ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF PUBLIC HEALTH

EVALUATION OF EFFICIENCY OF MORINGA STENOPETALA AND OPUNTIA FICUS INDICA FOR HOUSEHOLD WATER TREATMENT

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A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCE, SCHOOL OF PUBLIC HEALTH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PUBLIC HEALTH

December 2020

Addis Ababa University

College of Health Sciences

School of Public Health

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ACKNOWLEDGMENTS

First and foremost, I would like to thank Almighty God, who is the source of all knowledge and wisdom, for His protection and provision of strength, patience, and good health.

Next, my cordial appreciations have gone to my Advisors Mr. Worku Tefera and Dr. Abera Kumie whose priceless suggestions, guidance, counseling continuous encouragement, and unreserved support in each step to review my work.

I would like to thanks also Jimma University Environmental Health Department for allowing me their laboratory and equipment's to do my experiment.

Laboratory technician Mr. Seyoum Derib was very cooperative and committed to doing the best experiment by giving his valuable time. I have impressively appreciated him.

I wish to leave on record my heartfelt thanks to my wife Mrs. Tigist Gehano for her unfailing support, love, encouragement, and unlimited advice.

Finally, I would like to thank Addis Ababa University for funding this study and St.Paul's Hospital Millennium Medical College for sponsoring this scholarship.

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ABBREVIATIONS AND ACRONYMS

APHA- American Public Health Association

ANOVA- Analysis of Variance

CESCR -Committee on Economic Social and Cultural Rights

DO-Dissolved Oxygen

EDHS- Ethiopian demographic health survey

EDTA- Ethylendiaminetetraacetic acid

EPA- Environmental Protection Agency

gm/l- gram per liter

IDSA- International Disease Society of America

HWTS-Household water treatment system

JMP- Joint Monitoring Program

Mg/l- Milligram per liter

ML- Milliliter

M.Stenopetala- Moringa Stenopetala

M.Oleifera- Moringa Oleifera

NGOs-Non-governmental organization

NTU- Nephelometric Turbidity Unit

SDGs- Sustainable Development Goals

SSF- Slow sand filtration

SODIS – Solar disinfection system

SNNPR-South nation nationalities people region

SPSS- Statically Package for social science

OFI-Opuntia ficus indica

TDS.-Total Dissolved Solid

UNDP- United Nations Development Program

UN-United Nation

UNICEF- United Nation International Children's Emergency Fund

UV- Ultraviolet

WHO- World Health Organization,

ABSTRACT

Background: Advanced water treatments that involve a series of steps are unthinkable in rural areas, where a dispersed population is found. It is also extremely costly for investment in developing countries. For this reason, we investigate the comparative performance of natural plant coagulants in removing turbidity and microbial load as compared to alum and chlorine for household water treatment, with the additional benefit of preventing water-borne diseases.

Objectives: The main objective of this study was to investigate the efficiency of Moringa Stenopetala and Opuntia Ficus Indica cladodes as natural coagulants for household water treatment

Methods: Batch coagulation and microbial reduction experiments were carried out on surface river waters found in Jimma, Ethiopia with having initial different turbidities. The seeds of Moringa stenopetala (Baker f.), and Opuntia ficus indica cladodes were used for this study. The turbidity removal efficiency and microbial quality were tested using a jar test, and Membrane filtration techniques respectively. Relevant parameters affecting the effectiveness of coagulation (optimal dose of coagulants, pH, storage duration/conditions, temperature, and conductivity) were also investigated.

Results: The turbidity of river water were 32.1, 78.1, 132 and 78NTU, and their optimum doses for M.stenopetala was 20mg/l, 40mg/l, 50mg/l, 70mg/l, and for OFI cladodes were 50mg, 100mg/l.100mg/l and 0.5gr/l respectively. Their turbidity removal efficiency was found to be from 87% to 98.99% when treated with M. Stenopetala, and from 68% to 96.01% when treated with OFI at optimum doses in 3h settling time. About 97% of all types of microbial load removals were observed for both M. stenopetala and OFI.

Conclusion: The findings showed that M. stenopetala plant species meet the requirements of drinking water quality in terms of microbial standards and maximum allowable limit of turbidity (≤5 NTU) if they are used for household water treatment, but the result of OFI was insufficient to fall within guideline values.

Keywords: Coagulation, Turbidity, M.stenopetala, Opuntia ficus indica, Microbial load reduction, Household water treatment

1. INTRODUCTION

1.1 Background

Water is a matter of life and death, very literally. A pillar of socio-economic growth is equitable access to clean water and sanitation (1). The human right to water grants everyone the right to appropriate, clean, reasonable, physically available, and accessible water for private and domestic use. (2). Access to clean water is not only essential for good health, but also adequate livelihoods, integrity, and the prospect of economic development and education. The lack of access to appropriate quantities of clean water contributes to human misery and the depletion of human potential, which is both ethically and economically indefensible (3).

According to the latest Global Burden of Disease Study, about 2.39 billion diarrheal cases occurred globally and an estimated 1.31 million deaths annually(4), with higher incidence and case-fatality ratios in lower and middle-income countries such as Africa and south-east Asia,(5). Unsafe drinking water also contributes to more than 25 million cases and 250,000 deaths annually of enteric diseases(6, 7). The burden is greatest in low-income populations with poor access to safe water, sanitation, and urgent medical care(8, 9).

More than 2.1 billion people worldwide lacked safe drinking water services, around 423 million used unimproved sources and 25% of the world's population consumed microbiologically polluted water, and 2.3 billion people went without basic sanitation. (10). Rural residents and those from deprived and disadvantaged communities are less likely to have access to better water and sanitation services and are less likely to enjoy on-site piped water(11).

In Africa, one-third of the population does not have access to clean water, and nearly two-thirds do not have access to sanitation, leading to widespread suffering from waterborne diseases that cause productivity losses (12). As demonstrated by repeated outbreaks of waterborne illnesses in both rural and urban areas, water quality is a major concern. In Ethiopia, where acute watery

diarrhea broke out in crowded and unsanitary conditions of urban and rural areas in 2015, millions of people are also believed to be at risk of cholera (13).

Although piped water is an important long-term solution for the provision of clean water, the implementation in rural areas of developing countries is very costly and challenging (14). It is also assumed that improving the quality of drinking water at the household level is effective in combating infectious diarrhea. (15). It is also reported that household-level water treatment can reduce diarrhea by 71 % (16).

To ensure that they do not pose a health risk to the user, all surface water and some groundwater need treatment before consumption. Microbiological, chemical, and physical contaminants may be responsible for health risks to consumers from low-quality water (17). Therefore, it needs to have some sort of treatment systems that are designed to remove these contaminants from raw water. Small-scale water supplies in rural areas around the world are the cornerstone of a potable water(18). Small scale water treatments are used in emergencies, temporary settlement areas, at the household level, and in areas where the municipality is not well organized is very important to reduce the problem of water-borne disease through the utilization of different methods of treatments (15).

Household water treatment methods include coagulation, flocculation, and sedimentation filtration (ceramic and biosand), chemical disinfection, solar water disinfection (SODIS), and combined methods that have appeared to be widely used for household water treatment techniques in developing countries including Ethiopia(7). In terms of efficiency, affordability, and future sustainability for microbial water treatment, chlorination has been among the most promising(19, 20). But, advanced water treatment methods have been developed to treat microbial and chemical contaminants from polluted water. These include treatment methods include ozonation, deep bed filtration, reverse osmosis, depth filtration, membrane filtration, conventional water treatments, and UV light (21). The drawbacks of these methods are that they are expensive and thus cannot be afforded by low-income rural people (22). Therefore, there is a need for the finding of cost-effective and locally available indigenous plants for household water treatment and this is the main objective of this work.

1.2. STATEMENT OF THE PROBLEM

Unsafe drinking water is a major concern, as 75% of all diseases in developing countries are caused by contaminated drinking water, especially in rural areas of developing countries (23). According to the Ethiopian Demographic and Health Survey (EDHS), access to safe water in the rural part of Ethiopia is improved 57%, unimproved 43%, and surface water 19%(24). Without access to clean water, people in Ethiopia rely on surface water sources, such as ponds, streams, and rivers, and most of them are located at great distances from their households (up to six hours in some rural areas), where women and children are heavily burdened.

In the background of developing countries, the use of advanced technology and various chemicals for their water treatment is challenging (25). Also, because a significant population of Ethiopia resides dispersed in rural areas, it is difficult for them to obtain a piped water system. The gradual replacement of such chemical coagulants with alternative coagulants, ideally from natural and renewable sources, is desirable for these reasons.

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Many effective coagulants of plant origin have already been identified. Some of the common ones include Moringa oleifiera, Maerusa Subcordata, Moringa Stenopetala, Hydrolyzed Cassava, Cactus, Hyacinth Bean peels, Opuntia dillenii and Opuntia ficus indica are some of the natural coagulants that have been used over the years in several parts of the world including Ethiopia (23, 26-31). Even so, there were few studies have been done in evaluating their efficiency of *M. Stenoptala,* there was no study evaluating the efficiency of *Opuntia Ficus indica cladodes* in removing turbidity and microbial contaminants, and there was no agreements among those researches on optimal dosage and pH. So the main aim of this study was to evaluate the comparative efficiency of *M. stenopetala and Opuntia Ficus Indica* in terms of turbidity and microbial load reduction.

1.3. THE RATIONALE OF THE STUDY

Water security is crucial to meeting the Sustainable Development Goals of the United Nations (SDGs). However, the world is rapidly facing water shortages, and an estimated 4 billion people do not have enough access to clean and secure water (32). Providing clean water is a fundamental need for human life, and to preventing water-borne diseases; however, in Ethiopia nearly half of rural households residents (43%) have no access to an improved source of drinking water, and in both urban (88%) and rural (92%) areas report that they do not treat their water before drinking (33, 34). Although piped water is an important long-term solution in providing safe water, it is very expensive and challenging to implement in rural areas of developing countries(14). Due to that, there are numerous deaths and illnesses caused by waterborne pathogens.

Therefore, there is a need to investigating the new approaches that are continually being examined: need to be durable, lower in overall costs, and more efficient in the removal of the contaminants. So, the main aim of this study was to find our affordable water treatment technology that enhances increased coverage to safe drinking water, and above all to promote the use of inexpensive coagulants that can be used in remote places where piped water is unthinkable, with additional benefits of preventing water-borne diseases in a community.

1.4. SIGNIFICANCE OF THE STUDY

It is known that so many diseases can be transmitted by drinking water contaminated by human or animals' excreta particularly faeces and physico-chemical contaminants: therefore, it is requiring treatment before consumption to ensure that they do not represent a health risk to the user. The uses of advanced water treatment technology in rural parts of Ethiopia is difficult hence, this study intended to evaluate the efficiency of natural plant coagulants for household water treatment

The finding of this study assumed that it generates information that can help different organizations and offices in the study area to provide and promoting locally available, cost-effective and safe household water treatment materials. It will provide a range of background information; case studies and lessons learned and will give ideas for addressing issues relating to small-scale water supplies' national programs. Also, the finding of this study can be used as baseline data for further study.

