E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia Science & Technology Asia
Vol.30 No.2 April - June 2025 Page: [268-281]
Original research article

Molecular Docking of Laccase from *Trametes Versicolor* with Ligand Substrates from Textile Dyes and Antibiotics Impacting the Environment

Ugochukwu Okechukwu Ozojiofor[1,*], Mohammed Sani Abdulsalami[1], Nkechi Eucharia Egbe[1], Ahmed Ali Haroun[1], Peter Maitalata Waziri[2], Abba Umar Hassan[1], Kingsley Onuh[1]

[1]*Department of Biotechnology, Faculty of Science, Nigerian Defence Academy, Kaduna 800283, Nigeria*
[2]*Department of Biochemistry, Kaduna State University, Kaduna 800283, Nigeria*

Received 13 February 2025; Received in revised form 7 May 2025
Accepted 27 May 2025; Available online 18 June 2025

ABSTRACT

Environmental pollution from textiles and pharmaceuticals is a primary source of concern for various environmental protection agencies. The nature of synthetic dyes and the increasing rate of antibiotic resistance by bacteria have made them a major health concern. Laccases are capable of degrading organic pollutants, including phenols, dyes, bisphenol, polyaromatic hydrocarbon (PAHs) and pharmaceuticals. This study focused on assessing the toxicity and binding interaction of eight ligands of environmental importance with laccase from a fungus, *Trametes versicolor*, via an in-silico approach. The canonical SMILES of the ligands were retrieved from the PubChem database and the 3D structures were obtained using UCSF Chimera 1.18. The 3D structure of laccase was retrieved from the Protein Data Bank (PDB) and blind docked with the ligands. Discovery Studio 4.5 software was used to observe the different bonding interactions between the enzyme and the docked ligands. The ADMET study of the ligands was done using their canonical SMILES on the admetlab3.0 servers. The binding energies of laccase with Indigo carmine (IC), Malachite green (MG), Remazol brilliant blue R (RBBR), Direct red 75 (DR75), Ciprofloxacin (CIP), Amoxicillin (AMO), 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), and Guaiacol were −6.6, −7.0, −7.0, −7.4, −6.8, −7.0, −6.4, and −5.2 kcal/mol, respectively. All the dyes showed high probability for toxicity with values close to 1 and also environmental toxicity, with Malachite green (MG) showing the highest probability with bioconcentration factors (BCF), 50 percent growth inhibition concentration of *Tetrahymena pyriformis* (IGC_{50}), 96-hour fathead minnow 50 percent lethal concentration (LC_{50}FM), and 48-hour *Daphnia magna* 50 percent lethal

***Corresponding author:** ozojiofor.uo@nda.edu.ng **doi:** 10.14456/scitechasia.2025.40

concentration (LC_{50}DM) of 1.683, 3.858, 4.567, and 5.446. The active site of the enzyme that interacted with the eight ligands was found to contain one of these eleven (11) amino acid residues, namely, HIS, GLN, ASP, ALA, PHE, SER, LEU, ARG, VAL, PRO, and GLU in the docked complexes.

Keywords: Antibiotics; Dyes; Molecular docking; Toxicity; *Trametes versicolor*

1. Introduction

Laccases (p-diphenol: dioxygen oxidoreductase, EC 1.10.3.2), a family of copper-containing polyphenol oxidases found in bacteria, insects, fungi, and plants, are examples of multicopper oxidases (MCOs) [1]. In 1883, Yoshida discovered laccases in the exudates of the *Rhus vernicifera*, a Japanese lacquer tree [2].

A broad spectrum of substrates, including organic pollutants, anilines, aryl diamines, methoxy-substituted phenols, benzenethiols, aromatic diamines, hydroxyindiols, inorganic/organic metal compounds, and other compounds, can be oxidised by laccases by reducing molecular oxygen to water [3, 4]. Pathogenesis, delignification, decolorisation, depigmentation, and pigmentation are among the physiological processes that have been linked to fungal laccases, which have been the focus of much research [1].

Fungi belonging to the deuteromycetes, ascomycetes, and basidiomycetes groups are known to produce laccases. White-rot fungi that belong to the class of basidiomycetes are the most effective laccase producers and lignin biodegraders, according to Arora and Sharma [5]. Laccase research has made considerable use of two model fungi: *Trametes versicolor* and *Pleurotus ostreatus*. *Phlebia radiata*, *Cerrena unicolor*, *Polyporus brumalis*, *Fomes fomentarius*, *Panus rudis*, and *Cyathus bulleri* are other recognised basidiomycetes that generate laccases [6, 7].

Textile effluents are the most significant pollutants in terms of toxicity, volume, and discharge [8]. They contain a high concentration of organic and inorganic toxic compounds, and even after treatment, about 80% of the dyes still end up in aquatic environments without going through the proper chemical changes [9]. The aromatic structure, photostability, high heat resistance, and xenobiotic nature of these dyes prevent microbial enzymes from breaking them down [10]. Although the secondary products of anaerobic dye reduction, such as aromatic amines produced by biodegradation, can be hazardous, Guta et al. [8] showed that dye toxicity to mammals and aquatic organisms is rather low. The main health issue with dye discharge into aquatic environments is the possibility of mutagenic, carcinogenic, teratogenic, and genotoxic effects, which have been reported in some animal studies [8]. They are carcinogenic because they produce ions that attach to DNA and RNA and cause tumours and mutations. Water resource quality evaluation has received a lot of attention lately due to the increased emergence of many recently found substances with bioactive and bioaccumulative qualities, such as pharmaceuticals, which could contribute to pollution [11].

According to UNESCO [12], emerging contaminants (ECs) are a broad group of pollutants that are becoming increasingly prevalent. These include substances that are found in the environment, both natural and synthetics chemicals, and microbes that may be harmful to both human health and the ecosystem but are not routinely monitored or regulated. Controlling the contamination that ECs produce is therefore a new challenge for water quality worldwide. Because of their extensive use, physicochemical properties, and

capacity to be partially removed by conventional wastewater treatment processes, pharmaceuticals are therefore among the most important ECs [13].

