STUDY THE POTENTIAL OF PLANT DERIVED NANOPARTICLES FOR BIODEGRADATION OF PHARMACEUTICAL WASTE EFFLUENT

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

By

Prabhjot Singh Jassal

Registration Number: 41600127

Supervised By

Prof. (Dr.) Joginder Singh Panwar

Department of Microbiology (Professor)

Lovely Professional University

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

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DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Study the potential of plant derived nanoparticles for biodegradation of Pharmaceutical waste effluent**” in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Joginder Singh Panwar, working as Professor, in the School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

Signature of the scholar
Name of the scholar: Prabhjot Singh Jassal
Registration No.: 41600127
Department/school: Biotechnology
Lovely Professional University,
Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Study the potential of plant derived nanoparticles for biodegradation of Pharmaceutical waste effluent**" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the School of Bioengineering and Biosciences, is a research work carried out by Prabhjot Singh Jassal, 41600127, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

Signature of the supervisor

Name of supervisor: Dr. Joginder Singh Panwar

Designation: Professor

Department/school: School of Bioengineering and Biosciences

University: Lovely Professional University, Punjab, India.

ABSTRACT

Effluents released from pharmaceutical industries need to be treated in order to control the antibiotic pollution. The pharmaceutical wastes are not easily removed in the wastewater treatment plants. One reason being, some of these antibiotics are not easily degradable in the normal treatment system. They inhibit the biological organisms in the treatment system. Especially tetracycline, sulfathiazole, and ampicillin are representative antibiotics found in this wastewater.

The pharmaceutical waste contains many prescribed as well as other counter medicines in the form of capsules, tablets, aerosols creams and liquid syrups. If this problem continues then the organisms may become resistant and antibiotics lose their purpose. So there is an urgent need to degrade the antibiotics present in our environment. In the past few years people are focusing on the advanced oxidation process (AOPs) for the treatment of recalcitrant antibiotic. Among AOPs, Ozonation and Fenton's oxidation are the most tested methodologies but the high cost of equipment, energy requirement of the process and most importantly their environment unfriendly nature constitutes its major drawbacks. Hence, proposed plan is to look for some more economical and eco-friendly process to target this problem. The use of plant extracts may hold greater promise for rapid biodegradation of antibiotics present in pharmaceutical effluents. Also, the option of synthesizing nanoparticles, from plant extracts native at the contaminated sites is a novel approach for antibiotic released in the pharmaceutical effluents.

The current investigation is mainly aimed to solve the environmental issues related to pharmaceutical effluents. As this issue has been extremely important in current scenario related to multiple drug resistant and release of recalcitrant compounds in the environment. This has laid negative effects on the community around its vicinity. The community living around pharmaceutical effluent discharge sites are directly and indirectly associated with their negative effect. Increasing concern of these pharmaceutical effluents is not only its acute toxicity but also its genotoxic and mutagenic effects.

For this study, exploration of pharmaceutical effluent discharge site near Baddi, Himachal Pradesh was done for species abundance and plant identification, capable of nanoparticles synthesis. Quadrat method was used for calculating the most flourishing plant species in and around the pharmaceutical effluent discharge site. Plant samples of *Parthenium hysterophorous* and *Saccharum spontaneum* growing near the pharmaceutical effluent polluted sites along Baddi, Himachal Pradesh, India (latitude: 30.94263^o N and longitude: 76.77187^o E) were collected as a substrate for green synthesis. The plant materials thus collected were taxonomically authenticated by Systematic Botany Discipline, Forest Research Institute, Dehradun. As per the results of Quadrat method, abundant growth of *Parthenium hysterophorous* and *Saccharum spontaneum* in or around pharmaceutical effluent discharge site signifies their capability to tolerate and associate with the various antibiotics.

Simultaneously, collection of pharmaceutical effluent samples was done from the industrial area of Baddi, Himachal Pardesh, India and was subjected to physicochemical characterization for various parameters such as pH, Chemical oxygen demand (COD), Biological oxygen demand (BOD), Total dissolved solids (TDS), Total suspended solids (TSS) and the presence of respective antibiotics. The collected effluent was recorded with light grey color, neutral pH (7.11±2), COD (282±2 mg L^{-1}), BOD (98±2 mg L^{-1}), total dissolved solids (1820±2 mg L^{-1}) and total suspended solids (130.5±2 mg L^{-1}). Certain amendments have been made in context to effluent standards and emission standards for the bulk drug producing pharmaceutical industries as per the Environment (Protection) Second Amendment Rules, 2021. Results of wastewater analysis confirmed that the parameters such as pH, BOD, and COD, Total dissolved solids, Total suspended solids were above the standard prescribed limits of central pollution control board for effluent discharged into surface water. Pharmaceutical effluent was further characterized to check the presence of respective antibiotics in the sample. To undergo this, the sample was submitted to SAIF Lab Chandigarh for LCMS analysis. As per the literature review, the observed peaks at m/z=332 and m/z=288, m/z=245 and m/z=231 confirms the presence of ciprofloxacin and the other peaks observed at m/z=362, m/z=318 and m/z=261 confirms the presence of ofloxacin in the sample.

Collected and taxonomically authenticated plant species from the wastewater discharge site were then used for the preparation of ethanolic plant extracts which acts as substrate for the synthesis of TiO_2 nanoparticles. It is worth highlighting that X-ray diffraction, Fourier-transform infrared spectroscopy, and field emission scanning electron microscope techniques were used to characterize the synthesised TiO_2-NPs.

Further studies were done to investigate the effect of green synthesized TiO_2-NPs on photocatalytic degradation of ofloxacin and ciprofloxacin with respect to nanoparticle concentration, antibiotic concentration, response time and pH.

To check the effect of nanoparticles concentration on ofloxacin and ciprofloxacin degradation, 10ppm solution of each antibiotic were prepared and mixed with different concentrations of TiO_2 nanoparticles ranging from 1ppm to 13ppm. The results showed that maximum degradation of ofloxacin and ciprofloxacin through *Parthenium hysterophorus* derived TiO_2-NPs occur at 9ppm. In comparison to this maximum degradation of ofloxacin and ciprofloxacin through *Saccharum spontaneum* derived TiO_2-NPs occur at 11ppm. One way ANOVA on ofloxacin degradation with varying concentration of *Parthenium hysterophorus* derived TiO_2-NPs yielded P<0,0001, which suggests very significant effect of nanoparticle concentration on degradation of ofloxacin. Similar results were obtained for degradation of ciprofloxacin under varying nanoparticle concentration.

To investigate the impact of different concentrations of antibiotics on degradation, solutions with antibiotics concentrations ranging from 10-60ppm were prepared and mixed with the nanoparticle concentration which showed highest degradation (i.e. 9ppm Ofloxacin and 11ppm Ciprofloxacin). The solutions were then subjected to photocatalytic degradation for three hours. The results showed that the addition of nanoparticles enhanced degradation of the antibiotic. The highest degradation was observed with PhNP, achieving 97% degradation of Ofloxacin and 96% degradation of Ciprofloxacin, while for SSNP achieving 96% degradation of Ofloxacin and 95% degradation of Ciprofloxacin at 10ppm concentration of antibiotics. Results of Two way ANOVA suggested that, as we increase the antibiotic concentration keeping the nanoparticle concentration constant, the percentage degradation of ofloxacin decreases very significantly ($P<0.0001$). Similar results were obtained during comparison of ciprofloxacin degradation under PhNP with varying ciprofloxacin concentration, where also with increase in ciprofloxacin concentration by keeping the nanoparticle concentration constant the percentage degradation of ciprofloxacin decreases very significantly ($P<0.0001$).

To check the effect of varying time on antibiotic degradation, the study was done to determine the optimal duration for the degradation of ofloxacin and ciprofloxacin using green synthesised TiO_2-NPs. It is observed that the degradation of antibiotics is more pronounced in the mixture containing antibiotics and nanoparticles, as compared to solution containing only antibiotics. In the case of *Parthenium hysterophorous* and *Saccharum spontaneum* derived nanoparticles, the percentage of degradation for ofloxacin and ciprofloxacin increased from initial values to higher values upon increased exposure time. The results of Two way ANOVA for assessing effect of time on percentage degradation of ofloxacin and ciprofloxacin revealed, that there is significant effect of incubation time on both ofloxacin and ciprofloxacin degradation for both PhNP and SsNP. $P<0.0001$ shows that the degradation of antibiotic is significantly increased with time of incubation.

To investigate the effect of varying pH on degradation studies, 10ppm solution of each antibiotic was prepared, and the pH was adjusted within a range of 4 to 9 prior to the addition of nanoparticles. The results demonstrated an increase in degradation efficiency within the pH range of 4 to 6. However, degradation slowed down beyond pH 6 due to the instability of antibiotics in nearly neutral condition. At pH 5, antibiotics acquire a positive charge in an acidic solution, whereas the photocatalyst carries a negative charge. This results in an affinity between the positively charge antibiotic molecules and negatively charged photocatalyst surface, leading to higher degradation efficiency. Conversely, at neutral pH in alkaline solutions, both the photocatalyst and antibiotic acquire a negative charge, thereby limiting the degradation efficiency.

The current study is focused on an eco-friendly green approach for the effective degradation of pharmaceutical wastewater compared to the other conventional

methods already available for antibiotic degradation, which lead to environmental pollution because it comes up with the release of toxic by-products. Our study used the environmentally friendly green approach to efficiently degrade residual antibiotics in the pharmaceutical effluent, which releases CO_2 and H_2O as environmentally friendly by-products. Moreover, nowadays, researchers are using TiO_2 suspensions along with H_2O_2 (another chemical) to escalate the photocatalytic degradation process, but it decreases the sanity of the process's eco-friendly nature. Investigation of the current study reports better or comparable antibiotic degradation through *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs, because of their well-known nature for secreting allelochemicals having medicinal properties under stress conditions even without H_2O_2 usage. Its presence in the pharmaceutical effluent polluted sites signifies the capability of the plant derived nanoparticles to tolerate and associate with the degradation of various antibiotics.

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Date: 1/6/23 Prabhjot Singh Jassal

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EC	Electrical Conductivity
TDS	Total Dissolved Solid
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
PPCP	Poly Propylene Co-Polymers
PCs	Pharmaceutical Compounds
HGT	Human Gene Transfer
ARGs	Antibiotic Gene Resistance
WWTP	Waste Water Treatment Plants
CAS	Conventional Activated Sludge
HRT	Hydraulic Retention Time
RO	Reverse Osmosis
NF	Nano Filtration
AOP	Advanced Oxidation processes
WAO	Wet Air Oxidation
SCWO	Super Critical Water Oxygen
TOC	Total Organic Carbon
PDT	Photo Dynamic Therapy
SERS	Surface Enhance Raman Scattering
SPR	Surface Plasmon Resonance
CAS	Chemical Abstract Service
DSSC	Di-Sensitive Solar Cells

SMX	Sulfa Methaxazole
TMP	Trimethoprim
STPs	Sewage Treatment Plants
PhNP	Parhenium hysterophorus derived nanoparticles
SsNP	Saccharum spontaneum derived nanoparticles
TiO_2-NPs	Titanium oxide nanoparticles
CPFX	Ciprofloxacin
OFX	Ofloxacin
LCMS	Liquid chromatography-mass spectrometry

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Chapter 1

INTRODUCTION

1 INTRODUCTION

Water is a major significant portion of our global economy**.** 71% portion of the earth is covered with water, despite that the availability of water is decreasing day by day. This is due to the restricted supply of water for human consumption, as seawater cannot be utilized for daily uses. River water is utilized for a variety of reasons, including agriculture, human consumption, and industry. The presence of abundant river water is not solving the problem of the water crisis because the water is getting polluted day by day by industries, agriculture, and human waste (Sharma and Bhattacharya, 2017). The chemicals as well as pesticides used in agriculture play a major role in polluting the environment. Farmers are using pesticides to increase the quantity of the crop but they are also affecting the environment by causing soil, water, and air pollution. When pesticides are used on crops or some other plants, they get washed away by rain or by irrigation and enter nearby rivers or lakes. The pesticides in water can harm aquatic plants and animals, disrupt food chains, and can result in human health issues with polluted water consumption. The pesticides can persist for longer time (even for years) and can accumulate in the tissues of organisms, including humans. Calcium and magnesium which are present in pesticides cause the hardness of the water. The soil gets polluted by pesticides or chemicals and pollutes the underground water which is a major share of drinking water. The Great Lakes of North America and the Great Barrier Reef in Australia are two coastal areas that have been harmed by agricultural operations (Parris and Kevin, 2011).

Day by day escalation in the technical field can leads to industrial development, which is majorly affecting the water quality. Industrial development will results in environmental toxicity i.e. majorly water toxicity, which is used for human consumption. Industrial effluents, solid waste and other hazardous wastes form the major chunk of wastes released by industries resulting in environmental contamination. The waste is gathered and discarded in open areas close to rivers, and ponds which results in water pollution. When the concentration of the chemicals used in industries crosses the defined limit then it tends to cause harmful effects to the environment as well as humans. These chemicals become toxic and create a bad effect on water bodies which results in affecting the water economy. Metals like cadmium, mercury, lead, arsenic, chromium, copper, and nickel are found in water in tiny amounts but can cause a substantial impact on the aquatic environment as well as on human life. Pollution caused by these heavy metals degrades water quality by changing water parameters like pH, EC, TDS, BOD, and COD which results in altering natural processes. Most of the infections in underdeveloped nations are caused by polluted water. Day by day increase in textiles, tanneries, pharmaceuticals, and other chemical businesses cause a massive quantity of waste generated on daily basis which is discharged into rivers, and seas ultimately resulting in heavy water pollution (Islam et al., 2012).

Pharmaceutical and personal care goods are becoming the leading causes of water contamination. The release of pharmaceutical compounds (PCs) into the water had led to various changes in the environment. The increased usage of PPCPs (polypropylene copolymers) has resulted in water contamination since PPCP effluents put into water induce genetic alterations in plants, animals, and humans. PCs exposure not only causes genetic alterations, but it also causes changes in inherited features and affects human behavior. For example, the transformation of male fish into female fish due to estrogen production in the body of male fish is the result of PCs present in water.

Most potent pharmaceutical pollutants which showed detrimental consequences include: Antidepressant, Analgesic and Antibiotics (Samal et al., 2022). Out of these, antibiotics are the most commonly used pharmaceutical product, whose concentrations in fresh water were found to be greater than 10,000 tons per year. Antibiotics are chemicals or drugs which are used to treat or stop the growth of bacterial infections. Different classes of antibiotics are used to treat various kinds of infections. For example:

- **Penicillin:** For the treatment of various kinds of skin diseases.
- **Cephalosporins:** For the treatment of Gonorrhoea, pelvic inflammatory diseases, and Urinary tract infections.
- **Tetracyclines:** They have anti-inflammatory properties which mainly treat chest, urethral and pelvic infections.

- **Macrolides:** They have both anti-inflammatory and immunomodulatory properties. They are useful in the treatment of strains of bacteria that are resistant to penicillin.
- **Fluoroquinolones:** They help in the treatment of prostatitis, and severe cases of salmonellosis and shigellosis.
- **Sulphonamides:** They treat conditions like pneumocystis pneumonia and nocardiosis.
- **Glycopeptides:** They treat infections caused by gram-positive bacteria. (Hutchings et al., 2019).

These classified antibiotics have different end products which led to different kinds of contaminations in water. Certain limits are defined for accepted antibiotic concentrations to be present in water bodies :**Metronidazole (**0.05-0.01 mg/l); **Sulfamethoxazole** (0.08-0.01 mg/l); **Norfloxacin** (0.1-0.01 mg/l) ; **Ciprofloxacin (**0.1-0.01 mg/l) ; **Ofloxacin** (0.1-0.01 mg/l); **Ceftriaxone (**20-1 mg/l) (Roy et al., 2021).

1.1 Ways of fresh water contamination through antibiotics

There are many ways by which antibiotics reach the freshwater (Figure 1)

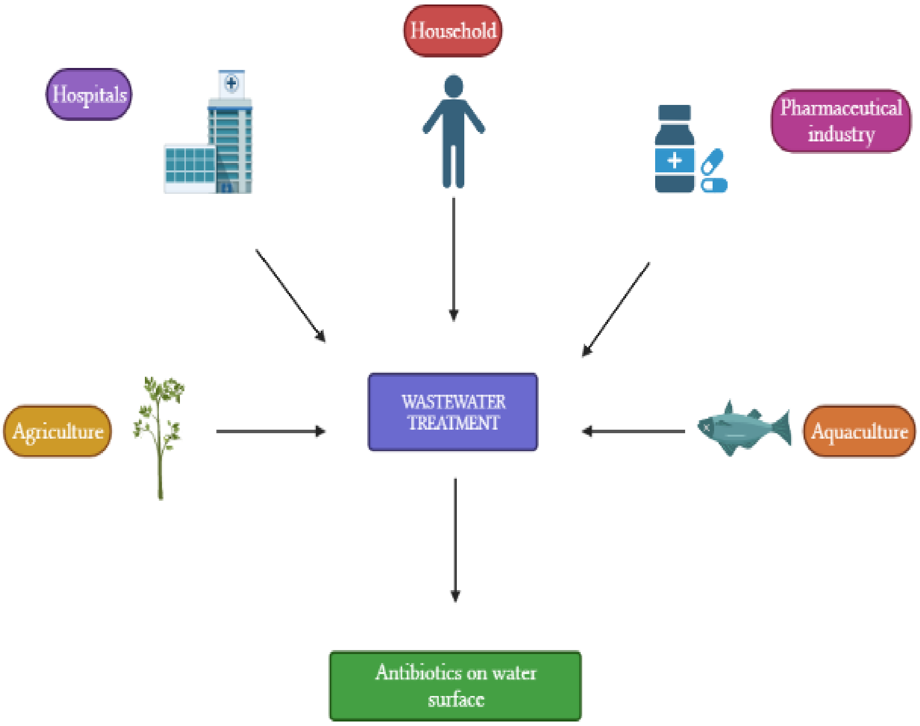


Figure 1: Different ways of freshwater contamination through antibiotics

Through effluents that come from Wastewater treatment plants- The disposal of effluents from wastewater plants in both developed and underdeveloped countries contributes to a disturbed aquatic ecology by increasing but not limiting the oxygen demand. This also led to the promotion of hazardous and algal blooms over the surface of the water. When this water is used for irrigation purposes or human consumption this will lead to harmful effects on both humans as well as the environment. These effluents from the industries mostly cause non-communicable diseases (Xiao et al., 2018).

Chemical manufacturing plants- As technologies are developing, there are different methods to do things with chemicals, for example for faster growth of plants, and for food preservation chemicals are used, but as we are all aware that the plants or the food produced or preserved through the chemicals do not acquire any beneficial characteristics. If the plant is not getting enough time to grow then how it will acquire the nutrients which are required ultimately humans are consuming the plants in many ways which will lead to harmful effects on the human body. Not only disturbing the human body these chemicals which are also released into the water are also affecting the water environment (Kumar and Mohan, 2019).

Aquaculture and Animal husbandry- As we are aware chemicals are being used for the overproduction of the desired products, so many chemicals are also used to boost aquaculture productivity. Anaesthetics, flesh pigments, and therapeutic compounds are the chemicals that are used in aquaculture production. Many bioactive chemicals are used for productivity, but intense use of these chemicals it had been led to illnesses in aquaculture production. The immense use of chemicals in water has become the primary source of water contamination. More significantly, bioactive chemicals have been proven to remain in human and animal tissues for an extended period after they consume them (Mavraganis et al., 2020). Different types of diseases are caused by polluted water as depicted in Figure 2.

1.2 Water Related Diseases

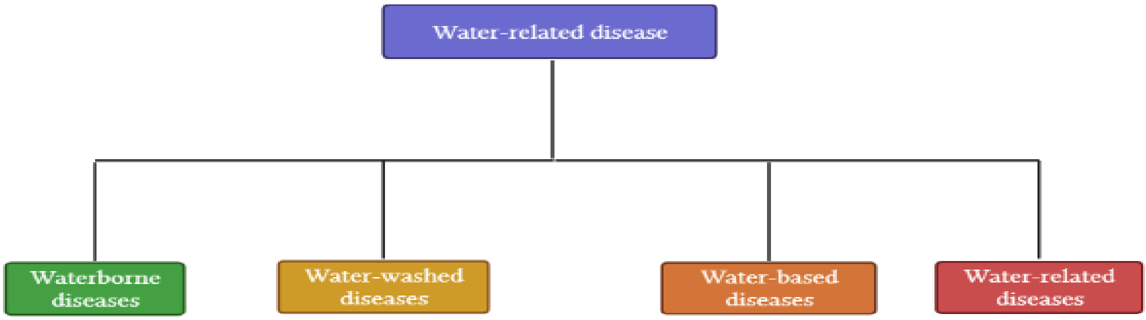


Figure 2: Different types of polluted water related diseases.

Waterborne diseases: These are the diseases which are caused by direct drinking of water which contain pathogenic microorganisms. When this water is used for preparation of food then it will lead to food borne diseases. It includes diseases like Cholera, Typhoid, Amoebic and bacillary dysentery, Diarrheal diseases.

Water-washed diseases: These kind of diseases are caused by poor personal hygiene and with skin or eye contact with contaminated water. It includes Scabies, Trachoma and flea, Lice and tick-borne diseases.

Water-based diseases: These diseases are caused by parasites found in intermediate organism living in contaminated water. It includes Dracunculiasis, Schistosomiasis, Helminths.

Water-related diseases: Caused by insect vectors, especially mosquitoes, that breed in water. It includes Dengue, Filariasis, Malaria, Onchocerciasis, Trypanosomiasis, Yellow fever (Gleick and Peter, 2002).

1.3 Antibiotic Resistance

Despite of knowing the presence of antibiotics in fresh water it is important to determine the concentration of antibiotics present in water, because on that basis, we can determine its impact on environment, human and aquatic life. As stated earlier about the importance of antibiotic concentrations, presence of antibiotics above those defined levels becomes harmful in every way possible. The major challenge faced with increased antibiotic concentration is: Antibiotic resistance.

Antibiotic resistance develops when bacteria evolve and become resistance to previously effective antibiotics. This happens when bacteria gain genetic alterations that allow them to withstand antibiotic exposure. It is becoming a global issue as more germs develop resistance to conventional antibiotics. This is leading to major infections which are hard to cure and can even lead to death also. Antibiotic resistance is increasing due to variety of factors, including antibiotic abuse and misuse, a lack of novel antibiotic research, and inadequate infection control measures in hospital settings. Some experts believe that these emerging pollutants may endanger human health by increasing antibiotic resistance in diseases-causing microorganisms. There is concern that people may be exposed to strains of bacterial infections that have evolved resistance and hence cannot by destroyed by currently available antibiotics. The pathogens can also be resistant against combination of antibiotics (Raghav et al., 2013). Several factors which leads to antibiotic resistance includes:

Overuse of Antibiotics: Usage of antibiotics is driving the emergence of resistance. Studies have shown that there is link between use of antibiotic and spread of resistance bacterium strains. The resistance can occur due to horizontal gene transfer between bacteria species or it can be due to naturally through mutation. Antibiotics are available without any prescription in many other countries and because of this they are freely available, numerous, and inexpensive, encouraging overuse. In which country they are restricted, online shopping of these medications are increased.

Inappropriate Prescribing: Inappropriate prescribing of antibiotics can also contribute to antibiotic resistance. Incorrectly administered antibiotics have dubious therapeutic value and expose patients to possible antibiotic-related problems. Sub-inhibitory and sub-therapeutic antibiotic doses can encourage the development of antibiotic resistance by promoting genetic modifications such as changes in gene expression, HGT, and mutagenesis. Modifications in antibiotic-induced gene expression can boost virulence, whereas increased mutagenesis and HGT promote antibiotic resistance and dissemination (Ventola and C. Lee, 2015).

Extensive Agricultural Use: Antibiotic usage in agriculture is frequently cited as a significant contribution to the clinical issue of resistant illness in human medicine.

Yet, there are at least three routes by which antibiotic resistance in agriculture might pose a concern to humans.

Humans can be infected with disease resistant microorganisms of agriculture origin when they consume bacterial contaminated food of agricultural origin in the form of meat or water, but the infection doesn't pass to the other person.

Using any of these methods, a human gets infected or colonised with a resistant bacterium, which results in ongoing transmission with human, with few of these individuals getting unwell. This situation represents a breach of the 'species barrier' by a microorganism which can be directly harmful to humans or a commensal capable of causing diseases.