2. LITERATURE REVIEW

2.1 Plants under investigation

2.1.1 Morniga Stenopetela

Moringa is a drought-resistant and salt-tolerant multipurpose farm tree primarily cultivated in South Ethiopia. There are 13 species divided into three categories of morphology (stem structural forms), namely the slender, the bottle, and the tree or shrubs/bushes. Moringa stenopetala is indigenous to East Africa, connecting South Ethiopia, North Kenya, and West Somalia, a region characterized by unreliable rain and frequently affected by drought, and is known throughout Ethiopia by various vernacular names such as "Haleko" in Gofa, "Shelagda" in the Konso language and "Shiferaw" in Amharic(35)

M. stenopetala is mostly known for its importance as a nutritious vegetable food crop in the terraced fields of Konso, Ethiopia. In this way, it is similar to its Indian relative, *Moringa oleifera.* The leaves, flowers, and green pods of *M. stenopetala* are eaten as a staple vegetable and are rich in proteins and Ca, Fe, and P (35). *Moringa stenopetala* is a favorite and main component of the daily meal of the Konso, Gamo,and Gofa people in southern Ethiopia (36). In the Konso area,Moringa leaves are eaten almost every day like spinach together with cereal balls or prepared as Kurukufa (local name of food prepared with Shelagda). Shelagda is particularly important as human food because the leaves, which have high nutritional value, persist throughout the year, including the dry season when few other sources of green vegetables are available. Over 5 million people consume *Moringa stenopetala* as a vegetable(37).

M. Stenopetala is also used as a companion plant for shading capsicum and sorghum crops, and also in folk medicine. The clarification and purification of water to make it potable is another use. For coagulating materials in suspension, a powder produced by grinding the seeds is found to be more effective than the seeds of the closely related horseradish tree (M. oleifera), which is used for this purpose in India (35). This is due to the high content of a water-soluble cationic protein in Moringa seeds that is capable of minimizing turbidity. For water treatment, drinking water clarification, oil extracted from Moringa can be used. The use of natural coagulants as replacements for existing chemical coagulants, including aluminum sulfate and ferric chloride, is

followed in developing countries. One of the natural coagulants is the water-soluble extract of Moringa's dry seeds. The mechanism of coagulation by Moringa is not well understood and different authors have attributed it to existence of proteins and non-protein flocculating agents (38).

Several researchers have examined the extracts of seeds from the M. oleifera tree be one of the most powerful clarifiers(39, 40). Since the early 1970s, experiments have been performed to assess its water treatment efficiency after witnessing women in Sudan using their seeds to purify the turbid Nile water (41). From then onwards, M. Oleifera has been widely researched as a coagulant and disinfectant in the water treatment process (42, 43) both at the laboratory and full scale. Several studies have confirmed that M. Oleifera can both act as a coagulant and a disinfectant, therefore, replacing the commonly used aluminum sulphate. Few works of literature also found the efficiency of M. Stenopetala for water treatment at the optimal dose, PH, and temperature (26, 44). According to Amagloh and Benang (2009) mature seed extracts of *Moringa oleifera* are more effective in turbid waters than immature seed extracts.

Figure 1-The image of leave and seeds of M, Stenopetala

2.1.2. Opuntia ficus indica

Opuntia ficus-indica (L) Mill, commonly known as prickly pear because of its rounded cladodes with fixed spines and small hair-like prickles and its belongs to the family Cactaceae which contains about 130 genera and nearly 1500 species. It is widely distributed in Mexico and all American hemispheres as well as in Africa and the Mediterranean basin(45). In Ethiopia, one of the vernacular names of Opuntia ficus-indica is Papaldhotta (Konsigna), the name given by the community that uses this plant for the management of diarrhea and household water treatment (46). In drought conditions, when grasses and other forage crops are no longer edible, the Opuntia cactus remains green and is used as an emergency feed crop for ranging livestock and for other emergencies like firefighting and hidden places for community protection from enemies.

Fruits of Opuntia ficus-indica embrace several essential ingredients, such as taurine, amino acids, readily absorbable carbohydrates, minerals, vitamin C, and soluble fibers and serves as an important source of nutrients and food. They can be consumed as fresh vegetables, added to casseroles, cooked, canned, or used in salads, syrups, alcoholic drinks, fruit juices, and in cheese production (47). The fruit, as well as cactus stem, are used to prepare value-added products, such as jam, squash, wine, pickle, body lotions, shampoo, creams, etc (45). However, the application of this plant for water treatment is quite new in comparison with other natural coagulants like Moringa oleifera and Nirmali (48). A few studies have examined the use of the cactus as a natural biodegradable coagulant instead of those chemicals in the coagulation/flocculation process. So the main aim of this study is to evaluate the contaminant removal performance of *M. stenopetala and Opuntia Ficus Indica cladodes* in terms of turbidity and microbial load reduction.

Figure 2. The image of Opuntia Ficus Indica species (Captured by PI in Konso)

2.2 PH optimization

pH is the measure of the intensity of acidity or alkalinity of water. The WHO allowable limit of pH in drinking water range from about 6.5 to 8.5 is suggested. In the high PH range, water may deposit some of its mineral content (49).

During coagulation, the pH has a profound effect on efficacy during the period of destabilization. The pH regulates both the speciation and solubility of the coagulant and also influences the speciation of the contaminants. An excessive amount of coagulant may be needed for high alkalinity water to lower the pH to the optimal pH ranges (alum pH 6 to 7, iron 5.5 to 6.5) (50). Moa reported that the optimum pH for the extracts of *M. stenopetala and opuntia ficus indica* were pH = 8, with a maximum turbidity reduction of 84%. At a pH of 9, the turbidity reduction efficiency decreased to 19%(23). A similar optimum pH of 8 was previously reported for *M. oleifera(51).* However, as shown elsewhere, the maximum turbidity removal efficiency of other natural coagulants occurred at various pH levels. The optimum pH for turbidity removal (maximum reduction of 80%) using crude extracts of *M. oleifera* seeds was 6.5 (52). The turbidity removal efficiency of *J. curcas* was highest at an acidic pH of 3, with turbidity removal of 99% (53). The most appropriate pH for extracts of the *Phaseolus vulgaris* (common bean), *Castanea sativa* (chestnut), *Quercus robur* (acorn), and *C. latifolia* was 10 (54).

Similarly, extracts of *M. oleifera* and *Opuntia* sp. performed well at a pH of 8 or above (55). In kaolin suspensions, more negatively charged ions are present at a pH above 7, improving charge neutralization. In comparison, the kaolin particles are less negatively charged at a pH lower than 7, causing increased repulsion effects between poly-electrolytes and particles, decreasing the efficiency of coagulation (56). Differences in the experimental conditions (the nature of water, method of extraction solvent, etc.) and the particular coagulant used may be attributable to the presumably contradictory findings in the literature on the effect of pH in turbidity removal efficiency (57).

2.3 Dose optimization

One of the most important parameters considered, determining the optimal condition for the performance of coagulants in coagulation and flocculation is coagulant dose. The coagulant dosage suggests the concentration of M. stenopetala seed and Opuntia ficus indica cladodes extract in the water. This difference is important to note because a lot of the seed mass was separated during the filtration step when preparing the extract. Insufficient dosage or overdosing would lead to poor efficiency in flocculation. Therefore, to reduce the dosing cost and sludge formation, it is necessary to determine the optimal dose and also to obtain optimal treatment efficiency. The effect of coagulant doses (0.01 gm/L to 0.03 gm/L) on the removal of reactive and direct dyes using Moringa stenopetala and opuntia ficus indica coagulant and flocculation time is 30-45min(23, 26, 27, 40, 58).

Various literature studies have stated that moringa and Opuntia ficus indica species can minimize turbidity, although their performance varies. The necessary dosage depends on turbidity ranges, meaning that the required optimum coagulant dosage also increased as the initial turbidity of the water sample increased (26). According to Ambelu, *M. stenopetala* powder reduced turbidity both in synthetic and natural surface water by their optimum dose. In higher turbidity ranges than lower and medium turbidity waters, the reduction of turbidity by natural coagulants has been more effective and the reduction increases with increasing doses (23, 26, 27, 59).

The residual turbidity decreases within a certain dosage of natural coagulants, which is referred to as the optimized dose, resulting in increased turbidity over and above the optimum (60). For instance, the result of Benti et al showed the extracted dose from *M. stenopetala* was ranged

from 0.01 gm/L to 0.03 gm/L and turbidity reduction efficiency ranges 72 % to 98.55 %, and for very high turbidity the optimum dose found for initial turbidity of 500 NTU and 1000 NTU was 0.07gm/L removal efficiency 99.41% and 99.01% which was the similar finding by Megersa., (23, 26). Increasing the coagulant dose above a certain limit does not enhance the removal of turbidity; in fact, this has significantly increased the residual turbidity of the coagulated sample(39). Muyibi and Evison (61)explained this as overdosing resulted in the saturation of the polymer bridge sites and caused destabilization of the destabilized particles due to an inadequate number of particles to create more interparticle bridges Researches indicated that Plant coagulants even showed a better coagulation effect than synthetic coagulant counterpart e.g Alum (59, 62).

Scholars also found that *M. stenopetala* has also antimicrobial properties; the seeds of the tree are used to clarify muddy water(35). According to Megersa et al, M. stenopetala showed excellent results in microbial load reduction (total coliform, fecal coliform, E. coli, and heterotrophic bacteria) was about 99.9 % of microbial load removal(63). Benti also observed the same result with the optimal dose of 0.01 gm/L to 0.07 gm/L for both natural and synthetic water after treating with M. stenopetala coagulants and there is also no any literature found they have an impact on physic-chemical properties of water(23).

Works of literature also show that the extracts of Opuntia Ficus Indica(OFI) had an outstanding flocculation capacity and the optimal dosage was vary from 3mg/l to 80mg/l with a turbidity removal efficiency of 63% to 98% (29, 30, 64, 65). One study conducted in Mexico indicated that OFI reduced turbidity by 98% for a range of initial turbidities at a PH of 10 using synthetic water(55). Another study which was used wastewater shows that the efficient removal of the turbidity is 93.09%, but the obtained water is still considered slightly cloudy water with optimal concentration a variable dose of the coagulant was added (ranging from 0.3 to 2 g L_1). Each dose is added into the beakers and is mixed at a high speed of 100 rpm for 2 min(66).

The effect of different operating parameters such as initial turbidity, pH, different salts, storage time, storage condition, and dosages was also studied by some scholars (31). The study showed that the initial turbidity had a significant effect on the determination of optimum dosages, and coagulant dosages from the lowest dosage of 10 mg/l to the maximum 60 mg/l showed

significant turbidity removal at low to medium initial turbidity of model turbid water and an optimum dose of 80 mg/l was obtained with 98% of turbidity removal. On the other way factors such as natural properties of a solution such as pH and electrolyte directly affect the behavior of both turbid water and bio coagulant and their removal efficiency of bio-coagulant showed that the maximum turbidity removal of 98% and minimum turbidity removal of 79% were obtained at pH=7 and pH=10, respectively(67).

Since the bio-coagulant consists of a wide variety of sugar and various organic compounds, leading to enzymatic attacks, it appears to degrade over time. To observe the effect of this degradation on its coagulation efficiency with time, the observer performed coagulation/flocculation experiments for up to 60 days and the results were stated that turbidity removal was greater than 90% after 7 days and more than 80% even after 15 days. After 20 days of storage, a dramatic decrease in turbidity removal was observed and a plateau was reached with turbidity removal of 40 percent after one month. Also, no improvement in turbidity removal was observed for up to 60 days, and decreases in efficiency over time could be due to enzymatic polysaccharide degradation(68). So the main aim of this study is to evaluate the comparative contaminant removal performance of *M. stenopetala and Opuntia Ficus Indica* in terms of turbidity and microbial load reduction.

Conceptual framework

The conceptual framework for this study shows how the particular variables in the study connect and identify the variables required in the research investigation. This was developed after reviewing different literature about factors that have been contributing to the efficiency of natural coagulants for household water treatment such as dosage, PH, the shelf life of seeds, characteristics of water, agitation speed, and straining period. This conceptual framework tells us the boundary of the topic, used for selecting appropriate data collection methods, for data analysis, also as a referent point for the discussion of the literature, methodology, and results. It also is seen as an abstract representation of the relationship between the study variables.