Antibiotics are used for human, veterinary, and animal health, and they are among the most often used pharmaceuticals globally. This is one of the factors contributing to resistance to these treatments. Since non-metabolized antibiotics cannot be removed by water treatment systems, they end up in the environment and persist [14]. Antibiotics like trimethoprim, tetracyclines, sulfamethoxazole, penicillins, sulfonamides, and quinolones have all been shown to be degraded by laccases. The most often used enzyme in research on the biodegradation of several micropollutants and antibiotics is the laccase from *T. versicolor* [7].

The aim of this study is to understand the potential of laccase from *T. versicolor* to biodegrade eight compounds impacting the environment adversely through their interactions with the enzyme and to predict their environmental toxicity via In-silico studies. To understand how *T. versicolor* laccase interacts with compounds that have adverse effects on the environment, molecular docking was employed. The study's findings could help future experimental research on the treatment of industrial wastewater by theoretically forecasting the possible environmental effects of several other newly discovered contaminants on fungal laccases, particularly *T. versicolor* laccase.

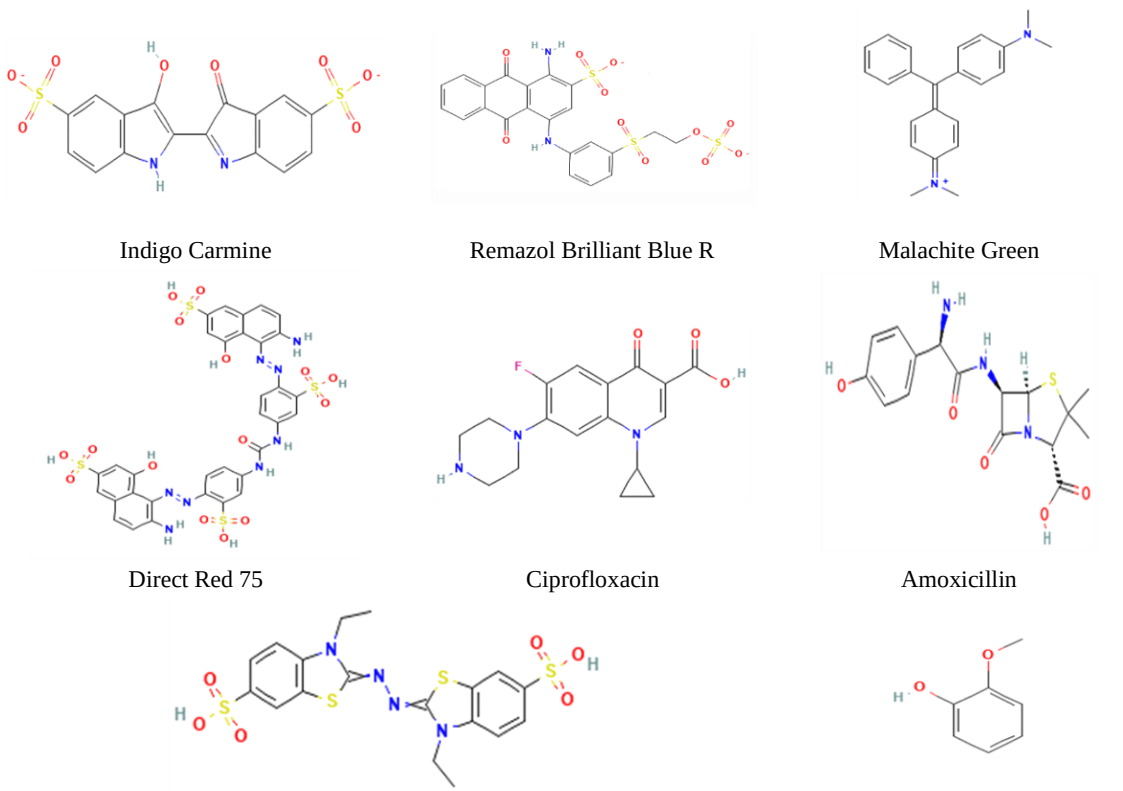


Fig. 1. Chemical structure of the compounds investigated.

