Vidyodaya J. of Sci. (2001) Vol. 10, pp 167-182

Use of seeds of *Moringa oleifera* to clarify turbid waters and wastewaters

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Received on: 05.25.01
Accepted on: 05.20.02

Abstract

Ability of seed extracts of *Moringa oleifera* to clarify turbid waters and wastewaters was investigated. Mature seed extracts of *Moringa* were comparatively more effective than immature seed extracts. Mature seed powder at 50 mg/20 ml reduced the turbidity (NTU) by 95% within 2h. It was the aqueous extract of *Moringa* seeds that was really effective in clarifying turbid waters, not the insoluble fraction. A positive relationship between the protein content and purifying ability of seed extract was observed. It was also found that extracts of mature *Moringa* seeds have an ability to clarify textile dye solutions. All four solutions of textile dyes namely Terasil Blue 3RL-02, Terasil Navy GRL-C, Terasil Red R and Terasil yellow 4G were clarified by *Moringa* seed extracts. Studies on pH and the conductivity showed that pH is slightly reduced and conductivity is rapidly increased when they were treated with *Moringa* seeds whilst conductivity was found to be dependent on treatment time and temperature. The clarifying ability of *Moringa* seed was also investigated against paper factory effluent but no significant effect was observed.

Mature seed extracts of *Moringa* significantly reduced the bacterial growth of polluted waters. The effectiveness was directly proportional to the amount of seeds used. An antimicrobial activity was found in crude aqueous extracts of *Moringa* seeds.

Key words: *Moringa* seeds, wastewaters, turbidity, antibacterial activity

1. Introduction

Conventional methods used for purification of water include coagulation, sedimentation, filtration, aeration and also chemical treatment. The use of coagulants such as alum is one of the commonest methods employed and it reduces the repulsive force between particulate matter, encouraging

particle collision and floc formation. Removal of suspended materials is usually followed by the removal of enteric pathogens by treatment of water with chlorine. The objective of all these treatment processes are to obtain a clear, bright, odourless, palatable and hygienically safe water.

The use of natural material of plant origin has also been attempted in several parts of the world to clarify turbid waters due to high cost of chemical treatment or non-availability of modern treatment technology. Jahn (1981) has reviewed methods used in traditional water purification, often in tropical developing countries. One such material of plant origin that has been investigated by several previous workers is the seed of *Moringa oleifera* (Ndabigengesere et al., 1995). Although a considerable amount of information has been generated, certain areas seem to lack information that can be of importance in practical applications.

The objective of the present investigation was to throw some light on some factors that affect the final quality of treated water such as degree of maturity of *Moringa* seeds with special reference to clarification and bacterial growth. In addition, the use of *Moringa* seeds in clarification to textile factory effluent was also investigated.

2. Materials and methods

Preparation of seed powder, seed coat and fruit-flesh of *Moringa oleifera*: Mature and immature pods of *Moringa* were collected during July - August period from the trees in Galgamuwa irrigation site area. Seeds were dried in an oven at 60^{0}C for 4-5 days. Seed coats were removed and the white kernel was crushed to a powder, using an electric grinder and sieved through a 500 mm pore-sized sieve. The same method was followed for seed coat and fruit-flesh.

Turbid water: Turbid water samples prepared by dissolving the clay soil in tap water (5g/20ml), was allowed to stand for 3-4 hours, and then filtered through a muslin cloth. A cream-coloured highly turbid water sample was obtained by this method.

Effect of mature and immature seeds of *Moringa* on turbid water: a Preliminary test to see the effect of *Moringa* seeds on clarification of turbid waters was carried out by addition of 100 mg of seeds powder to 10 ml of turbid water; it was then kept at room temperature and observed at regular intervals. A control was also carried out. Different amounts of seed pow-

der of mature *Moringa* seeds (i. e. 5mg, 10mg, 15mg, 25mg, 50mg, 75mg, 100mg, 125mg, 150mg) were put into boiling tubes containing 20 ml of turbid water and mixed well. Three replicates were used for each treatment. A control was also used without treating with seed powder. They were kept at room temperature for 2 h, and the turbidity was measured using turbidity meter.