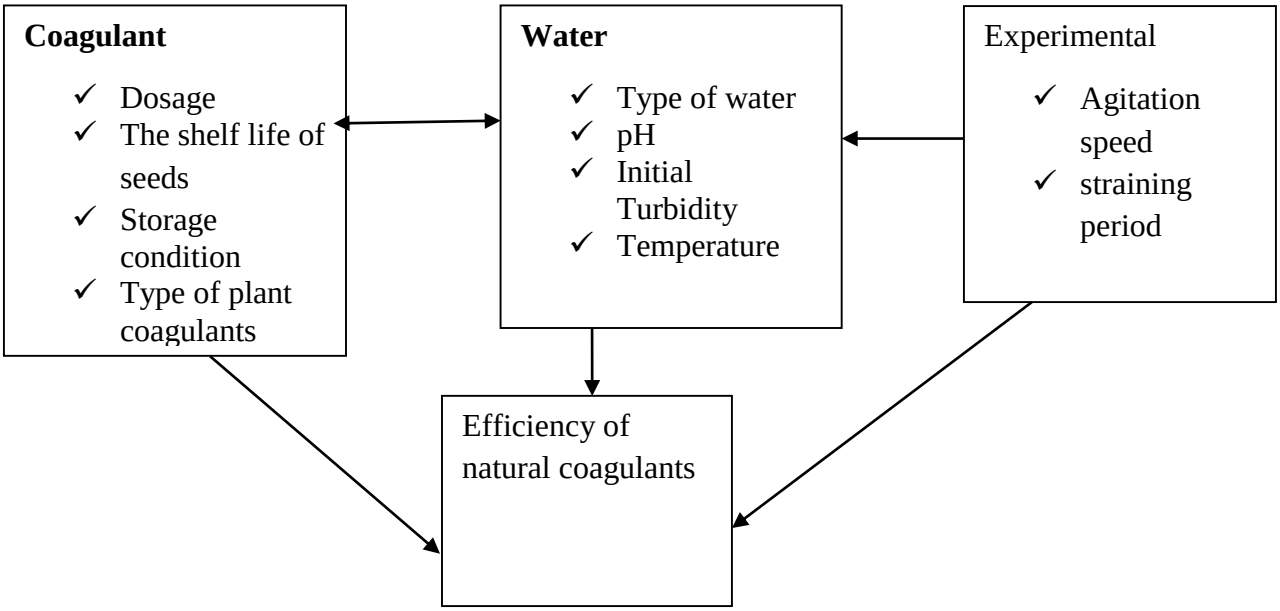

Figure 3- Conceptual framework of factors that determining the efficacy of M. Stenopetala and Opuntia Ficus Indica as natural coagulants for household water treatment (23, 26-31, 53, 58)

3. Objective

3.1. General objective

To evaluate the efficiency of Moringa Stenopetala and Opuntia Ficus Indica for household water treatment

3.2. Specific Objectives

- To determine the optimum dosage of natural coagulants
- To determine the optimum PH of natural coagulants
- To determine the physic-chemical and microbial quality of the resulting water

4. Methods and Materials

4.1. Study design and period

A laboratory-based experimental study was conducted from August to October 2020 at the laboratory of the Jimma University Environmental Health Science and Technology Department.

4.2. Materials and chemicals

Jimma University Environmental Health laboratory protocol was used and based on this protocol the materials, and chemical are included analytical scales, refrigerator, and oven, measuring cylinder, blenders, sieves, desiccators, Petri dish, stir bar, pipettes, Erlenmeyer flask, evaporator, filter paper, mortars, pestles, spatula, and tong. Materials were used in this study are the M. Stenoptala seed and Opuntia ficus indica, distilled water, technical ethanol 70%, sodium thiosulphate, sodium hypochlorite, MacConkey agar, M-FC Broth, eosin ethylene blue agar sulfuric acid, sodium hydroxide, EDTA, Eriochrome Blank T, calcon, potassium dichromate, silver nitrate, phenoldisulfonic acid, ammonium hydroxide, hydroxylamine hydrochloride, phenolphthalein indicator, mixed bromocresol green indicator, and others(69).

4.3. Preparation of native plant (m. stenoptala) coagulants

The plants used traditionally for water purification (seed of *M. stenopetala)* by the local community was collected from selected rural areas found in SNNPR (Konso, Jinka, and Arbaminch). The plant materials were identified by comparison with the already preserved specimens kept in the laboratory, and the husk from the seeds of *M. stenopetala* was first removed manually and good quality seeds were selected for the experiment. The identified mature dried seeds were prepared by soaking them in distilled or piped water for an hour and washed by distilled water then it was dried in outdoor temperature for three days. Afterward, the seeds were powdered using a mortar and pestle or coffee powdering machine and pestle then grained by plant grinder with the pore size of 212 micrometer in diameter to make it similar size and stored in sterilized container for future use.



Figure 4The image of the seeds of M,stenopetala and powdering mechanism

4.4. Preparation of native plant (opuntia ficus indica cladodes) coagulant

After collecting the cladodes (flattened stems) of prickly pear fruit or opuntia ficus indicas, and they were brushed for two minutes underwater. The skin and flesh were removed from each other and the powder was prepared by soaking into the water for an hour and washed distilled or piped water then will be dried in an outdoor temperature on the top of house for three days. Afterward, powdered using a mortar and pestle then grained by plant grinder with the pore size of 212 micrometer in diameter to make it similar size and stored in sterilized container for future use.

4.5. Water sampling

The experimental study was carried out in the laboratory of Jimma University Environmental Health Science and Technology Departments. The natural surface water samples were collected in or around Jimma. In this study, four turbidity ranges were considered for the experiment, namely; low turbidity of Awetu (32.1 NTU), medium turbidity of Kito (78.1 NTU), high turbidity of Gibe (132 NTU) and very high turbidity (378NTU) considering the turbidity ranges used by Miller *et al.* (2008).The turbidity of the sample was measured before the experiment using a multi-parameter instrument. The samples were divided into six: two samples were used as a control (one was turbid water without coagulants and positive control or Alum) and the other four samples were with different doses of coagulants of *M. stenopetala, and Opuntia Ficus indica* to test their performance in coagulation and microbial reduction.

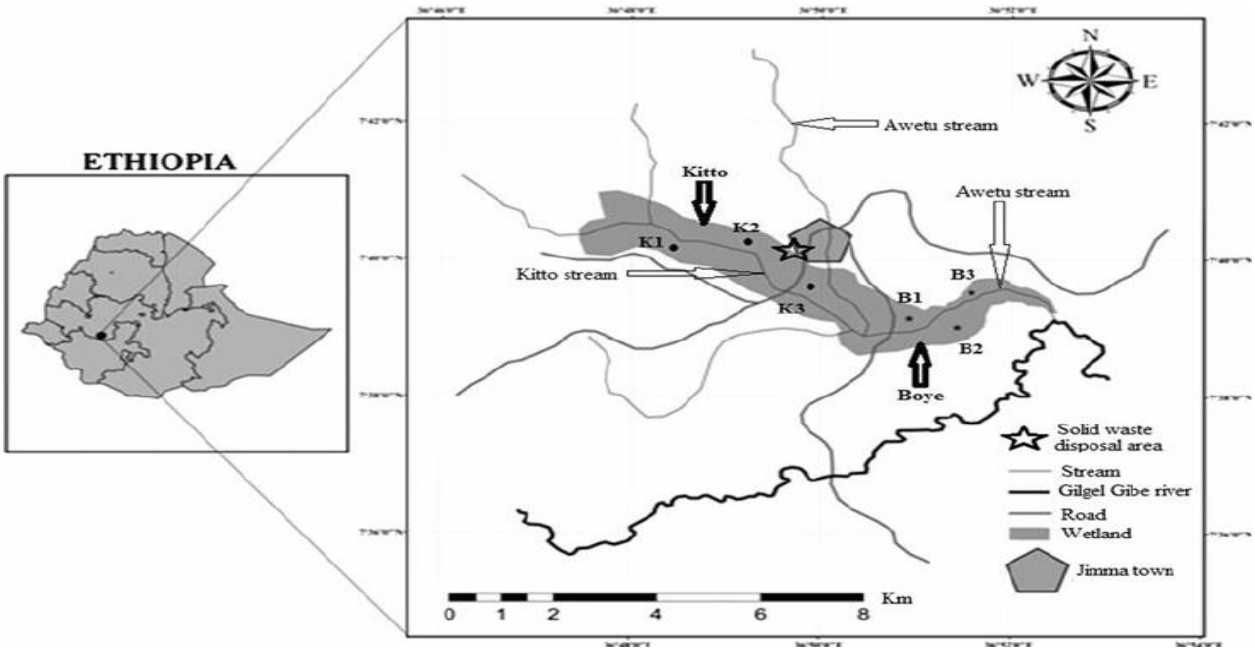

Figure 5: Location of map of the study rivers in Jimma City (Awetu, Kitto, Boye and Gibe rivers),

Figure 6: Sample water collection at Awetu river (Captured by PI)

4.6. Jar Test Operation

The most commonly used laboratory technique for coagulation is the jar test. Throughout the experiment with powder coagulants of native plant species, a traditional jar test apparatus was used to achieve a standardized agitation rate. It was done as a batch test, accommodating a series of six beakers and six-spindle steel paddles together. We used both positive (alum-treated turbid water) and negative (coagulant-free turbid water) controls. The other four samples were treated with different doses of coagulants of M. stenopetala and Opuntia fics indica with a dose range of 10 to 100 mg/l for M.stenopetala and from 50mg/L to 0.5gr/L for OFI cladodes. Before operating the jar test the natural surface water sample was mixed homogeneously. Then, the water samples ought to be measured for physicochemical; to represent an initial concentration. After the desired amount of coagulant is added to the water sample, agitation was taking place, which consisted of (170 rpm) for two minutes followed by 40 rpm for 20 min. After the agitation was stopped, the suspensions were allowed to settle first for 30 minutes, and an effective dose at which the maximum turbidity removal achieved was recorded for three hours at a constant interval of 30 minutes. The supernatant of the water sample was withdrawn using a pipette from the middle of the beaker for Physico-chemical parameters measurement (turbidity, pH, conductivity, and temperature) after treatment. All tests were performed at room temperature in the range of 20–25 °C and for different turbidity ranges. The removal efficiency of the analyzed parameters was determined by the formula below:

$$\text{Removal parameter in percentage} = \frac{100(Ci - Cf)}{Ci}$$

- ***Ci-*** represents the concentration of the parameter in the raw water.
- ***Cf-*** represents the concentration of the same parameter in the treated water.

