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Biological treatment of hospital and textile wastewaters using laccase-rich crude extracts from *Trametes villosa*

Tratamento biológico de efluentes hospitalares e têxteis usando extratos brutos ricos em lacase de *Trametes villosa*

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ABSTRACT

Hospital and textile wastewater represent distinct sources of environmental contamination, characterized by complex mixtures of micropollutants, including pharmaceuticals, dyes, and phenolic compounds. Widespread use, inadequate disposal, and the limited efficiency of conventional wastewater treatment processes have led to the accumulation of these micropollutants in aquatic environments. The structural complexity of these effluents poses a significant challenge to standardizing effective wastewater treatment methodologies. Oxidative enzymes, such as laccases, have emerged as potential tools for effluent remediation, improving water quality and reducing contamination. This study evaluated the application of an enzyme extract containing laccases produced by *Trametes villosa* for the treatment of hospital and textile wastewater. Analysis of hospital effluent samples by liquid chromatography coupled to mass spectrometry showed a significant reduction in antibiotic concentrations (97% for pyrazinamide, 74% for rifampicin, 66% for sulfamethoxazole, and 64% for trimethoprim) after enzymatic treatment compared with the control. Phenolic compounds were reduced by 64% in hospital effluents and by 98% in textile effluents. The chemical oxygen demand increased after enzymatic treatment in the hospital sample, while only a slight reduction was observed in the textile sample. The textile effluent treated with 1,000 U L^{-1} of laccase showed an 18% decrease in the visible spectral region and a 23% reduction in the maximum absorbance peak. In the phytotoxicity test with *Lactuca sativa*, effluents reduced seed growth, and enzymatic treatment reduced their toxicity. Thus, enzymatic treatment showed promising results as a sustainable strategy for treating hospital and textile wastewater.

Keywords: emerging contaminants; oxidoreductases; white-rot fungi; antibiotics; effluents, laccase.

RESUMO

Águas residuais hospitalares e têxteis representam fontes distintas de contaminação ambiental, caracterizadas por misturas complexas de micropoluentes, incluindo fármacos, corantes e compostos fenólicos. O uso generalizado, o descarte inadequado e a eficiência limitada dos processos convencionais de tratamento de águas residuais levaram ao acúmulo desses micropoluentes em ambientes aquáticos. A complexidade estrutural desses efluentes representa um desafio significativo para padronizar metodologias eficazes de tratamento de águas residuais. Enzimas oxidativas, como as lacases, surgiram como ferramentas potenciais para a remediação de efluentes, melhorando a qualidade da água e reduzindo a contaminação. Este estudo avaliou a aplicação de um extrato enzimático contendo lacases produzidas por *Trametes villosa* no tratamento de águas residuais hospitalares e têxteis. A análise de amostras hospitalares por cromatografia líquida acoplada à espectrometria de massas demonstrou redução significativa das concentrações de antibióticos (97% para pirazinamida, 74% para rifampicina, 66% para sulfametoxazol e 64% para trimetoprima) após o tratamento enzimático, em comparação com o controle. Os compostos fenólicos foram reduzidos em 64% nos efluentes hospitalares e em 98% nos efluentes têxteis. A demanda química de oxigênio aumentou após o tratamento enzimático na amostra hospitalar, enquanto, na amostra têxtil, observou-se apenas leve redução. O efluente têxtil tratado com 1.000 U L^{-1} de lacases apresentou diminuição de 18% na região espectral visível e redução de 23% no pico máximo de absorção. No teste de fitotoxicidade com *Lactuca sativa*, os efluentes reduziram o crescimento das sementes, e o tratamento enzimático diminuiu a toxicidade das amostras. Assim, o tratamento enzimático mostrou-se promissor como estratégia sustentável para o tratamento de águas residuais hospitalares e têxteis.

Palavras-chave: contaminantes emergentes; oxidorredutases; fungo da podridão branca; antibióticos; efluentes; lacase.

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Introduction

Emerging contaminants in wastewater have been identified as a critical environmental and health issue. The various chemical compounds present in the effluents can be found in concentrations ranging from a few nanograms to several micrograms per liter, with some being biologically active and having the potential to cause damage to ecosystems (Methneni et al., 2021; Li et al., 2022; Ramírez-Morales et al., 2024).

Every day, thousands of liters of effluents containing pollutants such as drugs, dyes, naphthol, nitrates, acetic acid, soaps, and metals flow through sewage treatment plants. When not properly treated, they are released into the aquatic environment, contaminating both surface and groundwater (Wijeyaratne and Wickramasinghe, 2020; Li et al., 2022; Yang, W., et al., 2025).

Conventional wastewater treatment technologies, such as sedimentation, coagulation, flocculation, and activated sludge, were designed to remove most of the suspended solids, dissolved organics, and nutrients from wastewater; however, not all chemicals can be removed through conventional wastewater treatment methods (Domingues et al., 2020; Wijeyaratne and Wickramasinghe, 2020; Ramos et al., 2021; Azanaw et al., 2022).

An ecological and promising alternative (or even complementary) to conventional chemical treatments is bioremediation, which involves using various organisms such as plants, algae, fungi, and bacteria, or their enzymes, to convert toxic contaminants into less toxic chemical derivatives than their initial state (Rizzo et al., 2019; Ceretta et al., 2021; Zdarta et al., 2021; Pandey et al., 2023).

