SUDAN UNIVERSITY OF SCIENCE AND TECNOLOGY

College of Graduate Studies

A Comparative study between the Efficiency of Moringa Oleifera Seed Cake and Poly Aluminum Chloro-hydrate for Removal of Water Turbidity

دراسة مقارنة بين كفاءة مستخلص بذرة المورنقا اولفيرا و بولى الومنيوم كلورو هيدرات لإزالة عكورة المياه

A Thesis Submitted in the Fulfilment of the Requirements the Degree of Doctor of Philosophy in Chemistry

BY

Elhassein Ahmed Adam Hamad

M.Sc. in Chemistry, University of Gezira

B.Ed. (Hons.) in Chemistry and Biology, University of Western Kordofan

Supervisor: Prof. Elmugdad Ahmed Ali

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DEDICATION

This work is dedicated
To

My parents,

Brothers, sister

and

Friends

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First of all, I render grateful thanks to Allah for offering me assistance, health and patience to complete this work. No words would rightfully express my deepest sincere and gratitude to my supervisor Prof. Elmugdad Ahmed Ali. I would like to thank him also for his continuous support and encouragement.

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ABSTRACT

Moringa oleifera (MO) is a pan-tropical, multipurpose tree whose seeds contain high quality vegetable oil (oil content up to 40% by weight). It contains water-soluble proteins that act as effective coagulants for water and wastewater treatment. The aim of the present study were to determine the physicochemical characteristics, phytochemical screening and the concentrations of elements in Moringa oleifera seeds. Laboratory jar test procedures was used for coagulation studies on experimental runs using actual high and low turbidity water from Blue Nile in Khartoum. The water extracts fibers part of Moringa oleifera seeds and aluminum chloro-hydrate (ACH) were applied to a sequential water treatment comprising coagulation, flocculation and sedimentation.

To optimize the efficiency of the seeds, the oil was removed by extraction with n-hexane. The analysis showed that the oil, protein, moisture, fiber and ash contents were 40.39%, 37.06%, 2.24%, 8.24% and 3.68%, respectively. The elements in mg/kg dry matter were analyzed by inductive coupled plasma and optical emission spectroscopy (ICP-OES) spectrometer. The seeds contained major elements P, K, Mg and Ca (8561, 5903, 4203 and 1240 mg/kg respectively) and minor elements (Fe, Zn, Na, Mn, Al, Cu, Ba, Ni, Cd and Pb). The photochemical analyses by gas chromatography and mass spectroscopy (GC-MS) showed presence of alkaloids, phenolic and antioxidants compounds.

Microbiological and physicochemical analyses were carried before and after treatment for low and high turbidity. The results showed that Moringa Oleifera has the ability to reduce turbidity up to 4.95 NTU below the World Health Organization’s (WHO) and The Sudanese Standards and Metrology Organization (SSMO) guideline value of 5 NTU for drinking water. It reduced the total coliform by 85% after 24 hours. The studies showed also the conformity of pH, alkalinity, electric conductivity and total dissolved solids with WHO and SSMO standards for drinking water. There was a decrease in the concentration of nitrate and phosphate, with increase in phosphate, but within permissible values. Moringa Oleifera showed remarkable ability to remove high turbidity and reduce total coliform with recommended for cost effective, eco-friendly, nontoxic, simple water treatment method for rural people.

The studies showed that no changes appeared on physicochemical parameters when the raw water of high and low turbidity were treated by aluminum chloro-hydrate sulfate, and below the World Health Organization’s (WHO) and The Sudanese Standards and Metrology Organization (SSMO) recommended guidelines.

المستخلص

المورينقا أولفيرا هي شجرة مدارية، متعددة الأغراض والتي تحتوي بذورها على زيوت نباتيه ذات جودة عالية (محتوى الزيت يصل إلى 40% بالوزن) و بروتين قابل للذوبان في المياه يعمل كمادة التخثر الفعال لمعالجة المياه ومياه الصرف الصحي. وهدفت الدراسة الحالية لإختبار الخصائص الفيزوكيميائية، دراسة الكيمياء النباتيه و تحديد تركيز العناصر لبذور المورينقا أولفيرا.

تم إجراء طريقة جهاز الجك المختبرى لدراسة التخثر بواسطة تجربة للمياه عالية ومنخفضة العكورة من مياه النيل الأزرق بالخرطوم.

تم إستخدام مستخلص بذرة المورنقا و كبريتات كلورو هيدرايت الألمونيوم للمعالجة المتتالية للمياه تتضمن التخثر و إندماج الجزيئات الصغيرة و الترسيب.

وجد أن محتوى الزيت، البروتين، الرطوبة، الالياف و الرماد لبذرة المورنقا أولفيرا 40.39% 37.06%, 2.24%,8.24% و 3.68% على التوالى.

تم تحليل العناصر في المادة الجافة بواسطة مطياف البلازما المذدوج الحثى و مطيافية الانبعاث البصرى و وجد أن البذور تحتوى على عناصر الفسفور و البوتاسيوم و الماغنسيوم والكالسيوم الرئيسية بتركيز 8561، 5903، 4203 و 1240 ملغم / كغم على التوالى.

و تليها عناصر الحديد و الزنك و الصوديوم و المنجنيز و الالمونيوم و النحاس و الباريوم و النيكل و الكادميوم و الرصاص الثانوية.

التحليل الكيميائى الضوئى بواسطة جهاز (كروماتوغرافى الغاز-مطياف الكتلة) أظهرت وجود مركبات الفينولات، الالكلويدات و مواد مضادة للأكسدة.

إختبارات الكائنات الدقيقة و الفيزوكيميائية تم تحليلها قبل و بعد المعالجة للمياه ذات العكورة العالية والمنخفضة، النتائج أظهرت بأن بذور المورنقا أولفيرا لديها القابلية على خفض نسبة العكورة دون 4.95 (وحدة العكورة النفلومترية) أقل من الحد المسموح به لمنظمة الصحة العالمية و هيئة المواصفات و المقاييس السودانية 5 (وحدة العكورة النفلومترية) لمياه الشرب.و خفضت المجموع الكلى للبكتريا القولونيه بنسبة 85% بعد مرور 24 ساعة.

أظهرت الدراسة أن درجة الحموضة، القلوية، الموصلية الكهربائية و الأملاح الكلية الذائبة متطابقة مع مواصفات منظمة الصحة العالمية وهيئة المواصفات و المقاييس السودانية لمياه الشرب.

أظهرت الدراسة ايضا انخفاض في تركيز النترات والفوسفات، مع زيادة في الكبريتات، لكنه يقع ضمن الحد المسموح به.

أظهرت بذور المورنقا أوليفيرا قدرة ملحوظة لإزالة عكورة المياه العالية و المنخفضة وتقليل نسبة المجموع الكلى للبكتريا القولونية، مع التوصية بأنها منخفضة التكلفة، صديقة للبيئة، وغير سامة، وبسيطة فى طريقة معالجة المياه لسكان الريف.

الدراسة أظهرة بأن ليس هنالك تغيير للخصائص الفيزوكيميائية فى المياه عالية و منخفضة العكورة عند معالجتها بواسطة كبريتات كلوروهيدرايت بولى الومنيوم و تقع تحت قيم منظمة الصحة العالمية وهيئة المواصفات و المقاييس السودانية لمياه الشرب الموصى بها.

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ACH	Aluminum Chloro-hydrate
ALUM	Aluminum Sulphate
AOAS	American Oil Chemists' Society
BN	Blue Nile
COD	Chemical Oxygen Demand
EDTA	Ethylene Di Amine Tetra Acetic Acid
EC	Electrical Conductivity
FTU	Formazin Turbidity Units
GC-MS	Gas Chromatograph Mass Spectrometer
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
MO	Moringa Oleifera
MOC	Moringa Oleifera Coagulant
NIST	National Institute of Standards and Technology
NTU	Nephelometer Turbidity Unit
PAC	Poly-aluminum Chloride
PH	Potential of Hydrogen
RW	Raw Water
SSMO	Sudanese Standard and Metrology Organization
TPC	Total Plate Count
TDS	Total Dissolved Solid
UNICEF	United Nation Children Emergence Fund
WHO	World Health Organization

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CHAPTER ONE

Introduction & Literature Review

1-1 Introduction

Water safety and quality are fundamental to human development and well-being. Providing access to safe water is one of the most effective instruments in promoting health and reducing poverty. Developing countries including Sudan face potable water supply problems (Lilliehöök, 2005). Blue Nile has large seasonal variation, and the water is highly turbid; fine negatively-charged particulate matter is suspended in water (cloudiness) in addition to suspended material such as silt, mud, heavy metals, bacteria and other microbes (Narayasamy and Saud, 2014) (Michael, 2010). Chemical or natural material treatments are required for their removal or reduction to acceptable limits (Carty et al., 2002). Many coagulants are widely used in conventional water treatment processes: inorganic, synthetic organic polymers and natural coagulants (Vieira et al., 2009). The most commonly used primary coagulants are aluminum sulphate (Alum), aluminium chlorohydrate (ACH), ferric chloride and polyaluminum chlorides (PAC) (Mumuni et al., 2013). There are several disadvantages of the application aluminum such as Alzheimer's disease linked with high aluminum residuals in treated water due to reactions with the OH– and alkalinity of water, production of large sludge volume, reduction of pH and low efficiency in coagulation of cold water (Jahn, 1988) (Aho and Agunwamba, 2014). Synthetic organic polymers have strong carcinogenic properties (Basra et al., 2014), which contain contaminant formulations during the manufacturing process, such as residual monomers and other reaction by-products (Muyibi and Alfugara, 2003). On the other hand, sludge disposal to the environment is of concern (Patil and Sadgir, 2013). Aluminium chlorohydrate (ACH) is a group of specific aluminium salts having the general formula $Al_nCl(3n-m)(OH)_m$, in water purification; this compound is preferred in some cases because of its high charge, which makes it more effective at destabilizing and removing suspended materials than other aluminium salts such as aluminium sulfate, aluminium chloride and various forms of polyaluminium chloride and polyaluminium chlorisulfate, in which the aluminium structure results in a lower net charge than aluminium chlorohydrate (Yonge, 2012). The pH of minimum solubility for ACH is substantially higher than that of alum allowing it to be effective at higher pH values without increasing the dissolved aluminum residuals of the water(Nansubuga et al., 2013). The natural material of plant origin has been attempted in several parts of the world to clarify turbid waters due to high cost of chemical treatment or non-availability (Moramudaii et al., 2001). Moringa Oleifera was originally an ornamental tree in the Sudan, planted during British rule (Schwarz, 2001). The women of Sudan have used the seeds from the Moringa Oleifera as clarified tree; Sudanese named (Rauwãq tree) (Jahn et al., 1986) for water treatment by swirling the seeds in cloth bags with water for a few minutes and allowing it to settle for an hour (Qureshi et al., 2013,). Moringa Oleifera is naturalized species from the monogenus family Moringaceae (Pirro et al., 2016). The tree grows rapidly from seeds or cuttings up to 4m in height, flowering and fruiting have been observed within 12 months of planting out. Two harvests of pods are possible in a single year (Ravikumar and Sheeja, 2012a). It has been experienced that the "level of polyelectrolyte present in the kernels is substantially less during the wet season; therefore, it is recommended that the seeds should be harvested during the dry season (Michael, 2010). Recent estimates has suggested that, for a spacing of 3m, a likely annual seed yield is 3 to 5 tons per hectare (Foidl et al., 2001). The seeds consist water soluble cationic protein (Vilaseca et al., 2014), characterized as basic polypeptides with a molecular weight between (6 to 16 Kilo Dalton) and an isoelectric pH of 10 to 11 (Michael, 2010). Moringa seeds contain flavonoids; alkaloids act as antimicrobial properties which able to flocculate gram-positive and gram-negative bacterial cells (Renata et al., 2013). The seed of Moringa contains vegetable oil. The presence of oil along other organic compounds in crude extract increases the content in organic matter of the treated water (Gidde et al., 2011). Also forms film coating which inhibit the contact with surface of reaction and thus reduces floc formation (Malusare and Milind, 2011). This fact represents a disadvantage for its application at full-scale water treatment and highly recommends purification of crude extract (Katayon et al., 2004). The seed has been termed a potential heavy metal removing agent due to its oxygen and nitrogen donating carboxylate and amino groups.

1-2 Background

Access to clean and wholesome water is a right to all without any prejudice whatsoever. Because water is required for countless uses, in addition to the fact that it sustains life. Water in sufficient quantity and good quality is essential for life. However, in spite of the above fact, it is unfortunate that at least one third of the population in developing countries has no access to safe drinking water (Hutton et al., 2004).

1-3 Chemistry of Water

Water is a simple molecule with two hydrogen atoms and an oxygen atom. Its molecular weight is 18. Water has unique features that lead it to behave differently from other compounds of similar molecular weight. The water molecule is dipolar because it has a negatively charged side and a positively charged one. Polarity leads to water to have high freezing and boiling points, large latent heat requirements for phase changes between ice and liquid and between liquid and vapor, temperature dependent density, a large capacity to hold heat, and good solvent action. The physical properties of water influence water quality.

i) Structure of Water

A water molecule consists of two hydrogen atoms covalently bonded to one oxygen atom (Fig. 1.1). The angle formed by lines through the centers of the hydrogen nuclei and the oxygen nucleus is 105°. The distance between hydrogen and oxygen nuclei is 0.96 x 10-8 cm. An oxygen nucleus is heavier than a hydrogen nucleus, so electrons are pulled relatively closer to the oxygen nucleus. This gives the oxygen atom a small negative charge and each of the hydrogen atoms a slight positive charge resulting in polarity of the water molecule (Fig. 1.1).

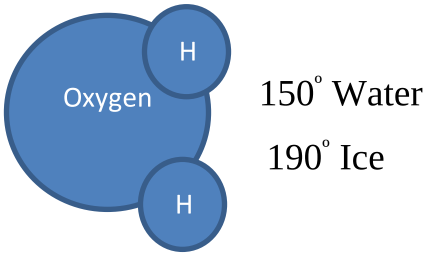


Figure 1.1 Bond angle of water and ice

Molecules attract each other through van der Waals forces. Negatively charged electrons of one molecule and positively charged nuclei of another molecule attracts. This attraction is almost, but not completely, neutralized by repulsion of electrons by electrons and nuclei by nuclei, and van der Waals forces are weak. Electrostatic van der Waals attractions between molecules increase in intensity with increasing molecular weight, because the number of electrons and nuclei increases as molecular weight increases. The positively charged side of a water molecule attracts the negatively charged side of another to form hydrogen bonds (Fig. 1.2). Hydrogen bonding is illustrated here in two dimensions, but hydrogen bonding actually, is three-dimensional. One water molecule can be bonded to another with the axis of attraction extending in any direction. Hydrogen bonds are not as strong as covalent bonds or ionic bonds, but they are much stronger than van der Waals attractions. The degree of molecular attraction in water is much greater than in nonpolar substances. Because of hydrogen bonding, water actually has the structure (H_2O)n rather than H_2O. The number of associated molecules (n) is greatest in ice and decreases as temperature increases. All hydrogen bonds are broken in vapor, and each molecule exists as a separate entity H_2O.

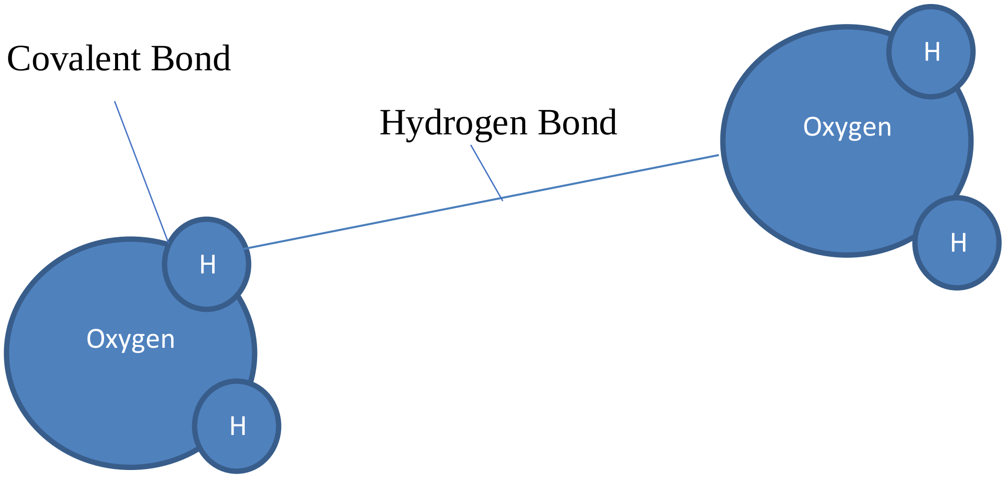


Figure 1.2 Hydrogen bonds of water

ii) Hydrogen bonding

The H_2O molecule is electrically neutral, but the positive and negative charges are not distributed uniformly. This is illustrated by the gradation in color in the schematic diagram here. The electronic (negative) charge is concentrated at the oxygen end of the molecule, owing partly to the nonbonding electrons (solid blue circles), and to oxygen's high nuclear charge which exerts stronger attractions on the electrons. This charge displacement constitutes an electric dipole, represented by the arrow at the bottom; you can think of this dipole as the electrical "image" of a water molecule.
As we all learned in school, opposite charges attract, so the partially-positive hydrogen atom on one water molecule is electrostatically attracted to the partially-negative oxygen on a neighboring molecule. This process is called (somewhat misleadingly) hydrogen bonding. Notice that the hydrogen bond (shown by the dashed green line) is somewhat longer than the covalent O—H bond. This means that it is considerably weaker; it is so weak, in fact, that a given hydrogen bond cannot survive for more than a tiny fraction of a second.

iii) The polarity of water

Water has a simple molecular structure. It is composed of one oxygen atom and two hydrogen atoms. Each hydrogen atom is covalently bonded to the oxygen via a shared pair of electrons. Oxygen also has two unshared pairs of electrons. Thus, there are 4 pairs of electrons surrounding the oxygen atom, two pairs involved in covalent bonds with hydrogen, and two unshared pairs on the opposite side of the oxygen atom. Oxygen is an "electronegative" or electron "loving" atom compared with hydrogen.

Water is a "polar" molecule, meaning that there is an uneven distribution of electron density. Water has a partial negative charge near the oxygen atom due the unshared pairs of electrons, and partial positive charges near the hydrogen atoms.
An electrostatic attraction between the partial positive charge near the hydrogen atoms and the partial negative charge near the oxygen results in the formation of a hydrogen bond as shown in the illustration.

Many other unique properties of water are due to the hydrogen bonds. For example, ice floats because hydrogen bonds hold water molecules further apart in a solid than in a liquid, where there is one less hydrogen bond per molecule. The unique physical properties, including a high heat of vaporization, strong surface tension, high specific heat, and nearly universal solvent properties of water are also due to hydrogen bonding. The hydrophobic effect, or the exclusion of compounds containing carbon and hydrogen (nonpolar compounds) is another unique property of water caused by the hydrogen bonds. The hydrophobic effect is particularly important in the formation of cell membranes. The best description is to say that water "squeezes" nonpolar molecules together.

1-4 Blue Nile

The Blue Nile with a catchment approximately 300,000km^2 drains the N– E Ethiopian Plateau. The source is Lake Tana, which a heart shaped body of water contributes only about 7% of its annual discharge and most water is collected in route through the Ethiopian highlands from many tributaries the largest of which is the Diddesa Khor. The Sudan, Ethiopian border marks the end of the Ethiopian highlands and the beginning of the Sudan plains. Inside the Sudan south of Sennar the Blue Nile is joined by two seasonal tributaries “the Dinder and the Rahad” river.

The Blue Nile flood season extends during the period June – October, which it contributes about 68% of the Nile water budget. Because of its rocky origin and high speed. The Blue Nile transports annually millions of tons of fertile soil, which causes high turbidity values in water and siltation of reservoirs.

Many coagulants are widely used in conventional water-treatment process for turbidity removal during potable water production. These coagulants may be classified as inorganic, synthetic organic polymer, and natural polymer. These coagulants are used for various purposes depending on the chemical characteristics of the water to be treated. Aluminums salts the most widely used coagulant in water treatment (Muyibi et al., 2004).

However, recent studies have pointed out several serious drawbacks in using alum salts such as Alzheimer's disease and similar health related problems associated with residual alum in treated waters, beside production of large sludge volumes. There is also the problem of reaction of alum with natural alkalinity present in water leading to reduction of pH and a low efficiency in coagulation of cold waters (Thakur and Choubey, 2014). Ongoing studies to produce more effective aluminum coagulants, such as polyaluminum chloride (PAC), have not corrected all the drawbacks mentioned above. Ferric salts and synthetic polymers have also been used as coagulants but with limited success because of similar disadvantages manifested in the use of aluminum salts.

In addition to these problems, chemicals used for water treatment in developing countries constitute a high percentage of annual running expenditure of water treatment companies. The costs of these chemicals have also been increasing at an alarming rate because local manufacturing companies cannot cope with the demand for these chemicals in other industrial applications. Therefore, the shortfall has to be imported with scarce convertible foreign currency (Arnoldsson and Bergman, 2007). These problems force many water treatment companies to resort to under-dosing of chemicals so as to meet the increasing water demand. The result is the supply of poor quality water, especially during the rainy season, when suspended solids concentration and other pollutants in surface water are very high.

On the other hand, naturally occurring coagulants are usually presumed safe for human health. Some studies on natural coagulants have been carried out and various natural coagulants have been produced or extracted from microorganisms, animals, or plants (Kaggwa et al., 2001).

1-5 Moringa Oleifera

Moringa oleifera Lam. (Moringaceae) is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands. Because *M. oleifera* has been naturalized in many tropic and sub-tropic regions worldwide, the plant is referred to by a number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mother’s Best Friend" (Muyibi and Evison, 1995).

The *Moringa* tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement.

The *Moringa* plant has been consumed by humans throughout the century in diverse culinary ways. Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value (Muyibi et al., 2003).

Epidemiological studies have indicated that *M. oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities and the seeds can be used for water treatment due present of polyelectrolytes. The investigation of the different parts of the plant is multidisciplinary, including but not limited to nutrition, ethnobotany, medicine, analytical chemistry, photochemistry and anthropology (Katayon et al., 2004).

1-5-1 Botanical Background of Moringa Oleifera

Moringa is a tree ranging in height from 5-12 m with an open umbrella-shaped crown, straight trunk (10-30 cm thick) and a corky, whitish bark. The plant (depending on climate) has leaflets 1-2 cm in diameter and 1.5-2.5 cm in length. The tree produces a tuberous tap root which explains its tolerance to drought conditions. Originally considered a tree of hot semi-arid regions (annual rainfall 250-1500 mm), *Moringa* is adaptable to a wide range of environmental conditions from hot and dry to hot, humid, wet conditions (Okuda et al., 2001).

The tree is tolerant to light frosts, but does not survive as a perennial under freezing conditions. *Moringa* grows more rapidly, reaching higher heights, when found in well-drained soils with ample water, but tolerates both sandy soils, heavier clay soils and water limited conditions. The tree can be established in slightly alkaline soils up to pH 9 as well as acidic soils as low as pH 4.5, and is well suited for a wide range of adverse environments that would not be suitable for other fruit, nut and tree crops (Hutton et al., 2004).

Cultivation entails collection of seeds from the tree, development of plantlets in the greenhouse for 2 to 3 months and transplantation of mature stems (1-1.5 m long) to the main fields.

The seeds contain much of the plant’s edible oil which is used as a cooking oil for frying and as a salad oil for dressing. The fatty acid compositions of solvent and enzyme-extracted oil from *M. oleifera* seeds showed 67.9% oleic acid in the solvent extract and 70.0% in the enzyme extracts. Other prominent fatty acids in *Moringa* oil include palmitic (7.8% and 6.8%), stearic (7.6% and 6.5%), and behenic (6.2% and 5.8%) acids for the solvent and enzyme-extracted oils, respectively (Arnoldsson et al., 2008).

Due to the high monounsaturated to saturated fatty acid ratio, *Moringa* seed oil could be considered an acceptable substitute for highly monounsaturated oils such as olive oil. *Moringa* oil utilized as a frying oil can be a healthy alternative to other commonly used oils such as palm, canola and soybean oil when comparing fatty acid composition. Oils with high amounts of monounsaturated (oleic type) fatty acids are desirable due to an association with decreased risk of coronary heart disease (Rebecca et al., 2006). The whole seeds can also be eaten green, roasted or powdered, and steamed in tea and curries. The pods and seeds, often referred to as Moringa kernels, have a taste that ranges from sweet to bitter and are most popularly consumed after frying to get a peanut-like taste (Ndabigengesere and Narasiah, 1998b).

The research on *Moringa oleifera* for water treatment in Sudan is very limited, and the present study seeks to investigate the chemical constituents, active ingredients and optimum conditions for effective water treatment by the use of Moringa oleifera seeds as coagulant for turbidity and remove coliform bacteria compared to poly aluminum chloride.

1-6 Some Physical and Chemical Characteristics

Consumers of drinking water tend to base their perceptions of drinking-water quality on easily observed parameters such as visual appearance, taste and odour. This can lead to ingestion of water that is microbiologically or chemically unsafe, but appears clean. Conversely, a water of high microbiological and chemical quality with regard to health impacts may still appear dirty or have an unpleasant taste or odour, and can be rejected by consumers. The supply of aesthetically unpleasant water can and often does lead to the use of less safe, but more appealing, water resources; or may compel users to invest in costly (and possibly unreliable) alternatives such as bottled water or domestic water treatment devices. Thus, even in the absence of a direct threat to public health, water suppliers should seek to produce and deliver drinking water that is acceptable to consumers. The concentration at which a parameter is objectionable to users will vary according to social, economic and cultural considerations. Therefore, WHO does not set guideline values at specific levels, but rather indicates a typical concentration that might give rise to complaints from consumers (UNICEF, 2008).

The constituent physical and chemical characterizations to be discussed include the following: turbidity (physical), color (physical), taste (physical) temperature (physical), nitrate (chemical), sulfate (chemical), pH (chemical), phosphorus (chemical), and alkalinity (chemical) and odor (physical).

1-6-1 The Jar Test

In practice, irrespective of what coagulant or coagulant aid is used, the optimum dose and pH are determined by a jar test. This consists of four to six beakers (such as 1000 ml in volume) filled with the raw water into which varying amounts of dose are administered. Each beaker is provided with a variable-speed stirrer capable of operating from 0 to 100 rpm.

Upon introduction of the dose, the contents are rapidly mixed at a speed of about 60 to 80 rpm for a period of one minute and then allowed to flocculate at a speed of 30 rpm for a period of 15 minutes. After the stirring is stopped, the nature and settling characteristics of the flocs are observed and recorded qualitatively as poor, fair, good, or excellent. The lowest dose of coagulant and pH that produce the desired flocs and clarity represents the optimum. This optimum is then used as the dose in the actual operation of the plant (Sincero and Sincero, 2002).

1-6-2 Turbidity

Turbidity is a measure of the extent to which suspended matter in water either absorbs or scatters radiant light energy impinging upon the suspension (Sincero and Sincero, 2002).

i) Sources of Turbidity

It is caused by the presence in water of particulate organic and inorganic matter, such as clay, silt, colloidal particles, and microscopic organisms. Soil particles constitute the major part of the suspended material in most natural waters (WHO, 2014) The discharge of untreated industrial and domestic effluents also adds great quantities of turbidity (Maiti, 2004); consequently surface waters are generally more turbid than groundwater. Raw water turbidity has been reported to range from less than 1 to more than 1000 NTU whereas it is usually <1 NTU in filtered waters (Rice et al., 2012).

ii) Health effect of turbidity

Turbidity has no direct health impact but consumption of highly turbid water may constitute a health risk due to the risk of microbial contamination. The major problem associated with turbidity is its effect on disinfection because it can protect pathogenic microorganisms from the effects of disinfectants, stimulate the growth of bacteria in distribution systems, and increase chlorine demand (UNICEF, 2008).

iii) Measuring of turbidity

The original measuring apparatus that measures turbidity, called the Jackson turbidimeter, was based on the absorption principle. A standardized candle was placed under a graduated glass tube housed in a black metal box so that the light from the candle can only be seen from above the tube. The water sample was then poured slowly into the tube until the candle flame was no longer visible. The turbidity was then read on the graduation etched on the tube. At present, turbidity measurements are done conveniently through the use of photometers (Koren, 1991). A beam of light from a source produced by a standardized electric bulb is passed through a sample vial. The light that emerges from the sample is then directed to a photometer that measures the light absorbed. The readout is calibrated in terms of turbidity. The unit of turbidity is the turbidity unit (TU) which is equivalent to the turbidity produced by one mg/L of silica (SiO_2). SiO_2 was used as the reference standard. Turbidities in excess of 5 TU are easily detected in a glass of water and are objectionable not necessarily for health but for aesthetic reasons. A chemical, formazin, that provides a more reproducible result has now replaced silica as the standard. Accordingly, the unit of turbidity is now also expressed as formazin turbidity units (FTU).

