Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
Microbiology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the research across the whole spectrum of the subject. These including bacteriology, virology, mycology and parasitology. In addition, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.12 (1)pp. 79- 90 (2020)*

Egypt. Acad. J. Biolog. Sci., 12(1): 79-90 (2020)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/



Biodegradation of Malachite Green by White-Rot Fungus, *Pleurotus Pulminarious*

Zubbair N. A.[1], Ajao A. T.[2*], Adeyemo E. O.[3] and Adeniyi D. O.[4]

1 -Department of Science Laboratory Technology, Biology/Microbiology Unit, Kwara State Polytechnic, Ilorin, Kwara State, Nigeria.

2-Department of Biosciences and Biotechnology, Microbiology Unit, Kwara State University, Malete, Kwara State, Nigeria.

3-Department of Science Laboratory Technology, Chemistry/Biochemistry Unit, Kwara State Polytechnic, Ilorin, Kwara State, Nigeria.

4-Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria.

E.mail:abdullahi.ajao@kwasu.edu.ng- nzubbair@gmail.com –
elija_adeyemo@gmail.com - modeleadeniyi@gmail.com

ARTICLE INFO

Article History
Received: 28/4/2020
Accepted:12/6//2020

***Keywords*:**
Ecofriendly, Malachite green, Laccase, Biodegradation, *Pleurotus pulminarious*

ABSTRACT

Wide spectrum of the industrial applications of Malachite green dyes and its lethal effect on the living organisms become major source of concerns as this causes damage to tissues, hindered growth and development system, developmental abnormalities, and mutagenic/carcinogenic potentials. This study evaluated the degradation of malachite green by laccase producing *Pleurotus pulminarious*. The pH, Total Protein, and laccase activities were used to monitor the progress of the degradation. The laccase activity and protein contents increased as the biodegradation process progressed with the highest Laccase activities and extracellular protein values of 0.2867±0.00 and 14.872±0.43 respectively at pH 3.9 and decreased progressively with increase in pH. Metabolites obtained after decolorizations were analyzed with GC-MS and FTIR studies and confirmed the biodegradation of dye. GC-MS analysis identified metabolites that were produced after the degradation of MG. This study demonstrated complete degradation of MG into low molecular weight compounds such as 3-Benzylhexahydropyrrolo[1,2-a] pyrazine-1,4-dione,3-Isobutylhexahydropyrrolo [1,2-a] pyrazine-1,4-dione, 3-Aminoacetophenone, N,4,4-Trimethylcyclohexanamine, 2,3,4-Trimethylhexane. It was concluded that the organism posses multifunction of having medicinal values, production of industrially important laccase, and for the treatment of industrial effluent containing malachite green.

INTRODUCTION

Dyes have proven to be a key environmental threat and a serious public health problem in spite of its numerous industrial applications (Nourein *et al*., 2017). Several manufacturing sectors use large volume of water for the production, thereby generating large quantities of dye-containing wastewater which can present a significant environmental threat to the environment constituting threats to biodiversity and integrity of marine ecosystems (Kurade *et al*., 2016; Rashid *et al*., 2016; Saeed *et al*., 2016; Nouren *et al*., 2017).

Dyes are chromophore compounds which are highly visible even at low concentrations (1 mg L^{-1}) and could cause visual pollution and disruption to the environment and water supplies (Ayed *et al*., 2017). Physicochemical methods have been the common strategies for the decolorization and detoxification of dyes with peculiar limitations such as low performance, high expense, poor quality and intrusion by other wastewater constituents, attention has been shifted from most of the available physiochemical methods for treating wastewater-containing dye (Banat *et al*., 1996), due to the generation of large quantities of sludge that may constitute a secondary pollutant (Du *et al*., 2011, Charturvedi and Verma, 2015; Al-Jawhari and Al-Mansor, 2017).

In an attempt to find an alternative solution, an environmentally safe, reliable and cost-effective method to handle wastewater-containing dyes (Hazrat, 2010; Ayed *et al*., 2017), Biological approaches, however, are the most attractive alternative solution suitable to treat dye-containing wastewater due to the following attributes: cheap, wide range of applicability, low running cost, non-toxic end product development and environmentally safe (Forgas *et al*., 2004; Pandey *et al*., 2007; Al-Fawwaz and Abdullah, 2016).

Though bacterial dye treatment is inexpensive but there is a high chance of toxic aromatic amine formation (Vyrides *et al*., 2014; Adnan *et al*., 2015). Alternatively, Fungi have demonstrated high adaptability, effective removal and mineralization due to their enzymatic machinery (Ciullini *et al*., 2008; Tana *et al*., 2014) and as such the use of fungi is a potential option for replacing or supplementing existing treatment (Fu and Viraraghavan, 2001 ; Dos santos *et al* ., 2004). Laccase enzyme plays a crucial role in the mycodegrading capability of fungi (Fu *et al*., 2001; Olukanni *et al*., 2010). Laccases are useful biocatalysts for a broad range of biotechnological applications, including decoloration, detoxification of a wide range of pollutants, bleaching of pulp, and decoloration of black liquor (Giardina *et al*., 2010).

Malachitegreen (MG) is a water-soluble cationic dye made of triphenylmethane used in fabric coloring (Zhou et al., 2015). It has a wide variety of applications both in the food and healthcare field (Chowdhury et al., 2011).

MG is one of the Azo dyes known to have one or more azo bonds (-N = N-), typically connected to lateral OH and/or SO_3 groups of benzene or naphthalenic rings (Garcia-Segura and Brillas, 2016).

MG has a wide toxicity spectrum affecting microorganisms and higher eukaryotes (Tamayo-Ramos *et al*., 2012). Carcinogenesis, mutagenesis, chromosomal disruption, teratogenicity, and respiratory toxicity have also been recorded (Daneshvar *et al*., 2007; Al-Fawwaz and Jacob, 2011; Al-fawwaz and Abdullah, 2016).