In this context, laccases (EC 1.10.3.2), one of the ligninolytic enzymes produced by white-rot fungi, are excellent choices, considering their substrate versatility, low catalytic requirement, and ability to oxidize various aromatic compounds and metals (Mate and Alcalde, 2017; Khatami et al., 2022; Thathola et al., 2024). Laccases are multicopper oxidase enzymes produced by different organisms, though basidiomycetes have laccases with high redox potential, facilitating the abstraction of electrons from substrates (Leontievsky et al., 1997; Frasconi et al., 2010; Debnath and Saha, 2020).

Previous studies have demonstrated the applicability of fungal laccases in the bioremediation of drugs (Lloret et al., 2010; Yang et al., 2017; Parra Guardado et al., 2019; Meiczinger et al., 2022), textile industry dyes (Daâssi et al., 2014; Iark et al., 2019; Bilal et al., 2021), and pesticides (Jin et al., 2016; Chen, X., et al., 2019; Bhatt et al., 2023), as well as natural and artificial hormones in aquatic environments (Chen, M., et al., 2019; Yang, Q., et al., 2025).

When it comes to the treatment of drugs or hospital effluents, laccases have been applied in recent years in various treatment processes, including the removal of individual drugs at specific concentrations in aqueous solutions, synthetic hospital effluents, or pre-treated hospital effluents, both in crude extract form and purified or immobilized forms (Ahmad et al., 2025; Chen et al., 2025; Singh et al., 2025). However, studies evaluating the use of laccases in the treatment of real hospital effluents are still scarce and recent (Alokpa et al., 2025a; 2025b). Additionally, only a few wastewater parameters are typically monitored during enzymatic treatment. Some studies mention the application of laccases in wastewater, but do not describe the performance of the enzyme in real hospital wastewater (Mojtabavi et al., 2023; Ghose et al., 2024; Hassan et al., 2025). Thus, the evaluation of the treatment of real hospital effluents with laccases is still a relevant topic in the environmental and biotechnological contexts, and several studies with different forms of laccases and treatment methods are needed to clarify the real potential of enzymatic wastewater treatment, including efficacy and cost (Alokpa and Cabana, 2025).

The potential application of laccases in textile effluent treatment has also generated numerous studies describing the removal of individual dyes in aqueous solutions, both in the presence and absence of chemical mediators. Some studies have evaluated free or immobilized laccases in real textile effluents (Khlifi et al., 2010; Benzina et al., 2013; Motamedi et al., 2021; Kalia et al., 2023; Pandey et al., 2023), but few have used crude extracts or laccases of *Trametes* sp. in the absence of chemical mediators (Ramayanam, 2025).

Therefore, this paper describes the bioremediation capacity of real textile and hospital effluents by using crude enzymatic extract containing laccase produced by the fungus *Trametes villosa*.

Materials and methods

Fungal strain and maintenance

A fungal strain of *Trametes villosa* (available upon reasonable request) from the Culture Collection of the Biotechnology Laboratory of the Federal University of Technology — Curitiba, Paraná (PR), Brazil, was used to produce the enzymatic extract containing laccase. The fungus was cultivated on Petri dishes containing potato dextrose agar (PDA) culture medium (Kasvi, São José dos Pinhais, Brazil) for seven days in an incubator at 28°C. After growth, mycelial discs with a diameter of 12 mm were removed from the Petri dishes and used to inoculate a semisolid medium ideal for laccase production.

Laccase production

The enzymatic extracts were produced through a medium composed of a solution of mineral salts, copper sulfate, and agro-industrial residues (sugarcane bagasse, rice husk, and grape pomace), prepared according to Modkovski et al. (2021). This semisolid medium was poured into an Erlenmeyer flask and autoclaved to inoculate the previously cut fungal mycelium (mycelium agar discs on PDA plates), with four plugs added per flask. The flasks were then incubated for seven days at 28°C. After incubation, the media were subjected to vacuum filtration and centrifugation (paper Framex, 11 cm in diameter) for 10 minutes at 15,000 rpm; the supernatant yielded a crude enzyme extract.

Laccase activity in the enzymatic extract was determined in a UV-visible spectrophotometer (BEL Engineering, model M-51, Monza,

Italy) at 420 nm, following the methodology described by Hou et al. (2004) by oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, ≥98% purity, Sigma-Aldrich, St. Louis, MO, USA), in 50 mM sodium acetate (pH 5.0), for 5 minutes at 40°C. Enzyme activity (U) was defined as the amount of enzyme required to oxidize 1 µmol of the ABTS substrate per minute. After measurement, the crude enzymatic extract was stored at -20°C until use in subsequent experiments.

Samples of the effluents and enzymatic treatment

The textile effluent sample was collected from a textile industry located in Curitiba, Brazil. The hospital effluent under study was collected at the Regional Hospital of Lapa de São Sebastião, in Curitiba, kindly provided by the Environmental Contaminants Laboratory of the Federal University of Technology — Paraná (UTFPR).

The samples containing hospital and textile effluents were treated with the enzyme extract adjusted to initial concentrations of 500 and 1,000 U L^{-1} of laccase activity in a total reaction volume of 30 mL (effluent + enzyme) for each activity. The bottles containing the samples were placed in the shaker incubator at room temperature (±23°C) under agitation at 120 rpm for 1, 2, and 24 hours. After incubation, the treated effluents were stored at -20°C for further analysis. Controls containing the effluent and denatured enzymatic extract (prepared by boiling for 5 minutes) were evaluated under the same conditions in duplicates.

