ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH

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A thesis submitted to the school of Public health, Addis Ababa University in partial fulfillment of the requirements of the degree of master in Public Health

Advisors: Dr. ABERA KUMIE (PhD)
Dr. MIRGISSA KABA (PhD)

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Abbreviations and Acronyms

AAU	Addis Ababa University
CFU	Colony forming unit
DHS	Demographic Health Survey
EPHI	Ethiopian Public Health Institute
EPA	Environmental Protection Agency
gm.	Gram
HCL	Hydrochloric Acid
hr.	Hour
Km	Kilometer
NGOs	Non-Governmental Organizations
mg/l	Milligram per liter
ml	Milliliter
MO	Moringa Olifeira
MPN	Most probable Number
NH_4OH	Ammonium Hydroxide
NTU	Nephelometric Turbidity Unit
TSA	Triptosoya agar
SPH	School of Public Health
UNICEF	United Nations International Children Educational Fund
WHO	World Health Organization

Summary

Background: Among all liquids, water is a unique substance and one of the most important natural resources. In the absence of safe and adequate drinking water supplies, diseases such as diarrhea, skin and eye infections are common. Treatment methods were thus required in order to protect the health of consumers. Currently seed of tropical plant Moringa Olifeira is being recommended as cost effective technology for water treatment in developing countries. The pH at which coagulation occurs is the most important parameter for proper coagulation performance as it affects the surface charge of colloids.

Objective: The present study was carried out to determine effect of water pH on turbidity removal and bacterial load reduction using Moringa Olifeira for water treatment**.**

Methods: During this study, ground water from open well and tap water were collected for treatment by Moringa Olifeira seeds in powdered form. Primarily the minimum dose of Moringa Olifeira for effective treatment was identified. Then coagulation ability of Moringa Olifeira seed extract was assessed at different pH values (4, 5, 6, 7, 8, and 9) of water. Parameters which may influence coagulation effectiveness of Moringa Olifeira were kept under control during experimental procedure.

Result: The result showed that 60mg of Moringa Olifeira is the optimum dose to treat water with 167NTU initial turbidity and 310 total coliforms. The effect of water PH on total coliforms reduction and turbidity removal effectiveness of Moringa Olifeira was studied and its removal effectiveness is also found to be 90.32% and 90.34% respectively. The pH evaluated has statistically significant effects on the turbidity removal and total coliform reduction with the *p-values* much lower than $0.05(1.2*10^{-11})$.

Conclusion: Effectiveness of Moringa Olifeira in reducing turbidity and bacterial load is significantly affected by water pH values. Therefore effect that could arise from variation in the pH of water should be considered. Sequentially, since Moringa Olifeira does not guarantee impurity removal as World Health Organization guidelines, additional disinfection of coagulated water to remove pathogens is necessary in order to achieve standard quality before the water is drunk.

1. Introduction

1.1. Background

Among all liquids, water is a unique substance and one of the most important natural resources. But its amount in nature is fixed, 1500 million cubic kilometers. The amount of fresh water available for use is only 0.3 percent (1). Pure water is clear, colorless, tasteless and odorless fluid. Microorganisms find their way in to water and depend upon circumstances to prosper or die (2). The treatment processes include purely physical methods such as screening and simple sedimentation, purely chemical methods such as adsorption and ion exchange, and physicochemical techniques in which contaminants are altered chemically to enhance their removal by physical processes (3). Clarification processes in modern practice are typically physicochemical techniques which are intended to remove microorganisms, turbidity and color-including the humid materials (4).

Increased demand and intensive use of water (as caused by population increases) often create the need for additional water treatment because water in new source areas tends to be of lower quality**.** The use of natural coagulants for drinking water treatment is usually presumed cost effective, nontoxic and safe for human health (5). One of these alternatives is Moringa Olifeira (MO) seeds which are a branched tree that grows 6-10 meter tall and grows abundantly in south west of Ethiopia. It is known by different vernacular name such Shiferaw, Halako, cabbage tree etc. Among 14 species of Moringa tree, Moringa Olifeira (MO) is well studied with regard to potential medicinal uses, water purification and the identification of compound of potential therapeutic importance which is native to India (6). Coagulation with Moringa Olifeira (MO) is used for both physical and chemical purification of turbid raw water. At very high turbidity the water can no longer be adequately treated by using filters. Coagulants such as MO have to be applied to transform water constituents into forms that can be separated out physically (7). They can clarify not only highly turbid muddy water, like the majority of traditional plant materials, but also waters of medium and low turbidity, which may appear milky and opaque or sometimes yellowish

or grayish (8). As a coagulant, Moringa Olifeira is environmentally friendly, and unlike alum, does not significantly affect the pH and conductivity of the water after the treatment. It also produces sludge four to five times less in volume than the chemical sludge produced by alum coagulation. Thus the purified proteins are more effective coagulants than alum (9).

1.2. Statement of the problem

In rural and developing countries people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water. Because they lack knowledge of proper drinking water treatment and they cannot afford costly chemical coagulants. In the absence of safe and adequate drinking water supplies, diseases such as diarrhea, skin and eye infections are common (10). It was indicated that 88 percent of diarrheal diseases are attributed to unsafe, inadequate sanitation and hygiene which have an estimated annual incidence of 4.6 billion episodes and 2.2 million deaths every year globally (11). More than 50 countries, more of them from developing countries still report cholera to WHO (12). Only 54 percent of households in Ethiopia have access to an improved source of drinking water and only 8 percent of households have an improved toilet facility, not shared with other households (3)

Therefore the importance of water quality preservation and improvement is necessary in life and increasing continuously. Concerning conventional coagulants earlier research findings of Crapper et al. (1973) and Miller et al. (1984) showed that the chemicals used for water purification are difficult to access because of high cost and low availability. They are also hazardous that can alter the pH of the water to dangerously low or high levels. Their residues impose health problems on consumers over long time or if an error occurs in their administration during the treatment process. These reports suggested that a high level of aluminum in the brain is a risk factor for Alzheimer's disease (9). As an alternative to conventional coagulants, MO seeds can be used as a natural coagulant (primary coagulant) in household water treatment as well as in the community water treatment systems to make the water potable for household consumption (7). The seeds from MO were found to be fast acting plant

coagulant which can be used without health risks, according to toxicological tests, especially at the low doses required for water treatment (5). Not only were the seed have health benefits, but it has a major advantage because it can be produced locally and would save foreign exchange and generate farm and employment income (8). There are many parameters that affect coagulation performance. Among the common parameters like water pH, coagulant type, dose etc. the pH at which coagulation occurs is the most important parameter for proper coagulation performance as it affects the surface charge of colloids (13-16). Most importantly no studies conducted in Ethiopia were found to assess the effect of water pH on MO effectiveness hence the findings from this study can provide the basis for appropriate intervention & plan to minimize excessive cost of chemical coagulant and prevalence of water borne disease in the country.

