Moringa oleifera iron nanoparticles for nitrate removal in surface water

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DECLARATION

I Tshepiso Moremedi declare herewith that the dissertation entitled, "*Moringa oleifera* iron nanoparticles for nitrate removal in surface water", which I herewith submit to the North-West University as completion of the requirements set for the Master's degree, is my own work and has not already been submitted to any other university.
I understand and accept that the copies that are submitted for examination are the property of the University.

Signature of candidate Moremedi

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Signed at NWU (Mafikeng) this 16 day of October 2017.

ABSTRACT

The availability of clean potable water is a life threatening problem in South Africa, especially in rural areas. They rely on surface and groundwater as the main sources of water for drinking and other domestic purposes without any pre-treatment. The quality of these sources, however, becomes worse every day due to contaminants from industrial effluent, agricultural runoffs and other human activities.

The main aim of this study was to fabricate iron nanoparticles (FeNPs) using *Moringa oleifera,* and also to investigate their effectiveness in nitrate (NO_3^-) removal from surface water and ground water. The importance of fabricating nanoparticles using plant extracts is being emphasized globally because traditional methods are costly, toxic and not eco-friendly. The green synthesis of FeNPs using extracts of *Moringa oleifera* seeds and leaves were prepared by mixing different ratios of plant extracts with iron chloride solution, and characterized using Dynamic light scattering, UV–visible spectroscopy (UV-vis), X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

FTIR revealed the presence of functional groups such as OH, CH and CO in both plant materials and green synthesized FeNPs. The UV-vis spectrum of *Moringa oleifera* leaves (MOL) based FeNPs and *Moringa oleifera* seed (MOS) based FeNPs showed absorption peaks at 210 and 240 nm respectively. The HRTEM of MOS-FeNPs showed the particle size in the range of 2.6 -6.2 nm and 3.4-7.4 nm for MOL-FeNPs which varied from the DLS results; MOS-FeNPs(151-224 nm) and MOL-FeNPs (250-474nm). In addition, an adsorption study of NO_3^- ion removal from surface water was also investigated. Batch adsorption experiments showed that 43% and 48% of nitrate was removed by *Moringa oleifera* extracts (seeds and leaves) compared to the 85% and 26% using MOS-FeNPs and MOL-FeNPs, respectively. Furthermore, the antibacterial activity studies using disc method illustrated that the maximum zone of inhibition against Escherichia coli was observed by MOS-FeNPs (17 mm) and followed by MOL-FeNPs (15 mm). The results of the study revealed these nanoparticles as a possible alternative method to nitrate removal in surface water and that they have dual properties as coagulant and antibacterial agents.

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DEDICATION

This study is dedicated to my late mother (Matshidiso Eunice Mooketsi), grandmother (Thembiswa Gladys Moremedi).

RESEARCH CONTRIBUTIONS

Tshepiso Moremedi, Lebogang Katata-Seru, Nitrate removal in surface water using *Moringa oleifera* iron nanoparticles, 7th International Symposium on Nanotechnology: Occupational and Environmental Health, 18 – 22 November 2015, Limpopo, South Africa (Poster presentation).

Tshepiso Moremedi, Lebogang Katata-Seru, Oluwole Aremu, "Nitrate reduction using green synthesized iron nanoparticles by *Moringa oleifera* seeds and leaves extracts",article submitted to the *Journal of Chemical Engineering* for peer-review.

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LIST OF ABBREVIATIONS

DLS	Dynamic light scattering
E.coli	*Escherichia coli*
ED	Electrodialysis
EPA	Environmental Protection Agency
FeNPs	Iron nanoparticles
FTIR	Fourier transform infrared spectroscopy
MCL	Maximum contaminant level
MHA	Muller hinton agar
MO	*Moringa oleifera*
MO-FeNPs	*Moringa oleifera* iron nanoparticles
MOL	*Moringa oleifera* leaves
MOL-FeNPs	*Moringa oleifera* leaf based iron nanoparticles
MOS	*Moringa oleifera* seeds
MOS-FeNPs	*Moringa oleifera* seed based iron nanoparticles
NPs	Nanoparticles
PDI	Poly dispersity index
RO	Reverse osmosis
UN	United Nations
UV-Vis	Ultra-violet visible
WHO	World Health Organization
XRD	X-ray diffraction
ZOI	Zone of inhibition

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CHAPTER 1

INTRODUCTION

1.1 General background

Water is essential to all known forms of life and makes up to 70 percent of the body mass (Lisha *et al.*, 2009). Regrettably, the quality of our water resources is continuously changing due to population growth, drought and demands from a variety of users (Savage and Diallo, 2005). Groundwater and surface water serve as the main sources of drinking water in rural communities and urban areas; however the availability of potable water is still a major concern (Fatombi *et al.*, 2012). Furthermore, pollution of surface water and groundwater from a variety of sources has really contaminated the quality of these water sources, reducing the supply of clean water (Coetser *et al.*, 2007, Foley *et al.*, 2005, Kemper, 2004).

According to the latest report by World Health Organization (WHO), 663 million people rely on unimproved water sources, of which over 159 million depend on surface water, and millions of people die annually from diseases such as diarrhoea, cholera etc. (WHO, 2015). In addition, it has been predicted that by 2030, between 75 and 250 million people in Africa will be living in high water stressed areas due to climate change. Sub-Saharan Africa has the highest number of water stressed countries of any region, even when compared to South-Eastern Asia and Southern Asia (UN Water Report, 2014).

Water contamination caused by high nitrate (NO_3^-) concentration has become a problem, posing serious threats to drinking water supplies such as surface water and groundwater, since it promotes eutrophication (Eneji *et al.*, 2013). Moreover, when nitrate ions are present in drinking water, they are toxic to humans and animals (Kassaee *et al.*, 2011). Various techniques for treating NO_3^- have been employed, such as adsorption, reverse osmosis, chemical precipitation, ion exchange and electrocatalysis. Their application is however limited due to incomplete pollutant removal, high energy consumption and toxic sludge production (Ali *et al.*, 2014). Among the afore mentioned techniques, adsorption is the most popular method, due to its low operational cost, low maintenance and simplicity (Dąbrowski, 2001). On the contrary, the achievement of adsorption has certain limitations, due to the lack of suitable adsorbents of high adsorption capacity at commercial levels (Ali, 2012). To overcome these limitations, extensive research has been focused on developing new possible methods using nanotechnology (Pendergast and Hoek, 2011).

A number of researches have been focused on the removal of water contaminants using iron nanoparticles (FeNPs) (Ryu *et al.*, 2011, Hwang *et al.*, 2011), because iron metal is non-toxic, abundant, cheap and easy to produce. Iron has been used for the removal of various surface water contaminants, including chlorinated compounds, pesticides, heavy metals, nitrates and dyes. Additionally, FeNPs have received attention in the field of nanotechnology due to their higher surface area and high reactivity (Fu *et al.*, 2014). There are different methods which have been used for synthesis of nanoparticles, which include chemical synthesis like reduction in solutions, sono-chemical which is a physical synthesis route, and recently plants have also been employed also via biological synthesis (Geethalakshmi and Sarada, 2010). The use of plants towards synthesis of nanoparticles offers different advantages of eco-friendliness, non-toxicity and cost effectiveness (Kavitha *et al.*, 2013).

In this study, *Moringa oleifera* extracts (seeds and leaves) will be applied for the first time to synthesize FeNPs by a green synthesis method. These nanoparticles will be assessed further using various characterization techniques, such as dynamic light scattering (DLS), Ultra-Violet visible spectroscopy (UV-vis), Fourier Transform Infrared spectroscopy (FTIR); and X-ray diffractometry (XRD). In addition, their efficiency in NO_3^- removal will be evaluated and their antibacterial activity will be investigated.

Moringa oleifera (MO) is a non-toxic tropical plant found throughout India, Asia, and Sub-Saharan Africa. The plant consists of seeds containing edible oil and a water soluble substance which has coagulation properties for aspects of water treatment such as alkalinity, turbidity and water hardness (Yin, 2010). MO also acts as an antibacterial agent against different bacteria and fungi (Mangale *et al.*, 2012).

However, its behaviour on removal of inorganic pollutant; for example NO_3^-, has not yet been given enough attention.

1.2 Problem statement

The quality of water is increasingly diminishing as a result of factors including population growth, climate change, and economic inflation in developing countries. Correspondingly, access to clean and healthy water is one of the biggest problems faced by many communities in rural areas. Another challenging issue is the relatively high cost of treatment methods such as ozonation, biological degradation and conventional methods used for water treatment, along with health problems associated with the use of these techniques (Mohmood *et al.*, 2013). Furthermore, these methods fail to remove some of the pollutants from aqueous media (Mamba et al., 2007). Thus efforts to mitigate this problem cannot be overemphasised.

Mahikeng town in the North West province, for example, has been reported to depend on groundwater or borehole water. However, the water quality in these sources is poor due to high levels of NO_3^-, and this results in changes in the characteristics of the water, such as colour, odour and taste (Van Vuuren, 2013). Therefore, there is a need for effective water treatment methods which do not compromise the health of individuals.

1.3 Aim and objectives

The main aim of this study was to fabricate MO-FeNPs through the biosynthesis method and also to investigate their effectiveness in NO_3^- removal from surface water. Therefore, the objectives of the study were to:

- Prepare plant extracts from *Moringa oleifera* (leaves and seeds).
- Synthesize FeNPs using these plant extracts.
- Characterize the nanomaterials, using Malvern Zetasizer, UV-Vis spectroscopy, FTIR spectroscopy, and XRD.

- Study the adsorption performance of nanoparticles on nitrate removal from surface and groundwater.
- Evaluate their antibacterial activities against *Escherichia coli* (E.coli).

1.4 Outline of the dissertation

The dissertation consists of five chapters, outlined as follows:

Chapter 1: Provides a brief introduction of challenges relating to the quality of drinking water quality globally, and the aim and objectives of the study.

Chapter 2: Deals with a review of the literature relevant to the study.

Chapter 3: Describes the experimental methods employed.

Chapter 4: Presents the results, and a discussion of the synthesized nanoparticles is also presented in this chapter.

Chapter 5: Gives the conclusions drawn from the findings of this research and recommendations for future studies.

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CHAPTER 2

LITERATURE REVIEW

2.1 Overview of nitrates in the environment

Nitrate is the end product of the oxidation of ammonia when (Wedin and Sorensen, 2013). NO_3^- is an oxyanion of nitrogen in which nitrogen is found in the oxidation state of +5. Under oxidizing conditions nitrite (NO_2^-) is converted into nitrate, which is the most stable positive oxidation state of nitrogen and more common in the aquatic environment than NO_2^- (WHO, 2011). NO_3^-, a common naturally occurring ion in the nitrogen cycle, as shown in Figure 2.1, promotes eutrophication and causes serious effects to drinking water sources (Anand et al., 2015b). Nitrogen-containing fertilizers and animal or human organic wastes can raise the concentration of NO_3^- in drinking water (Bhatnagar and Sillanpää, 2011).

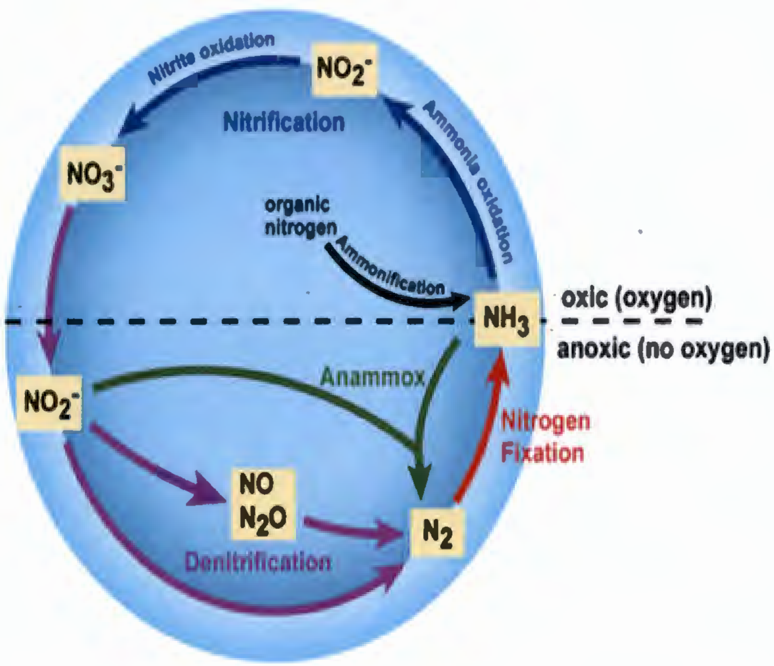

Figure 2.1: Schematic diagram of nitrogen cycle (Bhattacharya *et al*., 2013)

The high water solubility of NO_3^- leads to contamination of water sources, imposing threats and ecological disorders (Nuhoglu *et al.*, 2002). The high concentrations of NO_3^- in potable water merge into potential risks to the environment and public health; such as in water bodies (Bhatnagar amit *et al.*, 2011). They stimulate algal growth promoting eutrophication; and ingestion of plants or water containing high content of NO_3^- by cattle is said to lead to acute poisoning within 30 min to 4 hrs (Romano and Zeng, 2009). Furthermore, high levels of NO_3^- in drinking water taken by humans cause adverse effects, such as methemoglobinemia, also known as blue-baby syndrome, in infants and stomach issues in adults (Majumdar and Gupta, 2000, Tate and Arnold, 1990). Table 2.1 shows the effects of nitrates at different doses on human beings.

