Green Synthesis of Iron Nanoparticle from *Nephrolepis auriculata* for photocatalytic degradation of Methylene Blue

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

Submitted by:

Roopal Pal

2K19/MSCBIO/14

Under the supervision of

Dr. Navneeta Bharadvaja

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

MAY, 2021

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

<u>CANDIDATE'S DECLARATION</u>

I hereby certify that the work which is presented in the research work entitled "Green Synthesis of Iron Nanoparticle from *Nephrolepis auriculata* for photocatalytic degradation of Methylene Blue" in fulfilment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of biotechnology, Delhi technological university, Delhi is an authentic record of my own work, carried during a period from 7-jan-2021 to 28-may-2021, under the supervision of Dr. Navneeta Bharadvaja.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been communicated in Scopus indexed journal with the following details:

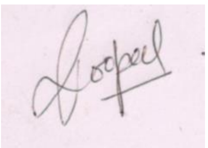
Title of this paper: Environmental remediation using Phyto-nanoparticles: An overview on synthesis, characterization, and remediation potential

Author names: Roopal Pal and Dr. Navneeta Bharadvaja

Name of Journal: Journal of Photochemistry and Photobiology B: Biology

Status of paper: Communicated

Date of paper communication: 28/5/2021



2K19/MSCBIO/14, Roopal Pal

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

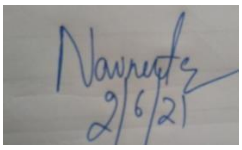
Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project dissertation titled "**Green Synthesis of Iron Nanoparticle from *Nephrolepis auriculata* for photocatalytic degradation of Methylene Blue**" which is submitted by Roopal Pal, 2K19/MSCBIO/14, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record for the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 2 June 2021



Dr. Navneeta Bharadvaja

(Supervisor)

Assistant professor

Department of Biotechnology

Delhi Technological University

Prof. Pravir kumar

Head of Department

Department of Biotechnology

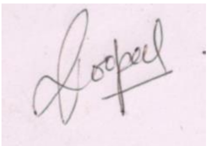
Delhi Technological University

ACKNOWLEDGEMENT

It is a privilege to express my profound sense of gratitude and indebtedness to my mentor Dr. Navneeta Bharadvaja, Assistant Professor in the Department of Biotechnology, Delhi Technological University for her valuable guidance and consistent encouragement during the progress of the project work. The dissertation would not be completed within a short period without her insightful suggestions and support.

I would also like to take the opportunity to acknowledge the contribution of Prof. Pravir Kumar, Head of Department, Department of Biotechnology, Delhi Technological University for allowing us to use the department facilities and his full support and assistance during the development of project. I would not miss the opportunity to acknowledge the contribution of all faculty members of the department for their cooperation and assistance during the development of project. I am highly thankful to Mr. Chhail Bihari and Mr. Jitendra Singh for their support.

I am equally grateful and wish to express my wholehearted thanks to respected lab seniors, Mr. Lakhan Kumar, Mr. Sidharth Sharma, and Ms. Harshita Singh for their kind support and help in the course of my research work. I would like to thank Anupam, Shruti, Neha, and Gagan and all my friends for their love and support. On a personal note, I wish to express my gratitude and affection to my family for their constant love and support.



Roopal Pal

2K19/MSCBIO/14

ABSTRACT

Ever increasing pollution in environment is alarming; phytonanoremediation is an environment friendly approach to deal with emerging pollution crisis. Greener technologies involvement give remediation process an edge over other methods via being cost effective, less time, energy and labor consuming. Synthesizing iron nanoparticles from *Nephrolepis auriculata* is a feasible approach for bioremediation of pollutants. Optimizing several key factors like pH, temperature, and agitation helps with regulating the degradation efficiency of iron nanoparticles to degrade toxic dyes like methylene blue. Synthesized nanoparticles are investigated with characterization techniques like UV-Vis spectrophotometer to evaluate their absorbance peak. This project aims to assess potential of green synthesized iron nanoparticle from *Nephrolepis auriculata* to degrade hazardous industrial dye like methylene blue.

KEYWORDS: Phyto-Nano remediation, Phyto-Nanoparticles, Methylene Blue, Photocatalytic Degradation, Iron Nanoparticles.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

Over last few years, due to improved living standards and consumer demand, attention to environmental pollution has increased. The removal of hazardous pollutant like industrial dye waste water effluent, heavy metals, oil spills, excessive use of biocides like pesticides and herbicides, pharmaceutical products, PAHs represents a serious threat to environment. Increasing pollutants are enormous and are gradually increasing due to human activity are generally found in water [1]. Utilizing green methods to combine with metal salt precursor in order to synthesize nanoparticles has an added benefit for environmental remediation. Metallic NPs like gold, silver, iron etc., have unique properties than rest of the nanoparticles based on their magnetic properties. For nanoparticle synthesis, using plant extract is favored over solvent extract. Phytocompounds rich plant extract enables to achieve stability of reduced metal ion nanoparticles thus serving as reducing and capping agents. Use of engineered nanoparticles is increasing in due their properties [2]. With respect to bulk materials, nanoparticles possess features at nanoscale like increased surface area to volume ratio, mechanical strength, optical, chemical as well as morphological properties, With respect to large scale materials. Surface properties of nanoparticles are adjustable with ample structure stability and enhanced capability to adsorb, enhanced specificity, ability to degrade pollutants and reuse of synthetic materials, and attempts have made to reduce environmental contaminants. As awareness for alarming increase in environmental pollution has been spreading fast, thus innovation for emerging techniques to remediate hazardous pollutants like industrial dyes like methylene direct discharge in water bodies is a necessity. Industrial dye waste water effluent discharged into water bodies accounts for main cause of water pollution. The ever increasing population, high demand of industrialization to match the increasing population attracts urbanization which seems to be generating enormous waste. Toxic effluent discharge in water bodies affects environment as well as deteriorates human health. Dyes and dye intermediates like benzene, toluene etc. contaminates surface and ground water.