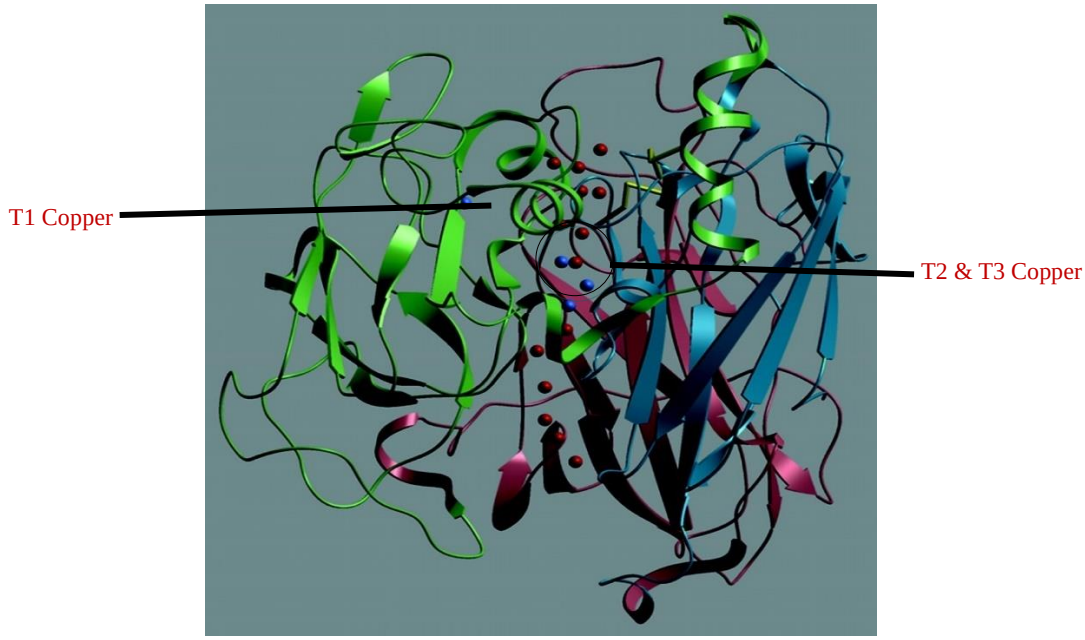


Fig. 2. 3D structure of laccase from *T. versicolor* retrieved from PDB (1GYC) showing the two channels leading to the T2/T3 cluster. Water molecules are depicted as red spheres, and copper ions are depicted as blue spheres [15].

2. Materials And Methods

2.1. Ligands

The four textile dyes used were: Remazol Brilliant Blue R (RBBR), Indigo Carmine (IC), Direct Red 75 (DR75), Malachite Green (MG), two antibiotics, Amoxicillin (AMO), Ciprofloxacin (CIP), and two laccase substrates (ABTS and Guaiacol) as controls. Figure 1 shows their chemical structures while Figure 2 shows the 3D structure of laccase.

2.2 Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) study of the dyes and pharmaceuticals

The canonical SMILES used for the ADMET study were obtained from the PubChem website (pubchem.ncbi.nlm.nih.gov) for the dyes (Direct Red 75, Remazol Brilliant Blue R, Indigo Carmine, and Malachite Green), pharmaceuticals (Ciprofloxacin and Amoxicillin), and the laccase substrates (ABTS and Guaiacol). The ADMET evaluation was done using prediction servers and the online web-based predictive tool SwissADME (http://www.swissadme.ch/index.php) which enables calculations and predictions for 21 physicochemical properties, 19 medicinal chemistry properties, 34 ADME endpoints, 36 toxicity endpoints, and 8 toxicophore rules (751 substructures) [16]. The toxicity factors assessed by the ADMET server were the AMES toxicity, carcinogenicity, drug induced liver injury (DILI), bioconcentration factors (BCF), 50 percent growth inhibition concentration of *Tetrahymena pyriformis* (IGC_{50}), 96-hour fathead minnow 50 percent lethal concentration ($LC_{50}FM$), and 48-hour *Daphnia magna* 50 percent lethal concentration ($LC_{50}DM$) [17].

2.3 Molecular Docking between Laccase, Dyes, and Pharmaceuticals

Molecular dockings between laccase, dyes, pharmaceuticals, and laccase substrates were operated with the AutoDock tools [18].

2.3.1 Ligand Preparation

The 3D models of the dyes, pharmaceuticals, and laccase substrates were obtained from the PubChem database (http://www.pubchem.ncbi.nlm.nih.gov) in SDF format [19], and optimized in BIOVIA Discovery Studio 4.5 Visualizer, and an online SMILE translator was then used to convert them into the Protein databank (PDB) format. The ligand was prepared by the addition of hydrogen, charges, and fixing of torsion and saved in the Chimera software in PDB format.

2.3.2 Receptor preparation

The 3D structure of laccase (PDB: 1GYC) were retrieved from PDB (https://www.rcsb.org), and the original ligands and water molecules were removed from the laccase protein using PyMOL [20].

2.3.3 Predictions for the Binding Site

The optimised protein was taken for binding site detection and to identify the key residues in the target protein that are responsible for ligand binding and are present in the active site [21] using Biovia Discovery Studio and a DoGSiteScorer (Zentrum für Bioinformatmatik: Universität Hamburg-Proteins Plus Server) [22].

2.3.4 Molecular Docking Studies

To determine the target site of the protein, blind docking was used, and the grid box was positioned at 40 points for X, Y, and Z dimensions, 1.0 Å for spacing; number of binding modes of 10, an energy range of 4, and an exhaustiveness of 8 were the grid box's parameters [17]. The interaction between the enzyme and ligands were studied via docking studies using AutoDockVina (version 1.1.2) and AutoDock Tools (ADT) of the MGL software package. After loading all 8 compounds, the PDB file was converted into a PDBQT format using ADT, which uses an incremental buildup algorithm to guide the flexible placement of ligand in the binding region [23]. In Vina Wizard (Version v1.2.3), the grid box (40×40×40 $Å^3$ with default grid spacing) was preserved at X: -41.3237, Y:16.2124, and Z:48.1140 for 1GYC. Following docking, the results were generated, and the best conformational pose with a high docking score was selected for analysis [23]. A total of ten bound conformations of each ligand were created, and the conformation with the best binding energy was used to visually understand the interaction between protein and Ligand molecules. To visualize the docked protein-ligand interaction, Discovery Studio software was used to observe the different bonding interactions between the enzyme and bound ligands.

3. Results and Discussions

Each dye used in this study is a representative of each class of textile dyes, namely; anthraquinone (RBBR), azo (DR75), Indigoid (IC), Triphenylmethane (MG), the antibiotics are two of the most consumed antibiotics [24], while ABTS and Guaiacol are well known substrate of laccase [25]. The ADMET properties of the eight ligands are presented in Tables 1-2. The in-silico studies showed the environmental toxicity of the four dyes and their potential carcinogenicity. The pharmaceuticals and controls showed lower carcinogenicity potential and did not violate the Lipinski rule of five as expected for a drug/compound but they did show some level of environmental toxicity. The dyes showed high probability for toxicity with values close to 1 and also environmental

toxicity, with malachite green showing the highest probability for BCF, IGC_{50}, $LC_{50}FM$, and LC_{50} DM of 1.683, 3.858, 4.567, and 5.446, respectively. Molecular docking was used to explore the interaction mechanism between laccase and the ligands. The result of the molecular docking of the ligands against laccase protein is shown in Table 3. All eight ligands bound to the substrate binding pocket of the laccase. The resolution of the PDB structure of the laccase from *T. versicolor* was 1.90Å, and the Ramachandran plot shows that 0.7% of the residues are visible in the disallowed regions with the enzyme having 499 amino acid residues, as shown in Table 4. The interactions between the ligands and enzymes showed hydrophobic and hydrogen bonding with several amino acids at the binding pocket of the enzymes (Figs. 3-4).

