from natural surface water using laboratory experiment. In the laboratory study water sample with turbidity of 64NTU was analysed and treated with the three coagulants produced from the Moringa oleifera seed namely: the shell- blended (without oil extraction) seed powder, de-oiled seed powder and the purified protein (polymer) powder. Jar test was conducted to determine the optimum dosages of the coagulant needed in the treatment of raw surface water using the three different coagulants as named above. The concentration of the dose selected for the jar tests were; 30mg/l, 50mg/l, 70mg/l, 90mg/l, 100mg/l and 120mg/l for all three coagulants. The optimum dosages for the shell- blended, de-oiled seed powder and the purified protein powder were found to be 100mg/l, 100mg/l and 90mg/l respectively. After treatment with the optimum coagulant dosage, the water samples were analysed for different physiochemical parameters such as the pH, total solids, turbidity, alkalinity, MPN and SPC. Protein powder had the highest turbidity removal efficiency with a percentage turbidity removal of 92.3% followed by de-oiled powder with a percentage turbidity removal of 83.25% and shell blended powder with a percentage turbidity removal of 75.68% at optimal dosage. The total coliform present in the raw water sample which was measured quantitatively was beyond the W.H.O limit. All three coagulants were able to reduce the total coliforms to a level which conforms to the standard set by the W.H.O. Protein polymer powder had the highest reduction efficiency at optimal dosage followed by the de-oiled powder which was then followed by shell-blended coagulant powder. This study has shown that Moringa oleifera seeds are highly effective in the reduction of the turbidity and faecal coliforms of turbid surface water. In the overall investigations, the results obtained were seen to be comparable to inorganic coagulants of alum and ferric chloride. The use of Moringa oleifera coagulant in combination with alum and ferric chloride showed that reduced usage of inorganic salts to an average of 60% could be achieved. Bactericidal activity seemed to be evident through analysis of E.coli viability in the water and sludge treated by the Moringa oleifera coagulant. Moringa oleifera seed extract was found to have no significant effect on pH or alkalinity of the water. The residual turbidities measured during most of the test runs were seen to satisfy Guideline for Drinking Water Supplies. Findings of this research paper lend support to earlier works recommending the use of Moringa oleifera as alternative natural material for water treatment.

Keywords: Moringa Oleifera Seed Extract, (Shell-Blended, De-Oiled Powder, Purified Protein (Polymer) Powder), Optimum Dosages, Total Solids, Turbidity, Alkalinity,

1. Introduction

1.1 Background

Water is used for several purposes by humans but the level of purity of the water being consumed is very crucial since it has a direct effect on health. 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 world's poorest. Poor water quality is a key cause of poor livelihood and poor health with 80% of all diseases in developing countries being water related (OECD, 2006). The Millennium Development Goal number 7 and target 10 addresses the need to find better solutions/alternatives to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation. Due to limited alternatives, 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.

The most widely applied conventional water treatment technology consists basically of aeration, coagulation, flocculation, sedimentation, filtration and disinfection. When particles are slow to settle or are non-settling, the process is speeded up by coagulation and flocculation through the addition of certain chemicals known as coagulants. These processes are effective at removing fine suspended particles that attract and hold bacteria and viruses to their surface. They can remove up to 99.9% of the bacteria and 99% of the viruses from water supplies (CRC, 2003).

However there are constraints encountered in the use of chemical coagulants, such as scarcity of foreign currency for importation and inadequate supply of chemicals. The conventional method of water purification using aluminium sulphate (alum) and calcium hypochlorite puts pressure on the nation's 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. Hence, they resort to sources such as dams, dug outs, streams, rivers, and lakes. Water from these sources is usually turbid and contaminated with microorganisms that cause many diseases including guinea worm and bilharzia. According to Postnote (2002), waterborne diseases are one

of the main problems in developing countries; about 1.6 million people are compelled to use contaminated water and more than a million people (of which two million are children) die from diarrhea each year.

Earlier research findings of Crapper et al. (1973) and Miller et al. (1984) showed that the chemicals used for water purification can cause serious health hazards if an error occurs in their administration during the treatment process. Although aluminium 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 aluminium ions in the drinking water (Jekel, 1991). These reports suggested that a high level of aluminium in the brain is a risk factor for Alzheimer's disease. However, Davis (2006) found no conclusive evidence linking aluminium with Alzheimer's disease. More so, large non-biodegradable sludge volumes are produced containing residual aluminium sulphate needing treatment facilities to prevent further contamination into the environment. Also, studies by researchers (Letterman and Driscoll, 1988; Mallevialle et al., 1984; Miller et al., 1984) have raised doubts about the advisability of introducing aluminium into the environment by the continuous use of aluminium sulphate as a coagulant in water treatment. There is therefore the need to investigate the use of non-chemicals which would be available locally in most developing countries.

As a consequence of the above mentioned drawbacks, there was a need to develop alternative, cost effective and environmentally friendly coagulants. The use of natural materials of plant origin to clarify turbid water is not a new idea (Bina, 1991; Folkard et al., 1989; Jahn, 1986, 1988; Kaser et al., 1990; Sani, 1990; Sutherland et al., 1992) cited by Ndabigengesere et al. (1995) and Madsen et al. (1987). A number of effective coagulants from plant origin have been identified and which are: Nirmali (Tripathi et al., 1976); Okra (Al-Samawi and Shokralla, 1996); Red bean, Sugar and Red maize (Gunaratna et al., 2007), Moringa oleifera (Jahn, 1988) and a natural coagulant from animal origin which is Chitosan. Natural mineral coagulants have also been used including fluvial clays and earth from termite hills.

Among all the plant materials that have been tested over the years, powder processed from the seeds from Moringa oleifera has been shown to be one of the most effective as a primary coagulant for water treatment and can be compared to that of alum (conventional chemical coagulant) (Madsen et al., 1987; Oslen, 1987; Postnote, 2002). It was inferred from their reports that the powder has antimicrobial properties.

1.2 Alternative natural materials used for water treatment

In laboratory and field tests, seed of Moringa Oleifera (MO) have shown promise as a coagulant in the clarification of turbid water (Folkard et al., 1999; Kalibbala, 2007; Ndabigengesere et al., 1995; Sutherland, 2001). The seeds contain water soluble positively charged proteins that act as an effective coagulant however the crude Moringa extract (though efficient in removal of turbidity) increased the organic load in the treated water (Ndabigengesere and Narasiah, 1998). Earlier studies have found Moringa to be non-toxic (Grabow et al., 1985), and recommended it use as a coagulant in developing countries (Barth et al., 1982; Bhole, 1987; Jahn, 1988; Müller, 1980) cited by Ndabigengesere et al. (1995) and Olsen (1987).