Table 2. 1: Effects of nitrate on human beings (WHO, 2011)

Nitrate range (mg/L)	**Adverse effects**
0-6	No adverse effects
6-10	Rare instance of Methaemoglobinaemia in infants; no effects in adults. Concentrations in the range generally well tolerated.
10-20	Methaemoglobinaemia may occur in infants. No effects in adults.
>20	Methaemoglobinaemia occurs in infants. Occurrence of mucous membrane irritation in adults

The environmental risks nitrates pose led to the implementation of the standard limits for NO_3^- concentration in water. The Environmental Protection Agency (EPA) limit is 10 mg/L (EPA, 2009), also in South Africa the recommended limit is 10 mg/L (The Water Wheel, 2008). The development of an inexpensive yet efficient method of NO_3^- removal still remains a major challenge.

There exist a number of different processes, currently employed for removal of pollutants from water. These treatment methods include coagulation, flocculation, sedimentation, filtration, disinfection, UV and zone treatment (Figure 2.2) (Shrimali and Singh, 2001). These processes however, have disadvantages attributed to them which limit their usage as affective treatment techniques. Traditional chemical methods such as coagulation use lots of chemicals and generate large amounts of sludge which is expensive to dispose (Segneanu *et al.*, 2013). Disinfection by chlorine, the most used treatment method (Ge *et al.*, 2006), produces disinfection by-products which are of concern.

Physical methods involve sedimentation and filtration, where no changes are implemented on the chemical state of the water. Instead contaminants (solids) are removed by a sedimentation process, whereby settlement of solids is allowed by means of gravity, and filters can also be used as mediums to remove entrained solids (Reed et al., 1995).

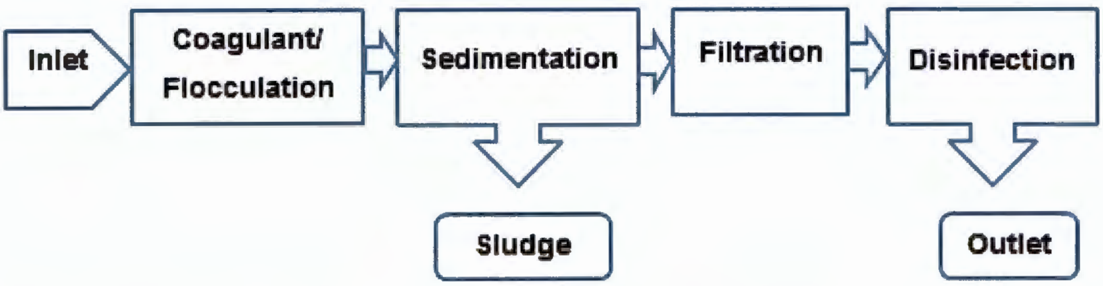

Figure 2.2: Schematics of conventional water treatment process

A number of methods are available for reducing NO_3^- concentrations to levels which comply with the regulations for NO_3^- in drinking water (USEPA, 2001). These include biological denitrification processes, ion exchange (IX), reverse osmosis (RO), electrodialysis (ED), and chemical denitrification. Among these methods, the first four have been used in industrial applications (Luk and Au-Yeung, 2002, Samatya et al., 2006). Biological denitrification and IX as NO_3^- removal methods have been suggested by WHO (WHO, 2011), while IX, RO, and ED are approved by EPA as the best available technologies for removing NO_3^- (USEPA, 2004). The technologies have their own strengths and limitations as shown in Table 2.2.

Table 2. 2.: Different technologies for nitrate removal (Jensen *et al.*, 2012)

Technology	**Advantages**	**Disadvantages**
Ion exchange	Years of industry experience, multiple contaminant removal, selective NO_3^- removal, financial feasibility, ability to automate	Potential for nitrate peaking, high chemical use, potential for disinfection byproduct formation
Reverse osmosis	High quality product water, multiple contaminant removal, desalination, feasible automation	Membrane fouling, scaling, energy demands, waste disposal, operational complexity
Electrodialysis	Multiple contaminant removal, limited to no chemical usage, long lasting membranes, lower waste volume	Energy demands, operational complexity, waste disposal
Chemical denitrification	Conversion of NO_3^- to other nitrogen species, multiple contaminant removal, high water recovery	Risk of nitrite formation, reduction to ammonia, pH, temperature dependence
Biological denitrification	High water recovery, low sludge waste, less expensive operation, limited chemical input, multiple contaminant removal	No waste brine, NO_3^- reduction rather than transfer to a waste stream, high water recovery, potential for multiple contaminant removal

2.2 Water treatment using nanotechnology based materials

The increase in human population has led to a lot of pressure exerted on water sources to meet water demands. It is suggested that with nanotechnology, cost effective and efficient solutions for improving water quality can be provided. Nanotechnology is an important field of modern research dealing with design, synthesis and manipulation of particle structure ranging from approximately 1-100 nm in one dimension" (Street *et al.*, 2014). This technology offers a range of novel nanomaterials (Table 2.3), that could provide solutions for the treatment of all kinds of waters including surface water (Theron *et al.*, 2008).

Table 2.3: Nanomaterials currently being evaluated as functional materials for water purification (Ahmed *et al.*, 2014)

Applications	**Representative nanomaterials**	**Nanomaterials Properties**
Adsorption	Carbon nanotubes	High specific surface area, highly assessable adsorption sites, tunable surface chemistry, easy reuse
	Nanoscale metal oxide	High specific surface area, short intraparticle diffusion distance, more adsorption sites, easy reuse, some are superparamagnetic
Membranes and membrane processes	Nano silver	Strong and wide-spectrum antimicrobial activity, low toxicity to humans
	Nano titanium dioxide	Photocatalytic activity, hydrophilicity, high chemical stability
	Nano magnetic	Tunable surface chemistry, superparamagnetic
Photocatalysis	Nano titanium dioxide	Photocatalytic activity in UV and possibly visible light range, low human toxicity, high stability, low cost
	Fullerene derivatives	Photocatalytic activity in solar spectrum, high selectivity
Disinfection and microbial control	Carbon nanotubes	Antimicrobial activity, fiber shape, conductivity
	Nano titanium dioxide	high chemical stability, low human toxicity, cost

Sadeghi-Kiakhani *et al,* (2013) reported a highly efficient bioadsorbent for the removal of anionic compounds such as dye from textile wastewater by preparing a combined chitosan-dendrimer nanostructure. The bioadsorbent is biodegradable, biocompatible, and nontoxic. They achieve removal rates of certain dyes up to 99%

In another study, Elmi *et al.*, (2014) synthesized ZnO nanoparticles by mechano-chemical and solgel methods. The nanoparticles were successfully used as antimicrobial agent in wastewater treatment, preferably with UV disinfection to improve water quality

Generally, two approaches are involved in producing nanomaterials, either a top to bottom approach or a bottom up approach can be used (Figure 2.3). In the bottom to top approach, nanoparticles are synthesized using chemical and biological techniques (Elghanian *et al.*, 1997, Hurst *et al.*, 2006) by self-assembly of atoms to new nuclei which grow into a particle of nanoscale (Kavitha *et al.*, 2013). On the other hand, in top to bottom approach, nanoparticles are generally synthesized by evaporation-condensation using a tube furnace at atmospheric pressure. Nanoparticles synthesized by chemical routes will be preferred in future, due to the ability to control of their size and shape (Revaprasadu and Mlondo, 2006).

The use of chemical or physical methods, for instance, the reaction of iron salts with sodium borohydride and vacuum sputtering, are expensive and require special equipment (Wang *et al.*, 2014a). Hence, plant mediated synthesis is actively being practiced by researchers for its positive advantages, like being less time consuming and more cost effective.

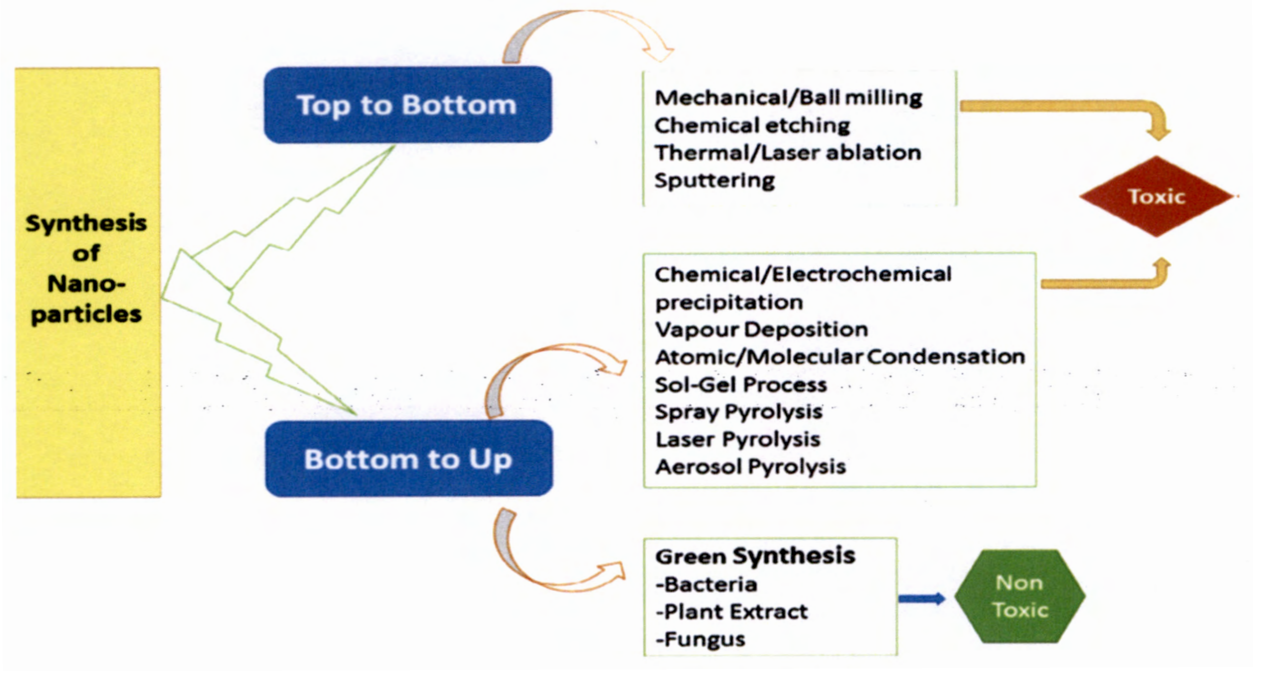

Figure 2.3: Different approaches synthesizing metal nanomaterials (Ahmed *et al.*, 2016).

2.3 Green synthesis of nanoparticles using plant extracts and its applications

The growing demand in chemical industries due to the benefits of nanomaterials and their preparation includes chemicals such as solvents, reagents and template materials. Such chemicals have caused harmful products in the past (Singh *et al.*, 2016). To reduce the unwanted products, the green chemistry concept has become prominent in science field (Ahmad *et al.*, 2011).

Synthesis of nanoparticles using plant extracts like chemical fabrication techniques uses the bottom-up strategy, whereby the main reaction is the reduction of the metal salts to form nanoparticles (Figure 2.4) (Veerasamy *et al.*, 2011). Plant based fabrication, as an alternative to traditional methods, could be the best because external experimental conditions like high energy and pressure are not required and the active biological component, like protein itself, acts as a reducing and capping agent, reducing the total cost of the synthesis process (Senapati *et al.*, 2005).

