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Treatment of Drinking Water Using *Moringa Oleifera* Lim.: An Application Of Bioremediation

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ABSTRACT

Sindh is that province of Pakistan that receives the downstream water of River Indus. Due to paucity of fresh groundwater the river water is used as a source of drinking water at the coastal villages of Sindh. As a matter of fact water of River Indus is turbid and it reaches to the villages via canal system that is not involving lined canals. With the result that the problem of turbidity further aggravates. In this study turbid water carried from these villages was treated by coagulation-flocculation techniques, with *Moringa oleifera* seeds as a coagulant and compared with that of the water treated with alum. Various dosages of the crude 5% water extract of dry, shelled and non-shelled *Moringa oleifera* seeds were used. Measurements of pH, conductivity and turbidity concentrations showed that coagulation with *Moringa oleifera* seeds works well as compared to coagulation done by alum and moreover, it did not significantly affect the quality of the treated water. However, concentration of organic matter in the treated water increased considerably with the dosage of *Moringa oleifera* solution which might exert a chlorine demand and also act as precursor of trihalomethanes during the disinfection with chlorine. In order to combat with that problem *Moringa oleifera* seeds were also tested for antimicrobial activity and we find significant zone of inhibition against pathogenic microorganisms like *E.coli* etc. This study concludes that *Moringa oleifera* seeds are a good source of drinking water treatment chemically as well as microbiologically.

Keywords: *Moringa oleifera*, Drinking water treatment, Bioremediation.

1. INTRODUCTION

Every year, billions of people lack availability of safe drinking water which eventually turns into a big reason of mortality especially among young age groups from fatal diseases like diarrhea [1]. The situation persists and it will continue to cause substantial loss of human live unless it is seriously dealt with at all levels. In the developing country drinking water treatment plants are expensive, the ability to pay for services is minimal and skills as well as technology are scarce. In order to alleviate the prevailing difficulties, approaches should focus on sustainable drinking water treatment systems that are low cost, robust and require minimal maintenance and operational skills. Locally available materials can be exploited towards achieving sustainable save potable water supply. Natural material like *Moringa oleifera* can minimize or avoid the concern and significantly reduce treatment cost if available locally. *Moringa oleifera* is a tropical plant belonging to the family *Moringaceae*. Up to now, fourteen different species have so far been identified, all of them possessing varying degree of coagulation activity [2]. *Moringa oleifera* is the most widespread species, which grows quickly at low altitudes in the whole tropical belt, including arid zones [3]. It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil. However, in Sudan it has been traditionally used in water purification [4]. These multiple uses of the *Moringa oleifera* plant have greatly promoted its widespread application.

Moringa oleifera seeds possess effective coagulation properties [5-6] and that they are non toxic to humans or animals and also biodegradable. They are quite efficient in reducing turbidity and microorganisms. The active component of the dried crushed seeds (powder) of *Moringa oleifera* is a soluble protein; a nature cationic polyelectrolyte that causes coagulation. The extract of its crude seeds is commonly used for water purification at household level [7]. Muyibi and Evison reported that *Moringa oleifera* could achieve turbidity removal between 92 to 99 % but its coagulation effectiveness also depends

on the initial turbidity levels in water [8]. It is very effective for high turbidity water and shows similar coagulation effects as alum. The use of *Moringa oleifera* as a primary coagulant is more appropriate for surface waters with excessive turbidity particularly during the rainy season.

The purpose of this study is to evaluate the effectiveness of *Moringa oleifera* seeds as an alternative natural material for drinking water treatment. A small volume of coagulation assay method was developed through a simple experiment of coagulation and antibacterial activity. The efficiency and properties of *Moringa oleifera* as a natural coagulant were studied and compared with alum, which is presently the most widely used industrial coagulant. It is environmental friendly, and unlike alum, does not significantly disturb the pH and conductivity of the water after the treatment. Additionally, sludge produced by coagulation with *Moringa oleifera* is not only innocuous but also four to five times less in volume than the chemical sludge produced by coagulation using alum. So, as a coagulant, *Moringa oleifera* may be a potentially viable substitute to alum.

2. MATERIAL AND METHODS

This study focuses to ascertain the efficiency of *Moringa oleifera* seeds as antimicrobial agent as well as coagulating agent.

2.1. Antimicrobial activity

2.1.1. Microbial strains

Pure culture of bacterial stains including *B.subtilis*, *E.coli*, *S.aureus* and *Pseudomonas* were obtained from Oxiod LTD Basingstoke, Hampshire, England.

