LACCASE MEDIATED BIOREMEDIATION OF PHARMACEUTICAL POLLUTANTS: MOLECULAR DOCKING AND SYNTHETIC PATHWAY PREDICTION OF CARBAMAZEPINE

Thesis submitted

in partial Fulfilment of the requirements for the

Degree of

MASTER OF TECHNOLOGY

in

BIOINFORMATICS

by

Monica Joshi

(2K22/BIO/02)

Under the Supervision of

Prof. JAI GOPAL SHARMA

Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Shahbad Daulatpur, Main Bawana Road, Delhi, 110042, India

May, 2024

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to Professor Jai Gopal Sharma for his invaluable guidance, support, and mentorship throughout the journey of this thesis. His expertise, encouragement, and insightful feedback have been instrumental in shaping my research and academic growth. I am truly grateful for his unwavering dedication, patience, and belief in my abilities.

I would also like to extend my deepest appreciation to my parents for their endless love, encouragement, and unwavering support throughout my academic pursuits. Their belief in meand their sacrifices have been a constant source of inspiration. I am grateful for their unwavering faith in my abilities and for always being there to provide guidance and encouragement.

Furthermore, I would like to express my gratitude to the faculty members of the department for their knowledge sharing and for providing a conducive learning environment. Their passion for teaching and dedication to fostering academic excellence have had a profound impact on my education**.**

This thesis would not have been possible without the support and contributions of these individuals, and I am deeply grateful for their involvement in my academic journey.

DELHI TECHNOLOGICAL UNNIVERSITY

(Formerly Delhi college of engineering)
Shahabad Daulatpur, Main Bawana road, Delhi, India

CANDIDATE'S DECLARATION

I Monica Joshi hereby certify that the work which is being presented in the thesis entitled "LACCASE MEDIATED BIOREMEDIATION OF PHARMACEUTICAL POLLUTANTS:MOLECULAR DOCKING AND SYNTHETIC PATHWAY PREDICTION OF CARBAMAZEPINE" in partial fulfillment of the requirements for the award of the Master's degree in Bioinformatics, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period of January to Junne under the supervision of Prof. Jai Gopal Sharma.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other institute.

MONICA JOSHI

This is to certify that the student has incorporated all the corrections suggested by the examiners in the thesis and the statement made by the candidate is correct to the best of our knowledge.

Signature of Supervisor **Signature of External Examiner**

DELHI TECHNOLOGICAL UNNIVERSITY

(Formerly Delhi college of engineering)
Shahabad Daulatpur, Main Bawana road, Delhi, India

CERTIFICATE

Certified that **Monica Joshi (2k22/BIO/02)** has carried out their search work presented in this thesis entitled "**Laccase mediated bioremediation of pharmaceutical pollutants: molecular docking and synthetic pathway prediction of carbamazepine**" for the award of Master of Technology from department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies result of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Prof. Yasha Hasija	**Prof. Jai Gopal Sharma**
Head of Department	**Supervisor**
Department of Biotechnology	**Department of Biotechnology**
Delhi Technological University	**Delhi Technological University**

ABSTRACT

Environmentally Persistent Pharmaceutical Pollutants (EPPPs) along with Personal Care Products (PPCs) poses great challenges to human health and aquatic ecosystem. Numerous reports have indicated their presence in the river, surface water, ground water and wastewater treatment plants (WWTPs) of India. The tendency of pharmaceutical products to remain active in the waterbodies even in the low concentrations, make them very dangerous for the human health and the ecosystem. Conventional WWTPs are not able to completely degrade these pollutants and hence, there is an urgent need to develop effective ways for the bioremediation of these pollutants from the ecosystem. Several studies have reported, key enzymes that are able to biodegrade these pollutants. Laccases, multicopper oxidases from microbes like bacteria and fungi are shown to eradicate some concentration of pharmaceutical pollutants. Hence, study of interaction of laccases with different pharmaceutical pollutants in necessary. In this study, we have screened pharmaceutical pollutants and studied their interactions with the target enzyme. After that we predicted the synthetic pathways of biodegradation for target compound, carbamazepine. This study will help us in predicting the potential targets. This research by leveraging in-silico tools will help also help us in predicting biodegradation pathways for various recalcitrant pollutants.

Key word – in-silico analysis, predictive bioremediation, molecular docking, laccase enzyme.

TABLE OF CONTENT

List of Tables

List Of Figures

List of Symbols and abbreviations

1. EPPP: Environmentally Persistent Pharmaceutical Pollutants
2. PPC: Personal Care Products
3. WWTP: Wastewater Treatment Plant
4. EPA: Environmental Protection Agency
5. EPMC: Emerging Pharmaceuticals Manufacturing Countries
6. NPEO: Nonylphenol Polyethoxylate Surfactants
7. NSAID: Nonsteroidal anti-inflammatory drugs
8. API: Active Pharmaceutical Agents
9. MOA: Mechanism of Action
10. ARB: Antibiotic Resistant Bacteria
11. QSAR: Quantitative Structure-Activity Relationship
12. BIOWIN: Biodegradation Probability Program for Windows
13. OASIS: Open-source Assistance System for the Identification of organic pollutants in environmental Samples.
14. EPI: Estimation Programs Interface
15. PTID: Pesticide Target Interaction Database
16. MBGD: Microbial Genome Database
17. PPS: Pathway Prediction System
18. ADT: Auto Dock Tools
19. PDB: Protein Data Bank
20. IJP: 3,5-dimethoxy-4-oxidanyl-benzoic acid
21. CASTp: Computed Atlas of Surface Topography of Proteins
22. KEGG: Kyoto Encyclopedia of Genes and Genomes
23. RMSD: Root Mean Square Deviation

CHAPTER-I
INTRODUCTION

Pharmaceutical and personal care products have become an essential part of our lives[1]. They include prescribed drugs for humans and animals, hygiene products like creams, deodorants and also disinfectants. Pharmaceutical products have always played an important role in the human society by controlling spreading of the diseases, and hence improving the life expectancy of humans[2]. Hence, pharmaceutical industries rapidly expanded along with the expansion of the global population[1]. However, lately these industries have also become the source of contamination in aquatic environment. Despite stringent regulations, these industries often discharge effluents containing active elements of drugs into the ecosystem leading to contamination. Currently, wastewater treatment plants are not able to completely eradicate these small active elements and so, they are directly discharged into nearby water streams without any treatment[2]. Hence, post use-PPCPs are causing significant threat to both humans and the ecosystem. Contamination by these PPCPs affects every water body including groundwater also[1]. Figure 1 depicts how PPCPs reaches to the entire ecosystem [3].

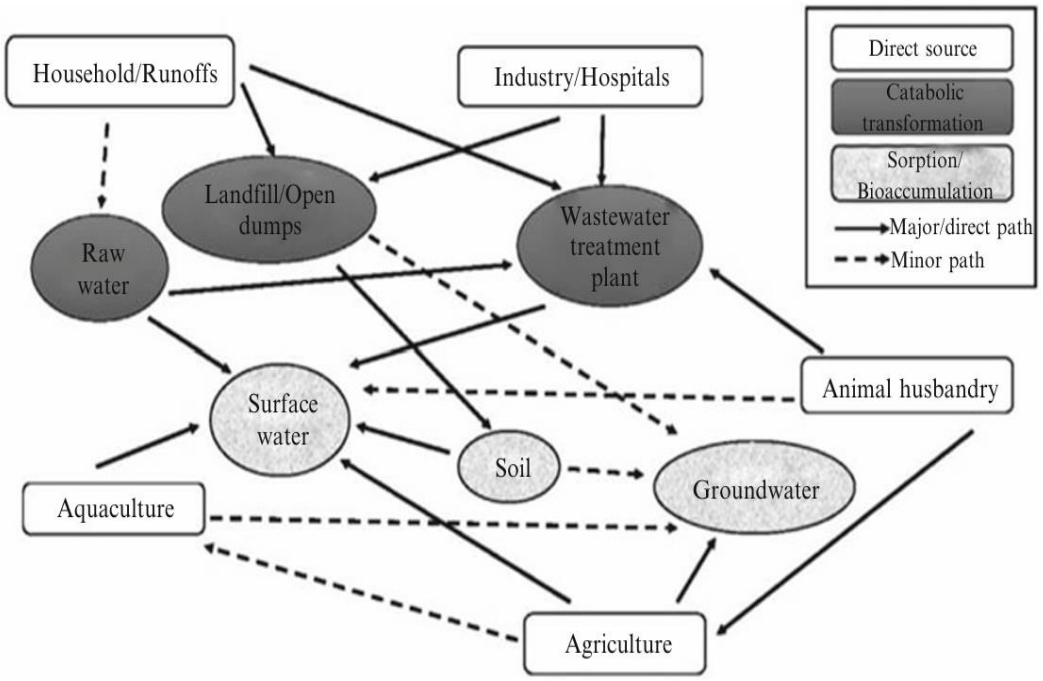


Figure 1- PPCPs pathways in the environment. (El-Gammal et al. 2022)

As PPCPs are personal used products so they are frequently found in faeces and urine. Hence, among many sources of PPCPs, discharge from the domestic sewage is

considered the main source of environmental contamination. PPCPs in microbial organisms can disrupt their metabolic pathways which could affect their enzymatic properties, degradation properties and also give rise to antibiotic resistance in them. Hence, there is an urgent need to find effective ways for bioremediation of these PPCPs as existing systems are incapable of treating majority of pharmaceutical compounds [4] [5].

Many studies have reported that biological treatment of these pharmaceutical contaminants could be a better way than a physical treatment; because by using biological ways we can modify the metabolites which would be degraded by the microbes present in the environment. Whereas, in physical treatment pharmaceutical pollutant will be converted from aquatic phase to solid phase[4].

It has been long established that the intrinsic degradation property of microbes can be utilised in degradation of environmental pollutants. This intrinsic property is applied in bioremediation process where, microbes via their metabolic processes degrades and converts the pollutants into less toxic substances. Despite this impressive ability of microbes, bioremediation has many limitations like, incomplete breakdown of organic pollutants, low efficiency of microbial remediation and remediation process only effective for biodegradable contaminants[6]. Hence, present methodologies of bioremediation and biodegradation is not completely effective in mitigation of hazardous pollutants. It is because we cannot determine comparative toxicity, environmental consequences and complete information on overall biologically transformed compounds with conventional bioremediation technologies[7]. These limitations of microbial bioremediation processes can be improved by the synthetic biology, which could be achieved by using molecular and computational tools that creates the new genetic architecture of a microbe. This new genetic architecture consists of a series of new components (gene promoters, transcription factors, enzymes etc.) that together build new metabolic pathways with end products that could be studied and even re-build [8].

For bioremediation of pharmaceutical pollutants, we can successfully predict their chemical properties, metabolic pathways of degradation by using in-silico tools. We can also simultaneously evaluate degradation specificity of many substrates. This prediction for bioremediation of pharmaceutical pollutants can be done by molecular docking studies. With the help of in-silico now, we can evaluate many things like microbial interactions, biodegradation methods, chemical structures of metabolites, toxicity levels and the interrelationships of microorganisms with each other [9]. Various studies have reported that use of molecular docking have given new insights in the field of bioremediation. Another important thing in the field of bioremediation is, to develop high accuracy pathway prediction algorithms for the better understanding of degradation pathways of many pollutants. Result of these tools is based on various aspects like, substrate features, sites of enzyme binding, structural modifications in enzyme-substrate pairs and the distance between substrate and product in a degradation pathway [10].

1.1 Thesis outline

Pharmaceutical products cause great damage to the aquatic system because of their tendency to persistently remain in the ecosystem. Conventional wastewater treatment systems are not able to eradicate them completely. The aim of this study is to identify potential targets through molecular docking for degradation by the laccase enzyme of *Bacillus subtilis.* Then, pathway prediction tool is utilised to predict the biodegradation pathway of the target carbamazepine.

CHAPTER -2
LITERATURE REVIEW

PPCPs are defined as “any substance used by consumers for personal health or cosmetic purposes or by agribusiness to enhance the growth or health of livestock” by the US Environmental Protection Agency (EPA). Nowadays, there has been a growing interest in the field of PPCPs and their impact on human and environmental health. It has been established that Asian nations such as India, Bangladesh, Pakistan, and China are the Emerging Pharmaceuticals Manufacturing Countries (EPMCs), not the western developed countries. Furthermore, these Emerging Pharmaceuticals Manufacturing Countries (EPMCs) are also home of the world’s biggest pharmaceutical consumers. More than 60% of the world's supply of active pharmaceutical chemicals, such as organic acids, antibiotics, and biotech goods, comes from China. Meanwhile, 20% of the world's supply of generic medications comes from the Indian pharmaceutical industry [11]. Following the consumption of pharmaceutical compound into the human body, they undergo various biotransformation processes that made them more soluble, helping in their excretion from the body. Therefore, wastewater treatment plants (WWTPs) may receive them either in metabolized form or unmetabolized form [12].

Due to the high toxicity of pharmaceutical compounds and the inability of wastewater treatment plants in treating them, there is an urgent need for the efficient wastewater treatment technology. It has been found that microorganisms can eradicate these pharmaceutical pollutants. Hence, microbial wastewater treatment has become an emerging solution to this problem. Studies have reported that various species of white-rot fungus and bacteria, like *Rhodobacter sphaeroides*, as well as combined bacterial and fungal therapies, can effectively eradicate these pollutants from the ecosystem. These can do so by aiding in degradation and mineralization of pharmaceutical pollutants into harmless molecular compounds [12]. For example, it has been discovered that Bacillus bacteria contribute to floc production, a microbial aggregation that accelerates the breakdown of nonylphenol polyethoxylate surfactants (NPEOs). As their only source of carbon and energy, Bacillus helps two other bacteria grow on NPEOs. The breakdown of NPEOs is aided by this mechanism [3]. List of some commonly found pharmaceuticals compounds and their occurrence in environment is listed in Table 1.

Table 1 - Commonly used pharmaceutical compounds that are also listed as emerging contaminants. (R. B. González-González et al.)

TYPE	ENVIRONMENTAL OCCURRENCE
Anti-inflammatory analgesics	Effluent & drinking water
Ibuprofen	Surface, drinking water, river, and effluent

Paracetamol	Hospital effluent and drinking water
Diclofenac	Effluent & drinking water
Naproxen	Effluent & drinking water
Ketoprofen	Effluent & drinking water
Ciprofloxacin	Effluent & drinking water
Sulfamethoxazole	Effluent & drinking water
Erythromycin	Effluent & drinking water
Ofloxacin	Surface, drinking water, river, and effluent
Levofloxacin	Effluent
Oxaliplatin	Predicted Effluent
Cisplatin	Predicted Effluent
Tamoxifen	Hospital Effluent
5-fluorouracil (5-FU)	Hospital Effluent
Metformin	Hospital Effluent
Carbamazepine	Surface, drinking water, river, and effluent
Fenofibrate	Effluent
Atenolol	Effluent

2.1 Pharmaceutical Pollutants

Various researches have indicated that wastewater is the home to various classes of pharmaceutical pollutants. These pharmaceutical classes of pollutants include antibiotics, antiepileptics, anticoagulants, analgesics and anti-inflammatories[13].

Nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, antidepressants, antihypertensives, lipid regulators, hormones, and anticonvulsants are among the commonly utilized medications, according to current demographic trends. In clinical practice, NSAIDs are among the most commonly recommended drugs. Antibiotics are employed to treat and prevent those infections caused by bacteria that are associated with chronic diseases, burns, cancer treatment, surgeries and transplantations. The

main pharmaceutical treatment for patients with depression is antidepressant medication. Anticonvulsants are used to treat seizures. These drugs also aid in discomfort and anxiety relief and enhance the quality of sleep [14].

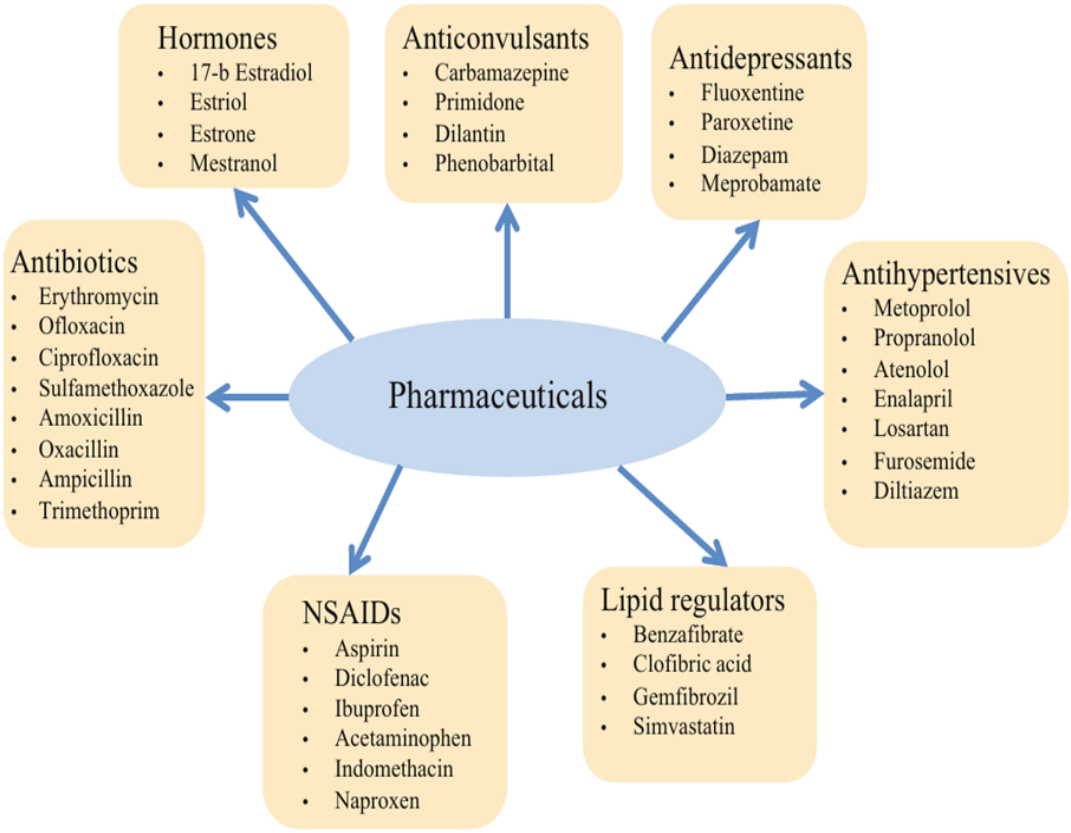


Figure 2-Classes of pharmaceuticals present in environment. (Chhaya, T. Raychoudhury et al.)

Table 2- Names, compound classifications, and WWTP effluent concentrations from the literature for the PPCPs. (El-Glammal et al.)

PPCP	Classification	WWTP effluent concentration (ng per litre)
Chlorophene	Antiseptic	750
Biosol	Antiseptic	250
Biphenylol	Antiseptic	900
Diclofenac	NSAID	110
Fluorouracil	Anticancer	Not detected
Ibuprofen	NSAID	1900
Triclosan	Antiseptic	800

Three main factors that contributes to the complicated problem of pharmaceutical residues contaminating our ecosystems: the manufacture of pharmaceuticals, animal and human excretion, and inappropriate medication disposal [2].

The industry itself, where these compounds are produced on a large scale, is the first major source of pollution related to pharmaceuticals. Manufacturing facilities frequently discharge effluents directly into wastewater streams in spite of strict laws. When suitable treatment techniques are either not followed or are inadequate, these effluents—which are heavy in APIs—find their way into aquatic habitats. This problem is made worse in areas where pharmaceutical production is concentrated, leading to extreme localized pollution[15].

Human and animal excretion of pharmaceutical residues is the second major source of pharmaceutical pollution, and it affects almost every household. It is far more widespread. The body does not completely metabolize all APIs that are ingested; a sizable portion are eliminated through urine and feces, which eventually find their way into sewage systems. The use of veterinary medications exacerbates this problem because residues from these drugs might enter the environment directly through the application of manure to agricultural land. Because of these factors, human and animal waste is a common and difficult source of pharmaceutical contamination to address[2].

Finally, improper disposal of not required drugs also causes significant damage to the ecosystem. Unused medications are routinely dumped in the family trash or poured down the sink or toilet. Because of this approach, these chemicals end up directly in home wastewater or landfill leachate, without any chance of getting treatment from wastewater plant. These untreated are also one of the biggest reasons of deteriorating quality of environmental health. When one considers the quantity of drugs utilized globally in addition to this relatively insignificant conduct, it becomes evident that pharmaceutical pollution is a significant and widespread issue [2].

High levels of pharmaceuticals have been found in a number of Indian water bodies, such as rivers, groundwater, hospital effluents, and wastewater treatment facilities (WWTPs). The research revealed alarming results, showing that antibiotic concentrations (sulfamethoxazole, ofloxacin, ciprofloxacin, norfloxacin, and amoxicillin) in Indian wastewater treatment plants (WWTPs) were higher than those found in Europe, Australia, North America and other Asian countries. Additionally, high concentrations of carbamazepine, ibuprofen, trimethoprim, atenolol, caffeine and acetaminophen were detected. Discharge of Post -use PPCPs into the water bodies also contaminates the terrestrial systems. Veterinary drugs also enter the environment through treatments for pasture animals, aquaculture, and manure application from farm facilities. Currently, over 4,000 pharmaceuticals are employed for various reasons, making the risks posed by PPCPs innumerable. Although PPCPs are typically found in low amounts in aquatic environments, their biological activity presents significant health risks. It has also been found that these compounds tend to accumulate in many non-target species [16].

2.2 Impact of Pharmaceuticals on human health and ecosystem

Among the emerging pharmaceutical pollutants, antibiotics constitutes one of the major classes that have harmful effects on both the humans as well as animals. Antibiotics have a widespread use therefore they are very commonly found in many environmental spaces. Depending on the drug class, 40–90% of prescribed antibiotics are excreted in their active form through urine and faeces, eventually finding their way into the environment and polluting plants, waterways, and soils. Later on, this same contaminated waterway is used irrigation to irrigate the crops leading to agro-ecosystem contamination. The extensive use of antibiotics in animal husbandry further leads to more contamination. Another major worry is the incorrect disposal of unused drugs, which involves flushing them into sewage systems [17]. Hence, there is an urgent need for the analysis of sources from which antibiotics are being discharged, Figure 3.

Worldwide researchers have reported finding antibiotics in quantities measured in micrograms per Liter (μg/L) in sewage treatment plant effluents, surface water, groundwater, marine environments, hospital discharges, and even drinking water in recent years [18]. Human and animal excretion of partially digested medicines is the main source of environmental antibiotics. Waste from pharmaceutical production procedures and the disposal of leftover antibiotics are two more important sources. Furthermore, it is known that commercial institutions, such as hospitals, and residential care facilities, as well as residential areas, such as private homes, dorms, hotels, and other lodgings, contribute antibiotics to municipal wastewater [18]. Some concentrations of antibiotics and the sources is reported in table [19].

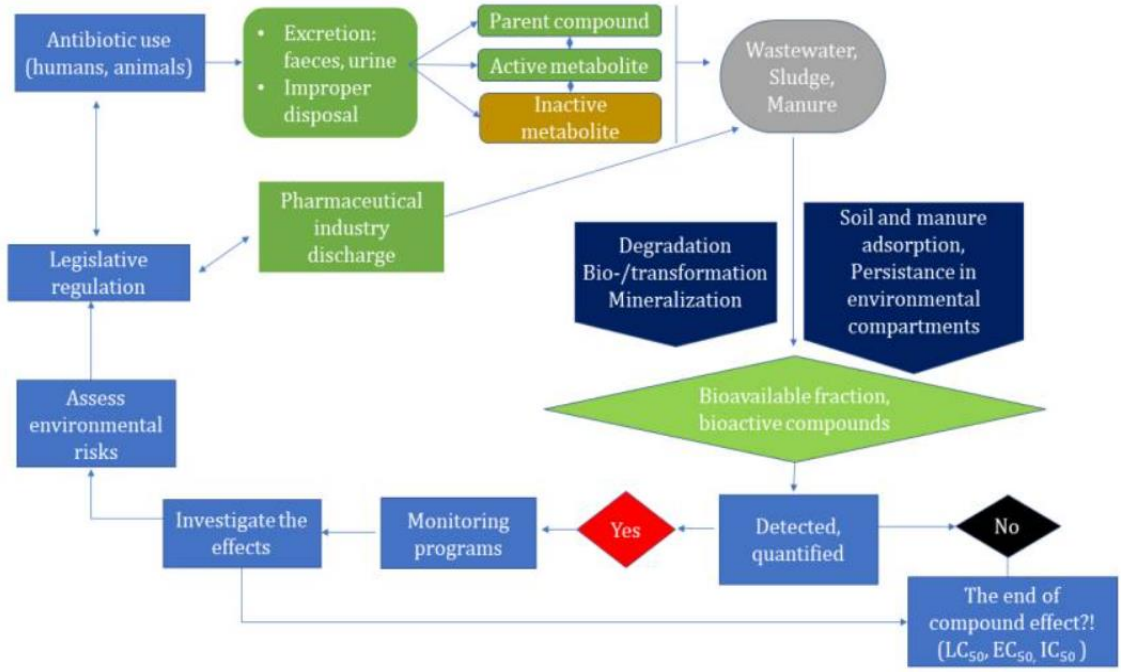


Figure 3 - Antibiotics in the environment from discharge to analysis and environmental risk analysis

Table 3 – MOA of selected drugs

Antibiotic	Mechanism of Action	Water Source	Use	Country	Amount/concen
Clarithromycin	Inhibition of proteins synthesis	Hospital wastewater	Respiratory-tract, skin and soft-tissue infections.	Switzerland	1280 ± 840 ng L^{-}
Erythromycin	Inhibition of proteins synthesis	Hospital wastewater	Prevent bacterial protein synthesis.	Portugal	575 ng L^{-1}
		Aquaculture-- ponds		Taiwan	57.4 ng L^{-1}
Ofloxacin	Inhibition of DNA replication	Hospital wastewater	Bacterial exacerbations-of chronic bronchitis	Portugal	6543 ng L^{-1}
		Domestic wastewater		China	2794 µg m^{-3}
Sulfamethoxazole	Inhibition of dihydrofolate synthesis	Hospital wastewater	Urinary tract infections	South Korea	25300 ng L^{-1}

2.2.1 Amoxicillin

Amoxicillin comes into the category of penicillin. It has a β-lactam structure in it. It works successfully against a variety of diseases caused by gram-positive and gram-negative bacteria. Because of its pharmacological and pharmacokinetic properties, amoxicillin is used in the treatment and prevention of bacterial infections that affect the gastrointestinal, urinary, cutaneous, and respiratory systems [18]. Furthermore, amoxicillin is also employed for diseases in animals. Additionally, it also serves as a role of growth promoter for various farm animals, including fish, cattle, dogs, cats, pigeons etc. The structure of the amoxicillin is depicted below, figure 4.

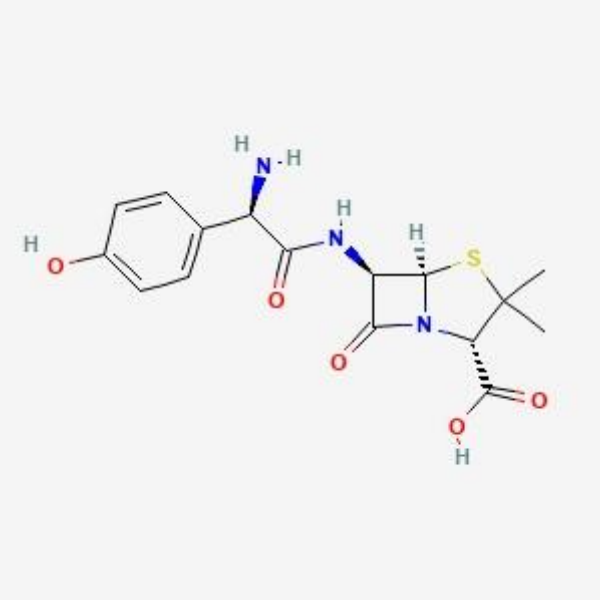


Figure 4 - molecular structure of amoxicillin

Antibiotics can cause severe allergic reactions in species that are susceptible to them, including humans, if they end up in drinking water. Antibiotic-resistant bacteria (ARBs) can arise from soil and water polluted with antibiotics, even at low concentrations. Approximately 75% of antibiotics that animals consume are eliminated through their urine and faeces. Furthermore, amoxicillin's breakdown products, or metabolites, are frequently more persistent and dangerous than the parent drug [20]. Bacteria in the aquaculture environment and bacteria in the terrestrial environment, such as animal microbes and human diseases, have been found to exchange genes for antibiotic resistance lately. Consequently, the aquatic environment's exposure to antibiotics may cause human infections that are a component of its microbiota to develop resistance [18]. bioaccumulation of amoxicillin in fish tissues, which can be eaten and have negative effects on humans such as immunoallergic reactions and can cause the Fish tissues include genes resistant to germs. Research on bacteria, such as Clostridium, Lactobacillus, Escherichia coli, and Enterococcus, has demonstrated that in reaction to the stress of drugs such as ciprofloxacin, erythromycin, penicillin, amoxicillin, and sulfamethoxazole, alter the metabolic processes of the bacterium [20].

Wastewater treatment plants are the hotspots for the spreading of antibiotic resistance genes in the ecosystem. Several studies have supported the fact that the environment inside the wastewater treatment plants help in proliferation of antibiotic resistance bacteria and antibiotic resistance genes. The antibiotics come into the category of recalcitrant pollutants and hence it’s very difficult to eradicate them [20]. Antibiotics, other pharmaceutical residues, and heavy metals in the wastewater remain in constant

contact with bacteria throughout wastewater treatment, which could put selection pressure on resistance genes [21].