1.3.Rational and significance of the study

Even though use of Moringa Olifeira has an added advantage over the chemical treatment of water and it is a potentially viable substitute to alum, not much has been done using Moringa Olifeira as a coagulant in water purification system. The optimum dose of coagulant, effect of water characteristics such as water pH on treatment effectiveness of coagulant and the pH at which high coagulation performance achieved are also not well recognized and current findings of different researches on optimum dose determination and effect of water pH are controversial. Besides to this the effect of water pH on treatment effectiveness of MO has not been studied and evidence based data are scarce in our country for planning and targeting appropriate measures/interventions to treat water using natural coagulant Moringa Olifeira.

Therefore, through such controversial findings, raising the question about optimum dose and pH effect is important. This study aims to fill the gaps through determination of optimum dose and the effect of pH value on MO effectiveness to purify water. It provides guidelines and an explanation on the most suitable pH value that should be used to achieve the maximum removal of turbidity and pathogenic bacteria. It will also have significant input in planning

and targeting appropriate measures/interventions to treat water using natural coagulant Moringa Olifeira through consideration of pH influence on performance, so that problem from consuming water with its impurities will be managed using limited resource available. Furthermore, the output of this study can serve as a baseline for further investigation on similar topic.

2. Literature Review

The quality of water resources is deteriorating exponentially due to contamination resulted from geometrical growth of world population, domestic and agricultural activities, modern industrialization and civilization, and other geological, environmental and global changes (17). Unless water becomes acceptable and palatable to induce its use, consumers may decide to use water of doubtful quality from a nearby unprotected stream, well or spring. Thus it is necessary to focus on treatment techniques that produce potable water or water that is both safe and pleasing (18,19).

Among the methods developed and being used from time to time, physical and chemical purification system is the most commonly used technologies for Drinking water treatment (20). However chemical clarification methods like flocculation, coagulation and sedimentation are often inappropriate because of the high cost and low availability of chemical coagulants. Therefore the use of natural materials of plant origin to clarify turbid water is introduced as option by earlier studies (21-26).

It is generally accepted that Moringa Olifeira seeds works as a coagulant. It treats water on two levels, acting both as a coagulant and an antimicrobial agent. Findings support recombinant proteins both removing microorganisms by coagulation as well as acting directly as growth inhibitors of the microorganisms (21). Therefore application of this low-cost MO protocol is recommended for simplified point of use, low risk water treatment where rural and per-urban people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water (8).

2.1. Effective dose of coagulant Moringa Olifeira for turbidity removal and total coliform reduction

Coagulant dose adjustment can be used for better coagulation and more decrease in turbidity. Accordingly different studies identified optimum dose of Moringa Olifeira for effective water treatment. The table below gives a rough guide to determine the dose requirement.

Table 1:- Turbidity level and Recommended dose of coagulant Moringa Olifeira (27).

Raw water turbidity (NTU)	Dose range (mg/l)
<50	10-50
50-150	30-100
>150	50-200

It was found that 60mg/l Moringa Olifeira seed powder is sufficient to reduce turbidity from water with initial turbidity of 410 NTU close to 10 NTU (8). Other studies also indicate that a dose of 10 mg/l of Moringa Olifeira seed extract could reduce primary turbidity 500NTU to 6.2NTU with removal effectiveness of 98.7%, where optimum dose of 20 mg/l seed reduces initial turbidity of 50 NTU to 6 NTU with removal effectiveness of 88% (28). Concerning determination of optimum dose for total coliform reduction, treating water with both the 10.0 and 12.0 gm. /l of coagulant MO dose gave a lower count of 2 for the Most Probable Number (MPN) of total coli forms per 100 ml for water. This indicate that the process of flocculation removes about 90 -99% of bacteria which are normally attached to the solid particles while the control treatment had the highest counts of 17 coli forms/100 ml (36). It is also indicated in other study that 150mg/l of MO is optimum dose to reduce initial total coliform of $4\times10^5\pm0.57$ SPC/100ml and 1600 ±0.57 MPN/100ml to 1×10^2 ±0.57 SPC/100ml and 5 ±0.57 MPN/100ml respectively (5).

2.2. Turbidity removal with coagulant Moringa Olifeira and effect of pH control

Turbidity is the measure of relative clarity of a liquid which makes water cloudy or opaque. It is an optical characteristic of water and is an expression of the amount of light that is scattered by material in the water when a light is shined through the water sample. Material that causes water to be turbid includes clay, silt, finely divided inorganic and organic matter, algae, soluble colored organic compounds, and plankton and other microscopic organisms (4). Turbidity is measured by shining a light through the water and is reported in Nephelometric

turbidity units (NTU).Turbidity can be classified based on Dosage Rates as, Low turbidity (NTU<50), Medium turbidity (NTU 50-150), High turbidity (NTU 150-250) and Extreme turbidity (NTU >250) (4).

As a coagulant, Moringa is non-toxic and biodegradable. It is also environmentally friendly and unlike alum, does not significantly affect the pH and conductivity of the water after the treatment. So, as a coagulant, MO may be a potentially viable substitute to alum (21). Moringa Olifeira turbidity removal was found to increase with increasing initial turbidity. The lowest turbidity removal of 83.2% was observed at 50 NTU, whilst the highest of 99.8% was obtained at 450 NTU (22). In general the technique highlighted should result in a turbidity reduction of 80.0% to 99.5% by adsorption and neutralization of the colloidal charges (29).

In line with WHO guideline for Turbidity 5 NTU for domestic purpose, water treated with MO is potable without any further treatment (30).

The removal of pollutants causing turbidity by coagulation depends on the dose of coagulants and other characteristics of the water including pH, temperature and ionic strength (31).

pH is the term used to express the intensity of the acid or alkaline condition of water (14).The pH at which coagulation occurs is the most important parameter for proper coagulation Performance as it affects the surface charge of colloids. Therefore controlling the pH of raw water is more effective in turbidity removal and may be applied in water treatment plants according to the nature of raw water in these plants and considerable number of jar tests to these water because the optimum pH may become different on each type of water (15, 16). Concerning effect of water pH on MO effectiveness it was it was found that natural coagulant Moringa Olifeira as a coagulant requires no pH adjustment (21). Unlike the above study it was stated that coagulant Moringa Olifeira need pH adjustment and it produce appreciable reduction of turbidity at 6-8 pH and it was declined at 10 to 12 pH for sample water with initial turbidity of 150 NTU as indicated on the follows (Table 2).

Table 2:- Percentage turbidity removal of Moringa Olifeira from sample water with initial turbidity of 150 NTU, with optimum dosage of 100mg (32).

