"Copper Oxide Nanoparticle Synthesis by Green Synthesis Approach using *Neolamarckia cadamba* And Its Characterization"

A DISSERTATION

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

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

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CANDIDATE'S DECLARATION

I, Muskan Garg, 2K20/MSCBIO/18 hereby certify that the work which I presented in the Major Project entitled 'Copper oxide nanoparticle synthesis by green synthesis approach using *Neolamarckia cadamba* and its characterization' in fulfillment of the requirement for the award of the Degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own, carried out during a period from **feb-2022 to -2 May-2022**, under the supervision of Dr. Jai Gopal Sharma.

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.

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CERTIFICATE

I hereby certify that the Project dissertation titled "**Copper oxide nanoparticle synthesis by green synthesis approach using *Neolamarckia cadamba* and its characterization**" which is submitted by **Muskan Garg**, **2K20/MSCBIO/18**, 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 recordfor the project work carried out by the student under my supervision. To the best of myknowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 6 May, 2022

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CONTENTS

List of Figures

ABBREVIATIONS

UV-	**Ultra violet radiations**
CuNPs/ CuONPs-	**Copper Oxide Nanoparticles**
N. cadamba-	***Neolamarckia cadamba***
UV-Vis spectroscopy-	**Ultraviolet Visible spectroscopy**
NPs-	**Nanoparticles**
MeO-	**Metal oxides**
Ag-	**Silver**
Au-	**Gold**
FTIR-	**Fourier transform infrared spectroscopy**
SEM-	**Scanning electron microscopy**
XRD-	**X ray diffraction**
EDX-	**Energy dispersive X-Ray Spectroscopy**
DLS-	**Dynamic light scattering**

ABSTRACT

Green synthesis of nanoparticles using plants is aa very active and advancing field in technical, economic and research sector. Nanoparticles are one of the mostprominent bioactive components that have different applications. They possess enhanced physical and chemical properties that includes melting point, thermal conductivity, electrical conductivity, catalysis, light absorptivity and wettability than large particles due to their highly small size in between 1 nm to 100 nm. These particles have length of 1 nm to 1000 nm in any one direction and 1 nm to 100 nm diameter. This size results in changing their physical and chemical properties as well as provide several unique properties. These are available in different forms such as nanosized wires, nanosized plates, nanofibers, and quantum dots. On the basis of composition, they are classified into different forms such as carbon-based nanomaterials, iron-based nanoparticles, polymer-based nanoparticles and nanocomposites. Nanoparticles can be synthesized using different approaches such as physical, chemical and biological. Physical and chemical approach are not suitable for production of nanoparticles because of the high cost and the toxicity resulted due to these approaches. Biological or green synthesis of nanoparticles has been proven to be cheap, effective, easy and non-toxic approach. In this project green synthesis approach was used for the preparation of copper oxide nanoparticles using *Neolamarckia cadamba.*

CHAPTER 1. INTRODUCTION

1.1General Introduction

The present project focuses on the CuO NPs synthesis by green synthesis approach. The role of nanomaterials in medicinal field as well as in other sectors has also been reviewed. There are two processes that are used in synthesis of nanoparticles that are top to down approach and bottom to up approach. Green synthesis approach is one of the following approaches included in bottom-up approach. Nanotechnology is one of the most recent emerging technologies. The word "nano" comes from the Greek word "nannos." The word nannos refers to extremely small.

Nanoparticles (NPs) are extremely small particles that range in size from 1 to 100 nanometers. They are extremely mobile and chemically active due to their small size, which increases surface area. The nanosize atoms present in metals and nonmetals as well as molecules both differ dramatically from the parent bulk material. They have a wide range of practical uses due to their characteristics, including medicine, industry, environmental cleanup, and so on (Jayadev, A. et al., 2021).

MeO nanoparticles are synthesized by involving various physical and chemical processes; however, traditional methods such as sol-gel, reduction of large particles into smaller by chemical and by using hot water, requires use of large amount of money and environmentally unfriendly because they produce harmful substances as end products. Thus, eco-friendly technologies for the manufacture of metal oxide nanoparticles, such as the use of biological extracts such as from plants, microbes, and algae, have piqued interest. Plant extract was preferred over other biological approaches because of its ease of handling, accessibility, economical, and suitability with medicinal applications such as medicine administration, treatment of various types of cancer, resistance to bacterial and fungal infections, and insecticide toxicity (Amin, F. et al., 2021).

Because metal nanoparticles are commonly employed in locations where people come into contact with them, the need for environmentally acceptable nanoparticle production processes that do not rely on harmful chemicals is developing all the time. Synthesis of copper nanoparticles by using plant extracts or microbes is appealing for a variety of reasons: copper is extremely conductive and less expensive than Ag and Au metals. Copper oxide nanoparticles are useful in various applications such as they are used in destroying harmful pathogens and in toxic gas detectors, in electrochemical cells, materials that shows superconductivity at high temperature, in photovoltaic cells and other uses (Altikatoglu, M. et al., 2017).

Because of their unique crystal shapes and large surface areas, copper oxide NPs are particularly important antibacterial particles. Copper oxide NPs have a lengthy shelf life when compared to other organic antibacterial agents since they are robust and stable (Amin, F et al., 2021).

It is critical to design a green nanoparticle synthesis technology that is clean, dependable, biocompatible, inexpensive, and nontoxic. Because plants contain a vast number of bioactive chemicals, many plant parts or complete plants have been used for production of Copper nanoparticles (Murthy, A C. H et al., 2018).

Alkaloids, terpenoids, flavonoids, polyphenols, sugars, proteins, and other phytoconstituents in plant extracts have a wide spectrum of reductive capabilities and operate as reducing and stabilizing agents during green synthesis (Phang, Y. K. et al., 2021).

Neolamarckia cadamba, often known as burlflower tree, kadamba tree or kadamb, is a member of the rubiaceae family. This tree has been known as miraculous tree because of its fast growth and huge medicinal and industrial uses. Every part of this has its own importance and uses such as its leaves are used in treatment of diabetes, injuries, pain and swelling. Its bark is used in treatment of ulcers, its flowers are used in treatment of skin diseases, its roots are used in treatment of lower abdomen related disorders and its aerial parts are used in treatment of stomach related issues. Its bark is used in manufacturing of furniture, paper and pulp.

The goal of this study was to biosynthesize Copper nanoparticles from *Neolamarckia cadamba* extract and characterize the particles using UV-VIS spectrophotometer, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX), Dynamic light scattering (DLS), and X-ray diffraction (XRD) techniques (Jayadev, A. et al., 2021)

1.2 ORGANIZATION OF THESIS

The following thesis title as ‘Copper oxide nanoparticles synthesis by green synthesis approach using *Neolamarckia cadamba* and its characterization” is a reviewed information gathered from various research and review article. The thesis focuses on the production of nanoparticles through green approach. The thesis alsocontains information on the uses of nanoparticles in different industries.

The chapter1 ie. Introduction of thesis briefs about the nanoparticles, their structural features, their physical, chemical and biosynthesis, their role in therapeutics, role of nanoparticles in other industries and the brief about green synthesis,

The chapter 2 is a review of literature which contains a broad knowledge about the nanoparticles and its types. It summarizes various plants and their polyphenols being useful for treating skin diseases. It also contains the invitro production of polyphenols through plant tissue culture techniques and the various types of abiotic elicitors that can be used in plant tissueculture for enhancement of polyphenols and future prospects.

The chapter 3 is a proposed methodology for the project and briefs about the extraction process, estimation techniques that can be used for detection of nanoparticles.

The chapter 4 contains result, discussion and conclusion part.

CHAPTER 2. REVIEW OF LITERATURE

2.1 NANOPARTICLES

Nanoparticles are defined as the materials that has changes in various parameters from its bulk or molecular counterparts. They are considered as the building blocks for nanotechnology. These nanosized particles have unique and desirable physical and chemical features in terms of different applications when compared to their bulk counterparts (Kumar, R. R. et al., 2012 and Rajiv, P. et ., 2013). Nanoparticles if divided at a size of atoms then it possess atom like properties because it possess high surface energy per it's weight than particles bigger in size. divided to near atomic size, which is due to their greater surface area, more fraction of surface atoms, and because of valence and conduction bands which have very large band gaps. This causes them to be more reactive to other molecules. Nanoparticles are fascinating to scientists because they have unique properties like size, dispersion, and shape that aren't found in larger particles (Nagajyothi, C. P. et al., 2014; Salam, H. A. et al., 2014; Khodashenas, B and Ghorbani, H. R. 2015; Gharagozlou, M. et al., 2015).

Classification of NPs

The number of dimensions are used as the classification criteria of nanoparticles. These are zero-dimensional (0-D), One-dimensional (1-D), Two-dimensional (2-D) and Three-dimensional (3-D)

Structural features and properties of nanoparticles

These are small molecules having a length of approximately 1 nm to 1000 nm. On reduction in size of nanoparticles their properties are modified. Their colour, electrical conductivity, mechanical properties such as crystal structure, optical properties, such as absorption and emission get changed. Nanoparticles possess greater surface area than their respective bigger material. Some material also faces changes in magnetism on their conversion into nanoparticles. Nanomaterials possess more enhanced quantum effects than their bulkier substitute and it also depends on the nature of semiconductor used. Nanoparticles shows enhanced thermal and electrical conductivities and enhanced mechanical properties. Nanoparticles have been proven to be more efficient catalyst than their bulkier parts. There are different nanoparticles that interferes with body of small microorganisms and acts as antiviral, antibacterial and antifungal. The nanomaterials have also made their place in various applications such as they are used for enhancing activities of various equipment (Baig et al., 2021).

Synthesis of Nanoparticles

Nanoparticles such as carbon-based nanoparticles and metal-based nanoparticles are synthesized by two different approach that is top to down approach and bottom to up approach. Top to down approach involves breakdown of bulk particle into nano sized particles (Baig et al., 2021). In top to down approach there are many methods included such as mechanical breakage of bulk materials, acid mediated conversion, laser beams, energetic plasma and gas bombarding and electro expulsion.

Use of physical or chemical methods for synthesis of nanoparticles results in various issues such as utilisation of harmful materials for synthesis of nanoparticles, difficulty in experimental procedures and synthesis of harmful by products. While green nanoparticle synthesis approach includes simple procedures, with no harmful byproducts formation, easily scalable approach and less costly. It thus helps in minimizing the use of hazardous and redundant compounds in the solid, liquid, and gaseous states (Sankar, R. et al., 2014 and Annavaram, V. et al., 2015).

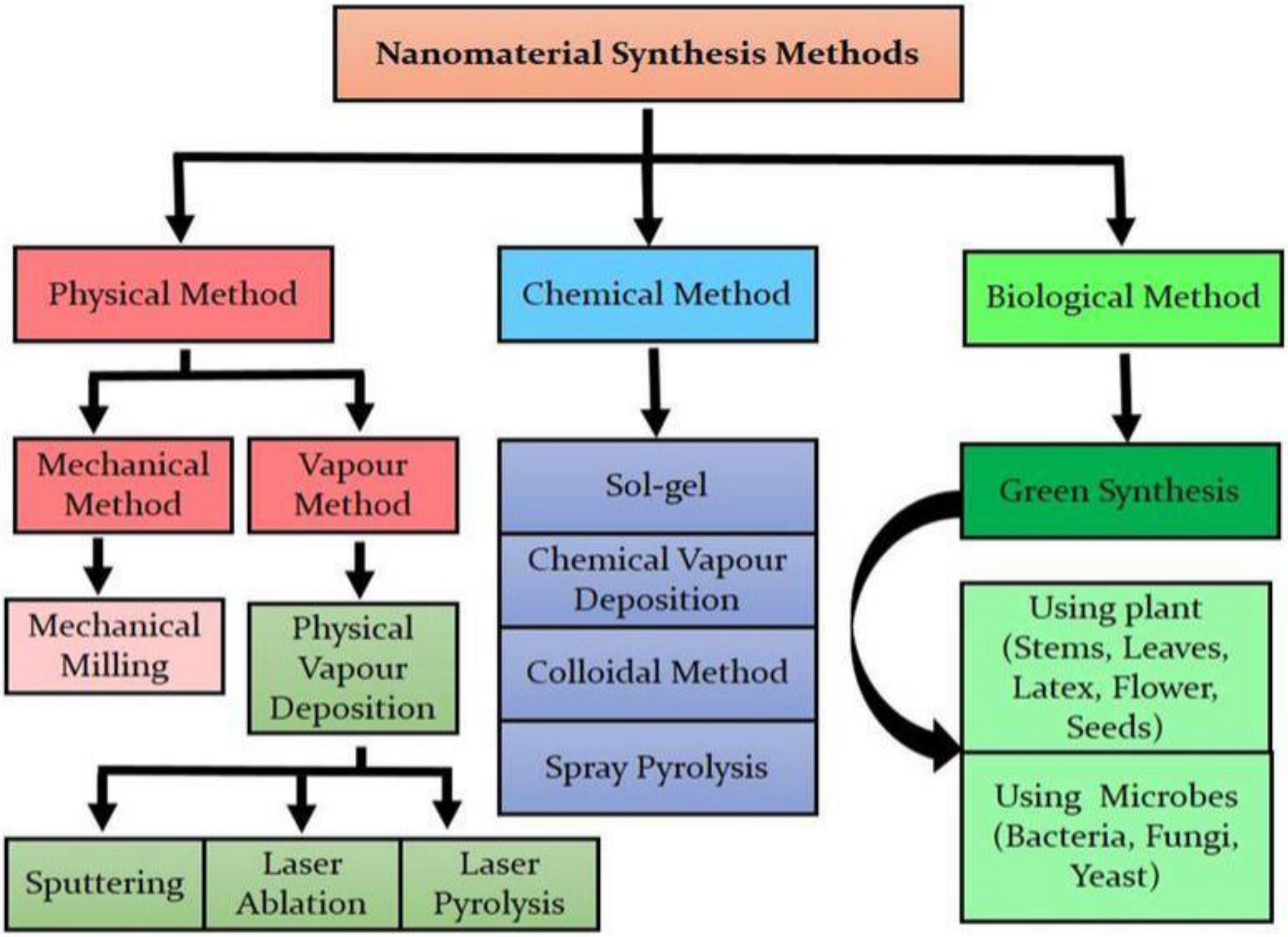

Fig 1. Methods of nanoparticles synthesis

Physical synthesis

For nanomaterials synthesis using physical processes different methods are used such as sputtering, evaporation, pulsed laser deposition, ultrasonication, microwave assisted synthesis, ball milling, spray pyrolysis processes, etc However this process involves major drawbacks such as including difficulty in collection of nanoparticles after synthesis, need of high energy resulting in wastage of energy, difficult procedures, requirement of high temperature, need of high vacuum, and requirements of expensive equipment, pollution caused during synthesis (Wang, T. et al., 2014).