The use of Moringa has an added advantage over the chemical treatment of water because it is biological and has been reported as edible. However, not much has been done using Moringa as a coagulant in water purification system. The cost of this natural coagulant would be less expensive compared to the conventional coagulant (alum) for water purification since it is available in most rural communities where treated water is a scarce resource. It is in this light that this research review was carried out to confirm the effectiveness of powder extracted from mature-dried Moringa oleifera seeds which is commonly available in most rural communities.

1.3 Characteristics of the coagulant component from the MO seed

In recent years the use of the MO seed for water treatment applications is gaining popularity and ongoing research is attempting to characterize and purify the coagulant component (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995, Okuda et al., 2001a). The nature and characteristics of the component, which has coagulation and antimicrobial effects has been reported differently by a number of researchers. It was described as a water –soluble cationic peptide with isoelectric point (pI) above 10 and molecular mass of 6.5kDa (Gassenschmidt et al., 1995), and 13 kDa (Ndabigengesere et al., 1995). The theoretical pI of the protein as estimated from the amino acid sequence is 12.6 (Brion et al., 2002). On the other hand, a non-protien and non-polysaccharide coagulant compound, with molecular mass of 3kDa, has been identified from salt extract solution (Okuda et al., 2001a). In the subsequent research, the coagulant was purified using anion exchange resin.

More than one coagulant peptide has been isolated from the seed and the sequence of one of them has been established (Gassenschmidt et al., 1995). This suggests that a number of coagulant proteins are present, which may differ in one or more amino acid residues. A recombinant form of MO protein was expressed in E.coli and it was found to have good flocculation and antimicrobial properties (Broin et al., 2002; Suarez et al., 2003). Seeds from different sources (geographic locations) exhibit varying coagulation performance (Narasiah et al., 2002), which may have to do with differences in the protein content and development of the seed. The complete array of proteins from the seed that poses the coagulation and antimicrobial property has not been fully identified.

The coagulation mechanism of the MO coagulant protein (MOCP) has been explained in different ways. It has been described as adsorption and charge neutralization (Ndabigengesere et al., 1995); Gassenschmidt et al., 1995) and inter-particle bridging (Muyibi and Evison, 1995a). The

coagulation mechanism of the non-protein organic compound was attributed to enmeshment by net-like structure (Okuda et al., 2001b). MOCP can be extracted by water or salt solutions (commonly NaCl). The amount and effectiveness of the coagulant from salt and water extraction methods vary significantly. In crude form, salt extract shows better coagulation performance than the corresponding water extract (Okuda et al., 1999). This may be explained by the presence of higher amount of soluble protein due to salting –in phenomenon. However, purification of MOCP from the crude salt extract may not be technically and economically feasible.

1.4 Comparison between coagulant chemicals and Moringa oleifera (MO) seed extract

Comparative coagulation studies between alum and MO have also been studied. Compared to the commonly used coagulant chemicals, MO has a number of advantages:

- *it is of low cost*
- *it produces biodegradable sludge*
- *it produces lower sludge volume*
- *it does not affect the pH of the water*.

The above listed advantages make MO a consumer and environmentally friendly low cost alternative with significant potential both in developing and developed countries.

Apart from turbidity removal MO also possesses antimicrobial properties (Olsen, 1987; Madsen et al., 1987). The mechanism by which MO acts upon microorganisms is not yet fully understood.

Broin et al. (2002) reported that a recombinant MO protein was able to flocculate gram-positive and gram negative bacterial cells. In this case -microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water (Casey, 1997). On the other hand, MO may also directly act upon microorganisms and result in growth inhibition.

For example, Sutherland et al. (1990) reported that MO could inhibit replication of bacteriophage. Caceres et al. (1991) also observed growth inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aurues*. Most of the reports on the antimicrobial effect of MO are based on crude extract (Olsen, 19 87; Madsen et al., 1987; Eilert et al., 1981). From the crude extract it is difficult to identify the exact nature of the component that carries out the effect. Eilert et al. (1981) attributed the antimicrobial effects to the compound 4(D -L-Rhamnosyloxy) benzyl isothiocyanate synthesized by the plant. Others have also reported antimicrobial effects of recombinant (heterologous) form of MO protein expressed in *E. coli* (Broin et al., 2002; Suarez et al., 2003). Reports on the antimicrobial effects of the protein purified from MO seed are very rare. As the effects of a recombinant and natural proteins may differ, antimicrobial studies of the purified protein (from the seed) are deemed necessary. The flocculation effect on colloidal particles and cells as well as the growth inhibition on microorganisms imply that the protein from MO may be very effective for both coagulation and disinfection in water treatment.

2. Current Research Work On Natural Materials

2.1 Laboratory Research Works Carried Out By Abaliwano et al., (2008), Ghebremichael, (2004) And Muyibi et al., (2004)

2.1.1 Preparation of MO seed extracts

According to the laboratory research works carried out by Abaliwano et al., (2008) and Ghebremichael, (2004), the MO seeds were obtained from Nigeria. Seeds were stored at room temperature. The seeds were shelled manually and the kernel ground to a fine powder using a kitchen blender. Oil was removed by mixing the seed powder in 95% ethanol (5%, w/v). This was mixed with a magnetic stirrer for 30-45 min and subsequently separation of the residue from the supernatant was done by centrifuging for 45 min at 3000 rpm. The supernatant was decanted and the residual solid was dried at room temperature. From the dried sample, 5% (w/v) was mixed with 1400ml of ammonium acetate (10mM, pH 6.8).The suspensions were stirred for 30 minutes using a magnetic stirrer and the separation of the solids from solution done by centrifuging at 3000 rpm for 45 minutes. The filtrate was termed as the crude extract.

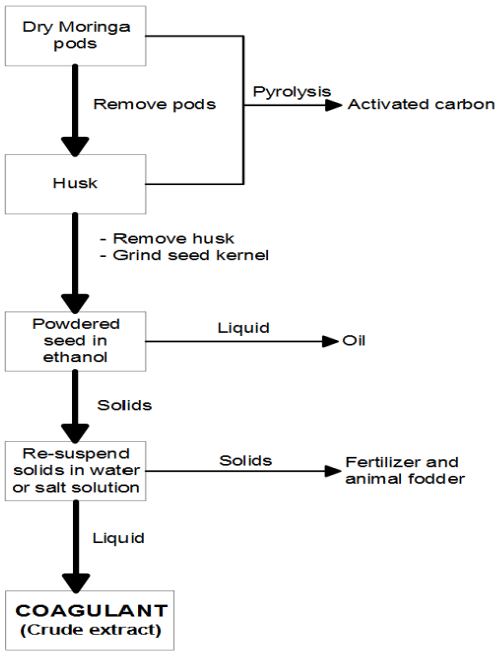


Fig. 2.1 Typical extraction of the coagulant component from the seeds.

