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RESEARCH ARTICLE

# Mycodecolorization Activity of *Pleurotus Citrinopileatus* for Chemically Different Textile Dye Under Varied Aromatic Amino Acids and Trace Elements

**A.K. Srivastava[*1], S.K. Uppadhyay[2] and S.K. Vishwakarma[1]**

[1]Department of Biotechnology, M.H.P.G. College, Jaunpur – 222003 INDIA
[2]Depatment of Environmental Science, V.B.S. Purvanchal University, Jaunpur – 222003 INDIA

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## ABSTRACT

In the present study, ligninolytic enzymes laccase (benzenediol: oxygen reductase EC; 1.10.3.2) and Manganese Peroxidase (Mn(II): hydrogen-peroxide oxidoreductase EC; 1.11.1.13) activity and of White Rot Fungi (WRF) *Pleurotus citrinopileatus* were enhanced with the application of trace metal i.e. Copper and Manganese at 25 ppm and 50 ppm followed by aromatic amino acids (Phenylalanine, Tryptophan and Tyrosine) at 0.02 µM and 0.4 µM. Laccase and MnP activity were 213.42U and 202.28U respectively, observed at 300ppm of Methyl Red supplemented with Tyrosine (0.2µM) followed by treatment of Tryptophan (198.45U and 195.16U) and Phenylalanine (195.85U and 188.15U). Maximum Laccase and MnP activity (Tyrosine treated) were revealed maximum decolorization of Phenol Red and Methyl Red (84.14% and 78.20%) followed by Phenylalanine (80.92% and 73.80%) and Trypatophan (71.22% and 70.12%). The negative correlation of Laccase and MnP activity was observed with a higher concentration (>50ppm) of trace metal in the medium, while at 25ppm of copper supplemented medium increase three-fold of Laccase activity (585.56U) as tyrosine medium and similarly, Manganese (25ppm) inosculated medium revealed three-fold more MnP activity (478.95U). A lower amount of Cu hoists Laccase and MnP activity which decolorized 300ppm of Methyl Red and Phenol Red with maximum percent (92.3% and 88.15%) followed by Mn. Thus, Laccase and MnP enzymes both play an important role in decolorization of dyes, and its activity was enhanced with the application of lower concentration of trace metals followed by aromatic amino acids.

## 1) INTRODUCTION

The dye industry is an exigent division of the chemical industry. Manufacture and use of synthetic dyes is a multibillion dollar industry and these synthetic organic with multiple aromatic ring either fused or connected covalently and modified with various hydrophilic functional group such a amine, carbonyl and hydroxyl group to produce desired color and affinity to the material being dyed [1]. Azo dyes, which are the gigantic and most varied group of aromatic synthetic compounds with one or more –N=N– groups, applied in a number of industries such as textile, food, cosmetics, and paper printing [2, 3]. All dyes do not interlace to the fabric depending on the class of the dye and about 10-15% of the dyes released into the wastewater during dyeing process [1, 3]. Dye wastewater is inscribed by extreme vacillation at many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, color, and salinity etc (REF). Decolorization is simply the evanescence of color in wastewater without the actual breaking apart of the dye molecules, which does not mean degradation of the complex dye molecules while degradation is the perdition of the large dye molecule to smaller components along with the breakdown of the chromophore. Chromophore groups of dyes may be destroyed; the intermediate product may be more toxic than original compounds and could present epochal issues for receiving water bodies [4]. Mineralization refers to organic compounds are converted into inorganic compounds *i.e.* nitrate, carbon dioxide and water which approaches complete detoxification and minimized secondary pollution. The various technologies of dye removal, such as adsorption on inorganic or organic matrices, decolorization by photocatalysis or by oxidation processes, microbiological or enzymatic decomposition have been sublime by earlier researchers for the effective treatment of dyes from wastewater to decrease their impact on the environment [2, 5]. These methods are quite expensive and consume high amounts of chemical and energy and also deviate from eco-friendly sustainable agenda. The effectiveness of microbial decolorization depends on the adaptability and the activity of the selected microorganisms [1, 6, 7]. It is well known that bacteria degrade azo dyes reductively under anaerobic conditions to colorless aromatic amines and these should be degraded further because of may be toxic, mutagenic and carcinogenic to human and animals [8]. Mycoremediation is a proper and green solution of