Table 1. ADMET prediction of the physicochemical and medicinal chemistry properties of the ligands.

Properties	**Physicochemical Property**					**Medicinal Chemistry**		
Ligands	**MW**	**Density**	**nHA**	**nHd**	**TPSA**	**NP score**	**Lipinski Rule violation**	**PAINS**
Indigo Carmine	466.367	1.166	10	4	174.19	0.029	0	0 alert
Malachite Green	364.9	0.875	2	0	6,25	-0.41	0	0 alert
Remazol Brilliant Blue R	579.99	1.166	13	3	229.96	-0.693	1	2 alerts
Direct Red 75	990.04	1.169	23	12	400.55	2.156	1	1 alert
Ciprofloxacin	331.4	1.041	6	2	74.57	0.725	0	0 alert
Amoxicillin	365.1	1.076	8	5	132.96	0.942	0	0 alert
ABTS	514.01	1.181	10	2	143.32	-1.052	0	0 alert
Guaiacol	124.05	0.949	2	1	29.46	0.504	0	0 alert

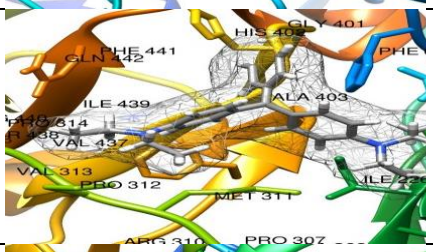
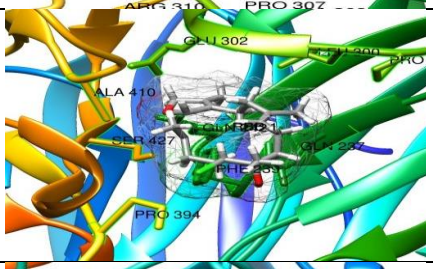
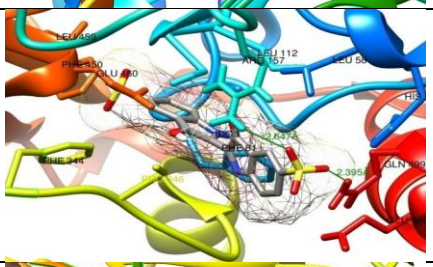
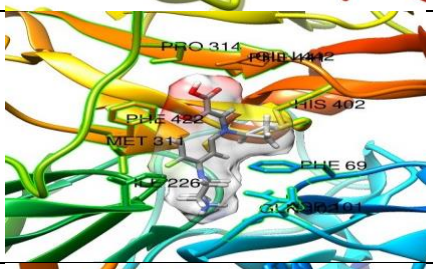
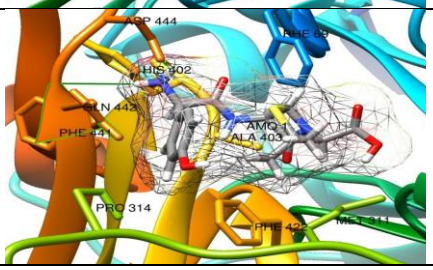
Key: nHA: No. of hydrogen bond acceptors; nHD: No. of hydrogen bond donors; NPscore: Natural Product score; TPSA: Topological polar surface area; PAIN: Pan assay interference compounds.

Table 2. ADMET prediction of the cellular and environmental toxicity of the ligands.

Properties	**Toxicity**			**Environmental Toxicity**			
Ligands	**AMES toxicity**	**Carcinogencity**	**DILI**	**BCF**	**IGC_{50}**	**$LC_{50}FM$**	**$LC_{50}DM$**
Indigo Carmine	0.637	0.523	0.982	0.423	2.916	3.788	3.953
Malachite Green	0.709	0.816	0.97	1.683	3.858	4.567	5.446
Remazol Brilliant Blue R	0.985	0.717	1.0	0.549	1.668	2.08	3.706
Direct Red 75	0.709	0.816	0.97	0.121	2.686	3.563	3.605
Ciprofloxacin	0.761	0.191	0.996	0.161	2.938	3.535	4.359
Amoxicillin	0.13	0.08	0.853	0.374	3.107	3.859	4.773
ABTS	0.708	0.96	0.97	0.797	3.343	4.197	4.452
Guaiacol	0.498	0.699	0.248	0.616	2.582	3.788	3.079

Key: BCF: Bioconcentration factor; DILI: Drug induced liver injury; IGC_{50}: *Tetrahymena pyriformis* 50 percent growth inhibition concentration; $LC_{50}FM$: 96-hour fathead minnow 50 percent lethal concentration; $LC_{50}DM$: 48-hour *Daphnia magna* 50 percent lethal concentration.

Table 3. Specific interactions of the best docking binding position of the laccase-ligands tested.