2.1.2. General procedure

Strains of bacteria were grown on Nutrient agar media. Inoculums for each strain with $1x\ 10^5$ colony forming unit/ml were used and antimicrobial activity of seed extract were determine by disc diffusion method. The zone of inhibition was measured in millimeter using zone reader [9]. Microscopy was performed on Nikon (Japan) microscope with Nikon FDX-35 fitted camera.

2.1.3. Preparation of Antimicrobial stock solution

Take *Moringa oleifera* seed (powder) 10g and add it in 100ml ethanol (95% conc.). Then mix and leave for 10 to 15 minutes. Decant the supernatant and repeat same procedure two times then evaporate ethanol at 90 °C for 25 min with the help of hot plate in safety cabinet.

2.1.4. Antimicrobial assay

Whatman no. 42 filter paper was cut into disk shape having 6.0 mm diameter and then was autoclaved at 121^0C temperature and 15 psi pressure. Mean while six different concentrations of 100mg/ml, 200mg/ml 400mg/ml, 500mg/ml, 700mg/ml and 1000 mg/ml were prepared by diluting known volume of stock solution into known volume of distilled water then the autoclaved disks were soaked into these concentrations of *Moringa oleifera* seed extract and dried using dry heat oven for 2 hrs at 40 ^{o}C. At the end, each disk absorbed 0.01ml of *Moringa oleifera* seed extract. 250mg/ml tetracycline antibiotic was used as control. Followed, a lawn of each bacterial strain was developed by spreading pure culture (1,000,000 cfu/ml) on the surface of nutrient agar contained in Petri plate. For spreading the culture on the agar plate autoclaved cotton swabs were used, subsequently the dried disks were applied on the lawn and the plates were incubated at 37 ^{0}C for 24 hrs, the same procedure was carried out using tetracycline as a control and finally, the zone of inhibition found against each bacterial strain was compared (Table 1) to the zone of inhibition found using standard drug.

2.2. Coagulating activity

2.2.1. Preparation of stock solution

The raw material (shelled and non shelled seed) was ground to powder in a domestic blender. The powder was added in the appropriate proportion (5 %) into the solvent the whole mixture was stirred for 30 minutes. The mixture was then filtered firstly through Whatman no. 42 filter paper and then through a 0.45 micro meter nylon membrane. Water is used as solvent to extract the hydrophilic active agents of *Moringa* in the coagulation process.

2.2.2. Application of coagulant on water sample

Turbid water sample was collected from a canal at Shah Bandar and onsite pH, conductivity, dissolved oxygen and turbidity were estimated that reveal the following results before testing;

Table 1: Results of canal water sample before testing.

Parameter	Value
Turbidity (NTU)	100
pH	7.8
Conductivity (us/cm)	4.86
Dissolve oxygen (mg/L)	7.6

Different doses (2ml/L, 5ml/L, 10ml/L, 20ml/L and 30ml/L) of stock solution (Prepared by using both shelled and non-shelled seeds) and alum solution were tested on the sample of canal water and the results of shelled an non shelled seeds were compared with alum.

2.3. Statistical analysis

Paired t-test statistic was used to compare the efficiency of the three treatments. Significance of test statistic is evaluated using p-value less than 0.05. If the p-value is greater than 0.05 the test has significant difference in it. Minitab software was used to do the statistical analysis.

3. RESULT AND DISCUSSION

3.1. Antimicrobial activities of seed extract of *Moringa oleifera*

The results of antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* and *Vibrio* were presented in table 2 and fig 1-5. *Moringa oleifera* showed significant activity against *Bacillus subtilis,Staphylococcus aureus* and *Escherichia coli* whereas, no activity was found against *Pseudomonas* and *Vibrio*. The present study was conducted to obtain preliminary information on the antibacterial activity of *Moringa oleifera* seed powder. The antibacterial activity of the *Moringa oleifera* seed extract is affected predominantly some important enzymes and bacterial cell wall or membrane [10].

3.2. Coagulating activity

Moringa oleifera is an effective natural coagulant which can be used in water treatment in two principal crude forms; shelled or non-shelled dry seeds. The action of *Moringa oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds. In coagulation *Moringa* hardly have an effect on the pH and conductivity. While alum changes the pH and conductivity of water samples after treatment.

During the experiment, coagulant property was manifested by formation of visible flocs (non shelled seed) easily recognized by naked eye following flow agitation of the turbid water. It is observed (Fig 7 and Table 2) that shelled seeds extract works well than non shelled seeds if we compared with alum. Moreover, *Moringa oleifera* (Non-shelled) was more effective at higher doses than shelled seeds.