The other method of measurement is by light scattering. This method is used when the turbidity is very small. The sample "scatters" the light that impinges upon it. The scattered light is then measured by putting the photometer at right angle from the original direction of the light generated by the light source. This measurement of light scattered at a 90-degree angle is called nephelometry. The unit of turbidity in nephelometry is the nephelometric turbidity unit (NTU) (Omar and Jafri, 2015).

iv) Environmental significance

Turbidity is an important consideration in public water supplies for three major reasons: -

1- Aesthetics - turbidity in drinking water is automatically associated with possible wastewater pollution.

2- Filterability - Filtration of water is rendered more difficult and costly when turbidity increases.

3- Disinfection - many of the pathogenic organisms may be encased in the particles and protected from the disinfectant.

1-6-3 The pH of Water

pH is one of the most fundamental symbols for water treatment specialists. It is the negative logarithms of hydrogen ion concentration which gives the acidity and alkalinity of water. The pH in water treatment is one of the most important parameters which may have a negative or positive effect. In coagulation, there is a range of pH for each coagulant in which an effective coagulation takes place; outside this range the coagulation process will not happen. In adsorption, neutral or higher pH was found to be the optimum, while in low pH no adsorption will take place (Boyd, 2015). The dissociation of water molecule into hydrogen and hydroxyl ions is best represented by the equilibrium reaction:

H_2O ‹------------------------› $H^+ + OH^-$

Hence: $pH = -\log [H^+]$

Dissociation of water into hydrogen and hydroxyl ions is governed by the dissociation constant (K_w).

$K_w = [H^+] [OH^-] = 10^{-14}$

In these equations hydrogen ion (H^+) is used for simplicity rather than the more correct hydronium ion (H_3O^+). If the concentration of hydrogen ion is equal to hydroxyl ion the solution is said to be neutral and the pH is equal to 7.0. The pH scale is an indicator of the balance between hydrogen and hydroxyl ions. It is not a quantitative measure of the acid or alkaline substances in water. Other ions like dissolved carbon dioxide, bicarbonate, and carbonate can affect the acidity or alkalinity of water (Michael, 1996).

1-6-4 Colour

It is another physical characteristic by which the quality of water can be judged(Cheremisinoff, 2001). Color is the perception registered as radiation of various wavelengths strikes the retina of the eye. Even pure water is not colourless; it has a pale green-blue tint in large volumes. Water takes on color when foreign substances such as organic matter from soils, vegetation, minerals, and aquatic organisms are present. Color can also be contributed to water by municipal and industrial wastes (Cheremisinoff, 2001). For example, Iron oxides cause reddish water and Manganese oxides cause brown or brackish water. This color may be objectionable not for health reasons but for aesthetics (Omar and Jafri, 2015).

It is necessary to differentiate between true colour due to material in solution and apparent colour due to suspended matter (Tebbutt, 1997).

Color in water is classified as either true color or apparent color. Water whose color is partly due to dissolved solids that remain after removal of suspended matter is known as true color. The term ‘‘apparent color’’ includes not only color due to substances in solution, but also that due to suspended matter. Apparent color is determined on the original sample without filtration or centrifugation (Rice et al., 2012). True color is the most difficult to remove. Measured by comparing a water sample with standard color solutions or colored glass disks, one color unit is equal to the color produced by a I mg/L solution of platinum. Water's color affects its marketability for domestic and industrial use. While coloured water does not present a safety issue, most people object to obviously coloured water. Colored water can affect laundering, food processing, papermaking, manufacturing, and textiles (Sincero and Sincero, 2002).

i) Environmental significance

Coloured water is not aesthetically acceptable to the general public. True colour caused by organic compounds may exert chlorine demand during disinfection process and thereby seriously reduce the effectiveness of chlorine as a disinfectant (Cheremisinoff, 2001).

1-6-5 Taste and Odor

Unpleasant tastes and odours can arise from inorganic or organic compounds in water sources, occurring naturally or as a result of human activity. An unpleasant taste or odour may indicate a failure of drinking-water treatment, and should be investigated to ensure that microbial and chemical quality of the water is not compromised.

A major cause of taste and odour complaints is chemical disinfection. Utilities with large distribution systems may apply large chlorine doses to ensure a residual throughout the distribution system. In community or household settings, it can be difficult to ensure a consistent chlorine dose while maintaining the desired residual, typically near 0.5 mg/L (Sincero and Sincero, 2002).

Above a residual free chlorine concentration of between 0.6 and 1.0 mg/dm^3 there is an increasing likelihood of complaints from consumers. Chloramines can also give rise to taste and odour problems.

A second major cause of taste and odour problem is dissolved inorganic compounds, especially metals. Naturally occurring iron and manganese commonly occur in groundwater; these may react with oxygen after exposure to air to form insoluble precipitates. Either as dissolved ions or as small particles, iron and manganese give a strong metallic taste to water. Iron is usually not detectable by users below 0.3 mg/dm^3, and in some cases higher concentrations are acceptable. Manganese levels below 0.1 mg/dm^3, well below the health-based GV of 0.4 mg/dm^3 are usually acceptable to users (Mohsin et al., 2013).

Metals can also enter drinking water from pipes and fittings. Although copper can give rise to taste problems, the taste should be acceptable at the health-based provisional guideline value of 2 mg/dm^3. Zinc levels above 3 mg/dm^3 can impart an undesirable astringent taste to water. Drinking water usually contains much lower levels of zinc, though older galvanized plumbing materials can leach zinc. There is no health-based GV for zinc.

Sulfate in drinking water can cause a noticeable taste above concentrations of about 250 mg/dm^3. In the absence of oxygen and free chlorine, bacteria can convert sulfate to hydrogen sulfide, which causes a distinctive "rotten-egg" odour at concentrations as low as 0.05 mg/dm^3. There are no health-based GVs for sulfate or sulfide (Omar and Jafri, 2015).

The third major class of taste and odour compounds is organic material. Bacteria and fungi in surface water reservoirs can produce a number of organic compounds that can impart unpleasant earthy/musty odours to water. Geosmin and 2-methylisoborneol (MIB) are of particular concern, since they can cause acceptability problems at trace levels (below 10 ng/dm^3, or 0.000010 mg/dm^3). These compounds are most likely to be found in drinking water following algal bloom events.

Finally, synthetic organic compounds can impart tastes and odours to water. For many organic compounds, health-based GVs are below taste and odour thresholds. However, a number of low-molecular weight hydrocarbons found in petroleum oils and solvents may impart a very unpleasant "diesel-like" odour to water at levels well below health-based GVs (Spellman, 2013).

1-6-6 Temperature

Temperature is one of the most important parameters in natural surface water systems because such systems are subject to great temperature variations; other than this, temperature is not commonly used for water quality evaluation (Chapra, 2008).

Surface water and groundwater are affected by temperature both naturally and artificially. Heat or temperature change in surface waters affects the solubility of oxygen in water, the rate of bacterial activity, and the rate at which gases are transferred to and from water, as well as the health of the fish population (Boyd, 2015).

The temperature of water affects the efficiency of treatment units. For example, in cold temperatures, the viscosity increases. This, in turn, diminishes the efficiency of settling of the solids that the water may contain because of the resistance that the high viscosity offers to the downward motion of the particles as they settle (Boyd, 2015). Cold water requires more chemicals for efficient coagulation and flocculation. High water temperatures can increase chlorine demand because of increased reactivity, as well as increased levels of algae and other organic matter in the raw water (Sincero and Sincero, 2002). High water temperatures can increase chlorine demand because of increased reactivity, as well as increased levels of algae and other organic matter in the raw water (Carr and Neary, 2008).

1-6-7 Total Dissolved Solids

The major chemical parameters of concern in water treatment are total dissolved solids (TDS).

Solids in water occur either in solution or in suspension. The solids in the water that remain after filtration and evaporation as residue are called total dissolved solids, or TDS. Dissolved solids may be organic or inorganic, and come from water's contact with substances in soil, on surfaces, and in the atmosphere. Organic dissolved constituents come from decayed vegetation, and from organic chemicals and gases(Boyd, 2015).

Water has the aptitude to dissolve an extensive variety of inorganic and some organic mineral deposits or salts such as potassium, calcium, sodium, bicarbonates, chlorides, magnesium, sulfates etc. These dissolved minerals, gases, and organic constituents may cause physiological effects, as well as color, taste, and odor problems.

There is no contract have been developed on bad or optimistic effects of water that exceeds the WHO standard of maximum permissible level is 1,000 ppm. A total dissolved solid (TDS) in drinking water originates in numerous ways from sewage and urban industrial wastewater etc. Hence, TDS test is mostly an indication to control the general quality of the water (Mohsin et al., 2013).

Dissolved solids can be removed from water by distillation, electro dialysis, reverse osmosis, or ion exchange. It is desirable to remove these dissolved minerals, gases, and organic constituents because they may cause psychological effects and produce aesthetically displeasing color, taste, and odors. While it is desirable to remove many of these dissolved substances from water, it is not prudent to remove them all. This is the case, for example, because pure, distilled water has a flat taste. Further, water has an equilibrium state with respect to dissolved constituents. If water is out of equilibrium or under saturated, it will aggressively dissolve materials with which it comes into contact. Because of this problem, substances that are readily dissolvable are sometimes added to pure water to reduce its tendency to dissolve plumbing (Spellman, 2013).

1-6-8 Alkalinity

Alkalinity is an important water quality parameter, because it measures the water's ability to neutralize acids. Alkalinity constituents in natural water supplies are bicarbonate, carbonate, and hydroxide ions, mostly the carbonates and bicarbonates of sodium, potassium, magnesium, and calcium. Alkalinity also occurs naturally from carbon dioxide (from the atmosphere and as a by-product of microbial decomposition of organic material) and from mineral origins (primarily from chemical compounds dissolved from rocks and soil) (John, 1990).

In natural water, most of the alkalinity is due to CO_2

1- The free CO_2 dissolves in water to form carbonic acid (H_2CO_3), which further dissociates into H^+ and HCO^{3-} . The HCO^{3-} further dissociates into H^+ and CO^{3-}.

$CO_2 + H_2O \rightarrow H_2CO_3$ (dissolved CO_2 and carbonic acid)

$H_2CO_3 \rightarrow H^+ + HCO^-$ (bicarbonate)

$HCO^{1-} \rightarrow H^+ + CO_3^{2-}$ (carbonate)

Where the quantities in parenthesis are concentration in mg/dm^3 as $CaCO_3$.

Alkalinity in water acts as a buffer that tends to stabilize and prevent fluctuations in pH. In fact, alkalinity is closely related to pH, but the two must not be confused. Total alkalinity is a measure of the amount of alkaline materials in the water. The alkaline materials act as the buffer to changes in the pH. If the alkalinity to too low (below 80 ppm) there can be rapid fluctuations in pH (i.e., there is insufficient buffer to the pH). High alkalinity (above 200 ppm) results in the water being too buffered. Thus, having significant alkalinity in water is usually beneficial, because it tends to prevent quick changes in pH that interfere with the effectiveness of common water treatment processes. Low alkalinity also contributes to water's corrosive tendencies (John, 1990).

Alkalinity is useful in waters that it provides buffering to resist changes in pH. It is normally divided into caustic alkalinity above pH 8.2 and total alkalinity above pH 4.5. Alkalinity can exist down to pH 4.5 because of the fact that HCO^{3-} is not completely neutralized until this pH is reached (Tebbutt, 1997). The principal problem with alkaline water is the reactions that occur between alkalinity and certain substances in the water. In addition, alkalinity levels affect the efficiency of certain water treatment processes, especially the coagulation process (Spellman, 2013).

While highly alkaline waters do not seriously affect human health, elevated alkalinity can cause an objectionable bitter taste. In treatment, alkaline water can cause problems with the reactions that occur between alkalinity and certain substances in the water that can foul water system appurtenances (DeZuane, 1997).

i) Environmental significance of alkalinity

Alkalinity in itself is not harmful to human beings, still the water supplies with less than 100 mg/dm^3 are desirable for domestic use. In large quantities, alkalinity imparts bitter taste of water.

The other applications of alkalinity data are:

1. Chemical coagulation process: alkalinity must be present in excess to neutralize the acid released by the coagulant.
2. Water softening process.
3. Corrosion control.
4. To know the buffer capacity of water.
5. pH changes due to aeration of water: It is common practice to aerate water for the removal of dissolved CO_2, as CO_2 is acidic gas, its removal tends to decreases pH (alkaline). Thus, high alkaline water tends to have high pH after aeration and low alkaline water tend to have low pH after aeration.
6. Alkalinity of boiler water is of interest to the environmental engineers (Carr and Neary, 2008).

1-6-9 Electrical Conductivity

Conductivity is the measurement of the ability of a solution to carry electric current. Pure water is normally not a good conductor of electricity, the increase of dissolved salts in water increase its conductivity. As such the conductivity of water sometimes used for indicating the degree of its purification or pollution.

Acids, bases and salts in water make it relatively good conductor of electricity and such substances are called electrolytes. The electrolyte in solution dissociates with positive (cations) and negative (anions) ions and imparts electrical conductivity. Thus, the higher concentration of electrolyte in water, the more is its electrical conductance (i.e. less the resistance to the flow of electricity). This gives a rapid method to get an idea about dissolved solids in water.

The important ions that impart conductivity in water are:

i) anions: Cl^-, S^{2-}, CO_3^{2-}, HCO^- and NO_3^-

ii) cations: Ca^{2+}, Mg^{2+}, Na^+ and K^+

Conductivity is temperature dependence; usually 25C° is the reference temperature. According to WHO standards EC value of drinking water quality should not exceeded 400 µS/cm and the conductivity of potable waters varies generally from 50 to 1500 µ mhos/cm (Idries, 2013).

Conductivity is customarily reported in micromhos per centimeter (mhos/cm). In the International System of units (SI) the reciprocal of ohm is the Siemens (S) and Analysis of water and effluents conductivity is reported as mill siemens per meter (mS/m).

I mS/m = 10 J.lS/cm = 10 J.lmhos/cm and I J.lS/cm = I J.lmhos/cm

Freshly distilled water has a conductivity of 0.5 to 2 J.lmhos/cm, and this increases after a few weeks of storing up to 2 to 4 J.lmhos/cm. This increase is caused by absorption of atmospheric CO_2.

i) Environmental significance of conductivity

1. Used for detection of impurities in water.
2. Used for quantitative measurement of ionic constituents dissolved in water, which are important for boiler feed water and cooling water etc.
3. Used for checking correctness of water analysis as there is a distinct relationship between conductivity and total dissolved solids (TDS) (Boyd, 2015).

1-6-10 Hardness

Softening is the term given to the process of removing ions that interfere with the use of soap. These ions are called hardness ions due to the presence of multivalent cations, mostly calcium and magnesium. In natural waters, other ions that may be present to cause hardness but not in significant amounts are iron (Fe^{2+}), manganese(Mn^{2+}), strontium (Sr^{2+}), and aluminum (Al^{3+}).

Hard water is generally considered to be those waters that requires considerable amounts of soap to produce a foam and that also produce scale in hot-water pipes, heaters, boilers, and other units in which the temperature of the water is increased. The hardness of waters varies from place to place. In general, surface waters are softer than groundwater's. The hardness of water reflects the nature of geological foundation with which it has been in contact (Boyd, 2015).

i) Types of hardness

Two basic types of hardness are associated with the ions causing hardness, Carbonate and noncarbonated hardness. When the hardness ions are associated with the ions in water, the type of hardness is called temporary, otherwise, the type of hardness is called permanent.

Carbonate hardness was formerly called temporary hardness, because it can be removed by boiling and is caused by dissolved Ca and Mg bicarbonate. Removal process of Ca and Mg bicarbonate is as follows:

$Ca(HCO_3)_2 + \text{heat} \rightarrow CaCO_3\ \text{(ppt)}\downarrow + CO_2 + H_2O$

$Mg(HCO_3)_2 + \text{heat} -+ MgCO_3\ \text{(ppt)}\downarrow + CO_2 + H_2O$

Carbonate hardness is especially important since it leads to scaling. Noncarbonate hardness was formerly called permanent hardness, because it cannot be removed by boiling. Non-carbonate hardness cations are associated with sulphate, chloride and nitrate ions of Ca and Mg (Carr and Neary, 2008).

The following lists the general classification of hard waters:

Soft	<50 mg/dm^3 as $CaCO_3$
Moderately hard	50–150 mg/dm^3 as $CaCO_3$
Hard	150–300 mg/dm^3 as $CaCO_3$
Very hard	>300 mg/dm^3 as $CaCO_3$

A very soft water has a slimy feel. For example, rainwater, which is exceedingly soft, is slimy when used with soap. For this reason, hardness in water used for domestic purposes is not completely removed. The advantages of hard water help tooth and bone growth. Also, hard water scaling reduces toxicity of lead oxide in pipelines made of lead. The other negative aspect of hard water versus soft water is the natural capacity of hard water to produce scale in hot water pipes, boilers and heaters. Therefore, surface raw water is softer than ground water because of more rains, less contact with soil minerals. but economic disadvantages of a hard water include increased soap consumption and higher fuel costs. The hardness of waters, expressed in mg/dm^3 $CaCO_3$ (calcium carbonate).

According to WHO guide line, the maximum permissible limit of total hardness should not be exceeded 300mg/dm^3 as $CaCO_3$ (Spellman, 2013).

ii) Environmental significance hardness

1- Soap consumption by hard-water causes economic loss to water consumers. Precipitated foam by hard water adheres to surfaces of tubs, sinks etc. and may stain clothing, dishes and other items.

2- Mg hardness, particularly associated with sulphate ion, has a laxative effect in person unaccustomed to it.

3- Carbonate hardness precipitates lead to scaling in boiler, which causes considerable economic loss.

4- Determination of hardness serves as a basis for routine control of softening process (Boyd, 2015).

1-7 Anions

1-7-1 Nitrate (NO_3^-)

Nitrate (NO_3^-) is a compound of nitrogen and oxygen that is found in many everyday food items such as spinach, lettuce, beets, and carrots. There are usually low levels of nitrates that occur naturally in water but the majority run-off from, animal feedlots, wastewater and sludge, septic systems, and nitrogen fixation from the atmosphere by legumes, bacteria, and lightning.

Nitrate (NO_3^-) is formed in waters by oxidation of ammonia compounds (by aerobic nitrifying bacteria, e.g. nitrosomonas) or by reduction of nitrate (by facultative anaerobic denitrify bacteria, e.g. Pseudomonas). Such oxidation and reduction may occur in wastewater treatment plants, water distribution system, and natural waters. As an intermediate stage in nitrogen cycle, it is unstable. Very high nitrite levels are usually associated with waters having unsatisfactory microbiological activity (Maiti, 2004).

Nitrate in water is colorless, tasteless, and odorless. Therefore, it can only be detected using chemical analysis. Nitrate is objectionable for causing what is called methemoglobinemia (infant cyanosis or blue babies) in infants.

Generally, the ground water has high nitrate concentration than surface water because of the percolating sewage, industrial waste, chemical fertilizers, leaches from solid waste landfills, septic tank effluents to the ground water. Whatever may be the reason the high concentration of nitrate is harmful to humans, particularly for infants.

The nitrite has a great affinity for hemoglobin than the oxygen and it replaces oxygen in the blood. The deficiency of oxygen causes suffocation. The color of the skin of the infants becomes blue so it is termed as blue baby disease. The medical name is methemoglobinemia, this disease is a fatal disease and it takes place when the concentration of nitrates is more than 50 mg/l according to WHO guideline (WHO, 2011).

1-7-2 Sulfate (SO_4)

The sulfate ion is one of the major anions occurring naturally in water. It produces a cathartic or laxative effect on people when present in excessive amounts in drinking water. Its SMCL is 250 mg/dm^3.

Sulfate mainly derived from the dissolution of salts of sulfuric acid and abundantly found in almost all water bodies. High concentration of sulfate may be due to oxidation of pyrite and mine drainage etc. Sulfate concentration in natural water ranges from a few to a several hundred mg per liter but no major negative impact of sulfate on human health is reported (Drinan and Spellman, 2012). However, most people get the majority of their dietary sulphates through food and not from the water. High sulphate levels (1000 mg/L) have been shown to have a laxative effect on humans and can cause mild gastrointestinal irritation. According to (WHO, 2004) guidance level the maximum permissible limit of sulphate in drinking water supply is limited to 250mg/l**.**

1-7-3 Phosphate (PO_4^{3-})

Phosphates in surface waters mostly originated from sewage effluents, which contains phosphate, based synthetic detergents, from industrial effluents, or from land runoff where inorganic fertilizers have been used in farming. Ground water usually contains insignificant concentrations of phosphates, unless they have become polluted. Phosphorous one of the crucial nutrients for algal growth and can contribute significantly to eutrophication of lakes and reservoirs (United States. Environmental Protection Agency. Office of Water, 2002).

i) Biological water quality characteristics

The greatest risk to public health from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant (WHO, 2011). The idea to estimate water quality is to use non-pathogenic and easy detectable microorganisms as indicators of contamination in drinking water. From their presence or absence, it can be inferred whether or not there is a risk for health (Mattelet, 2006).

While most bacteria we encounter are harmless, waterborne pathogenic bacteria transmit diseases that cause common symptoms of gastrointestinal disorder. Bacteria in waters can cause illnesses such as typhoid (Salmonella typhi), cholera (Vibrio cholerae) and diarrhea (Giardia lamblia). Faecal coliforms and streptococci indicate that wastes from humans or animals contaminate the water. Faecal streptococci are the most resistant group of bacteria, and are often analysed together with total coliforms as an indication of a total bacteriological status. Coliform bacteria can be removed from the water by chlorination. There are two main approaches to water quality monitoring for pathogen detection (Spellman, 2013).

The first one is to measure directly the concentration of the pathogen agent in water. This method is accurate when specific disease-causing waterborne agents are detected directly for the determination of water quality. It is practically impossible to take into account the broad range of pathogen organisms present in contaminated water. In addition, this type of method is relatively expensive and therefore difficult to implement in developing countries. The manipulation of such organisms often requires intensive training and is time-consuming.

The second approach based on "microbial indicators" has several advantages compared to the first method and is most commonly used for the microbial monitoring of water.

The role of the microbial indicators in drinking water is to use them as an index of faecal pollution and therefore the results are used for the assessment of the health risk (Dirar, 1986).

1-8 Total coliform Bacteria

i) Total coliforms

Total coliforms are a group of closely related bacteria (family Enterobacteriaceae) that have been used for many decades as the indicator of choice for drinking water. The group is defined as aerobic and facultatively anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment the milk sugar lactose to produce acid and gas within 48 h at 35°C. Few bacteria other than coliforms can metabolize lactose; for this reason, lactose is used as the basis for identification (the hydrolysis of o-nitrophenyl-β-d-galactopyranoside, or ONPG, is also used for identification in some coliform tests). The total coliform group includes most species of the genera Citrobacter, Enterobacter, Klebsiella, and Escherichia coli. It also includes some species of Serratia and other genera. Although all coliform genera can be found in the gut of animals, most of these bacteria are widely distributed in the environment, including water, and waste water. A major exception is E. coli, which usually does not survive long outside the gut, except perhaps in the warm water associated with tropical climates (Letterman, 1999). Total coliforms are used to assess water treatment effectiveness and the integrity of the distribution system. They are also used as a screening test for recent fecal contamination. Treatment that provides coliform-free water should also reduce pathogens to minimal levels. A major shortcoming to using total coliforms as an indicator is that they are only marginally adequate for predicting the potential presence of pathogenic protozoan cysts/oocysts and some viruses, because total coliforms are less resistant to disinfection than these other organisms. Another shortcoming is that coliforms, under certain circumstances, may proliferate in the biofilms of water distribution systems, clouding their use as an indicator of external contamination. Coliforms are also often not of fecal origin. Despite these drawbacks, total coliforms remain a useful indicator of drinking water microbial quality, (Payment et al., 2003).

1-9 Drinking water quality standards

There are various drinking water standards which can be followed. One of the most widely used standards is that established by World Health Organization (WHO). The WHO has set guidelines for the lower and the upper limits of certain inorganic compounds usually found in drinking water. Table (1.1) explains the maximum admissible concentration of physicochemical and bacteria as specified by the World Health Organization (WHO) and the Sudanese Standards and Metrology Organization (SSMO).

Table 1.1: physicochemical and bacteria parameters with WHO & SSMO standard

No.	Parameters	Method	WHO	SSMO
Physical Parameters				
1	Turbidity	Nephelometer	5 NTU	5 NTU
2	pH	pH meter	6.5–8.5	6.5-8.5
3	Temperature (Cº)	JENWAY 4320	-	Acceptable
4	EC	EC meter	2000 µs/cm	2000 µs/cm
5	TDS	TDS meter	1000 mg/dm^3	1000 mg/dm^3
6	Taste	Volunteer	Acceptable	Acceptable
7	Odour	Volunteer	Acceptable	Acceptable
Chemical parameters				
8	Total alkalinity	Titration	200 mg/dm^3	200 mg/dm^3
9	Total hardness	Titration	300 mg/dm^3	300 mg/dm^3
10	Hardness as Ca^{2+}	Titration	150 mg/dm^3	150 mg/dm^3
11	Nitrate (mg/dm^3)	Spectrophotometer	50 mg/dm^3	50 mg/dm^3
12	Sulphate (mg/dm^3)	Spectrophotometer	250 mg/dm^3	250 mg/dm^3
13	Phosphate(mg/dm^3)	Spectrophotometer	200 mg/dm^3	200 mg/dm^3
Bacteriological Parameters				
14	TPC/100 cm^3	Quantitative	Not detectable	Not detectable

1-10 Water Treatment Processes

Untreated surface waters contain clay, minerals, bacteria, inert solids, microbiological organisms, oxidized metals, organic color producing particles, and other suspended materials. Some of the microbiological organisms can include Giardia cysts, pathogenic bacteria, and viruses. Oxidized metals include iron and manganese. All of these materials can inhibit disinfection, cause problems in the distribution system, and leave the water cloudy rather than clear. The purpose of coagulation is to remove these particles. The ability of particles to remain suspended in water is a function of both the particle size and specific gravity.

Turbidity particles can range in size from molecular to 50 microns. Particles which are greater than one micron in diameter are considered silt, and settle out due to their relatively large size and density without the need to coagulate in a matter of seconds or minutes. Colloidal material ranges in size from 0.001 to one micron in diameter. These materials require days to months for complete settling. Since retention times in the water treatment process are generally less than twelve hours, the rate of settling of these colloidal particles must be increased in the water treatment process. This is accomplished in the coagulation process when tiny particles agglomerate into larger, denser particles which will settle more quickly. These tiny colloidal particles have a very large surface area to mass ratio, and this factor is important in keeping the particles suspended for long periods of time. In fact, the surface area to mass ratio is so high that electric charges and ionic groups become important in keeping the particles suspended. Two types of colloids exist. These are hydrophobic or water hating colloids, and hydrophilic or water loving colloids. Hydrophilic colloids form suspensions easily, and can be difficult to remove. These colloids can, however, react chemically with the coagulants commonly added to water under proper conditions. Examples of hydrophilic colloids would be organic color forming compounds. Hydrophobic colloids do not easily form suspensions. The reactions between hydrophobic colloids and the coagulants commonly added to water are largely physical rather than chemical. Examples of hydrophobic colloids would be clays and metal oxides. Coagulation, generally followed by filtration, is by far the most widely used process to remove the substances producing turbidity in water. These substances which normally produce turbidity consist largely of clay minerals and microscopic organisms and occur in widely varying sizes, ranging from those large enough to settle readily to those small enough to remain suspended for very long times.

