ASSESSMENT OF THE EFFECT OF WEST AFRICAN EBONY (*DIOSPYROS MESPILIFORMIS)* SEED EXTRACTS ON SELECTED BACTERIAS OF UPPER RIVER BENUE SURFACE WATER

ABSTRACT

This study was conducted to investigate the effects of *Diospyros mespiliformis*; crude seed, mucilage and seed extracts on some surface water quality parameters and total bacteria count. The experiment was design as a factorial experiment in Complete Randomized Design (CRD). Sample water was collected from Upper River Benue in Jimeta Bridge. *Diospyros mespiliformis* purchased at Jimeta Market, Yola Adamawa State. The water sample was collected in Jerry cans (50 litters) transported to wet laboratory, Department of Fisheries, School Agriculture, Modibbo Adama University of Technology Yola where the following physico-chemical parameters were analyzed Electrical Conductivity, Total Dissolve Solid, Ionic Concentration, Temperature, Biological Oxygen Demand and Turbidity. However, Total Bacterial Count was analyzed from microbiology laboratory of same University. Data obtained were analyzed using Analysis of Variance (ANOVA). Result obtained shows the effect of *Diospyros mespiliformis* treated surface water on the Physicochemical Parameters and effect of *Diospyros mespiliformis* Crude Seed, Mucilage and Seed Extracts on Total Bacteria Count. The following bacteria *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* were used during the studies with *E. coli* has highest total bacteria count of 233 and 22 recorded lowest in seed extract, *B. subtilis* recorded it highest of 164 and 42 reveal as lowest in the seed mucilage while S. aureus has highest of 100 with it lowest of 32 recorded in mucilage. Further study on the efficacy of *Diospyros mespiliformis* crude seed, mucilage and seed extracts on contaminant water treatment should be done.

Key Words: *West African Ebony, Seed Extracts, Upper River Benue and Surface Water*

1. INTRODUCTION

The quality of any water system reflects the inter-play of abiotic conditions existing in such environments [1]. Despite the improvement in water treatment processes, it is untenable and unbelievable under all situations that waterborne diseases still kill on the average of 22,000 people every day in developing countries while millions suffer the debilitating effects of these diseases [2]. Children bear the greatest health burden associated with unsafe water supplies through preventable diseases like diarrhoea. In developing countries about 2 million people die every year due to diarrhoeal disease; most are children of less than 5 years of age [3]. Other water related diseases reported in Nigeria are trachoma, schistosomiasis, ascariasis, trichuriasis,

ancylostomiasis (hookworm), malaria and encephalitis [4]. Therefore, water from all sources must have some form of purification before consumption because portable water should be free from contaminants. Water quality analysis is also important to protect the natural ecosystem [5]. *Diospyros mespiliformis* (kanya) Hochst (Ebenaceae) is used in ethnomedical practice against malaria in northern Nigeria [6]. This research work was limited to physico-chemical parameter and the sensitivity test of *Diospyros mespiliformis* on surface water treatment. There is evidence that the use of extracts from plant species in water treatment may possess both coagulating and antimicrobial properties. Naturally occurring coagulants are usually presumed safe for human health. Also, Production of biocompost/ biofertilizer from sludge produced after treatment with natural coagulants may be harmful. There are increasing human and agricultural activities in Upper River Benue which may be responsible for polluting the aquatic environment and affect the fishery resources the information that was obtained from this study served for ecology, conservation, sustainability and management of fisheries resources of water body.

2. Materials and Methods

2.1 Experimental Location (Sample Area)

The water sample was collected from Upper Benue River Adamawa Yola. Adamawa State is located within the climate of Northern Guinea Savannah zone and lies between latitude 8^0 and 11^0N and longitude 11.5^0 and 13^0E and climate is tropical with two distinct seasons; the experiment was conducted at wet laboratory in the Department of Fisheries Modibbo Adama University of Technology Yola.

2.2 Experimental Design

The experiment was design as a factorial experiment in Complete Randomized Design (CRD). The crude seed, mucilage and seed extracts were randomly assigned to three (3) treatments with one control and was replicated three times each to determine the effectiveness of the samples. Treatment one is the control containing 0g concentration, treatment 2, 3 and 4 constitute 5g, 10g and 15g respectively. Twenty eight (7L rubber bucket) was used for the experimental set up. The maximum experimental duration was twelve weeks in every week a liter of water samples from each replication was taken to the laboratory for analysis.