Moringa oleifera seeds did not affect signifcantly the pH value, which remained almost constant at 8 for dosages tested. In contrast, the pH value decreased from 8 to 4 for alum which means that in practical terms, further chemical addition is necessary in order to correct the pH of the finished water. *Moringa oleifera* seeds, both shelled and non-shelled, did not significantly change the conductivity of the treated water, which remained constant for all dosages tested. In contrast, the conductivity increased considerably with the increasing dosage of alum. This increase in conductivity is caused by sulphate ions remaining in the treated water. Furthermore, the volume of sludge produced in case of *Moringa* is considerably less than alum [11]. Finally, it is concluded that *Moringa oleifera* is a viable substitute of traditional drinking water treatment agents, as it contains both coagulation and antimicrobial activities.

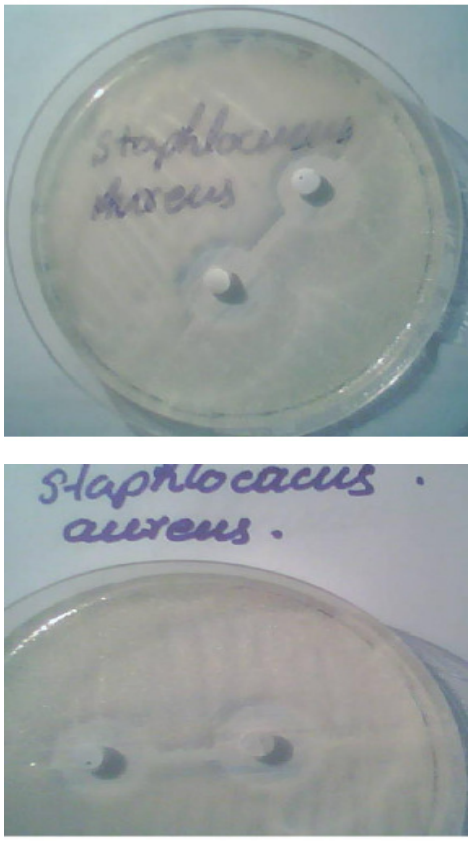

Figure -1: *Staphylococcus aureus* (ATCC) 25923

3.3. Statistical analysis

Figure 6 depict the results of paired t-test performed to evaluate the statistical significance between groups of treatments.

The entire three tests showed that there is significant difference between each test as the p-value is more than 0.05 in each test. The most significant (p-value = 0.925) difference is between shelled and alum treatment as shelled seed is a good coagulating agent as compared to alum.