Textile dyes: Samples of textile dye powder were obtained from A. Baur & Company, Colombo, and the samples were prepared by dissolving 60 mg of powder in 11 of tap water. The dyes used were Terasil Red R, Terasil Yellow 4G, Terasil Blue 3RL-02 and Terasil Navy GRL-C

Effect of mature seeds of *Moringa* on textile dyes: Different amounts of seed powder of mature *Moringa* seeds, (i. e. 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg and 200mg) were introduced into boiling tubes containing 20 ml of textile dye sample and mixed well. Three replicates were used for each treatment. A control was also used without treating with seed powder. these samples were kept at room temperature for 2 h. After centrifugation, the absorbance of supernatants were measured using a UV-spectrophotometer at 370 nm, which is λ max for red dye.

Effect of soluble and insoluble fractions of the mature seed extract of *Moringa*, on textile dyes: Aqueous seed extracts (25 to 200 mg) prepared as above were centrifuged to obtain soluble and insoluble fractions. They were added separately to Terasil Red R textile dye solutions. Absorbencies were read at 370 nm.

Effect of treatment with *Moringa* seeds on pH and conductivity:

Conductivity: The effect of temperature on conductivity of water, treated with *Moringa* seeds was investigated. 200 mg of seed powder was put into boiling tubes, each containing 20 ml of muddy water, textitle dye water and tap water, respectively. Untreated samples were used as controls. Three series of water samples were prepared, one of which was kept at room temperature. One series was kept at 0^0C (in ice box) and the other at 50^0C (in water bath). The conductivity of these samples was measured with two replicates for each measurement, at 0, 30, & 50^0C, after 11/2h of the treatment.

pH: 200 mg of seed powder was put into boiling tubes, each containing 20 ml of turbid water, textile dye water and tap water, respectively. Untreated samples were used as controls. All tubes were kept at room temperature. pH was measured after 1 1/2h.

Effect of mature seeds of *Moringa* on paper factory effluent: 100 mg of seed powder of mature seeds was added to 10 ml of paper factory effluent obtained from paper factory at Embilipitiya and it was kept at room temperature under observation. A control was also carried out.

Determination of protein content: Protein content of *Moringa* seed kernel was determined using total nitrogen content which was determined by Micro-kjeldhal method

Determination of the bacterial count of Beira lake water: Viable cell number was counted to estimate the number of living bacteria in a given sample. To obtain a colony count of bacteria, dilution plate technique was used. 1 ml of well-mixed water sample (Biera lake water) was placed in 9 ml of sterile distilled water. This first dilution tube was labelled as 10^{-1} (or 1/10). Further dilutions were prepared in the same way.

0.1 ml of each sample was withdrawn and contents transferred to a MacCartney bottle containing nutrient agar medium (15ml) at 45°C, mixed well and poured immediately into a sterile petri-dish. These plates were incubated at 30°C for 24 h. after which the number of colonies were counted.

Effect of mature seeds of *Moringa*, on the bacterial count of Beira lake water. 200 mg of the seed powder was mixed with 20 ml of Beira lake water and the mixture was allowed to settle for 1 1/2 hours at room temperature (30°C). Then, the series of dilution was made, as mentioned earlier, up to 10^{-3}. At the same time, another series of dilution was made, without treating with the seed powder, as a control. All the plates were poured in same way described earlier, microbial growth of the treated samples was compared with the untreated ones.

Effect of different amounts of mature seeds of *Moringa* on bacterial count of Beira lake water.

Series of samples were prepared as follows. 0.4g, 0.8g, 1.2g, 1.6g, 2.0g, 2.4g, and 3.0g of seed powder in 20 ml of Beira lake water, separately. These samples were kept under aseptic conditions at room temperature for

1 1/2 hours. A control was also used, without treating with seed powder. Bacterial inoculation was done as mentioned earlier. Agar plates were incubated at 30°C for 24 h. The microbial growth, in relation to the different amount of seeds, were compared with the control.

Examination of Beira Lake water for coliform group of bacteria.

Five boiling tubes of double strength MacConky broth (5ml each), and 10 test tubes of single strength MacConky broth (5ml each), each tube containing a Durham tube, were autoclaved and cooled to 45°C. 10ml, 1.0 ml and 0.1 ml of water samples were introduced into the 5 boiling tubes with 10ml MacConky broth, 5 test tubes with 5ml of MacConky and the other 5 test tubes with 5ml of MacConky. These tubes were incubated at 37° c for 24 h. The test tubes which show acid together with sufficient gas in the Durham tube to fill the concavity was considered to be presumptive positive. Eijkman test was carried out and the production of acid and gas in MacConky's broth at 44°C were taken to indicate the presence of *E. coli* type.

