Comparison of the Effectiveness of *Moringa oleifera* (*Moringa*) Seeds and *Strychnos potatorum* (*Ingini*) Seeds as Natural Coagulants for Turbid Water Purification

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Abstract

Lack of quality drinking water is a burning and life-threatening problem especially in the dry zone in Sri Lanka. Many inorganic coagulants and synthetic organic polymers widely used in conventional water treatment processes. Recent studies have indicated a number of serious drawbacks linked to the use of those coagulants and flocculants. Therefore, there is a rising interest to develop cost-effective, easier and environmental friendly processes of water purification with the usage of natural coagulants. Therefore, the present study is focused to study the effectiveness of water extracts of *Moringa oleifera* (*Moringa*) seeds and *Strychnos potatorum* (*Ingini*) seeds in turbid water purification. The dry matter content, protein content, fat content and ash content of the seed powder of *M. oleifera* and seed paste of *S. potatorum* were determined. The coagulation activity was assessed by the jar test using natural low, medium and high turbid water. The water samples were treated with seed extracts of *M. oleifera* and *S. potatorum* and the physicochemical markers evaluated were pH, turbidity, total solids, total dissolved solids, total suspended solids, electrical conductivity and the biological marker was total plate counts. The turbidity was reduced up to 92% by the dosage of 200 mg/l of *M. oleifera* and it was effective for low turbid water (Turbidity of 25-35 NTU) than the *S. potatorum*, The turbidity was reduced up to 95% by the dosage of 300 mg/l of *M. oleifera* and it was effective for medium turbid water (Turbidity of 40-50 NTU) than the *S. potatorum*. The turbidity was reduced up to 97% by the dosage of 4 mg/l of *S. potatorum* and 95% turbidity reduction was observed by the dosage of 400 mg/l of the *M. oleifera* in high turbid water (90-120 NTU). The optimum dose of different seed extracts revealed a pronounced efficiency for removing TPC, TS, TDS and TSS. There was no effect on pH and EC of the water samples treated with these natural coagulants. *M. oleifera* was effective for low turbid water than the *S. potatorum* while both coagulants were effective for high turbid water. However both natural coagulants, better water turbidity removal efficiencies were noted at high turbidity compared to low turbidity.

Keywords: *Moringa oleifera, Strychnos potatorum, Water purification, Natural coagulants*

INTRODUCTION

Water is undoubtedly the most imperative element among the natural resources and the access to safe and clean drinking water is a major concern all over the world. Lack of quality drinking water is a burning and life-threatening problem especially in the dry zone in Sri Lanka. Producing potable water from surface water or ground water usually involves one or several treatment steps for removing unwanted substances. Since the contamination possibilities are high, surface water generally requires more treatments than ground water.

Many coagulants and flocculants are widely used in conventional water treatment processes to remove turbid agents in the form of suspended and colloidal material. These coagulants can be classified as inorganic coagulants (e.g. aluminium and ferric salts) and synthetic organic polymers (e.g. polyacrylamide derivatives and polyethylene imine) (Ghebremichael *et al.*, 2005). Aluminium

salts are cheap and are the most widely used coagulants in water and waste water treatment all over the world. Regarding the application of synthetic polymers, the presence of residual monomers is undesirable because of their neurotoxicity and strong carcinogenic properties. All of these coagulants have in common the ability of producing positively charged ions when dissolved in water, which can contribute to charge neutralization (Muyibi *et al.*, 2003). Recent studies of Ghebremichael (2005) have indicated a number of serious drawbacks linked to the use of aluminium salts such as Alzheimer's disease associated with high aluminium residuals in treated water, excessive sludge production during water treatment and considerable changes in water chemistry. In addition, the use of alum salts is inappropriate in some developing countries because of the high costs of imported chemicals and low availability of chemical coagulants (Ghebremichael *et al.*, 2005).

Therefore, in recent years many researches started to develop cost-effective, easier, and environmental friendly processes of water clarification with the usage of natural coagulants which can be produced or extracted from microorganisms, animal or plant tissues. These coagulants should be biodegradable and are presumed to be safe for human health.

