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Bioremediation of toxic metal ions from coal washery effluent

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ABSTRACT

This investigation has dealt with bioremediation of heavy metal ions from the coal washery effluent (CWE) by *Pleurotus florida* (*P. florida*). The CWE was characterized as per the guidelines of American Standard and Testing Methods. The surface characterization of *P. florida* was done by scanning electron microscopy, Fourier-transform infrared spectroscopy, and energy-dispersive X-ray analysis. Metal ion concentration in fruit body and substrate (paddy straw) was determined by inductively coupled plasma optical emission spectrometry. The fitness functions of exponential and linear extension growth models were evaluated mathematically (goodness of fit) at boundary conditions at $t = 0$ and $t = t$. The metal toxicity stress markers produced in *P. florida* (control and CWE exposed) were estimated through standard protocols described in various references (which have been described later in the manuscript). The physicochemical characterization of CWE showed its acidic nature (pH 5.1 ± 0.26) together with contamination of toxic heavy metals like Pb, Cr, Cd, Zn, As, Mn, Ni, and Ti. The surface characterization of *P. florida* showed that the surface of *P. florida* was rough, heterogeneous in nature together with negatively charged functional groups like carboxyl, hydroxyl, ketonic, and esters. The elemental composition of *P. florida* revealed the abundance of carbon and oxygen in biomass compared to other elements such as nitrogen and phosphorus. Growth modeling of *P. florida* revealed the fact that experimental and theoretical values of linear extension growth rate K_r (mm d^{-1}) constant were not only close to each other but also they ranged from 1.36 to 2.21 mm d^{-1} and 1.41 to 2.03 mm d^{-1}. This showed the supremacy of the linear growth extension model over the exponential rate model. The metal toxicity stress markers like metallothionein (35.21 μg g^{-1}), superoxide dismutase (9.4 U mg^{-1}), lipid peroxidase (2.5 nmol mg^{-1}), catalase (2.15 Pkat mg^{-1}) and reduced glutathione (28.09 μg g^{-1}) had higher level expression in the fruit bodies of *P. florida* grown in CWE compared to control. The simultaneous increase of metallothionein concentration from 2.50 to 35.21 μg g^{-1} with an increase in the concentration of metal ions in the term of CWE concentration (25%–100%) in media showed the bioaccumulation of metal ions in the intracellular space. The maximum heavy metal uptake capacity of *P. florida* was found as 10.65, 1.12, 13.46, 3.0, 1.21, 19.11, 0.47, and 0.23 μg g^{-1} for Pb, Cr, Cd, Zn, As, Mn, Ni, and Ti respectively. The maximum heavy metal removal from substrate (paddy straw) was found 99.53% (Cd), 70.85% (Cr), 77.77% (Ni), 76.23% (Zn), 42.63% (Mn), 52.10% (Pb), 49.07% (Ti), and 51.66% (As). The negatively charged rough surface, high amount of carbon and oxygen in biomass, and the induced production of intracellular metal stress markers on exposure to heavy metals illustrated the immense bioaccumulation ability of *P. florida* in CWE.

Keywords: Bioremediation; Toxic metals; *Pleurotus florida*; Coal washery effluent, Antioxidant activity

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1. Introduction

Coal is one of the major sources of energy in India as well as in other developing countries due to its low cost and easy availability. Cleaning coal generates a lot of toxic chemicals, dust, and other hazardous materials that pollute the environment. Coal washery effluent (CWE) is directly discharged into various water bodies like rivers, ponds, lakes, etc. Effluents containing heavy metal ions are considered to be non-biodegradable as well as persistent in the environment as a toxic pollutant. One of the major problems with CWE is that it contains a large number of toxic heavy metal ions like lead, chromium, arsenic, manganese, iron, cobalt, aluminum, nickel, and copper, etc. These heavy metal ions deteriorate the quality of water and cause several diseases like cancer, Minamata, pulmonary edema, loss of vision, a neurological and nephrological disorder in humans [1].