Preparation of crude aqueous extract of *Moringa* seeds: Appropriate amounts of powdered mature seeds of Moringa were dissolved in appropriate volume of sterile distilled water and kept under sterile condition at room temperature for 2 hours. These solutions were filtered through a membrane-filter apparatus, using Whatman 0.2 μm Nitro-cellulose filter paper.

Antibacterial activity of crude aqueous extract of mature seeds of *Moringa* against *E.coli* and *Proteus* sp.:

Using 3 g/20 ml (2 hrs) as original sample, dilutions were made up to 10^{-4}. 100 mg of Streptomycin was dissolved in 1 ml of sterile distilled water and used as positive control. The sterile distilled water was used as negative control. 10^4 cells/ml cultures were prepared and nutrient agar plates were inoculated with 0.1 ml of these cultures and well-spread using a sterile glass spreader. Watman No 1 sterile paper disks (width-6 ml) were saturated with each dilution of each culture, sterile distilled water and the streptomycin solution separately. These saturated paper disks were transferred on to the seeded nutrient agar plates. These plates were incubated at 37° c for 24 h. Antibacterial activity against *E.coli* and *Proteus* sp. was recorded by measuring the diameter of the inhibition zone and were used as positive and negative controls respectively. Antibacterial activity was tested against *E.coli* and *Proteus* sp. The same procedure was followed as men-

tioned earlier. Plates were incubated at 37°C for 24 h. and the relative effectiveness of each dilution of each culture was recorded by measuring the diameter of the inhibition zone.

Statistical analysis: Results were analysed statistically using Tukey's multiple comparison procedure.

3. Results

Effect of *Moringa* on turbid water: Neither seed coat nor fruit flesh of *Moringa* had any effect on turbidity even after 5 d. A slight precipitate was observed in the solution where seed coat was added whilst fruit flesh produced a large precipitate. When kernel of mature seeds (50g/20 ml) was added turbidity of the sample was clarified noticeably (Fig 1) within an hour. Considerable amount of precipitate was also observed to settle down. Immature seeds however took about 5 h for a decrease in turbidity comparable to that of mature seeds. In the case of immature seeds, comparatively large quantities of seed powder were used, as small amounts of immature seeds are not significantly effective on the turbidity of turbid water sample. A minimum dosage of 125 mg/20 ml of immature seeds was required to clarify turbid water in a similar period of time (i.e. 1 h) to that of mature seeds. Like in the case of mature seeds, any increase in dosage of immature seed had apparently no additional effect on clarification (Fig 2).

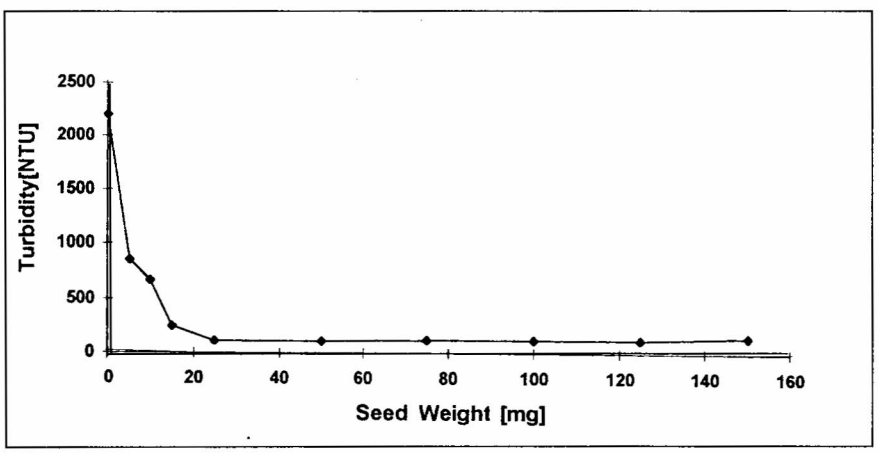

Fig.1. Effect of mature seeds of *Moringa* on turbidity of turbid water.