2.1.2 Purification of the MO coagulant protein

From the study of Ghebremichael, (2004) research laboratory works; it was shown that, the MO coagulant protein was purified using CM sepharose cation exchanger (IEX) with bead size 45-165um. Crude extract sample was added to the IEX matrix that had been equilibrated with ammonium acetate buffer and mixed. The seed protein was adsorbed to the IEX matrix. After settlement for between 1-2 hours, the supernatant was decanted and the IEX matrix washed with ammonium acetate three times to remove the non adsorbed protein and other impurities.

The adsorbed proteins were then eluted using a high molar concentration of NaCl (0.6M). A sodium chloride (NaCl) solution was added to the IEX matrix and mixed for

approximately 30 minutes and settlement done for up to 1 hour. The supernatant was extracted using a pipette so as not to interfere with the settled particles. This was the purified coagulant from the 1st elution. A second elution was done after the first eluent.

2.1.3 Coagulation test

The jar test is a widely used method to evaluate coagulation-flocculation processes (Kawamura, 1991). Abaliwano et al., (2008) conducted a coagulation tests using the jar test experiment of Phipps & Bird having a base floc illuminator. Six beakers were filled with 1 litre of the sample water and agitated at a rapid mixing speed of 120 rpm for 1 minute upon addition of the coagulant and slow mix at 40 rpm for 20 minutes. This was followed by 1 hour of sedimentation. Comparative tests were run under the same conditions as described above but using alum and ferric chloride.

Also, from Muyibi et al., (2004), the experimental program followed in their study consisted of two parts. Firstly, a sample of raw water was collected from the overhead tank and jar tests carried out to determine the effective dose for each experimental run. The stock solution of Moringa oleifera required for the 3-hour experimental run was prepared according to the effective dose obtained from the jar tests. The flow rate was set and the dosing pump calibrated to the required flow rate. During each run water samples were taken every 30 minutes from the overhead tank (initial sample), settling tank and from the treated water. Turbidity, pH, and alkalinity were measured for both raw water and treated water from the filter outlet according to standard guidelines and procedures (APHA et al., 2004). The filter head losses at different levels in the manometer tubing were also measured and recorded every 30 minutes.

2.2 Turbidity Removal Studies of Research Works Carried Out By Abaliwano et al., (2008), Ghebremichael, (2004) and Muyibi et al., (2004)

The purified Moringa oleifera coagulant protein (MOCP) and the crude Moringa oleifera extract demonstrated adequate coagulation capacity. The formation of flocs and decrease in turbidity indicate coagulation activity of the Moringa oleifera coagulants. Since the active agent of the Moringa oleifera is believed to be positively charged cation, these results point to a mechanism whereby the positively charged part of the coagulant associates with and neutralizes negative charges on the surface of the particles in the river water. Thus the mechanism of charge can be described as neutralization and adsorption as suggested by (Ndabigengesere et al., 1995). Enhanced by mixing, particle interaction between the differently charged particles takes place and flocs are formed which settle out of the water.

2.2.1. Low turbidity (< 50 NTU)

Muyibi et al., (2004), was able to establish that Moringa oleifera performed very well as a primary coagulant in the removal of turbidity from water with low initial turbidity in contrast to the results obtained from previous studies (Ndabigengesere et al.,1998; Muyibi et al.,1995; Kasser et al., 1990; Sutherland et al., 1992) which all concluded that since the bioactive constituents in Moringa oleifera seed extract is a low molecular weight short chain polyelectrolyte, it would be inefficient in the removal of turbidity from water with low initial turbidity. A possible reason which may be postulated for this observation is the method of preparation of Moringa oleifera used which involved extraction of oil of up to 25% of kernel weight (83% oil removal) from the powdered seed kernel before using the residual cake as primary coagulant. All previous studies in which Moringa oleifera seed extract was used as primary coagulant, no oil was extracted from the seed kernel before application.

2.3 Comparison of MOCP with Alum and Ferric chloride (by Abaliwano et al., 2008)

The coagulation activities of Moringa oleifera, alum and ferric chloride were compared using the jar tests apparatus. Results indicated that the coagulation activities of the alum, ferric chloride and Moringa oleifera coagulant were similar for high turbid waters (>100 NTU). Moringa oleifera was found to be less effective for low turbid waters.

MOCP did not significantly affect the pH-value which remained almost constant at 7.9 for all dosages tested. This is line with previous study which has shown that the use of Moringa oleifera does not cause alteration in pH (Ndabigengesere and Narasiah, 1998). In contrast the pH value decreased for both alum and ferric chloride with increase in dosage due to a series of hydrolytic reactions which produce hydrogen ions and hence the pH of the water is reduced.

The Electrical conductivity (EC) of both alum and ferric chloride shows only a slight increase with increase in dosage. In contrast, the conductivity of the water treated with MOCP increased significantly with increase in dosage. This increase is attributed to the presence of NaCl ions used during the purification stage for the process of elution. Dissolved Organic Carbon (DOC) was observed to increase with increased MOCP dosage. This is in contrast to previous research works which have found no increase in the DOC with the addition of the purified Moringa oleifera coagulant at the optimum dosage (Ghebremichael, 2004; Ndabigengesere and Narasiah, 1998; Okuda et al., 2001). The DOC increases only slightly with MOCP dosages at the optimum dose of 7mg DOC/L but as dosage increased beyond this the residual DOC value rises significantly. The rapid increase after a dosage of 7 mg DOC/L could be attributed to overdosing of coagulant and therefore having a residual of the coagulant protein in the treated water hence increasing the DOC value.

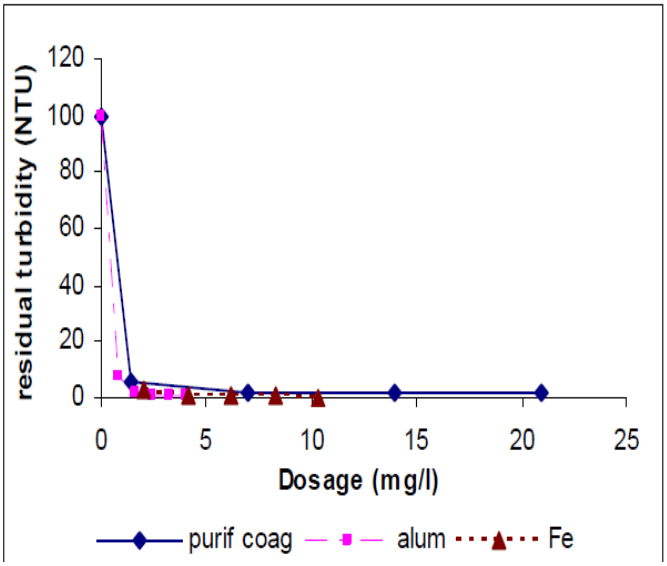


Fig. 2.2: Comparison of MOCP, alum and ferric chloride (conducted by Abaliwano et al., 2008)