S.No.	PH	turbidity removal by MO
2	4	33
3	6	45
4	8	80
5	10	73
6	12	68

It was also showed that that MO has high coagulation activity for synthetic turbid water with 50 mg kaolin per liter at pH 8 or more and little coagulation activity below pH 7 as indicated as follows (33).

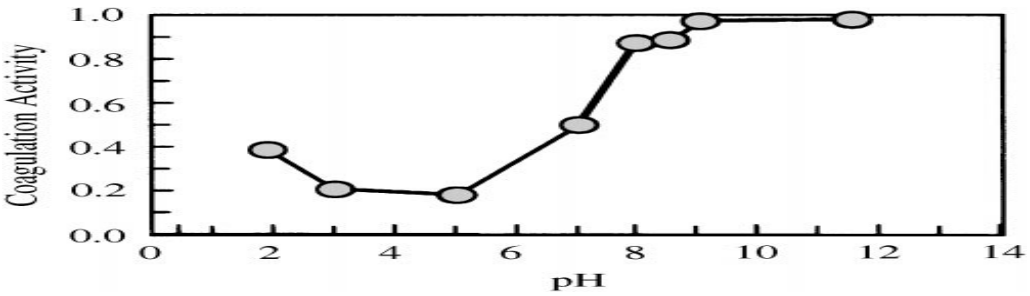

Figure 1:- Effects of pH on coagulation activity of Moringa Olifeira

2.3. Bacterial load reduction with coagulant Moringa Olifeira and effect of pH control

Concerning bacterial load reduction, not all technologies are highly effective in reducing all classes of water borne pathogens (bacteria, viruses and protozoa). Therefore, careful consideration of the health-based target microbes to control in a drinking water source is needed when choosing among these technologies. The most common technology of treating water for contamination is to use one of various chemical agents(chlorine, bromine, iodine, potassium permanganate, copper and silver ions, alkalis, acids and ozone (20). However chemical disinfectants for drinking-water are hazardous that can alter the pH of the water to dangerously low or high levels. Their residues also impose health problems on consumers over long time (34, 35). A low-cost technology option for

drinking water purification involving plant extracts of different parts of the Moringa Olifeira vegetable tree were examined for their antibacterial properties. It is suggested that bud and seed extracts can be used for water treatment, avoiding the risk of contamination by water-borne pathogens and promoting an indigenous solution to disease control and environmental management (34).

In line with WHO guideline for Microbial load which is 10 Total count/100ml of water respectively for domestic purpose, water treated with MO is potable without any further treatment (30).

Treating water by the seed gave a lower count of 2 for the Most Probable Number (MPN) of total coli forms per 100 ml with both the 10.0 and 12.0 g environmental management. This indicate that the process of flocculation removes about 90 -99% of bacteria which are normally attached to the solid particles while the control treatment had the highest counts of 17 coli forms/100 ml (36). Similarly Asian countries like India drastically reduce the incidence of waterborne diseases by treating water with Moringa Olifeira seed. These encouraging findings support the recommendation that of the seed is a cheap and easy alternative for purifying water, especially in remote areas(37).

However other studies disagree with the above conclusion. Based on these findings the MO protocol can produce potable water of higher quality than the original source, but is unable to guarantee (100%) virus- and/or bacteria-free water immediately after treatment or storage (38). Based on study conducted in India MPN was found in the range 500 – 1200 coli forms/ml to all samples after the treatment which indicates above the limits of WHO standards. This gives direct proof of dangerous impurities in water and therefore treated Samples were indicated to be bacteriologically not safe for drinking purpose (5).

Concerning effect of water pH of water on bacteriological load reduction it was difficult to find researches conducted specifically on this topic. However Findings support recombinant proteins both removing microorganisms by coagulation as well as acting directly as growth inhibitors of the microorganisms (21). Hence MO remove microorganisms by coagulation, the effect of pH value on coagulation can be also considered for bacteriological load

reduction. Therefore it is found that *Moringa* as a coagulant requires no pH adjustment. Unlikely Other study stated that MO has high coagulation activity at pH 8 or more and little coagulation activity below pH 7 (33).

3. Objectives

3.1 General Objective

To determine the effect of water pH on turbidity removal and bacterial load reduction using Moringa Olifeira for water treatment.

3.2 Specific Objectives

- To determine the minimum dose of Moringa Olifeira for effective removal of water turbidity and reduction of bacterial load/total coli forms.
- To determine effect of pH control on turbidity removal effectiveness of Moringa Olifeira.
- To determine effect of pH control on bacteria load/total coli forms reduction effectiveness of Moringa Olifeira.

4. Research hypothesis

- PH value of water has effect on effectiveness of Moringa Olifeira seed to treat water turbidity at specific dose of Moringa Olifeira.
- PH value of water to be treated has effect on effectiveness of Moringa Olifeira seed to reduce bacterial load at specific dose of Moringa Olifeira.

5. Methods

5.1 Study Design

An experimental study design was followed at room temperature in order to determine the effect of pH on effectiveness of MO for treating turbid and untreated pathogenic water.

5.2 Study Area and Period

The study was conducted in laboratory of Ethiopian Public Health Institute (EPHI) from the period June to August 2015. Ground water from open well for turbidity removal analysis was collected from Addis Ababa, Gulele Sub city, Woreda 09 and tap water was taken for bacterial load analysis. The ground water was taken for its high natural turbidity and sampling area was selected for its convenience to take sample within short time during sample transportation to EPHI where lab procedure was done. The unchlorinated tap water was used for bacterial load analysis and it was selected over chlorinated water for the purpose of controlling effect of chlorine on effectiveness of MO for microbial load reduction and ease of preparing synthetic contaminated water.

5.3 Sample collection

13 liters of Sample water for turbidity removal analysis was collected once from open well found in Gulele sub city Woreda 9, on June 01, 2015 at 8 am. Sample was collected from a well found at about 3 km from laboratory and transported using car for analysis within 45 minutes, with light proofing plastic container. Turbidity was measured immediately at sampling site within 5 minutes after sampling and in lab before starting laboratory procedure.

13 liters of tap water was taken and contaminated with standard McFarland (total coliform concentration of 10^8) for bacterial load reduction analysis. Professionals who are working in EPHI water quality analysis unit were involved in this study as sample collectors and laboratory assistants after taking short term training by principal investigator.

5.4 Study Variables

- Turbidity
- Microbial load
- PH of the water
- Temperature of the water
- Dose of MO
- Contact time

5.5 Materials

- Turbidity meter(AQUA LYTIC Turbidity meter, AL450T-IR)
- Analytical Balance(Mettler Toledo balance by fisher)
- pH meter(Accument pH meter by fisher)
- Volumetric Glassware – pipettes
- Incubator(Isotemp Incubator by fisher)
- Glassware – Beakers, Measuring Cylinders,
- Sampling bottles – plastic and glass
- Plates
- Magnetic stirrer(corning stirrer by fisher)
- Flask
- Acid (HCl)
- Base (NaOH)
- TSA(Triptosoya Agar)
- Stop watch
- MO seed
- Mortar with pestle

5.6 Operational definition

Bacterial load: - The amount of bacteria which form colonies after incubation under specified conditions taken before and after treatment with MO.