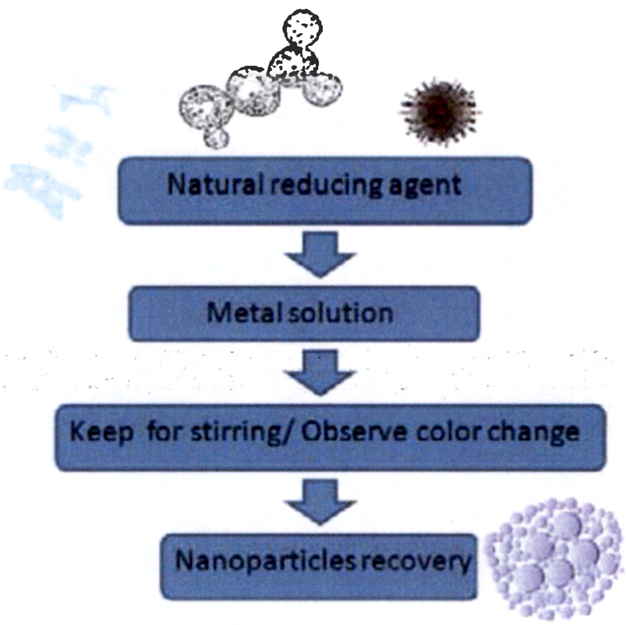

Figure 2.4: Schematic diagram of synthesis of nanoparticles from different sources (Ingale and Chaudhari, 2013)

The main mechanism considered for the process is plant assited reduction due to phytochemicals (Kozma *et al.*, 2015). The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides (Prabhu and Poulose, 2012). In the fabrication of nanoparticles using plant extracts, the extract is simply mixed a metal salt solution at room temperature, and the reaction completes within minutes (Mittal *et al.*, 2013).

Currently, a number of plants are being researched for synthesis of nanoparticles for their application in water treatment use as shown in Table 2.4. According to previous studies (Sathyavathi *et al.*, 2011, Prasad and Elumalai, 2011), only *Moringa oleifera* leaves have been used for preparation of nanoparticles for their antimicrobial activity, but not its seeds (Prasad and Elumalai, 2011, Elumalai *et al.*, 2015). Therefore, in this research work, MOS for the first time will be used for the development of nanoparticles which will further be used as an application for water treatment.

Table 2.4: Some of the plant extracts used for nanoparticles synthesis

Plant	Nanoparticle	Size	Reference
Acalypha Indica	Silver	20 - 30 nm	(Krishnaraj *et al.*, 2010)
Ocimum sanctum	Silver	4 - 30 nm	(Mallikarjuna *et al.*, 2011)
Calotropis procera	Silver	150 - 1000 nm	(Babu and Prabu, 2011)
Moringa oleifera leaves	Silver	57 nm	(Prasad and Elumalai, 2011)
Vitex negudo	Gold	10 - 30 nm	(Zargar et *al.*, 2011)

The green synthesis of FeNPs and silver nanoparticles (AgNPs) has been done using aqueous sorghum extracts as both reducing and capping agents. The produced NPs effectively catalyzed H_2O_2 degradation which made them useful for environmental remediation and treatment of hazardous waste (Njagi et al., 2010).

The leaf extract of *Croton sparsiflorus* has been used to synthesis AgNPs and the assemblies of spherical NPs seemed to be effective against *S. aureus,* E. coli, and *B. subtilis* (Kathiravan *et al.*, 2015). In another experiment a hot water extract of olive leaf was used for the production of AgNPs. The authors confirmed that the produced NPs possess good antibacterial activity against drug resistant bacterial isolates (Khalil et al., 2014).

2.4 Overview of *Moringa oleifera* and its applications

Moringa oleifera (MO) also known as drumstick tree, is a small deciduous tree of 2.5–10 m in height (Anand et al., 2015a). It is reported to contain minerals, proteins, vitamins, various phenolic compounds, amino acids and very important β-carotene, which are usually found in high amounts within the whole plant (Anwar *et al.*, 2007). This plant carries many phytochemicals (Figure 2.5) such as zeatin, kaempferom, quercetin with various combinations, and also has medicinal effects on curable diseases such as cardiac and circulatory diseases etc. (Makonnen et al., 1997), and also has antipyretic, antioxidant, antidiabetic, antiulcer, anti-inflammatory, and antiepileptic properties (Pal *et al.*, 1995).

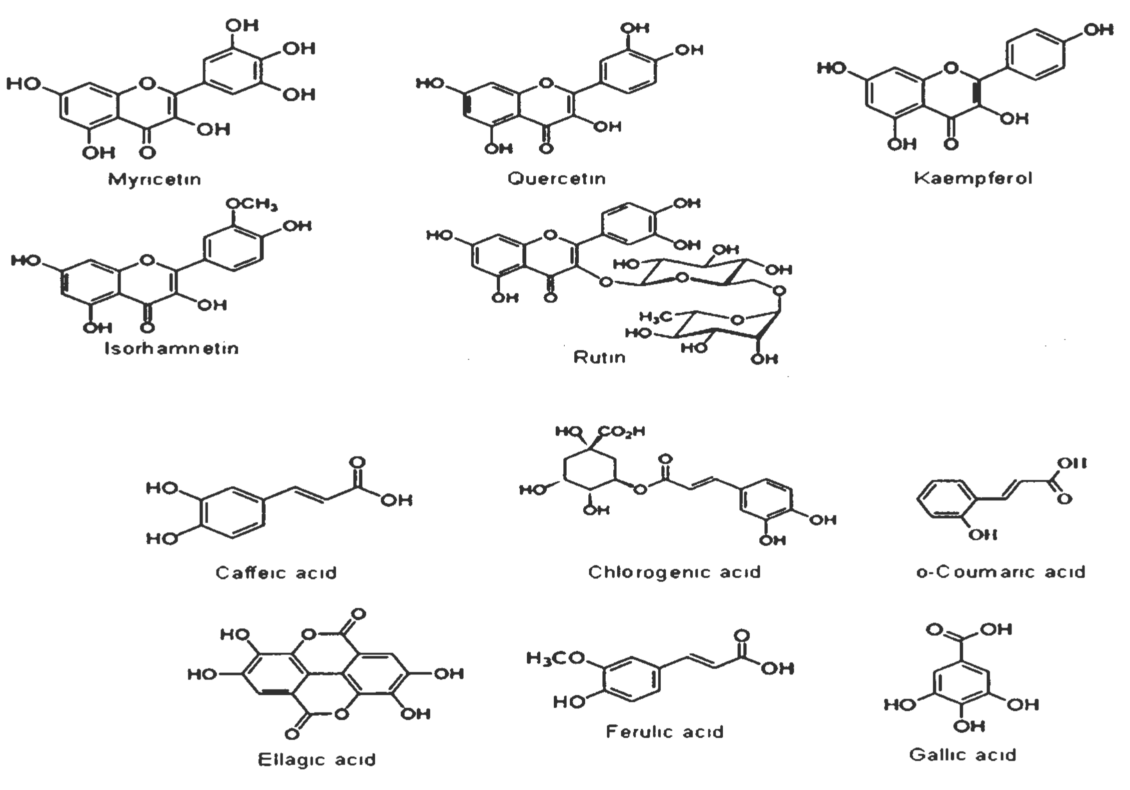

Figure 2.5: Chemical structure of some bioactive compounds found in *Moringa oleifera* (Leone *et al., 2015)*

The seeds from this plant, as indicated in Figure 2.6, act as water purifiers against Gram positive and Gram negative bacterial cells in the powder form (Kawo, 2007). The coagulating agent found in MOS is identified as dimeric cationic proteins having a molecular weight of 13 kDa and an isoelectric point between 10 and 11. The MO coagulant protein was identified as a heterogeneous mixture of sixty amino acid residues (Ndabigengesere *et al.*, 1995).

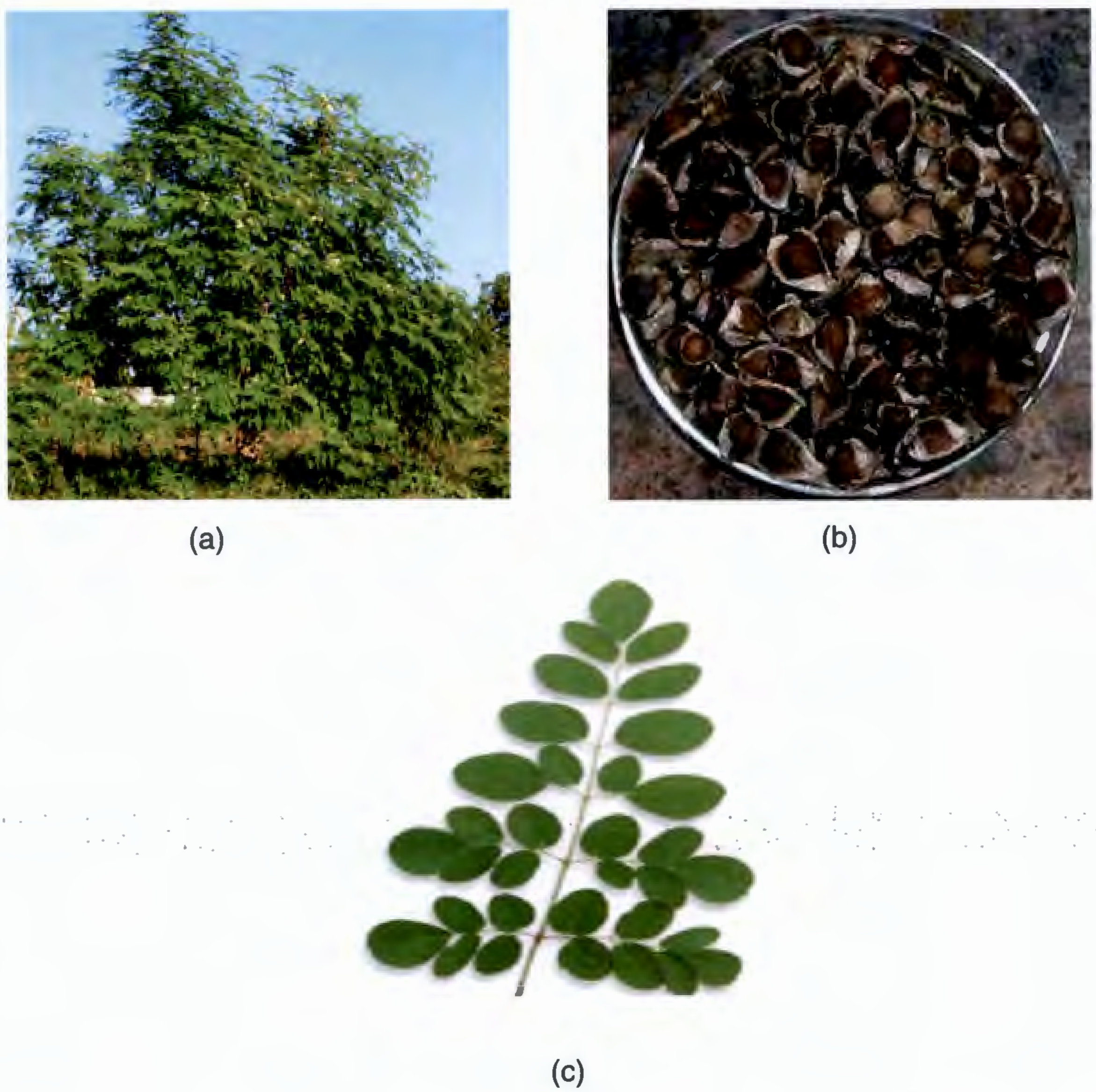

Figure 2.6: (a) *Moringa oleifera* tree, (b) *Moringa oleifera* seeds with coating, (c) *Moringa oleifera* leaves (Mangale *et al.*, 2012).

The mechanism of MO protein during the coagulation process is supposed to be that positively charged proteins and negatively charged particles (clay, silt, bacteria etc.)

bind. This leads to formation of flocs with a net-like structure due to particle collision (Figure 2.7) (Ndabigengesere *et al.*, 1995; Gassenschidt *et al.*, 1995).