MG is banned in many countries and is still used in many places around the world because of its effectiveness, low cost, and availability, and thus continues to present environmental, food safety and human health risks (Yang *et al*., 2015). There is considerable interest in developing an alternative approach that is environmentally safe, effective, and low-cost to degrade this kind of recalcitrant molecules (Wang *et al*., 2012). This study evaluated the capability of *Pleurotus pulminarious* for the detoxification and degradation of MG.

MATERIALS AND METHODS

Organisms and Culture Conditions:

Pleurotus pulminarious white-rot fungal strain was collected from the Federal Institute of Industrial Research Oshodi in Lagos State, Nigeria. The mycelium was cultivated on the potato Dextrose Agar (PDA) slant, subculture periodically, and maintained 4 ^{0}C.

Chemicals:

The common name of the dye was used. The analytical grade of Malachite Green (MG) and other chemicals used were obtained from Sigma (U.S.A.).

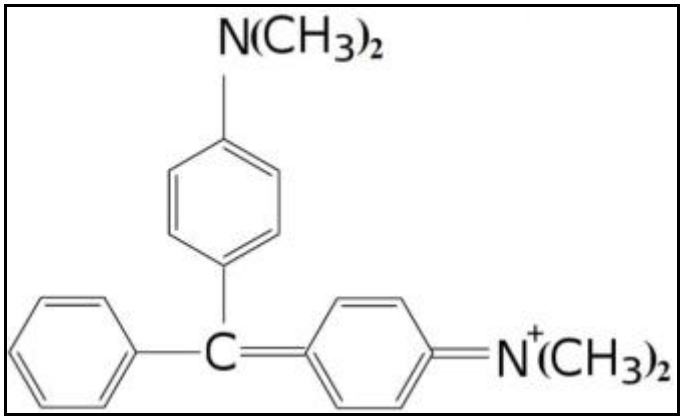


Fig.1. Chemical structure of Malachite green

Decolorization Protocol:

The decoloration of dyes in the liquid phase was evaluated using the PDB (Potato Dextrose Broth) medium containing 150 mgl−1 of green malachite dyes. In 250 mL Erlenmeyer flasks, hundred milliliters of potato dextrose broth were prepared and the pH was adjusted to 5.8 with 0.1 N HCl or 0.1 N NaOH. MG stock was prepared in sterile distilled water at a concentration of 1 mg mL^{-1}, filter-sterilized, and stored at 4 ºC for further use. A 5 mycelial agar plugs (5 mm diameter) were inoculated into Flasks containing 100 ml of liquid medium obtained from the edge of mycelia of actively growing selected fungus.

These flasks were then incubated The inoculated flasks were held at 37 ºC for 8-10 days under static conditions (Two milliliters of the aliquot were taken from the cultures periodically and then centrifuged at 25 ºC for 2 minutes (4000 rpm) (Barapatre *et al*., 2017). The following parameters were monitored: Extracellular Laccase activity assay (Collins and Dobson, 1997)

Determination of protein content Singh and Abraham (2013) and Analysis of MG degradation by GC-MS and FTIR spectroscopy over a period of the experiment as earlier described in previous work (Zubbair *et al*., 2019).

RESULTS AND DISCUSSION

The mean values of pH of the experimental culture medium during biodegradation of MG by *Pleurotus pulminarious* shown in Table 1 ranged from 3.90 ±0.12) to 10.5 ± 1.20, where highest pH value was recorded after 288 hours of incubation. The pH tends to increase as the incubation progressed. Both the extracellular protein and Laccase activities with the initial mean values of 14.872mg/mL and 0.2867 U/mL after 48 hours of incubation respectively and decreased progressively. There is a relationship between the pH and other monitoring indices.

Laccase activities found to be highest at pH of 3.9 with laccase activities of 0.2867±0.00. The results showed that the acidic medium favoured Laccase activities and protein secretion. Laccase activities were not detected at the pH of 8.6 after 288 hours of incubation.

In the control of the Malachite Green IR spectrum revealed the following observations. The peaks in the range 400-2900 cm-1 display the existence of –OH, -NH-, -C-H (amides and amines).

The peaks in the range of 2700-2000cm^{-1} accounted for the presence of Nitriles, azide compounds in the sample while peak at 1650 cm^{-1} represents aromatic ketones. The peaks in the following range 1500-600cm-1 are associated with specific peaks in the fingerprint region for the aromatic metabolite mono-substituted and para-disubstituted benzene rings.

Noticeable variations in the fingerprint region of MG's FTIR spectra and its metabolites (4000-400 cm-1) are shown

in Figure 1. The new peaks at 3363 cm^{-1} for -OH stretching vibrations of the FTIR spectrum of biodegraded products shown in Figure 2 illustrated the formation of the hydroxylated metabolites, similarly, the sharp peaks at 2947 cm-1, 2908cm-1 cm-1, and 2831 cm-1 for -CH stretching by asymmetric CH2 groups, while the marginal peak at 1651 cm-1 corresponds to the C-O stretch of ketones. The peaks at 1111 cm^{-1} and 1026cm^{-1} correspond to aromatic C-N stretching vibration. The peak at 1450cm^{-1} depicts -CH_2 scissoring and 1450cm^{-1} and 1411cm^{-1} for aromatic group with a support to the peak at 1651 cm-1 for the C=C stretching of the benzene rings. Also, the peak at 1130cm^{-1} cm-1 is for aromatic C–N stretching vibrations. Moreover, new peaks were also detected at 1211 cm^{-1} and 1111cm^{-1} (C-C). The results of the FTIR study, therefore, suggest that the notable identified chemical groups in the MG metabolites are -OH, -C = O and -NH_2, which substantiate the degradation. The reduction of peaks along the fingerprint region such as 950, 850, 800, 750, and 600 cm^{-1} can be attributed to the loss of aromaticity of the metabolites (Chaturvedi and Verma, 2015).

