

## DYE DECOLORIZATION AND BIOMASS DEGRADATION BY *Pleurotus pulmonarius* AND *Pleurotus ostreatus*

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**ABSTRACT**

Uncontrolled release of dyes and coloring materials as industrial effluents is one of the primary causes of environmental pollution. The present work was conducted to evaluate two fungal strains, *Pleurotus pulmonarius* and *Pleurotus ostreatus* in terms of their biodegradation ability of different dyes. The influence of pH on laccase activity and dye adsorption, phenol oxidase activity, ecotoxicity, and biomass degradation ability of cigarette butts (filters) was also included. In case of *Pleurotus pulmonarius*, the highest dye degradation activities was observed in methylene blue, while *Pleurotus ostreatus* showed the highest dye degradation activity in crystal violet. Percent dye degradation activity was significantly higher in *Pleurotus pulmonarius* than *Pleurotus ostreatus* (in case of methylene blue and crystal violet). It was seen for both the strains pH 5-7 was an optimum showing maximum dye adsorption (in case of methylene blue and crystal violet) and laccase activity. At higher pH values, the adsorption ability decreases. But both strains decolorized basic fuchsin at high pH (pH 7-9) only. Both the fungal strains were found to have extracellular phenol oxidase activity. The present study showed that after the bioremediation the dye solution had insignificant ecotoxicity. Biomass of cigarette filters was significantly reduced with *Pleurotus pulmonarius* while in case of *Pleurotus ostreatus*, the effect was less significant.

**Keywords:** *Pleurotus pulmonarius*, *Pleurotus ostreatus*, textile dyes, cigarette filter

**INTRODUCTION**

Dyes and coloring pigments have enormous applications in food industries, cosmetics, papers, plastic and textiles industries. Some dyes are potential threats to animals and other living organisms because they are toxic and carcinogenic. Although several efforts are being made for making people aware about environmental protection, industrial effluents are still mostly released into soil and natural water bodies. Uncontrolled release of such chemicals deteriorates the quality of the soil and water. There are several reports on the toxicity of textile dyes (Suryavathi *et al.*, 2005). Non-biodegradability of the textile dyes is also a major concern that needs to be addressed (Eichlerová *et al.*, 2006). Physical or chemical treatments for dye removal are expensive and/or produce large amounts of toxic byproducts (Fatima *et al.*, 2015; Asgher & Bhatti, 2012). Bioremediation is considered to be one of the most effective techniques because of its low running costs and production of relatively harmless byproducts (Djelal & Amrane, 2013). Biological methods for removal of dyes generally involve absorption technique and enzymatic degradation of the dyes (Harms *et al.*, 2011). Mycoremediation is the use of various fungi in removal of pollutants (Djelal & Amrane, 2013). Several uses of mushrooms were described by researchers, because of their great importance in the field of biotechnology (Sekan *et al.*, 2019; Xiao *et al.*, 2022). Fungi could be used as an alternative and cost-effective measure to eliminate the textile dyes from the wastewater (Abdel-Raheem & Shearer, 2002).

Lignin degrading fungi or white rot fungi are known to degrade petroleum hydrocarbons (Thapa *et al.*, 2012). Oyster mushrooms or Species of *Pleurotus*, which are known to be the most important edible mushrooms in the world, are commercially important and have roles in the field of biotechnology and environment (Thapa *et al.*, 2012; Adenipekun & Lawal, 2012). They mainly possess laccase (Lac) and Mn-peroxidase (MnP) which degrades lignin and cellulose and promotes their growth on a variety of agricultural wastes (Agrahar-Murugkar & Subbulakshmi, 2005).

On the other hand, different studies revealed that about  $4.5 \times 10^6$  million cigarette butts (CBs) are disposed per year (Bonanomi *et al.*, 2015; Novotny *et al.*, 2009). They are often disposed of on beaches and parks (Ariza *et al.*, 2008). CBs can pose serious problems to animals including our ecosystem (Novotny *et al.*, 2011). Smoked tobacco contains a substantial amount of nicotine, ammonia, hydrogen cyanide, acetaldehyde, benzene, pyridines etc. (Hoffmann, 1997). CBs are composed of compressed, plasticized cellulose acetate, and the presence of several acetate substitutions causes a barrier between the cellulose microbes for

biodegradation. Due to their slow degradation rate, they accumulate in the environment (Rustemeyer, 2004; Curtis *et al.*, 2017).

The present study was conducted to compare the biodegradation ability of different dyes of the fungal strains, *Pleurotus pulmonarius* and *Pleurotus ostreatus*. The influence of pH on dye degradation and laccase activity, phenol oxidase activity, ecotoxicity and bacterial toxicity were evaluated. Additionally, the fungal strains were also used for the biomass degradation of CBs.

