THE USE OF MORINGA OLIFERA SEED AS A NATURAL PLANT COAGULANT IN REMOVAL OF CLAY PARTICLES AND E.COLI

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Abstract:
Natural plant coagulants play an important role in the provision of potable water to rural communities in the developing world. The use of *Moringa oleifera* has been studied as a primary coagulant to remove clay particles and faecal indicator bacteria. These tests were carried out using artificial water and kaolin as model suspensions to represent the wide range of natural turbid waters which occur in developing countries. A study of electrophoretic mobility showed that *Moringa* seeds act as a cationic polyelectrolyte. Bacterial removal of 1-3 log units (90-99.9%) was obtained within the first 1-2 hours of coagulation with the *Moringa* suspension. It was found that the coagulation efficiency of *Moringa* was affected by certain physico-chemical factors. Coagulation efficiency increased in water with high turbidity and increasing calcium and magnesium concentrations; it decreased in the presence of organic matter.

Key Words
Moringa oleifera, natural plant coagulant, flocculation.

Introduction

Many people in rural areas of developing countries are obliged to draw their water from surface sources, which are frequently highly turbid and contaminated. Individual householders in Africa have used ground-up seeds for centuries to treat their drinking water. In municipal water treatment plants, such a use of indigenous plant-derived materials to effect destabilisation of suspended material can reduce the amount of alum required, thus producing savings, perhaps in already scarce foreign currency, and reductions in transport costs (Folkard, 1987).

The crushed seeds of the horse-radish tree *Moringa oleifera* seed on coagulation of bacteria from the turbid waters of hafirs and rivers has been reported (Jahn & Dirar, 1979; Grabow *et al.*, 1985; Madsen *et al.*, 1987). However, the effects of various physico-chemical parameters which affect the coagulation process are not clear. The present study was undertaken to re-evaluate the potential of *Moringa ofeifera* seed in removal of bacterial cells and clay particles in light of modern coagulation concepts.

Materials and Methods

The materials in the present investigation pertain to artificial water, clay suspensions, microorganisms and preparation of coagulants.

Preparation of Artificial Water

In order simulate chemical water characterisitcs in the Nile River, and standardize its quality, artificial water was made by dissolving calcium chloride ($CaCl_2$) and magnesium sulphate ($MgSO_4$) in distilled water, to produce a water with an approximate calcium concentration of 0.5 mmol/l and a magnesium concentration of 0.3 mmol/l. Alkalinity was provided by the addition of sodium bicarbonate ($NaHCO_3$) to produce an alkalinity of 200 mg/l $CaCO_3$.

Clay Suspension

The kaolin clay used (Hyroc TL, supplied by ECC International, Ceramic Division UK) was shown by x-ray diffraction to be pure. It is known to have a cation exchange capacity of about 7.0 meq/100 gm. Its density is 2.6 gm/ml. A few grams of the powder were weighed out and dried in an oven at 105°C for three hours. It was removed and cooled in a desiccator for 30 minutes. 50 ml. of distilled water were added to wet the powder and it was left to stand overnight. Distilled water was then added to the clay and was thoroughly mixed in a high speed domestic food blender. The mixture was made up to 1.5 liters, left for four hours for the coarser particles to settle, and one liter of the supernatant suspension was transferred to a 20 liter plastic container.

Preparation of Bacteria

E. coli type strain NCTC 9001 was used as test bacteria in all artificial contamination experiments. A nutrient broth culture was grown at 37° C overnight to approximately 10^8 viable bacteria/ml. 50 ml. of the broth culture was placed in a previously sterilized container and centrifuged at 3500 rpm for 30 minutes. The supernatant liquid was decanted and 25 ml of sterile phosphate buffer solution pH 7 added to the bacterial pellet and gently mixed. Enumeration of bacteria was carried out using the pour plate method with MacConkey Agar (Oxoid CM 7). Duplicate plates were incubated, inverted at 37 ± 0.5° C for 24 hours, after which time all red colonies were counted (DHSS, 1984). Plates with 30-300 colonies were used as determinates of the count.