Coarser components, such as sand and silt, can be removed from water by simple sedimentation. Finer particles, however, will not settle in any reasonable time and must be flocculated to produce the large particles that are stable. The enormous increase of surface area for a given weight of solids as the particles become smaller and more numerous is an important property of colloids. Substances producing colour, as distinct from turbidity, consist either of colloidal metallic hydroxides, iron for example, of organic compounds having a much smaller particle size. These substances too can be removed by coagulation, which serves to agglomerate the very small particles into sizes, which are settleable or can be removed by filters.

1-10-1 Coagulation

Coagulation is a process which enables small particles suspended in water to stick to each other, forming larger particles that can be more easily removed through sedimentation or filtration.

When chemical coagulants are added to the water, they form small solid particles (flocs), which aggregate (flocculate) and settle out of suspension. Following sedimentation, the remaining suspended solids are removed by rapid sand filtration.

The most common coagulants are alum and ferric salts (chloride or sulfate). In some cultures, plant products are used as natural coagulants. The crushed seeds of moringa trees (Moringa oleifera lam and Moringa peregrina fiori) are particularly effective coagulants, comparing favorably with alum in terms of removal of turbidity. A dosing rate of 200 mg crushed seeds per litre of water is suggested as optimal (UNICEF, 2008).

1-10-2 Sedimentation

Sedimentation is the removal of suspended solids by gravity. Sedimentation, or gravity settling, is often the first stage of surface water treatment, with the goal of removing coarse suspended solids that might clog filters or reduce disinfection efficiency. This is especially needed in tropical areas where rivers often have high silt loads (UNICEF, 2008).

1-10-3 Filtration

Filtration is the passing of water through a porous material. Filtration can improve both physical and microbiological quality (UNICEF, 2008).

1-10-4 Disinfection

The filtration reduces number of pathogens present in water, but none of them is 100% effective, especially against viruses. And with any treatment technology, there is the possibility of failure, which may go undetected. Accordingly, water should always be disinfected after other treatment.

Disinfection (physical or chemical) is the most effective and reliable way to ensure that any pathogens present in drinking water are removed to acceptable levels (UNICEF, 2008).

1-11 The Coagulation Process

Coagulation is accomplished by the addition of ions having the opposite charge to that of the colloidal particles. Since the colloidal particles are almost always negatively charged, the ions which are added should be cations or positively charged. The coagulating power of an ion is dependent on its valency or the magnitude of the charge. A bivalent ion (+2 charge) is 30 to 60 times more effective than a monovalent ion (+1 charge). A trivalent ion (+3 charge) is 700 to 1000 times more effective than a monovalent ion (Gregory, 1981). Typically, two major types of coagulants are added to water. These are aluminum salts and iron salts. The most common aluminum salt is aluminum sulphate, or alum. When aluminum sulphate is added to water, the aluminum ions enter into a series of complicated reactions. The aluminum ions become hydrated, meaning that water molecules attach themselves to the aluminum ions. In addition, anions present in the water, such as hydroxide and sulphate ions can be attached to the aluminum ions (Spellman, 2003). These reactions result in large, positively charged species having aluminum ions at their center. These particles may have charges as high as +4. Following these reactions, a second type of reaction occurs, called flotation. This reaction involves the bridging of two or more of these large species to form even larger, positively charged ions. A typical species can contain eight aluminum ions, twenty hydroxide ions, and will have a +4 charge. Iron salts behave in a similar manner when added to water.

Once these large polymeric aluminum or iron compounds are formed, the magnitude of their high positive charge allows these species to rapidly move toward the colloid, where they are adsorbed onto the negatively charged surface of the turbidity particle. The coagulant compounds can penetrate the bound water layer because of their high positive charge (Pontius and Clark, 1999). This rapid adsorption results in the compression of the electrical double layer, and results in the colloid becoming coated with the coagulant compounds. The net result of this process is that the electrical charges on the particle are reduced. The suspension is now considered to be destabilized, and the particles can be brought together through, among other forces, Brownian movement, and will be held together by the van der Waals forces. An additional process occurs which assists this process. As the coagulant continues to undergo the hydrolyzation reactions, progressively larger masses of flocculent material are formed. These compounds can become large enough to settle on their own, and tend to trap turbidity particles as they settle. This is commonly referred to as sweep floc. As the coagulation reactions and destabilization are occurring, the zeta potential at the surface of the colloid is also found to be reducing. Typically, the zeta potential for naturally occurring water may be in the range of 10 to 25 millivolts. As the reactions occur, this zeta potential will be reduced to approximately 5 millivolts. These figures are only examples of what might be considered typical waters. Since all waters exhibit a specific set of characteristics, these numbers will vary. It is interesting to note that the zeta potential does not have to be reduced to zero in order for coagulation to occur, because the forces of attraction can become predominant before complete destabilization occurs. Hydrophilic colloids participate in the coagulation process in a slightly different way. These colloids tend to attract water molecules to their surfaces. This is also a hydration process, and the water molecules act as a barrier to contact between particles. Also attached to the surfaces are hydroxyl, carboxyl, and phosphate groups, all to which are negatively charged. Coagulant products react chemically with the negatively charged groups attached to the hydrophilic colloids, forming an insoluble product which is electrically neutral and destabilized (UNICEF, 2008).

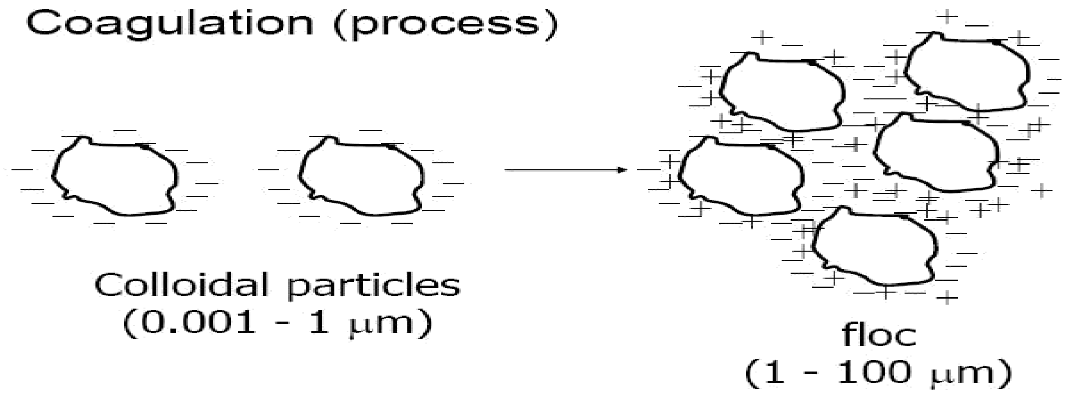


Figure 1.3 figure of coagulation and floc formation processes

1-11-1 Purpose of Coagulation

Untreated surface waters contain clay, minerals, bacteria, inert solids, microbiological organisms, oxidized metals, organic color producing particles, and other suspended materials. Some of the microbiological organisms can include Giardia cysts, pathogenic bacteria, and viruses. Oxidized metals include iron and manganese. All of these materials can inhibit disinfection, cause problems in the distribution system, and leave the water cloudy rather than clear. The purpose of coagulation is to remove these particles. The ability of particles to remain suspended in water is a function of both the particle size and specific gravity (Carty et al., 2002).

Turbidity particles can range in size from molecular to 50 microns. Particles which are greater than one micron in diameter are considered silt, and settle out due to their relatively large size and density without the need to coagulate in a matter of minutes. Colloidal material ranges in size from 0.001 to one micron in diameter. These materials require days to months for complete settling. Since retention times in the water treatment process are generally less than twelve hours, the rate of settling of these colloidal particles must be increased in the water treatment process. This is accomplished in the coagulation process when tiny particles agglomerate into larger, denser particles which will settle more quickly. These tiny colloidal particles have a very large surface area to mass ratio, and this factor is important in keeping the particles suspended for long periods of time. In fact, the surface area to mass ratio is so high that electric charges and ionic groups become important in keeping the particles suspended (Carty et al., 2002). Two types of colloids exist. These are hydrophobic or water hating colloids, and hydrophilic or water loving colloids. Hydrophilic colloids form suspensions easily, and can be difficult to remove. These colloids can, however, react chemically with the coagulants commonly added to water under proper conditions. Examples of hydrophilic colloids would be organic color forming compounds. Hydrophobic colloids do not easily form suspensions. The reactions between hydrophobic colloids and the coagulants commonly added to water are largely physical rather than chemical. Examples of hydrophobic colloids would be clays and metal oxides. Coagulation, generally followed by filtration, is by far the most widely used process to remove the substances producing turbidity in water. These substances which normally produce turbidity consist largely of clay minerals and microscopic organisms and occur in widely varying sizes, ranging from those large enough to settle readily to those small enough to remain suspended for very long times. Coarser components, such as sand and silt, can be removed from water by simple sedimentation. Finer particles, however, will not settle in any reasonable time and must be flocculated to produce the large particles that are settleable. The enormous increase of surface area for a given weight of solids as the particles become smaller and more numerous is an important property of colloids. Substances producing colour, as distinct from turbidity, consist either of colloidal metallic hydroxides, iron for example, or of organic compounds having a much smaller particle size. These substances, too, can be removed by coagulation, which serves to agglomerate the very small particles into sizes, which are settleable or can be removed by filters (DeZuane, 1997).

The process of coagulation may also find use, although not always, in the softening of hard water with lime or lime and soda ash. Softening is more properly a precipitation process, and coagulation is used to obtain a more rapid and complete settling of the precipitated hardness components. The dosage of coagulant depends on several parameters such as type and concentration of contaminants, pH, temperature etc. It also depends on the way the coagulant is added. Rapid stirring ensures adequate mixing, and so does dosing below the surface (Gregory, 1981).

1-11-2 Factors Affecting Coagulation

Factors that affect the coagulation include alkalinity, pH, temperature, ions concentration and composition, coagulant dosage are discussed below.

i) Alkalinity

Alkalinity in raw water can limit the pH reduction. It refers to the acid-neutralizing capacity of water, and is a general indication of a water buffering capacity. Alkalinity and pH are related as higher alkalinity water has higher pH. For low alkalinity waters, coagulants addition may consume all of the available alkalinity, reducing the pH for effective treatment. High alkalinity waters may require high coagulant to reduce the pH favorable for coagulation. Acid addition may be used to adjust the pH of waters high in alkalinity, however, this process may be difficult for some water utilities that must deal with influent variations in both coagulant dosage and alkalinity (as $CaCO_3$). Waters containing very low alkalinity may be adjusted to an optimum pH through addition of coagulation (DeZuane, 1997).

ii) pH

The pH at which coagulation occurs, is the most important for proper coagulation performance and it affects the surface charge of colloids, charge of the dissolved-phase coagulant species, surface charge of floc particles, and coagulant solubility (DeZuane, 1997).

iii) Temperature

Temperature is an important parameter for water treatment, treated by coagulation-flocculation method. Low temperature affects coagulation and flocculation processes by altering coagulant solubility, increasing water viscosity and retarding the kinetics of hydrolysis reactions and particles flocculation. Higher coagulant dosage, the addition of flocculation or filter aids, longer flocculation times and lower flotation, sedimentation, and/or filtration rates are often required at lower temperature especially, when alum used. Sedimentation processes are most affected (Noel et al., 2002).

iv) Dosage of coagulants

Different water samples have different quality of impurities. As a result, the coagulant dosages have to be adjusted to allow for variations in water impurities as well as the type of coagulant used. The amount of coagulant addition is an important factor and the effectiveness of colloidal destabilization is also directly proportional to the amount of coagulant added. For every coagulant, there exists an "optimal dosage" for a specific water chemistry and particle composition at which the coagulation of particles is optimized. An under-dosage of coagulant may provide insufficient destabilization to promote particle aggregation or destabilization may cause the sample to appear cloudy with no floc. With an over-dosage of coagulant, the particles themselves may be re-stabilized as the concentration of counter-ions increases, fragile, fluffy and dance flocs formation take place. These flocs may not settle well when the stirrer is turned off. The suitable dosages of coagulant would produce good flocs, which are heavy and tight, and would settle, and sweep coagulation may occur well once the stirrer is turned off. However, coagulant dosages cannot be calculated, they have to be determined experimentally by jar test (Noel et al., 2002).

1-11-3 Some Chemicals Used in Coagulation and Flocculation

Chemicals used in coagulation and flocculation are referred to as either primary coagulants or as coagulant aids. The coagulant aids can be used to condition the water to add density to slow-settling flocs or toughness so the floc will break up for the use of the primary coagulants. Primary coagulants cause the particles to become destabilised and begin to clump together. The reagents currently used in coagulation and flocculation are inorganic products, natural polymers and synthetic polymers. The most widely used coagulants are aluminium or iron salts and less frequently used coagulants are cationic polyelectrolytes. Aluminium and iron salts are used because they are effective, relatively low cost, widely available and easy to handle, store and apply. Aluminium sulphate is still used widely even though there has been much concern over the possible effects to human health, such as Alzheimer's and possible have strong carcinogenic properties (Noel et al., 2002).

i) Aluminium salts

When aluminium sulphate is added to water, the aluminium ions enter into a series of complicated reactions. The aluminium ions become hydrated, meaning that water molecules attach themselves to the aluminium ions. In addition, anions present in the water, such as hydroxide and sulphate ions can attach to the aluminium ions. These reactions result in large, positively charged complexes having aluminium ions at their centre. These particles may have charges as high as +4. Following these reactions, a second type of reaction occurs, called flotation, where metal ions form polymeric oxides in aqueous solution. This reaction involves the bridging of two or more of these large molecules to form even larger, positively charged ions. A typical molecule can contain eight aluminium ions, twenty hydroxide ions, and will have a +4 charge. Iron salts behave in a similar manner when added to water (Gregory, 1981).

The basic reaction when aluminium (Al^{3+}) is added to water is the formation of a precipitate of aluminium hydroxide with the release of some acidity.

$Al^{3+} + 3H_2O \leftrightarrow Al(OH)_3 + 3H^+$

These hydrolysis reactions are strongly influenced by pH i.e. the higher the pH the greater the tendency for the formation of hydroxylated species. The uncharged hydroxide $Al(OH)_3$ has a very low solubility in water and hence forms a precipitate optimally around a neutral pH. A similar reaction occurs when ferric salts are added to water, although they are less soluble than $Al(OH)_3$ but precipitates over a greater range of pH. Aluminium sulphate (liquid or solid) is added in solid form as blocks, as granulated or kibbled alum and also in liquid form. The chemical formula of pure aluminium sulphate is $Al_2(SO_4)_3 \cdot 18\ H_2O$ (Flynn, 2009).

ii) Iron Salts

Iron salts can also be used for coagulation; they work on much of the same principals as aluminium.

$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$

There are two main types of iron salts that are commonly used ferric sulphate and ferric chloride. Ferric sulphate is supplied as a red-brown powder or as granules.

Its chemical formula is $Fe_2(SO_4)_3.\ 9H_2O$. It is mildly hygroscopic but is difficult to dissolve and the solution is corrosive to aluminium, concrete and most steels. In a manner similar to the reaction with aluminium sulphate, the formation of a ferric hydroxide floc is the result of the reaction between the acidic coagulant and the natural alkalinity of the water, which usually consists of calcium bicarbonate, as expressed by the equation below, with the insoluble products (precipitates).

$Fe_2\ (SO_4\ )_3 + 3Ca\ (HCO_3\ )_2 \leftrightarrow 2Fe\ (OH\ )_3 + 3CaSO_4 + 6CO_2$

(Sincero and Sincero, 2002).

iii) Polymeric Coagulant

Polymeric materials used as flocculants/retention aids are mostly water-soluble linear polymers 23-27 of very high molecular weight. The cationic and anionic counterparts of these polymers are known as polyelectrolytes with many characteristic features of their own. Some of the nonionic water-soluble polymers at times bear some ionic groups as a result of inadvertent hydrolysis (Gregory, 1981).

iv) Natural Polymers

Natural polymers include those that are biological in origin or are derived from proteins, tannins, starch, or starch products such as cellulose derivatives and alginates. Unlike synthetic polymers, natural polymers are based on renewable organic biomass and are biodegradable, non-toxic and inexpensive. While natural polymers may be less expensive than synthetic polymers on a tonnage basis, they require higher dosages than synthetic polymers. Natural polymers are more susceptible to microbiological attack, so the storage life of natural polymers is less than that of other coagulants and flocculants. Natural polymers discussed in this section include chitosan, moringa oleifera, and guar (Raghuwanshi P.K., 2002).

Chitosan, a biopolymer extracted from shellfish exoskeletons, is the most commonly used natural polymer in storm water applications. The coagulation efficiency of chitosan is affected by both the pH of the water and the properties of clay particles in the raw water.

Moringa oleifera seeds have been used since time immemorial for water clarification in many third world countries. The seeds are more effective in higher turbidity applications (greater than 50 NTU) than in lower turbidity applications. They contain water-soluble proteins that carry a positive charge and have been studied for use in water treatment as a coagulant (Ravikumar and Sheeja, 2012b). A number of articles have been written in recent years on Moringa oleifera seed use in water and wastewater treatment in tropical regions where this tree is native. While there are no known storm water treatment applications of Moringa oleifera seeds, the effectiveness of this natural flocculant at reducing turbidity in surface waters indicates that it has potential for this application (Ndabigengesere and Narasiah, 1998a).

Guar gum (derived from the seeds of the guar plant) and other polysaccharides (i.e., complex carbohydrates) have been chemically modified and grafted with synthetic polymers, to increase their flocculation efficiency. Synthetic polymers were grafted onto the backbones of the natural polymers to combine the positive characteristics of synthetic and natural polymers. An extensive of biopolymer based flocculants (mainly chemically modified and grafted polysaccharides) has shown that they have effectively treated effluents from various industries, flocculating clay suspensions and suspended solids. Based on laboratory studies, chemically-modified products of guar gum and grafted copolymers have performed better than polyacrylamide based flocculants and potentially could replace synthetic flocculants. However, additional research and field studies are still needed (Ali et al., 2010).

Other natural polymers that have been studied for general water treatment applications include mimosa bark extract and valonia extract.

1-12 Moringa Oleifera Tree

1-12-1 General Description of Moringa Oleifera Tree

Moringa oleifera (Figures 1.4 and 1.5) is a perennial plant that grows very fast, with flowers and fruits appearing within 12 months of planting. They grow up to a height of 5-12 meters and pods 30-120 cm long and are harvested up to two times a year in India. The tree prefers lowlands in hot semiarid conditions with sandy or loamy soils but is known to adapt to new conditions quickly. It tolerates light frost, a soil pH of 9 and can live in areas with annual rainfall of up to 3000 mm. Today it can be found on elevations up to 2000 m in Zimbabwe (Pradhan, 2013).

With its origin in India and Pakistan M. oleifera was brought to the Africa continent and Sudan in particular for ornamental purposes during the colonial era.

The women of Sudan soon discovered the abilities of the tree and have used the seeds for water treatment since the beginning of the 20th century. The natural coagulant found in Moringa oleifera is present in 6 of the 14 species of Moringa growing in Africa, Madagascar, India and Arabia. Moringa oleifera is the only one of the species in the botanic family that is present in tropical and subtropical regions around the world, and is therefore the most famous (Ferreira et al., 2011).

The different purposes of the tree are many as all parts of the tree are used. Oil extracted from the seeds is used for working machinery, cosmetics, cooking and soap. The press cakes, what is left after the oil extraction, is used as soil fertilizer. Pods and leaves are used for eating both by humans and animals, as they contain a lot of vitamins. Using the tree as a vegetable is the main reason that it has been cultivated in large scale in India, but this is yet the only commercialized part of the tree. Different parts of the tree are used in traditional medicine for treating diarrhea and epilepsy among others, and some even claim to be treating tumors. The wood pulp can be used for papermaking and the tree itself can be used as a fence, natural windbreaks or fuel (Banerji et al., 2009).

In Sudan, there are more than 20 species of plants used for water purification during the flood season. In southern Blue Nile and western Sudan, roots and parks of hanza tree (Bosciasengalensis) which is also used in Northern Nigeria. In River Nile province, crushed fava beans (Viciafaba) are used. In Northern state, they also use fava beans besides Fenugreek seeds (Trigonellafoenumgraecum.L). In western Sudan, they use the leaves of guava tree (Psidiumguajava), peas (Pisumsativum), lentils (Lensculinaris), Indian almonds (Terminaliachebula), lupine beans (Lupinustermiskorsk), palm dates seeds (Phoenix dactylifera), rhizomes of sledge grass (Cyperusrotundus), and branches of tamarisk tree(Tamarixnilotica) (Jahn et al., 1986).

Table 1.2 Classification of Moringa Oleifera tree

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	Oleifera

(Mekonnen, 2003).

Figure 1.4 Fruiting of Moringa oleifera

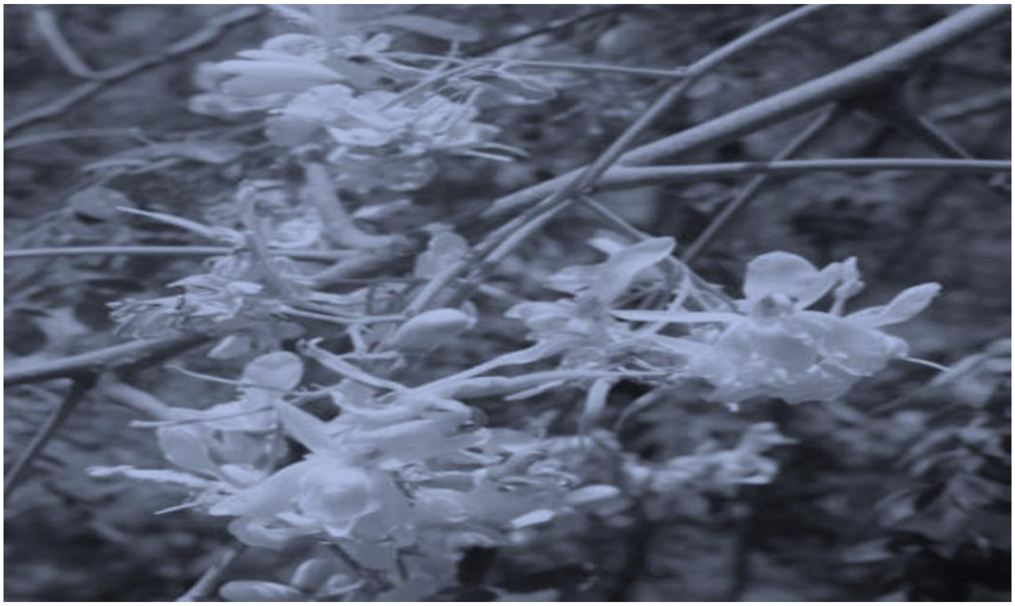


Figure 1.5 Flowers of Moringa oleifera

Figure 1.6 Leaves of Moringa oleifera

1-12-2 Uses of Moringa

i) Moringa as food

Moringa oleifera can be found in the wild but domestically it is cultivated throughout the plains, especially as hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams. The plant is an essential food commodity which has myriad attention as the natural nutrition of the tropics. Parts of the plant such as the leaves, fruits, flowers and immatures pods, also called long green pods, are used in many countries especially in India, Pakistan, Philippines, Hawaii and many parts of Africa as a highly nutritive vegetable and nutritional supplementation (Anwar et al., 2007). The tender leaves taste like watercress and, along with the flowers, is eaten cooked or raw. The green pods with seeds have a similar flavour to that of asparagus when cooked. The seeds from ripe fruits have a peanut flavour when fried. In certain parts of the world, these parts are eaten as fresh vegetables and can be frozen or canned. Combined with curries, the parts of the plant are commonly prepared with chicken or sea food as a Soup (Anwar and Bhanger, 2003). Moringa as animal feed and plant growth enhancer Moringa leaves are also fed to livestock as well as pigs, chickens and rabbits and can be fed to fishes as food. A study in Fiji reports significant weight gain over traditional fodder when 50% of the fodder contained Moringa (Ferreira et al., 2011). (Muyibi and Evison, 1995) reported that the inclusion of Moringa oleifera in cassava based broiler diets up to 5% is possible without negatively affecting productivity or haematological indices. Foidl and Reyes reported that adding Moringa leaves to cattle feed increased their daily weight gain by 32%. The milk production of cattle increased by 43% after being supplemented with 15-17 kg of fresh Moringa leaves and milk production increased further by 58% and 65% when cows were fed with 2kg and 3kg of dry matter of Moringa as supplement per day respectively. The adoption of Moringa as animal feed especially in third world nations will greatly improve the weight of animals as well as the production of animal milk which can boost the dietary protein supply in the population (Ferreira et al., 2011). Juice extract from green matter of the plant has been tested and is effective as a plant growth enhancer in the production of crops like soybeans, corn, turnips, black beans, red beans, white beans, cowpeas, bell peppers, chia, sunflowers, mung beans, onions, coffee, tea, chilli peppers, melons and sorghum. Crops sprayed with green matter juice extract of the plant comparatively resulted in increased crop size. The juice extracts were discovered to stimulate the growth of the plants roots enhancing the uptake of soil nutrient as demonstrated in sugarcane production. Leaf extracts have been found to increase Rhizobium root nodulation, nodule weight, and nitrogenase activity in mung bean (Vigna mungo (L.) HEPPER) when applied to seeds or as a root dressing (Padmapriya et al., 2014).

ii) Medicinal uses of Moringa

Myriad of medicinal properties have been attributed to parts of the plant. The high-quality protein of the leaves has led to its widespread use by doctors, healers, nutritionists and community leaders, to treat malnutrition and a variety of illnesses (Dwivedi et al., 2007).

The seeds of Moringa are considered to be antipyretic, the plant also contains numerous phytochemicals, some of which are of high interest due to their medicinal properties. Current studies demonstrate that isothiocyanates have antitumor activity in cancers of the lung, breast, skin, and pancreas (Kalkunte et al., 2006; Satyan et al., 2006) and this particular plant family is rich in a fairly unique group of glycoside compounds called glucosinolates and isothiocyanates. Small proteins/ peptides were isolated from the leaves of Moringa oleifera possessing antifungal and antibacterial activity (Arulselvan et al., 2016).

iii) Using of Moringa Oleifera As Water Treatment

The knowledge that seeds from the Moringa oleifera tree can purify water is not new; the seeds have been used for generations in countries like India and Sudan (Lilliehöök, 2005). Women of Sudan have used the technique of swirling seeds in cloth bags with water for a few minutes and letting it settle for an hour. This procedure is today recommended by different agencies (PACE and ECHO etc.) for people with limited access to clean water.

The required area for cultivation of Moringa when used for drinking water treatment is dependent on the raw water and dosage. With a production of 3 kg seed kernels per tree per year and a dosage of 100mg/dm^3, 30 000 litres of water can be treated from one tree. By assuming tree spacing of 3 m^2, an area of 1 ha can treat 30 000m3 annually (Lilliehöök, 2005).

After oil extraction from M. oleifera seeds, the residue pressed cake contains water soluble proteins that act as effective coagulants for water purification. One to two seeds per liter are required for water purification. Seed powders are mixed with water, after hours, the water is filtered to get purified water. The charged protein molecules can serve as nontoxic natural polypeptide to settle mineral particles and organics in the purification of drinking water, vegetable oil, depositing juice (sugarcane) and beer (Mumuni et al., 2013).