Parameters evaluated in the samples of both effluents before and after enzymatic treatment

Total phenolic compounds

The phenolic compounds were quantified in the samples using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965), with some modifications. In a 1 mL Eppendorf, 600 µL of ultrapure water, 50 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), 200 µL of sample, and 150 µL of 15% Na_2CO_3 were added; the samples were protected from light for one hour, and then measured in a spectrophotometer (BEL Engineering, model M-51, Monza, Italy) at 760 nm.

2.4.2 Chemical oxygen demand

The COD assay was performed using a spectrophotometer's closed reflux colorimetric method, following the description in 5220B (APHA, 2017). The sample digestion was performed at 150°C for 2 hours in a digestion block, and the assay was performed by spectrophotometry at 600 nm to measure Cr^{3+} absorbance.

Phytotoxicity with Lactuca sativa

The phytotoxicity of the samples after enzymatic treatment was verified using a germination test with lettuce seeds (*Lactuca sativa*). The study was adapted according to Charles et al. (2011) and Hofstätter et al. (2024). The seeds used in the experiment were purchased from commercial establishments. The variety of lettuce used was American lettuce, which has a germination percentage of 97%.

Eighteen lettuce seeds were evenly distributed on Petri dishes containing 80 g/m² qualitative filter paper with an average pore size of 14 microns. Next, the seeds were placed in contact with 3.0 mL of samples of the treated effluents at their respective concentrations of 0, 6.25, 12.5, 25, 50, and 100%, where 0% represents a positive control with distilled water, and for the negative control, glyphosate at 5%. The experiments were conducted in triplicate. The plates were incubated in a biochemical oxygen demand (BOD) incubator for 120 hours at 22±2°C. The germination index (GI) and the relative growth index (RGI) were calculated using the Priac, Badot, and Crini equation (Priac et al., 2017). RGI values were used to evaluate the toxicity of the effluent and interpreted as: lack of significant effect or non-significant effect (NSE): >0.8 and <1.2; Inhibitory effect (I): between 0 and 0.8; (S) Stimulation of root elongation: higher than 1.2 (Young et al., 2016).

Spectrophotometric scan of the textile effluent before and after enzymatic treatment

The spectrophotometric analysis of the textile effluent (treated and untreated enzymatically) was conducted using the scanning method in the visible region. The textile effluent was tested before and after treatment at two initial enzymatic activity concentrations, 500 and 1,000 U L^{-1}, in a total volume of 15 mL. The samples were placed in a shaker incubator at 25°C with a shaking speed of 120 rpm for 24 hours.

Each sample was scanned using a UV-visible spectrophotometer (BEL Engineering, model M-51, Monza, Italy) over 450–750 nm. After reading, the spectral area of each sample was integrated to determine the percentage of color reduction in the treated samples compared to the control. The maximum absorbance peak of the effluent was also determined spectrophotometrically and used to evaluate the effectiveness of the enzymatic treatment.

Evaluation of the hospital effluent by liquid chromatography coupled to mass spectrometry

The hospital effluent was collected from a facility mainly devoted to tuberculosis treatment; therefore, the monitoring of the enzymatic treatment process focused on antibiotics (including rifampicin) and caffeine (due to its widespread consumption and presence in hospital wastewater). The enzyme activity used in the experiment was 500 U L^{-1} based on preliminary tests that showed no substantial improvement in pollutant removal when higher enzyme activities were applied. Therefore, this activity was chosen as the optimal operating concentration, as it provided effective treatment while ensuring economic viability.

The samples were filtered through 0.22 µm PTFE syringe filters (Kasvi, São José dos Pinhais, PR, Brazil), and the concentrations of antibiotics and caffeine in the laccase-mediated reaction mixtures were determined by High-Performance Liquid Chromatography coupled with Mass Spectrometry (LC-MS), using Shimadzu model 8045 equipment

(equipped with Poroshell C18 column). The chromatographic separation was performed using an HPLC system equipped with a quaternary pump (G1311B), autosampler (G1329B), and column compartment (G1316A) coupled to a triple quadrupole mass spectrometer (Ultivo QQQ). The mobile phase consisted of (A) 5 mM ammonium acetate in ultrapure water and (B) methanol (LC-MS grade), the flow rate was 0.25 mL min^{-1}, and the injection volume was 10 µL. The separation was performed by gradient elution from 10 to 60% B over 15 min, maintained at 60% B until 17 min, then returning to 10% at 18 min and maintaining the initial conditions until 22 min for column rebalancing.

Mass spectrometry was performed using an electrospray ionization (ESI) source operating in positive mode. Nitrogen was used as the collision gas at 350°C, with a flow rate of 12 L/min, a nebulizer pressure of 35 psi, and a capillary voltage of ±3000 V. Quantification was performed in Multiple Reaction Monitoring (MRM) mode (Table 1).

Statistical analysis

Analysis of variance (ANOVA) was used to calculate the means of the effluents before and after enzymatic treatment, and the Tukey test was used to compare the means. The ANOVA was conducted using the statistical software PSPP. Statistical significance was determined by p-values lower than 0.05.

Results and discussion

The results of this study are divided into the stages of production of the crude extract of *T. villosa* containing laccases, its application, and the main effects on hospital and textile effluents (Figure 1).

Parameters such as drug concentration (only in hospital effluent), color (only in textile effluent), phenolics (both effluents), chemical oxygen demand (both effluents), and phytotoxicity (both effluents) were monitored and presented in the following sections.

Table 1 – Multiple Reaction Monitoring transitions and optimized MS parameters for target compounds.