Ligand PubChem CID	Complexes	Enzyme-Ligand Conformational pose	Docking affinity (Kcal/mol)
408099	Direct Red 75-laccase		-7.4
12450	Malachite Green-laccase		-7.0
17409	Remazol Brilliant Blue R-laccase		-7.0
2723854	Indigo Carmine-laccase		-6.6
2764	Ciprofloxacin-laccase		-6.8
33613	Amoxicillin-laccase		-7.0

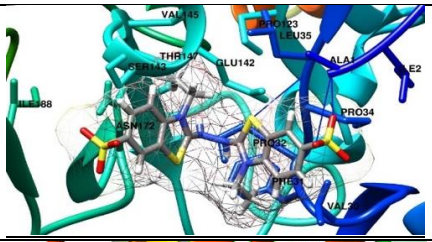
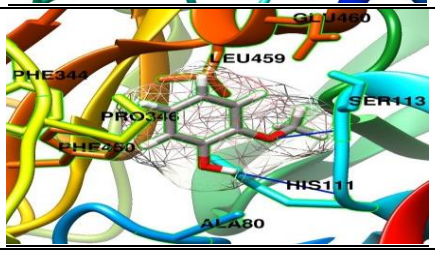
9570474	ABTS-laccase (control 1)		-6.4
460	Guaiacol-laccase (control 2)		-5.2

Table 4. Data from Ramachandran plot of laccase from *T. versicolor* (PDB:1GYC).

	Number	Percentage
Residues in most favorite regions [A, B, L]	361	86.8 %
Residues in additional allowed regions [a, b, l, p]	52	12.5 %
Residues in generously allowed regions [~a, ~b, ~l, ~p]	2	0.5 %
Residues in disallowed regions	1	0.2 %
Number of non-glycine and non-proline residues	416	100 %
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	39	
Number of proline residues	42	
Total number of residues	499	

The results showed that RBBR dye displayed pi-alkyl interaction with amino acids Ala410, Phe23, and Leu300, as well as carbon-hydrogen bonding with Ser427. The docking result for DR75 dye showed some pi-pi stacking interactions of the aromatic ring of DR75 with Phe69, pi-anion with Asp444, pi-alkyl with Ala403, and an attractive charge with Gln442. Indigo carmine (IC) was observed to interact with laccase through Gln499 and Arg157 via hydrogen bond, unfavourable negative-negative interaction with Glu496, and with Pro246 and Ala80 via pi-interaction. Malachite green (MG) interacted with the amino acids Arg440 and Val407 via hydrogen bond and with Val313, Phe422 and Pro420 through pi-alkyl interaction. For the pharmaceuticals, CIP interacted with Asp101 and Gln442 through hydrogen bond interactions and with Ala403, Phe69, and His402 via pi-alkyl interaction while AMO interacted with His402, Gln442, Asp444, and Ala403 through hydrogen interactions and Pro314 via pi-alkyl interaction. The interaction of guaiacol with the enzyme was via both hydrogen and hydrophobic with Ser113. The binding energies of laccase with IC, MG, RBBR, DR75, CIP, AMO, ABTS, and Guaiacol were −6.6, −7.0, −7.0, −7.4, −6.8, −7.0, −6.4, and −5.2 kcal/mol, respectively. The active site of the enzyme which interacted with the eight ligands were found to contain one of the eleven (11) amino acid residues, namely, HIS, GLN, ASP, ALA, PHE, SER, LEU, ARG, VAL, PRO, and GLU, which were involved in the hydrophobic interactions and hydrogen bonding of the docked complexes.

The in-silico ADMET studies revealed the environmental toxicity of the dyes, pharmaceuticals, and control as well as their potential carcinogenicity. The pharmaceuticals and controls showed lower carcinogenicity potential and did not violate the Lipinski rule of five as expected for a drug, but showed some level of environmental toxicity as observed in their BCF, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$ values. This is consistent with research finding that, because of their slow rate of absorption and metabolism, pharmaceuticals are emerging pollutants that are becoming widespread in the environment and are increasingly finding their way into water bodies [26, 27].

The bioconcentration factor (BCF) is defined as the ratio of a pollutant's concentration in an organism, absorbed through respiratory surfaces, to its concentration in water under steady-state conditions. This parameter is widely used to evaluate the potential for secondary poisoning and to assess risks to human health through bioaccumulation in the food chain [28]. The 48-hour *Tetrahymena pyriformis* IGC_{50} represents the concentration of a pollutant in water (measured in mg/L) that inhibits the growth of 50% of *Tetrahymena pyriformis* populations after 48 hours of exposure [29]. Similarly, the 96-hour fathead minnow LC_{50} indicates the pollutant concentration in water (in mg/L) that results in 50% mortality of fathead minnows within 96 hours [29, 30], while the 48-hour *Daphnia magna* LC_{50} refers to the concentration causing 50% mortality of *Daphnia magna* within 48 hours [31].

These metrics are critical for assessing the environmental toxicity of pollutants, particularly in aquatic ecosystems. Notably, a BCF value greater than 1 suggests that the pollutant accumulates to a higher concentration in biota than in the surrounding water, highlighting its bioaccumulative potential. All the dyes showed high probability for toxicity with values close to 1 and also environmental toxicity, with MG showing the highest probability with a bioconcentration factor, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$ of 1.683, 3.858, 4.567, and 5.446, respectively, which is in agreement with the work of Srivastava et al. [32] who reported that in aquatic organisms like fishes and some mammals, that MG has been linked to mutagenesis, carcinogenesis, chromosomal fractures, teratogenicity, respiratory toxicity, organ damage, and aberrant development. Laccase's active site is located near four copper ions, with substrate binding and oxidation occurring at T1 Cu. Mostly through hydrogen bonding and binding to laccase protein, dyes interact with amino acid residues surrounding T1 Cu [33]. The binding site between the dye and laccase in the molecular docking test was determined to be in the T1 Cu surface binding cavity (SBC), which has an acidic aspartic acid and a highly conserved histidine [34]. The outcomes demonstrated that, at the enzyme's surface binding cavity (SBC), RBBR dye displayed pi-alkyl interaction with polar amino acids, Ala410, Phe23, and Leu300, and hydrogen bonding with Ser427.