In recent times, there has been an increasing trend to find some indigenous cheaper material for wastewater treatment. Since the conventional procedure of wastewater treatment has some disadvantages, such as incomplete metal removal, high cost and high energy requirements, biological materials have been recognized as cheap substitutes for wastewater treatment. Current studies report that Moringa seeds and pods are effective sorbents for removal of heavy metal and volatile organic compounds in the aqueous system (Eman et al., 2014). It can be added in oxidation lagoons of wastewater treatment units to coagulate algae as well. The algae are removed by sedimentation, dried and pulverized, and then used as protein supplement for livestock. The unique characteristic of Moringa seeds could be a possible solution for the developing countries which are suffering from lack of clean water for irrigation (UDAYASRI et al., 2004).

iv) Moringa oleifera as biodiesel derived from seed oil

Biodiesel is a RE (renewable energy) source derived alternative fuel for diesel engine application that is produced through the transesterification of vegetable oil, animal fats or waste vegetable oil with alcohol in the presence of catalyst. Moringa oleifera seeds can be used for biodiesel production by transesterification using magnesium oxide (MgO) as a solid base catalyst (Nawi, 2015). As the result, about 60% yield of biodiesel was produced using 0.5 w.t % catalyst concentration and 12:1 methanol to oil ratio. In conclusion, biodiesel production by using Moringa oleifera seeds oil appears to be an acceptable alternative fuel as well as renewable source of energy for the future use (Rashid et al., 2008).

1-12-3 Active Agent of Moringa Oleifera Seeds

Extraction of the coagulants can be done in several ways. Most of them, including recommendations for domestic use, follow the pattern: dried seeds are grinding, with or without shells, using either a kitchen blender or a mortar. The powder is mixed with a small amount of water and the solution is stirred and filtered. The filtered solution is called a crude extract or stock solution and could be used for treating water without further preparation (Ndabigengesere and Narasiah, 1998b).

Several studies show that salt water and/or tap water is more efficient as solvent for the active agents as compared to distilled water. (Moramudaii et al., 2001) showed that the coagulation capacity with NaCl was up to 7.4 times higher with Moringa extract than with distilled water. This is based on the assumption that the coagulating protein is more soluble in water with high concentration of ions. Other studies have focused on purifying the active agent as much as possible and producing a stable protein powder without excessive organic matter. Two separate studies show that the active agents could be purified from the extract using a cation exchanger, leading to reduced levels of COD in the treated water. A more low-tech way of reducing the organic content is to extract the oil from the seeds with an organic solvent (Ghebremichael et al., 2005).

i) Characteristics of the active agents

The coagulant in the seeds was first confirmed by the German scientist Samia Alazharia Jahn (Jahn et al., 1986). The active agent is believed to be a protein, but the exact form of the protein is not yet known. Recent researchers have identified proteins of sizes ranging from 3 to 60 kDa, all possessing coagulating ability, which means that the Moringa seeds probably contain several different proteins that may act as coagulants (Okuda et al., 2001). The protein(s) act as cationic polyelectrolytes, which attach themselves to the soluble particles and create bindings between them, leading to large flocs in the water. Stirring and mixing accelerates the electrostatic flocculation, and the flocs condense (Sincero and Sincero, 2002).

1-13 Objective of Study

i) Main objective:

The main objective of the study was to determine the suitability of *Moringa oleifera* as an alternative and cheap local coagulating material in the improvement of water quality in terms of some physicochemical and microorganisms in Blue Nile water.

ii) Specific objectives:

1. Isolation, purification and characterization of the active constituents in *Moringa oleifera* seeds.

2. Determine the amount of seeds required for efficient treatment of the various turbidity levels in the Blue Nile.
3. Applying the isolated, purified active constituents of *Moringa oleifera* seeds to reduce microorganisms.

4. Evaluate and compare the performance and effectiveness of *Moringa* seeds coagulant as a replacement to Poly-Aluminum Chlorohydrate Sulfate (ACH) in water treatment.

CHAPTER TWO

MATERIAL & METHODS

2-1 Study Area

The Blue Nile with a catchment approximately 300,000km^2 drains the N– E Ethiopian Plateau. The source is Lake Tana, which a heart shaped body of water contributes only about 7% of its annual discharge and most water is collected in route through the Ethiopian highlands from many tributaries the largest of which is the Diddesa Khor. The Sudan, Ethiopian border marks the end of the Ethiopian highlands and the beginning of the Sudan plains. Inside the Sudan south of Sennar the Blue Nile is joined by two seasonal tributaries “the Dinder and the Rahad” river.

The Blue Nile flood season extends during the period June – October, which it contributes about 68% of the Nile water budget. Because of its rocky origin and high speed of the Blue Nile transports annually million tons of fertile soil, which causes high turbidity values in water and siltation of reservoirs.

2-2 Materials

2-2-1 Chemicals

All the chemicals and reagents used in the experiments were of analytical grade, and the chemicals are:

- Nitric Acid (HNO_3), United Kingdom.

- Hydrochloric Acid (HCl), United Kingdom.

- Ammonium Chloride (NH_4Cl), United Kingdom.

- Concentrated Ammonia Solution (NH_4), United Kingdom.

- Disodium salt of Ethylene diamine tetra acetic acid United Kingdom.

- Erichrome black T ($C_{10}H_{12}O_8CaN_2Na_2.2H_2O$), United State of America.

- Sodium Hydroxide (NaOH), United Kingdom.

- n-Hexane solvent (C_6H_{14}), United Kingdom.

- Sodium Chloride (NaCl), United Kingdom.

- Sodium Nitrate ($NaNO_3$), United Kingdom.

- Methyl orange indicator, United Kingdom.

- Poly-Aluminum chloro-hydrate Sulfate 50% solution, obtained from Summit Chemical Specialty Products (Thailand) Co., Ltd.

- M- Endo media powder, United State of America.

2-2-2 Equipment

The equipment used in the research are listed as follows:

- Jar test, J.P SELECTA S.A. company, Barcelona, Spain.
- pH meter from HANNA company, United State of America.
- JENWAY/4320 conductivity meter, JENWAY company, United Kingdom.
- DREL/2010 spectrophotometer from Hach company, Canada.
- 2100P turbidimeter from Hach company, Canada.
- ICP-MS - 7510, Shimadzu, Japan.

- GC-MS-TQ8040, Shimadzu, Japan.

- Soxhlet apparatus, HANNA company, United State of America.
- Rotary evaporator, Switzerland.

2-3 Methods

2-3-1 Collection of Moringa Oleifera Seeds

The Seeds of Moringa Oleifera used in this study were collected from a farm in Ad-Damazin, Blue Nile State, Sudan and keep for studies.

2-3-2 Preparation of Moringa Oleifera seed powder

The seeds collected from the farm were de-shelled and the endocarps were air dried at ambient temperatures (25°C) for a period of seven days before milling. Direct sunlight was avoided to prevent degradation of some of the plant photochemical constituents. The white kernel was crushed to a powder, using an electric grinder and sieved through a 500 µm stainless steel sieve. The fine powder obtained was stored in a sterile air-tight container in a dark place to prevent oxidation.

2-3-3 Physicochemical Analysis of Moringa Oleifera Seed

The oil, moisture, protein, fiber and ash contents were determined according to American Oil Chemists' Society (AOAS) method (William, 1980).

2-3-3-1 Determination of Oil Content

The soxhlet extraction method was used to extract the oil from Moringa Oleifera seeds. About 10 g of seeds powder were weighted accurately and transfered into an extraction thimble. A 170 cm^3 of n-hexane solvent was poured into round bottom flask. After set up the soxhlet apparatus, the solvent was heated for 45 min and the oil was extracted. The soluble extracts were filtered and evaporated in rotary evaporator at 50C° and pressure of 175 mbar). Then the percentage of oil contain was determined.

2-3-3-2 Determination of Moisture

The initial moisture content of the seed kernels was determined using the oven method. 5 g of seeds were taken in a tarred aluminum dish, then dryed in an air oven at 100 ±20 ^{0}C for 6 hours; the dish was cooled in a desiccator and weighed. The drying was replicated three times and the average value of moisture content was calculated and recorded.

2-3-3-3 Determination of Total Ash

About 5 g of sample was weighed in a tarred silica/platinum dish and transferred to muffle furnace. The sample was ashed at a temperature of 550 ±10 C° for 1 hour until the ash was free of carbon, the dish was cooled in a desiccator and weighted. The method was replicated until the difference between two successive weights is less than 1 mg, and the lowest weight was recorded.

2-3-3-4 Determination of Crude Fiber

The sample was boiled for 35 minutes in dilute sulphuric acid. After filtration and washing, the sample was boiled again for 35 minutes in diluted potassium hydroxide. After filtration, washing and drying the residue was ashed at 500C°. The difference in weight before and after ashing called crude fiber.

2-3-3-5 Determination of Crude Protein

1 g of M. Oleifera seeds powder was weighed into Kjeldahl flask. 0.5 g catalyst system made up of anhydrous sodium sulphate, copper (II) sulphate and selenium dioxide in the ratio 98:1:1 was added to the sample in the flask. 12 cm^3 of conc. suphuriuc acid was added and the mixture boiled for 1 hour at 420C°. The solution was transferred into 100 cm^3 volumetric flask and diluted to 100 cm^3. 10 cm^3 of the solution was placed in the Kjeldahl distillation apparatus (markehan still), and 20 cm^3 of 40% NaOH was added. The mixture was distilled and 20 cm^3 of 2% boric acid containing screened methyl orange indicator was added and titrated with standard HCl to end point, Then the percentage nitrogen was calculated.

2-3-4 Determination of Photochemical Compounds

2-3-4-1 Extraction and Isolation of M. Oleifera Seeds For GS-MS Analysis

The seeds were dehusked and air dried at room temperature. Then grinded into a fine powder (500μm) using a scientific electric blender. After oil extracted, the 50 g of the deoiled seed powder were soaked by ethanol 80%. The obtained filtrate was concentrated using a rotary evaporator at 45 °C. The extract acidified with conc. H_2SO_4 to remove water, after that the pH adjusted to 10 by ammonium hydroxide and extracted two times using chloroform followed by dichloromethane solvent. The obtained extract was kept for GC.MS analysis.

2-3-4-2 GC-MS Analysis

GC-MS analysis of the extracts was carried out in a GC-Ms system (Figure 2.1) equipped with split/splitless injector and auto-sampler attached to a polar 5-MS (5% phenyl polymethyl siloxane) capillary column (Rtx-5MS; 30 m×0.25 mm I. d. and 0.25-μm film thickness) and fitted to Mass Detector. The flow rate of the carrier gas, helium (He) was set to be at 50 ml.min−1 in splitless mode. The injector temperature was adjusted at 280 ºC, while the detector temperature was fixed to 280 ºC. The column temperature was kept at 70 ºC for 1 min followed by linear programming to raise the temperature from 200 ºC to 250 ºC (at 8 ºC min−1 with 2.5 min hold time), and 200 ºC to 250 ºC (at 10 ºC min−1 with 2 min hold time). The transfer line was heated at 280 ºC. Total run time was 27.2 min. Mass spectra were acquired in scan mode (70 eV); in the range of 50 to 550 m/z. Twenty microliter each of the extracts (250 mg/ cm^3 stock) were further diluted in 2 cm^3 of methanol. One microliter of this diluted sample was injected for GC-MS analyses.

2-3-4-3 Identification of Compounds

Interpretation of mass spectra was conducted using the database of the National Institute of Standards and Technology (NIST, USA). The database caters for more than 62,000 patterns of known compounds. The spectrum of the extracts was matched with the spectrum of the known components stored in the NIST library.

Figure 2.1 Gas Chromatograph Mass Spectrometer

2-3-5 Determination of Elements

2-3-5-1 Moringa Oleifera Seeds Preparation

A mass of 0.1 g ground seed powder was placed in a microwave digestion vessel and concentrated nitric acid (8cm^3) and hydrogen peroxide (2cm^3) were added. Digestion was carried out for about 30 min in the microwave. After digestion, the samples were transferred to a 25 cm^3 volumetric flask and made up to volume with deionized water. The solutions were directly analyzed for metal content using ICP-MS and results were expressed as ppm (mg/kg) of dry weight.

2-3-5-2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

A prepared solution containing analyte element was aspirated into the plasma generated by inductive coupled plasma source. The atomized elements produced characteristic emission spectra lines, which are separated by a simultaneous optical spectrometer. The intensity of spectral line of an element is proportional to concentration.

Figure 2.2 Inductively Coupled Plasma Mass Spectrometer -7510

2-3-6 High and Low Turbidity Water Treatment

2-3-6-1 Location of Study

The study was carried out in Khartoum Station Water Corporation, Soba Station laboratory, Sudan.

2-3-6-2 Water sample collected

The water for study was collected from the point of Soba area in Khartoum state, Blue Nile that flow from Ethiopia. The raw water was first collected in a plastic container of 5 liters and kept for use. The plastic containers were cleaned by washing with soap and tap water. The containers were disinfected with (1 + 1) HNO_3 and finally rinsed with double distilled water. The glass containers were washed by soaking in Aqua Regia (3 parts conc. HCl and 1 Part HNO_3) and followed by a thorough wash with tap water and finally with distilled water.

2-3-6-3 Preparation of Moringa Oleifera Seed Coagulant

i) Work solution

Stock solution was prepared by dissolving 1.5 % w/v of moringa oleifera seed powder into 300 cm^3 distilled water, mixed with a magnetic stirrer for 60 minutes and left to settled. The solution was filtered through a Whatman No 1 filter paper, the resultant was a clear, milk-like liquid. Each time fresh solution was prepared for the experimental work. From the prepared stock solution, 1.5g, 2g, 3g, 4g and 5g % w/v concentration were used for the optimization study.

2-3-6-3 Jar tests

Coagulation test was carried out using jar test to evaluate coagulation activity at several dose levels of Moringa Oleifera seed coagulant (MOSC). The study involved rapid mixing, slow mixing and sedimentation in a batch process. Jar Test was carried out using the jar apparatus with six paddles. Five glass beakers of 1000 cm^3 capacity were filled with the water samples, during this agitation, various amounts (20, 40, 60, 80 and 100 cm^3) of MOSC stock solution was added to five beakers respectively, and agitated for 2 min at 150 rpm, the rapid mix stage helped to disperse the coagulant throughout each container. The mixing speed was reduced to 30 rpm and was kept for 30 mints for slow mixing. This slower mixing speed helped in promoting floc formation by enhancing particle collisions which led to larger flocs. The process of mixing was followed closely to activate the coagulant properties and to avoid a risk of secondary bacteria growth during long flocculation process. After sedimentation for 40 mints, the water was filtered through filter paper Whatman No. 1. Utmost care was taken in process of settling since the sediment at the bottom contained the impurities, and only the clear water on the top was used in order not to allow the sediment to re-contaminate the cleare water.

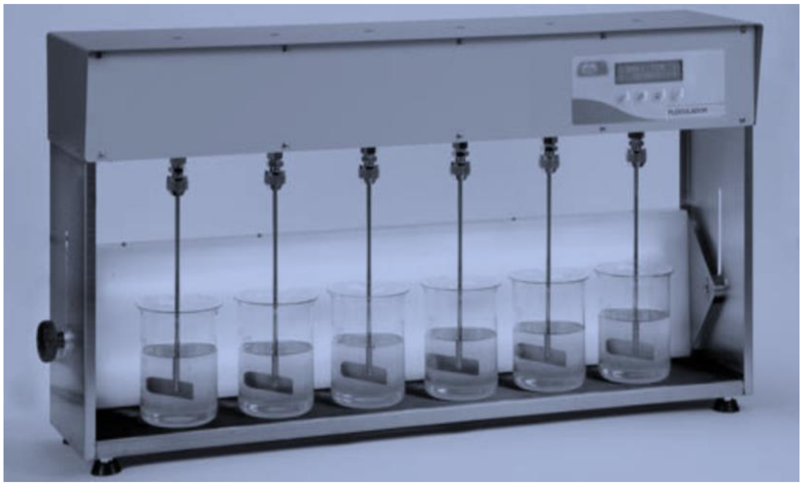


Figure 2.3 Jar test equipment

2-3-7 High and Low Water Turbidity Treated Analysis

After 40 mints of sedimentation of the treated water, supernatant samples were collected from each of the 6 beakers for physicochemical and biology analyses. The parameters described below were measured on the supernatant in each jar. For each coagulant and turbidity level, three identical jar tests were carried out in order to obtain statistically reliable results. However, some of the parameters were only measured during one of these three jar tests, due to restricted time and cost of materials.

2-3-7-1 Physicochemical Analysis

i) Determination of turbidity

Turbidity was measured with a 2100P turbidimeter from Hach. The initial turbidity was measured 3 times during the raw water stirring, and the average value from the three measurements was used as starting value of raw water (RW). After the sedimentation phase, samples for turbidity measurement were collected from the supernatant using a standard pipette. The sample beaker was washed once with distilled water and twice with the supernatant before recording the turbidity. Each measurement took 1-2 minutes including washing. In order to eliminate any differences in turbidity due to different sedimentation times, samples were taken from jars 1-6 into separate beakers before measurements were taken in the following order: (RW, 20, 40, 60, 80 and 100) cm^3.

ii) Determination of pH:

pH value was measured by pH meter. The pH meter was calibrated by the standard solutions of pH 4.7 and 9 at 26°C, and the pH values of samples were then recorded.

iii) Determination of Total Dissolved Solids and Conductivity

A 50cm^3 well-mixed sample was transferred into a beaker. The TDS/Conductivity meter probe was immersed in sample and its conductivity and TDS were recorded. This was after calibration with 0.01N KC1.

iv) Determination of temperature

This was determined at the time of analysis. An 50cm^3 of water sample was transferred into a 100m1 beaker and its temperature was recorded with 0 – 600 ºC thermometer. All samples were analyzed at room temperature.

v) Determination of alkalinity

-Method

A 50 cm^3 sample was transferred sample into a conical flask. Two drops of methyl orange indicator were added and the resulting mixture was titrated against the standard 0.1M HC1 solution to the permanent pink colour at pH 4.5. The following equation was used in the calculation.

Alkalinity mg ($CaCO_3$)/dm^3 = $\frac{\mathrm{V\ x\ M\ x\ 50.000}}{\mathrm{cm3\ of\ sample}}$

Where V = cm^3 of titrant

M = Molarity of HCl

vi) Determination of total hardness

A 50 cm^3 sample was transferred into a conical flask. To this was added a portion of ammonium chloride buffer solution, followed by 30mg enrichrome black T indicator crystals. The resulting solution was titrated with 0.01M EDTA solution with continuous stirring until the end point was reached. The end point is reached when the last reddish tinge disappeared.

Calculation: (Total hardness) mg/dm^3 $CaCO_3$ = $\frac{\mathrm{V\ x\ W\ x\ 1000}}{\mathrm{m1\ of\ sample}}$

Where V = cm^3 of titrant

W = mg $CaCO_3$ equivalent to 1 cm^3 EDTA titrant

2-3-7-2 Anion Analysis

i) Determination of nitrate

DREL/2010 spectrophotometer was used for the nitrate analysis. It adopts Cadmium reduction method by using powder pillow. 500 nm wavelength was set for high range nitrate nitrogen (NO_3^--N). After the correct wavelength was dialed in, the display quickly showed zeroing then mg/dm^3 NO_3^--N HR. 25cm^3 of the sample was poured into the sample cell. One Nitra Ver 5 Nitrate reagent powder pillow was added into the contents of the cell (the prepared sample). The prepared sample in the cell was vigorously shaken until the timer beeps in one minute. When the timer beeps, a five-minute reaction period began. Another sample cell was filled with 25mL of the sample (the blank). After the 5-minutes reaction, the spectrophotometer displayed mg/L $NO_3^- -$N HR. The blank was placed into the cell holder then the light shield was closed. The display showed zeroing 0.0 mg/dm^3 $NO_3^- -$N HR. The stopper was removed. The prepared sample was placed into the cell holder and the light shield closed tightly. The display showed the result in mg/L nitrate nitrogen $NO_3^- -$N after pressing the ―Read.

ii) Determination of phosphate

DREL/2010 spectrophotometer was used for the phosphate analysis. It is also called Orthophosphate (PhosVer 3) (Ascorbic Acid) method. 890nm wavelength was set for Phosphorus PO_4^{3-}. After the correct wavelength was dialed in, the display quickly showed zeroing then mg/L PO_4^{3-} PV. A 10- cm^3 Cell riser was inserted into the cell compartment. 10 - cm^3 of the sample was poured into a clean sample cell. One PhosVer 3 Phosphate Powder Pillow was added into the contents of the cell (the prepared sample). The sample was swirled immediately to mix; a blue colour formed showing the presence of phosphate. When the timer beeps, a two-minute reaction period began. Another sample cell was filled with 10 cm^3 of the sample (the blank). After the 2-minutes reaction time, the spectrophotometer displayed mg/dm^3 PO_4^{3-} PV. The blank was placed into the cell holder then the light shield was closed. The display showed 0.00 mg/dm^3 PO_4^{3-} PV. The stopper was removed. The prepared sample was placed into the cell holder and the light shield closed tightly. The display indicated the amount of mg/dm^3 PO_4^{3-} after pressing the ―Read‖ button.

iii) Determination of sulphate

DREL/2010 spectrophotometer was used for the sulphate analysis. It is Sulfa Ver 4 method by using sulfate powder pillow. A 450nm wavelength was set for sulphate SO_4^{2-}. After the correct wavelength was dialed in, the display quickly showed zeroing then mg mg/L SO_4^{2-}. 25 cm^3 of the sample was poured into a clean sample cell. One Sulfa Ver 4 Sulphate Reagent Powder Pillow was added into the cell containing the sample and swirled to get dissolved in the cell white turbidity developed which shows the presence of sulphate. When the timer beeps, a five-minute reaction period began and the prepared sample in the cell was allowed to stand undisturbed. Another sample cell was filled with 25 cm^3 of the (the blank). After the 5-minutes reaction, the spectrophotometer displayed mg/dm^3 SO_4^{2-}. The blank was placed into the cell holder then the light shield was closed. The display showed zeroing 0.0 mg/dm^3 SO_4^{2-}. The stopper was removed. The sample was placed into the cell holder and the light shield closed tightly. The display showed the result in mg/L sulphate (mg/dm^3 SO_4^{2-}) after pressing the ―Read‖ button.

2-3-7-3 Analysis of Total coliform bacteria

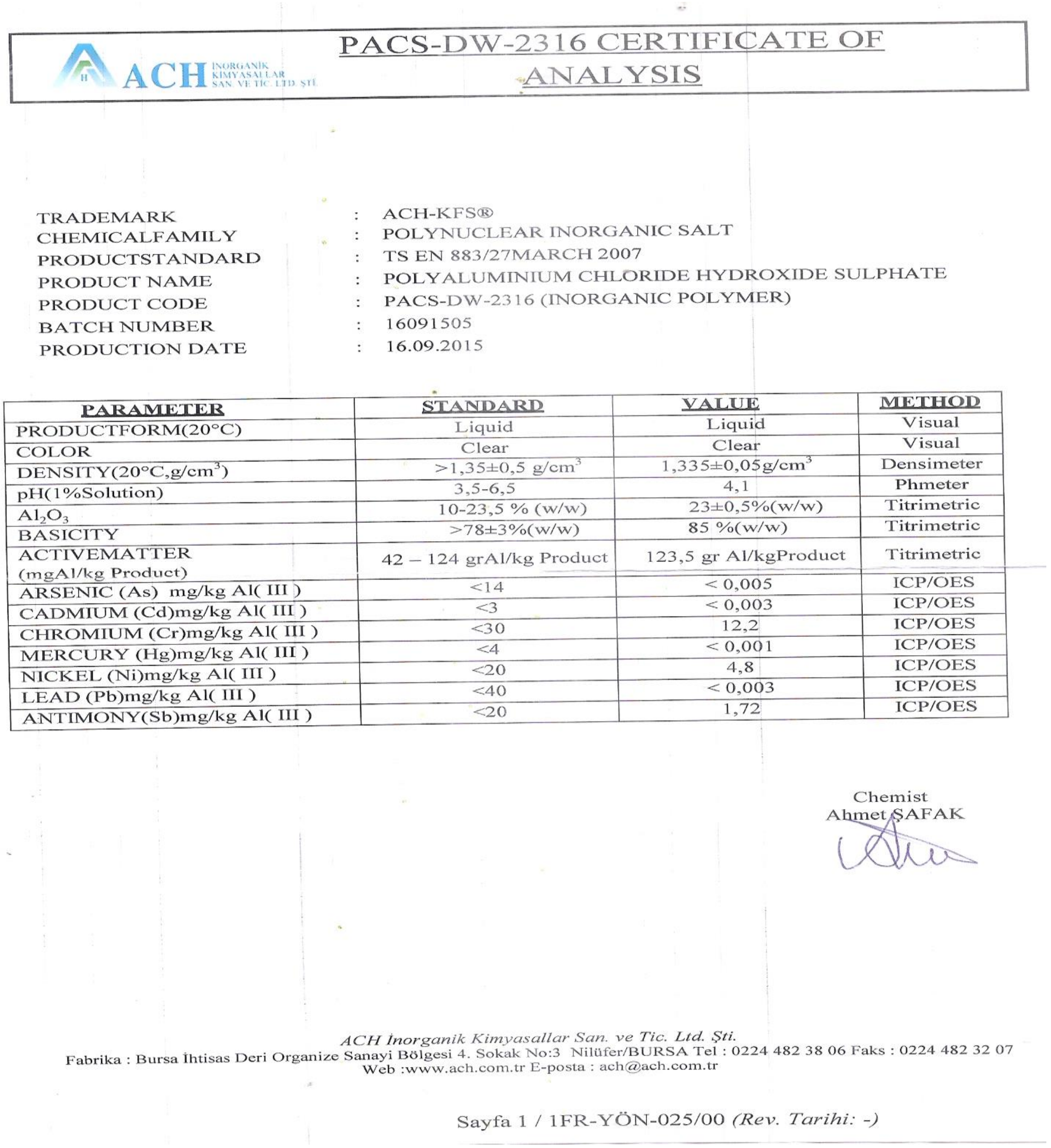
The total coliform bacteria was easily enumerated and detected. Supernatant samples were taken from raw water and the 3 of 5 beakers after 1-hour treatment with a concentration of 1.5 %w/v and dosages (20, 60 and 100 cm^3). The same experiment was carried out with concentrations of (2, 3, 4 and 5) % w/v with the same corresponding dosages as indicated above.

The M-Endo media was prepared by dissolving 20.5g of the M- Endo media powder in 1litre of distilled water, autoclaved and allowed to cool. Sample were poured under pressure vacuum, then surface of the funnel rained with portion of sterile distilled water. The paper placed on the pre-prepared pad saturated with $3cm^3$ of media, and inoculated for 24hrs in an electro-thermal incubator. The growth of the microorganisms was then observed and counted per cm^3 (number of microorganisms per ml of water samples).

CHAPTER THREE

3-1 Results & Discussion

3-1-1 analysis of PolyAluminium Chloride Hydride Sulphate



ACH İNORGANİK KİMYASALLAR SAN. VE TİC. LTD. ŞTİ.