Horizontal gene transfer introduces resistance genes originating in agriculture settings into human diseases. Antibiotic usage in humans then selects the ensuing resistant lineages (Isaacson and Torrence, 2002). Following are the examples of various drug resistance bacteria's which act as infectious agents for the onset of multiple human and animal diseases.

- Multidrug-resistance *Acinetobacter*.
- Drug-resistance *Campylobacter*.
- Fluconazole-resistant *Candida*.
- Extended spectrum beta-lactamase-producing Enterobacteriaceae.
- Vancomycin-resistant *Enterococci*.
- Multidrug-resistant *Pseudomonas aeruginosa*.
- Drug-resistant non-typhoidal *Salmonella*.
- Drug-resistant *Salmonella Typhimurium*.
- Drug-resistant *Shigella*.
- Methicillin-resistant *Staphylococcus aureus*.
- Drug-resistant *Streptococcus pneumonia.*
- Drug-resistant tuberculosis (Ventola and C. Lee, 2015).

Now we should discuss about the ways by which we can get solution of this problem which we are facing in today's world. As we know, that antibiotic pollution is stepping up day by day and results in escalation of serious global issues, many policies are implanted in several countries to solve this problem, but the situation is out of control right now. So now the researchers are looking for some new environment friendly ways to reduce antibiotic pollution. On the same time, they are also thinking of some ideas to degrade the antibiotics which are already there in aquatic environment. So, there are several methods used for degradation of antibiotics.

1.4 Methods of Antibiotic Degradation

- **Physical Methods-** Antibiotics can be removed from the aquatic environment using physical methods that are physical adsorption, membrane filtration, and precipitation.

  Physical Adsorption: In adsorption the particles get attached to surface of the adsorbent. So here the antibiotic molecules will be attached to the adsorbent by the means of intermolecular forces. The most used adsorbents are: Activated carbon, modified activated carbon and sieve pore structured substances. Both activated carbon and modified carbon have high adsorption rates. The adsorption efficiency of any adsorbent is related to pH of the solution. This method is low cost and easy to handle but the drawback is that due to weak intermolecular forces, the antibiotics which are adsorbed can easily escape. (Liu et al., 2021)

  Membrane Filtration: This technology uses micro-porous membranes to catch antibiotics and purify water through reverse osmosis. Membrane separation is used along with some other method in order to remove antibiotics from the aquatic environment. It is a physical process which doesn’t involve any kind of chemical use. The main drawback is that due to minute size of antibiotics particles the filtration pores can be clogged, after that the membrane needs to be changed which makes the process less cost effective. (Bayan et al., 2021)
- **Chemical oxidation:** As the name suggests itself, the process uses chemicals which in result produces free radicals via chemical reactions which ultimately react with antibiotics causing bond breakage. This method is not so in use because it causes a lot of secondary pollution. (Wang et al., 2020)
- **Biodegradation:** Biodegradation means use of microorganisms, enzymes, or microbes for breakage of antibiotics which are present in environment. But as we know that long exposure of antibiotics to microorganisms can lead to antibiotic resistance bacteria , so this process is also have some loss ends. (Reis et al., 2020)

- **Photodegradation:** It is the deterioration of a target chemical in the presence of light (natural or artificial) by photon absorption at a certain wavelength. The molecule is oxidised and hydrolysed as a result of the combined action of light and air.

  It is mainly consists of three steps:

1. Photon absorption
2. Excitation and
3. Reaction

   When a photocatalyst absorbs photons with energies higher than its band gap, electrons in the valence band become excited and move to the conduction band, creating a hole in the valence band. As a result, the photogenerated electrons and holes become separated and migrate towards the surface of the photocatalyst, where they can initiate secondary reactions with adsorbed materials. The photogenerated holes have ability to directly attack and degrade harmful substances, including antibiotics.

At the end based on the drawbacks of other conventional methods used for antibiotic degradation, “**Photocatalytic degradation**” is the most important conventional method to use, as it is easy to operate and highly efficient and is also environment friendly (Bai et al., 2022). Photocatalytic degradation process requires a photon absorber, which can be a nanoparticle, because it can be created artificially or can be found naturally also. They perform a specialized function that is why they are used across wide variety of industries. Nanoparticle is a particle which exhibit size range from 1 to 100 nm and have some specialized physical and chemical properties. Different properties of nanoparticles include:

- Super paramagnetic properties depending on their size.
- Their capacity to absorb solar radiation is much higher than in thin films of continuous bulk material sheets.
- They have enhanced diffusion at high temperature due to high surface area to volume ratio (Khan et al., 2019).

Present study is focused on the identification of most flourishing plant species around the pharmaceutical effluent discharge site along Baddi, Himachal Pradesh, India (latitude: 30.94263° N and longitude: 76.77187° E). Collected plant samples were taxonomically authenticated by Systematic Botany Discipline, Forest Research Institute, Dehradun, whose plant extracts were further used for the green synthesis of TiO_2 NPs. Hence, these green synthesized TiO_2 nanoparticles were considered to assess the photocatalytic degradation of Ciprofloxacin and Ofloxacin.

Chapter 2

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

Water is used for different purposes including industrial, domestic and agricultural purposes which lead to contamination and release of undesired compounds into the water. The problems are more often seen in Agriculture Industry where the most common constituent's compounds of soil surface such as, glyphosate, organophosphates, polychlorinated biphenyls and organochlorines is discharged into the marine environment. Furthermore, the non-degradable, inorganic materials have severely damaged the surface water and its aquatic life. These practices have disturbed the water cycle hence posing a global threat and effecting the environment, ecosystem, wildlife and human health. (Kayode-Afolayan, 2022).

Pesticides and Fertilizers also contribute to the contamination of Water. Pesticides are useful to remove bacteria, pests and other microorganisms. Water is immediately contaminated by pesticide-containing chemicals and degrades its quality. If pesticides are used excessively or improperly, the agricultural ecosystem could be endangered (Haseena M. et al., 2017). Only 60% of chemical fertilizers are used in soils; the remaining 40% will leak and participate in groundwater contamination. Lakes and other water bodies are becoming eutrophicated as a result of excessive phosphate run off. Ingestion of contaminated water, limitation to access of sanitation, contact with harmful water, and lack of management of water resources and systems, especially in agriculture, are all connected to adverse health effects in humans and animals staying in its vicinity (Kamble, 2014).

Because of the combination of rising temperatures and lower freshwater flows, climate change is expected to have an impact on both the quality and quantity of water. Reduction in flow of fresh water because of the upstream users and the increase in demand of water also cause the increased concentration of contaminants in fresh water stream, resulting in degradation of river water quality, and also create financial hazards.

The quality of water is negatively impacted by both climate change and increasing urbanization, particularly informal development. This could potentially have significant economic consequences for the communities that rely on these fresh water streams, directly through their use and indirectly through water-reliant industries like farming (Cullis, JD et al., 2019). River water quality is seriously threatened by human activities when population levels rise above a certain point. Urban development is singled out as one of the main contributors to surface water contamination in Asian nations. To conserve improved river water ecosphere of the nation while achieving its development goals as a country by keeping a check on its water bodies system is necessary (Liyanage and Yamada, 2017). The majority of the domestic sewage that is discharged into rivers is untreated. Toxicants, solid waste, plastic litter, and microbiological pollutants are all present in domestic sewage.

Cause of water pollution are hazardous substances. The primary source of water pollution is various industrial wastewater that is discharged into rivers without being treated. Surface water and ground water contamination are caused by hazardous material released by enterprises. Dependent on the nature of the industry is contamination. Metals that are toxic enter the water and lower its quality. Industries are responsible for 25% of pollution, which is more hazardous (Haseena M. et al., 2017).

Poor management of wastewater treatment is also a major reason of fresh and underwater contamination. A wastewater treatment facility needs the right technology, enough funding, and a strong governance structure to operate efficiently and continuously. Unfortunately, these systems' administration and upkeep are rarely examined. Treatment facilities may be unable to continue operating if there is insufficient funding for operation and maintenance (O&M) and an incompetent governance structure (Liang and Yue, 2021).

Pharmaceutical Industries in India contributes to its economy significantly. India is a fast-developing nation with a large population. The Indian medicines industry is now well-known on a global scale due to the high quality and reasonable prices of its products. Indian pharmaceutical industries is right now one of the rigorously growing industries with the growth rate of 2.4 percent in terms of value and 10 percent in aspect of volume worldwide. India exports 20 percent of all kinds of generic medicines globally. India exported medicines worth USD 16.89 billion in 2016 and exported pharma products worth USD 24.62 billion in 2020 (Chakraborty P., 2020).

Pharmaceutical plants and their production has increased over the years. There are different types of pharmaceutical industries with different production scale which has led to the adverse contamination of fresh water. The pharmaceutical wastewater is high in concentration of antibiotic, high biological toxicity, complex composition, high Chroma, and high sulphate content. Pharmaceutical wastewater has a high concentration of salts. In addition, it is hazardous to microorganisms and difficult to biodegrade (Guo et al., 2017).

There is a town in Himachal Pradesh called Baddi. It is one of the fastest growing Town of Himachal Pradesh and is also known as Baddi Industrial Town (BIT). Baddi has recently developed into a manufacturing hub for Indian and international businesses. The Indian government has also chosen to connect Baddi with the rail system in order to give manufacturing amenities and to increase commerce.

There are currently about 1000 pharmaceutical and medical businesses in Baddi. There are also more than 200 printing and packaging businesses. There are countless additional factories of different sizes (Kumar, 2019). The Baddi – Barotiwala Industrial Estate ocuupies 3,500 Acres of land. In this area there are 50 large and roughly more than 300 small industries and the final destination of all industrial and domestic waste coming out of them is Sirsa river (Chauhan et al., 2013).

This waste contains some amount of hard metals traces and complex pharmaceutical structures in low to high concentrations. Continuous usage of this contaminated water may cause both chronic and immediate damages (Patneedi and Prasadu, 2015) such as bioaccumulation in cells, reproductive harm and suppression of cell division (Wang et al, 2021). According to numerous studies, fish exposed to wastewater effluents can exhibit aberrant behavior in reproduction. In addition, fish exposed to residues of contraceptive medications, as per the range of their quantities found in the environment exhibit sharp declines in successful reproduction, indicating fluctuations in their population-levels. (Patneedi and Prasadu, 2015)

2.1 Major Pharmaceutical Effluents / Contaminants found in water bodies

Contamination of water bodies has become an alarming danger to aquatic as well as human life over the period of time. One of the major contamination is caused by pharmaceutical effluents that are discharged in the water bodies along with other contaminants. Pharmaceutical effluents include chemical residues of antibiotic and untreated drugs. Complete degradation or treatment does not occur in the human body thus resulting in the excretion in transformed form or unaltered form (Madikizela, 2017).

Treatment of these pharmaceutical effluents remains undefined and difficult because of their composition of unmanageable substances such as organic matter (active pharmaceuticals) and non-degradable nutrients such as phosphates, sulphates, nitrates (Kayode-Afolayan, 2022). A study was conducted in four African countries related to pharmaceutical effluents- Mozambique, Ghana, South Africa, Kenya in which some of the antibiotics were detected and quantified.

Various types of antibiotics detected in wastewater along with their respective concentrations are depicted in table-1(Madikizela, 2017).

Table 1. Various types of antibiotic contaminants and their respective concentrations in wastewater.

S.No.	**Antibiotic detected**	**Concentration(ng L^{-1})**
1.	Azithromycin	0.6 – 27
2.	Chlortetracycline	3.9 – 225
3.	Clarithromycin	0.11 – 439
4.	Dehydroerythromycin	0.17 – 2800
5.	Doxycycline	2.5 – 50
6.	Lincomycin	0.17 – 241
7.	Minocycline	2.9 – 315
8.	Oxytetracycline	1.4 – 1793

9.	Roxithromycin	0.52 – 11
10.	Tetracycline	0.85 – 465
11.	Sulfamethoxazole	0.11 – 53828
12.	Salfamethizole	0.11 - 0.42
13.	Sulfadimidine	0.69 – 330
14.	Sulfanerazine	0.23 - 0.31
15.	Sulfapyridine	0.73 – 153
16.	Sulfathiazole	0.32 – 3971
17.	Tetracycline	0.85 – 465

Increased level of these pharmaceutical effluents (antibiotics) in the environment result in advancement of ARGs(Antibiotic Resistance Genes) as outcome of natural selection and also add to the formation of the habitat as a stock for the further spread and expansion of ARGs to pathogens through food and water cycle (Khan et al., 2013).

2.2 Conventional Methods used for the treatment of Pharmaceutical wastewater

Antibiotic degradation / Bioremediation of pharmaceutical effluents (antibiotics) is a necessity to reduce the occurrence of antibiotic resistant genes (ARG) for the efficient treatment of wastewater through the wastewater treatment plant (WWTP) from the pharmaceutical industries hence, making the water consumable from water bodies. The standard contaminated water bio-rectification methods utilized by pharma industries for the removal of antibiotic wastewater are not very efficient (Phoon et al., 2020). There are many standard methods of wastewater treatment and antibiotic degradation which includes biological, chemical and advance treatment processes.

2.2.1 Biological Treatment- This method of Treatment is one of the traditional method used for treating the pharmaceutical wastewater. Biological treatment is of two types – Aerobic and Anaerobic treatment processes. Aerobic Treatment is most commonly used treatment process which consist of prolonged aeration activated sludge process, membrane bioreactors, membrane bioreactors and activated sludge with granular activated carbon. (Peng et al., 2004)

2.2.2 Activated Sludge Process - One of the frequently used aerobic method for the treatment of pharmaceutical wastewater which is found efficient against several categories of pharmaceutical and industrial wastewater is the activated sludge process. This method is cost efficient which makes it feasible for many purposes and hence called Conventional activated sludge treatment (CAS). This method of bioremediation majorly depends on two factors - Hydraulic Retention Time(HRT) and the heat or temperature. The other parameters which impact the effectiveness of the method are the existence of Biological oxygen demand, Chemical oxygen demand, Organic matter, pH, non-biodegradable matter. Some of the antibiotics (majorly estrogens) like Ibuprofen, bezafibrate, naproxen exhibit higher efficiency of removal of waste. Sulfa drugs / antibiotics like sulfamethxazole, diclofenac, and carbamezapine exhibit lower removal efficiency (Deegan et al., 2011).

2.2.3 Membrane Bioreactors (MRBs): Membrane Bioreactors are now frequently used for the bioremediation of industrial, pharmaceutical and various other wastewater treatments. This method is also preferred frequently due to its economic and technical advantages and easy to carry out. And it also attains high sludge retention time (HRT) in the smaller reactor volumes which provides a technical advantage (Gadipelly et al., 2014). These bioreactors use the combination of membrane filtration unit (microfiltration or ultrafiltration) along with a suspended growth bioreactor. These bioreactors are categorically designed into chambers to facilitate an environment that is biologically active. Suspended growth bioreactors are the bioreactors in which biomass develops in assembly. In Membrane bioreactors, membrane serves the action of solid-liquid dissociation unit. It also holds the biomass in the bioreactor before ejecting the treated wastewater into the surrounding. The preferred choice of membrane unit is the ultrafiltration membrane unit due its high specificity. In the membrane bioreactor, micro-organism concentrations can reach up to 20 mg/L (Radjenovic et al, 2007). The potential for larger biomolecules to degrade is amplified by this higher concentration of biomass.

2.2.4 Advanced Treatment Process-To increase the effectiveness of removing contaminants by secondary treatment, leading pharmaceutical waste treatment can be thought as the initial treatment of pre-treatment process. Advanced Treatment is of different types, which includes carbon adsorption, membrane separation, membrane technology.

2.2.4.1 Carbon Adsorption- As a kind of adsorbent Activated carbon offers various benefits. It possesses significant amount of area, multilayer pore structure, high capacity of adsorption and a stable chemical structure. As a result, it is frequently utilized as a catalyst carrier or adsorbent to remove contaminants. Industrial effluents must be treated with activated carbon since they are toxic and challenging to release in accordance with regulations. Additionally, it is a crucial method for the improved treatment of pharmaceutical wastewater. The two distinct forms of activated carbon adsorption are chemical adsorption and physical adsorption.

Physical adsorption - Physical adsorption has no specificity to adsorbate and is reversible. After being saturated with adsorbates, activated carbon is simple to fuse. On the other hand, chemical adsorption only adsorbs one or a small number of unique adsorbates, which is permanent and challenging to desorb. When activated carbon is saturated for cyclic use, regeneration returns its adsorption property.

The benefit of this method is that it removes the analytes rather than creating possibly more hazardous metabolites. Nevertheless, this method just allows the transfer of pollutants to a new phase where they are more prevalent or concentrated rather than their actual elimination.

Adsorption effectiveness is correlated with the adsorbent parameters, particularly porosity, surface area, and pore diameter. The effectiveness of the technique also depends on the amount of dissolved organic matter present in any natural water, which is a consideration in addition to the trace contaminants to be worried about or even the adsorbent materials. Organic matter directly competes for the available adsorption sites or surface, which is one of its principal effects.

Because of its wide applicability, capacity to be recycled, and superior treatment results, this approach is frequently used for latest treatment. However, a number of limitations, such as prohibitive relative costs, subpar regeneration effectiveness, and challenging operation, limit utilization (Yiping and Yu, 2010).

Chemical adsorption only adsorbs one or many unique adsorbates, which is difficult and irreversible to fuse. When activated carbon is saturated for cyclic use, regeneration returns its adsorption property. Because of its broad application, capacity to be recycled, and superior treatment results, this approach is frequently employed for advanced therapy. however, numerous drawbacks, including as poor regeneration efficiency, high relative prices and complicated operation, restrict use (Yiping and Yu, 2010).

Some of the other employed methods under the category of advance treatment processes are coagulation, sedimentation and flotation.

2.2.4.2 Coagulation: During coagulation, chemical agents are applied to wastewater, changing stable pollutants into unstable and precipitable ones. Coagulation's process is intricate. Learning how to compress and eliminate bound water around a water loving colloid is the key to efficient advanced treatment of pharmaceutical wastewater. Therefore, it is essential to consider the flocculent's characteristics, which are related to the coagulation process. Polymers and salts made of inorganic metals are commonly employed as flocculants. With this technique, harmful organic matter, and chromaticity can be eliminated. In the interim, it can enhance the potential of pharmaceutical wastewater to degrade (Guo et al., 2017).

2.2.4.3 Floatation: Secondary effluent suspended particles can be removed by flotation as well. By infusing air into wastewater, the method produces a lot of little bubbles, creating flocs that float and are less dense than wastewater. In order to separate, it can also float to the top of effluent.

2.2.4.4 Membrane separation - Membrane separation is the process of employing semipermeable membrane separating medium to allow a component of water to pass through a membrane only when a certain driving force is applied across it. Achieve the target substance's separation, purification, and concentration from the mixture in this method. The treatment of wastewater uses a variety of membrane separation techniques. such as reverse osmosis, electro-dialysis, microfiltration, and ultrafiltration.

***Microfiltration-** The dissociation process in microfiltration is done by the activity of the sieve separating the membrane, which is based on the same concept as classical filtration. The little difference is that Micro-Filtration's pore size is smaller. Using this technique, SS and bacteria in wastewater may be successfully removed.

***Ultrafiltration-**The ultrafiltration membrane serves as the filter medium, and the pressure differential between the membranes on each side serves as the ultrafiltration driving force. Water, inorganic ions, and small molecules flow through the membrane surface when water moves across it at a specified level of pressure, while other macromolecules are trapped. The primary purpose of this technique is to remove macromolecules and colloids from wastewater. While using this approach, it is important to make sure the membrane has enough membrane flux and can be readily dismantled, changed, and cleaned.

***Reverse Osmosis** - RO membranes fall into two categories: aromatic polyamide and cellulose ester. Tube, frame, plate, and roll, and hollow fibre types are some of its component forms.

The RO technique may be used to remove a range of organic substances and dissolved inorganic ions. Additionally, it is highly effective in removing salt and reusing water. This approach, however, necessitates a thorough preparation of the supply water. Nano filtration (NF) has been presented as a novel technique with the development of RO method.

***Precipitation-** Heavy metal ions chemically react in precipitation procedures to create non-soluble precipitates, which are then further removed from the water with the help of sedimentation process or filtrate. By precipitating metals as hydroxide at a high pH or by precipitating sulphide, wastewater can be treated to eliminate metals. Typically, precipitation is employed to remove radioactive materials, phosphorus compounds, and metal ions. Hydroxide treatment is the most often utilized precipitation method because to its relative simplicity, automatic pH control and low cost. The creation of vast amounts of relatively low density sludge, which poses dewatering and disposal issues, is the primary drawback of hydroxide precipitation (Zinicovscaia, 2016).

2.2.5 Advance Oxidation Processes (AOP): Due to the limited biodegradability of the majority of these species, the routinely employed wastewater treatment techniques are insufficient to completely remove pharmaceutical compounds. These contaminants may end up in the treated effluents that are released into waterways. AOPs are effective for treating different organic contaminants and harmful substances since they completely destroy the pollutants' chemical structures, including developing concerns like detrimental chemicals, dyes, pesticides, and naturally occurring poisons.

AOPs are a group of water oxidation treatments used in hospitals, industrial settings, and WWTPs to treat toxic effluents (Phoon et al, 2020). Broadly speaking, advanced oxidation processes are aqueous-phase oxidation techniques that depend on the engagement of highly reactive species, primarily but not completely hydroxyl radicals, in the processes that result in the oxidation of the target pollutant.

The two primary advance oxidation processes are microwave therapy and ultrasonic irradiation, which generally work at frequencies about 2450 MHz in either a monomode or multimode vessel. Electro-oxidation, the Fenton and Photo-Fenton processes, moist air oxidation, and ultraviolet (UV) or solar irradiation are further recent advance oxidation processes in this area. Depending on the chemical composition of the pharmaceutical effluent and the desired treatment outcome of destruction or transformation, the advance oxidation process may be utilized alone or in conjunction with other physiochemical and biological processes.

Fenton Oxidation - According to Equation 1, the Fenton oxidation is an efficient AOP that creates HO radical dots by catalyzing the breakdown of hydrogen peroxide (H_2O_2) by ferrous iron (Fe^{2+}). The target organic pollutant is then oxidized by the produced HO• (Equation 2). Fast breakdown of H_2O_2 by Fe^{2+} in the first stage of the reaction produces significant amounts of HO radical dots and changes Fe^{2+} into Fe^{3+} (Equation 3). Fenton oxidation is mostly explained by these straightforward steps, however the actual process is far more intricate and involves numerous more reactions. These processes can typically be divided into three categories: initiation, propagation, and termination reactions. (Bello et al, 2019)

$H_2O_2 + Fe^{2\pm} \rightarrow Fe^{3+} HO^{\bullet} + OH^{-}$ Eq.1

$OH^{\bullet} + \text{organic} \rightarrow \text{Products}$ Eq.2

$HO^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^{-}$ Eq.3

Either one of three below mentioned mechanisms can be used by the $HO^{\cdot}$ with organic substances and/or the Fenton catalyst for waste removal.

(i) Hydrogen addition

(ii) Hydrogen abstraction

(iii) Electron Transfer

According to research findings, even though increasing the wastewater's temperature is supposed to speed up the redox reaction, it nearly never affects the effectiveness of COD removal in Fenton's oxidation. At room temperature, the oxidation took 10–20 times as long to complete as it did at 50 °C, which took a few minutes. Although pH 3.5 generated marginally better outcomes, there were no appreciable variations in treatment effectiveness when evaluated with the initial pH range of 3.0-4.5. This result is consistent with current research that indicates the absolute pH for Fenton's oxidation is in the range of 3-5 and irrespective of the type of wastewater. Studying wastewater from five different artificial dyeing processes, it was discovered that a pH level below 3.5 is ideal for Fenton's oxidation. (Tekin et al., 2006)

Ozonation - The oxidizing agent ozone is well known and has been well investigated for tetracycline breakdown. It has advantages over other treatments, such as the fact that ozone quickly breaks down to oxygen in water after ozonating organic and inorganic contaminants, producing no chemical sludge in the process. (Gharbani and Mehrizad, 2014) Determining ozonation's function in TC degradation requires a thorough understanding of its mechanism. Ozonation can occur directly with liquefied ozone or indirectly with the production of OH radicals. (Mohsin and Mohammed, 2021)

Ozone usually targets the double bonds, aromatic rings, and amine functional groups of organic compounds like TC when it is present in aqueous form. Via direct 1,3-dipolar cycloaddition, ozone interacts with C=C, C=N, N=N, and in-situ formation of •HO. Highly reactive species are formed during the decomposition of ozone, especially at alkaline pH values (Glaze, 1987).