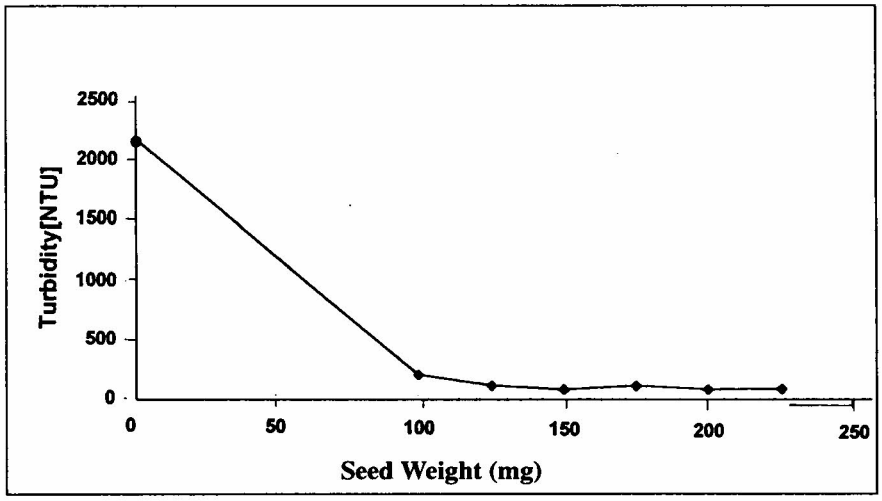

Fig. 2. Effect of immature seeds of *Moringa* on turbidity of turbid water.

When the aqueous phase and the residue obtained by centrifugation of seed extract, were added separately to turbid water, a gradual clarification of turbidity was observed with the aqueous phase up to 75 mg after which increasing amounts of seed had no noticeable effect on turbidity (Fig 3). In the case of residue, although there was a slight reduction in turbidity up to 75 mg, further increases did not have any effect on turbidity (Fig 3).

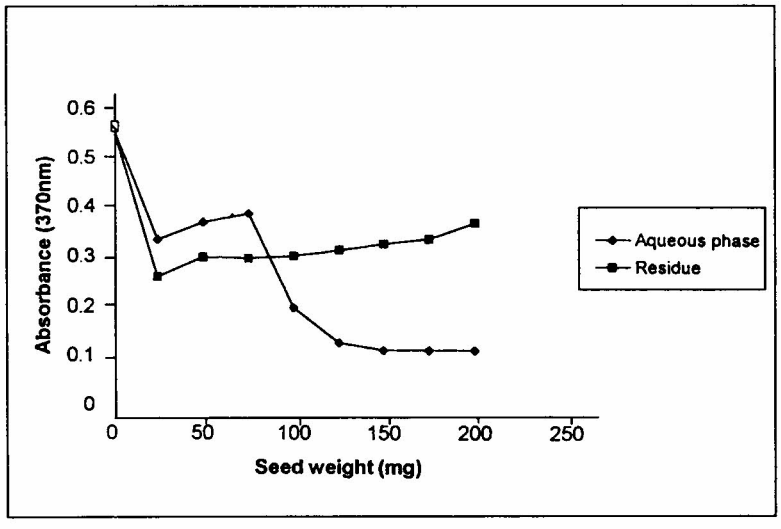

Fig. 3. Effect of aqueous phase and residue of seed extract on absorbance of textile dyes.

Effect of mature seeds of *Moringa* on paper factory effluent: No effect on colour of effluent, was observed even after 2 weeks of addition of *Moringa* seed powder to paper factory effluents.

Effect of *Moringa* seeds on textile dye solutions: In initial experiments, the solutions of all 4 textile dyes, namely Terasil Red dye, Terasil Yellow dye, Terasil Blue dye and Terasil Navy dye, were clarified by the addition of *Moringa* powder. 25 mg of *Moringa* seed powder brought about a sharp decrease in absorption of Terasil Red dye. (Fig 4). Addition of soluble fraction obtained by centrifugation of aqueous extracts of *Moringa* showed that it is the soluble fraction that is effective in clarification of textile dye solutions (Fig 5). Though there was some indication of a slight clarification initially up to about 75 mg of seed residue, increasing amounts had resulted in an increase in absorption instead of a decrease (Fig 6). Aqueous fraction on the other hand clarified the dye solution with increasing amounts resulting in a decrease in absorption. However, after addition of 150 mg, further increases in the amount of aqueous fraction did not produce any further reduction in absorption.