* Corresponding Author: **A.K. Srivastava**
*Email address:* srivastavabhishek4@gmail.com

eradication and reduction of the existing environmental pollutant (9). The use of lignin-degrading white rot fungi (WRF) has been attracted scientific attention to degrade a wide range of recalcitrant organic compounds. Their lignin modifying enzymes (LME), that is MnP (Mn (II): hydrogen peroxide oxido-reductase EC; 1.11.1.13), LiP (1,2- bis(3,4-dimethoxyphenyl) propane-1,3-diol; hydrogen peroxide oxidoreductase, EC 1.11.1.14,) and laccases (benzenediol: oxygen reductase EC; 1.10.3.2), are directly involved in the degradation of not only lignin in the natural lignocellulosic substrates [10, 11] but also various xenobiotic compounds [12, 13] including dyes [14-18]. *Pleurotus* species are gaining popularity in mycoremediation because of their capability of rapid growth and presence of highly efficient enzymatic machinery. Ligninolytic enzymes of the WRF are usually express during secondary metabolism, when carbon and nitrogen source become limiting [19] and their expression is usually inducible. Therefore, the effect of aromatic amino acids at different concentration is admitted topic which will be useful knowledge for the development of a low cast culture medium. Certain trace elements (Cu, Mn, Zn, Fe, Mo and Ni etc.) are essential for the fungal metabolism at optimized concentration, while they could be toxic or induced stress at beyond of their optimum concentration for organisms [20]. Thus, during evolution WRF species developed various effective enzymatic and non-enzymatic system, such as Laccase and Manganese peroxidase, as response to that stress factor [21]. Whether form and concentration of the aromatic amino acids and trace elements could make an impression on the decolorization of Methyl Red and Phenol Red and also on the activity of Laccase and Manganese peroxidase during observation by *P. citrinopileatus* was the questions that provided the goal for the present research work.

## 2) MATERIALS AND METHODS

***Cultures and their maintenance***

The pure cultures of *P. citrinopileatus* used in present experiments was procured from Directorate of Mushroom Research, Solan and Indian Agricultural Research Institute, New Delhi. Throughout the study, the stock culture was maintained on potato dextrose agar (PDA) slants at 27± 2 $^0$C and sub-cultured at regular interval of three weeks.

***Production of enzymes***

The experiment on production of ligninolytic enzymes was carried out in potato dextrose broth medium (20% peeled potato and 2% dextrose). Double distilled water was used for preparation of the medium and pH was adjusted at 6.0 by using N/10 NaOH or N/10 HCl. Incubation was carried out at 25$^0$C in BOD incubator in cotton plugged 250 ml Erlenmeyer flask containing 100 ml of media. Each flask inoculated with 1 mm in diameter of agar pieces of *Pleurotus* species and improved dikaryons from actively growing area on potato dextrose agar plate.

***Extraction of extracellular enzymes***

Samples of substrate were collected at regular interval of 5 days and extracted in phosphate buffer (pH 6.0) for ligninolytic enzymes. Filtrate of extraction was used for enzyme assay.

***Decolorization studies in liquid media***

The mycodecolorization experiments were done in potato dextrose broth medium supplemented with Methyl Red and Phenol Red 300 mg/l. Each inoculated with *P. citrinopileatus* in 250 ml Erlenmeyer flask containing 100 ml media and incubated in stagnant condition in BOD incubator at 25$^0$C. The disappearance of Methyl Red and Phenol Red was detected spectrophotometrically (Elico 164-SL) at $\lambda_{max}$ 526 nm and 557nm, respectively. Results were reported as the mean value of percent dye decolorization (% DD) of triplicate (22).