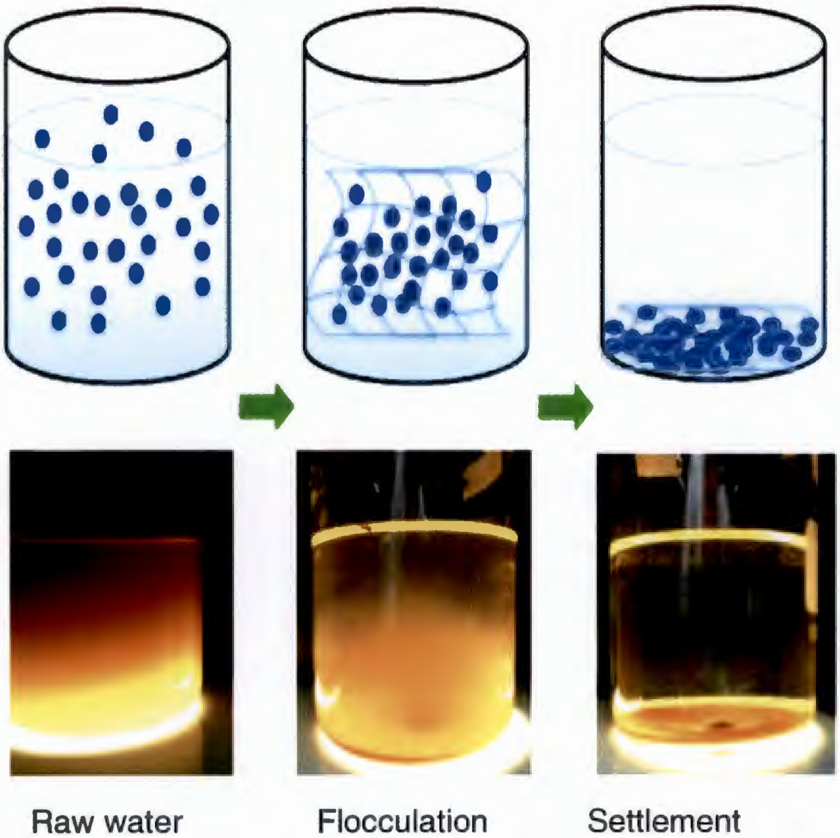

Figure 2.7: Coagulation mechanism of *Moringa oleifera* protein

Moringa oleifera extract possesses significant properties in the reduction of sludge volume and bacteria in contaminated waters (Muyibi and Evison, 1995), without any effect on the water pH, conductivity and alkalinity, therefore making MO protein attractive for water treatment (Suarez *et al.*, 2003). Besides being non-toxic, the protein is entirely biodegradable (Šćiban *et al.*, 2009). The active component can be extracted from seeds by the use of water or salt solutions, usually sodium chloride (Okuda *et al.*, 1999).

Folkard *et al*, (1993) evaluated the performance of MO in the flocculation of turbid water with alum. They found that MO gave equivalent performance to alum in the clarification of highly turbid waters. There was, however, a limit to the effectiveness of the seeds on low turbidity waters (Folkard *et al.*, 1993). In another study, Sani (1990) reported 93% reduction in turbidity within 2 hrs settling period for initial turbidities ranging from 205 - 986 nephelometric turbidity unit using MO dosages of 40 - 400 mg/L. A general rule of thumb is that powder from one Moringa seed to two liters of water is a good amount when water is slightly turbid; and to one liter when water is very turbid. The powdered seeds can be stored but the paste needs to be fresh for purifying the water.

Moringa oleifera have also been studied for removal of heavy metals from aqueous solutions. For instance, Mataka *et al*, (2010) showed that with an initial cadmium concentration of 7 mg/L, MOS powder, at a dose of 2.50 g/100 mL, a reduction of 58% concentration was achieved. The increase in initial cadmium (II) concentration resulted in a decrease of percentage removal of the cadmium (II). For a fixed dose of the biomass the total available binding sites are limited, therefore binding the same amount of metal ions. This resulted in percentage removal decrease of metal ion.

Kalavathy and Miranda, (2010) prepared activated carbon from MO wood for removal of copper (Cu), nickel (Ni) and zinc (Zn) from synthetic wastewater. Effects of pH, contact time, carbon dose, adsorbate concentration and temperature on the removal of metal ions were also studied. The MO wood charcoal was found to be a promising adsorbent for the removal of Cu, Ni and Zn from aqueous solution. These studies show that every part of the MO tree is very useful.

There are currently scarce studies related to regarding NO_3^- improving ground water quality using MOS plant. Hence, this study is evaluating the treatment for the removal of this ion in surface water and ground water with the MOS extract and finally using it as a reducing agent to form FeNPs.

2.5 Current research approach

Currently, FeNPs have been prepared using different plant extracts at room temperature or by the hydrothermal route, simply by mixing salt solution and plant extract. The most commonly used plant material for synthesis of FeNPs is tea extracts (Herlekar *et al.*, 2014). Some of the studies already done are discussed.

Kassaee *et al.* (2011) fabricated FeNPs through a novel modified arc discharge method for the removal of nitrate from water and concluded that during acidic/low pH levels, maximum removal is achieved. The efficiency of FeNPs also for the removal of NO_3^- from water appeared twice that of rather impure FeNPs, produced through reduction of iron sulphate by sodium borohydride (Kassaee *et al.*, 2011).

Another study reported by Wang *et al.* (2014b) where FeNPs were synthesized using green tea (GT-Fe) and eucalyptus leaves (EL-Fe) extracts, regarded as cleaner productions that can be used for the efficient removal of NO_3^-. FeNPs were reactive towards NO_3^- in comparison to the traditional chemically prepared Fe_3O_4 NPs. The biologically produced NPs showed significant in situ remediation of waste water especially in NO_3^- contaminated sites. Furthermore, the study suggested that NO_3^- removal can be increased by adjusting the initial pH level to about 8.4.

Wang *et al.* (2014a) They also reported that 72 % of total nitrogen, 30 % of total phosphorus, 85 % of chemical oxygen demand (COD) were removed respectively by the produced NPs. Thus NPs may play great role in treatment of waste water. The reason for very low phosphorus removal was assigned to the absence of calcium, magnesium, or aluminum, which act as precipitating agents.

2.6 Concluding remarks

In view of the literature cited in this chapter, with the emergence of new physical and chemical methods for the synthesis of FeNPs, the concerns for environmental contamination have been increased. It is therefore of outmost interest to us to develop cost-effective and easy to recover FeNPs, in an attempt to fill the gaps within the conventional methods of NO_3^- recovery from aqueous solutions.

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CHAPTER 3

METHODOLOGY

This chapter discusses how the MOS-FeNPs and MOL-FeNPs were fabricated. The prepared NPs were further characterized using different analytical instruments.

3.1 Materials

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$, 99.9%) was purchased from Sigma Aldrich (South Africa, Johannesburg); *Moringa oleifera* powdered leaves were procured from Garuda naturals health shop (Pty) Ltd (South Africa, Pretoria), *Moringa oleifera* seeds were bought from *Moringa* South Africa (South Africa, Durban). Distilled water obtained from the Milli-Q Elga System was used in all experiments. Nitrate stock solution was obtained from Merck (South Africa, Johannesburg). All chemical reagents used were of analytical grade.

3.2 Experimental methods

3.2.1 Preparation of *Moringa oleifera* seed extract

Moringa oleifera seed (MOS) extract was prepared using a modified method similar to Garcia-Fayos *et al* (Garcia-Fayos et al., 2016). Briefly; the seeds were separated from the pods and ground into fine powder using a mortar and pestle, followed by oil removal for the reduction of organic content in aqueous media. The seeds were defatted by mixing the seed powder in 95% ethanol (5%, w/v) and stirred for 45 min using a magnetic stirrer. The supernatant was dried then separated by centrifugation for 45 min at 3000 rpm and the settled residual at room temperature for 24 hrs. Different concentrations of MOS powder were mixed in 100 mL of 0.1 M sodium chloride or distilled water at room temperature using a magnetic stirrer for 60 min and left to settle for 20 min. The MO crude extract was then filtered through Whatman No.1 filter paper and stored in the fridge for further use.

3.2.2 Preparation of MOL extract

Moringa oleifera leaf (MOL) extract was done using a procedure by Elumalai *et al* (Elumalai and Velmurugan, 2015). Leaf powder of 4 g was weighed and transferred into 100 mL of distilled water. The mixture was boiled for 20 min at 60 °C; during which a light yellow solution was formed, and was then left to cool at room temperature. Furthermore, the yellow coloured extract was filtered and stored in a refrigerator.

3.2.3 Synthesis of iron nanoparticles using *Moringa oleifera* extracts

Green synthesis method adapted from Wang *et al* was utilised for the preparation of NPs *(Wang et al., 2014b).* For this purpose, MO extracts of different concentrations were mixed with 0.1 M $FeCl_3$ solution with various volume ratios of 1:1, 1:2, 1:3 and 1:4; and constantly stirred for 30 min. A change in colour from faint yellow to reddish brown and finally black after a certain period of time indicated the formation of FeNPs.

3.3 Instrumentation

3.3.1 Dynamic light scattering

The average particle size and particle size distribution of the nanoparticles (expressed as polydispersity index, PDI) were determined by dynamic light scattering (Malvern Zetasizer Nano ZS; Malvern Instrument, Malvern, United Kingdom) at 25 °C. Nanoparticles were filtered by using PTFE 0.2 µL pore size membrane to remove dust particles before analysis. All experiments were measured in triplicate.

3.3.2 Ultraviolet-visible spectroscopy

The absorption spectra of FeNPs and MO extracts were analysed using a UV-visible spectrophotometer (Perkin Elmer, Lambda 35, 54 Singapore). All samples were diluted ten times before the analysis. Distilled water was used as a blank reference.

3.3.3 X-Ray diffraction analysis

For the crystallinity phase of the nanoparticles, X-Ray diffraction analysis were performed using Shimadzu 6000 with Cu-Kα radiation source with wavelength of

0.154 nm and was operated at 40Kv/30 mA over 2θ range of 2 to 800. The scanning speed was maintained at 50 min^{-1}.

3.3.4 Fourier Transform Infrared spectroscopy

Fourier Transform Infrared spectroscopy analysis of FeNPs, MOL and MOS was carried out using a Perkin Elmer spectrum 100 FTIR spectrophotometer (USA). The samples were placed onto a sample holder and scanned over the range of 4000-400 cm^{-1}.

3.3.5 Transmission electron microscopy

The morphology of the optimized nanoparticles was analysed using TEM (TEM, JEOL JEM-200, Japan). The nanoparticles were stained with uranyl acetate solution before observation.

3.4 Water sampling

Water samples were collected directly into the sterile 500 mL Duran Schott glass bottles from surface water and borehole, after letting both water sources run for a minute. The samples were labelled properly and transported on ice to the laboratory for analysis. Aliquots of the samples were used for NO_3^- tests.

3.5 Batch adsorption analysis

The adsorption of nitrate using FeNPs, MOL and MOS extracts, was investigated by a modified batch adsorption (Poguberović *et al.*, 2016) under laboratory conditions, at room temperature (23 ± 1°C), on a water bath shaker at speed of 150 rpm.

Adsorption studies were obtained by preparing the required concentration of the NO_3^- solution which was achieved by dilution of NO_3^- stock solution. Fixed amounts of FeNPs (0.5 mL) and MO extracts were added respectively to 10 mL nitrate solutions containing different initial concentrations in the range of 5–20 mg/L. The mixture with an initial pH of 2.5 was added into a 20 mL beaker and left to react for 24 hrs. The reaction mixture was centrifuged for an hour at 3500 rpm and the supernatant was collected. The concentration of the NO_3^- was then analyzed using

Spectroquant® Prove Spectrophotometer 300. Adsorption of NO_3^- by nanoparticles, MOS and MOL extracts were calculated from the following formula

$$\% \text{ Removal} = \frac{c_0 - c_f}{c_0} \qquad (1)$$

Where c_0 is the initial metal concentration (mg/L) and c_f is the final residual concentration of NO_3^- at the end of the adsorption period, directly measured by UV-vis spectroscopy.

The obtained NPs were further applied to groundwater and surface water in order to evaluate the adsorption of NO_3^-.

3.6 Antibacterial activity studies

The antibacterial activities of plant extracts and FeNPS were evaluated against Gram negative *Escherichia coli* (E.coli) O157 obtained from NWU microbiology research group using a modified paper disc method by Prakash *et al.* (Prakash et al., 2013). Briefly, isolates were grown on nutrient agar at 37 °C for 18 to 24 hrs. The bacterial suspensions were then swabbed on the MHA plates using sterile cotton swabs. Sterile Whatman No 1 paper discs at 6 mm dimension were impregnated with MOS-FeNPs and MOL-FeNPs. The discs with MOS and MOL extracts located on the plates were maintained as references. These discs were gently pressed in MHA plates and incubated in inverted position for 24 hrs at 37° C to determine the zone of inhibition.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of MOS-FeNPs and MOL-FeNPs

The MOS extract were effectively used to fabricate the FeNPs. On mixing the MOS extract with aqueous $FeCl_3$ solution, a colour change from yellowish translucent to reddish brown was observed after about 30 minutes (Figure 4.1) and the pH reduced, which may be an indication of MOS-FeNPs formation. In this experiment it was observed that the pH changed from high acidic to low acidic (1.49 - 0.78).