Using phytonanoremediation for degrading pollutants is a beneficial alternative. This project aims to evaluate efficiency of Fe-NP synthesized from *Nephrolepis auriculata* for their photocatalytic degradation activity for methylene blue.

1.1 NANOPARTICLES

Over time, nanotechnology has received great attention. The foundation of nanotechnology is nanoparticles. They exist at nanoscale in the range of 1nm – 100nm and are composed of organic or inorganic matter like carbon and metals respectively. As compare to the bulk material, nanoparticles possess wide range of distinct optical, mechanical and physical properties at Nano-level. These properties are accounted by the fact of having larger surface area to the volume ratio resulting into greater reactivity or stable chemical reactivity. Characteristics like these extend applications of nanoparticles in various fields. Presently, various metallic nanomaterials such as gold, silver, and copper are being produced. They are being used for various purposes ranging from medical treatments to energy storage, cosmetic, cloth industry [3]. Nanoparticles are classified on the basis of three criteria : On the basis of their origin, On the basis of their size and on the basis of their chemical composition. On the basis of origin nanoparticles are classified on two categories like natural and anthropogenic. On the basis of size, nanoparticles can be in the range of 1nm to 10nm to 10nm, 10nm to 100nm and above 100nm range. On the basis of chemical composition they can be categorized as, when nanoparticles made from inorganic matter, when they are made from organic matter [4]. Bottom up method as well as top-down method are the two main methods of nanoparticle synthesis. The constructive approach that works by adding up of smaller particles is known as bottom up method. Biosynthesis of nanoparticle is a bottom up approach, other than that pyrolysis, Sol-gel etc. Biosynthesis is an eco-friendly method of producing nanoparticles that are safe and bio-degradable. This procedure uses plants, bacteria, and fungi to produce the nanoparticles, in place of chemicals for reducing and capping metal ions. Synthesis via green method increases efficiency and reactivity of nanoparticles [5]. Top down approach is opposite of bottom up approach, working in opposite direction, breaking down of larger material into Nano size particles. For top down approach, methods like lithography, sputtering and milling etc., are commonly used.

After nanoparticle synthesis, it is of prime importance to make sure that the newly synthesized particles are in the range of nanometers, Characterization is the way to ensure that nanoparticles have been synthesized. Characterization is a method of generally understanding the morphology and chemical properties of newly synthesized particles. Characterization is a basic process which is necessary condition for building up scientific understanding of nanoparticles for the ease of today's research and for future perspective as well. Characterization is a process utilized for studying the properties of materials. This step involves conducting various tests and calculations related to the properties of materials like mechanical strength and thermal properties. Recent and more improved technologies continue to emerge, adding more of so techniques that are being used from centuries. Characterization assists to assess the elemental composition as well as morphology of the synthesized nanoparticles and helps for assessing the end result [6].

On the basis of the optical characteristics, techniques like UV-Vis spectroscopy, Raman spectroscopy. On the basis of structural properties like morphological analysis, techniques like scanning electron microscopy, scanning tunneling microscope etc. Technique like XR-crystallography is done for understanding the crystal structural analysis. To evaluate nanoparticle's composition of elements, a technique like ED-spectroscopy is performed. For understanding structural properties like size and shape, SEM and TEM are preferred. For metallic nanoparticles iron nanoparticles, their magnetic properties are assessed by techniques like four-probe measurement [7].

1.2 <u>ENVIRONMENTAL CRISIS</u>

During the twentieth century, the rise of the human population coincided with industrialization. It initiated a paradigm shift in how society is organized. This profound change in society has led to the generation of an immense amount of waste. The revolution started with the rise of per-capita income. It has raised the standard of living and provided a better living environment for humans. The rise of population and urbanization has created an enormous quantity of waste which needs to have utmost priority of concern for all. The rapid pace of setting up of industries in urban and rural regions has now prompted many to formulate strategies to save the basic components of life. This project focuses on providing an elaboration of the eco-friendly environmental remediation technique and its importance. The deterioration of the environment has detrimental effects on human health. It has also affected the productivity of ecosystems. The pollution of the environment has detrimental effects on human health. This is evidenced by the presence of bacteria and viruses in the environment. Due to the nature of the industrial effluents that it produces, dyeing is regarded as one of the most hazardous industries that can cause pollution. It is evaluated that the global consumption of dye will reach around 9.0 million tons by 2020. There were around 280k tons of textiles release that was made to directly go open in surroundings every year..

Hazardous Chemicals like benzene and xylene are used for making dyes. The chemicals used for making these are also known as binding agents, organic coupling material, dye-ash, as well as rest supplementary chemicals. Industrial effluents from textile industry are often mixed with various xenobiotic substances such as dyes and chemicals. They can exhibit various toxicity characteristics such as residual color, high COD, and low BOD values. As industrial effluent is cause of excessive chemicals, it can cause various environmental problems. These chemicals can also be harmful to the environment. The untreated wastewater contributes to the depletion of the environment's natural resources and can lead to the formation of new water bodies [8]. The discharge of untreated also raw contaminated water into fresh water resources has detrimental effects on the environment and human well-being. Raw and ill-treated effluent can have longterm and shortterm effects on surroundings and human health. Because of presence of pollutants in the water, freshwater sources have been negatively affected by the consumption of wastewater. Amount and composition of waste water effluent pollutants are crucial factors that decides effect of pollutants on surroundings and medical condition of human [9].