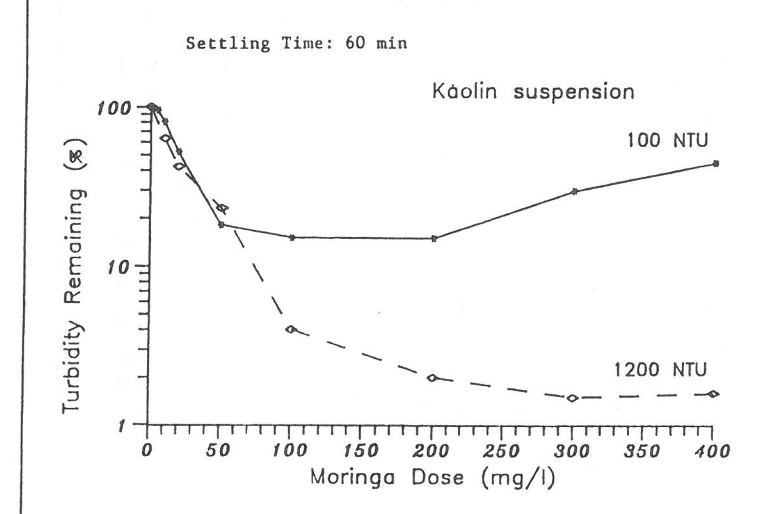
Preparation of *Moringa* Suspension

The outer seed casing was first removed and one gram of the kernels accurately weighed. The kernals were then thoroughly crushed by mortar and pestle and a small amount of distilled wated added to make a fine paste. The contents were then transferred to a beaker, and made up to 100 ml. This solution was stirred on a magnetic stirrer for a short time before being filtered through a fine muslin cloth to remove the larger particles.

Experimental Procedure

Conventional jar test experiments were carried out using a 4-jar test apparatus manufactured by Patterson Candy International. The water was first mixed at 120 r.p.m. for two minutes, then the coagulant dose was added to the water in the form of a suspension, and the mixture flash-mixed at the same mixing rate for two minutes. The rotational speed was then reduced to slow stirring speed at 20 minutes. The electrophoretic mobility of particles was determined by microelectrophoresis. A Particle Micro-Electrophoresis Mark II, Apparatus, manufactured by Rank Brothers of Cambridge, was used in this study. Turbidity readings were carried out using a Hach model 2100A turbidimeter.