Electrochemical Degradation- The electrochemical techniques for removing harmful organic chemicals use a powerful, adaptable, affordable, simple, and clean technology. When an electrolyte is present, oxidation over anodes such as graphite, TiO_2, Ru or Ir oxides, boron-doped diamond, or Ti-based alloys takes place. By using a process called direct anodic oxidation, which involves first adsorbing the pollutants on the surface of the anode and further oxidizing them via anodic electron transfer, the contaminants can be eliminated electrochemically. On the other hand, the reaction is categorised as indirect whether the molecules are broken down in the liquid volume through the use of species exhibiting electrical activity like metallic redox couples like Ag(II), Ce(IV), Mn(III), or Fe(III), or strong oxidants like H_2O_2, O_3, persulfate, percarbonate, and chlorinated species), which serve as middlemen for the transfer of electrons between the organic compounds and the electrode. (Homem and Santos, 2011)

Factors affecting Electrochemical Degradation-

The type and structure of material of the electrode, the experimental setup, and the electrolyte composition all play a role in the process selection. This kind of system is typically employed to stop electrode fouling.

The effectiveness of indirect oxidation is largely influenced by the diffusion rate of secondary oxidants into the solution, pH, and temperature, while direct oxidation is dependent on the compound diffusion rates towards the anode's active sites, electrode catalytic activity and the applied current. (Saracco et al, 2000)

Photo-catalysis- The breakdown or dissociation of chemical substances brought on by light, whether it be artificial or natural, is known as photolysis. Direct and indirect photolysis are two extensively used photo-induced reactions. In the initial scenario, organic chemicals absorb ultraviolet radiation and may either self-decompose or react with other water matrix components.

Indirect photolysis happens when photosensitizers like oxygen and peroxyl or hydroxyl radicals are photo-degraded. Ozone, external hydrogen peroxide addition, homolytic hydrogen peroxide cleavage (producing hydroxyl radicals), or photolysis of humic and inorganic chemicals found in the water matrix should all produce these oxidants. Indirect photolysis is more important for the pollutants' half-life even if both direct and indirect processes might occur at the same time (Giokas and Vlessidis, 2007). The efficiency of photolysis is influenced by the target molecule's absorption spectrum, light frequency and intensity, H_2O_2 and O_3 concentration (if used), and type of matrix. Certain elements found in natural waterways can either scavenge (organic matter) or create oxidant species (inorganic and humic elements, such as dissolved metals), which can either inhibit or facilitate the process (Kummerer, 2009).

This method has been shown to be inefficient than those where radiation is mixed with ozone or hydrogen peroxide as catalyst. One potential strategy to break down antibiotics in a natural aquatic environment is photolysis under solar illumination rather than mercury vapor lamps (I greater than 280 nm) (Kummerer, 2009).

Ultrasound Irradiation- The application of ultrasound irradiation for wastewater treatment is a relatively new approach. On the sono-chemical degradation of medicinal substances, there is not a lot of literature.

When liquids are exposed to high-intensity acoustic radiation at frequencies that cause cavitation, sonochemical processes are triggered (25 kHz). Consequently, cavitation allows for the simultaneous release of radicals and the concentration of ultrasound's distributed energy into micro-reactors. Ultrasonic irradiation has been used to eliminate several estrogenic chemicals from tainted water, with an 80–90% COD reduction in 40–60 minutes of treatment (Mendez-Arriaga et al., 2009). The method is best suited for the treatment of double-phase wastewater containing less-solubility organic matter.

Recently, wastewaters were cleaned of pharmaceutical compounds utilising a combination of biological treatment and hydrodynamic cavitation. When the hydrodynamic cavitation/hydrogen peroxide processes, UV treatment, and attached-growth biomass biological treatment were combined, Clofibric acid was removed with an efficiency of more than 90% while diclofenac and carbamazepine were removed with an efficiency of more than 98%. The remaining chemicals were lowered to undetectable levels (LOD). Diclofenac, ibuprofen, naproxen, and ketoprofen had the highest overall removal contributions from biological treatment; clofibric acid had the highest removal contributions from UV treatment; and carbamazepine had removal contributions from both hydrogen peroxide process/hydrodynamic cavitation and biological treatment that were comparable (Zupanc et al., 2013).

Wet Air Oxidation-Waste streams that are too concentrated for biological treatment and too diluted for incineration can benefit from wet air oxidation (WAO). The process may be defined as the process of oxidation of inorganic as well as organic substances in a water-based solution or suspension utilizing air or oxygen at elevated pressures and temperatures. Wet oxidation typically occurs between 180°Celcius and 2 Megapascals and 315°Celcius and 15 Megapascals. Chemical oxygen demand (COD) elimination may typically be between 75 and 90% during residence durations that range from 15 to 120 minutes. Without the release of Nitric oxide, Hydrochloric acic, Sulphur dioxide, furans, dioxins, coal ash, etc. insoluble organic matter is transformed into uncomplicated soluble organic compounds that are then oxidised and ultimately converted to carbon dioxide and water (Debellefontaine and Foussard, 2000).

The whole mineralization of the wastewater stream is not possible with wet air oxidation, in contrast to Super Critical Water Oxygen (SCWO), because some low molecular weight oxygenated molecules (particularly propionic and acetic acids, acetaldehyde, methanol, and ethanol) are resistant to oxidation. For instance, acetic acid elimination is often insignificant at temperatures below 300°C. Ammonia is easily converted from organic nitrogen molecules and is also relatively stable in wet air oxidation conditions.

As a result, wet air oxidation is a preliminary treatment for liquid wastes that necessitates further processing of the process's liquid and gas streams (Luck, 1999). Salt solubility decreases at high temperature ranges, which are inside the sub-critical area for water. Corrosion may have been an issue for early Zimpro sludge treatment processes because of precipitated salts. High pressures are needed to keep the state of water in a liquid form since the WAO process must be carried out in the aqueous phase. Moreover, pressurization raises the amount of dissolved oxygen present, which accelerates the rate of oxidation (Hii K. et al., 2014).

Hybrid Technologies- Hybrid technologies combine one or more traditional and cutting-edge treatment methods to completely eliminate pharmaceutical pollutants. Because no single-treatment technology is capable of removing all chemicals, hybrid approaches are required. In order to treat refractory pollutants and lower the expenses of the treatment process, a number of hybrid methods have been developed (Ternes et al., 2002). In essence, the method uses the standard filtration stage to eliminate any solid matrix, and then it eliminates the sludge for burning. The different combinations of technologies are then used to treat the clear wastewater.

According to cited research, the removal of amoxicillin involves combining Fenton and reverse osmosis. Then, the authors utilized a liquid-liquid extraction to get rid of a number of organic components (this stage removed 50% of the Total organic carbon). The degradation was then improved (Total organic carbon elimination to 38%) by Fenton oxidation. Lastly, the researchers employed a filtration system (reverse osmosis), removing 11% of total organic carbon and improving the effluent biodegradability to 99%. In order to remove imidazoles, simultaneous use of ozonation and adsorption was employed. The researchers found that simple ozonation produce some by-products that were very toxic, accompanied with 90-100% degradation and 10-20% mineralization of amoxycillin. Along with speeding up the elimination rate, activated carbon also decreases the toxicity of the by-products and the total organic carbon removal by around 30% during the ozonation process. Combining these two procedures enables the treatment of high organic matter water matrices, which is not achievable when ozonation is employed alone (Gadipelly et al, 2014).

2.3 Green Synthesis of Metallic Nanoparticles- In later years, researchers primary focus has shifted to the creation of effective methods of metal nanoparticle production using green chemistry They have looked into methods for producing well-characterized nanoparticles that are environmentally beneficial.

The method that is most commonly addressed is the utilization of organisms to create metal nanoparticles. Plants appear to be the most suitable among these creatures for the task of producing nanoparticles on a large scale through biosynthesis. (Iravani, 2011). Compared to microbes, plants make nanoparticles that are more stable and synthesis them at a faster rate. In addition, the nanoparticles are more varied in size and shape than those made by other creatures. The advantages of employing plants and products made from them for the biosynthesis of metallic nanoparticles have sparked researchers' interest in researching the processes of metal ions absorption and bio reduction by plants as well as the possible mechanisms of metal nanoparticle formation in plants. (Kamran et al, 2019).

In general, organic and inorganic nanoparticles can be distinguished. Semiconductor nanoparticles are included in inorganic nanoparticles (like ZnS, ZnO, CdS).

While organic nanoparticles contain carbon nanoparticles, metallic nanoparticles (such as Ag, Au, Al, Cu,) and magnetic nanoparticles (such as Fe, Co, Ni) are also present (like fullerenes, carbon nanotubes, quantum dots) (Dikshit et al., 2021). As they offer outstanding qualities with practical flexibility, Gold and Ag (noble metal) nano particles are gaining popularity. Ag-Nano Particles have a considerable surface zone, which contributes to their notable biological reactivity, catalytic properties, and atomic behaviour when compared to bigger particles having the same chemical composition.

2.3.1 Green Synthesis of Gold Nano Particles-

Gold nanoparticle (GNP) based biomedical applications have recently attracted a lot of scientific attention. Deoxyribonucleic acid labelling, photothermal ablation, fluorescence labelling, tissue engineering, contrast agents for magnetic resonance imaging, and other imaging techniques are only a few of the many potential biomedical uses that have been investigated.

The synthesis, stabilisation, and functionalization of the Gold Nano Particles are the subjects of much research. Sodium citrate, cetyl-trimethyl ammonium bromide and transferrin are the most often utilized stabilizing agents, whereas other amines, antibodies, oligonucleotides, lipids, and peptides are frequently used as functionalizing species. The relatively simple synthesis of Gold Nano Particles, good control over their shapes, optical properties, sizes and their excellent biocompatibility are the main factors driving interest in them. Repeated doses can result in hazardous levels of Gold-NPs build-up in the body. The majority of Gold Nano Particle investigations are therefore still in the preclinical stage.

Characteristics of Gold Nano-particles:-

1. They are more compatible biologically.
2. Gold is chemically inert.
3. Gold nanoparticles show optical characteristics like plasmon resonance.
4. Microscopic probes for studying cancer cells are made of gold nanoparticles.
5. Gold nanoparticles build up in malignant cells and exhibit cytotoxicity, causing the specific cell and cell-specific receptor to undergo apoptosis or necrosis.
6. The links between the gold and sulphur give these a remarkable level of stability.
7. Their photophysical capabilities can be used to release drugs in far-off locations. (Tomar et al., 2013)

Applications of Gold Nano-particles:-

- Photodynamic therapy (PDT) - Photodynamic therapy, also known as PDT, is recognized as a critical treatment for oncological disorders as well as certain skin or infectious diseases. PDT uses photosensitive molecules as light-sensitizing compounds and a laser. Singlet oxygen and extremely active free radicals produced with the energy of photosensitizers cause tumour cells to undergo apoptosis or necrosis.

  The key characteristics of Au NPs that have been used in photodynamic treatment are efficient fluorescence quenching and surface plasmon resonance (SPR) absorption. Moreover, gold conjugation makes it easier to enter cells because of its propensity to attach to thiols, disulfides, and amines. (Elahi et al., 2018)
- GNP as sensors - Gold nanoparticles can be employed for (active) sensor applications in addition to (passive) labelling. In a sensor, its purpose is to selectively detect the presence of analyte molecules and to generate a readout that shows the analyte's concentration. Changes in the optical characteristics of gold nanoparticles, for instance, can serve as a sign of analyte presence when an optical read-out is employed. Gold particle-based sensors could have a significant influence on diagnostics because of their small size (Sperling et al., 2008).
- Plasmonic Photothermal Therapy - One of the most promising areas of research for the treatment of cancer and infectious diseases at the moment is photothermal damage to cells. The core of this phenomenon is as follows:

  When exposed to the appropriate light, GNPs absorb the most energy in the visible or near-IR region and become extremely hot. If they are inside or close to the target cells in this situation (which is possible by conjugating gold particles to antibodies or other molecules), these cells are killed (Dykman and Khlebtsov, 2012).
- Drug Delivery - For a very long period, chemicals have been delivered into cells using gold nanoparticles. The molecules are adsorbed onto the

surface of the Au particles for this purpose, and the entire conjugate is then injected into the cells (Sperling et al, 2008). In order to improve the complex's anchoring and penetration into the target cells, target molecules (such as cetuximab) are added while creating a delivery system. Also, the use of multimodal delivery systems was suggested, in which GNPs were loaded with a variety of medicines (both hydrophilic and hydrophobic) and auxiliary compounds. The majority of researchers have found that GNP-conjugated anticancer preparations are very effective (Dykman and Khlebtsov, 2012)

2.3.2 Green Synthesis of Silver Nanoparticles

Due to their potential uses in plasmonics, optoelectronics, antimicrobial activities DNA sequencing, biological sensors, surface-enhanced Raman scattering (SERS), energy production, clean water technology, information storage, and biomedical applications, Ag NPs have generated a huge interest in their formation. Due to the formation of nanoparticles, which has shown the promise of nanotechnology during the past ten years, we have seen significant advancements in that field. (Rafique et al, 2017)

The traditional processes for making NPs are costly, unfriendly and hazardous to the environment. In order to circumvent these problems, researchers have pinpointed the exact green routes, or the sources of naturally occurring substances and their byproducts, that can be used for the synthesis of NPs. Using microorganisms such as fungi, bacteria, actinomycetes (prokaryotes) and yeasts (eukaryotes) is one way to classify "green synthesis," while using plants and plant extracts is another (Chitsazi et al, 2016) .

Characteristics of Silver Nano-particles:-

1. Studies have revealed that the valence band and conduction band of Ag NP are relatively close to one another, allowing electrons to travel freely. (Panja et al, 2021).
2. In many applications nowadays, Nano silver with a size range of 1 to 100 nanometers plays a significant role in nanotechnology. (Fahmy et al, 2019)

3. Unsaturated membrane fatty acids' cis-trans isomerization is strongly influenced by Ag+ and Ag NPs. (Hamad et al, 2020).
4. Silver nanoparticles have a very large surface area because of their incredibly small size, due to which they have a high surface energy and morer potential reactive sights.

Applications of Silver Nano-particles :-

- The free electrons of silver subsequently give rise to the SPR (Surface Plasmon Resonance) under luminescence of light (Panja et al, 2021). Usually, the size, shape, and dispersion of the nanoparticles have an impact on the SPR peaks' width and location.
- Furthermore, surface-enhanced Raman scattering employs AgNPs (SERS). According to reports, they can increase SERS's efficiency by up to 1014–1015 times, enabling the recognition and quantification of single molecules (Qian and Nie, 2008).
- With a vast variety of antibacterial properties, nano-silver can prevent the development and flourishment of both Gram-negative and Gram-positive bacteria (Yiping and Yu, 2010)
- Moreover, Nano silver works well as a fungicide. Several common fungal strains, such as Aspergillus fumigatus, Saccharomyces cerevisiae, Mucor, , and Candida tropicalis, can be eliminated by Ag NPs (Wright et al., 1999).
- Dental prosthesis, implantology and restorative and endodontic dentistry are only a few of the dental specialties where Ag NPs have been used. Silver nanoparticles hold a significant position in restorative, regenerative, and multipurpose biomedicine using nanomaterials because of their special qualities that make them practical for several sectors of genuine interest in contemporary society. (Burdusel et al, 2018)
- Many antibacterial and antimycotic applications exist for Silver-NPs. Ag-NPs have been widely used as an antibacterial coating in medical applications, wound dressings, cardiovascular implants, and agriculture engineering. Deodorants, food, preservation products, socks, room sprays,

water filters, soaps and sanitization systems are just a few examples of consumer byproducts that utilize Ag-NPs in the wider field of nanotechnology (Saha et al., 2010).

2.3.3 Green Synthesis of Titanium Dioxide TiO_2-NPs

There has been a lot of interest in the synthesis of inorganic metal oxide nanoparticles in physical, biological, chemical, mechanical, optical, medicinal, and engineering sciences, where cutting-edge methods are being created to examine and work with single atoms and molecules. The remarkable features of metal oxide and metal nanoparticles, including their antibacterial, magnetic, electrical, and catalytic activity, are caused by their high surface to volume ratio. Due to their potential oxidation strength, lack of toxicity, and high photo stability, TiO_2 nanoparticles among the many metal oxide nanoparticles have numerous uses in dye-sensitive solar cells (DSSC), air and water purification, and other fields.

Metal oxide nanoparticles were traditionally created using a variety of physical and chemical processes. Non-sputtering, reduction, the sol-gel method, Solvothermal, and electrochemical approach are some of the more popular synthetic techniques. However, these techniques are pricey, hazardous, high-pressure, energy-intensive, and potentially expensive (Sundrarajan and Sundrarajan & Gowri, 2011).

Because of the biocompatibility, eco-friendly products, and long-term economic sustainability of trustworthy biosynthetic and environmentally friendly approaches, also to prevent harmful consequences during their application, especially in the medical industry, these factors have gained great importance. Reduction/oxidation is the primary reaction that occurs during the bottom-up biosynthesis of nanoparticles. In 2004, 2000 tons of engineered nanoparticles were estimated to have been produced; by 2020, that number is expected to rise to 58,000 tons (Maynard et al., 2006).

Typically, the synthesis of metal and metal oxide nanoparticles is carried out by plant phytochemicals having antioxidant or reducing characteristics. The three environmentally favourable and green chemistry perspectives for the synthesis of nanoparticles are the selection of the reducing agent, solvent medium, and non-toxic substance for nanoparticle stabilization.

Using plant extracts compatible with the green chemistry principles, such as *Parthenium hysterophorous* and *Saccharum spontaneum*, nanoparticle synthesis was accomplished. The use of plant extract has advantages over other biosynthetic methods, including being readily available, safe to handle, and having a wide range of viable metabolites. (Sivaranjani and Philominathan, 2016)

Titanium Dioxide Nano-particles (Titania)

Titanium Dioxide Nano-particles are micro in size, hence possesses high surface area. It also shows the magnetic nature. Titanium dioxide is also known as. Titanium (IV) oxide or titania It is prominent that titanium oxide nanoparticles can stop bacterial growth and the continuing development of new cell structures. TiO_2 is now produced in excess of four million tons annually, and this chemical is used in a variety of common products.

Chemical properties of Titanium Dioxide-

- It has a chemical symbol (TiO_2)
- Chemical Abstracts Service (CAS) number 1317-80-2
- Titanium belongs to group 4 while Oxygen belongs to group 16
- Electronic structure of Titanium is [Ar] $3d^2$ $4s^2$ and Oxygen [He] $2s^2$ $2p^4$
- Configuration of Titanium and Oxygen in TiO_2 is 59.93 and 40.55 respectively.
- Molar mass is 79.9378 g/mol
- Density is 4.23 g/cm^3
- Melting Point of TiO_2-1,843° C
- Boiling Point of TiO_2 -2,972° C

Applications of TiO_2 nanoparticles (Figure 3)

- TiO_2 have an excellent photo catalytic property, that’s why it is used in antiseptic and antibacterial products.
- Titanium dioxide possesses degrading properties against germs and organic contaminants.
- Titanium dioxide is an ultra violet radiation resistant chemical.
- It is used in production of cosmetics products like skin milk, suns cream, day and night cream.

Figure 3: Applications of TiO_2 Nanoparticles.

Due to titanium dioxide's small crystal size, a large specific surface area, and highly porous structure, research on the photocatalytic application of TiO_2 and its composite material has recently attracted significant interest. Titanium Dioxide nano materials are the most commonly used photo catalyst due to its antibacterial properties minimal toxicity, highly oxidizing nature and its cost effectiveness.

The cosmetics industry, which has used TiO_2 nanoparticles in sunscreen products for 20 years, provides evidence of their low toxicity. Several investigations have demonstrated that human exposure to micro- or nano sized TiO_2 particles does not cause (photo) mutagenesis or (photo) geno toxicity (Senic et al, 2011). Most importantly, there are no differences between micro and nano TiO_2 in the body's recognition, distribution, or removal of it. It contains highly oxidizing photo generated holes, and the photo generated electrons are sufficiently reducing to form superoxide from dioxygen. The principal categories of indoor airborne contaminants are encouraged to oxidize at ambient temperatures without the need for any chemical additives.

Pure TiO_2, however, is only active under ultraviolet irradiation (below 5% of the solar spectrum) because of its high bandgap, which restricts its usage within visible light (around 45 percent of the solar spectrum). Thus, it is crucial to create active photo-catalysts for visible light that have a comparatively lower band gap (Ayati et al, 2014) .To achieve visible light photo-catalytic activity, a variety of surface modification approaches are used, such as the use of metals and nonmetals for doping, dye sensitization, and heterogeneous composites using synthetic and natural dyes. Among them, dye-sensitization is a process that shows promise in a number of areas, such as the creation of solar cells and the elimination of toxins from actual wastewater (Hussain et al, 2010).

2.4 Plants used for preparation of TiO_2NPs

Parthenium hysterophorous

A plant species that is among the most invasive in the globe is *Parthenium hysterophorus* L. (Family: Asteraceae), also known as congress weed or parthenium weed. Although traditional weed control techniques are routinely employed to manage this troublesome plant, there is currently more interest in examining its usefulness potential as a cutting-edge management strategy (Ahsan et al, 2020). Sesquiterpene, lactones, and phenolics, which are the plant's most well-known secondary metabolites and which contribute to its allelopathic potential, cytotoxic activity, and allergenic reactions, are among those that cause allergic reactions. However according to studies (Patel, 2011), *Parthenium hysterophorus* biochemical also have medical benefits, including anti-inflammatory, antibacterial, antifungal, and anticancer properties.

Parthenium hysterophorus leaf extracts have been used to create NPs, and more research has been recommended to create products with added value that are significant for biomedicine. With the aid of microwave irradiation and *P. hysterophorus* leaf extract, titanium dioxide nanoparticles have been created. However, no research has been done on the optimal production of Ag NPs using this plant, and no comprehensive therapeutic evaluation of the NPs in this form has been carried out (Adkins et al, 2019).

Saccharum spontaneum

Utilizing aqueous extracts from one of the promising herbal plants, *S. spontaneum,* a cost-efficient and secure methodology was developed for the production of stable silver nanoparticles. The *S. spontaneum* plant, often known as wild sugarcane, is widely distributed in South Asia, East Africa, and the Mediterranean. Infections of the urinary tract, diarrhea, obesity, hemorrhoids, and renal calculi are only a few of the conditions that are treated with the plant extract decoction. For breastfeeding moms to increase milk production, S. spontaneum root decoction is used in Ayurveda medicine (Martinez-Castanon, 2008) (Cheavegatti-Gianotto et al, 2011).Because it acts as a reductant and stabilising agent, the plant extract is therefore very safe and appropriate for the creation of TiO_2 nanoparticles. As a result, the TiO_2 nanoparticles (TiO_2NPs) created by *S. spontaneum* were studied for both gram-positive and gram-negative bacteria, it has a bactericidal effect (Mikawlrawng et al., 2014).

By adjusting the parameters of reaction time, temperature, concentration, and pH, metal nanoparticles can be produced in a variety of morphologies, including spherical, triangular, rod, hexagonal, wires, and cubes. It's interesting to note that according to Banerjee et al., the reaction duration affects the morphology of the Titanium nanoparticles.

Spherical nanoparticles at first, then trigonal or rod-shaped ones, were created. For the first time, the medicinal plant *S. spontaneum* was used as a reductant and coating (capping) agent to produce TiO_2 nanoparticles, and the MTT assay was carried out using MG63 osteoblast like cells in order to assess the cell viability. To the best of our knowledge, there are very less reports available for the production of TiO_2 NPs with multiple shapes utilizing plant extracts. (Ripa et al., 2009) (Yilmaz et al., 2011) (Mikawlrawng et al., 2014).