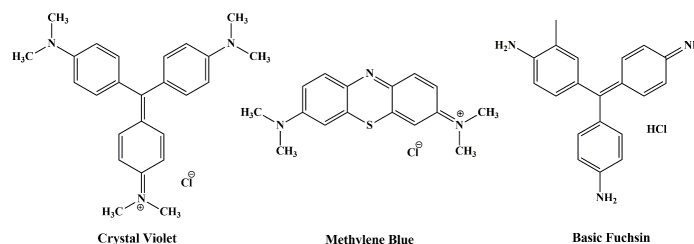
**MATERIALS AND METHODS**

**Collection and maintenance of fungal strains**

*Pleurotus pulmonarius* and *Pleurotus ostreatus* were collected from Ramkrishna Mission, Sargachi, Murshidabad, Berhampore, West Bengal, India were cultured on Potato Dextrose Agar (mentioned as PDA, hereafter)(Himedia, Mumbai, India).

**Dye Degradation**

**Dyes used:** Molecular structure of the dyes are given (Scheme 1). (1) Basic fuchsin : (**BF** hereafter, Molecular formula:  $C_{20}H_{20}N_3HCl$ ), (2) Crystal violet : (**CV** hereafter, Molecular formula:  $C_{25}H_{30}ClN_3$ ) and (3) Methylene blue: (**MB**, Molecular formula:  $C_{16}H_{18}ClN_3S$ ).



**Scheme 1** Chemical structure of the dyes used

**Decolorization of dyes using liquid medium**

Mycelia of *Pleurotus pulmonarius* and *Pleurotus ostreatus* were taken from Petri plates and then cultured in potato dextrose broth (PDB, hereafter) for 7 days. 1 ml of culture was taken into 5ml PDB containing 0.01% of respective dye (such as

**BF, MB and CV**). The test tube containing *Pleurotus pulmonarius* culture was marked as “A”, the test tube with *Pleurotus ostreatus* was marked as “B”, Test tube which had only dye was used as control (C) and the test tube containing only PDB was blank. All the test tubes were kept under stationary conditions for 10 days. Each experiment was done thrice. Growth and decolorization of dye were checked every day. After 10 days, the filtrates from the cultures were extracted and absorbance was taken using spectrophotometer. Maximum absorbance of the said dyes were taken as **BF**-550 nm, **MB**-665 nm, **CV**-579 nm wavelength respectively (Park et al., 2007).

$$\text{Pdd \%} = \frac{\text{Absorbance (c)} - \text{Absorbance (i)}}{\text{Absorbance (c)}} \times 100$$

“Pdd” denotes “Percent dye decolorization”

“Absorbance(c)” denotes “Absorbance control”

“Absorbance(i)” denotes “Absorbance inoculated”

#### Change of pH during the dye decolorization

1ml of culture of *Pleurotus pulmonarius* and *Pleurotus ostreatus* mentioned earlier was taken into 5ml PDB containing 0.01% of respective dye (such as **BF, MB and CV**). Besides 1ml of culture of the mentioned fungal strain were also inoculated into the PDB (without any dye) separately. All the test tubes were kept under stationary conditions for 10 days. Each experiment was done thrice. Change in pH and decolorization of dye were checked every day. After 10 days, the filtrates from the cultures were extracted and used for spectrophotometric analysis as stated above (Park et al., 2007).

#### Role of pH on adsorption of dye and laccase activity

Role of pH on the adsorption of dye was studied by the standard method described (Zhou et al., 2011). 1ml of culture of *Pleurotus pulmonarius* and *Pleurotus ostreatus* mentioned earlier was taken into 5ml PDB containing 0.01% of respective dye (such as **BF, MB and CV**) with initial pH range of 2 to 9. All the test tubes were kept under stationary conditions for 10 days. Each experiment was done thrice. Growth and decolorization of dye were checked every day. After that 100 µl of supernatant of the respective culture was added to 5 mM 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (dissolved in 0.1 M, pH 5.0 sodium acetate buffer) to determine the activity of Laccase enzyme followed by the method of Bourbounnais et al. (1995). All the test tubes containing the mixture were kept at 30° C for 2 min. Absorbance was estimated at 420 nm. On the other hand, filtrates from the cultures were also extracted and used for spectrophotometric analysis.

#### Detection of extracellular phenol oxidase activity

Extracellular phenol oxidase producing ability was tested using PDA plate with the supplementation of 10 mM ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] as substrate (Floch et al., 2007). Two fungal strains were inoculated at the centre of the plate separately and kept incubated in the dark. PDA plate containing ABTS only was used as control. Fungal strain producing colored zone surrounding the colony was treated as phenol oxidase positive (Flochet et al., 2007; Sinsabaugh, 2010).