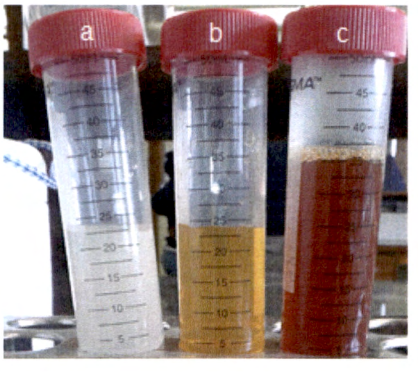

Figure 4.1: Colour change images of (a) MOS extract, (b) $FeCl_3$ solution and (c) MOS-FeNPs

The FeNPs were formed when MOL extract was added and left to stir for a few minutes. Figure 4.2 illustrates the difference in colour of the MOL extract and synthesized NPs. The formation of MOL-FeNPs was observed through a colour change from greenish to black as indicated by Figure 4.2. The time required for the reduction was less compared to MOS-FeNPs (2 minutes). More or less similar type of colour change was obtained by (Shah *et al.* 2014) and (Pattanayak and Nayak 2013)The colour change arises from the excitation of the surface plasmon resonance phenomenon and is typically of FeNPs (Shankar *et al.*, 2004)

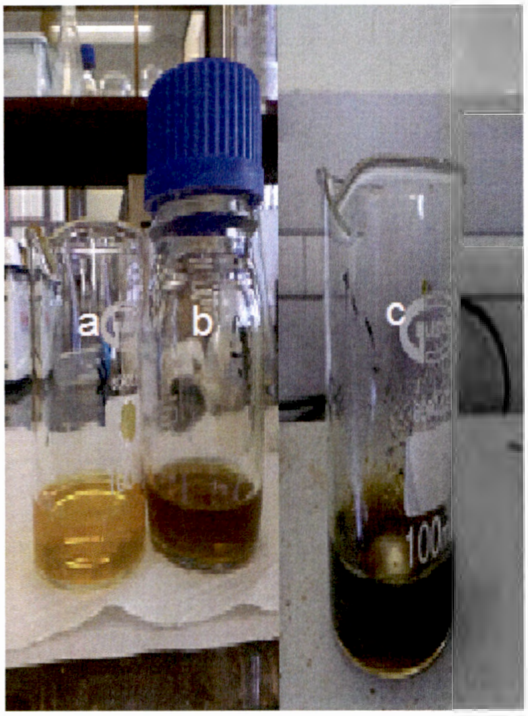

Figure 4.2: Colour change images of (a) $FeCl_3$ solution, (b) MOL extract and (c) MOL-FeNPs

4.2 Particle size and PDI analysis

The average particle size and PDI results of FeNPs prepared using MOS are given in Table 4.1. The results indicated that all the ratios (1:1, 1:2, 1:3, and 1:4) had an effect on the particle size. Smaller average particle size was obtained and PDI was obtained by 1:2 ratio, 151±2.916 nm and 0.202±0.004 nm respectively. Possible reasons for size variations include: concentration of extract (Huang et al., 2014), because it plays a key role in reducing and capping performance, and pH alterations could lead to agglomeration by over nucleation (at low pH) and instability of nanoparticles (at high pH) respectively.

Table 4.1: Size and PDI of Synthesized FeNPs from MOS extract

Plant sample		
MOS ratios	**Particle size (nm)**	**PDI**
1:1	224±62.31	0.459±0.173
1:2	151±2.916	0.202±0.004
1:3	211±2.858	0.373±0.017
1:4	138±4.565	0.219±0.001

MOL was also used in various ratios (1:1, 1:2, 1:3, and 1:4) to fabricate FeNPs. It was observed that the size of the NPs were within a range of 250 - 474 nm (Table 4.2.). The MOL extract produced NPs of appreciable size. Similar ranges in size were also obtained by different researchers (Kuang *et al.*, 2013, Wang *et al.*, 2014b).

Table 4.2: Size and PDI of Synthesized FeNPs from MOL extract

Plant sample		
MOL ratios	**Particle size (nm)**	**PDI**
1:1	306.5±9.263	0.677±0.103
1:2	250.1±7.211	0.763±0.035
1:3	474.9±8.045	0.571±0.010
1:4	273.9±1.856	0.441±0.010

Further characterization was done on the nanoparticles with the best PDI, because it is a parameter which predicts the heterogeneity of the sample. Therefore from the above results, nanoparticles formed by ratios of 1:2 and 1:4 were chosen for further analysis because of optimized results, while $FeCl_3$, MOS and MOL were used as controls.

4.3 UV-vis spectroscopy

UV-vis spectroscopy is an important technique to establish the formation of nanoparticles in aqueous solution. Figure 4.3 shows the UV spectra of MOS extract, MOS-FeNPs and after reaction with Fe (III). The characteristic peaks at 230 - 310 nm were observed, due to the excitation of surface plasmon vibrations in the FeNPs, which are identical to the characteristic UV vis spectrum of metallic iron (Pattanayak and Nayak, 2013).

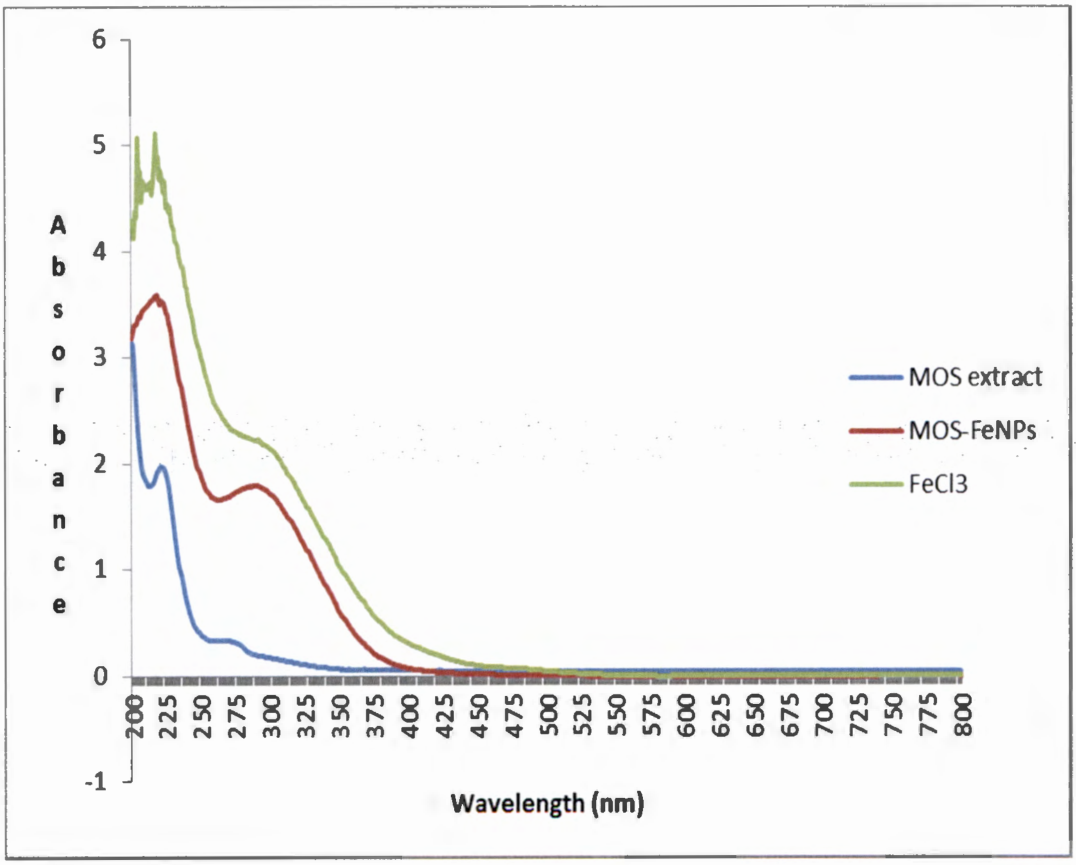

Figure 4.3: UV-visible absorption peak of MOS extract, MOS-FeNPs and $FeCl_3$

The results of the UV spectra obtained for MOL extract, $FeCl_3$ and MOL-FeNPs are illustrated in Figure 4.4 and the peak obtained for synthesized FeNPs using MOL extract were in the range of 200-300nm. Absorption peaks observed in the study and wavelength from literature for synthesizing zero valent iron nanoparticles using *Mangifera indica* leaf extract was 216-256 nm (Dhuper *et al.*, 2012) which is in good agreement with the present study.

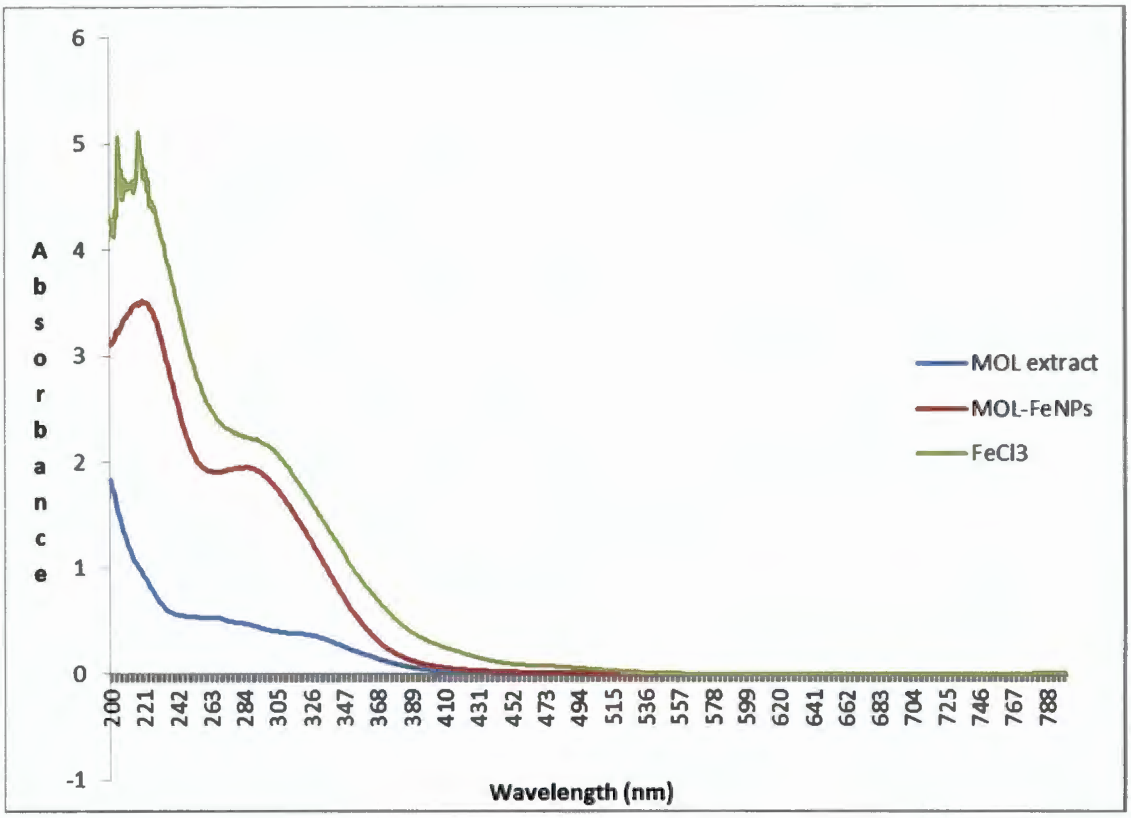

Figure 4.4: UV-visible absorption peak of MOL extract, MOL-FeNPs and $FeCl_3$

4.4 FTIR spectroscopy

The presence offunctional groups on the surface of FeNPs, MOS, MOL and $FeCl_3$ were identified by FTIR spectoscopy. Figure 4.5 gives three spectra of the MOS, $FeCl_3$ as well as the MOS-FeNPs. For MOS a band at 3280 cm^{-1} is assigned to the O-H stretching vibration and the contribution for the vibration stretching of the N-H bond of the amide group. These bands are due to the protein and fatty acids found in MOS (Reddy *et al.*, 2012). The band at 2918 cm^{-1} characterizes the band of the C-H bond of the alkane group. The two region bands at 1615 cm^{-1} and 1408 cm^{-1} are attributed to C-O bond stretching. Meanwhile, the FTIR spectra of MOS-FeNPs also displayed stretching vibration at 3519 cm^{-1} for O-H, at 1615 cm^{-1} for the C=C and 1049 cm^{-1} for C-O-C absorption peak (Wang et al., 2014b). Formation of FeNPs can be confirmed by a band at 567 cm^{-1}. Similar results were obtained by (Luo *et al.* 2016).

