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# BACTERIOLOGICAL AND PHYSCIO – CHEMICAL EVALUATION OF WATER TREATED WITH SEED POWDER OF *MORINGA OLEIFERA* LAM

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***ABSTRACT***
***Bacteriological and physico-chemical evaluation of water coagulated with Moringa oleifera seed powder preparations on Challawa river water intake station was conducted for a period of ten months (November, 2004 – August, 2005). Four graded doses of M. oleifera seed powder suspension (800mg/L, 880mg/L, 960mg/L and 1040mg/L) were employed for water treatment using standard methods. Results of bacteriological analysis on raw water samples using 9 – tube, 3 dilution technique, coliform estimations ranged between 150MPN/100ml and 1100MPN/100MPN and faecal coliform 86 MPN/100ml and 171 MPN/100ml, while M. oleifera treated water samples had counts ranging between 43MPN/100ml and 460 /100ml as well as 33MPN/100ml and 171 MPN/100ml for coliforms and faecal coliforms respectively. Aerobic mesophilic bacterial count on raw and M. oleifera treated water samples showed a range of 2.5 x $10^3$ cfu/ml to 9.3x$10^3$cfu/ml and 1.0x$10^1$cfu/ml to 9.3 x $10^2$ cfu/ml respectively. Escherichia coli, Proteus spp, Salmonella spp, Klebsiella spp and Shigella spp were detected in the raw water. Results of physico – chemical parameters of raw water samples from Challawa showed that turbidity ranged between 384NTU and 898 NTU, alkalinity 45mg/L and 70mg/L, pH 6.8, temperature 18°C and 25°C, sulphate ions 89mg/L and 195mg/L, nitrate ions 45mg/L and 162.5mg/L, phosphate ions 0.92 mg/L and 12.25mg/L Dissolved Oxygen (DO) 5.6mg/L and 38.4mgl, 5 – day Biological Oxygen Demand ($BOD_5$) 0.8mgl and 12mg/L. M. oleifera treated water samples had values for turbidity fluctuating between 14 NTU and 25NTU, alkalinity 30 mg/L and 43 mg/L, pH 7.0 and 7.2, temperature 20°C and 27°C, sulphate ions 19mg/L and 68mg/L, nitrate ions 24 mg/L and 68.0mg/L and phosphate ions 0.33 mg/L and 1.62mg/L. Statistical analysis of the results revealed significant difference between the varying seed powder doses in relation to bacterial recovery and most of the physico – chemical parameters at P<0.05. The implications of the results are discussed in relation to water sanitation and human health and some recommendations presented.***

***Keywords: Bacteriology, Challawa, Physico-chemistry, Seed, Water treatment***

## INTRODUCTION

About 1.3 billion people still lack safe drinking water and more than 6 million children die from diarrhea in developing countries every year (Lithierland, 1995). Only 61% of people in developing countries are estimated to have access to a potable water supply, greater in urban than rural areas, and 36% to sanitation, greater in urban than in rural areas (WHO, 1998). However, in many parts of the world river water which is used for drinking purposes can be highly turbid. The turbidity is conventionally removed by treating the water with expensive chemicals, many of which are imported at great cost and these are frequently unavailable. Large water treatment centres to the water purification by adding coagulants to the water such as aluminium sulphate (alum) (Sureshsundar and Sugirtharan, 2003).