#### Seed germination bioassay

The influence of treated dye (dye after bioremediation) and dye solution without bioremediation was observed on germination of Gram seed. 3% hydrogen peroxide solution was used to sterilize Gram seeds and distilled water was used to rinse 6-7 times to remove hydrogen peroxide. Filter papers (sterile) were used to soak with respective treated dye on sterilized petri plates and then Gram seeds were kept on the filter paper. Soaked filter paper with dye without any treatment and distilled water (sterilized) was used as control. The experiment was carried out three times. Any changes regarding the seed germination were observed for four days at room temperature.

#### Bacterial toxicity

The toxicity of the bioremediated dyes by these two fungal strains against *Staphylococcus aureus* and *Escherichia Coli* was tested using mycelial filtrate, treated, and untreated dye solution on the nutrient agar plate using the agar well diffusion method by measuring zone of inhibition with a duration of 48 h at 30° C (Kanmani et al., 2011).

#### Biomass degradation assay

Mycelia of *Pleurotus pulmonarius* and *Pleurotus ostreatus* were taken from Petri plates and then cultured in potato dextrose broth for 7 days and then transferred to PDA plate. Cigarettes of common brands were purchased and artificially smoked. A biomass degradation assay was performed to detect the capability of these fungal strains to degrade the cigarette filter on PDA plate containing the growth of the

respective fungal strain. Cigarette butt on PDA plate without any fungal growth was used as the control. All the plates were kept at 30°C in an incubator for 4 weeks. Any weight change was observed from day to day.

#### Statistical Analysis

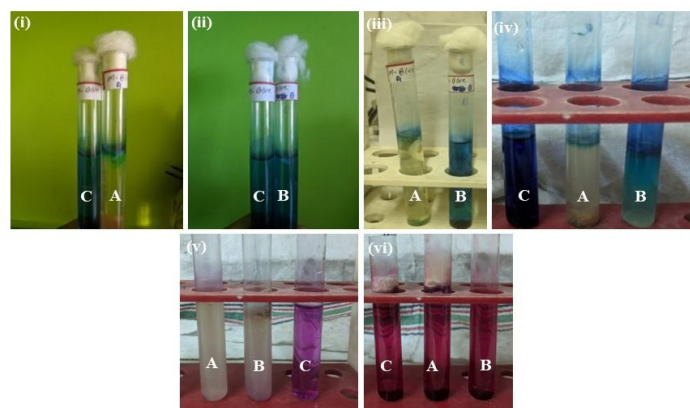
Mean and SD (standard deviation) were estimated after repeating each of the quantifications for three times. One tailed Student's t test was used to interpret the data. P < 0.05 was regarded as the level of significance to establish the significant differences between the means.

## RESULTS AND DISCUSSION

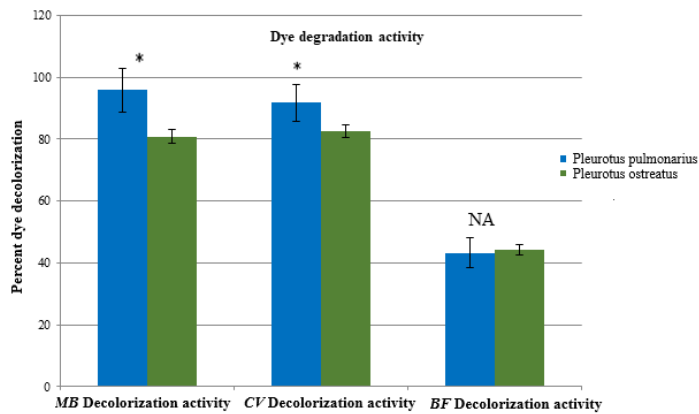
#### Decolorization of dyes

In the present study dye degradation ability of different fungal strains were primarily checked. Decolorization of the dyes was first observed after 12 hours and 24 hours of inoculation of *Pleurotus pulmonarius* in **MB** and **CV** respectively. But decolorization of the dyes was first observed after 24 hours and 48 hours of inoculation of *Pleurotus ostreatus* in **CV** and **MB** respectively. Negligible change was found in the color of **BF** after 10 days of inoculation of both the fungal strains (Figure 1a) in the same condition used for **MB** and **CV**. In case of *Pleurotus pulmonarius* the highest dye degradation activities were observed with **MB** (95.9 %), followed by **CV** (91.7 %) and least in **BF** (43.2 %) while *Pleurotus ostreatus* showed the highest dye degradation activity in **CV** (82.6 %) followed by **MB** (80.8 %) and least in **BF** (44.3 %) (Figure 1b). Percent dye degradation activities of *Pleurotus pulmonarius* (in case of **MB** and **CV**) were significantly higher (P < 0.05) than that of *Pleurotus ostreatus* (Figure 1b). Besides this, it could decolorize **MB** and **CV** more rapidly than *Pleurotus ostreatus*. Decolorization of **MB** by *Pleurotus pulmonarius* and *Pleurotus ostreatus* was consistent with several reports (Leo et al., 2019; dos Santos Bazanella et al., 2013; Akar et al., 2009; Maaset al., 2018). Morales-Alvarez et al. (2016) suggested that *Pleurotus ostreatus* was able to decolorize **CV** which was observed in this study. Decolorization of azo dye by *Pleurotus ostreatus* was also shown by Fiana et al. (2023).