Chemical synthesis

Chemical methods for synthesis of nanoparticles includes various ways such as sol-gel, co-precipitation, chemical reduction, hydrothermal, and polyol processes, among others. Chemical methods requires use of different surfactants and stabilizers to control size, shape and morphology of different kinds of nanoparticles which are often toxic and hazardous to environments. It also includes formation of toxic by products alongwith synthesis of nanoparticles (Ivanova, T. 2013; Hu, J., 2014). Because of this people are relying on biological synthesis of nanoparticles which is also called green synthesis method.

Biological synthesis of nanoparticles

Green chemistry also known as sustainable chemistry, entails the creation and implementation of compounds and processes that limit or eradicate the utilization and formation of toxic compounds to human health and the environment. This includes raw materials, reagents, solvents, finished goods, and by-products. For this manufacturing process, it also covers the utilization of sustainable raw materials and energy sources (Doble, M. and Kruthiventi, A. K. 2007]. There are twelve principles in this field. The use of the twelve principles of green chemistry in the production of nanoparticles is a relatively new problem in terms of sustainability (Duan, H., 2015). As a result, when the development of nanotechnology could benefit from a greener strategy that promotes the clean, dependable, biocompatible, benign, and eco-friendly procedure to synthesize nanoparticles, the integration of green chemistry principles into nanotechnology is vital (Jagtap, U. B. and Bapat, V. A. 2012].

In nanotechnology, which is the combination of nanotechnology and green chemistry (Anastas, P. T. and Horvath, I. T. 2012). Green production of nanoparticles has gotten a lot of attention. Green synthesis of nanoparticles has piqued interest over physical and chemical synthesis because it is more environmentally friendly and has fewer side effects. Plant extracts are the most widely used methods of green, non-toxic, eco-friendly nanoparticle production and also have the added benefit of being widely distributed, easily accessible, safe handling, and also contains different metabolites. This approach does not require pressure at a high range, high energy, high amount of heat, or harmful compounds in green synthesis (Annavaram, V. et al., 2015).

2.2 Copper oxide nanoparticles

Copper oxide nanoparticles have powdered texture and blackish to brownish color.

Copper oxide nanomaterials have gotten more attention than the other transition metal oxides because of their unique characteristics. CuO (Cupric Oxide) and Cu2O (Cuprous Oxide) are two major copper oxide compounds. Cuprous oxide has a monoclinic crystal structure with all three unequal axes and behaves like a semiconductor because of an indirect band gap of 1.21 – 1.51eV and is a p-type direct band gap II – VI semiconductor (Ogwu et al. 2007). Cupric oxide has a monoclinic structure and semiconductor behavior with an indirect band gap of 1.21 – 1.51eV. (Rehman et al. 2011).

As its starting material is found in plentiful amount in nature, it's low cost manufacturing procedures, nontoxic nature to environment with generally acceptable electrical and optical properties, cupric oxide (CuO) is being studied on a large scale as transition metal oxides as a p-type semiconductor with a narrow band gap (1.2 eV in bulk). Because Cu(II) ions are substantially more stable in ambient conditions, CuO is found to have high stability than Cu2O, making it more essential in practical applications. (Zhang et al. 2010). Antimicrobial drugs are the most common application for CuO nanoparticles. They are employed in hospitals because of their antibacterial potential to kill 99.9% of Gram-positive and Gram-negative bacteria within 60 minutes of application only if doses are given correctly [40]. Solar cells, catalysis, sensors, electrode materials, magnetic storage media, lithium ion batteries, and high Tc superconductors are all possible applications.

2.2 *Neolamarckia cadamba*

2.2.1 Introduction

Neolamarckia cadamba is also known by the name burlflower tree, kadamba tree or kadamb. It is a very important traditional medicinal plant as it can be used in treatment of various diseases such as dysentery fever and snake bites. Its leaves, barks and flowers are good sources of alkaloidal compounds, phytochemicals, cadambines which are used in treatment of patients with high blood sugar levels, high lipid levels, anxiety, injuries and various skin diseases. It is also known as gems tree and it is used widely in land restoration purposes (Huang et al., 2020).

It is a tall, multipurpose, evergreen that grows widely in tropical and subtropical regions including India, Indonesia, China etc. it can not tolerate low temperature and is attacked easily by insects. It is also known by the name miraculous tree declared in World Forestry Congress organized in 1972. It is called miraculous tree because it has fastest growth than other trees. Its wood is also used in various applications such as in paper and pulp manufacturing industries, in furniture manufacturing as plywood and fiberboard. Other than this it is also a very good renewable resource of biofuel production to minimize the use of fossil fuels and the pollution caused during burning of fossil fuels (Lu et al., 2021). *Neolamarckia cadamba* shows breeding through cross pollination and need more than five years for seeds to grow into tree that give flowers (Huang et al., 2020). For the growth of kadamb tree there must be presence of highly acidity (low pH) in soil (Dai et al., 2020). This plant belongs to Rubiaceae family and contains twenty-two chromosome pairs. This tree contains woods having light weight and moderately fine to medium texture. Thus these properties makes this plant highly useful in manufacturing of different furniture (Eng W H et al., 2021).

Figure 2: *Neolamarckia cadamba*

BOTANICAL DETAILS OF *Neolamarckia cadamba*

Botanical details

Kingdom: Plantae

Phylum: Angiosperms

Order: Gentianales

Family: Rubiaceae

Genus: *Neolamarckia*

Species: *N. cadamba*

Bionomial name- *Neolamarckia cadamba (Roxb.) Bosser*

Common name: Burlflower tree, kadamba tree, kadamb

2.2.2 APPLICAIONS OF *Neolamarckia cadamba*

2.2.2.1 MEDICINAL USES OF *Neolamarckia cadamba*

Leaves - Its leaves are used in treatment of wounds and areas affected with severe pain and swelling. Diabetes can also be cured by consuming cadamba leaves.

Bark - Its bark is used in treatment of fever, blood diseases such as ulcers in mouth and in gums related diseases.

Roots - Its roots are used in treatment of urinary tract infections, renal infections and glycosuria. Its roots are also used in treatment of fever, colic and muscle pain relief.

Flowers – Its flowers are used in treatment of skin diseases such as dark spots and pimples. Its flowers are also used in perfumes.

Aerial parts – its aerial parts are used in treatment of diarrhea and irritable bowel syndrome.

2.2.2.2 PHARMACOLOGICAL ACTION OF Neolamarckia cadamba

Antidiabetic activity- Neolamarckia cadamba leaves contains flavonoids that has insulin like activity and also promotes secretion of insulin in body thereby reduce high blood sugar levels. Cadamba bark also contains a god quantity of flavonoids, quinines, triterpenoids, saponins and tannins which are affective against high blood sugar levels. Its barks also contain different phenolic compounds such as pyrocatechol, isopropyl myristate etc, that have anti diabetic effects. The roots of *N. cadamba* also used in treatment of high blood sugar.

Antipyretic activity- Neolamarckia cadamba bark can be used in treatment high fever thus exhibiting antipyretic activity. Its leaves and barks also have anti-inflammatory and analgesic effects.

Antidiarrheal activity- *N. cadamba* flowers have shown to have antidiarrheal activity by reduction in interstitial fluid accumulation.

Diuretic and laxative activity- *N. cadamba* bark has been shown to have diuretic activity by increasing urine secretion and laxative activity.

Antihepatoxic activity- N. cadamba contains chlorogenic acid (CGA) responsible for reducing hepatic toxicity or liver toxicity and protection of liver.

Hypolipidemic activity – Consumption of root extract of N. cadamba have been shown to decrease bad cholesterol, lipidperoxides etc.

Antiproliferative and antioxidant activity- it has been found that Neolamarckia cadamba inhibit lipid peroxidation and increase superoxide dismutase and catalase enzyme action. The bark of N. cadamba possess phenolic compounds that have antiproliferative and antioxidant activities.

Antimicrobial, **antiparasitic**, **antifilarial**, **antimalarial and anthelminthic activity**- This tree contains antibacterial and antifungal activities against different forms of bacteria and fungi. It also has anthelmintic activity against different worms such as earth and tapeworm.

Antivenom and anticancer activity- this plant also has been shown to be used in treatment of snake bites and different forms of cancer such as breast, colon and esophageal cancers (Verma et al., 2018).

2.2.3 Application of *Neolamarckia cadamba* in Nanoparticle synthesis

Neolamarckia cadamba has been used in synthesis of different kinds of nanoparticles as a green synthesis approach because of presence of different secondary metabolites that act as reducing and stabilizing agents. For example, in one experiment iron oxide nanoparticles were synthesized using leaf extract of cadamba tree. Leaves extract of cadamba tree reduced ferric ions at first and then precipitate $Fe2O3$. These nanoparticles were subjected to FTIR analysis and UV-visible spectroscopy to find their existence (Mandloi, 2021). These nanoparticles have immense importance in targeted drug delivery, as photocatalyst and water purifier. In another study flower extract of Neolamarckia cadamba was used to synthesize silver nanoparticles. These synthesized nanoparticles were characterized using UV-Vis spectrophotometric analysis, FTIR, TEM and XRD analysis. These particles had good photocatalytic activities (Balaprasad, A et al., 2015).

CHAPTER3. METHODOLOGY

Aim: The main purpose behind this project was to synthesize copper oxide nanoparticles by green synthesis approach using Neolamarckia cadamba and its characterization.

Process of synthesis

PROCESS OF SMALL SCALE SYNTHESIS OF NANOPARTICLES

Plant material collection and processing

Neolamarckia cadamba leaves were collected from campus of Delhi Technological University (DTU), Delhi. Leaves were washed with tap water thoroughly and dried on blotting paper. Then, these leaves were sundried for 5 days to minimize moisture content. Dried leaves were crushed into fine powder using mortar and pestle.

Figure 3: *Neolamarckia cadamba* leaves

3.1.2 Preparation of *Neolamarckia cadamba* leaf extract

Powdered leaves were weighed using weighing meter 4 gm of leaf powder was added 100 ml Millipore water. This resultant mixture was boiled for 5-10 minutes on heating mantle at 80-degree Celsius temperature. The obtained leaf broth was cooled to room temperature. This mixture was filtered using muslin cloth first and then by Whatman filter paper. Residues were discarded and filtrate was stored at 4 degrees Celsius in refrigerator covered with aluminum foil.

3.1.3 Stock solution preparation of copper sulphate

To avoid the tedious process of weighing individual salts each time, it is best to prepare a concentrated solution of desired chemicals. These are called as stock solutions. They are made in distilled water and after use stored at room temperature.

To prepare stock solution 24.968 gm cupric sulphate was weighed using weighing balance. This was dissolved properly in 100 ml milliQ using magnetic stirrer.

Figure 4: Copper sulphate stock solution

3.1.3 SYNTHESIS OF WORKING SOLUTION

All the chemicals used in synthesis of nanoparticles were of AR Grade and were purchased from renowned companies. Green synthesis approach was used to obtain copper oxide nanoparticles. From the stock solution one batch was prepared having concentration of 10 mM. For 10mM concentration 0.9 ml of stock solution was added in 90 ml miliq (10mM) and stirred on magnetic stirrer. This solution had aqua blue colour. To this aqua blue solution cadamba leaf extract was added dropwise until the colour of solution changed from aqua blue to green. This green colour indicated formation of nanoparticles. This solution was stirred for 1 hours. This solution was incubated overnight at room temperature.

Figure 5 Synthesis of working solution

3.1.4 SYNTHESIS OF NANOPARTICLES

After incubation the solution was subjected to spectrophotometric analysis.

This solution was centrifuged at 4 degrees Celsius for 10 minutes at 10,000 rpm in 50 ml falcons. Pellet was collected and supernatant was discarded. Pellet was first washed with miliq for 2 times and then with 10 ml acetone for 2 times. Pellet was dried in hot air oven for 20 minutes (overnight preferable). This dried pellet was dissolved in acetone by vertexing and collected in Petri plate. From the Petri plate dried pellet was scratched off and collected very carefully in a plastic vial.

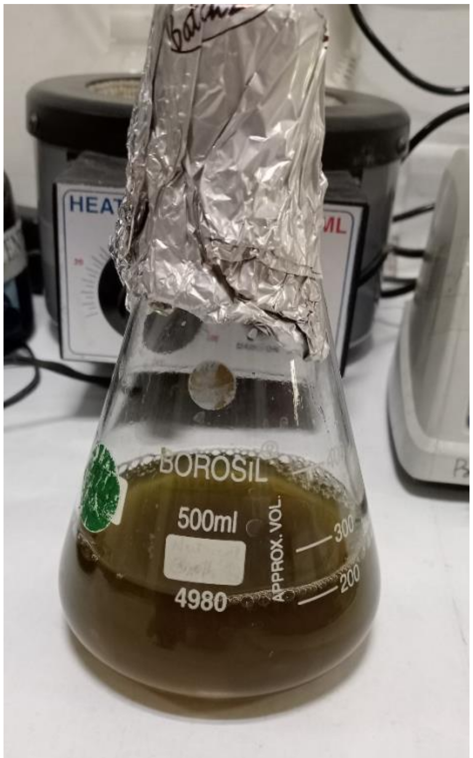

Figure 6: Synthesis of Nanoparticles solution

3.2. Characterization

For characterization all the reagents used were from Fischer Scientific Chemicals. Absorption of nanoparticles were measured using Eppendorf spectrophotometer. Nanoparticles were characterized by measuring FTIR spectrum using Perkin Elmer Instrument and FTIR accessories were taken from PCI analytics with prepared copper oxide nanoparticles in the form of KBr pellets in the range of 4000-400 cm1 (Vinosha et al., 2017). Morphological characteristics of prepared copper oxide nanoparticles were determined by SEM ZESS Instrument EVO 18 special edition. Prepared copper oxide nanoparticles stability and size was determined by using Zetasizer and their pH was determined by HACH device (R. Khan et al., 2018). XPERT-PRO X-Ray Diffractometer was used for XRD characterization.

