M.TECH (Industrial Biotechnology) [MOHITA CHUGH] 2021

Green Synthesis of Silver Nanoparticles from Chlorella minutissima: Reaction Optimization and Nanoremediation of Heavy Metals & Dyes.

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE
OF

Master of Technology
In
INDUSTRIAL BIOTECHNOLOGY

Submitted by

MOHITA CHUGH
(2K19/IBT/03)

under the supervision of

Dr. NAVNEETA BHARADVAJA

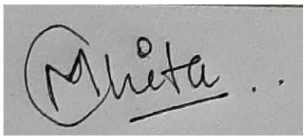
DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Bawana Road, Delhi-110042

JUNE, 2021

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I hereby certify that the work which is presented in the research work entitled "**Green Synthesis of Silver Nanoparticles from *Chlorella minutissima*: Reaction Optimization and Nanoremediation of Heavy Metals & Dyes**" in fulfilment of the requirement for the award of Degree of Masters of Technology in Industrial Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 7-jan-2021 to 28-June-2021, under the supervision of Dr. Navneeta Bharadvaja. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been published and communicated in various journal under my name with the guide.



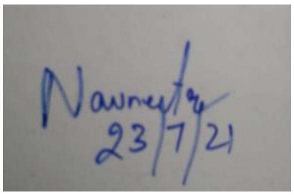
Place: Delhi
Date: **23/7/21**

MOHITA CHUGH
2K19/IBT/03

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Bawana Road, Delhi-110042

CERTIFICATE

This is to certify that the Project dissertation titled "**Green Synthesis of Silver Nanoparticles from *Chlorella minutissima*: Reaction Optimization and Nanoremediation of Heavy Metals & Dyes**" which is submitted by Mohita Chugh, 2K19/IBT/03, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record for the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.



Place: Delhi
Date: 23/7/21

Dr. Navneeta Bharadvaja
SUPERVISOR

Prof. Pravir Kumar
HEAD OF DEPARTMENT

ABSTRACT

The development of a remediation technique that is environment friendly, efficient and economical for the abatement of heavy metals and dyes will be an important step towards the treatment of water pollutants. For this, silver nanoparticles were synthesized from green algae *Chlorella minutissima* extract. For the enhancement of silver nanoparticles synthesis, various optimization studies were carried out where different cell disruption techniques (sonication, heating and autoclave), various range of pH (4 to 12) of reaction mixture, various salt and biomass concentration along with contact time were studied. Later, characterization techniques like visual detection and UV-Visible spectroscopy are used for the characterization of silver nanoparticles. In the end, the application of these silver phyconanoparticles were assessed in the remediation of six different heavy metals and 5 different dyes. It is observed that it can remediate more than 96% of EY, MO and EB and more than 87% CV and MB within 5 minutes and 65.18% nickel, 45.83% copper, 42% mercury, 33.84% cadmium within 1 hour and are less efficient in the case of cobalt (4.5%) and chromium (0.5%). These results have shown the potential of silver nanoparticles in the field of remediation of heavy metals and dyes. This project aims to develop a more economical, sustainable and promising tool for the remediation of heavy metals and dyes.

ACKNOWLEDGMENT

A formal statement of acknowledgment will hardly meet the needs of justice in the matter of expression of deeply felt sincere and allegiant gratitude to all who encouraged and helped me in many ways throughout the dissertation of Master of Technology. It is my privilege to express my profound sense of gratitude and indebtedness to my supervisor Dr. Navneeta Bharadvaja, Assistant Professor in the Department of Biotechnology, Delhi Technological University for her valuable guidance and consistent encouragement during the progress of the project work. The dissertation wouldn't be completed without her insightful suggestions and support. A special thanks to my senior, Mr. Lakhan Kumar for his constant support and immense faith on me. I have completed this project because of his guidance, inspiration, motivation provided by him. I am equally grateful and wish to express my wholehearted thanks to respected Head, Prof. Pravir Kumar, Prof. Jai Gopal Sharma and technical staff for their kind support. I would also like to thank other lab members Mr. Sidharth Sharma, Ms. Harshita Singh, Ms. Anuradha and Ms. Deepti Bharadwaj for their support and help in the course of my research work. I was prevailed enough to experience a sustained enthusiasm and involved interest from their side. This fuelled my enthusiasm even further and encouraged me to boldly step into what was totally dark and unexplored expanse for me. On a personal note, I wish to express my gratitude and affection to my family for their constant love and support.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBEREVIATIONS

List of Abbereviations:	
AgNp	Silver Nanoparticles
Np	Nanoparticles
MO	Methyl Orange
EY	Eosin Yellow
EB	Evans Blue
CV	Crystal Violet
MB	Methylene Blue
RE	Removal Efficiency
Cm	*Chlorella minutissima*
Cm.AgNp	*Chlorella minutissima* Silver Nanoparticles

CHAPTER 1 NTRODUCTION

1.1 HISTORY

Globally, water pollution is one of the major problem due to its hazardous effects towards the ecosystem [1]. Water pollutants like heavy metals, dyes and other pollutants are the most toxic compounds present in the wastewater. These pollutants are naturally as well as man-made. Naturally, they are present in the environment through rocks, weathering or volcanic eruption, etc. Man-made activities like wastewater of different industries such as textile, fertilizers, dyes, batteries, etc. [2] release this wastewater directly to water bodies which is toxic to ecosystem [3]. Industries release heavy metals such as Pb, Ar, Hg, Cd, Cr, Cu and Ni [4]. Heavy metals are non-biodegradable in nature. Dyes like methylene blue, crystal violet, methylene red, etc. are released directly from different textile industries. In India, very less wastewater treatment set-ups are available and because of this wastewater released from industries is directly enters the environment and have hazardous effects towards eco-system. They show harmful effects in renal, neurological, reproductive, and immunological systems [5]. For the remediation of these water pollutants, conventional and modern techniques are used. Conventional methods which are used for wastewater treatment precipitation, ion exchange, osmosis, etc. These physical and chemical methods are not efficient, eco-friendly and economical as compared to biological methods as they utilize toxic chemicals as well as requires energy for the abatement of dyes. To overcome this, scientists are now moving towards the biological methods as they are eco-friendly as well as more effective as compare to physicochemical techniques [6].

1.1.1 Heavy Metals

Heavy metals are present on earth's crust and soil. They have a relatively high density as compared to water [7]. Earlier, it was considered that heavy metals can't be fully removed from environment and only converted into the less-toxic form. There are some heavy metals which are important in biological reaction which are known as micronutrients or trace elements like copper, lead, selenium, iron, etc. [8]. The world health organization has directed some common heavy metals that are toxic to public health which are Cr, Pb, Al, Ni, Mn, Hg, Fe, Cu, Co, Ar, Zn, Cd, Se, etc. The never-ending use of heavy metals gives rise to global concerns, i.e., environment pollution, and they are a threat to human lives. Heavy metals are the most toxic as they get accumulated in the food chain, and even in low concentrations show hazardous effects [9].

1.1.2 Dyes

Industries like paints, pigments and textiles release dyes into the water stream. Dyes are aromatic synthetic compounds and have toxic impacts on the ecosystem. Synthetic dyes are used in numerous sectors like in food, leather, cosmetics, electronic industry, pharmaceuticals, printing, plastic, etc. These dyes are seen by the naked eyes in the water bodies but are very difficult to abate because of their aromatic structure. More than 3000 dyes are used in the textile, printing and various other purposes and effluent of these industries are released in an uncontrolled manner and pollute the environment [10]. These have harmful impact towards aquatic system because they decrease the light deep inside the sea. Thus, reducing photosynthetic activity. Many physical, chemical and biological methods are used for the remediation of dyes like precipitation, sedimentation, flocculation, adsorption, ion-exchange, bioremediation, etc.

1.2 HAZARDOUS EFFECTS OF CONTAMINANTS

Heavy metals and dyes are used for anthropogenic purposes in an indiscriminate way caused the presence of them in the environment in a substantial amount, which has started to alter the biological cycles and geochemical cycles. The abiotic components like soil, air, and water are severely affected by the overuse of these water pollutants that cause soil and water pollution, which are undesirable to be used. The plants, humans, and microbes make the biotic components which are also affected due to their presence in the environment.

Their accumulation into the environment has the tendency to get magnified at each level of the food chain, so they are needed to be transformed into some other forms [11]. The prolonged exposure of mammals to these contaminants through different media like skin contact, inhalation, and consumption unknowingly lead to various diseases. This wastewater is used in agricultural fields and other different domestic purposes which has the capacity to affect the entire human population along with pollution at various levels like soil and water [12]. It reduces soil respiration and affects the soil microbes, which further affects different microbial processes as well as enzyme's activity in the soil. Dyes present in the water streams have harmful impact towards aquatic system because they decrease the light deep inside the sea. Thus, reducing photosynthetic activity [13].

The water pollutants toxicity in plants can hamper their capacity of nutrition absorption, delayed germination, induced genotoxicity, oxidative stress and less enzymatic activity. The premature falling of leaves and the different above conditions will cause a low rate of photosynthesis and respiration, homeostasis which leads to lesser growth and development in plants. The higher quantity of dyes and heavy metals is fatal to plants which may cause diseases like yellowing of leaves in case of wheat and paddy due to chromium, growth inhibition due to lead, nickel, cadmium and chromium. Dyes are responsible for the altering of pH [14] [15].

Human and animal contact different infections after prolonged exposure to water pollutants. Several million of human populations throughout the world are suffering from water toxicity associated diseases. Dyes in the end leads to the release of heavy metal which act as a complexing agent in them. The presence of heavy metals has reported causing intermittent ovulation cycles or menstrual disorders, lesser sperm count, spontaneous abortions, birth defects and premature death which can lead to infertility [16] [17]. The risk associated with heavy metals in the case of children is more prevalent [9]. These health hazards may lead to disorders like muscular dystrophy, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and cancer as well [12]. The water pollutants and their toxicity will continue to affect the entire biosphere so we need to take measures to reduce dependency on them and their remediation at the source[18].

1.3 PHYCOREMEDIATION

Abatement of water pollutants with the help of biological agents is known as bioremediation. Bioremediation carried out by algae is known as Phycoremediation. The term microalgae refer to the aquatic microscopic photosynthetic species. These species accumulate water pollutants, CO_2, and other extracellular polysaccharides and are the source of O_2 in the marine environment. They produce a large amount of biomass that can be further utilized in green energy and other useful by-products [19]. Microalgae are evolved over five hundred million years and develop the power to abate the pollutants like dyes, heavy metals, organic pollutants, pathogens, etc. These pollutants act as their nutrients and generate a large amount of biomass [20] [21].

The algae can abate contaminants majorly by three main processes which are biodegradation, bioaccumulation and biosorption as shown in **fig.1**. These techniques use bio-binding or bio-removal methods. Biosorption is a process where contaminants like heavy metals and dyes bind to the cell surface of the biomass [22]. On the other hand, bioaccumulation is the accumulation of pollutants inside the cell [23]. This process uses the metabolic process for remediation. Thus, this process is carried out by the living biomass only. Another technique that is used by algae for the abatement of pollutants like dyes and organic compounds is biodegradation. In biodegradation, pollutants are degraded with the help of cell metabolism or enzymes [24].

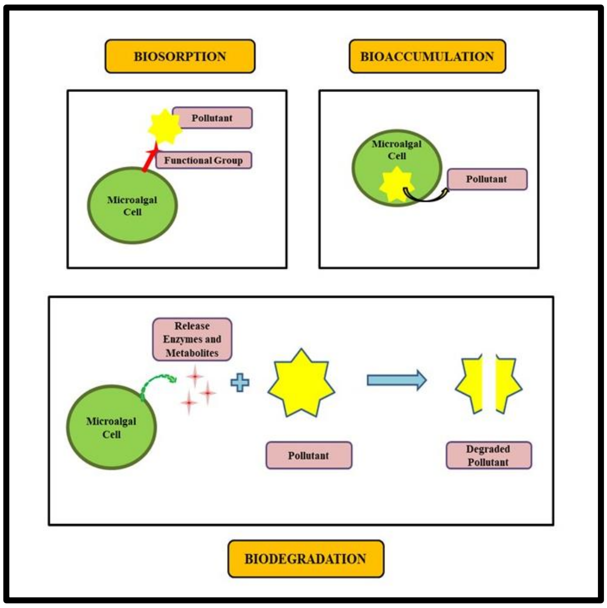


Fig. 1: Mechanism of action shown by Microalgae

Abatement of various pollutants varies according to the microalgal species and their ability to remediate the pollutants. Thus, the design of the cultivation system is one of the most important parameters in the experimental setup for remediation. Important parameters which determine the optimal remediation rate are the pH, temperature, media, concentration of the salt as well as biomass, light, type of system, agitation speed, area and energy. It is observed that algae are not able to show the complete remediation [25]. This lead environmentalist to shift their research toward the more advance technique of remediation i.e. nanoremediation where remediation of water pollutants takes place with the help of nanoparticles. Nanoparticles are tiny substances sized between 1 to 100 nanometers in diameter. Recently, nanotechnology has achieved a huge amount of attention as it has unique physical, chemical and magnetic properties. Due to these unique properties, they are used in various fields like medical, environment, automobiles, interior designing, biotechnology and various other emerging fields [26].

Nowadays, many scientists believe that nanomaterials are a promising remediators for the abatement of water pollutants [27]. On the other hand, the synthesis process of the nanoparticles requires the use of many harmful chemicals which pollutes the environment. To overcome this, nanobiotechnology i.e. production of nano-sized particles from biological agents has gained a lot of interest in research especially in the area of environment. As it will reduce the use of harmful chemicals and synthesized the nanoparticles in an eco-friendly way. [28]. Remediation carried out with the help of algal nanoparticles is known as phyconanoremediation. Out of all the biological agents like plants, fungi, algae or bacteria; algae come with an added benefit as they are rich in proteins, carbohydrates, lipids that leads to the production of various commercial products like biofuel, medicines, pigments, natural dyes, etc [29]. Thus, leftover biomass after the synthesis of phyconanoparticles can be further used for the production of commercial by-products. Till now, it is observed that algae are capable of producing the nanomaterial and these green nanoparticles have many applications in various fields like they are antibiotic, anti-parasitic, anti-inflammatory and very few studies are available on the water pollutants phyconanoremediation [30]. So, the objective of this study is to explore phyconanotechnology and its application for the abatement of heavy metals and dyes.

1.4 OBJECTIVES

The objective of this project is to develop inexpensive and simple process that would treat industrial wastewater to discharge standards. A phyconanoremediation set-up was adopted for this work.

The study aims to achieve following objective:

1. Synthesis of algae-based silver nanoparticles.
2. Optimization of synthesis parameters of silver phyconanoparticles.
3. To study heavy metal remediation from phyconanoremediation set-up.
4. To study the dye abatement from phyconanoremediation set-up.
5. Develop an efficient, environment-friendly and economical wastewater treatment technique.

CHAPTER 2 REVIEW OF LITERATURE

2.1 PHYCONANOPARTICLES: A POTENTIAL SOLUTION

Nanoparticles are the best material for remediation as they have high surface activity, large surface-to-volume ratio and unique physical as well as chemical characteristics. Type of nanoparticles and their physical, chemical and magnetic properties plays a major role in the abatement of water pollutants [31]. These tiny units help in the remediation of the maximum amount of heavy metals, dyes or organic pollutants within few minutes [32]. With all the above-mentioned benefits, physical or chemical synthesized nanoparticles comes with one major disadvantage that they are not environment-friendly [3]. Various challenges such as low detection, expensive, adverse environment and ecological impacts should be considered before the synthesis of these nanoparticles. These challenges are overcome by biological synthesis of nanoparticles. This green synthesis of nanoparticles will be considered environment-friendly and economical because it doesn’t let the use of harmful chemicals and energy. In green synthesis, nanoparticles are produced using plants, microbes like bacteria, fungi and algae [30]. Particles Synthesized using algae are known as phyconanoparticles and this technique is known as phyconanotechnology. Phyconanoremediation can be more effective, environment-friendly and economical technique as algae are rich in proteins, lipids, pigments, and other bioactive compounds. These metabolites are used by various industries like nutraceuticals, medical, cosmetics, biofuel and food industries. Additionally, it is observed that green nanoparticles don’t require a capping agent as biomolecules present in the algae acts as a capping agent, which will further decrease the use of toxic chemicals [33]. From the last ten years, many studies are being conducted in the field of biological synthesis of nanoparticles but very few were conducted using microalgae and macroalgae. These biological sources can produce various nanoparticles using different metal precursors. They can produce silver, iron, copper, gold and metal-oxide nanoparticles [34]. Still, very few studies are available in this area of remediation. Metal-based nanosized particles are extensively utilized for the abatement of water pollutants as they exhibit higher adsorption capacity [35]. To make this phyconanoremediation more economical, one can use the left-over biomass for the production of various commercial by-products like biofuel, pigments, biofertilizer, nutraceuticals, cosmetic products, therapeutic proteins and other medicines [36].