Inorganic salts like alum and ferric chloride have been found to leave a residual of alum and iron in the treated water. Driscoll and Letterman (1988) reported that approximately 11% of the aluminum input (through raw water and $Al_2(SO4)_3$) remained in the finished water as residual aluminum and was transported through the distribution system without any significant loss. Mesdaghinia et al.(2005) found that residual metal concentration due to under or overdosing resulted in significant deterioration of water quality with respect to residual aluminum and iron concentrations. Resultant sludge volume from sedimentation of Moringa oleifera treated water was approximately 5 times less than that of alum and ferric chloride. This is in correlation with research by Ndabigengesere et al. (1995) who found the sludge production of alum to be 4 to 5 times higher than that of Moringa oleifera. The cost of sludge disposal and treatment may be dependent on the volume of sludge. It can then be argued that MOCP sludge would be more economical to treat than either alum or ferric sludge. A further advantage is that MOCP sludge is biodegradable and can be reused as a fertilizer provided heavy metals are absent in the water being treated.

2.4 Anti bacterial effect of Moringa oleifera

The ability of the Moringa oleifera coagulant to remove bacteria from water was tested in the jar test experiments with spiking of sample water (Delft canal) with E.coli bacteria (Abaliwano et al., 2008). 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 Moringa oleifera coagulant dosage unlike alum where the bacterial count in the sludge remained fairly constant with increased dosage. This may be an indication of bactericidal activity of Moringa oleifera although further investigation is required to verify the mechanism of action (Abaliwano et al., 2008). Previous study by Suarez et al. (2003) demonstrated the ability of a recombinant Moringa oleifera protein to decrease the viability of gram-negative or gram-positive bacteria cells and to mediate the aggregation of negatively charged particles in suspension, such as bacterial cells, clay or silicate microspheres.

2.5 Summary of findings by Muyibi et al., 2004

1. Effective doses of 20 and 30 mg/l of Moringa oleifera for low (< 50 NTU) moderate turbidity (<100 NTU) and 50 – 80 mg/l for high turbidity (>100 NTU) feed water respectively removed an average 96 % of the initial turbidity of the raw water.
2. The effective dose of Moringa oleifera used for the test runs was obtained from the jar test results. For low turbidity it was 20 mg/l, moderate turbidity 30 mg/l and for the high turbidity the dosage varied from 50 to 80 mg/l depending on the initial turbidity.
3. Analysis of the water treated in the pilot plant showed that Moringa oleifera did not significantly affect the pH or alkalinity after treatment.
4. The maximum filter head loss, after which the breakthrough occurred, was 24 cm at the depth of 40 cm. The corresponding operation time was 18 hours.
5. The residual turbidities measured during most of the test runs satisfied the guideline for Drinking Water Supplies.

2.5 Summary of findings by Abaliwano et al., 2008

1. The purification by ion exchange matrix showed better coagulation activity in terms of turbidity removal with dosages 5 times lower than the crude Moringa oleifera extracts. The MOCP could effectively remove more than 95% of turbidity for high turbid waters.
2. The use of MOCP for coagulation purposes investigated through a number of jar test experiments found the following factors highly significant; the initial turbidity of the water as percentage removals for high turbid waters (>100 NTU) were greater than those for waters of lower turbidity (<50 NTU). The performance was seen to improve with increased mixing time.
3. Increasing dosage of Moringa oleifera seed coagulant leads to decrease in turbidity up to the optimum dose after which the residual turbidity increases due to floc re-stabilization.
4. On the quality of water treated by Moringa oleifera seed coagulant, the following were noted; the pH of the water was not affected by the addition of the coagulant; The volume of sludge produced was considerably less as compared to alum and ferric chloride; there was a gradual increase in the EC of the water treated by MOCP as a result of the use of NaCl in the purification process; The DOC value increased significantly with the use of the crude Moringa Oleifera, the increase of DOC with the MOCP was not as significant up to the optimal dosage and then increased gradually with increased dosage due to an overdose of coagulant.

5. The MOCP was found to be effective as a coagulant aid with alum and ferric chloride and its use could reduce the use of alum by almost 60%.
6. The bacterial quality of water treated with MOCP was similar to that with alum treatment although MOCP was seen to effect bactericidal activity.

3. Materials and Methods

3.1 Collection of Materials

- The seeds of the *Moringa oleifera* plant were collected from Songhai farm at Sapele in delta state. The total weight of the seed collected was 236.4gramms.
- The raw water sample used for this experiment was collected from Ikpoba dam reservoir, Benin City Edo State immediately after rainfall, with an initial turbidity 64NTU. The pH and temperature were taken at collection site, and then after the experiment

3.2 Experimental Procedure

The procedures that were used in the project are given below:

3.2.1 Preparation of Coagulant.

Moringa oleifera seeds were obtained. The seeds were allowed to dry properly under direct sunlight for three days. Mature seeds showing no signs of discoloration, softening or extreme desiccation were used (Ndabigengesere and Narasiah, 1998). The seed coating was first removed. The seed kernels were ground using a mortar to fine powder and the powder was sieved through a 60 micro-metre stainless steel sieve. The grounded Moringa oleifera seed powder was then divided into three equal parts by weight. One part was without oil extraction (shell-blended) while the remaining parts were de-oiled: (one part de-oiled with protein polymer purified) and was used for the treatment of the raw water.

3.2.2 Extraction of Seed Oil from the Moringa Seed

The seed oil was extracted from the Moringa oleifera seed using the Soxhlet extractor. The procedure for the extraction is as follows:-

- The grounded sample of the Moringa oleifera seed was prepared and 48.8g was weighed.
- 450 ml of 95% ethanol was poured into a round bottomed flask of the Soxhlet extractor with boiling chips.
- The Soxhlet apparatus was then set for the extraction.
- After about an hour of extraction, the round bottom flask was heated in a water bath of the concentrator apparatus.
- The solvent was then removed.
- The solid was then dried at room temperature.

The dried solid was then divided into two equal parts by weight. One part was kept for treatment of the raw water while the other part was then to extract the protein polymer.

3.2.3 Extraction of Polymer

The dried de-oiled Moringa Oleifera powder was used for the extraction of the polymer. The extract was then added to 3% sodium chloride solution. The suspension was then fed into a centrifuge machine and then rotated at a speed of 415 rpm for 20mins.

Extract produced was then filtered by Whatman filter paper No. 45 and brown coloured Sodium Chloride extracts was then collected.

The extract was further heated so that no white precipitation was formed at the bottom of the solution.