LARGE SCALE SYNTHESIS OF NANOPARTICLES

1. PREPARATION OF PLANT EXTRACT

36gm plant sample was weighed using weighing balance. To this 900ml miliq was added. This sample solution was then heated for 20 minutes on heating mantle at 80 degrees Celsius. After heating the sample solution was left undisturbed to cool at room temperature. This solution was then filtered into a beaker by using a funnel and muslin cloth. This solution was again filtered using Whatman No.1 filter paper. This filtered solution was then stored in refrigerator at 4 degrees Celsius.

Figure 7: Neolamarckia leaf extract

2. Preparation of working solution

The same stock of copper sulphate prepared for small scale synthesis was used in large scale synthesis of copper oxide nanoparticles. For these two batches of different concentration was prepared that were 10mM and 50mM. For 10mM 9ml from stock of copper sulphate was taken and dissolved in 900ml miliq in a beaker. Similarly for 50mM 45ml from stock of copper sulphate was taken and added in 900ml miliq. To these solutions plant extract was added while stirring dropwise until the colour changed from aqua blue to green. After formation of green colour these solutions were stirred for 2 hours. After that these solutions were incubated overnight at room temperature having beaker covered with aluminium foil.

3. SYNTHESIS OF NANOPARTICLES

After overnight incubation next day OD was taken using UV-Vis spectrophotometer. These solutions were centrifuged at 10,000 rpm for 10 min at 4 degrees Celsius in 50ml falcons. Pellets were collected and supernatants were discarded. The collected pellets were washed with miliq for 2 times using centrifuge at 10,000 rpm at 4 degrees Celsius for 10 minutes. After this these pellets were washed with 10ml acetone for 2 times using centrifuge at 10,000 rpm for 10 minutes at 4 degrees Celsius. These pellets were dried in hot air oven for 20 minutes at 45 degrees Celsius. These dried pellets were collected in petri plate by addition of acetone to remove pellet from falcons and by vortexing. These pellets were left to evaporate acetone. After evaporation of acetone the pellets were scratched from petri plate and collected in a plastic vial.

Figure 8 and 9: Synthesized and centrifuged nanoparticles

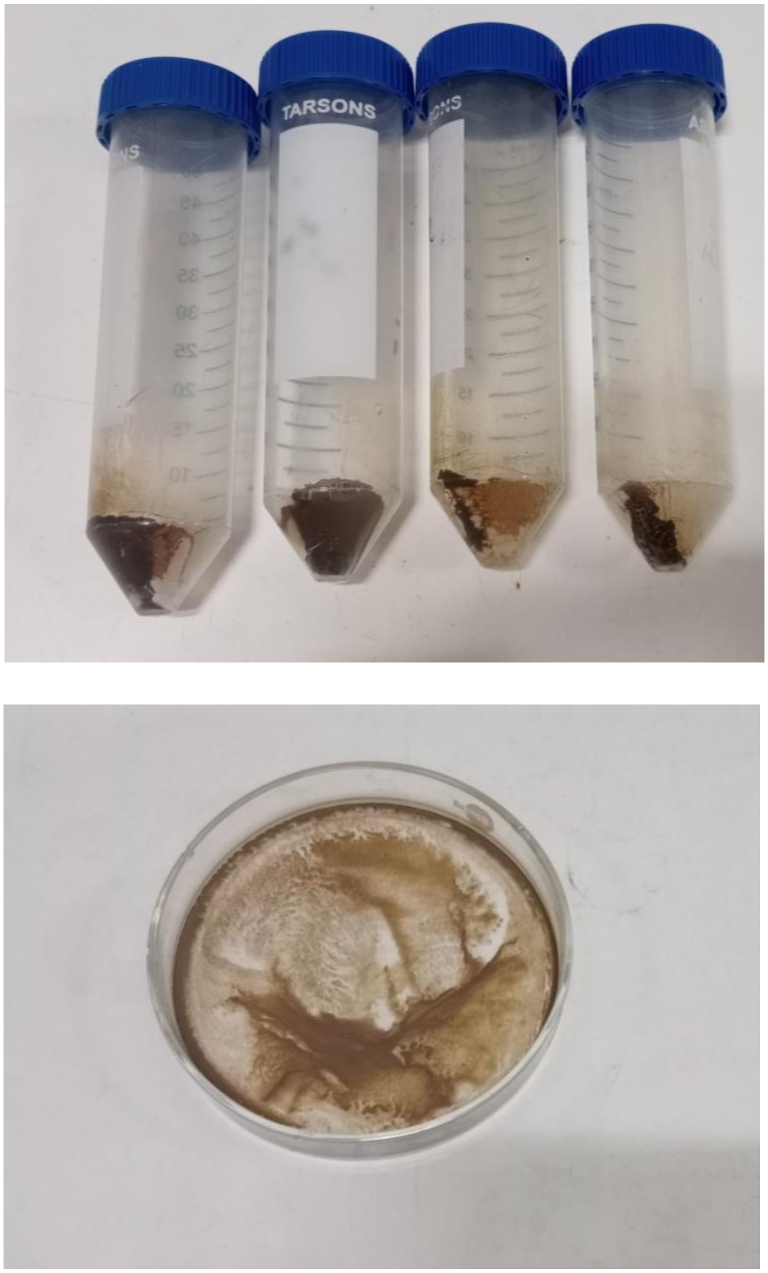

Figure 8: Collected nanoparticles in Petri plate

Figure 9: Dried Nanoparticles collected in vials

4. CHARACTERIZATION OF NANOPARTICLES

These nanoparticles were subjected to different characterization to confirm the formation of copper oxide nanoparticles such as UV-Vis spectroscopy, FTIR, XRD, EDX and SEM.

CHAPTER 4 RESULT

Nanoparticles synthesis using green synthesis approach such as plant extracts have various advantages over other commonly used approaches as well as it makes the synthesis of nanoparticles easy that is very tedious during cell culture monitoring for large scale nanoparticles synthesis (Ghotekar 2019). In this experiment aqueous leaf extract of *Neolamarckia cadamba* has proven to be highly effective and cheap methodology to nucleate nanoparticles. In this experiment, CuO NPs were combined by utilization of *Neolamarckia cadamba* leaf extract extricated arrangement as a dissolvable rather than natural solvents. In combination of nanoparticles, plants concentrate might act both as decreasing and covering specialists (Baig et al., 2021). The synthesis of Copper oxide Nanoparticles could be confirmed by using various analytical techniques to confirm the synthesis of nanoparticles.

4.1 UV–Visible Analysis

The prepared copper oxide nanoparticles were subjected to characterization by using UV-Visible absorption spectrophotometer using a Perkin Elmer, Waltham, USA, instrument. The UV-visible spectrum of Copper Oxide Nanoparticles obtained by using *Neolamarckia cadamba* leaf extract is demonstrated in figure 1. A strong absorption band was observed in the range of 200-210 nm that confirmed the presence of Copper NPs in solution. As the plant extract composition increases it results in shift in absorption spectra, which represents to the formation of copper oxide nanoparticles. The absorption spectrum becomes more stronger on increasing concentration and the peak representing highest absorption is obtained at around 205 nm. This spectra attributes to the formation of Copper oxide nanoparticles using Neolamarckia cadamba. Also, there was no change in the absorption spectra even after prolonged storage. The obtained spectra in result enlightens that synthesized nanoparticle were symmetrical and spherical in nature.

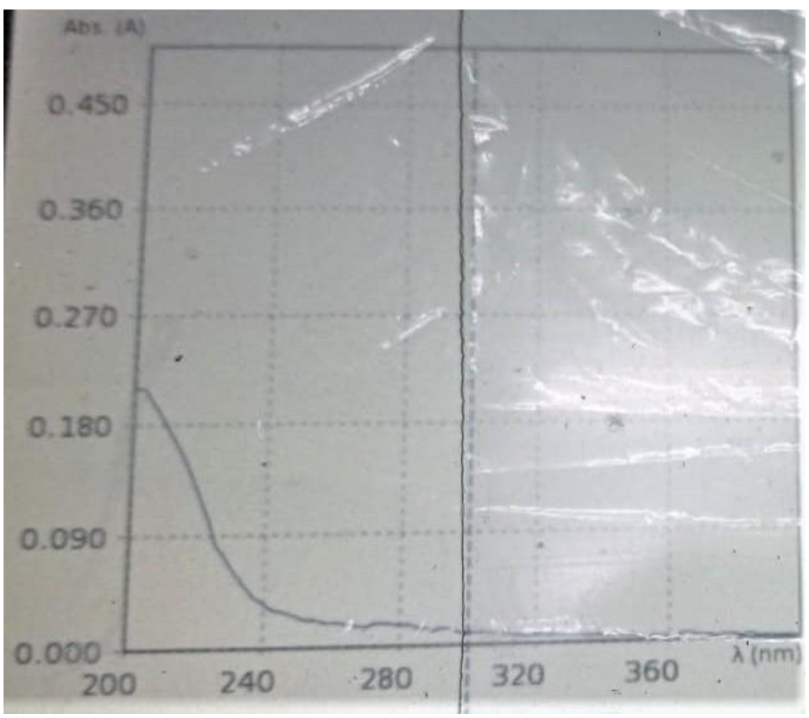

Figure 10: UV-Vis Spectra

4.2 XRD Study

Bruker D8 EA United States of America instrument was used for X ray diffraction. Before this the nanoparticles were lyophilized by freezing in a vial that had liquid nitrogen. Lyophilization were done of nanoparticles for 24 hours (Searles, 2010). X ray diffraction analysis was done to obtain crystalline structure of nanoparticles. In xrd analysis different peaks were obtained in different intensities for nanoparticles. In this maximum peak was observed at 600 nm. The XRD analysis has been shown in figure 3. The diffraction peaks were weak in the starting, the diffraction peaks became strong later and that weakened afterwards. These different peaks of XRD analysis corresponded to the previous data of Copper oxide nanoparticles synthesized in other experiments.

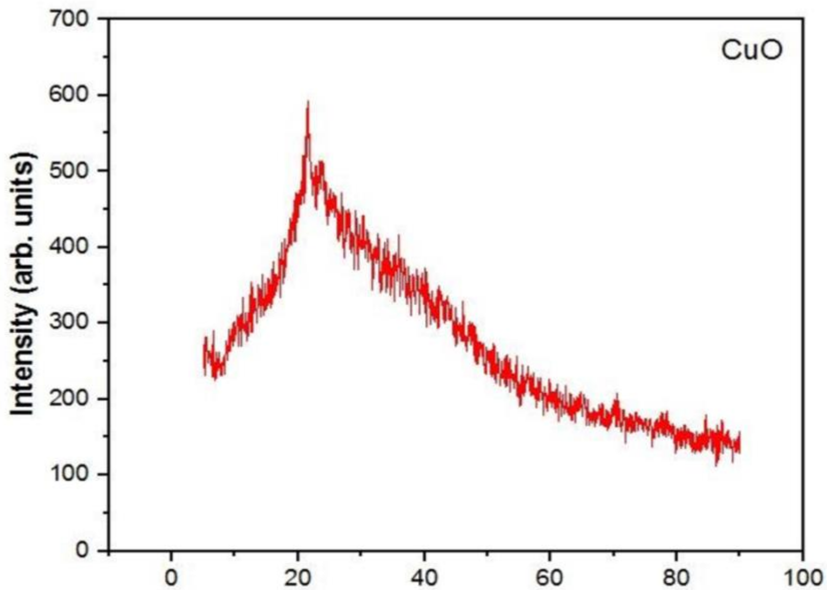

Figure 11: XRD Analysis of CuO NPs

4.3 FTIR Analysis

FTIR analysis is conducted to identify functional groups present in plants and nanoparticles. Different peaks were obtained from FTIR analysis. Peak at 3450 and 3270 cm corresponds to O-H. FTIR analysis is conducted to identify functional groups present in plants and nanoparticles. Different peaks were obtained from FTIR analysis. Peak at 3450 and 3270 cm corresponds to O-H functional groups vibrations. Peak at 2900 cm corresponds to uniform C-H stretch. Peak at 1650 cm corresponds to C=O functional groups. Peak at 1500 cm corresponds to uniform C-C stretch. Peak corresponding to O-H functional groups present in polyphenols is observed at 1400 cm and 1395 cm. Peak corresponding to C-O-C functional groups present in phenolic groups is observed at 650 cm. Peak corresponding to -OH functional groups present in phenolic groups is observed at 800cm.

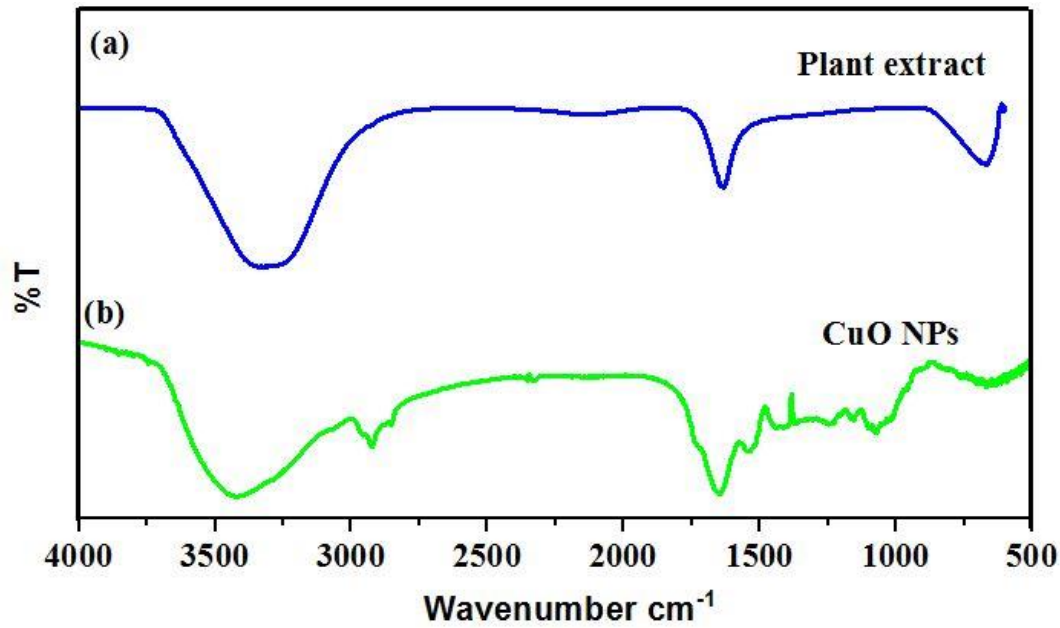

Figure 12: FTIR Analysis of CuO NPs

4.4 SEM and EDX Analysis

SEM and EDX are very important techniques that can be used to obtain the size, shape and other important characteristics of prepared nanoparticles. Scanning electron microscopy was used to obtain size and structure of copper Oxide nanoparticles (Reddy, 2017). The figure shown below represents the data collection with SEM analysis. The shape obtained of copper Oxide nanoparticles was rod and spherical. EDX spectrum of synthesized copper Oxide nanoparticles was obtained that showed peaks. These peaks were similar to titanium and gold peaks in EDX spectrum.