The use of natural materials of plant origin to clarify raw turbid water is not a novel idea. Natural coagulants have been used in household level for centuries in traditional water treatment process in tropical rural areas. Nowadays, there are reports that describe the natural coagulants from *Ingini* (*Strychnos potatorum*) seed and different leguminous species (Rajendran *et al.*, 2013). Another plant material which has recently received the greatest degree of attention is the seed of *Moringa oleifera* (Rajendran *et al.*, 2013). However, there were no reports on clear application of these plant materials for water treatments.

Therefore, the present study is focused to study the effectiveness of water extracts of *M. oleifera* seeds and *S. potatorum* seeds to reduce the level of turbidity and bacteriological contaminants from water and to make the water treatment process easier, healthier and environmental friendly for household applications.

MATERIALS AND METHOD

The experiments were carried out at the Research laboratory, Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna. *Moringa* and *Ingini* seed (fresh form) were purchased from village vender in Hambanthota, Southern Province, Sri Lanka. The best seed extraction procedures of natural coagulants were determined based on the performance in clarification of turbid water by conducting many trials. *Moringa oleifera* seed pods were allowed to dry naturally to a brown color. The shells were removed manually. The seed kernels were ground to a fine powder using a domestic blender and sieved through 250 µm stainless steel sieves. Then 1 g of seed powder was weighed and dissolved in 50 ml of distilled water to make 20 g/l stock solution. The stock solution was stirred for 10 minutes using magnetic stirrer, and finally filtrated through a Whatmann filter no. 40. A fresh solution was prepared every day in order to avoid ageing effects.

Strychnos potatorum seeds due to their hard structure, could not be powdered in a grinder. Therefore, the fresh seeds were soaked for 5 days in clean water and then mixture was blended to a paste-like solution and was filtered through a Whatmann filter no. 40. By determining the dry matter content, the strength of the stock solution was determined. The stock solution of 200 mg/l was prepared in daily basis for clarification studies.

Proximate composition of seed coagulants

The dry matter content, protein content, fat content and ash content of the *M. oleifera* seed powder and *S. potatorum* seed paste were determined following the standard methods. The dry matter content was determined by drying the samples in oven at 103°C until a constant weight was obtained. The protein content was determined by Kjeldahl method and the total ash content was determined by keeping the samples at 505°C for 5h until it becomes ash using muffle furnance. The

fat content of the samples was determined using Soxhlet fat extraction apparatus.

Jar test experiments to test the coagulant effect on turbidity reduction

Jar tests were carried out to determine the effective dosage of each coagulant which is able to purify the water samples. Five glass beakers of 1000ml capacity were filled with water of different turbidities obtained from the shallow wells. Each beaker was filled with 500ml of test water with identical turbidity. One beaker was used as the control while the other four were treated with each plant extract in turn with concentrations ranging from 100mg/l to 450mg/l of *Moringa oleifera* and concentrations ranging from 1mg/l to 4.5mg/l of *Strychnos potatorum*. The coagulants were mixed with turbid water at a high speed of 200rpm for10min by magnetic stirrer followed by slow mixing at 40rpm for 5min. The rapid mixing after adding a coagulant is important to obtain a uniform dispersion of the coagulant and also to increase the opportunity for particle-to-particle contact. Subsequent gentle mixing cements the microscopic coagulated particles into larger flocs. Then the content of the jars left to settle for 1hr. After sedimentation, supernatants were collected from each sample for water quality determination at 1hr, 2hr and 24hr to compare the efficiency of coagulation. The turbidity levels were measured as NTU units (Nephelometric Turbidity Units). The water samples collected for experiments had the initial turbidity of 25NTU, 45.44NTU and 118.34NTU for low, medium and high turbid water, respectively. For each coagulant and each turbidity level, three identical jar tests were performed in order to obtain statistically reliable results. If the optimal dosage was not found in the jar test, a new test with new dosage was carried out until the optimum was found.