2.3 Experimental Process

The general methodology that was employed during the course of this research the seed was dehusk, dry then subjected to extraction. The extracts were used in the analysis as presented in the figure below.

Figure 1: Flow chart of water treatment method for *Diospyros mespiliformis* seed. Adapted from [7].

2.4 Extraction of *Diospyros mespiliformis* Mucilage

The samples was separately extracted using methanol via soxhlet extraction method. 50g each of the samples was weighed and transferred into 250 ml soxhlet extraction chamber. About 150 ml of methanol was added to the boiling flask. The setup was mount over a heating mantle and then coupled with condenser, to which cold water was allowed to circulate. The extraction lasted for about 3hours at the boiling point of methanol, and the extract was recover through distillation in rotary evaporator machine.

The mucilage was then scoop from the ethanol phase. The recovered mucilage was wash with ethanol. After approximately 10 minutes, the ethanol was decant and the mucilage was spread on sterile Petri dishes and dried in an oven at 50^0C. Once brittle, the mucilage was grounded with a mortar and pestle which was stored in a seal container at room temperature.

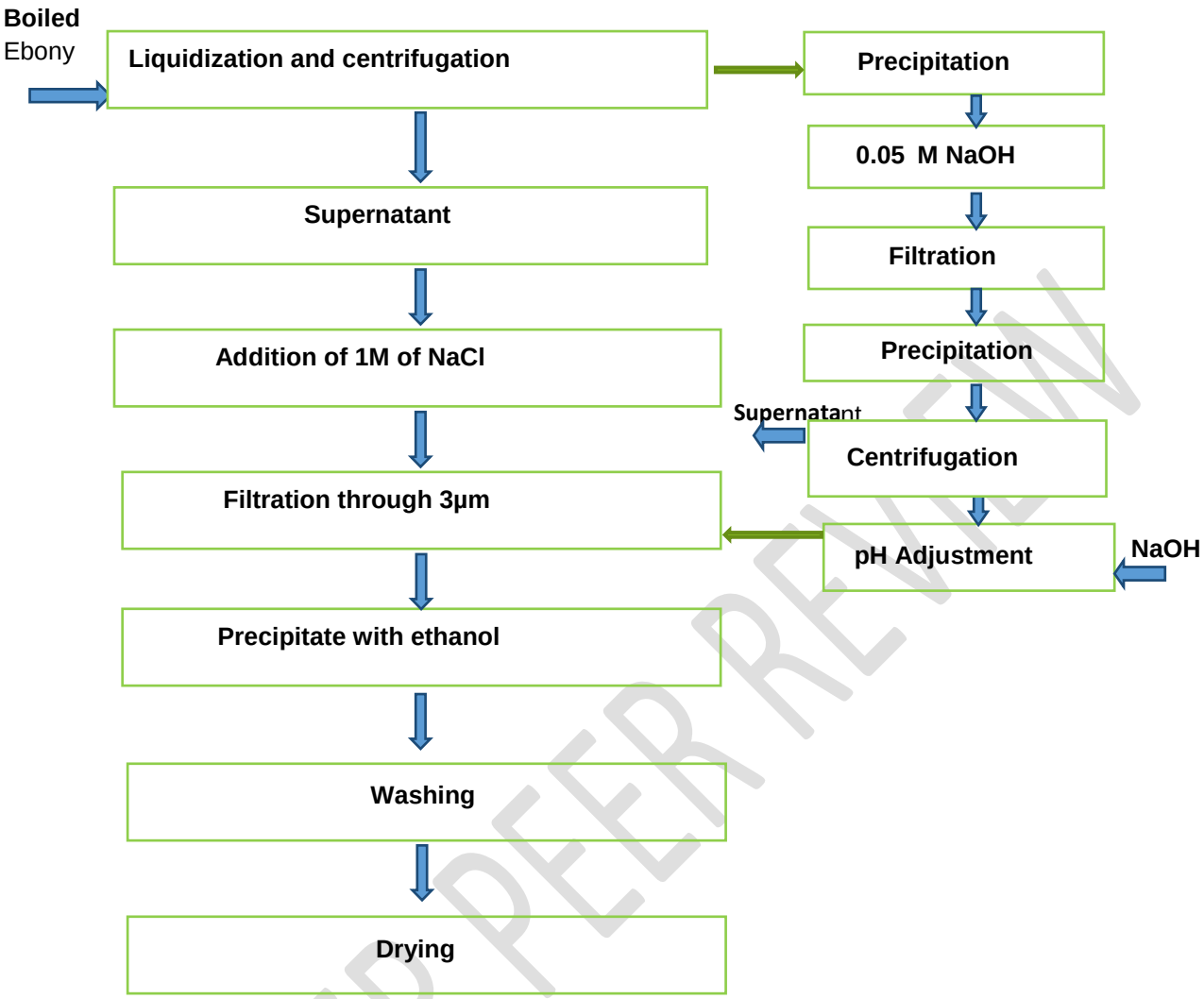

Figure 2: Process of block diagram for the preparation of *Diospyros mespiliformis* mucilage. Adapted from [8].

To isolate the precipitate, which has been previously classified as pectin, the solids retained from the centrifugation step were transferred to a beaker and covered with 0.05M NaOH solution containing 75% sodium metaphosphate. This solution acted as calcium sequestering agent use to promote the release of the mucilage pectin from the cell walls of the *Diospyros*, as was previously outlined by [9]. After 30 minutes of stirring, the pH reduce to 2.0 with HCL, and the suspension was centrifuged at 125 rpm for 10 minutes. Upon centrifugation, the supernatant was discarding and the precipitate was cover with deionized water and the pH may increase to 8.0 with 1.0 M NaOH. Filters was created from cloth sheets with large pore sizes and the *Diospyros* suspension was filtered to remove large solids from the mucilage solution. The filtrate was collected and the solids discard. Ethanol was added to the filtrate (1:1 v/v) to isolate the mucilage from the water. Upon initial