Therefore, it is important to remove these toxic metal ions before discharging into the natural water sources. Various conventional methods such as membrane filtration, chemical precipitation, electrodialysis, reverse osmosis, and electrochemical precipitation have been used for the removal of toxic metal ions from the domestic and industrial wastewaters [2]. These methods are not cost-effective at large scale, leads to the generation of secondary chemical sludge [3] and are less effective when less concentration of heavy metal is present in the effluent [4].

On the contrary, bioremediation is an eco-friendly, specific, and cost-effective technique that is involved in converting/removing harmful organic/inorganic pollutants into the less toxic form [5]. Bioremediation methods such as bioleaching, biological stabilization, animal remediation, composting, phytoextraction, phytotransformation, phytostimulation, phytostabilization, phytovolatization, rhizofiltration, and microbial and fungal bioremediation have been used tremendously for the removal of heavy metal ions from contaminated sites [6].

Among the above-mentioned techniques, fungal bioremediation is more advantageous compared to other methods [6,7]. The fungus can adapt easily in their surrounding environment and are capable to decompose organic/inorganic materials under natural conditions [8]. They can be cultivated under highly stressed conditions such as extreme pH, temperature, and salt concentration [9]. Macro-fungi such as white-rot fungi have the ability to uptake massive amounts of toxic metal ions in their fruit bodies, this property of fungi (mushroom) makes them appropriate for extraction of heavy metal ions from the contaminated sites [10].

Pleurotus spp. of mushrooms (macro-fungi) is more advantageous in terms of heavy metal removal compared to other mushroom species. Hence, *Pleurotus* spp. has been considered superior for the treatment of contaminated water and soil [11,12]. Additionally, *Pleurotus* spp. has a unique quality of effortless cultivation on various types of solid substrates (biomasses) under extreme environmental conditions. They are a good source of proteins, vitamins, minerals, nutrients, and also have many therapeutic applications like immune-stimulatory, anti-inflammatory, anti-oxidant, and anti-cancerous [13–16].

Apart from this, the cell wall components of mushroom play important role in the heavy metal removal due to the presence of heavy metal-binding sites and these active binding sites are responsible for the accumulation of metal ions in intracellular space [17]. The waste materials such as agricultural waste and plant residues containing cellulose, hemicelluloses, and lignin are fragmented by extracellular enzymes of *Pleurotus florida* (*P. florida*) species like lignin peroxidase, cellulase, and laccase. These enzymatically degraded materials are taken up by the fungal cell followed by intracellular digestion by the enzymatic system. The aforementioned enzymatic system in spite of its structural complexity and heterogeneity has shown that this organization is non-specific in nature, which is also responsible for the degradation of a variety of toxic compounds including polycyclic aromatics, polychlorinated biphenyls and dioxins [7,18].

An intracellular enzymatic system such as metallothionein (metal-binding proteins), superoxide dismutase (SOD), lipid peroxidase, reduced glutathione (GSH) and catalase of *Pleurotus* species play important role in the intracellular accumulation of toxic metal ions within the cell [19]. These intracellularly enzymatic systems of *P. florida* is responsible for counteracting reactive oxygen species (ROS) and minimizing metal toxicity. The activity of these enzymes increases linearly with heavy metal exposure with cells. Therefore, these enzymes are needful in minimizing the heavy metal toxicity and helpful in enhancing the accumulation of heavy metals in the intracellular space [20]. Toxic metallic components present in the mushroom substrate also interact with the extracellular enzymes and enter into the fungal cell in different ways such as diffusion, through outer cell transporter such as phosphate transporters, sulfate transporter, etc. The uptake of metals from the liquid environment is the simplest situation. *Pleurotus* species can bio-accumulate heavy metal ions from the substrate in their mycelia [21]. The detailed mechanisms of heavy metal interaction with fungal mycelium have been explained in Fig. 1. Fig. 1 shows the schematics of metal ion removal in the term of bioaccumulation by mushroom mycelia.