The use of natural materials of plant origin to clarify turbid surface waters is not a new idea. However, of all the plant materials that have been investigated over the years, the seeds of *M. oleifera* have been shown to be one of the most effective primary coagulant for water treatment (Jahn, 1986; Folkard *et al.,* 1986). Madsen *et al.* (1987) carried out coagulation and bacterial reduction studies of turbid Nile water in the Sudan using *M. oleifera* seeds and observed turbidity reduction of 80 – 99.5% paralleled by a bacterial reduction of 1 – 4 log units (90 – 99.9%) within the first 1 – 2 hours of treatment. Sani (1990) carried out Jar test with *M. oleifera* as the primary coagulant using water from four different sources (viz two surface and two shallow wells) with turbidities from 100 – 300 NTU and 80 to 150 NTU respectively and hardness from 180 – 300 mg/L as $CaCO_3$. It was observed that in addition to turbidity reduction of 99 – 99%, the hardness was also reduced to between 60 – 70% after coagulation and two hours settling. Studies report that *Moringa* seeds are effective sorbets for removal of heavy metals and volatile organic compounds in the aqueous system (Akhtar *et al.,* 2006; Sharma *et al.,* 2006).

Ghebremichael *et al.* (2005) reported that the coagulant protein showed both flocculating and antibacterial effects of 1.1 – 4 log reduction. With samples of high turbidity, the *M. oleifera* extract was found to have similar coagulation activity as alum. According to Broin *et al.* (2002) seeds of the tropical tree, *M. oleifera* contain small storage proteins able to flocculate particles as well as Gram-positive and Gram-negative bacteria.

In view of the reported potentials of the seeds of *M. oleifera,* it is still pertinent to conduct further investigation on the coagulation and flocculation activity of plant using a polluted water source such as the Challawa River with the objective of assessing the potential of *M. oleifera* seed in reducing pollution from water bodies.

## MATERIALS AND METHODS

### Study Area and Sample Collection Sites

Challawa River is the second largest river system after Kano River in Kano State. It meets Kano River at Tamburawa, some 20km from Kano closed settled zone. It flows to the northeast where it finally joins Rive Hadejia. The main Kano water works is situated in the Challawa River. The river provides sources of water for agricultural, industrial and domestic activities in Kano metropolis and some surrounding neighboruing communities. Infact, one of the major industrial estates is located at Challawa, which largely depends on the water works for its water needs. Similarly, significant parts of the urban Kano population rely heavily on the water works for their domestic needs (Ibrahim, 2003).

Based on geomorphologic characteristics, the river falls within basement complex, which comprises rocks of pre – Cambrian era such as gneiss, schist and quartzite (Olofin, 1980).

### Collection and Identification of Plant Materials

Dried mature seeds of *M. oleifera* were collected from Municipal and Gaya Local Government Areas of Kano State, Nigeria. The identification was established at Herbarium of Biological Sciences, Bayero University, Kano with the aid of treatise or regional flora (Dutta, 1979) and by comparison with herbarium sheets of the authentic species.

### Water Collection and Analysis

The analysis was conducted fortnightly for a period of ten months (November, 2004 – August, 2005). The samples were collected and handled in accordance with the method of APHA (1998). Physico – chemical and bacteriological analyses of the water samples were carried out within one week of collection. However, sterile, de – ionized, distilled water was used for control treatments.

### Preparation of *M. oleifera* Seed Suspension

Good quality *M. oleifera* seeds were selected and their wings and coats removed and crushed in a mortar. Two grams (2g) of the seed powder was weighed and then mixed with 100ml of distilled water in a screw – capped glass bottle, shaken vigorously for 1 minute and allowed to stand for 10 minutes. This suspension was filtered through a piece of muslin cloth, and the filterate was used within 1 hour.

Turbidity removal was performed by adding 40ml, 44ml, 48ml and 52ml of the seed filterate extract per litre of water (given a final concentration of 800mg/L, 880mg/L 960mg/L and 1040mg/L respectively). This mixture was agitated vigorously with a spatula for 2 minutes, stirred slowly for an additional 5 minutes and then left to stand for an hour (Madsen *et al.,* 1987).

### Physico – chemical Analysis

The physico – chemical parameters of the raw and *M. oleifera* treated water monitored. These parameters comprise of turbidity, alkalinity pH, temperature, Dissolved Oxygen, $BOD_5$, sulphate, nitrate and phosphate ions. Temperature of the raw water samples was determined using a mercury – in – glass thermometer (Indian type) at the point of collection while turbidity was determined using turbidimeter (hachratio/XR). Sulphate, nitrate and phosphate ions were determined using portable spectrophotometer (DR/20 – 10 model). The same procedure was followed for water samples treated with graded doses of *M. oleifera* seed suspension (APHA, 1998).