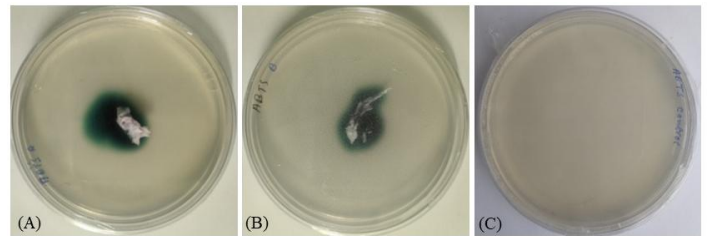
Decolorization of dye depends on the biodegradation of the chromophore present in the dyes (Rani et al., 2014). It was suggested that few fungi possess important exoenzymes such as peroxidases and phenol oxidases having the reduction ability of azo dyes (Duránet al., 2002). In the present study both the strains were also found to have phenol oxidase activity and this may be responsible for reduction of the said dyes (Figure 1c). Biodegradation of chromophore may be attributed to phenol oxidases through a nucleophilic reaction with water on phenolic ring carbon which has the azo linkage (Camarero et al., 2005). Phenol oxidase is reported to decolorize azo dyes by the free radical mechanism and nonspecifically with the production of phenolic compounds. Thus, toxic aromatic amines generation might be avoided (Wong & Yu, 1999). In this study *Pleurotus pulmonarius* is more efficient than *Pleurotus ostreatus* in terms of degradation of dyes, which may be due to biosorption by the fungal biomass as they produce more stem than *Pleurotus ostreatus* (Stamets 2000). Not only exoenzymes but also the mycelia of the fungi also attribute the decolorization of the dyes through absorption and adsorption by providing a large cell-surface ratio on the mycelium (Fu & Viraraghavan, 2002; Przysła et al., 2018). Decolorization might involve physical forces, precipitation, entrapment in inner spaces of fungal mycelium, exchange of ion and hydrogen bonds formation (Yeddou-Mezenner, 2010).



**Figure 1a** Percent dye degradation by *Pleurotus pulmonarius* (shown by A) and *Pleurotus ostreatus* (shown by B). Control (shown by C). (i) and (iii) Decolorization of Methylene blue after 12 hours and 24 hours of inoculation of *Pleurotus pulmonarius* respectively; (ii) and (iii) No decolorization of Methylene blue after 12 and 24 hours of inoculation of *Pleurotus ostreatus* respectively; (iv) decolorization of Methylene blue after 48 hours of inoculation of *Pleurotus pulmonarius* and *Pleurotus ostreatus* respectively; (v) Decolorization of Crystal violet after 6 days of inoculation of *Pleurotus pulmonarius* and *Pleurotus ostreatus* respectively; (vi) There was no change in color of Basic fuchsin after 10 days of inoculation.



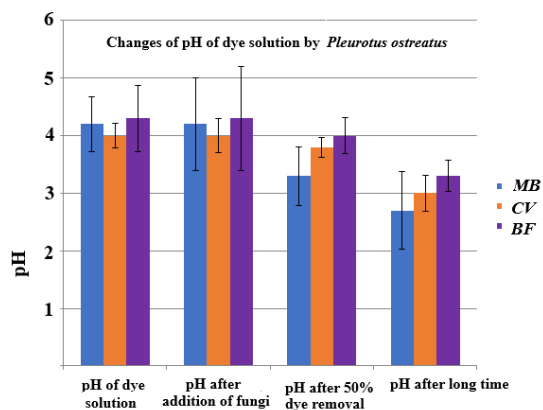
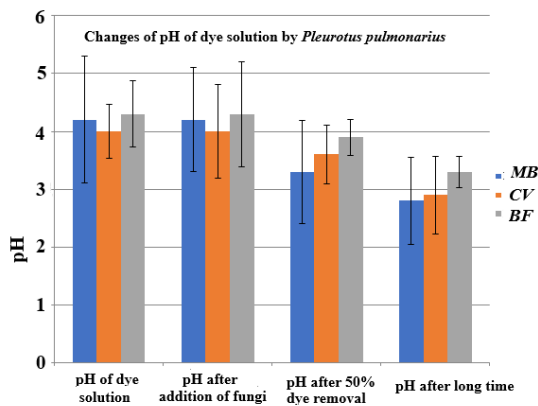
**Figure 1b** Percent dye degradation between *Pleurotus pulmonarius* and *Pleurotus ostreatus*. Values are expressed in Mean  $\pm$  SD. One tailed Student's t test was used to analyze the data. P values  $\leq$  0.05 was regarded as the level of significance to establish the significant differences between mean. “\*” means significant and “NA” means not significant. Percent dye degradation activities of *Pleurotus pulmonarius* (in case of Methylene blue and Crystal violet) were higher than that of the *Pleurotus ostreatus*.