- *Surface interaction of heavy metal during bioaccumulation*: functional groups present on the surface of the fungal cell namely OH^-, $-PO^{3-}$, $-SO^{2-}$, and $-NH_2$ interact with the metal ions and expedite the uptake of these metal ions into the cell. A diverse complex process like ion-exchange, adsorption, crystallization, reduction, and precipitation is involved in the interaction of heavy metal ions with fungal cells. These mechanisms are also influenced by metallic concentration in the medium, physiochemical behavior of metals, and types of media used for fungal growth [22,23].
- *Metal uptake*: membrane transporters of the fungal cell are responsible for the uptake of essential metal ions. These transport systems (nonspecific type) also interact with other non-essential metals ions and uptake them into the cell [23,24].
- *Mobilization of heavy metal ions*: fungal cells produce diverse groups of chemicals such as citric acid and oxalic acids and these chemicals are responsible for the mobilization process of metal ions. Citric acid works as

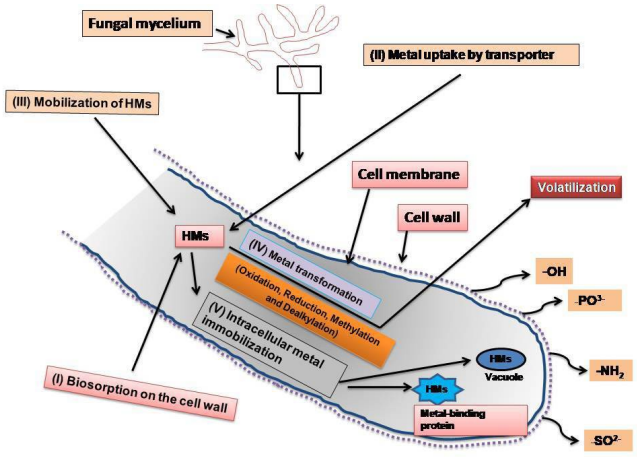


Fig. 1. The schematic diagram represents the mechanisms of uptake and accumulation of heavy metals within *P. florida* cells.

a chelating agent which forms a complex with metallic components and oxalic acid forms insoluble product known as oxalate [22,23].

- *Metal transformations*: biotransformation reactions occur inside the fungal cell. Biotransformation reactions involve mechanisms like methylation, oxidation, and reduction. These mechanisms also reduce the toxicity of heavy metal ions [23,24].
- *Heavy metal immobilization within cells*: immobilization of heavy metal ions generally follows two processes, which are mediated by cytoplasmic protein and compartments of vacuoles. The cytoplasmic proteins which are involved in the immobilizations are metallothionein, a –SH rich peptide. Vacuole have an important role in the storage of secondary metabolites and also regulates the intracellular metallic concentration. Metallothionein can bind with metal ions and minimize the toxic effects of metals on the fungal cell [23,24]. These mechanisms make *P. florida* a suitable option for bioremediation of heavy metal ions from industrial effluent.

