EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 8
1059-1070
Year: 2017

DECOLORIZATION OF DIFFERENT FOOD DYES BY 12 TYPES OF FUNGI AND TOXICITY EVALUATION AFTER FUNGAL TREATMENT

Shaileyee Das*[1] and Biswajit Das[2]

[1]Pandora House Value Chain Management, EnvyPure Department, Kolkata, West Bengal, India.
[2]Banshberia Water Treatment Plant, Hoogly, West Bengal, India.

***Corresponding Author: Shaileyee Das**
Pandora House Value Chain Management, EnvyPure Department, Kolkata, West Bengal, India.

Article Received on 19/06/2017 **Article Revised on 09/07/2017** **Article Accepted on 30/07/2017**

ABSTRACT
The objective of this study was to investigate the capacity of decolorization and detoxification of the food dyes Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, Allura Red, Ponceau 4R, Carmoisine, Erythrosine, Amaranth, Brown HT, Brilliant Blue FCF, Patent Blue V, Indigo Carmine, Green S, Fast Green FCF, Black PN and the mixture of the all dyes (MXD) by 12 types of fungi (Irpex lacteus, Aspergillus species, Penicillium geastrivorus, Datronia sp., Myrothecium roridum , Polyporus arcularius , Fomitopsis feei , Pleurotus ostreatus , Trametes versicolor , Trametes hirsuta , fomes fomentarius , Ganoderma lucidum). The dye Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, Allura Red, Ponceau 4R, Carmoisine, Erythrosine, Amaranthin liquid media decolorize totally after 5-days incubation, and the others Brown ht, brilliant blue fcf, patent blue v, indigo carmine, green s, fast green fcf, black pn the liquid media was 100% decolorized by penicillium geastrivorus, irpex lacteu, myrothecium roridum, polyporus arcularius within 8 days of incubation. initially the dye decolorization involved dye adsorption by the biomass followed by degradation. the acute toxicity after fungal treatment was monitored with the microcrustacean daphnia pulex and measured through effective Concentration 50% (EC50). Irpex lacteus, Aspergillus species, Penicillium geastrivorus, Datronia sp., Myrothecium roridum , Polyporus arcularius , Fomitopsis feei , Pleurotus ostreatus , Trametes versicolor , Trametes hirsuta , fomes fomentarius , Ganoderma lucidum all were reduced efficiently the toxicity of dyes Brilliant Blue FCF before the treatment were non toxic (EC50=> 100%) while the dye Brown HT was moderately acutely toxic (EC50=1-10%), and also Indigo carmine was minute acute toxic dye (EC50=10-100%). Except these three types of dyes , all dyes were acute toxic (EC50=<1%). Despite of MXD being constituted also by the toxic dyes, the low toxicity of the mixture is probably due to fact that they were in a low concentration (33.3 mg L-1). After the fungal treatment, 12 types of fungi efficiently reduced the toxicity of mixed dye, the 24h-EC50 value was increased from 10.93% (minor acutely toxic) to 40 – 52.3% (minor acutely toxic). Thus, these 12 types fungi were efficient to decolorize different food dyes and the mixture of them with a significant reduction of their toxicity. In addition this investigation also demonstrated the need of toxicological assays associated to decolorizatio experiments.

KEYWORDS: Fungi, Food Dyes, Decolorization, Detoxification.

INTRODUCTION
Food dye processing industry is a very important sector in the Indian economy, with considerable growth in the last years. As a outcome, there is an boost of environmental contamination caused by the large amount of dyes involved in the food dye manufacturing process that are discharged in the liquid effluents. Approximately 10-15% of the food dyes are released into the environment. Azo dyes, sulphur dyes are the main chemical class of dyes with the greatest variety of colors; therefore they have been extensively used by the food industry. These dyes are characterized by one or more azo group linkages (R1-N=N-R2), sulphur group linkages (R1-S-R2) and by aromatic structures. The biological effects of sulphur dyes and azo dyes after biotransformation have been shown to be highly toxic, and in some cases these compounds are more carcinogenic and mutagenic than the original dye stuffs.

There are many successful wastewater treatments; on the other hand these technologies are highly expensive. Conventional biological treatments that have been applied, resulting certain drawbacks. There are some textile dyes are relatively resistant to microbial degradation. Also in anaerobic treatment, anaerobic microorganisms are when degrading some dyes resulting in production of some aromatic amines that may be toxic and carcinogenic. Therefore, in recent years, there have

been intensive researches on fungal decolorization of textile wastewater but not studied on food dyes. The use of fungi can be a promising alternative to replace or supplement current treatments.

Several fungi are capable of adsorbing pollutant compounds through their highly oxidative and non-specific ligninolytic enzymes, which are also responsible for the decolorization and degradation of many different dyes. The white rot fungi, members of the Basidiomycetes, as Trametes versicolor, Trametes hirsuta, Irpex lacteus are enormously efficient for textile dye degradation.

Studies on non-basidiomycete fungi that adsorb dyes are reduced; nevertheless these fungi are also very competent for metabolizing a wide range of compounds, particularly by demethylation and oxidation. Aspergillus species, Penicillium geastrivorus, P. ochrochloron were found to be successful for removing textile dyes from liquid media.[1,2]

Despite of the competence of biological treatments by anaerobic and aerobic treatment, in some cases microorganisms can transform dyes into compounds more toxic than the original compound, consequently there is a need to assess the toxicity of the end product after the biological treatment. For this purpose some toxicological assays can be applied, including tests using microcrustacean Daphnia spp., routinely used to determine toxicity of chemicals for the establishment of environmental health standards due to their small size, short life cycle, high reproduction rates and their key ecological role in the aquatic food chains. Thus, the aims of the present study were to investigate the ability of Irpex lacteus, Aspergillus species, Penicillium geastrivorus, Datronia sp., Myrothecium roridum, Polyporus arcularius, Fomitopsis feei, Pleurotus ostreatus, Trametes versicolor, Trametes hirsuta, fomes fomentarius, Ganoderma lucidum to decolorize fifteen commonly use food dyes, as well as the fortitude of their toxicity after fungal treatment using the ecotoxicological assay with the microcrustacean Daphnia pulex.

Figure 1: 12 Types of Fungi Which Are Used For the Food Dye Adsorption In Our Study.

