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"Catalytic Degradation of Dyes by Metallic Nanoparticles biosynthesized from leaf extract of *Centella asiatica*"

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CANDIDATE'S DECLARATION

I, **Swati Raina**, Roll No. **2K16/IBT/10**, student of M.Tech Industrial Biotechnology, hereby declare that my project dissertation titled **"Catalytic Degradation of Dyes by Metallic Nanoparticles biosynthesized from leaf extract of *Centella asiatica"*** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology is an original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the project dissertation entitled **"Catalytic Degradation of Dyes by Metallic Nanoparticles biosynthesized from leaf extract of *Centella asiatica*"** which is submitted by **Swati Raina (2K16/IBT/10) Department of Biotechnology,** Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), in the partial fulfilment of the requirement for the reward of the degree of Master of Technology, is an authentic record of the candidate's own 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.

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ABSTRACT

During last decades disposal of dye based pollutants is a severe concern that challenges our environment. Dyes are a major class of synthetic organic compounds released by many industries such as textile, plastic, paper, food, and tanneries, pharmaceutical and cosmetic industries. These dyes are carcinogenic and highly stable in the water moreover conventional methods are unable to degrade these dyes posing a threat to the mankind and nature. Physical and Chemical methods employed for the degradation of the dyes again use harmful and toxic chemicals. In this study AgNPs and CuNPs were synthesized with the plant extracts of *Centella asiatica* using the metallic salts for the respective Metallic nanoparticle. The nanoparticles synthesized were characterized with UV-Vis spectrophotometer and SEM analysis. The UV-Vis gave the characteristic SPR peak for silver at 420 nm and 323 nm for copper. SEM images revealed the size in the range of 30-50 nm for silver and 10-30 nm for copper nanoparticles. Metal (NPs) have received great attention due to their catalytic role in the degradation of organic dyes, as they acts as a redox catalyst in the degradation of dyes by electron relay effect between donor and acceptor molecules. Thus in the present study we have exploited tis property for dye degradation of MO, MR, PR, and EY with photocatalysis and chemical catalysis with $NaBH_4$.

Keywords: *Centella asiatica,* Silver Nanoparticles, Copper Nanoparticles, Photocatalytic dye degradation, catalytic dye degradation, Methyl Orange, Methyl Red, Phenol Red and Eosin Y.

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Swati Raina

CONTENTS

LIST OF TABLES AND GRAPHS

LIST OF GRAPHS

LIST OF FIGURES

LIST OF ABBREVIATIONS

SEM	Scanning Electron Microscope
MO	Methyl Orange
MR	Methyl Red
PR	Phenol Red
EY	Eosin Y
NPs	Nanoparticles
Ag	Silver
Cu	Copper
nm	Nanometer
mM	Milli Molar
$AgNO_3$	Silver Nitrate
$CuSO_4$	Cupric Sulphate
$NaBH_4$	Sodium Borohydride
ml	Milli litre
°C	Degree Celsius
ppm	parts per million
rpm	rotation per minute
kV	kilo Volt
pH	potential of Hydrogen

CHAPTER 1

1.1 INTRODUCTION

The unforeseen activities of the human beings, industrializations and rapidly increasing urbanization have deteriorated the environment to extreme conditions. The pollution has raised to its life-threatening bars especially the water and air pollution. The effluents from various industries are blamable for this environment depletion, waste water generated from the dye industries has a major role to play in the effluent generation as dyes are used in production of innumerable consumer products such as textiles, plastics, foods, papers, paints, inks, etc. which results in substantial environmental pollution and toxicity to the living organisms and subsidize to biological hazards like eutrophication (Safavi and Momeni 2012). Several organic dyes are used in the industries for instance Methyl Orange, Methylene blue, Methyl Red, Congo Red, Eosin Y, Direct black, Crystal violet, and some other dyes like 4-nitrophenol, phenol red (Bogireddy, et al., 2015), Indigo, Bromophenol Blue (Khan, et al., 2016), Acridine orange (Kumar, et al., 2016), Methyl Violet 6B (Sinha, et al., 2014), Reactive blue. These dyes that are running down the pipes of the industries are a potential threat to our ecosystem and to the water bodies. The complete expulsion of the effluent is essential to minimize the water pollution which is a very cumbersome task as they are highly stable in the water and owing to their complicated structure it's difficult to eradicate such dyes. The approaches and methods that have been practiced in the past employing physical, and chemical remediation processes, those have their part of the shortcomings. On the other side the biological and the green approach utilizing the suitable nanocatalyst in the presence of sunlight and or chemicals offers a potential method for the complete elimination of pollutants from the environment.

Nanoparticles have started a new era of research in nanobiotechnology because their size and shape can be controlled, synthesis is rapid and easy moreover, has remarkable catalytic, optical, magnetic, and electronic, thermal properties which has potential applications in various fields. The extraordinary properties of the nanoparticles have appreciably enhanced the research fields due to their unique and controllable shape and size, making nanobiotechnology a promising field (Ravindran *et al.,* 2013). It has

innumerable applications due to strong optical absorbance and surface bio-conjugation related to surface plasmon resonance effect (Netala et al., 2016) and has been exploited in the design and development of many biomedical equipment like magnetic resonance imaging (MRI), computed tomography (CT) scanning and X- ray. Amongst all the explicable properties of the silver nanoparticles here we are exercising with one of the potential use of nanoparticles as a catalytic tool for the degradation of the dyes.

Amongst the various noble metals acting as nanoparticles most explored and potential nanoparticle that has been known for a long time is silver nanoparticles and copper nanoparticles. Silver nanoparticles and copper nanoparticles exhibit excellent catalytic properties owing to their shape, size and composition including their environment and also these properties depend upon the spatial ordering of particles. Nanoparticles have great application in the catalytic degradation due to the electron relay effect in which the electron relay occurs from the donor to the acceptor. The nanoparticles possess a large surface area which act as a substrate for the electron transfer reaction. Just before the electron transfer reaction, both reactants are adsorbed on the metal surface. Subsequently, the reactant gains an electron and is reduced. Thus, in all the catalytic reactions, nanoparticles performs as a competent catalyst due to the electron transfer process (Ghosh, et al., 2002). Silver is a well-known antimicrobial agent, owing to its strong toxicity against a broad spectrum of microorganisms; bacteria, fungi, protozoa and certain viruses due to this property silver based compounds have been broadly used in many bactericidal applications (Ravindran, et al., 2013). Silver nanoparticles have emerged itself as potential antifungal agent against *Candida albicans* (K.J Kim, et al., 2009), *Candida nonalbicans* and *Candida tropicalis* (Netala, et al., 2016) also fungal infections are often found in the immunocompromised patients going through chemotherapy and HIV infections (Ashavani Kumar, et al., 2008). Textile dye waste water degradation by the photocatalytic method (Kavitha, et al., 2014), methyl violet 6B dye (Sinha, et al., 2014) is a new upcoming application of the Silver nanoparticles. Silver nanoparticles are exploited as a catalyst for the removal of toxic dyes (Sinha, et al., 2014). Copper nanoparticles also exhibit all the basic properties of the nanoparticle family and it can be more extensively utilized in the catalysis application due to its cost-effective trait compared to the silver, gold and platinum nanoparticles. Oxides of copper nanoparticles are thermodynamically stable and have been used in various processes owing to its ease of availability and suitable heterogenous recyclable catalyst in various effluent and toxin

removal programs and protocols. Copper nanoparticles have significant antimicrobial properties, some papers have reported for better antimicrobial properties in copper nanoparticles as compared to the silver nanoparticles against *E.coli*

Nanoparticles synthesis methods foremost involve physical and chemical synthesis. These methods employ the use of hazardous chemicals, low material conversions and have high energy requirements. These process a potential threat to the environment and biological risks, this renders for the need to develop an alternative environmentally friendly process for nanoparticles synthesis without using toxic chemicals which brings in light the green synthesis approach.

Biosynthetic methods employing green chemistry exploits the use of either microorganisms or plant extracts which have emerged as simple and viable alternative to the chemical and physical methods.The plant extracts have been an exclusive choice for numerous researchers as they are natural producers of reducing and capping agents that play a vital role in the nanoparticles synthesis. Also, green synthesis is safe for therapeutic use as well as water treatment plants and unlike the microbial synthesis it is less complex and has almost no scope of contaminations. They offer advantages such as environment friendly, single step process, cost-effective and possess almost no toxicity. The extracellular and intracellular biosynthesis of metallic nanoparticles utilizing microorganisms such as bacteria, fungi and yeast etc., are eco-friendly method but the process involving in their cultivation of biomass and the added on downstream processing of nanoparticles seems to be a cumbersome task and can pose a potential risk of contamination. There have been reports to produce nanoparticles from various plant extracts. Here in this report we have come forward with the nanoparticles synthesized from *Centella asiatica*.

Centella asiatica is a creeping plant which belongs to family of Apiaceae and commonly known as “gotu kola” and “Mandukaparni”. *Centella asiatica* is an herbaceous creeper and perennial, with leaves shaped as kidney beans and found in regions of India, Sri Lanka, Madagascar, South Africa, Australia, China, and Japan. This plant has various pharmacological activities like antipyretic, analgesic and anti-inflammatory anti-cancer, anti- depressant to name a few and its used in the treatment of epilepsy, schizophrenia and cognitive dysfunction, diarrhoea, cholera, measles, jaundice, leukorrhoea, haematemesis, hepatitis, urethritis, toothache, syphilis, smallpox, neuralgia, rheumatism,

toothache and varices. These pharmacological activities are due to the presence of wide variety of phytocomponds such as triterpenoids, phenols, flavonoids, phytosterols, and free amino acids. These phytocompounds are predominantly responsible for the synthesis of the nanoparticles as the play the key role being the capping and stabilizing agents for the nanoparticles.

OBJECTIVES

- Biosynthesis of Silver and Copper Nanoparticles from the leaf extracts of *Centella asiatica* with different metallic salt concentrations.
- Characterization of the Metallic nanoparticles.
- Catalytic Dye degradation by the Nanoparticles photo catalytically and by using reducing agent $NaBH_4$
- Comparison between the dye degradation percentage using the different methods of dye degradation (photo catalysis and by $NaBH_4$)

1.2 REVIEW OF LITERATURE

Disposal of dye-based effluents is a serious concern among many issues that challenge our environment fraternity. Despite the major role played by natural and synthetic dye products in making our world colorful and spectacular, its uncontrolled discharge is a major cause of the non-aesthetic pollution that leads to the destruction of ecosystem. Numerous industries like textile, cosmetics, paper, food, plastic and pharma utilize enormous amounts of synthetic dyes (Sannino, D. et al., 2013), the effluents and discharge from the aforementioned industries result in significant environmental pollution and also extreme health hazards. Several health issues are encouraged due to this discharge of organic pollutants like cancer, skin irritation, disorders of blood, poisoning of the CNS and damage to kidney and liver of humans and animals. People have reported and shown in studies that many of the dyes used in the textile industries are cancer causing and mutagenic and hence, harmful to the nature. The major contributor to this waste is the textile dye employed in the textile industries, studies have shown that the industry just devours about 60% of the total dye production for coloring the fabrics and after they are utilized 15% of these dyes are wasted and run off into the water bodies. Some dyes that affect the environment through waste water effluents are cationic dye, cationic fluorescent dye and anionic mono azo dye. Methylene Blue (MB), phenothiazine cationic dye, commonly used for coloring paper, dyeing cottons, wools, etc. can cause harmful effects on living things such as difficulties in breathing, vomiting, diarrhoea and nausea. Azo dyes are commonly used as colorants in several industries such as food, pharma, textile, paper, leather and paint industries (Rajesh, et al., 2016). The carcinogenic azo group and delocalization of π electrons in the azo dyes are accountable for its charming colors. They are readily soluble in water and other polar solvents. Owing to its solubility, the effluents of food, textile and leather industries are highly contaminated with azo dyes, which lead to the environmental pollution such as bioaccumulation in aquatic organisms and critical health problems to living organisms (Golka, et al., 2004). Hence, the removal of such carcinogenic azo compounds from water is biologically and environmentally important. The removal methods such as H_2O_2 oxidation, AgNPs assisted reductive-degradation, adsorption, electrochemical degradation, and bio degradation have been successfully employed and well documented in the literature

(Forgacs, et al., 2004; Bokare, et al., 2008; Singh, et al., 2015). Among them, silver and copper nanoparticles (AgNPs) assisted catalytic reductive-degradation of azo dyes has been attracted researchers for its simplicity and high reaction rates. The representative anionic azo dye, Methyl Orange (MO), is a well-known carcinogenic substance and a major effluent from textile and food industry. Methyl Red being an azo dye as a class are a concern for their potential mutagenicity and carcinogenicity. The derivatives of the dye have been detected in the urine of workers exposed to direct azo dyes. In an epidemiological study of silk dyers and painters indicated a strong association with the bladder cancer. Azo dyes are widely used in the industries and through the water stream line may enter some food chains and eventually reach the human beings. The enzymes present in the liver cleave the azo dyes in to aromatic amines and addition of a nitro group to these aromatic amines directly convert them to mutagens. Phenol Red (PR) is a dye used in the textile industry as well as a pH indicator in laboratory and in cell culture lab. So, the effluent produced in the sum from all these sources is huge, to contribute in the effluent for the environment pollution bar to raise up. Eosin Y (EY), the demonstrative fluorescent anionic dye displays yellow-green fluorescence and is used in the fields of dyeing, printing, leather, printing ink and fluorescent pigment, etc. (Meenakumari and Phillip, 2015). The dyes flowing from the industries dissolve in the water sources in a fair amount of concentration range from 10 ppm to 200 ppm which is a significant amount to pollute the water bodies worldwide (Gonawala & Mehta, 2014). Hence, the dyes coming from these textile industries should be treated before releasing in the water bodies and treatment of dye effluents should be a mandatory part of waste water treatment. Apart from affecting the human life and the animals these dyes also affect the aquatic life when released in the water bodies, as they change the chemical composition of the water by forming stable bonds and forms toxic compounds which influences the biological pathway and affects the flora and fauna (Predescu, et al., 2012).

The mixing of the dye with the water bodies is a major environmental concern as it decreases the dissolved oxygen in the water and also decreases the sunlight penetration due to the colored water which hinders in the aquatic life thriving.

Dyes can be of many different structural varieties like acidic, basic, disperse, azo, anthraquinone based and metal complex dyes. Chemical reduction using reducing agents like sodium borohydride, thiourea dioxide and sodium hydrosulfide is an effective decolorization technique for many dyes (Meenakumari and Phillip, 2015). Numerous

methods of water treatment for dye removal have been utilized like physical, chemical and biological methods. Reduction of these dye compounds using conventional processes is generally not economical, ineffectual and time consuming (Wesenburg, et al., 2003). The conventional methods include adsorption, reduction and membrane filtration, electrochemical treatment, chemical oxidation and anaerobic treatment etc. the ultimate result of these traditional methods are merely transfer pollutants from the liquid to the solid phase which may cause secondary pollution and require further treatment. Therefore, researchers have mainly focused on reduction, decolorization or detoxification of these compounds. Chemically stable dye pollutants make it difficult for the traditional methods to eradicate these dyes and hence these methods are ineffective. Moreover these dyes are of a complex structure hence it's difficult for microorganisms to degrade them and hence these dyes are not effectively degraded by traditional and conventional biological treatments (Joseph and Mathew, 2015). Lately, nanotechnology application has been utilized in the area of waste water treatment. Reductive degradation of hazardous dyes with metal nanomaterials is a convenient degradation process because of their unique physiochemical and electronic properties which are not present in bulk materials. The metallic nanomaterials are adaptable materials that can be used various in applications such as biosensors, waste water treatment, remediation, chips, electronic systems, medical devices and personal care products. Silver and Copper nanoparticles are noble catalysts and hence have application in catalyzing many reduction reactions. The rate by which the catalysis will happen depends upon the nanoparticles and its spatial arrangement of the particles also depends upon the size of the nanoparticles. The nanoparticles catalytic activity is size dependent, the smaller the size the more is the nanoparticle effective and more potential is its use is in the reduction of the hazardous dyes or for effluent treatment and for the other numerous applications of the nanoparticles. The catalytic arena of nanoparticle application has undergone remarkable growth in last years and it has bought a new revolution in the application field of nanoparticles. Metallic nanoparticles possess tremendous chemical and physical properties like surface to volume ratio, reactivity agglomeration or aggregation state, defect structure helping the formation of holes and electrons, which gives it dominance over the bulk materials (Davis and Klabunde, 1982). Nanoparticles are such efficient catalysts due to their size and reactivity and this property of nanoparticle has served the purpose of various researchers in the effective removal of dyes.