Coagulation: - A process by which impurities present in water are destabilized by MO

Coagulant: - a chemical or MO which is employed during coagulation process.

Dose: - The milligram of MO seed added in to specified volume of water for investigation purpose.

Flocculation: - A slow mixing technique which promotes the binding together of destabilized particles.

pH: - The intensity of the acid or alkaline condition of water

Recombinant protein: - protein that has been made artificially using scientific method that involve combining parts of protein from different living things

Turbidity: - A measure of the extent to which light is either absorbed or scattered by suspended matter in the water before and after treatment with MO.

Tap water: - Unchlorinated and microbial free water supplied to a tap (valve) from ground source with water pump, to push water to reservoir container through the plumbing. m

5.7 procedure of laboratory analysis

As ground water from open well found in Gulele sub city, Woreda 9, taken it brought to Ethiopian Public Health Institute. Before any further investigation one liter of sample was added to each 6 beakers for each bacterial load reduction and turbidity removal. The optimum dose of MO was then determined using jar test. Following this the minimum dose that gives best result in both bacterial load reduction and turbidity removal (60mg/l) was determined as optimum dose for parameters. Turbidity was measured with turbidity meter. Standard plate count (NMKL no 44/2004 method) was used for measuring total coliform count. The pH of the water was also adjusted as required by adding HCl or NaOH in order to determine impurity removal effectiveness of MO seed at different pH values. 6 liters of water for each bacterial load reduction and turbidity removal were taken. Then the water sample with different pH values (**4, 5, 6, 7, 8 and 9**) was treated with predetermined dose of MO (**60mg/l**) to determine its effectiveness. The characteristics like Temperature of water (**25**

^{0}c), duration of MO seed storage (**1 year**), contact time (**90 minutes**) and others which may influence coagulation effectiveness of Moringa Olifeira were kept under control during experimental procedure using checklist. Sample water for bacterial load reduction analysis was prepared by contaminating unchlorinated Tap water using standard McFarland with total coliform concentration of 10^{8} and serial dilution was done. In determining the number of total coli forms present in each of the treated water samples, TSA (Triptosoya Agar) and red violet were used as the medium for the bacteria growth and medium for inhibition of gram positive bacteria growth respectively. The plates were then incubated for 48 hr. at 37°C to allow replication of indicator organism after which total coliforms were counted using colony counter or microscope. The measurements of turbidity and total coliforms were determined before and after treatment procedure. The experiment was duplicated 4 times with the same procedure in order to keep the consistency and reliability of the result and the mean of the each result were taken for analysis, presentation and interpretation purpose. The results obtained were then compared with other studies and WHO guideline standards. The detail description of lab procedures for both Jar test and microbial load analysis and preparation of coagulant are annexed (Annex B, Annex C and Annex D)

5.8 Statistical Analysis

One way Analysis of variance (ANOVA) was performed to determine if pH value of water have a significant effect on turbidity removal and bacterial load reduction.

5.9 Data quality assurance

The quality of data kept by using checklist and following the necessary procedures recommended by WHO in order to get the intended results. In addition to the training given to the sample collectors and lab assistants sample was collected under supervision of principal investigator. Besides this, the experiment was duplicated 4 times with the same procedure in order to keep the

consistency and reliability of the result and the mean of the each result were taken for analysis, presentation and interpretation purpose.

5.10 Ethical Consideration

The ethical approval and clearance was obtained from school of public health. Permission was obtained from Gulele sub city Woreda 9 Administration. The purpose of the study was also explained to community leaders and they were informed that the information obtained will not be disclosed to third body without their willingness. Permission was also asked from EPHI both in verbal and using formal letter of support with ethical clearance obtained from AAU to get material support to smoothen the investigation.

5.11 Dissemination of the results

The final report or finding containing all the appropriate recommendations will be distributed to those concerned bodies especially water authorities including NGOs that work on water-related services in order to consider and popularize the low-cost water treatment protocol using MO seed through controlling pH value of water to be treated. The result of this study will be also used by interested individuals who want to study on the topic of interest.

6. Results

The results presented in this thesis cover the measurements carried out from June to August 2015. First, the determination of optimum dose of Moringa Olifeira on the removal of turbidity and total coli forms reduction was studied on the ground water collected from Gulele Sub city Woreda 09. Consequently the effect of water PH on effectiveness of Moringa Olifeira was studied using predetermined dose of MO (60mg/l). The results are shown as following:-

6.1. Determination of optimum Moringa Olifeira dose

6.1.1. Determination of optimum dose of Moringa Olifeira for turbidity removal for sample water at room temperature (25^0c), with initial turbidity of 167 NTU.

Regarding the jar test to determine the optimum dose of Moringa Olifeira, 45, 60, 75, 90, and 105 mg of MO were analyzed per one liter of untreated water with initial turbidity of 167NTU. After 90 minutes turbidity was reduced to 19.84(88.12%), 16.13(90.34%), 17.32(89.63%), 21.64(87.04%), 20.96(87.45%) for 5 samples respectively. The control group (without addition of MO seed) gives turbidity with mean value of 80.16(52 %) as shown below (Table 3).

Table 3:- Determination of effective dose of MO for turbidity removal for sample water at room temperature (25^0c), with initial turbidity of 167 NTU.

Dose of MO (mg/l)	Turbidity removal effectiveness	
	Residual turbidity {C.I=95%}	% of Turbidity Removal {C.I=95%}
Control (no MO)	80.16	52
45	19.84	88.12
60	16.13	90.34
75	17.32	89.63
90	21.64	87.04
105	20.96	87.45

6.1.2. Determination of optimum dose of Moringa Olifeira for reduction of total coliforms for sample water at room temperature (25^0c), with initial total coli forms count of 310 SPC/100ml.

With the same doses stated for turbidity, the total coli form bacteria removal effectiveness was analyzed using plate count method and residual total coli forms were registered with mean values of 120(61.29%), 80(74.19%), 90(70.69%), 110(64.52%) and 110(64.52%) respectively after incubation at 37degree Celsius for 48 hrs. The control group (the sample without addition of MO) gave total coliforms of 280(9.68%) as shown below (Table 4).

Table 4:- Determination of effective dose of MO for total coli forms reduction for sample water at room temperature (25^0c), with initial total coli forms count of 310 SPC/100ml.

Dose of MO(mg/l)	Total coliform reduction effectiveness	
	Residual total coli forms {C.I=95%}	% of total coli forms reduction {C.I=95%}
Control (no MO)	280	9.68
45	120	61.29
60	80	74.19
75	90	70.96
90	110	64.52
105	110	64.52

Both total coliforms reduction and turbidity removal effectiveness of coagulant Moringa Olifeira at different coagulant dose is shown as below (Figure 2).