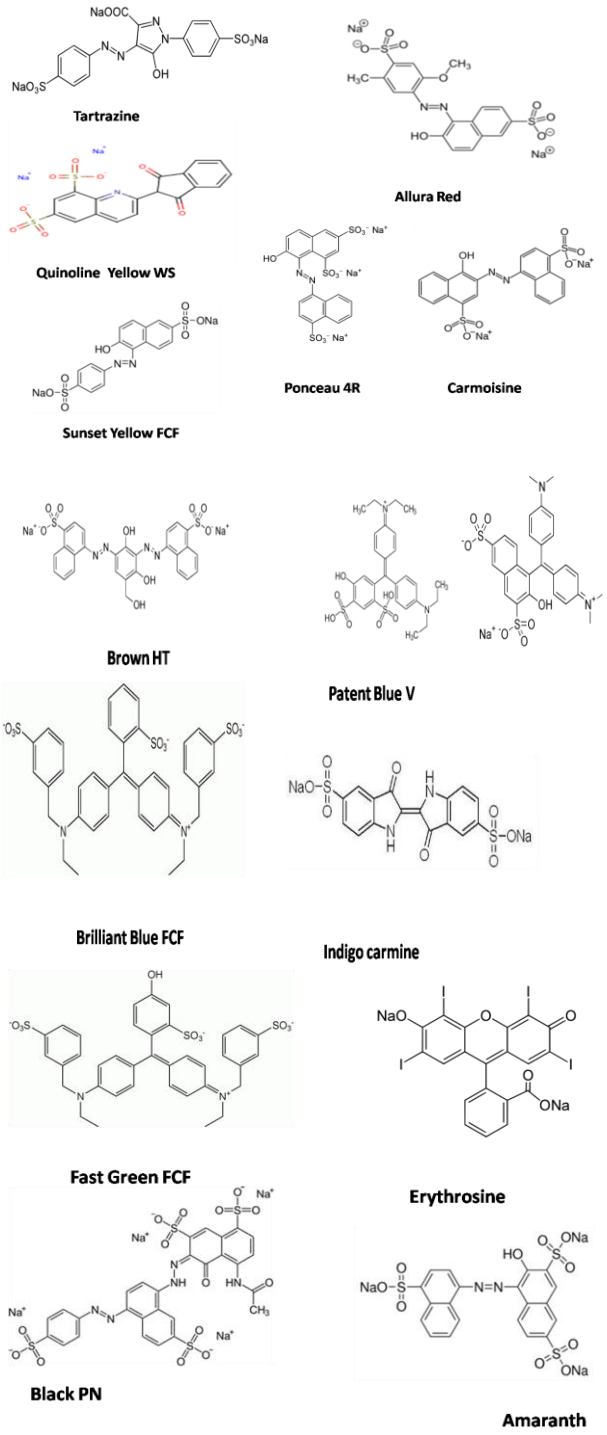
MATERIALS

a) Chemicals

Table 1: The dyes used in the investigation were.

Tartrazine		Brown HT	
Quinoline Yellow WS		Brilliant Blue FCF	
Sunset Yellow FCF		Patent Blue V	
Allura Red		Indigo Carmine	
Ponceau 4R		Green S	
Carmoisine		Fast Green FCF	
Erythrosine		Black PN	
Amaranth			

Solutions of these dyes were prepared by dissolving the dyes in distilled water then filtered through Watman filter paper membrane 0, 22 µm. These dyes were chosen based on their wide use in the food industry in India and throughout the world.



b) Microorganism

The fungus used in this work all were previously isolated from sediment collected from Hoogly district in West Bengal State, India, an area of estuarine habitat of Hoogly river, under the influence of different sources of industrial contamination. The culture has been maintained on malt extract agar at 4°C and preserved.

METHODS

a) Culture conditions

The individual fungus were inoculated into potato dextrose agar (PDA)containing the dyes separately in a final concentration of 200 mg L^{-1} and the three dyes mixed at a final concentration of 66.6 mg L^{-1} for each dye. The petri plates were incubated at 28ºC, after inoculation. Following incubation for 5 days 9 mycelial plugs (5 mm diameter) from the colony margin were used as inoculums, they were transferred to conical flasks containing 150 mL of potato dextrose broth (PDB). Following 2-day incubation at 28°C on a rotary shaker at 140 rpm, 100 mg L^{-1} of each dye was added and the mixture containing 33.3 mg L^{-1} of each dye was also added. Without fungi, control experiments were performed under the same conditions described above.

b) Decolorization

Aliquots of the fungal culture after 0, 2, 5, 7 and 14-day incubation following the addition of the dyes were centrifuged at 10.000 g for 10 min. Then the supernatant was diluted 1:10 with distilled water and were measured spectrophotometrically using a Shimadzu UV-1601 (Kyoto, Japan) spectrophotometer. The color removal was calculated as following:

Percentage Decolorization (%) = (Ab – Aa)/Ab X 100

where Ab is the absorbance of the initial dye solution (day 0) and Aa is the absorbance at cultivation time (2, 5, 7 and 14 days). All assays were conducted in triplicate in the dark and results were expressed as the mean values with the standard deviation calculated.[6,15,20]

c) Ecotoxicity

The freshwater microcrustacean Daphnia pulex was used for the acute toxicity determination and the methodology applied was based on OECD 202 protocol.[1,29,36,37] D. pulex was cultured in mineral water in a temperature controlled chamber at 23 ± 1 °C, with a light intensity of 40 µE m^{-2} s^{-1} and a 12:12 h light:dark cycle[55]. The daphnids were fed with the chlorophyte Ankistrodesmus falcatus that was cultured in MBL medium. The toxicity determination was conducted with the liquid media containing the dyes before and after the 14-day incubation with every fungus. The liquid media was filtered through Wattman filterpaper 0.22 µm and the supernatant was evaluated. The method applied was static using 20 neonates divided in two groups of 10 individuals for concentration-test. The mineral water was used as dilution water for the concentration-test and as control. The time of exposition was 24 hours under constant temperature of 24°C and in the dark.[55]

RESULTS AND DISCUSSION

a) Decolorization

The standard deviation of the triplicates of each dye and the dye mixture was calculated for the five periods of decolorization analyses (0, 2, 5, 7 and 14 days of incubation) and the deviation was not higher than 0.02% (data not shown). Therefore, the results were

reproducible for the three dyes tested and their mixture[6, 15, 20].

Penicillium geastrivorus, Irpex lacteu, Myrothecium roridum, Polyporus arcularius decolorized completely (100%) Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, Allura Red, Ponceau 4R, Carmoisine, Erythrosine, Amaranthin liquid media after 5-days incubation (Table 2, Figure 2). The dye was absorbed by the biomass; nonetheless, it was showed potential to observe visually a reduction of the dye adsorption from the 3rd to the 5th day of incubation.

In the presence of Brown HT, Brilliant Blue FCF, Patent Blue V, Indigo Carmine, Green S, Fast Green FCF, Black PN the liquid media was 100% decolorized by Penicillium geastrivorus, Irpex lacteu, Myrothecium roridum, Polyporus arcularius within 8 days of incubation (Figure 3) and it was observed the biomass adsorbing the blue dye. The color on the biomass was reduced gradually from the 5th until the 14th day of incubation when the biomass was completely free from the dye.