The silver and copper nanoparticle among all the metallic nanoparticles are the interesting ones in the nanobiotechnology family due to their exceptional electronic and optical properties and their strong hold over the strong toxicity against a broad spectrum of microorganisms. Furthermore, nanoparticles have a high surface to volume ratio, which gives them advantage over the bulk materials and can dramatically enhance the interaction between reactants and catalysts. A trail of methods are being developed for the synthesis of nanoparticles like polyol, photochemical, radio lytic, chemical, sonochemical and some biological methods employing microorganisms and plant extracts. Although the physical and chemical methods utilized in the production of nanoparticles involve hazardous and toxic chemicals, also they require harsh reaction conditions. Amongst all the production methodologies the most prevalent method is the chemical synthesis which requires chemical stabilizing and capping agents and these chemicals add up to the toxicity of the nanoparticles, contributing immense amount of chemicals in the process which ultimately poses huge environmental hazards and hence limits their utility. Immense research has been carried out in the synthesis of nanoparticles to reduce down the usage of toxic chemicals and to produce an environment friendly procedure to reduce the environmental threats (Joseph & Mathew 2015).

Metallic nanoparticles have been acknowledged for their catalytic properties in the area of degradation of organic dyes. Numerous papers have reported the catalytic role of nanoparticles in the dye degradation and amongst those AgNPs and CuO NPs also metal oxide like TiO_2 NPs and its composites have been widely studied, TiO_2 coated with silica gel beads (Zhang, 2013) were used as catalysts for methyl orange (MO) dye degradation, for other dyes like methylene blue (MB) and 4-NP reduction TiO_2 NPs were utilized (Naraginti, et al., 2015). In the synthesis of nanoparticles by chemical synthesis apart from the lengthy procedure additional steps as maintainace of high temperatures and calcinations are required (Zhao, et al., 2011). Although the conventional methods show promising nanoparticles of good catalytical properties and degradation rates (Joseph and Mathew 2015) but the utilization of harmful chemicals during the process contaminates the nanoparticles and the methods that do not contain these chemicals are time consuming and expensive. To avoid and overcome these obstacles the researchers prefer the green synthesis, of nanoparticles for the degradation purposes of organic pollutants. Green approach or the green synthesis involves the use of microorganisms (bacteria and fungi) and plant extracts. The production of nanoparticles using the plant extracts are preferred

over the microorganisms due to the maintainace of the cell cultures, risk of contaminations and difficult downstream processes. Various reports are present on the utilization of the plant extracts for fabrication of the NPs as the phytocompounds present in the extract help in reduction of the salt and also acts as the capping and stabilizing agents during the synthesis of nanoparticles. The fabrication involving the plant extracts are simple, easy and fast and also occur at ambient conditions. Amongst the metallic NPs, silver and copper nanoparticles have become the center of attraction in the application field owing to their optical properties as well as the catalytical applications. The nanoparticles act as a redox catalyst by the formation of superoxide which are responsible for the electron relay effect between the donor and the acceptor molecules (Bogireddy, et al., 2015). Several organic dyes are used in the industries for instance Methyl Orange, Methylene blue, Methyl Red, Congo Red, Eosin Y, Direct black, Crystal violet, and some other dyes like 4-nitrophenol, phenol red (Bogireddy, et al., 2015), Indigo, Bromophenol Blue (Khan, et al., 2016), Acridine orange (Kumar, et al., 2016), Methyl Violet 6B (Sinha, et al., 2014), Reactive blue. Hence, for the fabrication of these nanoparticles a safe and nontoxic, energy saving process has to be developed for the dye degradation in aqueous solutions under mild conditions and economically possible for industrial scale and environment friendly.

Recently, green synthesis of Nanoparticles by using an environmental friendly method involving plant extract has opened a new door to overcome these problems. Simplicity, economical friendliness, low cost, no need to use high pressure, energy, temperature and toxic chemicals are some of many advantages of these green synthesis methods. It has been found that the extracts of plants act both as reducing and capping agents in the synthetic process of the nanoparticles. There have been various reports on the production of silver nanoparticles from different plant extracts which includes *Aloe vera* (Chandran et al.,2006), *Datura metel L*. (Kesharwani et al.,2009), *Centella asiatica* (Roy and Bharadvaja 2017), *Jatropha curcas* (Bar et al., 2009), *Allium cepa* (Saxena et al., 2010), *Achyranthus aspera L*. (Daniel et al., 2011), *Dioscorea bulbifera L.* (Ghosh et al.,2012), *Citrus limon* (Mohapatra et al., 2015), *Plumbago zeylanica* (Roy and Bharadvaja 2017) etc. According to the literature *Centella asiatica* the medicinal plant is known to have several active constituents like triterpenoids and phenols that are responsible for stabilizing the nanoparticles acting as the capping agents.

Centella asiatica (CA), belongs to family *Umbellifere* (*Apiceae*). Popularly known as *gotu kola* is a clonal, herbaceous creeper found throughout in India growing in moist places up to an altitude of 1800 m. It is mostly found in tropical and subtropical countries growing in swampy areas, including parts of India, Sri Lanka, Pakistan also in Madagascar and South Africa, South pacific and parts of Eastern Europe. The whole plant is used for medicinal purposes (Singh, et al., 2002). It is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. In Ayurveda, CA is one of the main herbs for revitalizing the nerves and brain cells. Eastern healers relied on CA to treat emotional disorders, such as depression, that were thought to be rooted in physical problems (Hagemann, et al., 1996). In the Western medicine, during the middle of the twentieth century, CA and its alcohol extracts reported to have shown positive results in the treatment of leprosy (Baily, et al., 1945) and various health care problems. Pharmacological activities of this plant include epilepsy, schizophrenia and cognitive dysfunction, diarrhoea, cholera, measles, jaundice, leukorrhoea, haematemesis, hepatitis, urethritis, toothache, syphilis, smallpox, neuralgia, rheumatism, toothache and varices; and as an antipyretic, analgesic and anti-inflammatory anti-cancer, anti- depressant. The medicinal benefits of the plant owe to the phytocompounds present in the plant.

Centella asiatica is an Ayurvedic plant which is rich in phytocompounds such as Saponins also acknowledged as triterpenoids, Saponins are the principal active constituents of "gotu kola". These triterpenoids include asiaticosides and are responsible for the wound healing properties and vascular effects which inhibit the collagen production at the wound site (Singh, et al., 1969). The other components from *Centella asiatica* such as Brahminoside and brahmoside are thought to be responsible for the CNS actions and utero relaxant properties, but have no present conformations by lieu of clinical studies. One component in the crude extract is centelloside which is found effective in the venous hypertension treatments (Heidari, et al., 2007. Apart from the components that have pharmacological activities there are some constituents of *Centella asiatica* like sterols, flavonoids (Srivastava, et al., 1997), essential acid (0.1% with beta-chariophylen, trans-beta-pharnesen and germachrene D), abundant tannins (20-25%), free aminoacids (alanine, aspartate, glutamate, serine, aminobutyrate, lysine and treonine), phytosterols (campesterol, stigmasterol, sitosterol), fatty acids (linoleic acids, linolnelic, palmitic, oleic, and stearic acids), mucilages, resins, an alkaloid (hydrochotine), a bitter component

(vallerine), flavonoids (derivates of chercetin and kempferol). In addition to, all its medicinal benefits *Centella asiatica* has proven an appreciable source of nanoparticles due the presence of the active constituents that serve as the stabilizing and synthesizing agents for the production of the nanoparticles.

Nanotechnology is the field which deals with the fabrication of minute particles (< 100nm in size), and these particles because of their extreme small size serve as the principal block of various physical and biological systems (Sun et al. 2014). Since last decade, this arena of nano sized particle has achieved new heights because various research fellows from vivid fields have been attracted to their unique properties and applications (Nadhman et al., 2014). Some attractions in world of nanobiotechnology has been silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) (Kharissova et al., 2013) as these minute metallic particles possess potential use in various industries and applications such as catalysis, textile manufacture, and microelectronics and many more. The utilization of these nanoparticles is also in the biological applications which comprises of the biomolecular detection and biosensing, anti-bacterial (Niraimathi et al., 2013) food production, bio-labelling, therapeutics, anti- microbial treatment , drug delivery systems and waste water treatment, as well as biomedical equipment (Jagtap and Bapat 2013; Singh et al., 2010) and gene-based diagnostics/therapies (Thirumurgan et al., 2010). AgNPs are considered to be among the most potent antibacterial agents and catalysts whereas CuNPs have their potential in the catalytic, optical, electrical properties and activities, for a very long time the Cu and CuO have been used as algaecides and fungicides. Cu has been used in the water purifying systems as it owes to the antibacterial properties and antifouling agents which make them potent to be employed in the water treatment plants and effluent systems. The green chemistry employing the plant extracts has given rise to a technology in which nano products, which is biologically and environmentally safer or at least less harmful than currently employed conventional chemical and physical methods (Karn, 2008).

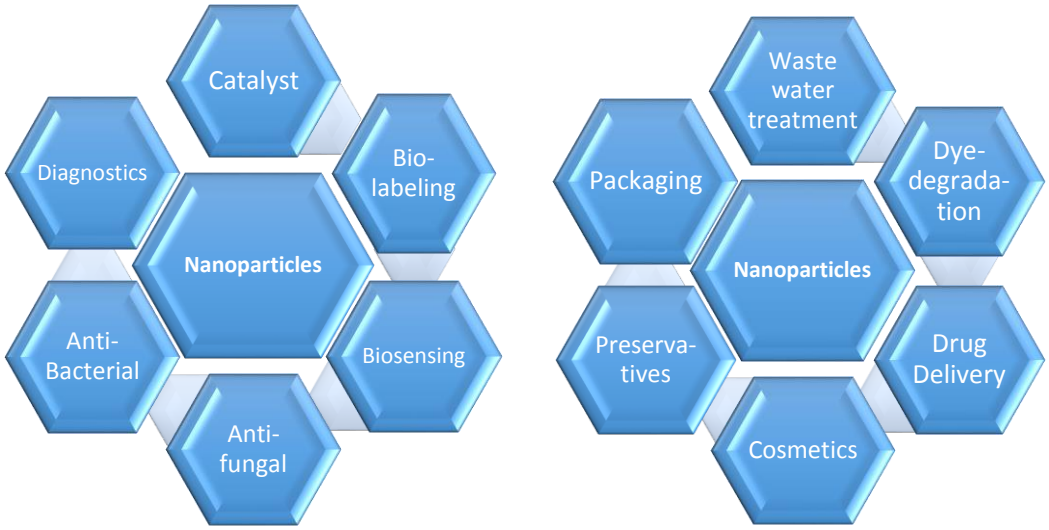


Figure 1: Applications of Nanoparticles

Applications of nanoparticles are inestimable owing to its strong optical properties related to surface plasmon resonance effect and has been exploited in the design and development of many biomedical equipment like magnetic resonance imaging (MRI), computed tomography (CT) scanning and X- ray. These metallic nanoparticles have the potential to replace conventional contrast-enhancing agents used in the imaging therapies. Due to its surface plasmon effect theses metallic nanoparticles show tremendous applications such as optical receptors in biosensors (Karimzadeh and Mansour 2010), sensors (Cobley et al., 2009), act as excellent catalysts in the chemical reactions (Edison and Sethuraman 2012), DNA detection and delivery, bio-analyzers (Ravindran et al., 2013), free radical scavenging (Ramamurthy et al., 2013), and antimicrobial agents (Netala et al., 2015). Due to the expeditiously growing science of producing and utilizing nano-sized particles, it requires more sophisticated synthesis approaches to challenge the inherent approaches available such as thermal decomposition (Yang et al., 2007), process employing microwave, gamma radiations, ultrasound irradiation, laser ablation (Simakin et al., 2004), electrochemical assisted (Hosseini and Momeni 2010), sonochemical synthesis (Salkar et al., 1999) and chemical reduction (Lee and Meisel 1982), photochemical (Callegari, et al., 2003), Langmuir Blodgett (Zhang, et al., 2006). Many of these nanoparticles synthesis methods involve the use of hazardous chemicals, low material conversions and have high energy requirements. These process a potential threat to the environment and biological risks, this renders for the need to develop an alternative environmentally friendly process for nanoparticles synthesis without using toxic

chemicals, these approaches are biosynthetic approaches also called green synthesis of nanoparticles.

Green synthesis or green chemistry is the emerging technology for the nanoparticle synthesis which exploits the use of either microorganisms or plant extracts and have emerged as a simple and viable alternative to the chemical and physical methods. These extracts have been an exclusive choice for numerous researchers as they are natural producers of reducing and capping agents that play a vital role in the nanoparticles synthesis. Also, green synthesis is safe for therapeutic use and unlike the microbial synthesis it is less complex and has almost no scope of contaminations. They offer advantages such as environment friendly, single step process, cost-effective and possess almost no toxicity. Apart from this extracellular and intracellular biosynthesis of metallic nanoparticles employed with bacteria, fungi and yeast etc., are eco-friendly method but cultivation of these biomass and downstream processing of nanoparticles seems to be a problematic task and pose a risk of contamination.

Amongst the various noble metals acting as nanoparticles most explored and potential nanoparticle that has been known for a long time are silver nanoparticles and copper nanoparticles. Silver nanoparticles exhibit excellent optoelectronic properties which depend on their shape, size, composition including their environment and also spatial ordering of particles. Silver is a well-known antimicrobial agent, owing to its strong toxicity against a broad spectrum of microorganisms; bacteria, fungi, protozoa and certain viruses due to this property silver based compounds have been broadly used in many bactericidal applications. The persistence of antibiotic resistant bacteria has exploited the anti-microbial properties of silver and silver-based compounds, including silver nanoparticles (Ravindran, et al., 2013). The antibacterial properties of silver nanoparticles are associated with the small size that enables the penetration through the cell membrane to affect the intracellular mechanism and release of reactive oxygen species and liberation of Ag+ ions to the environment which interact with the thiol groups of vital enzymes and inactivates them. Experiments reveal that DNA losses its replication ability on treatment with silver nanoparticles. Other studies state that there are some evident structural changes in the cell membrane as well as formation of small electron-dense granules formed by silver and sulphur (Morones, et al., 2005). Silver nanoparticles have emerged itself as potential antifungal agent, fungal infections are often found in the immunocompromised patients going through chemotherapy and HIV infections

(Ashavani Kumar, et al., 2008). Some reports show that silver nanoparticles exhibit tremendous anti-fungal effects against *Candida albicans* (K.J Kim, et al., 2009), *Candida nonalbicans and Candida tropicalis* (Netala, et al., 2016) by inhibiting the budding process and disrupting the cell membrane. The silver nanoparticles owing to the size dependent surface plasmon can detect a single molecule (Nie and Emory 1997). Thus, the detection of various biomolecular analytes using plasmonic sensors is an advancement of nanobiotechnology field (Potara, et al. 2012). This advancement has shown utilisation in the detection of immunoglobulins, peptides and proteins. There have been various reports on the production of silver nanoparticles from different plant extracts.

The size, composition and nanostructure can be employed to adjust the optical properties (Krutyakov**,** et al., 2008; Farland et al., 2003). The size dependent surface plasmon resonance contributes to surface enhanced Raman signals intense enough to detect a single molecule (Nie and Emory 1997). Thus, the detection of various biomolecular analytes using plasmonic sensors is an advancement of nanobiotechnology field (Monica Potara, et al., 2012). (Netala et al. 2015) synthesised the silver nanoparticles from the aqueous callus cultures of *Centella asiatica*. The 5-20 nm sized, crystalline nanoparticles were obtained Phenolic content present in the callus extract was reported to act as the reducing agent which was able to convert the Ag+ ions to AuNPs and proteins present in the extract stabilising and synthesising the NPs. In another study, the synthesis of silver nanoparticles was reported from fresh leaf extract of *Centella asiatica* nanoparticles sized 3-30nm (Vuong et al., 2017). Logeswari, et al., (2015) also reported that *C. asiatica* plant extracts showed the formation of silver nanoparticles and the average diameter of the synthesized nanoparticles was 28.4nm. (Rout, et al., 2013) reported spherical and cubic silver nanoparticles 30-50 nm size synthesis from $AgNO_3^-$ ions to AuNPs utilizing the active compound Chavicol. Roy and Bhardavaja 2017 have reported synthesis of AgNPs from three different extracts of *Centella asiatica* viz. ethanolic, methanolic and aqueous extracts of from different accessions forming spherical AgNPs. FTIR studies showed terpenoinds, long chain fatty acids and secondary amide derivatives were responsible for the nanoparticle synthesis and proteins as capping agents.