PACS-DW-2316 CERTIFICATE OF ANALYSIS

TRADEMARK	: ACH-KFS®
CHEMICALFAMILY	: POLYNUCLEAR INORGANIC SALT
PRODUCTSTANDARD	: TS EN 883/27MARCH 2007
PRODUCT NAME	: POLYALUMINIUM CHLORIDE HYDROXIDE SULPHATE
PRODUCT CODE	: PACS-DW-2316 (INORGANIC POLYMER)
BATCH NUMBER	: 16091505
PRODUCTION DATE	: 16.09.2015

PARAMETER	STANDARD	VALUE	METHOD
PRODUCTFORM(20°C)	Liquid	Liquid	Visual
COLOR	Clear	Clear	Visual
DENSITY(20°C,g/cm^3)	>1,35±0,5 g/cm^3	1,335±0,05g/cm^3	Densimeter
pH(1%Solution)	3,5-6,5	4,1	Phmeter
Al_2O_3	10-23,5 % (w/w)	23±0,5%(w/w)	Titrimetric
BASICITY	>78±3%(w/w)	85 %(w/w)	Titrimetric
ACTIVEMATTER (mgAl/kg Product)	42 – 124 grAl/kg Product	123,5 gr Al/kgProduct	Titrimetric
ARSENIC (As) mg/kg Al(III)	<14	< 0,005	ICP/OES
CADMIUM (Cd)mg/kg Al(III)	<3	< 0,003	ICP/OES
CHROMIUM (Cr)mg/kg Al(III)	<30	12,2	ICP/OES
MERCURY (Hg)mg/kg Al(III)	<4	< 0,001	ICP/OES
NICKEL (Ni)mg/kg Al(III)	<20	4,8	ICP/OES
LEAD (Pb)mg/kg Al(III)	<40	< 0,003	ICP/OES
ANTIMONY(Sb)mg/kg Al(III)	<20	1,72	ICP/OES

Chemist
Ahmet ŞAFAK

ACH İnorganik Kimyasallar San. ve Tic. Ltd. Şti.
Fabrika : Bursa İhtisas Deri Organize Sanayi Bölgesi 4. Sokak No:3 Nilüfer/BURSA Tel : 0224 482 38 06 Faks : 0224 482 32 07
Web :www.ach.com.tr E-posta : ach@ach.com.tr

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3-2-1 Moringa Oleifera Seed Analysis

i) Physicochemical Analysis

The percentage of the chemical constituents of Moringa Oleifera is showed in Table 3.1

Table 3.1 Physicochemical analysis of Moringa Oleifera seed

Constituents (%)	Moringa Oleifera
Oil content	40.39
Moisture content	2.24
Protein content	37.06
Fiber content	8.24
Ash content	3.68

From the table 3.1 the n-hexane extracted oil content of Moringa Oleifera seeds was found to be 40.39%, this highest oil yield of MO seeds might be attributed to the natural habitats and geoclimatic constraints. In general, MO seeds contain 35–42% oil. The remaining seed cake residue could be used in the water treatment. The presence of oil in MO seed has an effect on the coagulant and turbidity removal activities; therefore, MO seed's high oil content would have a negative impact on the turbidity water treatment process. The moisture content of seeds was found to be 2.24 %. The moisture usually depends on factors such as harvesting time as well as storage conditions. The protein and fiber was found to be 37.06% and 8.24% respectively. The ash content of the M. oleifera seed was determined to be 3.68 %. This indicates a high mineral content in the seeds.

ii) Photochemical Screen

a- Mass Spectrum of Moringa Oleifera Seeds

The fragmented ions were separated by the analyzer, according to their mass to charge ratio. GC-MS analyses of MO seeds show several complexes. Alkaloids, Flavonoids, Phenolic compound and antioxidant.

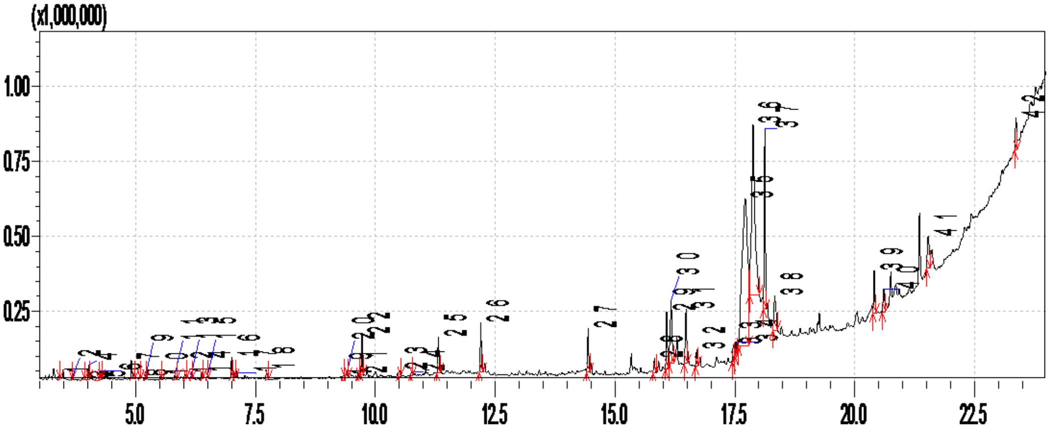

Figure 3-1 GC-MS chromatogram of M. Oleifera seed extracted

From the figure 3-1 GC method was optimized by varying the oven temperature. In current gradient oven temperature programming, a good resolution of the extracts has been seen in a relatively short duration of time. The fragmented ions were separated by the analyzer, according to their mass to charge ratio. GC-MS screen of M. Oleifera seeds show several complexes. Alkaloids, Phenolic compound and antioxidant compounds (Table 3.2).

Table (3.2) Identification of screen compounds used GC-MS

No.	**Name of compound**	**R. Time**	**Area %**	**Classification**
1	Pyrazine, 2,5-dimethyl-	3.394	0.23	Alkaloid
2	2-Pyrrolidinone, 1-methyl-	5.082	0.11	Alkaloid
3	1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene-	6.145	0.09	Alkaloid
4	2-methyl-pyrrolidinone	6.491	0.08	Alkaloid
5	Pyridine, 3-(1-methyl-2-yrrolidinyl)-	9.325	0.21	Alkaloid
6	Phenol, 2-methoxy-4-(2-propenyl)-	9.394	0.21	phenolic
7	2-Hydrazino-2-imidazoline	9.640	0.14	Alkaloid
8	Phenol, 2,4-bis(1,1-dimethylethyl)-	11.313	1.57	Phenolic
9	pipradrol	16.077	2.73	Alkaloid
10	pipradrol	16.702	0.73	Alkaloid
11	Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl-	20.408	1.89	phenolic

i) Identification of pyrazine, 2,5-dimethyl-

The EI mass spectrum of pyrazine, 2,5-dimethyl- MW 108 (Figure3.2). The base peak is found at m/z 108 corresponding to M [$C_6H_8N_2$]. The pyrazine, 2,5-dimethyl- appear at R.T 3.394 in total ion chromatogram.

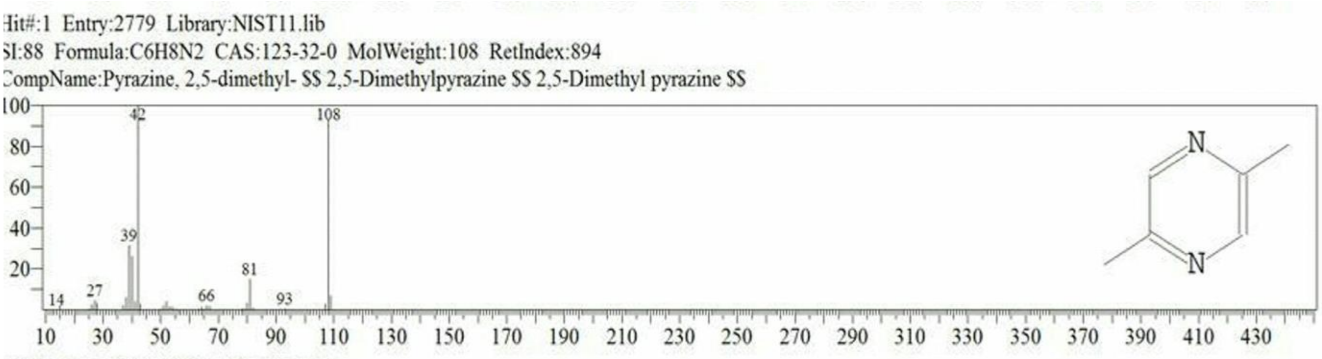

Figure (3.2) The mass spectrum analysis of pyrazine.

ii) Identification of 2-pyrrolidinone, 1-methyl-

The EI mass spectrum of 2-pyrrolidinone, 1-methyl- MW 99 (Figure 3.3). The base peak is found at m/z 99 corresponding to M [C_5H_9NO].

The 2-pyrrolidinone, 1-methyl- appear at R.T 5.082 in total ion chromatogram.

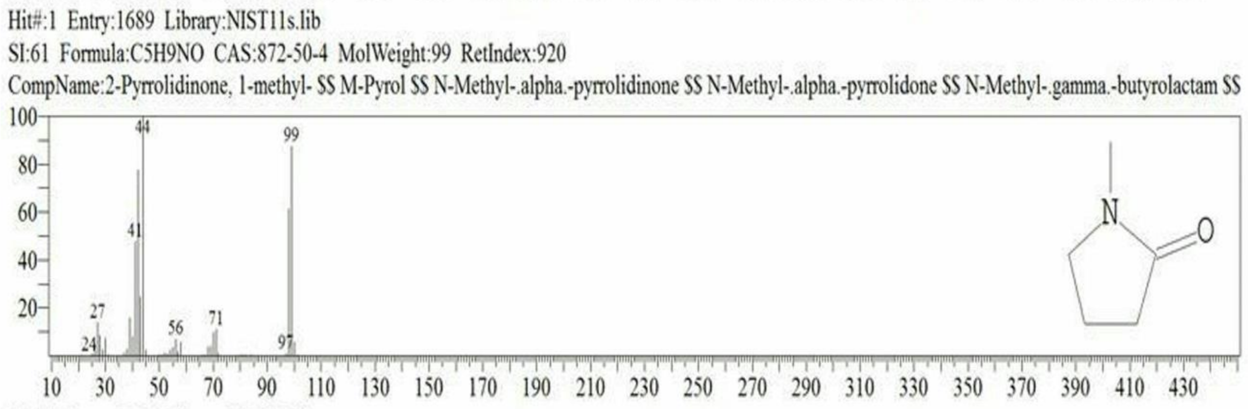

Figure (3.3) The mass spectrum analysis of 2-pyrrolidinone, 1-methyl-.

iii) Identification of 1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene-

The EI mass spectrum of 1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene- MW 138 (Figure 3.4). The base peak is found at m/z 82 corresponding to M $[C_8H_{14}N_2]$. The 1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene- appear at R.T 6.145 in total ion chromatogram.

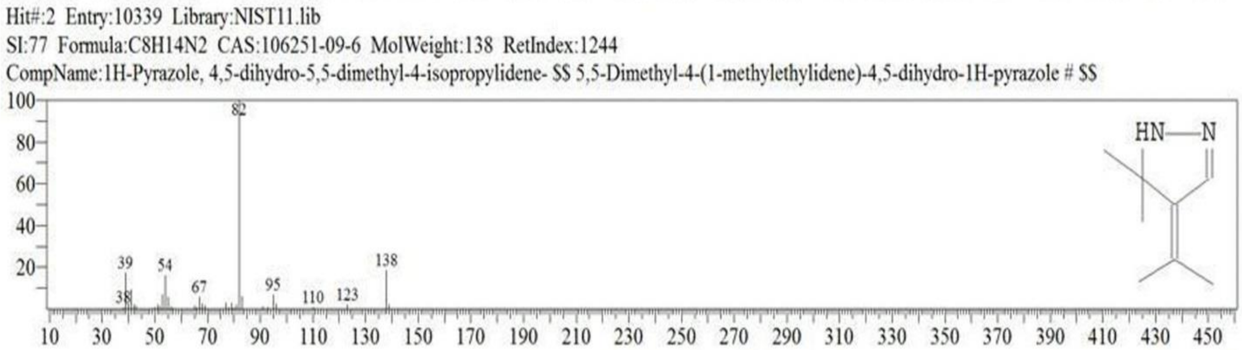

Figure (3.4) The mass spectrum analysis of 1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene-.

iv) Identification of 2-methyl-pyrrolidinone

The EI mass spectrum of 2-methyl-pyrrolidinone MW 85 (Figure 3.5). The base peak is found at m/z 70 corresponding to M $[C_5H_{11}N]$. The 2-methyl-pyrrolidinone appear at R.T 6.491 in total ion chromatogram.

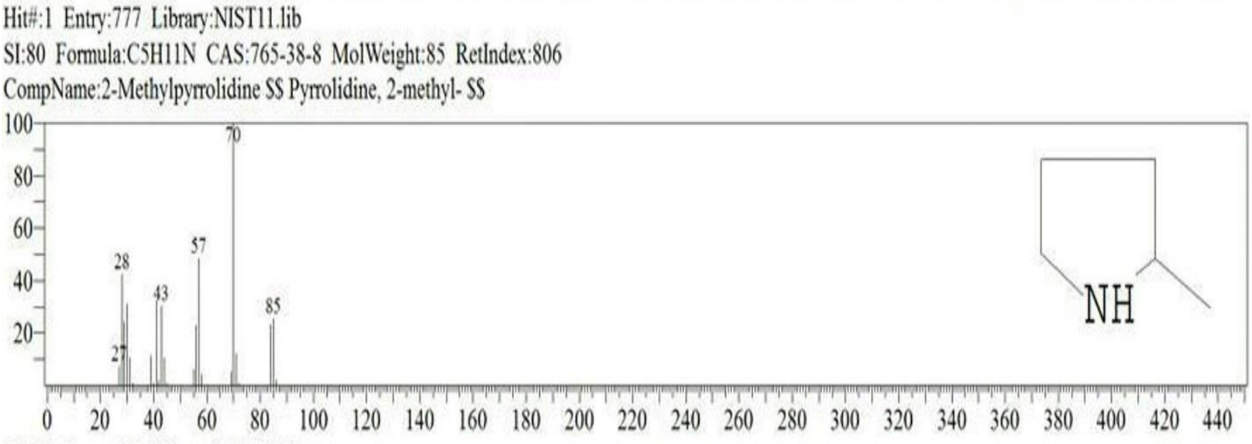

Figure (3.5) The mass spectrum analysis of 2-methyl-pyrrolidinone.

v) Identification of Pyridine, 3-(1-methyl-2-pyrrolidinyl)-

The EI mass spectrum of pyridine, 3-(1-methyl-2-pyrrolidinyl)- MW 162 (Figure 3.6). The base peak is found at m/z 100 corresponding to M [$C_{10}H_{14}N_2$]. The pyridine,3-(1-methyl-2-pyrrolidinyl)-appear at R.T9.325 in total ion chromatogram

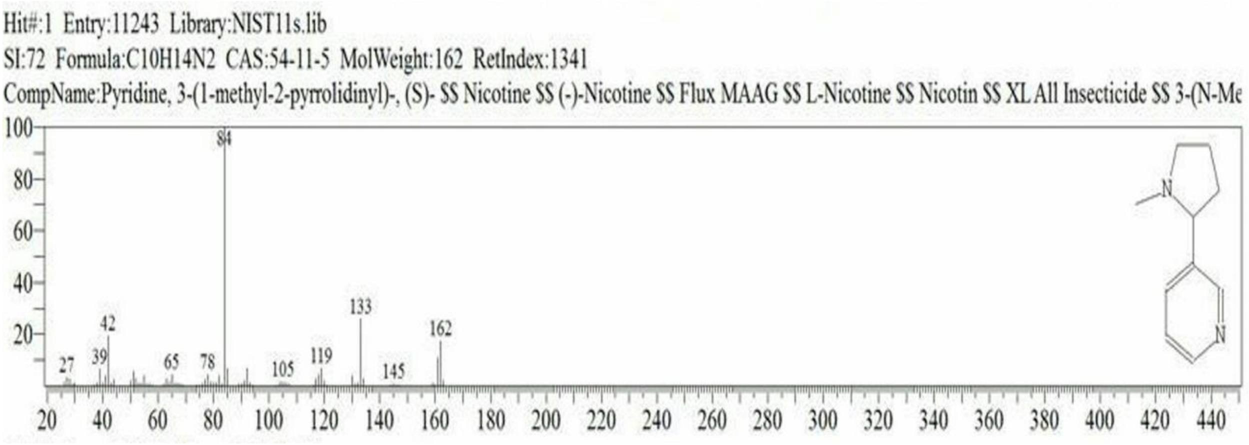

Figure (3.6) The mass spectrum analysis of pyridine, 3-(1-methyl-2-pyrrolidinyl)-.

vii) Identification of Phenol, 2-methoxy-4-(2-propenyl)-

The EI mass spectrum of Phenol, 2-methoxy-4-(2-propenyl)- MW 206 (Figure 3.7). The base peak is found at m/z 164 corresponding to M [$C_{12}H_{14}O_3$]. The Phenol, 2-methoxy-4-(2-propenyl)- appear at R.T 9.395 in total ion chromatogram.

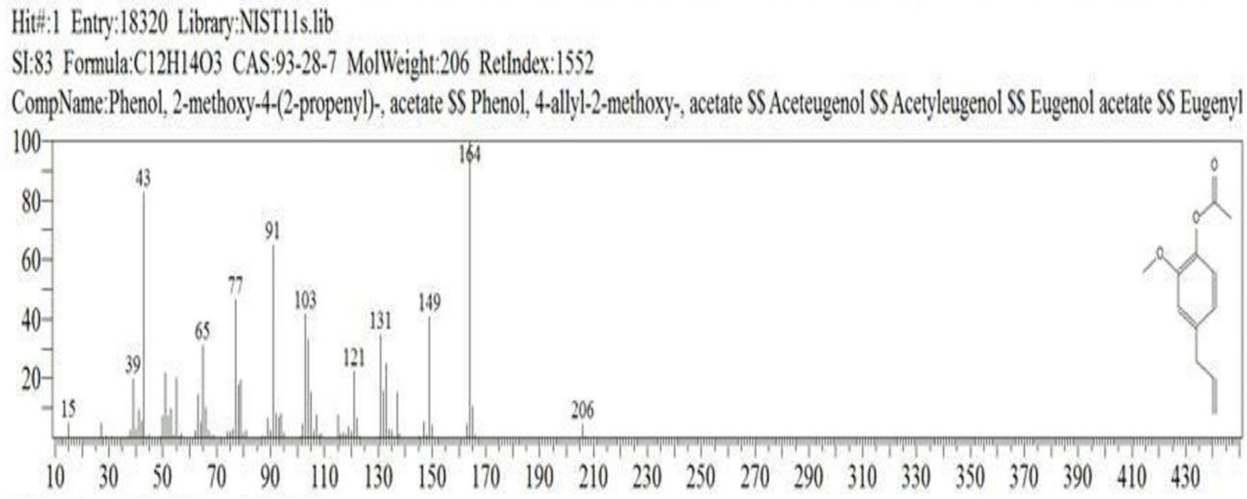

Figure (3.7) The mass spectrum analysis of Phenol, 2-methoxy-4-(2-propenyl)-.

vi) Identification of 2-Hydrazino-2-imidazoline

The EI mass spectrum of 2-Hydrazino-2-imidazoline MW 100 (Figure 3.8). The base peak is found at m/z 100 corresponding to M [$C_3H_8N_4$]. The 2-Hydrazino-2-imidazoline appear at R.T 9.640 in total ion chromatogram.

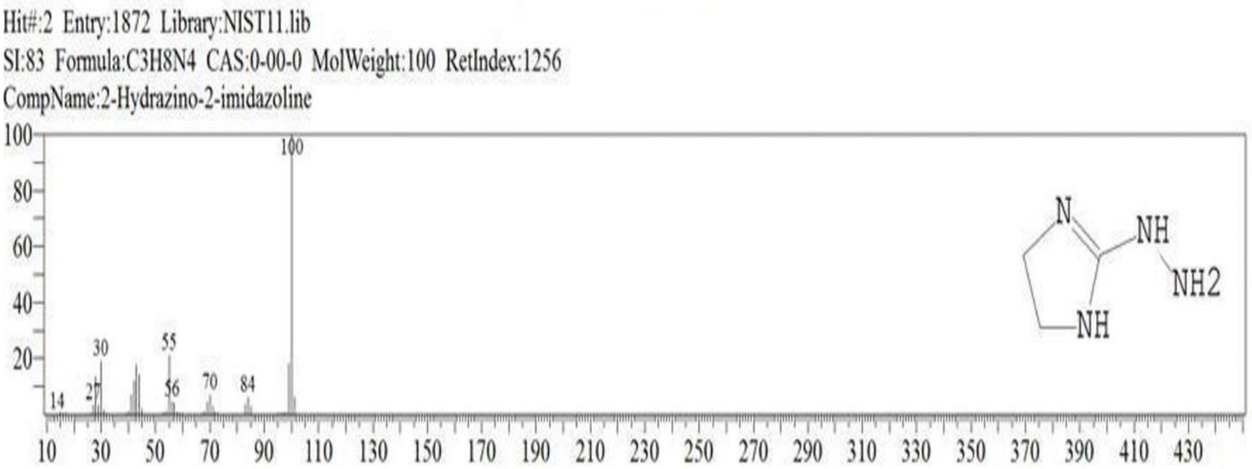

Figure (3.8) The mass spectrum analysis of 2-Hydrazino-2-imidazoline.

viii) Identification of Phenol, 2,4-bis(1,1-dimethylethyl)-

The EI mass spectrum of Phenol, 2,4-bis(1,1-dimethylethyl)- MW 206 (Figure 3.9). The base peak is found at m/z 191 corresponding to M [$C_{14}H_{22}O$]. The Phenol,2,4-bis(1,1-dimethylethyl)-appear at R.T 11.315 in total ion chromatogram.

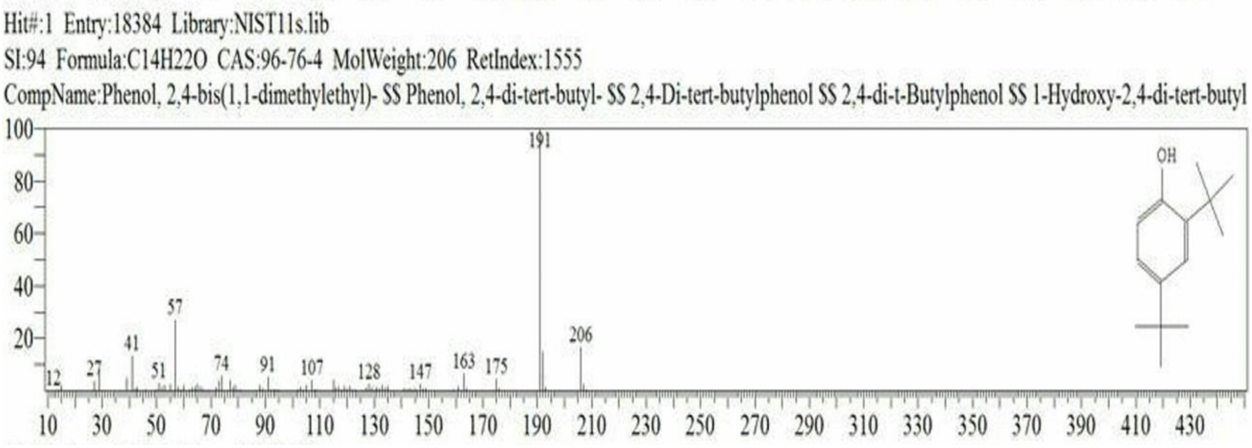

Figure (3.9) The mass spectrum analysis of Phenol, 2,4-bis(1,1-dimethylethyl)-.

ix) Identification of pipradrol

The EI mass spectrum of pipradrol MW 267 (Figure 3.10). The base peak is found at m/z 84 corresponding to M $[C_{18}H_{21}NO]$. The pipradrol appear at R.T 16.077 in total ion chromatogram.

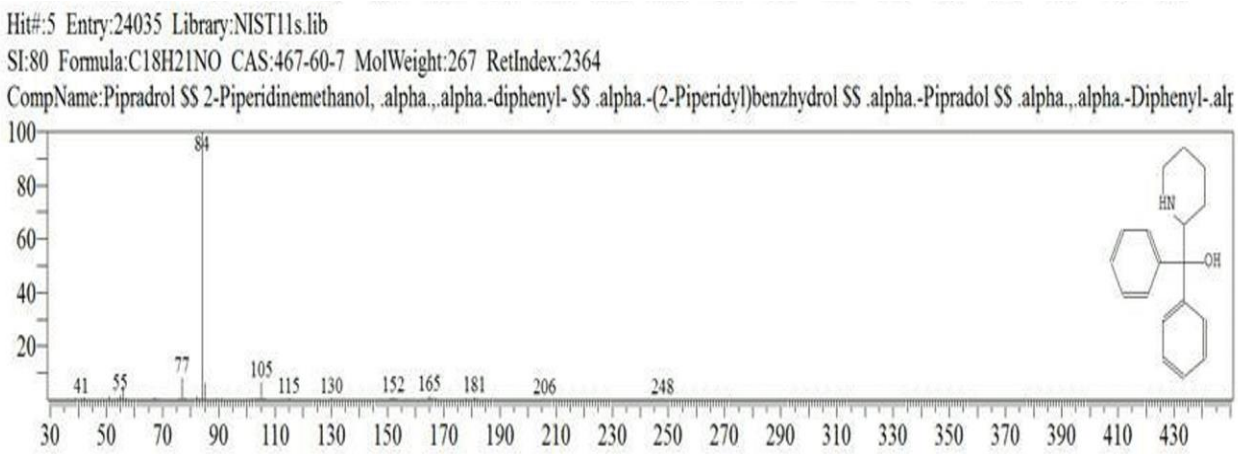

Figure (3.10) The mass spectrum analysis of pipradrol.

x) Identification of pipradrol

The EI mass spectrum of pipradrol MW 267 (Figure 3.11). The base peak is found at m/z 84 corresponding to M $[C_{18}H_{21}NO]$. The pipradrol appear at R.T 16.702 in total ion chromatogram.

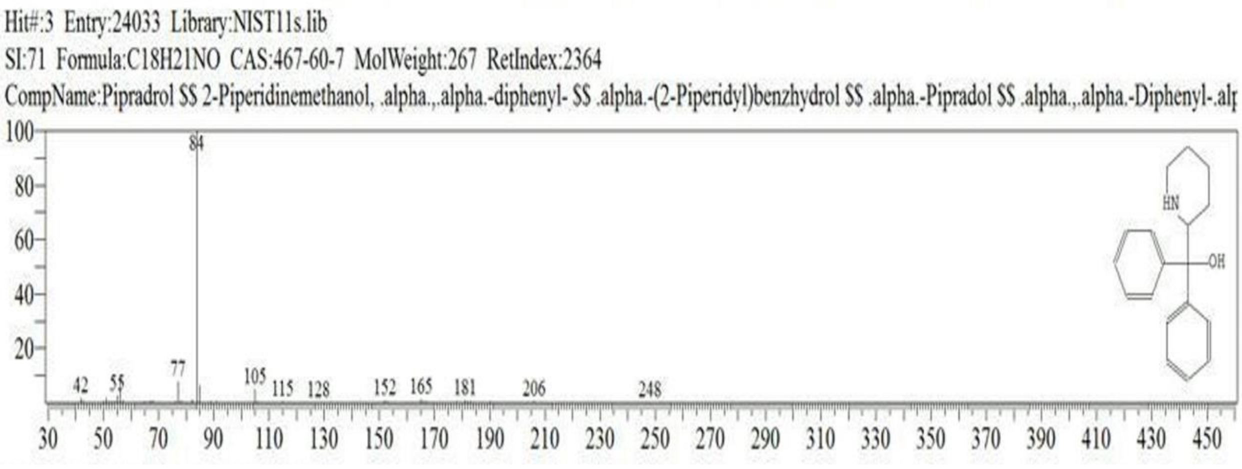

Figure 3.11 The mass spectrum analysis of pipradrol.

xi) Identification of Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl-

The EI mass spectrum of Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl- MW 340 (Figure 3.12). The base peak is found at m/z 177 corresponding to M $[C_{14}H_{22}O]$. The Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl- appear at R.T 20.405 in total ion chromatogram.