2.2.2 Tetracycline

One of the most often utilized classes of antibiotics worldwide is tetracycline. It is useful in both human and veterinary therapy due to its broad-spectrum efficacy against a variety of bacterial illnesses. Tetracycline is suggested for the treatment of infectious diseases such as pneumonia, infections of the bones and joints, skin infections, infections contracted through sexual contact, and infections of the gastrointestinal tract. Tetracycline is an effective medication against the pathogens known as "biothreats," which include *Yersinia pestis, Bacillus anthracis, and Francisella tularensis*. These pathogens are responsible for causing some of the deadliest diseases. For many infectious illnesses, tetracycline-class pharmaceuticals are typically the first-choice course of treatment. Like Amoxicillin, tetracycline pollution reason is due to its low metabolism in both human and animal organisms and its low stability [22]. The phytotoxic effects of tetracycline on plants that can result in chromosomal abnormalities, stoppage of growth, and decreased amounts of carotenoid and photosynthetic chlorophyll in plants [17]. The structure of tetracycline is depicted below, figure 5.

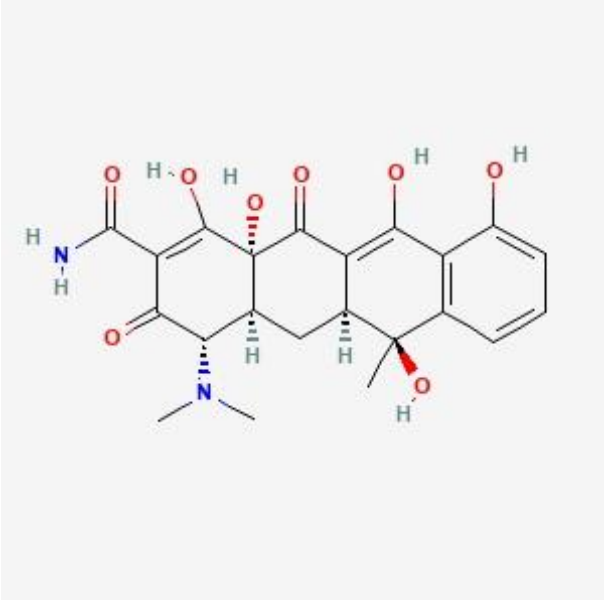


Figure 5 - molecular structure of tetracycline

Since pharmaceuticals chemical structure is naturally soluble in water, they cannot dissociate themselves in an aqueous solution. Pharmaceuticals are primarily designed to perform physiological and biochemical processes for the treatment of diseases and because of this characteristic, they are able to pass through biological barriers and remain in the human body throughout time. The ability of a pharmaceutically active substance to build up and have a harmful effect on non-target species raises serious problems. According to the published researches, PPCPs have significant effect

on the bacterial communities and other living species in both the terrestrial and aquatic environments. The majority of microbial communities have acquired resistance to antibiotics due to the widespread presence of antibiotics in waste water and natural water bodies. These antibiotics include ceftazidime, fluoroquinolones, bacitracin, sulphonamides, cefotaxime, tetracycline, cefepime, erythromycin, colistin, beta-lactams, amoxicillin, cefpodoxime, bacitracin, aztreonam, doxycycline, penicillins, trimethoprim, lincomycin, cephalosporin, chloramphenicol, and many more [16].

2.3 Biodegradation

Biodegradation of PPCP is the ability to get degraded via the microbes like bacteria and fungi present in the environment. Also, degradation of a compound can also occur co-metabolically. When there is complete transformation of a compound into water and carbon-dioxide then it is called mineralization. Mineralization can be the outcome of the degradation process. There is another type of biodegradation known as partial biodegradation in which, the molecules is converted into metabolites conjugated with sulfate or glucuronic [3]. The process of biodegradation is considered as most important process in the removal of most xenobiotics, including pharmaceuticals [23]. During the process of biodegradation of the pharmaceuticals, microbes in the environment came in contact with many active molecules [23].

Over the past few decades, it has been reported that several bacterial species have the ability to produce enzymes. Many of these bacterial enzymes have shown their role in the mitigation or elimination of complex environmental pollutants [24]. Enzymes are highly efficient biological catalysts and they can transform the toxic compounds into simpler non-toxic compounds. Thereby, bacteria utilized these enzymes in their metabolic cycle for the effective removal of the pollutants from the contaminant sites. Among the major classes of enzymes, oxidoreductases and hydrolases are the classes that have been most extensively researched for their property of degrading toxic compounds into environmentally safer compounds. This is due to the fact that these enzyme classes possess very high catalytic activity and ability to target wide ranges of substrates including recalcitrant pollutant compounds [25].

A pollutant biodegradability is based on its physical and chemical properties. Some pollutants are easily biodegradable because of their properties and some are not. It has been proved that those pollutants that have a very branched chemical structure are more susceptible to microbial degradation as they provide many sites for their microbial action to act on. Highly complex pollutants are termed as recalcitrant pollutants. Pollutants containing monocyclic aliphatic compounds in their structures are more biodegradable than the pollutants containing polycyclic aliphatic compounds. Furthermore, presence of functional groups in structure of pollutants also affects the biodegradability [3].

2.4 In-silico methods

With the increase in global population, there is also tremendous rise in the numbers of anthropogenic pollutants. Experimental bioremediation is a good way for assessing the toxicity and fate of these compounds but it is struggling to keep up with the rapid increase of these compounds. As a result, the in-silico method is becoming more and more popular as an appropriate replacement for the bioremediation study of the different environmental pollutants. This technique enables a marvellous fest - virtual screening. Through the virtual screening of interactions between pollutants and enzymes, we can solve the limitations of traditional bioremediation. Thus, Computational biology methodology provides a simpler and more effective method for identifying bioremediation targets than the wet lab methods. The in-silico techniques utilise specific microbial model systems that mimics biological conditions to predict the chemical qualities of target molecules, the metabolic degradation pathways for new emerging contaminants, and biotransformation processes. By predicting substrate specificity, it helps evaluate the chances of biodegradation. The extensive use of docking and molecular dynamics simulations has expanded to predicting bioremediation targets, offering new insights for improving current bioremediation practices. Visualizing the computational interactions between dyes and enzymes provides a deeper understanding of the biodegradation process. Relevant and new computational algorithms are utilized in molecular docking experiments to perform virtual screenings of protein-ligand interactions. Through substrate specificity prediction, it helps in determining the probability of degradation. The extensive use of docking and molecular dynamics simulations has expanded to predicting bioremediation targets, offering new insights for improving current bioremediation practices. Visualizing the computational interactions between specific pollutants and enzymes provides a deeper understanding of the biodegradation process which further aids in understanding the way an enzyme interacts with the ligand. Relevant computational algorithms are utilized in docking assays to perform virtual screenings of protein-ligand interactions [26].

These in-silico methods are able to accurately predict the chemical characteristics of xenobiotic compounds, the processes by which they degrade, and the biotransformation of novel recalcitrant compounds. Some of these in-silico tools for researching and understanding the fate and degradation mechanisms include molecular docking, quantitative structure-activity relationship (QSAR) modeling/algorithms, and homology or comparison modelling. Molecular docking has proven by many researchers to be an efficient technique for mitigating pollutants through biodegradation processes in recent times. Molecular docking has become an indispensable tool in environmental monitoring because it is a very relevant and economical technique that facilitates understanding the reaction processes of ligands with accurate enzyme binding. Through the use of molecular docking assays, the formation of robust and stable docked complexes between ligands (xenobiotics) and enzymes can be studied. Recently, numerous studies have focused on the application

of docking in bioremediation. Similar to docking studies, researchers all over the world are now able to more accurately identify potential degradation routes thanks to recent developments in pathway prediction tools and related databases. There are numerous novel approaches available right now in the field of bioremediation of environmental contaminants. Here are few instances: PathPred, MetaRouter, Envipath, BIOWIN, OASIS, EPI suite (Estimation Programs Interface); pesticide target interaction database (PTID); Biodegradation Network-Molecular Biology database (Bionemo); Biodegradative Oxygenases Database (OxDBases); Microbial Genome Database (MBGD); BioCyc and MetaCyc; EAWAG-BBD Pathway Prediction System (PPS). These innovative web resources are compatible with Linux and Windows operating systems and preserve heterogeneous knowledge about microbial bioremediation [27]. Figure represents A schematic diagrramtical overview of the in-silico bioremediation approach involves the following flow: starting with the screening of pollutants, followed by the prediction of degradation pathways on a specialized computer system. This process utilizes various tools and techniques, including databases, molecular docking, molecular dynamics simulation, and high-performance computing strategies [28].

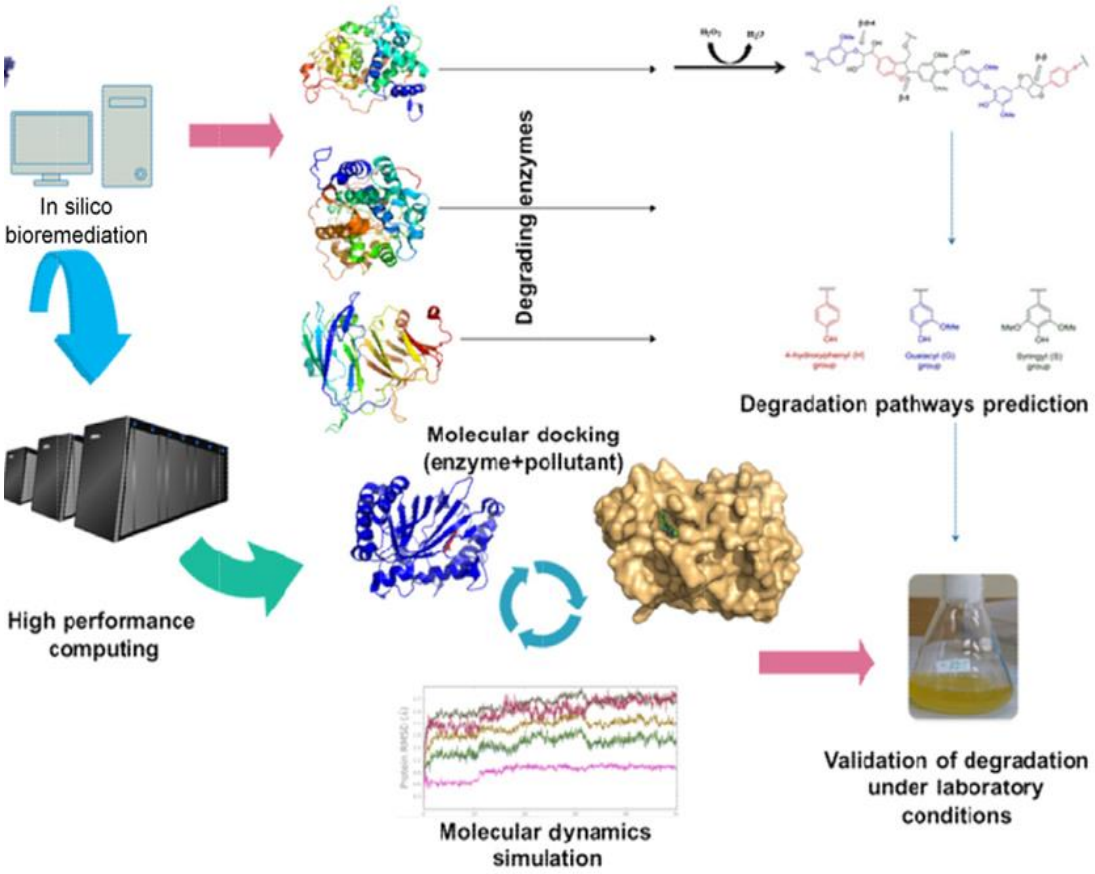


Figure 6 - Overview of how in-silico degradation method works

Microbial enzymes are the most important part of the bioremediation, this process is dependent on their functioning as they are sustainable, efficient, and some of them are fast. Therefore, it is crucial to screen for microbial enzymes capable of degrading pollutants. Molecular docking is used in the remediation of recalcitrant molecules to screen for enzymes or proteins capable of effectively degrading a targeted xenobiotic [10]. This is done by selecting the most favorable position with the lowest possible conformation energy during complex formation by assessing the binding positions of individual molecules in relation to one another [29]. For over a decade, molecular docking has been effectively used to do the biodegradation of pollutants that are increasingly released into the environment from industrial, anthropogenic, and natural sources during remediation [24]. Molecular docking simulates the atomic-level interactions between a contaminant and an enzyme or protein, enabling the description of xenobiotic behaviors at the targeted enzyme or protein's binding sites and elucidating the xenobiotic's catalytic activity. This method is a computational tool that accelerates the process of selecting and optimizing the enzyme characteristics for a particular xenobiotic. It is capable of accurately predicting how an enzyme will be bound to and perform when absorbing or degrading a pollutant, hence highlighting the relationship between an enzyme and a pollutant. Site-directed mutagenesis testing is guided by an identifiable active site and its binding properties, so that the outcomes may be confirmed in a lab setting.

We calculate the association of a target compound and a protein by investigating several docking points. If we do not know the docking locations, blind docking is done in that case [29]. Docking experiments is based on the famous lock and key hypothesis, according to which the ligand and receptor are both rigid bodies that fit into each other like a key into a lock. This was later improved by Koshland's induced fit theory, which said that a protein's active site is dynamically changing due to molecular interactions with ligands. This suggests that while doing docking experiments we should choose both ligands and receptors to be flexible, not rigid, as this will provide a more accurate description of the binding event. There are two steps in the docking process: 1. Estimating the ligand's structural information, orientation, and its location; 2. calculate the binding energy.

During the docking process, specialized computer algorithms are used to compute the potential active location or binding position in the enzyme structure from the protein sequences to find out the properties of the active site. These algorithms then aim to fit the ligand into the protein's or enzyme's active region and generate energy scores for the most favorable ligand-protein pairings. The most appropriate chemical pairings and the computed energy score can subsequently be used to predict the process of enzymatic breakdown of pollutants [30]. Hence, the docking technique is used to screen pollutants that bind with the suitable enzyme during degradation, thereby enhancing our understanding of catalysis process. In bioremediation, docking proves invaluable in saving time and costs, as it facilitates routine screening of environmental pollutants to identify the enzymes capable of degrading them.

Numerous pollutants have emerged as a result of human anthropogenic activities, posing serious risks to the environment and public health. The hazardous pollutants cannot be adequately addressed by current bioremediation techniques. They often lack the complex capacity to offer comprehensive information regarding biodegradation and compounds that have been modified altered. As a result, research into substitute methods for reducing the harmful impacts of pollution is always necessary. Several in-silico methods have been applied in bioremediation projects, together with computational frameworks. These methods seek to eliminate pollutants, evaluate toxicity, and estimate the probability of breaking down of recalcitrant pollutants[26]. The roles played by a few in-silico methods in predicting the dangerous chemicals' degradation routes were discussed below in the table 4.

Table 4 – Applications and MOA of selected computational techniques.