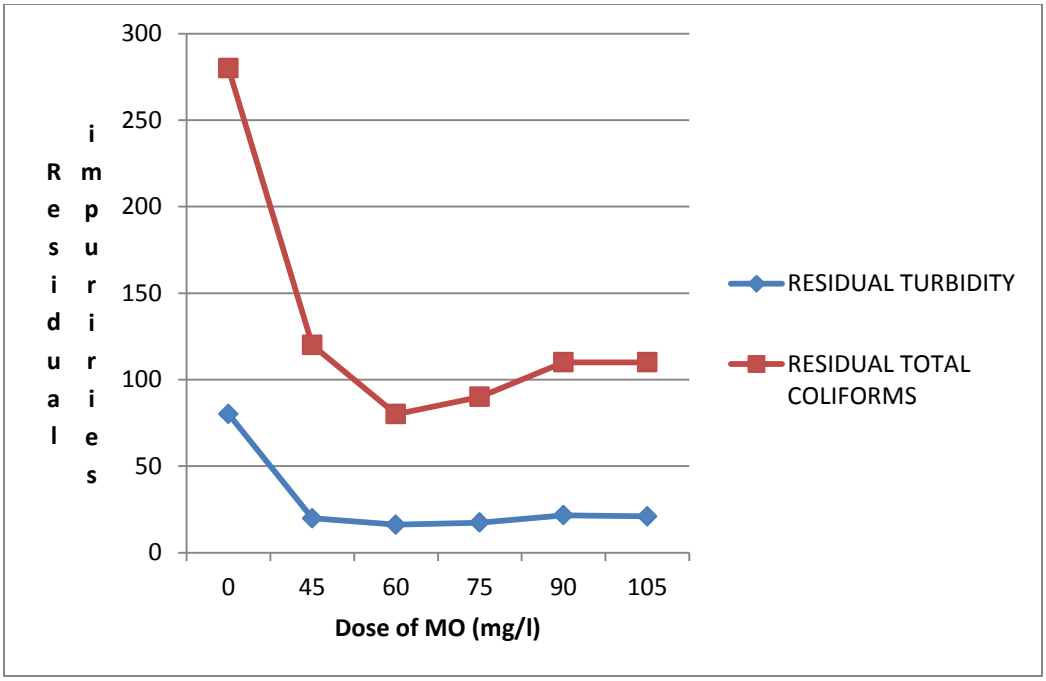

Figure 2:- Turbidity removal and Bacterial load reduction of coagulant MO at different coagulant dose for water at room temperature (25^0c), with 167NTU initial turbidity for turbidity removal analysis and water with 310 initial total coliform counts for bacterial load reduction analysis.

6.2. Determination of effect of pH value of water

6.2.1. Effect of pH value on turbidity removal effectiveness of Moringa Olifeira (60 mg/l), for sample water at room temperature (25^0c), with initial turbidity of 167 NTU.

During determination of the effect of water pH on total coli form reduction effectiveness of MO, the pH of water sample was varied to determine whether it is dependent or not on PH values of water. Based on this PH values were adjusted as 4,5,6,8 and 9 and analyzed by treating sample water with predetermined dose of MO seed for 90 minutes contact time. Accordingly initial turbidity of 167 NTU was analyzed and reduced to the mean values of 80.16(52%), 19.84(88.12%), 16.13(90.34%), 17.32(89.63%), 21.64(87.04%), 20.96(87.45%) respectively as shown below (Table 5).

Table 5: Effect of pH on turbidity removal for sample water at room temperature (25^0c), with initial turbidity of 167 NTU using predetermined optimum dose (60mg/l) of coagulant for each pH.

Parameter (pH value)	Turbidity removal effectiveness	
	Remaining turbidity/{C.I=95%}/	**% of Turbidity Removal**{C.I=95%}
4	16.55	90.08
5	10.43	93.75
6	14.10	91.56
7.2(control)	16.13	90.34
8	11.1	93.35
9	14.53	91.30

6.2.2. Effect of water pH on total coliform reduction effectiveness of Moringa Olifeira, for water at room temperature (25^0c) and initial total coliform 310 SPC/100ml

Concerning effect of pH value on total coli forms reduction using predetermined MO dose, Different trials were carried out on sample with initial total coli forms of 310 varying the pH level as 4, 5, 6, 8 and 9. Accordingly total coli forms reduction was analyzed and reduced to 67.5(78.23%), 30(90.32%), 45(85.48%), 105(66.13%), and 75(75.81%) respectively. The control group (without pH adjustment=7.02) gave total coli forms of 80(74.19%), respectively as shown below (Table 6).

Table 6: Effect of water pH on total coliforms reduction effectiveness of Moringa Olifeira (60 mg/l), for water sample at room temperature (25^0c) and initial total coliform count 310 SPC/100ml.

Parameter (pH value)	Total coliform reduction effectiveness	
	Remaining total coli Forms {C.I=95%}	**% of total coliforms reduction** {C.I=95%}
4	67.5	78.23
5	30	90.32
6	45	85.48
7.2(control)	80	74.19
8	105	66.13
9	75	75.81

Both total coliforms reduction and turbidity removal effectiveness of coagulant Moringa Olifeira at different coagulant dose is shown as below (Fig. 2).