2.2 SYNTHESIS OF PHYCONANOPARTICLES

Synthesis of nanoparticles majorly takes place with the help of these two approaches i.e. top-down and bottom-up methods. In the top-down method, bulk material is broken down into nanoparticles. Contrarily, various nanosized materials are assembled together for the synthesis of nanomaterials. This approach is known ad bottom-up approach. Nanoparticles can be produced by using physical, chemical and biological methods. Due to the physicochemical and mechanical properties, scientists have a keen interest in this emerging field. Conventional methods of synthesizing nanoparticles are physical and chemical methods [37]. In physical methods, the most common method used for the synthesis are pyrolysis and attrition [38]. But these methods generate a very less amount of nanoparticles with the help of a large amount of energy, which makes the process expensive [39].

To overcome these challenges, researchers move towards the chemical method of production. The frequently used chemical methods of production are chemical reduction, electrochemical and photochemical reactions [40]. Here chemicals like borohydride, potassium bitartarate, hydrazine, etc. are used as a reducing agent along with the stabilizing agents. The major drawback of the chemical method is that they use toxic chemicals which leads to the production of hazardous nanoparticles along with the by-products. These methods are not environment friendly. However, scientists, discover the environment-friendly method of synthesis which is biological methods. In this method, green synthesis of nanoparticle occurs with the help of biological sources like plants, algae, enzymes, bacteria and fungi [41]. This biological synthesis makes the process more economical as well as eco-friendly as it eliminates the use of high energy, toxic chemicals, high temperature and pressure which makes this process more suitable for the production of nanoparticles.

Synthesis takes place with the help of two pathways; intracellular and extracellular synthesis. In intracelluar synthesis, nanoparticles that are synthesized inside the cell depend upon the metabolic pathway of algae where cell organelles or enzymes reduced the metal ions into zerovalent state. On the other hand, in extracellular synthesis, algae secrete enzymes that helps in the reduction of metal ions into the nanoparticles [32]. Basic procedure for synthesis of nanoparticles involves these four basic simple steps [42]:

1. Preparation of the algal extract from algal biomass in water by using cell disruption methods like heating or sonication.
2. Preparation of metal precursor salt stock.
3. Mix the salt and extract. Incubate the mixure for some time in continuous rotation at optimum temperature.
4. Purification of nanoparticles is performed with the help of centrifuge and followed by washing with ethanol. After that, dry these particles at 80°C for three hours. **Fig.2** explains the basic procedure for the synthesis of phyconanoparticles.

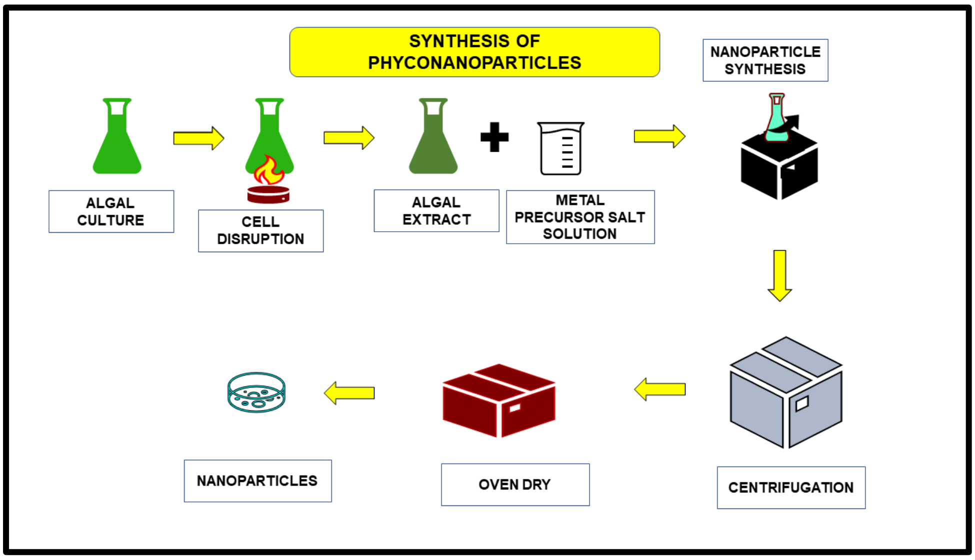


Fig. 2: Synthesis of Phyconanoparticles

Different nanoparticles are synthesized using different micro and macro algae. Few examples of green synthesis of nanoparticles from algae along with their optimum parameters are mentioned in the table 1.

Table 1
Phyconanoparticle synthesis and its parameters

Nanoparticles	**Algae**	**Salt concentration and Algal Extract Volume: Salt Solution**	**Cell Disruption:**	**Reaction Temperature**	**Reaction p^H**	**Agitation speed**	**Reaction duration /period**	**References**
Gold Nanoparticles	Gelidiella acerosa	1mM Gold Chloride	Heating at r 60 °C	37°C	-	-	20 minutes	[43]
Silver nanoparticles	C. vulgaris	10 mM AgNO3	-	25°C	5, 7 and 11	5000 rpm;	48 h	[44]
palladium nanoparticles	Chlorella vulgaris	1 mM $PdCl_2$	Drying	60°C	-	Magnetic Heater Stirrer	10 mins	[45]

Silver nanoparticle	Chlorella minutissima	1 mM Silver Nitrate	Heating (100°C for 30 min)	50°C for 25 mins	-	-	25 mins-2 h	[42]
silver nanoparticles	Sargassum longifolium	1 mM silver nitrate	Boiling (60∘C for 10 min.)	Room Temp	6.2, 6.8, 7.8 and 8.4	120 rpm	1 to 32 h	[46]
Silver Np	Caulerpa racemosa	10^{-3} M silver nitrate	-	room temperature	-	-		[47]
Silver Np	Padina sp.	0.01 M silver nitrate	Heating at 60 °C for 20 min	60 °C	-	-	48 h	[48]
Fe3O4 NP	Padina pavonica (Linnaeus)	0.1M $FeCl_3$	Boiling for 15 min	Room Temperature water	-	-	1 h 30 mins	[49]

2.3 **CHARACTERIZATION OF NANOPARTICLES**

Various techniques are used for the characterization of shape, size, structure, composition and magnetic properties of nanoparticles. It is observed that these methods provide the information of shape, size, surface and elemental composition of the nanoparticles. Initially, the first type of identification is visual characterization. In this, a color change is observed like silver nanoparticles changes its colorless solution of reaction mixture to brown color. Depending upon the concentration of nanoparticles, color varies. It is observed that a higher concentration of silver nanoparticles results in dark brown color [46]. Another technique that can be used to identify the synthesis of nanoparticles is UV-Vis spectroscopy. It is observed that when radiation hits the particles, a transition of electrons is observed from higher to lower energy levels. The formation of silver nanoparticles is observed at around 400-450 nm [50]. UV-Vis spectroscopy helps in the identification of size, concentration and aggregation of nano-sized particles [51].

A More advanced technique that is used for the analysis is the electron microscope. Many types of electron microscopes are used to determine morphology and structural characteristics. The most common is the Scanning Electron Microscope (SEM) [52]. Another technique is Transmission Electron Microscopy (TEM) which also provides a detailed understanding of structure as well as morphology of nanoparticles. SEM, TEM and energy dispersive X-ray give the elemental composition of nanoparticles [53]. Another technique which doesn't harm the sample is X-ray diffraction. It helps in the understanding of structural, compositional as well as the interaction of molecules with the metallic nanoparticles. It is used in the identification of crystallinity and size of nanoparticles [54]. Some spectroscopic techniques are also used for size determination and other features. Techniques like Fourier transform Infrared (FTIR), Raman spectroscopy (RS), X-ray photon electron and X-ray absorption spectroscopy are used for the size determination. Other optical absorption spectroscopic techniques are used to identify the mass concentration of nanoparticles in a sample. Another technique is Photoluminescence, which is a non-destructive technique and helps in the determination of elemental composition of nanoparticles [55].

Mass spectroscopy provides data related to molecular mass, elemental structure and surface properties. Inductively coupled plasma- Mass spectroscopy (ICP-MS) helps in the detection and quantification of nanoparticles. It helps in the size determination of nanoparticles [56]. Fluorescence correlation spectroscopy gives a detailed understanding of the binding kinetics of nanoparticles [57]. Zeta potential provides details of electric charge on the surface of nanomaterial sample [58]. Surface area is one of the most important feature of nanomaterial. As higher the surface area, higher will be the remediation capacity of pollutants by the nanoparticles. It is measured by Brunauer Enmett- Teler (BET) [59]. Magnetic property of the nanoparticle is identified by using Vibrating Sample Magnetometer (VSM). In the presence of a magnetic field, when vibrations are created, an embedded coil induces current. Other methods which are used for the identification of magnetic properties of nanomaterials are electron paramagnetic resonance spectroscopy (EPR) and superconducting quantum interference device (SQUID) [60]. These are few techniques which are used for the characterization of nanoparticles.

2.4 PHYCONANOREMEDIATION OF DYES

It is observed that phycoremediation is not able to achieve complete remediation and consider to be an expensive method of treatment. To overcome this many advanced techniques, like remediation of dyes with the help of phyconanoparticles, can achieve more than 90% of remediation within 5 to 30 minutes and make algae a potential candidate for remediation. Algae-based nanoparticles are used for the abatement of dyes which makes this technique eco-friendly as well as more efficient [41]. Nanoparticles show maximum removal efficiency due to their unique physical, chemical and magnetic properties. They have numerous adsorption sites because of these special features. These nano-sized particles help in the excellent abatement of dyes within few hours [32].

Rajkumar et al reported that *Chlorella vulgaris* synthesize silver nanoparticles of size 5-20 nm can remediate 96.51% methylene blue within three hours [61]. Silver phyconanoparticles are also able to remediate 84.60% of methyl red [62], methyl orange [63], congo red [63]. On the other hand, gold phyconanoparticles are also used in the photocatalytic degradation of dyes. Ramakrishna et al observed that *Sargassum tenerrimum* can synthesize 5-45 nm gold nanoparticles which are used for the abatement of Rhodamine B and Sulforhodamine 101 [64].

2.5 PHYCONANOREMEDIATION OF HEAVY METALS

Heavy metals like Cd, Cu, Cr, Ni, Co and Hg are abated by nanoparticles. Silver nanoparticles are extensively used in medical industries because of their unique characteristics. These particles are extensively used as anti-inflammatory, anti-biotics, dye removal and heavy metal removal [65]. Silver nanoparticles can remediate mercury, cadmium, chromium, cobalt, lead, etc. [66][67]. It is observed that remediation ability of silver nanoparticles is dependent on the reduction potential of heavy metals [66]. Thus, it can be concluded that for every different heavy metal, a different type of nanomaterial is required. Attasi and Nsiah reported that 20 nm silver nanoparticle is able to remediate 92.92% lead and 53.34% cobalt [68]. On the other hand, El-Tawil et al observed that silver-quartz nanocomposite enhances the removal efficiency of mercury to 96% within 60 mins [69].

Iron nanoparticles are able to remediate various heavy metals like Cr, Hg, Cu, Ni, and Cd [70][71]. Yang et al state that zerovalent iron nanoparticles reduce heavy metals by using the reduction mechanism [66]. Huang et al observed that every nanoparticle have different abatement mechanism for different kind of heavy metals [72]. Different mechanism which are observed are redox reaction, precipitation, co-precipitation and adsorption. Pumice nanoscale zero-valent iron nanoparticle of 20.2 nm diameter is able to remediate 107.1mg/g mercury and 106.9 mg/g chromium [71]. Metal oxide nanoparticle like Iron oxides Geothite is able to remediate V, Mn, Ni, Co, Zn, Th, etc. [66] [73]. Metal nanoparticles like silver, palladium, gold, iron nanoparticle and so forth are used for wastewater treatment.

Many algal species like *Sargassum muticum*, *Turbinaria ornate*, *Sargassum polycystum*, *Turbinaria conoides*, *Gilidiella acerosa* , *Sargassum wightiigrevilli*, *Padina pavonica*, *Colpmenia sinusa* are able to synthesize different size of silver nanoparticles [74]. Still, very few studies for the removal of heavy metals with algal based silver nanoparticles are reported. Silver and gold nanoparticles synthesized by Nannochloropsis sp and C. vulgaris are able to remediate 66.10-68.86% lead [75]. Graphene Oxide nanoparticles synthesized by three different algal strains are able to remediate more than 82% of lead within 30 mins [76]. Palladium Nanoparticles show excellent remediation ability of arsenic as well as lead**.** Chlorococcum sp. MM11 synthesizes 20-50 nm and spherical iron nanoparticles which are able to remediate 92% of chromium [77].

CHAPTER 3 MATERIALS AND METHODOLOGY

3.1 MATERIALS

Chlorella minutissima was acquired from the Plant Biotechnology Laboratory, Delhi Technolgical University, New Delhi, India. Silver nitrate and other Chemicals required for the preparation of BG-11 were obtained from the same lab. All the glasswares were thoroughly washed with lab detergent and treated with sulfuric acid, then washed with deionized double distilled water and finally dried prior to use. Milli-Q water was used for the preparation of required solutions.

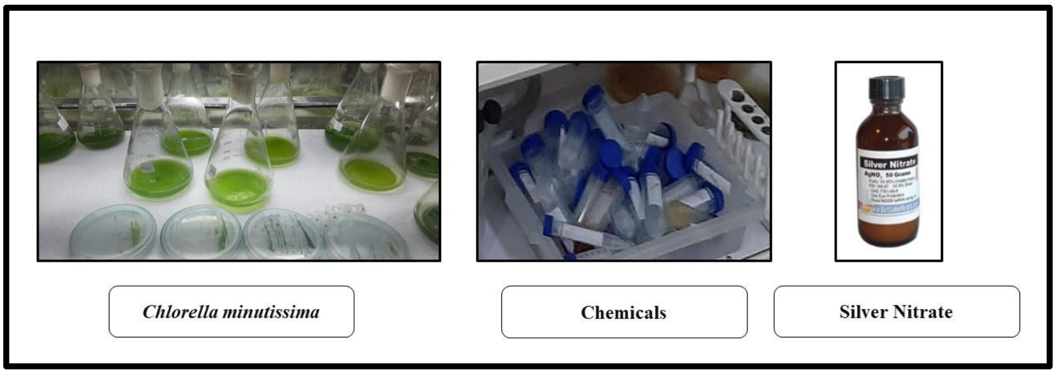


Fig. 3: Material Required for the synthesis of silver phyconanoparticles

3.2 CULTIVATION OF MICROALGAE

Chlorella minutissima was cultivated in 1 L flask with 750 ml volume of BG-11 (Ph 8-9). The flask was kept at 25°C, with photoperiod of 16 hr light : 8 hr dark with 50-100 μmol photons m^{-2} s^{-1} . Microalgal biomass was collected by using centrifuge at 5,000 rpm, 4°C for 10 minutes. Pellet was cleaned by washing with ddH_2O and repeat this process thrice and supernatant was discarded. Wet biomass was dried in glass plates in hot air oven at 60°C for 24 hours. This dried biomass was collected from the glass plates and converted into powdered form using mortar and pestle.

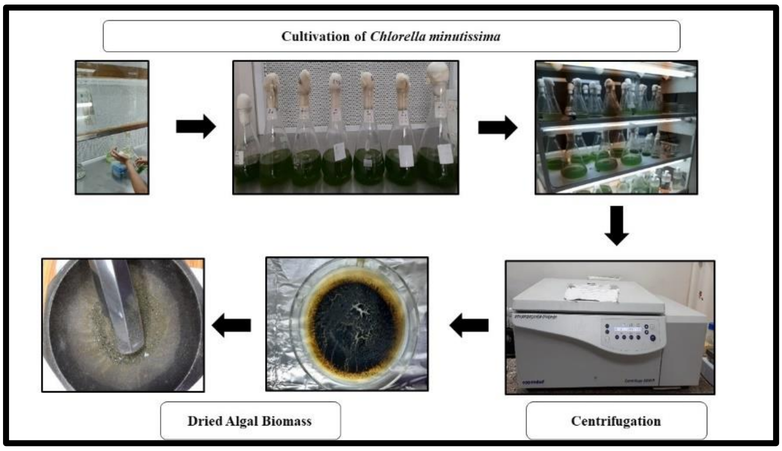


Fig. 4: Cultivation of Microalgae Strain

3.3 <u>ALGAL EXTRACT PREPARATION</u>

Dried powdered *Chlorella minutissima* was mixed with distilled water and kept for sonication for 30 minutes at 70 amplitude and 1 cycle. After that it is heated (100°C, 30 minutes) for the preparation of Cm extract. The algal extract was then centrifuged (10,000 rpm;10 minutes) and suspended solids were discarded and supernatant was used for nanoparticles synthesis.