Sr no.	**In-silico technique**	**Applications**	**Mode of action**
1	Molecular docking approach	**To predict** the binding properties of **proteins** and **ligands** involved in **in silico bioremediation processes. For** the **investigation of protein-protein interactions as well as** nucleic acid-protein interactions. **It is used** in **protein engineering. Determines** the mechanism of **enzymatic** reactions. Identification of **molecular docking sites** (blind docking).	Complementarity (the **docking** process **has** two **parts:** geometry and energy). **Determine** the most stable protein-ligand configuration.
2	Molecular dynamic simulation approach	**Information used to predict** changes and **modifications** in **the conformation** of **a** protein-ligand complex or enzyme **over** time.	It explores the multiple dynamic conformational changes of a protein to identify the most probable configuration. Simulation under different conditions can be compared to identify the active or inactive conformation.
3	Pathway Prediction System (PPS)	It is **used to predict** different **ways** of **converting** toxic **substances** or **pollutants into non-toxic substances according** to biotransformation rules.	It is **based** on **the identification of** functional groups **in organic molecules** that are **the** targets of **microbial degradation reactions.**

4	Biodegradation Network Prediction Software (BNPS)	It is used **to predict possible** metabolic **links** and metabolic **processes** for bioremediation.	Identifies the routes to the biodegradation network with the use of reaction rule
5	Metabolic engineering	**Identify** millions of **viruses** through **biomolecule** and DNA engineering.	It improves the **bioremediation** process of **engineered microorganisms and increases** their **degradation capacity.**

CHAPTER -III
METHODOLOGY

Enzymes including laccases have the potential of eliminating pharmaceutically active compounds, and this has drawn a lot of attention—especially in recent years. Laccase mediated degradation follows a series of processes: first a compound is broken down by a laccase, an electron is moved from the molecule to the laccase. This electrostabilized the copper atom in the active core of the enzyme. The Copper molecule then undergoes regeneration by way of a coupled reaction with molecular oxygen that produces water. Elements including the existence of linked bonds, heterocyclic or anaromatic rings, electron-donor groups, and readily oxidizable substituents are important in determining how much degradation occurs [31].

3.1 Preparation of enzyme and reference molecule

Microbial and fungal enzymes, laccases have been found in various literature for their importance in removal of pharmaceutical products from the aquatic system [31]. In this research, protein sequence of of CotA laccase complexed with syringic acid (ID – 7Y8B) from Bacillus subtilis subsp. subtilis str. 168 is used. 3D structure of laccase fig was retrieved in PDB format from the protein data bank (https://www.rcsb.org). The laccase molecule was then cleaned of all residual ligands and water molecules using PyMOL software, and the receptor molecule was then modified by adding hydrogen atoms using AutoDock Tools (ADT) software.

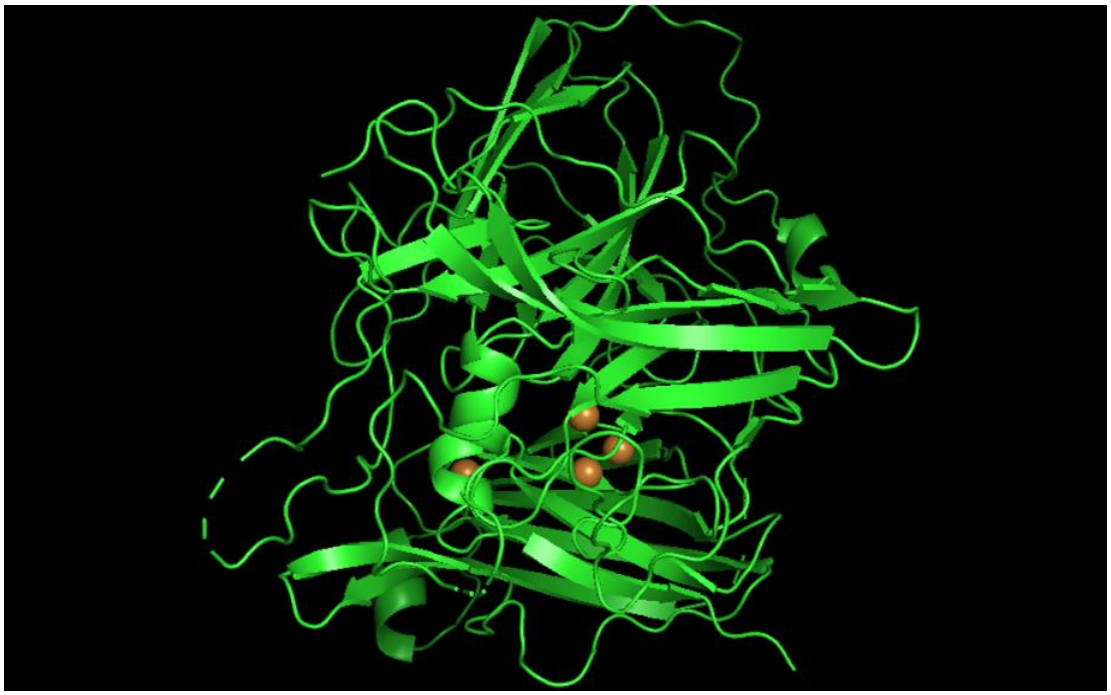


Figure 7-3D structure of laccase enzyme

IJP which was the ligand (3,5-dimethoxy-4-oxidanyl-benzoic acid) was used as a reference molecule for the validation of the autodocking studies. Its 3D structure was retrieved in SDF format which we later convert into PDB format by the online tool, online smiles translator, nci.nih.gov/translate/. The 3D structure files of all the pharmaceutical pollutants were also retrieved in SDF format which later converted into the PDB format. All pollutants and reference were converted into the PDB format before the autodocking.

Below is the figures which illustrated the 2D and 3D structure of the IJP as well as its chemical characteristics.

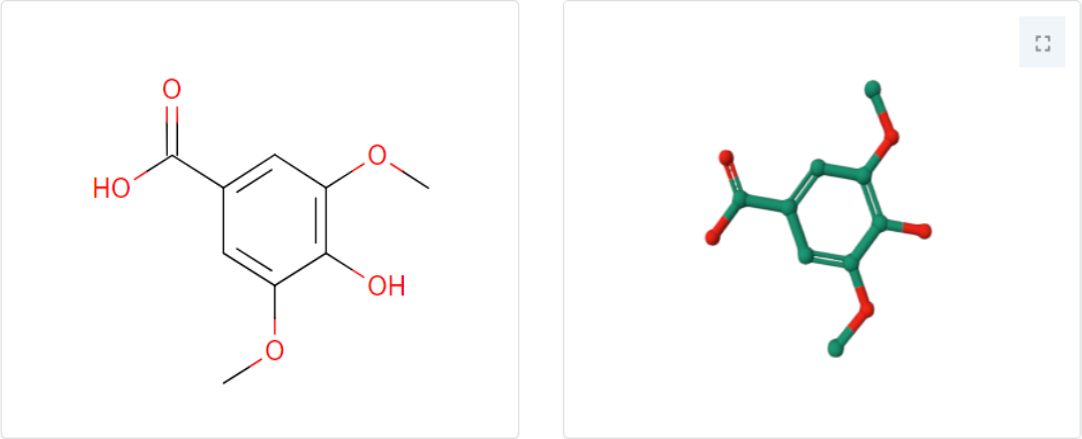


Figure 8 - 2D and 3D structure of reference molecule

Chemical Component Summary	
Name	3,5-dimethoxy-4-oxidanyl-benzoic acid
Synonyms	Syringic acid
Identifiers	3,5-dimethoxy-4-oxidanyl-benzoic acid
Formula	$C_9 H_{10} O_5$
Molecular Weight	198.173
Type	NON-POLYMER
Isomeric SMILES	COc1cc(cc(c1O)OC)C(=O)O
InChI	InChI=1S/C9H10O5/c1-13-6-3-5(9(11)12)4-7(14-2)8(6)10/h3-4,10H,1-2H3,(H,11,12)
InChIKey	JMSVCTWVEWCHDZ-UHFFFAOYSA-N

Figure 9 - summary of chemical components of the reference molecule

3.2 Analysis of active site of protein

The CASTp (Computed Atlas of Surface Topography of Proteins) (http://sts.bioe.uic.edu/castp/index/.html?1ycs) is an online platform utilized in this

research for identifying, outlining, and computing the geometric and topological attributes of protein structures. This is used for the active site analysis of our protein structure. After the analysis of active site of the protein, molecular docking of the enzyme protein with various ligands is performed. The figures below illustrated the active binding region in protein and the residuals that have involvement in active binding sites (highlighted by grey colour).

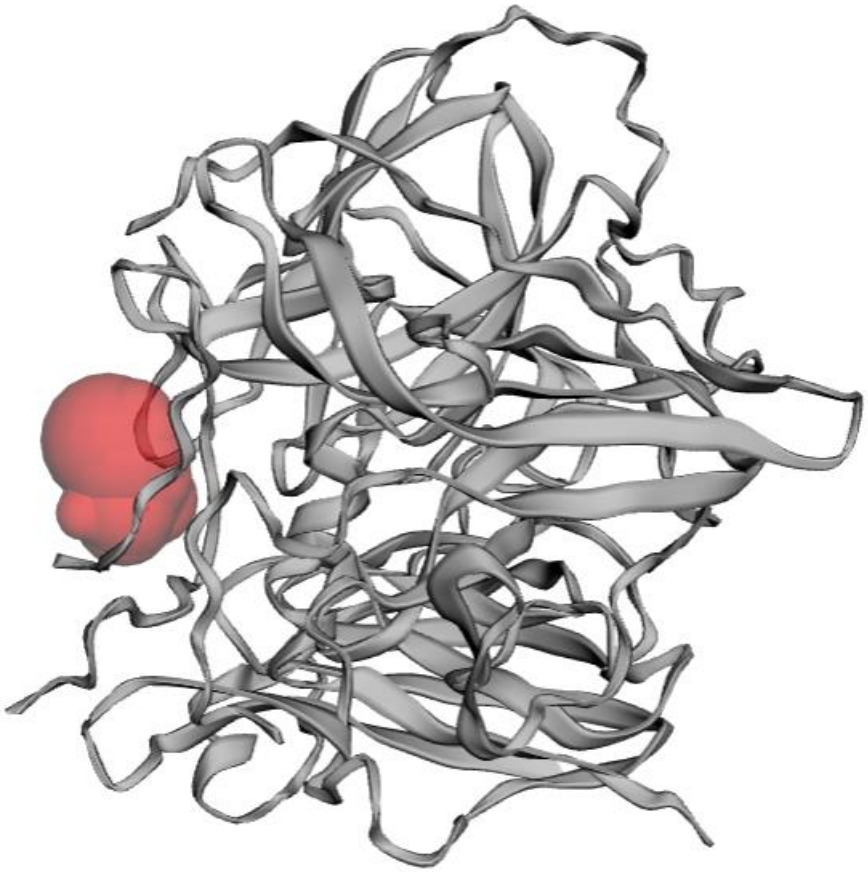


Figure 10 - active binding site in protein

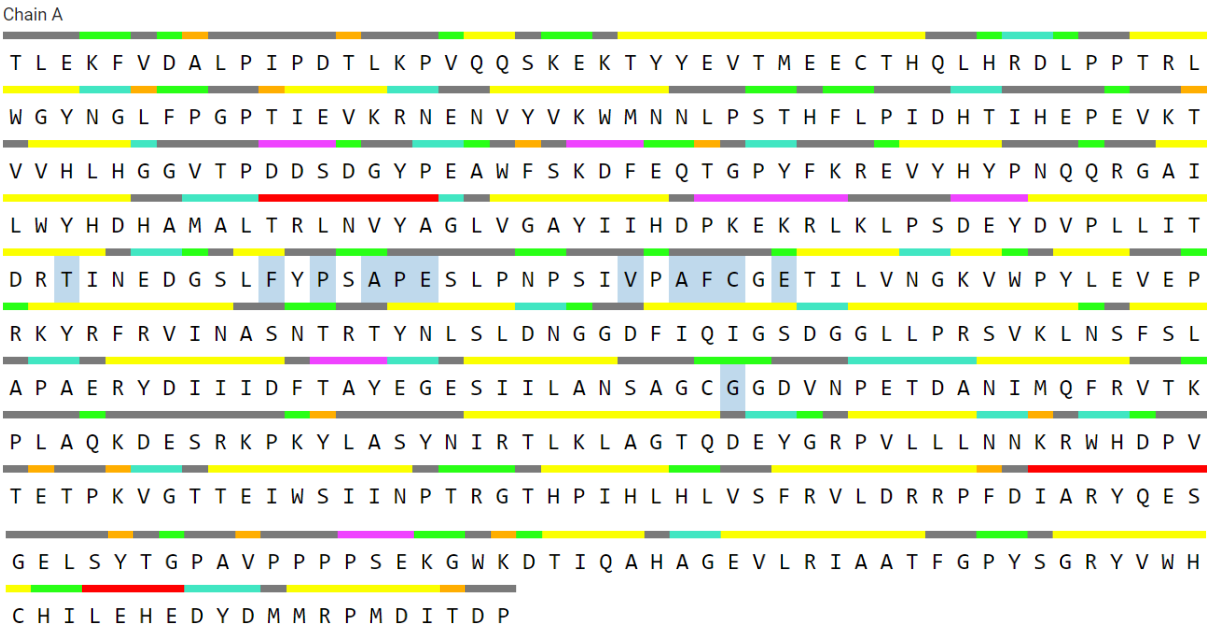


Figure 11- grey residues that are involved in active binding

3.3 Molecular Docking

Laccase which is the one of the important enzymes in the process of biodegradation of pharmaceutical pollutants was subjugated to the molecular docking analysis. But before doing this, validation of our autodocking method is important. Hence, before the virtual screening of our pollutant substrates through the process of molecular docking, we tested our docking process with the re-docking of our reference molecule IJP. We did this to check the proper coordinates of our docking pose of the molecule. Our process was validated when we observed that the laccase crystal structure was superimposed by the reference docked molecule.

We used AutoDock Vina for the docking experiments. The grid centre for the docking study was set to X=9.871, Y=0.414, Z=3.751 with grid box size of 40,40 and 40 for X,Y and Z coordinates respectively. The exhaustiveness level was set at eight as a standard. Both the receptor and the ligand were prepared by allocating polar hydrogen atoms and Kollamen charges. The pollutant compounds were set flexible during the molecular docking process, while the enzyme stayed stable. For every ligand, this software produced nine distinct bound conformations based on the ligand's propensity for binding. For further examination, the conformation with the least binding energy was selected as the ideal one. The PyMol software was used to study the 3D interaction of enzyme-substrate complex.