Fig. 1 Removal of Kaolin with Moringa Suspension



Results and Discussion

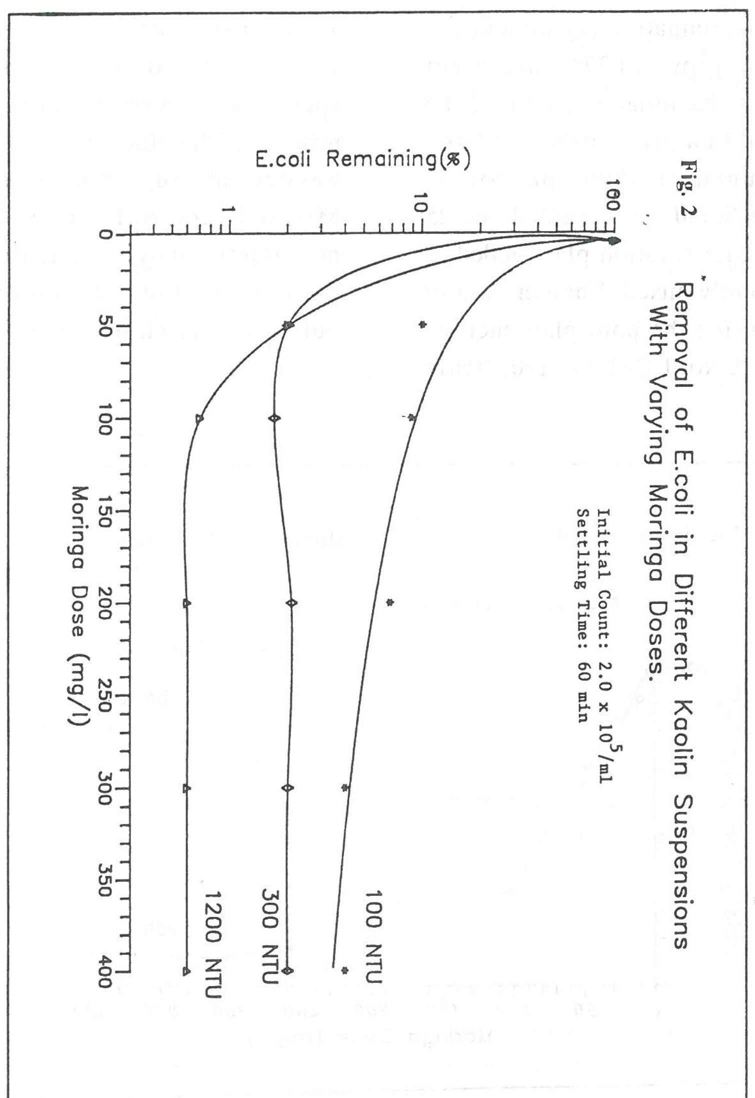
Coagulation of Clay Particles

Figure 1 shows the residual turbidity after one hour sedimentation with varying coagulant dose. The removal of the clay shows that the higher the clay concentration, the more extensive is the removal. This indicates the significance of the collision effect which is clearly greatest for the highest concentration of clay. The significance of orthokinetic particle collision to aggregation has been extgensively demonstrated by research workers (Gemmell, 1963; Stamberger, 1962).

Figure 1 shows the optimum dose is much more clearly defined at the lower turbidity of 100 NTU, when overdosing leads to restabilsation and an increase in turbidity. This agrees with work carried out by Black and Vilaret (1969), Folkard (1967), and Robinson (1974). Black andVilaret (1969) reported that a much narrower optimum range was found with monodispersed latex suspensions, and it was thought that the wider range with clay was probably due to the variations in clay particle sizes and to their characteristic shape and surface structure.

Coagulation of *E. Coli*

Bacterial reduction using *Moringa* seed suspension was investigated with water to which were added varying concentrations of kaolin to give turbidities of 0, 100,300, and 1200 NTU. *E. coli* removal of over 90 percent was achieved during the first hour of sedimentation in most experiments. The maximum removal was 99.5 percent at *Moringa* doses of 200-400 mg/l in high turbidity water (1200 NTU) (Figure 2).



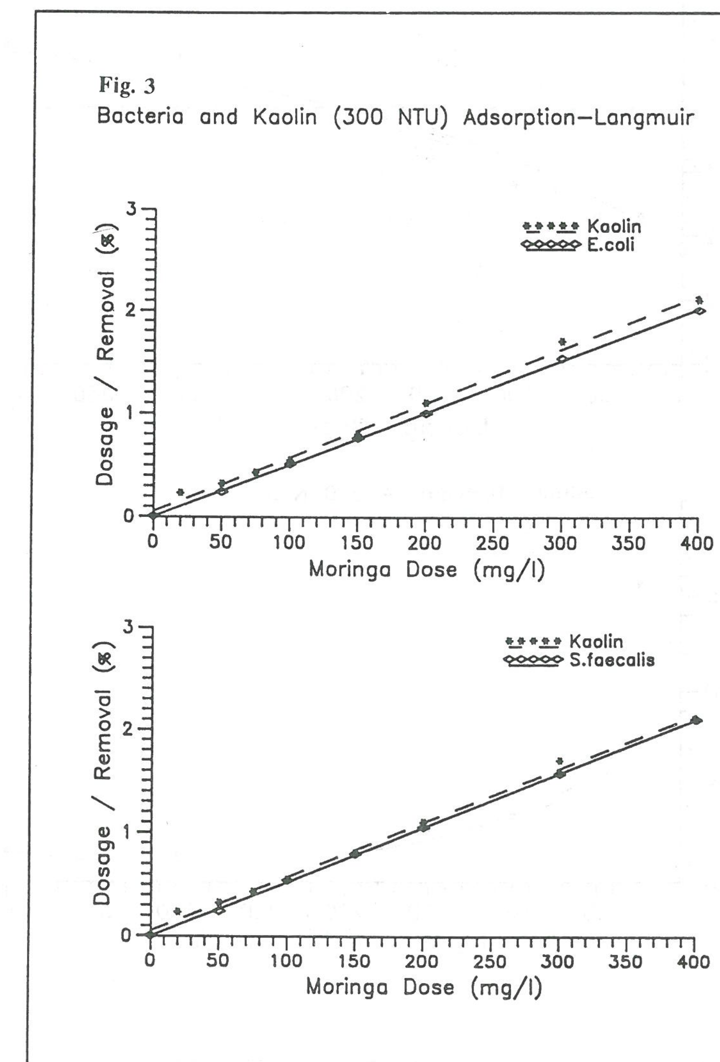
However, as can be seen from the figure, the removal efficcciency of *Moringa* is considerably affected by the concentration of kaolin clay suspension. The rate of orthokinetic flocculation is first order with respect to the concentration of particles, the velocity gradient, and the floc volume fraction O'Melia, 1972). He concluded that the removal of bacteria and other microorganisms from water and wastewater by coagulation usually therefore requires the presence of other colloidal particles. The regrowth of bacteria during the first 24 hours of coagulation was observed. The effect of environmental factors influencing the regrowth has been reported (Bina, 1991).