1.3 PHYTO-NANOREMEDIATION ON RESCUE

The pollutant cleaning process is primarily called remediation. When pollutant degradation processes involves biological agents which is less toxic in nature, such processes are called as bioremediation and if in same processes plants are incorporated, it is called phytoremediation. Nano phytoremediation is a type of environmental pollutant remediation technology that combines two emerging fields like nanotechnology and plant technology. Nanoparticles have crucial benefits to offer like absorption and adsorption for reducing environmental pollutants [10].

The environment must be cleaned of all sorts of environmental contamination, in order to conserve deteriorated environmental condition and to protect human health. Clean surroundings is very important to us, because we rely heavily on clean air for breathing and clean water for drinking, as well as for agriculture and industry. Regrettably, several environmental water sources like underground water are precious resources but also constantly damaged by pollutants created by anthropogenic activities [11]. In order to save the environment, environmental sustainability must be maintained, which means protecting, conserving and restoring the environment while maintaining environmental quality for a long time. Therefore, more and more efforts are encouraged and researched and developed to remediate contaminated sites, as well as for reducing the capital investment of the purification methods. Over several past years, due to the high efficiency and precision of nanomaterials, the use of nanomaterials to treat wastewater has become very important. Properties like chemical reactivity, larger surface to volume ratio, cost effectiveness, less energy consuming and ease of reusability of nanoparticles makes them desirable alternative as component for wastewater treatment. Traditional method of nanoparticle synthesis like physical and chemical methods the use of volatile chemicals makes them hazardous to environment and is a cause of secondary pollution, whereas eco-friendly NP synthesis from plants is cost efficient. Plants produce ample amount of phytocompounds like flavonoids, terpenoids and several other antioxidants that are serves by reduction and stabilization of metal solution, capping agents for nanoparticles synthesis [12].

CHAPTER 2 REVIEW OF LITERATURE

Nanoparticles exists in nanoscale dimension, that is, less than 100 nm. Several properties like larger surface area to volume ratio, energy absorption, magnetic, optical properties etc can distinguish them from bulk materials [13]. They are roughly divided into two categories, one is, Organic nanoparticles, are the ones that can be synthesizes from dead organic matter like vegetable or fruit peel and are usually C-nanoparticles like fullerenes. Another group is known as inorganic nanoparticles, that can be metallic and magnetic in nature for example iron nanoparticles, zinc oxide nanoparticles [10].

Fe-NPs are found in metallic, bimetallic, trimetallic or oxides forms and several studies have reported them as potent pollutant remediation agents. Fe-NP has been found to have more than 16 forms of Fe oxides. They are uesd as anti-microbial material since a long time and they have been used to degrade dyes through adsorption. Other than that, FE-NP have been used to act on nitrobenzene, pharmaceutical products, industrial effluent, heavy metal contamination, and serve as heterogeneous catalysts in environmental processes. The appearance of Fe-NP as environmental pollutant remediation agents is because of their magnetic properties, dual redox ability when reacts with water [14]. Plant based Iron nanoparticles synthesis requires plant parts like leaves, roots, fruits and flower for extraction of phytocompounds from them and to be used as reducing agents. Plant produces many biologically active phytocompounds like flavonoids, terpenoids, saponin and polysaccharides etc., some of them are easily solubilized in water while are solubilized in organic solvents. Apart from plant extract, metal salt presursors like ferric chloride and ferrous chloride are required that is to be reduced and stabilized by active biological reducing agents. Iron present in Fe^{3+} and Fe^{2+} form is reduced to zero valent iron (Fe^{0}) in order to synthesize Fe-NP [15].

Large group of phytocompounds like polyphenols structure contains ketone as well as hydroxyl functional group that serves the main function by chelating the reactive metal ion. Another large class of phytocompounds like Flavonoid, act as reducing agent for NP synthesis by losing electrons. For example, Gold nanoparticle synthesis from *Elaeis guineensis* leaf extract, flavonoid served as reducing agents for nanoparticle synthesis [16].

In the nanoparticle synthesis process, optimizing of many factors that affect their generation like temperature, pH, amount of plant extract and metal salt and there ratio is necessary. A study was conducted to understand the factors affecting the synthesis of Fe-NP from tea plant for dye degradation, using Ferric sulphate as metal salt precursor, and several conditions were sorted out to synthesize nanoparticles like varied temperature and pH. For example, by enhances the concentration of plant extract and shifting pH to alkaline, degradation efficiency of Fe-NP was reduced. On the other hand, enhancing temperature resulted higher efficiency of nanoparticles

Nanoparticles (Size/Shape)	**Plant Material**	**Plant extract : Metal salt ratio**	**Reaction: Temp/Agitation/**	**Application**	**References**
Tin oxide (SnO_2) NP (8nm dia, spherical)	*Vitex agnus-castus* fruits extract	$SnCl_2$ (80ml, 0.01M)	60° C (6hrs)/35,000 rpm	**Dye removal** (Complete photodegradation of RhB after 190 min) **Heavy metal ion removal** (Adsorptive removal of Co^{2+} at adsorbent dosage=0.12 g/L At pH7)	[17]
Zero Valent Silver (AgNPs)	*Ficus Benjamina* leaves extract	Leaves extract: $AgNO_3$ (1Mm)	25° C,/stirred for 40 mins	**Heavy metal removal** (removal of Cd	[18]