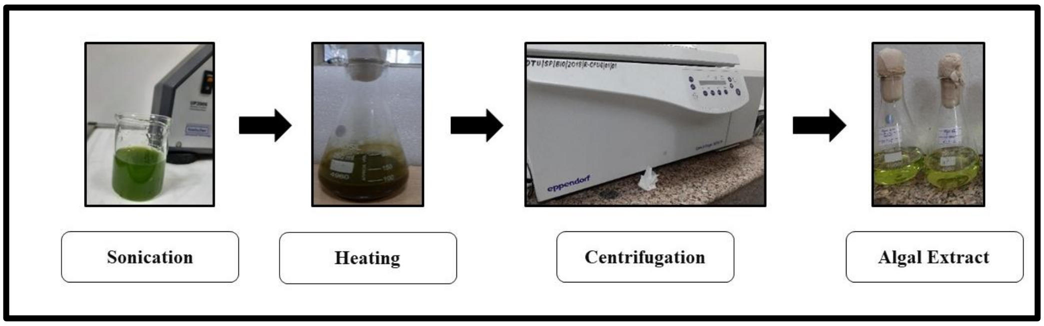


Fig. 5: Algal Extract Preparation

3.4 <u>SYNTHESIS OF Cm.AgNp</u>

Green synthesis of AgNp were carried out by adding 10 ml of algal extract in 90 ml $AgNO_3$ colloidal solution and kept at rotatory shaker, 25°C overnight. The reaction mixtures changes its color from colorless to yellowish brown which indicated silver nanoparticles synthesis.

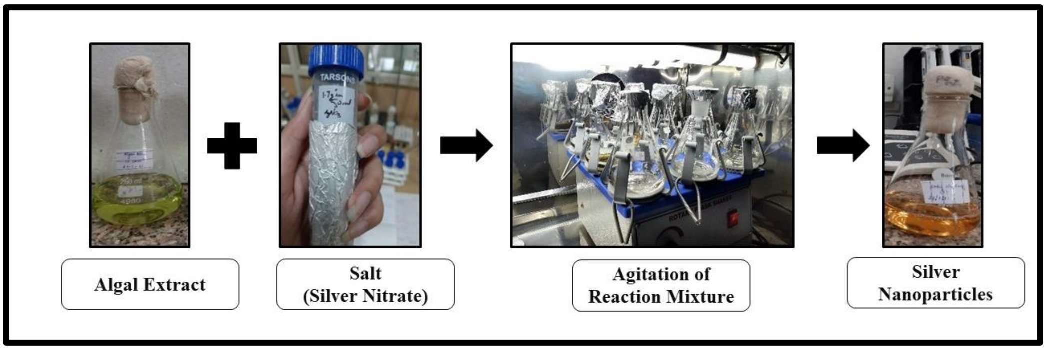


Fig. 6: Synthesis of silver Nanoparticles

3.5 <u>CHARACTERIZATION OF Cm.AgNp</u>

3.5.1 Visual Characterization:

This is the first characterization technique which helps in the identification of silver nanoparticles synthesis. The color change is observed after mixing the algal extract with the salt.

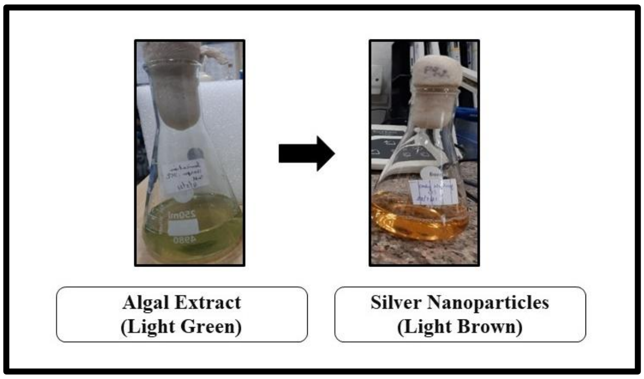


Fig. 7: Visual Characterization of Silver Nanoparticles

3.5.2 UV-Visible Spectroscopy:

The bioreduction of silver ions was recorded using UV–Visible spectrophotometer full scan (wavelength ranging from 200 to 700 nm.

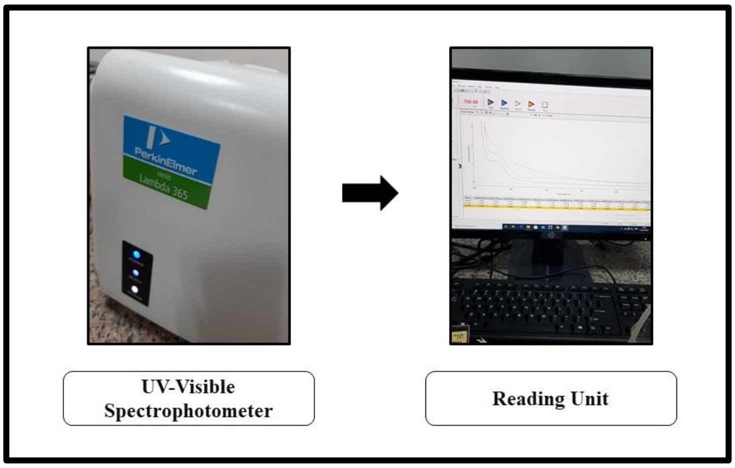


Fig. 8: UV-Visible Characterization Technique for Silver Nanoparticles

3.6 OPTIMISATION OF REACTION CONDITIONS FOR Cm.AgNp

3.6.1 Effect of Cell-Disruption Technique:

Different Cell-disruption techniques were performed to know the optimum cell-disruption technique for maximum production of silver nanoparticles. Experiments were conducted using, autoclave, heating (100°C for 30 minutes) and sonication (70 amplitude, 1 cycle for 30 minutes) to know the best technique for the cell-disruption.

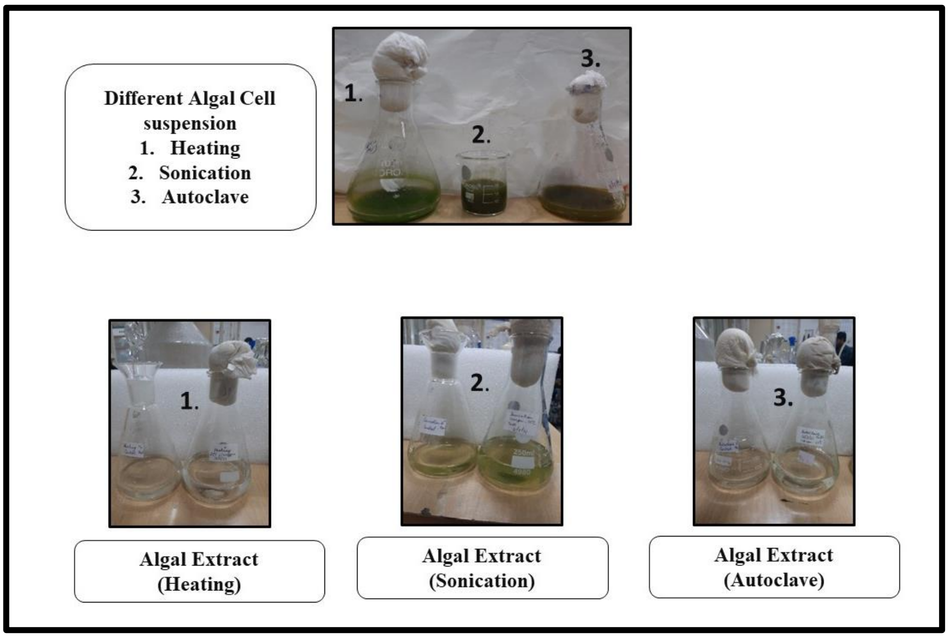


Fig. 9: Initial stage of Cell-Disruption Technique

3.6.2 *Effect of Salt Concentration*

Different silver nitrate concentrations are used to obtain optimum salt concentration for the maximum synthesis of AgNp. Experiment was conducted at eight different concentrations i.e. 0.1mM, 0.5mM, 1mM, 5mM, 10mM, 20 mM, 50 mM and 100mM.

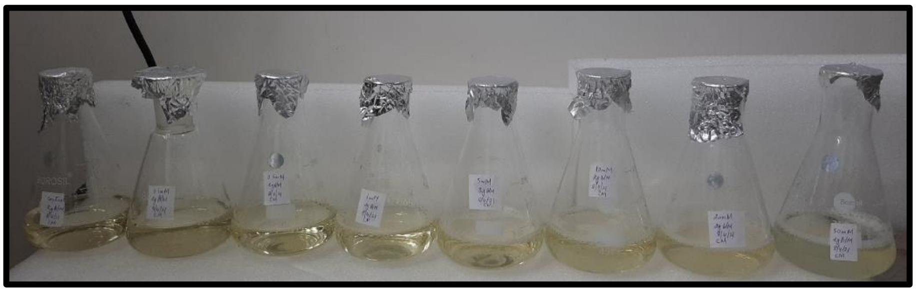


Fig. 10: Different Salt concentration at zero hour.

3.6.3 Effect of Biomass Concentration Variation

Different dried *Chlorella minutissima* biomass concentrations were used to obtain optimum biomass concentration for the maximum synthesis of silver nanoparticles. Experiment was conducted at eight different concentrations i.e. 1g, 2g, 3g, 4g and 5g.

3.6.4 *Effect of pH*

Different pH range from 4 to 12 of reaction mixture were prepared using 1N NaOH and 1M HCl and this study is carried out to know the effect of different pH on the synthesis of silver nanoparticles.

Fig. 11: Different pH of reaction mixture at zero hour

3.6.5 *Effect of Contact Time*

To find out the effect of contact time on the synthesis of green silver nanoparticles. Experiment was conducted for 72 hrs and readings were recorded at different time intervals i.e. 1 hr, 2 hrs, 4 hrs, 24 hrs, 48 hrs and 72 hours.

3.7 PURIFICATION OF Cm.AgNp

The mixture obtained was centrifuge at 10,000 rpm, 25°C for 10 min and discard supernatant. The obtained pellet was washed with ethanol for three times.

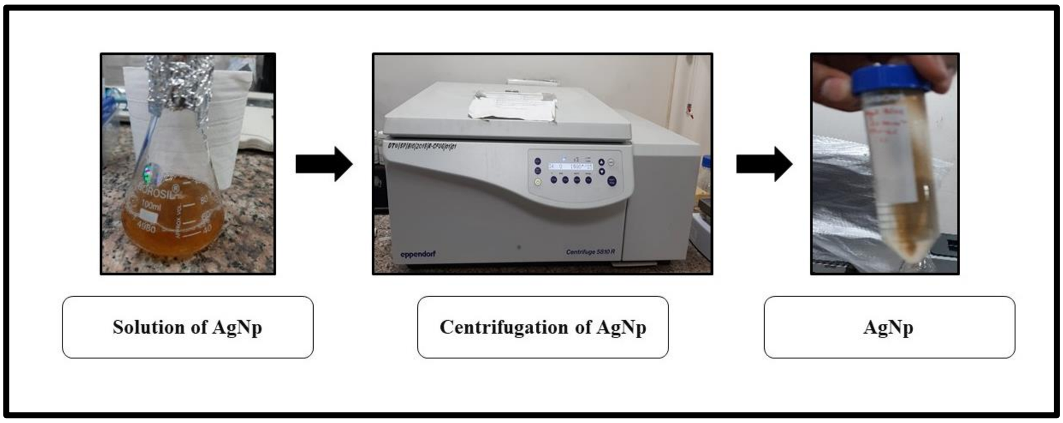


Fig. 12: Purification of Silver Nanoparticles

3.8 DRYING OF Cm.AgNp

These particles are dried at 80°C for 3 hours and then grinded into the powder using mortar and pestle.

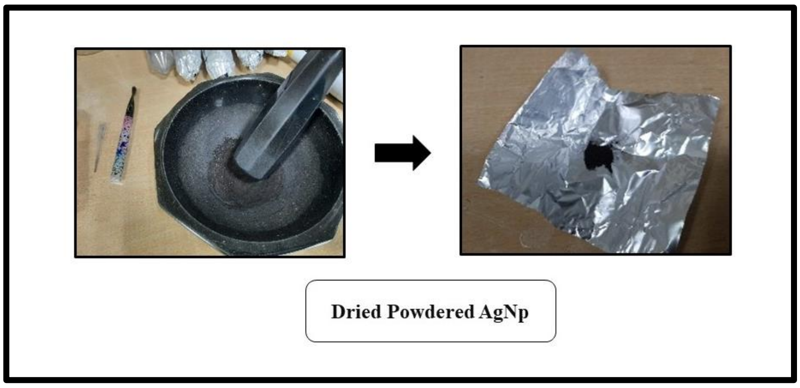


Fig. 13: Drying of Silver Nanoparticles

3.9 APPLICATION OF PHYCONANOPARTICLES

3.9.1 Photocatalytic Degrdation of Different Dyes

The photocatalytic degradation of five different dyes MB, MO, CV, EY and EB of concentration 10^{-4}M is carried out along with 3ml of sodium borohydrate (NaBH4) solution (1.74 mM) at constant light, 120 rpm, 25°C for 1 hour with the help of 10 mg of silver nanoparticles. Readings are taken after centrifugation at 5 minutes and at 1 hour with the help of UV-Visible Spectrometer.

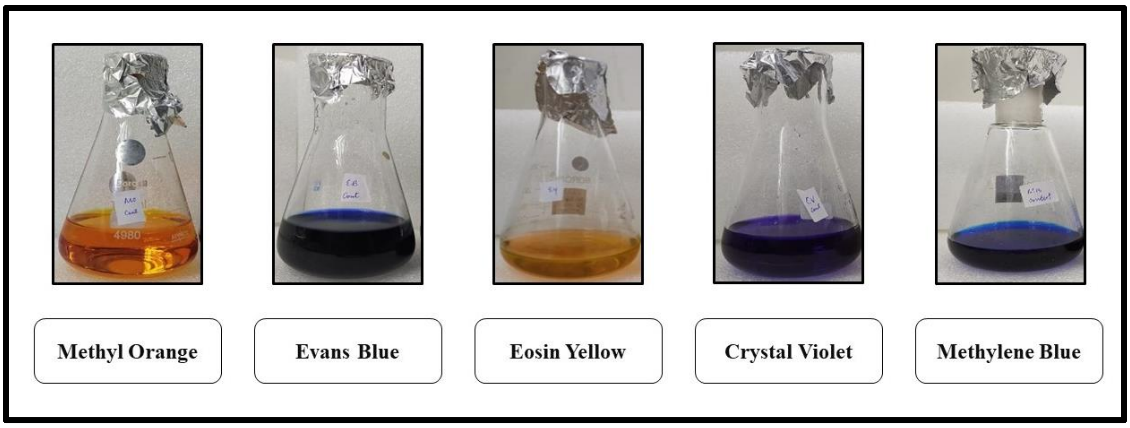


Fig. 14: Different Dyes at zero hour

3.9.2 <u>Phyconanoremediation of Different Heavy Metals</u>

All batch adsorption experiments were conducted by mixing 10 mg adsorbents (silver phyconanoparticles) with a known volume of 5g/L mercury, chromium, cobalt, nickel, cadmium and copper with 120 rpm, 25°C for 3 hours. Readings were estimated after centrifugation at the interval of 1 hour for 3 hours by using UV-Visible spectrometer.