Chemical processes that occur in the presence of light and a photo catalyst are known as photo catalysis in chemistry. A semiconductor called a photo catalyst accelerates the rate of reaction by its presence. Numerous uses for photo catalysis include antimicrobial, deodorizing, air purification, antifogging, self-cleaning, and water purification. (Ameta et al, 2018)

Photo catalyst – Photo catalysts are the solids which promotes reactions when light is present and are not fully consumed during the reaction. These solids are essentially semiconductors. An ideal photo catalyst has the following properties-

(i) It should be photoactive
(ii) It should be chemically and biologically inert
(ii) Should utilize visible and Ultraviolet light
(iv) It should be Photo stable
(v) Should be inexpensive
(vi) Should be non-toxic

Titanium DioxideTiO_2, Tungsten Trioxide(WO_3), Cadmium Sulphide(CdS), Strontium Titanate($SrTiO_3$), Iron Oxide(Fe_2O_3) can be used as Photocatalysts. (Bhatkhande et al, 2002).

2.5 Photo catalytic Degradation of Antibiotics (Sulfamethoxazole, Trimethoprim)

In human medicine, the synthetic antimicrobial sulfamethoxazole (SMX) is frequently given to treat bronchitis and urinary tract infections. Additionally, it is utilised in veterinary medicine to promote animal growth as well as to cure and prevent illnesses.

For the prevention and treatment of respiratory or gastrointestinal tract infections in cattle, pigs, and poultry, trimethoprim (TMP) is mostly used as a preventive and treatment for urinary tract infections. In fact, the usage of trimethoprim and sulfonamides in veterinary medications is common. The presence of these two antibiotics in the effluents of STPs has been reported in several investigations, which highlights the fact that they are not entirely destroyed after treatment.

The degradation of sulfamethoxazole and trimethoprim by TiO_2 in suspension with the help of photo catalysis. As the name suggest photo catalysis works under a spectrum of wavelengths less than 400 nanometers, cause the semiconductor surface to produce e/h+ pairs that can redox react with organic compounds to degrade them, or they can react with the water molecules to produce hydroxyl radicals, which can also be in-charge results in degrading the organic pollutants present in the medium. Titanium dioxide is the most common catalyst used, and water is often the reaction's medium. These technologies' key benefits are –

(I) Cheapness/Affordable Price of TiO_2
(II) TiO_2 is very stable across a wide variety of pHs and working conditions.
(III) It has a very low selectivity which means it can degrade a large variety of toxins.
(IV) With TiO_2, total mineralization can be accomplished.

Chapter 3

HYPOTHESIS

3. HYPOTHESIS

In recent years, antibiotics have become a common byword among researchers, medical experts, and biotechnology and pharmaceutical companies as a potential key for treating various diseases. This huge use of antibiotics is now a major source of antibiotic pollution and an emerging concern for the environment. Antibiotic pollution has led to the major problem of antibiotic resistance to the organisms in our surroundings. Waste released from different pharmaceutical industries, companies and hospitals must be treated to eliminate antibiotic pollution. Pharmaceutical waste includes over-the-counter and prescription medicines released to water bodies and surroundings. This antibiotic waste is hard to treat through biological wastewater treatment plants because they suppress the microorganisms in treatment systems. Several conventional methods have been used for the treatment of antibiotic pollution from more than a decade, but the problem with these conventional methods is that leads to too much of secondary pollution. Therefore, present study is focused on an eco-friendly green approach by using plant derived TiO_2-NPs growing native to effluent discharge site, for the efficient degradation of residual antibiotics in pharmaceutical effluent, which releases CO_2 and H_2O as environmentally friendly by-products.

The project aimed to develop an eco-friendly green approach which can efficiently reduce the pharmaceutical waste from the environment. In the field of interdisciplinary result with exciting new development and using it to gain the fundamental knowledge is essential to overcome this issue by facing present technologies and also with respect to social, legal and ethical issues involved in this method and in near future this technique will go long way in eradicating this ill-famed problem from the environment. Degradation of antibiotics through metal oxide nanoparticles produced from plants growing native to the pharmaceutical effluent discharge site come up with its eco-friendly application.

Chapter 4

AIM AND OBJECTIVES

4. AIM AND OBJECTIVES

4.1 Background

The toxicity caused by the pharmaceutical effluent discharge pollution, with special reference to antibiotics in the environment needs an efficient treatment to reduce the pharmaceutical waste. Hence, biodegradation technique which is sustainable, cost effective and eco-friendly used nowadays to eradicate this issue. Most flourishing plant species growing native to the pharmaceutical effluent discharge sites were selected to synthesise metal oxide nanoparticles to check their possible potential for the degradation of antibiotics with an eco-friendly green approach.

4.2 Objectives

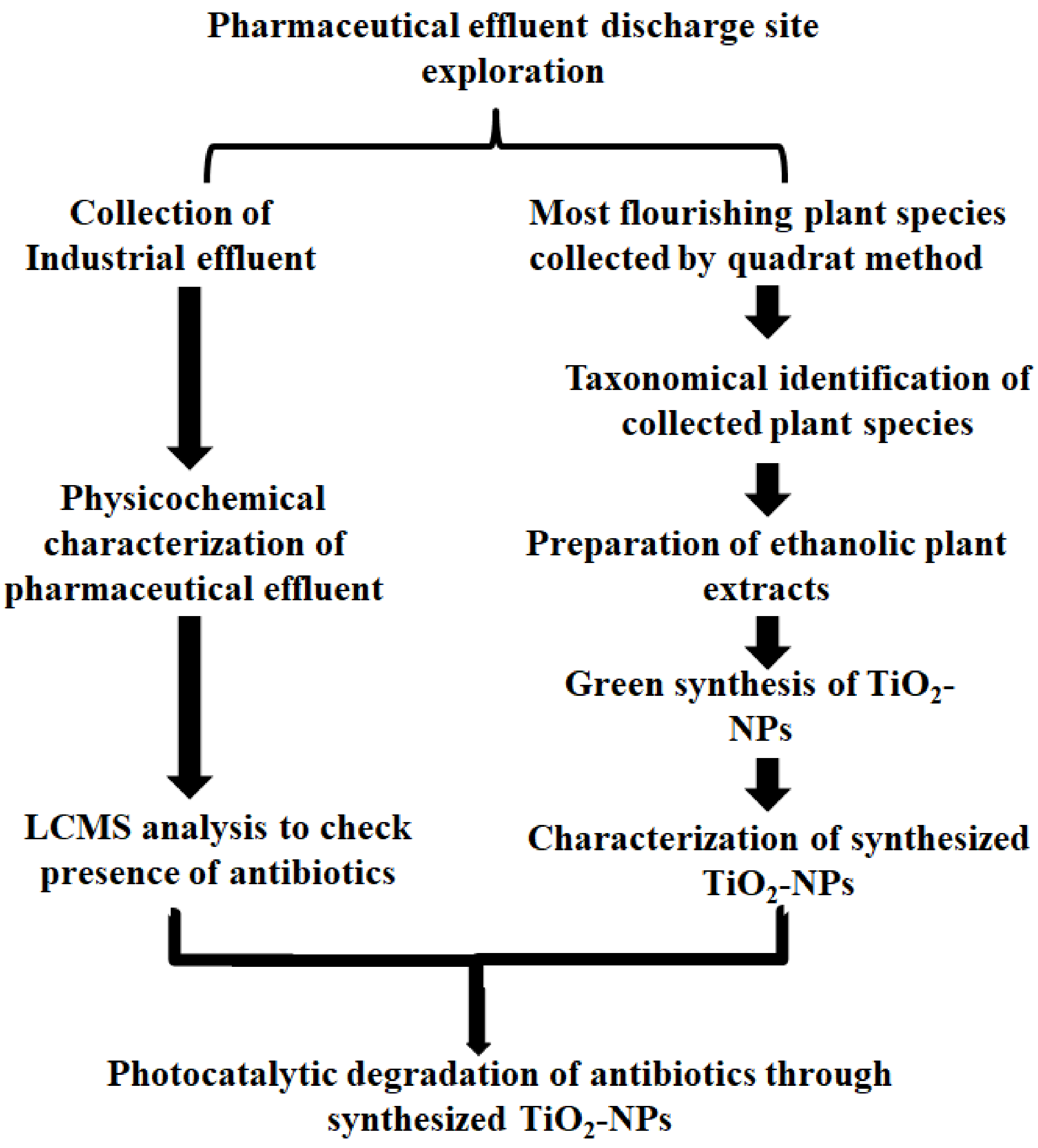
1. To explore sites and identify plants capable of nanoparticles synthesis around Pharmaceutical effluent discharge near Baddi region (Himachal Pradesh)
2. Physico-chemical characterization of the pharmaceutical effluents
3. Preparation and characterization of plant derived nanoparticles
4. To study the biodegradation potential of most prevalent pharmaceutical effluent contaminant by plant derived nanoparticles.

Chapter 5

MATERIALS AND METHODS

5 MATERIALS AND METHODS

WORK PLAN



5.1 Materials

Titanium Tetraisopropoxide (97% v/v, $C_{12}H_{28}O_4$ Ti, 284.22 g mol^{-1}) from Central Drug House, India , Ethanol (70%), Ciprofloxacin (98% w/w,$C_{17}H_{18}FN_3O_3$,331.34 g mol^{-1}) from Sigma-Aldrich, Ofloxacin (98% w/w, $C_{18}H_{20}FN_3O_4$, 361.4 g $mol^{-1)}$ from TCI chemicals, NaCl (99.5%) and Phosphate buffer from Hi-Media, India. Double distilled water was used for preparation of all solutions. A UV chamber fitted with 2 UV tubes (each tube of 15W power) was used to perform photocatalytic experiments. To carry out the effective treatment of ofloxacin and ciprofloxacin the synthetic solution of antibiotics was kept at a distance of 10 cm.

5.2 Site Exploration, Collection and taxonomical identification of Plant Material

Exploration of site for species abundance and plant identification, capable of nanoparticles synthesis around Pharmaceutical effluent discharge site near Baddi, Himachal Pradesh was done and plant population density was calculated by Quadrat method (Mousaei Sanjerehei, M., 2011).

Plant samples of *Parthenium hysterophorous* and *Saccharum spontaneum* growing near the pharmaceutical effluent polluted sites along Baddi, Himachal Pradesh, India (latitude: 30.94263^o N and longitude: 76.77187^o E) were collected as a substrate for green synthesis. The plant materials thus collected were taxonomically authenticated by Systematic Botany Discipline, Forest Research Institute, Dehradun.

5.3 Collection and Physicochemical characterization of Pharmaceutical effluent

Pharmaceutical effluent of CPFX and OFX producing batch was collected from the pharmaceutical industry situated in the industrial area of Baddi, Himachal Pardesh, India. The pharmaceutical effluent was collected in pre-rinsed and dried amber colored glass bottle (5 L) and stored in a refrigerator at 4^{o}C temperature. The initial characterization of pharmaceutical effluent was done for pH, Chemical oxygen demand (COD), Biochemical oxygen demand (BOD) and Total dissolved solids (TDS). Further to check the presence of respective antibiotics, the sample was subjected to LCMS through Q-Tof micro mass spectrometer for analytical studies.

The treatment of CPFX and OFX in synthetic wastewater was investigated using the optimized conditions for the degradation of CPFX and OFX. **Chemical oxygen demand (COD)** was determined in accordance to Indian Standard protocol IS:3025 (Part 58). **Biochemical oxygen demand (BOD)** was determined in accordance to Indian Standard protocol IS:3025 (Part 44). **Total dissolved solids (TDS)** was measured with the help of evaporation method, by desiccating the solids obtained after filtration of collected pharmaceutical effluent sample in an oven at 103ºC for 24 hours. Difference between the final weight of the dry solids with filter paper and blank filter paper gives total amount of dissolved solid present in the sample.

5.4 Preparation of plant extract

To begin with, the plant material was subjected to shade drying at ambient temperature for duration of seven days. Subsequently, the dried plant material was pulverized into a fine powder using a mortar and pestle. Thereafter, an extract of the plant material was prepared using a Soxhlet apparatus. Specifically, 25 grams of the powdered plant material was placed in the Soxhlet extractor along with 250 mL of 70% ethanol solvent and subjected to extraction at a temperature of 50℃ for a continuous period of 8 hours. The resulting extract was identified as an ethanolic plant extract.

5.5 Green Synthesis of TiO_2-NPs

Initially, we combined 50 mL of titanium tetraisopropoxide and 20 mL of ethanolic plant extract in a flask and subjected the flask to rotary shaking at 50℃ for a period of 5 hours. Following this, the resulting nanoparticles were isolated by centrifuging the mixture at a speed of 5000 rpm for 15 minutes. The separated nanoparticles were subsequently dried in a hot air oven at a temperature of 500℃ for a duration of 5 hours to obtain the final product.(Ahmad et al., 2020)

5.6 Characterization of Synthesized TiO_2-NPs

Various methods used for the characterization of synthesized TiO_2 nanoparticles are listed as follows.

5.6.1 *X-Ray Diffraction (XRD) analysis of TiO_2-NPs*

The crystallinity, as well as phase information of synthesized TiO_2-NPs, was determined by X-ray diffraction study. X-ray diffraction of our synthesized nanoparticles was taken by a very advanced D8 Eco Advance Brucker XRD instrument. The dried powder of synthesized nanoparticles is used in the diffraction experiment. The 2θ range of 20–80° was used for recording the diffractogram.

5.6.2 *Fourier-transform infrared spectroscopy (FTIR) analysis of TiO_2-NPs*

FTIR was conducted at a standard room temperature using a FTIR spectrometer (Shimadzu 8400S) equipped with a potassium bromide (KBr) beam splitter. To prepare the sample a slim layer was spread onto KBr at a ratio of 100:1. Following placement of the KBr-sample mixture in the sample holder, FT-IR spectra were logged at 500–4000 cm^{-1}.

5.6.3 *Field Emission Scanning Electron Microscopy (FE-SEM) analysis of TiO_2-NPs*

A FE-SEM is a powerful tool that utilizes a highly focused beam of electrons to scan over a sample's surface and generate high-resolution images.

The morphological features of synthesized titanium dioxide nanoparticles were examined by using JSM-7610F FE-SEM, operating at various magnifications ranging from 10000X to 45000X. For sample preparation, powdered sample is sticked on to the double sided carbon tape and further fixed it on the holder. Sticked powder sample is being gold coated for making the material conductive and then the prepared sample is inserted in FE-SEM.

5.7 Photocatalytic Degradation of Ofloxacin and Ciprofloxacin by synthesized TiO_2-NPs

The current investigation aimed to evaluate the impact of distinct factors, such as reaction duration, nanoparticle concentration, antibiotic concentration and pH on the mineralization of antibiotics. Additionally, the comprehensive examination of the degradation mechanism showed that photocatalysis plays a significant part in minimizing harmful waste. This occurs when the molecules of water confine the holes (H^+) present in the aqueous medium. Later, OH^- radicals are created, functioning as potent oxidizing agents and transforming the organic chemicals into gaseous and water by-products (Kanakaraju et al., 2023). Given the improved efficacy of this approach in practical settings, TiO_2 nanoparticles offer a viable solution for eliminating recalcitrant contaminants from industrial wastewater.

5.7.1 *Effect of nanoparticles concentration on degradation studies*

Antibiotics solution was exposed to various concentrations of nanoparticles, including 1ppm, 3ppm, 5ppm, 7ppm, 9ppm, 11ppm, and 13 ppm and then subjected to photocatalytic degradation for duration of 3 hours. Measurements were recorded at the end of the 3-hour period. The antibiotics solution without nanoparticles was used as a control and was used as a control and was exposed to similar treatments (Ahmed et al., 2004). The absorbance was measured at 340nm. The experiment was carried out in triplicates. The percentage of degradation was determined using the formula: $(C_o-C)/C_o*100$, (Nosrati et al., 2012) where C_o represents the initial concentration of the antibiotic at the zero hour and C represents the concentration of the degraded antibiotic at a given time.

5.7.2 *Effect of antibiotic concentration on degradation studies*

Various concentration of antibiotics, including 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, and 60ppm, were mixed with that concentration of nanoparticles that showed the most significant photocatalytic degradation. The mixtures were then exposed to UV light for a period of 3 hours, and the measurements were taken at the end of the 3-hour period. The antibiotic solution without nanoparticles was used as a control and was exposed to similar treatments (Ahmed et al., 2004). The absorbance was measured at 340nm. The experiment was carried out in triplicates. The percentage of degradation was determined using the formula: $(C_o-C)/C_o*100$, (Nosrati et al., 2012) where C_o represents the initial concentration of the antibiotic at the zero hour and C represents the concentration of the degraded antibiotic at a given time.

5.7.3 *Effect of contact time on degradation studies*

The concentration of nanoparticles and antibiotics that demonstrated the highest photocatalytic degradation as reported, were mixed together and exposed to UV light for a duration of 6 hours. Measurements were taken every hour and the antibiotic solution without nanoparticles was used as control and subjected to comparable treatments (Ahmed et al., 2004). The absorbance was measured at 340nm. The experiment was carried out in triplicates. The percentage of degradation was determined using the formula: $(C_o-C)/C_o*100$, (Nosrati et al., 2012) where C_o represents the initial concentration of the antibiotic at the zero hour and C represents the concentration of the degraded antibiotic at a given time.

5.7.4 *Effect of pH on degradation studies*

A 10 ppm concentration of the antibiotics solution was prepared, and the pH was adjusted to a range of 4 to 9 (with concentrated Phosphoric acid and NaOH) before the addition of nanoparticles. The concentration of nanoparticles which showed the highest degradation potential were mixed with prepared antibiotic concentration (i.e. 10 ppm) and the resulted mixture was subjected to photocatalytic degradation for the optimized time period at which photocatalytic degradation yielded best results. Measurements were taken at the time that exhibited the best photocatalytic degradation. The antibiotic solution without nanoparticles served as a control and underwent similar treatments (Ahmed et al., 2004). The absorbance was measured at 340nm. The experiment was carried out in triplicates. The percentage of degradation was determined using the formula: $(C_o-C)/C_o*100$, (Nosrati et al., 2012) where C_o represents the initial concentration of the antibiotic at the zero hour and C represents the concentration of the degraded antibiotic at a given time.

5.8 Statistical analysis of the process

The acquired data were subjected to statistical analysis to determine the impact of different variables on the extent of antibiotic degradation. The statistical software package GraphPad PRISM 9.0 was utilized to perform one-way and two-way ANOVA.

Chapter 6

RESULTS AND DISCUSSION

6 RESULTS AND DISCUSSION

The primary goal of this research is to investigate the degradation pathway via photocatalytic activity, which plays an important role in the reduction of hazardous wastes by trapping holes (H^+) in the aqueous environment and producing hydroxyl radicals (OH^-). These radicals are powerful oxidizers that degrade organic pollutants into water and gaseous byproducts. For this ethanolic extracts of the plant samples of *Parthenium hysterophorus and Saccharum spontaneum* was selected in the undertaken studies considering its environmental friendly, safe, uniform and cost-effective green approach. *Parthenium hysterophorus* is a common plant weed found flourishing around the barren infertile areas under stress conditions. This plant is well known for secreting allelochemicals having medicinal properties (Jaiswal et al., 2022). On the other hand *Saccharum spontaneum* is having its own importance in our traditional medicine systems, whose extracts can be used for the treatment of sexual, respiratory, gynaecological and mental disorders (Pandey et al., 2015). Their presence in the pharmaceutical effluent polluted sites signifies the capability of the plant to tolerate and associate with the various antibiotics. The influence of several parameters such as response time, antibiotic concentration, nanoparticle concentration and pH was investigated.

6.1 Site Exploration, Collection and taxonomical identification of Plant Material

Pharmaceutical effluent discharge site situated near CETP, was explored (as depicted in Figure 4) and most frequent plant species found along the banks of Sarsa river in Baddi, Himachal Pardesh, India was collected as shown in Figure 5.

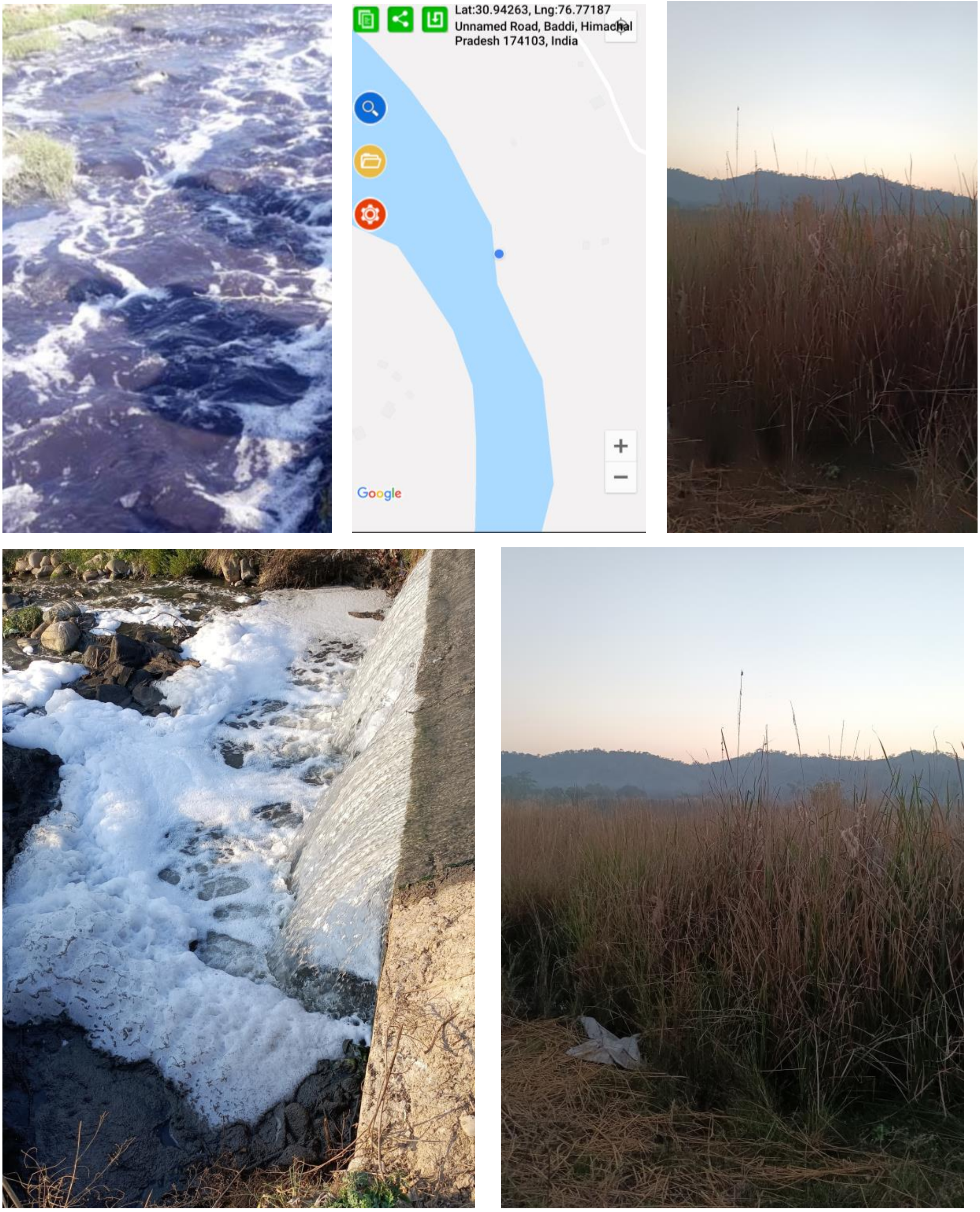


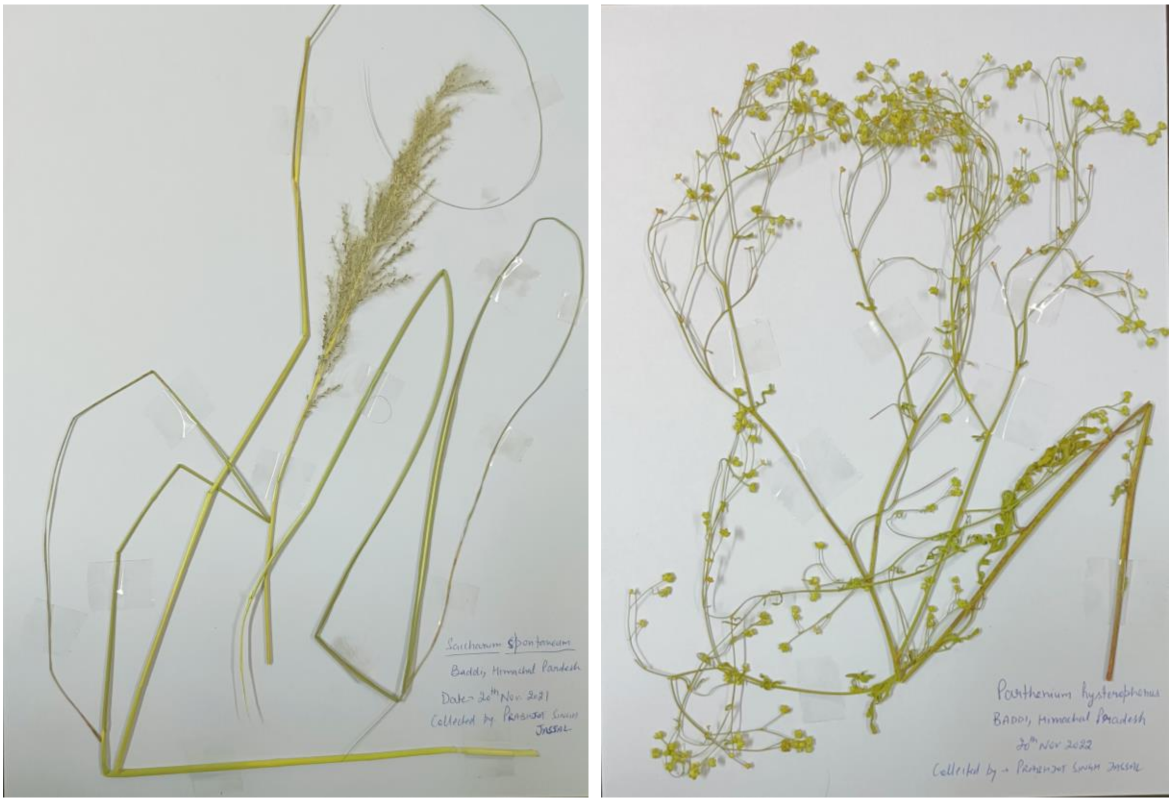
Figure 4: Pictures of pharmaceutical effluent discharge site Baddi, Himachal Pradesh, India (latitude: 30.94263º N and longitude: 76.77187º E)

Figure 5: Pictures of two most prevalent plant species growing in and along the pharmaceutical effluent discharge site.