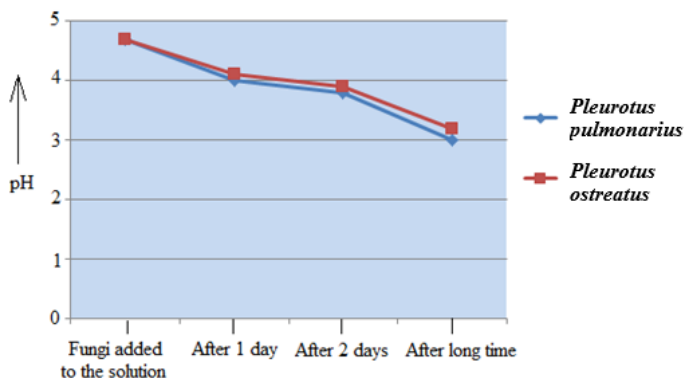
**Figure 1c** Phenol oxidase detection assay on media containing ABTS. A: *Pleurotus pulmonarius*; B: *Pleurotus ostreatus*; C: Control. Both the strains were found to have phenol oxidase activity

**Study of pH changes during the dye decolorization**

A study to understand the pH change during dye degradation was performed with all the dyes (Figure 2a and Figure 2b). Initial pH of all the dyes were found to be in the range 4.0-4.3. After the addition of *Pleurotus pulmonarius*, pH of the medium started decreasing. During 50 % removal, it was 3.3 for MB, 3.8 for CV and 4.0 for BF (Figure 2a). Similar trend was also observed for pH variation study in case of *Pleurotus ostreatus* (Figure 2b) These results suggests that the negatively charged cell wall of the fungal strains attracts the cationic dye molecule and H<sup>+</sup> ion is left unadsorbed due to which pH is gradually decreasing. The decreased pH might also be due to acid production by the fungal strains (Cheng et al., 2016). The pHs of the fungal strains were also noted as control experiments. It was observed that the pHs of the strains were found to be 4.7, both for *Pleurotus pulmonarius* and *Pleurotus ostreatus*, and with time it decreased (Figure 2c). This is in accordance with the previous known literature (Zhou et al., 2011; Akar et al., 2009).



**Figure 2a and 2b** pH variation of the dye solution after (a) *Pleurotus pulmonarius* was added (b) *Pleurotus ostreatus* was added. Values are expressed in Mean  $\pm$  SD. pH of the dye solution was found to be reduced after inoculation of *Pleurotus pulmonarius* and *Pleurotus ostreatus* respectively



**Figure 2c** pH of PDB without dye after addition of the fungal strains. pH of PDB was found to reduce after inoculation of *Pleurotus pulmonarius* and *Pleurotus ostreatus* respectively

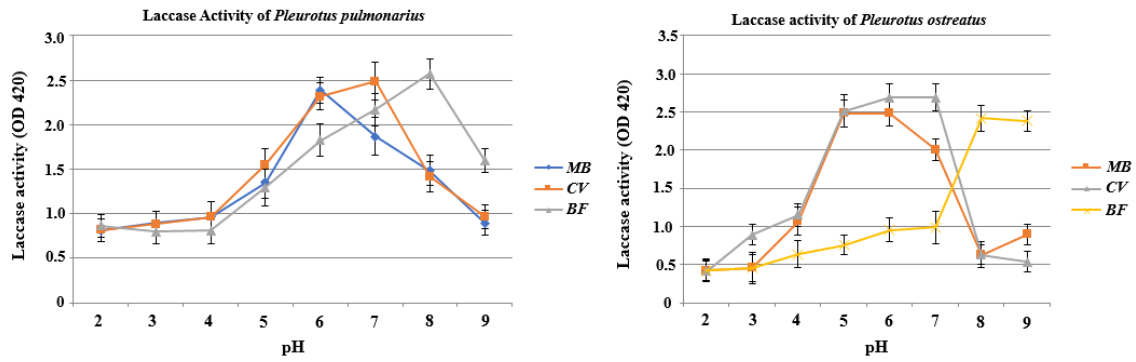
**Effect of pH on dye degradation and laccase enzyme activity**