Table 1: Changes in pH, Protein and Laccase Activities during Biodegradation of Malachite green by *P.pulminarious*

Time (Hours)	pH	Extracellular Protein (Mg/ml)	Laccase Activities(U/ml)
48	3.90 ± 0.12^{d}	14.872 ± 0.43^{a}	0.2867 ± 0.00^{a}
96	5.00 ± 0.00^{c}	13.834 ± 0.72^{ab}	0.2767 ± 0.01^{a}
144	5.20 ± 0.33^{c}	13.651 ± 0.30^{ab}	0.2648 ± 0.00^{ab}
192	6.10 ± 0.11^{ab}	10.174 ± 0.00^{b}	0.2317 ± 0.02^{b}
240	7.80 ± 0.00^{b}	8.21 ± 0.67^{c}	0.00197 ± 0.00^{c}
288	10.50 ± 1.20^{a}	2.56 ± 0.00^{d}	ND

Values are presented as Mean ± SEM (n = 3). All groups are compared to each other at $P < 0.05$. Values with the same superscripts along the same column are not statistically different from each other. ND; Not detected

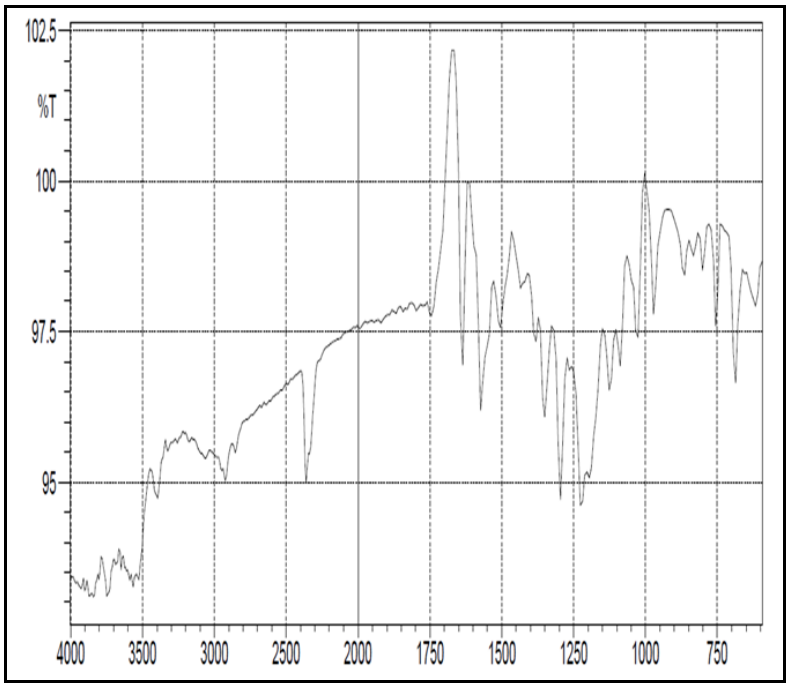


Fig. 1: FTIR spectral of non degraded malachite green dye (control)

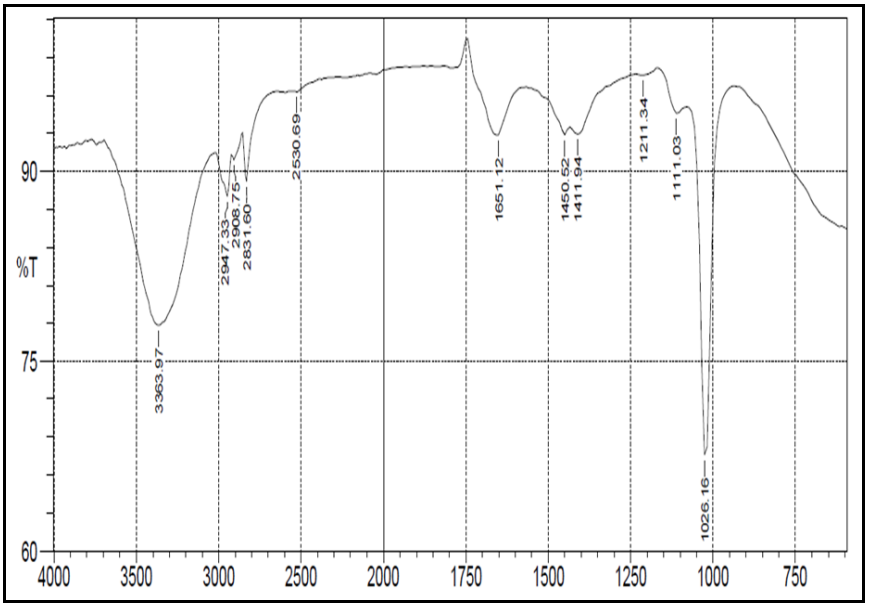


Fig. 2: FTIR spectral of degraded malachite green dye

Similarly, the sharp peaks at 2947 cm-1, 2908cm-1 cm-1, and 2831 cm-1 for -CH stretching by asymmetric CH2 groups, while the marginal peak at 1651 cm-1 corresponds to the C-O stretch of ketones. The peaks at 1111 cm^{-1} and 1026cm^{-1} correspond to aromatic C-N stretching vibration. The peak at 1130cm^{-1} cm-1 is for aromatic C–N stretching vibrations. Moreover, new peaks were also detected at 1211 cm^{-1} and 1111cm^{-1} (C-C). The results of the FTIR study, therefore, suggest that the notable identified chemical groups in the MG metabolites are -OH, -C = O and -NH_2, which substantiate the degradation. The reduction of peaks along the fingerprint region such as 950, 850, 800, 750, and 600 cm^{-1} can be attributed to the loss of aromaticity of the metabolites (Chaturvedi and Verma, 2015).