Most flourishing plant species at the wastewater site was collected with help of Quadrat method. Quadrat is a square area which is marked with boundaries for studying the population size and density of plants. A $1m^2$ square made of plastic pipes was used to make a quadrat which was placed on a ground at 10 different places to infer the species abundance/ plant population density as shown in Table 2. Table values indicates, plant species A and B to be most flourishing across pharmaceutical effluent discharge sites, with highest plant population density values viz. 5.3 and 3.4 respectively among the evaluated plant species. Collected specimens are prepared and then submitted to Systematic Botany Discipline, Forest Research Institute, Dehradun for taxonomical identification as shown in Figure 6. Submitted plant samples are then taxonomically identified as *Saccharum spontaneum* (A) and *Parthenium hysterophorus* (B) as shown in Figure 7.

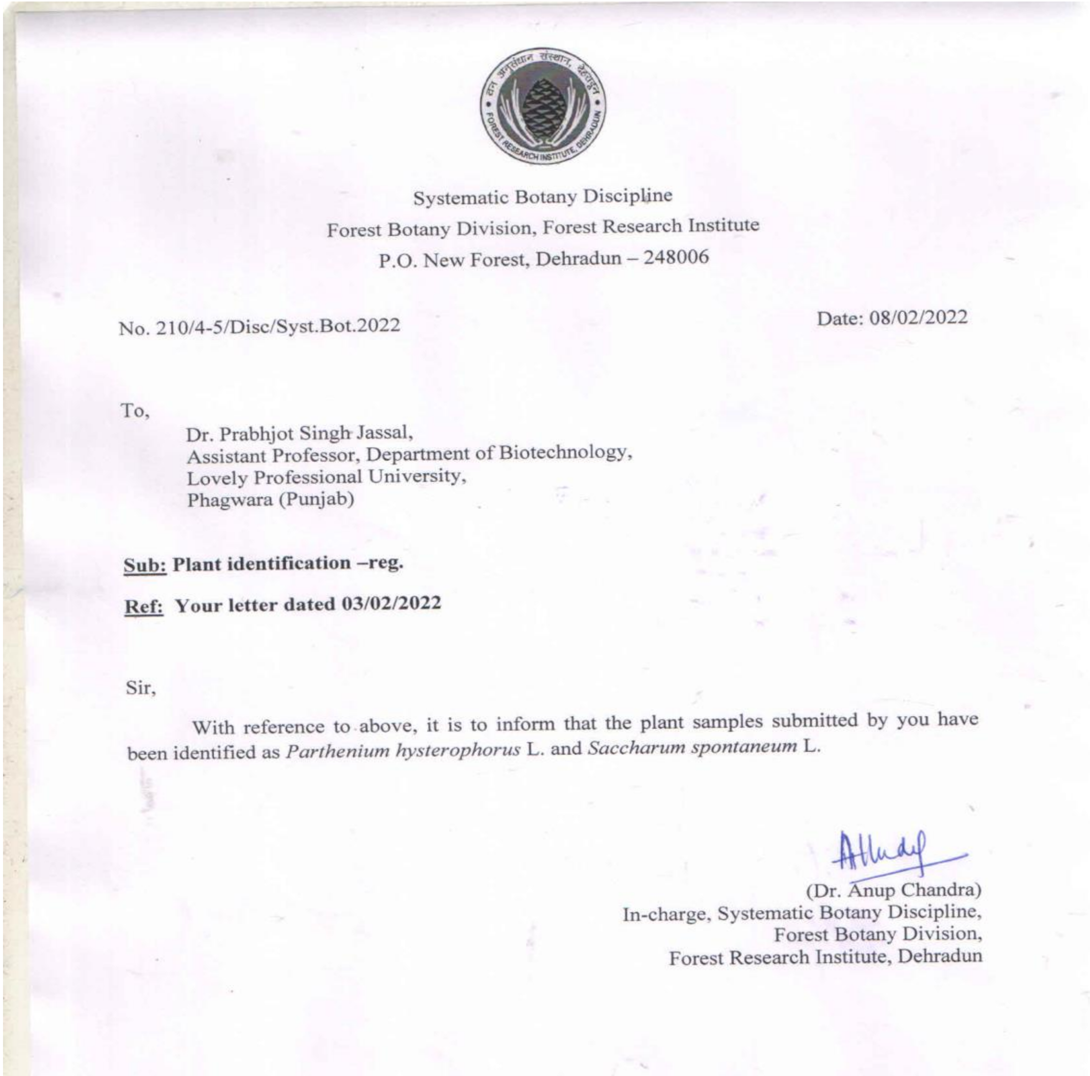
Table 2: Quadrat method for calculating plant population density around Pharmaceutical effluent discharge site near Baddi, Himachal Pradesh.

	Plant Species	A	B	C	D	E
	I	6		2		
	II	5	2			
	III	11				
	IV	10				
	V		7	3		
	VI	10	4		2	
	VII	5	3	1	3	
	VIII	6	3	2	2	
	IX		6	3	3	1
	X		9			
Total No. of Individuals (S)		53	34	11	10	1
Total Number of Quadrats studied (Q)		10	10	10	10	10
Plant populationDensity (D) D=S/Q		5.3	3.4	1.1	1	0.1



(A) (B)

Figure 6: Prepared plant samples collected from pharmaceutical effluent discharge site, submitted to Systematic Botany Discipline, Forest Research Institute, Dehradun for taxonomical identification.



Systematic Botany Discipline
Forest Botany Division, Forest Research Institute
P.O. New Forest, Dehradun – 248006

No. 210/4-5/Disc/Syst.Bot.2022

Date: 08/02/2022

To,
Dr. Prabhjot Singh Jassal,
Assistant Professor, Department of Biotechnology,
Lovely Professional University,
Phagwara (Punjab)

Sub: Plant identification –reg.

Ref: Your letter dated 03/02/2022

Sir,

With reference to above, it is to inform that the plant samples submitted by you have been identified as *Parthenium hysterophorus* L. and *Saccharum spontaneum* L.

(Dr. Anup Chandra)
In-charge, Systematic Botany Discipline,
Forest Botany Division,
Forest Research Institute, Dehradun

Figure 7: Plant identification certificate received from Systematic Botany Discipline, Forest Research Institute, Dehradun for taxonomical identification. Submitted plant species are identified as *Saccharum spontaneum* (A) and *Parthenium hysterophorus* (B).

6.2 Sample Collection and Physicochemical characterization of pharmaceutical effluent

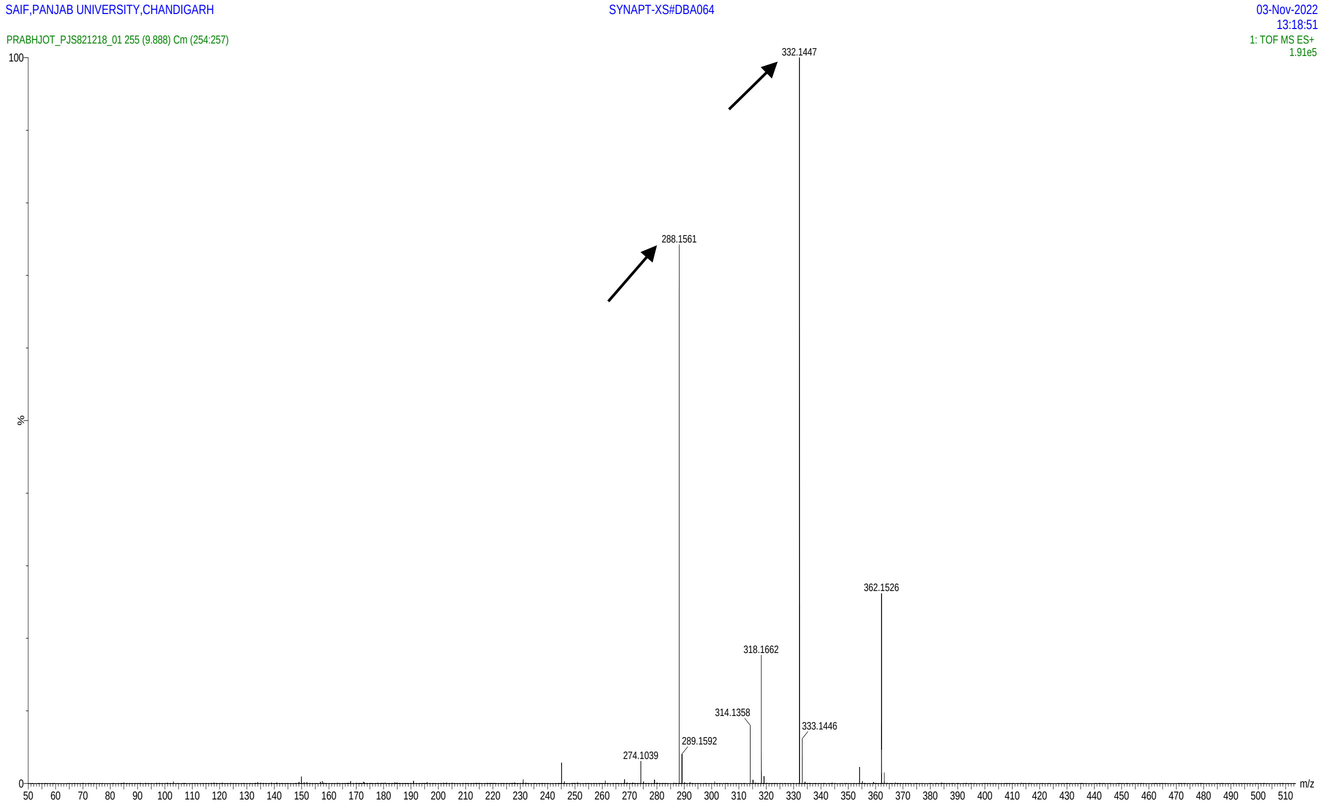
Pharmaceutical sample collected from the industrial area of Baddi, Himachal Pardesh, India was subjected to physicochemical characterization for various parameters such as pH, Chemical oxygen demand (COD), Biochemical oxygen demand (BOD), Total dissolved solids (TDS), Total suspended solids (TSS) and the presence of respective antibiotics. Standard protocols were used to perform physico-chemical analysis of the collected wastewater. The pharmaceutical wastewater was collected in pre-rinsed and dried amber colored glass bottle and was subjected to testing within 3 days of collection. Standardized procedures were used for the examination of collected wastewater. List of physical parameters analysed were described in Table 3.

Table 3: Physicochemical characterization of pharmaceutical wastewater

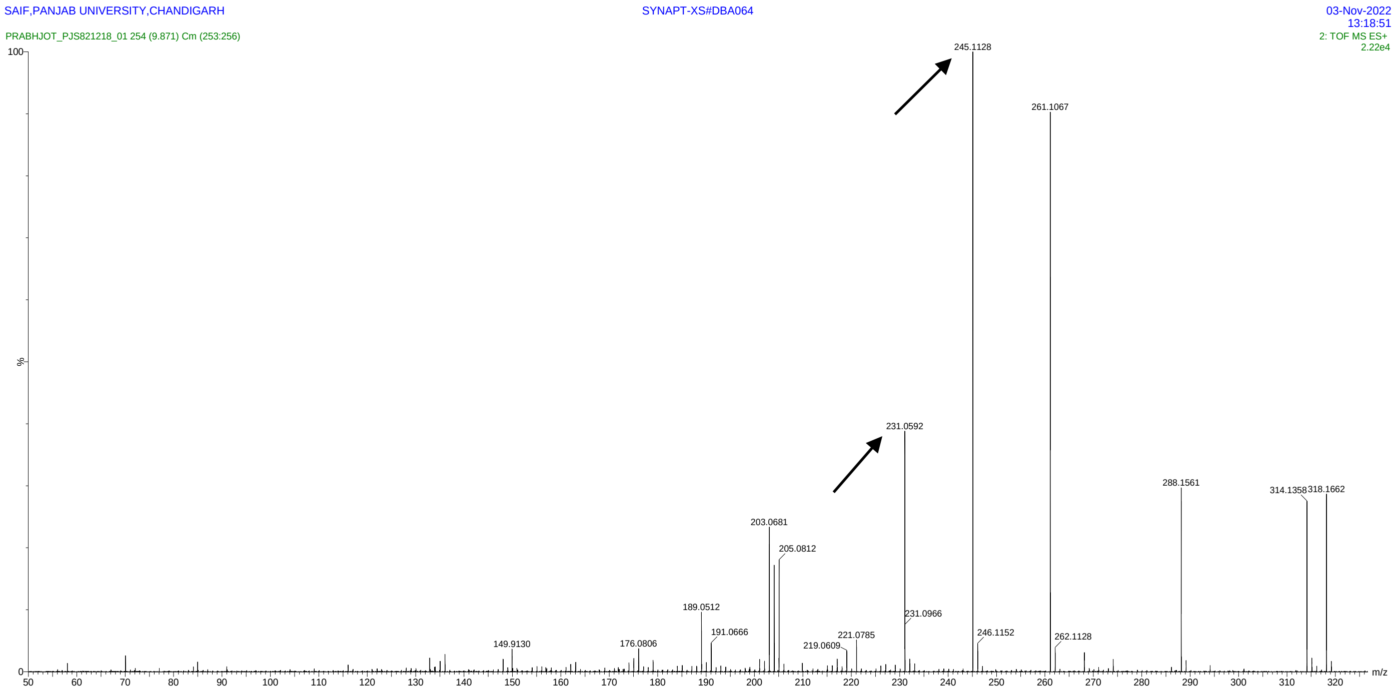
Sr.No.	Parameters	Test Results	Standard Value
1.	pH value	7.11±2	6.0-8.5
2.	Chemical Oxygen Demand (mg/l)	282 ± 2	250
3.	Biochemical Oxygen Demand (mg/l)	98±2	30
4.	Total Suspended Solids (mg/l)	130.5±2	100
5.	Total Dissolved Solids (mg/l)	1820±2	500

The collected effluent was recorded with light grey color, neutral pH (7.11±2), COD (282±2 mg L^{-1}), BOD (98±2 mg L^{-1}), total dissolved solids (1820±2 mg L^{-1}) and total suspended solids (130.5±2 mg L^{-1}). As per the Environment (Protection) Second Amendment Rules, 2021, certain amendments have been made in context to effluent standards and emission standards for the bulk drug producing pharmaceutical industries. The wastewater analysis results confirmed that the parameters such as pH, BOD, and COD, Total dissolved solids, Total suspended solids were above the standard prescribed limits of central pollution control board for effluent discharged into surface water.

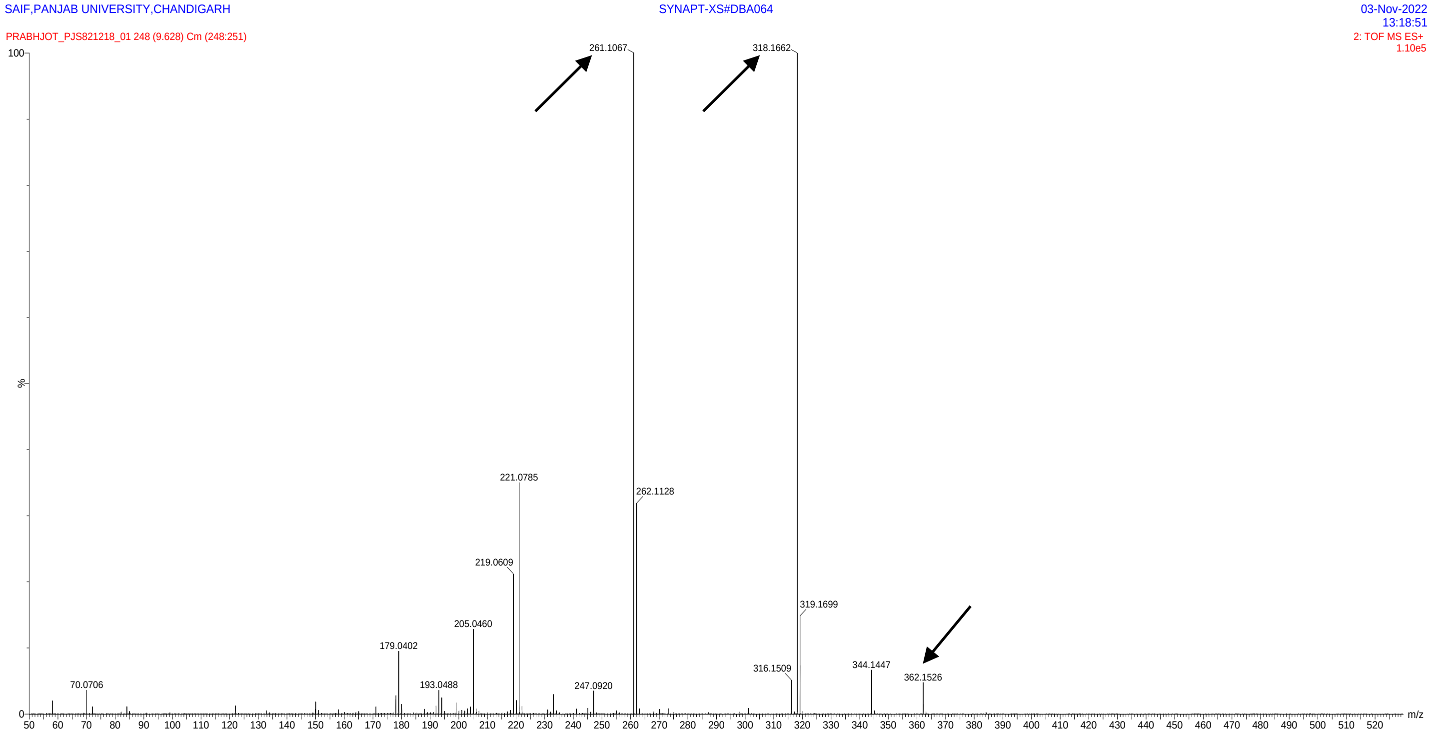
Further characterization of pharmaceutical effluent was done to check the presence of respective antibiotics in the sample. For that, the sample was submitted to SAIF Lab Chandigarh for LCMS analysis. As per the literature review, we have observed the following peaks in LC-MS spectra of the sample as shown in Figure 8. According to the literature, the peaks observed at m/z=332 and m/z=288 (Figure 8a), confirms the presence of ciprofloxacin in the sample. Further splitting of the antibiotic occurs in the sample and results in observed peaks at m/z=245 and m/z=231(Figure 8b), which further evidently confirms the presence of ciprofloxacin (Zhang et al, 2016).



(a)



(b)



(c)

Figure 8: LCMS spectra of the pharmaceutical effluent where observed peaks at m/z=332 and m/z=288 (Fig. 8a); m/z=245 and m/z=231(Fig. 8b) confirms the

presence of ciprofloxacin and the other peaks observed at m/z=362, m/z=318 and m/z=261(Fig. 8c), confirms the presence of Ofloxacin in the sample.

Splitting of Ciprofloxacin (m=331) in sample occurs as follows.

1 $m/z = 332 \rightarrow [m+H]^+$

2 $m/z = 288 \rightarrow [m-CO_2]+ H^+$

3 $m/z = 245 \rightarrow [m-CO_2 - C_2H_5N]+ H^+$

4 $m/z = 231 \rightarrow [m-CO_2 - C_2H_5N - CH_2]+ H^+$

44 43 14

Along with that, other peaks observed at m/z=362, m/z=318 and m/z=261 (Figure 8c) in LCMS spectra, confirms the presence of Ofloxacin in the sample as suggested by Byrro, R.M.D. et al, 2012.

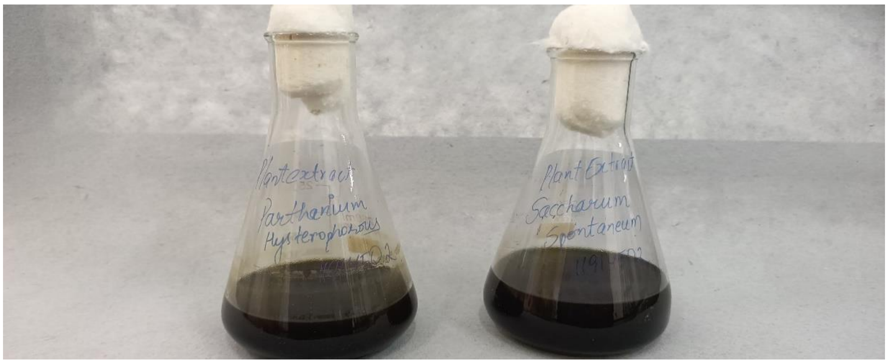
Splitting of Ofloxacin (m=361) in sample occurs as follows.

1. $m/z = 362 \rightarrow [m+H]^+$
2. $m/z = 318 \rightarrow [m-CO_2]+ H^+$
3. $m/z = 261 \rightarrow [m-CO_2 – C_2H_4N]+ H^+$

44 57

6.3 Preparation of plant extract

Plant samples of *Saccharum spontaneum* and *Parthenium hysterophorous* were collected from the pharmaceutical effluent polluted sites along Baddi, Himachal Pradesh, India. Ethanol was used as the solvent for the extract preparation. The powder form of the plant was prepared using a Mortar and pestle. Followed by the ethanolic plant extract preparation using Soxhlet Apparatus as shown in Figure 9.



(a) (b)

Figure 9: Ethanolic Plant Extracts of *Parthenium hysterophorous* (a) and *Saccharum spontaneum* (b).

6.4 Green Synthesis of TiO_2 Nanoparticles

TiO_2 nanoparticles were prepared using the ethanolic plant extract of *Parthenium hysterophorous* and *Saccharum spontaneum.* 50 mL of Titanium tetraisopropoxide was mixed with 20 mL of ethanolic plant extract for the preparation of nanoparticles. After that the sample was kept in a shaker incubator at 50°C for 5 hours. Further the process was followed by centrifugation at 5000 rpm for 15 minutes. The supernatant formed was discarded and the pellet was washed thrice with 70% ethanol. Then the pellet was taken out in the Petri dish and sealed with parafilm. After that the nanoparticles were dried in a hot air oven at 500°C for 5 hours as depicted in Figure 10. (Ahmad et al., 2020).