Experiments to test the effect of coagulants on physicochemical quality of water

Total solids (TS), Total suspended solids (TSS), Total dissolved solids (TDS), pH, electrical conductivity (EC) and total plate counts (TPC) were the water quality parameters measured. Turbidity was measured using a 2100P turbid meter (Hach, USA). The pH values and EC values were measured using Hanna pH/EC combo waterproof tester, USA.

TS was determined by measuring the volume of the water sample, evaporating the water in the sample and weighing the residue. The sample was evaporated in oven at 103°C for 3hr. Total solids content of water is the weight of residue expressed as milligrams per unit volume of water sample. TSS was determined by weighing a filter paper; the water sample was then filtered using that filter paper and the wet paper was then dried in the oven at 103 to 105°C and recorded the weight. TSS content of the water is the increase in weight of filter paper and it was expressed as milligrams per unit volume of water sample. TDS was determined by filtering the water sample using a filter paper and evaporating the filtrate in the oven at 103°C for 3hr. TDS content of water was expressed as milligrams per unit volume of water sample.

Experiments to test the effect of the coagulant on microbiological quality of water

The plate count agar was prepared according to standard procedure and then it was sterilized at 121 °C for 20 min in autoclave. The 10^{-1} dilution was prepared by mixing the 1 ml of water sample with 9 ml of MRD (maximum recovery diluents). Using separate sterile pipettes, serial decimal dilutions of 10^{-2}, 10^{-3} and 10^{-4} were prepared by transferring 1 ml of previous dilution to 9 ml of diluents. Then 12-15 ml of plate count agar (cooled to 45±1°C) was poured into to each plate and the plates were allowed to solidify. Then the appropriately marked petri dishes were inoculated with 1 ml of each dilution separately and in duplicates. Then the inoculated petri dishes were incubated at 37 °C for 48 hrs. The colonies were counted using the colony counter and it was recorded as the total plate count.

RESULTS AND DISCUSSION

Proximate composition of seed coagulants

The proximate composition of *M. oleifera* seed powder consisted of 34.1% protein, 15.5% lipids, 95% dry matter, 11.8% ash. The proximate composition of *S. potatorum* seed paste consisted of 38.4% protein, 1.02% lipids, 4% dry matter, 3.2% ash.

Jar test experiments to test effect on turbidity of water

Coagulation and flocculation efficiency of *M. oleifera* seed extract was compared with that of *S. potatorum* seed extract. The optimum dosage is the minimum dosage of coagulant corresponding to the lowest residual turbidity or the highest coagulation activity.

It was revealed from Table 1 that the processed *M. oleifera* gave the turbidity removal of 92%, 95%, and 95% for the treatment of raw water with low, medium and high turbidity by using the optimum dosage of 200, 300 and 400mg/l respectively. Residual turbidity reduces below 5 NTU in water treated at the optimum dosage of coagulant. The turbidity of potable water should be below 5 NTU which is the standard set by world health organization, WHO. In contrast to the findings of present study, the findings of Eman *et al.* (2009) revealed that *M. oleifera* dose of 0.05, 0.15 and 0.3mg/l were found effective in reducing turbidity up to 95.5%, 98.5% and 99.3% in low, medium and high turbid water, respectively. But the investigated optimum doses of present study were in line with the study by Folkard *et al.* (2000) which had earlier reported that *M. oleifera* seed powder dosages between 10-200 mgl^{-1} were effective for turbidities ranging between < 50 and > 150 NTU. These results supports the findings of Choubey *et al,* (2012) regarding *M. oleifera* which showed that the effectiveness of *Moringa oleifera* for turbidity removals is 97% for high turbid water and lower removals of 86% for low turbid water. According to Ndabigengesere *et al.* (1995) *Moringa oleifera* produced the best results with an average percentage in turbidity reduction of 96 ± 2% for water of relatively high initial turbidity.

The turbidity was increased when overdosing the coagulant extract than the optimum dosage at each turbidity level. As adapted from Ghebremichael *et al.* (2005) the overdosing resulted in the saturation of the polymer bridge sites and caused re-stabilization of the destabilized particles due to insufficient number of particles to form more inter-particles bridges.