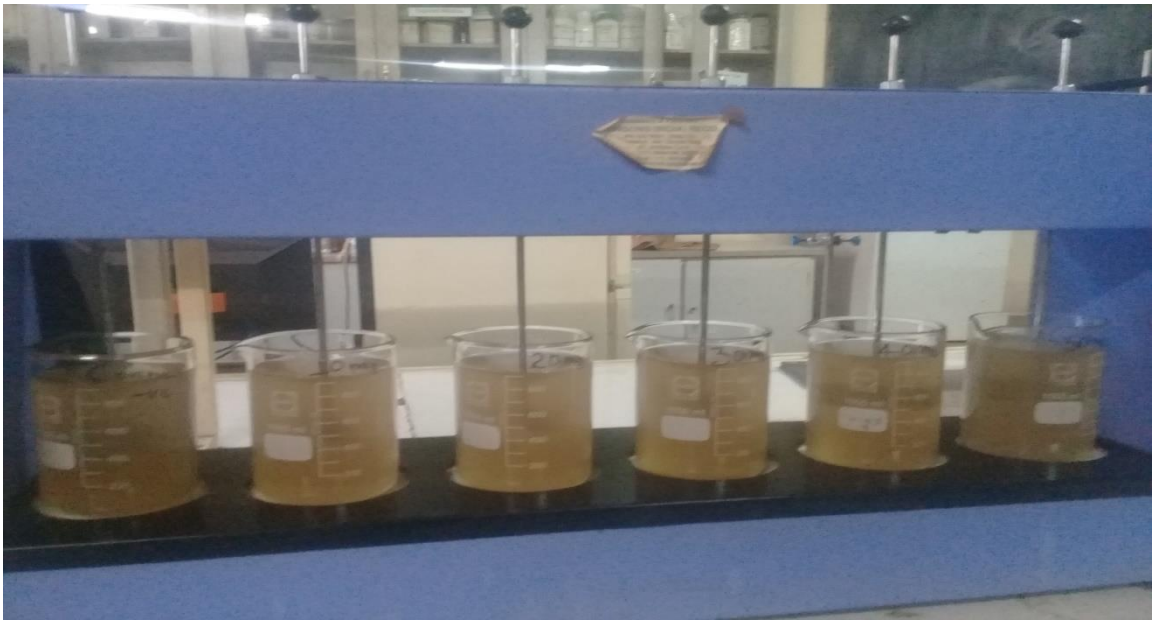

Figure 7: The image of Jar test (captured by PI at the laboratory)

4.7. Microbial culture test

The samples were serially diluted up to 10-3 mg/l for natural surface water. Then, 0.1 ml of each diluent of 10-1 to 10-3 mg/ l was plated aseptically onto nutrient MacConkey agar for total coliform, M-FC Broth for fecal coliform, and eosin ethylene blue agar for Escherichia coli counts following the standard protocols as described by [36]. Incubation was carried out at 37 °C for 24 h for total coliforms and 44.5 °C for 24 h for fecal and E. coli, and the plate's readings were performed following standard microbiological procedures of Jimma University, and APHA (2005).

4.8. Sampling and analysis of treated water

Filtrate sampling and analysis were carried out following the standard methods and procedures (APHA, 2005). By using this procedure sample was taken after treatment. All reagents and chemicals were used of analytical grade and distilled de-ionized or piped water was used in all preparations and analyses. The pH, electrical conductivity, and temperature were measured using a multi-parameter prob. Turbidity was measured using a portable turbidity meter. Total coliform, fecal coliform, and E. coli of water samples were tested using a membrane filtration technique following standard protocols APHA, 2005.

4.9. Operational definitions

The optimum dosage -is the minimum dosage corresponding to the lowest residual turbidity value of <5NTU

Efficiency- turbidity removal abilities of natural coagulants

4.10. Quality control

The procedure of the experiments was done consistently throughout the whole study to minimize the sources of error and all equipment calibrated. Standard procedures were prepared and followed strictly to assure quality assurance of laboratory investigation. Replicate analysis of each parameter was done following the standard protocol to get a satisfactory result. For microbiological analysis of the filtrate, controls were used for each analysis.

4.11. Data management

During the lab experiment period, the principal investigator checked for completeness and consistency laboratory results registration forms through a close follow up of the procedures. The data were entered into Microsoft excel registration forms, and cross-tabulation was done to check the completeness and accuracy of the entered data and then the data was exported to statistical package for social sciences (SPSS) version 25 for analysis.

4.12. Statistical Analysis

Results were expressed descriptively. The experimental results were analyzed using the Excel software or Statistical Package for Social Sciences (SPSS), version 25 a. The analyzed data was presented using tables and figures.

4.13. Ethical consideration

Before starting the study, ethical clearance was obtained from the research ethics committee of Addis Ababa University School of Public Health. A formal letter was written to all concerned bodies and permission was secured at all levels.

4.14. Dissemination of findings

After data analysis and interpretation, the necessary information was disseminated to concerned bodies such as the School of Public Health, Addis Ababa University Health Science Library, Konso Zone Health office, and research publication office.

5. RESULTS

Four natural surface water samples were collected from (Awetu, Kito, Boye, and Gibe) rivers. The initial turbidity, pH, conductivity, and temperatures of the sampled waters were measured before the experiment. The results revealed that the initial turbidity value of all sampled water ranged between 32.1 in Awetu to 378 in Boye, the pH value ranged from 7.86 to 8.49. The results of the conductivity measured at (µs/cm) also ranged from 71.5 in Awetu to 152.8(µs/cm) in Kito water sampled, the temperature was ranged from 20.6 to 24.2 while the microbial loads were present in highly in all samples.

Table 1-The Physicochemical and biological characteristics of river water

Parameters of natural water	Surface water sample site name			
	Awetu	Kito	Boye	Gibe
Turbidity (NTU)	32.1	78.1	378	132
Electric conductivity (µS/cm)	71.5	152.4	116.5	92.4
pH	8.31	7.86	7.92	8.49
Temperature (°C)	20.5	22.9	24.2	22.9
TC(Colony count per 100ml)	$367x10^2$	$330 x10^2$	$556 x10^2$	$254 x10^2$
FC(Colony count per 100ml)	$137 x10^2$	$127 x10^2$	$338 x10^2$	$72 x10^2$
E.coli(Colony count per 100ml)	$87 x10^2$	$107 x10^2$	$143 x10^2$	$54 x10^2$

Note.TC total coliform, FC fecal coliform, NTU nephelometric turbidity unit

5.1. The efficiency of natural coagulants on the turbidity of river waters

The turbidity removal efficiency of powder of Moringa stenoptela seeds and Opuntia ficus indica cladodes in river water was tested and has been checked with that widely used inorganic chemical alum. The optimum dosage was identified after 30 min to 3 h of settling time with an interval of 30min measuring time, the removal efficiency of the M. stenopetala seeds and cladodes of opuntia ficus indica was slightly different, and with an increase in the contact time, up to 3h to 12h, the turbidity removal efficiency of M. stenopetala plant coagulants was nearly as effective as that of alum at all doses however, it was impossible to achieve 5 NTU using opuntia ficus indica cladodes. Most of the suspended particles were settled within 30min, and afterward the effect somewhat limited, this may because of large particles was removed within short period of time, and small particles was settling slowly

The seed powder of M .stenopetala reduced the turbidity of Awetu river by 87%. 85.76%, 83.98%, 83.73% and 82.58% at optimum dose of 20mg/l, 30mg/l, 40mg/l, 50mg/l and 60mg/l respectively. It was very efficient at 20mg/l with a turbidity reduction of 87%. The powder of Moringa stenopetala reduced turbidity from 32.1 to 4.1nephelometric turbidity unit (NTU). The percentage reduction relative to negative controls at an optimal dose was 71%, and 40.3% relative to positive control as shown in table 2.

Table 2-The turbidity removal efficiency of M.stenopetala powder on Awetu river water with initial turbidity of 32.1NTU

Name of River	Dosage	Initial turbidity (NTU)	Settling time					
			30min	1h	1.5h	2h	2.5h	3h
Awetu	10mg/l	32.1	10.1	10.01	10.0	9.8	9.5	8.95
	20mg/l	32.1	6.19	5.8	4.95	4.6	4.15	4.1
	30mg/l	32.1	13.51	8.71	6.76	6.5	6.0	4.57
	40mg/l	32.1	12.4	8.5	8.23	7.85	6.99	5.14
	50mg/l	32.1	12.8	11.06	10.29	7.87	7.13	5.22
	60mg/l	32.1	14.06	14.33	8.40	6.95	5.8	5.59
	70mg/l	32.1	16.18	11.80	10.31	8.49	6.99	6.84
	80mg/l	32.1	19.8	11.25	11.00	9.30	8.80	8.25
	90mg/l	32.1	13.50	12.6	9.36	7.37	7.42	6.47
	100mg/l	32.1	13.71	13.63	10.69	10.6	8.12	7.81

					7		
-ve control	32.1	28.7	28.3	28.2	27.6	27.2	27.01
+ve control	32.1	19.8	18.20	18.01	18.1	17.7	17.1

On the other hand, the Opuntia ficus indica Cladodes reduced the turbidity of Awetu river from 32.1 to 9.81 NTU, which it was reduced the turbidity by 68% at an optimal dose of 50mg/l as shown in figure 8. Although significant turbidity reduction is observed in both plants, the turbidity reduction efficiency of M.stenopetala was greater as compared to that of the opuntia ficus indica cladodes and alum.

The turbidity removal efficiency of M.stenopetala and OFI cladodes at the optimum dosage of 20mg/l and 50mg/l, respectively on Awetu river as comparing with alum and negative control are shown in figure 7 below.

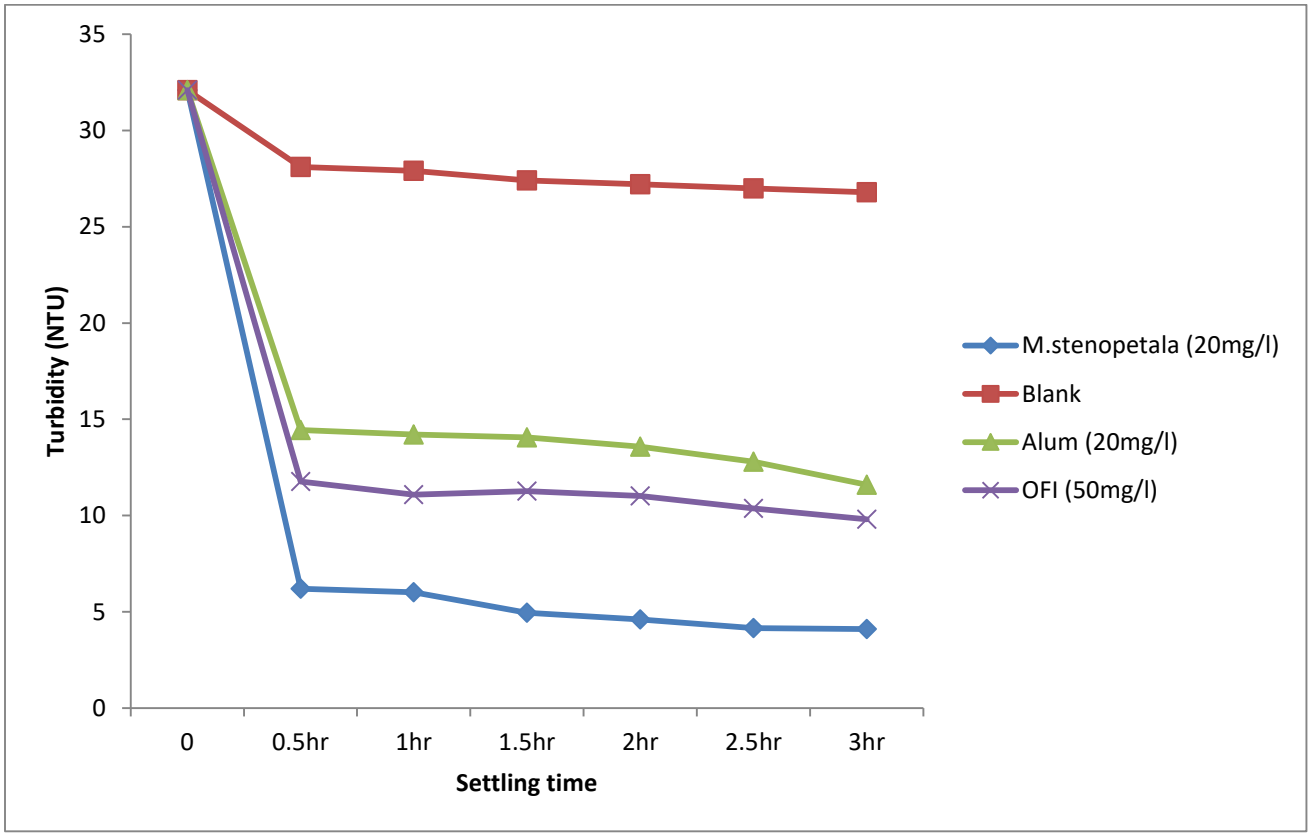

Figure 8: Turbidity removal efficiency of M.stenopetala at the optimum dosage of 20mg/l on Awetu river water at initial turbidity of 32.1NTU

Kito River had initial turbidity of 78.1NTU, and M.stenopetala seed was efficient from 20mg/l to 80mg/l, and very efficient at the optimum dose of 40mg/l, and they reduced the turbidity to 4.15NTU (94.68%), while cladodes of opuntia ficus indica reduced the turbidity to 10.31NTU at the optimal dose of 100mg/l.