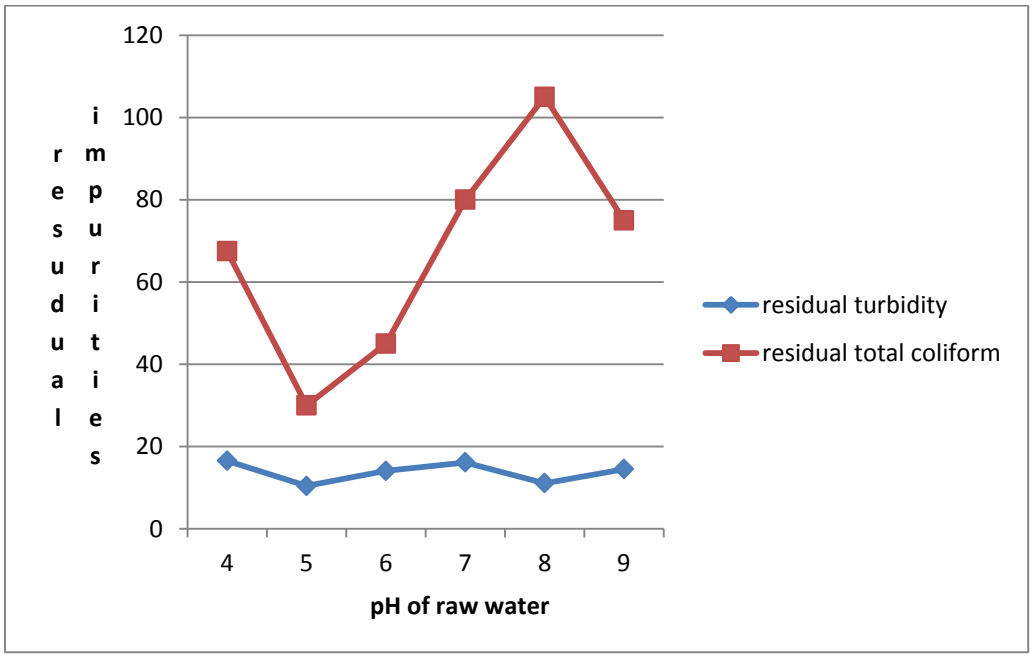

Figure 3: Turbidity removal and Bacterial load reduction of coagulant MO at different water pH for water with 167NTU initial turbidity and 310 initial total coliform count using 60mg/l MO powder.

6.3. Statistical Analysis

ANOVA was executed and one parameter was studied at four levels on the ground and tap water using predetermined optimum coagulant dose of 60mg/l, whether this parameter has a significant effect on turbidity removal and total coli form reduction.

Parameter	levels
pH	4,5,6,7,8,9

Accordingly the pH evaluated has statistically significant effects on the turbidity removal and total coliform reduction since the *p-values* were much lower than 0.05 (P=0.000 for both turbidity removal and total coliform reduction) at 95.0% confidence interval.

Table 7: Analysis of variance for turbidity removal and total coliform reduction.

	TURBIDITY REMOVAL					TOTAL COLIFORM REDUCTION				
Source of variation	Sum of Squares	Degree of freedom	Mean square	F-Ratio	P-Value	Sum of Squares	Degree of freedom	Mean square	F-Ratio	p-Value
Between	99.01	3	33	275	$P<1.2*10^{-11}$	14225	3	4741	137.94	$P<1.2*10^{-11}$
Within	1.92	16	0.12	_	_	550	16	34.37	_	_
total	100.93	19	_	_	_	14775	19	_	_	_

7. Discussion

7.1. Optimum dose of Moringa Olifeira for turbidity removal and microbial load reduction

The removal of contaminant by coagulation depends on the nature and concentration of coagulants. Therefore coagulant dose adjustment can be used for better coagulation and more decrease in turbidity bacterial load. Jar test was conducted to determine the optimum MO dose that gives minimum residual turbidity and bacterial load. As a result 45,60, 75, 90 and 105mg of MO seed powder was analyzed per one liter of turbid water with initial turbidity of 167NTU for turbidity analysis and synthetic water with initial total coliform count of 310 for bacterial load analysis, after 90 minutes contact time.

The finding of this study showed that 60 mg/l of Moringa seed powder was the optimum dose to reduce the initial turbidity of 167NTU to 16.13 NTU with 90.34% removal effectiveness and initial microbial load of 310 to 80 with reduction effectiveness of 74.19%. In agreement with this, it was indicated that 30-100 mg/l of coagulant dose is optimum to treat water with initial turbidity of 50-180 NTU (32). Similarly other finding showed that 60mg/l of MO seed powder is sufficient to reduce initial turbidity of 410 NTU close to 10 NTU which is in agreement with this study (8). On the other hand it was found in another study that a dose of 10 mg/l of Moringa Olifeira seed extract could reduce primary turbidity of 500NTU to 6.2NTU with removal effectiveness of 98.7%, where optimum dose of 20 mg/l seed reduces initial turbidity of 50 NTU to 6 NTU with removal effectiveness of 88% (28). According to the above study 10 mg/l and 20mg/l of coagulant MO is optimum dose for the initial turbidity of 500 NTU and 50 NTU respectively. In comparison with the finding of this study the above study showed optimum dose much lesser than this study finding. This may be due use of salt solution to extract coagulant in the above study where powder is directly added for this study, difference in characteristics of turbid water since synthetic turbid water with kaolin was used

in the above study where this study uses natural turbid water. The source of variation may also attribute to improper grinding of seed.

7.2. Effect of water pH on Turbidity removal with coagulant Moringa Olifeira

Removal of specific contaminants in coagulation processes may be effected by such factors as pH and alkalinity. As the result turbidity removal and total coliform reduction effectiveness of MO was done at different pH using 60 mg/l coagulant dose. Accordingly this study revealed that pH value of water has significant effect on turbidity removal effectiveness of coagulant Moringa Olifeira With p value of $p<1.2*10^{-11}$. The remarkable achievement of 93.75% turbidity removal was registered at PH 5, where the control group (without adjustment: pH=7) gave 90.34% turbidity removal.

On the other hand the study conducted in Dinsha Institute of Management and Technology to determine effectiveness of MO at different pH values of water indicated that Natural coagulant Moringa Olifeira produce appreciable reduction of turbidity at 6-8 pH. According to the study done in Hiroshima University, Department of Environmental Science and Faculty of Engineering, it was found that MO has high coagulation activity at pH 8 or more and little coagulation activity below pH 7. In addition to these, study conducted in University de Sherbrook, Department of Civil Engineering and Biology, also showed that Natural coagulant Moringa Olifeira as a coagulant requires no pH adjustment. Unlike the above studies this study found that water pH has significant effect on coagulation ability MO and it is more effective at acidic media to reduce turbidity from raw water. This variation is may be due to the fact that coagulant Moringa Olifeira contains positively charged particles. Since the action of Moringa Olifeira as a coagulant is because of water soluble cationic proteins in the seeds through mechanism of adsorption and neutralization of charges, the state of the coagulant agent can enhance the turbidity removal process at lower pH(acidic media) (21).

7.3. Effect of water pH on total coliform reduction with coagulant Moringa Olifeira

Concerning effect of water pH on bacteriological load reduction this study showed that similar result with turbidity removal that pH value of water has significant effect on total coliform reduction effectiveness of coagulant Moringa Olifeira With p value of $p<1.2*10^{-11}$. The remarkable achievement of 90.32% total coliform reduction with residual total coliform 30 SPC/100ml. This finding is in agreement with USEP standards which state that water with less than 1×10^{6} SPC/100ml is safe for drinking (5). Here it was difficult to find researches conducted specifically on effect of water pH on bacteria load reduction for comparison. However finding from study conducted in University de Sherbrook, Department of Civil Engineering and Biology support recombinant proteins both removing microorganisms by coagulation as well as acting directly as growth inhibitors of the microorganisms. Since MO remove microorganisms by coagulation, the effect of water pH on turbidity removal and the best pH value for high turbidity removal can be also considered as it is also for bacteriological load reduction.