When the fungus was cultivated in the presence of the mixture of the every dyes, MXD, it decolorized 100% of the culture supernatant after 10 days (Figure 3) when the biomass visually had adsorbed most of the dyes. From the 5th day till the 10th day of incubation there was a gradual reduction of the color on the biomass until complete disappearance. During the present experiment it was demonstrated the efficiency of Penicillium geastrivorus, Irpex lacteu, Myrothecium roridum, Polyporus arcularius to decolorize the different kinds of dyes with differences in the decolorization ability regarding each dye tested, which might be dependent on dye structure. Relatively small structural differences can markedly affect decolorization.[6,15,20]

Yet the relationship between the molecular structure of the dyes and their decolorization by fungi is still unclear. The decolorization of the liquid media was confirmed by the disappearance of the characteristic peak of these dyes during the spectrophotometric experiments. In the meantime the dyes were adsorbed by the biomass and from visual observation they gradually disappeared from its surface. Visual observation of the biomass is an important aspect in dye decolorization investigation[6, 15, 20], since it allows the distinction of biomass adsorption from microbial degradation. Therefore, the reduction of color on the biomass observed in Figure 3 with the mixed dyes indicates a potential dye degradation by Penicillium geastrivorus, Irpex lacteu, Myrothecium roridum, Polyporus arcularius.[55]

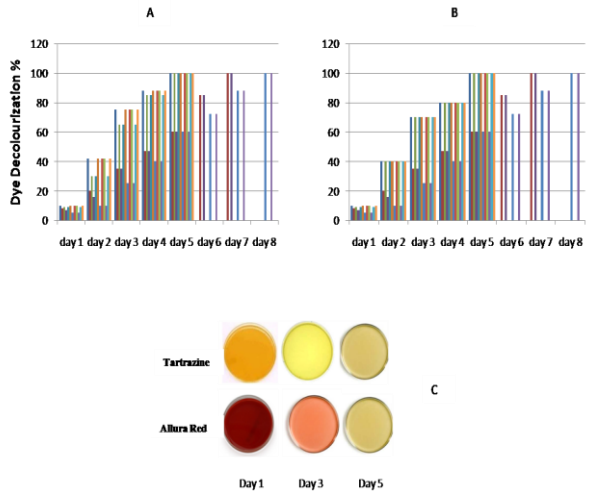


Figure 2: A) % of Tartrazine Dye Degradation by 12 types of fungi, B) % of Allura Red Dye Degradation by 12 types of fungi, C) Visual observation of the dyes Tartrazine and Allura Red decolorized by Irpex lacteu, after 1 day (1), 3 days (2), 5 days (3)of incubation.

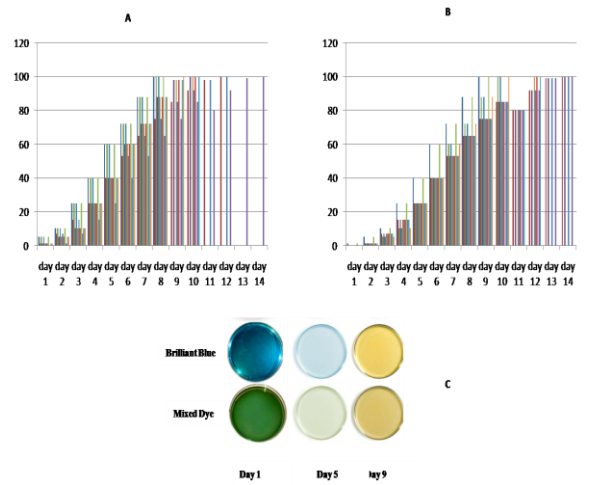


Figure 3: A) % of Brilliant Blue Dye Degradation by 12 types of fungi, B) % of Mixed Dye Degradation by 12 types of fungi, C) Visual observation of the dyes Brilliant Blue and Mixed dye decolorized by Irpex lacteu, after 1 day (1), 5 days (2), 9 days (3)of incubation.

b) Ecotoxicity

The effluents from food industries even after the treatment can remain toxic and mutagenic; nonetheless they are released into the environment any way. Consequently, the inefficiency of this process leads to the need of toxicological assays after effluent treatment. Thus, in the present investigation the toxicological tests were performed using daphnids since they have been found to be sensitive and used as important models to evaluate the toxicological implications that may result from azo dyes to the environment[1, 29, 36, 37]. The 24h-EC50, obtained with the acute toxicity test using Daphnia pulex, of the liquid media before and after the fungal treatment with 12 types of fungi was determined

(Table 3). The dyes Brilliant Blue FCF before the treatment were nontoxic (EC50=> 100%) while the dye Brown HT was moderately acutely toxic (EC50=1-10%), and also Indigo carmine was minute acute toxic dye (EC50=10-100%). Except these three types of dyes, all dyes were acute toxic (EC50=<1%). Despite of MXD being constituted also by the toxic dyes, the low toxicity of the mixture is probably due to fact that they were in a low concentration (33.3 mg L-1). After the fungal treatment, 12 types of fungi efficiently reduced the toxicity of mixed dye, the 24h-EC50 value was increased from 10.93% (minor acutely toxic) to 40 – 52.3% (minor acutely toxic) (Table 3).

The fungus also reduced the toxicity of the all dyes which were acutely toxic before fungal treatment. After fungal treatment they were shown minor acute toxicity (EC50= 10-100%). In Brilliant Blue FCF after fungal treatment was not increased the toxicity.

The toxicological assay using daphnids showed a significant reduction of toxicity after dye decolorization 12 types of fungi, indicating that this process corresponds to an actual detoxification of the dyes. Even though the detoxification in some treatments is not always reduced while the decolorization takes place[55], as it happened in the present study with the food dye, there are very few studies that have evaluated the toxicological level after dye or effluent treatment. Therefore, based on these findings, the need of this kind of evaluation has been confirmed.[1,29,36,37]

Table 2: Total time taken for 100% decolorization of dyes.