3.2.4 Purification of the Polymer

The heated crude protein extract was then poured into the dialysis tube and kept for 12hrs in the beaker containing cold water which was kept in ice bath. After the dialysis process was completed, salts were removed from the dialysis tube into the surrounding water solution and white protein remained inside the tube, which was removed out from the tube by rinsing with deionised water. This separated protein was homogenized with cold acetone for delipidization in a homogenizer to remove lipids. After delipidization the protein was then dried at room temperature.

3.2.5 The Coagulant Activity Test

The optimum dosage of the Moringa oleifera crude extracts was then determined by the jar test procedure. The jar test was performed using the shell blended, de-oiled and purified protein powder. The Aqua lytic jar test apparatus was used for the experiment. The jar test procedure was performed using these following steps:-

- Six (6) beakers were arranged on the jar test apparatus (1L capacity).
- Each beaker contained raw water with turbidity of 64NTU.
- 30mg, 50mg, 70mg, 90mg, 100mg and 120mg of the Moringa Oleifera shell blended powder was added to the raw water samples in the 1L beaker.
- The solution was then mixed for ten seconds using a stirrer at a high speed, mixing speed was then dropped to about 100rpm and this speed was further maintained for the next two minutes. The speed was then reduced to about 60rpm and maintained for another three minutes. The speed was further reduced to about 20rpm and mixing was continued at this speed for the next fifteen minutes (15mins).
- After stirring the samples were left to settle for the next one hour.

This procedure is then repeated for the de-oiled powder and the purified protein powder extracts. The jar test experiment was also repeated for both the de-oiled powder and the protein purified powder.

3.2.6 Turbidity Measurement

After 1 hour of sedimentation of the treated water, supernatant samples were collected from each of the 6 beakers to check the turbidity of the treated water samples for each of the three different coagulants used. This was necessary to determine the optimum coagulant dosage of the blended shell, de-oiled and purified protein powder required. The optimum dosage is the dosage of the Moringa Oleifera coagulant corresponding to the water sample with the least residual turbidity after treatment with the coagulant.

3.3 Physio-Chemical Analysis of the Treated Water Sample

3.3.1 Turbidity

Turbidity was measured with a 2100P turbidimeter from Hach. The initial turbidity was measured 3 times on the raw water while stirring, and the average value from the three measurements was used as starting value of raw water (RW). After the sedimentation phase, samples for turbidity measurement were collected from the supernatant using a standard pipette. The sample beaker was washed once with distilled water and twice with the supernatant before recording the turbidity. Each measurement took 1-2 minutes, washing included. In order to eliminate any differences in turbidity due to different sedimentation times, samples were taken from jars 1 to 6 into separate beakers before measurements were taken in the following order: (RW, 30, 50, 70, 90, 100 and 120) ml.

3.3.2 Total Dissolved Solids (TDS)/ Conductivity Determination

A 50ml well-mixed sample was measured into a beaker. The WTW TDS/Conductivity meter probe was immersed in sample and its conductivity and TDS recorded. This was after calibration with 0.01N KC1.

3.3.3 Alkalinity Determination.

0.1M HC1: A 2.1m1 solution of 12M concentrated HC1 was added to a 200m1 of distilled water in a 1000m1 volumetric flask. To this mixture was added more distilled water until it got to the 1000m1 mark.

0.05N Na_2CO_3 solution: A litre of the carbonate solution was prepared by dissolving a 4.5g of dried Na_2CO_3 in double distilled water and transformed into a 1L volumetric flask. The solution was made to the mark with double distilled water.

Standardization of HC1: The approximate 0.1M HC1 prepared was titrated against 40m1 of 0.05N Na_2CO_3 diluted with 60mL of water. The acid was added until a pH of 5 was reached. The solution was boiled for 5 minutes and cooled in a desiccator at room temperature. The titration was then continued to the pH inflection point. 50mL sample was measured into a conical flask. Two drops of methyl orange indicator was added and the resulting mixture titrated against the standard 0.1M HC1 solution to the permanent pink colour at pH 4.5.

3.3.4 Total Hardness Determination

3.3.4.1 Reagents

The determination of the total hardness of water is based on a complexometric titration of calcium and magnesium with an aqueous solution of the disodium salt of EDTA at pH value of 10. The buffer solution was prepared by dissolving 16.9g of ammonium chloride (NH_4Cl) in 143mL of conc. Ammonium hydroxide solution (NH_4OH). This was diluted to 250mL with distilled water.

0.01M Sodium salt of EDTA: A 0.01M solution of disodium salt of EDTA were prepared by dissolving 3.7222g of the salt in distilled water and diluting to 1000m1. To this 780mg of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) was added.

3.3.4.2 Method

A 50m1 sample was measured into a conical flask. To this was added a portion of ammonium chloride buffer solution and followed by 30mg enrichrome black T indicator crystals.

The resulting solution was titrated with 0.01M EDTA solution with continuous stirring until the end point was reached. The end point is reached when the last reddish tinge disappeared.

Calculation:

Formula used for the calculation of hardness in mg/l $CaCO_3$

$$\frac{A \times B \times 1000}{\text{ml sample}}$$

A = final reading minus initial reading = ml of titrant.

B = mg $CaCO_3$ equivalent to 1.00ml EDTA titrant.

A reagent blank without the sample was performed.

3.3.5 Total Bacterial Count.

The different seeds extracts from the different samples at different concentration in mg/l (30, 50, 70, 90, 100 and 120) was each made into a suspension and introduced into 1 liter each of raw water. A liter of raw water was kept aside as control. Another 1 liter of distilled water was also kept as control. The water samples were stirred and allowed to settle and observed after 22 hours. The same procedure was repeated using alum. Total bacterial count of the raw water was recorded before and after application of seeds and alum. Total bacterial count was carried out by pour plate method, and oxoid agar was used as follows:

Water sample was diluted into three dilutions 10^{-1}, 10^{-2} and 10^{-3}; 1.0ml from 10^{-2} and 10^{-3} dilutions were transferred into sterile Petri-dish. Water and the agar were mixed thoroughly by gentle rotation (clockwise and counterclockwise, and rocking back and forth). The agar and the contents was allowed to solidify and incubated at 37°C for 24 hours. Average bacterial count from the triplicate plates was taken, and the bacterial content of the water was recorded from the known dilutions and multiplied by the dilution factor as shown by the formula below.

Equation = (C/V x M) where: C = mean colony count

V = volume of plate

M = dilution e.g. (10^{-2} and 10^{-3}).

4. Results And Discussion

4.1. Observations

4.1.1 Turbidity of Surface Water before and After the Jar Test

The turbidity of the water sample before and after treatment with various doses of the shelled blended, oil-extracted and

purified polymer powder of the Moringa oleifera seed are given in the table below:

Table 4.1: The turbidity of the water sample before and after treatment with the moringa oleifera coagulants.