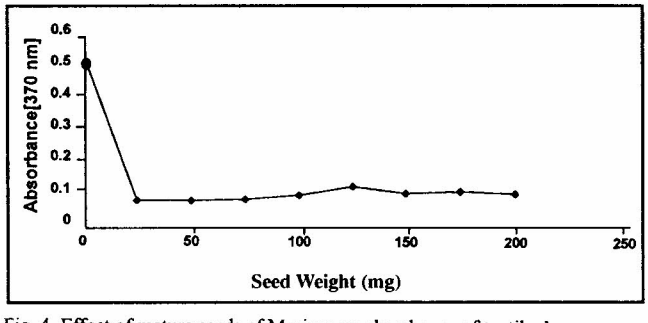

Fig. 4. Effect of mature seeds of Moringa on absorbance of textile dyes.

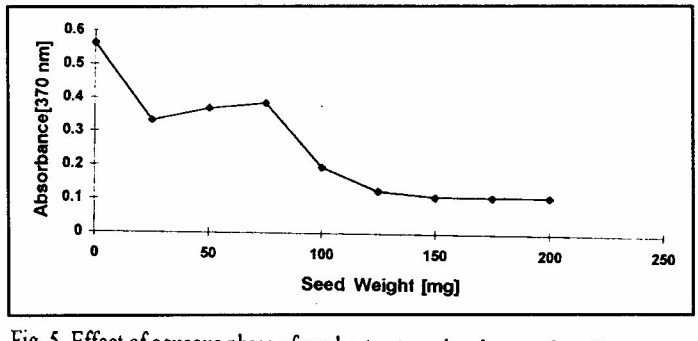

Fig. 5. Effect of aqueous phase of seed extract on absorbance of textile dyes.

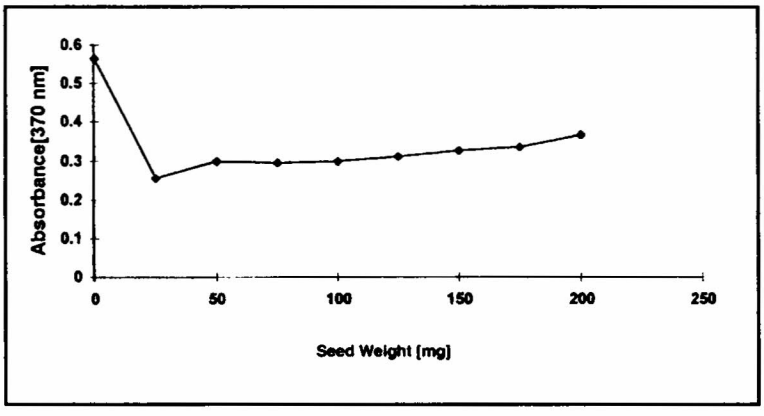

Fig. 6 Effect of residue of seed extact on absorbance of textile dyes.

Effect of treatment with *Moringa* seeds on pH and conductivity: Treatment of turbid water, textile dye solution and tap water with *Moringa* seeds did not result in a significant change in pH though there was a slight reduction in pH in al three cases (Table 1). Conductivity of *Moringa* seed treated turbid water and tap water was found to increase with time (Table 2). This increase was quite marked in textile dye solution. Conductivity of *Moringa* seed treated turbid water, textile dye solution and tap water decreased noticeably (i.e. to half of the value at room temperature) at 0°C whilst it almost doubled in all 3 treatments at 50°C (Table 3).

Table 1 Effect of treatment with *Moringa* seeds on pH of different water samples

Sample type	Untreated	Treated
Turbid water	5.95	5.70
Dye water	6.14	5.81
Tap water	6.88	6.52

Table 2 Effect of treatment of water with *Moringa* seeds on conductivity, for different periods of time

Sample type		Conductivity (µs)		
		After 1.5 h.	After 3 h	After 4.5 h
Turbid water	U	69	69	69
	T	435	468	582
Dye water	U	74	74	74
	T	473	503	643
Tap water	U	65	65	65
	T	442	487	586

* U = Untreted, T = Treated, µs = Micro Siemens

Table 3 Effect of treatment of water with *Moringa* seeds on conductivity, at different temperatures

Sample type		Conductivity (µs)		
		O^{0}C	30^{0}C (r.t)*	50^{0}C
Turbid water	U	35	69	125
	T	234	435	810
Dye water	U	40	74	130
	T	243	473	900
Tap water	U	32	65	122
	T	239	442	800

* r.t. = room temperature, U = untreated T = Treated

Protein content: Mature seeds of *M. oleifera* was found to have a higher protein content (42.5%, dry wt. basis) compared to immature seeds which had a protein content of 30.9% (Table 4).