contact, mucilage begins moving to the ethanol phase. The mucilage was left to precipitate. The mucilage was washing in ethanol and dried on sterile Petri dishes in an ovenat 500C. The mucilage was then be ground with a mortar and pestle and stored in sealed containers on the bench top at room temperature.

2.5 Physico-chemical Parameters

The following methods were adapted

2.5.1 pH Measurement

The pH of the sample was measure using a calibrated Jenway 3505 pH meter. A volume of 200 mL of the supernatants obtain from the rubber bucket containing the treatments was measure. The pH meter probe was then be insert making sure it did not touch the rubber bucket. The pH reading was then be taken from the LCD display after it had stabilized.

2.5.2 Conductivity Measurement

The samples use for the pH measurements was used for the conductivity test. A calibrated Jenway 4510 Conductivity meter was use. The conductivity meter probe was then be insert making sure it did not touch the rubber bucket. The reading was recorded from the LCD display after it had stabilized.

2.5.3 Total Dissolved Solid Measurement

A calibrated Jenway 4510 total dissolved solid meter was use. The total dissolved solid meter probe was then be insert making sure it did not touch the rubber bucket. The reading was recorded from the LCD display after it had stabilized.

2.5.4 Determination of Biological Oxygen Demand (BOD)

Preparation of the seed dilution water was made and the dilution was calculated as required. For instance, raw water estimated BOD was 400mg/L and the dilutions may be 1/100 and 1/200. The final effluent- estimated BOD was 20 mg/L and the dilutions may be 1/5 and 1/2. The sample was diluted with seeded dilution water in 2 liters volumetric flasks. For instance, 1/100 dilution 20 ml sample in 2 lit. The diluted sample was transferred to a number of BOD bottles (4 bottles for each dilution). The bottles was incubated for 5 days (120 hours) at 200C. The treated surface water dilution was prepared and shared into four bottles and then incubated for 3-5 days at 200C. The residual dissolved oxygen was determined after incubation period.