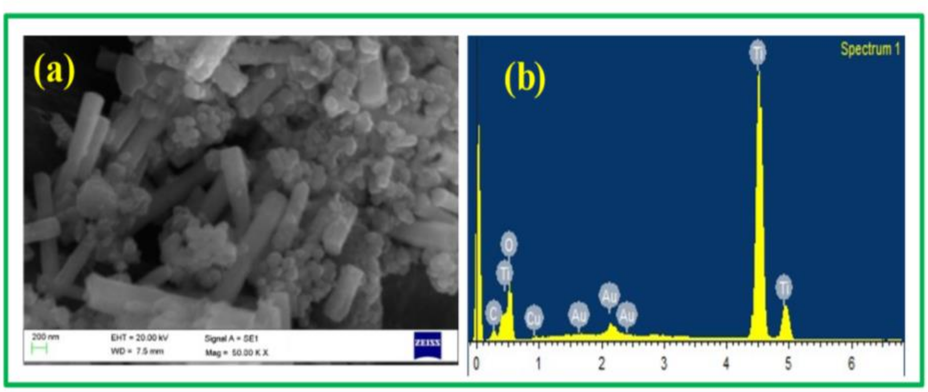

Figure 13: (a) SEM and (b) EDX analysis of CuO NPs

4.5 DLS Analysis

DLS or dynamic light scattering analysis was performed to determine nanoparticles size distribution. All the nanoparticles were of average size of 18.98nm. It is a characterization method used to find the average size of all the particles present in a suspension. This characterization method is also termed as photon correlation spectroscopy and is highly recommended to find diffusion action of nanoparticles or other particles in a solution (Stetefeld. J et al ., 2016).

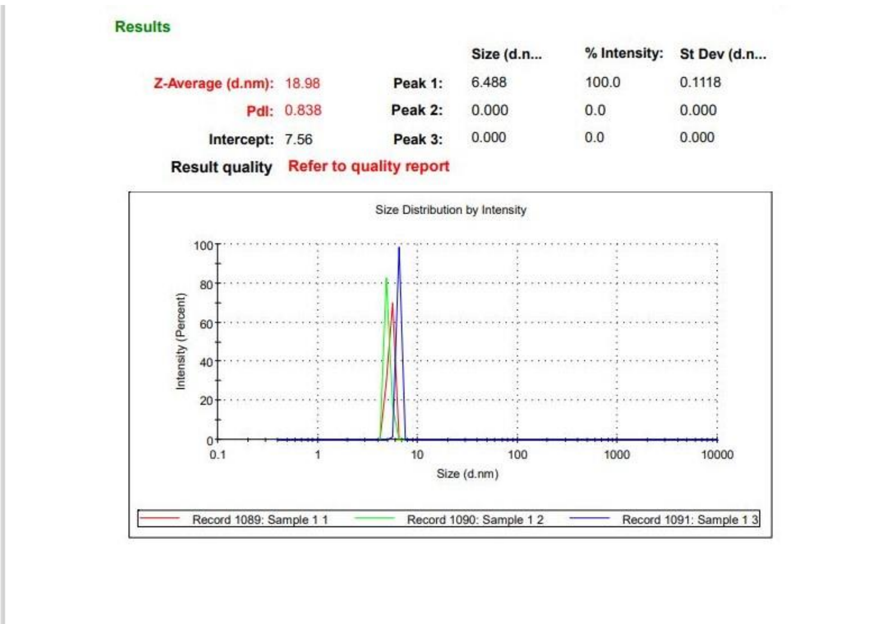

Figure 14: DLS analysis of copper oxide nanoparticles

5. CONCLUSION

Fusion of copper Oxide or any other nanoparticles can be done by using different regularly used materials and technology. In this study fusion of nanoparticles were conducted using green Synthesis approach by utilization of leaf extract of Neolamarckia cadamba which is a highly economic and eco-friendly approach. In this synopsis I have focused on economic, easy and perfect approach for synthesis of nanoparticles that is via green Synthesis approach. In this synopsis I have focused on economic, easy and perfect approach for synthesis of nanoparticles that is via green Synthesis approach using Neolamarckia cadamba leaf extract that acts as a lessening and balancing out agent. Copper Oxide nanoparticles when combined with plant became more steady involving decreased discharge of nanoparticles. On increasing time intervals, the hydrodynamic size and accumulation increased. However, size of copper Oxide nanoparticles blended with plant showed reduction in size and in total size expansion were obtained. Copper Oxide nanoparticles combined with plant were less dissolvable than designed copper Oxide nanoparticles. Copper Oxide nanoparticles combined with plant also had decreased rate of discharge than designed copper Oxide nanoparticles. Copper Oxide nanoparticles combined with plant were more steady than designed copper Oxide nanoparticles. Copper Oxide nanoparticles combined with plant were less toxic than designed copper Oxide nanoparticles. Because of non-toxicity copper Oxide nanoparticles combined with plant are used in various applications.

However, whether designed copper Oxide nanoparticles or copper Oxide nanoparticles combined with plant harm provided by these particles due to disintegration remains unclear. However, characterization of copper Oxide nanoparticles synthesized by green Synthesis approach through UV-Visible spectrophotometer, XRD, dye degradation, SEM, EDX, DLS and FTIR shows their different potential application in different fields.

6.Discussion

Nanoparticles have become very important for both plants and humans. Life. They have various role in different fields such as biology, physics, chemistry, electronics, mechanics, agriculture, waste management, pollution control and disease treatment. The shape, size, structural appearance and structural dimensions of nanoparticles make them enable to be used in different applications. These particles are synthesized using different processes in which physical and chemical process results in damage to environment because the heat, gases and chemical released into the environment are highly toxic in natute. Thus green synthesis approach is being widely used. This approach makes nanoparticles synthesis easy, cheap, non toxic, and efficient. Exposure to these pollutants have been found to resulting in damage to eyes, throat, nose and brain. Thus, green biosynthesis of nanoparticles is a feasible, non-toxic, lees costly and eco-friendly. It is a part of bottom-up approach in which plant extracts, bacterial culture or fungi are used as raw material that leads to redox reaction for synthesis of nanoparticles. No capping or stabilizing agent is required in green synthesis approach as plant secondary metabolites itself acts as stabilizing and capping agents method.

In green synthesis of nanoparticles, most commonly medicinal plants are used as reducing agents for synthesis of vdifferent kinds of nanoparticles such as copper, silver, gold, zinc and iron nanoparticles. These biologically derived metal NPs have different applications as cytotoxic agent against cancerous cells, acts as antioxidant, antimicrobial compounds and anticoagulant agents. As plant uses secondary metabolites for reduction and stabilizing these nanoparticles these nanoparticles are often found with different secondary metabolites such as flavonoids and alkaloids. Copper oxide nanoparticles have been utilized in various important applications specifically in biology than other metal nanoparticles. They are being used in giant magnetic resistant materials, sensors, catalysts, optical, electrical, gas sensors, superconductors, transforming solar energy and for preparing organic-inorganic nanostructure composites. They have been found as potent antibacterial, antifungal, cancer resistant and antioxidant agents.

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A REVIEW ON MARINE OIL SPILLS- POTENTIAL CAUSES, IMPACTS AND CURRENT REMEDIATION APPROACHES

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ABSTRACT

Oil spills have been a great trouble for the environment for many years. Oil spills are environmental calamities that occur due to release of surplus amounts of oil in the environment. Spills occurring in marine environments involve more potential risks than other locations because most people are directly dependent on marine ecosystems for water, food, nutrition and employment. Oil spills are caused by accidental breakage of tankers, pipelines, refineries, improper storage facilities, and during extraction and transportation of oil. These spills pollute the environment and cause potential harm to living organisms. Thus, immediately after occurrence of an oil spill, these should be cleaned up to make the environment suitable, which is often a costlier process. Methods that can be applied in remediation are: physical, chemical, thermal, biological and nanoparticle mediated remediation. Physicochemical methods cause secondary harm to marine life and environment that is rare in biological and nanoparticles mediated remediation of oil spills. Bioremediation employs the inherent capability of microbes for uptake of hydrocarbons present in oil for their energy source. Nanoremediation involves use of nanoparticles which can remove a wide range of hydrocarbons present in oil spills. But bioremediation and nanoremediation can be combined together in which nanoparticles help microbes to increase their bioremediation potential. This technology is called nano enhanced bioremediation. In this review we will critically analyze oil spills, their causes and impacts on marine environment, and different remediation technology and their effectiveness to clean oil

KEYWORDS: Marine oil spills; causes; impacts; bioremediation; nanoremediation; nano-enhanced bioremediation

1. INTRODUCTION

Oil spills have become a very common way of water pollution affecting biotic and abiotic components of the environment (Othumpangat & Castranova, 2014). There are many reasons that result in oil spills. But leakage of oil from offshore drilling platforms and transporting tankers carrying oil from one place to another are the main causes of oil spills. Other than these, oil spills are caused by natural disasters (seepage of crude oil and gases from earth and sea depth) which accounts as the biggest source of oil release into ocean (Othumpangat & Castranova, 2014), (Ivshina et al., 2015), (Britannica, n.d.). Oil which is released from natural seeps is a slower process that provides enough time to the environment to adapt while on the contrary oil spills resulting from human activities spread into water very rapidly and cause environment pollution quickly (NOAA, 2021). Marine oil spills were a big problem till the 1960s because of super tankers that were used for transporting petroleum oil in volume more than 0.5 million metric tons of oil from one place to another via sea routes. Although the allowed volume for shipping oil has been restricted with many government regulations. But in the present scenario, the frequency of occurrence of small oil spills has become very high, that can be as high as 1 million tons of petroleum oil getting spilled per year (Britannica, n.d.). As per international statistics, most oil spills occurring on a daily basis are not in large volume (Ivshina et al., 2015). Oil spill spreads widely to form a layer of oil on water surface or to form emulsions and affects the natural properties of seawater, seashore and nearby sensitive ecosystems. Hence it is necessary to clean oil immediately to prevent harm to the environment. This layer of oil over the surface of water blocks sunlight from penetrating into the water column where marine biota are present generally. Phytoplankton do not receive enough sunlight to perform photosynthesis due to the layer of oil. Lack of photosynthesis creates food scarcity that can affect different organisms interconnected via a food chain. Oil is composed of different hydrocarbons such as cyclic and non-cyclic saturated hydrocarbons, unsaturated cyclic hydrocarbons, non-hydrocarbon components containing nitrogen, Sulphur and oxygen along with some trace metals and toxins with varying proportion in different kind of oils (Colvin et al., 2020). Oil spreads on water from different processes that involve production of toxic by-products. The toxins present in oil are consumed by humans and animals, then it may result in development of health hazards. Oil can coat the wings of birds or the furs of sea otters, producing problems during flying and insulation from cold temperatures (Britannica, n.d.). Marine animals inhale or consume oil directly from water or from sediments which results in health problems such as growth reduction, damaged immune response, damaged nervous system, damaged lungs, damaged liver, reduced metabolism, heart problems, loss in reproductivity, and death in many cases. Humans can consume oil in the form of contaminated water or contaminated marine food that causes serious health issues (Ivshina et al., 2015). Oil spills also disturbs the country's economy by affecting several businesses such as traveling and tourism, fishing, transporting companies via sea routes, agriculture etc. (Dhaka & Chattopadhyay, 2021). Other than this oil spill clean-up requires investment of huge amounts of money by responsible companies and authorities for example in prestige oil spill about $36,097 million in 2002 and $770.58 million in 2002-2004 was spent for cleaning of oil spill (Penela-Arenaz et al., 2010). Some amount of oil can also reach the shoreline within some time period and harm living organisms and sensitive ecosystems (Zengel et al., 2015). It is necessary to clean oil within water to prevent sensitive ecosystems from contamination at seashore and to minimize investment of money (4-5 times higher than oil spill cleaning in open sea), time and workforce (Dhaka & Chattopadhyay, 2021). These oil spills can be cleaned up by physical, mechanical, chemical, thermal, biological and nanoparticles-based remediation methods. Physical remediation involves use of oil booms, skimmers and sorbents. Oil booms prevent oil spread and confine it in a small space which can be collected by use of skimmers or solidifiers or can be burned in situ. Chemical remediation includes use of dispersants and sorbents. Dispersants break down oil into small droplets which can be cleared via natural attenuation or microbes. Sorbent uptakes oil particles either on their outer surface or inside their body or both which can be removed with mechanical methods such as squeezing oil or burning in situ. Thermal remediation involves burning of oil in situ collected via oil booms or via sorbents. Bioremediation involves use of microbes for degradation of oil which can be enhanced through biostimulation and bioaugmentation. Nanoparticles mediated remediation involves use of nanoparticles which have very small size, high surface area and high oil spill remediation potential. All of these strategies aim to achieve three goals: first, to prevent oil from spreading over longer distances; second, to protect highly sensitive ecosystems; and third, to effectively remove oil from the environment without causing secondary harm. However, efficiency of these remediation technologies is largely affected by many environmental conditions, oil characteristics and water conditions such as wind, temperature, water currents, waves pattern, oil viscosity, area where spill occurs (onshore or offshore), volume of oil and its properties and sensitivity to debris and marine ecosystems (Pete et al., 2021). These oil spill remediation strategies alone do not provide 100% efficiency. Different technologies are used together for cleaning of any oil spill in marine water in a sequential manner such as in the prestige oil spill there were three technologies utilized that are physical and chemical methods to extract maximum oil and bioremediation to clean remaining oil. In recent scenarios principles of different remediation techniques are being combined to get more pronounced remediation such as nano enhanced remediation which uses nanoparticles to enhance bioremediation potential of microbes. Oil spills and the problems raised due to oil spills are managed by different units of a country through the formulation of plans and policies. The Indian government has also developed many contingency plans for the management of oil spills (Tewari & Sirvaiya, 2015). Other than this, new and cost-effective methods are being developed for efficient remediation of oil spills without generating additional secondary harm. In this review we will critically analyze causes of oil spills and its impacts on environment, bioremediation and nanoremediation for oil spills management and combination of both technologies.