Based on WHO guideline value total coliform count and turbidity for drinking water should be less than 10 MPN/100ml and 5 NTU respectively (35). The variation of this study result in optimum coagulant dose determination, pH effect and coagulation effectiveness of MO from permissible limit and other studies stated above may also be attributed to the following facts.

The present paper includes some aspects not studied before: 1) Unlike other studies, this investigation is carried out with real water, rather than synthetic turbid water with kaolin clay, in order to obtain the most reproducible results possible for ground water treatment as possible. It is also considered that working with real water has a specific advance to know exact coagulation ability of Moringa Olifeira seed extract (39). This is because material that causes water to be naturally turbid includes clay, silt, finely divided inorganic and organic matter, algae, soluble colored organic compounds, plankton and other microscopic organisms (4). Hence synthetic turbid water with kaolin clay

solution does not contains all suspended material in natural turbid water, using synthetic turbid water to determine coagulation ability of MO may have limitations. 2) Direct addition of powder was done in this study where the coagulant is extracted using salt solution in other studies. In this case the coagulation effectiveness may be not solely as the result of coagulant MO since salt solution extraction was more efficient than distilled water in extracting Moringa's active coagulant ingredient (40).

3) During laboratory procedure factors such as temperature of raw water, stirring time, agitation speed, settling time, contact time and duration of Moringa Olifeira seed storage which may influence Moringa Olifeira effectiveness were kept under control using checklists. The variation in coagulation effectiveness may also attributed to difference in initial turbidity hence the coagulation process using MO is best for high turbid water.

8. Strengths and Limitations of the study

8.1. Strengths of the study

1. Sample collection and lab procedures were done together with experienced professionals who have been working in EPHI in a way those results and findings of the study could be used for further advanced and evidence based implementation of the result.

8.2. Limitations of the study

1. Scarcity of reference materials, particularly from developing countries would make the comparison mainly with locally available materials.

9. Conclusion

Within the scope of treating water for human consumption, these results show very interesting data (90.32% total coliforms and 93.75% turbidity removal using 60 mg/liter of natural clarifier MO). However, since Moringa Olifeira does not guarantee impurity removal as WHO guidelines, additional disinfection of coagulated water to remove pathogens is necessary in order to achieve standard quality before the water is drunk

The study revealed that MO is significantly affected by water pH values and the best result was registered at pH 5. From this it is possible to say effect that could arise from variation in the pH of water should be considered. So that it is possible to increase benefit from naturally available local coagulant MO in treating turbid and bacteriologically contaminated water. Careful control of coagulant dosage is also needed to give optimum coagulation.

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10. Recommendations

Towards improvement of accessibility of safe drinking water for community using low cost and locally available technology principal investigator of this study highly recommends the following activities for concerned bodies:

1. The protein purification technique used here is simple and rapid and can be easily scaled up for production of large quantities of coagulant protein for households and water treatment plants applications through consideration of water pH and subsequently using appropriate disinfection method, since conventional treatment methods are expensive.
2. Government should design and implement community capacity development strategy to change community knowledge, attitude and practice on internalization of household water treatment with MO through focusing on rural and peri-urban population drinking highly contaminated water without using any treatment method.
3. In line with community capacity development cultivation and distribution of the plant should be encouraged by the government as much as possible.
4. Further study should be designed on other factors affecting MO impurity removal effectiveness to get better result.

11. References

1. Moeller.D.W. Environmental Health. Third Edition ed. USA: Harvard University press; 2004.
2. Duerden B. Microbiology and parasitic infection. seventh ed. Singapore:1995.
3. CSA. Ethiopian demographic health and survey. Addis Ababa: 2011.
4. Environmental Protection Agency and United States Geological Survey. Turbidity and health problems [cited 2014 October18]; Available from: <http://water.usgs.gov/edu/turbidity.html>.
5. Mangale Sapana M. Chonde Sonal G. RPD. Use of Moringa Oleifera (Drumstick) seed as Natural Absorbent and an Antimicrobial agent for Ground water Treatment Research Journal of Recent Sciences. 2012;1(3):31-40.
6. Lemma H. The antibacterial activity and water clarifying property of Moringa olifeira seed extracts.Ethiopia: 2014.
7. Schwarz D. Water Clarification using Moringa oleifera. 2000.
8. Legesse W. Environmental Quality control-I for Environmental Health Student. second edition ed. Ethiopia: 2005.
9. Suleyman A. M, Charles A. Okuofu, . Coagulation of low turbidity surface waters with Moringa oleifera seeds. . International Journal of Environmental Studies. 1995;48(3-4):263-73.
10. UNICEF. Water sanitation and Health. 2010.
11. Peavy HS, Rowe, D.R.,Tchnobanoglous, G. Environmental Engineering. Singapore: Mc Graw Hill; 1985.
12. Salvato JA. Environmental Engineering and Sanitation. 4th ed. New York: John Wiley and Sons; 1992.
13. Uyak V. TI. Disinfection by-product precursors reduction by various coagulation techniques in Istanbul water supplies. Journal of Hazardous Materials 2007;141:320-8.
14. Edzwald JK, Van Benschoten J. E. Aluminum coagulation of natural organic matter. Springer-Verlag 1990:341-9.

15. Yang Z. L. GBY, Yue Q. Y, Wang Y. . Effect of pH on the coagulation performance of Al-based coagulants and residual aluminum speciation during the treatment of humic acid-kaolin synthetic water. Journal of Hazardous Materials. 2010.

16. Yan M. WD, Yu. J., Ni. J., Edwards M., Qu. J. Enhanced coagulation with polyaluminum chlorides,: Role of pH/Alkalinity and speciation. Chemosphere. 2008;71:1665-73.

17. V.K. Gupta F, FWIF. Environmental Water Advances in Treatment,Remediation and Recycling. Elsevier BV. 2013;75(1953):589-94.

18. Sawyer C. MCp, Perkin G. Chemistry for Environmental Engineering and Science. 5th ed. Singapore: Mc Graw Hill 2003.

19. Pierce JJV, A.A. and Weiner, R.F. Environmental pollution and control. 6th ed. USA: Butterworth-Heinemann; 1998.

20. Nicholas P. C, P.D.N.P. Handbook of Water and Waste Water Treatment Technologies. USA: Butterworth-Heinemann; 2002.

21. Anselme Ndabigengesere BGT, K. Subba Narasiah. Active Agents and mechanism of coagulation of turbid waters using Moringa olifeira. Elsevier Science Ltd. 1995;29(2):703-10.

22. Bina B. Investigation into the use of natural plant coagulants in the removal of bacteria and bacteriophage from turbid waters: University Newcastle upon Tyne; 1991.

23. Folkard GK, Sutherland, J.P, Jahn, S.A.A. . clarification with Natural coagulants and dissolved air flotation. Water - lines. 1986;5(2):23-6.