2.5.5 Turbidity Measurement

The turbidity measurement of the sample water was done using a HACH 2100P direct reading potable turbidimeter. It is a multipurpose turbidimeter that was configured to read turbidity at the wavelength of 750 nm specified for measuring turbidity. Distilled water was first poured into a 10 ml sample cell and inserted into the turbidimeter. The calibration button was pressed and the instrument was then calibrated. Initial calibration of the 2100P turbidimeter was based on formazin, the primary standard for turbidity [10]. Each of the samples to be read was poured into a 10 ml sample and inserted into the turbidimeter. The turbidity of the samples were displayed on the LCD panel of the instrument in Nephelometric Turbidity Units (NTU). After each reading, the spectrophotometer was calibrated again with the distilled water before being used on the next sample the reading was taken for a period of four weeks.

2.6 Microbial Analysis

Microbial analysis were carried based on the procedure described by [11].

2.6.1 Total Viable Counts (TVC)

Using serial dilution (10-3) technique to identify bacterial count. 1ml from each treatments was pipeted and poured into the media containing about 20 ml of melted nutrient agar after solidification in each set of the petri dish, the plates were inverted and incubated at 370C for 24hours. Colonies was count by making the colonies on the opposite side of the plates on its position in the colonies counter apparatus.

The average count per plate = $\frac{n+n+n}{3}$ = average colonies

Equation:

$$c = \frac{n}{vd}$$

2.7 Statistical Analysis

The experiment were conducted in triplicate to ensure reproducibility of results. The final results was the average of the three. Data obtained was analyzed using Analysis of Variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Effect of *Diospyros mespiliformis* treated surface water on the physicochemical parameters.

Table 1: Promulgate the result of *Diospyros mespiliformis* (West African Ebony) Crude Seed, Mucilage and Seed Extracts on physicochemical parameters of the treated surface water.

Physicochemical parameters; the Electrical conductivity of *D. mespiliformis* mucilge recorded the highest mean value of 291 µS with the control has the lowest of 111 µS, the Total Dissolve Solid recorded; 137 highest in *D. mespiliformis* mucilage and 51 lowest in it control, pH, Temperature and BOD, 7.51 in seed extract with it lowest 6.42 in crude seed, 28.12^{o}C highest in crude seed, 26.45^{o}C lowest in *D. mespiliformis* mucilage, 4.12 highest in seed extracts, 2.98 lowest recorded in *D. mespiliformis* mucilage respectively. It is imperative that *Diospyros* extracts has significant effects on the above water parameters.

3.2 Effect of *Diospyros mespiliformis* (West African Ebony) crude seed, mucilage and seed extracts on total bacteria count.

Table 2: Confided the total viable count (cfu/ml), *E.coli* with the highest colonies forming unit of 233 (cfu/ml) as control, 22.16 (cfu/ml) in *D. mespiliformis* (West African Ebony) seed extracts, while *B. subtilis* and *Staphylococcus aureus* has 164 (cfu/ml) highest, 42.16 (cfu/ml) lowest in *D. mespiliformis* mucilage and 100 (cfu/ml) for control, 32.66 (cfu/ml) in *D. mespiliformis* respectively. There was tremendous reduction in the amount of total bacteria count as shown in the table.

Table 1: Physicochemical parameters of *Diospyros mespiliformis* treated surface water

Parameters	Crude seed (T1)				Mucilage (T2)				Seed extracts (T3)				LSD
	T1x	T1a	T1b	T1c	T1X	T1a	T1b	T1c	T1X	T1a	T1b	T1c	
	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	
EC	111^{f}	151^{e}	157^{de}	197^{c}	111^{f}	174^{cd}	236^{b}	291^{a}	111^{f}	162^{de}	185^{c}	256^{b}	23.19^{***}
TDS	51^{g}	71^{ef}	70^{f}	92^{cd}	51^{g}	82^{de}	111^{b}	137^{a}	51^{g}	75^{ef}	98^{c}	120^{b}	11.92^{***}
pH	7.01^{c}	7.01^{c}	6.42^{f}	6.60^{e}	7.01^{c}	6.76^{d}	6.97^{c}	6.90^{cd}	7.01^{c}	7.31^{b}	7.41^{a}	7.51^{a}	0.11^{***}
Temp.	27.14^{a}	28.12^{b}	26.45^{c}	26.48^{c}	27.14^{a}	26.45^{c}	26.49^{c}	26.50^{c}	27.14^{a}	26.51^{c}	26.49^{c}	26.48^{c}	0.15^{***}
BOD	3.54^{ab}	3.42^{ab}	3.24^{ab}	3.04^{ab}	3.54^{ab}	2.98^{b}	3.17^{ab}	3.24^{ab}	3.54^{ab}	3.22^{ab}	4.12^{a}	3.57^{ab}	1.11***
Turb.	61^{a}	42^{b}	41^{bc}	45^{b}	61^{a}	29^{ef}	32^{de}	31^{e}	61^{a}	25^{f}	28^{ef}	37^{cd}	5.00^{***}