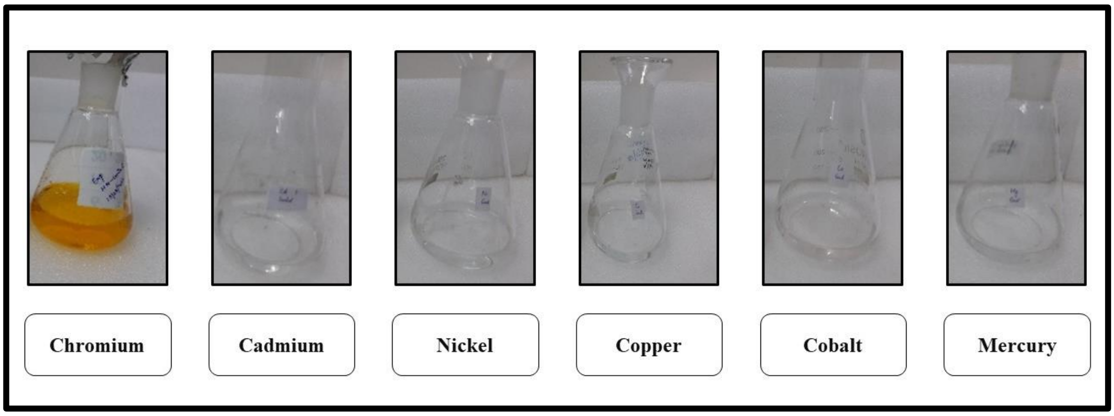


Fig. 15: Different Heavy Metals at Zero hour

3.10 <u>REMOVAL EFFICIENCY</u>

Based on the spectrophotometer data, the RE of metal ions were calculated by using the formulae:

Removal Efficiency = $\{(C_i - C_f)/ C_i\} . 100$

Where, C_i: Initial metal ion concentration (mg/L), and

C_f: Final concentration of metal ion in the solution (mg/L)

CHAPTER 4 RESULTS AND DISCUSSION

4.1 BIOSYNTHESIS OF Cm.AgNp

When silver nitrate is added to the Cm extract, bioreduction of silver ions starts within few minutes. This can be easily observed when colorless or light green reaction mixture changes to light brown color and as the contact time increases intensity of color also increases. This change in color indicates the biosynthesis of silver nanoparticles as shown in (Fig.16). This happens due to surface plasmon resonance. Similar results of color change for the biosynthesis of AgNp was also reported by Raghuwanshi et al. using *Chlorella minutissima* [42].

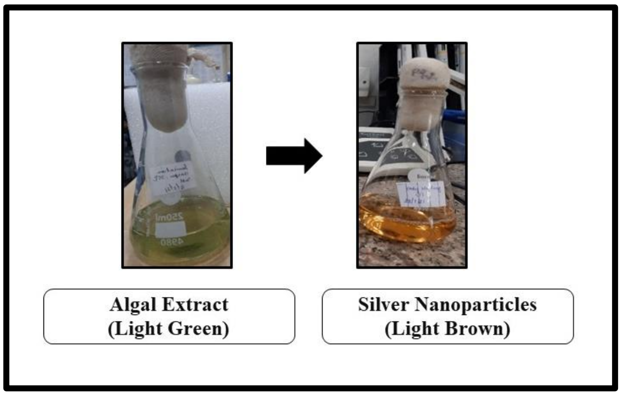


Fig. 16: Biosynthesis of silver Nanoparticles.

4.2 CHARACTERIZATION OF Cm.AgNp

4.2.1 *Visual Characterization*

Initially, the first type of identification is visual characterization. In this, a color change is observed like silver nanoparticles changes the colorless solution of reaction mixture (silver nitrate and extract) into light brown color. Depending upon the concentration of nanoparticles, salts, biomass concentration or pH, color varies. It is observed that a higher concentration of silver nanoparticles results in dark brown color as shown in the fig.17. The similar results of visual characterization are also observed by Rajeshkumar et al. using Sargassum longifolium [46].

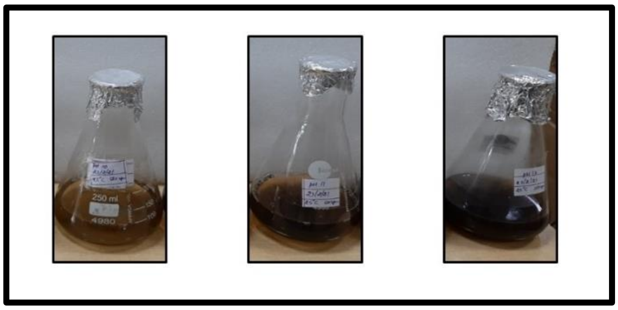


Fig. 17: Visual Characterization of Silver Nanoparticles

4.2.2 *UV- Visible Spectroscopy*

Another technique that can be used for the identification of nanoparticles is UV-Visible spectroscopy. It is observed that when radiation hits the particles, a transition of electrons is observed from higher to lower energy levels. The formation of silver nanoparticles is observed at around 400-450 nm as shown in the fig.18. Similarly, Al-Qahtani also showed synthesis of silver nanoparticles at 420 nm using Ficus tree (Ficus Benjamina) [67].

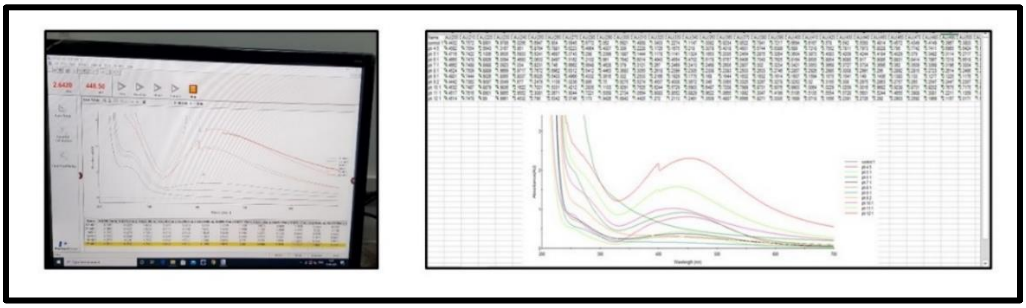


Fig. 18: Results of UV-Visible Spectroscopy

4.3 Optimisation of Reaction Conditions for Cm.AgNp

To know the optimum parameters for the maximum synthesis of silver phyconanoparticles, different reaction conditions are performed in different experimental set-ups.

4.3.1 *Effect of Cell-disruption Method*

It is observed that maximum cell-disruption is carried out in the autoclave as compared to sonication and heating as shown in the fig.9 but maximum synthesis of silver nanoparticles is carried out in the heating> sonication > autoclave as shown in fig.19. Thus, autoclave is not an optimum technique for the cell disruption. Therefore, in the further experiments to get the maximum cell-disruption for the synthesis of maximum amount of AgNp combination of sonication along with the heating technique is carried out.

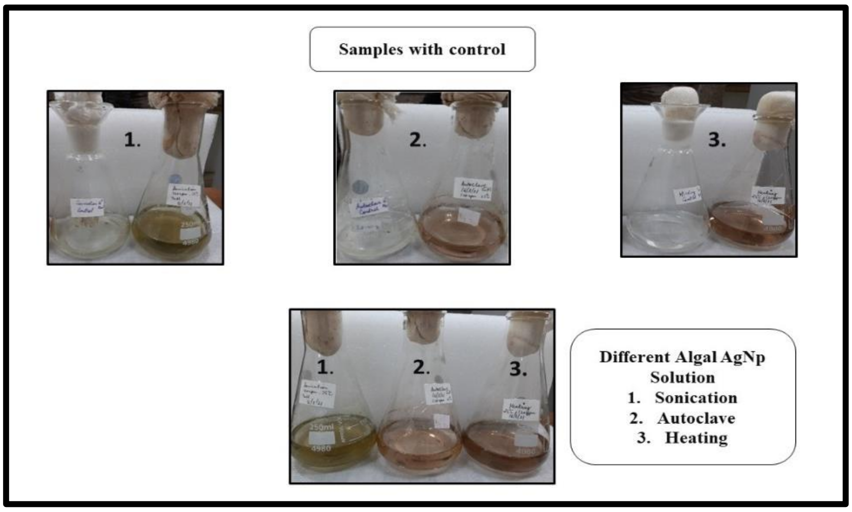


Fig. 19.1: Pictures of Cell-Disruption Results

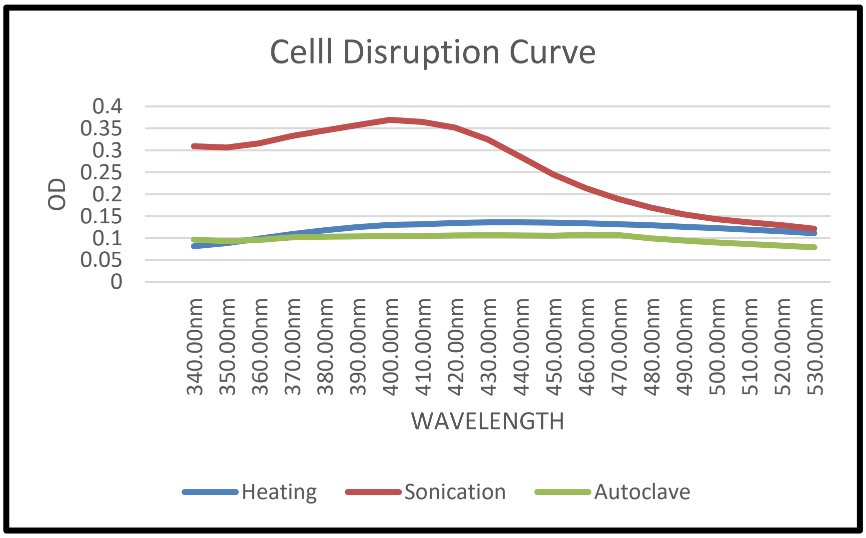


Fig. 19.2: Graphical Result of Cell-disruption Technique

Fig. 19: Results of Cell-disruption Technique

4.3.2 Effect of Salt-Concentration Variation

Different salt concentrations are used for the synthesis of silver nanoparticles. It is observed that 0.1mM silver nitrate is not a good amount for the synthesis of nanoparticles, as after 48 hours, no synthesis is observed. Other different concentrations like 0.5mM, 1mM, 5Mm, 10mM, 20 mM, 50 mM and 100mM leads to synthesizes of algal based silver nanoparticles. When 10 ml of 100mM $AgNO_3$ is added to the algal extract, within few minutes, precipitation reaction takes place and it can be clearly seen that this concentration is also not good for the synthesis of the silver nanoparticles as it leads to the instant agglomeration of silver nanoparticles.

The best salt concentration which gives excellent results is 5mM and 10mM $AgNO_3$. It is observed that it start synthesises within 2 hours. It is observed that as the concentration of the salt increases, the reduction time for silver ions or synthesis of silver nanoparticles decreases. Thus, it can be stated that concentration of salt is directly related to synthesis of silver nanoparticles as shown in fig. 20 but further increase in the concentration (after 50mM) of metal precursor will lead to the agglomeration of silver nanoparticles. Similarly, Uzair et al. also stated the same results for the effect of reactant concentration on the synthesis of silver nanoparticles [78].

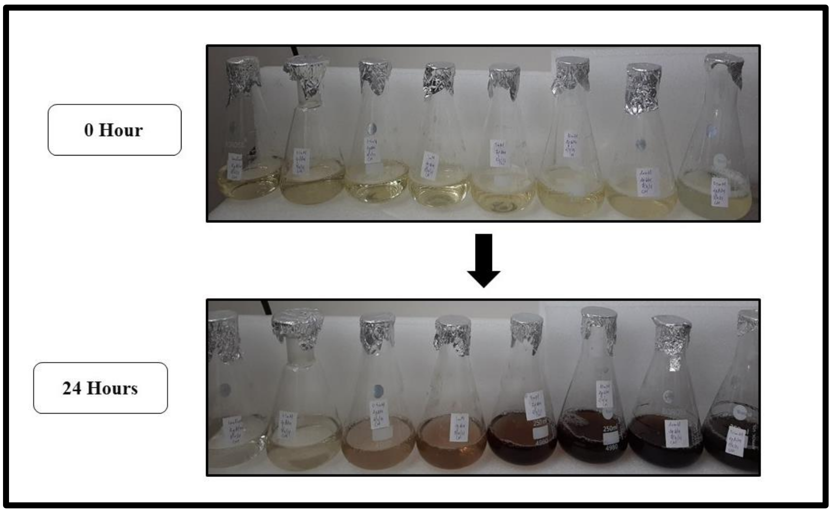


Fig. 20.1: Pictures of Salt Variation Experiments

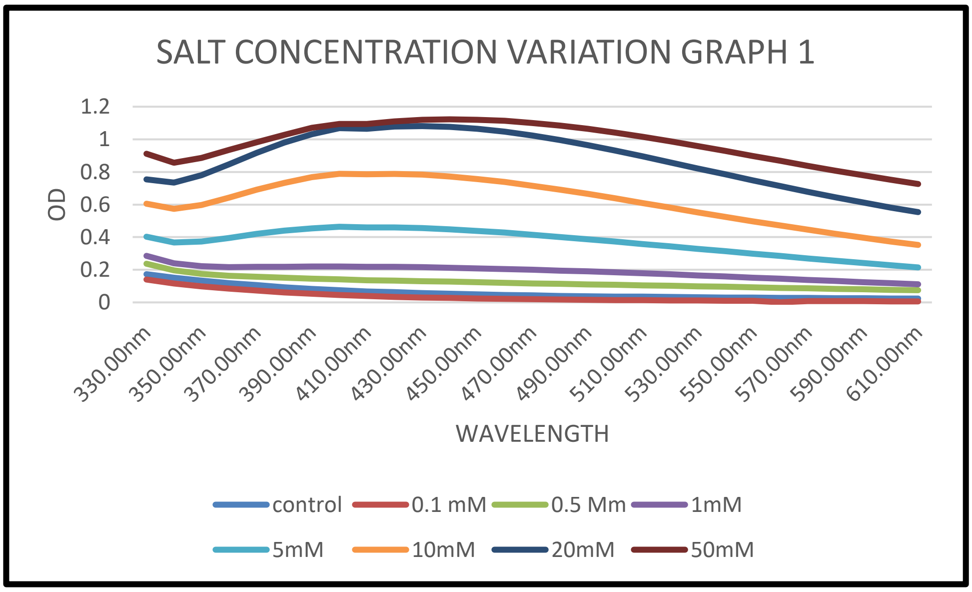


Fig. 20.2: Graphical Result of Salt Variation Experiments

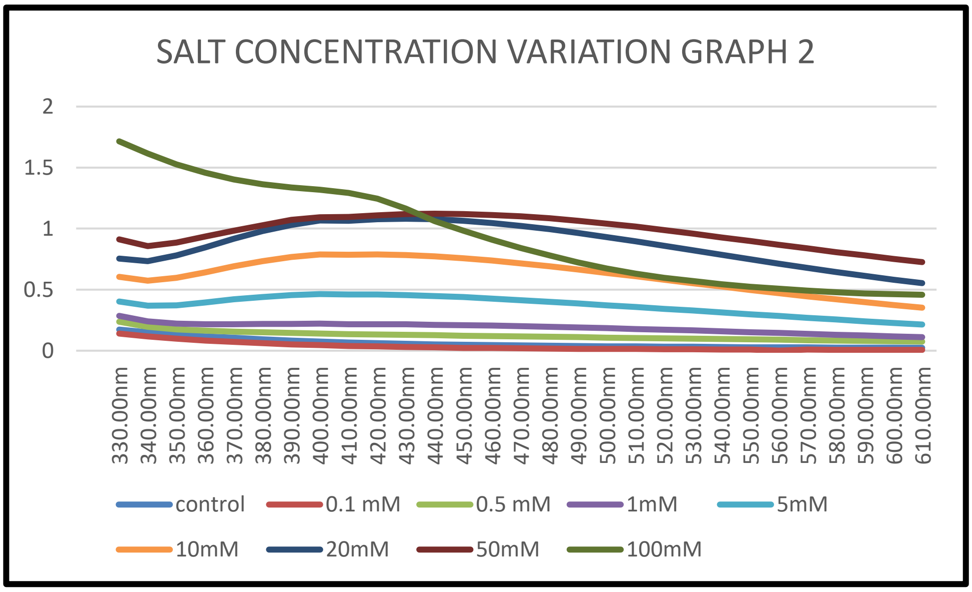


Fig. 20.3: Graphical Result of Salt Variation Experiments
Fig. 20: Results of Salt Variation Experiments

4.3.3 Effect of Biomass Variation

Different biomass concentrations are used for the synthesis of algal based silver nanoparticles and it is observed that as the concentration of biomass increases leads to the increase in the production of more silver nanoparticles within less time as shown in the figure. The intensity of the surface plasmon band increases as we increase the biomass concentration. As we increase the algal biomass concentration, there is a blue shift which was also studied by Aboelfetoh et al. This indicates the decrease in the size of nanoparticles, with the increase of biomass concentration [63].