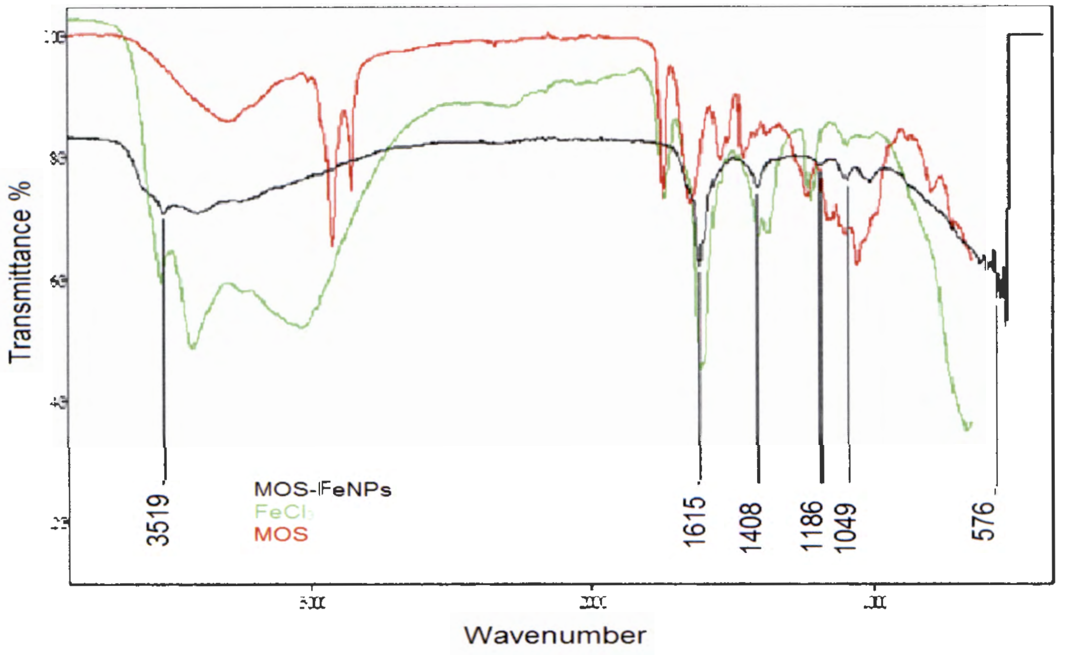

Figure 4.5: FTIR spectra of MOS-FeNPs, MOS extract and $FeCl_3$ solution

The FTIR spectra indicated by Figure 4.6 showed the bands present at 3421 cm^{-1}, 2362 cm^{-1} and 1601 cm^{-1} due to O-H stretching, C-H, and C=O respectively of MOL-FeNPs. These regions around 3270 cm^{-1} (O-H), 2900 cm^{-1} (C-H) and 1700 cm^{-1} (C=O) can also be observed on the spectra of MOL. MO is reported to be enriched with phytochemicals such as amino acids, alkaloids, flavonoids and phenolic compounds (Elumalai *et al.*, 2015), hence the presence of these peaks were observed. The peak at 565 cm^{-1} confirms that FeNPs were obtained.

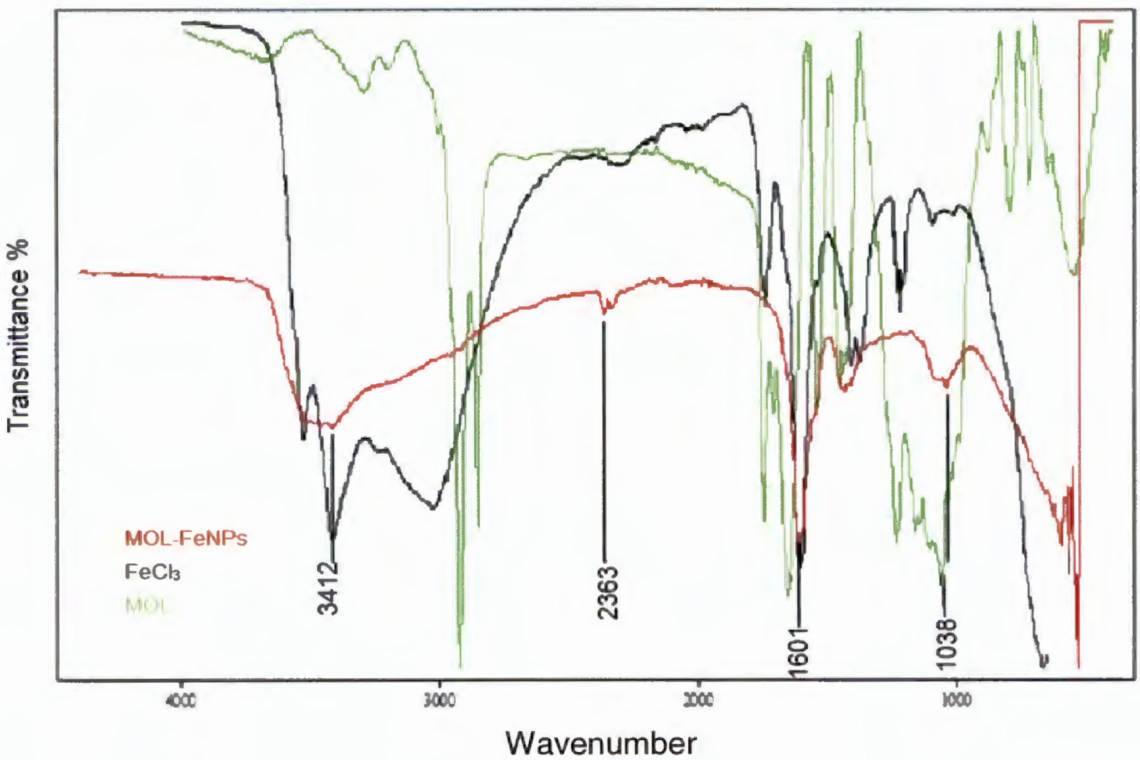

Figure 4.6: FTIR spectra of MOL-FeNPs, MOL extract and $FeCl_3$ solution

4.5 X-ray diffraction

A powerful tool for showing the crystalline and amorphous region is the use of XRD spectroscopy. Figure 4.7 presents the XRD patterns of MOS, MOS-FeNPs, MOL and MOL-FeNPs. From Figure 4.7, results revealed a characteristic peak at around 2θ of 45° on the MOS-FeNPs which corresponds to zero valent iron (Wang, 2013, Huang et al., 2014). Similar results were observed in recent reports were FeNPs were produced by tree leaf extract (Machado *et al.*, 2015) and green tea (Hoag et al., 2009). This peak indicates that the green technique produces amorphous NPs (Njagi *et al.*, 2010).

No peaks were observed on the MOL-FeNPs analysis. The peaks of MOL formed at 2θ of around 20° and 25° are due to the high content of oils and proteins within the material's composition, with a mass of around 69% (Araújo *et al.*, 2013).

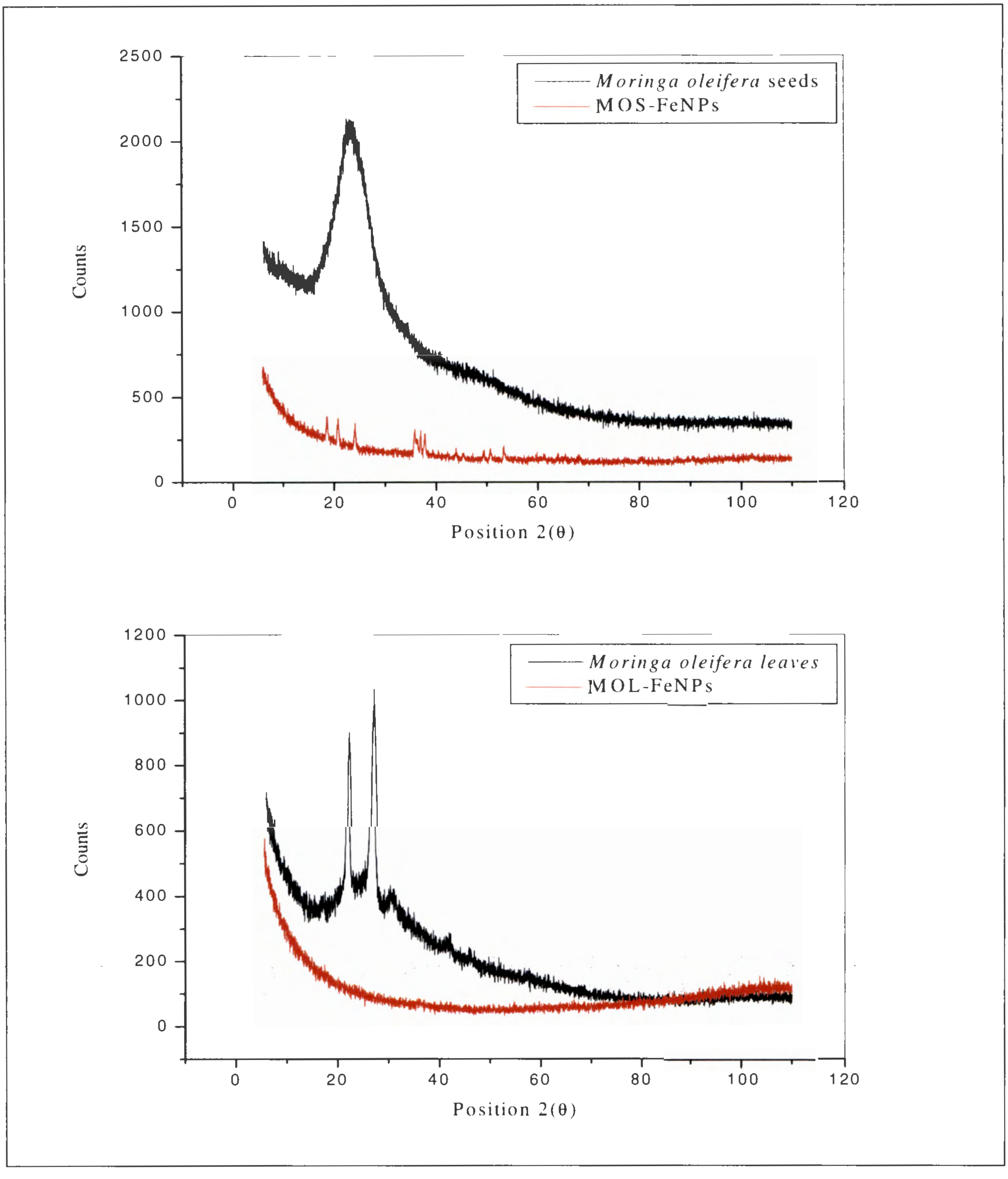

Figure 4.7: XRD pattern of MOS, MOS-FeNPs, MOL and MOL-FeNPs

4.6 TEM analysis

The size and morphology of synthesized FeNPs was analyzed using HRTEM. The HRTEM image of synthesized MOS-FeNPs (Figure 4.8) suggested that some of the nanoparticles were in spherical shape on thick surface layer, this could be due to the thickening layer of MOS (Figure 4.8a), and the size range was found to be between 2.6 -6.2 nm. The sizes measured by HRTEM analysis was very lower than that measured by DLS analysis.