2. OIL SPILLS AND THEIR FATE

Oil spills occur due to uncontrollable release of oil on land and water. It can be petroleum or vegetative in nature. Soybean oil spills and unrefined sunflower oil spills were some of the disastrous non petroleum oil spills in the past. This form of oil coats animal bodies and plants resulting in death of animals because of hypothermia, dehydration, diarrhea, starvation and suffocation by clogging in the nostrils, throat and gills of animals and depletion of oxygen. They result in fouling of water and readily burns if ignited. These spills undergo oxidation and polymerization and sink at the bottom due to increase in density and bind on the surface of sediments resulting in smothering. Oxidation and weathering of vegetative oil results intermediates generation that are dangerous to marine life (Oceanic et al., n.d.). Crude oil is non-renewable fossil fuel found in nature formed by degradation of animals and plants remains at high temperatures and pressures by natural process within the earth sediments in billions of years. Crude oil exists in either liquid or gaseous form in earth sediments or on earth surface in tar sands. Petroleum oil is prepared from crude oil in refineries which separates different parts of crude oil (Singh et al., 2020). There are different causes of oil spills which are man made mistakes and natural disasters. Oil spills pollute the environment, pose danger to marine life as well as wildlife and impact economic development of a country.

After oil spillage it undergoes different weathering processes that results in decrement in oil volume. Importance of each weathering process is decided by conditions of environment, spill location and oil composition. Weathering results in changes in oil characteristics at each process and its distribution to other parts than spill sites.

2.1. Spreading- Oil starts spreading immediately after spillage in different directions by the action of winds and currents. It changes its morphology and spreads on the top layer of water, and forms an oil layer of a few millimeters in thickness, called an oil slick (Singh et al., 2020). After some time, this oil slick spreads into a thinner layer regarded as an oil sheen that can move easily towards shore due to the action of winds and currents (Ivshina et al., 2015); (Ndimele et al., 2018). Further spreading results in conversion of oil slick into patches and droplets (Singh et al., 2020), (Oil and Petroleum Products Explained, 2021). The speed of spread of oil depends on the viscosity of oil. Light and less viscous oil spreads faster than heavy and more viscous which moves slower (Oil and Petroleum Products Explained, 2021); (Keramea et al., 2021).

2.2. Dispersion- Dispersion of oil is the breakdown of an oil layer into tiny droplets by the action of wind and waves. These oil droplets form either the oil in water emulsions or recombine to produce secondary oil layers. The large droplets resurface to their primary region (Singh et al., 2020). These droplets can be readily degraded by microorganisms present in marine environments. Some of these oil droplets get dissolved within the water column due to its solubility which is affected by environmental conditions such as temperature (oil dissolves faster at high temperature than at low temperature), composition of oil (majority of oil does not dissolve in water), wind, and turbulence of water. Dispersion of the oil layer can be promoted by utilization of chemical dispersants (Keramea et al., 2021).

2.3. Evaporation- Evaporation is the fastest method of weathering, resulting in drop of a considerable number of evaporative constituents such as gasoline, low molecular weight hydrocarbons, lighter polycyclic aromatic hydrocarbons into the atmosphere. This results in enhancement in density and viscosity of oil and decrease in oil toxicity for water bodies. The rate of evaporation depends upon temperature, wave action and wind velocities. Heavy oil can only undergo very low evaporation around ~10% while light oil can evaporate around 75% or more. Sometimes the evaporation process can be harmful if oil spills occur close to a heavily populated area (Singh et al., 2020); (Keramea et al., 2021).

2.4. Emulsification- Emulsification involves mixing of oil in water by production of oil and water emulsions according to wave action and wind velocities. An increase in viscosity results in increase in emulsification that results in decrement in evaporation. These emulsions remain for a long time in water and create risk to the environment by decreasing dispersion. Emulsification rate for heavy oil is higher than light weight oil thereby heavy oil forms more stable emulsion (Keramea et al., 2021).

2.5. Photooxidation- Photooxidation, a very slow process, involves oxidation of oil hydrocarbons via the action of sunlight. The resulting products are oxygenated, soluble in water and possess high resistivity to biodegradation. This process depends upon the oil type and thickness of the oil layer. Tar balls are formed from a thick oil layer by partial oxidation that may settle at bottom or move towards shoreline (Keramea et al., 2021).

2.6. Sedimentation- Sedimentation involves sinking of oil towards the sea bottom and deposition over sediments. Heavy oil being more dense sinks to the bottom faster than light oil (Keramea et al., 2021).

2.7. Biodegradation- Biodegradation involves breakdown of oil into simpler products by microbes for instance bacteria, fungus, algae etc. It is the key process in weathering. Microorganisms require proper environmental conditions including temperature (high temperature preferred), pH and nutrients for uptake of hydrocarbons present in oil spills (Oil and Petroleum Products Explained, 2021). All of these processes are essential to reduce the risk of toxicity caused by oil (Keramea et al., 2021).

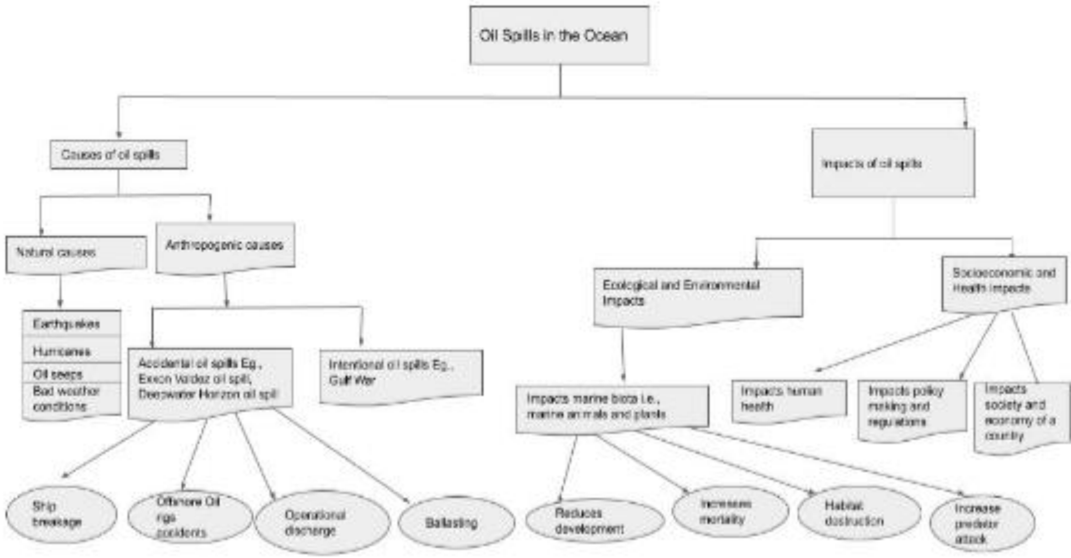
Different oil spills have taken place in the past and are still continuing in present. Details about some of these oil spills have been given in table 1.

Table. 1 Name of oil spills, occurrence, location, quantity, type of oil, shoreline covered and impact on animals

S. No.	NAME OF SPILL, DATE OF OCCURRENCE AND ITS LOCATION	QUANTITY, TYPE OF OIL, SHORELINE AND EFFECT ON ANIMALS	REFERENCES
1.	Deepwater Horizon, Mississippi River delta, United States of America, 20 April, 2010	6,27,000 tons, 6500 to 1,76,000 km² area affected, 150 dolphins killed	(Deepwater Horizon – BP Gulf of Mexico Oil Spill, 2022); (Othumpangat & Castranova, 2014)
2.	World War 2 oil spill, 15 January, 1942, Eastern America coastal waters, North Carolina, United States of America	4,84,200 tons, Crude oil and its products, 50 miles US coastline, killed >50,000 seabirds and many otters	(NOAA, 2022); (Singh et al., 2020)
3.	Ixtoc I, 3 June, 1979, Campeche Bay, Gulf of Mexico	4,75,000 tons, Crude oil, 261 km shoreline	(Soto et al., 2014); (Singh et al., 2020)
4.	Atlantic Empress, Island of Tobago, Caribbean Sea, West Indies, 19 July, 1979,	2,87,000 tons, Crude oil, 10 miles shoreline	(Clements, 2010); (Singh et al., 2020)
5.	ABT Summer, S. Atlantic off Angola, May 1991	2,67,000 tons, Crude oil,	(Clements, 2010)
6.	Castillo de Bellver, off South Africa, August 1983	2,52,000 tons, Crude oil, 1500 birds killed	(Clements, 2010)
7.	Amoco Cadiz, Brittany coast, France, 16 March, 1978	2,23,000-2,22,000 tons, light crude and heavy fuel oil, 320 km shoreline, 4572 birds killed	(Clements, 2010); (Othumpangat & Castranova, 2014)
8.	Gulf war, Persian Gulf, January 1991	~1,50,000 tons, Crude oil, 12 km shoreline	(Little et al., 2021)
9.	Odyssey, N. Atlantic, off Nova Scotia, Canada, 10 November, 1988	1,32,000 tons, Crude oil	(Clements, 2010)
10.	Sanchi, off Shanghai, East China Sea, Jan 2018	1,36,000 tons, Condensate	(Little et al., 2021)
11.	Irene's Serenade, Navarino Bay, Greece, February 1980	1,00,000 tons, Crude oil	(Little et al., 2021)
12.	Sea Empress, Pembroke, Wales, UK, February 1996	72,000 tons, Crude oil, 200 km shoreline, 3495 birds killed	(Clements, 2010)
13.	Prestige, Atlantic, off Finisterre Galicia South coast, NW Spain, 19 November, 2002	63,000 tons, Heavy Fuel Oil, 240-3000 km shoreline, 9348-22,000 birds killed	(Albaiges et al., 2006); (Little et al., 2021)
14.	Exxon Valdez, Prince William Sound, Alaska, USA, 24 March 1989	37,000 tons, Crude oil, 800 km shoreline, 2,50,000 birds killed, 3000 sea otters killed, 300 harbor seals killed, eagles and some whales	(Clements, 2010); (Othumpangat & Castranova, 2014)
15.	Hebei Spirit, Republic of South Korea, 7 December, 2007	11,000 tons, Crude oil, 8 km area affected offshore	(Noh et al., 2019)

CAUSES AND IMPACTS OF OIL SPILLS

Fig. 1 Flowchart depicting the causes and impacts of marine oil spills



3.Causes of oil spills

Mishaps during oil transport, oil extraction and processing, and other reasons all lead to the contamination of the marine surroundings by oil. Mishaps, on the other hand, contribute to a minor percentage of the oil that makes its way into the environment. Besides marine ecology, marine oil spills and their consequences have a negative influence on associated activities such as trawling, tourism, marine agriculture, and the oil business. Oil spills are a risk of industrial activity, and they can have disastrous consequences for the region's flora and fauna. According to studies, manual errors account for 30–50% of all oil spills, while defective machinery or total faulty equipment account for 20–40% of all leaks (Dhaka & Chattopadhyay, 2021).

Oil spills can be classified into two different types of causes:

3.1. Natural causes- Natural calamities such as earthquakes, severe weather, and hurricanes trigger the disposition of crude oil and gas into the seas (Ndimele et al., 2018). Oil spills are created very often because of erosion of rocks within the sea depth. Hurricane Ivan was responsible for the collapse of an oil-producing vessel off the Louisiana coast. This resulted in oil leakage of 300–700 barrels per day (What Is Oil Spill? N.d.).

3. 2. Anthropogenic causes- Oil spills are caused by anthropogenic reasons like land runoffs, unintended releases from tanks and containers, pipeline wreckage, shipping traffic, releases from carrying ships, boring machine operational problems, and accidental leaks from oil tankers while clean-up operations are underway (Singh et al., 2020).

It can also be classified into two categories:

3. 2.1 Accidental oil spills- This could be due to the destruction of oil-carrying ships (grounding, colliding), which could dump millions of tons of fuel into the sea. A well-known example of an unintended oil disaster is the Exxon Valdez oil spill that took place in 1989. Offshore oil rig accidents also fall into this category, with the Deep-Water Horizon oil oilfield explosion being a well-known case. Accidents involving the processing and storage of crude oil and their byproducts are also included in accidental oil spills. Oil is released into marine environments as a result of operational discharge from ordinary vessel maintenance and cleaning, as well as ballasting (Dhaka & Chattopadhyay, 2021). Despite the fact that the number of oil tanker spills has declined in recent decades, contamination risks remain. A shipwreck off the coast of Sanchi in 2018 is a recent example. The Sanchi oil leak, which happened in January 2018, was the world's and also the most polluting oil tanker accident. The Sanchi was transporting 1,36×1000 tons of distillate at the time of the disaster, which sank after 8 days of blasts at sea. Ship burns, explosions, capsizing, and even drowning can occur as a result of tanker oil leaks, jeopardizing crew safety and generating huge financial losses for shipping companies and cargo owners (Chen et al., 2019). According to the International Tanker Owners Pollution Federation Limited (ITOPF) ship operation data, ship operations associated with small and medium sized oil leak incidents (< 700 tons of oil spillage) involve transporting, refueling, and other different activities. Other operations involve loading and unloading ballast water, cleaning cabins, and navigating. Anchoring in inland/restricted sites, harboring in open waters, sailing in inland/restricted regions, navigation in open waters, trying to load, refueling, and other ship operations all contribute to large-scale oil leak disasters (> 700 tons of oil spillage) (Chen et al., 2019).