In this study, the GC – MS analysis identified the metabolites of MG in neutral fractions by contrasting the mass spectra with data in the NIST98 library and by interpreting the fragmentation patterns independently. 5 Metabolites, including3-Benzylhexahydropyrrolo [1,2-a]pyrazine-1,4-dione, 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione, 3-Aminoacetophenone, N-4,4-Trimethylcyclohexanamine, 2,3,4-Trimethylhexane were found to be resulting metabolites. The mass spectral characteristics of metabolites are m/z= 128, 135, 141, 210, and 244 respectively. While retention times are 7.433, 15.400, 8.333, 16.508, and 23.317 minutes respectively. There are many other metabolites' chromatographic peaks in GC–MS chromatograms which are ignored as a result of their simplicity. The GC degradation products of Malachite green dye showed the presence of several peaks (Figs. 3 & 4). The peaks represent the number of compounds present in the dye, before and after degradation. The degraded dye contains a total of 22 peaks compared to the non-degraded dye which has a total of 30 compounds present indicating that some compounds have been degraded.

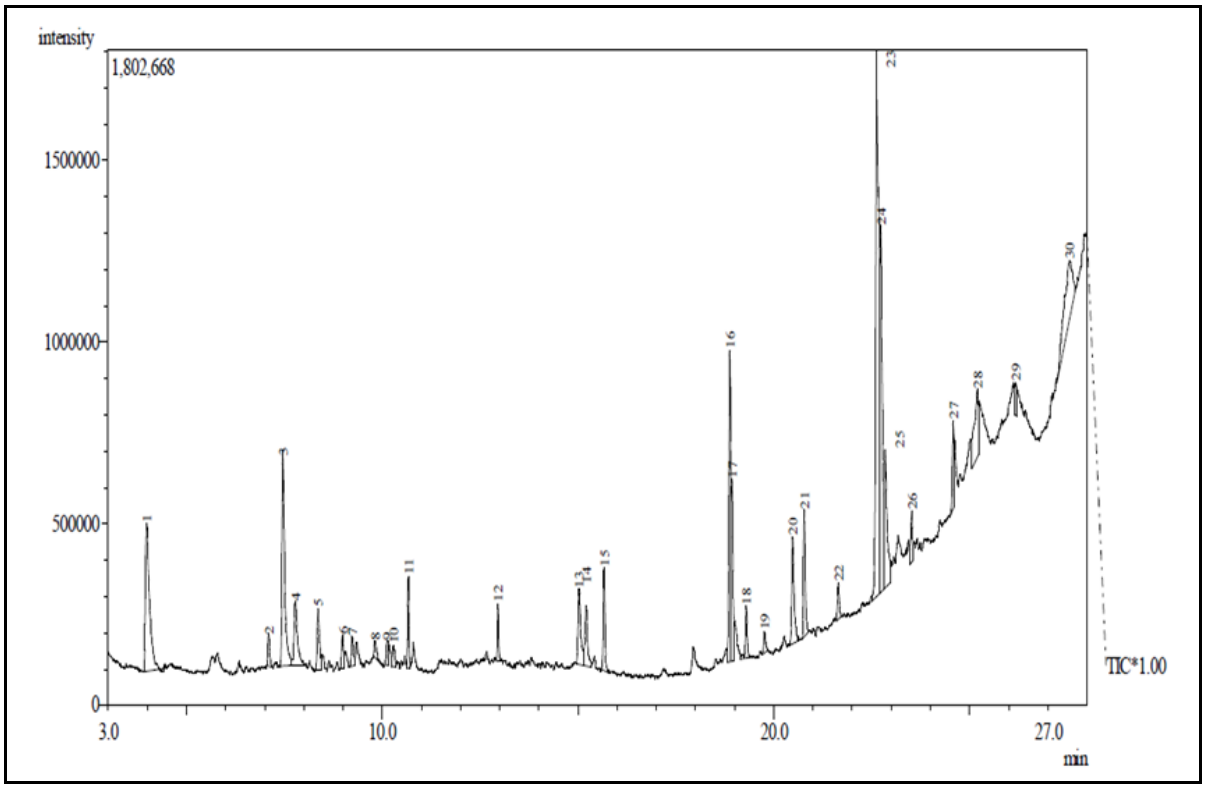


Fig.3: GC-MS Fingerprint of non-degraded malachite green dye (control)