Quantitative Adsorption

A consideration of adsorption indicated that some of the isotherm equations may be used for quantitative expression of the results. It was found that the data obtained on removal of bacterial cells fitted the Langmuir equation and plotted as a straight line. In the experiments carried out, the coagulant *(Moringa* suspension) is the adsorbate and the adsorbent surface is that of the bacterial cells or clay (kaolin). To test the fit of the data to the Lanmuir equation, it is converted to:

$$\frac{C}{x/m} = \frac{1}{axb} + \frac{C}{a}$$

The parameter x/m is represented in this case by percentage removal, and concentration by dosage. The results of the polymer adsorption experiments for *Moringa* onto *E. coli* and kaolin are shown in Fig. 3 for doses from 0 to 400 mg/l. The adsorption data follow a Langmuir isotherm, with the saturation adsorption increasing as the concentration of *Moringa* increases. Similar results were found by Treweek and Morgan (1977) in adsorption of the cationic polymer, polyethylamine of varying molecular weights, onto *E. coli.*



Effect of *Moringa* on the Zeta Potential of Particles

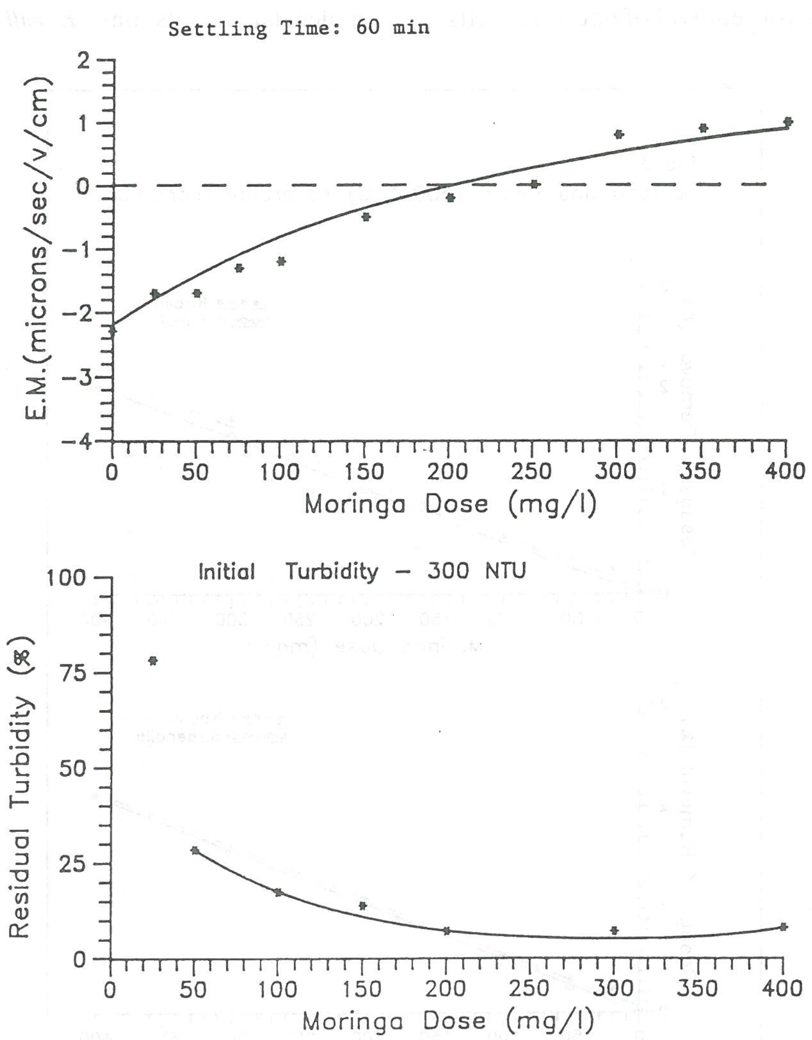
Suspensions of *Moringa* seeds, as suggested in literature, were found to have positive charges. In order to support the theory that the action of *Moringa* seeds was similar to a synthetic cationic polyelectrolyte electrophoretic mobility, determinations were carried out for various coagulant seed dosages. Samples of water containing clay, *E. coli*, and suspensions of *Moringa* seeds were examined under the microscope of the electrophoresis apparatus.