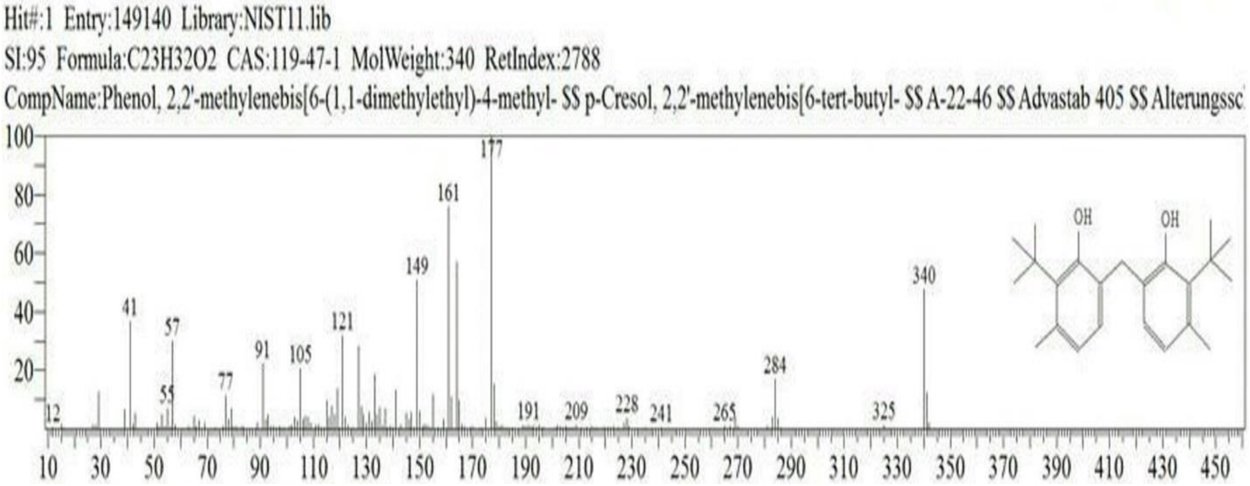

Figure (3.12) The mass spectrum analysis of Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl-.

iii) Elements Content in Moringa Oleifera Seeds

Table 3.3 Concentration of major and minor elements in Moringa Oleifera seeds

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Elements	**Pb**	**Zn**	**P**	**Mg**	**Mn**	**Ni**	**Na**	**Fe**	**K**	**Cu**	**Ca**	**Cd**	**Ba**	**Al**
Concentration (mg/kg)	<0.015	35.12	8561	4203	11.98	0.2797	12.46	37.50	5903	4.933	1240	0.1121	4.273	8.152

From the table 3.3 both major elements (P, K, Mg and Ca) and minor elements (Pb, Cd, Ni, Ba, Cu, Ba, Al, Mn, Na, Zn and Fe) were present in seeds of M. Oleifera. Among the micro nutrients, lead (Pb) had the lowest concentration

(<0.015 mg/kg). This was followed by Cd, Ni, Cu, Ba, Al, Mn, Na, Zn and Fe (0.1121, 2797, 4.273, 4.933, 8.152, 11.98, 12.46, 35.12 and 37.50 respectively). Phosphorus (8561 mg/kg) had the highest concentration followed by the K (5903mg/kg), Mg (4203 mg/kg) and Ca (1240 mg/kg). The concentration of P, K, Mg and Ca was highest compared to other elements among the macro nutrients. This might be due to the highest concentration in plants absorbed from soil.

3-1-3 Physicochemical Analysis of Raw and Treated Water

3-1-3-1 Influence of MOC on High Turbidity

Table 3.4 shows the influence of *Moringa oleifera* coagulant (MOC) on turbidity. From the table, Moringa oleifera coagulant (MOC) concentrations of 1.5% (w/v) and 2% (w/v) rapidly reduced the turbidities at 3977 NTU to 10-13 NTU rang at a volume of 100mL. With the same MOC concentrations a decrease in dosage from 20 to 40 cm^3 showed reduction in turbidities at 3977 NTU to 4-8 NTU. The table also reveals that MOC concentration of 3, 4 and 5 % (w/v) could reduce the turbidities of 3977 NTU to 7-10 NTU at 20mL. It is also evident from the table that, with MOC concentrations of (3, 4, 5) % (w/v) one can obtain residual turbidities of 12-18NTU at 3977NTU at increased dosages of 40cm^3 to 60cm^3 per liter of turbidity water. Therefore, decreasing concentrations and volumes of MOC reduces turbidity. Hence levels of particulate matter or sediments in turbidity water reduced appreciably.

Table 3.4 Mean high turbidity of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	3977	3977	3977	3977	3977
20	4.95	6.42	7.58	9.93	9.98
40	6.57	8.20	12.8	14.4	14.9
60	7.65	11.6	13.8	20.7	18.7
80	9.40	12.0	17.6	23.8	28.4
100	10.80	13.4	22.0	30.3	35.3

3-1-3-2 Influence of MOC on Low Turbidity

Table 3.5 shows the influence of Moringa oleifera coagulant (MOC) on turbidity. From the table, *Moringa oleifera* coagulant (MOC) concentrations of 1.5% (w/v), 2% (w/v) and 3% (w/v) rapidly reduced the turbidities at 63.7 NTU to 7.1-9.9 NTU rang at a volume of 20mL. With the same MOC concentrations increase in dosage from 20 to 40cm^3 showed reduction in turbidities at 63.7 NTU to 9.7-10.6 NTU. The table also reveals that MOC concentration of 4 and 5 % (w/v) could reduce the turbidities of 63.7 NTU to 14.6-20.1 NTU at 20 cm^3. It is also evident from the table that, with MOC concentrations of (1.5 and 2) % (w/v) one can obtain residual turbidities of 9.7-20.1NTU at 63.7 NTU at increased dosages of 40cm^3 to 1000cm^3 per liter of turbidity water. Therefore, decreasing concentrations and volumes of MOC reduces turbidity. Hence levels of particulate matter or sediments in turbidity water reduced appreciably.

Table 3.5 Mean turbidity of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	63.70	63.70	63.70	63.70	63.70
20	7.10	8.10	9.90	14.60	20.10
40	9.70	10.60	12.30	17.50	23.30
60	12.30	12.10	14.40	21.40	25.80
80	14.80	14.60	17.00	33.54	29.90
100	16.10	20.10	32.00	40.33	36.60

From the table 3.4 at high turbidity 3977, the concentrations of 1.5g ($20cm^3$) shown rapidly reduced of the turbidities from 3977 NTU to 4.95 (99.88%) NTU which below in WHO and SSMO standard 5 NTU. From the table the concentration of 2g,3g,4g and 5g at (20 ml) reduce the turbidity from the 3977 NTU to 6.42NTU (99.84%), 7.58 NTU (99.81%), 9.93NTU (99.75%) and 9.98 NTU (99.74%) respectively. This indicate that the higher initial turbidity the higher the reduction in turbidity, due to increase in suspended particles available or adsorption and colloidal charge neutralization. The net effect is an increase in particle collision frequency and agglomeration rate.

It is evident from the table that at concentration of 3g, 4g and 5g with MO $60cm^3$, $80cm^3$ and $100cm^3$ the turbidity increased; This can be explained by the fact that overdosing resulted in the saturation of the polymer bridge sites and caused destabilization of the destabilized particles due to insufficient number of particles to form more inter-particle bridges.

The high positive charge and small size suggest that the main destabilization mechanism may might be adsorption and charge neutralization.

From the table 3.5 at low turbidity 63.70NTU, the concentrations of 1.5g ($20cm^3$) shown rapidly reduced of the turbidities from 63.70 to 7.10NTU (88.86%). From the table the concentration of 2g,3g,4g and 5g at ($20cm^3$) reduce the turbidity from the 63.70 to 8.10NTU (88.90%), 9.90NTU (84.84%), 14.60NTU (75.67%) and 20.10NTU (47.66%) respectively.

It is evident from the table that at concentration of 1g,2g, 3g, 4g and 5g with MO $40cm^3$, $60cm^3$, $80cm^3$ and $100cm^3$ the turbidity increase; This can be explained by the fact that overdosing resulted in the saturation of the polymer bridge sites and caused destabilization of the destabilized particles due to insufficient number of particles to form more inter-particle bridges. We concluded that use of Moringa oleifera for drinking water treatment may not be appropriate since turbidity of raw water for drinking water is usually low. This therefore suggests that M. oleifera will be good in treating Nile water that has high turbidity.

3-1-3-3 Influence of MOC on pH of High Turbidity

Table 3.6 shows influence of Moringa oleifera coagulant (MOC) on pH. From the table, it could be observed generally that the pH of raw water (RW) does not show a remarkable change in pH for all samples with different dosages (20, 40, 60, 80, 100, and 120) cm^3/dm^3. This finally shows that RW and samples treated with different concentrations and dosages not show a significant change in pH. The pH for raw water (RW) and treated water is still within the WHO and SSMO value of 6.5 to 8 of drinking water.

Table 3.6 Mean pH of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	8.10	8.10	8.10	8.10	8.10
20	8.00	7.93	7.70	7.66	7.60
40	7.90	7.90	7.66	7.64	7.57
60	7.88	7.87	7.62	7.50	7.55
80	7.80	7.50	7.60	7.48	7.40
100	7.75	7.40	7.55	7.44	7.35

3-1-3-4 Influence of MOC on pH of Low Turbidity

Table 3.7 shows influence of Moringa oleifera coagulant (MOC)on pH. From the table, it could be observed generally that the pH of raw water (RW) does not show a remarkable change in pH for all samples with different dosages (20, 40, 60, 80, 100, and 120) cm^3/dm^3. This finally shows that RW and water treated with different concentrations and dosages not show a significant change in pH. The pH for raw water (RW) and treated water is still within the WHO and SSMO value of 6.5 to 8 of drinking water.

Table 3.7 Mean pH of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	7.90	8.10	7.90	7.90	8.00
20	7.90	7.90	7.70	7.80	7.60
40	7.80	7.80	7.60	7.40	7.30
60	7.80	7.80	7.60	7.10	7.20
80	7.70	7.60	7.30	7.00	7.00
100	7.80	7.30	7.20	6.90	6.80

It was found that the pH values of the raw high turbidity water samples were similar compared to those obtained after treatment with M. oleifera. It is evident from the table 3.5 and 3.6 during the concentration of M. oleifera the changed of the pH decrease. This decrease in pH could be explained by the fact that the solution was become more acidic due to presence of sulphate in the Moringa Oleifera seeds produce sulphuric acid which lowered the pH level. but these values were within the acceptable range of 6.5-8.5 (SSMO and WHO). Although pH has no direct effect on health, its indirect action on physiological processes cannot be over emphasized.

3-1-3-5 Influence of MOC on Conductivity of High Turbidity

Table 3.8 shows the effect of Moringa oleifera coagulant (MOC) on conductivity. From the table, it is observed generally that change in concentrations of MOC at 20cm^3 do not affect the conductivity of raw water (RW). The conductivity increases slightly from a dosage of 40cm^3/dm^3 to 80 cm^3/dm^3 in all the treatments. But this increase does not affect the quality of the water since the changes do not exceeded the WHO and SSMO permissible limit of 1000µs/cm for waters (WHO 2006). The conductivity increases above 1000µs/cm at dosage of 100ml in all the treatment, which effect in the quality of treated water.

Table 3.8 Mean conductivity of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	260	260	260	260	260
20	412	429	443	465	418
40	596	613	629	642	655
60	781	832	847	860	872
80	947	965	983	995	1000
100	113	1139	1147	1155	1167

3-1-3-6 Influence of MOC on Conductivity of Low Turbidity

Table 3.9 shows the effect of Moringa oleifera coagulant (MOC) on conductivity. From the table, it is observed generally that change in concentrations of MOC at 20mL do not affect the conductivity of raw water (RW). The conductivity increases slightly from a dosage of 60cm^3/dm^3 to 100cm^3/dm^3 in all the treatments. But this increase does not affect the quality of the water since the changes do not exceeded the WHO and SSMO permissible limit of 1000µs/cm for waters (WHO 2006). The conductivity increases above 1000µs/cm at dosage of 100ml in all the treatment, which effect in the quality of treated water.

Table 3.9 Mean conductivity of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	130	134	125	140	136
20	170	193	280	360	400
40	250	330	390	490	550
60	380	410	520	622	710
80	440	470	601	740	860
100	500	554	710	824	1000

From the table 3.8 and 3.9 initial electric conductivity of raw water was high. The conductivity increases slightly in all the treatments during coagulation due to solubility of mineral. Also, Moringa Oleifera have lower molecular weight water soluble proteins which carry positive charge. But this increase does not affect the quality of the water since the changes do not exceed the WHO and SSMO permissible limit of 1000µs/cm for drinking water.

3-1-3-7 Influence of MOC on Total Dissolved Solids of High Turbidity

Table 3.10 shows the influence of M. Oleifera coagulant (MOC) on TDS. The table shows that increase in concentration of MOC does increase the TDS. But increase in coagulant dosage does not affect the TDS levels in the water. It is within the WHO and SSMO value of 1000mg/L-2000mg/dm^3 for drinking water (WHO 2006).

Table 3.10 Mean Total Dissolved Solids of raw and treated water
at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	125.8	125.8	125.	125.8	125.
20	247	257	25	260	36
40	357	368	37	386	39
60	468	500	47	505	53
80	568	611	60	599	61
100	679	686	69	711	72

3-1-3-8 Influence of MOC on Total Dissolved Solids of low turbidity

Table 3.11 shows the influence of M. Oleifera coagulant (MOC) on TDS. The table shows that increase in concentration of MOC does increase the TDS. But increase in coagulant dosage does not affect the TDS levels in the water. It is within the WHO and SSMO value of 1000mg/dm^3 for drinking water (WHO 2006).

Table 3.11 Mean Total Dissolved Solids of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	86.40	85.70	76.80	89.00	87.00
20	109.00	123.00	179.00	230.00	256.00
40	160.00	211.00	250.00	313.00	352.00
60	243.00	262.00	333.00	398.00	454.00
80	282.00	301.00	385.00	473.00	550.00
100	320.00	354.00	454.00	527.00	640.00

From the table 3.10 and 3.11 the TDS concentrations gradually increased for both thigh and low turbidity water with increasing concentrations. This was probably due to increased levels of inorganic substances such as calcium, magnesium, bicarbonates, chlorides and sulphates. TDS values were however higher in ACH treated water than Moringa treated water due to higher levels of aluminium adding on to the inorganic level.

But this increase in the TDS below within WHO and SSMO value of 1000mg/dm^3 for drink water.

3-1-3-9 Influence of MOC on Alkalinity of High Turbidity

Table 3.12 shows the effect of M. oleifera coagulant (MOC) on alkalinity. Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3 clearly shows that MOC does not affect the alkalinity of the water.

Table 3.12 Mean total alkalinity of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	160.75	160.75	160.75	160.75	160.75
20	90.83	120.1	121.3	123.1	125.9
40	110.21	122.7	121.6	125.0	128.5
60	113.90	123.1	124.5	126.3	130.0
80	115.55	125.3	126.3	127.6	131.7
100	118.71	126.4	127.1	129.3	133.4

3-1-3-10 Influence of MOC on Total Alkalinity of Low Turbidity

Table 3.13 shows the effect of M. oleifera coagulant (MOC) on alkalinity. Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3 clearly shows that MOC does not affect the alkalinity of the water.

Table 3.13 Mean total alkalinity of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	122.51	122.51	122.51	122.51	122.51
20	119.84	118.00	105.34	106.19	105.73
40	117.72	112.70	98.67	100.03	90.11
60	114.77	110.16	88.52	93.36	68.14
80	111.32	107.31	86.33	86.67	74.82
100	109.59	105.48	84.17	73.34	66.75

The presences of alkalinity in high turbidity water before and after treatment was given in table 3.12 for analysis. Alkalinity during the present research work was observed to be 160.75 mg/dm^3 for Nile water. At various doses of M. oleifera seed powder, it was observed that the alkalinity reduced after the treatment at 20cm^3 and 40cm^3 of 1.5, 2, 3, 4 and 5 doses. But at higher dose of 60, 80 and 100cm^3 of 1.5, 2, 3, 4 and 5 doses of Moringa seed, the alkalinity was slowly increased. The alkalinity was present in the range of 113.90-133.43 mg/dm^3 which was within limits of WHO standards.

This can be explained by the fact that the coagulant lies in the presence of water soluble as cationic proteins, and that the basic amino acids present in the protein of Moringa accept a proton from water and release a hydroxyl group making the water alkaline.

The presence of alkalinity in low turbidity water before and after treatment is given in table 3.12 for analysis. Alkalinity during the present research work was observed to be 122.51 mg/l for Nile water. At various doses of M. oleifera seed powder, it was observed that the alkalinity reduced after the treatment 1.5, 2, 3, 4 and 5 doses of Moringa seed, the alkalinity was slowly increased. The alkalinity was present in the range of 119.84-66.75 mg/dm^3 which was within limits of WHO standards.

This decreased can be explained by the fact that the coagulant lies in the presence of water soluble proteins as cationic proteins, and the basic amino acids present in the protein of Moringa accept a proton from water and release a hydroxyl group making the solution alkaline.

3-1-3-11 Influence of MOC on Total Hardness of High Turbidity

Table 3.14 shows influence of M. Oleifera coagulant (MOC) on Total hardness. Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3.

Table 3.14 Mean total hardness of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	192.11	192.11	192.11	192.11	192.11
20	120.30	120.48	124.91	128.31	140.80
40	122.17	124.33	128.61	134.70	142.21
60	124.62	132.18	132.75	141.11	145.88
80	126.07	136.14	132.98	146.73	146.62
100	128.50	139.60	140.37	148.00	150.07

3-1-3-12 Influence of MOC on Total Hardness of Low Turbidity

Table 3.15 shows influence of M. Oleifera coagulant (MOC) on Total hardness.

Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3.

Table 3.15 Mean total hardness of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	110.00	118.51	115.77	116.94	12043
20	122.11	124.00	126.67	129.55	112.00
40	132.06	128.00	140.00	134.70	136.00
60	136.39	132.43	146.67	136.11	148.32
80	136.44	136.80	157.33	140.73	144.94
100	138.50	139.60	160.00	148.00	156.64

Table 3.14 and 3.15 show range and mean values of total hardness of raw and treated water at high and low turbidity. Raw high turbidity water with total hardness levels of 192.11 mg/dm^3 gave a total hardness level of 110.00 and 150.07 mg/dm^3 at coagulant dosage of 20-100 cm^3/dm^3, respectively. And Raw low turbidity water with total hardness levels of 110.00 mg/dm^3 gave a total hardness level of 122.11 and 156.65 mg/dm^3 at coagulant dosage of 20-100 cm^3/dm^3 respectively. From this result MOC dosage does not affect total hardness with increase in concentration and dosage as it is confirmed in the following concentrations (1.5, 2, 3, 4,5) %w/v on Table 3.14 and 3.15.

3-1-3-13 Influence of MOC on Hardness as Ca^{2+} of High Turbidity

Table 3.16 shows influence of M. Oleifera coagulant (MOC) on Total hardness. Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3.

Table 3.16 Mean total hardness as Ca^{2+} of raw and treated water at high turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
RW	86.66	86.66	86.66	86.66	86.66
20	60.00	91.17	99.34	105.3	110.0
40	89.33	80.64	106.0	109.4	112.4
60	73.33	97.66	111.4	113.3	115.6
80	57.33	110.7	115.7	115.8	118.5
100	96.00	105.9	117.5	119.0	122.5

3-1-3-14 Influence of MOC on Hardness as Ca^{2+} of Low Turbidity

Table 3.17 shows influence of M. Oleifera coagulant (MOC) on Total hardness. Raw water (RW) and treated water with 1.5 to 5 % weight per volume of MOC dosed with 20-100 cm^3/dm^3.

Table 3.17 Mean total hardness as Ca^{2+} of raw and treated water at low turbidity

	Different conc. of MOC (%w/v)				
Dosage(cm^3/dm^3)	**1.5**	**2**	**3**	**4**	**5**
Raw water	75.89	75.89	75.89	75.89	75.89
20	60.00	91.17	76.66	53.33	80.15
40	89.33	80.64	84.00	57.33	88.53
60	73.33	97.66	80.00	64.00	108.92
80	57.33	110.73	74.66	72.00	120.48
100	96.00	105.94	86.66	70.66	140.36

Table 3.16 and 3.17 show range and mean values of hardness as Ca^{2+} of raw and treated water at high and low turbidity. Raw high turbidity water with total hardness levels of 86.66 mg/dm^3 gave a hardness as Ca^{2+} level of 60.00 and 122.50 mg/dm^3 at coagulant dosage of 20-100 cm^3/dm^3 respectively. And Raw low turbidity water with hardness as Ca^{2+} levels of 75.89 mg/dm^3 gave a hardness as Ca^{2+} level of 60.00 and 140.36 mg/dm^3 at coagulant dosage of 20-100 cm^3/dm^3 respectively. From this result MOC dosage does not affect hardness as Ca^{2+} with increase in concentration and dosage as it is confirmed in the following concentrations (1.5, 2, 3, 4,5) %w/v on Table 3.16 and 3.17.

3-1-4 Anion Analysis

3-1-4-1 Influence of MOC on Anions of High Turbidity

Table 3.18 show range and mean values of anions (Nitrate-nitrogen, phosphate and sulphate). Raw water with nitrate levels of 26 mg/dm^3 gave a nitrate level of 3.80 and 5.63 mg/dm^3 at coagulant dosage of 20-100 ml/cm^3, respectively. From this result MOC dosage does not affect nitrate with increase in concentration and dosage as it is confirmed in the following concentrations (1.5, 2, 3, 4 and 5) %w/v on Table 3.18.

In the case of phosphate MOC showed a corresponding decrease with increase in concentration of MOC. This is seen in Table 3.18.

Table 3.18 Mean anions of raw and treated water at high turbidity

Conc.% (w/v)	**Dosage(cm^3/dm^3)**	NO_3^- **(mg/dm^3)**	PO_4^{3-} **(mg/dm^3)**	SO_4^{2-} **(mg/dm^3)**
1.5	RW	26.00	1.58	13.00
	20	5.63	1.31	17.00
2	RW	26.00	1.58	13.00
	20	5.30	1.34	19.00
3	RW	26.00	1.58	13.00
	20	4.7	1.61	21.00
4	RW	26	1.58	13.00
	20	4.5	00.54	32.00
5	RW	26.00	1.58	13.00
	20	3.8	00.79	9.00

3-1-4-2 Influence of MOC on Anions of Low Turbidity

Table 3.19 show range and mean values of anions (Nitrate-nitrogen, phosphate and sulphate). Raw water with nitrate levels of 4 mg/dm^3 gave a nitrate level of 2.35 and 3.2 mg/dm^3 at coagulant dosage of 20-100 cm^3/dm^3 respectively. From this result MOC dosage does not affect nitrate with increase in concentration and dosage as it is confirmed in the following concentrations (1.5, 2, 3, 4,5) %w/v on Table 3.18. In the case of phosphate MOC showed a corresponding decrease with increase in concentration of MOC. This is seen from Table 3.18. Levels of sulphate increase with increase in concentration of MOC. The increase amount of phosphate in the treated water is as a result of the MOC, but do not affect the quality of water as it does not increase above the unacceptable levels.

Table 3.19 Anions of raw and treated water at low turbidity

Conc.% (w/v)	**Dosage(cm^3/dm^3)**	NO_3^- **(mg/dm^3)**	PO_4^{3-} **(mg/dm^3)**	SO_4^{2-} **(mg/dm^3)**
1.5	RW	4.00	00.62	10.00
	20	3.20	1.10	17.00
2	RW	4.00	00.62	10.00
	20	3.35	1.34	19.00
3	RW	4.00	00.62	10.00
	20	3.07	1.39	20.00
4	RW	4.00	00.62	13.00
	20	2.46	1.43	23.00
5	RW	4.00	00.62	10.00
	20	2.35	1.52	24.00

3-1-5 Bacterial Analysis

3-1-4-1 Influence of MOC on Total Coliform of High Turbidity

Table 3.20 shows the effect of Moringa oleifera coagulant (MOC) on total coliforms. From the table MOC concentration of 5%w/v reduced total coliform to 3 at 100mL. MOC of lower concentrations (1.5, 2, 3 and 4) %w/v also reduced the total coliforms levels from 15-5. From these results, it is evident that effective reduction will be achieved at a dosage of 100mL/L of 4% w/v and 5% w/v concentration. Table 3.20 shows percentage reduction in total coliforms of raw water treated with MOC.

Table 3.20 Total coliform of raw and treated water at high turbidity

Conc. % (w/v)	**Dosage (cm^3/dm^3)**	**Total coliform($100cm^3$)**
1.5	RW	164
	20	18
	60	16
	100	15
2	RW	164
	20	18
	60	16
	100	13
3	RW	164
	20	17
	60	14
	100	10

4	RW	164
	20	15
	60	7
	100	5
5	RW	164
	20	7
	60	4
	100	3

3-1-5-2 Influence of MOC on Total Coliform of Low Turbidity

Table 3.21 shows the effect of Moringa oleifera coagulant (MOC) on total coliforms. From the table MOC concentration of 5%w/v reduced total coliform to 2 at 100cm^3. MOC of lower concentrations (1.5, 2, 3 and 4) %w/v also reduced the total coliforms levels from 7-3. From these results, it is evident that effective reduction will be achieved at a dosage of 100 cm^3/dm^3 of 4% w/v and 5% w/v concentration. Table 3.21 shows percentage reduction in total coliforms of raw water treated with MOC.

Table 3.21 Total coliform of raw and treated water at low turbidity

Conc. % (w/v)	Dosage (cm^3/dm^3)	Total coliform(100cm^3)
1.5	RW	72
	20	7
	60	7
	100	4

2	RW	72
	20	7
	60	6
	100	3
3	RW	72
	20	6
	60	6
	100	3
4	RW	72
	20	7
	60	5
	100	3
5	RW	72
	20	5
	60	3
	100	2

From the tables 3.21, the number of total coliform bacteria in raw high and low turbidity water was 164 and 72TCP respectively, From the results obtained, concentrations of 1.5 - 5% (w/v) gave 15-3 TCP and 15-5 TCP respectively reduction of total coliform within one hour. during coagulation, the number of bacteria decrease when the concentration increase. The seeds of the Moringa Oleifera show flocculated bacteria by low molecular weight cationic water-soluble proteins that attach themselves to suspended particles, including bacteria. But at the same time, it's not removed 100%. This can be explained by the concentration of photochemical compound which in the Moringa Oleifera seed coagulant.

3-1-6 Influence of Aluminum Chloro-hydrate

3-1-5-1 Influence of Aluminum Chloro-hydrate On High Turbidity Water

Table 3.22 Quality characteristics of treated water by (ACH) at high turbidity

Parameters	4 cm^3	6 cm^3	8 cm^3	10 cm^3	12 cm^3
Turbidity (NTU)	8.10	7.20	6.00	5.10	3.30
Coagulant activity (%)	99.79	99.82	99.85	99.87	99.91
Taste	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odour	Odorless	Odorless	Odorless	Odorless	Odorless
Colour	Colorless	Colorless	Colorless	Colorless	Colorless
pH	8.00	7.90	7.80	7.80	7.70
Temperature (Cº)	28.70	26.30	26.30	24.90	27.30
EC (µs/cm)	290.14	304.36	317.92	333.30	341.69
TDS (mg/dm^3)	132.40	144.51	153.77	167.93	180.83
Total alkalinity (mg/dm^3)	100.56	99.12	98.00	97.93	95.87
Total hardness (mg/ dm^3)	136.24	138.13	140.96	141.90	143.00
Hardness as Ca^{+2} (mg/ dm^3)	45.94	47.00	48.23	49.93	50.90
Sulphate (mg/ dm^3)	-	-	-	-	19
Phosphate (mg/ dm^3)	-	-	-	-	00.98
Nitrate (mg/ dm^3)	-	-	-	-	14.5
Total coliform TCP/100 cm^3	Not detected	-	Not detected	-	Not detected

3-1-6-2 Influence of Aluminum Chloro-hydrate at Low Turbidity Water

Table 3.23 Quality characteristics of treated water by (ACH) at low turbidity

Parameters	4 cm^3	5 cm^3	6 cm^3	7 cm^3	8 cm^3
Turbidity (NTU)	10.30	8.90	7.00	6.20	5.80
Coagulant activity (%)	83.83	85.40	89.01	90.26	90.89
Taste	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odour	Odorless	Odorless	Odorless	Odorless	Odorless
Colour	Colorless	Colorless	Colorless	Colorless	Colorless
pH	7.75	7.71	7.63	7.63	7.58
Temperature ($^{\circ}C$)	28.70	26.30	26.30	24.90	27.30
EC (μs/cm)	290.14	304.36	317.92	333.30	341.69
TDS (mg/dm^3)	132.40	144.51	153.77	167.93	180.83
Total alkalinity (mg/dm^3)	114.56	110.12	107.00	101.93	95.87
Total hardness (mg/dm^3)	136.24	138.13	140.96	141.90	143.00
Hardness as Ca^{+2} (mg/dm^3)	45.94	47.00	48.23	49.93	50.90
Sulphate (mg/dm^3)	-	-	-	-	15
Phosphate (mg/dm^3)	-	-	-	-	00.83
Nitrate (mg/dm^3)	-	-	-	-	11.5
Total coliform TCP/100 cm^3	Not detected	-	Not detected	-	Not detected

Table 3.22 reveals that 4mL and 6ml concentrations of aluminum chlorohydrate sulfatewill be very effective in removing turbidity from 3977 NTU to 5.10NTU (99.87%) and 3.30NTU (99.91%). It is evident from the table that when the concentration of aluminum chlorohydrate sulfate increased the turbidity decreased. This high efficiency can be explained by the fact that the Aluminum chlorohydrate sulfate or (ACH) typically refers to aluminum chloride coagulants which has

basicity values of approximately eighty-three percent. It has been suggested that the fraction of higher charged aluminum species, such as Al_{13}^{7+}, increases as basicity increases and that it is the primary hydrolysis product in coagulants with basicity greater than seventy percent.