***Parameter optimization***

***Aromatic Amino Acids***

The effect of aromatic amino acids on dye decolourisation was followed the method prescribed by Dhawan and Kuhad (23). The stock solution of Phenylalanine (PHE), Tyrosine (TYR) and Tryptophan (TRP) were sterilized by membrane filter and stored in dark brown amber bottle. These amino acidswere added to pre-sterilized 100 ml erlenmayer flask, containing 30 ml dye potato dextrose broth medium at various concentration viz. 0.2 µM and 0.4µM. The inoculum of *P. citrinopileatus* was inoculated and incubated at 27± 2 $^0$C for observing dye decolourization and ligninolytic eznymes (MnP and Laccase) activity.

***Trace Element***

To study the effect of trace elements like $CuSO_4.5H_2O$and $MnSO_4.H_2O$ at the concentration of 25 ppm and 50 ppm were selected for dye decolourization. These trace elements were added at above concentration in 100 ml erlenmayer flask containing 30 ml dye Potato Dextrose broth medium and *P. citrinopileatus* were inoculated and incubated in BOD incubator at 27± 2 $^0$C for observing dye decolourization and ligninolytic eznymes (MnP and Laccase) activity.

***Enzymatic study***

***Manganese Peroxidases (EC 1.11.1.13)***

Manganese peroxidase (MnP) activity was determined using guaiacol as substrate. The reaction mixture contained 0.2 ml of 0.5 M Na-tartrate buffer (pH 5.0), 0.1 ml of 1 mM $MnSO_4$, 0.1ml of 1mM $H_2O_2$, 0.25 ml of 1 mM guaiacol and 0.3 ml of crude enzymes. The oxidation of substrate at 30$^0$C was followed spectrophotometrically at $\lambda_{max}$ 465nm [24].

***Laccase (EC 1.10.3.2)***

Laccase activity was determined via the oxidation of o-methoxyphenol catechol monomethylether (guaiacol) as substrate. The reaction mixture contained 1 ml of 1mM guaiacol in 0.1M sodium phosphate buffer (pH 6.0) and 1ml of crude enzyme solution was incubated at 30$^0$C for 10min. The oxidation was followed by the increase in absorbance at $\lambda_{max}$ 495nm [25].

***Statistical analysis***

The statistical significance of the effect of aromatic amino acid and trace elements on Manganese peroxidase and Laccase activity in dye containing medium was analyse with One-way ANOVA at 95 percent probability level. All statistical analysis was conducted using MS-Excel and IBM SPSS Statistics (Version-25). The graph of dyes decolorization, effect of aromatic amino acids and effect of trace elements were created by using the Sigma Plot (Version-11) with the using of standard deviation and standard error (±SD and ±SE).

## 3) RESULT AND DISCUSSION

***Decolorization studies in liquid media***

The Methyl Red and Phenol Red decolorization by *P. citrinopileatus* under various treatments such as aromatic amino acids and trace elements at different concentration is depicted in Figure 1. The results demonstrated that decolorization was obviously inhibited by higher

concentration of both aromatic amino acids (0.4 µM) and trace elements (50ppm), although it was increased at its lower concentration i.e. 0.2µM (aromatic amino acids) and 25ppm (trace elements). Figure 1a shown that the maximum decolorization of Methyl Red with 25ppm Cu (90.30%) followed by Mn (85.88%), while the utmost decolorization 88.15% of Phenol Red (Figure 1b) is achieved with Cu, whereas Mn decolorize only 86.12% of dye.The decolorization profile of Methyl Red (Fig 1c) with 0.2 µM of TYR (78.20%), PHE (73.80%) and TRP (70.11%), whereas at this concentration maximum decolorization of Phenol Red (Fig 1d) was observed with TYR (84.14%) followed by PHE (80.92%) and TRP (71.22%).