Dyes	*Irpex lacteu*	*Aspergillus species*	*Penicillium geastrivorus*	*Datronia sp.*	*Myrotheciu m roridum*	*Polyporus arcularius*	*Fomitop sis feei*	*Pleurotus ostreatus*	*Trametes hirsuta*	*Fomes fomentarius*	*Ganoder ma lucidum*	*Trametes versicolor*
Tartrazine	100% in 5 days	100% in 7 days	100% in 5 days	100% in 7 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Quinoline Yellow WS	100% in 5 days	100% in 7 days	100% in 5 days	100% in 7 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Sunset Yellow FCF	100% in 5 days	100% in 7 days	100% in 5 days	100% in 7 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Allura Red	100% in 5 days	100% in 7 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Ponceau 4R	100% in 5 days	100% in 7 days	100% in 5 days	100% in 7 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Carmoisine	100% in 5 days	100% in 7 days	100% in 5 days	100% in 7 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days
Erythrosine	100% in 5 days	100% in 7 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Amaranth	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days	100% in 8 days	100% in 5 days	100% in 5 days
Brown HT	100% in 6 days	100% in 8 days	100% in 6 days	100% in 8 days	100% in 6 days	100% in 6 days	100% in 10 days	100% in 6 days	100% in 6 days	100% in 10 days	100% in 6 days	100% in 6 days
Brilliant Blue FCF	100% in 8 days	100% in 12 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 10 days	100% in 12 days	100% in 10 days	100% in 8 days	100% in 14 days	100% in 10 days	100% in 9 days
Patent Blue V	100% in 8 days	100% in 12 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 10 days	100% in 12 days	100% in 10 days	100% in 8 days	100% in 14 days	100% in 10 days	100% in 9 days
Indigo Carmine	100% in 7 days	100% in 10 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 10 days	100% in 12 days	100% in 10 days	100% in 8 days	100% in 12 days	100% in 8 days	100% in 7 days
Green S	100% in 7 days	100% in 10 days	100% in 7 days	100% in 8 days	100% in 7 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 7 days	100% in 12 days	100% in 8 days	100% in 7 days
Fast Green FCF	100% in 7 days	100% in 10 days	100% in 7 days	100% in 8 days	100% in 7 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 7 days	100% in 12 days	100% in 8 days	100% in 7 days
Black PN	100% in 8 days	100% in 12 days	100% in 8 days	100% in 10 days	100% in 8 days	100% in 10 days	100% in 12 days	100% in 10 days	100% in 8 days	100% in 14 days	100% in 10 days	100% in 9 days
Mixed dye	100% in 10 days	100% in 14 days	100% in 10 days	100% in 14 days	100% in 10 days	100% in 12 days	100% in 14 days	100% in 12 days	100% in 10 days	100% in 14 days	100% in 12 days	100% in 10 days

Table 3: Ecotoxicity study.

Samples	EC50 %	CI 95%	Classification	Samples	EC50 %	CI 95%	Classification
Tartrazine	30.8	27.2-34.8	Minor acutely toxic	**Brown HT**	9.5	8.6-10.4	Moderately acutely toxic
Irpex lacteu	105	98.7-112.4	Not toxic	**Irpex lacteu**	75.2	68.7-82.9	Minor acute toxic
Aspergillus species	56.9	50. 3- 62.6	Minor acute toxic	**Aspergillus species**	56.9	50. 3- 62.6	Minor acute toxic
Penicillium geastrivorus	108.5	98.7-112.4	Not toxic	**Penicillium geastrivorus**	68.5	68.7-82.9	Minor acute toxic
Datronia sp.	55.1	50. 3- 62.6	Minor acute toxic	**Datronia sp.**	55.1	50. 3- 62.6	Minor acute toxic
Myrothecium roridum	101.7	98.7-112.4	Not toxic	**Myrothecium roridum**	71.4	68.7-82.9	Minor acute toxic
Polyporus arcularius	52.5	50. 3- 62.6	Minor acute toxic	**Polyporus arcularius**	52.5	50. 3- 62.6	Minor acute toxic
Fomitopsis feei	54.7	50. 3- 62.6	Minor acute toxic	**Fomitopsis feei**	54.7	50. 3- 62.6	Minor acute toxic
Pleurotus ostreatus	111.1	98.7-112.4	Not toxic	**Pleurotus ostreatus**	78.1	68.7-82.9	Minor acute toxic
Trametes hirsuta	108.4	98.7-112.4	Not toxic	**Trametes hirsuta**	76.5	68.7-82.9	Minor acute toxic
Fomes fomentarius	51.9	50. 3- 62.6	Minor acute toxic	**Fomes fomentarius**	51.9	50. 3- 62.6	Minor acute toxic
Ganoderma lucidum	101.6	98.7-112.4	Not toxic	**Ganoderma lucidum**	79.3	68.7-82.9	Minor acute toxic
Trametes versicolor	102.8	98.7-112.4	Not toxic	**Trametes versicolor**	75.7	68.7-82.9	Minor acute toxic
Quinoline Yellow WS	50.8	47.2-54.8	Minor acutely toxic	**Brilliant Blue FCF**	99.4	98.7-112.4	Not toxic
Irpex lacteu	107	98.7-112.4	Not toxic	**Irpex lacteu**	105	98.7-112.4	Not toxic
Aspergillus species	76.9	70. 3- 82.6	Minor acute toxic	**Aspergillus species**	103.2	98.7-112.4	Not toxic
Penicillium geastrivorus	110.5	98.7-112.4	Not toxic	**Penicillium geastrivorus**	108.5	98.7-112.4	Not toxic
Datronia sp.	76.9	70. 3- 82.6	Minor acute toxic	**Datronia sp.**	101.3	98.7-112.4	Not toxic
Myrothecium roridum	103.7	98.7-112.4	Not toxic	**Myrothecium roridum**	101.7	98.7-112.4	Not toxic
Polyporus arcularius	76.9	70. 3- 82.6	Minor acute toxic	**Polyporus arcularius**	102.1	98.7-112.4	Not toxic
Fomitopsis feei	76.9	70. 3- 82.6	Minor acute toxic	**Fomitopsis feei**	99.6	98.7-112.4	Not toxic
Pleurotus ostreatus	111.1	98.7-112.4	Not toxic	**Pleurotus ostreatus**	107.9	98.7-112.4	Not toxic
Trametes hirsuta	110.4	98.7-112.4	Not toxic	**Trametes hirsuta**	108.4	98.7-112.4	Not toxic
Fomes fomentarius	76.9	70. 3- 82.6	Minor acute toxic	**Fomes fomentarius**	105.7	98.7-112.4	Not toxic
Ganoderma lucidum	103.6	98.7-112.4	Not toxic	**Ganoderma lucidum**	101.6	98.7-112.4	Not toxic
Trametes versicolor	104.8	98.7-112.4	Not toxic	**Trametes versicolor**	102.8	98.7-112.4	Not toxic
Sunset Yellow FCF	0.95	<1.0	acutely toxic	**Patent Blue V**	0.9	<1.0	acutely toxic
Irpex lacteu	27.9	20. 3- 32.6	Minor acute toxic	**Irpex lacteu**	26.9	20. 3- 32.6	Minor acute toxic
Aspergillus species	29.7	20. 3- 32.6	Minor acute toxic	**Aspergillus species**	28.7	20. 3- 32.6	Minor acute toxic
Penicillium geastrivorus	31.9	20. 3- 32.6	Minor acute toxic	**Penicillium geastrivorus**	30.9	20. 3- 32.6	Minor acute toxic
Datronia sp.	27.5	20. 3- 32.6	Minor acute toxic	**Datronia sp.**	26.5	20. 3- 32.6	Minor acute toxic
Myrothecium roridum	28.8	20. 3- 32.6	Minor acute toxic	**Myrothecium roridum**	27.8	20. 3- 32.6	Minor acute toxic
Polyporus arcularius	25.0	20. 3- 32.6	Minor acute toxic	**Polyporus arcularius**	24.9	20. 3- 32.6	Minor acute toxic
Fomitopsis feei	24.4	20. 3- 32.6	Minor acute toxic	**Fomitopsis feei**	23.5	20. 3- 32.6	Minor acute toxic
Pleurotus ostreatus	26.7	20. 3- 32.6	Minor acute toxic	**Pleurotus ostreatus**	25.6	20. 3- 32.6	Minor acute toxic
Trametes hirsuta	28.5	20. 3- 32.6	Minor acute toxic	**Trametes hirsuta**	27.2	20. 3- 32.6	Minor acute toxic
Fomes fomentarius	23.0	20. 3- 32.6	Minor acute toxic	**Fomes fomentarius**	22.9	20. 3- 32.6	Minor acute toxic