Additionally, we have also done pH dependant study of dye degradation by the fungi. Interestingly, it was seen that the pH at which laccase activity was found to be the maximum, also showed maximum dye degradation. It was seen for both *Pleurotus pulmonarius* and *Pleurotus ostreatus*, maximum dye degradation was

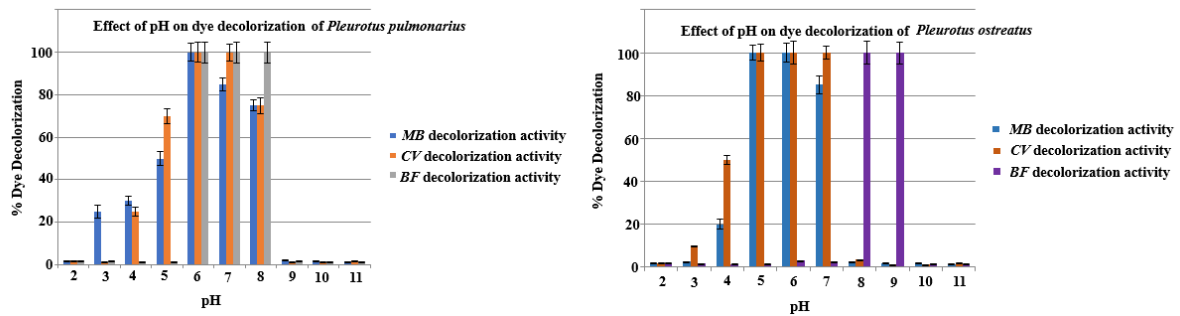
observed at pH 5-7 (in case of MB and CV). Maximum Laccase activity was also observed in the same pH range (Figure 2d, 2e, 2f, 2g). This is also in corroboration with earlier studied literature where fungal strains were used for dye removal in aqueous solution (Ankamwar, 2016; Hameed & Ahmad, 2009; Pathania et al., 2017;Kunjadia et al., 2016). On the other hand, for BF, maximum decolorization was observed at pH 7-9.

Mycoremediation using *Pleurotus pulmonarius* and *Pleurotus ostreatus* is occurring in a dynamic system and the adsorbents are stable in a range of pH. It was also revealed that the fungal strains are acid resistant and mycoremediation is suitable for conditions of waste water discharge (Sintakindi & Ankamwar, 2020; Li et al., 2020).

pH dependant dye removal study reveals that since cell wall in fungal strains are negatively charged, they should have efficient in dye removal. But, again, at very low pH, proton concentration is also high and there is significant competition between protons and dye molecules (Ankamwar, 2016; Hameed, 2009; Pathania et al., 2017; Abd El-Rahim et al., 2009; Rajhans et al., 2021). At a slightly higher pH, this competition decreases and we see an optimum result at pH 5-7. At higher pH values, the adsorption ability decreases. For BF, however, removal was better at pH 7-9 unlike the other dyes. This might be because of the higher amount of efficient electrostatic interaction of negatively charged surface of the fungus with the cationic dye. In an earlier work by El Haddad (2016) it was seen that the most optimum dye removal by mussel shell biomass waste was found to be at 9.2 for BF. Previously, maximum removal of the BF by Fusarium oxysporum was also reported at higher pH i.e., pH 10 (Batanaet et al., 2022). Another report suggested that hydrophobic nature of BF might cause maximum dye uptake (Basic fuchsin) at higher pH values (Radha et al., 2023).



**Figure 2d and 2e** Effect of pH on the laccase activity in adsorption of the dyes by *Pleurotus pulmonarius* and *Pleurotus ostreatus*. Values are expressed in Mean  $\pm$  SD. pH at which laccase activity was found to be the maximum, also showed maximum dye adsorption.