***Enzymatic study***

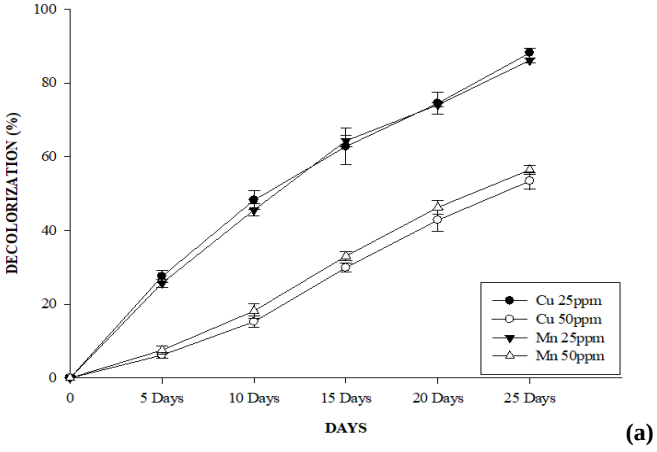
Trace elements and aromatic amino acids in general potent inhibitors of enzymes activity and from this point of view, their concentration play very important roles. Usually they become start to toxic for WRF in concentration only a few times greater than the required. The activity of Manganese peroxidase and Laccase were observed in Methyl Red and Phenol Red containing medium which was also supplemented with aromatic amino acids and trace elements at various concentration with significance; mentioned in Table 2 and 3. The effect of three aromatic amino acids (PHE, TYR and TRP) at 0.2µMsupport better MnP and Laccase in both Methyl Red and Phenol Red containing media. The maximum MnP and laccase enzymes activity was observed with TYR i.e. 202.28±1.20 U and 213.42±2.72 U, respectively followed by TRP (195.16±3.23 U and 198.45±1.57 U) and PHE (118.15±4.43 U and 195.85 U) in Methyl Red containing medium (Table 1). Table 2 showed that the maximum MnP activity (484.34±2.81 U) at 10 days with Mn whereas Cu support maximum laccase activity (489.02±2.06 U) also in 10 days in media inosculated with Methyl Red. While the maximum laccase activity (585.56±2.88 U) was shown with Cu in Phenol Red after 15 day interval. Dhawan and Kuhad, [23] observed that the various amino acids, their analogues and vitamins have shown stimulatory as well as inhibitory effects on laccase production by *Cyathusbulleri*. DL-methionine, DL-tryptophan, glycine and DL-valine stimulated laccase production, while L-cysteine monohydrochloride completely inhibited the enzyme production. Many reports vindicates that most WRF secret more MnP at a low nitrogen concentration than a high nitrogen concentration, MnP production by some WRF was higher in nitrogen- rich culture than in nitrogen-limited culture [26- 28]. Tychanowicz *et al.* [29] observed that the addition of 25mM $CuSO_4$ increased the level of laccase from 270 to 1,420 $U.L^{-1}$ and the fungus *P. Pulmonarius* showed high resistance to copper. Copper sulphate was adroitly used as an inducer to increase laccase production [30]. Metal ions like $CuSO_4$, $BaCl_2$, $MgCl_2$, $FeCl_2$ and $ZnCl_2$ having no effect on purified laccase from *Pleurotus* species, whereas $HgCl_2$ and $MnCl_2$ moderately decrease enzyme activity [31].

**Table1**.Impact of aromatic amino acids on MnP and Laccase activity in Methyl Red and Phenol Red containing media. Given values are means of three replicates ±SE**.** However, the bold figure given in parenthesis for given enzymes and treatments are the means ±SE across different sampling days.