Concentration (dosage) (mg/l)	Raw Water Turbidity (NTU)	Treated Water Turbidity(NTU)		
		Shell-Blended (without oil extraction)	Oil Extracted	Purified Polymer (Protein)
30	64	19.45	12.42	9.45
50	64	17.63	12.80	8.47
70	64	17.75	11.21	8.50
90	64	16.54	10.91	6.10
100	64	15.56	10.72	7.91
120	64	16.45	11.10	7.70

4.1.2 Optimum dosage of coagulant

The optimum dosage of the shelled blended, De-oiled and purified protein powder coagulant is presented in the table below:

TABLE 4.2: COAGULANTS OPTIMUM DOSAGES

Coagulant	Optimum Dosage(mg/l)
Shelled blended	100
De-oiled powder	100
Purified protein powder	90

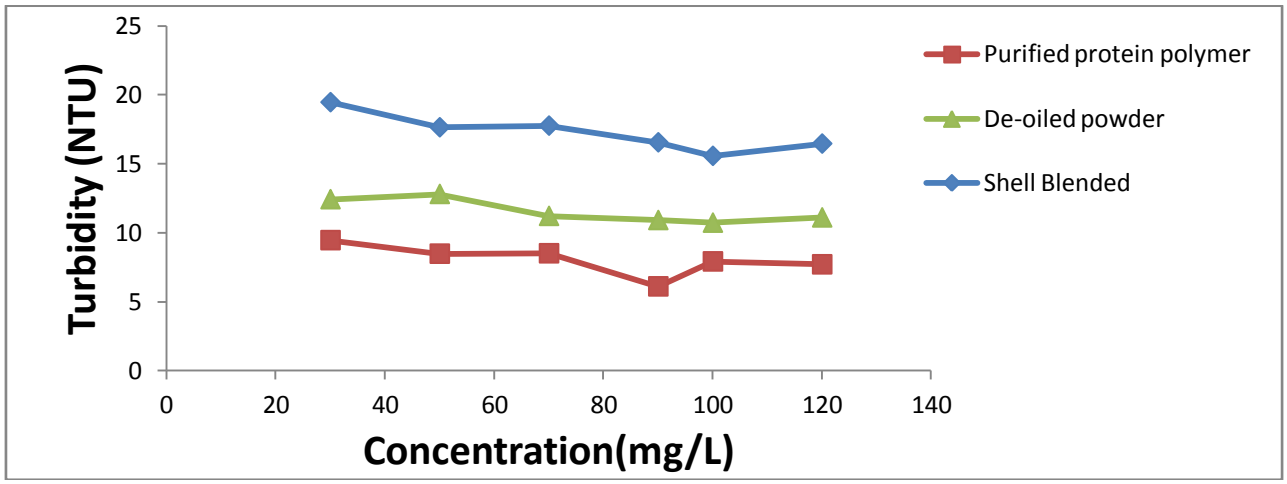


Fig 4.1 Turbidity of treated water with the different coagulants.

4.2 TURBIDITY REMOVAL STUDY

In the experiments carried it was observed in fig. 4.1, that the use of Moringa oleifera shell- blended seed powder showed decreased turbidity with increased dose from 30 to 50mg/land increased turbidity with increased dose from 50 to 70mg/land decreased turbidity from 70mg/l to 90mg/l up to the optimum dosage of 100mg/l and an increased turbidity with increased dose from 100 to 120mg/l. Residual turbidity did not meet the W.H.O standard of 5NTU. Shell-blended Moringa oleifera seed powder removed between 69.6%-75.6% of turbidity in the treated water when dosages of 30mg/l, 50mg/l, 70mg/l, 90mg/l, 100mg/l and 120mg/l was used in treating the raw water. The treated water sample for all dosages of the shell-blended Moringa Oleifera used in treating the raw water sample all had a residual turbidity which was found to be above the WHO limit for turbidity.

In the treatment with the de-oiled seed powder coagulant extract, there was a increase in turbidity with increased dosage from 30 to 50mg/l and decreased turbidity with increased dose from 50 to 70mg/l and decreased turbidity from 70mg/l to 90mg/l up to the optimum dosage of 100mg/l and an increased turbidity with increased dose from 100 to 120mg/l. Residual turbidity did not meet the W.H.O standard of 5NTU. De-oiled Moringa oleifera seed powder removed 80.6%-83.25% of the turbidity in the raw water sample.

After treatment with the purified protein powder of Moringa oleifera seed, there was a decrease in turbidity with increased dosage from 30 to 50mg/land increased turbidity with increased dose from 50 to 70mg/l and decreased turbidity from 70mg/l up to the optimum dosage of 90mg/l and an increased turbidity with increased dose from 90 to 100mg/l then a decrease in turbidity with increased dose from 100mg/l to 120mg/l. Residual turbidity did not meet the W.H.O standard of 5NTU. Purified protein powder Moringa oleifera seed powder removed 85.6-90.5% of the turbidity in the raw water sample. Additional treatment with

filtration process could be applied to the treated water to make it safe for human consumption.

TABLE 4.3 Physiochemical characteristics of the surface water before and after treatment with the optimum coagulant dosage of the shell-blended powder, de-oiled powder and the purified protein polymer powder.

S/No	Parameter	Before Treatment	After Treatment (Optimum Dosage)			W.H.O Standard
			Shell-blended	De-Oiled	Protein Powder	
1	pH	6.7	6.4	6.6	7.1	6.5-8.5
2	Turbidity (NTU)	64	15.56	10.72	6.10	5
3	Alkalinity (mg/l)	5.80	1.52	1.70	2.30	200
4	Total solids (mg/l)	40.92	35.85	28.45	18.70	500
5	MPN (cfu per 100ml)	29	10.3	7.2	6.1	10
	SPC	11.0	4.9	3.6	3.0	-