Ganoderma lucidum	27.7	20. 3- 32.6	Minor acute toxic	**Ganoderma lucidum**	26.5	20. 3- 32.6	Minor acute toxic
Trametes versicolor	26.8	20. 3- 32.6	Minor acute toxic	**Trametes versicolor**	25.6	20. 3- 32.6	Minor acute toxic
Allura Red	0.93	<1.0	acutely toxic	**Indigo Carmine**	89.4	88.7-102.4	Minor acute toxic
Irpex lacteu	17.9	10. 3- 22.6	Minor acute toxic	**Irpex lacteu**	105.6	98.7-112.4	Not toxic
Aspergillus species	19.7	10. 3- 22.6	Minor acute toxic	**Aspergillus species**	101.8	98.7-112.4	Not toxic
Penicillium geastrivorus	11.9	10. 3- 22.6	Minor acute toxic	**Penicillium geastrivorus**	107.4	98.7-112.4	Not toxic
Datronia sp.	17.5	10. 3- 22.6	Minor acute toxic	**Datronia sp.**	100.1	98.7-112.4	Not toxic
Myrothecium roridum	18.8	10. 3- 22.6	Minor acute toxic	**Myrothecium roridum**	100.3	98.7-112.4	Not toxic
Polyporus arcularius	15.0	10. 3- 22.6	Minor acute toxic	**Polyporus arcularius**	101.5	98.7-112.4	Not toxic
Fomitopsis feei	14.4	10. 3- 22.6	Minor acute toxic	**Fomitopsis feei**	99.3	98.7-112.4	Not toxic
Pleurotus ostreatus	16.7	10. 3- 22.6	Minor acute toxic	**Pleurotus ostreatus**	107.6	98.7-112.4	Not toxic
Trametes hirsuta	18.5	10. 3- 22.6	Minor acute toxic	**Trametes hirsuta**	108.2	98.7-112.4	Not toxic
Fomes fomentarius	13.0	10. 3- 22.6	Minor acute toxic	**Fomes fomentarius**	105.1	98.7-112.4	Not toxic
Ganoderma lucidum	17.7	10. 3- 22.6	Minor acute toxic	**Ganoderma lucidum**	101.3	98.7-112.4	Not toxic
Trametes versicolor	16.8	10. 3- 22.6	Minor acute toxic	**Trametes versicolor**	102.4	98.7-112.4	Not toxic
Ponceau 4R	0.93	<1.0	acutely toxic	**Green S**	50.8	47.2-54.8	Minor acutely toxic
Irpex lacteu	17.9	10. 3- 22.6	Minor acute toxic	**Irpex lacteu**	107	98.7-112.4	Not toxic
Aspergillus species	19.7	10. 3- 22.6	Minor acute toxic	**Aspergillus species**	76.9	70. 3- 82.6	Minor acute toxic
Penicillium geastrivorus	11.9	10. 3- 22.6	Minor acute toxic	**Penicillium geastrivorus**	110.5	98.7-112.4	Not toxic
Datronia sp.	17.5	10. 3- 22.6	Minor acute toxic	**Datronia sp.**	76.9	70. 3- 82.6	Minor acute toxic
Myrothecium roridum	18.8	10. 3- 22.6	Minor acute toxic	**Myrothecium roridum**	103.7	98.7-112.4	Not toxic
Polyporus arcularius	15.0	10. 3- 22.6	Minor acute toxic	**Polyporus arcularius**	76.9	70. 3- 82.6	Minor acute toxic
Fomitopsis feei	14.4	10. 3- 22.6	Minor acute toxic	**Fomitopsis feei**	76.9	70. 3- 82.6	Minor acute toxic
Pleurotus ostreatus	16.7	10. 3- 22.6	Minor acute toxic	**Pleurotus ostreatus**	111.1	98.7-112.4	Not toxic
Trametes hirsuta	18.5	10. 3- 22.6	Minor acute toxic	**Trametes hirsuta**	110.4	98.7-112.4	Not toxic
Fomes fomentarius	13.0	10. 3- 22.6	Minor acute toxic	**Fomes fomentarius**	76.9	70. 3- 82.6	Minor acute toxic
Ganoderma lucidum	17.7	10. 3- 22.6	Minor acute toxic	**Ganoderma lucidum**	103.6	98.7-112.4	Not toxic
Trametes versicolor	16.8	10. 3- 22.6	Minor acute toxic	**Trametes versicolor**	104.8	98.7-112.4	Not toxic
Carmoisine	0.93	<1.0	acutely toxic	**Fast Green FCF**	0.93	<1.0	acutely toxic
Irpex lacteu	27.9	20. 3- 32.6	Minor acute toxic	**Irpex lacteu**	15.9	10. 3- 22.6	Minor acute toxic
Aspergillus species	29.7	20. 3- 32.6	Minor acute toxic	**Aspergillus species**	16.7	10. 3- 22.6	Minor acute toxic
Penicillium geastrivorus	21.9	20. 3- 32.6	Minor acute toxic	**Penicillium geastrivorus**	10.9	10. 3- 22.6	Minor acute toxic
Datronia sp.	27.5	20. 3- 32.6	Minor acute toxic	**Datronia sp.**	15.5	10. 3- 22.6	Minor acute toxic
Myrothecium roridum	28.8	20. 3- 32.6	Minor acute toxic	**Myrothecium roridum**	16.8	10. 3- 22.6	Minor acute toxic
Polyporus arcularius	25.0	20. 3- 32.6	Minor acute toxic	**Polyporus arcularius**	15.0	10. 3- 22.6	Minor acute toxic
Fomitopsis feei	24.4	20. 3- 32.6	Minor acute toxic	**Fomitopsis feei**	14.4	10. 3- 22.6	Minor acute toxic
Pleurotus ostreatus	26.7	20. 3- 32.6	Minor acute toxic	**Pleurotus ostreatus**	16.7	10. 3- 22.6	Minor acute toxic
Trametes hirsuta	28.5	20. 3- 32.6	Minor acute toxic	**Trametes hirsuta**	18.5	10. 3- 22.6	Minor acute toxic