Table 3-The turbidity removal efficiency of cladodes of Opuntia Ficus Indica powder on Kito river water

Places		Initial turbidity	30min	1h	1.5h	2h	2.5h	3h
Kito	10mg/l	78.1	42.3	38.04	36.0	32	28.04	26.44
	30mg/l	78.1	39.4	34.2	32.1	27.7	24.01	22.6
	50mg/l	78.1	18.70	17.09	16.38	15.34	14.72	12.01
	100mg/l	78.1	13.6	12.51	11.75	11.31	10.77	10.31
	150mg/l	78.1	15.8	14.40	14.30	13.69	12.84	12.46
	200mg/l	78.1	17.3	16.92	15.50	14.40	13.61	13.28
	250mg/l	78.1	22.10	19.60	18.07	17.80	16.56	15.13
	500mg/l	78.1	26.96	20.50	18.86	18.04	17.7	17.04
	-ve control	78.1	73.1	72.8	70.4	70.1	69.3	68.8
	+ve control (100mg/l)	78.1	4.42	4.38	4.34	4.30	4.28	4.24

The turbidity removal efficiency of M.stenopetala and OFI powder on Kito river water at the optimum dose of 40mg/l and 100mg/l on Awetu river were presented in figure 8 below.

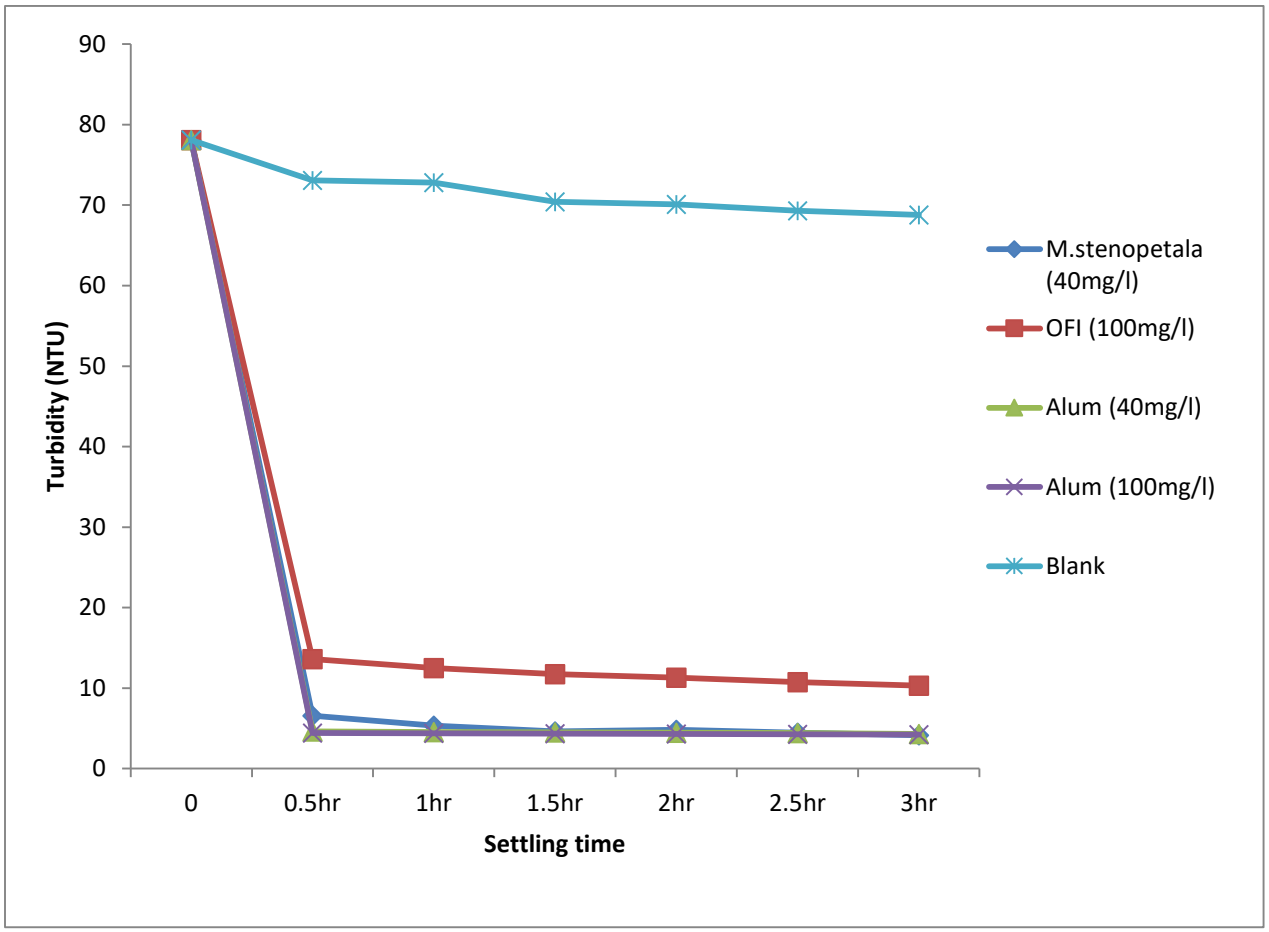

Figure 9: Turbidity removal efficiency of M.stenopetala (40 mg/l)& OFI (100mg/l) on Kito river water at initial turbidity of 78.1NTU

The average percentage turbidity removal for Boye river water by M. stenopetala, and cladodes of Opuntia ficus indica powder were 98.99%, and 96.01% for initial turbidity of 378NTU at the optimum dose of 70mg/L and 500mg/L respectively. The maximum turbidity removal efficiency of this study was observed in Boye river water. M.stenopetala reduced the turbidity of Boye to 3.61NTU at the optimum dosage of 70mg/l, which was more efficient than the same dose of alum, whereas Opuntia ficus cladodes decrease the turbidity 378 NTU to 15.08NTU.

Table 4-Turbidity removal efficiency of M.stenopetala at the optimum dosage of 70mg/l on Boye pond water

Sampling site		Settling time						
Boye	Dosage	0.5hr	1h	1.5h	2h	2.5h	3h	14hr
	10mg/l	119	114	121	120	98	97.2	87.6
	20mg/l	38.6	38.0	36.7	36.7	35.1	34.02	28.7
	30mg/l	9.94	8.86	8.24	8.20	7.69	5.52	3.75
	40mg/l	5.74	4.74	5.11	4.30	3.99	3.94	3.80
	50mg/l	5.42	4.91	4.69	4.16	4.10	3.86	3.75
	60mg/l	8.45	6.78	5.71	5.31	4.97	4.72	4.07
	70mg/l	5.82	4.56	4.35	4.24	4.14	3.81	3.61
	80mg/l	5.19	4.86	4.74	4.56	4.40	3.98	3.67
	90mg/l	6.09	5.26	4.97	4.90	4.87	4.83	4.72
	100mg/l	6.28	6.23	6.03	5.99	5.58	5.02	3.68
	-ve control	321	314	303	302	297	289	232
	+ve control (70mg/l)	49.2	49.0	44.0	42.3	40.2	39.8	36.3

The turbidity removal efficiency of M.stenopetala (70mg/l) and opuntia ficus indica (0.5g/l) on Boye river water with initial turbidity of 378NTU was shown in the figure below

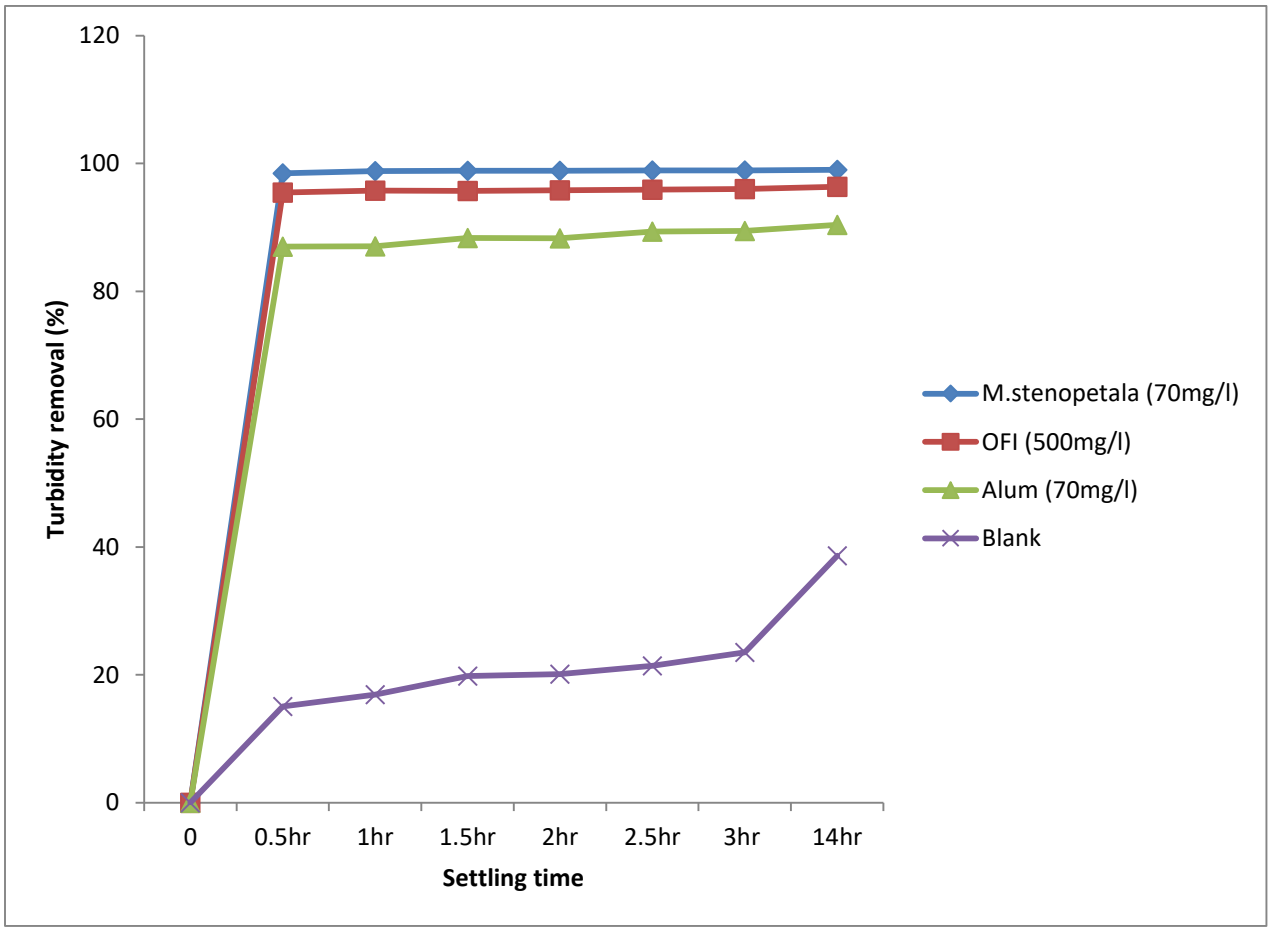

Figure 10 : The turbidity removal efficiency of M.stenopetala (70mg/l) and Opuntia ficus indica (0.5g/l) on Boye water