Table 3.23 reveals that 4mL and 5ml concentrations of aluminum chloro-hydrate were very effective in removing turbidity from 63.70 NTU to 6.20NTU (90.26%) and 5.80NTU (90.89). It is evident from the table that when the concentration of aluminum chloro-hydrate increase the turbidity decrease. This high efficiency can be explaining by Aluminum chloro-hydrate or (ACH) typically refers to aluminum chloride coagulants with the basicity values of approximately eighty-three percent. It has been suggested that the fraction of higher charged aluminum species, such as Al_{13}^{7+}, increases as basicity increases and that it is the primary hydrolysis product in coagulants with basicity greater than seventy percent.

Tables 3.22 and 3.23 show that the pH values of the raw high turbidity water samples were similar compared to those obtained after treatment with Aluminum Chloro-hydrate within the acceptable range of 6.5-8.5 (SSMO and (WHO).

Tables 3.22 and 3.23 show that the initial electric conductivity of raw water was high. The conductivity increased slightly in all the treatments during coagulation due to solubility of aluminum which carries positive charge. But this increase did not affect the quality of the water since the changes did not exceed the WHO and SSMO permissible limit of 1000µs/cm for drinking water.

Tables 3.22 and 3.23 indicate that the TDS concentrations gradually increased for both high and low turbidity water with increasing concentrations of Aluminum Chloro-hydrate, But this increase in the TDS was below within WHO and SSMO value of 1000mg/l for drink water.

The results, presented in tables 3.22 and 3.23 show clearly that Aluminum Chloro-hydrate sulfate had no effect on the alkalinity of the treated water, which were in agreement with WHO and SSMO standard 200 mg/dm^3.

Tables 3.20 and 3.21 show the effecs of Aluminum Chloro-hydrate sulfate on anions (Nitrate, phosphate and sulphate). High turbidity of raw water having nitrate and phosphate levels of 26 mg/L and 1.58mg/l gave a mean nitrate and phosphate levels of 14.5 mg/dm^3 and 0.98 mg/dm^3, respectively at coagulant dosage of 1.5g (20cm^3).

Low turbidity of raw water having nitrate and phosphate levels of 26 mg/L and 1.58mg/l gave a mean nitrate and phosphate level of 11.5 mg/dm^3 and 0.83 mg/dm^3, respectively, at coagulant dosage of 1.5g (20cm^3).

Tables 3.22 and 3.23 show MOSC did not affect in nitrate and phosphate levels with increase of dosage. This was confirmed in the case of concentrations of (2, 3, 4 and 5 % w/v).

3-2 Conclusion and Recommendations

The Moringa oleifera seed coagulant (MOSC) showed good coagulating properties, especially for treatment of very high turbidity waters. It did not affect the pH, alkalinity, TDS or conductivity of the water as same as aluminum chloro-hydrate except sulfate, but the effect was still the within permissible value. A sedimentation time of at least one hour with MOSC improved the treatment by reducing turbidity up to 99%.

Moringa oleifera seed coagulant (MOSC) could reduce the high and low turbidity close to the World Health Organization guideline and Sudanese Standard and Metrology Organization value of 5 NTU from raw water with average turbidities during test runs ranging from 60-4000 NTU.

High concentrations of MOC 4-5% (w/v) could reduce total coliform levels to below 90%, but that did not guarantee that the treated water would end up completely (100%) free of pathogenic germs. It is recommended to use the treated water within one day to prevent pathogen growth.

Moringa was found to be a sustainable, cheap solution for coagulation in water treatment compared to aluminum chloro-hydrate. Moringa oleifera seed can be produced locally at low cost at the household level or in small communal nurseries which is to be encouraged among the rural population instead of chemical coagulant of high cost. The harvest of a mature single tree (3 kg) will treat about 30000 liters of water.

Further research and development efforts in this area need to be done for microbiological disinfection compound to be used instead of chemical in the future.

The application of natural coagulants is bright for future because of their abundant source, low price, environment friendly, multifunction, and biodegradable nature in water purification as alternative to that of chemical and conventional coagulants for small to medium size water supplies.

Selection of the most suitable natural coagulants for purification of water at rural and household levels.

In developed country natural coagulants are suitable because they are relatively cost-effective compared to chemical coagulants, can be easily processed in usable form and do not needs more experience.

The process of settling is also important. The sediment at the bottom contains the impurities so care must be taken to use only the clear water off the top and not allow the sediment to re-contaminate the cleared water.

Although Moringa treatment does not remove 100% of water pathogens. It is acceptable for drinking only where people are currently drinking untreated, contaminated water.

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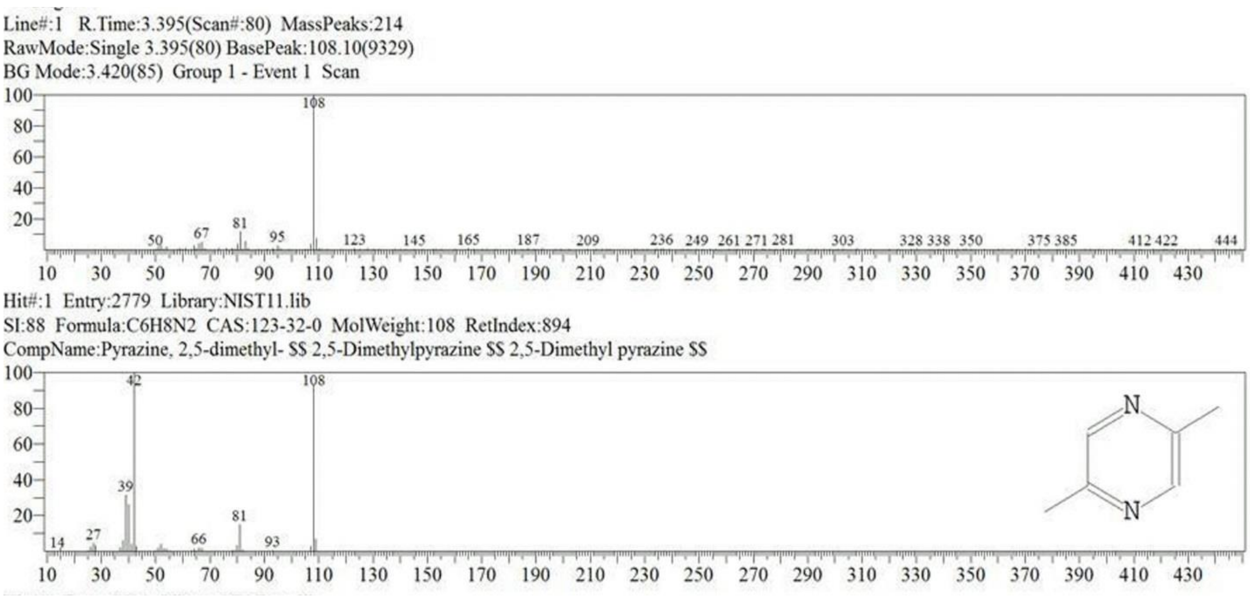
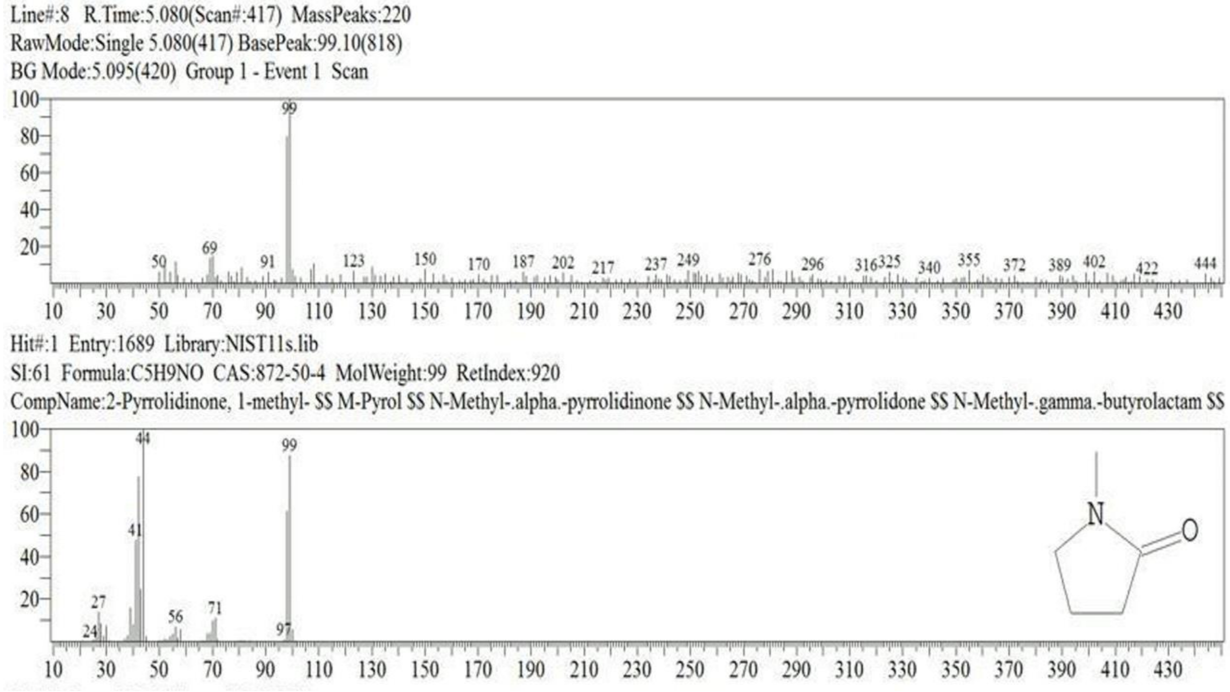
Appendix (A) Similarity search results and mass spectrum of n-hexane Moringa Oleifera seeds extracted compounds:



Line#:1 R.Time:3.395(Scan#:80) MassPeaks:214
RawMode:Single 3.395(80) BasePeak:108.10(9329)
BG Mode:3.420(85) Group 1 - Event 1 Scan

Hit#:1 Entry:2779 Library:NIST11.lib
SI:88 Formula:C6H8N2 CAS:123-32-0 MolWeight:108 RetIndex:894
CompName:Pyrazine, 2,5-dimethyl- $$ 2,5-Dimethylpyrazine $$ 2,5-Dimethyl pyrazine $$

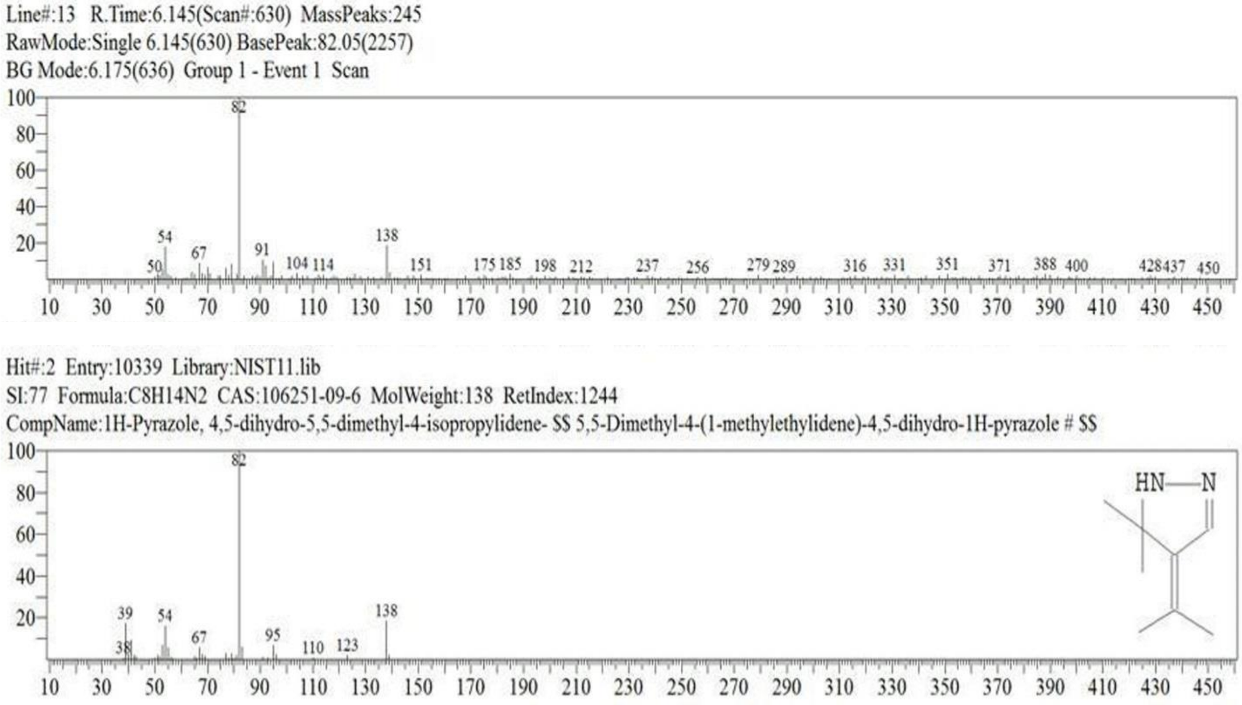
Appendix (1) Identification of pyrazine. (MW 108)



Line#:8 R.Time:5.080(Scan#:417) MassPeaks:220
RawMode:Single 5.080(417) BasePeak:99.10(818)
BG Mode:5.095(420) Group 1 - Event 1 Scan

Hit#:1 Entry:1689 Library:NIST11s.lib
SI:61 Formula:C5H9NO CAS:872-50-4 MolWeight:99 RetIndex:920
CompName:2-Pyrrolidinone, 1-methyl- $$ M-Pyrol $$ N-Methyl-.alpha.-pyrrolidinone $$ N-Methyl-.alpha.-pyrrolidone $$ N-Methyl-.gamma.-butyrolactam $$

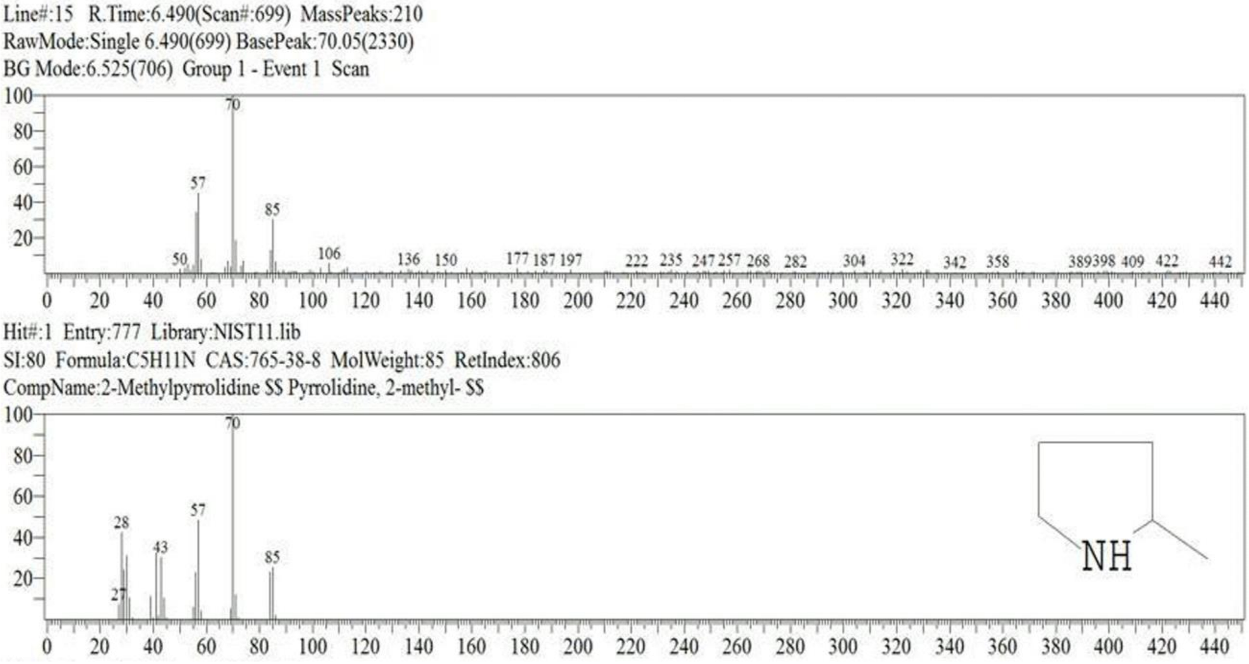
Appendix (2) Identification of 2-pyrrolidinone. (MW 99)



```
Line#:13  R.Time:6.145(Scan#:630)  MassPeaks:245
RawMode:Single 6.145(630) BasePeak:82.05(2257)
BG Mode:6.175(636)  Group 1 - Event 1  Scan
```

```
Hit#:2  Entry:10339  Library:NIST11.lib
SI:77  Formula:C8H14N2  CAS:106251-09-6  MolWeight:138  RetIndex:1244
CompName:1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene- $$ 5,5-Dimethyl-4-(1-methylethylidene)-4,5-dihydro-1H-pyrazole # $$
```

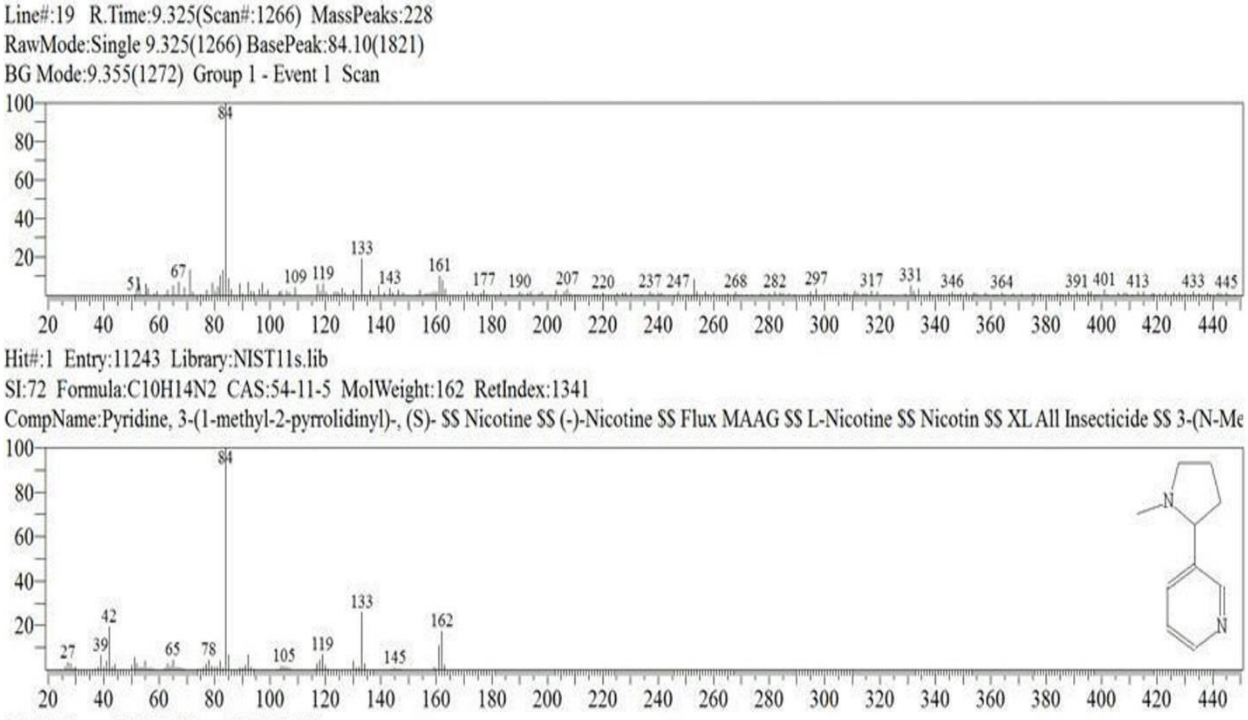
Appendix (3) Identification of 1H-pyrazole, 4,5-dihydro-5,5dimethyle1-4-isopropylidene-. (MW 138)



```
Line#:15  R.Time:6.490(Scan#:699)  MassPeaks:210
RawMode:Single 6.490(699) BasePeak:70.05(2330)
BG Mode:6.525(706)  Group 1 - Event 1  Scan
```

```
Hit#:1  Entry:777  Library:NIST11.lib
SI:80  Formula:C5H11N  CAS:765-38-8  MolWeight:85  RetIndex:806
CompName:2-Methylpyrrolidine $$ Pyrrolidine, 2-methyl- $$
```

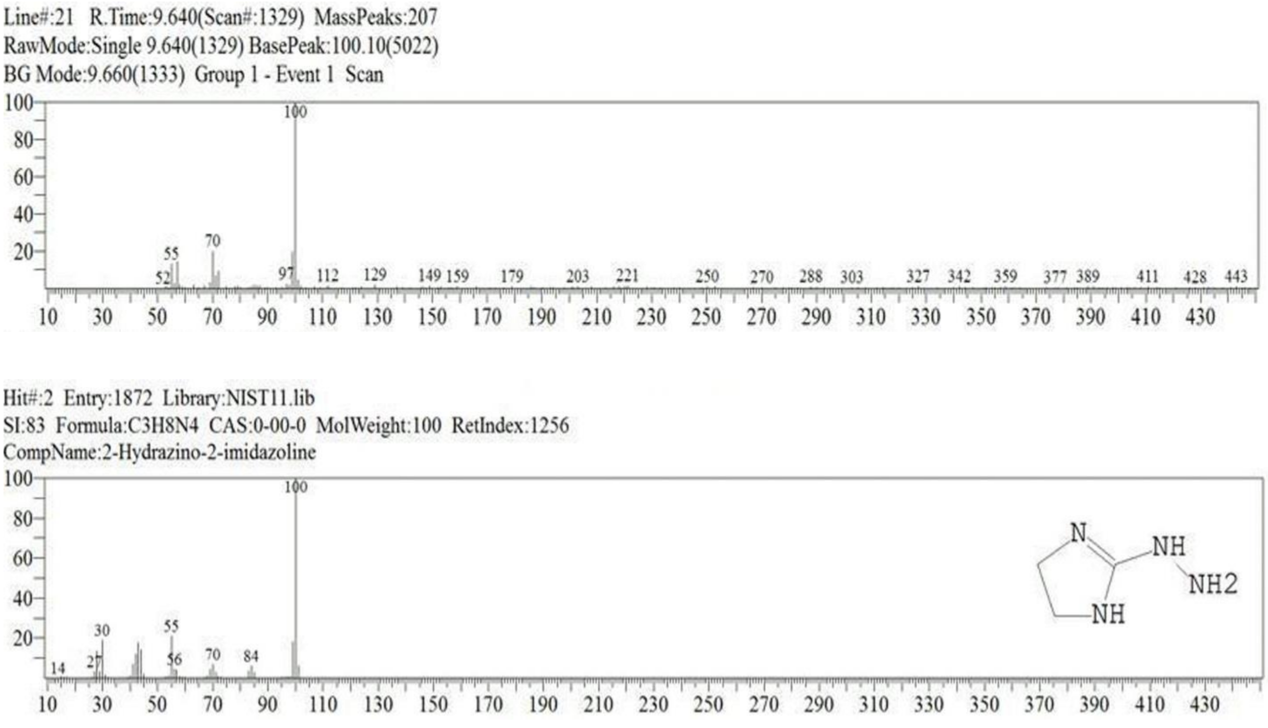
Appendix (4) Identification of 2-methyl-pyrrolidinone. (MW 85)



Line#:19 R.Time:9.325(Scan#:1266) MassPeaks:228
RawMode:Single 9.325(1266) BasePeak:84.10(1821)
BG Mode:9.355(1272) Group 1 - Event 1 Scan

Hit#:1 Entry:11243 Library:NIST11s.lib
SI:72 Formula:C10H14N2 CAS:54-11-5 MolWeight:162 RetIndex:1341
CompName:Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- $$ Nicotine $$ (-)-Nicotine $$ Flux MAAG $$ L-Nicotine $$ Nicotin $$ XL All Insecticide $$ 3-(N-Me

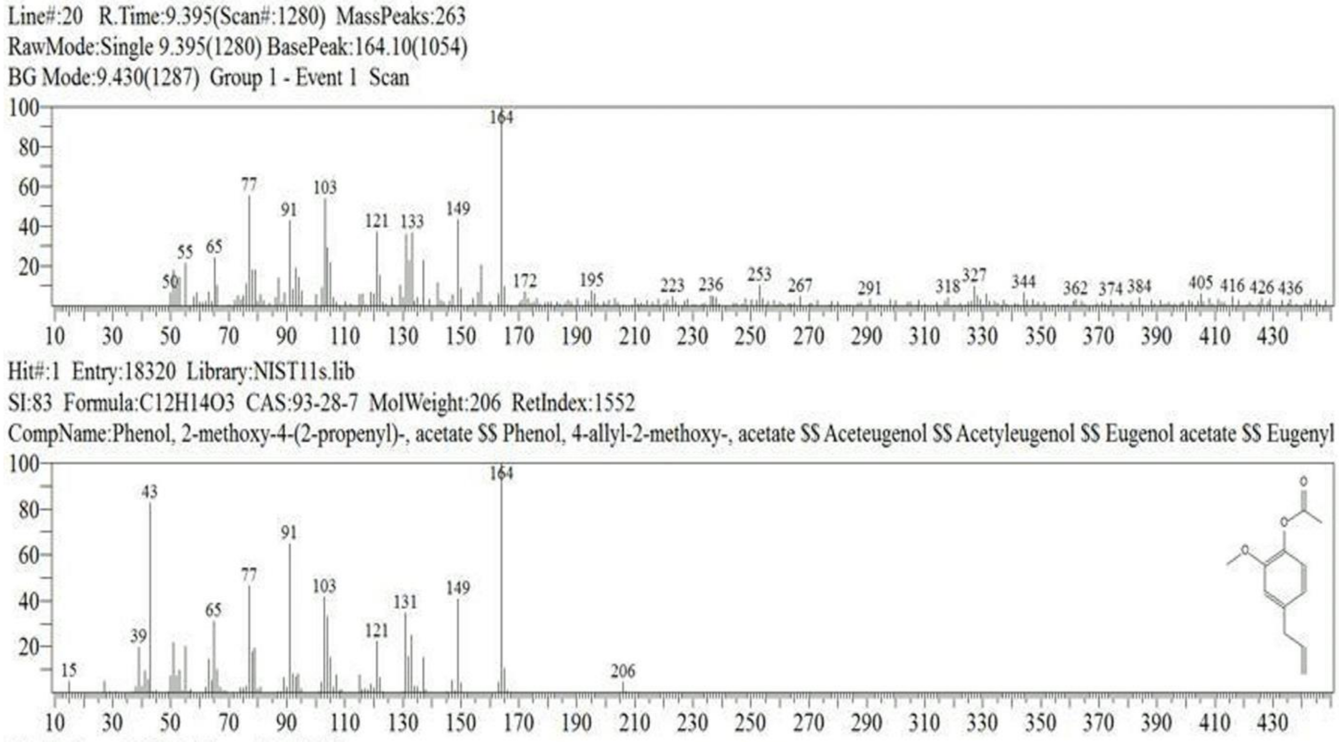
Appendix (5) Identification of Pyridine, 3-(1-methyl-2-pyrrolidinyl)- (MW 162)



Line#:21 R.Time:9.640(Scan#:1329) MassPeaks:207
RawMode:Single 9.640(1329) BasePeak:100.10(5022)
BG Mode:9.660(1333) Group 1 - Event 1 Scan