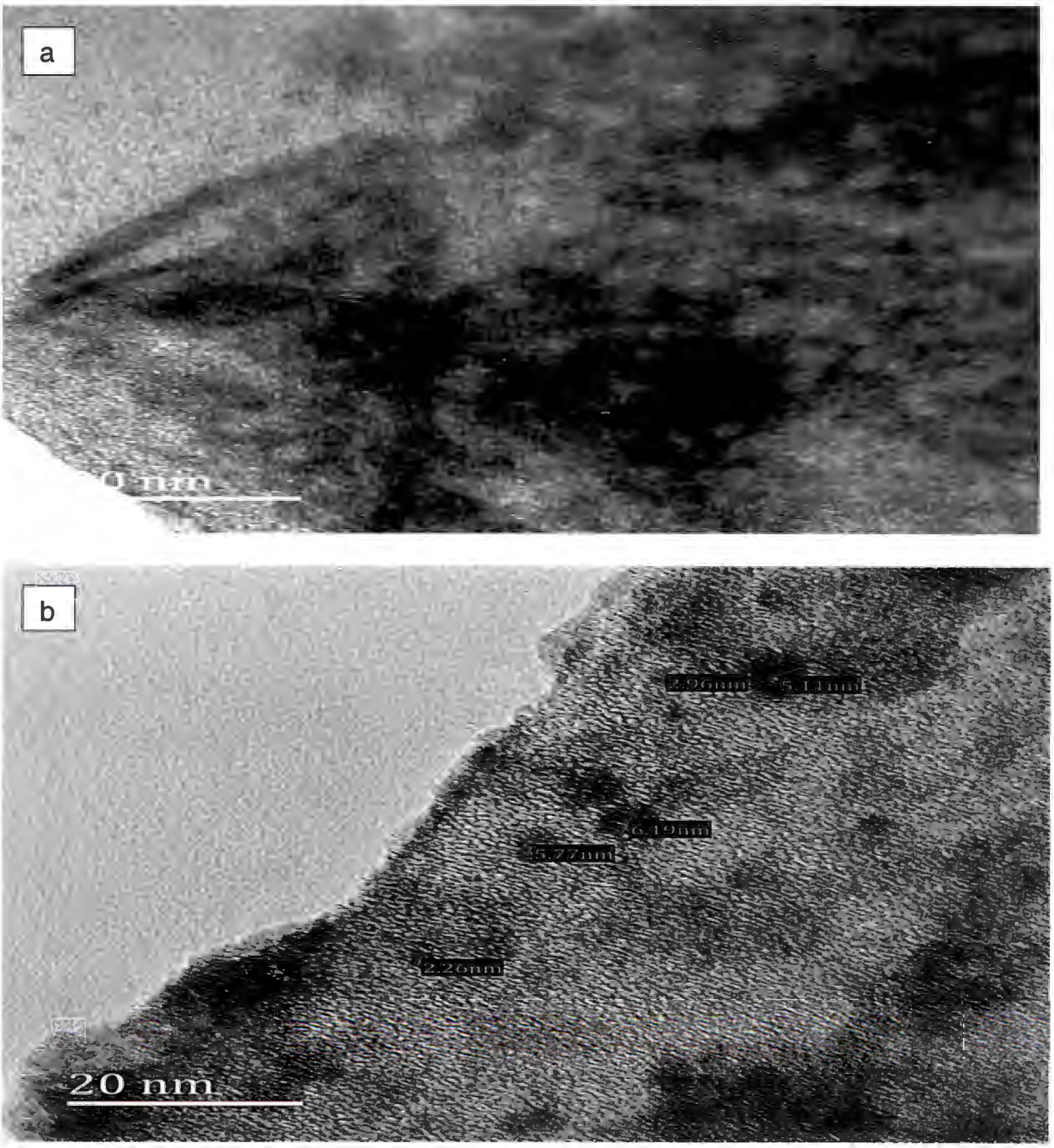

Figure 4.8: TEM images of (a) MOS and (b) MOS-FeNPs

Analysis of the MOL-FeNPs by HRTEM confirmed that they were in nanorange, in spherical shape and a diameter of 3.4-7.4 nm. A representative image is illustraed in Figure 4.9. Similar findings were reported by Yadav *et al.* by describing a spherical shape of FeNPs prepared from *Aloe vera* (Yadav *et al.*, 2016).

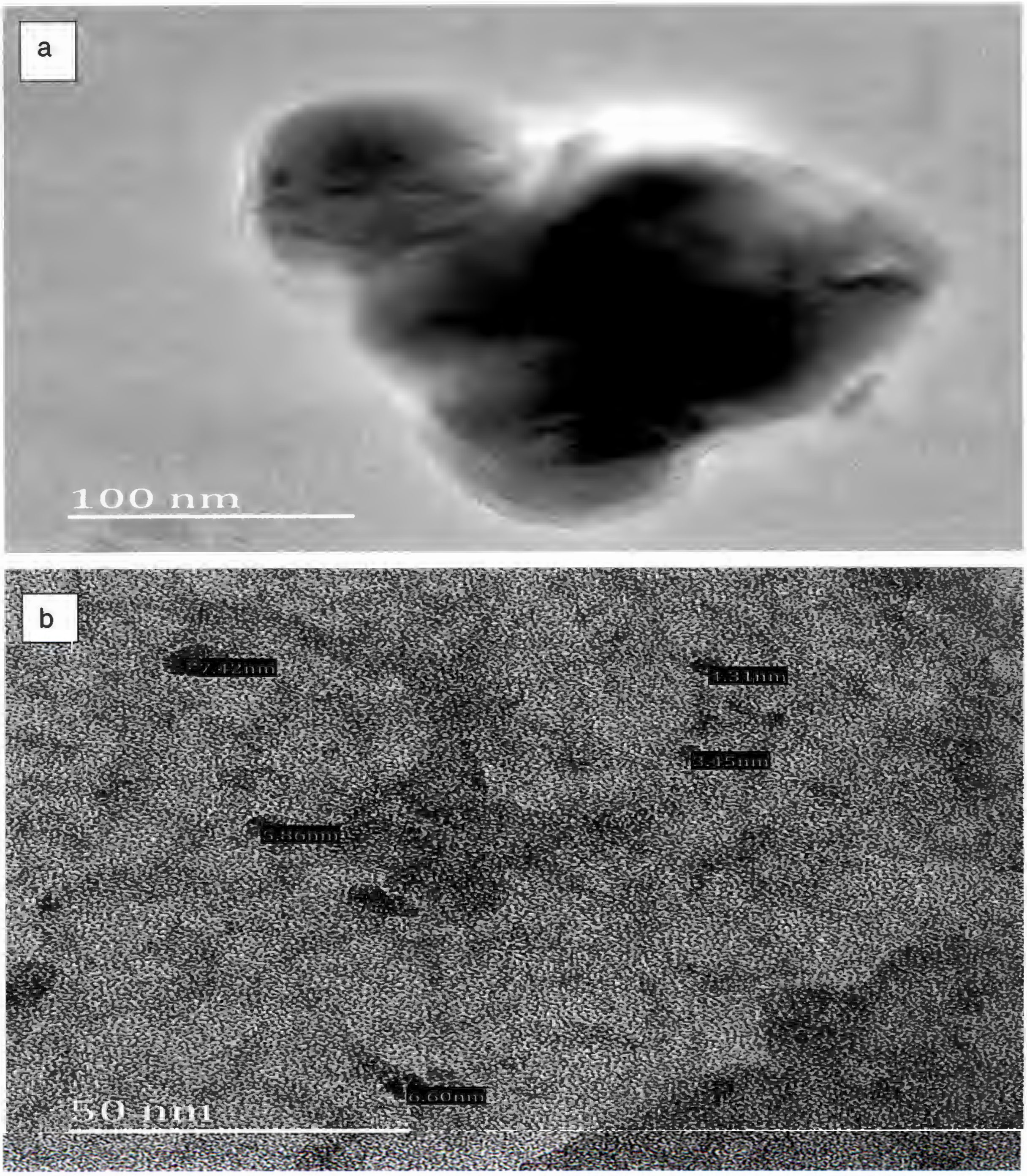

Figure 4.9: TEM images of (a) MOL and (b) MOL-FeNPs

4.7 Adsorption analysis

The removal efficiency of NO_3^- by MOS extract and MOS-FeNPs are presented in Figure 4.10. Previous reports have identified application of FeNPs in NO_3^- removal to be a promising technique (Wang *et al.*, 2014b). Different concentrations of NO_3^- (5, 10, 15 and 20 mgL^{-1}) were investigated. Dosage (0.5 mL) of different materials was fixed as well as contact time (24 hrs). The removal of NO_3^- increased as the pH of the solution decreased respectively with increase in concentration. The MOS-FeNPs were observed to be more effective than the extract. The removal percentages varied from 69% to 64% respectively.

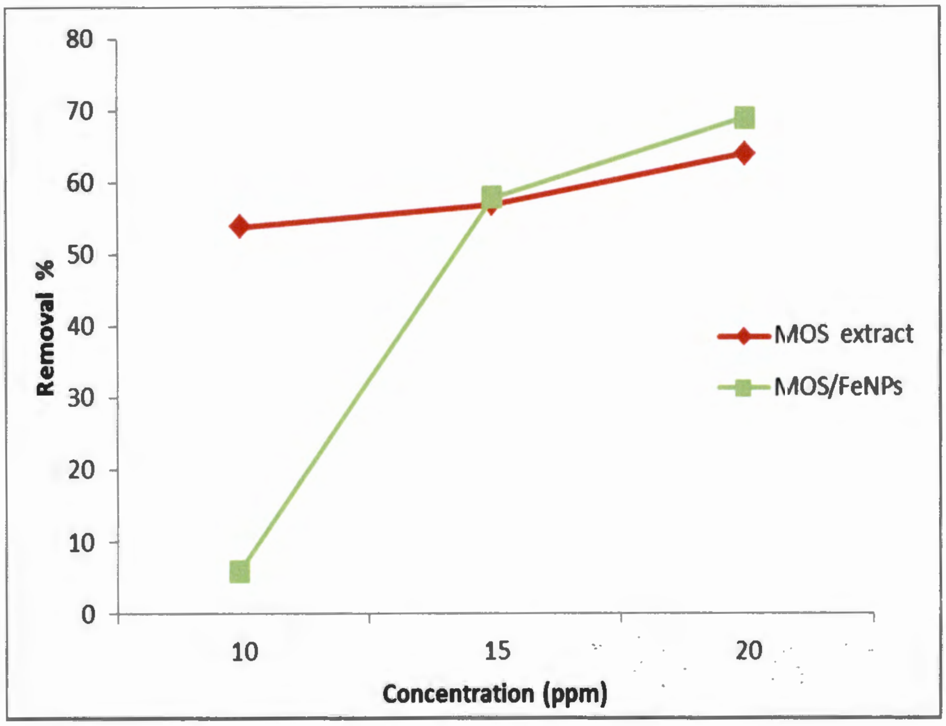

Figure 4.10: Removal of NO_3^- using MOS extract and MOS-FeNPs

MOL-FeNPs had the highest NO_3^- removal efficiency as compared to MOS-FeNPs, with removal efficiency of 76% (Figure 4.11). The results obtained are almost consistent with previous studies involving NO_3^- reduction using FeNPs (Liu *et al.*, 2014, Wang et. al., 2014b). From the overall results, MOL-FeNPs performed better than MOS-FeNPs.

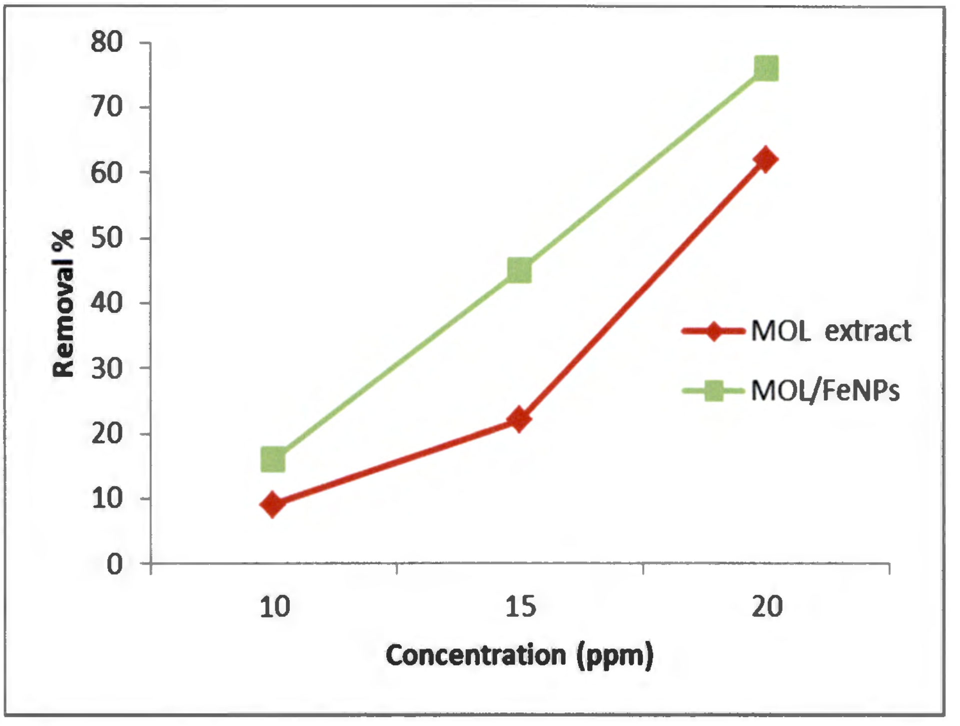

Figure 4.11: Removal of NO_3^- using MOL extract and MOL-FeNPs

To evaluate whether or not the prepared MOS-FeNPs and MOL-FeNPs can remove NO_3^- in real samples, tests were conducted using ground water and surface water collected from a bore hole at Seweding village and river water at Montshioa Stadt in North West province. Performances were done on both samples for comparison. Results in Figure 4.12 showed removal efficiency of 43% and 85% for MOS and MOS-FeNPs respectively in ground water, whilst 58% and 74% was eliminated in surface water.