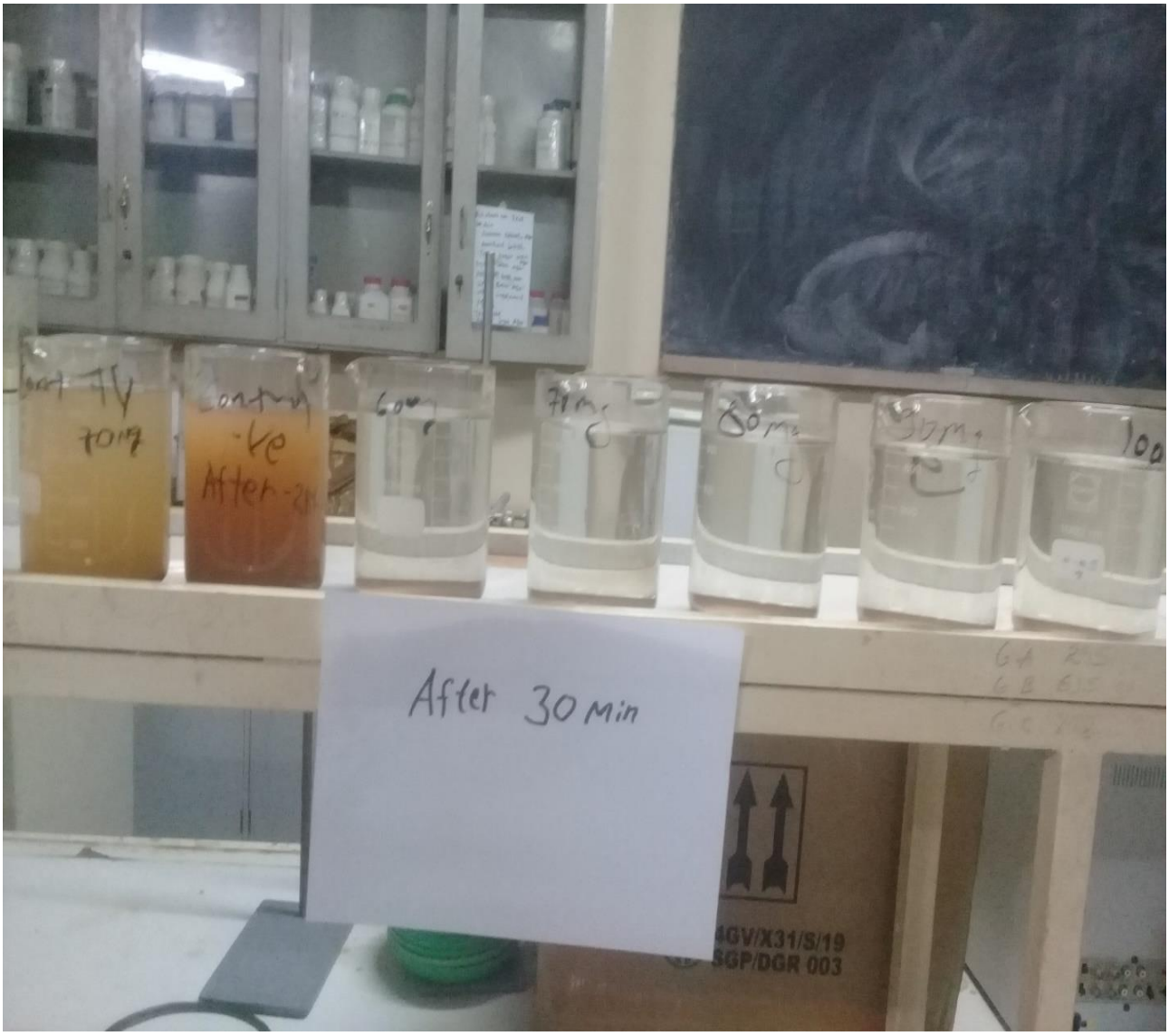

Figure 11: Resulting water after treating with 70mg/l of M.stenopetala on Boye water

The optimum dose found for Gibe river with initial turbidity of 132NTU was 50mg/l and 100mg/l for both M.stenopetala and opuntia ficus indica cladodes powder with a turbidity removal efficiency of 96.77% and 92.90% respectively.

Table 5-The results of Gibe river after treated with moringa stenopetala seed with initial turbidity of 132NTU

Places		Initial	30min	1h	1.5h	2h	2.5h	3h
Gibe	10mg/l	132	18.92	17.34	17.06	16.79	15.54	14.97
	20mg/l	132	12.43	12.08	11.65	11.28	10.43	9.02
	30mg/l	132	9.03	8.84	7.50	6.96	6.57	5.86
	40mg/l	132	7.47	7.21	6.16	5.68	5.46	5.25
	50mg/l	132	5.43	5.03	4.82	4.54	4.26	4.12
	60mg/l	132	6.92	6.38	6.16	5.82	5.28	4.94
	70mg/l	132	8.07	7.93	7.66	7.18	6.62	5.72
	80mg/l	132	12.04	11.89	10.52	9.20	7.23	6.03
	90mg/l	132	12.43	11.54	10.98	9.22	8.20	7.29
	100mg/l	132	12.38	12.04	11.56	10.28	9.45	8.05
	-ve control	132	128	128	126	124	122	122
	+ve control (50mg/l)	132	6.44	6.26	5.74	5.31	5.18	4.92

The powder of the pants reduced turbidity from 132NTU to 4.12 and 8.41nephelometric turbidity units (NTU). The percentage reduction relative to negative controls at an optimal dose was 89.2% and 85.53% as shown in figure11. However, M.stenopetala was almost equally effective as with both 50 and 100mg/l doses of alum

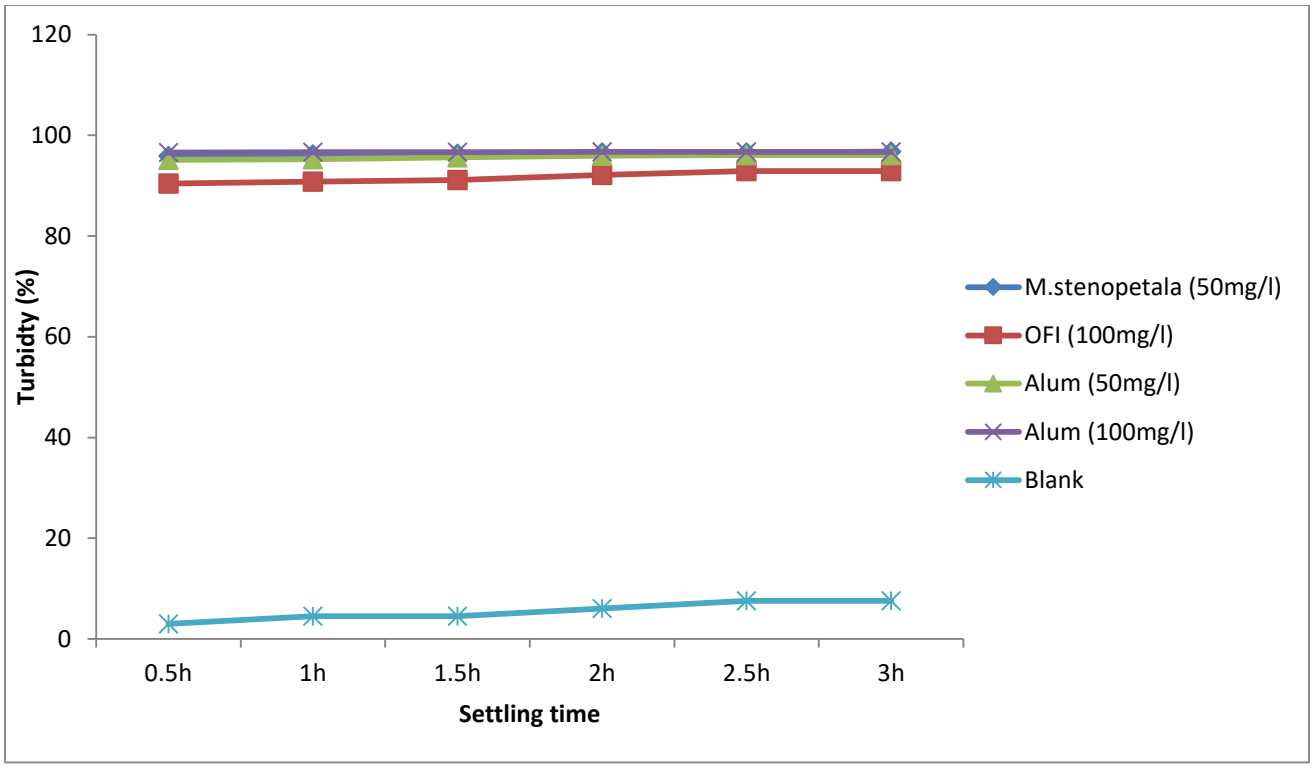

Figure 12 : The performance of natural coagulants on Gibe River at the optimum dosage of 50mg/l and 100mg/l

5.2. Effects of water pH on coagulation

The effect of pH was evaluating by adding Hcl and NaOH into the water to attain the pH we wanted. The turbidity removal efficiency of M. stenopetala and cladodes of Opuntia ficus indica were examined in surface water, with the pH varying from 5 to 10. As it can be seen from (Fig. 12) that the bio-coagulant showed significant turbidity removal almost over the entire pH range, but it was slightly lower at alkaline pH. The least reduction in turbidity removal was observed at basic pH for both of M. stenopetala and Opuntia ficus indica (Fig. 13). The optimum pH for the M. stenopetala was from pH=5 to 9, with turbidity reduction efficiency was from 98.65% to 99.05%, and the maximum turbidity reduction was at pH=8, which with almost the same as the actual pH (7.92) of water. At pH of 10, the turbidity reduction efficiency decreased to 94.55% (20.58NTU).

The optimum pH Cladodes of Opuntia ficus indica was pH = 7, with a maximum turbidity reduction of 96.66%. At a pH of 10, the turbidity reduction efficiency decreased to 87.77% (46.2NTU).