Means in same row with different superscripts are significantly different at (P = .05).

Key: T1x = Control, T1a = 5 mg L^{-1}, T1b = 10 mg L^{-1} and T1c = 15 mg L^{-}

Table 2: Effect of *Diospyros mespiliformis* treated surface water on total bacteria count

Bacteria	Crude seed (T1)				Mucilage (T2)				Seed extracts (T3)				LSD
	T1x (mg L^{-1})	T1a (mg L^{-1})	T1b (mg L^{-1})	T1c (mg L^{-1})	T1X (mg L^{-1})	T1a (mg L^{-1})	T1b (mg L^{-1})	T1c (mg L^{-1})	T1X (mg L^{-1})	T1a (mg L^{-1})	T1b (mg L^{-1})	T1c (mg L^{-1})	
E.Coli	233^{a}	200.50^{c}	180.50^{c}	122.17^{d}	233^{a}	70.83^{e}	50.66^{f}	42.33^{f}	233^{a}	28.83^{g}	26.50^{g}	22.16^{g}	12.43^{***}
Bacillus subtilis	164^{a}	121.67^{b}	100.67^{d}	111.50^{c}	164^{a}	59.66^{h}	43.33^{i}	42.16^{j}	164^{a}	89.33^{e}	83.66^{f}	94.50^{g}	5.49^{***}
Staphylococcus aureus	100^{a}	91.83^{b}	90.66^{b}	47.00^{e}	100^{a}	67.00^{d}	37.00^{f}	32.66^{f}	100^{a}	82.50^{c}	$87.66b^{c}$	35.66^{f}	5.98^{***}

Means in same row with different superscripts are significantly different at (P = .05).

Key: T1x = Control, T1a = 5 mg L^{-1}, T1b = 10 mg L^{-1} and T1c = 15 mg L

3.3 Physico-chemical Parameters

3.3.1 Electrical Conductivity

Electrical conductivity as shown in table one increase significantly. The permissible limit of conductivity reading of World Health Organization (WHO) for drinking water is 0-3000 µS and hence the result obtained are in the permissible limit as allowed. From the result conductivity increases with an increase in dosage, the conductivity reading also increased generally. The increase in conductivity reading may be as a result of ions formation in the water. Formation of the ions also contributed to the overall conductivity [12]. The reaction of the salt with the water is also one of the possible causes for the rise in the reading. The conductivity value also depend on the ions concentration in the water [13]. When *Diospyros mespiliformis* crude seed, mucilage and seed extracts was used, the impacts in the result revealed that the use of excess *Diospyros mespiliformis* above the ideal dosage lead to the rise in the conductivity reading.

3.3.2 Hydrogen Ion Concentration (pH)

According to World Health Organization (WHO) pH standard for drinking water ranges 6.0-8.5. Using *Diospyros mespiliformis* crude seed, mucilage and seed extracts, the pH reading remained fairly constant. Even though the initial surface water reading is acidic prior to analysis, there were change in the final pH reading which was still along the permitted range of standard for drinking water. The acidic (initial) reading of the surface water is probably caused by soil and water natural acidification which is a biogeochemical process happens very slowly by soil respiration, a process where soil organisms respire and produce carbon dioxide and the carbon dioxide dissolves in water giving away carbonic acid which is a type of weak acid formed in the solution [14]. Acidic water consumption is harmful known to the consumers. Among the consequences of supplying the water without treating their pH is corrosion of the plumbing that is used to channel the water from the water treatment plant to houses. The corrosive nature of acidic water causes metal ions such as iron, manganese, cooper, lead and zinc to leach into the water, causing elevated levels of toxic metals in the water. It may also cause aesthetic problems, such as a metallic or sour taste. Thus, using plant extracts such as *Diospyros mespiliformis* for water treatment may have an enormous advantages by eliminating the need for application of lime or bicarbonate which might be needed to raise the pH as seeing in the study there is significant different in the pH with seed extracts recorded highest 7.51, this reduces life threating effects and hence, it provides extra cost saving.