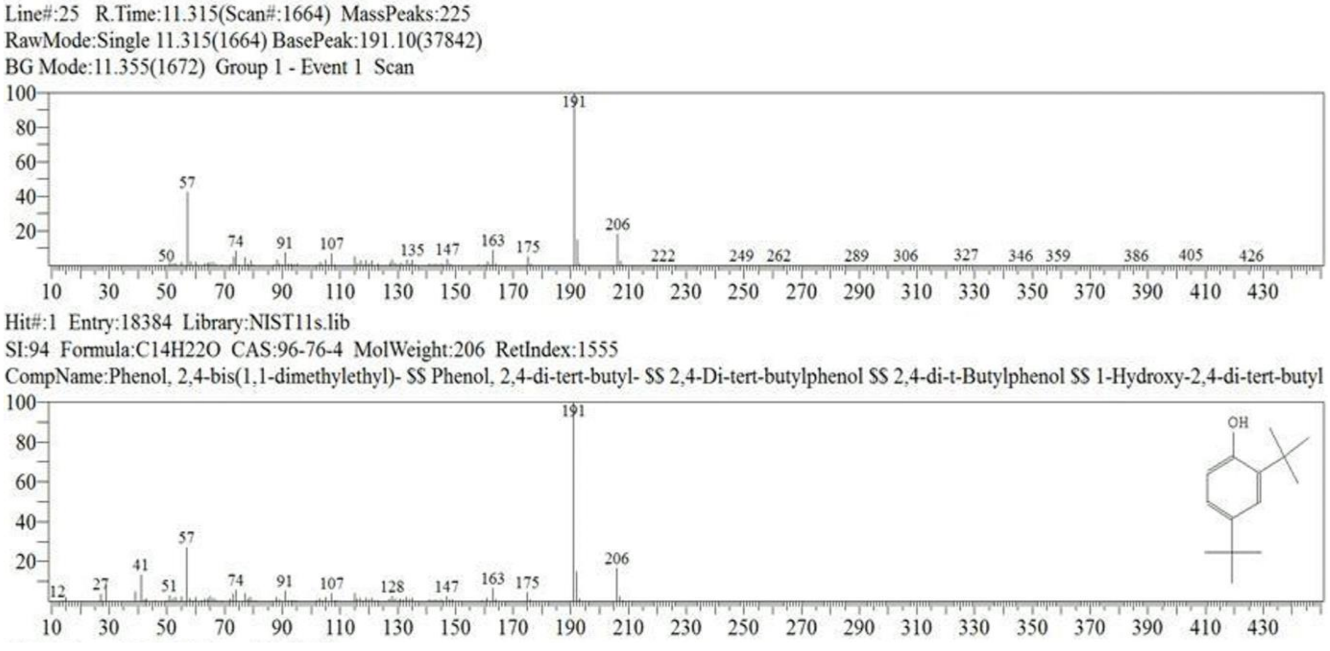
Hit#:2 Entry:1872 Library:NIST11.lib
SI:83 Formula:C3H8N4 CAS:0-00-0 MolWeight:100 RetIndex:1256
CompName:2-Hydrazino-2-imidazoline

Appendix (6) Identification of 2-Hydrazino-2-imidazoline. (MW 100)



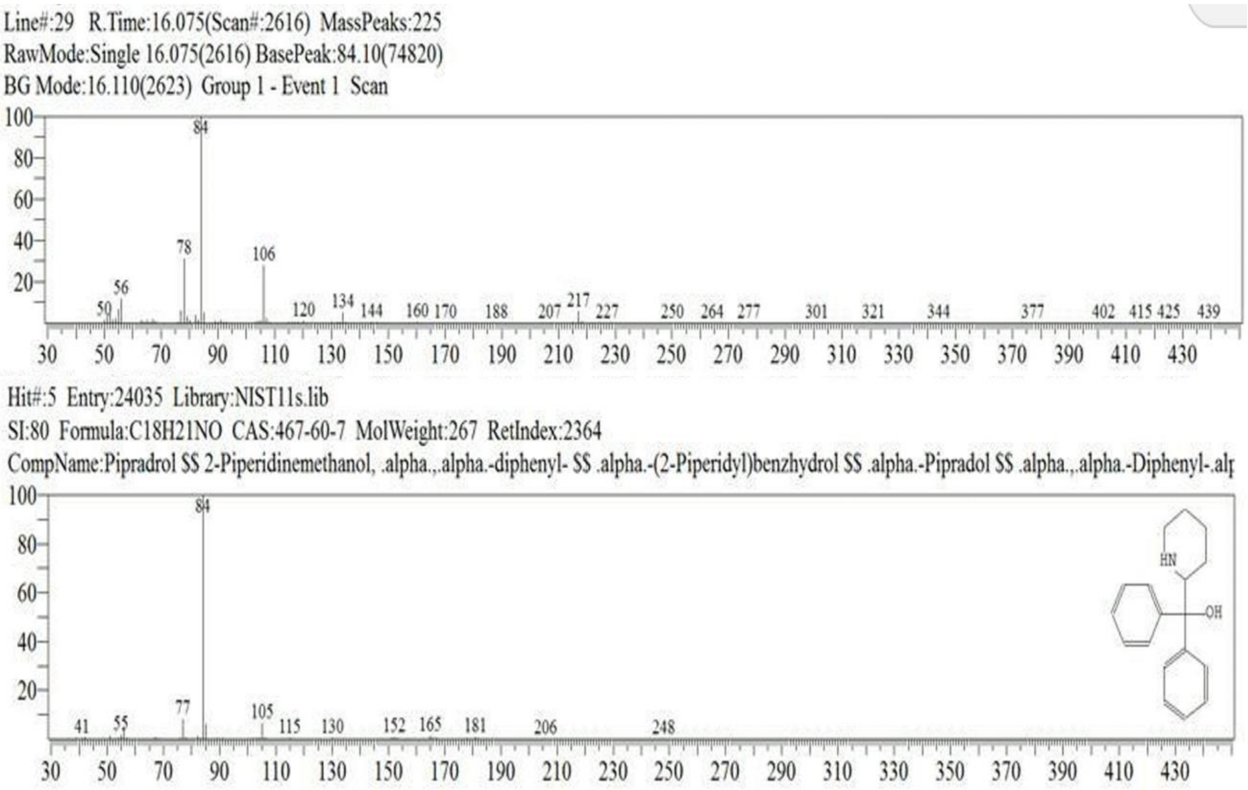
```
Line#:20   R.Time:9.395(Scan#:1280)  MassPeaks:263
RawMode:Single 9.395(1280) BasePeak:164.10(1054)
BG Mode:9.430(1287)  Group 1 - Event 1  Scan
Hit#:1  Entry:18320  Library:NIST11s.lib
SI:83  Formula:C12H14O3  CAS:93-28-7  MolWeight:206  RetIndex:1552
CompName:Phenol, 2-methoxy-4-(2-propenyl)-, acetate $$ Phenol, 4-allyl-2-methoxy-, acetate $$ Aceteugenol $$ Acetyleugenol $$ Eugenol acetate $$ Eugenyl
```

Appendix (7) Identification of Phenol, 2-methoxy-4-(2-propenyl)-. (MW 206)



```
Line#:25   R.Time:11.315(Scan#:1664)  MassPeaks:225
RawMode:Single 11.315(1664) BasePeak:191.10(37842)
BG Mode:11.355(1672)  Group 1 - Event 1  Scan
Hit#:1  Entry:18384  Library:NIST11s.lib
SI:94  Formula:C14H22O  CAS:96-76-4  MolWeight:206  RetIndex:1555
CompName:Phenol, 2,4-bis(1,1-dimethylethyl)- $$ Phenol, 2,4-di-tert-butyl- $$ 2,4-Di-tert-butylphenol $$ 2,4-di-t-Butylphenol $$ 1-Hydroxy-2,4-di-tert-butyl
```

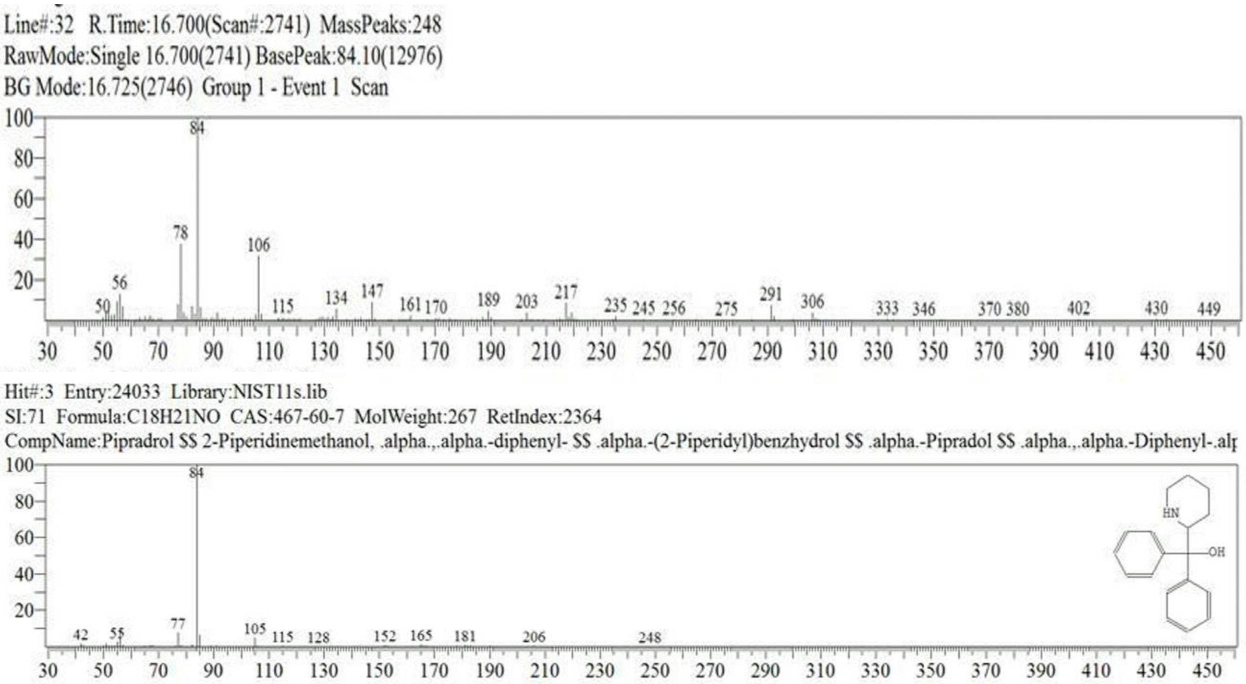
Appendix (8) Identification of Phenol, 2,4-bis(1,1-dimethylethyl)-. (MW 206)



```
Line#:29  R.Time:16.075(Scan#:2616)  MassPeaks:225
RawMode:Single 16.075(2616) BasePeak:84.10(74820)
BG Mode:16.110(2623)  Group 1 - Event 1  Scan
```

```
Hit#:5  Entry:24035  Library:NIST11s.lib
SI:80  Formula:C18H21NO  CAS:467-60-7  MolWeight:267  RetIndex:2364
CompName:Pipradrol $$ 2-Piperidinemethanol, .alpha.,.alpha.-diphenyl- $$ .alpha.-(2-Piperidyl)benzhydrol $$ .alpha.-Pipradol $$ .alpha.,.alpha.-Diphenyl-.alp
```

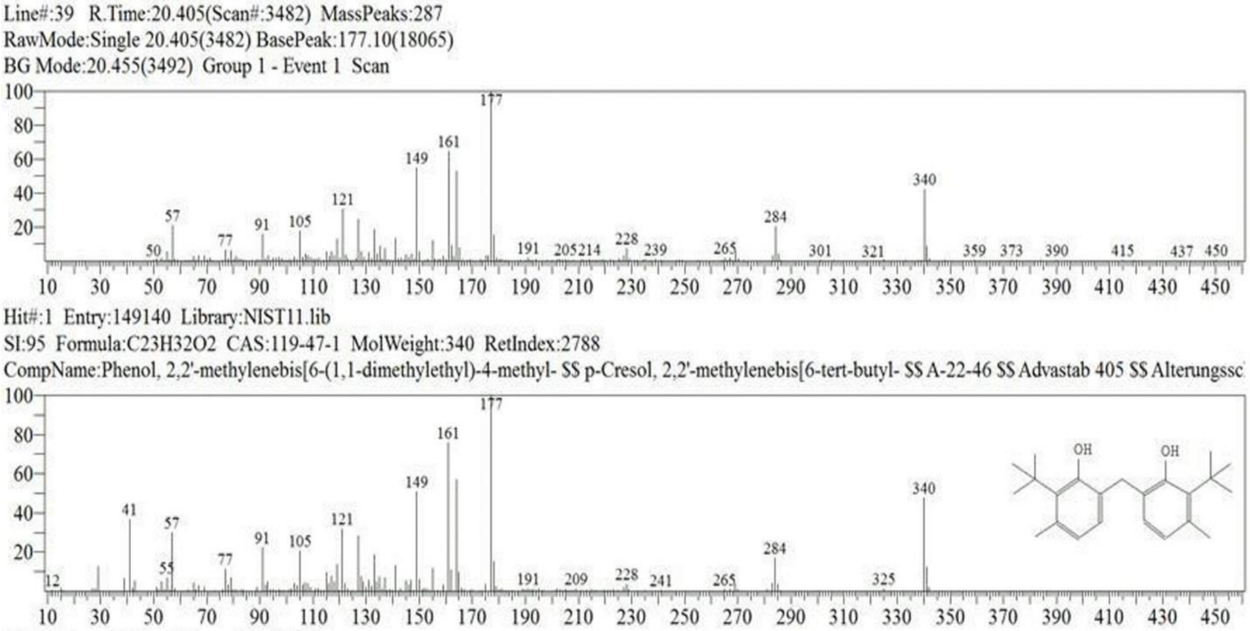
Appendix (9) Identification of pipradrol (MW 267)



```
Line#:32  R.Time:16.700(Scan#:2741)  MassPeaks:248
RawMode:Single 16.700(2741) BasePeak:84.10(12976)
BG Mode:16.725(2746)  Group 1 - Event 1  Scan
```

```
Hit#:3  Entry:24033  Library:NIST11s.lib
SI:71  Formula:C18H21NO  CAS:467-60-7  MolWeight:267  RetIndex:2364
CompName:Pipradrol $$ 2-Piperidinemethanol, .alpha.,.alpha.-diphenyl- $$ .alpha.-(2-Piperidyl)benzhydrol $$ .alpha.-Pipradol $$ .alpha.,.alpha.-Diphenyl-.alp
```

Appendix (10) Identification of pipradrol (MW 267)



```
Line#:39   R.Time:20.405(Scan#:3482)  MassPeaks:287
RawMode:Single 20.405(3482) BasePeak:177.10(18065)
BG Mode:20.455(3492)  Group 1 - Event 1  Scan
```

```
Hit#:1  Entry:149140  Library:NIST11.lib
SI:95  Formula:C23H32O2  CAS:119-47-1  MolWeight:340  RetIndex:2788
CompName:Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- $$ p-Cresol, 2,2'-methylenebis[6-tert-butyl- $$ A-22-46 $$ Advastab 405 $$ Alterungssc
```

Appendix (11) Identification of Phenol, 2,2-methylenebis(6-(1,1-dimethylethyl)-4-methyl. (MW 340)

Appendix (B): Treated water quality and characteristics of high turbidity

Moringa oleifera coagulant (MOC) treatment Dose (1.5 g)						
Dose 1.5g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		100 mg	200 mg	300 mg	400 mg	500 mg
Turbidity (NTU)	3977	4.95	6.57	7.65	9.40	10.80
Coagulant activity (%)	00.00	99.88	99.83	99.80	99.87	99.72
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
Color	Dirty brown	Colorless	Colorless	Colorless	Colorless	Colorless
PH	8.10	8.00	7.90	7.90	7.70	7.70
EC (µs/cm)	260.00	412.00	596.00	781.00	947.00	1131
TDS (mg/dm^3)	125.80	247.00	357.00	468.00	568.00	679.00
Total alkalinity (mg/dm^3)	160.75	90.83	110.21	113.90	115.55	118.71
Total hardness (mg/dm^3)	192.11	120.30	124.59	124.62	126.07	128.50
Hardness as Ca^{+2} (mg/dm^3)	86.66	60.00	89.33	73.33	57.33	96.00
Sulphate (mg/dm^3)	13	17.00				
Phosphate (mg/dm^3)	1.58	1.31				
Nitrate (mg/dm^3)	26	5.6				
Total coliform TPC/100 cm^3	164	18		16		15

Moringa oleifera coagulant (MOC) treatment Dose (2 g)

Dose 2g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		130.33 mg	260.67 mg	400 mg	530.33 mg	660.67 mg
Turbidity (NTU)	3977	06.42	08.20	11.60	12.00	13.40
Coagulant activity (%)	00.00	99.84	99.79	99.70	99.69	99.66
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
Color	Dirty brown	Colorless	Colorless	Colorless	colorless	colorless
PH	8.10	7.90	7.90	7.70	7.50	7.40
EC (µs/cm)	260.00	429.00	613.00	832.00	1024.00	1139.00
TDS (mg/dm^3)	125.80	257.00	368.00	500.00	611.00	686.00
Total alkalinity (mg/dm^3)	160.75	120.13	122.70	123.16	125.31	126.48
Total hardness (mg/dm^3)	192.11	120.48	124.33	132.18	136.14	139.60
Hardness as Ca^{+2} (mg/dm^3)	88.32	91.17	80.64	97.66	110.73	105.94
Sulfate (mg/dm^3)	13.00	19.00				
Phosphate (mg/dm^3)	1.58	1.34				
Nitrate-N (mg/dm^3)	26.00	5.30				
Total coliform TPC/100 cm^3	164	18		16		13

Moringa oleifera coagulant (MOC) treatment Dose (3 g)						
Dose 3g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		200 mg	400 mg	600 mg	800 mg	1000 mg
Turbidity (NTU)	3977	7.58	12.80	13.80	17.60	22.00
Coagulant activity (%)	00.00	99.81	99.68	99.65	99.56	99.45
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
Color	Dirty brown	Colorless	Colorless	Colorless	Colorless	Colorless
PH	8.10	7.70	7.60	7.60	7.60	7.50
EC (µs/cm)	260.00	443.00	629.00	847.00	1033.00	1147.00
TDS (mg/dm^3)	125.80	257.00	372.00	478.00	602.00	697.00
Total alkalinity (mg/dm^3)	160.75	121.34	121.67	124.52	126.33	127.17
Total hardness (mg/dm^3)	192.11	124.91	128.61	132.75	132.98	140.37
Hardness as Ca^{+2} (mg/dm^3)	79.20	76.66	84.00	80.00	74.66	86.66
Sulfate (mg/dm^3)	15.00	21.00				
Phosphate (mg/dm^3)	1.58	1.61				
Nitrate-N (mg/dm^3)	26.00	4.70				
Total coliform TPC/100 cm^3	164	17		16		14

Moringa oleifera coagulant (MOC) treatment Dose (4 g)						
Dose 4g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		266.67 mg	533.33 mg	800 mg	1066.67 mg	1333.33mg
Turbidity (NTU)	3977	9.93	14.45	20.70	23.80	30.30
Coagulant activity (%)	00.00	99.75	99.64	99.48	99.40	99.24
Taste	Not acceptable	acceptable	acceptable	acceptable	acceptable	acceptable
Odor	Odor	Odorless	Odorless	Colorless	Colorless	Colorless
Color	Dirty brown	Colorless	Colorless	Colorless	Colorless	Cloudy
PH	8.10	7.60	7.60	7.50	7.50	7.40
EC (µs/cm)	260.00	465.00	642.00	860.00	1046.00	1155.00
TDS (mg/dm^3)	125.00	260.00	386.00	505.00	599.00	711.00
Total alkalinity (mg/dm^3)	160.75	123.19	125.03	126.36	127.67	129.34
Total hardness (mg/dm^3)	192.11	128.31	134.70	141.11	146.73	148.00
Hardness as Ca^{+2} (mg/dm^3)	60.62	53.33	57.33	64.00	72.00	70.66
Sulfate (mg/dm^3)	13.00	32.00				
Phosphate (mg/dm^3)	1.58	00.54				
Nitrate-N (mg/dm^3)	26.00	4.5				
Total coliform TPC/100 cm^3	169	15		7		5

Moringa oleifera coagulant (MOC) treatment Dose (5 g)						
Dose 5g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		330.33 mg	666.67 mg	1000 mg	1333.33mg	1666.67 mg
Turbidity (NTU)	3977	9.98	14.90	18.70	28.4	35.30
Coagulant activity (%)	00.00	99.75	99.63	99.53	99.29	99.11
Taste	acceptable	acceptable	acceptable	acceptable	Not acceptable	Not acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odor	Odor
Color	Dirty brown	Colorless	Colorless	Colorless	Color	Color
PH	8.10	7.60	7.50	7.40	7.40	7.30
EC (µs/cm)	260.00	481.00	655.00	872.00	1063.00	1167.00
TDS (mg/dm^3)	125.80	362.00	395.00	532.00	615.00	722.00
Total alkalinity (mg/dm^3)	110.31	105.73	103.11	95.14	92.82	88.75
Total hardness (mg/dm^3)	192.11	140.80	142.21	145.88	146.62	150.07
Hardness as Ca^{+2} (mg/dm^3)	75.89	80.15	88.53	108.92	120.48	140.36
Sulfate (mg/dm^3)	13.00	9.00				
Phosphate (mg/dm^3)	1.58	00.79				
Nitrate-N (mg/dm^3)	26.00	22				
Total coliform TPC/100 cm^3	169	7		4		3

Appendix (C): Treated water quality and characteristics of low turbidity

Moringa oleifera coagulant (MOC) treatment Dose (1.5 g)						
Dose 1.5g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		100 mg	200 mg	300 mg	400 mg	500 mg
Turbidity (NTU)	63.70	7.10	9.70	12.30	14.80	16.10
Coagulant activity (%)	00.00	88.86	84.77	80.69	76.77	74.72
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odorless	Slightly
Color	Dirty brown	Colorless	Colorless	Colorless	Colorless	Colorless
PH	7.90	7.90	7.80	7.80	7.70	7.60
EC (µs/cm)	135	170.00	250.00	380.00	440.00	500.00
TDS (mg/dm^3)	86.40	109.00	160.00	243.00	282.00	320.00
Total alkalinity (mg/dm^3)	117.50	110.84	108.72	103.77	99.32	97.59
Total hardness (mg/dm^3)	110.00	122.11	132.06	136.39	136.44	138.50
Hardness as Ca^{+2} (mg/dm^3)	86.66	60.00	89.33	73.33	57.33	96.00
Sulfate (mg/dm^3)	10.00	17.00				
Phosphate (mg/dm^3)	00.62	1.10				
Nitrate (mg/dm^3)	4.00	3.2				
Total coliform TPC/100 cm^3	75	7		7		4

Moringa oleifera coagulant (MOC) treatment Dose (2 g)						
Dose 2g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		130.33 mg	260.67 mg	400 mg	530.33 mg	660.67 mg
Turbidity (NTU)	73.00	8.10	10.60	12.10	14.60	20.10
Coagulant activity (%)	00.00	88.90	85.45	83.42	80.00	72.47
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Not acceptable
Odor	Odor	Odorless	Odorless	Odorless	Odorless	Slightly
Color	Dirty brown	Colorless	Colorless	Colorless	colorless	Cloudy
PH	8.10	7.90	7.80	7.80	7.60	7.30
EC (μs/cm)	134.00	193.00	330.00	410.00	470.00	554.00
TDS (mg/dm^3)	85.70	123.00	211.00	262.00	301.00	354.00
Total alkalinity (mg/dm^3)	135.90	133.00	125.70	123.16	120.31	118.48
Total hardness (mg/dm^3)	118.51	124.00	128.00	132.43	136.80	139.60
Hardness as Ca^{+2} (mg/dm^3)	88.32	91.17	80.64	97.66	110.73	105.94
Sulfate (mg/dm^3)	9.00	16.00				
Phosphate (mg/dm^3)	00.66	1.08				
Nitrate (mg/dm^3)	4.50	2.00				
Total coliform TPC/100 cm^3	103	15		12		5

Moringa oleifera coagulant (MOC) treatment Dose (3 g)

Dose 3g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		200 mg	400 mg	600 mg	800 mg	1000 mg
Turbidity (NTU)	65.30	9.90	12.30	14.40	17.00	32.00
Coagulant activity (%)	00.00	84.84	81.16	77.94	74.00	50.08
Taste	Not acceptable	Acceptable	Acceptable	Acceptable	Not acceptable	Not acceptable
Odor	Odor	Odorless	Odorless	Odorless	Slightly	Slightly
Color	Dirty brown	Colorless	Colorless	Colorless	Cloudy	Cloudy
PH	7.90	7.70	7.60	7.60	7.30	7.20
EC (µs/cm)	125.00	280.00	390.00	520.00	601.00	710.00
TDS (mg/dm^3)	76.80	179.00	250	333.00	385.00	454.00
Total alkalinity (mg/dm^3)	120.00	103.34	96.67	86.52	84.33	82.17
Total hardness (mg/dm^3)	115.77	126.67	140.00	146.67	157.33	160.00
Hardness as Ca^{+2} (mg/dm^3)	79.20	76.66	84.00	80.00	74.66	86.66
Sulfate (mg/dm^3)	8.88	17.00				
Phosphate (mg/dm^3)	00.65	00.85				
Nitrate (mg/dm^3)	4.50	2.80				
Total coliform TPC/100 cm^3	120	15		3		2

Moringa oleifera coagulant (MOC) treatment Dose (4 g)

Dose 4g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		266.67 mg	533.33 mg	800 mg	1066.67 mg	1333.33mg
Turbidity (NTU)	60.00	14.60	17.50	21.40	33.54	40.33
Coagulant activity (%)	00.00	75.67	70.83	64.33	44.10	32.78
Taste	Not acceptable	acceptable	Not acceptable	Not acceptable	Not acceptable	Not acceptable
Odor	Odor	Odorless	Odorless	Odor	Odor	Odor
Color	Dirty brown	Colorless	Cloudy	Cloudy	Cloudy	Cloudy
PH	7.90	7.80	7.40	7.10	7.00	6.90
EC (µs/cm)	140.00	360.00	490.00	622.00	740.00	824.00
TDS (mg/dm^3)	89.60	230.00	313.00	398.00	473.00	527.00
Total alkalinity (mg/dm^3)	122.51	106.19	100.03	93.36	86.67	73.34
Total hardness (mg/dm^3)	116.94	129.55	134.70	136.11	140.73	148.00
Hardness as Ca^{+2} (mg/dm^3)	60.62	53.33	57.33	64.00	72.00	70.66
Sulfate (mg/dm^3)	10.91	21				
Phosphate (mg/dm^3)	00.57	00.69				
Nitrate (mg/dm^3)	4.20	1.30				
Total coliform TPC/100 cm^3	150	20		16		12

Moringa oleifera coagulant (MOC) treatment Dose (5 g)						
Dose 5g	Raw water	20 cm^3	40 cm^3	60 cm^3	80 cm^3	100 cm^3
		330.33 mg	666.67 mg	1000 mg	1333.33mg	1666.67 mg
Turbidity (NTU)	38.40	20.10	23.30	25.80	29.90	36.9
Coagulant activity (%)	00.00	47.66	39.32	32.81	22.13	4.69
Taste	Not acceptable	Not acceptable	Not acceptable	Not acceptable	Not acceptable	Not acceptable
Odor	Odor	Odor	Odor	Odor	Odor	Odor
Color	Dirty brown	Color	Color	Color	Color	Color
PH	8.00	7.60	7.30	7.20	7.00	6.80
EC (µs/cm)	136.00	400.00	550.00	710.00	860.00	1000.00
TDS (mg/dm^3)	87.00	256.00	352.00	454.00	550.00	640.00
Total alkalinity (mg/dm^3)	110.31	105.73	103.11	95.14	92.82	88.75
Total hardness (mg/dm^3)	120.43	112.00	136.00	148.32	144.94	156.64
Hardness as Ca^{+2} (mg/dm^3)	75.89	80.15	88.53	108.92	120.48	140.36
Sulfate (mg/dm^3)	9.10	18.00				
Phosphate (mg/dm^3)	00.54	00.46				
Nitrate (mg/dm^3)	4.70	3.90				
Total coliform TPC/100 cm^3	46.00	28.00		20.00		4.00