Fomes fomentarius	23.0	20. 3- 32.6	Minor acute toxic	**Fomes fomentarius**	13.0	10. 3- 22.6	Minor acute toxic
Ganoderma lucidum	27.7	20. 3- 32.6	Minor acute toxic	**Ganoderma lucidum**	17.7	10. 3- 22.6	Minor acute toxic
Trametes versicolor	26.8	20. 3- 32.6	Minor acute toxic	**Trametes versicolor**	16.8	10. 3- 22.6	Minor acute toxic
Erythrosine	0.93	<1.0	acutely toxic	**Black PN**	0.93	<1.0	acutely toxic
Irpex lacteu	17.9	10. 3- 22.6	Minor acute toxic	**Irpex lacteu**	11.9	10. 3- 22.6	Minor acute toxic
Aspergillus species	19.7	10. 3- 22.6	Minor acute toxic	**Aspergillus species**	12.7	10. 3- 22.6	Minor acute toxic
Penicillium geastrivorus	11.9	10. 3- 22.6	Minor acute toxic	**Penicillium geastrivorus**	10.9	10. 3- 22.6	Minor acute toxic
Datronia sp.	17.5	10. 3- 22.6	Minor acute toxic	**Datronia sp.**	12.5	10. 3- 22.6	Minor acute toxic
Myrothecium roridum	18.8	10. 3- 22.6	Minor acute toxic	**Myrothecium roridum**	13.8	10. 3- 22.6	Minor acute toxic
Polyporus arcularius	15.0	10. 3- 22.6	Minor acute toxic	**Polyporus arcularius**	15.0	10. 3- 22.6	Minor acute toxic
Fomitopsis feei	14.4	10. 3- 22.6	Minor acute toxic	**Fomitopsis feei**	12.4	10. 3- 22.6	Minor acute toxic
Pleurotus ostreatus	16.7	10. 3- 22.6	Minor acute toxic	**Pleurotus ostreatus**	11.7	10. 3- 22.6	Minor acute toxic
Trametes hirsuta	18.5	10. 3- 22.6	Minor acute toxic	**Trametes hirsuta**	14.5	10. 3- 22.6	Minor acute toxic
Fomes fomentarius	13.0	10. 3- 22.6	Minor acute toxic	**Fomes fomentarius**	11.0	10. 3- 22.6	Minor acute toxic
Ganoderma lucidum	17.7	10. 3- 22.6	Minor acute toxic	**Ganoderma lucidum**	10.7	10. 3- 22.6	Minor acute toxic
Trametes versicolor	16.8	10. 3- 22.6	Minor acute toxic	**Trametes versicolor**	12.8	10. 3- 22.6	Minor acute toxic
Amaranth	0.93	<1.0	acutely toxic	**Mixed dye**	10.93	10-20	Minor acute toxic
Irpex lacteu	17.9	10. 3- 22.6	Minor acute toxic	**Irpex lacteu**	47.9	40. 3-52.6	Minor acute toxic
Aspergillus species	19.7	10. 3- 22.6	Minor acute toxic	**Aspergillus species**	49.7	40. 3-52.6	Minor acute toxic
Penicillium geastrivorus	11.9	10. 3- 22.6	Minor acute toxic	**Penicillium geastrivorus**	41.9	40. 3-52.6	Minor acute toxic
Datronia sp.	17.5	10. 3- 22.6	Minor acute toxic	**Datronia sp.**	47.5	40. 3-52.6	Minor acute toxic
Myrothecium roridum	18.8	10. 3- 22.6	Minor acute toxic	**Myrothecium roridum**	48.8	40. 3-52.6	Minor acute toxic
Polyporus arcularius	15.0	10. 3- 22.6	Minor acute toxic	**Polyporus arcularius**	45.0	40. 3-52.6	Minor acute toxic
Fomitopsis feei	14.4	10. 3- 22.6	Minor acute toxic	**Fomitopsis feei**	44.4	40. 3-52.6	Minor acute toxic
Pleurotus ostreatus	16.7	10. 3- 22.6	Minor acute toxic	**Pleurotus ostreatus**	46.7	40. 3-52.6	Minor acute toxic
Trametes hirsuta	18.5	10. 3- 22.6	Minor acute toxic	**Trametes hirsuta**	48.5	40. 3-52.6	Minor acute toxic
Fomes fomentarius	13.0	10. 3- 22.6	Minor acute toxic	**Fomes fomentarius**	43.0	40. 3-52.6	Minor acute toxic
Ganoderma lucidum	17.7	10. 3- 22.6	Minor acute toxic	**Ganoderma lucidum**	47.7	40. 3-52.6	Minor acute toxic
Trametes versicolor	16.8	10. 3- 22.6	Minor acute toxic	**Trametes versicolor**	46.8	40. 3-52.6	Minor acute toxic

Note: EC50 – Effective Concentration; EC50<1 – Acutely toxic; EC50= 1–10% – Moderately acutely toxic; EC50= 10–100% – Minor acutely toxic; EC50>100%– Not acutely toxic.CI 95% - 95 % Confidence Intervals.

CONCLUSION

Evaluated 12 types of fungi were efficient to decolorize different kinds of dyes and the mixture of them by initially adsorbing them and subsequently degrading them, which led to the decolorization of the biomass. These funguses were also capable to detoxify food dyes and the mixture of the dyes tested. More studies regarding dye degradation and toxicity reduction by fungal treatment are necessary. Most importantly the results of this investigation demonstrated the great significance of toxicological assays associated with decolorization experiments.

ACKNOWLEDGMENTS

The present work was supported by Serampore Water Treatment Plant, Hoogly, West Bengal, India. The authors are indebted to Pandora House Value Chain Management (Kolkata, India) for donating the dyes used in the present investigation.