Several types of research have been done on the bioremediation efficiency of *Pleurotus* species. Arbanah et al. [25] performed the bioremediation experiment on the *Pleurotus ostreatus* (*P. ostreatus*) in the liquid broth medium containing 2.9 mg L^{-1} of hexavalent chromium. The authors reported 20.17% removal of hexavalent chromium from the laboratory wastewater. Vaseem et al. [12] also reported that *P. ostreatus* has excellent ability of bioremediating heavy metals from CWE. The authors performed bioremediation study on the agar plate medium containing Mn (7.3 mg L^{-1}), Zn (2.23 mg L^{-1}), Ni (0.89 mg L^{-1}), Cu (1.17 mg L^{-1}), Co (1.75 mg L^{-1}), Cr (0.56 mg L^{-1}), Fe (13.4 mg L^{-1}), and Pb (22.7 mg L^{-1}) and reported that mycelium of *P. ostreatus* can reduce the 57.2% Mn, 82.6% Zn, 98.0% Ni, 99.9% Cu, 99.3% Co, 99.1% Cr, 89.2% Fe, and 35.6% Pb from solid agar medium. Wu et al. [26] performed the removal of Mn and phenanthrene by *Pleurotus eryngii* in the potato liquid medium. The maximal removal of Mn and phenanthrene was obtained as 92.17% and 93.85% respectively with 0.6 gL^{-1} Tween 80. Melgar et al. [27] performed the bioremediation of Cu and Pb using *Agaricus macrosporus* in the liquid broth medium. The authors observed that *Agaricus macrosporus* was capable of uptake 76.64 mg g^{-1} (Cd), 68.80 mg g^{-1} (Hg), 72.78 mg g^{-1} (Pb) and 77.09 mg g^{-1} (Cu). Besides, the heavy metal removal property of *Pleurotus* sp. has emerging applications in the removal of dyes from wastewater which originates from textiles, coating, and construction industries [28,29]. Several types of research has been done to evaluate the removal efficiency of dye using *Pleurotus* sp. [30–33]. Kaur et al. [34] carried out the experiments on the removal of amido black 10B in potato dextrose broth (PDB). The authors reported that *P. florida* removed 80.19% amido black 10B dye from PDB using enzymes like laccase, manganese dependent peroxidase, and lignin peroxidase. Kunjadia et al. [35] estimated the level of ligninolytic enzymes (ligninolytic enzyme play a proactive role in the degradation of azo dyes in aqueous phase) in mushroom (*Pleurotus* spp.) grown in yeast dextrose broth medium containing coralene golden yellow, coralene navy blue and coralene dark red azo dyes, coralene navy blue and coralene dark red azo dyes. The authors reported an enhanced level of ligninolytic enzymes in the *Pleurotus* spp. exposed to dyes. Kuzhali et al. [36] executed a batch experiment on dye decolorization by *P. florida* and *Calocybe indica* in the liquid phase. The authors estimated the maximum decolorization of textile dye as 64.15% and 35.44% by *P. florida* and *Calocybe indica*, respectively. Bhattacharya et al. [37] did a batch study on the degradation of carcinogenic dye benzo[a]pyrene supplemented with 5 mM concentration of heavy metal salt in liquid broth medium. Authors reported 71.1% of benzo[a] pyrene degradation by enzymes namely laccase and manganese peroxidase of *P. ostreatus* in the presence of copper ions.

It is worth mentioning here that all these above-mentioned studies on mushroom mediated bioremediation of heavy metal and dyes were either performed on the Petri plates (solid well-defined medium) or in liquid phase comprising of liquid growth medium. Practically, these methods involve high capital as growth media cost is very high; hence these techniques cannot be scaled up to the commercial level. Additionally, these experiments do not mimic the conditions of real wastewater as most of the aforesaid investigations have utilized well-defined medium (solid or liquid) for the growth of mushrooms. Furthermore, the above-mentioned schemes are unable to treat bioremediation of heavy metal ions and dyes from solid substrate medium such as contaminated soil or a disposable solid waste. Besides these limitations, another drawback of these studies was the involvement of mycelial growth which was exploited for bioremediation and degradation of heavy metal ions and dyes. Gigantic mycelial growth is complicated to handle and consumes a bulky amount of growth medium during the remediation process.

With the above-mentioned facts, the present investigation aimed at bioremediation of heavy metal ions from CWE containing solid substrate medium (paddy straw) by *P. florida*. The present investigation is applicable to the removal of heavy metal ions present in the solid waste like agricultural residues, industrial solid waste, and contaminated soil sites. Another focus of this study is the development of eco-friendly-cum-cost effective bioremediation

of heavy metal ions from solid waste (in the present work, *P. florida* has been grown on the solid substrate (paddy straw) without any specific growth media). In the side-lines of the work, the physicochemical analysis of CWE such as pH, temperature, biological oxygen demand (BOD), electrical conductivity, dissolved oxygen and the concentration of heavy metals together with growth kinetic modeling and quantification of stress markers such as metallothionein, SOD, lipid peroxidase, reduced GSH and catalase activity has also been elucidated.