Compound	RT (min)	Ionization	Fragmentor (V)	Precursor (m/z)	Quantifier (m/z)	Qualifier (m/z)
PYZ	16.047	ESI+	100	124	107	81
Caffeine	8.186	ESI+	100	195.1	138.1	110.2
SMZ	4.396	ESI+	80	254	156	91
TMP	11.671	ESI+	100	291.2	230	123.1
RFC	14.648	ESI+	135	823.4	163.1	151

PYZ: pyrazinamide; SMZ: sulfamethoxazole; TMP: trimethoprim; RFC: rifampicin.

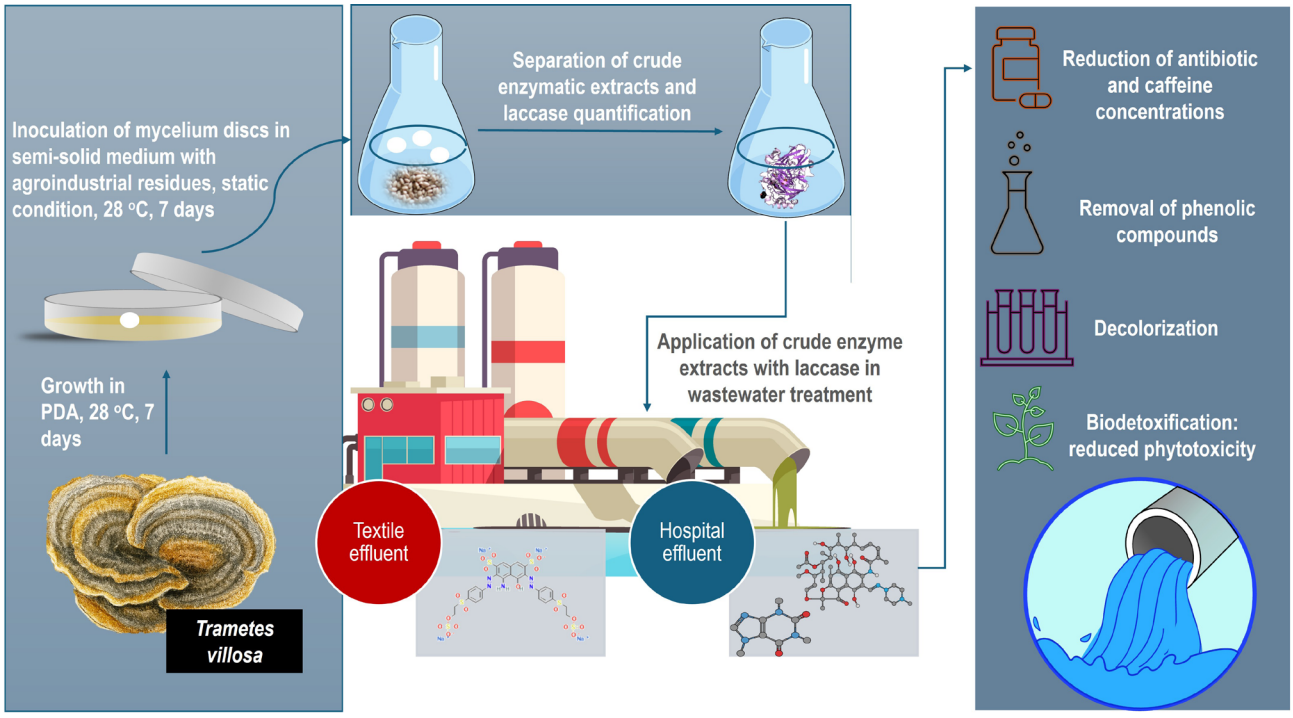


Figure 1 – Steps developed for the application of extracts containing laccases in the treatment of textile and hospital effluents on a laboratory scale, and the expected results.

Production of enzymatic extract

The semisolid culture medium proved efficient at inducing laccase production, yielding an average activity of 11,754±1.83 U L^{-1} in the crude extract. The production of laccase from the fungus *Trametes villosa* was previously evaluated by Modkovski et al. (2021), who obtained a laccase production with a maximum activity of 17,000 U L^{-1}.

The use of lignocellulosic residues in culture media can be beneficial for inducing the production of ligninolytic enzymes by white-rot fungi, as it simulates their natural environment. Furthermore, using rice husks, grape pomace, and sugarcane bagasse adds value to low-cost, widely available products that would otherwise be discarded, thereby minimizing the environmental impact of improper disposal and accumulation.

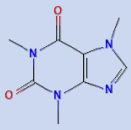
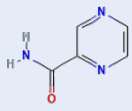
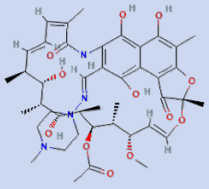
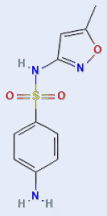
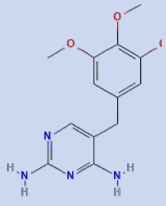
Effect of enzymatic treatment in hospital and textile effluents

Removal of pharmaceuticals and caffeine

The removal of antibiotics and caffeine through enzymatic treatment was evaluated in hospital effluents using liquid chromatography coupled with mass spectrometry. The samples were analyzed at 1, 2, and 24 hours, and the results were statistically evaluated, revealing significant differences in the means of the tested groups (Table 2).