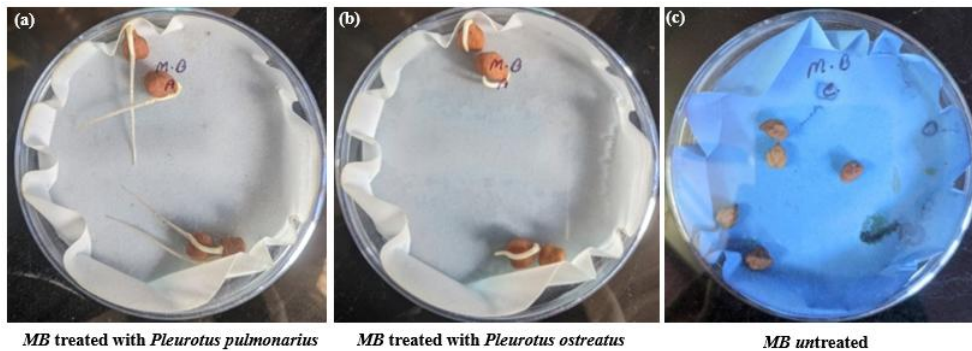


**Figure 2f and 2g** pH dependant study of dye adsorption of *Pleurotus pulmonarius* and *Pleurotus ostreatus*. Values are expressed in Mean  $\pm$  SD. pH 5-7 was an optimum showing maximum dye adsorption (in case of **MB** and **CV**). *Pleurotus pulmonarius* decolorized **BF** at pH 7-8. *Pleurotus ostreatus* decolorized **BF** at pH 8-9.

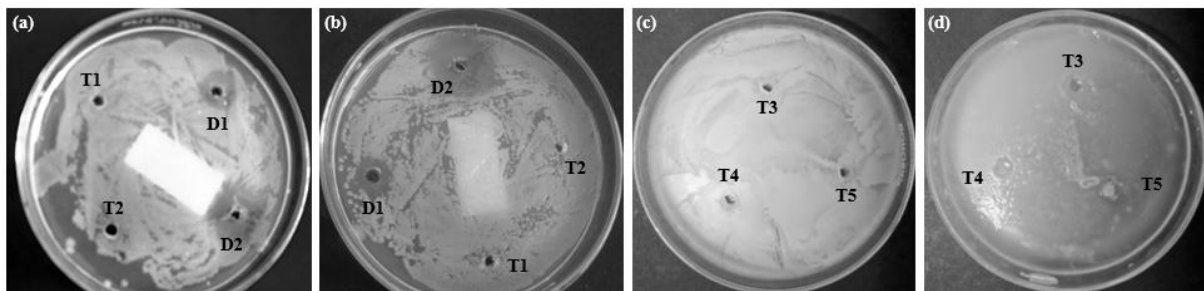
**Ecotoxicity**

In order to support the detoxification studies, seed germination assay and bacterial toxicity test were also performed. It was observed that most of the seeds germinated in a medium of treated dye (by the fungal strains) while seed germination was delayed in case of untreated dyes (Figure 3a). Hence it indicates

treated dye after bioremediation did not affect seed germination of Gram seeds. It can be thus safely concluded that significant amount of the dye molecule was destroyed the fungal strains. Beside this dye after bioremediation did not show any bactericidal activity against both Gram-positive and Gram-negative bacteria (Figure 3b). So, these fungi were not toxic and might be used as effective bioremediation agents.



**Figure 3a** Seed germination assay of Gram seed by treated and untreated dye

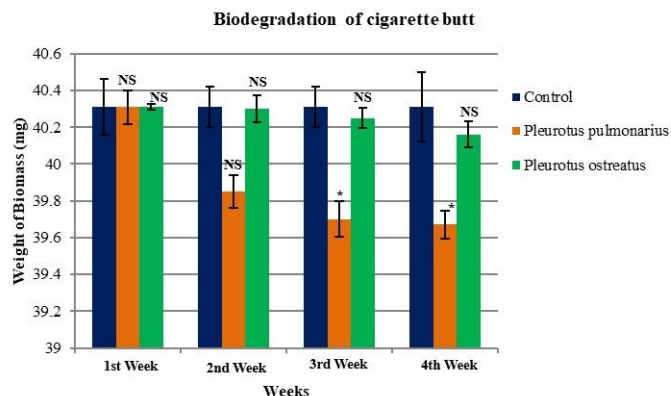


**Figure 3b** Bacterial toxicity of treated and untreated dye against *Staphylococcus aureus* and *Escherichia Coli*. (a) Plate of *S. Aureus*, T1: **CV** treated with *Pleurotus ostreatus*; T2: **MB** treated with *Pleurotus ostreatus*; D1: **CV**; D2: **MB**; (b) Plate of *E. Coli*, T1: **CV** treated with *Pleurotus ostreatus*; T2: **MB** treated with *Pleurotus ostreatus*; D1: **CV**; D2: **MB**; (c) Plate of *S. Aureus*, T3: **CV** treated with *Pleurotus pulmonarius*; T4: **MB** treated with *Pleurotus pulmonarius*; T5: **BF** treated with *Pleurotus pulmonarius*; (d) Plate of *E. Coli*, T3: **CV** treated with *Pleurotus pulmonarius*; T4: **MB** treated with *Pleurotus pulmonarius*; T5: **BF** treated with *Pleurotus pulmonarius*