CHAPTER 2

MATERIALS & METHODS

Silver nitrate (99.99%), Methyl orange, Methyl red, Phenol red, Eosin Y and Sodium borohydride were procured from Sigma Aldrich and Cupric Sulphate from Fisher Scientific. All glass wares were cleaned and rinsed several times with distilled water.

2.1. Preparation of extract

Centella asiatica was procured from NBPGR, New Delhi. The tubes of *Centella asiatica* were periodically sub cultured after an interval of 4-8 weeks. The green leaves of *C. asiatica* were collected from the freshly sub-cultured tubes, washed and then dried to remove excess water on them. After drying them, the leaves were weighed and then boiled in distilled water (1gm/ 10ml) in a sterilized conical flask of 250 ml volume for 20 min at 60°C. The extract obtained was filtered with Whatman Filter Paper No. 1 to remove the impurities and centrifuged at 10,000 rpm for 5 min which was stored at 4°C for further use in experiments.

2.2 Synthesis of Nanoparticles

2.2.1 Synthesis of Silver nanoparticles

Four concentration ratios of silver nitrate and plant extract have been synthesized by adding 1 mL of plant extract each to 50 mL of 1mM, 2mM, 3mM, 4mM silver nitrate solution. The formation of silver nanoparticles was indicated by the appearance of yellowish brown color with immediate addition of the plant extract to the silver nitrate solutions.

2.2.2 Synthesis of Copper nanoparticles

Copper nanoparticles were synthesized by adding 1ml of plant extract to different molar concentrations of 50 ml of Cupric sulphate ($CuSO_4.5H_2O$) at 1mM, 5mM, 20mM, 100mM to obtain copper nanoparticles of different concentrations and varied properties. The formation of copper nanoparticles was indicated by the change of color from colour

less to bluish green at immediate addition of the plant extract to the cupric sulphate solutions.

2.3 Preparation of Dyes and Sodium borohydride

1mM of the dye (Methyl orange, Methyl red, Phenol red, Eosin Y) is prepared in 50ml of distilled water. 50ml of Sodium borohydride (100mM) was prepared freshly before the experimental setup every time.

2.4 Characterization of Nanoparticles

2.4.1 UV-Vis Spectrophotometer

UV-Vis spectroscopic studies for determining optical properties of nanoparticles were carried out on Cary-300 UV-Vis by Agilent Technologies at Delhi Technological University, Chemistry Department. Silver nanoparticles were characterized at a wavelength range between 300-700nm and copper nanoparticles at 200-700nm at different time intervals from 0 hour to 72 hours after the addition of *Centella asiatica* plant extract to the silver nitrate and cupric sulpate solutions.

2.4.2 SEM

The study for the surface morphology of the nanoparticles, SEM images were visualized in a Carl Zeiss EVO 18 SEM, at an accelerating voltage of 20 kV. The suspension of nanoparticles, were added over glass coverslips, dried in an oven, mounted onto the designated SEM holders using conductive carbon tape and gold-coated before visualization. The images were taken at magnification of 50 KX with 200 nm bar.

2.5 Catalysis with Nanoparticles

2.5.1 Photo catalysis with Silver nanoparticles

1ml of 10^{-3}M Methyl orange (MO), Methyl red (MR), Phenol red (PR) and Eosin Y(EY) was measured separately and added with 1 mL each from different concentration (1mM, 2mM, 3mM, 4mM) of silver nanoparticle solution. The volume was made upto 5ml by adding distilled water to the solution of respective dye with the respective concentration of silver particle solution and kept for stirring in dark for 2hr to achieve an equilibrium between the dye and the nanoparticles. After the dark incubation, the solution was kept

in sunlight for 0-24 hours to check the degradation rate and degradation value at regular intervals of time. The dye degradation process is analyzed by the UV-Vis spectrophotometer (Cary 300 UV-Vis).

2.5.2 Chemical Catalysis of Dyes with silver nanoparticles

1 mL 100 mM sodium borohydride solution is added to 1 mL 10−3M methyl orange (MO), Methyl red (MR), Phenol red (PR) and Eosin Y(EY) respectively. Then 1 ml from different concentration (1mM, 2mM, 3mM, 4mM) of silver nanoparticles is added to each dye with sodium borohydride solution and stirred. The solutions are then made up to 5 mL using distilled water and vigorously stirred for some time. The degradation of dyes is indicated by the decolorization of the solution. Methyl orange, Methyl red and Phenol red become colorless in an oxidizing environment because of the presence of reducing agent (NaBH4) and the nanoparticles indicating the reduction of these dyes. The degradation rate of dyes were recorded upto 3hrs after that no significant reduction was observed. The reaction unsupported by the catalyst is a studied as a reference. All the degradation process was monitored using UV–visible absorption spectrophotometer (Cary 300 UV-Vis).

2.5.3 Photo catalysis with Copper nanoparticles

1ml of 10^{-3}M Methyl orange (MO), Methyl red (MR), Phenol red (PR) and Eosin Y(EY) was measured separately and added with 1 mL each from different concentration (1mM, 5mM, 20mM, 100mM) of copper nanoparticle solution. The volume was made upto 5ml by adding distilled water to the solution of respective dye with their respective concentration of copper particle solution and kept for stirring in dark for 2hr to achieve an equilibrium between the dye and the nanoparticles. After the dark incubation, the solution was kept in sunlight for 3 hours to check the degradation rate and degradation value at regular intervals of time. The dye degradation process is analyzed by the UV-Vis spectrophotometer (Cary 300 UV-Vis).

2.5.4 Chemical Catalysis with copper nanoparticles

1 mL 100 mM sodium borohydride solution is added to 1 mL 10−3M methyl orange (MO), Methyl red (MR), Phenol red (PR) and Eosin Y (EY) respectively. Then 1 ml from

different concentration (1mM, 2mM, 3mM, 4mM) of copper nanoparticles solution is added to each dye with sodium borohydride solution and stirred. The solutions are then made up to 5 mL using distilled water and vigorously stirred again for some time. The degradation of dyes is indicated by the decolorization of the solution. All the dyes precipitated and became colorless in an oxidizing environment because of the presence of reducing agent ($NaBH_4$) and the nanoparticles within 5-7 minutes of reaction indicating the reduction of these dyes. The reaction unsupported by the catalyst is a studied as a reference. All the degradation process was monitored using UV–visible absorption spectrophotometer (Cary 300 UV-Vis).

2.6 UV-Visible spectroscopy of the degraded dye:

The UV-Vis analysis of the degraded dye was done by aliquoting 1ml from the solution of dye and nanoparticle and analyzed the optical properties in the quartz cuvette keeping double distilled water as blank in the other quartz cuvette and measuring it within the respective wavelength range in which the nanoparticle is expected to show absorbance. Silver nanoparticles are analyzed in the wavelength range of 300-700 nm but for Copper nanoparticles the wavelength range is from 200-700nm. The result of the dye degradation was determined by the UV-Visible spectroscopy (Cary Series 300 UV-SPECTROMETER), by calculating the dye degradation percentage.

2.7 Percentage of Dye degradation:

The dye degradation percentage can be evaluated by calculating the difference between the initial concentration of the absorbance and the final concentration of the absorbance and dividing it with the initial concentration by using the given formulae:

$\{(C_o - C_t)/ C_o * 100\}$

Where C_o is the initial concentration of the dye and C_t is the concentration of dye after t hours of sun exposure.

Centella asiatica leaves

Plant extract

$AgNO_3$ / $CuSO_4$ solution added to the plant extract

Nanoparticles synthesized

Different dyes

2

Added NPs, stirred kept in dark for some time, then kept in sunlight for photocatalysis

1

$NaBH_4$ added to different dye concentrations

Nanoparticles added to Dye + $NaBH_4$

Degraded Dye

UV-Vis spectroscopy for result analysis

Calculation of % of dye degradation

Figure 2: Schematic representation of the synthesis and catalytic degradation of the dyes by Silver and Copper nanoparticles.

CHAPTER 3

RESULTS

3.1 Synthesis of Nanoparticles

3.1.1 Silver nanoparticle synthesis

Silver nanoparticles were synthesized from the *Centella asiatica* leaf extract by adding $AgNO_3$ solution of different concentrations (1mM, 2mM, 3mM, 4mM) and after immediate addition of the plant extract to the solutions of $AgNO_3$, colour change was observed from colorless to yellowish brown, which is characteristic of the production of silver nanoparticles.

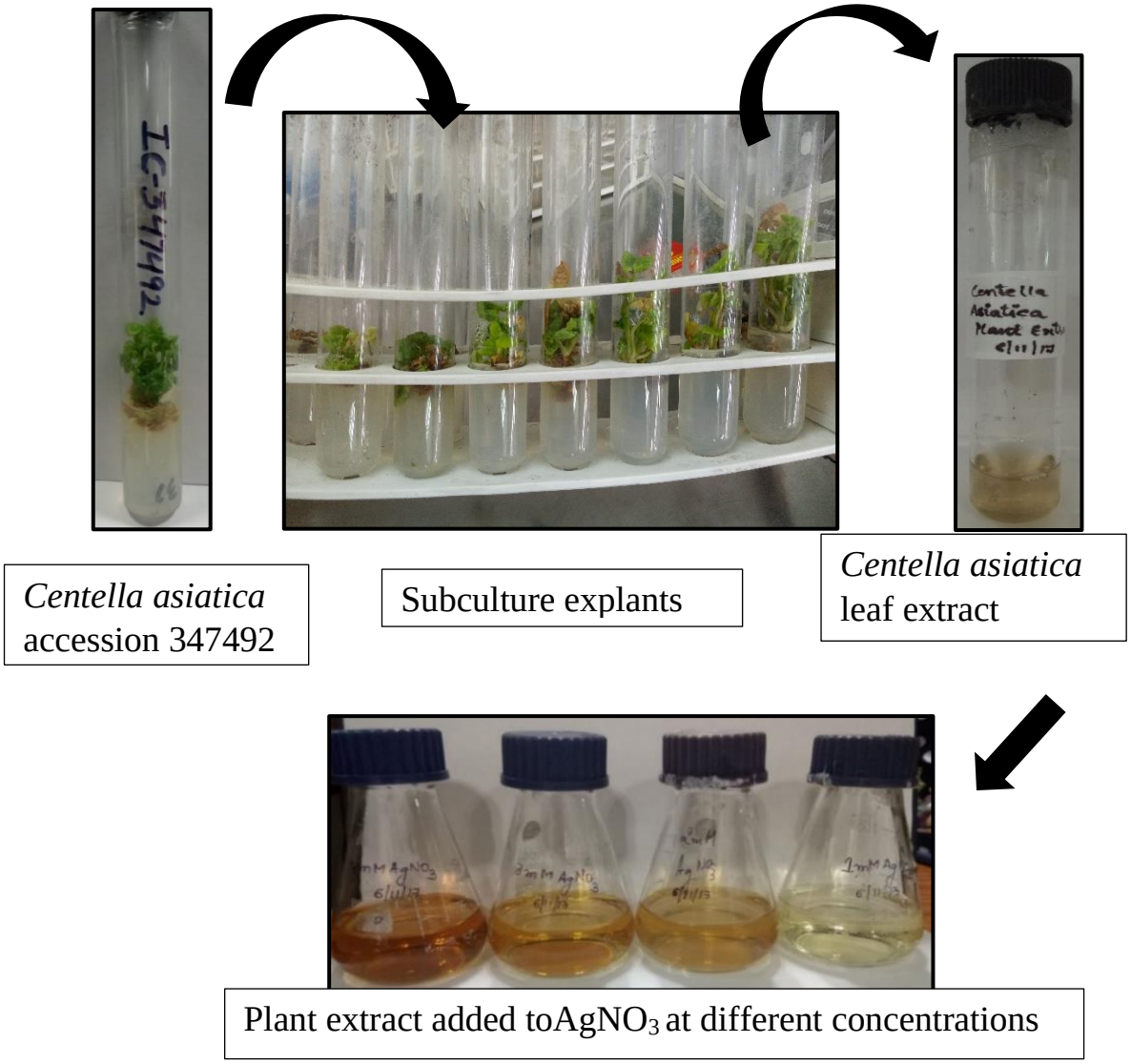


Figure 3: Synthesis of Silver nanoparticles from the leaf extracts of *Centella asiatica*

The colour difference in the solution was observed with the increasing concentration of the silver nitrate concentration, with the increasing concentration of the silver nitrate from 1mM to 4mM the intensity of the yellowish brown colour also increased inferring about the elevated concentration of nanoparticle formation.

3.1.2 Copper nanoparticle synthesis

Copper nanoparticles were synthesized by adding 1ml of *Centella asiatica* leaf extract to Cupric Sulphate ($CuSO_4.5H_2O$) solution of different concentrations (1mM, 5mM, 20mM, 100mM) and after addition of the plant extract to these solutions of Cupric Sulphate, immediate colour change was observed from colorless to bluish green, which is observed during the production of copper nanoparticles. The colour intensity of the solution was observed to be increasing with the increasing concentration of the cupric sulphate concentration from 1mM to 100mM. The colour intensity changed from light blue to bluish green moving from 1mM to 100mM inferring about the increasing concentration of copper nanoparticles and their sizes.

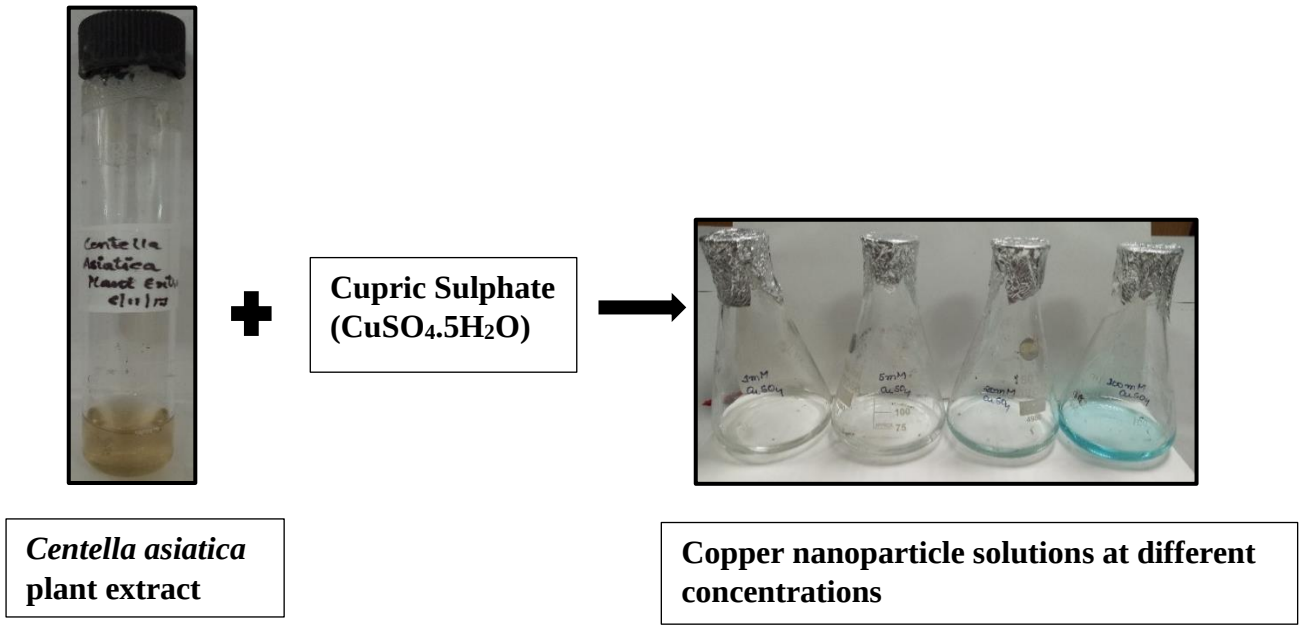
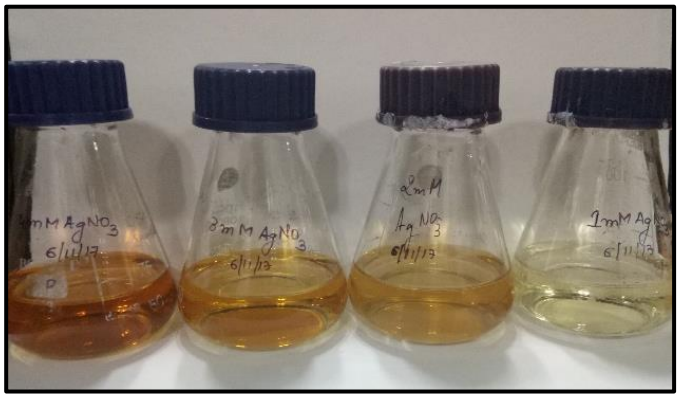


Figure 4: Synthesis of Copper nanoparticles from the leaf extracts of *Centella asiatica*

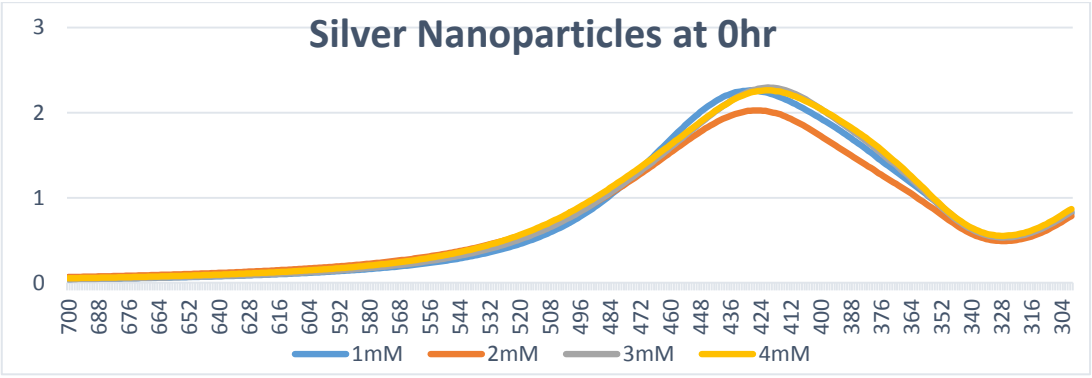
3.2 UV-Visible spectroscopy analysis

3.2.1 UV-Vis analysis of Silver Nanoparticles

The UV-Visible absorption spectra of the samples showed a highlighted peak in the range of 330-550nm which is characteristic of silver nanoparticles. Due to the surface plasmon resonance phenomenon, the AgNPs exhibited strong absorption peak in the visible range named SPR peak due to the surface plasmon excitation. The max SPR peak was observed at 430nm which decreased with the increasing concentration of the $AgNO_3$ (Table 2). The decrease in the SPR peak is evident of the decreasing particle size of the nanoparticles synthesized. The SPR peak and different molecules of the plant leaf extracts could be responsible for capping and stabilization of AgNPs formed. This particular SPR peak may also correspond for the spherical shape of AgNPs. The highest peak of absorbance was shown at 0^{th} hour at all the concentrations of $AgNO_3$.