Fig. 4 demonstrates the effect of *Moringa* doses on the electrophoretic mobility of kaolin. A gradual decrease in the electrophoretic mobility occurs as the concentration of coagulant increases. The positively charged *Moringa* suspension neutralizes the negatively charged particles, and eventually at a certain concentration level, a charge reversal is produced.

The isoelectric point was found to be a dosage of 200 mg/l which showed excellent correlation with the doses of *Moringa* suspension which yielded maximum turbidity and *E. coli* removal.

Fig. 4

Electrophoretic Mobility and Residual Turbidity(%) versus Moringa dosage.



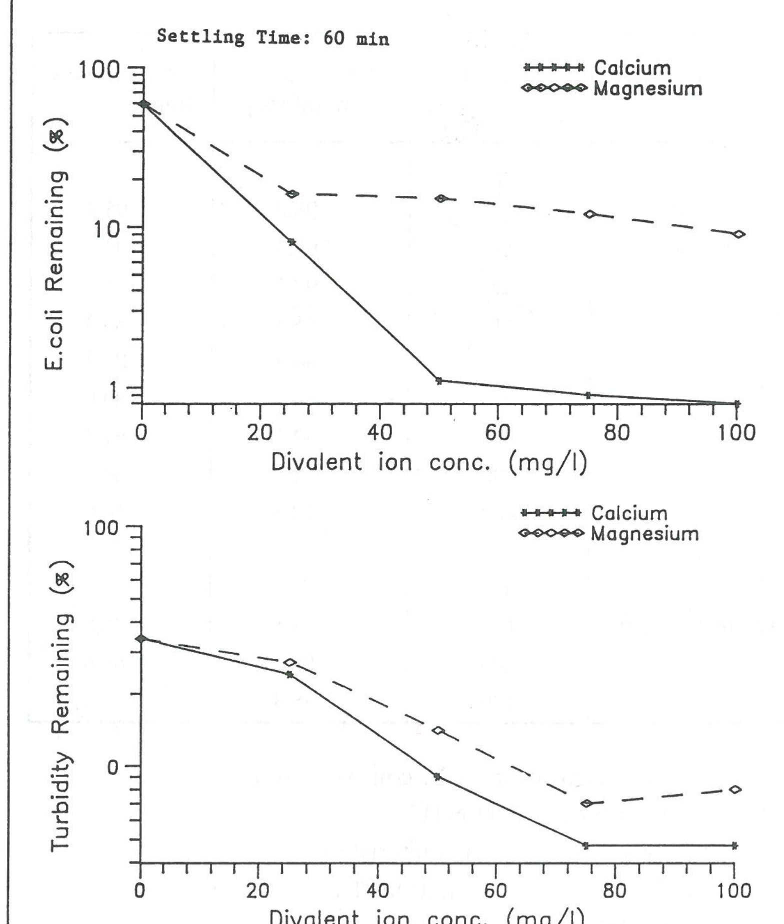
Similar results were reported by Narkis and Rebhun (1975) using PVMPI as a cationic polyelectrolyte on removal of humate, clay minerals and mixtures thereof. All of these mixtures were designed to simulate natural water quality. In each system, a zero electrophoretic mobility was recorded at optimum removal. From results obtained in the present investigation, it is possible to conclude that the action of *Moringa* as a cationic polyelectrolyte consists of not only polymer bridging, but it also acts as a weak electrolyte, which in turn can neutralize the charge of particles and subsequently cause coagulation.

Effect of Calcium and Magnesium on Particle Removal

Solution properties, particularly the presence of divalent metal ions such as calcium and magnesium, have a profound effect upon the determination of the mode of interaction between polymers and colloidal surfaces (Black et al., 1965). Five samples of kaolin suspension were taken with initial turbidity 300 NTU and alkalinity 200 mg/l. $CaCl_2$ and $MgSO_4$ were added to give calcium and magnesium ion concentrations of 0, 25, 50, 75 and 100 mg/l respectively. *E. coli* was added by flash mixing a few minutes before adding an appropriate dose of coagulant, and the jar test performed. The readings were taken after one hour sedimentation.