Jia et al. [35] reported that RBBR interacted with amino acid residues Asn208, Gln237, Asn264, Gly392, and Ala393 on laccase via H-bonding. The docking results for DR75 dye showed some pi-pi stacking interactions of the aromatic ring of DR75 with Phe69, pi-anion with Asp444, pi-alkyl with Ala403, and hydrogen bonding with Gln442 in the active site of the enzyme. IC was observed to interact with laccase through Gln496 and Arg157 via hydrogen bonding, an unfavourable negative-negative interaction with Glu496, and with Pro246 and Ala80 via pi-interaction. MG interacted with Arg440 and Val407 via a hydrogen

bond and with Val313, Phe422, and Pro420 through a pi-alkyl interaction. The abundance of pi-interactions between the four textile dyes and the enzyme is due to the aromatic nature of the dyes, which all contain at least one benzene ring.

Concerning the pharmaceuticals, CIP interacted with Asp101 and Gln442 through hydrogen bond interactions and with Ala403, Phe69, and His402 via pi-alkyl interaction, while AMO interacted with His402, Gln442, Asp444, and Ala403 through hydrogen interactions and Pro314 via pi-alkyl interaction. ABTS interaction was through hydrophobic means while guaiacol interacted via hydrophobic and hydrogen bonding with Ser113.

The pharmaceuticals were both found to exhibit hydrogen bonding with Gln442. This could be because the amino acid plays an important role in creating a binding pocket conducive to the hydrophobic interaction of the enzyme with diverse antibiotics [36]. Sánchez-SanMartín et al. [36] reported the predicted binding energies of AMO and CIP by molecular docking as −6.55 and −6.51 kcal mol^{-1}, respectively, while Buzzo et al. [37] reported binding energies for tetracycline, sulfisoxazole, and trimethoprim as −8, −6.7, and −6.1 kcal/mol. The findings from all of these works are in agreement with this study.

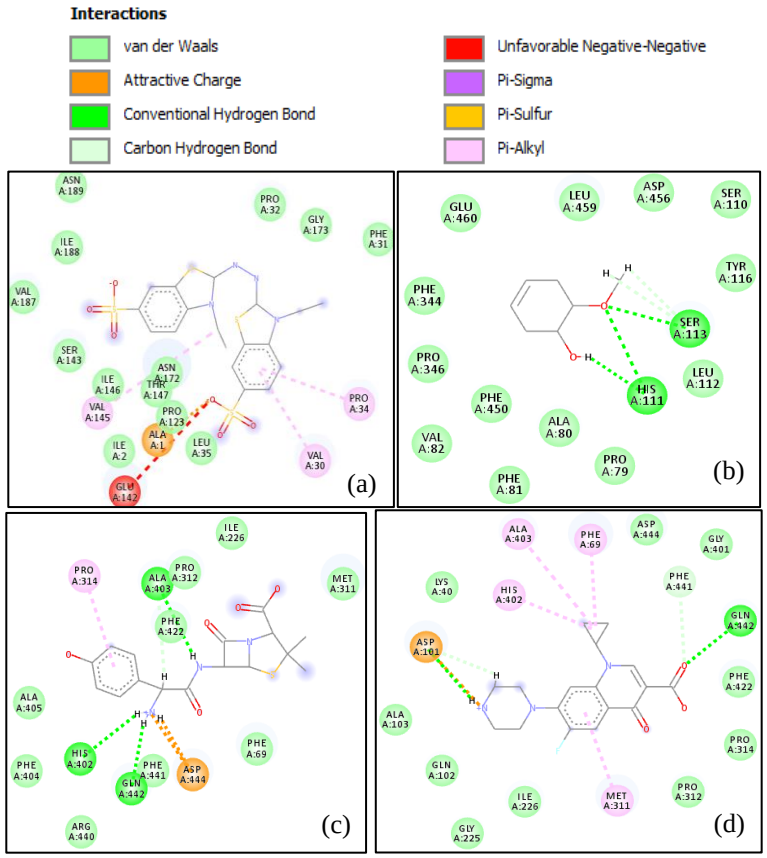


Fig. 3. 2D structures of molecular interactions between 1GYC and (a) ABTS, (b) Guaiacol, (c) Amoxicillin, and (d) Ciprofloxacin.

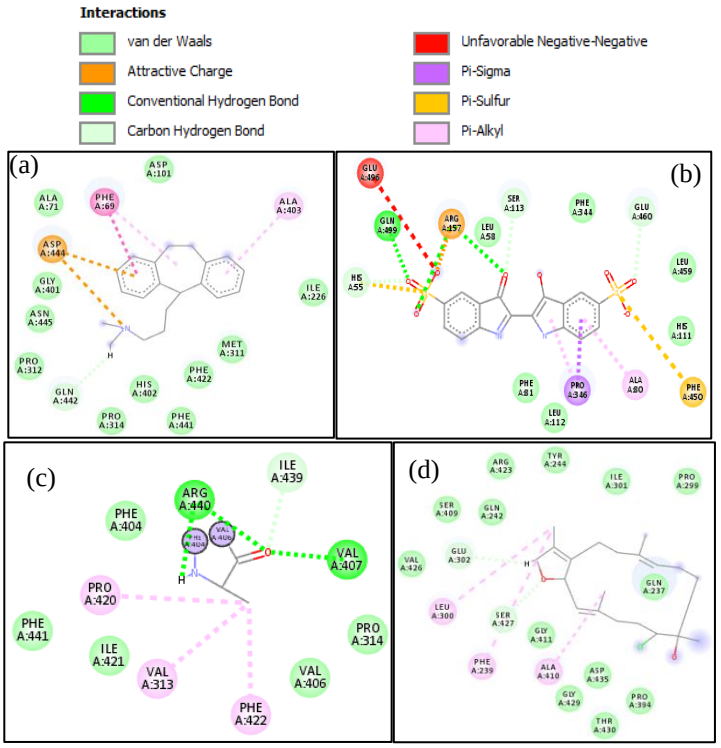


Fig. 4. 2D structures of molecular interactions between 1GYC and (a) Direct Red 75, (b) Indigo Carmine, (c) Malachite Green, and (d) Remazol Brilliant Blue R.