Nanoparticle (dendritic structure)		2: 25		(II) from polluted Water i.e. highest removal percentage at 40 min)	
zinc oxide Nanoparticles (65-80nm, irregular & complex)	*Sphagneticola trilobata*	Leaf extract : Zinc acetate dehydrates(0.1M) 25 ml : 1.3 ml	60° C/6000 rpm	**Heavy Metal remova** 100 mg/mL with 42.37 percent)	[19]
Zero valent iron nanoparticle (Varied sized NP in range of 50-80nm, spherical shaped)	*Eucalyptus globulus* leaf extract	Leaf extract :$FeSO_4 7H_2O$(0.1M) 1:1	37° C/stirred continuously	**Heavy metal removal** 400mg/l Chromium degrade (30mins)	[20]
Gold nanoparticles (AuNPs)	*Capsicum annum* L. leaf extract	Leaf extract: gold (III) chloride trihydrate (HAuCl4.3H2O)		**Heavy metal removal** percantge removed of Pd by 39.270 (AuNPs stopped growth of S. aureus, (yeast) at 0.112,	[21]

				0.055respt.	
zero valent iron nanoparticles (100 nm, irregularly shaped)	Damask Rose Garden Thyme	Leaf extract:$FeCl_2.4H_2O$(0.1M) 2.3		**Heavy Metal Removal** 100 mg/l Cr(VI) degraded (100%) at 0.2 g/l)	[22]
Silver Nanoparticles (AuNP) (AH - <30 nm) AI - <35nm PC - 50 nm	*Araucariia heterophylla* *Azadirachta indica* *Prosopis chilensis*	plant gum extract: AgNO3(3mM) 1:10	37° C/10,000 rpm for 5 min	**Antimicrobial Activity** AuNP kills gram positive and negative bacterias. **Anti-Cancer** (Decreased viability at 1mg/mL of AuNP) **Heavy metal removal** (Inefficient and Irregular adsorbing pattern)	[23]
Iron Nanoparticle	*eucalyptus* leaf extract	eucalyptus leaf extract: FeSO4 (0.05M)	80° C for 2 hours	**Heavy Metal Removal**	[24]

(FeNP) (size range between20 to 80 nm)		(1:2)		74.2% and 45.2%	
Cupric Oxide Nanoparticles (CuO-NPs) (15–20 nm,	*Calotropis procera*	Plant latex: $CuSO_4.5H_2O$ (0.2 M)	37°C, 10000 rpm for 10 min	**Heavy Metal removal** (5mg/l Cr removed at 18.84 g/l adsorbent dose)	[24]
Iron Nanoparticles (Fe-NPs) (40-7nm, spheroidal)	*Nephrolepis auriculata*	Plant extract:$FeCl_3$(0.1 M) (2:1)	37°C, Continuous stirring for 10 mins	**Heavy Metal Removal** (90.93% Cr(VI) removal)	[25]
Zero valent iron nanoparticles (20–30 nm)	*Catharanthus roseus* flower extract	Flower Extract :$FeNO_3$.H2O(0.01M) (1:1)	Constant stirring of mixture for 15 mins	**Heavy Metal removal** of Cr(VI) (10 ppm)by 1.6 g/L of ZVIN	[26]
Iron Oxide Nanoparticles	tangerine peel extract	tangerine peel extract: Fe3O4 (1:1)	Constant stirring for 20 min	**Heavy Metal Removal** 99% max degraded Pb(II) in 90 min	[26]
Zero valent Iron nanoparticles	*Eichhornia crassipes*	Leaf Extract : $FeCl_3$ (0.01M)	37° C/12,000 rpm for 10 mins	**Heavy Metal Removal** Cr(VI)- 89%	[27]

(20~80 nm,		(1:1)		degraded	
Iron Nanoparticle Approx. 40nm	Spearmint leaves	Extract: $Fe(NO_3)_3$ (10 mM)	30°C /200 rpm for 72 hrs/ dark	**Heavy Metal Removal(As(III) and As(V)** (Max absorption metals at 86.53 & 94.67 mg/g resp.	[28]
Maghemite nanoparticles (γ-Fe2O3) (20-40 nm, spherical, crystalline)	*Tridax* leaf extract	Leaf Extract : FeCl3(2 M) & FeSO4.7H2O (1M)	At room temp/mixing	**Fly Ash heavy metals (Pb, Cd) Removal** (85% Pb removed in 2hrs & Cd level were < Detection Level	[29]

Table 1. Various nanoparticles synthesis from plants under optimum conditions and their Application.

Addition of plant extract to metal precursor serves as the natural reducing agent and reduces Fe^{3+} to Fe^{0}, to investigate this reduction, the reaction mixture is monitored with UV-Vis spectrophotometer under 200nm to 800nm range. UV-Vis spectrophotometer works at the principle of detecting the light that passes through the sample. Optical properties of nanoparticles are easily understood with this technique.

NP	**Plant Source**	**Metal Salt: Plant**	**Reaction**	**Characterization**	**Reference**

		Extract	(agitation/temp)	Method & Result	
Iron oxide NP	Mentha Pulegium L.	Fecl3 (0.01, 0.04, 0.07, and 0.1 M): leaf extract 20ml:200ml 1:10	continuous stirr at 75 °C for 1 h	**UV–Vis spectra**; Abs. peak at 275-301nm **FT-IR spectrum**: peak at 510 and 594 cm− **SEM:** cubic and irregular shaped	[30]
Iron Oxide NP	Carica papaya	FeCl3.6H2O(50 mL of 0.1M): leaves extract 1:1	magnetic stir for 30 min	**FT-IR spec** : wavenumber range of 4000–400 cm−1. **EDX**: C 25% O 31% Fe 32% XRD: crystallite size 4.58 nm **TGA**: 25.41% weight loss at temp around 24.38 °C to 301.70 °C	[31]
Iron oxide NP	C. moschata	Fe (NO3)3.9H2O 0.1M : extract (1:2)		**FTIR**: band range 3000–3500 cm–1	[32]