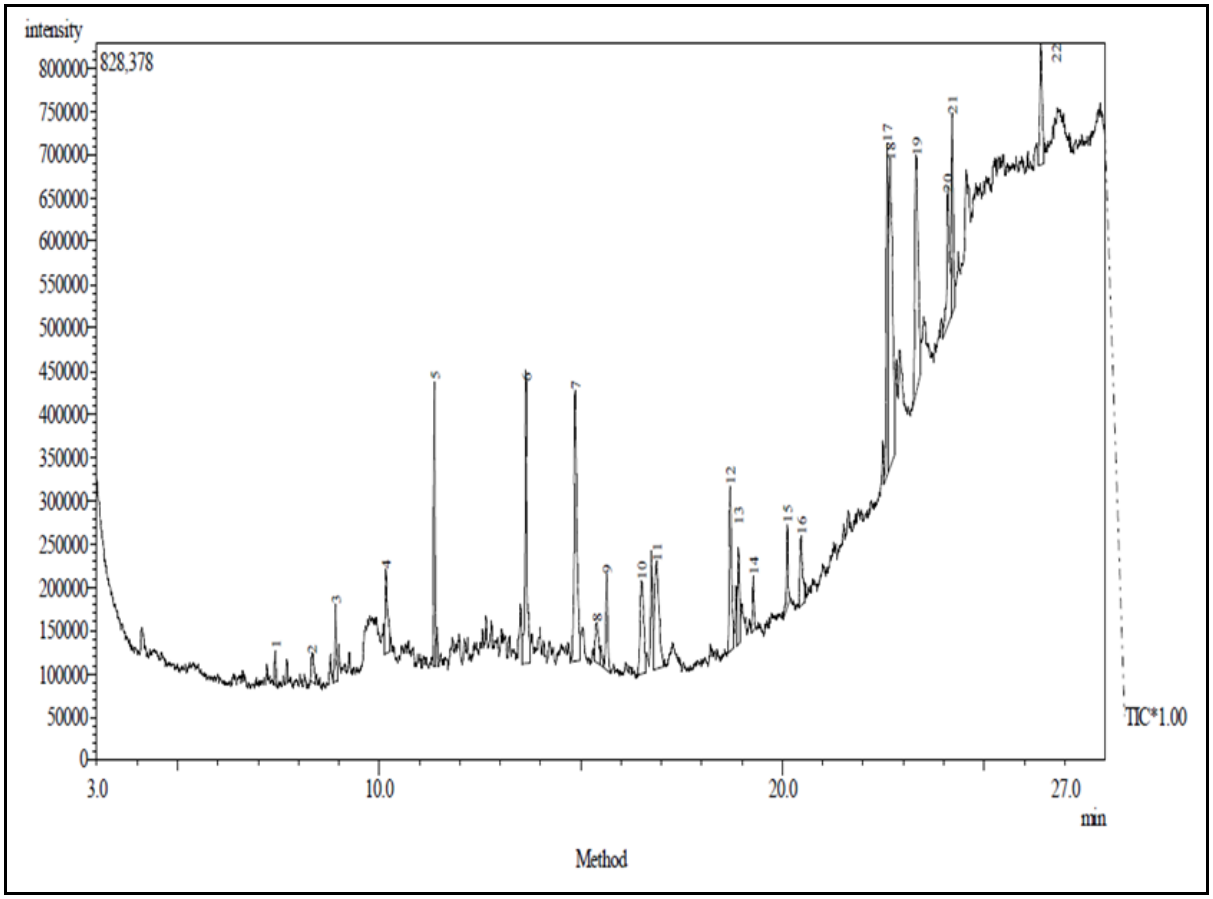


Fig.4: GC-MS Fingerprint of degraded malachite green dye.

The structures of the detected compounds were determined using the fragmentation method and the m / z values obtained through mass spectral analysis (Fig. 5). The result of the GC-MS analysis of the treated dye in comparison with the control dye revealed the ability of the *Pleurotus pulminarious* to degrade the components of the complex dye molecules as seen in terms of the reduction or total removal of some compounds and functional groups

Degradation of azo dyes has been documented involving aromatic cleavage, which was found to base on the identity of phenolic, amino, acetamide, 2-methoxyphenol, or other easily biodegradable functional groups of the ring substituents, leading to greater degradation (Paszczynski *et al*., 1991).