2. Materials and methods

2.1. Collection and characterization of CWE

Wastewater sample was collected from the effluent treatment plant of Northern Coalfields Limited, Kakri Project, Sonbhadra District, Uttar Pradesh, India. The CWE was collected in the month of December 2018. The temperature of the effluent was around 20°C. The color of the CWE was black and samples were collected in the dark amber coated bottles. The wastewater sample was stored as per the guidelines of the American Public Health Association (APHA), USA.

Physicochemical characterization and heavy metal analysis of CWE was carried out as per APHA standard methods. pH, electrical conductivity, and turbidity were measured by digital pH-cum-conductivity meter and nephelometer (Thermo Scientific make, Singapore). Total solids, dissolved solids, and suspended solids were analyzed using hot air oven (Equitron Medica Private Limited make, India) and furnace (Narang Scientific Work Pvt. Limited make, India) at various temperatures. BOD_5 and dissolved oxygen were also determined using the standard APHA method. Phosphate, ammonia, and nitrate concentration were determined using standard methods. The concentration of heavy metal was determined by using inductively coupled plasma optical emission spectrometry (ICP-OES), Perkin Elmer, Optima 7000 DV instrument make, USA.

2.2. Collection and preparation of the substrate for mushroom cultivation

Paddy straw waste was purchased from the local market, near the Sheer gate of Banaras Hindu University (BHU), Varanasi, India. The paddy straw was chopped into two to three inches size. The chopped paddy straw was soaked into hot water and left for 1 h for sterilization. After sterilization, the excess water was removed from the paddy straw.

2.3. Experimental design for bioremediation of CWE

2.3.1. Spawn preparation

Spawns were prepared in the glass bottles. The wheat grains were boiled for 35 min in the glass beaker of 2 L capacity. After boiling the excess water was discarded through filtration. Thereafter, 2% of calcium carbonate and 0.5% of calcium sulfate powder (on the dry weight basis) were mixed with the boiled wheat grains. The wheat grains were transferred to the glass bottles and autoclaved for 15 min at 121°C. The autoclaved grains were allowed to cool at room temperature and were inoculated with the active mycelium of *P. florida* grown on from potato dextrose agar (PDA) plate inside the laminar flow unit. Inoculated wheat grains were incubated at 27°C ± 2°C in a dark place for 15 d until the proper mycelium growth covered the grains. These mycelial covered grains were called as spawn and were used as inoculum for bioremediation.

2.3.2. Bioremediation of CWE

The range of process parameters studied in the present work was as follows: concentration of CWE: 0% to 100% (intermittent interval of 25% each) and growth period: 20–40 d (intermittent interval of 5 d each). Other than these, other parameters such as temperature (27°C ± 2°C), pH of CWE (7.5 ± 0.2) and dosage of spawn (30 mg g^{-1}) were kept constant throughout the study.

The CWE was filtered using Whatman filter paper No. 42 and also autoclaved to remove the microbial and other solid particle contamination. The various concentrations (100% CWE, 75% CWE, 50% CWE, 25% CWE, and 0% CWE) of CWE were prepared in double-distilled water. The 0% CWE contained 100% double distilled water and was taken as control. All dilutions were prepared in the triplet for further experiments. Sterilized paddy straw was dipped into the sterile CWE and double distilled water (control) in the separate beakers for 24 h.

After CWE treatment, the excess water was removed from the paddy straw and the straw was packed in polyethylene bags. Each polyethylene bag contained 500 g of paddy straw. 30 g of spawn was added to every bag. Finally, the inoculated bags were stored at optimized conditions (27°C ± 2°C) in the sterile box made up of perspex sheet (2.5 m × 1.5 m × 0.5 m; thickness of the sheet is 10 mm) and was incubated at optimum growth conditions (temperature (27°C ± 2°C), pH of CWE (7.5 ± 0.2) and dosage of spawn (30 mg g^{-1}). The lid of the box was movable (sliding) and to maintain the moisture content during the growth of *P. florida* over paddy straw the lid was removed every alternate day and then double-distilled water was sprayed on the surface of polyethylene bags. After spraying the lid was again closed. The box was kept in dark in an air-conditioned atmosphere.