Figure 5 shows that the presence of divalent cations in water can greatly increase the ability of *Moringa* suspension to aggregate the negative colloids.

Fig. 5 Effect of Divalent Ion Conc. on Coagulation of E.coli and Kaolin (300 NTU) with Moringa seed (200 mg/l).



Effect of Organic Matter on Particles' Removal

It is believed that the presence of extraneous organic matter of proteinaceous character will interfere with bacterial cell removal by coagulation and flocculation. The following experiment was carried out to determine the effect of different concentrations of organic matter on the efficiency of *Moringa* for the removal of *E. coli* and clay particles. Organic matter in the form of bacteriological peptone and wastewater effluent was added to the synthetic water prior to addition of *E. coli* and coagulant. Enumeration of *E. coli* and turbidity readings were carried out after one hour of sedimentation.

Table 1 shows the removal of *E. coli* and turbidity by *Moringa* suspension in the presence of bacteriological peptone and wastewater effluent. It can be seen that bacteriological peptone and wastewater effluent interfered with *E. coli* and kaolin clay removal by coagulation with *Moringa*. Table 1 shows that the effect of organic matter in reduction of *E. coli* with *Moringa* seed suspension is not significant up to concentrations of 150 mg/l of bacteriological peptone; above 150 mg/l increasing the bacteriological peptone concentration distinctly decelerated the coagulation process.

Gibbs (1983) observed that particles with natural organic coatings coagulated less rapidly than particles without natural organic coatings. He concluded that

TABLE 1

Removal of E. Coli By Coagulation and Sedimentation (60 min) with Moringa (200 mg/l) Seed Suspensions in the Presence of Organic Matter

Organic Matter	Concentration	* E. Coli Removal (%)	** Turbidity Removal (%)
	mg/l		
Bacteriological Peptone	0.0	98.2	95.3
	10	97.5	94.3
	25	97.2	91.7
	50	95.7	88.3
	75	94.7	81.3
	100	93.2	80.0
	150	92.3	69.3
	200	71.7	60.0
	400	18.3	10.0
	ml.l		
*** Wastewater Eff.	0.0	98.5	96.0
	200	80.0	66.6
	400	58.3	43.3

* Initial Concentration of E. coli 6.0 * 10/ml

** Initial Turbidity : 300 NTU

*** Wastewater effluent characteristics :

5- day BOD (mg/l)	12.0 mg/l
Suspended solids	18.0 mg/l

the natural organic coatings stabilized the particles. Morel (1983) found that even at the relatively high pH of 8, a dissolved organic concentration of a few mg/l was sufficient to saturate the available inorganic surface on a few mg/l of aquatic particles. He concluded that the surfaces of aquatic particles might be entirely covered by organic matter and hence exhibit the physical and chemical characteristics of organic matter.

It is also believed that soluble organic materials compete with bacterial cells for adsorption sites on soil particles (Bitton, 1975). These results indicate that the process of coagulation may not be expected to operate with high efficiency, if the raw wastewater contains significant amounts of organic matter.

Summary and Conclusions

1. The effect of coagulation on bacteria, and clay minerals can be interpreted in terms of adsorption concepts. The results of coagulation tests with these particles were quantitatively expressed by means of the Langmuir adsorption equation. This showed a marked similarity in behavior of the clay minerals and baceria.

2. Electrophoretic mobility determinations suggested that *Moringa* behaves as a cationic polelectrolyte causing coagulation partly by polymer bridging and partly by acting as a weak electrolyte.

3. The aggregation and thus removal of *E. coli* was directly proportional to the concentration of the clay particles in the suspension. Up to 99.5% removal of *E. coli* was achieved at *Moringa* doses of 200-400 mg/l in kaolin suspension of 1200 NTU.

4. The ability of *Moringa* to coagulate suspended particles was found to be greatly enhanced by the presence of divalent metal ions such as calcium and magnesium.

5. The presence of organic matter affected the coagulation efficiency of *Moringa*. The results indicated that the process of coagulation may not operate with high efficiency if the raw water contains excess organic matter. Final effluent added at 20% concentration and bacteriological peptone at >150 mg/l significantly reduced the removal of *E. coli* and turbidity.

6. These studies provide quantitative evidence, obtained under carefully controlled laboratory conditions, of the ability of *Moringa* to remove bacteria and turbidity from water at a household, small village, or a town treatment plant.

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