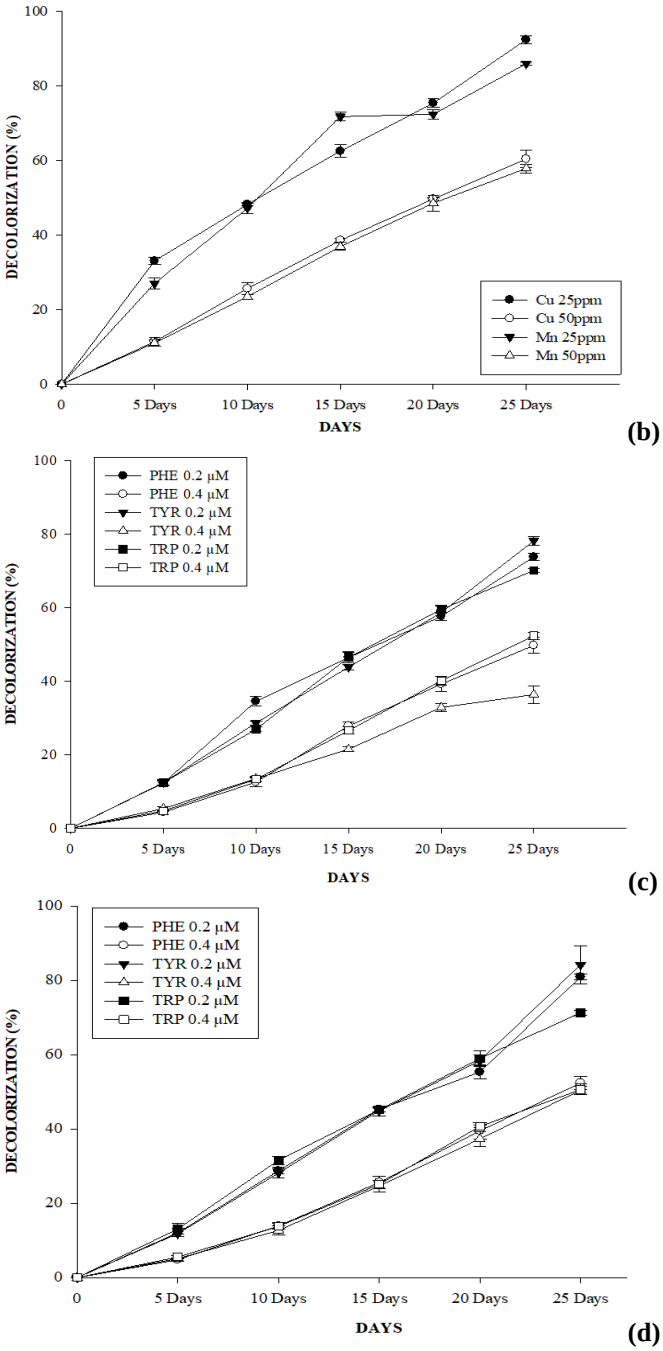
| Dyes | Treatments | Days | Enzymes | |
|---|---|---|---|---|
| | | | MnP | Laccase |
| Methyl Red | 0.2 µM PHE | 5 days | 41.14±1.24 | 41.20±0.99 |
| | | 10 days | 68.33±4.50 | 86.35±2.02 |
| | | 15 days | 188.15±4.43 | 195.85±2.57 |
| | | 20 days | 155.82±1.97 **(113.36±3.04)** | 116.69±1.81 **(110.02±1.85)** |
| | 0.4 µM PHE | 5 days | 22.10±1.61 | 18.41±2.76 |
| | | 10 days | 41.87±2.00 | 28.87±1.11 |
| | | 15 days | 58.67±1.11 | 53.70±2.67 |
| | | 20 days | 42.64±1.65 **(41.32±1.59)** | 59.12±0.98 **(40.03±1.88)** |
| | 0.2 µM TYR | 5 days | 41.86±1.69 | 46.05±1.47 |
| | | 10 days | 101.22±1.92 | 100.60±2.52 |
| | | 15 days | 202.28±1.20 | 213.42±2.72 |
| | | 20 days | 150.66±1.67 **(130.84±1.62)** | 164.56±2.13 **(131.16±2.21)** |
| | 0.4 µM TYR | 5 days | 18.52±1.28 | 16.33±1.03 |
| | | 10 days | 36.27±1.72 | 31.57±1.42 |
| | | 15 days | 51.98±1.93 | 41.56±1.10 |
| | | 20 days | 43.34±2.06 **(37.53±1.75)** | 38.00±1.21 **(31.86±1.19)** |
| | 0.2 µM TRP | 5 days | 30.96±0.80 | 30.64±0.80 |
| | | 10 days | 61.74±0.84 | 73.60±0.98 |
| | | 15 days | 195.16±3.23 | 198.45±1.57 |
| | | 20 days | 104.19±2.38 **(98.01±1.81)** | 156.17±2.33 **(114.71±1.42)** |
| | 0.4 µM TRP | 5 days | 20.36±0.99 | 20.30±0.71 |
| | | 10 days | 35.51±1.14 | 42.26±0.60 |
| | | 15 days | 50.46±3.63 | 54.27±1.85 |
| | | 20 days | 47.86±1.70 **(38.55±1.87)** | 41.09±0.65 **(39.48±0.95)** |