Iron Oxide NP	Laurus nobilis L.	0.1 M FeCl3 .6H2O : leaves extract 30ml/30ml (1:1)	Continuous stirring at room temperature	**UV-VIS** : Abs at 285nm FT-IR: Abs peak (600, 450) cm−1 **EDS spectra**: Fe 69% O 22% **TEM**: 8.03 ± 8.99 nm average particle size	[33]
Iron oxide NP	borassus flabellifer	$FeCl_3$ (0.2M) & $FeSO_4$ (0.1M) 2:1 : coat extract(25ML) (1:1)	Stirring at room temp	**XRD**: crystalline size 35 nm **UV-VIS** : Abs at 350nm **TGA**: weight loss between 0 °C–700 °C **SEM**: hexagonal shaped iron	[34]
Iron Oxide NP	Hibiscus rosa-sinensis	Ferric and ferrous chloride: Extract (1:1)	200 rpm/room temp	**UV-VIS** : Max abs. at 229nm **FITR**: abs peak 567.94 cm−1 **SEM**: average particle size of 65 nm	[35]

Iron Oxide NP	Pheonix dactylifera	Fecl3(0.4M) : Extract (1:1)	Heat at 70°C	**SEM:** Diameter 12.6 nm **FTIR**: peaks at 1653, 1633 and 1623 cm- 1.	[36]
Iron Oxide NP	Withania coagulans	FeCl3.6H2O & FeCl2.4H2O: Leaf extract (1:5)	Stirr at room temp	**UV-VIS** : 294nm **FTIR:** characteristic peaks at 666 cm−1 for bond vibration in Fe-O **TEM:** diameter of 10–15 nm	[37]

Table 2. Various green synthesized nanoparticles from plants with characterization techniques.

CHAPTER 3 MATERIALS AND METHODOLOGY

3.1 CHEMICALS

Ferric chloride was collected from Sisco Research Laboratories Pvt. Ltd., Methylene blue dye, distilled water

3.2 FERRIC CHLORIDE SOLUTION

0.1M of ferric chloride stock solution was prepared by adding 162.5 gm of ferric chloride to 1000ml of distilled water.

3.3 LEAF EXTRACT PREPARATION

Leaves of *Nephrolepis Auriculata* were washed twice under tap water as well as later with distilled water. Drying of leaves was done for sometimes and later heating of leaves was done at 30° Celsius for until completely dried to avoid damage to phytocompounds. After drying, fine powder of leaf was made in mortar pestle. The extract was obtained by preparing a stock solution of 30gm/L [38] by boiling dried leaf powder in distilled at 80° C for half an hr. After boiling, cooling down of mixture was done at 25° C then filter paper used to filter out the extract and remove supernatant. The filtrate could be stored in a flask at 4°C for a week [39].

Fig 1. ***Nephrolepis auriculata*** **Plant**

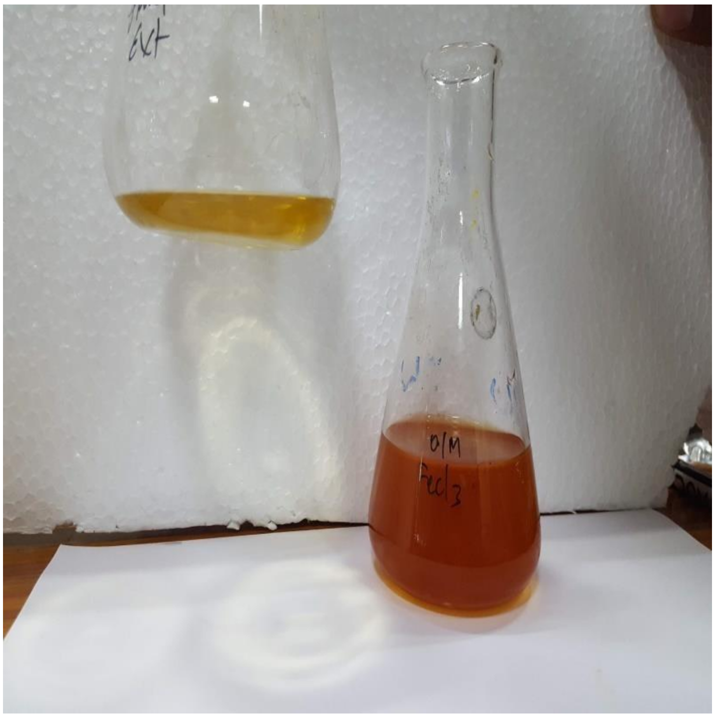


Fig 2. NA plant extract and 0.1M $Fecl_3$ solution.

3.4 NA-Fe-NP SYNTHESIS

Fe-NP was synthesized by adding 0.1M $FeCl_3$ in leaf extract in varied volume ratio at room temperature. Various ratios of metal salt and plant extract were: 1:2, 1:3, 1:4, 1:5, 1:9, 2:3, 3:7. Fe-NP synthesis was marked by finding at the bottom of the flask, precipitate of black colour, right after the addition of leaf extract to metal salt precursor solution of ferric chloride and was named as NA-Fe-NP.

Fig 3. Nanoparticle solution in different volume ratio.

3.5 NA-Fe-NP ISOLATION

To isolate newly synthesized, NA-Fe-NP mixture is rotated in centrifuge at 8000 rpm for 20 mins at 25° Celsius for separation of Fe-NP from the solution. Black palette at the bottom was collected and remaining supernatant was decanted. Separated nanoparticles were washed with ethanol and water in micro centrifuge at 10,000 rpm for 10 mins. Drying of synthesized and washed Fe-NP is 80°Celsius for 3 hrs. [40]. Dried NA-Fe-NP is carefully scrapped on petri dish and and grinded into fine particles. NA-Fe-NP are later stored in a glass bottle.