3.2.2 Intentional oil spills- Intentional releases of oil into maritime systems include untreated effluents from various businesses, fuel oil from service centers, illicit bilge water discharges, military actions, and other deliberate releases of oil into maritime systems. Approximately 10 million barrels of oil were spilled into the Arabian Gulf during the Gulf War of 1990–1991, wreaking havoc on the region's ecosystem (Dhaka & Chattopadhyay, 2021). Several oil spills have resulted from deliberate attacks by terrorists, vandals, or governments at war. A notable example is the 2015 Revolutionary Armed Forces of Colombia (FARC) attacks on Colombia's oil companies, which resulted in the spillage of approximately 400,000 tons of oil (What Is an Oil Spill? n.d.).

3. Impacts of oil spills

Marine oil spills have a significant impact on the marine ecosystem. Marine oil spills cause toxicity on organisms in after a short period of exposure to high concentrations of toxic oil components or after a long period of exposure to low concentrations (Dhaka & Chattopadhyay, 2021). Oil spills can result in extensive contamination, which has economic, environmental, healthcare, social, and societal implications. Damage to the fishing and tourism industries, animal extinction, and public health consequences are all instances of such threats (Colvin et al., 2020). When oil spills into coastal habitats, mangroves, and marine species protected zones, it disrupts the natural processes of the wetlands, mangroves, and sea, reducing biodiversity (Chen et al., 2019).

4.1. Ecological and Environmental Impacts

Almost all creatures are poisoned by oil. The harmful effect is determined by the oil's composition and concentration, as well as the species' sensitivity (Mosbech, 2002). Fresh crude oil contains a high concentration of volatile chemicals like benzene and toluene, which are harmful to humans and easily absorbed through the skin or plant membrane (Rates, 2015).

4.1.1. Impacts on fishes- Oil spills have a variety of effects on fish, including higher mortality, killing or affecting sublethal harm to fish eggs and larvae, such as structural defects, reduced eating and development rates, increased susceptibility to predators and food shortages, habitat destruction, decline of egg hatching capacity, slagging of gill structures, and impeded fertilization, expansion, development, eating, and breathing (Yuewen & Adzigbli, 2018). Adult fish exposed to high levels of oils may develop aeration problems, heart failure, a reduction in cardiac output, serious cardiotoxicity, and slowed swimming rates(Singh et al., 2020).

4.1.2. Impacts on Marine Invertebrates- Although oil may be found across the oceanic ecosystem, it cannot be kept out of the coasts, deep oceans, and estuarine communities that serve as habitat for most invertebrate species and have a severe impact on them by breaking their food webs and inducing acute and chronic toxicity. The sensitivities of invertebrates to oil vary depending on how they eat, how they react to ingested contaminants, and where they reside (Yuewen & Adzigbli, 2018). Tiny invertebrates, like amphipods, are particularly vulnerable to crude oil's analgesic effects, which include abnormal carbohydrate metabolism, high death rates, sluggish growth, sexual disability, hormone cycle disruption, and transcriptome abnormalities, among other things (Singh et al., 2020).

4.1.3. Impacts on Seabirds- Seabirds are well-known targets of oil spills in terms of mortality. Oil inhibits seabirds' ability to fly and protect themselves, making them particularly susceptible. Further consumption, such as through attempted feeding and ingesting foodborne illnesses, breathing and frequent contact with the oil droplets, leads to serious internal poisoning and significant fatality rates (Zhang et al., 2018). Because they dwell in cold water, Arctic seabirds are particularly sensitive to the loss of their plumage's insulating properties. In addition, spilt oil will retain its sticky and feather-destructive qualities in cool water for a longer time frame (Mosbech, 2002).

4.1.4. Impacts on Marine Mammals- The inability of mammals to detect oil, their ingestion of poisoned food, unintentional intake of sea tar residues, and physical contact with oil, which involves oil adhesion to skin, eyes, and other delicate vital organs, and also oral ingestion of volatile hydrocarbons when infringing, are the main threats. Infection of the lung membranes, lung obstruction, and bronchitis are all possible consequences of such interactions (B. Zhang et al., 2018). Surface oiling of juvenile sea lions frequently results in mortality since their fur has not grown enough to give protection in an oiled condition. Almost 10,000 newborn seals died after the shores of their island were tainted with oil following a massive oil spill in South (Rates, 2015).

4.1.5. Impacts on marine Planktons- Plankton (phytoplankton and zooplankton) are microscopic flora and fauna that live in water (Rates, 2015). Because of their weak locomotor ability, the majority of marine organisms are harmed. Because they are at the beginning of the food chain, they accumulate polycyclic aromatic hydrocarbons (PAHs) and pose a risk of biomagnification (Dhaka & Chattopadhyay, 2021). Oil exposure in planktonic copepods can result in instantaneous death, toxic effects, decreased eating, postponed egg production, poor birth rates, lower swimming rates, and reproductive problems (Singh et al., 2020).

4.1.6. Impacts on vegetation and marine environment- The oil forms a dense film on the surface of the sea that blocks light and prevents gaseous exchange. Not only will plants be unable to photosynthesize, but animals living beneath the afflicted area will notice that the supply of oxygen gradually drops and is no longer replaced by the environment. When plants are unable to photosynthesize, they die, causing a vicious cycle in the food chain that eventually affects all animals (Akhmetov et al., n.d.). Corals are poisonous when exposed to dissolved crude oils, resulting in suffocation or coating, reduced growth rates, lower lipid content, changed shape, lack of egg and larvae viability, bleaching, and discoloration (Singh et al., 2020).

4.2. Socioeconomic and Health Impacts

Oil spills have been found to have a variety of impacts, including pollution-related health problems, financial losses and lost productivity due to infrastructure damage, and negative effects on labor availability and the fishing industry (Issa & Vempatti, 2018).

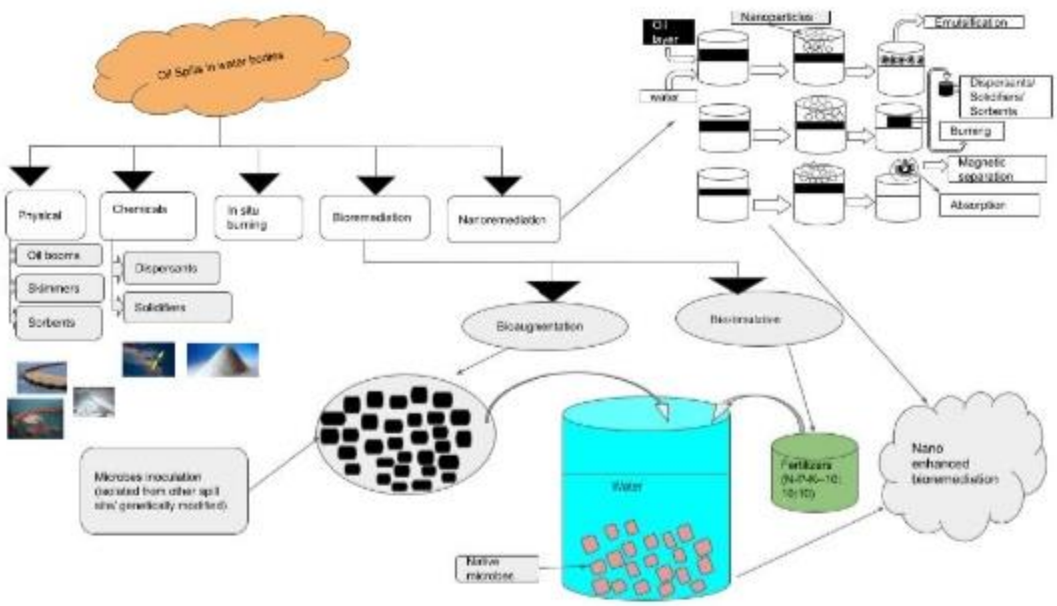
4.2.1. Impact on socioeconomics- The spill had an immediate impact on the seafood business, resulting in the closure of fishing zones as well as a likely drop in the quality of the seafood and price reductions. The tourism sector was harmed as a result of the perception of a damaged environment (Solo-Gabriele et al., 2021). Industrial processes that depend on water from the seas and oceans are harmed. All shipyard, construction, and research efforts have been halted. The authorities' indefinite closing, sealing off, and prohibition of enterprises and activities in these regions to clean up the oil spill has resulted in massive economic losses for resorts, hotels, cafes, and other seafront businesses and leisure activities (Dhaka & Chattopadhyay, 2021).

4.2.2. Impacts on Policy and Regulations- Oil spill response policies and laws differ greatly based on the region of the leak and the entity responsible. Every oil-producing nation has its own ecological restoration methods and regulations, though they are typically equivalent and follow well-established criteria. Developing nations are also required to clean up oil spills as soon as possible, and they typically have a response and contingency plan in order to do so. The Navy and other associated organizations in Peru, for example, have been deployed to respond as swiftly as possible (Zhang et al., 2018).

4.2.3. Impact on human health- The healthcare system's problems are divided into two categories. To begin with, there are direct physical toxic health impacts, which occur when toxic exposures cause acute, quick, and long-term health implications. Long-term health effects usually manifest a few years or decades after exposure begins, or they may persist as a chronic illness from the start. Second, there are the indirect mental impacts, which can be triggered by a variety of stressors such as physical health problems or concerns about them, socioeconomic damages, environmental damages, and a deterioration of trust in a "system" that permits such a spill to occur (Solo-Gabriele et al., 2021). Petroleum hydrocarbons (PHCs), volatile organic compounds (VOCs), poly-aromatic hydrocarbons (PAHs), and other flammable petroleum products can cause breathing problems, inflammation, discomfort, drowsiness, migraines, heart problems, liver and kidney damage, nervous and immune effects, and reproductive problems in humans (Singh et al., 2020).

5. Remediation approach

Fig. 2 Different remediation approaches in marine oil spills



5.1. PHYSICAL REMEDIATION

Physical remediation involves cleaning of oil present on the water surface and does not cause changes in the properties of water. This method involves different tools such as booms, skimmers, and sorbents (Simeonova, n.d.). This method results in oil recovery in its original form that can be used in different processes, like any oil.

Oil booms stop the spread of oil over a large distance by containing it. They are moved over the thickest portion of the oil spill at a very slow speed, and oil is collected by a vessel that takes up the oil and holds it at an angle between the boom and the body of the vessel (Tewari & Sirvaiya, 2015). Wind velocities of over 5 m/s, current velocities above 4 m/s, and wave heights of more than 1 meter result in pulling oil under boom (Hoang et al. 2018). There are many kinds of booms, but the most commonly used are fence, curtain and fire-resistant ones in the remediation of oil spills (Ventikos et al., 2004). Fire resistant booms are made of fire-resistant metals that can work at high temperature with burning oil. It can have designs of either fence boom or curtain boom. Fence booms have floatable fence structure and swim upright in water via external or internal buoyant force. Curtain booms have an oil containment skirt to collect oil under the water surface supported by floating chambers (Ndimele et al., 2018).

Skimmers are used for gathering oil collected by oil booms from superior layers of water that is stored in containers. There are different kinds of skimmers, categorized broadly into 3 classes: suction skimmers, weir and adhesion or oleophilic skimmers. Suction skimmers uptake oil via suction by floating heads and transfer it to a storage tank. Adhesion skimmers consist of belts, discs, and a continuous stretch of oleophilic substance to which oil gets stuck when moved over the oil layer. This oil is squeezed or scrapped and stored in an oil tank (Tewari & Sirvaiya, 2015). Weir skimmers are dams like equipment that uptake oil by the help of gravitational force (Ndimele et al., 2018).

Sorbents are the materials that clean spills by retaining oil on their surface, inside their bodies, or both (Tuan Hoang et al., 2018). The uptake of oil with the help of sorbents is the fastest and most commonly used method for remediation of small oil spills (Nyankson & Gupta, 2016). These materials have both oleophilic and hydrophobic properties. These are used after using oil skimmers to clean the remaining oil from the surface in the form of a semi-solid mass (Tewari & Sirvaiya, 2015); (Nyankson & Gupta, 2016). A sorbent may be absorbent, adsorbent, or a combination of both. An adsorbent retains oil about 3 to 15 times more than its weight over the surface of their body. An absorbent retains oil about 4 to 20 times more than their weight inside their body, causing it to expand by up to 50% in size (Ndimele et al., 2018). There are three different materials available to be used as oil sorbents: organic, inorganic or mineral, and synthetic (Hoang et al., 2018).

5.2. CHEMICAL REMEDIATION

Chemical mediated remediation involves the use of chemicals that change the chemical properties of oil. However, these chemicals react with oil, resulting in alteration in the properties of oil that is recovered using this method and cannot be used further (Allen & Brian, 2021). Chemical methods involve the use of 2 types of chemicals: dispersants and solidifiers. Dispersants consist of emulsifiers and/or solvents. Emulsifiers belong to surfactants that are surface active agents. Dispersants are surfactants that show properties like detergents as they contain ionic and non-ionic parts that provide both hydrophilic and oleophilic properties to the dispersant (Othumpangat & Castranova, 2014). These chemicals are spread on oil spills and mixed in water through wind or mechanically (Tewari & Sirvaiya, 2015). These chemicals lead to the dispersion of small oil droplets in water. The resultant oil droplets will be deteriorated by microbes and natural phenomena. The most disastrous oil spill of history was the Deepwater horizon spill, in which two dispersants namely COREXIT 9500A and COREXIT 9527A were used for treatment. COREXIT 9500A was used in higher quantities than COREXIT 9527A as COREXIT 9527 was found to be toxic to the marine environment during its utilization in the Exxon Valdez oil spill in 1989 (Othumpangat & Castranova, 2014).

Solidifiers are chemicals that inhibit the release of hydrocarbons from oil into water and air by thickening liquid oil spills that are removed mechanically or burned in situ (Pete et al., 2021). These are chemical compounds that are hydrophobic, dry, and semi-solid in nature. They interact with oil by van der Waal forces and decrease surface tension in air-water to produce an insoluble solid rubbery biomass (Allen & Brian, 2021). There are many types of solidifiers available such as raw flex and norsorex (Ndimele et al., 2018).

5.3. THERMAL REMEDIATION

Thermal remediation involves burning of oil with agents such as kerosene or other igniters within the sea on the spill site such as marshes, called “in-situ burning.” In-Situ is a Latin word used for on-site. However, an oil spill can be burned only if the oil slick has a thickness of more than 3 mm. Oil gets burned downward below the exterior of the slick at a speed of 3 millimeters per minute, which may be anywhere between 0.5 and 4 millimeters per minute according to the nature of oil and the amount to which it has emulsified. This method can involve fire resistant booms that concentrate oil spills within a small area and result in the confining oil spill into a thicker oil layer (Hoskin and Underwood, 2006). However, the fire causes harm to marine life as it can burn marine animals and marine plants (secondary fire). It affects human life severely as oil combustion results in the production of toxic gases. Oil combustion generates many toxins, of which PM2.5 is the most harmful pollutant for humans. This method should be used in calm seas and immediately after an oil spill occurs (Ndimele et al., 2018).