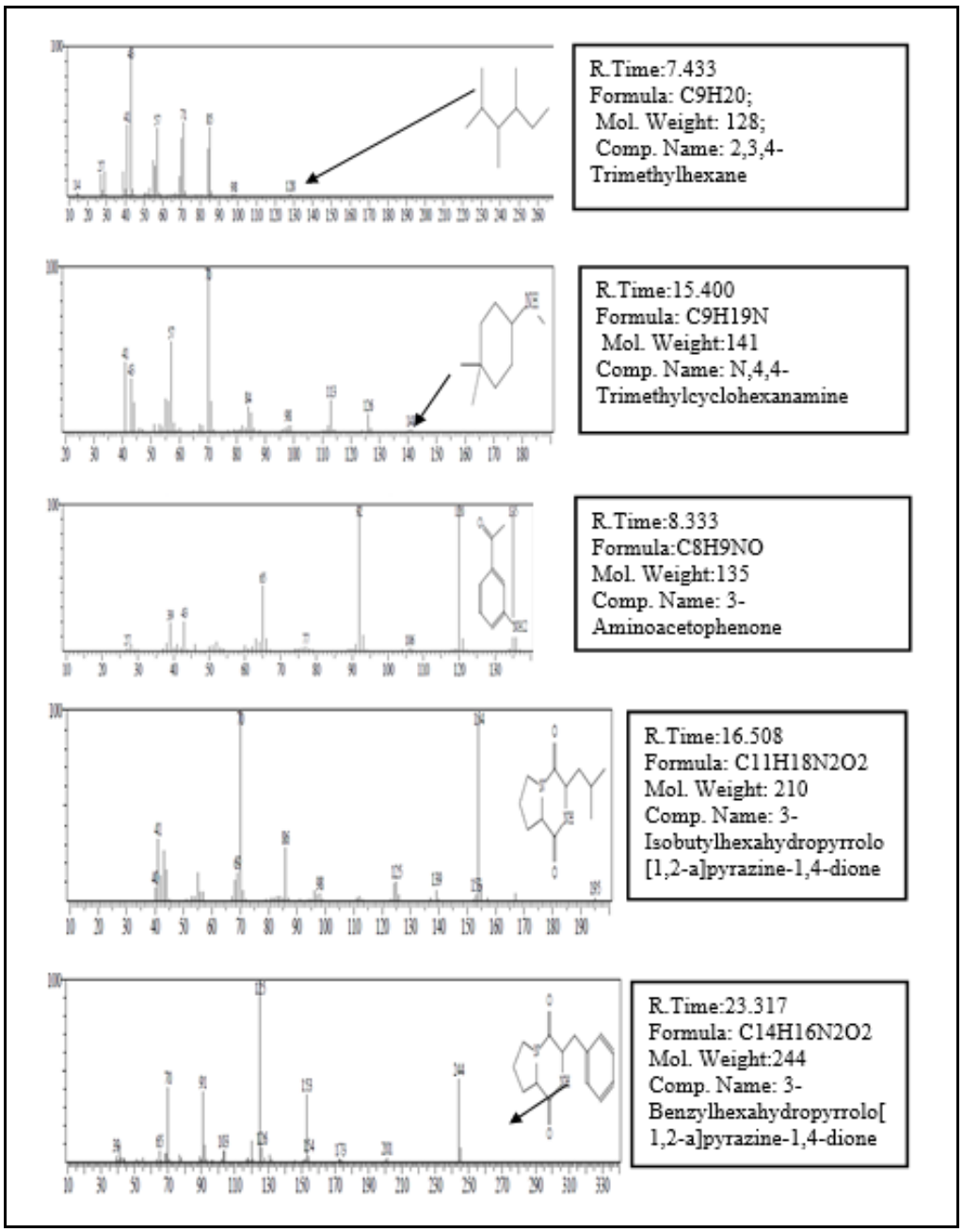


Fig. 5: Identification of MG biotransformation products by using GC–MS

The effect of laccase activity is indicated by the presence of aromatic amine in the degraded sample. It was found that the azo compounds with hydroxyl or amino groups are more likely to degrade than those with methyl, methoxy, sulfo, or nitro groups. Apparently, the presence of laccase accounted for the degradation process of the dye as it confirmed from the mass analysis report leading to the formation of different intermediate metabolites (Pandey *et al.*, 2007).

The ability of the isolates to secrete laccase enzyme demonstrated in the previous work can be attributed to its ability to degrade malachite green. It has been reported that laccase from white-rot fungi play a key role in the decoloration of textile effluents (Eichlerov´a *et al*., 2003). Some *Pleurotus* laccases have also been reported to have colour removal potential (Zilly *et al*., 2002). Highest laccase activity recorded at the pH of 3.90±0.12 observed in this study agreed with the work of Singh and Abraham (2013) who reported that fungal growth is optimal at low pH and that the maximum laccase activity of the fungus was found to be at optimum pH 4 with maltose and peptone as ideal carbon and nitrogen sources, also that the F11 strain was able to produce maximum protein content of 1.9 mg/ml at pH values 5 and 6 optimum pH being 5.5 with laccase activity at pH 4.In similar study Hou *et al*. (2004) demonstrated that laccase was the only ligninolytic enzyme activity identified in the supernatant when the fungus grew with or without shaking in liquid culture.

Arul Diana Christie and Shanmugam (2012) reported that their four fungal isolates demonstrated maximum activity yielded within the temperature range of 35 to 45 ^{0}C under acidic medium

Dye decoloration by ligninolytic fungi has been attributed in previous studies to extracellular laccase activity (Abadulla *et al*., 2000; Minussi *et al*., 2001; Salony *et al*., 2006),This claim was supported by Priyadarsini *et al*. (2011) who documented that the involvement of the laccase gene and the successful transformation of the gene into *E.coli* from a white rot fungus led to the higher percentage of decoloration which affirmed the role of the laccase. Besides extracellular enzymes, it is also possible that these fungi's dye decolorization activity may also be due to their mycelia's ability to adsorb/absorb the dye (Shahid et al. 2013). It was thought that decoloration by the fungal strain would also occur in the sequence of intermediates that generates similar color change to other work (Sharma and Sobti, 2000). An interpretation of the molecular biodegradation that occurred will include the chemical recognition of the breakdown products (Park *et al*., 2007), which informed the analysis of degraded products using GC-MS and FTIR rather than the phenomenon of color changes.

It can be concluded that *Pleurotus pulminarious* posses Genetic and enzymatic capability to degrade malachite green which can, be adopted for the remediation of textile effluent and production of industrially important laccase enzyme.

REFERENCES

Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Cavaco-Paulo, A. & Gübitz, G.M. (2000). Decolourization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Applied Environment Microbiology*, 66: 3357-3362.

Abdel Rahim, W.M.& Moawad, H. (2003) Enhancing bioremoval of textile dyes by eight fungal strains from media supplemented with gelatine wastes and sucrose. *Journal of Basic Microbiology*, 43 (5): 367-375.

Adnan, L.A., Abdull RahimMohd Yusoff, A.R., Hadibarata, T. & Ameer Badr Khudhair, A.B. (2014). Biodegradation of Bis-Azo Dye Reactive Black 5 by White-Rot Fungus *Trametes gibbosa* sp. WRF 3 and Its Metabolite Characterization. *Water Air Soil Pollution*, 225: 2119. http://dx.doi.org/10.1007/s11270-014-2119-2.

AI-Jawhari1, I.F.H. & AL-Mansor, K.J. (2017). Biological Removal of

Malachite Green and Congo red by Some Filamentous Fungi. *International Journal of Environment, Agriculture and Biotechnology*, 2(2): 723-731. http://dx.doi.org/10.22161/ijeab/2.2.21

Al-Fawwa A. T., & Jacob, J. H. (2011) "Removal of methylene blue and malachite green from aqueous solution by Chlorella and Chlamydomonas species isolated from a thermal spring environment." *International Journal of Integrative Biology*, 12(1): 36-41.

Al-Fawwaz, A.T. & Abdullah, M. (2016). Decolorization of Methylene Blue and Malachite Green by Immobilized *Desmodesmus* sp. Isolated from North Jordan. *International Journal of Environmental Science and Development*, 7(2): 95-99.

Arul Diana Christie, S. & Shanmugam, S. (2012) Analysis OF Fungal Cultures Isolated from Anamalai Hills For Laccase Enzyme Production Effect On Dye Decolorization, Antimicrobial Activity. *International Journal of Plant, Animal and Environmental Sciences.*,2(3): 143-148.