REFERENCES

1. Abadulla, E.; Tzanov, T.; Costa, S.; Robra, K.H.; Cavaco-Paulo, A.; Gübitz, G.M. Decolorization and detoxification of textile dyes with a laccase from Trametes hirsuta. Appl. Environ. Microbiol. 2000; 66: 3357-3362.
2. Ambrósio, S.T.; Campos-Takaki, G.M. Decolorization of reactive azo dyes by Cunninghamella elegans UCP 542 under cometabolic conditions. Biores. Technol. 3. Arora, D.S.; Chander, M, 2004; 91: 69-75.
3. Decolourisation of diverse industrial dyes by some Phlebia spp. and their comparison with Phanerochaete chrysosporium. J. Basic. Mycrobiol. 4. Bae, J.S.; Freeman, H.S, 2002; 44: 331-338.
4. Synthesis and evaluation of nongenotoxic direct dyes. Fibers Polym. 5. Boer, C.G.; Obici, L.; Souza, C.G.M.; Peralta, R.M, 2004; 3: 140-146.
5. Decolorization of synthetic dyes by solid state cultures of Lentinula (Lentinus) edodes producing manganese peroxidase as the main ligninolytic enzyme. Biores. Technol, 94: 107-112.
6. Cha, C.J.; Doerge, D.R.; Cerniglia, C.E. Biotransformation of malachite green by the fungus Cunninghamella elegans. Appl. Environ. Microbiol, 2001; 67: 4358-4360.
7. Chen, K.C.; Wu, J.Y.; Liou, D.J.; Hwang, S.C.J. Decolorization of the textile dyes by newly isolated bacterial strains. J. Biotechnol, 2003; 101: 57-68.
8. Conneely, A.; Smyth, W.F.; McMullan, G. Metabolism of the phthlocyanine textile dye remazol turquoise blue by Phanerochaete chrysosporium. FEMS Microbiol. Lett, 1999; 179: 333-337.
9. Coulibaly, L.; Gourene, G.; Spiros, A.N. Utilization of fungi for biotreatment of raw wastewaters. Afri. J. Biotechnol, 2003; 2: 620-630.
10. Das S., Mandal, S.C. Cytotoxicity of Flavonoids analyzed using Different Cell Lines from The Onion Pell (Allium cepa). NSHM Journal Of Pharmacy And Health Care Management, 2015; 6: 10-16.
11. Das S., Optimization of Ultrasound-assisted Extraction of Total Flavonoids and Antioxidant properties from Trigonella foenum-graecum Seeds with Response Surface Methodology. IJPSR, 2013; 4: 4308-4318.
12. Das S., Hepatoprotective Activity of Methanol Extract of Fenugreek Seeds on Rats. IJPSR, 2014; 5: 1506-1513.
13. Das S., Mandal, S.C., Synthesis And Therapeutic Activity of Silver Nanoparticles Using Bio-Based Protocol. The Pharma Review, 2014; 12: 124-128.
14. Dos Santos, A.Z.; Neto, J.M.C.; Tavares, C.R.G.; da Costa, M.G. Screening of filamentous fungi for the decolorization of a commercial reactive dye. J. Basic Microbiol, 2004; 44: 288-295.
15. El-Rahim, W.M.A.; Moawad, H. Enhancing bioremoval of textile dyes by eight fungal strains from media supplemented with gelatine wastes and sucrose. J. Basic Microbiol, 2003; 43, 367-375.
16. Fontenot, E.J.; Lee, Y.H.; Matthews, R.D.; Zhu, G. Pavlostathis, S.G. Reductive decolorization of textile reactive dyebath under methanogenic conditions. Appl. Biochem. Biotechnol, 2003; 109: 207-224.
17. Fraaije, M.W.; Mattevi, A.; Berkel, W.J.H.V. Mercuration of vanillil-alcohol oxidase from Penicillium simplicissimum generates inactive dimmers. FEBS Lett, 1997; 402: 33-35.
18. Fraaije, M.W.; Roubroeks, H.P.; Hagen, W.R.; van Berkel, W.J.H. Purification and characterization of an intracellular catalaseperoxidase. Eur. J. Biochem, 1996; 235: 192-198.
19. Fu, Y.; Viraraghavan, T. Fungal decolorization of dye wastewaters: a review. Biores. Technol, 2001; 79: 251-262.
20. Ghosh N, Ali A, Ghosh R, Das S, Mandal SC, Pal M. Chronic Inflammatory Diseases: Progress and Prospect with Herbal Medicine. Curr Pharm Des, 2016; 22(2): 247-264.
21. Hamilton, M.; Russo, R.C.; Thurston, R.V. Trimmed Spearman-Karber Method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol, 1977; 11(7): 714-719.
22. Hanazato, T.; Yasuno, M. Zooplankton community structure driven by vertebrate and invertebrate predators. Oecologia, 1989; 81, 450–458.
23. Hatvani, N.; Mécs, I. Effects of certain heavy metals on the growth, dye decolorization, and enzyme activity of Lentinula edodes. Ecotoxicol. Environ. Safe, 2003; 55: 199-203.
24. He, F.; Hu, W.R.; Li, Y. Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. Chemosphere, 2004; 57: 293-301.
25. Heinfling-Weidtmann, A.; Reemtsma, T.; Storm, T.; Szewzyk, U. Sulfophthalimide as major metabolite formed from sulfonated phthalocyanine dyes by the white-rot fungus Bjerkandera adusta. FEMS Microbiol. Lett, 2001; 203: 179-183.
26. Jin, X.C.; Liu, G.Q.; Xu, Z.H.; Tao, W.Y. Decolorization of a dye industry effluent by