Table 5. Laccase amino acid residues involved in ligand interactions.

Molecular Interactions	Investigated Ligands							
	Indigo Carmine	**Malachite Green**	**Remazol Brilliant Blue R**	**Direct Red 75**	**Ciprofloxacin**	**Amoxicillin**	**ABTS**	**Guaiacol**
Hydrogen Bond	Arg157 Gln499	Val407 Arg440	-	-	Gln442 Asp101	Asp444 Gln442 His402 Ala403	-	Ser113 His111
Hydrophobic interaction	Glu460 Phe450 Ala80 Pro346 Ser113 Glu496 His55	Ile439 Pro420 Val313 Phe422	Leu300 Ser427 Phe239 Ala410	Asp444 Phe69 Ala403 Gln442	Ala403 Phe69 His402 Phe441 Met311	Pro314 Phe422	Pro34 Val30 Val145 Glu142 Ala1 Leu35	Ser113

The active site of the enzyme that interacted with all the ligands was found to contain eleven (11) amino acid residues, namely, HIS, GLN, ASP, ALA, PHE, SER, LEU, ARG, VAL, PRO, and GLU, and all were involved in the hydrophobic interactions and hydrogen bonding of the docked complexes. Fu et al. [38] reported the presence of 18 amino acid residues (ALA, ARG, ASN, ASP, GLN, GLU, GLY, HIS, ILE, LEU, LYS, PHE, PRO, SER, THR, TRP, TYR, and VAL) in the active site of laccase from the fungus *Amylostereum areolatum*. This agrees with this study, as all eleven amino acids in this study were highlighted in their study. Molecular docking results showed DR75 has a stronger binding affinity with laccase than other dyes by interacting with the substrate binding cavity of the enzyme, this could partly explain the reported toxic nature of azo dyes, while amoxicillin showed a higher binding affinity than ciprofloxacin.

This in-silico study does not completely reflect the interaction of the ligands with the enzyme in a real experimental situation but can serve as a theoretical forecast on the possible environmental effects of several compounds.

4. Conclusion

In this study, almost all the ligands could bind to the laccase with similar binding affinity. DR75 (azo dye) bound to the enzyme with the highest binding affinity; this could account for the toxic nature of azo dyes. The antibiotics both interacted with Ala403, Gln442, and His402 in the binding pocket of the enzyme. All the dyes showed high probability for toxicity with values close to 1 and also environmental toxicity, with MG showing the highest probability with a bioconcentration factor, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$ of 1.683, 3.858, 4.567, and 5.446, respectively. Finally, through this investigation, it was concluded that the laccases from *T. versicolor* could be a viable choice for decolorization and degradation of the ligands. There is a need for further in-vitro and in-vivo study of the laccase from *T. versicolor* to support these in silico analyses.

References

[1] Forootanfar H, Rezaei S, Zeinvand-Lorestani H, Tahmasbi H, Mogharabi M, Ameri A. Studies on the laccase-mediated decolorization, kinetic, and microtoxicity of some synthetic azo dyes. J Environ Health Sci Eng. 2016;14(7):1-10.

[2] Kim JM, Park SM, Kim DH. Heterologous expression of a tannic acid-inducible laccase3 of *Cryphonectria parasitica* in Saccharomyces cerevisiae. BMC Biotechnol. 2010;10(1):18-26.

[3] Gasser CA, Ammann EM, Shahgaldian P, Corvini PFX. Laccases to take on the challenge of emerging organic contaminants in wastewater. Appl Microbiol Biotechnol. 2014;98:9931-52.

[4] Nunes CS, Kunamneni A. Chapter 7 – Laccases—Properties and applications. In: Nunes CS, Kumar V, editors. Enzymes in Human and Animal Nutrition. Cambridge, MA: Academic Press; 2018. p. 133-61.

[5] Arora DS, Sharma RK. Ligninolytic fungal laccases and their biotechnological applications. Appl Biochem Biotechnol. 2010; 160:1760-88.

[6] Yang J, Li W, Ng TB, Deng X, Lin J, Ye X. Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation. Front Microbiol. 2017; 8:832.

[7] Ozojiofor UO, Abdulsalami MS, Egbe NE, Haroun AA. Fungi laccases: Structure, functions, and potential application in the biodegradation of pharmaceutical micropollutants. Int J Adv Biotechnol Res. 2023;14(1):24-38.

[8] Guta R, Rijalu NE, Adugna B, Diriba AA. Smallholder market participation and its associated factors: evidence from Ethiopian vegetable producers. Cogent Food Agric. 2019; 6:1-15.

[9] Jamee R, Siddique R. Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach. Eur J Microbiol Immunol. 2019; 9:114-8.

[10] Sharma J, Sharma S, Soni V. Classification, and impact of synthetic textile dyes on aquatic flora: A review. Reg Stud Mar Sci. 2021; 45:101802.

[11] Gogoi A, Mazumder P, Kumar V, Chaminda GGT, Kyoungjin A, Kumar M. Occurrence and fate of emerging contaminants in water environment: A review. Groundw Sustain Dev. 2018; 6:169-80.

[12] UNESCO. UNESCO Project. Emerging Pollutants in Wastewater Reuse in Developing Countries, International Initiative in Water Quality (IIWQ). 2014. Available from: https://unesdoc.unesco.org//ark:/48223/pf0000235241.

[13] Zenker A, Cicero MR, Prestinaci F, Bottoni P, Carere M. Bioaccumulation and biomagnification potential of pharmaceuticals with a focus on the aquatic environment. J Environ Manage. 2014; 133:378-87.