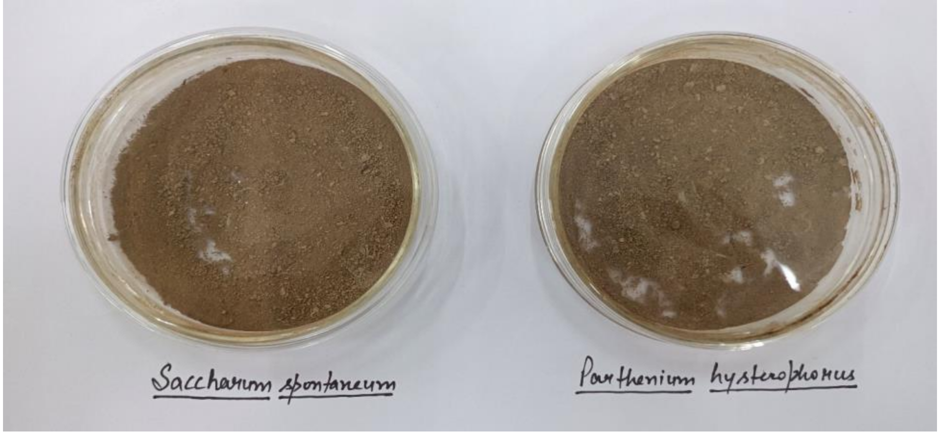


Figure 10: Synthesized TiO_2 nanoparticles by using the ethanolic plant extract of *Parthenium hysterophorous* and *Saccharum spontaneum*.

6.5 Characterization of Synthesised TiO_2-NPs.

6.5.1 *FTIR spectroscopy analysis of Parthenium hysterophorus and Saccharum spontaneum derived TiO_2-NPs.*

FTIR spectroscopy is used to calculate the various functional groups present in TiO_2 nanoparticles. FTIR spectra of TiO_2 for *Parthenium hysterophorus* and *Saccharum spontaneum* were taken between 500-4000 cm^{-1} range. For *Parthenium hysterophorus*, spectra of nanoparticles manifests absorbance peak located at 436.16 cm^{-1}, 1629.02 cm^{-1} and 2919.1 cm^{-1} as shown in Figure 11. The peak at 436.16 cm^{-1} in the spectra displays the stretching vibration of Ti-O, and the peak at 1629.02 cm^{-1} shows the stretching vibration of Ti-O-Ti. The peak at 2919.1 cm^{-1} demonstrates the stretching vibration of the O-H group (alcohol-phenol). The results are in accordance with as explained by Vembu et al., 2022.

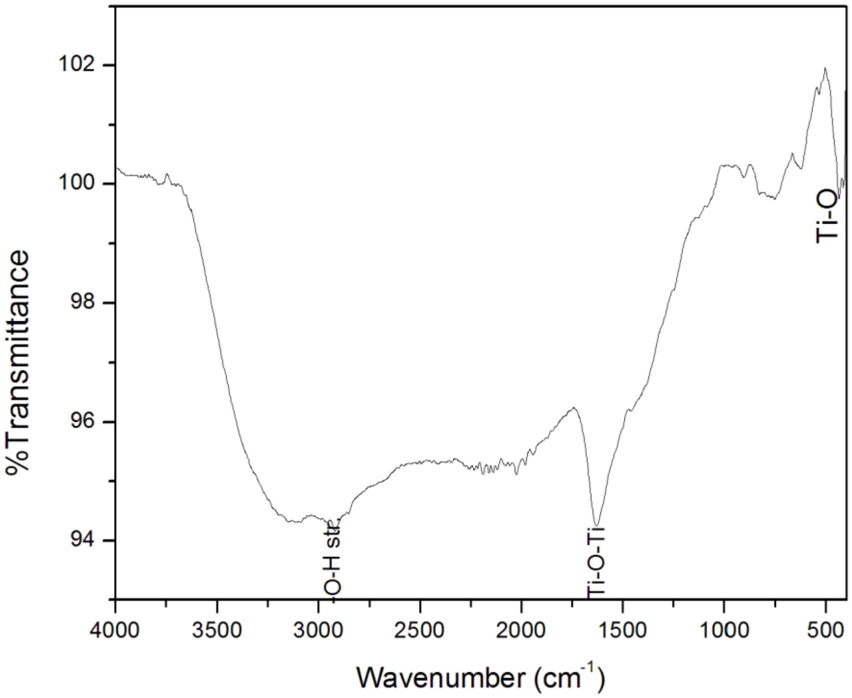


Figure 11: FTIR spectra of TiO_2-NPs synthesized from ethanolic leaf extracts of *Parthenium hysterophorus*

For *Saccharum spontaneum*, the spectra exhibited prominent absorbance peaks at approximately 443.89 cm^{-1}, 1544.12 cm^{-1} and 3023.38 cm^{-1} as shown in Figure 12. The peak observed at 443.89 cm^{-1} corresponds to the stretching vibration of Ti-O bonds, while the peak at 1544.12 cm^{-1} represents the stretching vibration of Ti-O-Ti bonds. Additionally, the peak at 3023.38 cm^{-1} indicates the stretching vibration of the O-H group, specifically alcohol-phenol functional groups. These findings provide valuable insights into the molecular structure and composition of TiO_2 nanoparticles (Vembu et al., 2022).

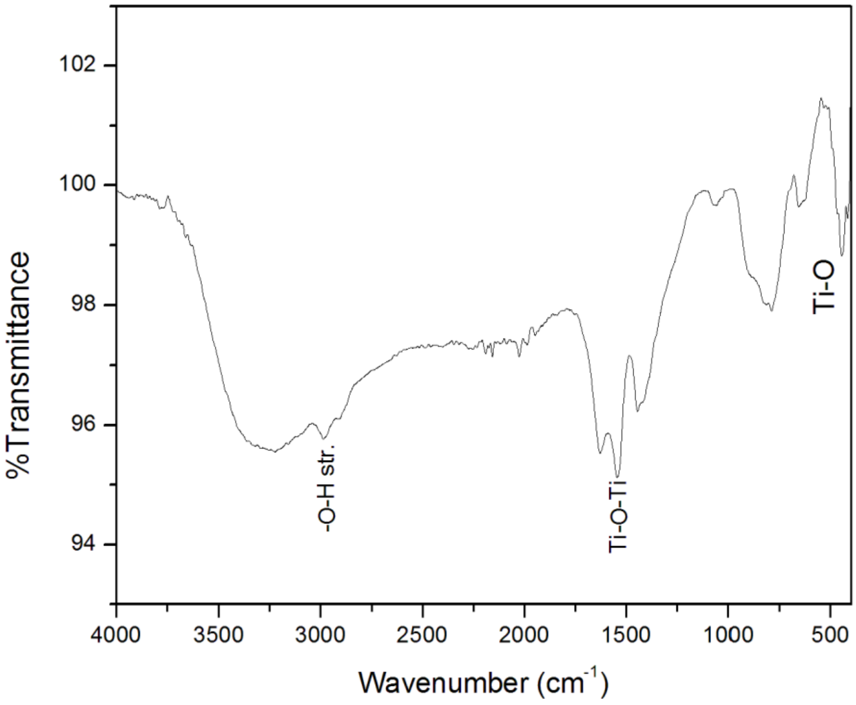


Figure 12: FTIR spectra of TiO_2-NPs synthesized from ethanolic leaf extracts of *Saccharum spontaneum*.

6.5.2 *X-Ray Diffraction (XRD) studies of Parthenium hysterophorus and Saccharum spontaneum derived TiO_2-NPs.*

The position of the detector is recorded in terms of the angle 2θ, and the intensity represents the number of X-rays detected at each angle, typically measured in counts. The graph illustrates that when X-rays are directed towards the titanium dioxide nanoparticles, 562 counts were detected at an angle of 42°. For *Parthenium hysterophorus* highest counts were observed around 42° and 26.59°, with relatively low fluctuations, and the counts remain constant at 400 throughout the range of 20°-40° as depicted in Figure 13. These results are in the range depicted by other researchers (Sharma et al., 2014; Jhuang et al., 2016; Theivasanthi et al., 2013) and it agrees with the JCPDS card no. 21-1272. By comparing the obtained XRD pattern of synthesized nanoparticles with the standard XRD patterns discussed by researchers (Haider et al., 2015), we are convinced that the rutile phase of titanium dioxide nanoparticles is formed in our sample. The XRD peaks were observed at 2θ values of 26.59° and 42°, which corresponds to the XRD pattern of the Rutile phase.

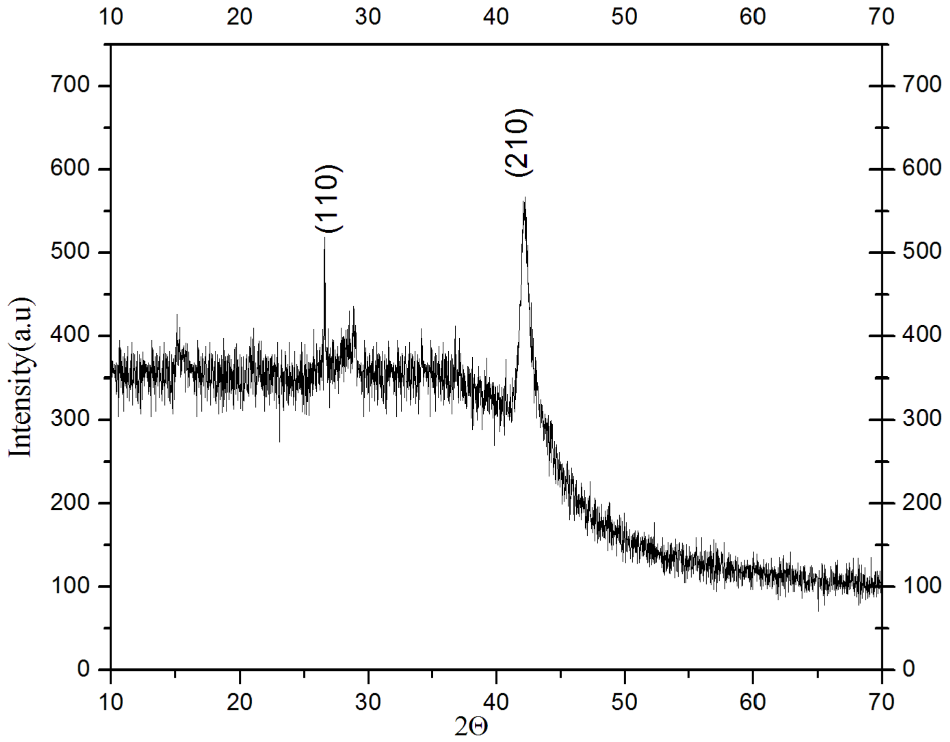


Figure 13: X-ray diffraction patterns of TiO_2-NPs synthesized from ethanolic leaf extracts of *Parthenium hysterophorus*.

In contrast to that, for *Saccharum spontaneum* the graph values revealed that when X-rays are directed towards the synthesized TiO_2-NPs, 588 counts were detected at an angle of 48°. The highest counts were observed around 48.01° and 25.4°, with relatively low fluctuations, and the counts remain constant at 300 throughout the range of 35°-45° as depicted in Figure 14. These results are in the range depicted by other researchers (Wang et al.,2020; Thamaphat et al., 2008; Theivasanthi et al., 2013) and it agrees with the JCPDS card no. 21-1272. By comparing the obtained XRD pattern of synthesized nanoparticles with the standard XRD patterns discussed by researchers (Haider et al., 2015), we are convinced that the anatase phase of titanium dioxide nanoparticles is formed in our sample. The XRD peaks were observed at 2θ values of 25.4° and 48.01°, which corresponds to the XRD pattern of the anatase phase as depicted in Figure 14.

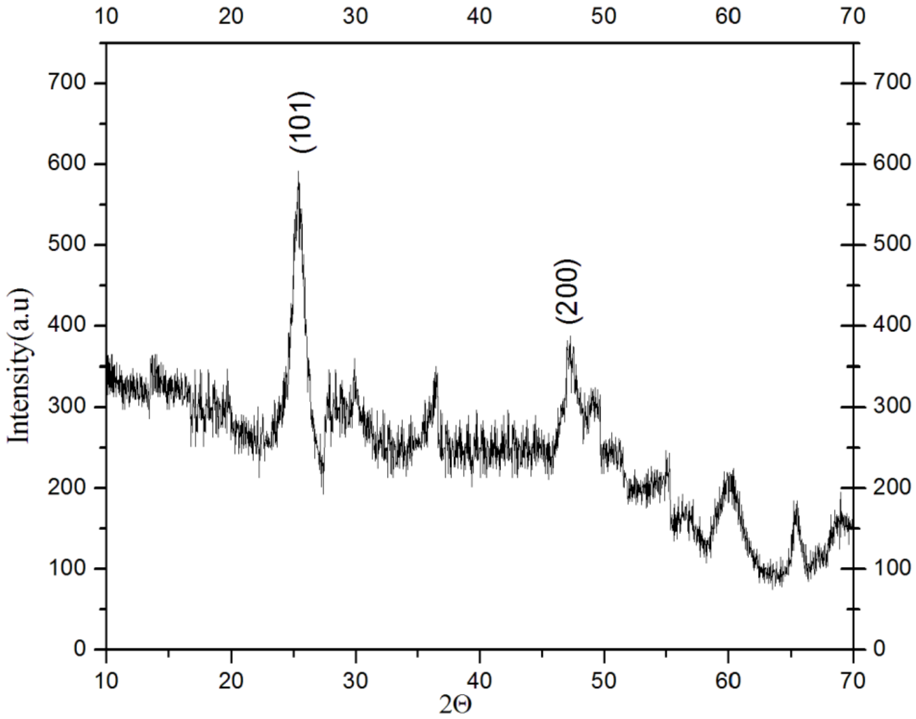
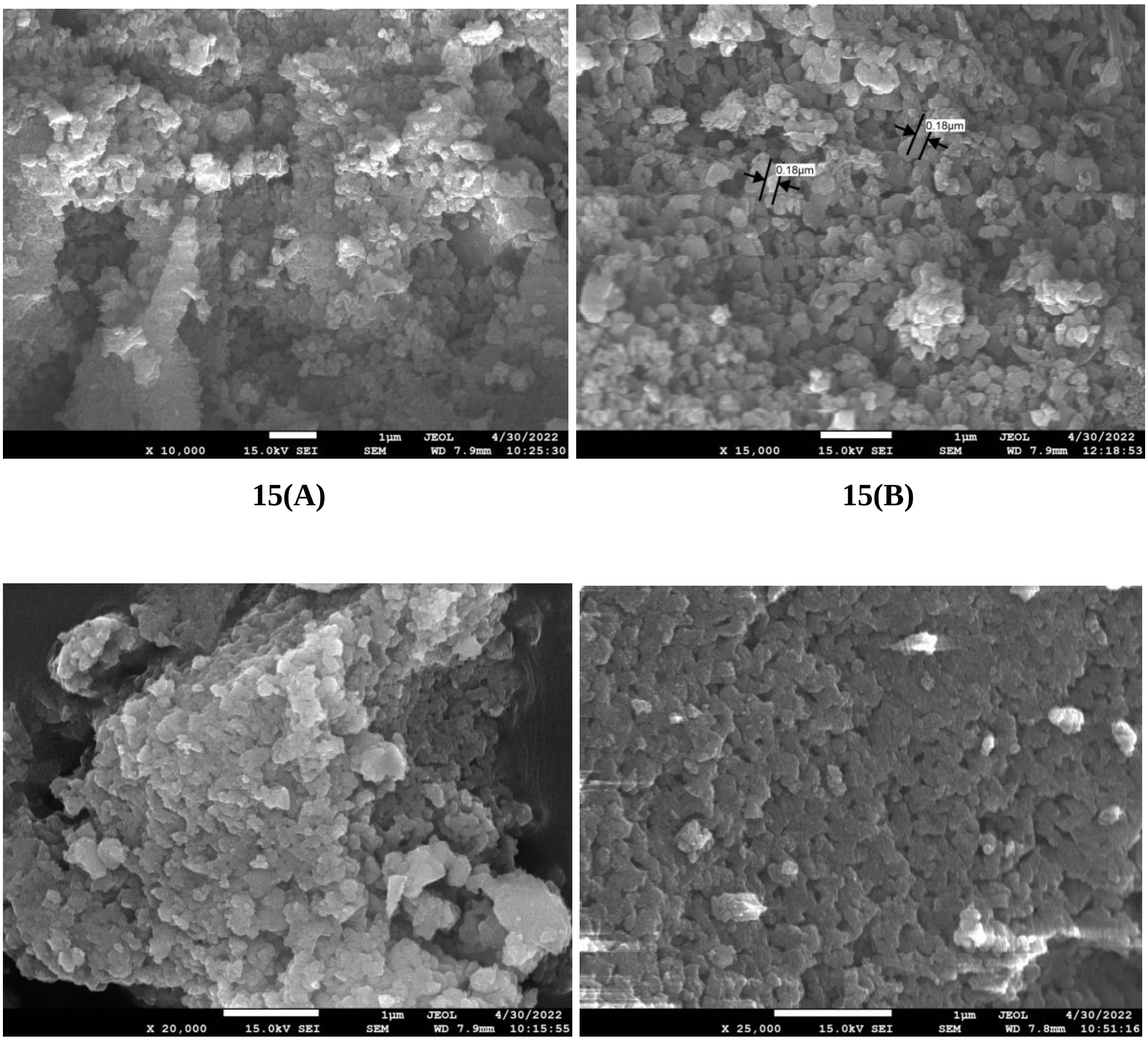


Figure 14: X-ray diffraction patterns of TiO_2-NPs synthesized from ethanolic leaf extracts of *Saccharum spontaneum*.

6.5.3 *Field Emission Scanning Electron Microscopy (FE-SEM) studies of Parthenium hysterophorus and Saccharum spontaneum derived TiO_2-NPs.*

The morphological features of *Parthenium hysterophorus* derived titanium dioxide nanoparticles were examined using FE-SEM, operating at various magnifications of 10000X

(Figure 15A), 15000X (Figure 15B), 20000X (15C) and 25000X (15D). The nanoparticles exhibited a range of shapes, including spherical and hexagonal, with rough and irregular morphologies. The average particle size was determined to be 180nm. In fact, around 200 nm TiO_2 size was reported by a few other research scholars (Haider et al., 2015; Papanicolaou et al., 2019).

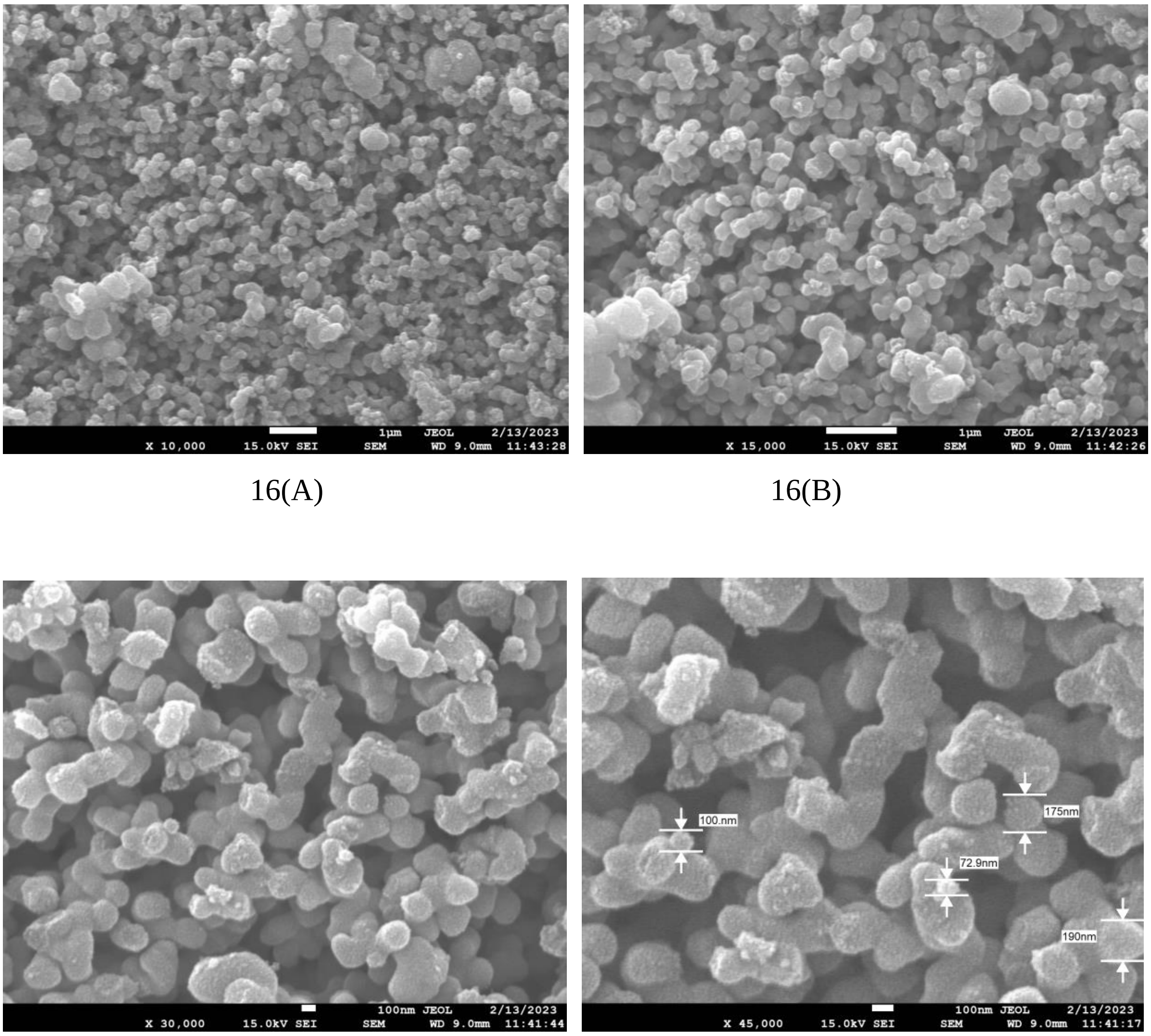


15(A) **15(B)**

15(C) **15(D)**

Figure 15: FESEM images of *Parthenium hysterophorus* derived TiO_2 NPs at 10000X (15A), 15000X (15B), 20000X (15C) and 25000X (15D). The shape of the synthesized nanoparticles ranges from spherical to hexagonal, where the average size of nanoparticles was found to be 180 nm.

Similarly, morphological features of *Saccharum spontaneum* derived titanium dioxide nanoparticles were examined using FE-SEM, operating at various magnifications of 10000X (16A), 15000X (16B), 30000X (16C) and 45000X (16D). In this case, the synthesized nanoparticles were found to be irregular to nearly spherical, with rough and irregular morphologies. The smallest and largest particle size was determined to be 72.9 and 190 nm respectively. In fact, similar TiO_2-NPs size was reported by a few other research scholars. (Bundele et al.,2023; Jonidi et al.,2023).



16(A) 16(B)

16(C) 16(D)

Figure 16: FESEM images of *Saccharum spontaneum* derived TiO_2 NPs at 10000X (16A), 15000X (16B), 30000X (16C) and 45000X (16D). The shape of the synthesized nanoparticles was found to be nearly spherical, where smallest and largest particle size was determined to be 72.9 and 190 nm respectively.

6.6 Photocatalytic degradation of of Ofloxacin and Ciprofloxacin by synthesized TiO_2-NPs

6.6.1 *Effect of nanoparticles concentration on Ofloxacin and Ciprofloxacin degradation*

The aim of the study was to investigate the degradation of ciprofloxacin and ofloxacin antibiotics when exposed to various concentrations of TiO_2 nanoparticles. Solution of 10ppm of each antibiotic were prepared and mixed with different concentrations of TiO_2 nanoparticles ranging from 1ppm to 13ppm. The resulting mixture was subjected to photocatalytic degradation by exposure to UV light for duration of 3 hours. Antibiotic solution without nanoparticles served as control. The degradation of antibiotics was measured by monitoring the absorbance at 340nm (Pascual-Reguera, Parras et al. 2004). The results showed that the degradation of antibiotics was more efficient in the presence of TiO_2 nanoparticles, which acted as photocatalysts under UV light to facilitate the removal of antibiotics from the solution as shown in Table 4.

The efficiency of degradation was calculated by formula:

$(C_o-C)/ C_o * 100$ (Nosrati et al., 2012)

C_o = initial concentration of the antibiotic at zero hour

C = concentration of the degraded antibiotic at 3rd hour

Table 4: Percentage degradation of Ofloxacin and Ciprofloxacin on different Nanoparticles concentration.