### Bacteriological Analysis

Pour – plate technique was employed in determining the number of clonies formed (cfu/ml) on plate count agar (oxoid), while 9 – tube, 3 – dilution (multiple tube fermentation) technique was used for determining the Most Probable Number (MPN) counts of coliforms and faecal coliforms of the water samples. Confirmatory tests were then carried out on positive MPN tubes to confirm the presence of *E. coli.* Biochemical test on isolates were made for characterization including gram staining, citrate utilization, indole production, urease and other tests (APHA, 1998). Same procedure was maintained for *Moringa* treated water samples.

### Statistical Analysis

Two – by – two chi – squared ($X^2$) test and One Way Analysis of Variance (Cope, 1990) were employed to statistically determine whether significant difference exist between the varying seed concentrations among the test parameters.

**RESULTS**

Table 1 presents results of average count of physico chemical parameters of raw and *M. oleifera* treated water from Nov. 2004 to August, 2005. Turbidity of raw water ranged between 384 NTU and 898 NTU, while that of moringa treated water fluctuated between 15.9 NTU and 33NTU. Alkalinity ranged between 45mg/L and 70mg/L as in raw water, while that of treated water ranged between 30mg/L and 43mg/L. pH of raw water ranged between 6.8 and 7.6 while for *Moringa* treated water ranged between 7.0 and 7.2. Temperature ranged between 18$^{o}$C and 25$^{o}$C while for treated water ranged between 20$^{o}$C and 27$^{o}$C. Sulphate ions ranged between 89mg/L and 195mg/L while that of treated water ranged between 19mg/L and 68mg/L. For phosphate ions ranged between 0.92mg/L and 12.25mg/L while that of treated water ranged between 0.33mg/L and 1.62mg/L. For nitrate ions, the raw water ranged between 45mg/L sand 162.5mg/L while that of *Moringa* treated water ranged between 24mg/L and 68mg/L. For control samples using sterile deionized water, turbidity was found to be 3NTU, Alkalinity 25mg/L, pH = 6.0 and Temperature 25$^{o}$C.

Table 2 presents mean bacteriological count of raw and *M. oleifera* treated water. The bacterial estimation count ranged between 2.5 x $10^3$ cfu/ml and 9.3 x $10^3$ cfu/ml. While that of *Moringa* treated water ranged between 1.2 x $10^1$ cfu/ml and 3.9 x $10^2$cfu/ml. For coliform estimation, the raw water ranged between 150 MPN/100ml and 1100 MPN/100ml while that of treated water ranged between 90 MPN/100ml and 258 MPN/100ml. Faecal coliform estimation showed that the range for raw was between 86MPN/100ml and 171MPN/100ml, while that of *Moringa* treated water ranged between 26 MPN/100ml and 140 MPN/100ml. The control sample had bacterial values less than one colony forming unit per milliliter, while that of coliform and faecal coliform was 0 MPN/100ml.

Table 3 shows mean values of bacteriological and physicochemical parameters due to effect of varying doses of *M. oleifera* on treated water. Bacteriological parameters reduced with increasing dose of the seed powder. Temperature and pH of the treated water was constant, even as the seed dosage was increased from the lowest to the highest concentration. Values of nitrate and phosphate ions increased with increasing dosage of the seed powder.