According to Schwarz (2000) the seed kernels of *M. oleifera* contain lower molecular weight water-soluble proteins which carry a positive charge. The chemical composition of basic polypeptides with molecular weights ranging from 6000 to 16,000 Da, containing up to six amino acids of mainly glutamic acid, methionine and arginine. The report further explains that when the seeds are crushed and added to water, the protein produces positive charges acting like magnets and attracting predominately negatively charged particles such as clay, silt, and other toxic particles. Under proper agitation, these bound particles then grow in size to form the flocculates which are left to settle by gravity. This accounted for the effectiveness of *Moringa* as a coagulant for raw water purification.

It was revealed from Table 2, that the processed *S. potatorum* gave the turbidity removal of 71%, 92%, and 97% for the treatment of raw water with low, medium and high turbidity by using the optimum dosage of 1.5 mg/l, 3 mg/l and 4 mg/l respectively. Residual turbidity did not reduce below 5 NTU in low and medium turbid water but it was below 5 NTU in high turbid water treated at the optimum dosage of coagulant.

In control test raw water had turbidity reductions of average 12%, 14.6% and 15% with low, medium and high turbidity, respectively indicating that just stirring the water and allowing it to settle can reduce turbidity of water to a certain extent.

The findings for *S. potatorum* are in contrast to the results found in Choubey (2013). Findings of Choubey (2013) prove that water turbidity can be reduced up to 90% with an optimum dosage of 4g/l of *S. potatorum* while 97% turbidity reduction was achieved with an optimum dosage of 4mg/l of *S. potatorum* in present study.

According to Tripathi *et al.* (1976) *S. potatorum* seed extract is an anionic polyelectrolyte which contains carboxyl (COO-) and hydroxyl (OH-) as main active groups. The presence of divalent cations in water can greatly increase the ability of anionic polyelectrolyte to aggregate negative colloids (Black *et al.*, 1965). The high turbid water tested in the present study, in addition to divalent cations may have trivalent aluminum, which might have enhanced turbidity removal by *S. potatorum* than the *M. oleifera.*

According to the results of both natural coagulants, at high turbidity better removal efficiencies were noted compared to lower initial turbidity. It may be due to the presence of a larger number of particles in the water which raises the chances of collision and flock formation and hence better settlement of the denser flocks that are formed.

Experiments to test the effect on physicochemical quality of water

The results relate to the physicochemical properties of treated water are summarized in Table 3 and Table 4. The treatment with *M. oleifera* and *S. potatorum* had no significant effect on the pH and EC of the treated water. However, this is not in agreement with the findings of Udensi *et al* (2011) for *M. oleifera* and the findings of Choubey (2013) for *S. potatorum*. For the water sample, the pH remained fairly constant at 6.9, 7.0 and 7.2 from an initial pH of 7.1, 7.1 and 7.4 of water samples with low, medium and high turbidity, respectively for treatments with optimum dosage of *M. oleifera* while those were 7.2, 7.3 and 7.3 for treatments with optimum dosage of *S. potatorum*, respectively. The pH values were found well within the desirable limits (6.0-8.0) for drinking water as specified by WHO. The observed independence of pH of *M. oleifera* and *S. potatorum* clarification would be an added advantage of those natural coagulants, especially in developing countries where a huge portion of currency is spent for importation of the chemicals to use in pH adjustments in conventional water treatment. In the literature there are evidences that the use of *M. oleifera* seed and *S. potatorum* seed as natural coagulants influenced the pH in water to a certain extent. Ndabigengesere *et al.* (1995) reported that the action of *M. oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds. This suggests that in water, the basic amino acids present in the protein of coagulants would accept a proton from water resulting in the release of a hydroxyl group making the solution basic. However, the findings of present study are in agreement with that of the Emelie *et al.* (2008). The findings in this study indicated that the increasing pH value is not necessarily contributed by the doses of *M. oleifera* and *S. potatorum* seed extractions in contrast to the results found in other studies of Udensi *et al.* (2011) and Choubey (2013) which confirmed that the pH increases by increasing the dose of *M. oleifera* and *S. potatorum* seed coagulants.