Table 4 Protein content (%, dry wt basis) of mature and immature seeds of *Moringa*

Sample	Immature seed	Mature seed
I	30.19	41.56
II	31.13	43.44
III	31.38	42.50
Mean	30.90	42.50

Bacterial count of Beira Lake water: Colony forming units of bacteria were counted as bacterial count in water body (Table 5), which was 3.8 x 10^6/ml.

Table 5 Bacterial count of Beira Lake water

Dilution	Bacteria
Stock	****
10^{-1}	****
10^{-2}	****
10^{-3}	***
10^{-4}	**
10^{-5}	*

* 1 - 30 colonies per plate
** 31 - 100 colonies per plate
*** 101 - 300 colonies per plate
**** > 300 colonies per plate
Mean bacterial count (per 0.1ml) = 38 x 10^4
CFU (Colony Forming Units) = 38 x 10^5/ml

Effect of mature seeds of *Moringa* on the bacterial count of Beira Lake water: 0.01g/ml concentration of *Moringa* seeds reduced the bacterial growth in Beira lake water while bacteria in 10^{-3} dilution of Beira lake water were totally controlled by the tested concentration 0.01g/ml).

Effect of different amounts of mature seeds of *Moringa* on bacterial count of Beira lake water: Significant reduction in growth of bacteria was not observed up to 0.08g/ml concentration whereas 0.1g/ml onwards counts showed a marked reduction in growth of bacteria (Table 6). Further 0.15g/ml showed complete inhibition of bacterial growth.

Table 6 Antibacterial activity of crude aqueous extracts of *Moringa oleifera* seeds.

Amount of seeds (mg)	Bacteria
400	****
800	****
1200	****
1600	****
2000	**
2400	*
3000	-

* = 1-30 colonies per plate
** = 31-100 colonies per plate
**** = > 300 colonies per plate

Beira Lake water for coliform bacteria: The most probable number of presumptive coliform was calculated as 1800+/100ml (Table 7).

*** Eijkman test**

All tubes showed acid together with sufficient gas in all Durham tubes.

Table 7 Coliform bacterial count in Beira Lake water

Volume of water (ml)	10.0	1.0	0.1
Positive tubes	5	5	5

* The most probable Number] = 1800+/100 ml.
of presumptive coliform]

Antibacterial activity of crude aqueous extract of mature seeds of *Moringa* against *E.coli* and *Proteus* sp.: Diameter of the inhibition zone increased with increasing concentration of seed extract for *E.coli* as well as *Proteus* sp. (Table 8). Even the lowest concentration used in this study showed a notable inhibitory effect on growth of bacteria.

Table 8 Effect of different concentrations of *Moringa* seed extracts on growth of *E.coil* & *Proteus* sp.

Sample type	Diameter of inhibition zone (mean, mm)	
	E.coil	*Proteus* sp
10^{-1}	18.50	19.50
10^{-2}	8.50	9.50
10^{-3}	6.75	7.50
10^{-4}	6.00	7.00
(+) control	50.50	42.00
(-) control	0.00	0.00

4. Discussion

The results of this study show that, seed coat and fruit-flesh of *Moringa oleifera* do not possess any coagulation activity, it is only the seed kernel that has such property. Similar results have been reported by, Ndabigengesere, et al (1994), who carried out extensive studies on *Moringa*, including the coagulation activity of various parts of *M. oleifera.* When coagulation activity of mature and immature *Moringa* seeds was compared, it was observed that there was a statistically highly significant ($p<0.05$) decrease in the turbidity of muddy water, subjected to mature *Moringa* seeds. Turbid water, subjected to treatment with immature seeds caused phenolic discoloration, whilst reducing the turbidity significantly. In this study, use of the dosage of 250 mg/L of mature seeds, (5 mg/20 ml) showed a 61% decrease of the turbidity within 2 hours. The same dosage has been used by Fagnon (1995) when treating Nile water by Jahn, and 30-200 mg/1.