Ayed, L., Bekir, K., Achour, S., Cheref, A. & Bakhrouf, A. (2017). Exploring bioaugmentation strategies for azo dye CI Reactive Violet 5 decolourization using bacterial mixture: dye response surface methodology. *Water and Environment Journal*, 31: 80–89.

Cha, C- J., Doerge, D.R., & Cerniglia, C.E. (2001) Biotransformation of malachite green by the fungus Cunninghamella elegans. *Applied Environment Microbiology*, 67: 4358–4360.

Chaturvedi, V. & Verma, P. (2015). Biodegradation of malachite green by a novel copper-tolerant *Ochrobactrum pseudogrignonense* strain GGUPV1 isolated from copper mine waste water. *Bioprocess*,2:42.http://dx.doi.org/10. 1186 /s40643-015-0070-8.

Chen, C. Y. Kuo, J. T. Cheng, C. Y. Huang, Y. T. Ho, I. H. & Chung, Y. C. (2009) Biological decolorization of dye solution containing malachite green by *Pandoraea pulmonicola* YC32 using a batch and continuous system. *Journal of Hazardous Materials.*, 172: 1439-1445.

Ciullini, I., Tilli, S., Scozzafava, A. & Briganti, F. (2008) Fungal laccase, cellobiose dehydrogenase, and chemical mediators: Combined actions for the decolorization of different classes of textile dyes. *Bioresource Technology*, 99: 7003–7010.

Cooper, P. (1995) Colour in Dyehouse Effluent, Alden Press, Oxford

Daneshvar, N. Ayazloo, M. Khataee, A. R. & Pourhassan, M. (2007) "Biological decolorization of dye solution containing malachite green by microalgae *Cosmarium* sp," *Bioresource Technology*, 98(6): 1-7.

Desai, C., Pathak, H.& Madamwar, D. (2010) Advance in molecular and "-omics" technologies to gauge microbial communities and bioremediation at xenobiotic/ anthropogen contaminated sites. *Bioresource Technology*, 101: 1558-1569.

Du, L. N., Wang, S., Li, G., Wang, B., Jia, X. M., Zhao, Y. H. & Chen, Y. L. (2011) Biodegradation of malachite green by *Pseudomonas* sp. Strain DYI under aerobic condition: Characteristics, degradation products, enzyme analysis and phytotoxicity. *Ecotoxicology*, 20(2): 438-446.

Eichlerov´a, I., Homolka, L. & Nerud, F. (2003) Decolorization of orange G by *Pleurotus ostreatus* monokaryotic isolates with different laccase activity. *Folia Microbiology*, 48: 775–779 CrossRef

Forgacs, E., Cserhati, T.& Oros, G. (2004) Removal of synthetic dyes from wastewaters: *a review of Environment International*, 30: 953–971.

Fu Y. & Viraraghavan, T. (2001) Fungal decolorization of dye wastewaters: A review. *Bioresour Technology*, 79: 251-262.

http://dx.doi.org/10.1016/S0960-8524(01)00028-1

Garcia-Segura, S. & Brillas, E. (2016) Combustion of Textile Monoazo, Diazo and Triazo Dyes by Solar Photoelectro-Fenton: Decolorization, Kinetics and Degradation Routes. *Applied Catalysis,* 181: 681–691.

Hazrat, H. (2010) Biodegradation of synthetic dyes: a review. *Water Air Soil pollution,* 213: 251-273.

Hou, H., Zhou, J., Wang, J., Du, C. and Yan, B. (2004). Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. *Proc. Biochemistry,* 39(11): 1415-1419.

Hofrichter, M. Vares, K. Scheibner, K. Galkin, S. Sipila, J. & Hatakka, A. (1999) Mineralization and solubilization of synthetic lignin by manganese peroxidases from *Nematoloma frowardii and Phkebia radiata*. *Journal of Biotechnology,* 67: 217–228.

Kalyani, D. C., Patil, P.S., Jadhav, J.P. & Govindwar, S.P. (2008) Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas* sp. SUK1. *Bioresour Technology*,99:4635–4641.PMID: 17765541.

Kovacic, P. & Somanathan, R. (2014). Toxicity of imine-iminium dyes and pigments: electron transfer, radicals, oxidative stress and other physiological effects, *Journal of Appied. Toxicology*, 34: 825--834.

Kurade, M.B., Waghmode, T.R., Patil, S.M., Jeon, B-H. & Govindwar, S.P. (2016) Monitoring the gradual biodegradation of dyes in a simulated textile effluent and development of a novel triple layered fixed bed reactor using a bacterium-yeast consortium, Chemical Engineering Journal. doi: http:// dx.doi.org/10.1016/ j.cej. 2016.09.028.

Minussi, R.C., de Moraes, S.G., Pastore, G.M. & Duran, N. (2001). Biodecolourization screening of synthetic dyes by four white-rot fungi in a solid medium: possible role of siderophores. *Letter of Applied Microbiology*, 33: 21-25.

Mukherjee, T. & Das, M. (2014) Degradation of Malachite Green by *Enterobacter asburiae* strain XJUHX-4TM, *Clean -- Soil Air Water*, 42: 849-856.

Nouren, S., Bhatti,H-N, Iqbal, M , Bibi, I., Kamal, S., Sadaf, S., Sultan, M., Kausar, A. & Safa, Y. (2017). By-product identification and phytotoxicity of biodegraded Direct Yellow 4 dye. *Chemosphere*, 169: 474-484.

Olukanni, O.D., Osuntoki, A.A., Keyalni, D.C., Gbenle, G.O. & Govindwar, S. (2010) Decolorization and biodegradation of reactive blue 13 by *Proteus mirabilis* LAG. *Journal of Hazardous Materials*, 184: 290-298. http://dx.doi.org/10.1016/j. jhazmat .2010 .08.035.