(i)



(ii)

Figure 5: (i) 0hr Reaction mixture of $AgNO_3$ and plant extract; (ii) UV-Vis spectrometer graph of AgNps at different concentrations of $AgNO_3$ at 0hr.

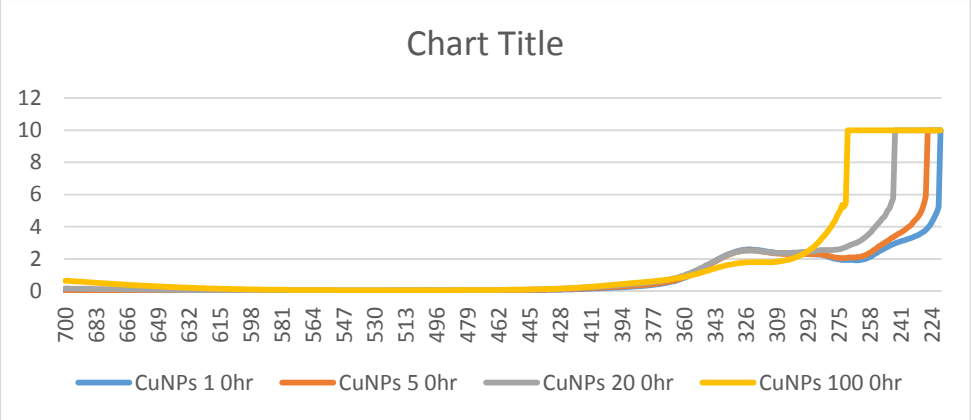
Table 1: Absorbance of $AgNO_3$, maximum absorbance and absorbance range of different concentration of AgNPs

Sample	Concentration of $AgNO_3$ (mM)	Range of Absorbance peak(nm)	SPR peak of AgNPs(nm)	Absorption Range
Sample 1	1mM	330-540	430	1.25-2.25
Sample 2	2mM	330-550	425	1.20-2.00
Sample 3	3mM	330-530	420	1.30-2.30
Sample 4	4mM	330-530	420	1.20-2.25

3.2.2 UV-Vis analysis of Copper Nanoparticle

Copper nanoparticles synthesized at different concentrations 1mM, 5mM, 20mM, 100mM give a highlighted absorbance peak in the range of 250-360nm which is the characteristic range of copper nanoparticle synthesis as stated in some previous studies. The absorbance range of the nanoparticle falls in the range of 0.8- 3.05 for 1mM, 5mM, 20mM copper nanoparticles but the absorption range of 100mM nanoparticles was less 0.9-1.8 because of the SPR effect, more the concentration of the nanoparticles lesser the size becomes. The maximum absorption of copper nanoparticles was seen at the wavelength of 323 nm at 0hr of every concentration and moreover maximum absorption was observed in 1mM concentration of the copper nanoparticle of all the concentrations.

(i)



(ii)

Figure 6: (i) 0hr Reaction mixture of $CuSO_4.5H_2O$ and plant extract; (ii) UV-Vis spectrometer graph of CuNPs at 0hr.

Table 2: Absorbance of CuNPs, maximum absorbance and absorbance range of different concentration of CuNPs

Sample	Concentration of CuNPs (mM)	Range of Absorbance peak(nm)	SPR peak of CuNPs(nm)	Absorption Range
Sample 1	1mM	360-255	323	0.81- 2.30
Sample 2	5mM	360-255	323	0.85- 2.59
Sample 3	20mM	340-250	323	0.89- 3.05
Sample 4	100mM	345-310	322	0.95- 1.81

3.3 SEM

The SEM images were visualized on the Carl Zeiss EVO 18, at an accelerating voltage of 20 kV, a magnification of 500X and a bar of 200nm. SEM images of silver nanoparticles showed spherical and agglomerated nanoparticles with estimated size range of 30-50nm.

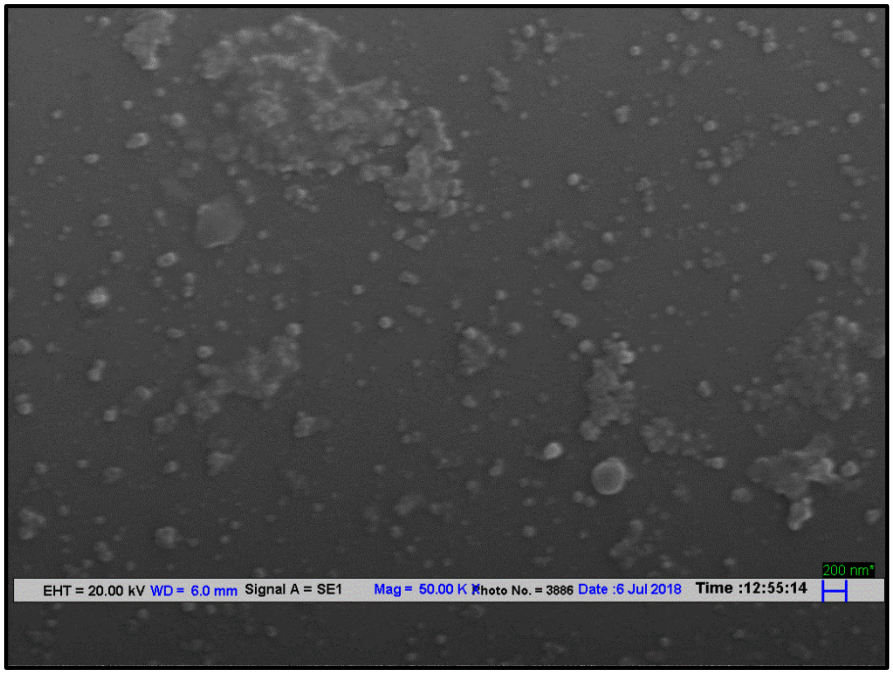


Fig 7: SEM image of Silver Nanoparticles by Carl Zeiss EVO 18 at IIT Delhi SEM Facility

The SEM images of the copper nanoparticles inferred that the shape of the nanoparticles was spherical and at 500X magnification it appeared as small particles and hence, we can say that the nanoparticle can fall under the range of 20-30nm size.

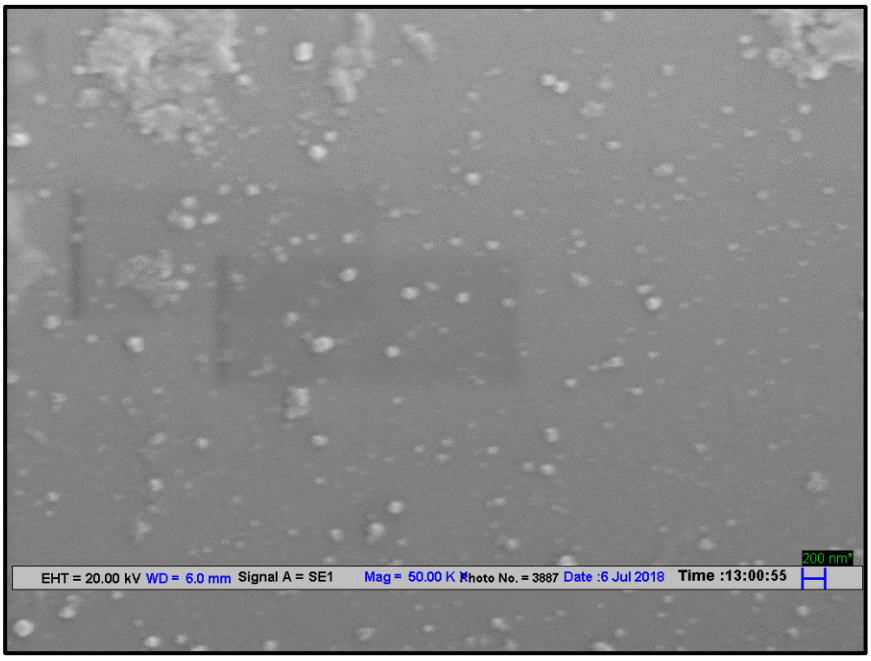


Fig 8: SEM image of Copper Nanoparticles by Carl Zeiss EVO 18 at IIT Delhi SEM Facility

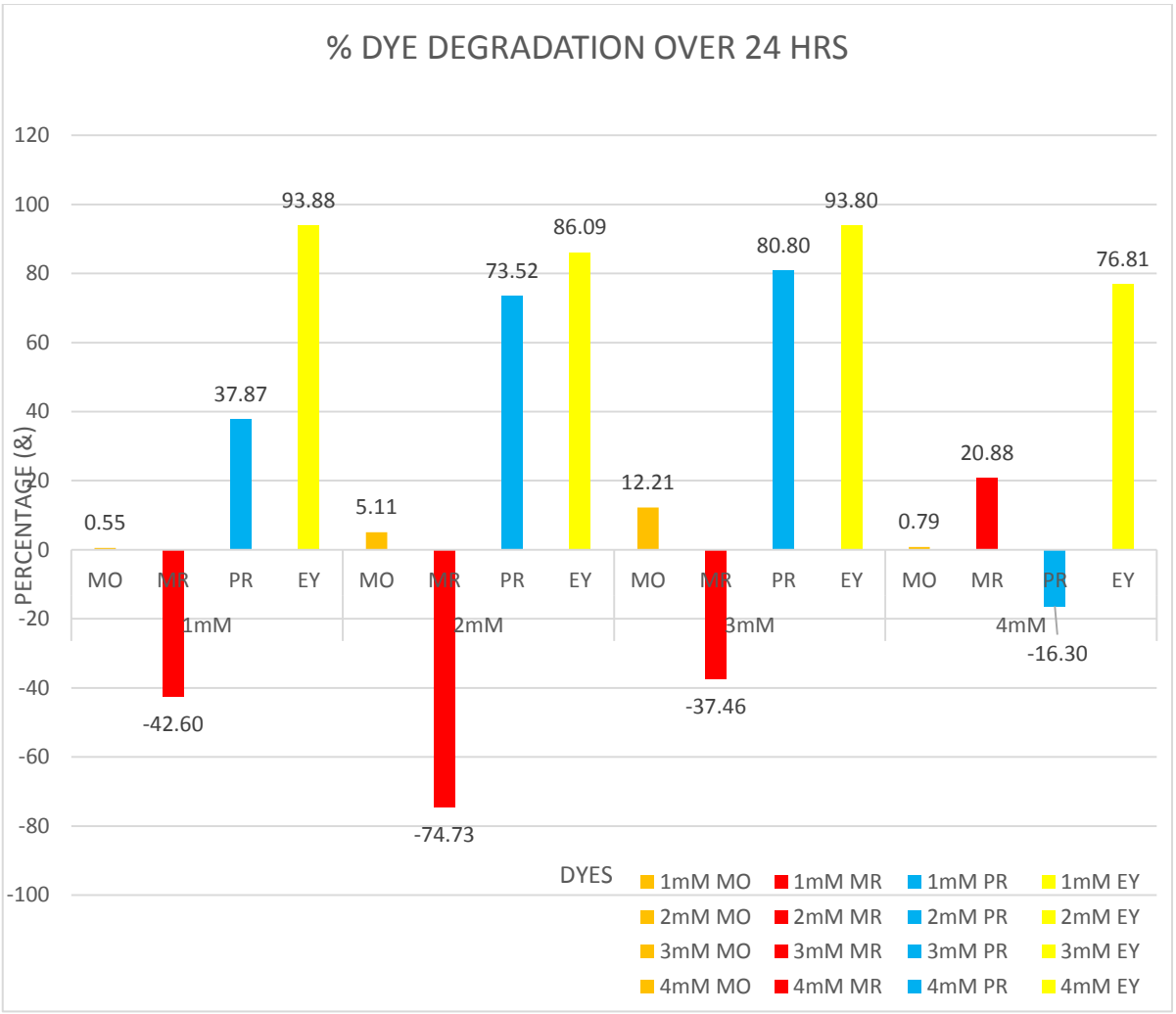
3.4 Catalytic Dye degradation

3.4.1 Catalytic degradation of Dye with Silver Nanoparticles

The synthesized Silver nanoparticles in this reported work are utilized in the dye degradation process which is one of the aspect of all the other applications of the nanoparticles and their wide horizon of properties. Different dyes i.e Methyl orange, Methyl red, Phenol red and Eosin Y are degraded by the metallic nanoparticles by 2 different methods, Photocatalytic degradation & Catalytic degradation by $NaBH_4$. Absorption spectra of the dyes with the nanoparticles under different conditions at periodic intervals were recorded. Control experiments were also setup which had no Nanoparticles in them and showed no changes in the dyes.

3.4.1.1 Photocatalytic Dye Degradation

In this method the dyes (Methyl Orange, Methyl Red, Phenol Red and Eosin Y) were degraded by Silver nanoparticles under solar irradiation. The degradation was visually observed most in Eosin Y, Phenol Red and Methyl Orange in decreasing order and almost negligible in Methyl Red. The gradual fading of dye colour was evident with the increasing exposed time up to 24 hrs. Absorption spectra of the dyes at periodic intervals were recorded by UV-Vis spectrophotometer. Maximum degradation was perceived by 2mM, 3mM silver nanoparticle solution for the Phenol red and Eosin Y and low levels of degradation was noticed in Methyl orange. Although almost no degradation was witnessed in Methyl red except the 4mM silver nanoparticle solution which showed degradation of Methyl red up to 20%. Eosin Y was the dye which was maximum degraded by all the concentration of nanoparticles and the dye percentage was as high as 93% by 3mM and lowest upto 76% by 4 mM. The extended data on the dye degradation by photocatalytic method is given in Graph 1.



Graph 1: Percentage of dye degradation by AgNps with photocatalytic method over 24 hours of sun exposure

In the Photocatalytic dye degradation method by Silver Nanoparticles best results were given by 2mM and 3mM aqueous solution of silver nanoparticles at 24 hours. Eosin Y degradation percentage by all the concentrations of silver nanoparticles was highest followed by Phenol Red, a slight degradation is also seen in the Methyl orange dye, but no degradation is shown by Methyl Red by this method.

Dye degradation percentage at 24 hours: EY>PR>MO>MR

Table 3: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 0hr and with different concentration aq. solution of silver nanoparticles.