3.6 PHOTOCATALYTIC DEGRATION OF METHYLENE BLUE

For Photocatalytic degradation of Methylene Blue with Fe-NP, Stock solution of methylene blue dye is prepared by weighing and adding 20mg methylene blue dye (20ppm) in 1000ml distilled water. In a beaker, 30mg of NA-Fe-NP is added to 50ml of MB Dye solution [41]. The solution is mixed thoroughly with magnetic stirring for 30 mins. After proper mixing of the mixture, solution is kept out undisturbed under direct sunlight for 4 hours. The progression of degradation is observed closely at pre decided time intervals and absorbance spectrum of the supernatant is taken initially at 0 min after an interval of half an hour for four times using UV-Vis spectrophotometer.

3.7 CHARACTERIZATION OF NA-Fe-NP

3.7.1 UV-VIS SPECTROPHOTOMETER

UV-Vis spectrophotometer usually employed for quantifying absorbed and scattered light while passing through the sample in absorbing species in the sample. UV-Vis spectrophotometer is used for optical analysis of nanoparticles and confirms the synthesis of Fe-NP by reading absorption peak of Fe-NP. Absorbance value was taken under 200-800nm range in UV-Vis Spectrophotometer.

CHAPTER 4 RESULTS

4.1 COLOUR CHANGE IN METAL SALT SOLUTION

In synthesis of NA-Fe-NP, on addition of NA leaf extract (30g/l) in 0.1 M ferric chloride solution. An instant colour change is observed in metal salt solution of brown colour to deep black within few seconds suggesting synthesis of iron NPs.

4.2 UV-Vis SPECTROPHOTOMETER

The green synthesized nanoparticles are observed to show absorbance peak in the range of 250nm to 300nm [42].

4.3 METHYLENE BLUE DEGRADATION BY NA-Fe-NP

Initially, Initial absorbance peak of methylene blue before the addition of iron nanoparticles is seen at 663nm. After addition of nanoparticles and upon sunlight exposure, with time the absorbance peak decreases indicating degradation of methylene blue dye [43].

4.4 DISCUSSION

Iron nanoparticle synthesized from plants was the choice of experiment due to their magnetic property along with high energy as well as an ability to easily isolate Fe-NP from MB dye solution under the influence of magnetic field. Green synthesis of NPs from plants reduces the use of harsh chemicals. Phytocompounds like flavonoids, terpenoids and saponin, tannins etc., act as reducing agent with the help –OH and –COOH functional groups reducing metal ions from Fe^{3+} to Fe^{0} existing in the compound. Stable bonding b/w functional group of phytocompounds and nanoparticles enables the control of NP size and provides stability. There is a scope for betterment in phytonanoparticle for environmental remediation by conducting more experiments at larger scale with optimized conditions for an attempt to standardize the process and its incorporation as industrial dye wastewater effluent treatment plant for commercial use while understanding more about nanoparticles related safety in human body

.

REFERNCES

[1] T. Rasheed, M. Bilal, F. Nabeel, M. Adeel, and M. N. Iqbal, “Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection , quanti fi cation , and treatment,” *Environ. Int.*, vol. 122, no. September 2018, pp. 52–66, 2019, doi: 10.1016/j.envint.2018.11.038.

[2] D. Ashwini and G. Mahalingam, “Green synthesized metal nanoparticles, characterization and its antidiabetic activities-a review,” *Res. J. Pharm. Technol.*, vol. 13, no. 1, pp. 468–474, 2020, doi: 10.5958/0974-360X.2020.00091.8.

[3] S. Hasan, “A Review on Nanoparticles : Their Synthesis and Types,” *Res. J. Recent Sci. Res . J . Recent . Sci . Uttar Pradesh (Lucknow Campus)*, vol. 4, no. February, pp. 1–3, 2014.

[4] N. Strambeanu, L. Demetrovici, D. Dragos, and M. Lungu, “Nanoparticles: Definition, classification and general physical properties,” in *Nanoparticles’ Promises and Risks: Characterization, Manipulation, and Potential Hazards to Humanity and the Environment*, Springer International Publishing, 2015, pp. 3–8.

[5] A. M. Ealias and M. P. Saravanakumar, “A review on the classification, characterisation, synthesis of nanoparticles and their application,” in *IOP Conference Series: Materials Science and Engineering*, Dec. 2017, vol. 263, no. 3, p. 032019, doi: 10.1088/1757-899X/263/3/032019.

[6] D. Titus, E. James Jebaseelan Samuel, and S. M. Roopan, *Nanoparticle characterization techniques*. Elsevier Inc., 2019.

[7] L. Kumar, V. Ragunathan, M. Chugh, and N. Bharadvaja, “Nanomaterials for remediation of contaminants: a review,” *Environmental Chemistry Letters*, vol. 1. Springer Science and Business Media Deutschland GmbH, p. 3, Mar. 15, 2021, doi: 10.1007/s10311-021-01212-z.

[8] http://dbtindia.gov.in/, “Mapping of Research Outcome on Remediation of Dyes, Dye Intermediates and Textile Industrial Waste | Department of Biotechnology.” http://www.dbtindia.gov.in/latest-announcement/mapping-research-outcome-

remediation-dyes-dye-intermediates-and-textile (accessed May 30, 2021).

[9] J. N. Edokpayi, J. O. Odiyo, and O. S. Durowoju, “Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa,” in *Water Quality*, InTech, 2017.

[10] A. Srivastav *et al.*, “Nano-phytoremediation of pollutants from contaminated soil environment: Current scenario and future prospects,” in *Phytoremediation: Management of Environmental Contaminants*, vol. 6, Springer International Publishing, 2019, pp. 383–401.