Concentration of nanoparticles	Percentage Degradation of Antibiotic with NPs derived from Ph		Percentage Degradation of Antibiotic with NPs derived from Ss	
	Ofloxacin	**Ciprofloxacin**	**Ofloxacin**	**Ciprofloxacin**
0ppm	35±0.21 Control	38±0.56 Control	35±0.21 Control	38±0.56 Control
1ppm	71±0.4	69±0.55	70±0.75	68±0.85
3ppm	76±0.43	79±0.1	78±0.78	77±0.36

5ppm	85±0.60	87±0.62	89±0.43	87±0.72
7ppm	89±0.36	90±0.4	90.5±0.55	91±0.43
9ppm	94±0.36	92±0.69	93±0.36	90±0.4
11ppm	82±0.26	95±0.60	87±0.60	93±0.55
13ppm	77±0.75	81±0.45	82±0.51	85±0.36

NPs: Nanoparticles

Ph: ***Parthenium hysterophorous***

Ss: ***Saccharum spontaneum***

Based on the tabulated data (Table 4), it was observed that the percentage of photocatalytic degradation in control group was found to be 35% for ofloxacin, and 38% for ciprofloxacin. In contrast, a significant increase in the degradation of antibiotics was noted when nanoparticles were utilized. For *Parthenium hysterophorus* derived TiO_2 nanoparticles, percentage degradation of ofloxacin is increased from 71% to 94%, while for ciprofloxacin, it is increased from 69% to 95%. However, in case of Ss derived TiO_2 nanoparticles, it is increased from 70% to 93% for ofloxacin and 68% to 94% for ciprofloxacin respectively.

The maximum degradation was observed in a mixture containing antibiotics and nanoparticles (derived from Ph and Ss) at concentration of 9ppm for ofloxacin and 11 ppm for ciprofloxacin. Beyond these concentrations, there was a decrease in the percentage of degradation, which led to a reduction in efficiency of antibiotic removal. This can be attributed to the increase in the surface area of the catalyst available for absorption and degradation. However, increasing the quantity of nanoparticles leads to an increase in the solution's opacity, which reduces the penetration of photon flux in the reactor and, consequently, photo-catalytic degradation (Mohd Azan et al., 2022).

6.6.1.2 *Percentage degradation of Ofloxacin and Ciprofloxacin with Parthenium hysterophorus derived* TiO_2*-NPs*

One way ANOVA on ofloxacin degradation with varying concentration of *Parthenium hysterophorus* derived TiO_2-NPs yielded $P<0.0001$, which suggests very significant effect of nanoparticle concentration on degradation of ofloxacin as shown in Figure 17. Similar results were obtained for degradation of ciprofloxacin under varying nanoparticle concentration. A very low p value ($P<0.0001$) suggested a strong effect of nanoparticle concentration over ciprofloxacin degradation as depicted in Figure 17.

6.6.1.3 *Percentage degradation of Ofloxacin and Ciprofloxacin with Saccharum spontaneum derived TiO2-NPs*

One way ANOVA on ofloxacin degradation with varying concentration of *Saccharum spontaneum* derived TiO_2-NPs yielded $P<0.0001$, which suggests very significant effect of nanoparticle concentration on degradation of ofloxacin as shown in Figure 18. Similar results were obtained for degradation of ciprofloxacin under varying nanoparticle concentration. A very low p value ($P<0.0001$) suggested a strong effect of nanoparticle concentration over ciprofloxacin degradation as depicted in Figure 18.

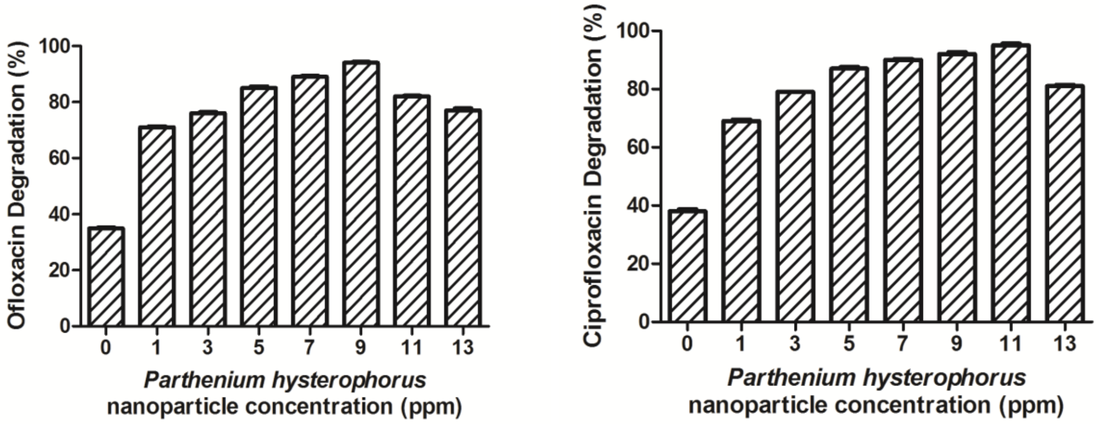


Figure 17: Percentage degradation of ofloxacin and ciprofloxacin with varying concentration of *Parthenium hysterophorus* derived TiO_2-NPs.

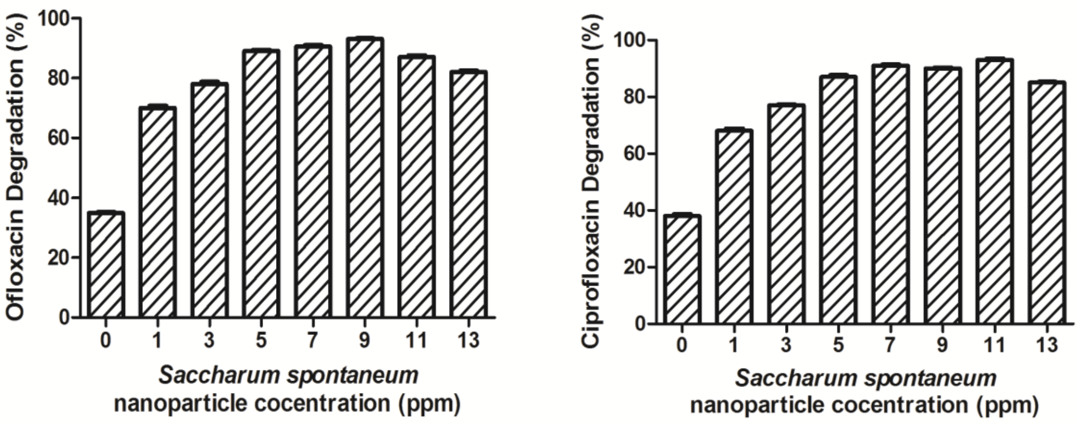


Figure 18: Percentage degradation of ofloxacin and ciprofloxacin with varying concentration of *Saccharum spontaneum* derived TiO_2-NPs.

6.6.1.4 *Comparison of percentage degradation of ofloxacin under varying concentrations of Parthenium hysterophorus and Saccharum spontaneum derived TiO_2 nanoparticles*

Two way ANOVA results revealed that ofloxacin is degraded with significant difference when treated with PhNP and SsNP separately as depicted in Figure 19. It means that there is a remarkable difference on photocatalytic degradation of ofloxacin when treated with PhNP and SsNP, where SsNP shows more degradation as compared to PhNP as analysed by very low P value ($P<0.0001$).

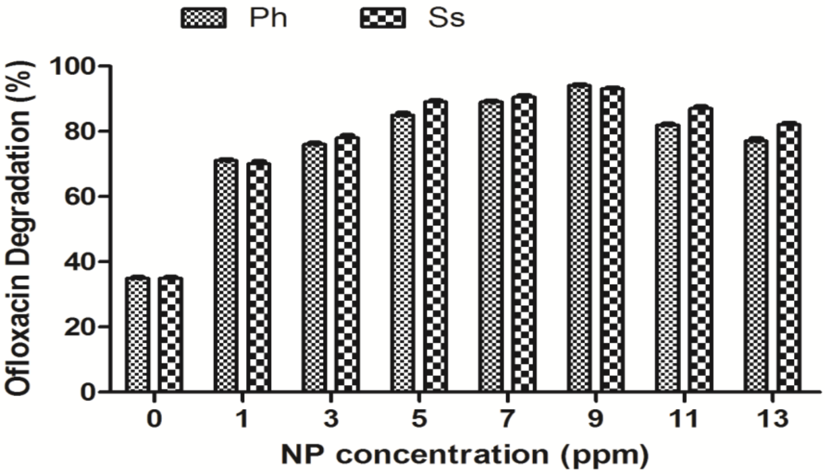


Figure 19: Comparison of ofloxacin degradation under different concentrations of *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs.

6.6.1.5 *Comparison of percentage degradation of ciprofloxacin under varying concentration of Parthenium hysterophorus and Saccharum spontaneum derived TiO2 nanoparticles.*

In contrast to the results obtained for ofloxacin degradation, we observed no significant difference on degradation percentage of ciprofloxacin when treated with PhNP and SsNP as shown in Figure 20. $P>0.05$ obtained from Two-way ANOVA discards any kind of significant variation in this study.

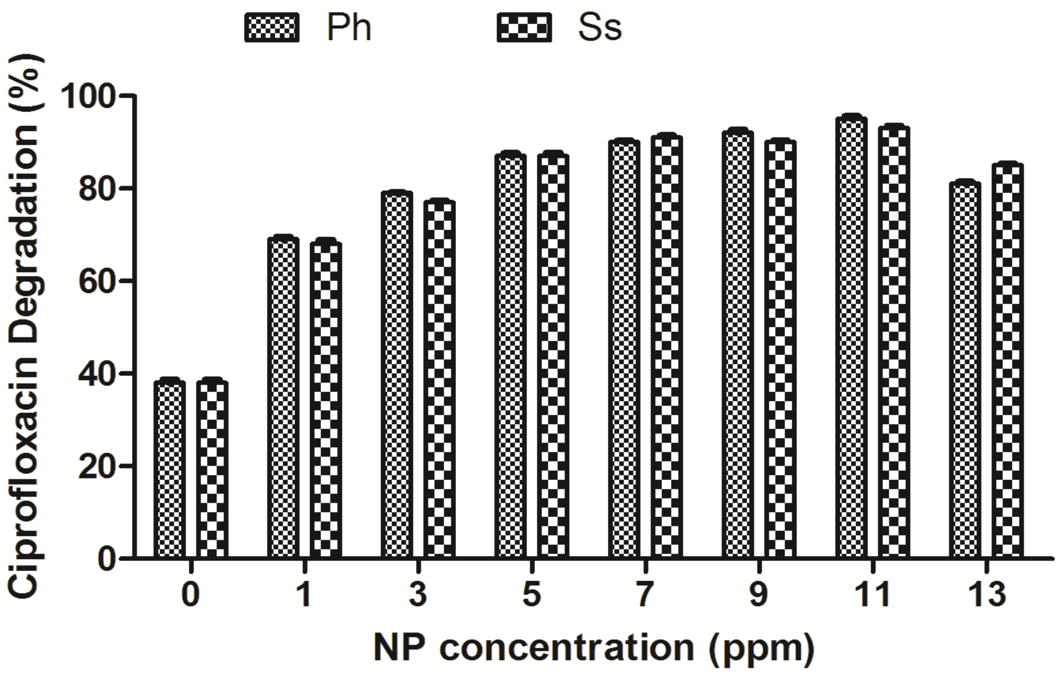


Figure 20: Comparison of ciprofloxacin degradation under different concentrations of *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs.

6.6.2 *Effect of varied antibiotic (Ofloxacin and Ciprofloxacin) concentrations on degradation studies.*

To verify this, we mixed the concentration of nanoparticles that showed the most significant degradation (as listed in Table 4) with varying concentrations of antibiotics (10ppm, 20ppm, 30ppm, 40ppm, 50ppm, and 60ppm), and subjected the mixture to photocatalytic degradation for 3 hours. The measurements were taken at 340 nm after 3 hours (Mohd Azan et al., 2022). The solution without antibiotic was served as control.

Again, the degradation efficiency was calculated by using the formula:

$(C_o\text{-}C)/\ C_o * 100$ (Nosrati et al., 2012)

C_o = initial concentration of the antibiotic at zero hour

C = concentration of the degraded antibiotic at 3rd hour

Table 5: Percentage degradation of Ofloxacin and Ciprofloxacin on different Antibiotic Concentration

Concentration of antibiotics	**Percentage Degradation of Antibiotic solution (Control)**		**Percentage Degradation of antibiotic with NPs derived from Ph**		**Percentage Degradation of Antibiotic with NPs derived from Ss**	
	Ofloxacin	**Ciprofloxacin**	**Ofloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Ciprofloxacin**
10ppm	39±0.41	37±0.7	97±0.62	96±0.65	96±0.52	95±0.7
20ppm	29±0.69	30±0.36	91±0.60	92±0.60	90±0.62	90±0.55
30ppm	23±0.4	21±0.36	80±0.36	86±0.65	82±0.36	82±0.60
40ppm	15±0.36	11±0.65	69±0.07	73±0.45	71±0.60	70±0.55
50ppm	09±0.4	9±0.43	51±0.66	64±0.7	60±0.52	59±0.60
60ppm	07±0.62	7.5±0.52	49±0.36	53±0.40	48±0.36	47±0.62

NPs: Nanoparticles

Ph: ***Parthenium hysterophorous***

Ss: ***Saccharum spontaneum***

The data presented in Table 5 indicates a reduction in the percentage degradation of both the control sample and the sample containing a mixture of antibiotics and nanoparticles with increase in antibiotic concentration.

The observed phenomenon can be attributed to the limited number of active sites on the photocatalyst at a specific concentration of nanoparticles. The photocatalytic degradation rate is higher at lower concentrations of antibiotics, owing to the finite quantity of active centres. As the concentration of antibiotics increases, the rate decreases due to an excess of antibiotics molecules in relation to the available active sites on the photocatalyst. 10ppm concentration of antibiotic exhibited highest exhibited highest photocatalytic degradation for both *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs.

The main factor leading to the observed trend is the increase in C/C_o (ratio of initial concentration to final concentration) with an increase in the initial antibiotic concentration. One possible explanation for this trend is that an increase in antibiotic concentration leads to the formation of additional active sites for reactive radical formation, assuming a constant concentration of nanoparticles and light irradiation. These reactive radicals are then consumed in the breakdown of the increasing amount of organic molecules, leading to a decrease in the efficiency of antibiotics decomposition (Nguyen et al., 2020). Various researchers have reported a corresponding trend in the deterioration of different antibiotics with different nanoparticles. (Kulkarni et al., 2016)

6.6.2.1 *Comparison of ofloxacin degradation under PhNP with varying ofloxacin concentration*

Results of Two way ANOVA suggested that, as we increase the antibiotic concentration keeping the nanoparticle concentration constant, the percentage degradation of ofloxacin decreases very significantly ($P<0.0001$), as depicted in Figure 21.

Similar results were obtained during comparison of ciprofloxacin degradation under PhNP with varying ciprofloxacin concentration, where also with increase in ciprofloxacin concentration by keeping the nanoparticle concentration constant the percentage degradation of ciprofloxacin decreases very significantly ($P<0.0001$), as depicted in Figure 21.

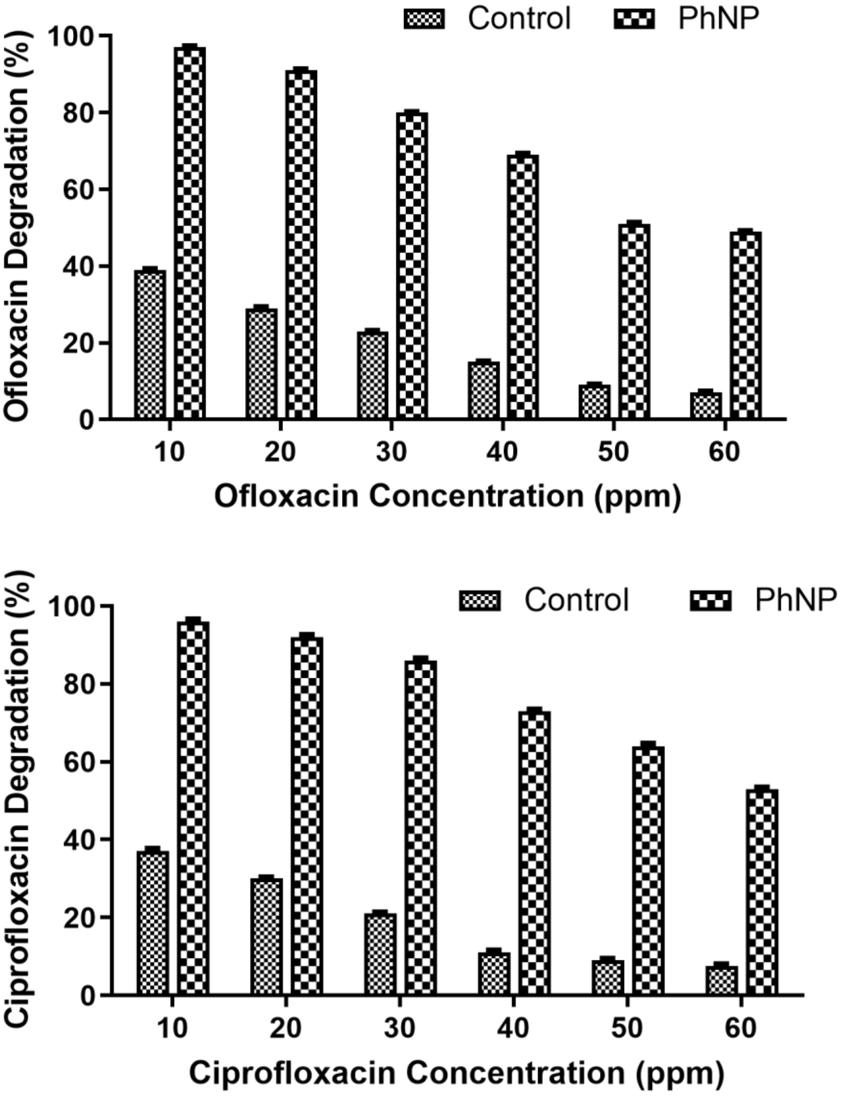
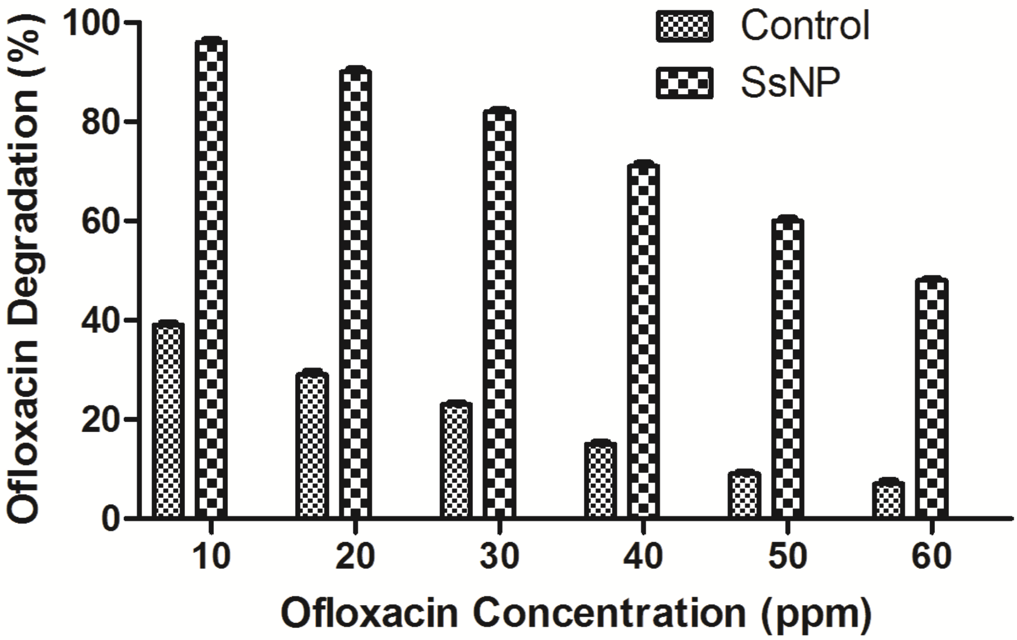


Figure 21: Comparison of ofloxacin and ciprofloxacin degradation under PhNP with varying antibiotic concentrations.

6.6.2.2 *Comparison of ofloxacin degradation under SsNP with varying ofloxacin concentration*

Results of Two way ANOVA suggested that, as we increase the antibiotic concentration keeping the nanoparticle concentration constant, the percentage degradation of ofloxacin decreases very significantly (P<0.0001), as depicted in Figure 22.

Similar results were obtained during comparison of ciprofloxacin degradation under SsNP with varying ciprofloxacin concentration, where also with increase in ciprofloxacin concentration by keeping the nanoparticle concentration constant the percentage degradation of ciprofloxacin decreases very significantly (P<0.0001), as depicted in Figure 22.



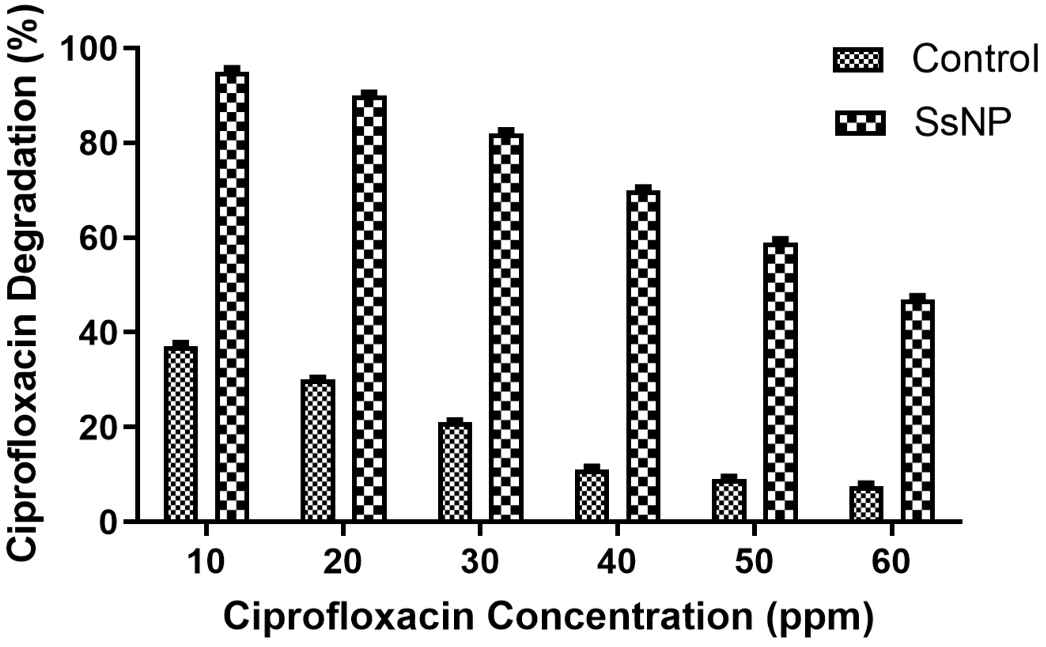


Figure 22: Comparison of ofloxacin and ciprofloxacin degradation under SsNP with varying antibiotic concentration.

6.6.3 *Effect of contact time on degradation studies.*

The nanoparticle concentrations that displayed the highest level of photocatalytic degradation in Table 4 and the antibiotic concentration that displayed the highest level of photocatalytic degradation in Table 5 were combined and the mixture was subjected to photo-catalysis for duration of 6 hours. Readings were taken at hourly intervals, with an antibiotic solution without nanoparticles used as a control as shown in Table 6. (Shafique Ahmad et al., 2004).

The efficiency of degradation was calculated by formula:

$(C_o\text{-}C)/\ C_o * 100$ (Nosrati et al., 2012).

C_o = initial concentration of the antibiotic at zero hour.

C = Concentration of the degraded antibiotic at 1st, 2nd, 3rd ……6th hour

Table 6. Percentage degradation of antibiotics with varying time

Time (h)	Percentage Degradation of Antibiotic Solution (Control)		Percentage Degradation of Antibiotic with NPs derived from Ph		Percentage Degradation of Antibiotic with NPs derived from Ss	
	Ofloxacin	**Ciprofloxacin**	**Ofloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Ciprofloxacin**
0	08±0.34	09±0.26	31±0.40	35±0.62	29±0.44	30±0.75
1	11±0.60	13±0.65	80±0.62	83±0.78	81±0.60	86±0.65
2	19±0.36	20±0.51	89±0.51	90±0.60	90±0.60	91±0.60
3	25±0.60	26±0.1	92±0.55	93±0.36	91±0.52	92±0.45
4	31±0.34	32±0.55	94±0.34	94±0.75	93±0.45	94±0.65
5	33.5±0.55	34±0.78	95.5±0.60	96±0.43	95±0.36	95±0.79

NPs: Nanoparticles

Ph: *Parthenium hysterophorous*

Ss: *Saccharum spontaneum*

It is observed that the degradation of antibiotics is more pronounced in the mixture containing antibiotics and nanoparticles, as compared to solution containing only antibiotics.