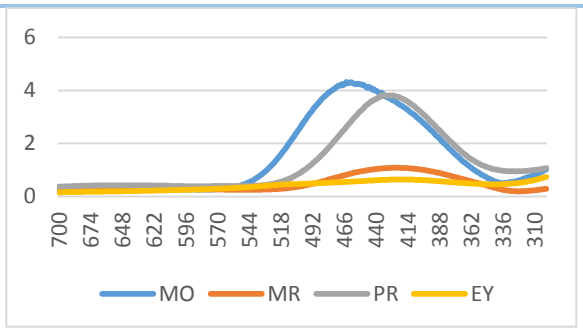
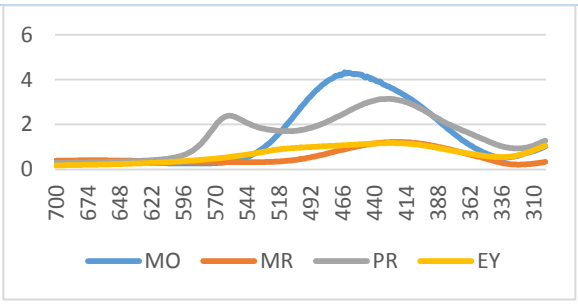
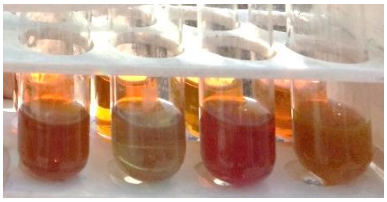
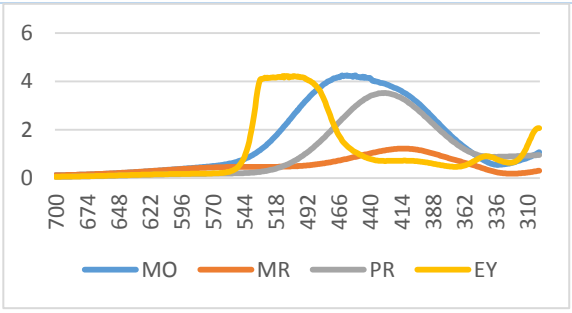
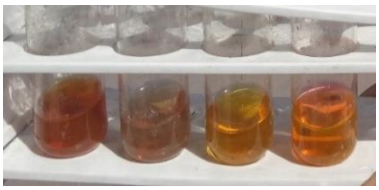
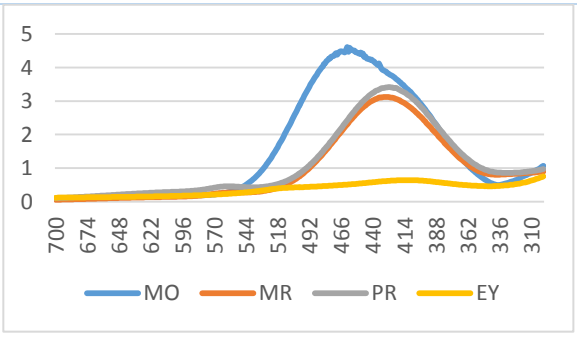
Conc. of aqueous solution of AgNps	Graph	Image
1mM		
2mM		
3mM		
4mM		

Table 4: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 2 hr and with different concentration aq. Solution of silver nanoparticles.

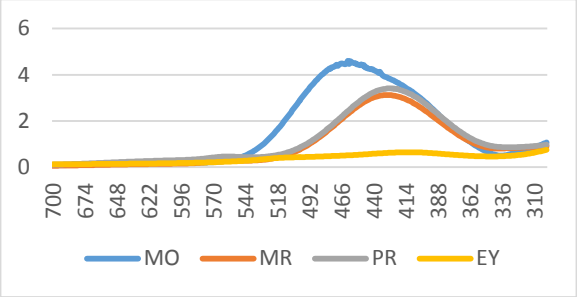
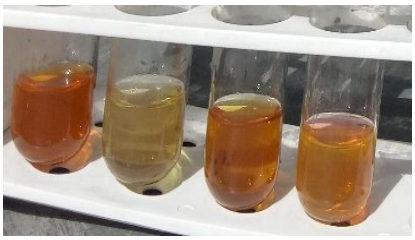
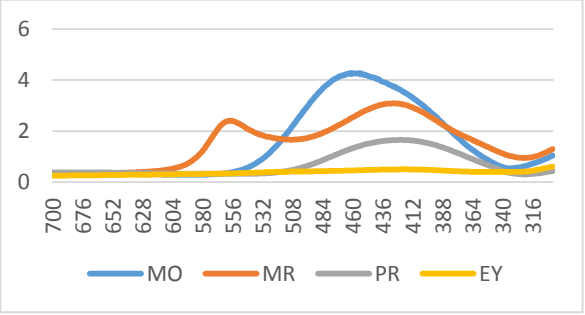
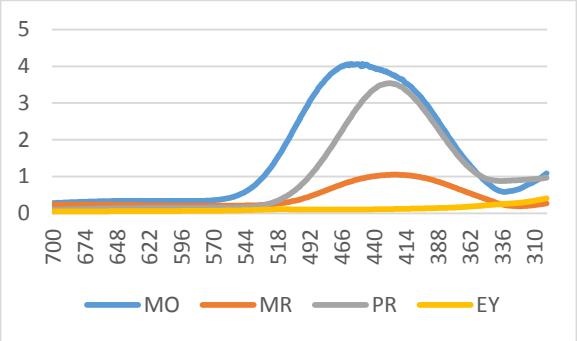
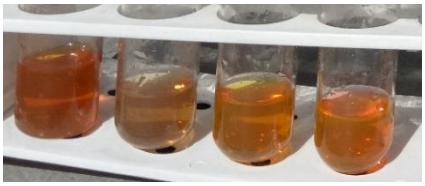
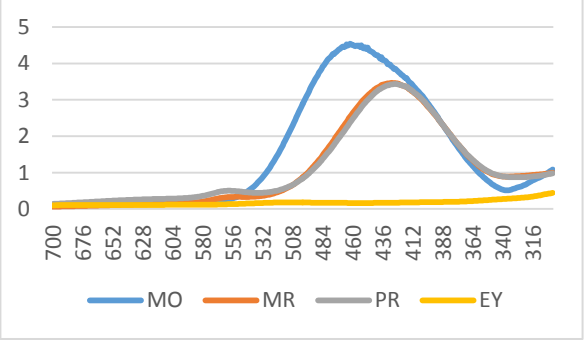
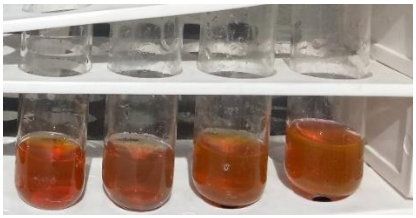
Conc. of aqueous solution of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

Table 5: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 6 hr and with different concentration aq. Solution of silver nanoparticles.

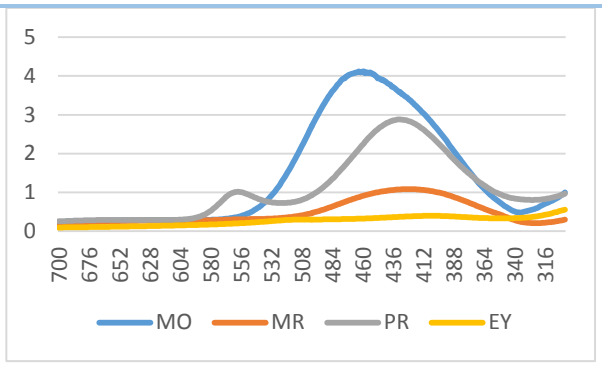
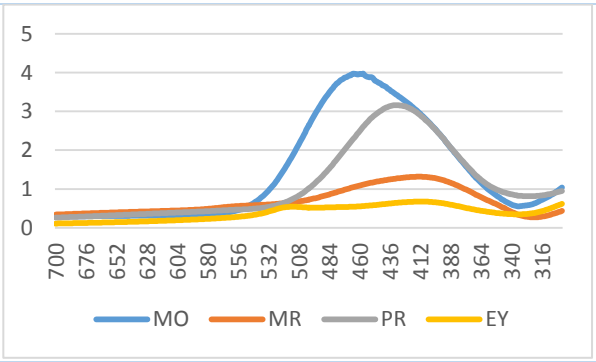
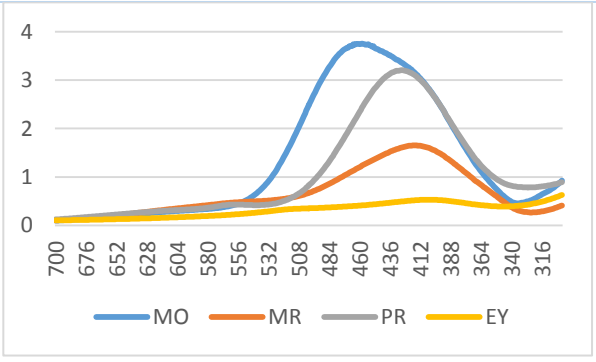
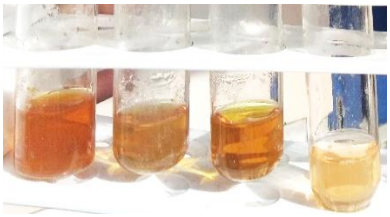
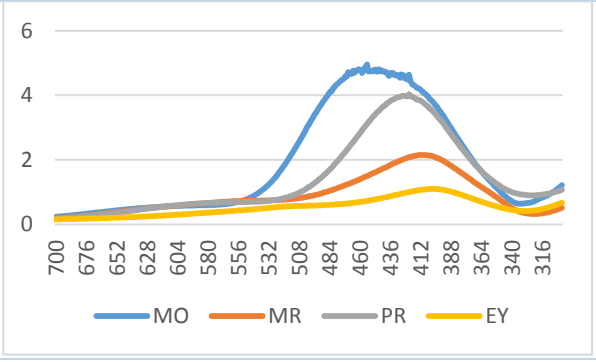
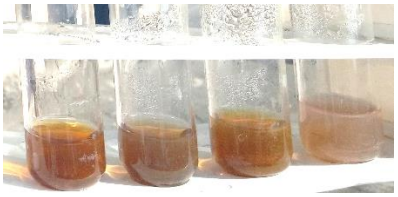
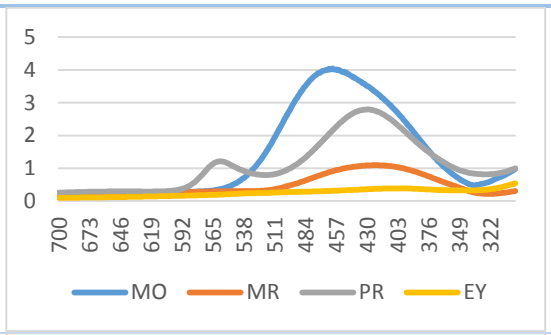
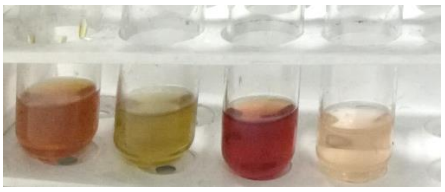
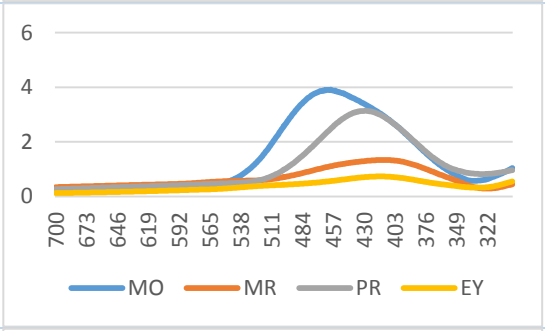
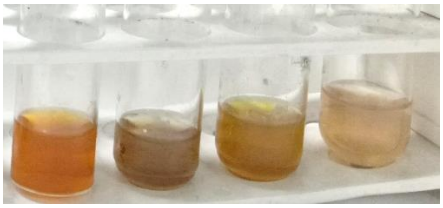
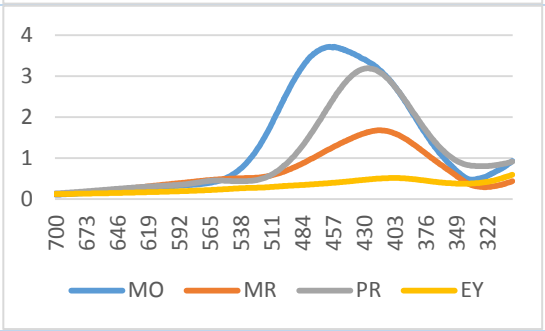
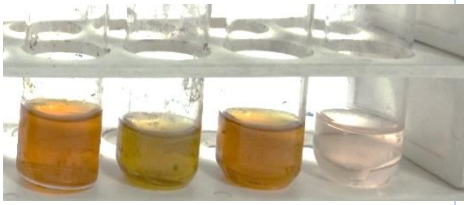
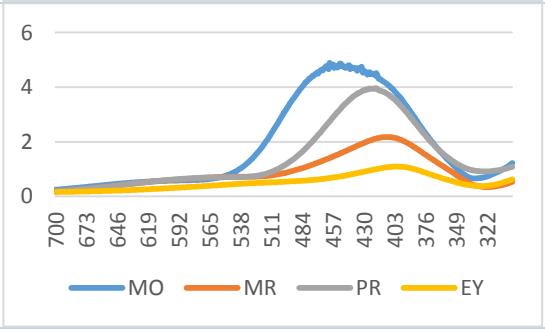
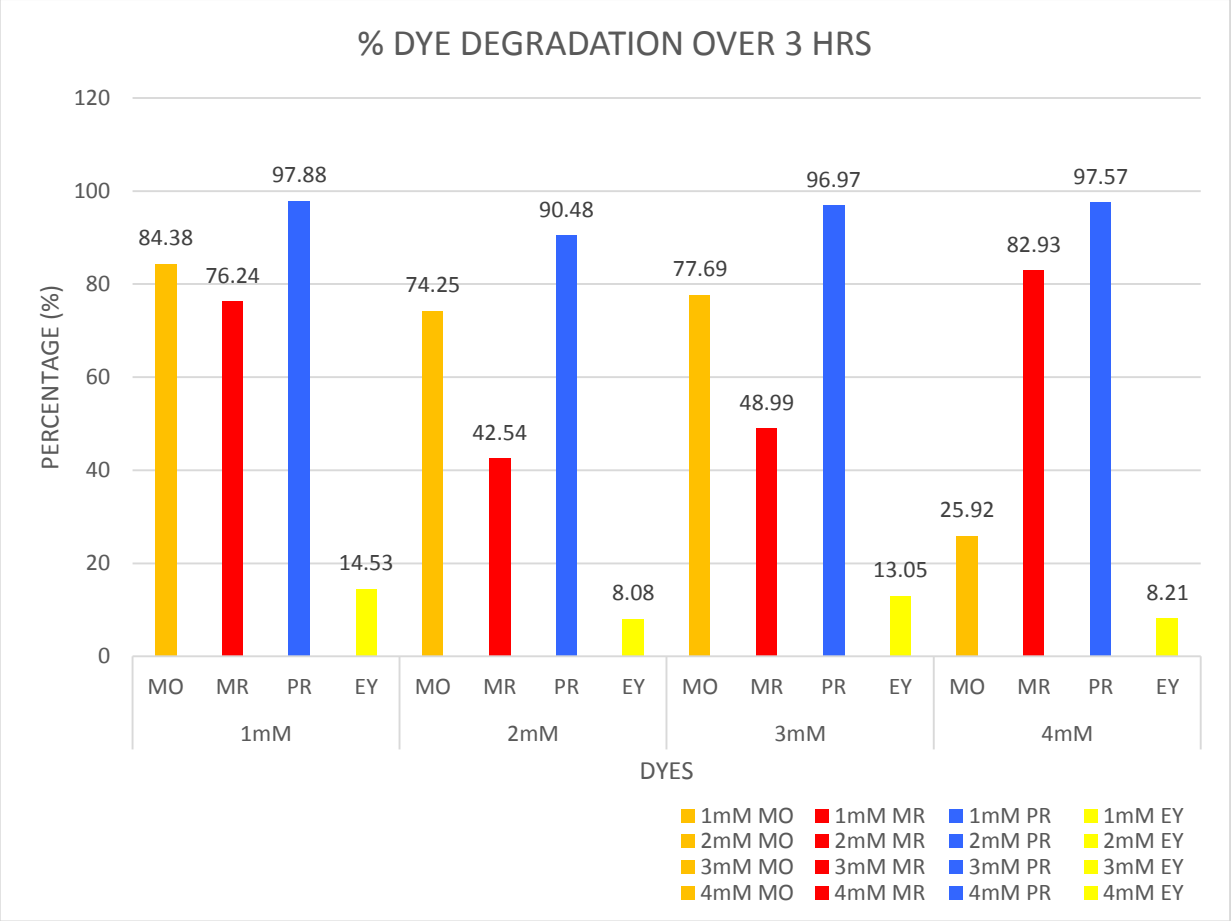
Conc of aq sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

Table 6: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 24 hr and with different concentration aq. Solution of silver nanoparticles

Conc of aq sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

3.4.1.2 Catalysis with Sodium Borohydride:

In this method the dyes (Methyl Orange, Methyl Red, Phenol Red and Eosin Y) were degraded by Silver nanoparticles using the reducing agent $NaBH_4$. The degradation of dyes is indicated by the decolorization of the solution. Methyl orange, Methyl red and Phenol red become colorless in an oxidizing environment due to the presence of reducing agent ($NaBH_4$) indicating the reduction of these dyes. The degradation was visually observed most in Phenol Red, Methyl Orange and Methyl Red and minimal change was observed in Eosin Y. The gradual fading of dye colour was evident with the increasing exposed time upto 3 hrs. Absorption spectra of the dyes at periodic intervals were recorded. Phenol red was most degraded by all the silver nanoparticle concentrations of all the dyes, with the dye degradation percentage of 90-97%. Highest degradation was perceived by the 1mm concentration of silver nanoparticles with 97.88% and least by 2mM concentration of AgNPs up to 90.48%. Following the impressive degradation of the phenol red, methyl orange stands second in degrading. Methyl orange was degraded finest by 1mm concentration of AgNPs by degrading it to 84.37% followed by 3mm and 2mM which degraded it upto 77% and 74% respectively and least by 4mM AgNPs. Methyl red being degraded most by 4mM and also 1mM by 82.9% and 76.2% respectively, also 2mM and 3mM did justice to its degradation but almost half of the previous concentrations which is upto 46%. Eosin Y was least degraded by all the silver concentrations, 1mM being the superlative among all the concentrations with 14% and 2mM being the least with 8%.