Seven pharmaceutical compounds were selected as ligands for autodocking studies – Aspirin, Ibuprofen, Ketoprofen, Naproxen, Diclofenac, Clofibric acid and Carbamazepine. Each of their 2D and 3D structures were downloaded from the

PubChem database, https://pubchem.ncbi.nlm.nih.gov/. Below figures shows the 2D structures of the selected pharmaceutical compounds –

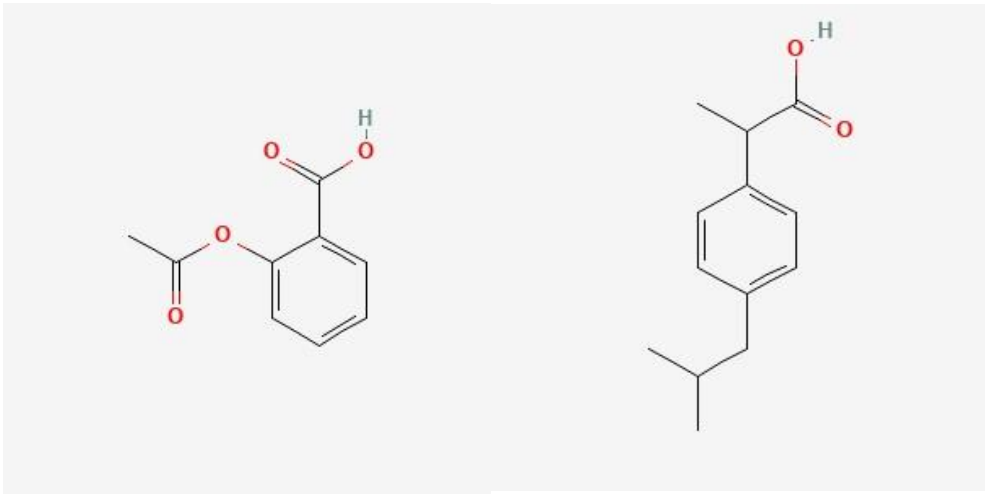


Figure 12 - Structure of Aspirin (left) and Ibuprofen (right)

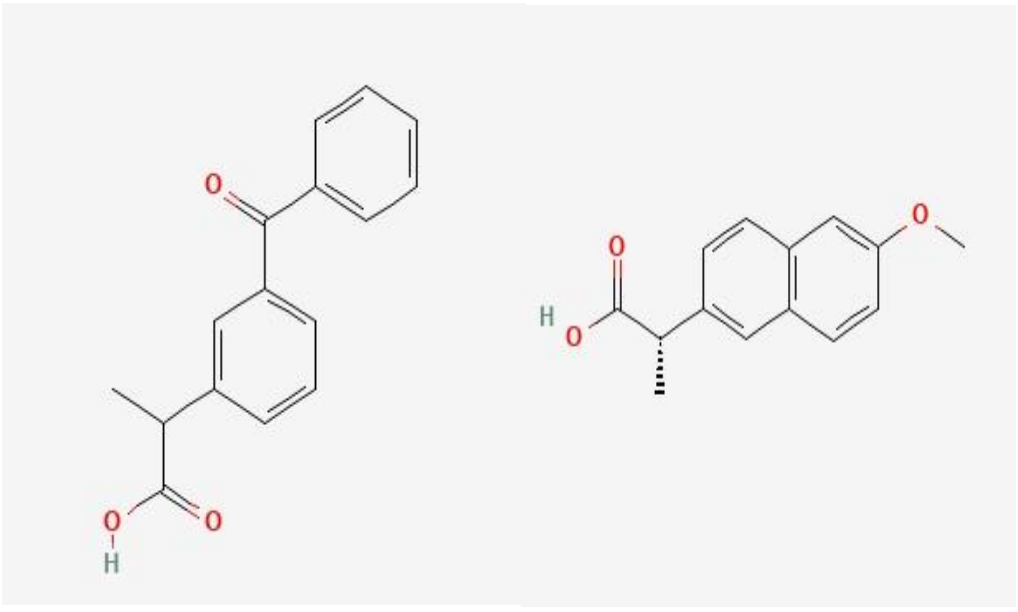


Figure 13 - Structures of ketoprofen (left) and Naproxen (right)

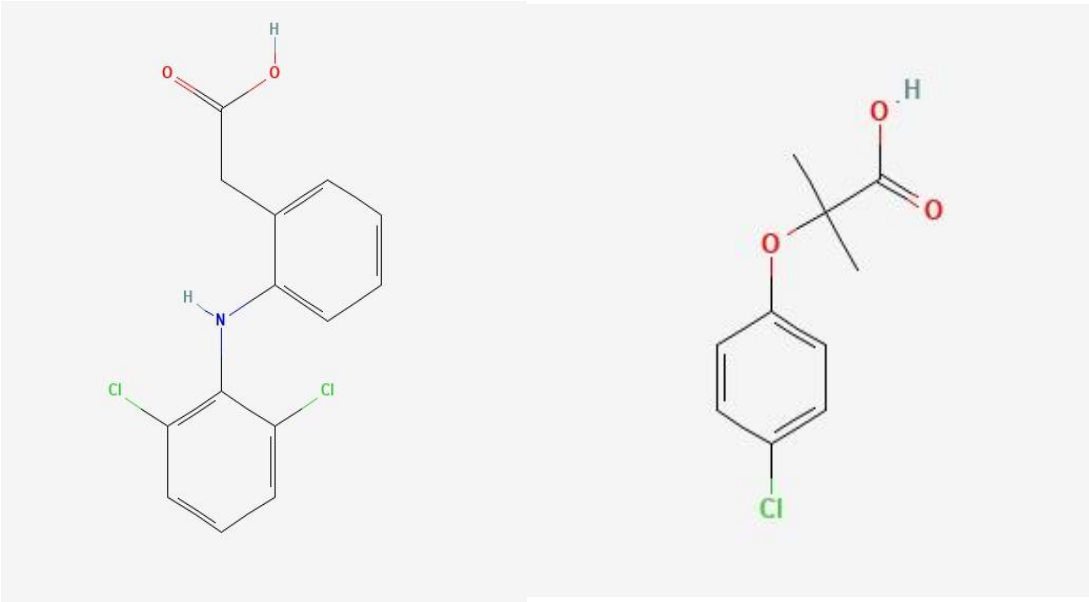


Figure 14 - structures of diclofenac (left) and clofibric acid (right)

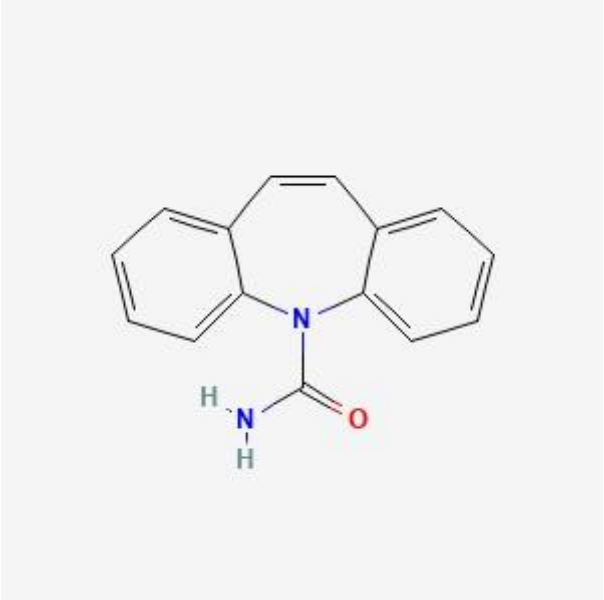


Figure 15 - structure of carbamazepine

3.4 Synthetic pathway prediction

After obtaining the structural information and the interaction between the protein molecule (enzyme) and ligands (pharmaceutical pollutants), prediction of potential biodegradation pathways was done using the database PathPred, https://www.genome.jp/tools-bin/pathpred/pathpred.cgi. PathPred uses the KEGG data for its knowledge-based prediction framework. This service provides possible reactions and the prediction of probability of a compound's transformation based on previously available data. The predicted or modified chemical molecules could be seen and is displayed as a graph in the form of a tree. Chances of accurate predictions of those pathways of the compounds are very high, whose identical structures are already present in KEGG compounds [26].

PathPred database predicts reference biodegradation pathways for two types of molecular compounds – 1. For the microbe-based breakdown of environmental pollutants and; 2. For the formation of secondary metabolites. On the basis of these requirements, the user can select any of the two compound types. Users predicting for the biodegradation or transformation of pollutants can use the following format as input – 1. MDL Mol file, 2. SMILES format and 3. KEGG compound identifier. Whether the enzymes for these reactions are known or unknown, the PathPred server provides new and possible reactions [26]. The below figure illustrates the homepage of the PathPred site –

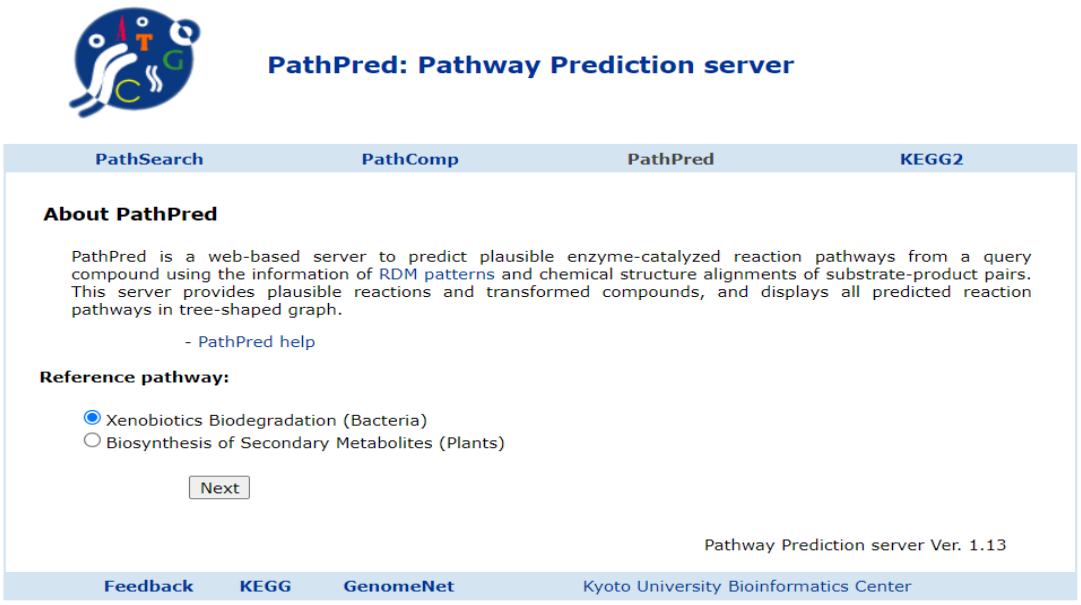


Figure 16 - Homepage of Pathway Prediction server

Elements are shown as compounds (nodes) and reactions (edges) in the projected pathway diagram. The compounds indicated in black with CX numbers are not found in the KEGG database, whereas the compounds labeled in blue with C numbers are those that are known to be involved in KEGG pathways. Nodes with light colors indicate that the same component is present in other places along the pathway. In the bi-directional prediction, the compound is indicated in red upon reaching the terminal. Predictions based on successive reference reactions within the KEGG pathway are indicated by consecutive edges of the same hue. The reaction score of the edge indicates the probability of the expected reaction, which is correlated with its thickness. In cases where the predicted pathway fails to bridge the initial and final compounds in the bi-directional prediction, the outcome is displayed as two separate trees [32].

To evaluate each anticipated pathway's plausibility, scientists have created two rating systems. Reaction score is used to find the probability of a predicted reaction by comparing the atoms of the query compound with those of the matched compound. Reaction score is based on the Jaccard coefficient. Since RDM atoms are thought to be very important for the reaction, this score is given three times the weight at those atoms in comparison to other atoms. The pathway score, on the other hand, represents the average of individual reaction scores within the pathway. To focus the search for possible transformation reactions, compounds with high route scores are given priority as queries for next prediction cycles. The similarity between generated compounds and those in other projected paths is additionally taken into account in bi-directional prediction situations. As a result, the ranking of compounds is determined by adding up the highest similarity score between molecules in the opposite direction and the pathway score [32].

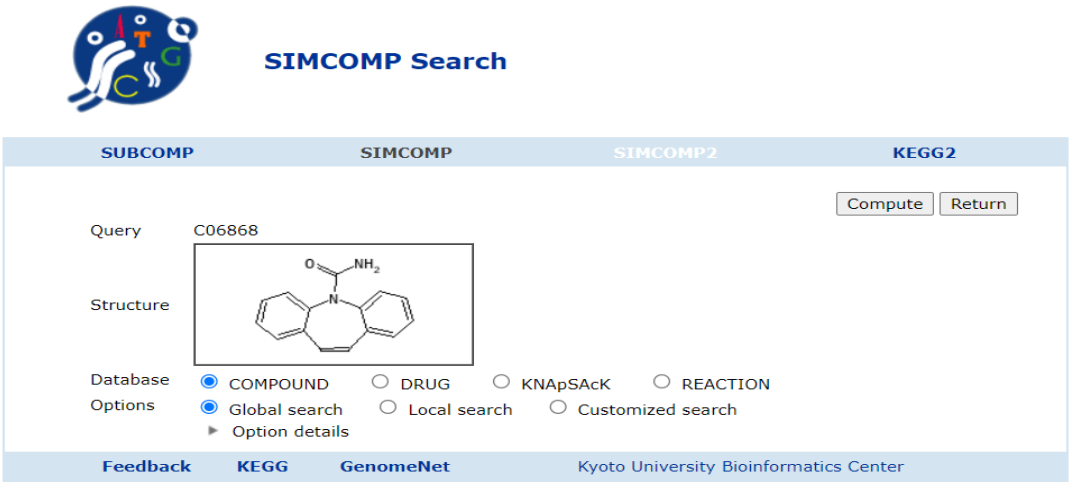


Figure 17- query compound

COMPOUND: C06868

Help

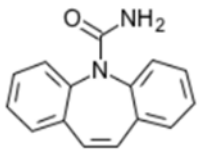
Entry	C06868 Compound
Name	Carbamazepine
Formula	C15H12N2O
Exact mass	236.095
Mol weight	236.2686
Structure	 C06868 Mol file KCF file DB search
Remark	Same as: D00252
Reaction	R08312 R08313 R08317
Pathway	map00982 Drug metabolism - cytochrome P450
Enzyme	1.14.14.1 1.14.14.-

Figure 18- compound search in KEGG

CHAPTER -IV
RESULT

The docking analyses of laccases were done with Aspirin, Ibuprofen, Ketoprofen, Naproxen, Diclofenac, Clofibric acid and Carbamezapine. The output of the docking analysis revealed the following things – energy and RMSD values (RMSD lower bound and RMSD upper bound). The predicted binding affinity is calculated in Kcal/mol. RMSD values are computed on the optimal mode only and it involves motion of two heavy atoms. RMSD value is the metric to check whether the docking simulation is accurately predicted or not. If the value of RMSD is ≤ 2 Å then, docking simulation is accurately predicted [33].

4.1 Results of molecular docking analysis

1. Aspirin

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000
2	-5.9	1.233	3.342
3	-5.6	15.561	17.708
4	-5.3	16.283	18.485
5	-5.2	16.633	19.105
6	-5.2	19.047	19.752
7	-5.2	7.732	9.965
8	-5.1	41.171	42.626
9	-5.0	22.542	24.604

Best binding affinity: -6.2 kcal/mol

Aspirin shows moderate binding affinity with consistent modes.