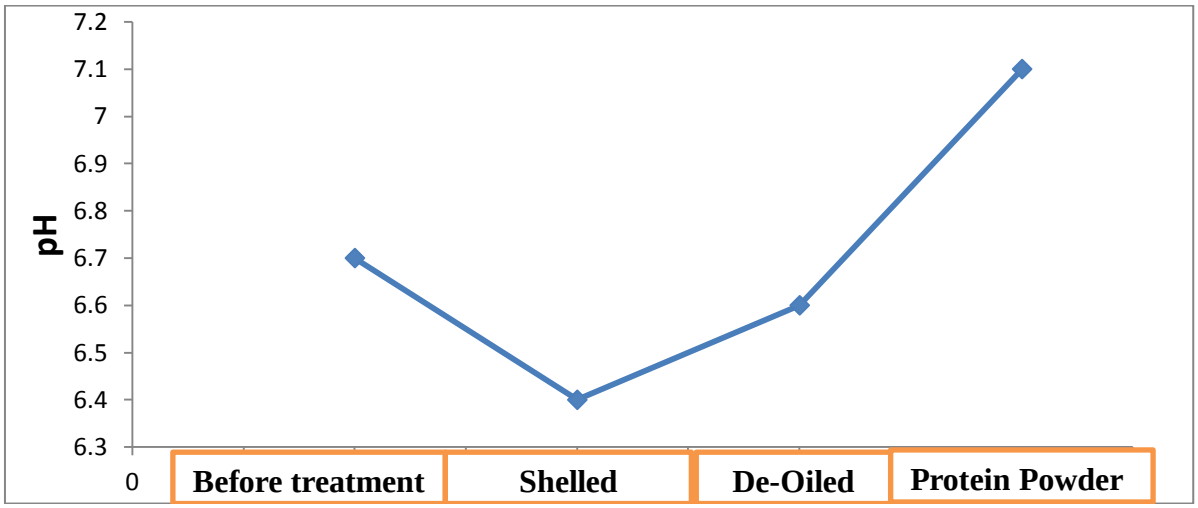


Fig 4.2. pH of the water sample before and after treatment with the optimum dosages of the shell-blended, de-oiled and purified powder coagulants.

In the analysis, at optimum dosages of the shell-blended, de-oiled and purified protein polymer coagulants, it was observed that after treatment with the coagulants, the pH of 6.7 for the raw water was decreased to 6.4 when treated with the optimum dosage of 100mg/l of the shell-blended coagulant. The pH was increased from 6.4 to 6.6 when the de-oiled seed coagulant was used at optimum dosage of 100mg/l (fig. 4.2). When the purified protein powder was used in treating the water sample the pH was increased to 7.1 at optimum dosage of 90mg/l.

The pH increases with increase in the turbidity removal efficiency of the coagulants with the shell-blended Moringa seed coagulant having the least pH of 6.4 followed by the de-oiled Moringa seed powder, which had a pH of 6.6 and then the purified protein powder with a pH of 7.1.It was reported that the action of Moringa oleifera as a coagulant lies in the presence of water soluble cationic proteins in the seeds (Jahn, 1988). This suggests that in water, the basic amino acids present in the protein of Moringa seed powder would accept a proton from water resulting in the release of a hydroxyl group making the solution basic. In essence the better the coagulating activity of the Moringa Oleifera coagulant used the treated water tends to be more basic.

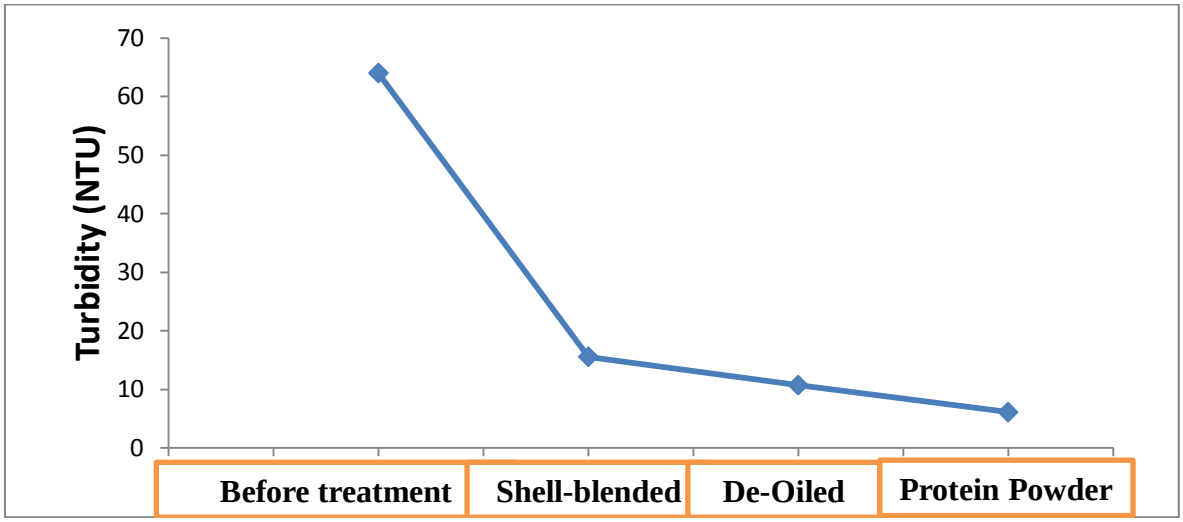


Fig 4.3 Turbidity of the water sample before and after treatment with the optimum dosages of the shelled, de-oiled and the purified protein powder.

The purified protein powder had the highest turbidity removal efficiency, followed by the de-oiled seed powder, and then the shelled blended Moringa seed powder at optimum dosages of the coagulants.

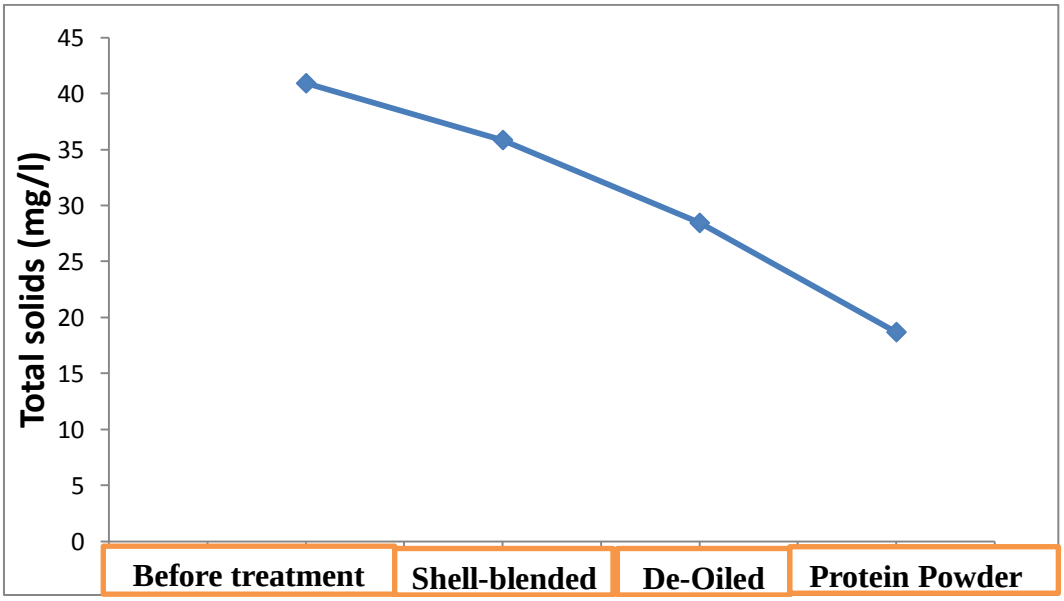


Fig 4.4 Total solid of the water sample before and after treatment with the optimum dosages of the shelled, de-oiled and the purified protein powder coagulant.