The degradation of complex mixtures such as hospital effluent occurs due to the low specificity of the laccase enzyme, since its catalytic mechanism has four copper atoms in its active site, characterized by the presence of one type 1 copper ion, one type 2 copper ion, and two type 3 copper ions (Figure 2) (Mehra et al., 2018).

Table 2 – Initial concentration of antibiotics and caffeine in hospital effluent samples before (Control) and after enzymatic treatment (µg/L). The data represent the average values and standard deviations of the chemical compounds in the hospital effluent, as evaluated by High-Performance Liquid Chromatography coupled with Mass Spectrometry (LC-MS). The means in the row followed by the same letter are not statistically different.

Compounds (µg/L)	Chemical structure	Calibration curve	R^2	LOD (µg/L)	LOQ (µg/L)	Control	1h	2h	24h
Caffeine		y=276.08x - 19.55	0.9975	0.31	0.95	1,398±196^a	395±35.6^b	416±5.5^c	414±41.3^c
PYZ		y=10.14x - 1.36	0.9967	0.50	1.51	37.2±0.4^a	2.4±1.4^b	1.4±0.9^b	0.9±0.2^b
RFC		y=4.87x - 13.06	0.9913	0.78	2.37	22.8±2.0^a	6.3±0.8^b	6.0±0.4^b	5.0±0.7^b
SMZ		y=365.14x + 60.69	0.9994	0.20	0.60	11.8±1.1^a	4.0±0.4^b	4.7±0.3^b	4.0±0.2^b
TMP		y=633.85x - 21.19	0.9989	0.10	0.30	10.5±1.1^a	3.2±0.11^b	3.3±0.03^b	3.2±0.09^b

Control: samples composed of the effluent and denatured enzymatic extract; TMP: trimethoprim; SMZ: sulfamethoxazole; PYZ: pyrazinamide; RFC: rifampicin.
The chemical structures of the compounds were retrieved from PubChem.
Source: Kim et al. (2025).

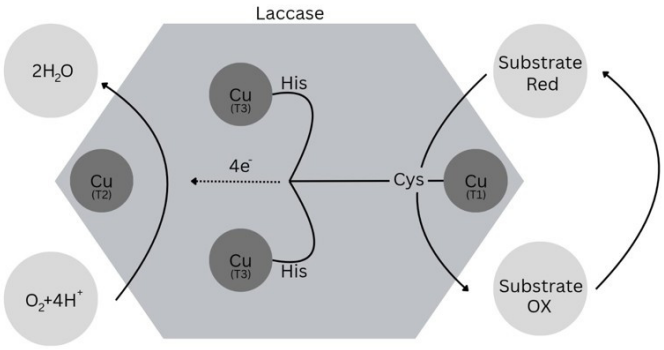


Figure 2 – The general mechanism of laccase catalytic activity.
Substrate Red: Reduced Substrate; Substrate OX: Oxidized substrate; His: histidine; Cys: cysteine.
Source: adapted from Rodríguez-Delgado et al. (2015).

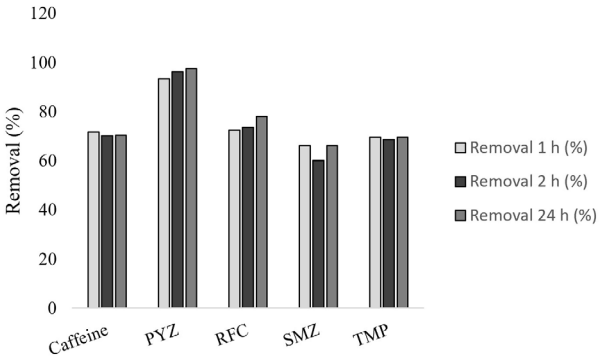


Figure 3 – Removal efficiency (%) of caffeine, pyrazinamide (PYZ), rifampicin (RIF), sulfamethoxazole (SMZ), and trimethoprim (TMP) by enzymatic treatment.

Type 1 copper is the primary electron acceptor during catalysis, where substrates are oxidized, along with the reduction of molecular oxygen to water by the transfer of four electrons. This atom has a trigonal coordination with two histidines and one cysteine, while eight histidine residues serve as ligands for type 2 and type 3 copper atoms (Janusz et al., 2020). This redox mechanism can generate reactive radical intermediates that undergo subsequent non-enzymatic coupling, rearrangement, or fragmentation reactions, which explains the simultaneous transformation of chemically distinct pharmaceutical products within the same treatment system.

Laccase can oxidize phenolic or amine compounds present in drugs, catalyzing the transfer of electrons to molecular oxygen and forming reactive radicals that can rearrange into oligomers with higher molar mass and lower toxicity (Shraddha et al., 2011).

Several studies have shown that fungal laccase, even in the absence of mediators, efficiently degrades antibiotics; its mechanism of action involves opening the aromatic rings of antibiotics (Legerská et al., 2018; Sánchez-SanMartín et al., 2024). The chemical structure of the drugs, as well as the presence of electron-donating or electron-withdrawing functional groups, can alter the rate of enzymatic degradation (Chmelová et al., 2024).

The enzymatic degradation of pharmaceutical products in hospital effluent primarily occurred within the first hours of the reaction, indicating rapid enzyme kinetics followed by a plateau phase, possibly due to substrate depletion or the formation of less reactive intermediates. The best degradation result observed in our experiments was the removal of pyrazinamide, which showed the highest susceptibility to enzymatic oxidation, reaching 93.5% removal after 1 hour and 97.6% after 24 hours (Figure 3), indicating a rapid transformation of its heterocyclic structure.