Oil spills remediation using physical, chemical or thermal methods is not efficient enough to promote stability in the environment and causes secondary harm to marine life. This led to development of new methods which can be applied on big oil spills, are cheap and environmentally friendly such as bioremediation and nanoremediation.

5.4. BIOREMEDIATION

Bioremediation is the application of microbes (bacteria, fungus or algae) and plants for the degradation of oil spills. Microbes and plants possess unique metabolic pathways for the degradation of complex oil into simpler products that allow them to survive in areas with heavy oil spills (Ndimele et al., 2018). Microorganisms have been used for many years for the treatment of wastewater, but for oil spill remediation, they were first used in the Santa Barbara oil spill in 1969 by George Robinson. This method has been shown to be the only method to clean oil spills effectively for oil spills covering a large area (Pete et al., 2021). The process of utilizing plants for the decontamination of water is called phytoremediation. These plants uptake organic contaminants, i.e., hydrocarbons, to utilize them in different pathways or store them inside shoots, leaves, and roots that filter contaminants. These plants require a high concentration of growth-limiting nutrients and oxygen (Ndimele et al., 2018). The potential of microbes to degrade hydrocarbons can be enhanced in two ways: biostimulation and bioaugmentation. In biostimulation, the growth of microbes is enhanced by supplying growth promoting nutrients, and in bioaugmentation, microbes are incorporated into the spill site to enhance the degradation of oil particles. Proper sunlight and oxygen, along with growth promoting nutrients, are required for growth of oil degrading microorganisms, which is inhibited due to use of physical (mechanical) methods as a primary step. Biodegradation is also affected due to chemicals used in chemical methods as a primary treatment by causing toxicity in microorganisms (Pete et al., 2021).

For example, there are many types of bacteria, fungi, blue green algae, algae and diatoms that consume hydrocarbons present in oil for food and life (EPA, 1999). Oil spill technology involving the use of microbes for cleaning oil spills is called microbial remediation technology. These microorganisms remove oil hydrocarbons in different ways, such as they may uptake hydrocarbons or cause changes in the chemical constitution of the originally present hydrocarbons such that the resultant products are less toxic than the original one. The bioremediation method is more efficient than other methods for many reasons, such as: environmental friendliness (the release of only CO2 and oxygen causes less harm to the environment), suitability for different climate situations (Hoang et al. 2018), cheaper than the other methods, and sustainable and economically viable (Ndimele et al., 2018).

In nature, mostly bacteria clean oil spills to relieve the stress caused by oil, to obtain carbon sources for development and multiplication, and to fulfil the need for energy via oxidation and reduction of compounds during respiration. These bacteria have evolved to degrade hydrocarbons present in oil, as found in areas with abundant natural hydrocarbons. Alkanindiges sp., is a rare bacteria found in soil whose abundance is dependent on environmental constraints. Some of the bacteria such as obligate hydrocarbonoclastic bacteria (OHCB) have been present in a minimum population in the absence of petroleum hydrocarbons in an environment that increases their concentration with the increase in concentration of oil spills. Some of these hydrocarbonoclastic bacteria can degrade multiple types of hydrocarbons and are called broad spectrum hydrocarbonoclastic bacteria. There are no bacteria present on earth that can degrade every hydrocarbon present in oil spills. This is determined by the type of catalytic enzymes found in bacteria, which determine which hydrocarbons the bacteria will degrade. Thus, it requires the use of many bacteria to eliminate all the hydrocarbons present in any oil spill (Xu et al., 2018). A microbial community consisting of different bacterial populations has a very necessary function during degradation of oil hydrocarbons during an oil spill. These bacteria break composite mixtures of different hydrocarbons present in oil into simpler carbonyl and acid residues (Saeki et al., 2009). The adaptation of microorganisms determines the rate of degradation of oil hydrocarbons into carbon and energy. Their abundance is maintained by the formulation of oil, oil concentration, environmental conditions and nutrients present at spill sites. There are different microorganisms that can degrade specific hydrocarbons and some of these microorganisms, the types of hydrocarbons degraded by them and the conditions required by them for degradation of hydrocarbons has been listed in the table 2.

Table. 2 Name of microorganisms and hydrocarbons degraded by them

S. No.	NAME OF MICROORGANISMS	Hydrocarbons	Reference
1.	Haloarchaea IM1011 (*Haloriensalis hydrocarbonoclasticus*)	Aliphatic Hexadecane 57±5.2%	(Zhao et al., 2017)
2.	*Haloferax* sp., *Halobacterium* sp., and *Halococcus* sp.	Crude oil 13% to 47% and n-octadecane 28 % to 67%	(Al-Mailem et al., 2010)
3.	*Haloarcula* sp. and *Haloferax* sp.	Heptadecane (32-95%)	(Tapilatu et al., 2010)
4.	*Dietzia* sp.	Aliphatic alkanes that vary from C12 to C40	(Chen et al., 2017)
5.	*Achromobacter xylosoxidans*	Monoaromatics and polyaromatics	(Xu et al., 2018)
6.	*Rhodococcus ruber* Ac-1513-D and *Rhodococcus erythropolis* 1514-D	Aliphatic alkanes and diesel	(Zhukov et al., 2007)
7.	*Proteobacteria* and *Actinomycetales*	Alkanes	(Xu et al., 2017)
8.	Brevibacterium	Asphaltenes and petroleum	(Hoang et al., 2018)
9.	Flavobacterium	Chlorine containing hydrocarbons	(Hoang et al., 2018)
10.	Pseudomonas	Chlorine benzoate	(Hoang et al., 2018)
11.	*Mycobacterium cosmeticum* byf-4	Benzene, toluene, ethylbenzene and o-xylene	(Zhang et al., 2013)
12.	Fungi (Aspergillus, Candida, Fusarium, Penicillium, and Trichoderma	n-alkanes and aromatics	(Pete et al., 2021)
13.	Algae (Amphora, Chlamydomonas, Chlorella, Dunaliella, Prototheca and Ulva	Aromatics	(Pete et al., 2021)
14.	Diatoms	Aromatics	(Pete et al., 2021)
15.	Blue Green Algae (Anabaena, Nostoc, Oscillators, Aphanocapsa, Microcoleus and Plectonema	n-alkanes and benzene containing hydrocarbons	(Pete et al., 2021)

5.4.1. BIOAUGMENTATION- In bioaugmentation, a microbial consortium is incorporated into the spill site to enhance degradation of oil particles. This is also called microbial seeding. A microbial consortium consists of several microorganisms capable of degrading different hydrocarbons present in oil spills with a higher degradation potential than indigenous microorganisms. These microbes are dissolved in stabilizing agents that can only be activated by stimulants (Ndimele et al., 2018). It involves amendment of the microbiota of spill sites by adding marine microorganisms that have hydrocarbon-degrading properties or by enhancing the quantity of compounds that are biodegradable in nature. It is common practice at places where native microbiota face problems in biodegradation of complex oil components, which is due to their extended lag phase or stress, or sites at which naturally occurring microorganisms fail to metabolize all the hydrocarbons present at spill sites due to their complex composition, or when the number of microorganisms capable of biodegradation of oil hydrocarbons is very small. These marine communities can be isolated from previous oil spill sites or they can be microorganisms modified through rDNA technology in order to build or enhance their biodegradation activity. This method is challenged by some problems, such as the added microorganisms having to compete with local microbes for food and shelter, and destroying local microbiota. This method can be effective only when there is enough information about the composition of spilled oil and local microbiota degradation capabilities. Species capable of establishing synergistic relationships and incorporation of microorganisms which show higher degradation than native microbiota and are genetically stable. They can degrade multiple types of hydrocarbons and can survive in harsh environments. These microorganisms can be immobilized on a carrier before being introduced to the spill site (Pete et al., 2021).

5.4.1.1. IMMOBILIZATION – It involves attaching microorganisms to the carrier which can be chitosan beads most commonly. Species that are mostly immobilized for breakdown of oil spills include Bacillus sp., and metagenomic clones. This method is mostly used for degradation of n-alkanes, benzo(K)fluoranthene and benzo(a)anthracene. Polyvinyl alcohol is used as a carrier for degradation of oil and grease in combination with bioaugmentation and biostimulation by using a mixed consortium of fungi, bacteria and yeasts. Immobilization is based on the same approach of formation of biofilms opted by microorganisms to protect themselves from stress and to form microbial communities. In immobilization microbial cells are attached artificially to the surface by three different ways: encapsulation, entrapment and adsorption. Microbes immobilized on a surface have limited movement and high degradation activity. These cells are found to have high growth rate, increased cellular number, increased biofilm formation and increased bioremediation potential. The carriers used for immobilization should be cheap, less damaging to the environment, unalterable and should promote reconstruction and restoration of microbial cells from media. There are many carriers available to be used in immobilization for example chitosan and organic polymers (Pete et al., 2021).

5.4.1.2. SURFACE MODIFICATION- Most of the microorganisms produce compounds like chemical surfactants which are biosurfactants. Biosurfactants attach outside of the cell surface to increase microbial cell capability to bind with oil droplets thereby increasing bioremediation. Surface modification technique also targets modification of microbial cell surface through attaching polyelectrolyte layers in sequential orders leading to production of a hydrophobic thin film. This film is used by microbial cells to bind with the oil droplets (Pete et al., 2021).

5.4.1.3. GENETIC ENGINEERING- Genetic engineering involves modification of microbial oil biodegradation capability by changing their DNA. This technique involves modification of genes responsible for oil degradation by enhancing their expression in cells or incorporation of oil degradation genes in organisms without any such genes naturally or it might involve modification of genes responsible for cell interaction with oil particles. It may involve modification of metabolic pathways involved in utilization of oil hydrocarbons into simpler products. But this technology favor introduction of genetically modified microbes that can negatively impact the genome of native microbiota which can be solved by development of suicidal microbes which itself die on completion of degradation of oil (Pete et al., 2021).

5.4.2. BIOSTIMULATION- Microbes require proper pH, temperature, elements that act as Electron acceptor and inorganic and organic nutrients for instance carbon, oxygen, nitrogen, phosphorus and potassium to carry out degradation of oil. Biostimulation is achieved by the introduction of different nutritional components and acceptor of electrons required for growth of native microorganisms in the spill site for enhancement of bioremediation by enhancing proliferation of microorganisms (Ndimele et al., 2018). Oleophilic fertilizers are a good source of nitrogen and phosphorus which are added to spill sites to enhance the degradation capabilities of microbes (Ron & Rosenberg, 2014). A study was conducted for 10 months to find efficiency of an oleophilic fertilizer S200 on Cantabrian coast present in North Spain one of the of prestige oil spill. This fertilizer could promote biodegradation of n-alkanes, cyclic hexanes and aromatic hydrocarbons. This study also showed enhanced isomeric selectivity for C1-phenanthrenes and dibenzothiophene (Jiménez et al., 2006). Biostimulation has been successfully used in the Exxon Valdez oil spill via addition of inipol EAP 22 and customblen (Team & Committee, 2007). Microbes face complications in uptake of growth limiting nutrients on their exposure to oil spills thereby resulting in decrease in population of microbes. These indigenous microbes have potential of degradation of hydrocarbons present in oil thus they should be present in high concentration on spill sites to promote fast remediation. It can be done separately or in combination of bioaugmentation and immobilization. It also utilizes nontronite flakes. It helps in increasing the bioremediation potential of Alcanivorax borkumensis for degradation of alkanes (Pete et al., 2021). Efficiency of biostimulation depends upon oil composition, native species and environmental conditions. As growth nutrients are not only required by hydrocarbon degrading microbes thus addition of fertilizers might also increase non required microbes. This problem can be checked out by using nutrients packed in targeted oil droplets that release nutrients only for hydrocarbon degrading microbes. In biostimulation there are chances of increment in microorganisms lag time which can delay biodegradation of oil hydrocarbons generally by two to four weeks.

Addition of dispersants on spill sites also helps in increasing bioremediation potential of indigenous microorganisms by breakdown of thick oil slick into small oil droplets via emulsification i.e., reducing oil water interfacial tension (Team & Committee, 2007). Microbes can uptake such readily bioavailable small oil droplets and degrade them thereby fastening oil degradation in marine water bodies. In a study bioremediation potential of Thalassolituus sp. Belonging to the oceanospirillales group by addition of dispersants. This study involved addition of COREXIT EC 9500 A in 3 water samples collected from Newfoundland, Labrador and Nova Scotia in summer and later fall winter along with nutrients, oil and condensate at 6°C to replicate summer and at 11°C to replicate winters. Degradation of hydrocarbons was found to be higher in summer than in winter. Normal alkanes were degraded faster than aromatic hydrocarbons. Dispersants enhanced degradation of normal alkanes (C10-C15) in winter than in summer. There was no impact on the rate of degradation of PAH and alkylated PAH (Tremblay et al., 2017). These surfactants can be chemical or biological in nature. Biosurfactants are produced by microorganisms in different forms such as sugar containing lipids, lipid containing polysaccharides, oligosaccharides and lipid containing peptides. These are produced mainly by bacteria. These kinds of surfactants are more efficient than chemical surfactants because of their biodegradability, less cytotoxicity and disperse oil far better than chemical dispersants. In a study a biosurfactant was produced by drying the broth culture of Gordonia sp., which had high biosurfactant producing potential. This biosurfactant was used in its crude form which was named as JE1058BS and was used in sea water contaminated with crude oil (Saeki et al., 2009).