| Phenol Red | 0.2 µM PHE | 5 days | 38.30±1.48 | 38.99±0.61 |
| | | 10 days | 72.25±1.10 | 78.15±1.43 |
| | | 15 days | 169.30±1.79 | 157.64±1.92 |
| | | 20 days | 94.77±1.37 **(93.65±1.44)** | 86.20±1.28 **(90.24±1.31)** |
| | 0.4 µM PHE | 5 days | 18.77±0.90 | 16.66±0.93 |
| | | 10 days | 41.84±0.32 | 38.09±0.92 |
| | | 15 days | 58.52±2.48 | 55.34±1.80 |
| | | 20 days | 49.19±1.96 **(42.08±1.41)** | 59.78±2.02 **(42.47±1.42)** |
| | 0.2 µM TYR | 5 days | 45.27±1.55 | 46.45±0.63 |
| | | 10 days | 89.57±2.05 | 83.46±1.23 |
| | | 15 days | 172.48±2.07 | 179.71±2.45 |
| | | 20 days | 93.31±2.83 **(100.16±2.13)** | 106.23±2.85 **(103.96±1.79)** |
| | 0.4 µM TYR | 5 days | 16.09±0.86 | 15.94±0.59 |
| | | 10 days | 33.33±0.69 | 32.62±1.17 |
| | | 15 days | 51.31±3.73 | 45.60±1.32 |
| | | 20 days | 41.15±1.31 **(35.47±1.65)** | 55.95±0.35 **(37.53±0.86)** |
| | 0.2 µM TRP | 5 days | 43.43±0.94 | 35.76±1.54 |
| | | 10 days | 74.49±2.10 | 78.75±0.71 |
| | | 15 days | 164.91±2.60 | 112.11±1.43 |
| | | 20 days | 101.44±1.62 **(96.06±1.81)** | 100.21±1.13 **(81.71±1.20)** |
| | 0.4 µM TRP | 5 days | 20.05±0.53 | 17.38±0.83 |
| | | 10 days | 40.55±0.52 | 35.74±2.14 |
| | | 15 days | 54.64±1.72 | 54.25±1.13 |
| | | 20 days | 42.35±2.15 **(39.40±1.23)** | 43.03±2.20 **(37.60±1.57)** |
| **ANOVA[a]** | | | **F= 2.461; P=0.021** | **F= 2.781; P=0.01** |