[11] K. D. Grieger, R. Hjorth, J. Rice, N. Kumar, and J. Bang, “Nano-remediation : tiny particles cleaning up big environmental problems,” *Iucn*, pp. 1–7, 2015.

[12] A. K. Patel, D. Gupta, A. Singh, V. K. Mishra, and N. K. Sharma, “Green-synthesized nanoparticles for treatment of wastewater: an environmentally sustainable pollution remediation technology,” in *Sustainable Environmental Clean-up*, Elsevier, 2021, pp. 29–70.

[13] S. K. Murthy, “Nanoparticles in modern medicine: state of the art and future challenges.,” *Int. J. Nanomedicine*, vol. 2, no. 2, pp. 129–41, 2007, Accessed: May 31, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/17722542.

[14] O. P. Bolade, A. B. Williams, and N. U. Benson, “Green synthesis of iron-based nanomaterials for environmental remediation: A review,” *Environmental Nanotechnology, Monitoring and Management*, vol. 13. Elsevier B.V., p. 100279, May 01, 2020, doi: 10.1016/j.enmm.2019.100279.

[15] Z. Xiao, M. Yuan, B. Yang, Z. Liu, J. Huang, and D. Sun, “Plant-mediated synthesis of highly active iron nanoparticles for Cr (VI) removal: Investigation of the leading biomolecules,” *Chemosphere*, vol. 150, pp. 357–364, May 2016, doi: 10.1016/j.chemosphere.2016.02.056.

[16] T. Ahmad, M. A. Bustam, M. Irfan, M. Moniruzzaman, H. M. A. Asghar, and S. Bhattacharjee, “Mechanistic investigation of phytochemicals involved in green synthesis of gold nanoparticles using aqueous Elaeis guineensis leaves extract: Role of phenolic compounds and flavonoids,” *Biotechnol. Appl. Biochem.*, vol. 66, no. 4, pp. 698–708, 2019, doi: 10.1002/bab.1787.

[17] J. Ebrahimian, M. Mohsennia, and M. Khayatkashani, “Photocatalytic-degradation of organic dye and removal of heavy metal ions using synthesized SnO2 nanoparticles by Vitex agnus-castus fruit via a green route,” *Mater. Lett.*, vol. 263, p. 127255, 2020, doi: 10.1016/j.matlet.2019.127255.

[18] K. M. Al-Qahtani, “Cadmium removal from aqueous solution by green synthesis zero valent silver nanoparticles with Benjamina leaves extract,” *Egypt. J. Aquat. Res.*, vol. 43, no. 4, pp. 269–274, 2017, doi: 10.1016/j.ejar.2017.10.003.

[19] A. M. Shaik, M. David Raju, and D. Rama Sekhara Reddy, “Green synthesis of zinc oxide nanoparticles using aqueous root extract of Sphagneticola trilobata Lin and investigate its role in toxic metal removal, sowing germination and fostering of plant growth,” *Inorg. Nano-Metal Chem.*, vol. 50, no. 7, pp. 569–579, 2020, doi: 10.1080/24701556.2020.1722694.

[20] V. Madhavi, T. N. V. K. V. Prasad, A. V. B. Reddy, B. Ravindra Reddy, and G. Madhavi, “Application of phytogenic zerovalent iron nanoparticles in the adsorption of hexavalent chromium,” *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 116, pp. 17–25, 2013, doi: 10.1016/j.saa.2013.06.045.

[21] M. F. Baran, H. Acay, and C. Keskin, “ Determination of Antimicrobial and Toxic Metal Removal Activities of Plant-Based Synthesized (Capsicum annuum L. Leaves), Ecofriendly, Gold Nanomaterials ,” *Glob. Challenges*, vol. 4, no. 5, p. 1900104, 2020, doi: 10.1002/gch2.201900104.

[22] M. Fazlzadeh, K. Rahmani, A. Zarei, H. Abdoallahzadeh, F. Nasiri, and R. Khosravi, “A novel green synthesis of zero valent iron nanoparticles (NZVI) using three plant extracts and their efficient application for removal of Cr(VI) from aqueous solutions,” *Adv. Powder Technol.*, vol. 28, no. 1, pp. 122–130, 2017, doi: 10.1016/j.apt.2016.09.003.

[23] A. V. Samrot *et al.*, “Bioactivity and Heavy Metal Removal Using Plant Gum Mediated Green Synthesized Silver Nanoparticles,” *J. Clust. Sci.*, vol. 30, no. 6, pp. 1599–1610, 2019, doi: 10.1007/s10876-019-01602-y.

[24] X. Weng, X. Jin, J. Lin, R. Naidu, and Z. Chen, “Removal of mixed contaminants Cr(VI) and Cu(II) by green synthesized iron based nanoparticles,” *Ecol. Eng.*, vol. 97, pp. 32–39, 2016, doi: 10.1016/j.ecoleng.2016.08.003.

[25] Y. Yi, G. Tu, P. E. Tsang, S. Xiao, and Z. Fang, “Green synthesis of iron-based nanoparticles from extracts of Nephrolepis auriculata and applications for Cr(VI) removal,” *Mater. Lett.*, vol. 234, no. Vi, pp. 388–391, 2019, doi: 10.1016/j.matlet.2018.09.137.

[26] L. P. Lingamdinne, J. R. Koduru, and R. R. Karri, “Green synthesis of iron oxide nanoparticles for lead removal from aqueous solutions,” *Key Eng. Mater.*, vol. 805 KEM, no. Iii, pp. 122–127, 2019, doi: 10.4028/www.scientific.net/KEM.805.122.