**DISCUSSION**

In this study, the effect of *M. oleifera* seed suspension showed a lot of potential interms of water treatment especially with respect to bacteriological and physico – chemical quality. Turbidity reduction is one of the major noticeable features in this research work. Addition of 800mg/L seed concentration to turbid raw water in this study showed that all turbidity of raw water samples were drastically reduced. This observation further confirms the work of Buba and Chaudhuri (2005) who reported turbidity reduction of turbid surface waters from 15 – 25 to 0.3 – 1.5 NTU. Madsen *et al* (1987) reported turbidity of 80 – 99% for turbid Nile water. No much modification seen in pH with the addition of seed concentrate. The same observation was made with the work of Schwarz (2000) who reported that, a seed of *M. oleifera* used in water treatment in his research work did not modify the water pH. However, high counts of sulphate, phosphate and nitrate ions in the raw water shows increase level of activities in the area during sampling, which might be due to agricultural activities, washing, bathing, animals, grazing e.t.c. With initial addition of seed concentrate (800mg/L) all the turbid samples were drastically reduced interms of the above ions mentioned. The above result in Table 3: showed that parameters tested in relation to the test coagulant. This observation might be related to the fact that the seed solution acts as natural cationic polyelectrolyte during treatment and thus acted as coagulant.

With regards to bacteriological investigations, aerobic mesophilic bacteria, coliform and faecal coliform counts were drastically decreased with the addition of seed powder of *Moringa oleifera.* Results of average count of bacteria showed significant difference ($P<0.05$) that existed between seed concentration as they relate to recovery of bacteria. Tauscher (1994) revealed that the coagulant properties of the seed are due to a series of low molecular weight cationic proteins. The seed kernels of *M. oleifera* contain significant quantities of low molecular weight (water soluble) proteins which carry a positive change when the crushed seeds are added to raw water. The proteins produce positive changes acting as magnets and attracting the predominantly negatively charged particles (such as clay, silk, bacteria and other toxic particles in water). The flocculation process occurs when the proteins bind the negative change forming flocs through the aggregation of particles which are present in water. These flocs are easy to remove by settling or filteration. This could be responsible for the considerable reduction in bacterial concentration with the addition of seed suspension. Presence of *Salmonella* spp, *Shigella sp, Klebsiella sp, Proteus sp* and *E. coli* showed the extent of contamination of the raw water. However, detection of *E. coli* which is one of the indicator organisms in all the water samples is an indication of faecal contamination especially with *E, coli* type I. As observed bacterial reduction in this study could also be related to the use of *M. oleifera* seed powder as primary coagulant for water treatment. Eilert *et al.* (1980); Eirlert *et al.* (1981) reported the presence of antibacterial agent, 4 – (alpha – L – rhamnosyloxy) benzyl isothiocynate, a glycosidic mustard oil. This antibacterial agent could be responsible for the elimination of some of the bacteria found in the raw water for this study.

**Table 1: Physicochemical characteristics of Raw and *M. oleifera* treated water samples**

| | Turbidity (NTU) | | Alkalinity (mg/L) | | pH | | Temp. ($^{O}$C) | | $SO_4^{2-}$ (mg/L) | | $PO_4^{-}$(mg/L) | | $NO_3^{-}$ (mg/L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Months** | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** |
| Nov. | 421 | 14 | 70 | 41 | 7.4 | 7.2 | 23 | 20 | 89 | 68 | 8.5 | 0.4 | 80.0 | 60 |
| Dec. | 392 | 16 | 70 | 43 | 7.0 | 7.2 | 25 | 26 | 96 | 62 | 2.4 | 0.9 | 45.5 | 30 |
| Jan. | 384 | 15.9 | 5043 | 7.2 | 7.2 | 7.2 | 23 | 24 | 107 | 22 | 1.5 | 1.3 | 70.0 | 26 |
| Feb. | 474 | 22 | 55 | 30 | 7.6 | 7.0 | 20 | 23 | 118 | 21 | 3.5 | 1.3 | 75.0 | 25 |
| Mar. | 394 | 19 | 45 | 35 | 7.2 | 7.2 | 22 | 27 | 125 | 23 | 1.5 | 0.9 | 50.0 | 27 |
| April | 425 | 25 | 50 | 33 | 7.2 | 7.2 | 21 | 23 | 160 | 40 | 0.92 | 0.33 | 45.0 | 24 |
| May | 520 | 21 | 65 | 43 | 6.8 | 7.0 | 20 | 23 | 175 | 22 | 10.3 | 1.62 | 150 | 68 |
| Jun | 735 | 22 | 60 | 34 | 7.2 | 7.0 | 18 | 22 | 180 | 24 | 9.3 | 0.8 | 155 | 55 |
| July | 880 | 21 | 50 | 43 | 7.2 | 7.0 | 20 | 25 | 195 | 29 | 12.25 | 1.9 | 162.5 | 61 |
| Control (sterile deionized $H_2O$) | 3.0 | | 25 | | 6.0 | | 25 | | - | | - | | - | |