Alharbi et al. (2019) evaluated the use of laccase from the fungus *Trametes versicolor* for the degradation of four pharmaceutical compounds individually over 48 hours. High removal rates were observed during the first 8 hours of the reaction, resulting in 100% removal of diclofenac, 95% removal of trimethoprim, 85% removal of carbamazepine, and 56% degradation of sulfamethoxazole. According to the authors, removing the four drugs tested individually was more effective than when tested in mixtures under the same conditions. This highlights the importance of working with real effluents, as various biotic and abiotic factors (including the initial concentration of each drug) can directly influence the degradation rate of these compounds.

Navada and Kulal (2019) evaluated the degradation of chloramphenicol (10 mg/mL), using laccases produced by *Trametes hirsuta* in the presence of the mediator ABTS (0.25 mM) and observed 82% degradation in 48 hours. According to Mojtabavi et al. (2023), the immobilized commercial laccase proved efficient at removing ciprofloxacin from aqueous samples after 3 hours of incubation.

Alokpa et al. (2025a) evaluated the application of free laccase (1,000 U L^{-1}) in the treatment of synthetic effluent and hospital effluent (filtrated), both spiked with 1 µg/L of various drugs, including one analgesic, five anti-inflammatory agents, and one anticonvulsant. They observed that drug removal rates were lower in real hospital effluent compared to synthetic effluent, and that more hydrophilic compounds, such as acetaminophen and mefenamic acid, were more sensitive to laccase oxidation. Indomethacin and ketoprofen exhibited the lowest removal rates (approximately 30–34%).

Al-Qaim et al. (2014) reported that caffeine is easily found in hospital effluents and may reach concentrations of 9,099 ng/L in effluents from wastewater treatment stations. Caffeine was among the most abundant compounds in hospital wastewater. The biological removal of caffeine was evaluated by Cruz-Morató et al. (2014) using the fungus *Trametes versicolor*, achieving a partial removal of the compound by approximately 38%. Our results showed a 72% removal of caffeine from hospital effluents using free laccase from a crude extract of *T. villosa*.

These results demonstrate that the laccase-containing enzymatic extract used in the present study efficiently removes pharmaceutical compounds, achieving high removal rates even without chemical mediators. Optimizing the process and/or immobilizing laccase for application in hospital effluent are targets for future research.

Removal of effluent color

The area of the visible spectrum (by spectrophotometric analysis in scan mode) of each effluent sample (before and after treatment) was integrated, and the presence of a maximum peak at 580 nm was determined. A spectral area reduction of 3.79% and a reduction in the maximum peak height at 580 nm of 6.81% were observed for samples treated with an initial activity of 500 U L^{-1} of laccase (LAC 500). In the effluent samples treated with an initial activity of 1,000 U L^{-1} (LAC 1,000), a spectral area reduction of 18.03% and a maximum peak height reduction at 580 nm of 23.76% were obtained. Therefore, a six-fold greater reduction was observed in the LAC 1,000-treated sample compared to the LAC 500-treated sample, demonstrating that the number of enzymatic units is proportional to the color removal in the treated effluent.

Effluents from fabric dyeing processes typically contain indigo dye derivatives, auxiliary chemicals, and azo- or anthraquinone-based dyes (Kalia et al., 2023).

The use of crude laccase extract and purified laccases has proven effective in degrading dyes with diverse structures (Kumar et al., 2024). Azo dyes contain a double bond between nitrogens (-N=N-), which bind to two conjugated aromatic systems in their molecules and represent the synthetic dyes most widely used by industry, while indigo dye contains a C=C bond connecting two heterocyclic rings with carbonyl groups, forming a chromophore composed of a conjugated system with four atoms in the carbonyl-double bond-carbonyl groups (Ge et al., 2024).

According to Wong and Yu (1999), anthraquinone dyes can serve as excellent substrates for laccase-mediated reactions and exhibit greater discoloration with increased enzymatic activity, corroborating our studies, which demonstrated greater degradation with increased enzymatic activity. Variations in dye degradation may be influenced by differences in the chemical structures of compounds in textile effluent.

Most of the studies reporting dyes biodegradation/decolorization by laccases from *Trametes* species describe the use of purified enzymes (free or immobilized), individual synthetic dyes, and chemical mediators (Wang et al., 2018; Bayramoglu and Arica, 2019; Bilal et al., 2021; Nouaa et al., 2025).

According to Khlifi et al. (2010), the decolorization of textile industry effluent by laccase from *Trametes trogii* without mediators was inefficient.

In this work, the crude enzymatic extract from *Trametes villosa* reduced effluent color; however, it is believed that effluent remediation can be optimized by immobilizing the enzyme or by using natural chemical mediators.

Evaluation of total phenolic compounds in effluents samples

Wastewater is a significant source of phenolic compounds and other pollutants, including pharmaceuticals and endocrine disruptors. These pollutants pose substantial environmental and health risks due to their toxicity and persistence (Rout et al., 2021; Ahmaruzzaman et al., 2024). Applying the crude laccase extract to the samples demonstrated moderate feasibility and efficacy for treating phenolic compounds in hospital wastewater. The average phenolic concentrations were 32.03 mg/L in the control sample and 12.75 mg/L in the treated sample, representing a 60.9% decrease in phenolic concentration after enzymatic treatment.