Graph 2: Percentage of dye degradation by AgNps with catalytic method using NaBH4 over 3 hours

In the method using reducing agent $NaBH_4$ in the aqueous solution of silver nanoparticles of concentration 1mM, 2mM and 3mM, 4mM showed a decent dye degradation percentage in MO, MR, PR but least in EY. 1mM and 3mM aqueous solution of silver nanoparticles showed best results in degrading the dyes in 3 hours but no degradation was observed after 3 hours. MO, MR, PR became colorless after 3 hours in almost all the varied concentrations of silver nanoparticles.

Dye degradation percentage at 3 hours: PR>MO>MR>EY

The dye degradation method involving the reducing agent $NaBH_4$ shows better degradation rates with Silver Nanoparticles in less time than the method exploiting only the Silver Nanoparticles with exposed irradiation.

Table 7: Dye degradation of MO, MR, PR, EY (from left to right) at 0 hour in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles.

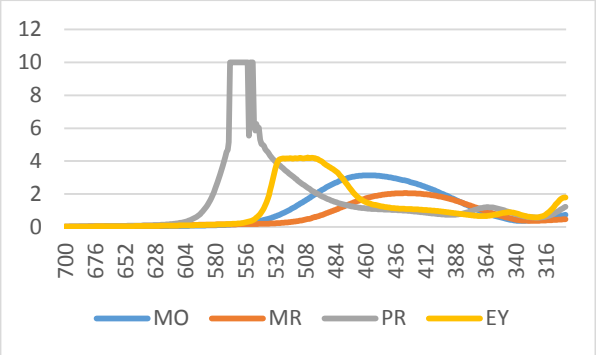
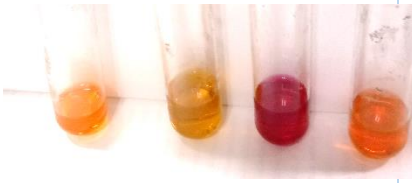
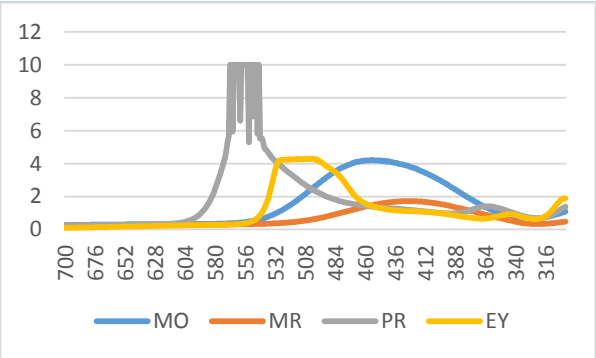
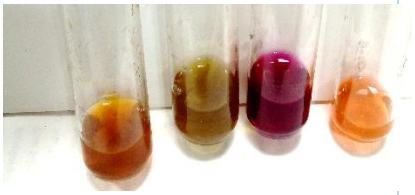
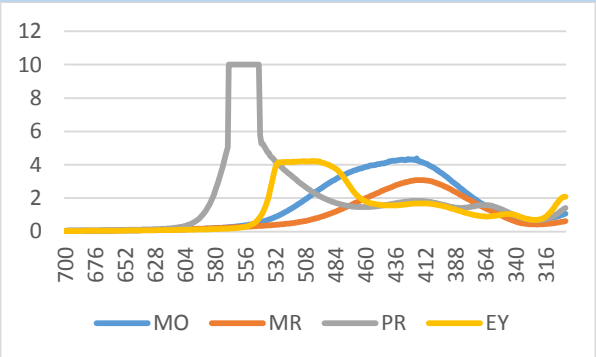
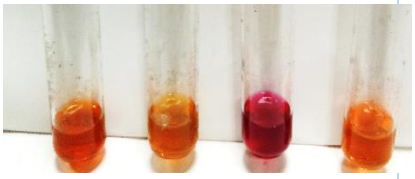
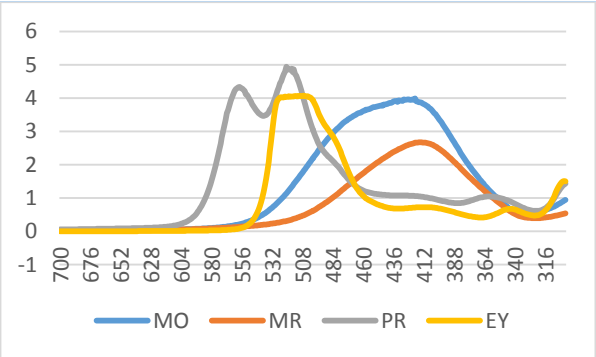
Conc of aq sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

Table 8: Dye degradation of MO, MR, PR, EY (from left to right) at 1hr in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles

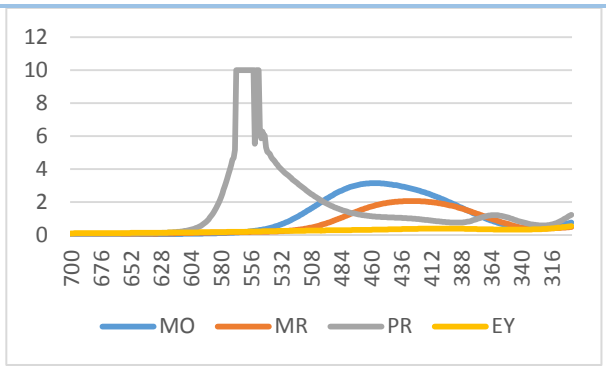
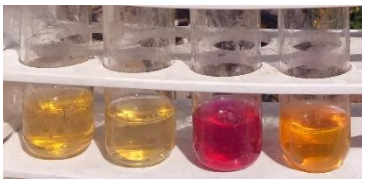
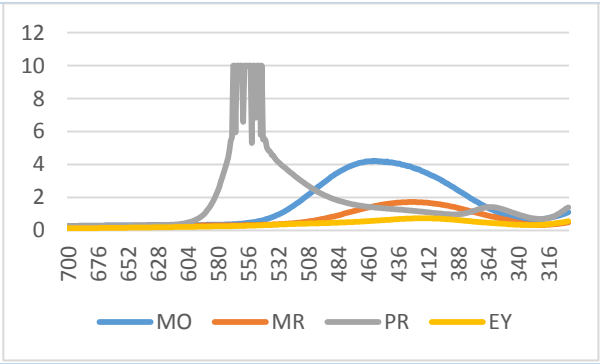
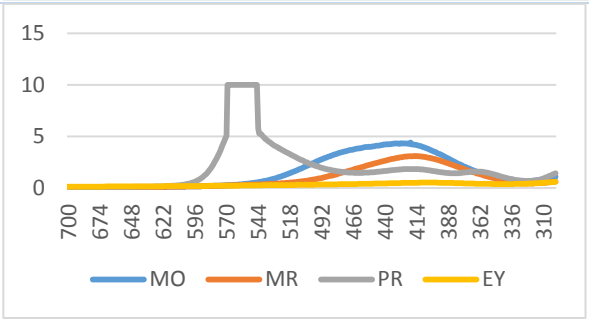
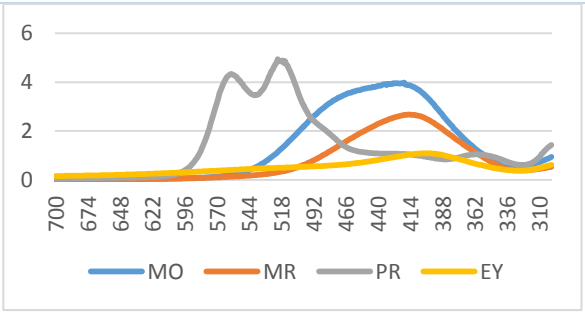
Conc. of aq. sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

Table 9: Dye degradation of MO, MR, PR, EY(from left to right) at 2 hr in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles.

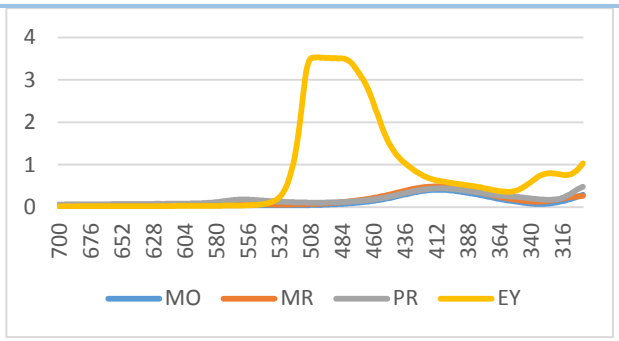
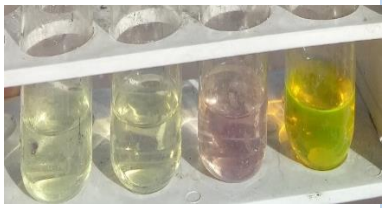
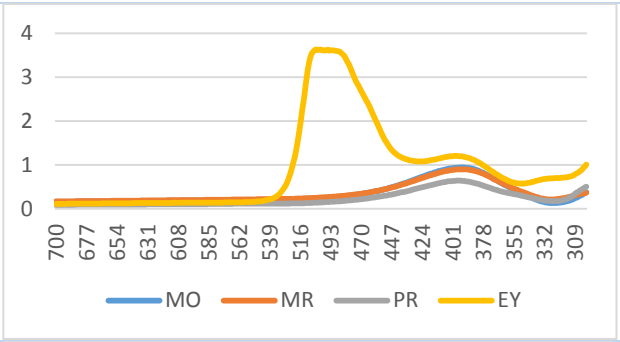
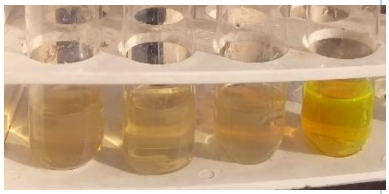
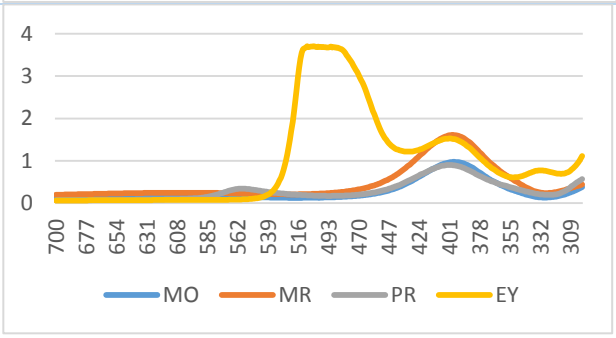
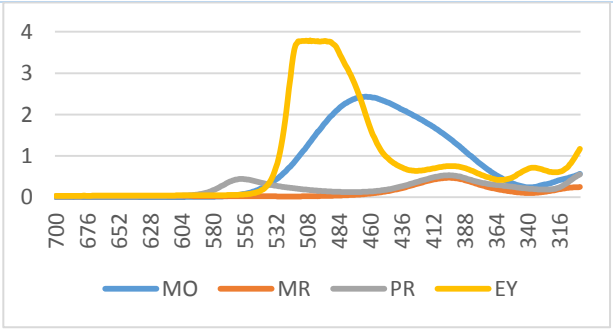
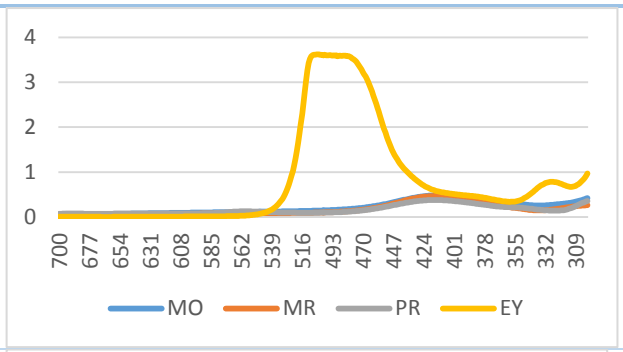
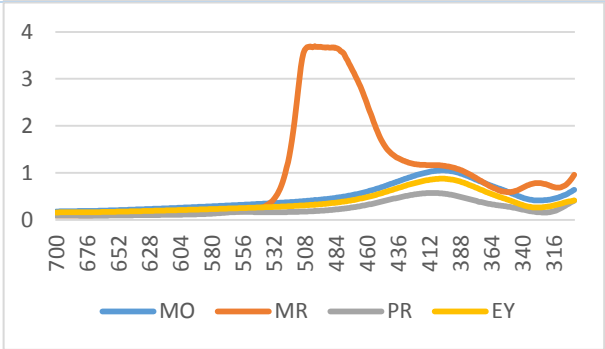
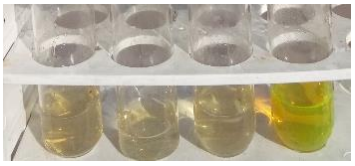
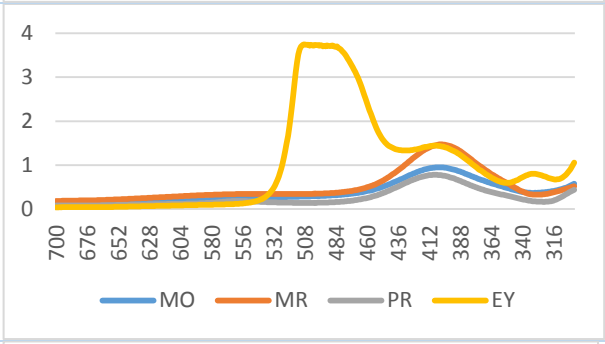
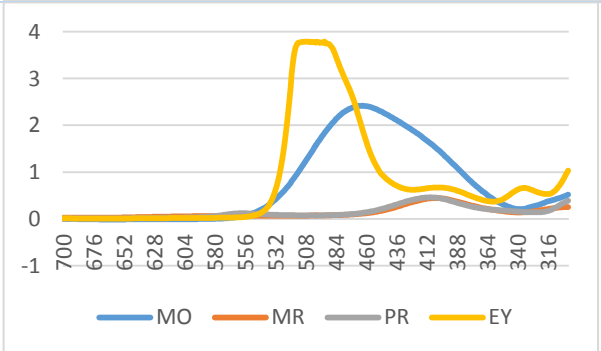
Con of aq sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

Table 10: Dye degradation of MO, MR, PR, and EY (from left to right) at 3 hour in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles

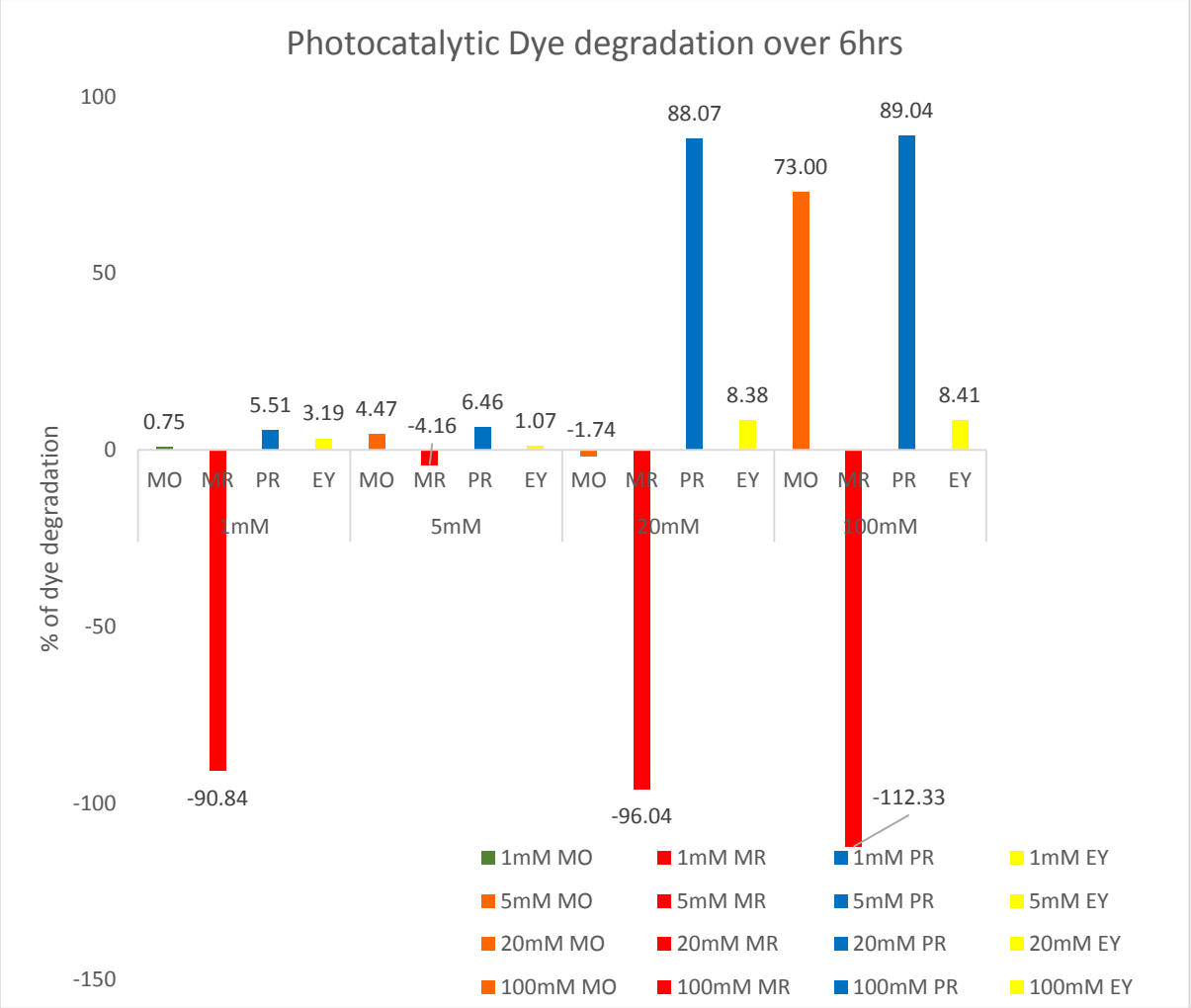
Conc. of aq. sol of AgNps	Graph	Images
1mM		
2mM		
3mM		
4mM		

3.4.2 Catalysis of Dye by Copper Nanoparticles

Different dyes like MO, MR, PR, and EY which are generally used in the textile industries are utilized here for the dye degradation study and performance analysis of the different concentration of copper nanoparticles by different methods viz. photo catalysis and by using chemical catalyst such as sodium borohydride working as a reducing agent for the catalysis process. The copper nanoparticles were perceived to show degradation by both the processes but at different pace and extent of degradation. The dye degradation percentage was determined by the UV-vis spectra of the dyes at different time intervals on addition of the required amount of copper nanoparticles to the dye for the photocatalytic method and in the case of chemical catalysis, sodium borohydride and copper nanoparticles with the respective dyes. Control experiments were also setup which had no Nanoparticles in them and showed no changes in the dyes.

3.4.2.1 Photocatalytic dye degradation

In this method of dye degradation we have the same set of dyes as for the silver nanoparticles viz., Methyl Orange, Methyl red, Phenol Red and Eosin Y. In this set of experiment, we changed the type of nanoparticle that we used, to see the comparison between the different types nanoparticles and their catalytic activity with the same set of dyes and same conditions. The best results are shown by 100mM CuNPs, it degraded methyl orange and phenol red upto a percentage of 73 and 89% respectively. Fair amount of degradation of phenol red was remarked by 20mM CuNPs. Almost negligible degradation of dyes was perceived by the photocatalytic method in just about all the CuNPs concentrations.



Graph 3: Percentage of dye degradation by CuNps with photocatalytic method over 24 hours of sun exposure

Table 11: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 0 hr and with different concentration aq. Solution of copper nanoparticles.

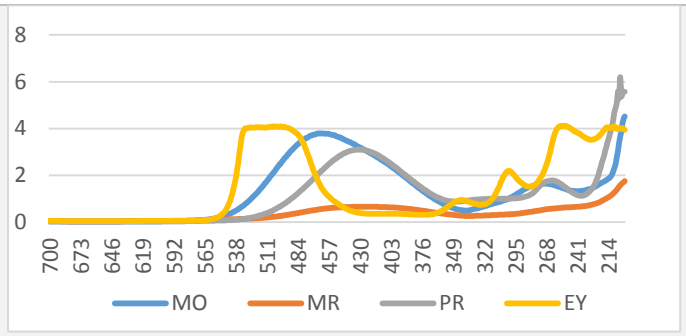
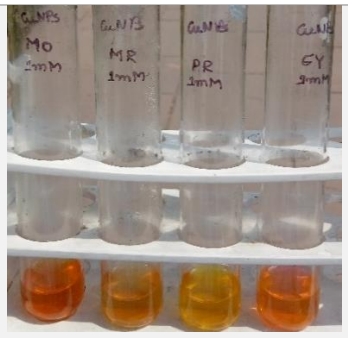
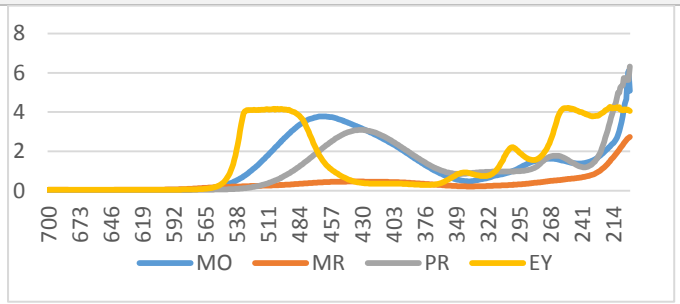
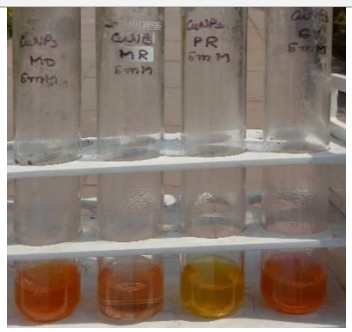
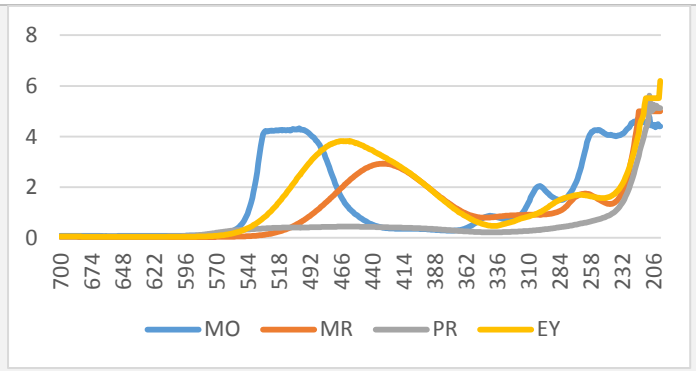
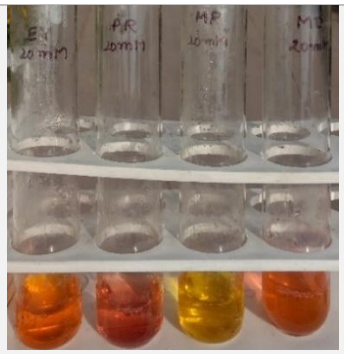
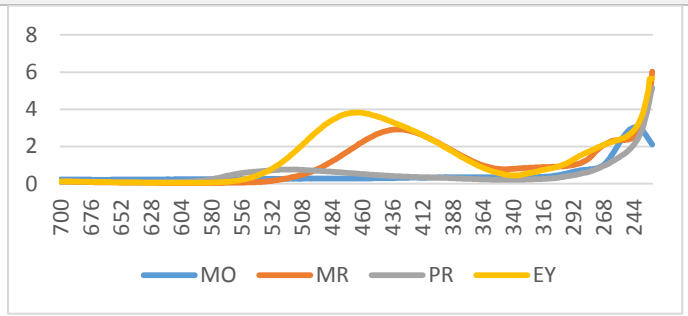
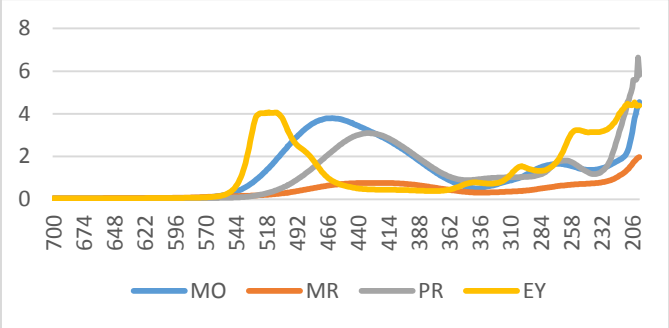
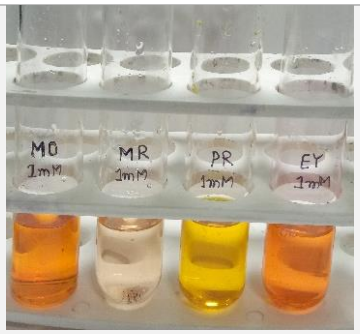
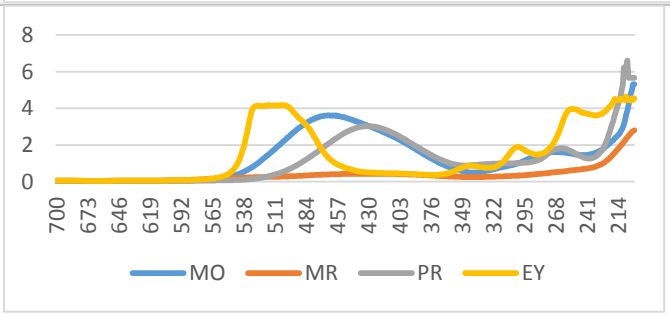
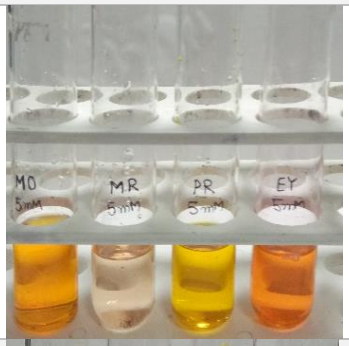
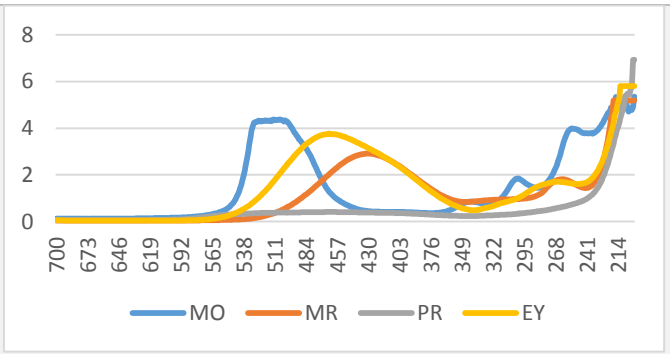
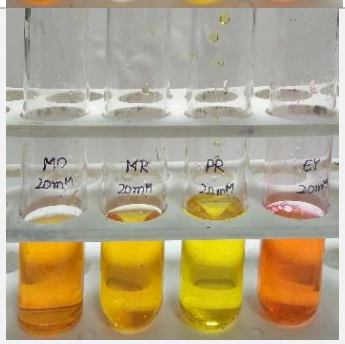
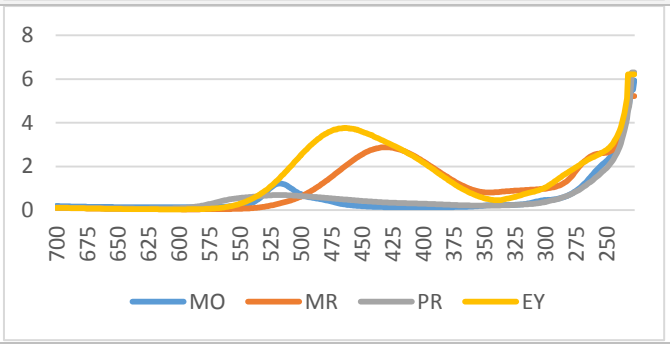
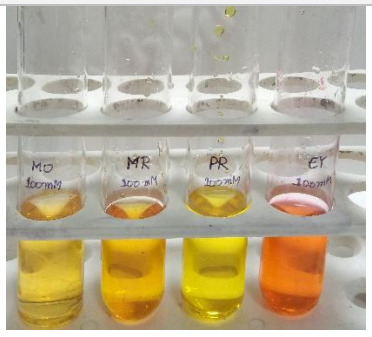
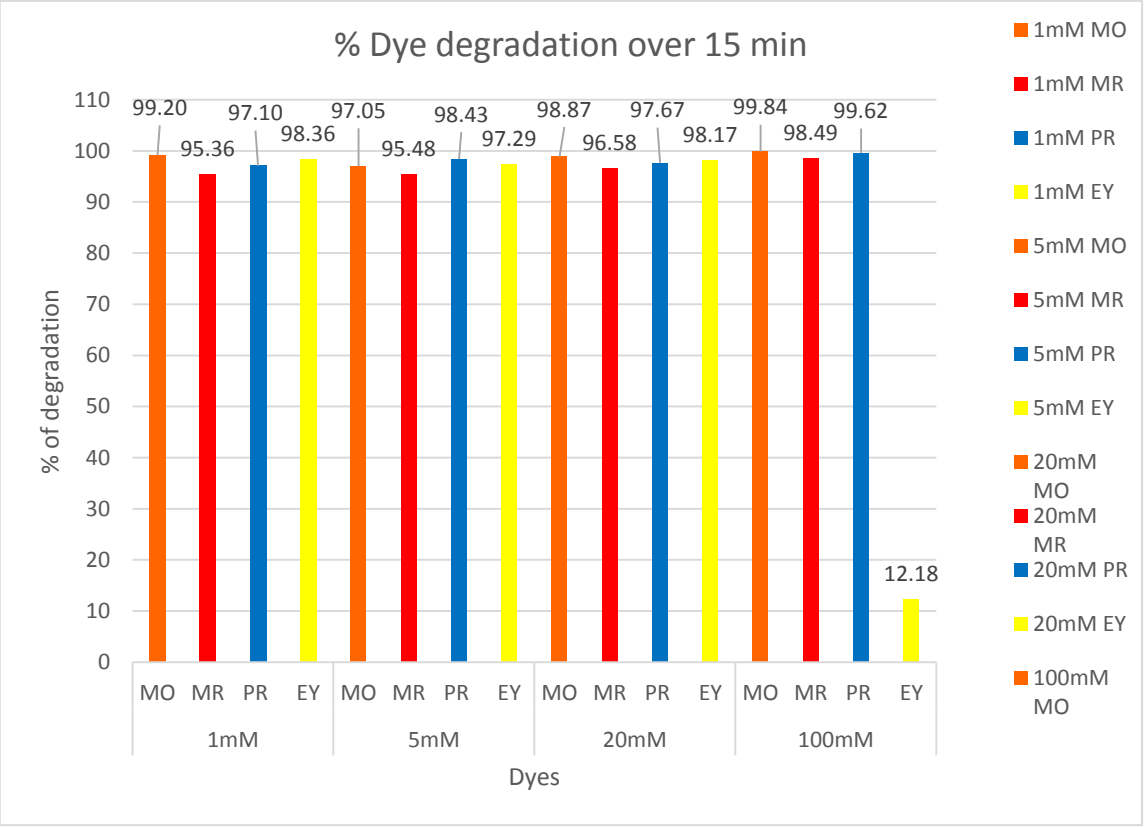
Conc. of aq. Sol. of CuNps	Graph	Image
1mM		
5mM		
20mM		
100mM		

Table 12: Photocatalytic dye degradation of Dyes (MO, MR, PR, EY) from left to right at 6 hr and with different concentration aq. Solution of copper nanoparticles.