**Table 2: Bacteriological characteristics of Raw and *M. oleifera* Treated Water samples**

| | Bacterial count (cfu/ml) | | Coliform count (MPN/100ml) | | Faecal coliform count (MPN/100ml) | |
|---|---|---|---|---|---|---|
| | **RW** | **TW** | **RW** | **TW** | **RW** | **TW** |
| November 2004 | $6.1x10^3$ | $1.2x10^1$ | 460 | 90 | 171 | 26 |
| December 2004 | $3.7x10^3$ | $3.1x10^1$ | 210+ | 132 | 152 | 67 |
| January 2005 | $8.2x10^3$ | $4.6x10^1$ | 1100 | 126 | 171 | 93 |
| February 2005 | $7.8x10^3$ | $2.7x10^1$ | 460 | 173 | 171 | 110 |
| March 2005 | $2.6x10^3$ | $8.8x10^1$ | 150 | 120 | 152 | 84 |
| April 2005 | $2.5x10^3$ | $2.8x10^1$ | 150 | 117 | 86 | 63 |
| May 2005 | $8.4x10^3$ | $2.7x10^2$ | 1100 | 258 | 171 | 140 |
| Jun 2005 | $9.3x10^3$ | $6.2x10^1$ | 1100 | 132 | 171 | 63 |
| July 2005 | $6.5x10^3$ | $3.9x10^2$ | 460 | 166 | 171 | 110 |
| August 2005 | $6.1x10^3$ | $6.6x10^1$ | 210+ | 183 | 752 | 110 |
| Control (Sterile deionized $H_2O$) | $<1.0x10^1$ | | 0 | | 0 | |

**Table 3: Mean bacteriological and physicochemical values of treated water sample due to varying doses of *M. oleifera***

| Seed dosage (mg/L) | Bacterial count (cfu/ml) | Coliform count (MPN/100ml) | Faecal coliform (MPN/100ml) | Turbidity (NTU) | Alkalinity | pH | Temp. ($^{O}$C) | $SO_4^{2-}$ (mg/L) | $NO_3^{-}$ (mg/L) | $PO_4^{-}$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 800 | $5.52 \times 10^1$ | 232 | 134 | 14.4 | 39 | 7.1 | 24 | 38 | 27.0 | 0.7 |
| 880 | $4.28 \times 10^1$ | 168 | 82 | 20.6 | 36 | 7.1 | 24 | 31 | 41.1 | 0.8 |
| 960 | $4.53 \times 10^1$ | 86 | 84 | 21.7 | 35 | 7.1 | 24 | 30 | 38.8 | 1.1 |
| 1040 | $2.45 \times 10^1$ | 73 | 53 | 22.3 | 42 | 7.1 | 24 | 28 | 36.3 | 1.5 |

## CONCLUSION

*Moringa oleifera* seed have been shown to be a potential substitute for chemicals used in water treatment. In this study, turbidity reduction was one of the best out – put where the level of suspended particles including bacteria (flocculates) was drastically reduced. Consequently, this method might reduce water borne diseases.