In case of *Parthenium hysterophorous* and *Saccharum spontaneum* derived nanoparticles, the percentage of degradation for ofloxacin and ciprofloxacin increased from initial values to higher values upon increased exposure time. This phenomenon is attributed to the increased production of hydroxyl radicals through the photocatalytic degradation of wastewater subjected to increased photoperiod. Furthermore, when TiO_2 nanoparticles are exposed to UV radiations, electrons are excited from the valence band to the conduction band, which contributes to the degradation of antibiotics (Ramy and Hashem, 2014). Other studies have reported lower degradation using nanoparticles or UV lamps (Jodat and Jodat 2014).

6.6.3.1 *Effect of time on Ofloxacin and Ciprofloxacin degradation under Parthenium hysterophorus derived TiO_2-NPs.*

The results of Two way ANOVA for assessing effect of time on percentage degradation of ofloxacin revealed, that there is significant effect of incubation time on ofloxacin degradation as shown in Figure 23. $P<0.0001$ shows that the degradation of antibiotic is significantly increased with time of incubation.

Similar results were obtained, while assessing the effect of time on percentage degradation of ciprofloxacin as depicted in Figure 23, where also the degradation of ciprofloxacin is significantly increased with incubation time as analysed by very low P value ($P<0.0001$).

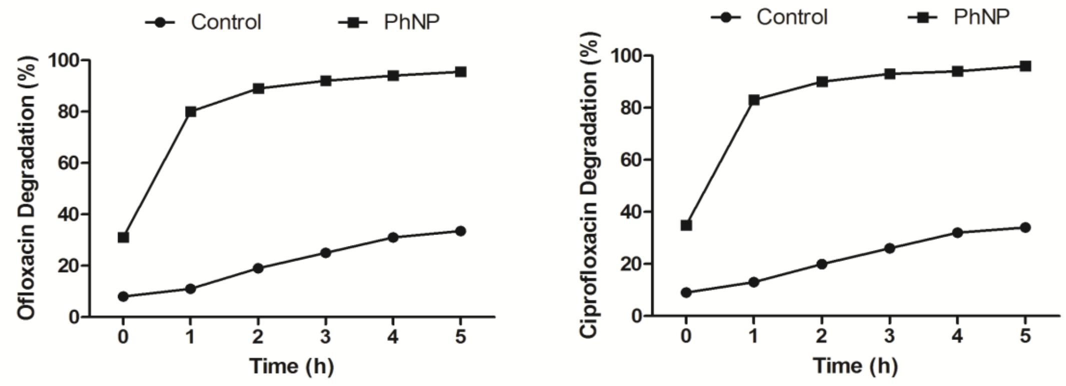


Figure 23: Effect of time on ofloxacin and ciprofloxacin degradation under *Parthenium hysterophorus* derived TiO_2-NPs.

6.6.3.2 *Effect of time on Ofloxacin and Ciprofloxacin degradation under Saccharum spontaneum derived TiO_2-NPs.*

The results of Two-way ANOVA for assessing effect of time on percentage degradation of ofloxacin revealed, that there is significant effect of incubation time on ofloxacin degradation as shown in Figure 24. $P<0.0001$ shows that the degradation of antibiotic is significantly increased with time of incubation.

Similar results were obtained, while assessing the effect of time on percentage degradation of ciprofloxacin as depicted in Figure 24, where also the degradation of ciprofloxacin is significantly increased with incubation time as analysed by very low P value ($P<0.0001$).

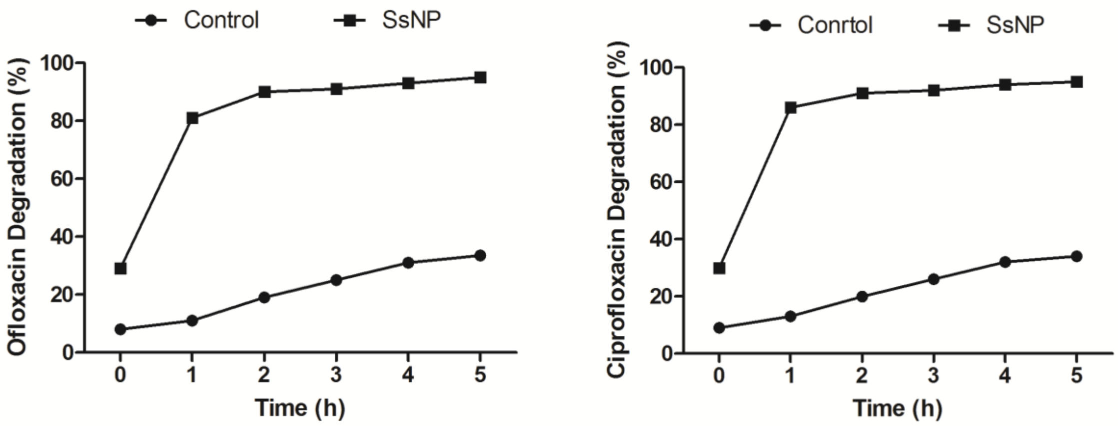


Figure 24: Effect of time on ofloxacin and ciprofloxacin degradation under *Saccharum spontaneum* derived TiO_2-NPs.

6.6.4 *Effect of pH on degradation studies*

A solution of antibiotic with a concentration of 10ppm was prepared respectively for ciprofloxacin and ofloxacin, and the pH was adjusted within a range of 4 to 9 prior to the addition of nanoparticles. The nanoparticles solution (exhibiting the highest degradation in Table 4 i.e. 9ppm for ofloxacin and 11ppm for ciprofloxacin) is then added to the prepared antibiotic solutions (10ppm for both ofloxacin and ciprofloxacin) of varied pH (pH range 4-9) and the resulting mixture was exposed to photocatalytic degradation for the duration of 6h (where the most efficient degradation of antibiotic occurs as discussed in Table 6). Readings were taken following this duration to assess the level of antibiotic degradation using the methodology previously reported (Ahmad et al., 2020). A control experiment was conducted using an antibiotic solution without the addition of nanoparticles and was subjected to the same treatments. It should be noted that the antibiotics remains stable in a pH range of 5 to 6, but after pH 6 there is a sudden fall in the percentage degradation of antibiotic as shown in Table 7. In order to investigate the effect of pH on antibiotic degradation, we varied the pH within a range of 4 to 9 prior to the addition of nanoparticles.

The degradation efficiency was calculated by using the formula:

$(C_o-C)/ C_o * 100$ (Nosrati et al., 2012)

C_o = initial concentration of the antibiotic at zero hour.

C = Concentration of the degraded antibiotic at 5th hour.

Table 7: Percentage degradation of antibiotics with varying pH

pH	**Percentage degradation of Ofloxacin**			**Percentage degradation of Ciprfloxacin**		
	Ab sol. (Control)	**Ab+ NPs Sol of Ss**	**Ab+ NPs Sol of Ph**	**Ab sol. (Control)**	**Ab+ NPs Sol of Ss**	**Ab+ NPs Sol of Ph**
4	35±0.7	87±0.58	86±0.65	32±0.45	90±0.60	89±0.65
5	43±0.79	91±0.69	93±0.60	48±0.72	92±0.7	92±0.55
6	51±0.78	94±0.62	95±0.75	50±0.36	95±0.45	96±0.45
7	45±0.65	90±0.65	91±0.62	39±0.65	87±0.62	90±0.65
8	30±0.62	85±0.79	83±0.45	35±0.36	82±0.43	83±0.43
9	26±0.65	79±0.36	77±0.62	27±0.65	71±0.65	75±0.65

NPs: Nanoparticles

Ph: ***Parthenium hysterophorous***

Ss: ***Saccharum spontaneum***

Ab: Antibiotics

The results presented in Table 7 demonstrate an increase in degradation efficiency within the pH range of 4 to 6, while maintaining a stable concentration of both antibiotic and nanoparticles with consistent light irradiation. However, degradation slowed down beyond pH 6 due to the instability of antibiotics in nearly neutral condition. At pH 5, antibiotics acquire a positive charge in an acidic solution, whereas the photocatalyst carries a negative charge. This results in an affinity between the positively charge antibiotic molecules and negatively charged photocatalyst surface, leading to higher degradation efficiency. Conversely, at neutral pH in alkaline solutions, both the photocatalyst and antibiotic acquire a negative charge, thereby limiting the degradation efficiency (Rozas et al., 2010).

6.6.4.1 *Effect of pH on Ofloxacin and Ciprofloxacin degradation under Parthenium hysterophorus derived TiO_2-NPs.*

The degradation percentage of ofloxacin and ciprofloxacin is significantly affected by change in pH as revealed by One way ANOVA ($P<0.0001$), Figure 25.

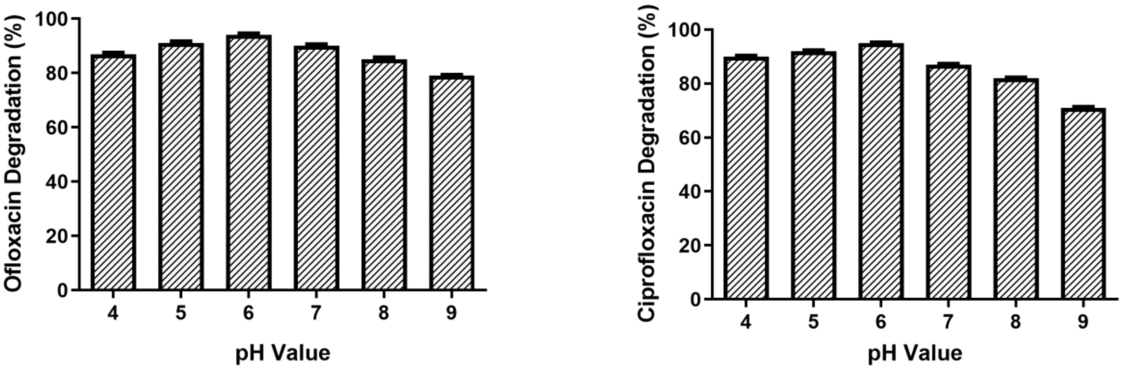


Figure 25: Effect of pH on ofloxacin and ciprofloxacin degradation under *Parthenium hysterophorus* derived TiO_2-NPs.

6.6.4.2 *Effect of pH on Ofloxacin and Ciprofloxacin degradation under Saccharum spontaneum derived TiO_2-NPs.*

For *Saccharum spontaneum* derived TiO_2-NPs, percentage degradation of ofloxacin and ciprofloxacin is also significantly affected due to change in pH as revealed by One way ANOVA ($P<0.0001$), Figure 26.

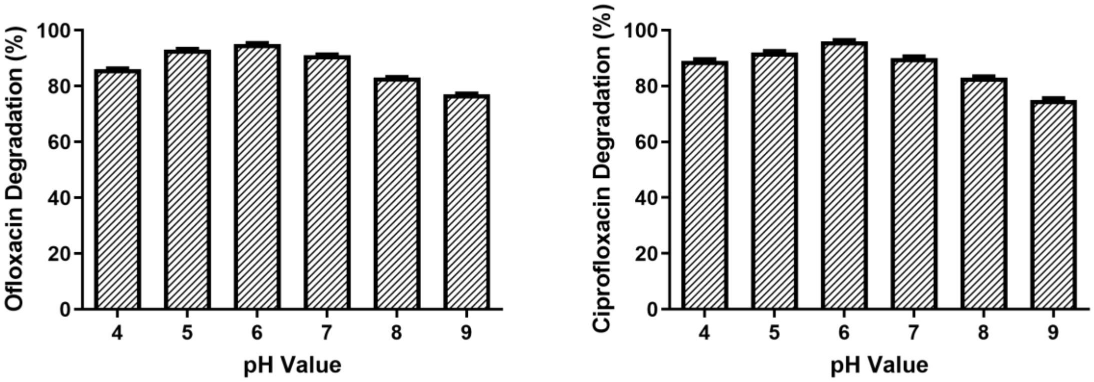


Figure 26: Effect of pH on ofloxacin and ciprofloxacin degradation under *Saccharum spontaneum* derived TiO_2-NPs.

Chapter 7

SUMMARY AND CONCLUSIONS

7 SUMMARY AND CONCLUSIONS

TiO_2-NPs were synthesised from *Parthenium hysterophorus* and *Saccharum spontaneum* growing near the pharmaceutical effluent polluted sites along Baddi, Himachal Pradesh, India (latitude: 30.94263° N and longitude: 76.77187° E). Average size of synthesized nanoparticles for *Parthenium hysterophorus* was found to be 180 nm where as for *Saccharum spontaneum* smallest and largest particle size was determined as 72.9 nm and 190 nm. Maximum ofloxacin degradation was observed at 9ppm i.e. 94% and 93% for both *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs, however in contrast to that maximun ciprofloxacin degradation was observed at 11ppm i.e. 95% and 93% for both *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs when subjected to photocatalytic degradtion for the duration of 3 hours. Following this with the varied antibiotic concentrations from 10-60 ppm, maximun degradation of both ciprofloxacin and ofloxacin was observed at 10 ppm (i.e. lowest antibiotic concentration), because an increase in antibiotic concentration leads to the formation of additional active sites for reactive radical formation, assuming a constant concentration of nanoparticles and light irradiation. These reactive radicals are then consumed in the breakdown of the increasing amount of organic molecules, leading to a decrease in the efficiency of antibiotics decomposition. Other optimized parameter for ofloxacin and ciprofloxacin degration was pH 6, which showed the best degradation potential of antibiotics among varied pH values. Ethanolic extracts of the plant samples of *Parthenium hysterophorus and Saccharum spontaneum* was selected in the undertaken studies considering its environmental friendly, safe, uniform and cost-effective green approach. *Parthenium hysterophorus* is a common plant weed found flourishing around the barren infertile areas under stress conditions. This plant is well known for secreting allelochemicals having medicinal properties (Jaiswal et al., 2022). On the other hand *Saccharum spontaneum* is having its own importance in our traditional medicine systems, whose extracts can be used for the treatment of mental disorders. Their presence in the pharmaceutical effluent polluted sites signifies the capability of the plant to tolerate and associate with the various antibiotics. Plant derived nanoparticles can provide an interface for efficient binding with pharmaceutical compounds (because of their organic nature) and thereby play a role in their corresponding neutralisation and biodegradation. Moreover photocatalytic degradation of pharmaceutical compounds through plant derived TiO_2-NPs is an environment friendly efficient method which come up with the release of non-toxic byproducts such as CO_2 and H_2O as compared to other conventional methods which releases secondary pollutants as a result of degradation. Hence, the green synthesis of TiO_2 nanoparticles was considered to assess the photocatalytic degradation of ofloxacin and ciprofloxacin. To our knowledge, we want to mention that photocatalytic degradation of ofloxacin and ciprofloxacin by using *Parthenium hysterophorus* and *Saccharum spontaneum* derived TiO_2-NPs has not been reported before.

Chapter 8

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8 BIBLIOGRAPHY

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9 Appendices

Table 1: ANOVA table for effect of PhNP concentration on percentage degradation of ofloxacin

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (PhNP conc.)	15527	7	2218	F (7, 16) = 9339	P<0.0001
Residual (within columns)	3.800	16	0.2375		
Total	15530	23			

Table 2: ANOVA table for effect of PhNP concentration on percentage degradation of ciprofloxacin

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (PhNP conc.)	16306	7	2329	F (7, 16) = 8791	P<0.0001
Residual (within columns)	4.240	16	0.2650		
Total	16311	23			

Table 3: ANOVA table for effect of SsNP concentration on percentage degradation of ofloxacin

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (SsNP conc.)	16426	7	2347	F (7, 16) = 6877	P<0.0001
Residual (within columns)	5.460	16	0.3413		
Total	16432	23			

Table 4: ANOVA table for effect of SsNP concentration on percentage degradation of ciprofloxacin

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (SsNP conc.)	16177	7	2311	F (7, 16) = 7834	P<0.0001
Residual (within columns)	4.720	16	0.2950		
Total	16181	23			

Table 5: ANOVA table for effect of NP concentration and NP type on ofloxacin degradation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	66.33	7	9.475	F (7, 32) = 32.74	P<0.0001
NP Type	31887	7	4555	F (7, 32) = 15742	P<0.0001
NP conc.	45.05	1	45.05	F (1, 32) = 155.7	P<0.0001
Residual	9.260	32	0.2894		

Table 6: ANOVA table for effect of NP concentration and NP type on ciprofloxacin degradation

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	44.25	7	6.321	F (7, 32) = 22.58	P<0.0001
NP Type	32439	7	4634	F (7, 32) = 16550	P<0.0001
NP conc.	0.7500	1	0.7500	F (1, 32) = 2.679	P=0.1115
Residual	8.960	32	0.2800		

Table 7: ANOVA table for effect of varying ofloxacin concentration with PhNP and control over ofloxacin degradation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	545.7	5	109.1	F (5, 24) = 416.2	P<0.0001
PhNP	7834	5	1567	F (5, 24) = 5975	P<0.0001
Ofloxacin conc.	24806	1	24806	F (1, 24) = 94600	P<0.0001
Residual	6.293	24	0.2622		

Table 8: ANOVA table for effect of varying ciprofloxacin concentration with PhNP and control over ciproofloxacin degradation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	369.6	5	73.91	F (5, 24) = 236.9	P<0.0001
PhNP	6142	5	1228	F (5, 24) = 3938	P<0.0001
Ciprofloxacin conc.	30369	1	30369	F (1, 24) = 97353	P<0.0001
Residual	7.487	24	0.3119		

Table 9: ANOVA table for effect of varying ofloxacin concentration with SsNP and control over ofloxacin degradation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	396.7	5	79.34	F (5, 24) = 309.8	P<0.0001
SsNP	6957	5	1391	F (5, 24) = 5433	P<0.0001
Ofloxacin Conc.	26395	1	26395	F (1, 24) = 103062	P<0.0001
Residual	6.147	24	0.2561		

Table 10: ANOVA table for effect of varying ciprofloxacin concentration with SsNP and control over ciproofloxacin degradation.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	525.3	5	105.1	F (5, 24) = 324.1	P<0.0001
SsNP	6902	5	1380	F (5, 24) = 4258	P<0.0001
Ciprofloxacin conc.	26814	1	26814	F (1, 24) = 82717	P<0.0001
Residual	7.780	24	0.3242		

Table 11: ANOVA table for effect of time on ofloxacin degradation with control and PhNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	76.25	5	15.25	F (5, 24) = 53.04	P<0.0001
Time	2660	5	532.0	F (5, 24) = 1850	P<0.0001
Control vs. PhNP	39402	1	39402	F (1, 24) = 137051	P<0.0001
Residual	6.900	24	0.2875		

Table 12: ANOVA table for effect of time on ofloxacin degradation with control and SsNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	120.3	5	24.06	F (5, 24) = 89.95	P<0.0001
Time	2352	5	470.5	F (5, 24) = 1759	P<0.0001
Control vs. PhNP	39701	1	39701	F (1, 24) = 148413	P<0.0001
Residual	6.420	24	0.2675		

Table 13: ANOVA table for effect of time on ciproofloxacin degradation with control and PhNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	107.0	5	21.40	F (5, 24) = 66.87	P<0.0001
Time	2177	5	435.4	F (5, 24) = 1361	P<0.0001
Control vs. SsNp	40000	1	40000	F (1, 24) = 125000	P<0.0001
Residual	7.680	24	0.3200		

Table 14: ANOVA table for effect of time on ciproofloxacin degradation with control and SsNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	170.3	5	34.05	F (5, 24) = 98.70	P<0.0001
Time	2206	5	441.3	F (5, 24) = 1279	P<0.0001
Control vs. SsNP	39800	1	39800	F (1, 24) = 115363	P<0.0001
Residual	8.280	24	0.3450		

Table 15: ANOVA table for effect of pH on ofloxacin degradation using PhNP.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (pH)	418.1	5	83.63	F (5, 12) = 208.8	P<0.0001
Residual (within columns)	4.807	12	0.4006		
Total	422.9	17			

Table 16: ANOVA table for effect of pH on ofloxacin degradation using SsNP.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (pH)	694.5	5	138.9	F (5, 12) = 353.1	P<0.0001
Residual (within columns)	4.720	12	0.3933		
Total	699.2	17			

Table 17: ANOVA table for effect of pH on ciprofloxacin degradation using PhNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (pH)	1125	5	224.9	F (5, 12) = 648.7	P<0.0001
Residual (within columns)	4.160	12	0.3467		
Total	1129	17			

Table 18: ANOVA table for effect of pH on ciprofloxacin degradation using SsNP

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (pH)	832.5	5	166.5	F (5, 12) = 499.5	P<0.0001
Residual (within columns)	4.000	12	0.3333		
Total	836.5	17			

Figure 1: Quadrat pictures for calculating plant population density.

List of Publications

1. **Prabhjot Singh Jassal** , Dapinder Kaur , Ram Prasad *, Joginder Singh* “Green synthesis of titanium dioxide nanoparticles: Development and applications”Journal of Agriculture and Food Research (2022): DOI:10.1016/j.jafr.2022.100361.
2. **Prabhjot S. Jassal***, Robinka Khajuria, Ronit Sharma, Pritam Debnath, Sonal Verma, Abey Johnson, Sumit Kumar “Photocatalytic degradation of ampicillin using silver nanoparticles biosynthesised by Pleurotus ostreatus” BioTechnologia (2020): DOI: 10.5114/bta.2019.90246
3. **Prabhjot Singh Jassal***, Dapinder Kaur, Manpreet Kaur, Disha Sharma “Level of antibiotic contamination in the major river systems: A review on South Asian countries perspective” Journal of Applied Pharmaceutical Science (2023): DOI: 10.7324/JAPS.2023.56748
4. **Prabhjot Singh Jassal***, Mahek Saini “Green Synthesis of Metal Oxide Nanoparticles: A Review” International Journal of Research and Analytical Reviews (2019).

List of Conferences

1. **Oral Presentation: Prabhjot Singh Jassal,** Joginder Singh. Characterization and potential applications of green synthesized TiO2 nanoparticles. **Achieved Third Prize**. International Conference on Sustainability: Life on Earth 2021.
2. **Oral Presentation: Prabhjot Singh Jassal,** Joginder Singh. Photocatalytic degradation of ofloxacin using Titanium dioxide nanoparticles green synthesized from *Saccharum spontaneum*. ICBB 2022.

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Certificate of Merit

*This is to certify that **Prof./Dr./Mr./Ms. Prabhjot Singh Jassal** of **Lovely Professional University** has participated in **Oral Presentation*** on the topic entitled **Characterization and Potential Applications of Green Synthesized Tio2 Nanoparticles** *and stood **Third** in International Conference on **Sustainability: Life on Earth 2021 (ICS-LOE 2021)** held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand, at Lovely Professional University, Punjab.*

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Prepared by
(Administrative Officer-Records)

Dr. Joydeep Dutta
Organizing Secretary, ICS-LOE, 2021

NSharma
Dr. Neeta Raj Sharma
Convener, ICS-LOE, 2021

Certificate No.257760

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Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Prabhjot Singh Jassal** of **Lovely Professional University** has participated in **Oral Presentation** on the topic entitled **Photocatalytic degradation of Ofloxacin using Titanium oxide nanoparticles green synthesised from Saccharum spontaneum** in the International Conference on **Bioengineering and Biosciences (ICBB-2022)** held on **18-19 November 2022** organized by the Department of Biotechnology, School of Bio-engineering and Biosciences in association with the Society of Bioinformatics for Experimenting Scientists (Bioclues) organized at Lovely Professional University, Punjab.

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Prepared by
(Administrative Officer-Records)

Himanshu Singh
Dr. Himanshu Singh
Organizing Secretary, ICBB, 2022

NSharma
Dr. Neeta Raj Sharma
Convener, ICBB, 2022

Figure 2: Certificates of Participation for attending International Conference on sustainability: Life on Earth 2021 and ICBB-2022