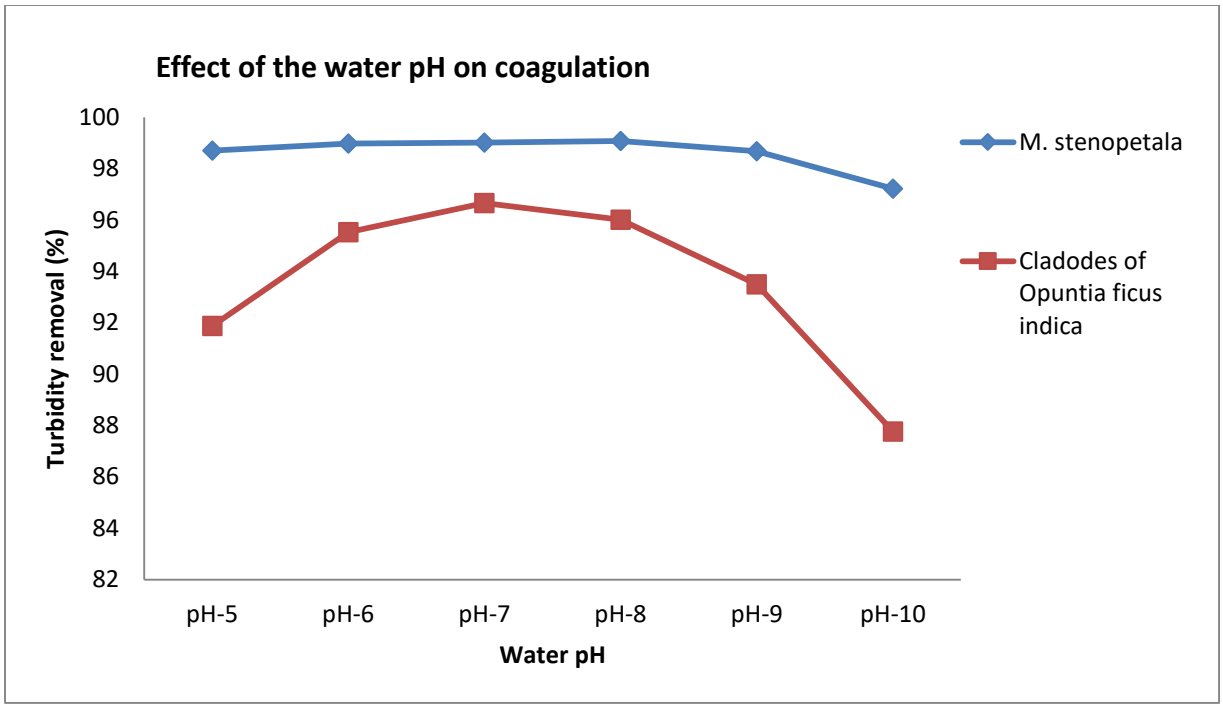

Figure 13 : The effect of the water pH on the efficiency of natural plant coagulants

Figure 14: The effect of pH on the performance of natural coagulants

5.3. The efficiency of natural indigenous plant coagulants as a disinfectant

With regards to microbial results, the colony counts were drastically decreased with both M. stenopetala and Cladodes of Opuntia ficus indica powder treatments for natural surface water (Table 6). As the results of average colony count of bacteria showed there was a slight difference between M. stenopetala and Cladodes of OFI powder treatment concerning all types of bacteria (Total coliform, feacal coliform, and E. coli bacteria), and M. stenopetala (99.99%) was almost the same as chlorine treatment in natural surface water. At present, more than 99% of microbial load removal was observed after treating the water using these two coagulants. The percentage microbial load reduction after treatment with M.stenopetala and Cladodes of OFI was ranged from 99% to 99.99% for the first 0.5 hours.

A higher percentage of microbial elimination could be observed for lower turbidity (99.99%) than higher-turbidity levels (99 %). This higher percentage of microbial load removal from low turbidity water than high-turbidity water could be due to the increment of suspended particles in high-turbidity water which protect microbes from the action of extracts.

Table 6- Microbial load reduction of natural coagulants on surface water

River	Initial microbial load (cfu x10 ^{2}/100ml)			Microbial load after treatment (cfu/100ml)											
				Negative control cfu x10 ^{2}/100ml)			M.stenopetala			Cladodes of OFI			Positive control		
	TC	FC	EC	TC	FC	EC	TC	FC	EC	TC	FC	EC	TC	FC	EC
Awetu	367	137	87	352	126	86	0	0	0	3	1	0	2	0	0
Kito	330	124	107	323	117	104	1	0	0	8	5	2	0	0	0
Boye	556	338	143	495	321	136	3	1	1	13	2	6	5	3	1
Gibe	254	72	54	244	67	53	0	2	0	4	1	2	0	0	1

pH of the medium is 7.01 for TC=total coliform, 7.02for FC=fecal coliform, 7.3 for EC=E.coli , (Checked before sterilization)

5.4. Physic-chemical quality of treated water/ effects of the coagulants on physic-chemical quality of water

The pH, temperature, and electric conductivity of the water after treatment using the effective doses of the two coagulants was not significantly changed, and it ranged between 7.8 to 8.42, 23.9 0C to 25.7 0C, and 83.9 (µS/cm) to 165.5(µS/cm) respectively which shows almost neutral. However, the same river water treated with alum decreased the pH from 8.31 to 5.18 and from 8.49 to 5.20 which make such treated water strongly acidic. When dissolved in water, the aluminum ions are hydrolyzed and it lowers the pH by increasing the concentration of H+. Most likely, the naturally occurring coagulants from plant materials possess a buffering property.

5.4. Effect of the storage duration and conditions on coagulation

After the seeds were powdered using a coffee machine or mortar and pestle and then, stored in sterilized in both open and closed containers at room temperature and 4°C for up to a month. Storage for up to a month at 4°C resulted in no significant differences in the turbidity reduction efficiency.

The results of storages were ranges from 89% to 99.22% when treated with M. Stenopetala, and from 69% to 97.08% after treated with OFI cladodes, which was slightly incremented turbidity removal potential in both open and closed containers.

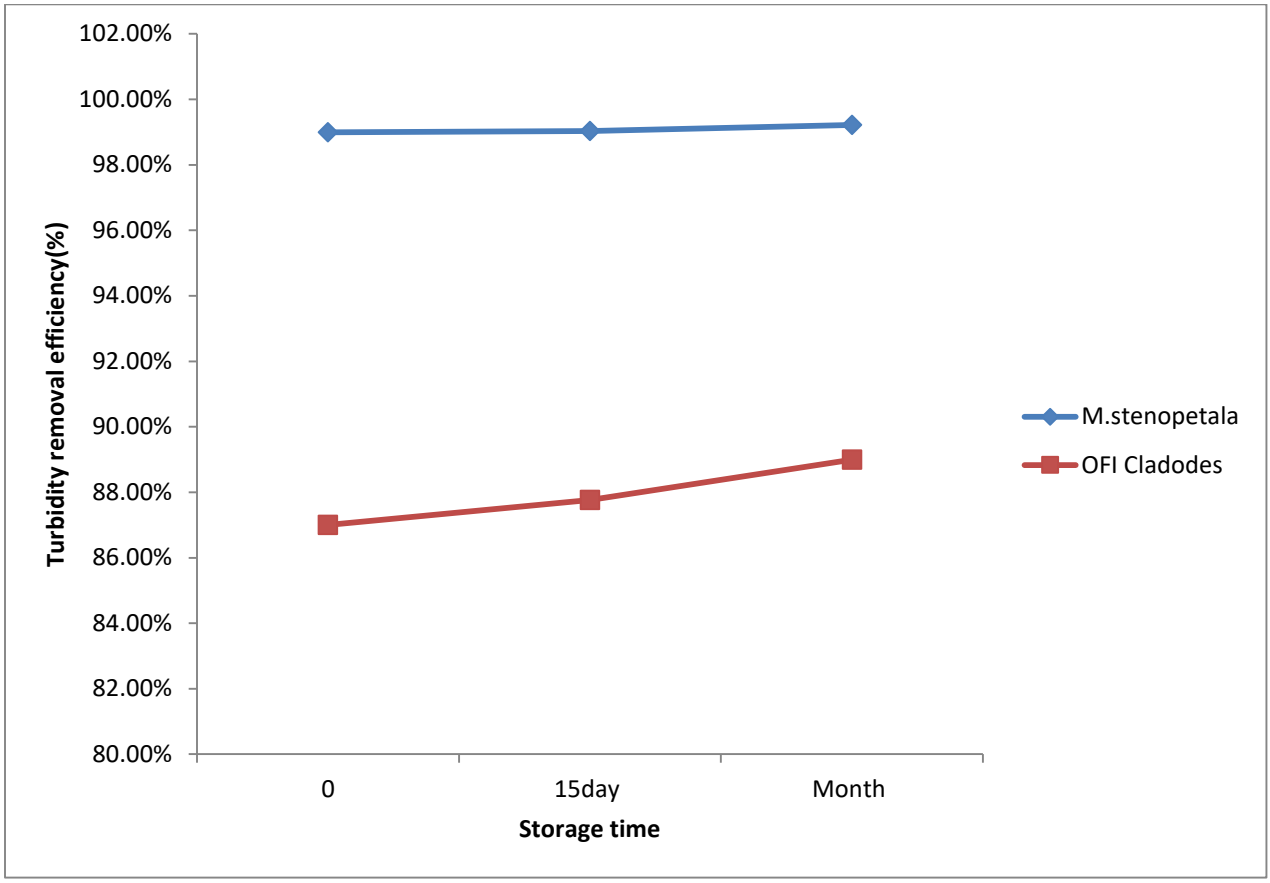

Figure 15: Turbidity removal efficiency of stored powder of natural coagulants

6. Discussion

This study was conducted to evaluate the turbidity removal efficiency of M.stenopetala seeds and cladodes of opuntia ficus indica for household water treatment and the possible underlying conditions. The results obtained from the study showed that both plants were reduced the turbidity and microbial load of surface water by their optimum dose. Both plant species work well in medium and very high turbidity water than lower. This is because turbidity increases with suspended particles, which can readily form interparticle bridges that enable them to settle down easily(70).

The optimum dosage is the minimum dosage corresponding to the lowest residual turbidity value of <5NTU(71). In the current study, we considered optimum dosage where residual turbidity was in the range of WHO guideline or the lowest residual turbidity even though the guideline could not be met. For instance, the optimum dosage for M. stenopetala and cladodes of opuntia ficus indica for Awetu River water was found to be 20mg/l and 50mg/l, for Kito river water 40mg/l and 100mg/l, for Boye water 70mg/l and 500mg/l, and Gibe river water 50mg/l and 100mg/l respectively. Their turbidity decreased from 32.1NTU to 4.1 NTU and 9.81 NTU in Awetu, from 78.1 NTU to 4.15 and 10.31 in Kito river water, from 378 NTU to 3.81NTU and 15.08NTU in Boye, and from 132NTU to 4.12NTU and 8.41NTU in Gibe river water, when treated with M. stenopetalan and Opuntia ficus indica respectively at their optimum dosages. This study showed that the water treated with M.stenopetala is in-line with WHO water quality standards of <5NTU, but it was impossible to achieve 5 NTU using Cladodes of opuntia ficus indica according to this study(14). The turbidity removal efficiency was found to be from 87% to 98.99% when treated with M. Stenopetala, and from 68% to 96.01% when treated with OFI respectively. Similar results were found, using seeds and extracts of the M. subcordata and M. stenopetala with different optimum dosages from 10mg/l to 100mg/l within six hours settling time by Moa, Ambelu, Megersa, and other authors (23, 26, 47, 63).

The turbidity removal efficiency of cladodes of opuntia ficus indica for Awetu, Kito, Boye, and Gibe was 68%, 86.79%, 96.01%, and 93.63% respectively, which was consistent with a previous study conducted by Choudhary et al. and other(30, 31, 72). The slight difference in efficiency with Moringa and other species of cactus is due to the characteristics and type of water or the type and amount of active coagulant components present. The differences in efficiency may be due to intraspecific variations of seeds, other than the variability between different plant species,

with the dosage and coagulation, the output of seeds of plants also differed according to the geographic regions where they are found (71).

For medium and highly turbid water turbidity reduction increases with increasing dosage, similar to the finding reported by Ambelu and Katayon(23, 31, 43). This evidence is different from those reported by Megersa et al(26), as they documented that the optimum dosage of M. Stenopetala is not increased with increasing initial turbidity. This difference might be due to unlike experimental setups, namely, the type of water used. Surface water characteristics, type, and size of particles, alkalinity, and other process variables may vary from river to river, which affects the performance of coagulants, unlike synthetic water samples.

The optimum dose of the coagulant found for effective removal of turbidity and microbial in this study was seen in the range 20mg/L to 70mg /L for M.stenopetala and for opuntia ficus indica the dose ranged from 50mg/L to 500mg/L. The pH, conductivity, and temperature of the water after treatment using the effective dose of the two coagulants were almost the same as that of water before treatment which shows almost neutral. The findings of this study were consistent with the finding of Vidal et al.(73).