2. Ibuprofen

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-5.7	0.000	0.000
2	-5.4	29.776	31.556
3	-5.4	30.157	31.879
4	-5.4	29.675	31.350
5	-5.3	1.185	2.002
6	-5.1	23.089	24.417
7	-4.9	29.974	30.696
8	-4.9	1.747	2.644
9	-4.8	30.010	32.098

Best binding affinity: -5.7 kcal/mol

Ibuprofen shows moderate binding affinity with consistent modes.

3. Ketoprofen

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.6	0.000	0.000
2	-6.3	20.494	23.498
3	-6.3	18.674	21.243
4	-6.1	20.939	24.930
5	-6.1	2.137	6.309
6	-6.1	20.285	23.443
7	-6.0	36.207	37.729
8	-5.7	1.816	2.509
9	-5.7	1.973	6.448

Best binding affinity: -6.6 kcal/mol

Ketoprofen shows a relatively strong binding affinity compared to aspirin and ibuprofen.

4. Naproxen

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.9	0.000	0.000
2	-6.4	1.689	2.663
3	-6.2	42.004	43.376
4	-6.1	17.324	21.276
5	-6.0	24.262	26.606
6	-5.9	17.470	20.618
7	-5.7	23.590	26.038
8	-5.7	33.092	37.053
9	-5.7	1.592	2.175

Best binding affinity: - 6.9 kcal/mol

Naproxen has one of the strongest binding affinities among the pollutants, with a stable primary binding mode. This suggests it interacts favourably with the laccase enzyme.

5. Diclofenac

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.7	0.000	0.000
2	-6.6	0.187	2.098
3	-6.3	2.440	3.141
4	-6.2	2.058	5.003
5	-5.9	35.932	38.029
6	-5.9	3.556	6.207
7	-5.8	1.632	2.575
8	-5.5	34.076	36.472
9	-5.5	33.757	36.142

Best binding affinity: -6.7 kcal/mol

Diclofenac also shows strong binding affinity and stability in its primary binding mode.

6. Clofibric acid

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.1	1.670	2.370
3	-5.5	23.702	24.678
4	-5.4	18.660	20.634
5	-5.4	24.128	25.107
6	-5.2	24.788	25.765
7	-5.1	18.090	20.318
8	-5.0	22.880	23.808
9	-4.9	32.626	34.791

Binding affinity: -6.5 kcal/mol

Clofibric acid also has a strong binding affinity, although slightly lower than naproxen and diclofenac.

7. Carbamezapine

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.0	0.000	0.000
2	-6.9	21.509	24.247
3	-6.8	24.250	26.903
4	-6.8	8.132	10.551
5	-6.7	1.523	2.217
6	-6.6	37.371	38.655
7	-6.6	1.879	4.334
8	-6.6	12.355	14.197
9	-6.4	36.987	39.434

Binding affinity: -7.0 kcal/mol

Carbamazepine exhibits the strongest binding affinity among the pollutants, indicating it has a highly favorable interaction with the laccase enzyme.

4.2 Structural and visualization analysis of enzyme and pollutants

Figure 19- interaction of laccase with the ligand asprin

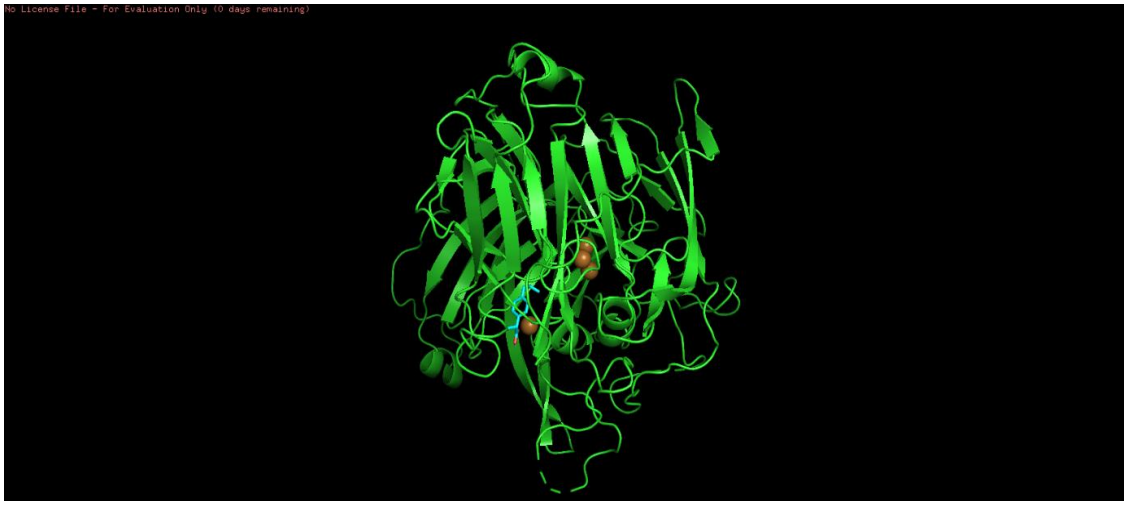


Figure 20 - interaction of laccase with ligand ibuprofen

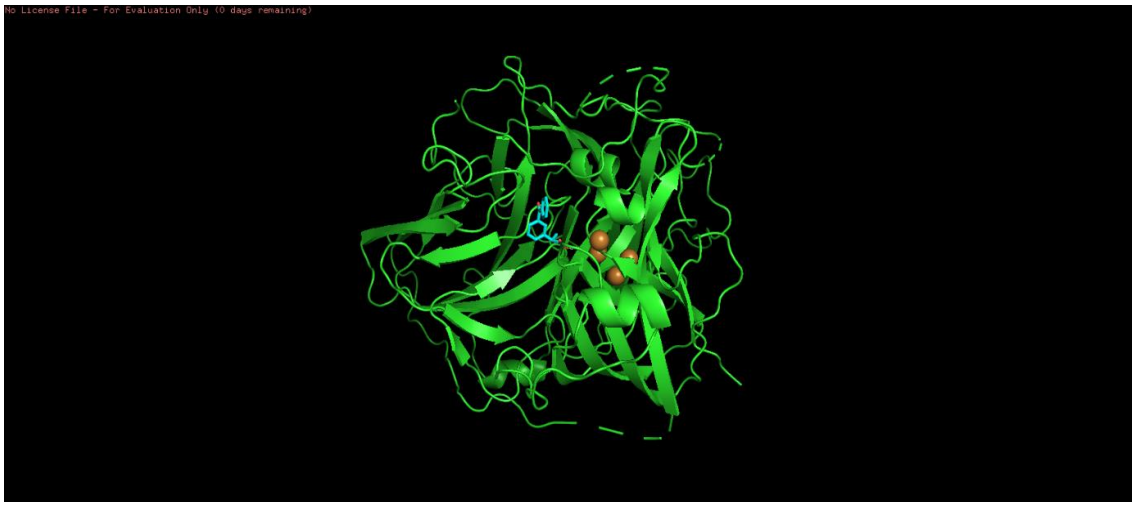


Figure 21 - interaction of laccase with ligand ketoprofen

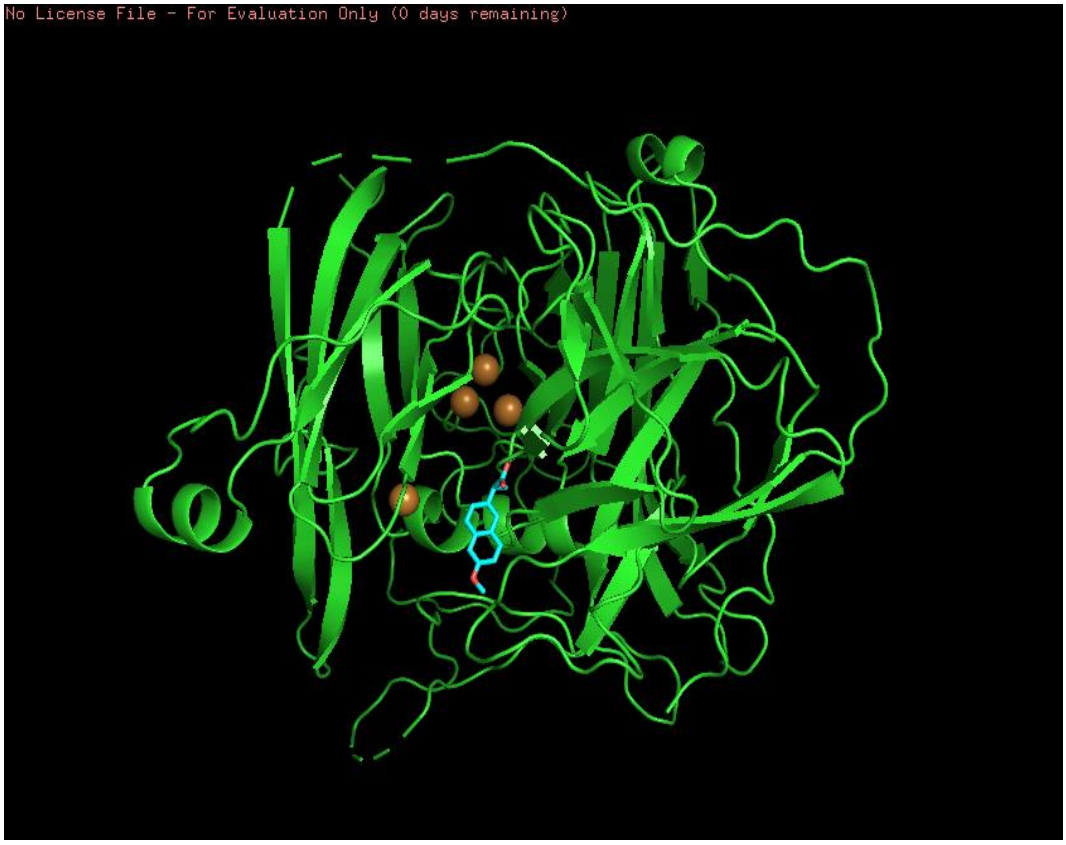


Figure 22 - Interaction of laccase with ligand naproxen

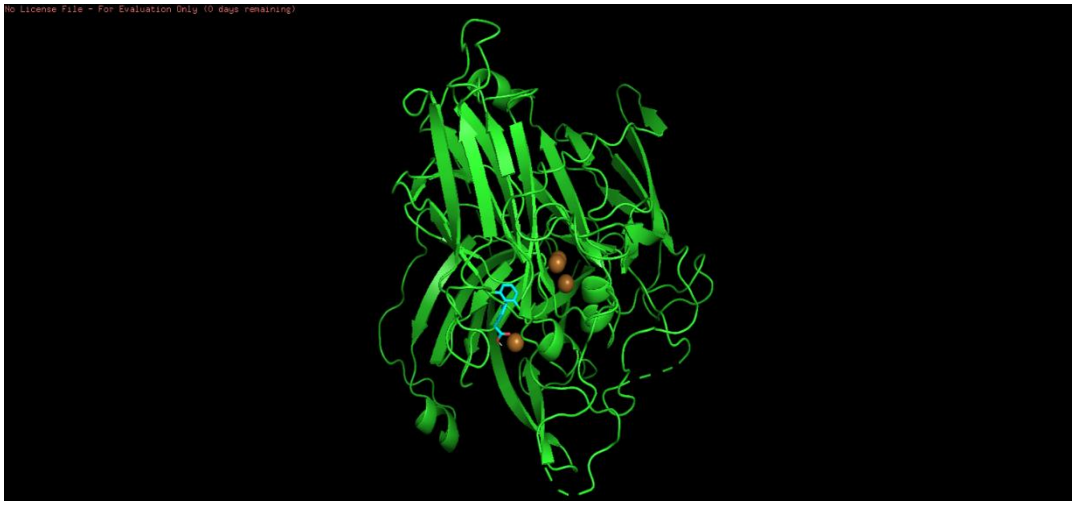


Figure 23 - interaction of laccase with ligand diclofenac

Figure 24 - interaction of laccase with ligand clofibric acid

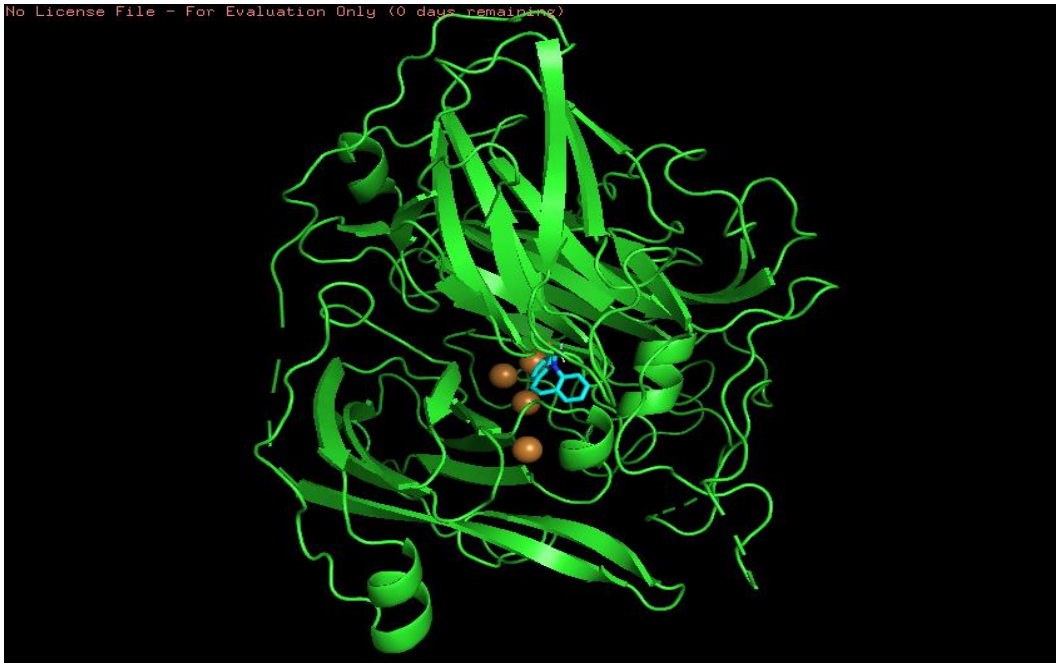


Figure 25 - interaction of laccase with ligand carbamazepine

4.3 Predicted pathways by PathPred

Following the molecular docking studies, pollutant carbamazepine showed the highest binding affinity of -7.0 kcal/mol against all the pollutants. This strong binding affinity suggests that there is a strong interaction and carbamazepine could be the potential substrate of the enzyme laccase. To further understand the molecular degradation pathways of carbamazepine pollutant, PathPred tool is used. In the below figure, pathway prediction tree is illustrated.