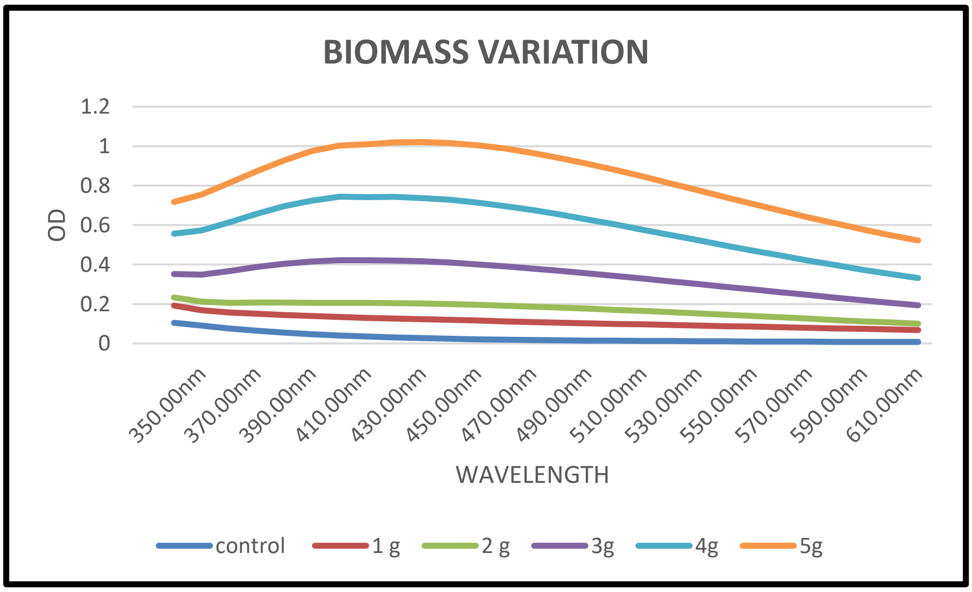


Fig. 21: Results of Biomass Variation Experiment

4.3.4 Effect of pH Variation

Different pH value from 4 to 12 of reaction mixture were prepared using 1N NaOH and 1M HCl and it is observed that all the range of pH leads to the synthesis of different color of the nanoparticles with different time frames and at different wavelength as shown in fig. 22. It can be visually seen that different colors of silver nanoparticles are produced at different range of pH. At pH 12, instant synthesis occurs within few minutes (2-3 minutes) and then reaction mixture is precipitated. All other pH ranges lead to the synthesis of different silver phyconanoparticles according to the their pH. It is observed that pH 4 gives reddish brown color as well as different UV-Visible peak. Similarly, pH 5 and 6 gives different color at different wavelength. pH 7, 8 and 9 shows dark yellowish color at different range of wavelength. It is observed that higher the pH leads to the synthesis of more dark brown nanoparticles as shown in the fig. 22. Thus, it can be stated that color intensity increases with increase in pH. Moreover, broader peaks are observed at lower pH as compared to higher due to the formation of anisotropic nanoparticles [46]. Thus, it can be concluded that different pH ranges leads to the formation of different nanoparticles in terms of shape, size and concentration.

Fig.22.1: Picture of pH Variation Experiments

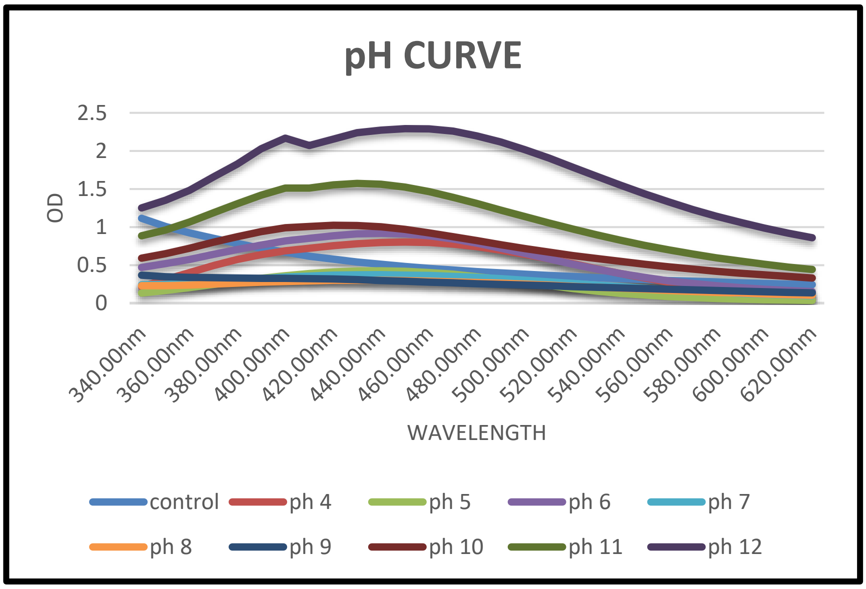


Fig.22.2: Graphical Representation of pH Variation Experiments

Fig.22: Results of pH Variation Experiments

4.3.5 Effect of Contact Time

It is observed that as the contact time between the salt and algal extract increases, intensity of the peak increases. Thus, it can be stated that as the reaction time increases, concentration of the phyconanoparticles also increases as shown in the fig.23. This study is also supported by Aboelfetoh et al., they stated that silver nanoparticles are very stable and doesn't lead to the aggregation of particles even after 5 months [63]. This might be due to the presence of capping agents like proteins, vitamins, or functional groups like hydroxyl present in the algae.

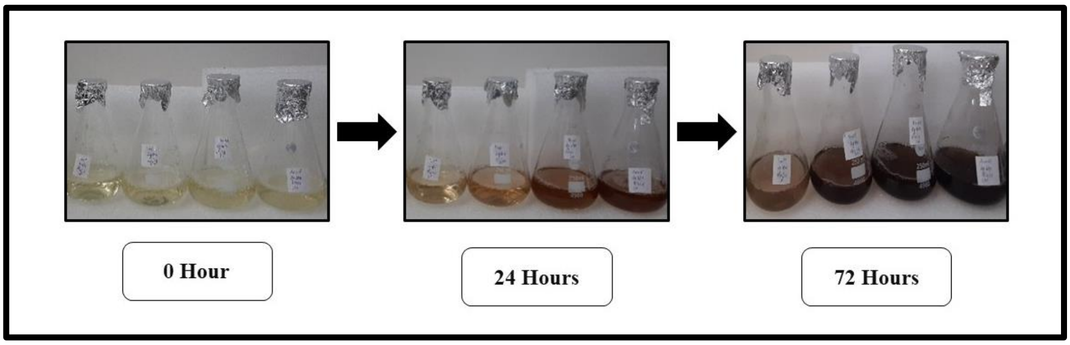


Fig. 23.1: Picture of Contact Time Experiments

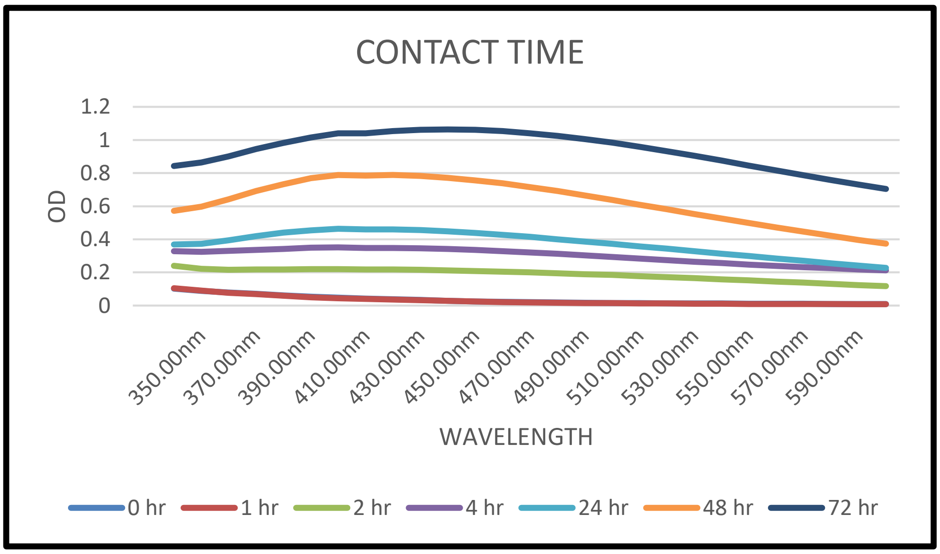


Fig. 23.2: Graphical Representation of Contact Time Experiments
Fig. 23: Results of Contact Time Experiments

4.4 APPLICATION OF PHYCONANOPARTICLES:

4.4.1 Photocatalytic Degradation of Dyes

The photocatalytic degradation of dyes is carried out in the presence of sodium borohydride at 25°C, 120 rpm for 1 hour. Its performance was assessed in the reduction of different dyes like EY, MB, EB, MO and CV. It is observed that as soon as silver nanoparticles were added, they are able to remediate more than 96% of EY, MO and EB and more than 87% in the case of CV and MB within 5 minutes. Various different algal based nanoparticles removal efficiency for dyes are 50.27% of 60 mg/L methyl orange [79], 95% degradation at 72 h for 10mg/L methylene blue [80], 85% for crystal violet [81], 90.12% for eosin yellow [82], and till now no studies were conducted by silver nanoparticles for the photocatalytic degradation of Evans Blue. Thus, it can be concluded that algal-based silver nanoparticle is more efficient for the degradation of these dyes. Further increase in time have not shown any significant change in the removal efficiency of these dyes. Similarly, Aboelfetoh et al. also observed photocatalytic degradation of congo red within short span of time and after that no significant change is observed by them [63]. This might be due to the apparent equilibrium at 1 hour.