**Table 2.** Impact of Trace Elements on MnP and Laccase activity in Methyl Red and Phenol Red containing media. Given values are means of three replicates ±SE**.** However, the bold figure given in parenthesis for given enzymes and treatments are the means ±SE across different sampling days.

| Dyes | Treatments | Days | Enzymes | |
|---|---|---|---|---|
| | | | **MnP** | **Laccase** |
| Methyl Red | **25 ppm Cu** | **5 days** | 294.21±2.74 | 297.60±3.74 |
| | | **10 days** | 310.60±2.76 | 489.02±2.06 |
| | | **15 days** | 357.91±2.49 | 422.49±1.15 |
| | | **20 days** | 293.33±2.20 **(314.01±2.5)** | 330.06±2.86 **(384.79±2.45)** |
| | **50 ppm Cu** | **5 days** | 42.67±1.97 | 49.17±1.04 |
| | | **10 days** | 74.97±1.54 | 63.86±3.01 |
| | | **15 days** | 53.79±0.59 | 75.87±2.19 |
| | | **20 days** | 37.13±1.49 **(52.12±1.40)** | 54.14±1.8 **(60.76±2.01)** |
| | **25 ppm Mn** | **5 days** | 335.85±3.16 | 276.09±3.89 |
| | | **10 days** | 484.34±2.81 | 340.19±2.64 |
| | | **15 days** | 412.15±1.86 | 280.06±2.40 |
| | | **20 days** | 355.87±3.83**(397.05±2.91)** | 192.29±2.10 **(271.16±2.76)** |
| | **50 ppm Mn** | **5 days** | 65.52±0.56 | 66.75±1.75 |
| | | **10 days** | 95.49±1.89 | 95.49±1.89 |
| | | **15 days** | 55.40±2.13 | 55.40±2.13 |
| | | **20 days** | 57.38±1.13 **(68.44±1.43)** | 57.38±1.13 **(68.75±1.73)** |
| Phenol Red | **25 ppm Cu** | **5 days** | 252.44±2.60 | 307.3±4.32 |
| | | **10 days** | 272.57±1.69 | 492.43±4.66 |
| | | **15 days** | 353.11±1.94 | 585.56±2.88 |
| | | **20 days** | 339.05±1.74 **(304.29±1.99)** | 348.77±4.17 **(433.51±4.01)** |
| | **50 ppm Cu** | **5 days** | 41.20±2.93 | 64.47±1.16 |
| | | **10 days** | 65.16±3.04 | 101.13±2.97 |
| | | **15 days** | 78.88±3.17 | 92.99±2.29 |
| | | **20 days** | 42.84±4.50 **(50.02±3.41)** | 67.70±1.28 **(81.57±1.93)** |
| | **25 ppm Mn** | **5 days** | 237.06±2.99 | 239.30±7.47 |
| | | **10 days** | 351.59±1.76 | 330.10±7.84 |
| | | **15 days** | 478.95±2.52 | 364.29±5.83 |
| | | **20 days** | 367.19±1.94 **(358.7±2.30)** | 260.60±8.60 **(298.57±7.43)** |
| | **50 ppm Mn** | **5 days** | 49.57±2.89 | 52.45±3.70 |
| | | **10 days** | 89.83±1.60 | 69.66±2.82 |
| | | **15 days** | 80.96±3.87 | 87.46±2.67 |
| | | **20 days** | 56.52±2.95 **(69.22±2.83)** | 56.94±3.17 **(66.63±3.09)** |
| **ANOVA[a]** | | | **F= 39.807; P=0.000** | **F= 24.215; P=0.000** |



**(a)**



**(b)** **(c)** **(d)**

**Figure 1**: Effect of metals **(a)** and Aromatic Amino acids (c) on Methyl Red decolorization; and **(b)** & **(d)** represent Phenol Red decolorization through metals and Aromatic Amino acids respectively at different days. [Phenylalanine (PHE), Tyrosine (TYR) and Tryptophan (TRP), Cu=Copper and Mn=Manganese]

## 4) CONCLUSION

Activity of ligninolytic enzymes laccase and Manganese Peroxidase of *Pleurotus citrinopileatus* were increase under optimized concentration of Copper and Manganese followed by aromatic amino acids. Both ligninolytic enzymes were capable to decolorized decolorized 300ppm of both azo dyes *i.e.* Methyl Red and Phenol Red.

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