3.3.3 Temperature

The initial temperature of the surface water was 27.14^{o}C and the highest temperature after the addition of *Diospyros mespiliformis* extracts is 28.12^{o}C. This is in accordance with the study from [15] where their finding was in line with this study. The study stated that after the addition of powdered *Moringa oleifera* seeds when used as coagulant in the water purification and treatment processes, the initial reading of the

groundwater sample of 28.40^{o}C only reached 29.00^{o}C at its highest. [16] In their research stated that temperature affects the coagulation efficiency rate since lower temperature usually takes longer for the particles for flocculation to takes place. From the study by [17] the reason why this occurred is explained by the decreasing speed of the hydrolysis reaction and precipitation rate of the cation hydroxides and increased in water viscosity as well. Increase in viscosity of the water usually lowers the sedimentation speed of the after coagulation particles that was formed and might increase the stability of the colloidal that was supposed to be removed as well.

3.3.4 Biological Oxygen Demand

The degree and extent of Dissolve Oxygen increase depends on the Biological Oxygen Demand (BOD) of the effluent in that, the lower the BOD, the higher the DO and vice versa. BOD is the most important variable in water pollution control since it indicates the actual level of biodegradable pollutants in the water [18]. The optimum biological oxygen demand obtained in this study is 4.12 with least mean of 2.98 this correspond with the work of [19] whose reported that water body with BOD 10mg/l is said to be definitely polluted, 5mg/l doubtful clean and polluted, 3.0mg/l average, and 1-2.0mg/l said to be considered clean. However, there is significantly different in means Biological Oxygen Demand obtained in this study which is stilled remained within the acceptable range.

3.4 Total Bacteria Count for Surface Water Treated with *Diospyros mespiliformis* Extracts

The optimum percentage reduction of *E. coli* bacteria in table 2 was 90.48% which is in harmony with the work of [20] who reported an illustrated detailed of antimicrobial agents of the seed extract from *Moringa oleifera* to remove a maximum of 93.2% for *E. coli* and 96.2% for *Bacillus subtilis*. [21] Also found that it is possible to remove the whole of the coliform bacteria from Hong River, belonging to an *Escherichia coli* family, by using the simple crushed *Moringa oleifera* seeds. [22] Also tested *Moringa oleifera* dried powder for purifying the river M'Poko surface water. *Streptococci*, *Clostridium*, and *E. coli* were reduced by 62%, 95%, and 47%, respectively. Also 74.30% and 67.34% for *Bacillus subtili s*and *Staphylococcus aureus* revealed from this finding.

4. CONCLUSION

The result from the finding revealed that seed extracts of *Diospyros mespiliformis* were very effective in its ability to increases the level of electrical conductivity, pH from acidic to basic form Total dissolve solid and reducing the bacteria growth. *Diospyros mespiliformis* extracts also were very effective in it ability for turbidity reduction. At maximum dose of 5 mg/l mostly observed to be more effective. *Diospyros mespiliformis* mucilage and seed extract are more effective to *S. aureus*, *E.coli* and *B. subtilis* when compared with *Diospyros mespiliformis* crude seed. Other significant finding show that extracts from plant did not affect pH of the water samples. This plant species have made the requirements of drinking water quality in terms of maximum permissible limit of turbidity (≤5 NTU).

REFERENCES

1. Ali E, Khairy H. Environmental assessment of drainage water impacts on water quality and eutrophication level of Lake Idku, Egypt. *Environmental Pollution* (Barking, Essex: 1987). 10.1016/j.envpol.2016.05.064.