Aspergillus fumigatus XC6. Appl. Microbiolo. Biotechnol, 2007; 74: 239-243.
27. Knapp, J.S.; Newby, P.S.; Reece, L.P. Decolorization of dyes by wood-rotting basidiomycete fungi. Enzyme Microb. Technol, 1995; 17: 664–668.
28. Malachová, K.; Pavlícková, Z.; Novótny, C.; Svobodová, K.; Lednická, D.; Musílková, E. Reduction in the mutagenicity of synthetic dyes by successive treatment with activated sludge and the ligninolytic fungus, Irpex lacteus. Environ. Mol. Mutagen, 2006; 47: 533–540.
29. Martins, M.A.M.; Queiroz, M.J.; Silvestre, A.J.D.; Lima, N. Relationship of chemical structures of textile dyes on the pre-adaptation medium and the potentialities of their biodegradation by Phanerochaete chrysosporium. Res. Microbiol, 2002; 153: 361-368.
30. Máximo, C.; Amorim, M.T.P.; Costa-Ferreira, M. Biotransformation of industrial reactive azo dyes by Geotrichum sp. CCMI 1019. Enzime Microb. Technol, 2003; 32: 145-151.
31. Novótny, C.; Dias, N.; Kapanen, A.; Malachová, K.; Vándrovcová, M.; Itävaara, M.; Lima, N. Comparative use of bacterial, algal and protozoan tests to study toxicity of azo and anthraquinone dyes. Chemosphere, 2006; 63, 1436-1442.
32. Novótny, C.; Svobodová, K.; Kasinath, A.; Erbanová, P. Biodegradation of synthetic dyes by Irpex lacteus under various growth conditions. Int. Biodet. Biodegrad, 2004; 54: 215-223.
33. OECD - Organization for Economic Co-Oeperation and Development. Revised Proposal for updating Guideline 202. Daphnia sp. Acute Imobilisation Test, 2000.
34. Özsoy, H.D.; Ünyayar, A.; Mazmanci, M.A. Decolourisation of reactive textile dyes Drimarene Blue X3LR and Remazol Brilhant Blue R by Funalia trogii ATCC 200800. Biodegradation, 2005; 16: 195-204.
35. Prigione, V.; Tigini, V.; Pezzella, C.; Anastasi, A.; Sannia, G.; Varese, G.C. Decolourisation and detoxification of textile effluents by fungal biosorption. Wat. Res., 2008; 42: 2911-2920.
36. Ramsay, J.A.; Nguyen, T. Decoloration of textile dyes by Trametes versicolor and its effect on dye toxicity. Biotechnol. Lett, 2002; 24: 1757-1761.
37. Saparrat, M.C.N.; Hammer, E. Decolorization of synthetic dyes by the deuteromycete Pestalotiopsis guepinii CLPS no. 786 strain. J. Basic Mycrobiol, 2006; 46, 28-33.
38. S. Das, S., Mandal, S.C. Effect of Process Parameters of Microwave Assisted Extraction (MAE) on Natural Product Yield from Onion Peel. IJPSR, 2015; 6: 3260-3275.
39. Sharma, P.; Singh, L.; Dilbaghi, N. Response surface methodological approach for the decolorization of simulated dye effluent using Aspergillus fumigatus fresenius. J. Hazard. Mater, 2009; 161: 1081–1086.
40. Shaul GM, Holdsworth TJ, Dempsey CR, Dostall KA. Fate of water soluble azo dyes in the activated sludge process. Chemosphere, 1991; 22: 107–119.
41. Shedbalkar, U.; Dhanve, R.; Jadhav, J. Biodegradation of triphenylmethane dye cotton blue by Penicillium ochrochloron MTCC 517 J. Hazard. Mater, 2008; 157: 472–479.
42. Singh, H. Mycoremediation: fungal remediation. WileyInterscience, New Jersey. 39. Stemberger, R.S. (1981). A general approach to the culture of planktonic rotifers. Can. J. Fish. Aquat. Sci, 2006; 38: 721–724.
43. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56:69–80. Tamaro M, Monti-Bragadin C, Banfi E. 1975. Mutagenic activity of anthraquinone derivatives used as dyes in a textile factory. Boll Ist Sieroter Milan, 2001; 26: 105–107.
44. Tan, N.C.G.; Prenafeta-Boldú, F.X.; Opsteeg, J.L.; Lettinga, G.; Field, J.A. Biodegradation of azo dyes in cocultures of anaerobic granular sludge with aerobic aromatic amine degrading enrichment cultures. Appl. Microbiol. Biotechnol, 1999; 51: 865-871.
45. Tien M, Kirk TK. Lignin peroxidase of Phanerochaete chrysosporium. Methods Enzymol, 1988; 161: 238–249.
46. Ulson de Souza, S.M.A.G.; Forgiarini, E.; Ulson de Souza, A.A. Toxicity of textile dyes and their degradation by enzyme peroxidase (HRP). J. Hazard. Mater, 2007; 147: 1073-1078.
47. Umbuzeiro, G.A.; Roubicek, D. A.; Rech, C. M.; Sato, M. I. Z.; Claxton L. D. Investigating the sources of the mutagenic activity found in a river using the Salmonella assay and different water extraction procedures. Chemosphere, 2004; 54: 1589-1597.
48. Verma, Y. Toxicity evaluation of effluents from dye and dye intermediate producing industries using Daphnia bioassay. The Internet Journal of Toxicology, 2008; 4(2). http://www.ispub.com/ostia/index.php?xmlPrinter=true&xmlFilePath=j ournals/ijto/vol4n2/dye.xml.
49. Villegas-Navarro, A.; Ramírez-M, Y.; Salvador-S, M.S.; Gallardo, J.M. Determination of wastewater LC50 of the different process stages of the textile industry. Ecotoxicol. Environ. Saf, 2001; 48: 56-61.
50. Wong PK, Yuen PY. Decolorization and biodegradation of methyl red by Klebsiella pneumoniae RS-13. Water Res, 1996; 30: 1736–1744.
51. Yamada-Onodera, K.; Mukumoto, H.; Katsuyaya, Y.; Saiganji, A.; Tani, Y. Degradation of polyethylene by a fungus, Penicillium simplicissimum YK. Polym. Degrad. Stab, 2001; 72: 323-327.
52. Yang, Q.; Yang, M.; Pritsch, K.; Yediler, A.; Hagn, A.; Schloter, M.; Kettrup, A. Decolorization of synthetic dyes and production of manganese-

dependent peroxidase by new fungal isolates. Biotechnol. Lett, 2003; 25: 709-713.
53. Yesilada, O.; Cing, S.; Asma, D. Decolourisation of the textile dye Astrazon Red FBL by Funalia trogii pellets. Biores. Technol, 2002; 81: 155-157.
54. Yu, Z.; Wen, X. Screening and identification of yeasts for decolorizing synthetic dyes in industrial wastewater. Int. Biodet. Biodegrad, 2005; 56: 109-114.
55. Y Verma. Toxicity Evaluation of Effluents from Dye and Dye Intermediate Producing Industries Using Daphnia Bioassay. The Internet Journal of Toxicology, 2007; 4(2), 1-7.
56. Zeng, G.M.; Yu, H.Y.; Huang, H.L.; Huang, D.L.; Chen, Y.N.; Huang, G.H.; Li, J.B. Laccase activities of a soil fungus Penicillium simplicissimum in relation to liginin degradation. World J. Mycrobiol. Biotechnol, 2006; 22: 317-324.
57. Zheng, Z.X; Levin, R.E.; Pinkham, J.L.; Shetty, K. Decolorization of polymeric dyes by a novel Penicillium isolate. Process Biochem, 1999; 34, 31–37.
58. Zbaida S, Levine WG. Characteristics of two classes of azo dye reductase activity associated with rat liver microsomal cytochrome P450. Biochem Pharmacol, 1990; 40: 2415–2423.
59. Zieger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests. V. Results from the testing of 311 chemicals. Environ Mol Mutagen, 1992 19(21): 2–141.