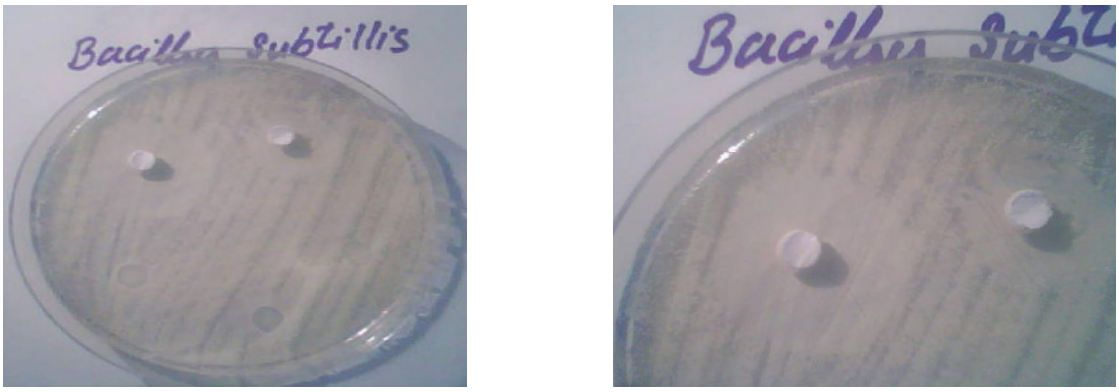

Figure - 2: ***Bacillus subtilis*** **(ATCC) 6633**

Figure - 3: ***Escherichia coli*** **(ATCC) 25922**

Figure - 4: ***Pseudomonas*** **(ATCC) 27853**

Figure - 5: V*ibrio*

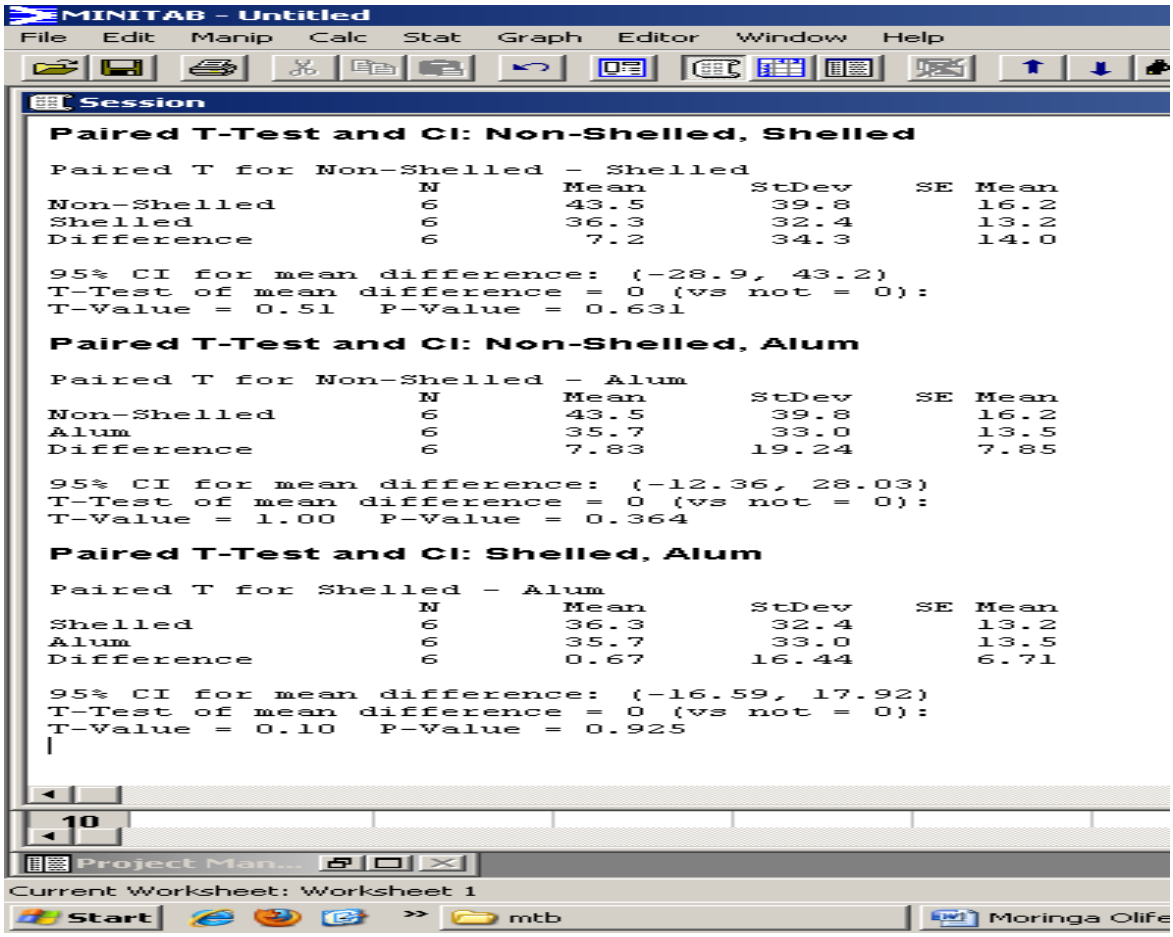

Figure - 6: Result of paired t-test statistic using Minitab software

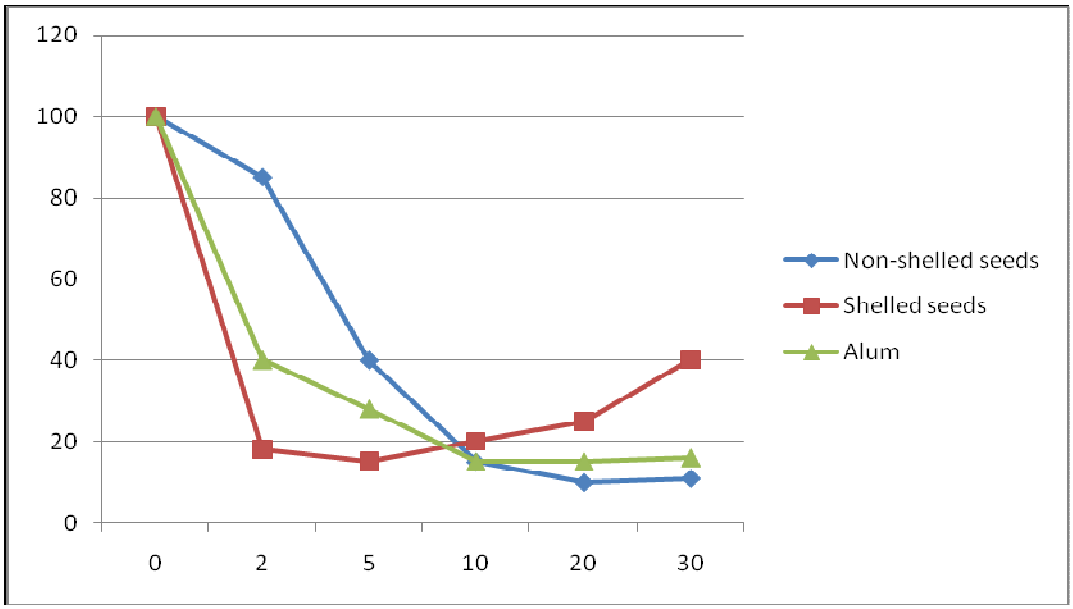

Figure - 7: Comparative graph showing residual turbidity levels using same doses of three different treatments.

Table - 2: Comparative table showing residual turbidity levels using same doses of three different treatments

Dosage (ml / L)	Residual Turbidity (Non-shelled seeds)	Residual Turbidity (Shelled seeds)	Residual Turbidity (Alum)
0.0	100	100	100
2.0	85	18	40
5.0	40	15	28
10.0	15	20	15
20.0	10	25	15
30.0	11	40	16

4. CONCLUSION

Finally, it is concluded that shelled seed has the highest coagulating tendency followed by alum and non-shelled seed. Moreover, seeds of *Moringa oleifera* also contain antimicrobial activity that aids in disinfection process of water treatment.

Those people how drink untreated water in any area of the country they are recommended to use *Moringa oleifera* seed for the treatment of drinking water.

Today, most pathogenic organisms are becoming resistant to antibiotics [12]. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Thus, *M. oleifera* could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

The only hurdle to the adoption of *Moringa* for water treatment seems to be the adequate supply of the seeds. As solution to this problem it is recommended that there will be intensive cultivation of *Moringa* tree in tropical countries, exactly like coffee or tea, two important cash crops grown and consumed all over the world.