## REFERENCES


Akhtar, M., Hassany, S.M., Bhanger, M.I., Iqbal, S. (2006): Absorption potential of *M. oleifera* pods for the removal of organic pollutants from aqueous solutions. *Journal of Hazardous Materials.* In print.

American Public Health Association (APHA) (1998): *Standard methods for the examination of water and waste water*, 20th edition. American water works assoc. and water environ. Federation, U.S.A Ports 1000 – 10,000 10145pp.

Broin, M., Santaella, C., Cuine, S. and Kokou, K. (2002): Flocculent activity of a recombinant protein from *M. oleifera* Lam seeds. *Applied Microbiology and Biotechnology,* 60(1-2):114 – 119

Buba, R and Chaudhuri, M. (2005): Home Water Treatment by Direct Filteration with Natural Coagulant *J. water Health.* 2005 Mar, 3 (I): 27 – 30.

Cope, G. (1990): Graph Pad Instat Version 1.15 University of Shelfield, U.K.

Dutta, A.C (1979): *Botany for Degree Students*. 5th edition Oxford University Press Publishers, UK. Pp 1 – 909.

Eilert, U, Wolters, B and Nahrstedt, A. (1980): Antibiotic Principle of Seeds of *Moringa oleifera Planta Medica* 39 (3): 235.

Eilert, U., Wolters, B. Nahrtedt, A. (1981): The Antibiotic Principle of seeds of *Moringa oleifera* and *M. stenopotala. Planta Medica* 42 (1): 55 – 61.

Folkard, G.K, Sutherland, J.P and Jahn, S.A.A (1986): Water clarification with Natural coagulants and dissolved air flotation. *Water – lines* 5 (2), pp 23 – 26.

Ghebremicheal, K.A., Gunaratna, K.R., Henriksson, H., Brumer, H. and Dalhammar, G. (2005): Jun: A simple purification and activity assay of the coagulant protein from *M. oleifera* seed. *Water Res.* 39(11):2338 – 44.

Ibrahim, S. (2003): Survey of some aspects of biodiversity and physicochemical characteristics of Challawa river water, Kano Nigeria. M.Sc thesis (unpublished. Department of Biological, Bayero University, Kano. Pp 72.

Jahn, S.A. (1986): Proper use of African Natural Coagulations for Rural water suppliers. *Manual* No. 191, Pub: GTZ Eschborn, Germany.

Litherland, S. (1995): Large scale water purification article: Trees for life. Inter Press Service, Washington DC.

Madsen, M. Schlundt, J. and El – fadil, E. Omer (1987): Effect of water coagulation by seeds of *M. oleifera* on bacterial concentrations. *J. Trop. Med. Hygiene* 90, 101 – 109.

Olofin, E.A (1980): Some aspects of Tiga Dam on the environment downstream in the Kano River basin. Ahmadu Bello University, Zaria, Nigeria Ph.D thesis (unpublished).

Sani, M.A. (1990): The use of 'Zogale' seeds for water treatment. B. Eng. Final year Report. Department of Civil Engineering, Bayero University, Kano, Nigeria.

Schwarz, D. (2000): Water Clarification using *Moringa oleifera.* Gate information service. Tech information W/gtz. http://www.gtz.del/gate/gateid.afd.

Sharma, P., Kumari, P., Srivastava, M.M. and Srivastava, S. (2006): Removal of cadmium from aqueous system by shelled *M. oleifera* Lam. seed powder. *Bioresource Technology.* 97:299 – 305.

Sureshsundar, S. and Sugirtharan, M. (2003): New choice in water purification; moringa seeds. http://www.dailynews.IK/2003.02/10/fea02.html.

Tauscher, B. (1994): Water Treatment of Flocculant Compounds of higher plants. *Plant research and Dev.*, 40:56 – 70.

WHO 1998, *World Health Report – Guidelines for Drinking water quality*: vol. III surveillance and control of community supplies, 2nd edition. WHO Geneva 1998.