To evaluate the effect of storage duration and condition, in this study the natural coagulants were stored in open and closed containers to month. There was a slight increment (of about 1% in both M.stenopetala and OFI) in the turbidity removal potential of the stored powder in both the open and closed containers after a month. The increase in the capacity to reduce turbidity was probably due to organic acids formed upon storage by the microbial decomposition of organic matter. This result was consistent with Moa's et al previous research, (26) and inconsistent with research was done on M.olifera(68). This difference may be due to different seed species and experiment setup.

This study revealed that significant turbidity removal was observed almost over the entire pH range, but it was slightly lower at alkaline pH for both M. stenopetala and Opuntia ficus indica (Fig. 12). The optimum pH for the M. stenopetala was from pH=5 to 9, with turbidity reduction efficiency was from 98.65% to 99.05%, and the maximum turbidity reduction was at pH=8, which with almost the same as the actual pH (7.92) of water. At pH of 10, the turbidity reduction efficiency slightly decreased to 94.55% (20.58NTU), which shows a similar detection

of reduction, but not in magnitude to the study done by Moa, which reported that at pH of 9 turbidity reduction efficiency decreased to 60%(74). The difference may be due to the fact that in the study the plant extracts were used to evaluate the effect of pH on the coagulants. The optimum pH for Opuntia ficus indica Cladodes was pH = 7, with a maximum turbidity reduction of 96.66%. At a pH of 10, the turbidity reduction efficiency decreased to 87.77% (46.2NTU). which was similar to the findings of Choudhary and Neogi who reported that the maximum turbidity removal of 98% and minimum turbidity removal of 79% were obtained at pH of 7 and 10 respectively(31).

In the current research, seeds from the plants M.stenopetala, and OFI cladodes demonstrated substantial antimicrobial activity by significantly reducing the amount of total coliform, fecal coliform and E.coli colony count on the natural surface water (Table 10). The seeds of the M.stenopetala plant demonstrated greater antimicrobial activity than the opuntia ficu indica cladodes concerning the efficiency of the plants for microbial load reduction. This is possibly attributable to the disparity in the degree of removal of turbidity and bioactive compounds as shown in the literature papers (75). Even so, the efficiency of OFI cladodes was lower than that of M.stenopetala it was less than 10CFU, which is grade B or lower risks for water-borne diseases. In this study, more than 99% of microbial load removal was observed after treating the water using these two coagulants. The percentage of microbial load reduction after treatment with M.stenopetala and OFI Cladodes ranged from 99% to 100% for the first 0.5 hours. This finding is in agreement with the finding of Megeressa, Ambelu, and Moa et al. (23, 26, 30, 31) who found the effect of M. subcordata and M.stenopetala for both synthetic and natural surface water was ranged from 97.6% to 99.9%, 96% to 99.9%, for in the first 0.5-hour process respectively. This might be due to the coagulation effect of M. stenopetala and OFI powder thus microbes may settle with other particles.

7. Strengths and limitations of the study

7.1. Strengths

A relatively large sample of water (over 250L from 4 rivers) was used with a lot of repetition. As far as we are aware, this is the first study to evaluate the efficiency of both M.stenopetala and opuntia ficus indica cladodes. Most of the previous studies used plant extracts, which is difficult to recommend for the community. Thus, this study provides useful information to purify turbid water at households using locally available natural plant coagulants.

7.2. Limitations

There were some limitations throughout the process of the study. One of the limitations of this study was, unable to test the combinations of both plants for coagulation and microbial load reduction, and initial ionic concentration of some heavy metal elements of river waters. Opuntia ficus indica extracts were as efficient as M.stenopetala, but this study did not extract mucilage rather used the cladodes directly using the cladodes. This study was limited in detecting differences across seasons.

8. Conclusion and Recommendation

8.1. Conclusion

The batch experimental results indicated that the uses of M.stenopetala seeds and Opuntia ficus indica cladodes were very effective in turbidity and microbial load reduction. At optimum dose, great reductions of turbidity were achieved, but above or below the optimum dose, there was a reduced turbidity removal efficiency of both M. stenopetala and OFI cladodes. Increasing the settling period to 3 to 12hr almost all the doses around or above the optimum doses was effective as the optimum doses.

The results showed that different optimum dosages were needed to treat river water samples. For example, the optimum dosage for M. stenopetala and opuntia ficus indica cladodes for Awetu River water was found to be 20mg/l and 50mg/l, for Kito river water 40mg/l and 100mg/l, for Boye water 70mg/l and 500mg/l, and Gibe river water 50mg/l and 100mg/l respectively, On the other hand, M.stenopetala was more effective than OFI cladodes, and as turbidity increases the removal efficiency of both plants increases. The pH, conductivity, and temperature of the water did not significantly change after treatment. However, turbidity and microbial removal efficiencies of OFI were insufficient to fall within WHO guideline values. Generally, both the microbial and turbidity reduction findings of M. stenopetala revealed that plant species can meet the requirements of drinking water quality in terms of microbial standards and maximum permissible limit of turbidity (≤5 NTU) if they are used for household water treatment, but in cases of OFI, the resulting water was low risk for water-borne diseases.

8.2. Recommendations

Based on the findings of the present study, the following recommendations are forwarded

For, Ministry of Health and Health bureaus

- They should promote the use of inexpensive natural coagulants for household water treatment that can be used in remote places where piped water is unthinkable.

For, further Researches:

- Further studies should be conducted to ascertain the precise mechanism of turbidity and microbial removal.
- Further studies are required to check the toxicity of these natural coagulants

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Annex 1

WHO guideline value for physical and chemical constituents of drinking water that may attack quality of drinking water.

Parameters	Guideline value
PH	6.5-8.5
Total suspended solid	1000mg/l
Total dissolved solid	1000mg\l
Turbidity	5NTU
Hardness	500 mg\l
Calcium hardness	65 mg\l
Chloride	200 mg\l
Fluoride	1.5 mg\l
Iron	0.3 mg/l
Nitrate	50 mg/l
Phosphate	200mg/l

Source: WHO guideline value for drinking water quality vol.3 Geneva 2011.

Annex-2

Bacteriological analysis
Membrane filters technique:

This Method describes a membrane filter (MF) procedure for the detection and enumeration of total coliform, fecal coliform (Escherichia coli) and enterococci bacteria in ambient water.

Procedures:

1. **Selection of sample size**: size of sample will be governed by expected bacterial density. In drinking water analysis, sample size will be limited only by the degree of turbidity or by the noncoliform growth on the medium. For regulation purposes, 100mL is the official sample size.
2. **Sterile filtration units**: use sterile filtration units at the beginning of each filtration series as a minimum precaution to avoid accidental contamination.
3. **Filtration of sample**: using sterile forceps, place a sterile membrane filter (grid side up) over porous plate of receptacle.
4. **Alternative single-step direct technique**: If the agar-based medium is used, place prepared filter directly on agar as described in preceding section, invert dish, and incubate for 22 to 24hours at 35 + 0.5OC. Differentiation of some colonies from either agar or liquid medium substrates may be lost if cultures are incubated beyond 24 hours.
5. **Counting**: To determine colony counts on membrane filters, use a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device, with a cool white fluorescent light source directed to provide optimal viewing of sheen. Samples of disinfected water or wastewater effluent may include stressed organisms that grow relatively slowly and produce maximum sheen in 22 to 24h.
6. **Calculation of coliform density**:

Compute the count, using membrane filters with 20 to 80 coliforms colonies and not more than 200 colonies of all types per membrane, by the following equation:

$$\text{(Total) coliform colonies /100mL} = \frac{\text{Coliform colonies counted X 100}}{\text{ML of sample filtered}}$$

Fecal coliform Membrane filter procedure using Membrane laurel sulfate broth procedure

1. Collect a sample of wastewater in a sterile 100-ml screw-capped bottle.
2. Dip the membrane filter forceps in ethanol, and burn off in the flame of the Bunsen burner. Using the now sterile forceps, transfer a sterile absorbent pad to each of three sterile Petri dishes.
3. Using a sterile 5-ml or 10-ml pipette, aseptically add 1.8 ml of sterile membrane lauryl sulfate broth to each of the three Petri dishes, so as to just saturate (but not flood) each absorbent pad. .
4. Dip the membrane filter forceps in ethanol and burn off in the Bunsen flame. Aseptically place sterile membrane filter in the membrane filtration unit .
5. Pour in about 20 ml of sterile quarter-strength Ringerís solution (Fig.2.3), and then add 5 mL of the wastewater sample to the membrane filtration unit using a sterile pipette.
6. Turn on the vacuum pump and, when all the liquid has been filtered through the membrane filter, switch off; a manual vacuum pump can also be used.
7. Aseptically transfer the membrane filter to a sterile Petri dish containing an absorbent pad just saturated with sterile membrane lauryl sulfate broth. It is best to do this by a rolling action, so as to avoid air bubbles between the membrane filter and the absorbent pad.
8. Repeat steps 4-7 twice. 9.
9. Place all three Petri dishes upside down in an incubator maintained at 44°C (± 0.5°C).

After incubation for 24 h, count the number of yellow colonies, irrespective of size, on each of the three membrane filters

10. Filter smaller volumes of the sample (or dilutions of it) if each membrane filters has more than 100 colonies growing on it, since it then becomes difficult to count them.

Membrane filtration method E. coli

Procedures

1. Prepare the M-TEC agar and urea substrate as directed in section
2. Mark the Petri dishes and report forms with sample identification and sample volumes.
3. Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base; the membrane filter is now held between the funnel and the base.
4. Shake the sample bottle vigorously about 25 times to distribute the bacteria uniformly and measure the desired volume of sample or dilution into the funnel.
5. For ambient surface waters and waste waters, select sample volumes based on previous knowledge of pollution level, to produce 20-80 E. coli colonies on the membranes.
6. Smaller sample size or sample dilutions can be used to minimize the interference of turbidity or high bacterial densities. Multiple volumes of the same sample dilution may be filtered and the results combined.
7. Filter the sample and rinse the sides of the funnel at least twice with 20-30mL of sterile rinse water. Turn off the vacuum and remove the funnel from the filter base.
8. Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the M-TEC agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane, if bubbles occur. Close the dish, invert, and incubate at 35 OC for 2 h.
9. After 2 h incubation at 35 OC, transfer the plates to Whirl-Pak bags, seal, and place inverted in a 44.5 OC water bath for 22-24 h.
10. After 22-24 h, remove the dishes from the water-bath. Place absorbent pads in new Petri dishes or the lids of the same Petri dishes, and saturate with urea broth. Aseptically transfer the membranes to absorbent pads saturated with urea substrate and hold at room temperature.
11. After 15-20 min. incubation on the urea substrate at room temperature, count and record the number of yellow or yellow-brown colonies on those membrane filters ideally containing 20-80 colonies.

12. Calculation of Results Select the membrane filter with the number of colonies within the acceptable range (20- 80) and calculate the count per 100 mL according to the general formula:

$$\text{E. coli/100 mL} = \frac{\text{No. E. coli Colonies Counted} \times 100 \text{ mL}}{\text{Volume in mL of Sample Filtered}}$$

Annex -3

The coliform classification applied to grade protection water supplies.

Grade A; Fecal coliform/100ml in all samples. That is, coliforms to WHO bacteriological guide values.

Grade B; 1-10 fecal coliforms\100ml.

Grade C; 11-50 fecal coliforms/100ml. that is, medium contamination with the significant waterborne disease risk.

Grade D; >50 fecal coliforms/100ml in all any sample i.e. grossly contaminated with high waterborne disease risk.

Source; Cheesebrough, M(1984) medical laboratory for tropical countries Vol. II