In contrast, when MOL and MOL-FeNPs were used, the percent removal was decreased, as the percent removal reduced to 26% and 48% respectively in ground water, whereas 52% and 70% was achieved in surface water treatment. Overall the results highlighted the potential of green synthesized FeNPs for NO_3^- reduction. However, on the basis of the aforementioned results, MOS-FeNPs showed better effectiveness in reduction of NO_3^- than MOL-FeNPs, in both samples. Wang *et al.* (2014b) achieved a percentage removal of 56% and 41% respectively, using FeNPs synthesized by green tea and eucalyptus leaves extracts. Based on the above, MOS-FeNPs and MOL-FeNPs with their effective removal ability represent promising alternatives for NO_3^- contaminated surface water treatment, and possibly for other types of aqueous media.

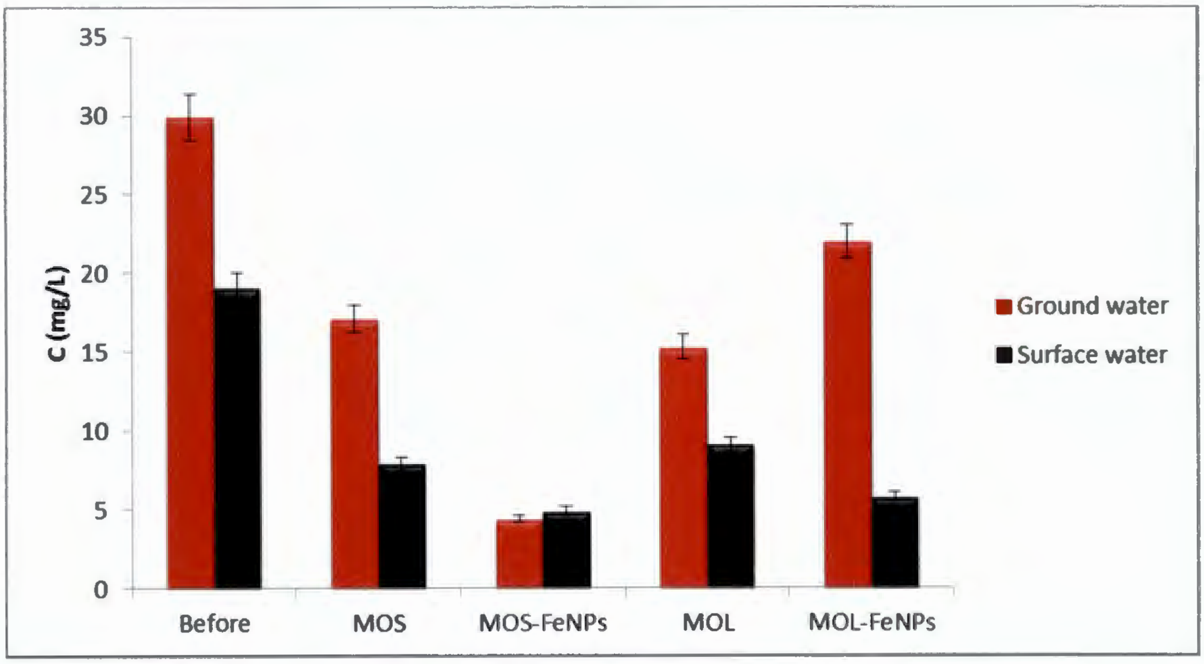

Figure 4.12: Effectiveness of MOS-FeNPs, MOS extract, MOL-FeNPs and MOL extract on surface water and ground water for NO_3^- removal

4.7 Antibacterial activity

Figure 4.13 shows the antibacterial effect of $FeCl_3$ solution, MOS, MOS-FeNPs, MOL and MOL-FeNPs on gram negative strain. The FeNPs exhibited good antibacterial activity against gram negative bacteria and the zone of inhibition is tabulated as shown in Table 3 and Figure 4.14. The effect of antibacterial activity is high in the case of MOS- FeNPs with maximum zone of inhibition (17 mm), because they are smaller in particle size (Carlson *et al.*, 2008), whereas MOS extract illustrated no inhibition.

Compared to MOS-FeNPs, the MOL-FeNPs showed the maximum zone of 15 mm and $FeCl_3$ solution 11 mm against E.coli. On the other hand, MOL extract did not exhibit any zone of inhibition.

Table 4.3: Zone of inhibition images of MOS-FeNPs, MOS extract, MOL-FeNPs, MOL extract and $FeCl_3$ against tested E.coli

Components	**Zone of inhibition (mm)**
MOL extract	0
MOL-FeNPs	15
MOS extract	0
MOS-FeNPs	17
$FeCl_3$	11

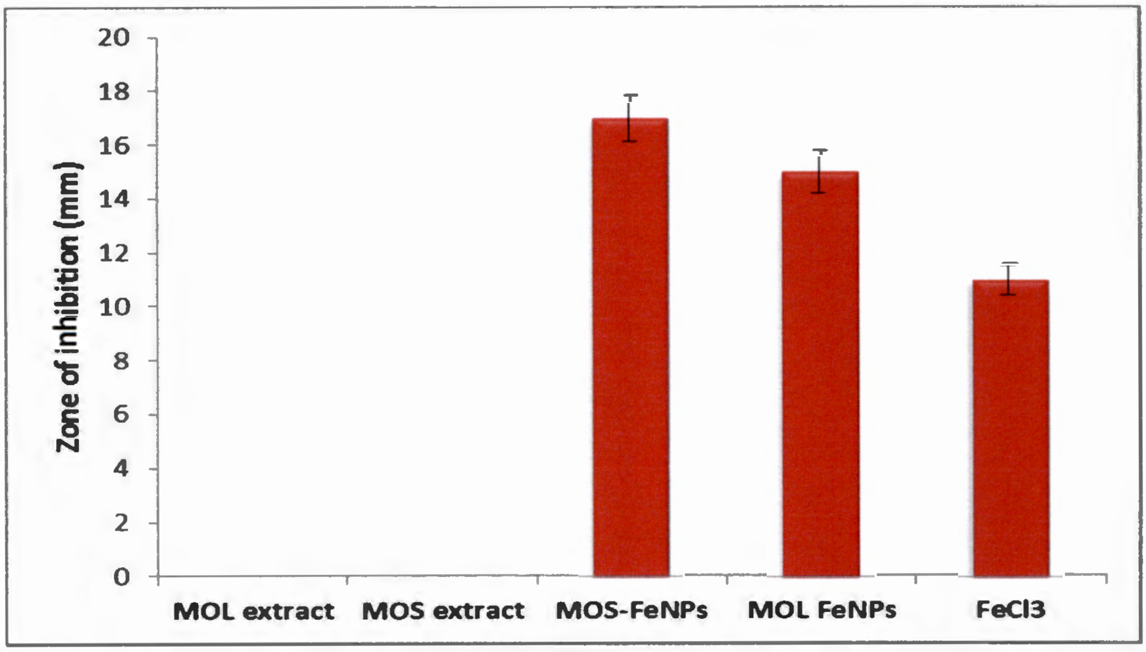

Figure 4.13: Antibacterial activity images of MOS-FeNPs,MOS extract, MOL-FeNPs, MOL extract and $FeCl_3$ against tested E.coli

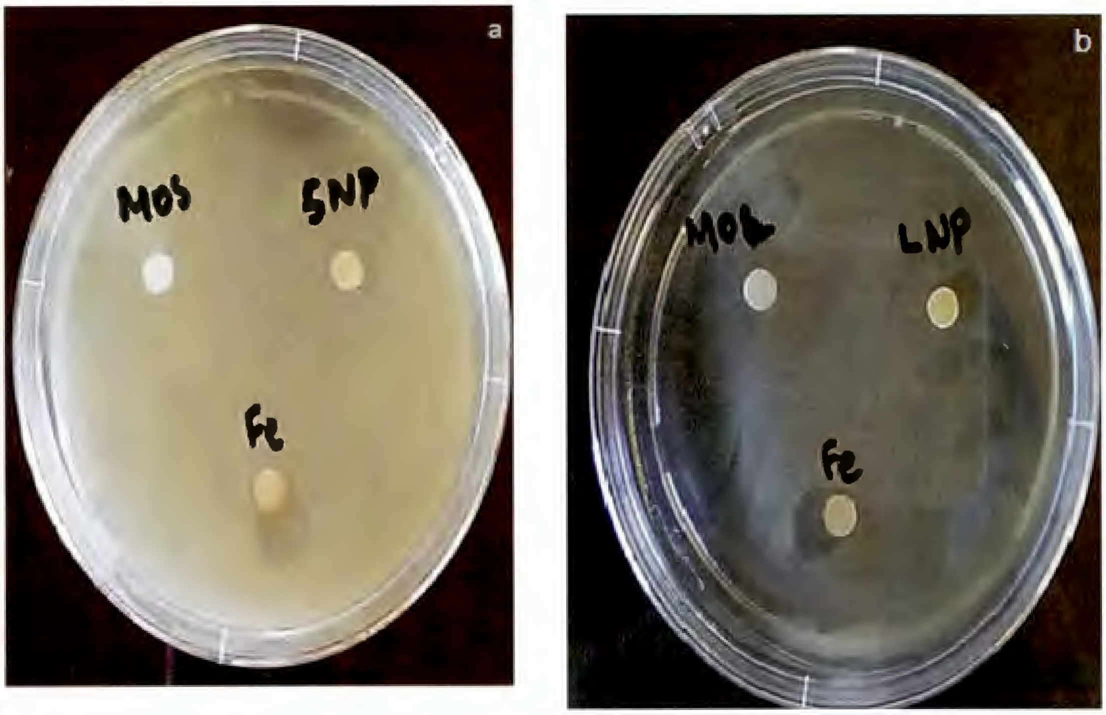

Figure 4.14: Zone inhibition of $FeCl_3$, MOS extract, MOS-FeNPs, MOL extract and MOL-FeNPs against E.coli

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The present work was focused on the development of a green method for the synthesis of FeNPs using MOS and MOL extracts. Usually, nanoparticles are fabricated using physical and chemical methods, which involve various hazardous chemicals and can cause environmental pollution. Green synthesis of nanoparticles using plant extracts is an emerging approach in nanotechnology field. The effectiveness of these nanoparticles was evaluated for surface water treatment by analyzing the parameters of NO_3^- removal and their antibacterial activity.

The UV-vis absorption peaks at 240 nm indicated the synthesis of FeNPs. The particle size analysis data showed that the MOS-FeNPs (1:2) were able to yield small mean particle size of 151±2.916 nm with small PDI of around 0.202.while for HRTEM, the particle size was very small (2.6 - 6.2 nm). FTIR studies confirmed the fabrication of the FeNPs by the action of different phytochemicals with their different functional groups present in the extract solutions. The XRD patterns confirmed that the nature of the synthesized nanoparticles was amorphous.

Adsorption studies with MOS-FeNPs showed 84% and 74% removal of NO_3^- in ground water and surface water respectively, and, the results confirmed the use of seeds for water treatment as in the literature. The NO_3^- adsorption uptake increased by decreasing the pH value of standard solutions, and as the initial concentration increased. The results show that even with high heterogeneity of nanoparticles, confirmed through characterization techniques, there is a great potential for the application of these nanoparticles in NO_3^- treatment.

The MOS-FeNPs and MOL-FeNPs also demonstrated positive antibacterial properties against gram negative strain. The MOL-FeNPs showed a smaller zone of inhibition against E.coli, while MOS-FeNPs demonstrated a large zone, suggesting that the latter were more effective in preventing bacterial growth. This suggests that these NPs have dual properties of coagulant and antibacterial activities, which is ideal for treating contaminated water.

5.2 Recommendations for future work

The following recommendations from results and conclusions drawn, may be suggested as follow up to this study:

1. The study showed tha the FeNPs were successfully fabricated using Moringa oleifera seeds and leaves extracts, however there is a need to consider parameters including; effect of concentration, temperature and stirring time for preparation process.

2. The phytochemical compounds which are involved in the reduction of metal ion should be identified.

3. Research efforts must concentrate on the improvement of monodispersity and nanoparticles.

4. Though MOS-FeNPs and MOL-FeNPs were effective in the removal of NO_3^-, an investigation needs to be done on the effect of contact time, effect of pH and effect of adsorbent dose on the adsorption of NO_3^- by these nanoparticles.