[14] Oulton RL, Kohn T, Cwiertn DM. Pharmaceuticals and personal care products in effluent matrices: a survey of transformation and removal during wastewater treatment and implications for wastewater management. J Environ Manage Monit. 2010; 12:1956-78.

[15] Piontek K, Antorini M, Choinowski T. Crystal structure of laccase from the fungus Trametes versicolor at 1.90-Å resolution containing a full complement of coppers. J Biol Chem. 2002; 277:37663-9.

[16] Adigun TO, Sulaiman FA, Na'Allah A, Alabi MA, Odo CE, Adebamiji ET, Oluwadare IA, Ozojiofor UO, Omoniyi AP, Joel WO, Aina KO, Alejolowo OO, Akiode SO, Adeegbe JF. Discovery of Eriodictyol as putative Exportin-1 inhibitor for non-small cell lung cancer therapy. Afr Sci. 2023;24(2):215-28.

[17] Lenin S, Ponthier E, Scheer KG, Yeo ECF, Tea MN, Ebert LM. A drug screening pipeline using 2D and 3D patient-derived in vitro models for pre-clinical analysis of therapy response in glioblastoma. Int J Mol Sci. 2021; 22:4322.

[18] Morriss-Kay GM. The evolution of human artistic creativity. J Anat. 2010; 216:158-76.

[19] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019;47(D1): D1102–D11.

[20] Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des.2010;24(5):417-22.

[21] Venkatachalam CM, Jiang X, Oldfield T, Waldman M. Ligand fit: A novel method for the shape-directed rapid docking of ligands to protein active sites. J Mol Graph Model. 2003;21:289-97.

[22] Volkamer A, Kuhn D, Grombacher T, Rippmann F, Rarey M. Combining global and local measures for structure-based druggability predictions. J Chem Inf Model. 2012;52:360-73.

[23] Suborna SA, Lubna FI, Hasan MH, Arabi II, Bhowmik P. Is the amino acid HIS458 and ASP/ASN206 of laccase from *T. versicolor* and *T. hirsuta* crucial for the decolorization of polycyclic aromatic textile dyes? An in-silico investigation. Eurasian J Sci Technol. 2024;5(1):106-24.

[24] Tadesse BT, Ashley EA, Ongarello S, Havumaki J, Wijegoonewardena M, González IJ. Antimicrobial resistance in Africa: a systematic review. BMC Infectious Diseases. 2017;17(1):616-30.

[25] Ding H, Wu Y, Zou B, Lou Q, Zhang W, and Zhong, J. Simultaneous removal and degradation characteristics of sulfonamide, tetracycline, and quinolone antibiotics by laccase-mediated oxidation coupled with

soil adsorption. Journal of Hazardous Material. 2016;307,350-8.

[26] Wang J, Wang S. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. J Environ Manag. 2016;182:620-40.

[27] Litwińska K, Bischoff F, Matthes F, Bode R, Rutten T, Gotthard-Kunze G. Characterization of recombinant laccase from *Trametes versicolor* synthesized by *Arxula adeninivorans* and its application in the degradation of pharmaceuticals. AMB Express. 2019;9:102-15.

[28] Raj B, Farrell JA, Liu J, El-Kholtei J, Carte AN, Acedo JN. Emergence of neuronal diversity during vertebrate brain development. Neuron. 2019; 108(6): 1058-74.e6.

[29] Cronin MT, Gregory BW, Schultz TW. Quantitative structure-activity analyses of nitrobenzene toxicity to Tetrahymena pyriformis. Chem Res Toxicol. 1998;11(8):902-8.

[30] Martin TM, Young DM. Prediction of the acute toxicity (96-h LC50) of organic compounds to the fathead minnow (*Pimephales promelas*) using a group contribution method. Chem Res Toxicol. 2001;14(10):1378-85.

[31] Guilhermino L, Diamantino T, Silva MC, Soares AM. Acute toxicity test with *Daphnia magna*: an alternative to mammals in the prescreening of chemical toxicity? Ecotoxicol Environ Saf. 2000;46(3):357-62.

[32] Srivastava A, Dangi LK, Kumar S, Rani R. Microbial decolorization of Reactive Black 5 dye by *Bacillus albus* DD1 isolated from textile water effluent: kinetic, thermodynamics & decolorization mechanism. Heliyon. 2022;8: e08834.

[33] Zhang Y, Hu P, Muhammad Y, Tang Y, Shao S, Gao Z. High-density immobilization of laccase on hollow nano-sphere NH2-MIL88(Fe) host with interfacial defects to improve enzyme activity and stability for remazol brilliant blue R decolorization. Chem Eng J. 2021; 405:127003.

[34] Cannatelli MD, Ragauskas AJ. Two decades of laccases: advancing sustainability in the chemical industry. The Chem Rec. 2017; 17:122-40.

[35] Jia Y, Huang Q, Zhu L, Pan C. Characterization of a recombinant laccase B from *Trametes hirsuta* MX2 and its application for decolorization of dyes. Molecules. 2022;27(5):1581-94.

[36] Sánchez-SanMartín J, Márquez SL, Espina G, Cortés-Antiquera R, Sun J, Blamey JM. Evaluation of antibiotic biodegradation by a versatile and highly active recombinant laccase from the thermoalkaliphilic bacterium *Bacillus sp*. FNT. Biomolecules. 2024; 14:369.

[37] Buzzo BB, Giuliatti S, Pereira PAM, Gomes-Pepe ES, Lemos EGM. Molecular docking of Lac_CB10: highlighting the great potential for bioremediation of recalcitrant chemical compounds by one predicted Bacteroidetes CopA-laccase. Int J Mol Sci. 2023; 24:9785-94.

[38] Fu N, Li J, Wang M, Ren L, Luo Y. Genes identification, molecular docking and dynamics simulation analysis of laccases from *Amylostereum areolatum* provides molecular basis of laccase bound to lignin. Int J Mol Sci. 2020; 21:8845-64.