[27] Y. Wei, Z. Fang, L. Zheng, and E. P. Tsang, “Biosynthesized iron nanoparticles in aqueous extracts of Eichhornia crassipes and its mechanism in the hexavalent chromium removal,” *Appl. Surf. Sci.*, vol. 399, pp. 322–329, 2017, doi: 10.1016/j.apsusc.2016.12.090.

[28] K. S. Prasad, P. Gandhi, and K. Selvaraj, *Synthesis of green nano iron particles (GnIP) and their application in adsorptive removal of As(III) and As(V) from aqueous solution*, vol. 317, no. Iii. Elsevier B.V., 2014.

[29] V. K. Yadav and M. H. Fulekar, “Biogenic synthesis of maghemite nanoparticles (γ-Fe2O3) using Tridax leaf extract and its application for removal of fly ash heavy metals (Pb, Cd),” *Mater. Today Proc.*, vol. 5, no. 9, pp. 20704–20710, 2018, doi: 10.1016/j.matpr.2018.06.454.

[30] A. Bouafia and S. E. Laouini, “Green synthesis of iron oxide nanoparticles by aqueous leaves extract of Mentha Pulegium L.: Effect of ferric chloride concentration on the type of product,” *Mater. Lett.*, vol. 265, p. 127364, Apr. 2020, doi: 10.1016/j.matlet.2020.127364.

[31] S. Hossen *et al.*, “Heliyon Green synthesis of iron oxide nanoparticle using Carica papaya leaf extract : application for photocatalytic degradation of remazol yellow RR dye and antibacterial activity,” *Heliyon*, vol. 6, no. May, p. e04603, 2020, doi: 10.1016/j.heliyon.2020.e04603.

[32] A. Carolina *et al.*, “Green synthesis of iron oxide nanoparticles for tartrazine and bordeaux red dye removal,” *J. Environ. Chem. Eng.*, vol. 8, no. 1, p. 103618, 2020, doi: 10.1016/j.jece.2019.103618.

[33] M. Jamzad and M. Kamari, “Green synthesis of iron oxide nanoparticles by the

aqueous extract of Laurus nobilis L . leaves and evaluation of the antimicrobial activity," *J. Nanostructure Chem.*, no. 0123456789, 2020, doi: 10.1007/s40097-020-00341-1.

[34] J. Sandhya and S. Kalaiselvam, "Biogenic synthesis of magnetic iron oxide nanoparticles using inedible borassus flabellifer seed coat : characterization , antimicrobial , antioxidant activity and in vitro cytotoxicity analysis Biogenic synthesis of magnetic iron oxide nanoparticles usin," *J. Photochem. Photobiol. B Biol.*, vol. 185, no. August 2018, pp. 262–274, 2020.

[35] S. A. Razack, A. Suresh, S. Sriram, and G. Ramakrishnan, "Green synthesis of iron oxide nanoparticles using Hibiscus rosa - sinensis for fortifying wheat biscuits," *SN Appl. Sci.*, vol. 2, no. 5, pp. 1–9, 2020, doi: 10.1007/s42452-020-2477-x.

[36] J. A. A. Abdullah, L. Salah Eddine, B. Abderrhmane, M. Alonso-González, A. Guerrero, and A. Romero, "Green synthesis and characterization of iron oxide nanoparticles by pheonix dactylifera leaf extract and evaluation of their antioxidant activity," *Sustain. Chem. Pharm.*, vol. 17, no. May, 2020, doi: 10.1016/j.scp.2020.100280.

[37] S. Qasim *et al.*, "Journal of Photochemistry & Photobiology , B : Biology Green synthesis of iron oxide nanorods using Withania coagulans extract improved photocatalytic degradation and antimicrobial activity," *J. Photochem. Photobiol. B Biol.*, vol. 204, no. January, p. 111784, 2020, doi: 10.1016/j.jphotobiol.2020.111784.

[38] Y. Yi, G. Tu, P. E. Tsang, S. Xiao, and Z. Fang, "Green synthesis of iron-based nanoparticles from extracts of Nephrolepis auriculata and applications for Cr(VI) removal," *Mater. Lett.*, vol. 234, pp. 388–391, 2019, doi: 10.1016/j.matlet.2018.09.137.

[39] V. B. Ramadhan, Y. L. Ni, E. Yanuar, and S. Suprapto, "Synthesis of copper nanoparticles using Ocimum tenuiflorum leaf extract as capping agent Synthesis Of Copper Nanoparticles Using Ocimum tenuiflorum Leaf Extract as Capping Agent," vol. 020067, no. December, 2019.

[40] M. S. H. Bhuiyan *et al.*, "Green synthesis of iron oxide nanoparticle using Carica papaya leaf extract: application for photocatalytic degradation of remazol yellow RR dye and antibacterial activity," *Heliyon*, vol. 6, no. 8, p. e04603, Aug. 2020, doi:

10.1016/j.heliyon.2020.e04603.

[41] S. Bishnoi, A. Kumar, and R. Selvaraj, “Facile synthesis of magnetic iron oxide nanoparticles using inedible Cynometra ramiflora fruit extract waste and their photocatalytic degradation of methylene blue dye,” *Mater. Res. Bull.*, vol. 97, pp. 121–127, Jan. 2018, doi: 10.1016/j.materresbull.2017.08.040.

[42] C. Justin, S. A. Philip, and A. V. Samrot, “Synthesis and characterization of superparamagnetic iron-oxide nanoparticles (SPIONs) and utilization of SPIONs in X-ray imaging,” *Appl. Nanosci.*, vol. 7, no. 7, pp. 463–475, Oct. 2017, doi: 10.1007/s13204-017-0583-x.

[43] M. Vanaja *et al.*, “Degradation of methylene blue using biologically synthesized silver nanoparticles,” *Bioinorg. Chem. Appl.*, vol. 2014, 2014, doi: 10.1155/2014/742346.