The growth of *P. florida* was observed till the end of the 40th day and samples of fruits bodies were collected at 20th, 25th, 30th, 35th, and 40th day from every dilution of CWE including control. The paddy straw substrate samples were also collected at zero-days and after bioremediation. No growth was recorded at the beginning of the 41st day. However, degradation of the cell mass was observed from the 40th day onwards. The samples of paddy straw and fruit body were collected till the end of the 40th day and samples were stored at –20°C.

2.4. Analysis of metallothionein concentration in P. florida fruit body grown in CWE containing substrate

The concentration of metallothionein in the fruit bodies was analyzed using the standard method of Griffith [38]. 600 mg of mushroom fruit bodies were homogenized in sulphosalicylic acid. The homogenized samples were

pellet down at 12,000 × g for 20 mins at 4°C and supernatants were recovered. Two separate solutions were prepared (solution A and B), the compositions of solution A was 100 mM sodium monohydrogen phosphate heptahydrate, 15 mM ethylenediaminetetraacetic acid (EDTA), 1.8 mM 5,5-dithiobis(2-nitrobenzoic acid) and 0.04% bovine serum albumin and solution B of 50 mM imidazole, 1 mM EDTA, 0.2% (w/v). The pH of both solutions was adjusted to 7.2. The metallothionein fraction in the supernatant was determined using 800 µl solution A, 640 µl of solution B, and 800 µl supernatant. The concentration of metallothionein in solution was determined at 412 nm.

2.5. Analysis of antioxidant enzymatic system in P. florida grown in CWE containing substrate

Antioxidant enzymes such as GSH, SOD, lipid peroxidase, and catalase were analyzed using standard methods described by Moron et al. [39], Das et al. [40], Ohkawa et al. [41], and Aebi [42]. The fruit bodies of mushrooms were homogenized in chilled 0.1 M phosphate saline buffer using mortar pistil. The homogenized samples were centrifuged at 10,000 rpm for 10 min. The supernatant was again centrifuged at 10,000 rpm for 10 min and the supernatant obtained was stored at –80°C.

GSH was estimated using the method described by Moron et al. [39]. The GSH was allowed to react with 5, 5-dithiobis nitrobenzoic acid which generates yellow-colored compound. The absorbance was measured at 412 nm. The SOD activity was measured using the standard method of Das et al. [40]. The lipid peroxidase was measured by the method described by Ohkawa et al. [41]. The catalase activity was estimated using the method suggested by Aebi [42]. All antioxidant enzymes were measured in the *P. florida* fruit bodies cultivated in CWE containing medium and also in control. The analysis was performed in mushroom samples collected from 0%, 25%, 50%, 75%, and 100% CWE containing medium from 20th to 40th days.

2.6. Growth modeling of P. florida in CWE

Autoclaved PDA media containing CWE in various concentrations (0%, 25%, 50%, and 100% of CWE) was dispensed into Petri plates of size 9 cm. Approximately 35–40 ml of sterile PDA was dispensed on each plate. The spores of *P. florida* were inoculated in the center of plates. The lids of the plate were closed and sealed with parafilm tapes and the lids were stroked with radii which crossed the inoculum site (ten radii were marked). To prevent desiccation of plates, routinely plates were packed in polyethylene bags and incubated at 28°C in dark in a BOD incubator (REMI Laboratory Instruments make, India). The mycelia growth of *P. florida* was marked under a compound microscope (10×) with a marker at every 1 to 2 d. At the completion of growth, the marks were measured from the point of inoculation with a ruler having a precision of 1 mm. The peripheral growth (P_i; in mm) was measured according to Trinci [43].

Growth kinetics calculation of *P. florida*: growth fitness functions shown in Eqs. (1) and (2) has been used in the present work to elucidate the growth kinetics of *P. florida* [44].

$$\beta = \beta_0 \exp^{\alpha.t} \tag{1}$$

$$\beta = \beta_0 + K_r \cdot t \tag{2}$$

where α and K_r are growth rates expressed as the growth rate (d^{-1}) and growth per day (mm d^{-1}), respectively. β and β_0 represent the growth at time t and time $t = 0$. For growth kinetics integration, the real values of t (at β) were substituted in linear functions rather than intercepts (t at β_0). The weight of these growth fitness functions was determined (Table 2) by comparing the experimental value of β (at time t) with the theoretical value which was calculated by the fitting of data approximately closure to the value of β.