In the treated water samples, the EC remained fairly constant at 0.02, 0.03 and 0.03dS/m from an initial EC of 0.02, 0.03 and 0.03dS/m of water samples with low, medium and high turbidity with optimum dosage of *M. oleifera*, respectively while those were 0.02, 0.03 and 0.03 dS/m for treatments with optimum dosage of *S. potatorum*, respectively. According to past research findings the use of *M. oleifera* seed and *S. potatorum* seed as natural coagulants influenced the EC in water to a certain extent. However the experimental results of Choubey (2013) showed that conductivity of the water treated with *S. potatorum* was not changed from that of raw water.

According to results indicated in the Table 5, Table 6 and Table 7, the treated water samples with *M. oleifera* seed extract, TS, TDS and TSS were found to be reduced in 58%, 57% and 57% respectively, in low turbid water where they were 57% for medium turbid water and 60%, 59% and 59% respectively, for high turbid water. According to results shown in Table 8, Table 9 and Table 10, the treated water samples with *S. potatorum* seed extract, TS, TDS and TSS were found to be reduced in 51%, 50% and 54% respectively, in low turbid water where they were 51%, 53% and 47% respectively, for medium turbid water and 52%, 52% and 50% respectively, for high turbid water. The experimental results of Choubey (2013) indicated that the performance of *S. potatorum* was highly significant on reduction of TDS up to 60% with an optimum dosage of 8g/l.

Experiments to test effect on microbiological quality of water

A significant removal of total bacteria was found after treatment of turbid water with natural coagulants (table 11 and 12). The reduction of total bacteria was about 97% when the water is treated with *M. oleifera* seed extract (Table 11) and it was about 89% when treated with *S. potatorum* seed extract (Table 12). This is in the agreement with the findings of Schwarz (2000) that the process of flocculation of *M. oleifera* removes about 90-99% of bacteria which are normally attached to the solid particles. It was seen that the *M. oleifera* seed extract and *S. potatorum* seed extract act as antimicrobial agents. Eilert *et al.* (1980) identified the presence of 4-α-rhamnosyloxy-benzyl isothiocyanate an active antimicrobial agent in the *M. oleifera* seed. In other hand, coagulating the solid matter in water may itself remove a good portion of the suspended bacteria.

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Tables

Table 1: Effect of *Moringa* extract on turbidity of water

Turbidity level	Dosage of *Moringa* (mg/l)	Initial Turbidity (NTU)	Residual Turbidity (NTU)	Turbidity Reduction (%)
Low	100	25.0	4.68	81.32
Low	150	25.0	4.39	82.57
Low	200	25.0	2.11	91.58
Low	250	25.0	7.07	71.79
Medium	200	45.44	4.4	92.03
Medium	250	45.44	3.3	93.97
Medium	300	45.44	2.91	94.73
Medium	350	45.44	4.45	91.94
High	300	118.34	15.99	86.5
High	350	118.34	13.74	88.39
High	400	118.34	6.22	94.74
High	450	118.34	17.67	85.05

Table 2: Effect of *Ingini* extract on turbidity of water

Turbidity level	Dosage of *Ingini* (mg/l)	Initial Turbidity (NTU)	Residual Turbidity (NTU)	Turbidity Reduction (%)
Low	1	25.0	8.5	66.17
Low	1.5	25.0	7.3	70.83
Low	2	25.0	8.37	66.57
Low	2.5	25.0	9.69	61.18
Medium	2	45.44	7.98	85.55
Medium	2.5	45.44	7.3	86.8
Medium	3	45.44	5.37	90.28
Medium	3.5	45.44	6.89	87.55
High	3	118.34	6	94.93
High	3.5	118.34	4.24	96.42
High	4	118.34	3.15	97.33
High	4.5	118.34	5.65	95.22