5. REFERENCES

1. Postnote, Access to water in developing countries, 2002:178. **www.parliament.uk/post/home.htm**
2. Jahn SAA, Using Moringa seeds as coagulants in developing countries, **J. Am. Water Works Assoc**.1988;80:45-50.
3. Morton JF, The horseradish tree, Moringa pterygosperma (Moringaceae) – a boon to arid *lands*, **Economic Botany,** 1991; 45: 318–33.
4. Jahn SAA, Hamid D, Studies on Natural Coagulants in the Sudan with special reference to Moringa oleifera seeds, **Water S.A**., 1979; 5:90-7.
5. Dorea CC. Use of *Moringa spp.* seeds for coagulation: A review of a sustainable option. **Water Sci. Technol. Water Supply.** 2006; 6; 219-227.
6. Narasiah ks, Vogel A and Kramadhati NN. Coagulation of turbid waters using Moringa oleifera seeds from two distinct sources. **Water Sci. Technol. Water Supply.** 2002; 2:83-8.
7. Muyibi SA, and Evison LM. Optimizing Physical Parameters affecting coagulation of turbid water with Moringa oleifera seeds, **Wat. Res**., 1995; 29:2689-5.
8. Taley S. Moringa oleifera for Water Purification—a Traditional Boon for Rural Peoples. **Stockholm Water Symposium**, Workshop Poster. Stockholm, Sweden. 2007.
9. **NCCLS,** Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard. 2002: 19087-1898.
10. Theis T, Wedde M, Meyer V and Stahl U, Antifungal protein from Aspergillus giganteus causes membrane permeabilization, **Antimicrob. Agents. Therop**., 2003;47:588-93.
11. Ndabigengesere A, Narasiah KS and Talbot G. Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera.* **War. Res.** 1995; 29: 703-710.
12. Chandarana H, Baluja S, and Chanda SV. Comparison of antibacterialactivities of selected species of zingiberaceae family and some syntheticcompounds. **Turk. J. Biol.** 2005; 29:83-97.