Hospital wastewater contains many compounds that are not fully removed by conventional treatment (Tao et al., 2025), posing an environmental risk. Therefore, alternative and effective treatments for hospital wastewater (which may be applied in conjunction) are in high demand. The biodegradation capacity of *T. versicolor* extracts was evaluated in a mixture of 30 organic contaminants by Nguyen et al. (2014), who observed the effective biodegradation of 14 phenolic compounds. Therefore, the crude extracts of *T. villosa* may be promising tools for removing phenolic compounds in complex mixtures, such as those observed in hospital effluents.

The enzymatically treated textile effluent samples demonstrated 98.5% removal of phenolic compounds, with an average concentration of 52.30 mg/L in the control and 1.37 mg/L in the treated sample. Similarly, the phenolic content of wastewater from a wine distillery was reduced using a *Trametes pubescens* growth culture (Strong and Burgess, 2008).

The laccase from Trametes versicolor was effective in transforming various phenolic compounds, both alone and in complex mixtures derived from olive mill wastewater (Canfora et al., 2008). The use of laccase has demonstrated its feasibility and efficacy in treating phenolic compounds in textile industry wastewater, reaffirming its effectiveness in transforming phenolic mixtures (both in simple and complex conditions) and highlighting its potential as an efficient tool for detoxifying phenolic wastes.

Chemical oxygen demand

The COD of the hospital wastewater sample showed a significant increase compared to the control sample, which had an average of 313.4 mg O_2 L^{-1}. After treatment, the chemical oxygen demand was 715.1 mg O_2 L^{-1} for the treated sample.

Cristóvão et al. (2009) also reported a significant increase in the COD of a simulated effluent containing reactive dyes (from 394 to 3,383 mg O_2 L^{-1}) after laccase treatment. By subtracting the enzyme values, the average of the treated effluent was similar to the initial values, due to the effluent's complexity.

A reduction in COD values was expected, as the treatment method is based on oxidation. However, the addition of the crude extract (containing proteins and other compounds produced by the fungus) and the presence of products and by-products with a high degree of complexity and unknown components in the effluents may have interfered with the COD assay, contributing to this unexpected result.

The analysis of COD in textile effluent samples demonstrated a minimal reduction from 497.2 mg O_2 L^{-1} in the control sample to 405.3 mg O_2 L^{-1} after enzymatic treatment.

According to Khouni et al. (2011), treatment with commercial laccase resulted in 98% decolorization of the studied textile effluent. However, despite improved color removal, enzymatic treatment did not directly affect COD removal.

In a study on the remediation of primary sewage sludge, it was observed that the enzymes cellulase and protease, tested individually, showed little or no impact on COD removal. Meanwhile, the mixture of the two enzymes resulted in a 97% reduction in total COD removal (Parmar et al., 2001).

Ecotoxicological assay

The average root length of the negative controls (in duplicate) was compared to that of the treatment samples using the Dunnett test, revealing a statistically significant difference ($p<0.05$) in the tested group.

In this test, a strong inhibitory effect was observed in seeds at higher concentrations before treatment. An unexpected hormetic effect on seed germination was observed at a 6.25% dilution of raw effluent. According to Rillig-Morales et al. (2025), exposure to low doses of chemicals stimulates the activation of compensatory stress response pathways, including antioxidant defense systems and increased metabolic activity. Hormetic effects can improve the performance and resilience of organisms to environmental stressors; however, as the amount of these stressors increases, the overall response tends to change dramatically. This may occur due to the cumulative negative impacts of previously beneficial stressors (Erofeeva, 2024). This is evident in our experiment, where we observe that the other dilutions showed an inhibitory effect.

After enzymatic treatment, a significant reduction in the phytotoxicity of hospital effluent was observed, as evidenced by the absence of an inhibitory effect on root elongation at a dilution of 12.5% of the treated effluent (Table 3). Only samples with 100% effluent still showed inhibitory effects; however, with the treated sample, a slight improvement in germination was observed.

Hillis et al. (2011) found that using lettuce (*L. sativa*) seeds was a valuable tool for assessing the phytotoxicity of antibiotics, and measuring root length (RL) was the most sensitive parameter to the effects of the evaluated antibiotics. The authors observed that the antibiotic levofloxacin was the most phytotoxic, and trimethoprim was the least phytotoxic (sulfamethoxazole was determined to have intermediate phytotoxicity).

Gerber et al. (2017) demonstrated that the germination of lettuce seeds in contact with raw effluent from pig slaughterhouses was lower than in the control treatment ($p<0.05$). Still, the authors found no difference in germination between lettuce seeds exposed to treated effluent and the control ($p<0.05$).

Gomes et al. (2019) verified the effects of ciprofloxacin on seed germination and root development of maize. They concluded that the antibiotic could accelerate germination by directly affecting the oxidative metabolism of seeds and roots, acting additively or synergistically to induce oxidative stress.

The results of the textile effluent tests showed that lettuce seed germination was inhibited after contact with a 12.5% dilution of the textile effluent at 100% concentration (Table 4). Enzymatic treatment significantly reduced seed growth inhibition in samples diluted at 12.5 and 25% of the effluent (RGI values).

Table 3 – Result of the phytotoxicity of hospital effluent on *L. sativa* seeds, before and after enzymatic treatment with laccase. The data presented are the values obtained after five days of germination in a biochemical oxygen demand (BOD) incubator. Comparative phytotoxicity (GI%) of textile and hospital effluents before and after treatment.