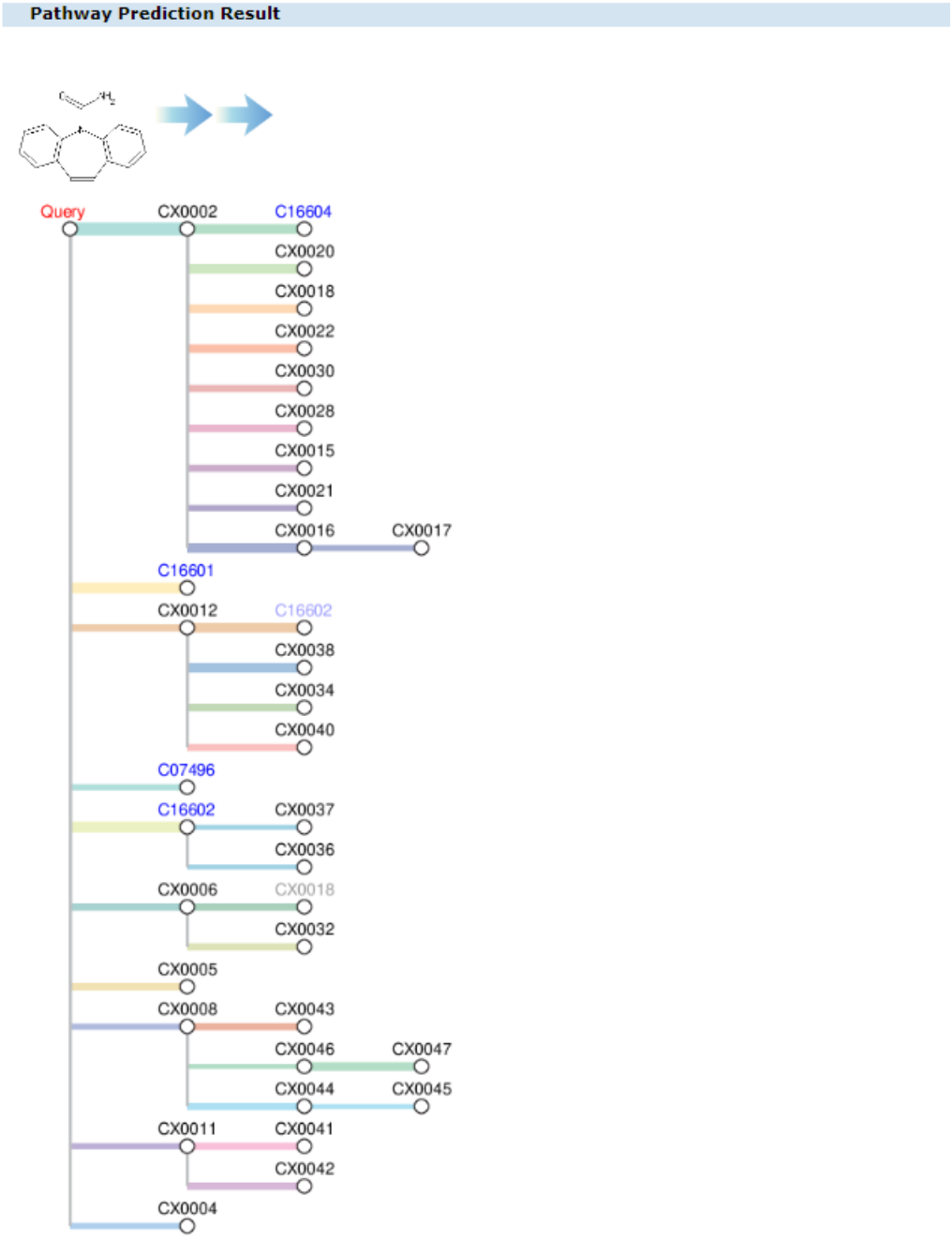


Figure 26 - the predicted pathway tree of Carbamazepine biodegradation

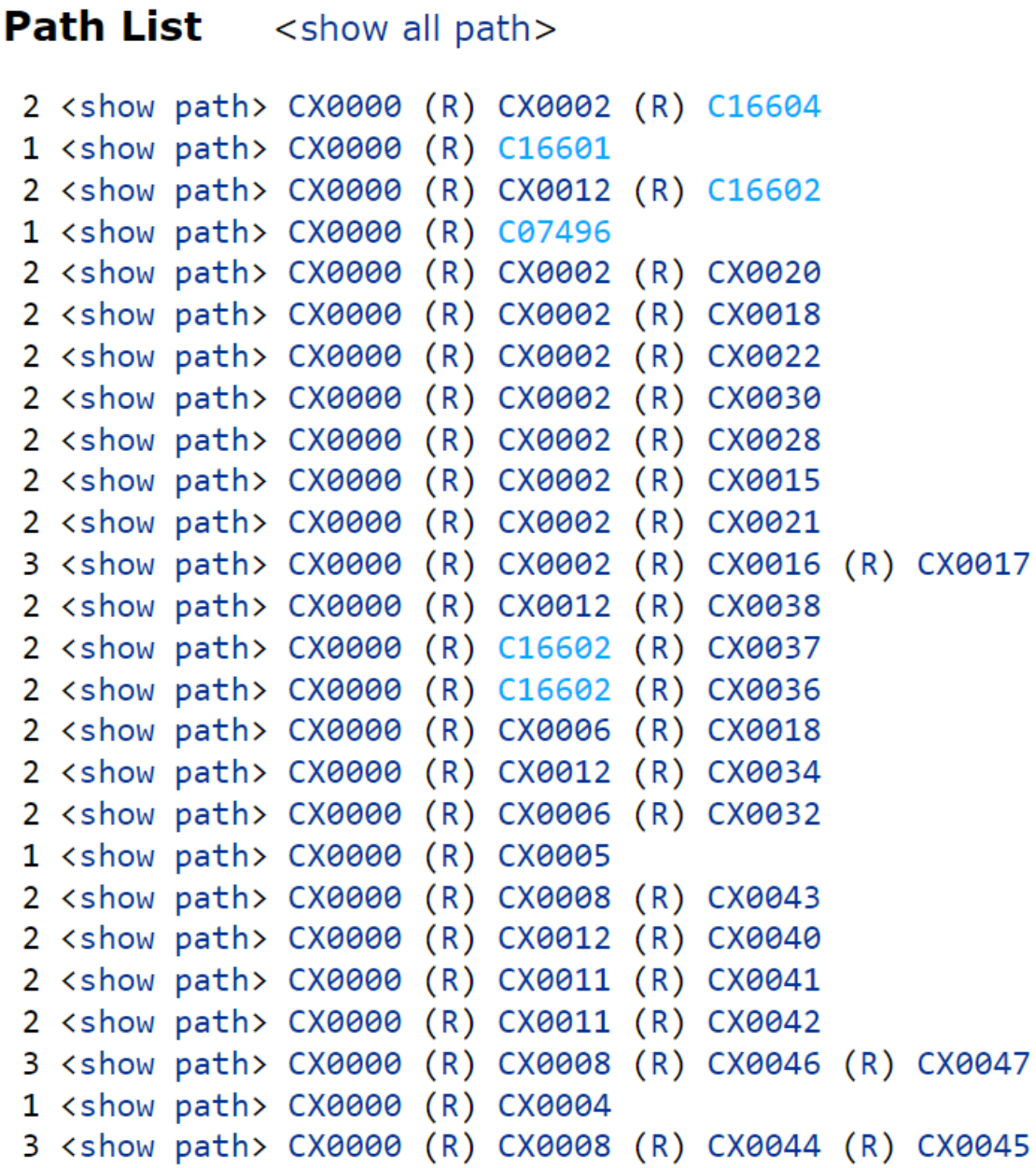


Figure 27 - Path list of biodegradations of carbamazepine

Herein, is biodegradation pathway prediction, from carbamazepine to 2-Hydroxyiminostilbene (C16604). Also, it shows other pathways that lead to the modification in the structure of carbamazepine - 3-Hydroxycarbamazepine (C16602) and 2-Hydroxycarbamazepine (C16601). In the below figure an example of pathway prediction is depicted.

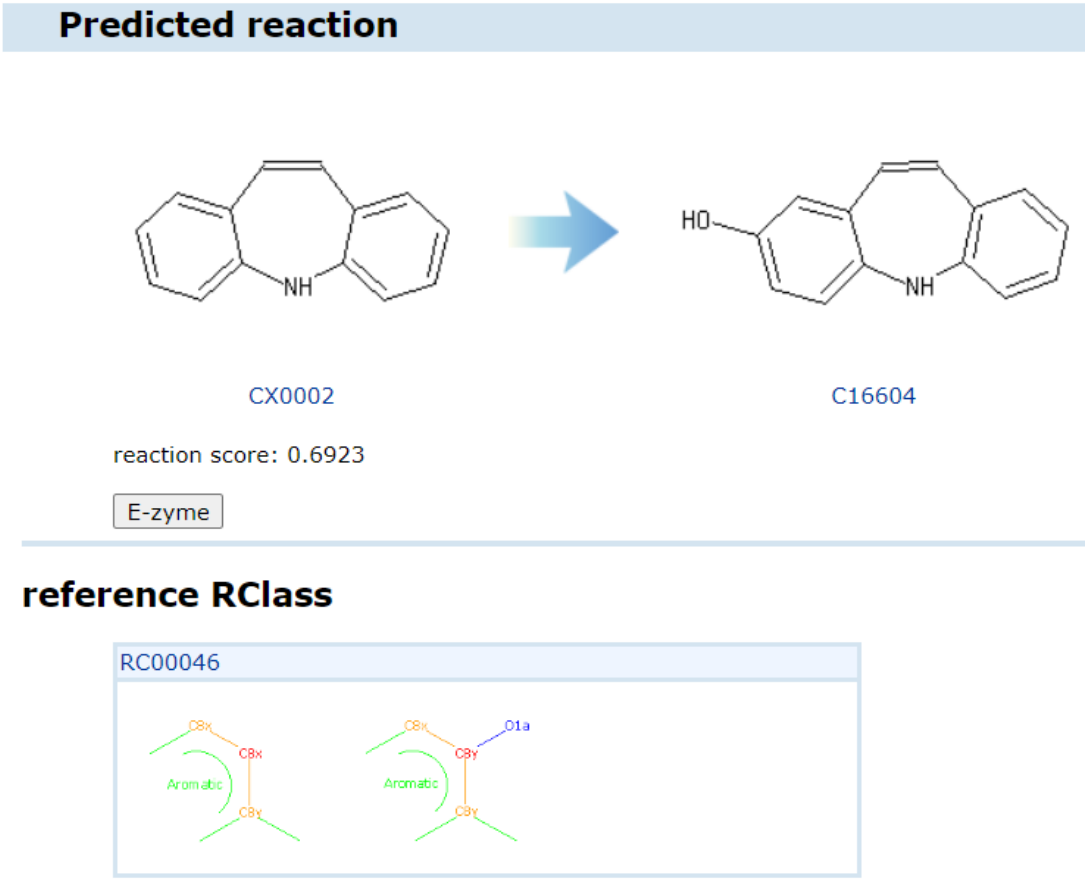


Figure 28 - Pathway from query compound to C16604 is depicted

With the help of the identification of metabolites and degradation pathways our understanding of the degradation process will be better.

CHAPTER -5
FUTURE PROSPECTS

The approach of in-silico mediated bioremediation using laccase enzyme mediated molecular docking and use of pathway prediction tools for the prediction of a biodegradation pathway of a targeted compound holds immense value. Using in-silico approach is more economical and sustainable to the world. With the rapid advancements in computational sciences, the quality and precision of results given by the molecular docking processes and pathway prediction tools will be greater. In near by using high-end computational algorithms and software we will be able to accurately predict the enzyme-substrate interactions and identify more pollutants that can be effectively biodegraded by laccase enzymes. By using these tools, we can study the dynamics of protein-ligand complex stability in bioremediation process. Using in-silico tools for the study not only saves the time but also accelerate the process of screening of compounds. By combining big data analytics and molecular docking approaches, we can analyse a much larger dataset of environmental pollutants and enzymes together, find out places that are hotspots for the pollution and also adapt our bioremediation strategy in a very minute level also to meets the requirements of local ecosystem and microbial communities present there. There is a need to Fastly develop high-throughput in-silico techniques so we can effectively analyse those enzymes from the various classes that have a role in bioremediation and biodegradation of pollutants. High-throughput will fasten the search of enzymes for our target pollutants.

Nowadays, many researchers have also started incorporating artificial intelligence and machine learning in the field of bioremediation and biodegradation. These technologies will further help in modifying the microbial pathways for the degradation. These techniques can also help in improving the enzyme structures. Furthermore, we will be able to explore and the knowledge of our understanding of microbial pathways will increase. We will be better in approaching the problem of biodegradation.

Synthetic biology-based biosensors are already there for the determination of real-time pollutant level in the environment. If we integrate in-silico technologies with these biosensors we will be able to get pollutant level throughout the time. This will aid in understanding the microorganisms and pollutant interaction and we will be able to use this knowledge as feedback and apply it in our computational models and more effectively mimic the biological surroundings around the pollutants.

Hence, by more incorporating in-silico bioremediation strategies we will be able to effectively solve this problem in economical ways. The future of molecular docking-based studies will improve as more technological advancements are coming in near future. Environmental pollution is a global problem and is cause of many health-related diseases, therefore, researchers all over the world are studying how technologies can be employed for bioremediation strategies.

CHAPTER-6
CONCLUSIONS

In this study we investigated laccase mediated bioremediation of seven selected pharmaceutical compounds – Aspirin, Ibuprofen, Ketoprofen, Naproxen, Diclofenac, Clofibric acid and Carbamazepine. The results of molecular docking, visualization analysis was presented. Predictive design of synthetic pathways of carbamazepine is also shown. Our findings suggest that laccase enzyme has the capability to degrade these pharmaceutical pollutants. This in-silico study also helps in exploring the enzyme-substrate interactions.

CHAPTER-7
REFERENCES

[1] M. A. El-Gammal, A. S. Elsaeidy, H. Ashry, and A. W. M. Jobran, "Biodegradation method of pharmaceuticals and personal care products," in *Handbook of Biodegradable Materials*, Cham: Springer International Publishing, 2022, pp. 1–39. doi: 10.1007/978-3-030-83783-9_49-1.

[2] B. I. Ashiwaju, C. G. Uzougbo, and O. F. Orikpete, "Environmental Impact of Pharmaceuticals: A Comprehensive Review," *Matrix Science Pharma*, vol. 7, no. 3, pp. 85–94, 2023, doi: 10.4103/mtsp.mtsp_15_23.

[3] M. A. El-Gammal, A. S. Elsaeidy, H. Ashry, and A. W. M. Jobran, "Biodegradation method of pharmaceuticals and personal care products," in *Handbook of Biodegradable Materials*, Cham: Springer International Publishing, 2022, pp. 1–39. doi: 10.1007/978-3-030-83783-9_49-1.

[4] M. Hejna, D. Kapuścińska, and A. Aksmann, "Pharmaceuticals in the Aquatic Environment: A Review on Eco-Toxicology and the Remediation Potential of Algae," *Int J Environ Res Public Health*, vol. 19, no. 13, p. 7717, Jun. 2022, doi: 10.3390/ijerph19137717.