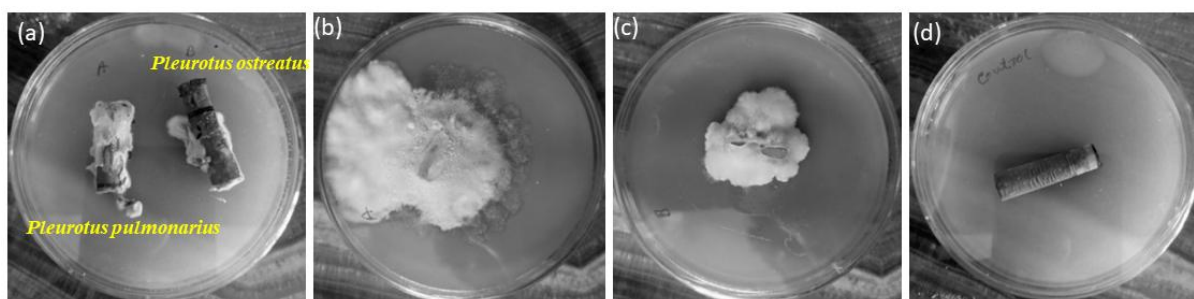
Dyes treated with *Pleurotus ostreatus* as well as *Pleurotus pulmonarius* did not show bacterial toxicity against both *Staphylococcus aureus* and *Escherichia Coli*.

### Biomass degradation

The result of the biomass degradation of cigarette filters or cigarette butts indicates that both *Pleurotus pulmonarius* and *Pleurotus ostreatus* have cellulose degradation activity. This may be due to their cellulase enzyme activity degrading the cellulose of the cellulose acetate which is a nonbiodegradable component of cigarette filters (Shafey et al., 2009). Several reports also suggested that the fungal group of *Pleurotus sp.* possesses cellulase activity (Cohen et al., 2002; Moyson & Verachert,1991). Updyke (2014) also showed the biomass degradation of cigarette filters by *Pleurotus ostreatus*. But biomass of cigarette butt was not significantly reduced after 4 weeks of inoculation of *Pleurotus ostreatus* in this study (Figure 4). On the other hand, *Pleurotus pulmonarius* is more effective in the reduction of biomass of the cigarette filter than the *Pleurotus ostreatus* as the biomass of cigarette butt was significantly reduced ( $P < 0.05$ ) after 4th week of inoculation of the former. But both of them degraded biomass of cigarette butt maximally after 7 month (Figure 5). Biodegradation by *Pleurotus pulmonarius* and *Pleurotus ostreatus* was also reported by Odigbo et al. (2023).



**Figure 4** Comparison of biomass degradation of cigarette butts between *Pleurotus pulmonarius* and *Pleurotus ostreatus*. Values are expressed in Mean  $\pm$  SD. One tailed Student's t test was used to analyze the data. P values  $\leq 0.05$  was regarded as the level of significance to establish the significant differences between mean. “\*” means significant and “NS” means not significant. Biomass of cigarette butt was significantly reduced ( $P \leq 0.05$ ) after 4th week of inoculation of *Pleurotus pulmonarius*. Biomass of cigarette butt was not significantly reduced after 4 weeks of inoculation of *Pleurotus ostreatus*.



**Figure 5** Biomass degradation of cigarette butts by *Pleurotus pulmonarius* and *Pleurotus ostreatus* after 4 week of inoculation (A). Maximum biomass degradation of cigarette butts by *Pleurotus pulmonarius* (B) and *Pleurotus ostreatus* (C) after 7 month of inoculation respectively. Cigarette butt on PDA plate without any fungal strain was used as control (D)

All the results of this suggest that both *Pleurotus pulmonarius* and *Pleurotus ostreatus* can be used for bioremediation of textile dyes while *Pleurotus pulmonarius* can be used for bioremoval of cigarette butts as their uses will be cost-effective with negligible environmental hazard.

### CONCLUSION

In the present study decolorization of methylene blue and crystal violet by *Pleurotus pulmonarius* was found to be faster than the decolorization of the dyes by *Pleurotus ostreatus*. But inoculation of both the fungal strains was found to show a negligible change in the color of basic fuchsin in the same condition used for Methylene blue and Crystal violet. But they decolorized Basic fuchsin at pH 7-9. The present study showed that the bioremediated dye had no ecotoxicity. On the other hand, it was found that *Pleurotus pulmonarius* was more effective in the significant reduction of the biomass of cigarette filters than *Pleurotus ostreatus*.

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