6.NANOPARTICLES BASED REMEDIATION- It is the most recent field of science being used in marine oil spills remediation. This has been used for many other purposes from a long period such as drug delivery, gene delivery, cancer treatment, wastewater treatment, heavy metal removal (arsenic, chromium, mercury, zinc, nickel), maintenance, catalysis, nano sensors and food industry. This technology has proved to be more efficient than other commonly used methods for remediation because of superparamagnetic, least cytotoxicity, small size, eco-friendly nature and high stability, recyclability, higher hydrophobicity, high oleophilicity and great adsorptivity of nanoparticles used in this method (Pete et al., 2021); (Singh et al. 2020). This technology involves use of engineered/manipulated nanoparticles/ nanomaterials (ENMs) or naturally occurring nanoparticles of size 1-100 nm for contaminants removal from soil, water and air. These particles are found to increase biodegradation activity of microorganisms by causing biostimulation or dispersion via photocatalysis thus are used in treatment of oil spills. These particles have large surfaces that favor more chemical reactions and removal of different kinds of hydrocarbons and other pollutants (Corsi et al., 2018). This technology involves development of nano emulsifiers, solidifiers and sorbents. Magnetic nanoparticles have been used mostly because they possess superparamagnetic ability, are less coercive and have a high susceptibility to magnetic fields.

6.1. NANOPARTICLES FOR REMEDIATION- Engineered/ manipulated nanoparticles/ nanomaterials (ENMs) or naturally occurring nanoparticles of size 10-9 are generally used for remediation of oil spills. These nanoparticles can be divided into different forms based on their composition:

6.1.1. Carbon based nanoparticles- These nanoparticles are composed of carbon fullerenes (above or ~C^{60}) and carbon nanotubes that involves multi-walled and single-walled nanotubes in the shape of hollow sphere, ellipsoid, and tubular (Del Prado-Audelo et al., 2021).

6.1.2. Metal based nanoparticles- These nanoparticles include quantum dots, nonzero valent iron (nZVI), gold nanoparticles, silver nanoparticles and different metal oxides nanoparticles (Del Prado-Audelo et al., 2021).

6.1.3. Polymer based nanoparticles- It includes dendrimers or other highly ordered and branched polymers capable of interaction with different functional groups (Del Prado-Audelo et al., 2021).

6.1.4. Nanocomposite- These materials are composed of a combination of more than one type of nanoparticles or nanoparticles with other bulk materials. These nanoparticles generally have size larger than 1-100 nm (Del Prado-Audelo et al., 2021).

The most commonly used nanoparticles are metal oxides, mainly nanoscale zero valent iron (nZVI), zeolite, carbon nanotubes and metal nanoparticles (Corsi et al., 2018). These nanoparticles can be further modified to have higher adsorption and better remediation of oil spills (Del Prado-Audelo et al., 2021).

6.2. NANOPARTICLES BASED EMULSIFIERS OR PICKERING EMULSIFIERS- Nanoparticles can be used as emulsifiers that form a stable emulsion at oil water interface. Nanoparticles get adsorbed on the interface between oil droplets and water as a monolayer to prevent clustering of droplets. A stable emulsion is formed when the angle at which water and nanoparticles contact is more than 90. These nanoparticles offer more steric hindrance than surfactants thereby having more stability and resistance to clustering. Stability of emulsion is affected by shape, size, area, concentration of nanoparticles and force existing at the interface. Nanoparticles bind on an interface existing between oil and water which requires both hydrophilic and hydrophobic interactions. As oil water interfaces possess negative charge nanoparticles must have positive charge to be adsorbed. There are different nanoparticles that can be used as pickering emulsifiers such as: natural clay nanoparticles, halloysite, halloysite clay nanotube (HNT), chitosan modified HNT, surfactants modified HNT, wax modified HNT, xanthan modified silica particles and biosurfactants modified nanoparticles (Pete et al., 2021).

6.3. NANOPARTICLES BASED SOLIDIFIERS- Nanoparticles can be used as oil herders/ solidifiers because they decrease air water interfacial tension by thickening the oil layer. Spreading of oil on water surface can be calculated by calculation of all three interfacial tensions present between air, oil and water. Oil spreads due to positive spreading coefficient and restricts in small sites due to negative spreading coefficient. Solidifiers tend to decrease spreading coefficient to prevent flow of oil. Simple oil herders facilitate physical removal of oil and in situ burning. Nanoparticles based oil herders are produced by combining amphiphilic molecules with nanoparticles such as lignin nanoparticles oil herder combined to 1-pentanol. These herders show irreversible adsorption through van der Waals interaction, hydrophobic and hydrophilic interaction between nanoparticles (Pete et al., 2021).

6.4. MAGNETIC NANOSORBENTS- Nanoparticles exhibiting superparamagnetic ability are called magnetic nanoparticles. They are synthesized from variety of materials such as iron, nickel, cobalt, manganese, magnetite, maghemite, mixed ferrite, molecular magnets with conformations of core shell, nano thread, ferrofluids, nanocomposites and magnetic nanocomposites. These magnetic nanoparticles are surface modified or bi functionalized for desired properties and specific response towards external magnetic fields. They can be in the form of simple mono component nanoparticles such as metallic nanoparticles, metal oxide, alloy or composite core shell, carbon nanotubes coated with magnetic nanoparticles. Composite nanoparticles have improvements in magnetic, physical, optical and thermal properties. Iron oxide nanoparticles are most commonly used magnetic nanoparticles because they are biocompatible, biostable, highly oleophilic, biodegradable, nontoxic, easy to synthesize and superparamagnetic approved by Food and Drug administration. They do not show magnetism in absence of magnetic fields but become magnetic as magnetic field is applied. These np are called

S.no.	NAMES OF NANOPARTICLES USED IN REMEDIATION	NAMES OF HYDROCARBONS REMOVED AND REMOVAL EFFICIENCY	REFERENCES
1.	Cellulose nanofibrils/ nanocellulose aerogels/ anisotropic cellulose-based wood sponges	n-alkane, ether, acetone, aromatics, alcohol, etc.	(Guan et al., 2018)
2.	Multi shell nano carbon scavengers (NCS)	Raw and distillate petroleum oil (91% removal in 1:3 [mg NCS per mg oil])	(Daza et al., 2017)
3.	Zn doped cobalt ferrite magnetic nanoparticles	Crude, gasoline, diesel and hydraulic oil. 13.72 ± 0.42-5.50± 0.53 g/g (crude), 14.99 ± 0.95-8.86 ± 0.42 g/g (diesel), 18.23 ± 1.01-8.06 ± 1.26 g/g (gasoline) and 10.58 ± 0.49-5.24 ± 0.31 g/g (hydraulic)	(Pete et al., 2021)
4.	Modified expanded graphite (EG)	(17.22%) engine, (10.47%) crude, (3.68%) diesel and (2.5%) gasoline in 2 hours (recovery 58-83%	(Xu et al., 2018)
5.	Magnetically modified activated carbon (MAC)	Diesel oil (97%)	(Nazifa et al., 2019)
6.	Myrrh gum coated iron oxide nanoparticles	Crude oil (95±1%)	(Atta et al., 2020)
7.	Fe_3O_4 SiO_2 MPS nanocomposite	Crude oil, gasoline and kerosene oil	(Kamgar et al., 2018)
8.	Polyethylene coated iron oxide (magnetite) and carbon nanotube (multiwalled)	Kerosene oil (71.2%)	(Abdullah & Al-Lohedan, 2021)
9.	Fe_3O_4 nanoparticles coated with SAS	Crude oil ~22.5% g/g (90%)	(Qiao et al., 2019)
10.	Nanocomposite Polystyrene/MWCNTs/magnetite (PS-MWCNTs-Fe) nanosorbent	Toluene	(Abdullah et al., 2021)
11.	Acetylene black (AB) and multi-walled carbon nanotube (MWCNT) as igniters	Crude oil combustion increased by 39.5% with acetylene and 31.1% with MWCNT	(Singh et al., 2020)
12.	Magnetically activated carbon nano hybrid material extracted from water hyacinth	Facile oil (80%)	(Shokry et al., 2020)
13.	Hollow amphiphilic crosslinked nanocapsules from sacrificial silica nanoparticle grafted with polycaprolactone-b-poly (ethylene glycol)	Pyrene	(Bentz et al., 2017)
14.	Polyethylene and polyvinyl chloride- blended polystyrene nanofibrous sorbents	Crude oil, motor oil and diesel	(Alnaqbi et al., 2020)
15.	Kenaf stalk grafted sorbent	Crude oil	(Salisu et al., 2019)

superparamagnetic iron oxide nanoparticles (SPIONs). They readily interact with different physical and chemical products such as inorganic and organic polymers, functional groups, ligands, non-metal, alloys, dyes, peptides and antibodies. Magnetic nanocomposites contain magnetic nanoparticles integrated with organic polymer, liquid, ceramic, carbon, liposomes, proteins that provide properties of both magnetic nanoparticles and matrix (Singh et al., 2020). These materials uptake oil through adsorption or adsorption or through combining both mechanisms together.

Table. 3 Names of nanoparticles used in oil spills remediation, names of hydrocarbons removed and removal efficiency

4. NANO-ENHANCED BIOREMEDIATION- In recent years both techniques nanoremediation and bioremediation are being used in combination for better remediation of oil spills which is called nano-enhanced bioremediation. This is because of potential of microbes and nanoparticles to clean oil in ocean effectively that get enhanced when combined together. This technique has shown to have highest remediation potential over all the other techniques. This technology works by using pickering emulsifiers for bacterial cell division on droplets, or coating microorganisms with magnetic nanoparticles targeting easy removal and nanoparticles loaded with nutrients targeting release of nutrients only at oil-water interface. In a study a hybrid catalytic system which combined a biocatalytic and a photocatalysis system was designed with the help of graphite-like carbon nitride compound with a bacterium that degrades hydrocarbons namely Acinetobacter sp., to eliminate hydrocarbons with 16 carbon atoms. It had 78.2% removal efficiency within 240 minutes (Ma et al., 2018).

7.1. PICKERING EMULSIFIERS INDUCED BIOREMEDIATION- Pickering emulsifiers can be used as an environment friendly alternative of chemical surfactants being nontoxic, stable and non-Newtonian at high temperature and pressure. Microbes are made to adsorb on oil droplets or oil in water interface stabilized through nanoparticles mediated emulsifiers. These microorganisms can produce biofilm when attached with these emulsifiers, as emulsifiers are hydrophobic in nature and provide large surface area and favorable conditions required for formation of biofilms. These emulsifiers can stabilize emulsion for a week that requires very less energy that is provided by ocean turbulence only (sonication not required). There are many pickering emulsifiers that can be used to increase bacterial proliferation such as clay nanoparticles based pickering emulsifiers, nontronite, section, halloysite, modified HNT and graphene oxide quantum dots (Pete et al., 2021).

In a study hydrophobized, silanized halloysite nanotubes were used as a pickering emulsifier which could enhance cell proliferation of A. borkumensis. About 0.5-1.0 % halloysite powder was added that resulted in micro emulsification of oil and increased A. borkumensis proliferation via increasing conversion of resazurin to resorufin that indicated enhanced bacterial cell proliferation (Pete et al., 2021).

7.2. MAGNETIC NANOPARTICLES INDUCED BIOREMEDIATION- Cells of marine microorganisms can be modified by attaching magnetic nanoparticles on their surface. These modified microbes show high affinity and interaction with oil droplets firmly. On binding of magnetic nanoparticles treated microorganisms with oil droplets these cells are removed from water by applying magnetic field. These bacteria are named as magneto responsive bacteria (Xu et al., 2018). There are many nanoparticles that can be used to create magneto responsive bacteria such as carbonized kaolinite sheets and polyvinylpyrrolidone coated magnetic nanoparticles in Alcanivorax borkumensis, Halomonas sp, Vibrio gazogenes and Marinobacter hydrocarbonoclasticus for degradation of hexadecane and crude oil (Pete et al., 2021). In a study a hydrocarbon degrading bacterial species Brevibacillus parabrevis was coated with PAH- modified magnetic nanoparticles to develop magneto responsive bacteria. These bacteria acted as pickering emulsifiers as they formed a pickering emulsion of 1:5 oil water mixture by attaching over tetradecane oil droplets. These were separated through application of magnetic force (Cheng et al., 2020).

7.3. NANOPARTICLES MEDIATED BIOSTIMULATION- Nanoparticles can also be used to favor biostimulation by loading them with different organic and inorganic nutrients and electron acceptors targeted to be released on oil water interface. This can help in reducing growth limiting nutrients uptake by non-hydrocarbon degrading bacteria and dilution of nutrients. There are many nanoparticles that can be used for targeted biostimulation such as mesoporous silica nanoparticles. These mesoporous silica nanoparticles had a gate which opened only on their reaction with alkanes which were found only on the oil water interface (Pete et al., 2021).

5. CONCLUSION AND FUTURE PERSPECTIVES

From the above discussion, it is clear that oil spills cause a large amount of damage to the environment, plants, animals, and humans in different ways. There are different methods that are able to clean oil spills efficiently, but not completely. Different oil spills need different methods for remediation. There are various approaches which are implemented at oil spill sites, with bioremediation and nano remediation being highly efficient. Classical methods involve utilization of devices such as skimmers, sorbents and oil booms, chemicals such as dispersants and solidifiers, and in situ burning. Remediation approach based on these traditional methods proves to be very expensive and labor intensive. These methods also result in secondary harms to the environment thus not applicable on a large scale. Bioremediation involves use of microbes which have a unique metabolic pathway for uptake and degradation of hydrocarbons. Nanoremediation involves use of nano sized materials capable of interacting with oil hydrocarbons which can be removed via external magnetic fields. These both approaches bioremediation and nanoremediation can be used on a large scale, being less expensive and easy to implement. However recent studies are focused on combining these two approaches together to have more enhanced remediation which is termed as nano enhanced bioremediation.

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AUTHORS' CONTRIBUTION

All authors conceived and designed the study. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agreed to be held accountable for the content therein.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

AVAILABILITY OF DATA

All datasets generated or analyzed during this study are included in the manuscript.

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CANDIDATE'S DECLARATION

I, Muskan Garg, 2K20/MSCBIO/18 hereby certify that the work which I presented in the Major Project entitled 'Copper oxide nanoparticle synthesis by green synthesis approach using *Neolamarckia cadamba* and its characterization' in fulfillment of the requirement for the award of the Degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own, carried out during a period from **feb-2022 to -2 May-2022**, under the supervision of Dr. Jai Gopal Sharma.

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.

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CERTIFICATE

I hereby certify that the Project dissertation titled "**Copper oxide nanoparticle synthesis by green synthesis approach using *Neolamarckia cadamba* and its characterization**" which is submitted by **Muskan Garg, 2K20/MSCBIO/18**, 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 recordfor the project work carried out by the student under my supervision. To the best of myknowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 6 May, 2022

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