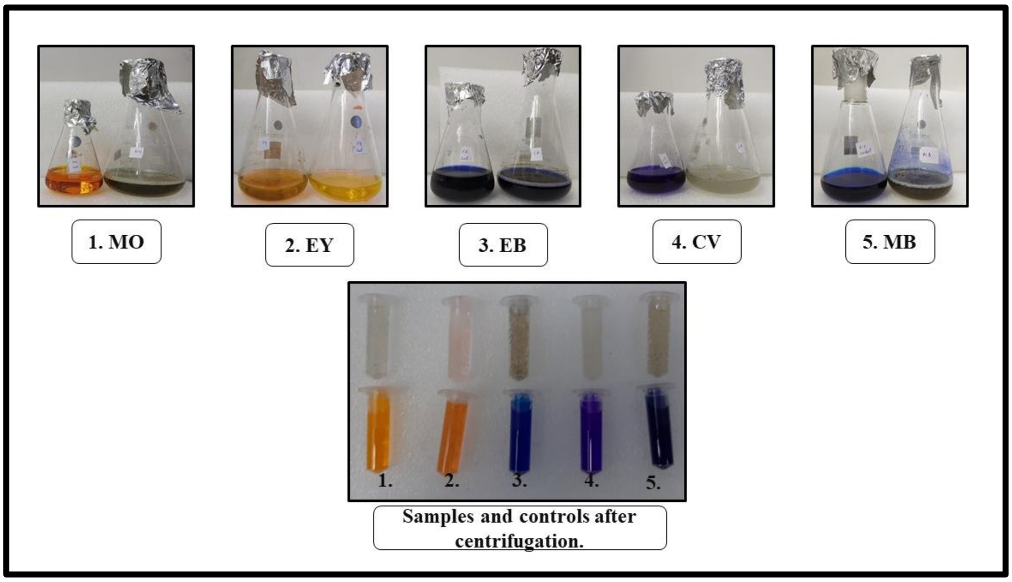


Fig. 24: Result of Photocatalytic Degradation of dyes

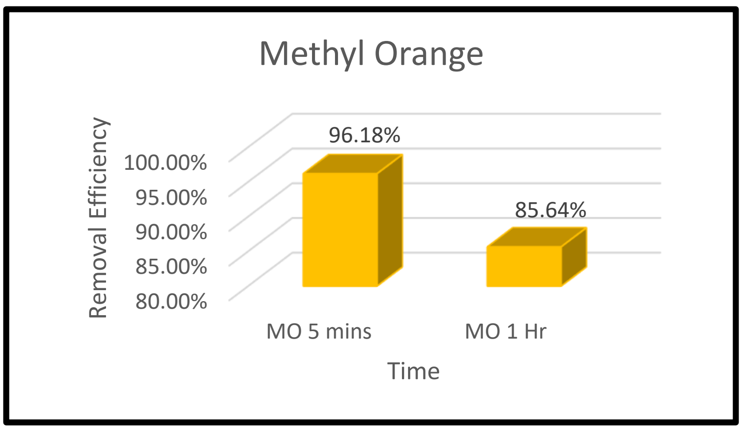


Fig. 24.1: Removal Efficiency of Methyl Orange

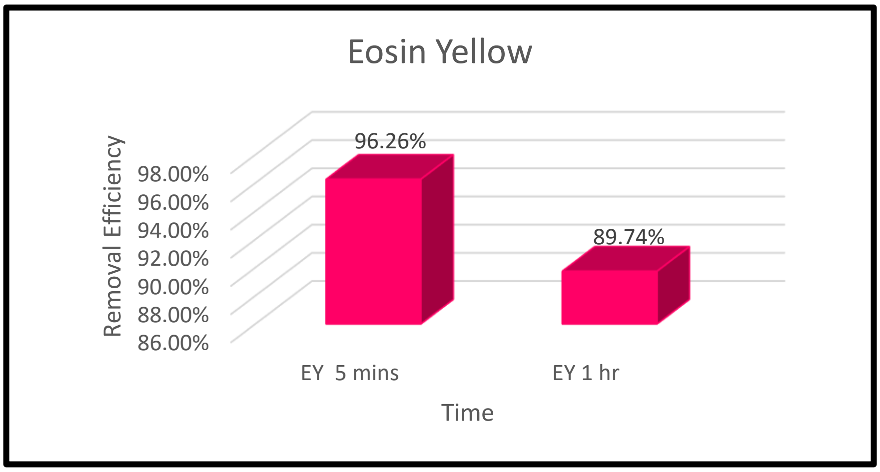


Fig. 24.2: Removal Efficiency of Eosin Yellow

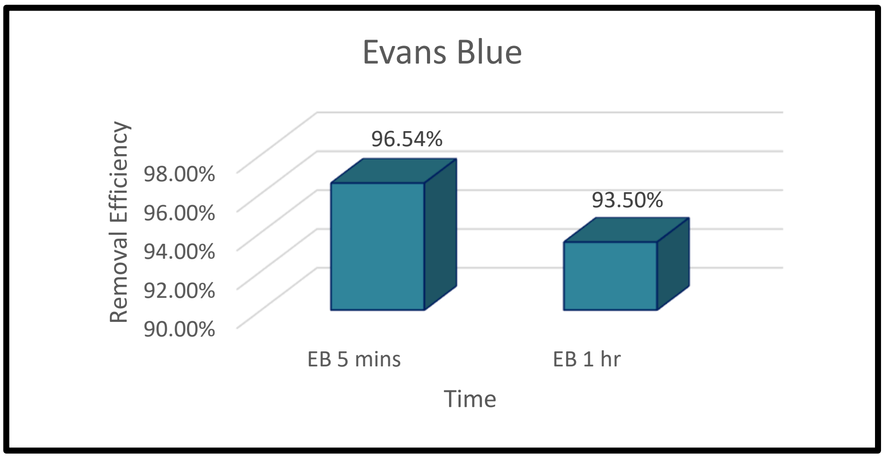


Fig. 24.3: Removal Efficiency of Evans Blue

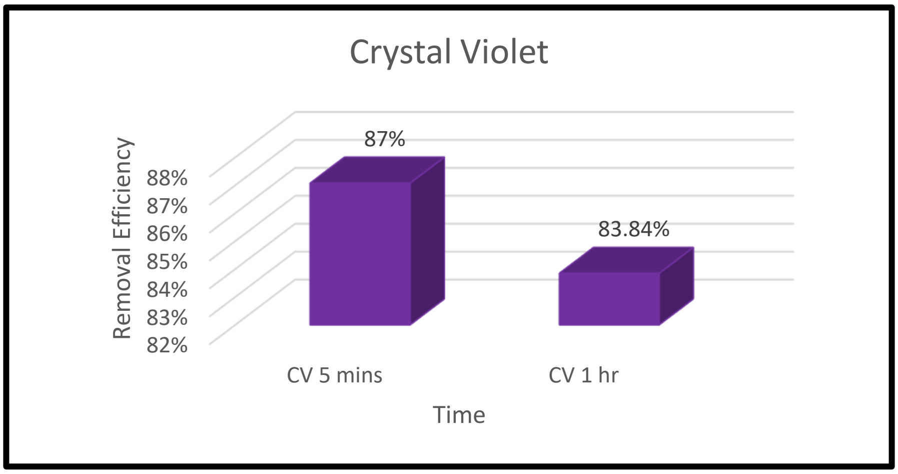


Fig. 24.4: Removal Efficiency of Crystal Violet

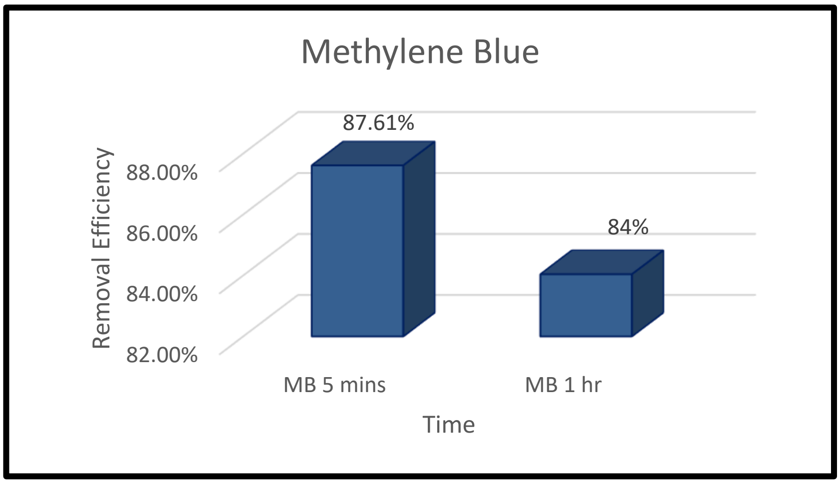


Fig. 24.5: Removal Efficiency of Methylene Blue

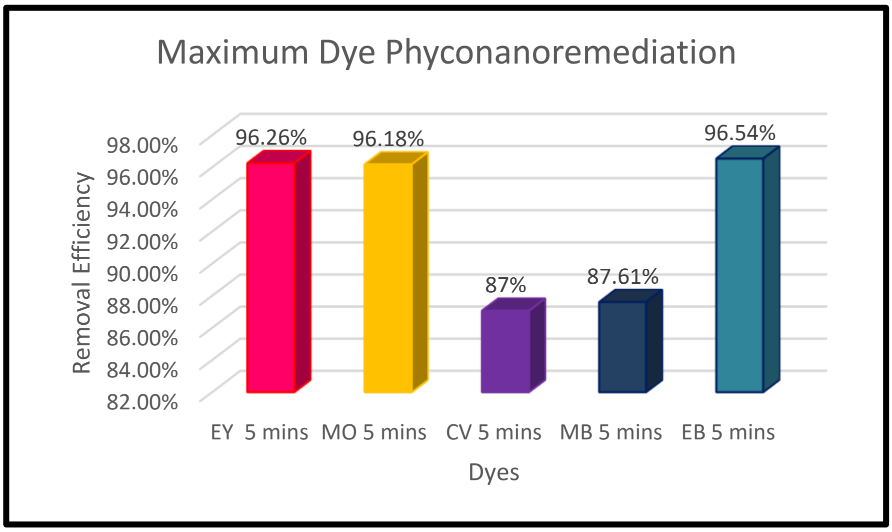


Fig. 25: Maximum Dye Phyconanoremediation

4.4.2 Phyconanoremediation of Heavy Metals

Algal-based AgNp were studied for the abatement of different heavy metals like Mercury, cadmium, nickel, copper, cobalt and chromium. It is also observed that removal of heavy metals takes place within 1 to 2 hours which is very less as compare to Phycoremediation. Silver nanoparticles can abate 65.18% nickel, 45.83% copper, 42% mercury, 33.84% cadmium and very less efficient for cobalt (4.5%) and chromium (0.5%). It is observed that silver phyconanoparticle is not able to remediate more than 0.5% chromium and 4.5% cobalt even after 24 hours. Thus, it can be concluded that Cm.AgNp are less effective for the remediation of cobalt and chromium but it is effective for nickel, copper, mercury and cadmium as it is able to remediate them within 1 hour. Further increase in time have not shown any significant change in the removal efficiency of these heavy metals, this statement is also supported by Al-Qahtani in the case of cadmium removal by Benjamina leaves based silver nanoparticles[67]. It can be due to the attainment of apparent equilibrium as stated by Aboelfetoh et al. [63]. It might be due to the dependence of remediation rate on several abatement factors like adsorbent dose, pH, temperature, etc. This removal efficiency can be enhanced by the use of biological compoments. Till now, no studies are conducted for the remediation of mercury, nickel, cobalt by algal based nanoparticles. Moreover no studies are reported for the remediation of these heavy metals by silver phyconanoparticles but other nanoparticles like iron-based nanoparticles [77], cadmium based nanoparticles [83], graphene oxide nanoparticles [76] are studied for the remediation of chromium, cadmium, and copper respectively. Thus, more studies are required to explore this area of research as it is showing excellent results in the phycocatalytic degradation of dyes.