Conc.of aqueous solution of CuNps	Graph	Image
1mM		
5mM		
20mM		
100mM		

3.4.2.2 Chemical catalysis of dyes by sodium borohydride

In this experimental setup, we perceived exceptional dye degradation in almost every dye achieving a maximum result of 99.84% in degradation of methyl orange at a concentration of 100mM and the least among the dyes that were degraded was 95.37% An exception dip in the dye degradation was observed with Eosin Y, in the concentration of 100Mm the dye degradation was 12.18%. The dye was precipitated within 5-7 min after addition of the nanoparticles in the solution of dye and sodium borohydride, the dye accumulated and formed a black precipitate at the bottom of the test tube. After centrifugation, every dye solution was crystal clear, drawing out the dye from the water.



Graph 4: Percentage of dye degradation by CuNps with catalytic method using NaBH4 over 15 min

Table 13: Dye degradation of MO, MR, PR, EY (from left to right) at 0 hour in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles.

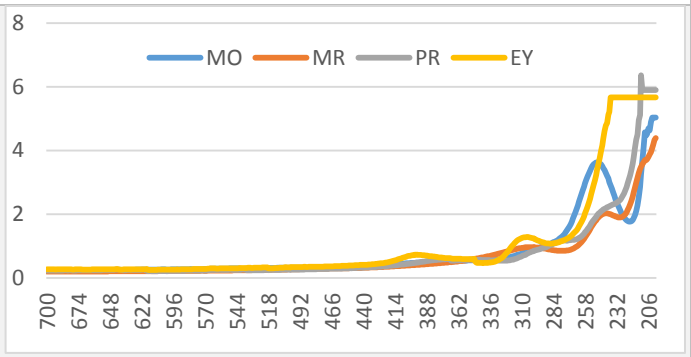
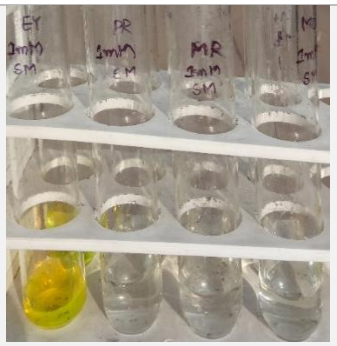
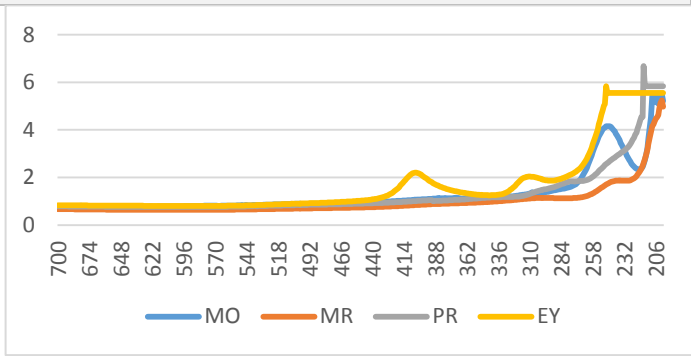
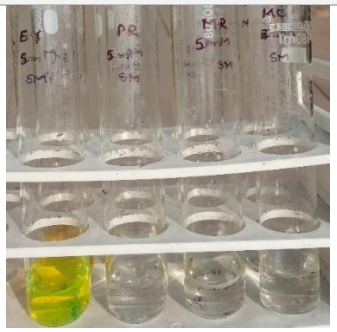
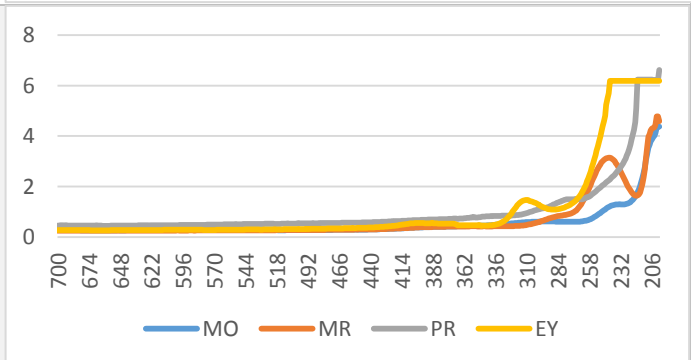
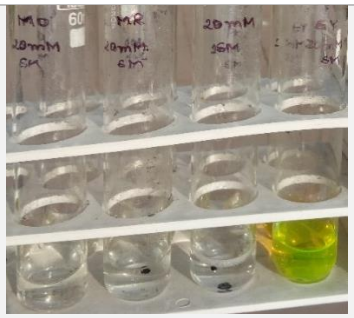
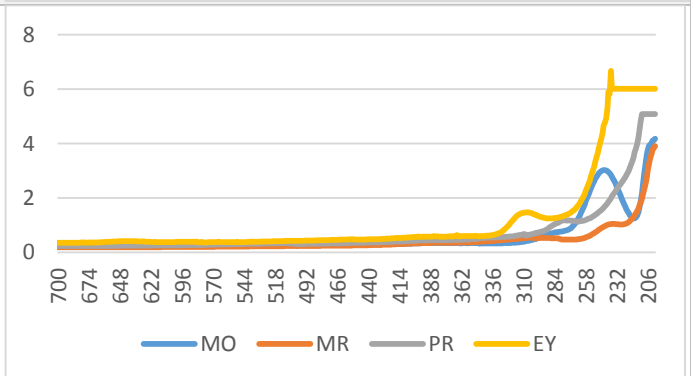
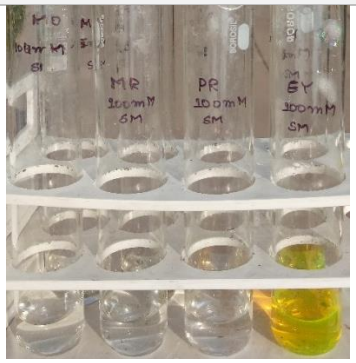
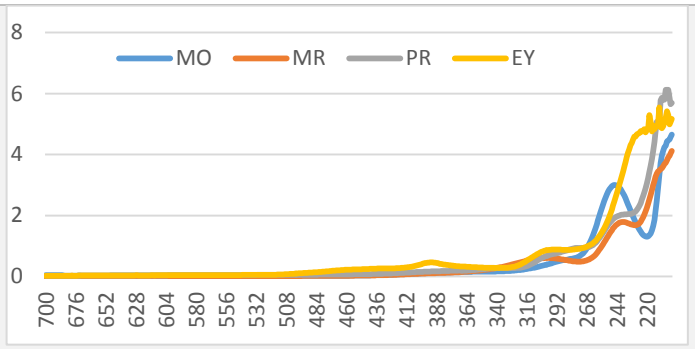
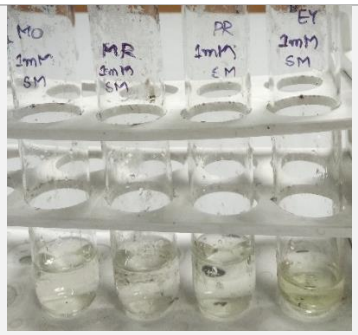
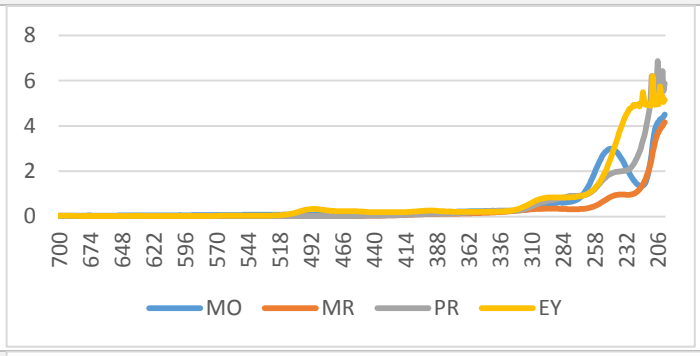
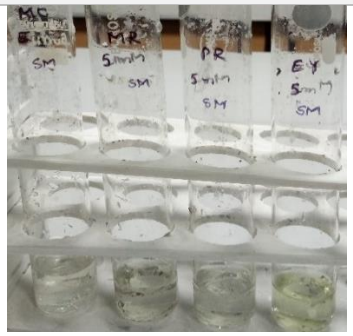
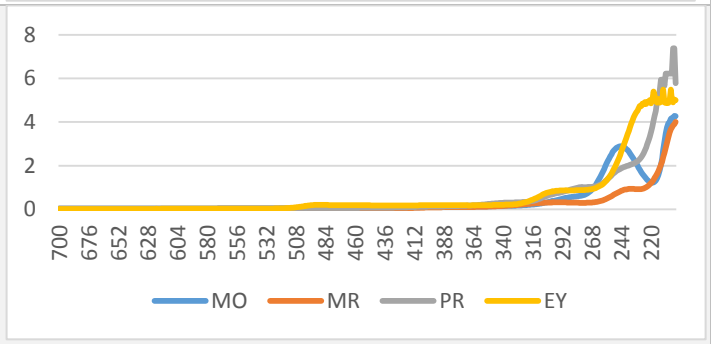
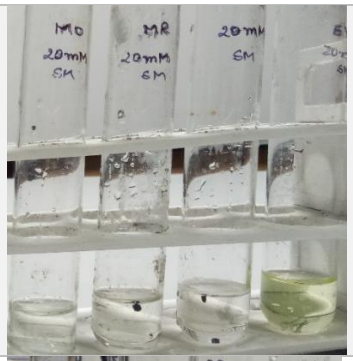
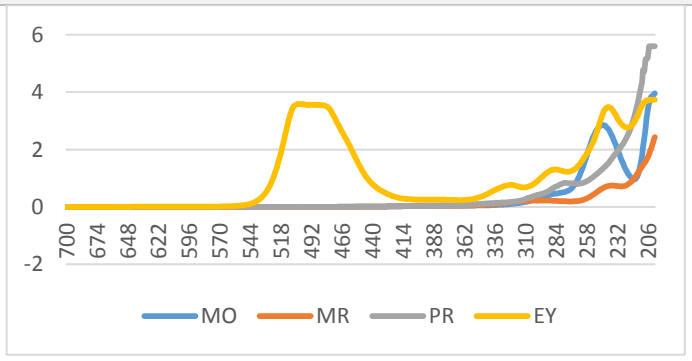
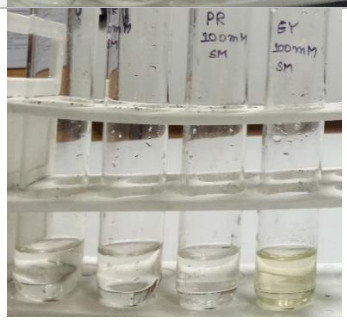
Concentration of aqueous solution of CuNps	Graph	Image
1mM		
5mM		
20mM		
100mM		

Table 14: Dye degradation of MO, MR, PR, EY (from left to right) at 15min in the presence of NaBH4 and with different concentration aq. Solution of silver nanoparticles.

Concentration of aqueous solution of CuNps	Graph	Image
1mM		
5mM		
20mM		
100mM		

3.2 DISCUSSION

The green approach engaging the plant extract for the synthesis of the metallic nanoparticles has an upper hand in comparison to the physical and chemical approaches as it is less engrossing the equipment demand, chemical demand and mandates less time and cost as well. Moreover, the green synthesis has no ill effects on the environment, thus can be utilized for the wide spectrum of applications in the field of nanoparticles without the any constrains with the contamination, cost, and time. Thus this approach has been utilized by a no of researchers (Netala, et al., 2016; Roy and Bhardvaja, 2017; Logeswari, et al., 2015)

Metallic nanoparticles have shown tremendous properties like optical, electronic and catalytically in various fields and thus have application in the arenas which employ these properties of the nanoparticles one of which is utilized here in this study, catalytic property of metallic nanoparticles. Metallic nanoparticles like silver and copper nanoparticles have a characteristic SPR range which reveals the fabrication of nanoparticles. Numerous have reported about the specific range of wavelength for both silver and copper which are characteristic for the synthesis of these nanoparticles. Karthik, et al., 2017, have perceived the silver nanoparticles SPR peak at 405-434 nm for different concentration of plant extracts and in this study we also acquired the peaks at 420-430nm for silver, inferring the production of silver nanoparticles at the specific conditions. Also Arya, et al., 2017; Bogireddy, et al., 2015; Joseph and Mathew, 2015 all have reported the characteristic SPR range in between 410-430nm. In the study involving copper nanoparticles, as reported by Nasrollahzadeh, et al., 2014, 2015 the SPR peak is observed at 350nm and 269nm in respective years and 265nm -285nm stated by Kumar, et al., 2015. Although many researchers have reported the maximum SPR peak from 550nm (Roy, et al., 2017), and 560nm by Lee, et al., 2013. There have been different observation s about the SPR peaks for copper nanoparticles but all in the range of 250-550nm. In our study the SPR range for copper NPs was 323nm.

Silver and copper nanoparticles have revealed great results in the dye degradation with sodium borohydride and moderate results with the photocatalytic method. The

nanoparticle synthesized by the green approach utilizing Centella asiatica and Silver nitrate, were employed for dye degradation application, Here in this study, the photocatalytic and chemical catalysis with Silver and copper nanoparticles is done with multiple dyes Methyl orange, Methyl Red, Phenol Red, and Eosin Y. Previously some papers have stated some similar research as Devi and Singh, 2014 reported the synthesis of CuO NPs from *Centella asiatica* and degraded Methyl orange by photocatalytic method. Joseph and Mathew, 2015 reported the degradation of Methyl orange and Rhodamine B with sodium borohydride, they perceived the degradation of dyes within 10 min of reaction with silver nanoparticles and showed dye degradation of 66.66% silver nanoparticles and $NaBH_4$ whereas in this study we got a dye degradation percentage of 77.69% with 3mM concentration of $AgNO_3$. MeenaKumari and Philip, 2014 have shown synthesis of silver and gold nanoparticles by green synthesis and utilized the nanoparticles for the degradation of textile dyes like Methylene blue, Methyl orange and Eosin Y by metallic nanoparticles and sodium borohydride and within 20 minutes a great reduction in dye colour was observed, in our study Methyl Orange, Methyl Red and Eosin Y was degraded to a significant rate in 30min -1 hour with Silver nanoparticle and sodium borohydride and with copper nanoparticles all dyes Methyl orange, Methyl red and Phenol red was degraded completely upto 97% in 5 minutes of time. Moreover all these papers have suggested the size of the nanoparticles to be less than 100nm and in this report we have perceived the silver nanoparticle size to be 30-50nm and for copper nanoparticles to be in between 20-30nm, which is a desirable size to qualify for a nanoparticle and various shapes have been witnessed in different reports, but here we observed spherical shaped silver and copper nanoparticles.

3.3 CONCLUSION

The release of the organic dyes is one of the major concerns of the environment as these dyes flow to the municipal waste water for treatment, due to their high stability in the water these unlike the municipal organic waste do not degrade and end up in the treated water which is used in the agricultural fields. These dyes in the water end up absorbing in the soil, disturbing the flora and fauna of the soil also these are taken up by the plants with the nutrients and minerals from the soil. The textile dyes or the dyes released in the water bodies from any industry are posing a threat to the environment as well as to mankind. Thus, a solution is to be found that can be used to eliminate this pollution at the first hand. Nanoparticles have proven to have vivid catalytic properties thus can be utilized here in this arena of water treatment. Metallic nanoparticles synthesized from green synthesis is the way to go for biosynthesis of nanoparticles competing with the other physical and chemical methods. Among silver and copper nanoparticles, copper nanoparticles stand a better chance at degrading the dyes as they gave better degradation percentage with sodium borohydride than silver nanoparticles with the same catalyst and also copper is economical in comparison to silver nanoparticles.

3.4 FUTURE PROSPECTS

The hazardous dyes that we embellish our clothes with, are a menace to us and the nature these dyes rotate back to us after entering the water bodies and then the food chain. Metallic nanoparticles like Copper, Silver nanoparticles or other hybrid combination of metallic nanoparticles can be explored which may give better results than a single nanoparticles alone also some other ecofriendly substances, substitutes of sodium borohydride can be looked for which can enhance the rate of degradation of the dyes Using other metallic nanoparticles can be cost effective with the same or a better efficiency. Degradation in controlled conditions can be done by regulating the temperature and the pH of the reactor where degradation occurs as temperature and pH play and important role in the degradation. Cheapest nanoparticle which gives the best catalytic activity should be searched for also focusing on the least side effects.

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