[5] K. Balakrishna, A. Rath, Y. Praveenkumarreddy, K. S. Guruge, and B. Subedi, "A review of the occurrence of pharmaceuticals and personal care products in Indian water bodies," *Ecotoxicol Environ Saf*, vol. 137, pp. 113–120, Mar. 2017, doi: 10.1016/j.ecoenv.2016.11.014.

[6] N. M. Jabbar, S. M. Alardhi, A. K. Mohammed, I. K. Salih, and T. M. Albayati, "Challenges in the implementation of bioremediation processes in petroleum-contaminated soils: A review," *Environmental Nanotechnology, Monitoring and Management*, vol. 18. Elsevier B.V., Dec. 01, 2022. doi: 10.1016/j.enmm.2022.100694.

[7] A. K. Singh, P. Chowdhary, and A. Raj, "In silico bioremediation strategies for removal of environmental pollutants released from paper mills using bacterial ligninolytic enzymes," in *Microorganisms for Sustainable Environment and Health*, Elsevier, 2020, pp. 249–285. doi: 10.1016/B978-0-12-819001-2.00013-9.

[8] E. L. Rylott and N. C. Bruce, "How synthetic biology can help bioremediation," *Current Opinion in Chemical Biology*, vol. 58. Elsevier Ltd, pp. 86–95, Oct. 01, 2020. doi: 10.1016/j.cbpa.2020.07.004.

[9] T. M. Nolte, K. Pinto-Gil, A. J. Hendriks, A. M. J. Ragas, and M. Pastor, "Quantitative structure–activity relationships for primary aerobic biodegradation of organic chemicals in pristine surface waters: starting points

for predicting biodegradation under acclimatization," *Environ Sci Process Impacts*, vol. 20, no. 1, pp. 157–170, 2018, doi: 10.1039/C7EM00375G.

[10] C. O. Adetunji *et al.*, "In silico approaches for xenobiotic polymers and their degradation mechanism," in *In silico Approaches to Macromolecular Chemistry*, Elsevier, 2023, pp. 479–501. doi: 10.1016/B978-0-323-90995-2.00003-5.

[11] A. Chakraborty *et al.*, "Pharmaceuticals and Personal Care Products as Emerging Environmental Contaminants: Prevalence, Toxicity, and Remedial Approaches," *ACS Chemical Health & Safety*, vol. 30, no. 6, pp. 362–388, Nov. 2023, doi: 10.1021/acs.chas.3c00071.

[12] R. B. González-González *et al.*, "Persistence, environmental hazards, and mitigation of pharmaceutically active residual contaminants from water matrices," *Science of The Total Environment*, vol. 821, p. 153329, May 2022, doi: 10.1016/j.scitotenv.2022.153329.

[13] N. Nassiri Koopaei and M. Abdollahi, "Health risks associated with the pharmaceuticals in wastewater," *DARU Journal of Pharmaceutical Sciences*, vol. 25, no. 1, p. 9, Dec. 2017, doi: 10.1186/s40199-017-0176-y.

[14] Chhaya, T. Raychoudhury, and S. K. Prajapati, "Bioremediation of Pharmaceuticals in Water and Wastewater," in *Microbial Bioremediation & Biodegradation*, Singapore: Springer Singapore, 2020, pp. 425–446. doi: 10.1007/978-981-15-1812-6_16.

[15] I. Pinto, M. Simões, and I. B. Gomes, "An Overview of the Impact of Pharmaceuticals on Aquatic Microbial Communities," *Antibiotics*, vol. 11, no. 12, p. 1700, Nov. 2022, doi: 10.3390/antibiotics11121700.

[16] P. Chaturvedi *et al.*, "Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants," *Environ Res*, vol. 194, p. 110664, Mar. 2021, doi: 10.1016/j.envres.2020.110664.

[17] S. I. Polianciuc, A. E. Gurzău, B. Kiss, M. G. Ștefan, and F. Loghin, "Antibiotics in the environment: causes and consequences," *Med Pharm Rep*, Jul. 2020, doi: 10.15386/mpr-1742.

[18] A. Elizalde-Velázquez, L. M. Gómez-Oliván, M. Galar-Martínez, H. Islas-Flores, O. Dublán-García, and N. SanJuan-Reyes, "Amoxicillin in the Aquatic Environment, Its Fate and Environmental Risk," in *Environmental Health Risk - Hazardous Factors to Living Species*, InTech, 2016. doi: 10.5772/62049.

[19] J. E. Sosa-Hernández *et al.*, "Sources of antibiotics pollutants in the aquatic environment under SARS-CoV-2 pandemic situation," *Case Studies in*

Chemical and Environmental Engineering, vol. 4, p. 100127, Dec. 2021, doi: 10.1016/j.cscee.2021.100127.

[20] K. K. Sodhi, M. Kumar, and D. K. Singh, "Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies," *Journal of Water Process Engineering*, vol. 39, p. 101858, Feb. 2021, doi: 10.1016/j.jwpe.2020.101858.

[21] C. Uluseker *et al.*, "A Review on Occurrence and Spread of Antibiotic Resistance in Wastewaters and in Wastewater Treatment Plants: Mechanisms and Perspectives," *Front Microbiol*, vol. 12, Oct. 2021, doi: 10.3389/fmicb.2021.717809.

[22] Y. Amangelsin, Y. Semenova, M. Dadar, M. Aljofan, and G. Bjørklund, "The Impact of Tetracycline Pollution on the Aquatic Environment and Removal Strategies," *Antibiotics*, vol. 12, no. 3, p. 440, Feb. 2023, doi: 10.3390/antibiotics12030440.

[23] A. Barra Caracciolo, E. Topp, and P. Grenni, "Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review," *J Pharm Biomed Anal*, vol. 106, pp. 25–36, Mar. 2015, doi: 10.1016/j.jpba.2014.11.040.

[24] A. K. Singh, P. Chowdhary, and A. Raj, "In silico bioremediation strategies for removal of environmental pollutants released from paper mills using bacterial ligninolytic enzymes," in *Microorganisms for Sustainable Environment and Health*, Elsevier, 2020, pp. 249–285. doi: 10.1016/B978-0-12-819001-2.00013-9.

[25] B. Zhu, Y. Chen, and N. Wei, "Engineering Biocatalytic and Biosorptive Materials for Environmental Applications," *Trends Biotechnol*, vol. 37, no. 6, pp. 661–676, Jun. 2019, doi: 10.1016/j.tibtech.2018.11.005.

[26] A. K. Singh, M. Bilal, H. M. N. Iqbal, and A. Raj, "Trends in predictive biodegradation for sustainable mitigation of environmental pollutants: Recent progress and future outlook," *Science of the Total Environment*, vol. 770. Elsevier B.V., May 20, 2021. doi: 10.1016/j.scitotenv.2020.144561.

[27] M. A. Malla, A. Dubey, A. Raj, A. Kumar, N. Upadhyay, and S. Yadav, "Emerging frontiers in microbe-mediated pesticide remediation: Unveiling role of omics and In silico approaches in engineered environment," *Environmental Pollution*, vol. 299, p. 118851, Apr. 2022, doi: 10.1016/j.envpol.2022.118851.

[28] A. K. Singh, P. Chowdhary, and A. Raj, "In silico bioremediation strategies for removal of environmental pollutants released from paper mills using bacterial ligninolytic enzymes," in *Microorganisms for Sustainable Environment and Health*, Elsevier, 2020, pp. 249–285. doi: 10.1016/B978-0-12-819001-2.00013-9.

[29] Z. Basharat, M. Bibi, and A. Yasmin, “Implications of Molecular Docking Assay for Bioremediation,” 2017, pp. 24–45. doi: 10.4018/978-1-5225-2325-3.ch002.

[30] S. Srinivasan, S. K. Sadasivam, S. Gunalan, G. Shanmugam, and G. Kothandan, “Application of docking and active site analysis for enzyme linked biodegradation of textile dyes,” *Environmental Pollution*, vol. 248, pp. 599–608, May 2019, doi: 10.1016/j.envpol.2019.02.080.

[31] D. Chmelová, M. Ondrejovič, and S. Miertuš, “Laccases as Effective Tools in the Removal of Pharmaceutical Products from Aquatic Systems,” *Life*, vol. 14, no. 2, p. 230, Feb. 2024, doi: 10.3390/life14020230.

[32] Y. Moriya *et al.*, “PathPred: an enzyme-catalyzed metabolic pathway prediction server,” *Nucleic Acids Res*, vol. 38, no. Web Server, pp. W138–W143, Jul. 2010, doi: 10.1093/nar/gkq318.

[33] A. Castro-Alvarez, A. Costa, and J. Vilarrasa, “The Performance of Several Docking Programs at Reproducing Protein–Macrolide-Like Crystal Structures,” *Molecules*, vol. 22, no. 1, p. 136, Jan. 2017, doi: 10.3390/molecules22010136.

LIST OF PUBLICATIONS

1. Review paper titled, “The utility of synthetic biology in treatment of industrial wastewater” has been Accepted with revisions in the journal titled, “Nature Environment and Pollution Technology”.

Submission of manuscript for publication

operations@neptjournal.com <operations@neptjournal.com> Mon, May 20, 2024 at 2:54 PM
To: "Dr. Jai Gopal Sharma" <sharmajaigopal@dce.ac.in>

Dear Author,

We are pleased to inform you that our referees have reviewed your manuscript titled “The utility of synthetic biology in treatment of industrial wastewater " and have recommended it for publication in the journal Nature Environment and Pollution Technology.

However, please note that your paper has been Accepted with revisions based on the recommendations of the reviewers. The review comments have been attached to this email for your perusal. We request you to revise the manuscript accordingly and send it back to us within five days. While making revisions, please use the Track Changes feature or highlight the text for easy identification.

Please use the attached file for the corrections as already some modifications are made to the document.

We appreciate your efforts and look forward to receiving the revised version of your manuscript.

Best regards,

Apurva Goel

Managing Editor

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of engineering)
Shahabad Daulatpur, Main Bawana road, Delhi, India

PLAGIARISM VERIFICATION

Title of the Thesis LACCASE MEDIATED BIOREMEDIATION OF PHARMACEUTICAL POLLUTANTS: MOLECULAR DOCKING AND SYNTHETIC PATHWAY PREDICTION OF CARBAMAZEPINE

Total Pages 43 Name of the Scholar MONICA JOSHI

Supervisor (s)

(1) Prof. Jai Gopal Sharma

(2) _____

(3)_____

Department BIOTECHNOLOGY, DELHI TECHNOLOGICAL UNIVERSITY

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

Software used: Turnitin Similarity Index: 10% Total Word Count: 8024

Date: 31/05/2024

Candidate's Signature **Signature of Supervisor(s)**

PAPER NAME

major thesis final.docx

WORD COUNT	CHARACTER COUNT
8024 Words	49181 Characters
PAGE COUNT	FILE SIZE
43 Pages	3.9MB
SUBMISSION DATE	REPORT DATE
May 31, 2024 12:06 PM GMT+5:30	May 31, 2024 12:07 PM GMT+5:30

10% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 5% Internet database
- 9% Publications database
- Crossref database
- Crossref Posted Content database

Excluded from Similarity Report

- Submitted Works database
- Bibliographic material
- Quoted material
- Cited material
- Small Matches (Less then 8 words)

How much of this submission has been generated by AI?

***2%**

of qualifying text in this submission has been determined to be generated by AI.

* Low scores have a higher likelihood of false positives.

Caution: Percentage may not indicate academic misconduct. Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Frequently Asked Questions

What does the percentage mean?
The percentage shown in the AI writing detection indicator and in the AI writing report is the amount of qualifying text within the submission that Turnitin's AI writing detection model determines was generated by AI.

Our testing has found that there is a higher incidence of false positives when the percentage is less than 20. In order to reduce the likelihood of misinterpretation, the AI indicator will display an asterisk for percentages less than 20 to call attention to the fact that the score is less reliable.

However, the final decision on whether any misconduct has occurred rests with the reviewer/instructor. They should use the percentage as a means to start a formative conversation with their student and/or use it to examine the submitted assignment in greater detail according to their school's policies.

How does Turnitin's indicator address false positives?
Our model only processes qualifying text in the form of long-form writing. Long-form writing means individual sentences contained in paragraphs that make up a longer piece of written work, such as an essay, a dissertation, or an article, etc. Qualifying text that has been determined to be AI-generated will be highlighted blue on the submission text.

Non-qualifying text, such as bullet points, annotated bibliographies, etc., will not be processed and can create disparity between the submission highlights and the percentage shown.

What does 'qualifying text' mean?
Sometimes false positives (incorrectly flagging human-written text as AI-generated), can include lists without a lot of structural variation, text that literally repeats itself, or text that has been paraphrased without developing new ideas. If our indicator shows a higher amount of AI writing in such text, we advise you to take that into consideration when looking at the percentage indicated.

In a longer document with a mix of authentic writing and AI generated text, it can be difficult to exactly determine where the AI writing begins and original writing ends, but our model should give you a reliable guide to start conversations with the submitting student.

MONICA JOSHI

+91-8700645886 joshimonica843@gmail.com

EDUCATION

M.Tech(Bioinformatics)	2022 ongoing	Delhi Technological University, Delhi	8.00 CGPA
B.Tech(Biotechnology)	2018-2022	Jaypee Institute of Information Technology, Sec 62, Noida	6.7 CGPA
Class XII (CBSE)	2017	KV 1 AFS Hindon, Ghaziabad	83.25%
Class X (CBSE)	2015	KV 1 AFS Hindon, Ghaziabad	9.4 CGPA

INTERNSHIPS

- Nano Science and Technology Consortium, Noida [July 2023].
  Successfully completed Next Generation Sequence Data Analysis using Galaxy Platform.
- Intern at DRDO INMAS, Delhi [July 2021- August 2021].
  Gained expertise in research methodologies, data analysis, and trend identification within the field of ORCS (Ozonated Radical Confined Space).

ACADEMIC PROJECT

- Study of Impact of Solar Panels on Environment.
  Established impact of air pollution on efficiency of photovoltaic cells in generating power.
- Designed and implemented a MySQL database system for the blood donation process.
- Implemented ANN (Artificial Neural Network) to predict heart disease occurrence based on comprehensive heart disease dataset.

ACADEMIC ACHIEVEMENTS

- Qualified NCERT Common Entrance Examination with State Rank of 9.
- Qualified GATE Entrance Examination with all India Rank of 2026.

TECHNICAL SKILLS

Python, SQL	MySQL, Tableau, Excel, Jupyter Notebook, Machine Learning (Basic)	Literature Review, Biological databases (such as Uniprot, NCBI), NGS, Western blotting, Scientific writing

RELEVANT COURSES

- Bioinformatics
- Advanced Proteomics
- OMICS in Medicine

EXTRA CURRICULAR ACTIVITIES

- Participated and give E-short presentation in ICABB in Jaypee University.
- Achieved participation certificates on other seminars.