Table 3: Effect of *Moringa* extract on pH and electrical conductivity of water

Turbidity level	Dosage of *Moringa* (mg/l)	pH - Initial	pH - After Treatment	EC – Initial (dS/m)	EC - After Treatment (dS/m)
Low	200	7.1	6.9	0.02	0.02
Medium	300	7.1	7	0.03	0.03
High	400	7.4	7.2	0.03	0.03

EC - Electrical Conductivity

Table 4: Effect of *Ingini* extract on pH and electrical conductivity of water

Turbidity level	Dosage of *Ingini* (mg/l)	pH - Initial	pH - After Treatment	EC – Initial (dS/m)	EC - After Treatment (dS/m)
Low	1.5	7.1	7.2	0.02	0.02
Medium	3	7.1	7.3	0.03	0.03
High	4	7.4	7.3	0.03	0.03

EC - Electrical Conductivity

Table 5: Effect of *Moringa* extract on total solid content of water

Turbidity level	Dosage of *Moringa* (mg/l)	TS (mg/l)- Initial	TS (mg/l) - After Treatment	TS Reduction (%)
Low	200	585.52	245.81	58.02
Medium	300	723.56	310.58	57.08
High	400	884.87	355.67	59.81

TS - Total Solids

Table 6: Effect of *Moringa* extract on total dissolved solid content of water

Turbidity level	Dosage of *Moringa* (mg/l)	TDS (mg/l)- Initial	TDS (mg/l) - After Treatment	TDS Reduction (%)
Low	200	398.32	169.15	57.53
Medium	300	548.64	237.92	56.63
High	400	705.29	291.73	58.64

TDS- Total Dissolved Solids

Table 7: Effect of *Moringa* extract on total suspended solid content of water

Turbidity level	Dosage of *Moringa* (mg/l)	TSS (mg/l)- Initial	TSS (mg/l) - After Treatment	TSS Reduction (%)
Low	200	398.32	169.15	57.53
Medium	300	548.64	237.92	56.63
High	400	705.29	291.73	58.64

TSS- Total Suspended Solids

Table 8: Effect of *Ingini* extract on total solid content of water

Turbidity level	Dosage of *Ingini* (mg/l)	TS (mg/l)- Initial	TS (mg/l) - After Treatment	TS Reduction (%)
Low	1.5	585.52	245.81	51.19
Medium	3	723.56	310.58	51.55
High	4	884.87	355.67	51.89

TS - Total Solids

Table 9: Effect of *Ingini* extract on total dissolved solid content of water

Turbidity level	Dosage of *Ingini* (mg/l)	TDS (mg/l)- Initial	TDS (mg/l) - After Treatment	TDS Reduction (%)
Low	1.5	398.32	199.15	50
Medium	3	548.64	257.92	52.99
High	4	705.29	341.73	51.55

TDS- Total Dissolved Solids

Table 10: Effect of *Ingini* extract on total suspended solid content of water

Turbidity level	Dosage of *Ingini* (mg/l)	TSS (mg/l)- Initial	TSS (mg/l) - After Treatment	TSS Reduction (%)
Low	1.5	187.2	86.66	53.7
Medium	3	174.92	92.66	47.02
High	4	169.58	83.94	50.5

TSS- Total Suspended Solids

Table 11: Effect of *Moringa* extract on total plate count of water

Turbidity level	Dosage of *Moringa* (mg/l)	TPC – Initial (cfu/100 ml)	TPC - After Treatment (cfu/100 ml)
Low	200	1.35×10^3	5.4×10^1
Medium	300	1.45×10^3	4.1×10^1
High	400	1.87×10^3	3.5×10^1

TPC - Total Plate Count

Table 12: Effect of *Ingini* extract on total plate count of water

Turbidity level	Dosage of *Ingini* (mg/l)	TPC – Initial (cfu/100 ml)	TPC - After Treatment (cfu/100 ml)
Low	1.5	1.35×10^3	1.25×10^2
Medium	3	1.45×10^3	1.8×10^2
High	4	1.87×10^3	1.85×10^2

TPC - Total Plate Count