Protein content of mature and immature seeds was also studied and the results show that, mature seeds contain more protein (42.5%) than immature seeds. (30.9%). The results of this study indirectly supports the reports of previous workers, that, there is a positive relationship in between the protein content and the coagulation activity of the seeds of *M. oleifera* (Ndabigesere et al, 1994). A similar report comes from M. A. P. (Micro Active Protein) in Sweden, which has been granted a US patent for the company's unique biological method for the purification of water, aqueous solutions and gases, based on the addition of natural protein extracted from farmed common blue mussles. (Anon, 1997). This also removes harmful bacteria, viruses and moulds. The results obtained in the present study for the protein content of mature *Moringa* seeds was fairly similar to the findings of several previous workers. According to Ndabigesere, et al (1994), the protein content is 37% and according to Duke's Phytochemical and Ethnabotanical Database (Anon, 1999) it falls between 38-40%. With regard to the method used for protein determination, some authors (Dahot et al, 1997) have used the Lowry method to estimate the protein content in *Moringa* seeds. However, in the present study, it was found to be unsatisfactory due to phenolic discoloration which interferes with colour development, in the case of immature seeds. To overcome this, Kjeldahl method was used in the present investigation, for the estimation of protein content in *Moringa* seeds.

The effect of *Moringa* seeds on textile factory effluents containing textile dyes has not been investigated previously. It was observed that, there was a statistically significant ($p<0.05$) decrease in the absorbency of textile dyes, subjected to mature *Moringa* seeds, indicating a removal of the dye. A comparative study of the coagulation activity in seed extract and the precipitate was also carried out using a textile dye sample. The results of this study show that, small or dosages up to 75 mg, the residue phase is comparatively more effective than the aqueous phase. But higher dosages than 75mg of residue phase, showed a gradual increase in absorbency, while the absorbency of the sample treated with aqueous phase showed a rapid decrease. (Figs 4 & 5). According to the previous studies by Ndabigengesere et al (1994), the specific protein, responsible for the coagulation activity, is water soluble. The effectiveness of the aqueous phase of the seed extract may be due to the availability of more protein in the aqueous phase. It is possible that up to 75mg, the amount of protein in the residue is higher than that of the aqueous phase, making it more effective than the aqueous phase.

The study on the effect of *Moringa* seeds, on bacterial count of Beira lake water, shows that for complete removal of bacteria (about 3.8 x 10^6 ml), 0.15g/ml of mature *Moringa* seed powder is required. The results indicate that, the reduction of the bacterial growth may be due to the antibacterial activity of *Moringa* seeds. Daihot et al (1977) and Caceres et al (1991) too have reported of antimicrobial activity in *Moringa* seeds in a preliminary study. It was found that *E. coli* was constantly present in this water body even in the highest dilution of water tested in this investigation. Antibacterial activity of seed extract against *Proteus* sp., has not been investigated by any previous worker.

Assay of antibacterial activity of aqueous extract of mature seeds against coliform bacteria display *Moringa* seeds posses sufficient antibacterial activity against *E. coli and Proteus* sp.. The results for the test of the measuring of two parameters, pH & the conductivity of the treated and raw water samples show that, treatment of different types of water samples with *Moringa* seeds doesn't significantly affect the pH, but causes only a slight reduction in pH. This is in agreement with the results of Ndabigengesere et al (1994). But the conductivity of the samples is highly affected by *Moringa* seeds. The conductivity of a solution containing a solute, usually depends on three factors, namely, amount of the ions, charge of the ions and the velocity of the ions in the solution. In this test, the dosage used was 200 mg/20 ml and, such a high concentration may provide a high amount of ions in the solution, and lead to high conductivity. Change in conductivity with temperature can be attributed to changes in velocity of the ions in the solution, at low and high temperature. Low temperature decreases the velocity of ions in the solution and cause conductivity decreases, while the high temperature, increases the velocity of ions and cause conductivity increases. Time was also found to be important, longer the treatment period, higher the amount of ions freed into the aqueous phase. (i. e. water sample). This assumption is further proved by the results of untreated samples. However, according to Ndabigengesere, et al (1994), conductivity is not so affected by the addition of *Moringa* seeds, they have used a concentration of 5% by weight (crude powder/solvent), since higher concentrations were cumbersome to filter. None of the treatments used, was effective in reducing the colour of paper factory effluent.

5. Acknowledgements

Authors wish to thank Mr. P. Dias and Miss Dilini Kulawansa of Department of Mathematics, USJ for assistance in statistical analysis. We also wish to thank Mr. P. Padmasiri, Chief Chemist, National Water Supplies & Drainage Board, Ratmalana for his help in turbidity measurements. Authors are also grateful to Technical Staff of Department of Botany, USJ for their assistance in laboratory work.

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