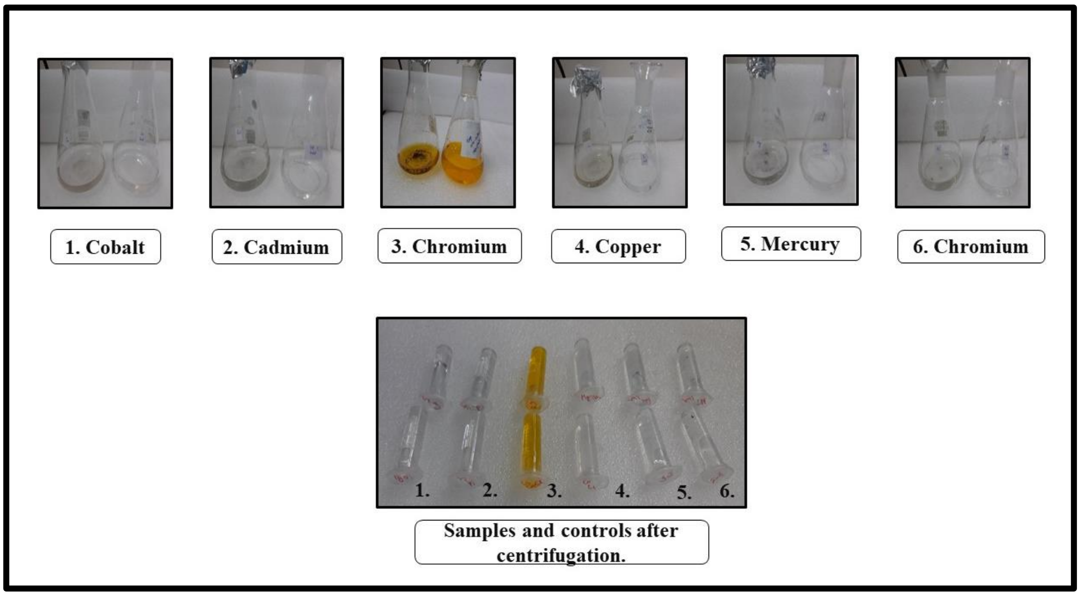


Fig. 26: Result of Phyconanoremediation of Heavy Metals

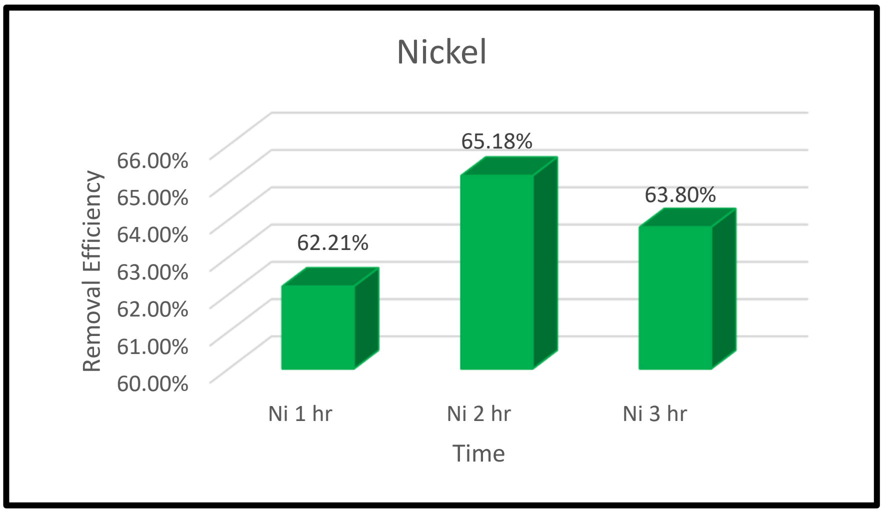


Fig. 26.1: Removal Efficiency of Nickel

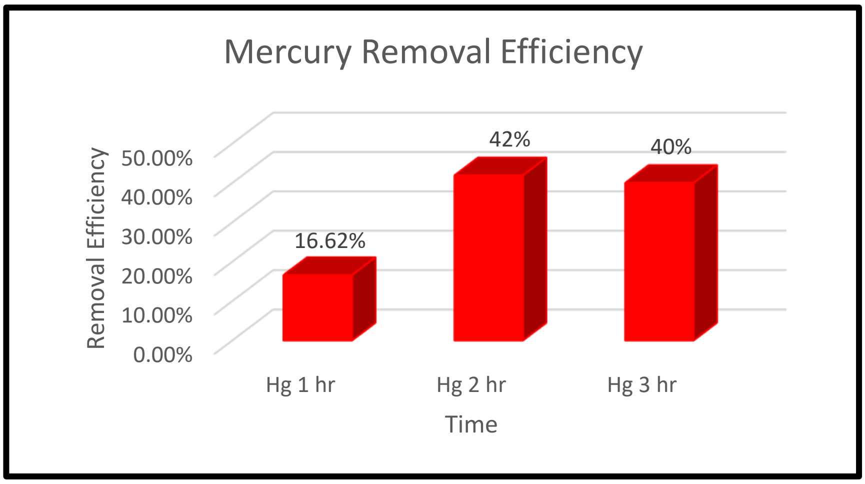


Fig. 26.2: Removal Efficiency of Mercury

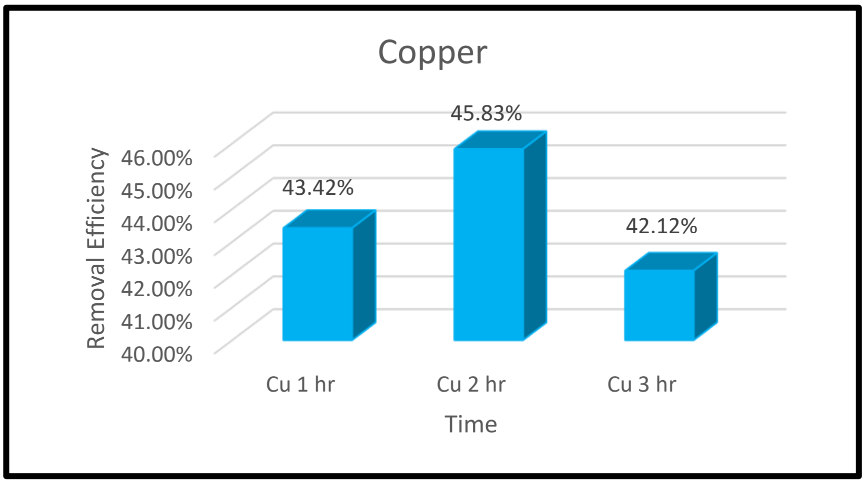


Fig. 26.1: Removal Efficiency of Copper

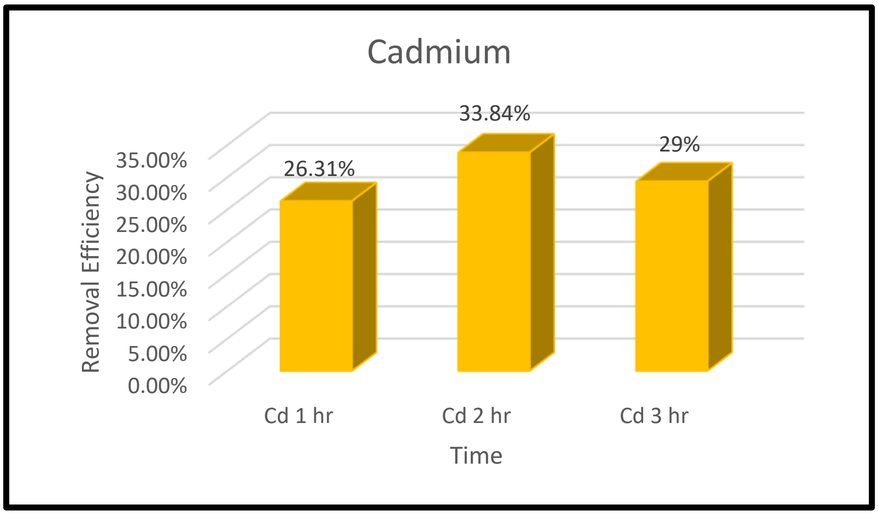


Fig. 26.1: Removal Efficiency of Cadmium

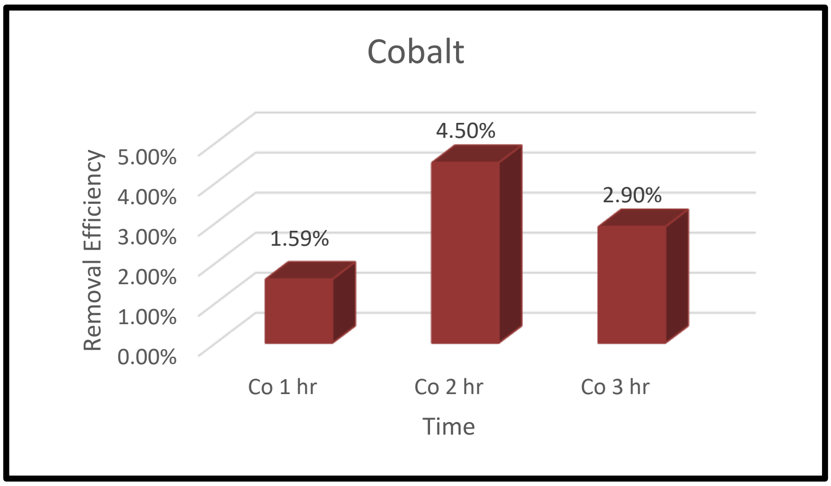


Fig. 26.1: Removal Efficiency of Cobalt

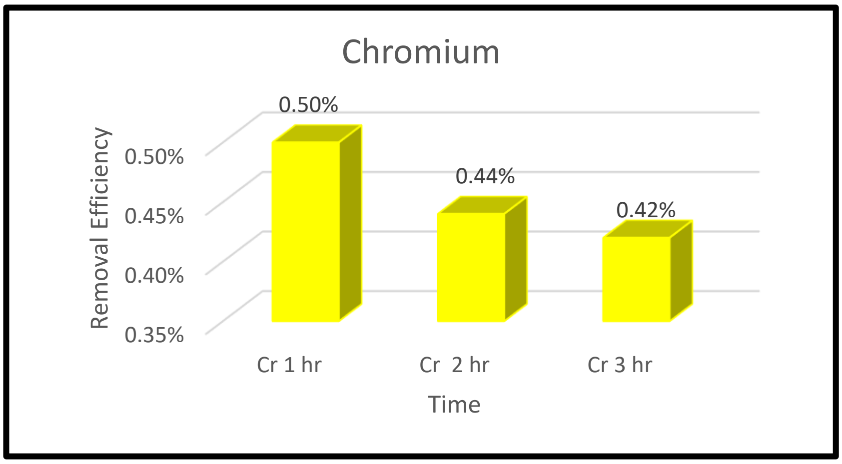


Fig. 26.1: Removal Efficiency of Chromium

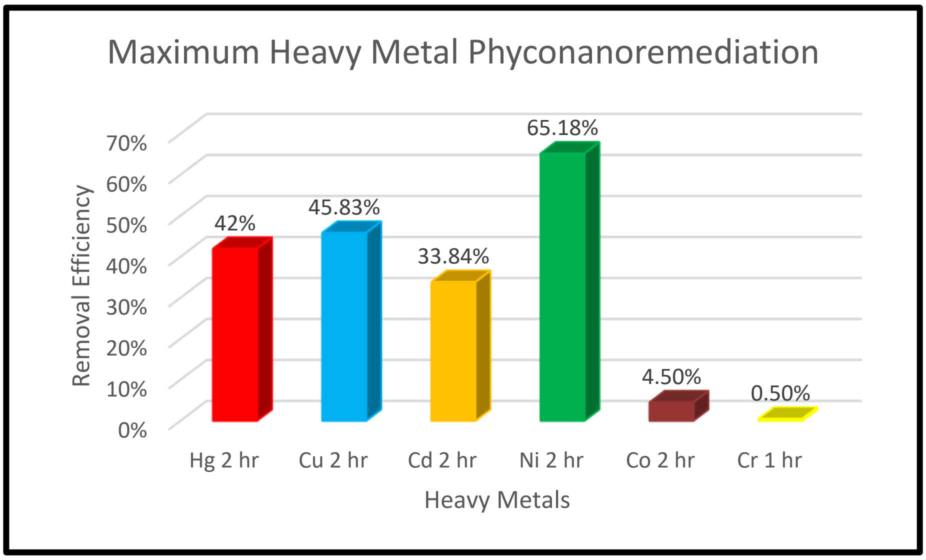


Fig. 27: Maximum Phyconanoremediation of Heavy Metals

4.5 <u>DISCUSSION</u>

This project aims to develop a technique that is more efficient and economical than bioremediation and it is observed that silver nanoparticles synthesized from *Chlorella minutissima* might prove to be an efficient remediator for different dyes and heavy metals. To produce maximum amount of Cm.AgNp, different optimization reactions were carried out to know the optimal kinetic parameters. It is observed that heating and sonication are the best cell disruption techniques that leads to the maximum production of AgNp. Different biomass concentrations (1g, 2g, 3g, 4g and 5g) were used in an experiment to study the effect of biomass concentration variation and it is observed that as we increase the biomass concentration, the intensity of surface plasmon band increases and the peak is shifted towards the lower wavelength and it indicates that the synthesis of smaller size and more concentration of silver nanoparticles takes place by increasing the biomass concentration. In the salt concentration experiment, it is observed that all the concentration except 0.1mM and above 50mM leads to the synthesis of silver nanoparticles and as we increase the concentration of salt up to 50 mM, the concentration of silver nanoparticles also increases. When particles are produced using 100 mM salt concentration, it is seen that instant agglomeration of silver nanoparticles takes place. On the other hand, when reaction mixture pH is changed from 4 to 12 pH, it is observed that different pH leads to the synthesis of different colors of silver nanoparticles at different peaks. It is seen that higher pH leads to the synthesis of more dark brown AgNp. Moreover, broader peaks are observed at lower pH. Thus, it can be concluded that different pH leads to the synthesis of different silver nanoparticles of different concentrations. In the last, it is observed that as the contact time between the reaction mixture increases, the intensity of the peak also increases, which means that the concentration of silver nanoparticles also increases. These reaction kinetics parameters will lead to the synthesis of maximum production of silver nanoparticles which can be further used for the remediation of heavy metals and dyes. It is observed that different dyes like EY, MB, EB, MO, and CV show maximum abatement within 5 minutes. On the other hand, heavy metals Hg, Cd, Ni, Cu, Co, and Cr; Cm.AgNp were effective for Ni>Cu> Hg>Cd and less efficient for Co and Cr. These results have shown the potential of Cm.AgNp and to make this technique more economical, in the future leftover biomass might be utilized for the production of various economical by-products as algae is rich in different bioactive compounds. This will make this technique more cost-effective, sustainable and a promising phyconanoremediation technique.

4.6 CONCLUSION

Phyconanoremediation is a promising technique for the remediation of heavy metals and dyes. Green algae *Chlorella minutissima* synthesize AgNp using its extract and metal precursor salt. For the enhancement of the rate of synthesis of AgNp, various kinetic studies were carried out like cell disruption technique, pH, contact time, biomass and salt concentration variations. Optimization of synthesis parameters is done by conducting varying cell disruption methods. In this, three different techniques i.e. sonication, heating, and autoclave are used and it is concluded that out of these three, heating and sonication are considered to be the best cell-disruption techniques and are able to synthesize maximum amount of AgNp. Other studies with different salt concentrations were conducted and it was observed that 0.1mM and 100mM are not able to synthesize AgNp, rest all the concentrations can synthesize the nanoparticles. Moreover, it is observed that as the salt concentration increases, synthesis of AgNp is also increased up to 50mM. In the next study, different biomass concentrations were observed and it was concluded that biomass concentration has a direct relation with the synthesis of AgNp. Different pH (4 to 12) of reaction mixture is prepared and all pH can synthesize the AgNp but leads to the development of different color, absorbance and shape of the peak with every different pH. It might be due to the synthesis of different shapes and sizes of AgNp. Additionally, it is observed that alkaline pH can synthesize AgNp within few minutes as compared to lower pH. Later, the relationship of contact time with the synthesis of AgNp is also studied where it is observed concentration of AgNp increases with the increase in the reaction time.

In the end, these AgNp are utilized for the remediation of heavy metals and dyes. It is observed that as soon as AgNp are added, they are able to remediate more than 96% of eosin yellow, methyl orange and evans blue dyes and more than 87% in the case of crystal violet and methylene blue within 5 minutes. Later, these particles are also studied for the abatement of heavy metals and they can abate 65.18% nickel, 45.83% copper, 42% mercury, 33.84% cadmium and are less efficient for cobalt (4.5%) and chromium (0.5%).

These result has shown the potential of AgNp in the remediation of water pollutants. Moreover, no studies are reported for the remediation of these heavy metals by AgNp. Thus, more studies in the field of phyconanoremediation, abatement mechanism, and production of different by-products along with the phyconanoremediation are required to make this bioremediation technique fast, eco-friendly and more cost-effective. This will make this technique more sustainable, efficient, economical as well as powerful commercial phyconanoremdiation tool.

4.7 FUTURE PROSPECTS

Phyconanoremediation is an environment-friendly technique but production of algal-based nanoparticles is an expensive process. It is observed that a large amount of biomass is required for the production of very small amount of nanoparticles, which will make this process time-consuming. To make this method more economical one can develop an integrated system where metals present in the wastewater can be used for the synthesis of algal nanoparticles and these particles will be further used for the remediation of water pollutants. This will eliminate the use of metal precursor from the synthesis process as wastewater consists of many metals which can be further reduced to metal-based nanoparticles as shown in **fig.4**.

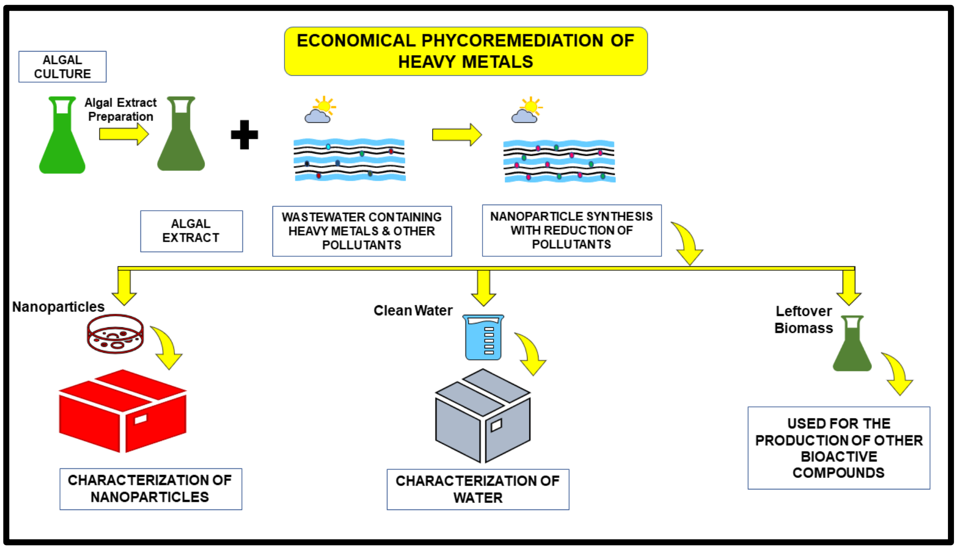


Fig. 28: Economical Phyconanoremediation of Water Pollutants

Another way to overcome this is to develop a commercial system where supernatant is used for the synthesis of nanoparticles and leftover biomass will be utilized for production of by-products like lipids, proteins, pigments, and other bioactive compounds. These bioactive compounds can be utilized in various industries such as biofuel, therapeutic proteins, automobiles and medicine in the future. One can re-use these nanoparticles. For this, desorption kinetics studies are required. As seen in algae, alteration in pH leads to the desorption of heavy metal might be applied here too. Another major limitation is that at present there is no such developed method for their disposal. When discharged into the environment, it is very difficult to detect them. Despite of excellent removal efficiency, the full abatement mechanism using phyconanoparticles is not known. Thus, studies in the field of removal mechanism, optimal algal nanoparticles synthesis, adsorption as well as desorption kinetics, recycling of nanoparticles, phyconanoremediation and production of by-products are highly required to make this technique a promising green solution for various environmental problems.

APPENDIX

1. GROWTH MEDIA FOR *Chlorella minutissima:*

BG-11 (1L) pH-9:

Table A 1: Composition of BG-11

Components	Concentration (g/L)
$NaNO_3$	1.5 g/L
K_2HPO_4	0.04 g/L
$MgSO_4.7H_2O$	0.075 g/L
$CaCl_2.7H_2O$	0.036 g/L
Citric Acid	0.006 g/L
Na_2CO_3	0.02 g/L
Na_2 EDTA	0.001 g/L
Ferric Ammonium Citrate	0.006 g/L
Trace Elements Solution	1mL

Table A2: Composition of trace elements of BG-11

Trace Elements Solution	Concentration (g/L)
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.\ 7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co\ (NO_3)_2.6H_2O$	0.0494

2. REMOVAL EFFICIENCY

$(C_i – C_e)/ C_i$ for each experimental set-up (Experiment 1, 2 and 3) is calculated. Then average of three experiment is taken. This average value is multiplied by 100 for the calculation of removal efficiency.

2.1 ***Removal Efficiency of Different Dyes***

Table A 3: Removal Efficiency of Dyes

Dyes	Time	Average	Removal Efficiency
Methyl Orange	5 minutes	0.961856	96.18%

	1 hour	0.856433	85.64%
Eosin Yellow	5 minutes	0.962612	96.26%
	1 hour	0.897431	89.74%
Evans Blue	5 minutes	0.96544	96.54%
	1 hour	0.935001	93.50%
Crystal Violet	5 minutes	0.87079	87%
	1 hour	0.838422	83.84%
Methylene Blue	5 minutes	0.876127	87.61%
	1 hour	0.840482	84%

2.2 ***Removal Efficiency of Different Heavy Metals***

Table A 4: Removal Efficiency of Heavy Metals

Dye	**Time**	**Average**	**Removal Efficiency**
Mercury	1 hour	0.166255	16.62%
	2 hours	0.41946	41.94%
	3 hours	0.400496	40.04%
Copper	1 hour	0.434201	43.42%
	2 hours	0.458367	45.83%
	3 hours	0.421298	42.12%
Cadmium	1 hour	0.263112	26.31%
	2 hours	0.338474	33.84%
	3 hours	0.293758	29.37%
Nickel	1 hour	0.622097	62.20%
	2 hours	0.651805	65.18%
	3 hours	0.63805	63.80%
Cobalt	1 hour	0.015981	1.5%
	2 hours	0.045069	4.5%
	3 hours	0.029092	2.9%
Chromium	1 hour	0.005081	0.50%
	2 hours	0.004479	0.44%
	3 hours	0.004266	0.42%

REFERENCES

[1] A. Hansda, V. Kumar, and Z. Usmani, "Phytoremediation of heavy metals contaminated soil using plant growth promoting rhizobacteria (PGPR): A current perspective," *Recent Res. Sci. Technol.*, vol. 6, no. 1, pp. 131–134, 2014.

[2] R. A. Wuana and F. E. Okieimen, "Heavy Metals in Contaminated Soils : A Review of Sources , Chemistry , Risks and Best Available Strategies for Remediation," *Int. Sch. Res. Netw.*, vol. 2011, pp. 1–20, 2011.

[3] R. Gupta and H. Xie, "Nanoparticles in Daily Life: Applications, Toxicity and Regulations," *J Env. Pathol Toxicol Oncol.*, vol. 37, no. 3, pp. 209–230, 2018.

[4] V. Padil and S. Rao, "Bioremediation of industrial toxic metals with gum kondagogu (Cochlospermum gossypium): A natural carbohydrate biopolymer," *Indian J. Biotechnol.*, vol. 10, pp. 113–120, 2011.

[5] K. Jomova and M. Valko, "Advances in metal-induced oxidative stress and human disease," *Toxicology*, vol. 283, no. 2–3, pp. 65–87, 2011.

[6] U. . Devasena and R. Thiruchelvi, "Synthesis of nanoparticles using algae and its application in dye degradation – A review," *Int. J. Res. Anal. Rev.*, vol. 6, no. 1, pp. 252–256, 2019.

[7] E. Lombi, F. J. Zhao, S. J. Dunham, and S. P. Mcgrath, "Phytoremediation of Heavy Metal – Contaminated Soils : Natural Hyperaccumulation," *J. Environ. Qual*, vol. 30, pp. 1919–1926, 2001.

[8] WHO, "Trace elements in human nutrition and health World Health Organization," 1996.

[9] M. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew, and K. N. Beeregowda, "Toxicity, mechanism and health effects of some heavy metals," *Interdiscip. Toxicol.*, vol. 7, no. 2, pp. 60–72, 2014.

[10] K. Piaskowski, R. Świderska-Dąbrowska, and P. K. Zarzycki, "Dye removal from water and wastewater using various physical, chemical, and biological processes," *J. AOAC Int.*, vol. 101, no. 5, pp. 1371–1384, 2018.

[11] H. Ali, E. Khan, and I. Ilahi, "Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and

bioaccumulation,” *J. Chem.*, vol. 2019, 2019.

[12] B. O. Anyanwu, A. N. Ezejiofor, Z. N. Igweze, and O. E. Orisakwe, “Heavy metal mixture exposure and effects in developing nations: An update,” *Toxics*, vol. 6, no. 4, pp. 1–32, 2018.

[13] S. Jiwan and K. Ajay, “Effects of Heavy Metals on Soil, Plants, Human Health and Aquatic Life,” *Int. J. Res. Chem. Environ.*, vol. 1, no. January, pp. 15–21, 2011.

[14] A. Emamverdian, Y. Ding, F. Mokhberdoran, and Y. Xie, “Heavy Metal Stress and Some Mechanisms of Plant Defense Response,” *Sci. World J.*, vol. 25, no. 4, pp. 1–18, 2015.

[15] R. Fryzova *et al.*, “Oxidative stress and heavy metals in plants,” *Rev. Environ. Contam. Toxicol.*, vol. 245, pp. 129–156, 2017.

[16] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, “Heavy Metal Toxicity and the Environment,” *Mol. Clin. Environ. Toxicol.*, pp. 133–164, 2012.

[17] M. O. Fashola, V. M. Ngole-jeme, and O. O. Babalola, “Heavy Metal Pollution from Gold Mines : Environmental Effects and Bacterial Strategies for Resistance,” *Int. J. Environ. Res. Public Health*, vol. 13, no. 1047, pp. 1–20, 2016.

[18] B. E. Igiri, S. I. R. Okoduwa, G. O. Idoko, E. P. Akabuogu, A. O. Adeyi, and I. K. Ejiogu, “Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review,” *J. Toxicol.*, vol. 2018, 2018.

[19] N. Bharadvaja and L. Kumar, “Algal biorefinery for extraction of bioactive compounds,” *Curr. Bioact. Compd.*, vol. 16, pp. 1–9, 2020.

[20] D. Bhardwaj, L. Kumar, and N. Bharadvaja, “A review on sources of dyes, sustainable aspects, environmental issues and degradation methods,” in *India 2020: Environmental Challenges, policies and green Technology.*, 2020, pp. 137–148.

[21] I. Priyadarshani, D. Sahu, and B. Rath, “Microalgal bioremediation : Current practices and perspectives,” *J. Biochem. Technol.*, vol. 3, no. 3, pp. 299–304, 2012.

[22] H. Zohoorian, H. Ahmadzadeh, M. Molazadeh, M. Shourian, and S. Lyon, “Microalgal bioremediation of heavy metals and dyes,” in *Algal Bioremediation*, Elsevier Inc., 2020, pp. 659–674.

[23] M. Chugh, L. Kumar, and N. Bharadvaja, “Bioremediation of Heavy Metals: A step towards environmental sustainanbility.,” in *India 2020: Environmental Challenges, policies and green Technology.*, vol. 66, 2020, pp. 77–90.

[24] L. Kumar and N. Bharadvaja, “Enzymatic bioremediation: a smart tool to fight

environmental pollutants," in *Smart Bioremediation Technologies*, Elsevier, 2019, pp. 99–118.

[25] M. Chugh, L. Kumar, and N. Bharadvaja, "Bioremediation of heavy metals: A step towards environmental sustainability.," in *India 2020 : Environmental Challenges, Policies and Green Technology*, 2020, pp. 77–90.

[26] M. A. Maurer-Jones, I. L. Gunsolus, C. J. Murphy, and C. L. Haynes, "Toxicity of engineered nanoparticles in the environment," *Anal. Chem.*, vol. 85, no. 6, pp. 3036–3049, Mar. 2013.