The Total Solids of the raw water sample was 40.92mg/l which conforms to the standard limits of the W.H.O. After the treatment of the raw water sample with the shell-blended Moringa oleifera seed powder at optimum dosage, the total solids were reduced to 35.85mg/l. When de-oiled Moringa Oleifera seed powder was used in treating the raw water sample, the total solids of the water sample was reduced to 28.45mg/l. When the purified protein powder was used in treating the raw water sample the total solids of the water sample was reduced to 18.70mg/l.

Moringa oleifera is known to be a natural cationic polyelectrolyte and flocculent with a chemical composition of basic polypeptides with molecular weights ranging from 6000 to 16,000 Daltons, containing up to six amino acids of mainly glutamic acid, methionine and arginine (Jahn, S.A.A, 1986).

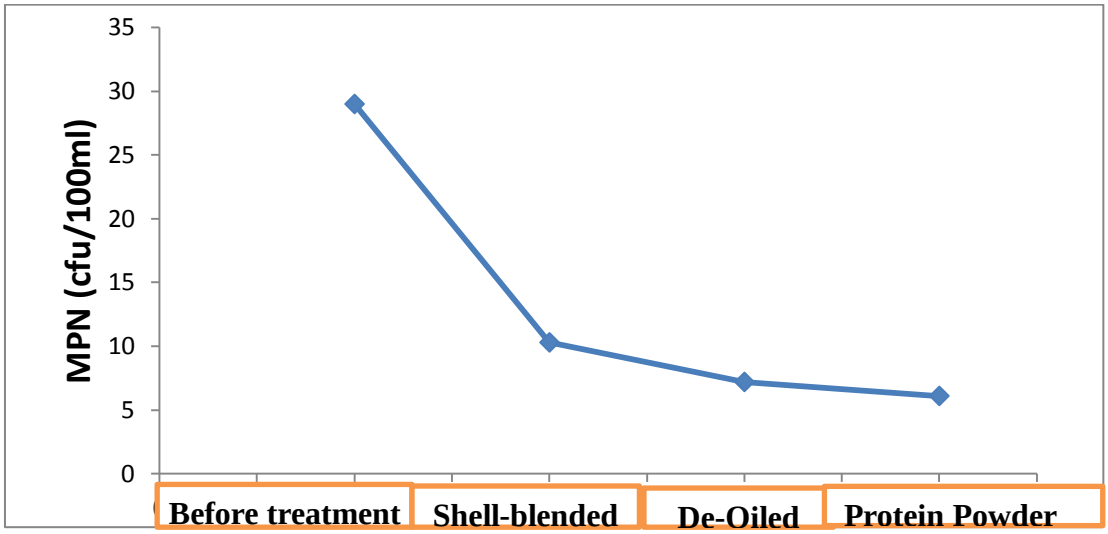


Fig 4.5 MPN (cfu/100ml) of the water sample before and after treatment with the optimum dosage of the shell-blended, de-oiled and purified protein powder of the *Moringa oleifera* coagulant.

MPN means total coliforms which are calculated quantitatively. The presence of coliforms indicates that the water is feacally contaminated and not safe for drinking purpose. Due to coli forms various waterborne diseases occur and therefore, MPN should be not exceed 10(cfui100ml) for drinking water. In the present study, it was observed that the initial MPN of the untreated water sample was 29 (cfu/ml), which was present beyond the limits of WHO standards. After the treatment of the water sample with the shell-blended Moringa oleifera seed powder, MPN / 100 ml coli form was decreased to 10.3cfu

per 100ml which was just within the WHO limit. When the de-oiled Moringa oleifera seed powder was used in treating the water sample the MPN was reduced to 7.2 cfu per 100ml which was within the WHO limit. When the purified protein powder was used in treating the raw water sample the MPN was reduced to 6.1 cfu/100ml which was within the WHO limit.

The presence of MPN within the WHO limit gives an indication that the impurities present in the water after treatment with the optimum dosage of the shell-blended, oil extracted and purified polymer powder are not harmful to humans. Therefore the treated water sample is safe for consumption.

5.0 Conclusion And Recommendation

The overall performances of treatment plants are not satisfactory and the main problems are attributed to inappropriate design and operation of the coagulation and filtration units. Pumice and Moringa oleifera (MO) were suggested as alternative natural material in dual media filtration and coagulation, respectively.

Moringa oleifera seeds acts as a natural coagulant, flocculent, absorbent for the treatment of drinking water. It reduces the total solids, turbidity, alkalinity, after the treatment. It also acts as a natural antimicrobial agent, active against the micro-organisms which are present in the drinking water and decrease the number of bacteria. The MPN test of the raw water sample had shown that the water sample isn't safe for human consumption due to the high level of coliform unit present in the water sample which was beyond the WHO limit for drinking water.

After the treatment of the water sample with all three Moringa oleifera coagulants namely; the shell-blended, de-oiled and the purified protein powder, the MPN test showed a reduction in the coliform unit present which was within the WHO limit for drinking water standard. The purified protein powder had the highest reduction efficiency of the total coliform unit present, followed by the oil extracted and then the shelled Moringa Oleifera seed powder respectively.

The turbidity of the water sample after treatment with all three coagulants from the Moringa oleifera seed powder reduced the turbidity of the water sample. The purified protein powder had the highest turbidity removal efficiency followed by the de-oiled and the shelled seed powder respectively. The oil content in the seed will form an emulsion or film coating which may inhibit the contact with the surface of reaction and thus reduce floc formation. This is the reason for showing maximum percentage reduction observed when coagulation has no oil content.

The residual turbidity of the water sample after treatment with the shell-blended, de-oiled and the purified protein powder showed turbidity beyond the WHO standard limit. This therefore implies that additional treatment such as bio-sand filtration must be applied to the water sample before it is assumed safe for human consumption. Moringa oleifera seed poses no toxic effects on humans and the environment.

It is an eco-friendly and cheaper method of purification of water and therefore can be used in the rural areas where no facilities are available for the treatment of drinking water. After the treatment of Moringa oleifera seed, sludge gets settled at the bottom of tank. Large scale treatment at village level produces large quantity of sludge which can be used as bio-fertilizers and it becomes an added advantage of this treatment.

Considering the fact that Moringa coagulum can be locally produced, its use in water purification should be encouraged. This is likely to reduce the high cost of the current water treatment systems.

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