Samples	Dilution (%)	RL (cm)	CV (%)	GI (%)	RGI	Effect
Negative Control	–	22.1±6.5	29.2	–	–	–
Before treatment	6.25	28.0±8.9	32.0	144.1	1.3	S
	12.5	18.2±5.9	32.4	77.7	0.8	I
	25	16.4±5.3	33.3	69.9	0.8	I
	50	12.3±4.8	38.8	52.6	0.6	I
	100	9.4±2.7	27.8	40.3	0.4	I
After treatment	6.25	23.7±9.7	41.1	107.1	1.1	NSE
	12.5	21.2±8.4	40.4	96.1	1.0	NSE
	25	25.1±7.7	30.6	113.7	1.1	NSE
	50	21.8±7.0	32.1	98.8	1.0	NSE
	100	13.3±5.2	39.1	59.9	0.6	I

NC: Negative control samples exposed to 5% glyphosate; RL: Root length; CV: Coefficient of variation; GI: Germination index; RGI: Relative growth index; RGI values were used to evaluate the toxicity of the effluent and interpreted as: No significant effect (NSE): >0.8 and <1.2; Inhibitory effect (I): between 0 and 0.8; (S) Stimulation of root elongation: higher than 1.2; -: Not calculated.

Table 4 – Result of the phytotoxicity of textile effluent on *L. sativa* seeds, before and after enzymatic treatment with laccase. The data presented are the values obtained after five days of germination in a biochemical oxygen demand (BOD) incubator.

Samples	Dilution	RL (cm)	CV (%)	GI (%)	RGI	Effect
Negative Control	–	22.1±6.5	29.2	–	–	–
Before treatment	6.25%	20.0±5.8	29	85.3	0.9	NSE
	12.5%	18.2±5.9	32.4	77.7	0.8	I
	25%	16.4±5.3	33.3	69.9	0.8	I
	50%	12.3±4.8	38.8	52.6	0.6	I
	100%	9.4±2.7	27.8	40.3	0.4	I
After treatment	6.25%	21.3±7.2	33.6	88.3	1.0	NSE
	12.5%	20.8±7.0	34.2	85.9	0.9	NSE
	25%	19.0±7.7	40.5	78.5	0.9	NSE
	50%	14.8±5.2	35.1	61.1	0.7	I
	100%	12.8 ±6.1	47.7	52.8	0.6	I

NC: Negative control samples exposed to 5% glyphosate; RL: Root length; CV: Coefficient of variation; GI: Germination index; RGI: Relative growth index; RGI values were used to evaluate the toxicity of the effluent and interpreted as: No significant effect (NSE): >0.8 and <1.2; Inhibitory effect (I): between 0 and 0.8; (S) Stimulation of root elongation: higher than 1.2; -: Not calculated.

Untreated textile effluent showed greater inhibition at higher concentrations (IG=40.3 at 100%), which is consistent with the presence of azo dyes, aromatic amines, and auxiliary chemicals commonly reported in textile wastewater. Reddy and Osborne (2020) showed that

dyes and industrial effluents (a mixture of residual dyes, metals, and other compounds) can significantly affect the growth and development of *Vigna radiata, Vigna mungo,* and *Cajanus cajan* seeds.

Germination tests with synthetic solutions confirmed that mixtures of metals are more toxic than metals considered separately, and that real effluent from industries was more harmful than synthetic solutions (Charles et al., 2011). In addition, particularly high levels of copper and nickel directly impacted the germination of *Lactuca sativa* seeds (Charles et al., 2011).

Cantele et al. (2017) demonstrated that the decolorization of synthetic dyes with crude laccase extract did not produce inhibitory effects on *L. sativa* seeds, corroborating the result obtained in this study.

The phytotoxicity of both effluents was significantly reduced after enzymatic treatment. Most of the treated samples showed GI≥80%, evidencing effective detoxification. The improvement was particularly evident in the textile effluent, where intermediate dilutions went from an inhibitory effect to no significant effect. This suggests a degradation or structural modification of the chromophore and aromatic compounds, reducing their phytotoxic potential.

Conclusion

This study demonstrated the effectiveness of crude enzyme extract containing laccase in removing pharmaceutical compounds (caffeine and antibiotics), phenolics, and dyes from effluents. However, the increase in COD of hospital effluent and the stimulation of lettuce seed growth after treatment with laccases were unexpected. The use of a crude, unpurified extract from agro-industrial waste, without mediators, reduces the process cost. One of the greatest challenges in environmental biotechnology today is the transition from laboratory-scale experiments to full-scale wastewater treatment systems. In hospital wastewater treatment plants, crude laccase extract could be applied as a tertiary treatment step in batch reactors or membrane enzyme reactors, providing sufficient contact time between the enzyme and contaminants. For this to occur, a more detailed evaluation of operational parameters such as enzyme stability, optimal dosage, hydraulic retention time, and the volume of enzyme extract required to treat large wastewater flows is necessary. Enzyme immobilization can be an effective strategy to improve process stability and reduce operating costs, facilitating practical implementation in wastewater treatment plants.

Authors' Contributions

Silva, M.: conceptualization; methodology; formal analysis; investigation; writing – original draft. **Liz**, M.: methodology; resources. **Ramsdorf**, W.: methodology; formal analysis; investigation; resources. **Barbosa**, E.: formal analysis; investigation. **Tesuka**, L.: formal analysis; investigation. **Lima**, N.: formal analysis; investigation. **Ferreira**, L.: formal analysis; investigation. **Haminiuk**, C.: writing – review and editing; funding acquisition; resources. **Maciel**, G.: conceptualization; methodology; formal analysis; investigation; writing – review and editing; funding acquisition; supervision.

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