24. Jahn SAA. Water clarification with Natural coagulants and dissolved air flotation. Water Journal of Analytical Science. 1986;1:40-1.

25. Jahn SAA. Using Moringaseeds as coagulants in developing countries. Journal of American Water Works Association. 1988;80:43-50.

26. Madsen MS, J. El - fadil, E. Omer Effect of water coagulation by seeds of M. oleifera on bacterial concentrations. JTrop Med Hygiene. 1987;90:101 - 9.

27. Lea M. Bioremediation of Turbid SurfaceWater Using Seed Extract from the Moringa oleifera Lam (Drumstick) Tree , Inc, . John Wiley & Sons. 2014:-1G.2.8.

28. M. Yarahmadi, M. Hossieni, B. Bina, M.H. Mahmoudian, A. Naimabadie and Shahsavani A. Application of Moringa Oleifer Seed Extract and Polyaluminum Chloride in Water Treatment. World Applied Sciences Journal. 2009; 7(8): 962-967.

29. Moringa olifeira and turbidity. [updated 2014 Monday, 17-Mar-2014 11:03:39 EDT; cited cited 2014 October 24]; Available from: http://moringaolifeiratrees.org/.

30. T. Nkurunziza JBN, E. N. Banadda, I. Nhapi. The effect of turbidity levels and Moringa oleifera concentration on the effectiveness of coagulation in water treatment. IWA Publishing. 2009; 59(8):1551-8.

31. Viessman WJ, Hammer, M.J. . Water supply and pollution control. seventh ed. New York: Collins publisher; 2005.

32. Sunita Singh Thakur* SC. Assessment of coagulation efficiency of Moringa oleifera and Okra for treatment of turbid water. Scholars Research Library. 2014; 6(2):24-30.

33. Tetsuji Okuda* AUB, Wataru Wishijimam, Mitsumasa Okadam Isolation and Characterization of Coagulant extracted from Moringa oleifera seed by salt solution. Elsevier Science Ltd. 2001; 35(2):405±10.

34. Ahmed.Toqeer KR, Hassan. Maqbool, Ayub. Najma, Scholz.Miklas, McMinn. William. Coagulation and disinfection in water treatment using MO. Environment research & development water supply. 2010;163(8):381-8.

35. WHO. Guideline for drinking water quality. second ed. Geneva1997.

36. Francis Kweku A. AB. Effectiveness of Moringa oleifera seed as coagulant for water purification. African Journal of Agricultural Research. 2009;4(1):119-23.

37. A.B.Olayemi ROA. Studies on traditional Water purification using Moringa olifeira seeds. African study Monographs. 1994. ;15(3):135-42.

38. Olsen* A-i. Low Technology Water purification by Bentonite clay and Moringa olefeira seed flocculation as performed in Sudanese Villages: Effects on Schistosoma Mansoni Cercariae. Pergamon Journals Ltd. 1987;21(5):517-22.
39. J. sánchez-martín, J. beltrán-heredia, peres J. A. Improvement of the flocculation process in water treatment by using *Moringa Olifeira* seeds extract Brazil journal of chemical Engineering. 2012; 29(03): 495 - 501,
40. M. Golestanbagh, I. S. Ahamad, A. Idris, Yunus R. Effect of storage of shelled Moringa Olifeira seeds from reaping time on turbidity removal. Journal of Water and Health. 2011; 09(3).

13. Annexes

Annex A– checklist for quality control during laboratory procedure

Parameters		Measurement value
For Raw water	PH	7.2
	Turbidity	167 NTU
	Total coli forms	310
	Temperature	25^0C
Factors during procedures	Stirring time	30 minutes
	Settling time	90 minutes
	PH of water	4, 5, 6, 7, 8, 9
	Incubation time	48 hours
	Incubation temperature	37 ^{0}c

Annex B– Preparation of coagulant

Moringa Olifeira- · Plant species - Genus Moringa

· Family- Moringaceae.

· Common Name_ Drum sticks [worldwide],

Shiferaw, Halako, [in Ethiopia]

Moringa Olifeira, known as Moringa, is native to north India but is now found throughout the tropics including southern part of the Ethiopia, mostly in Arba Minch,Konso, Borena zone. Moringa is full of nutrients and vitamins and is good in human food as well as in the food of animals. Moringa helps to clean dirty Water and is a useful source of medicines. Dried mature seeds of M. Olifeira will be collected from Arba Minch area of Wolaita Sodo and crushed in mortar after removal of their wings and coats. 2 kilograms of powder is then prepared for treatment.

Annex C– Procedures for Jar Test

1. Matured seeds will be removed from the pods, and shelled.
2. Then seed kernels will be crushed and sieved.
3. Different doses (45, 60, 75, 90 and 105 mg) of powdered seed will be prepared.
4. Sample water will be collected from open well.
5. Following 5 flaks will be filled with the water from the same source which has equal turbidity measure, 1 liter volume and PH 7
6. Water with adjusted PH (4, 5, 6, 7, 8, and 9) which has equal turbidity and volume as indicated above, will be prepared and each added to 5 flasks.
7. Then coagulant with different doses stated above will be mixed with water samples prepared and Stirred for 2 minute at 100 rpm.
8. Then the water will be slowly and regularly stirred for 20 minutes at 30 rpm.
9. After stirring the treated water will be covered and left to settle for at least one hour.
10. After an hour, sample will be taken for microbial load analysis using sterilized pipette and turbidity will be measured with turbidity meter as per standard methods.
11. Finally the dose and PH value that will give satisfactory results will be taken as effective dose and PH for turbidity removal.

Annex D–Procedures for Microbial Load Count Using plate count

1. The growth media TSA (Triptosoya Agar) and gram positive bacteria growth inhibitor (red violet) was prepared.
2. 100ml of tap water was taken and Contamination with standard McFarland With total coliform load concentration of 10^8 was done.
3. Serial dilution of sample water was carried out (10^2, 10^4, 10^6 …).
4. Equal volume (10 ml) of water sample from each diluted sample were taken and added to plates.
5. Suitable selective culture media TSA was added to each plate, mixed thoroughly, covered and left for an hour.
6. Red violet was added on each plate to inhibit gram positive bacterial growth.

7. All plates were transferred to incubator and incubated at 37^0c for 48 hr. to allow replication of the indicator.
8. The plates with countable colonies were identified and colonies of total coliforms were counted with colony counter and microscope.
9. Results were then expressed in number of total coliforms per 100 ml of sample after each colony grown on plates were multiplied by initial standard concentration where this sample was used as sample for further study.
10. Finally the dose and PH value that will give the lowest total coliform count was taken as effective dose and PH for pathogenic bacteria load reduction.

ASSURANCE OF PRINCIPAL INVESTIGATOR

Declaration

I, the under Signed, declare that this is my original work and has never been presented in this or any other university and that all the source material used for the thesis has been duty acknowledged.

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