Pandey, A, Singh, P. & Iyengar, L. (2007) Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation*59: 73–84.

Parshetti, G., Kalme,S., Saratale, G. and Govindwar, S. (2006) Biodegradation of Malachite Green by *Kocuri arosea* MTCC 1532. *Acta Chimica Slovenica.,* 53: 492-498.

Paszczynski, A. Pasti, M.B. Goszcznski, S. Crawford, D. L. & Crawford, R.L. (1991) New approach to improve degradation of recalcitrant azo dyes by *Streptomyces spp.* and *Phanerochaete chrysosporium*. *Enzyme Microb. Technol.*, 13: 378–384.

Plácido, J., Chanagá, X., Ortiz-Monsalve, S., Yepes, M. & Mora, M. (2016). Degradation and detoxification of synthetic dyes and textile industry effluents by newly isolated *Leptosphaerulina* sp. from Colombia. *Bioresour. Bioprocess*, 3: 6. *http:/dx. doi/*10.1186/s40643-016-0084-x.

Ponraj, M., Jamunarani, P., & Zambare, V. (2002) "Isolation and Optimization of Culture Conditions for Decolorization of True-Blue Using Dye Decolorizing Fungi'. *Asian Journal Of Experimental Biological Sciences*, 2(2): 270-277.

Priyadarsini, R. I., Bhuvaneswari, V. and Kumar, K.S. (2011) Isolation, Identification and Phylogenetic analysis of White Rot Fungus and Heterologous Expression of Gene Encoding Laccase. *Journal of Applied Sciences in Environmental Sanitation*, 6(1): 69 - 83.

Rashid, A., Bhatti, H.N., Iqbal, M. & Noreen, S. (2016). Fungal biomass composite with bentonite efficiency for nickel and zinc adsorption: a mechanistic study. *Ecological Engineering* 91: 459-471.

Rauf, M.A. & Ashraf, S. S. (2012) Survey of recent trends in biochemically assisted degradation of dyes. Chem. Eng. J., 209: 520-530.

Reiss, R., Ihssen, J. & Thony-meyer, L. (2011) *Bacillus punilus* laccase; a heat stable enzyme with a wide substrate spectrum. *BMC Biotechnol.*,11:9-14. http ://dx.doi.org/ 10.1186/ 1472-6750-11-9.

Robinson, T., McMullan, G., Marchant, R. & Nigam, P. (2001). Remediation of Dyes in Textile Effluent: A Critical Review on Current Treatment Technologies with a Proposed Alternative. *Bioresource Technology*, 77(3): 247-255.

Saeed, M., Adeel, S., Ilyas, M., Shahzad, M.A., Usman, M., Haq, E.-U. & Hamayun, M. (2016). Oxidative degradation of Methyl Orange catalyzed by lab prepared nickel hydroxide in aqueous medium. *Desalinat. Water Treatment*, 57: 12804-12813.

Shahid, A., Singh, J., Bisht, S., Teotia, P. & Kumar, V. (2013) Biodegradation of Textile Dyes by Fungi Isolated from North Indian Field Soil. *Environment Asia*, 6(2): 51-57.

Sharma, M. K. & Sobti, R.C. (2000) Rec. effect of certain textile dyes in *Bacillus subtilis*, Mutant. *Research*, 465: 27–38.

Singh, N. & Abraham, J. (2013) Isolation of laccase producing fungus from compost soil and partial characterization of laccase. *Advances in Applied Science Research*, 4(5): 91-98.

Srivastava, S., Sinha, R. & Roy, D. (2004) Toxicological effects of malachite green. *Aquatic Toxicoogy*, 66: 319-329.

Tamayo-Ramos, J.A., van Berkel, W.J. & de Graaff, L.H. (2012) Biocatalytic potential of laccase-like multicopper oxidases from *Aspergillus niger*. *Microb. Cell Fact.*, 11: 165. http://dx.doi: 10.1186/1475-2859-11-165 PMID:23270588.

Tana, L., Li, H., Ning, S. & Xu, B. (2014) Aerobic decolorization and degradation of azo dyes by suspended growing cells and immobilized cells of a newly isolated yeast *Magnusiomyces ingens* LH-F1, *Bioresour. Technology*, 158: 321-28.

Vilar, V. Botelho, C. & Boaventura, R. (2007) "Methylene blue adsorption by algal biomass-based materials: Biosorbents characterization and process behavior," *Journal of Hazardous Materials*, 147: 120-132.

Vyrides, I., Bonakdarpour, B. & Stuckey, D.C. (2014). Salinity effects on biodegradation of reactive black 5 for one stage and two stages sequential anaerobic aerobic biological processes employing different anaerobic sludge. *International Biodeterior. Biodegrad*ation, 95: 294-300.

Wang, N., Chu, Y., Wu, F., Zhao, Z. & Xu, X. (2017). Decolorization and degradation of Congo red by a newly isolated white rot fungus, *Ceriporia lacerata*, from decayed mulberry branches. *International Biodeterioration and Biodegradation*, 117: 236-244.

Zhang, C., Zhang, S., Diao, H., Zhao, H., Zhu, X., Lu, F., *et al*. (2013)

Purification and characterization of a temperature-and pH-stable laccase from the spores of *Bacillus vallismortis* fmb-103 and its application in thedegradation of malachite green. *Journal of Agriculture and Food Chemistry*, 61: 5468–5473. doi:10.1021/jf4010498PMID:23706133.

Zilly, A., Souza, C.G.M., Barbosa-Tessmann, I.P. & Peralta, R.M. (2002) Decolorization of industrial dyes by a Brazilian strain of *Pleurotus pulmonarius* producing laccase as the sole phenol-oxidizing enzyme. *Folia Microbiology*, 47: 273–277. CrossRef