[27] F. D. Guerra, M. F. Attia, D. C. Whitehead, and F. Alexis, "Nanotechnology for environmental remediation: Materials and applications," *Molecules*, vol. 23, no. 7, pp. 1–23, 2018.

[28] D. Bera, L. Qian, T. K. Tseng, and P. H. Holloway, "Quantum dots and their multimodal applications: A review," *Materials (Basel).*, vol. 3, no. 4, pp. 2260–2345, 2010.

[29] N. Arora, A. Patel, K. Sartaj, P. A. Pruthi, and V. Pruthi, "Bioremediation of domestic and industrial wastewaters integrated with enhanced biodiesel production using novel oleaginous microalgae," *Environ. Sci. Pollut. Res.*, vol. 23, no. 20, pp. 20997–21007, 2016.

[30] H. Bahrulolum *et al.*, "Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector," *J. Nanobiotechnology*, vol. 19, no. 1, pp. 1–26, 2021.

[31] V. N. Thekkudan *et al.*, "Review on nanoadsorbents: A solution for heavy metal removal from wastewater," *IET Nanobiotechnology*, vol. 11, no. 3, pp. 213–224, 2017.

[32] A. Saravanan *et al.*, "A review on biosynthesis of metal nanoparticles and its environmental applications," *Chemosphere*, vol. 264, pp. 1–16, 2021.

[33] S. Negi and V. Singh, "Algae: A potential source for nanoparticle synthesis," *J. Appl. Nat. Sci.*, vol. 10, no. 4, pp. 1134–1140, 2018.

[34] A. M. El Shafey, "Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review," *Green Process. Synth.*, vol. 9, no. 1, pp. 304–339, 2020.

[35] F. Ameen, K. Alsamhary, J. A. Alabdullatif, and S. ALNadhari, "A review on metal-based nanoparticles and their toxicity to beneficial soil bacteria and fungi," *Ecotoxicol. Environ. Saf.*, vol. 213, p. 112027, 2021.

[36] J. Hu *et al.*, "Using a mixture of microalgae, biochar, and organic manure to increase the capacity of soil to act as carbon sink," *J. Soils Sediments*, vol. 19, no. 11, pp. 3718–3727, 2019.

[37] T. Cele, "Preparation of Nanoparticles," in *Engineered Nanomaterials-Health and safety*, vol. 15, no. 1, 2013, p. 13.

[38] S. Chen *et al.*, “Photoluminescence of undoped and Ce-doped SnO 2 thin films deposited by sol-gel-dip-coating method,” *Appl. Surf. Sci.*, vol. 258, no. 7, pp. 3255–3259, 2012.

[39] F. LewisOscar, S. Vismaya, M. Arunkumar, N. Thajuddin, D. Dhanasekaran, and C. Nithya, “Algal Nanoparticles: Synthesis and Biotechnological Potentials,” in *Algae-Organisms for Imminent Biotechnology*, 2016, pp. 157–182.

[40] K. Balantrapu and D. V. Goia, “Silver nanoparticles for printable electronics and biological applications,” *J. Mater. Res.*, vol. 24, no. 9, pp. 2828–2836, 2009.

[41] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, “Synthesis of silver nanoparticles: Chemical, physical and biological methods,” *Res. Pharm. Sci.*, vol. 9, no. 6, pp. 385–406, Dec. 2014.

[42] N. Raghuwanshi, N. Arora, R. Varshney, P. Roy, and V. Pruthi, “Antineoplastic and antioxidant potential of phycofabricated silver nanoparticles using microalgae Chlorella minutissima,” *IET Nanobiotechnology*, vol. 11, no. 7, pp. 827–834, 2017.

[43] P. Senthilkumar, L. Surendran, B. Sudhagar, and D. S. Ranjith Santhosh Kumar, “Facile green synthesis of gold nanoparticles from marine algae Gelidiella acerosa and evaluation of its biological Potential,” *SN Appl. Sci.*, vol. 1, no. 4, pp. 1–12, 2019.

[44] M. Soleimani and M. Habibi-Pirkoohi, “Biosynthesis of silver nanoparticles using Chlorella vulgaris and evaluation of the antibacterial efficacy against Staphylococcus aureus,” *Avicenna J. Med. Biotechnol.*, vol. 9, no. 3, pp. 120–125, 2017.

[45] F. Arsiya, M. H. Sayadi, and S. Sobhani, “Green synthesis of palladium nanoparticles using Chlorella vulgaris,” *Mater. Lett.*, vol. 186, pp. 113–115, 2017.

[46] S. Rajeshkumar, C. Malarkodi, K. Paulkumar, M. Vanaja, G. Gnanajobitha, and G. Annadurai, “Algae Mediated Green Fabrication of Silver Nanoparticles and Examination of Its Antifungal Activity against Clinical Pathogens,” *Int. J. Met.*, vol. 2014, pp. 1–8, 2014.

[47] T. Kathiraven, A. Sundaramanickam, N. Shanmugam, and T. Balasubramanian, “Green synthesis of silver nanoparticles using marine algae Caulerpa racemosa and their antibacterial activity against some human pathogens,” *Appl. Nanosci.*, vol. 5, no. 4, pp. 499–504, 2015.

[48] P. Bhuyar, M. H. A. Rahim, S. Sundararaju, R. Ramaraj, G. P. Maniam, and N. Govindan, “Synthesis of silver nanoparticles using marine macroalgae Padina sp. and its antibacterial activity towards pathogenic bacteria,” *Beni-Suef Univ. J. Basic Appl. Sci.*, vol. 9, no. 1, 2020.

[49] H. Y. El-Kassas, M. A. Aly-Eldeen, and S. M. Gharib, “Green synthesis of

iron oxide (Fe3O4) nanoparticles using two selected brown seaweeds: Characterization and application for lead bioremediation," *Acta Oceanol. Sin.*, vol. 35, no. 8, pp. 89–98, 2016.

[50] F. Eya'ane Meva *et al.*, "Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from Megaphrynium macrostachyum leaf extract," *Rev. Bras. Farmacogn.*, vol. 26, no. 5, pp. 640–646, 2016.

[51] E. Tomaszewska *et al.*, "Detection limits of DLS and UV-Vis spectroscopy in characterization of polydisperse nanoparticles colloids," *J. Nanomater.*, vol. 2013, pp. 1–10, 2013.

[52] A. Rautela, J. Rani, and M. Debnath (Das), "Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms," *J. Anal. Sci. Technol.*, vol. 10, no. 1, pp. 1–10, 2019.

[53] L. Kumar, V. Ragunathan, M. Chugh, and N. Bharadvaja, "Nanomaterials for remediation of contaminants: a review," *Environ. Chem. Lett.*, no. 0123456789, 2021.

[54] G. A. Dorofeev, A. N. Streletskii, I. V. Povstugar, A. V. Protasov, and E. P. Elsukov, "Determination of nanoparticle sizes by X-ray diffraction," *Colloid J.*, vol. 74, no. 6, pp. 675–685, 2012.

[55] J. Abraham, B. Jose, A. Jose, and S. Thomas, "Characterization of green nanoparticles from plants," in *Phytonanotechnology*, Elsevier Inc., 2020, pp. 21–39.

[56] M. Tharaud, P. Louvat, and M. F. Benedetti, "Detection of nanoparticles by single-particle ICP-MS with complete transport efficiency through direct nebulization at few-microlitres-per-minute uptake rates," *Anal. Bioanal. Chem.*, vol. 413, no. 3, pp. 923–933, 2021.

[57] Z. Xu, X. Huang, C. Dong, and J. Ren, "Fluorescence correlation spectroscopy of gold nanoparticles, and its application to an aptamer-based homogeneous thrombin assay," *Microchim. Acta*, vol. 181, no. 7–8, pp. 723–730, 2013.

[58] M. K. Rasmussen, J. N. Pedersen, and R. Marie, "Size and surface charge characterization of nanoparticles with a salt gradient," *Nat. Commun.*, vol. 11, no. 1, pp. 1–8, 2020.

[59] W. B. Ayinde, W. M. Gitari, and A. Samie, "Optimization of microwave-assisted synthesis of silver nanoparticle by Citrus paradisi peel and its application against pathogenic water strain," *Green Chem. Lett. Rev.*, vol. 12, no. 3, pp. 225–234, 2019.

[60] J. F. D. F. Araujo, A. C. Bruno, and S. R. W. Louro, "Versatile magnetometer assembly for characterizing magnetic properties of nanoparticles," *Rev. Sci. Instrum.*, vol. 86, no. 10, pp. 1–7, 2015.

[61] R. Rajkumar, G. Ezhumalai, and M. Gnanadesigan, "A green approach for the

synthesis of silver nanoparticles by Chlorella vulgaris and its application in photocatalytic dye degradation activity," *Environ. Technol. Innov.*, vol. 21, p. 101282, 2021.

[62] S. Husain, S. Afreen, Hemlata, D. Yasin, B. Afzal, and T. Fatma, "Cyanobacteria as a bioreactor for synthesis of silver nanoparticles-an effect of different reaction conditions on the size of nanoparticles and their dye decolorization ability," *J. Microbiol. Methods*, vol. 162, no. December 2018, pp. 77–82, 2019.

[63] E. F. Aboelfetoh, R. A. El-Shenody, and M. M. Ghobara, "Eco-friendly synthesis of silver nanoparticles using green algae (Caulerpa serrulata): reaction optimization, catalytic and antibacterial activities," *Environ. Monit. Assess.*, vol. 189, no. 7, 2017.

[64] M. Ramakrishna, D. Rajesh Babu, R. M. Gengan, S. Chandra, and G. Nageswara Rao, "Green synthesis of gold nanoparticles using marine algae and evaluation of their catalytic activity," *J. Nanostructure Chem.*, vol. 6, no. 1, pp. 1–13, 2016.

[65] A. V. Samrot *et al.*, "Bioactivity and Heavy Metal Removal Using Plant Gum Mediated Green Synthesized Silver Nanoparticles," *J. Clust. Sci.*, vol. 30, no. 6, pp. 1599–1610, 2019.

[66] J. Yang *et al.*, "Nanomaterials for the removal of heavy metals from wastewater," *Nanomaterials*, vol. 9, no. 3, pp. 1–39, 2019.

[67] K. M. Al-Qahtani, "Cadmium removal from aqueous solution by green synthesis zero valent silver nanoparticles with Benjamina leaves extract," *Egypt. J. Aquat. Res.*, vol. 43, no. 4, pp. 269–274, 2017.

[68] I. K. Attatsi and F. Nsiah, "Application of silver nanoparticles toward Co(II) and Pb(II) ions contaminant removal in groundwater," *Appl. Water Sci.*, vol. 10, no. 6, pp. 1–13, 2020.

[69] R. S. El-Tawil, S. T. El-Wakeel, A. E. Abdel-Ghany, H. A. M. Abuzeid, K. A. Selim, and A. M. Hashem, "An Overview of the Algae-Mediated Biosynthesis of Nanoparticle," *Heliyon*, vol. 5, no. 9, pp. 1–9, 2019.

[70] S. M. Seyedi, H. Rabiee, S. M. S. Shahabadi, and S. M. Borghei, "Synthesis of Zero-Valent Iron Nanoparticles Via Electrical Wire Explosion for Efficient Removal of Heavy Metals," *Clean - Soil, Air, Water*, vol. 45, no. 3, pp. 1–20, 2017.

[71] T. Liu, Z. L. Wang, and Y. Sun, "Manipulating the morphology of nanoscale zero-valent iron on pumice for removal of heavy metals from wastewater," *Chem. Eng. J.*, vol. 263, pp. 55–61, 2015.

[72] P. Huang *et al.*, "Rapid magnetic removal of aqueous heavy metals and their relevant mechanisms using nanoscale zero valent iron (nZVI) particles," *Water Res.*, vol. 47, no. 12, pp. 4050–4058, 2013.

[73] T. Leiviskä, M. K. Khalid, A. Sarpola, and J. Tanskanen, "Removal of vanadium from industrial wastewater using iron sorbents in batch and continuous flow pilot systems," *J. Environ. Manage.*, vol. 190, pp. 231–242, 2017.

[74] R. Chaudhary, K. Nawaz, A. K. Khan, C. Hano, B. H. Abbasi, and S. Anjum, "An overview of the algae-mediated biosynthesis of nanoparticles and their biomedical applications," *Biomolecules*, vol. 10, no. 11, pp. 1–36, 2020.

[75] V. O. Adenigba, I. O. Omomowo, J. K. Oloke, B. A. Fatukasi, M. A. Odeniyi, and A. A. Adedayo, "Evaluation of microalgal-based nanoparticles in the adsorption of heavy metals from wastewater," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 805, no. 1, 2020.

[76] S. Ahmad *et al.*, "Algal extracts based biogenic synthesis of reduced graphene oxides (rGO) with enhanced heavy metals adsorption capability," *J. Ind. Eng. Chem.*, vol. 72, no. March 2019, pp. 117–124, 2019.

[77] V. Subramaniyam, S. R. Subashchandrabose, P. Thavamani, M. Megharaj, Z. Chen, and R. Naidu, "Chlorococcum sp. MM11—a novel phyco-nanofactory for the synthesis of iron nanoparticles," *J. Appl. Phycol.*, vol. 27, no. 5, pp. 1861–1869, 2015.

[78] B. Uzair *et al.*, "Green and cost-effective synthesis of metallic nanoparticles by algae: Safe methods for translational medicine," *Bioengineering*, vol. 7, no. 4, pp. 1–22, 2020.

[79] N. E. A. El-Naggar, R. A. Hamouda, A. A. Saddiq, and M. H. Alkinani, "Simultaneous bioremediation of cationic copper ions and anionic methyl orange azo dye by brown marine alga Fucus vesiculosus," *Sci. Rep.*, vol. 11, no. 1, pp. 1–19, 2021.

[80] M. Vanaja *et al.*, "Degradation of methylene blue using biologically synthesized silver nanoparticles," *Bioinorg. Chem. Appl.*, vol. 2014, 2014.

[81] G. Magnacca *et al.*, "Novel magnetite nanoparticles coated with waste-sourced biobased substances as sustainable and renewable adsorbing materials," *ACS Sustain. Chem. Eng.*, vol. 2, no. 6, pp. 1518–1524, 2014.

[82] E. Alzahrani, "Photodegradation of Eosin y Using Silver-Doped Magnetic Nanoparticles," *Int. J. Anal. Chem.*, vol. 2015, 2015.

[83] J. Jena *et al.*, "Biological sequestration and retention of cadmium as CdS nanoparticles by the microalga Scenedesmus-24," *J. Appl. Phycol.*, vol. 27, no. 6, pp. 2251–2260, 2015.

LIST OF PUBLICATIONS

1. Kumar,L., Ragunathan,V., **Chugh,M**.,Bharadvaja,N (2021). "Nanomaterials for remediation of contaminants: a review". Environmental Chemistry Letters. Springer DOI: 10.1007/s10311-021-01212-z
   Published In: Environmental Chemistry Letters. Springer. IF:8.7

2. **Chugh M**., Kumar L., Bharadvaja N. (2020) Bioremediation of Heavy Metals: A step towards environmental sustainanbility. In: India 2020: Environmental Challenges, policies and green Technology. pp 77–90.
   Published: India 2020: Environmental challenges, Policies and green Technology, Imperial Publications, ISSN: 978-81-932621-1-5, 2020

3. Gupta V, Singh M, **Chugh M**, et al (2018) Preliminary Studies of Vegetable Plants Growth Index Under Controlled Condition of Soilless System. Trends Biosci 11:3268– 3273
   Published In: Trends in Biosciences Journal volume 11, Issue 24, June 2018.

4. **Chugh, M**., Kumar, L., Bhardwaj, D., Bharadvaja, N. (2021). Bioaccumulation and detoxification of Heavy Metals: An Insight into the Mechanism. In Development in wastewater treatment research and processes: Innovative microbe-based applications for removal of chemicals and metals in wastewater treatment plants. ***Elsevier.***
   Accepted, Publication due in October.

5. Kumar, L., Khushbu, **Chugh, M**., Bharadvaja, N. (2021). Microbial remediation of tannery wastewater. In Development in wastewater treatment research and processes: Innovative microbe-based applications for removal of chemicals and metals in wastewater treatment plants. ***Elsevier.***
   Accepted, Publication due in October

6. **Chugh, M**., Kumar, L., Bharadvaja, N. (2021). Fungal diversity in the bioremediation of toxic effluents. In Development in wastewater treatment research and processes: Microbial degradation of xenobiotics through bacterial and fungal approach. Elsevier.
   Accepted, Under Printing