2. W. H. O. 4th ed. Geneva, Switzerland: Guidelines for Drinking-water Quality- World Health Organization; 2011.

3. W. H. O. 4th ed. Geneva, Switzerland: Guidelines for Drinking-water Quality- World Health Organization; 2011.

4. Sridhar MKC, Oloruntoba EO. Water, Development, Health and the Nigerian Millennium Development Goals, Chapter 5, International: T. Agbola, C.O. Olatubara, B. Wahab, L. Sanni and O. Ipingbemi (Eds) 2008;pp.83-112.

5. Patil PN, Sawant DV, Deshmukh RN. Physico-chemical parameters for testing of water- A review. International J Evirontl Sci. 2012;3(3):1194-1207.

6. Etkin NL. *Antimalarial plants used by Hausa in northern Nigeria. Tropical Doctor*, 1997;27: 12-16.

7. Thome A, Reginatto C, Cecchin I, Colla LM. Bioventing in a residual clayey soil contaminated with a blend of biodiesel and diesel oil. J Environtal Eng. 2014;140:1-6.

8. Thome A, Reginatto C, Cecchin I, Colla LM. Bioventing in a residual clayey soil contaminated with a blend of biodiesel and diesel oil. J Environtal Eng. 2014;140:1-6.

9. Turquois T, Rinaudo M, Taravel FR, Heyraud A. "Extraction of Highly Gelling Pectic Substances from Sugar Beet Pulp and Potato Pulp: Influence of Extrinsic Parameters on their Gelling Properties." *Food Hydrocolloids*, 1999;13: 255-262.

10. Lange B. Model 2100P Portable Turbidimeter: Instrument and procedure manual. 2004;pp. 2126, Hamsphire.

11. Diliello LR. *Method in food and Dry Microbiology Duncan, A.V.I* Publishing company west port Connecticut. 1982;PP.49-56.

12. Jowa T, Mguni LL. Treatment of low turbidity water using Poly-Aluminum Cloride (PAC) and Recycled Sludge: Case study Chinhoyi. Zimbabwe J Sci Technol. 2015;10:101-108.

13. Hendrawati IR, Yuliastri E, Rohaeti H, Effendi L, Darusman K. The use of surface *Moringa oleifera* seed powder as coagulant to improve the quality of wastewater and ground water. IOP Conference Series, 2016;31: 1-5.

14. Yusuf J, Yuakubu MB, Balarabe AM. The use of *Moringa oleifera* seed as a coagulant for domestic water purification. IOSR J Phar and Bio Sci. 2015;10:6-9.

15. Hendrawati IR, Yuliastri E, Rohaeti H, Effendi L, Darusman K. The use of surface *Moringa oleifera* seed powder as coagulant to improve the quality of wastewater and ground water. IOP Conference Series, 2016;31: 1-5.

16. Leon-Luque AJ, Barajas CL, Pena-Guzman CA. Determintion of the optimal dosage of aluminum sulfate in the coagulation-flocculation process using and artificial neural network. Int J Sci Development. 2016;**7**:346-350.

17. Krupinska I. Effect of the type of aluminum coagulant on effectiveness at removing pollutants from groundwater in the process of coagulation. Proceedings of the International Conference on Environmental Engineering. 2014;9:1-8.

18. Hammer MJ, Hammer MJJ. Water and waste water technology. Prentice- Hall Inc. 1996;p.519.

19. Ekubo AA, Abowei JFN. Review of some water quality management principles in culture fisheries, Resear J Appl Sci Eng and Technol. 2011;3(2):1342-1357.

20. Dasgupta S, Gundaa NSK, Mitra SK. Evaluation of the antimicrobial activity of *Moringa oleifera* seed extract as a sustainable solution for potable water. RSC Adv. 2016;6: 25918-25926.

21. Nguyen TH. Use of *Moringa oleifera* seeds as natural coagulant for water purification in Viet Nam. *Young Scientist*. 2016;132: 127–132.

22. Poumaye, N., Mabingui, J., Lutgen, P. and Bigan, M. Contribution to the clarification of surface water from the *Moringa oleifera*: case M'Poko River to Bangui, Central African Republic. Chemical Engeneering Research and Design. 2012;90:2346-2352.