2.7. Fourier-transform infrared spectroscopy analysis of P. florida fruit body

The Fourier-transform infrared spectroscopy (FTIR) spectra of both mushroom samples *P. florida* exposed CWE and *P. florida* grown in the distilled water were analyzed. The samples were prepared using the standard method suggested by Xu et al. [45] and Das and Guha [46]. The sample of mushroom fruit bodies was mixed with the photometric grade potassium bromide (KBr) in a ratio of 1:3. The spectra were analyzed between the range of 400–4,000 cm^{-1} (wavenumber). The FTIR spectra were analyzed using the JASCO-6300 FTIR instrument make, Japan.

2.8. Scanning electron microscopy–Energy-dispersive X-ray analysis

Scanning electron microscopy (SEM) (ZEISS EVO, Carl Zeiss Microscopy make, Germany) was performed to study the surface morphology of *P. florida* mushroom. The gold layer was coated on the sample. The gold coated sample was placed in the SEM equipment chamber and images were taken at a voltage of 20 keV. Energy-dispersive X-ray analysis (EDX) was used for the elemental analysis and this technique worked along with SEM analysis. The energy of the electron beam was kept in the range between 10–20 keV.

2.9. Analysis of heavy metal ions

Samples of paddy straw and fruit bodies were dried at 120°C in a hot air oven. Dried samples were powdered and 1 g of each sample was digested with diacid solution of nitric acid (HNO_3) and perchloric acid ($HClO_4$). The digestion process was also performed on a hot plate until the complete solubility of the sample was attained. After the acid digestion, the samples were diluted with doubled distilled water and heavy metal analysis was performed by ICP-OES.

The percentage of heavy metal removal from the paddy straw substrate was calculated using the following equation:

$$\text{Percentage removal}\left(\%\right) = \frac{\left(C_0 - C_e\right)}{C_0} \times 100 \tag{3}$$

where C_0 and C_e are the initial and final concentration of the heavy metal µg g^{-1} in the *P. florida* substrate medium.

2.10. Statistical data analysis

All experiments were carried out in the triplet (n = 3) and the average values (±standard deviation) of the data were used to plot the graphs. The experimental errors were also calculated and have been shown as error bars in graphs.

3. Results and discussion

3.1. Physiochemical characterization of CWE

The physiochemical analysis showed that CWE was heavily polluted (Table 1) with toxic heavy metals. The pH of CWE was acidic in nature. The acidic nature of CWE was due to the presence of various acids such as HCl, HNO_3, and sulfuric acid (H_2SO_4) used during the washing of coal [47]. The concentration of suspended solid was very high; it was much higher than the permissible limit of the United States Environmental Protection Agency (USEPA) [48] and World Health Organization (WHO) [49]. These suspended particles deposit on the bottom of the rivers and create disturbance in the movement and growth of the aquatic organisms. The suspended particles also affect the breeding of aquatic organisms, including an increase in mortality rate in fishes [50–52]. The nitrate and phosphate concentrations were much higher in the CWE. Higher concentrations of nitrate and phosphate play a major role in the development of algal bloom and which decreases the dissolved oxygen concentration in the water. Algal bloom enhances the production of ammonia and CO_2 which causes the death of aquatic organisms [53]. Heavy metals like nickel,

Table 1
Characterization of coal washery effluent

Parameters	CWE	WHO [49]	USEPA [49]
Temperature (°C)	26.8°C ± 0.40	25	40
pH	5.1 ± 0.26	6–99	5–9
Total solids (mg L^{-1})	5,353 ± 419	1,500	–
Total suspended solid (mg L^{-1})	731 ± 159	–	–
Turbidity (NTU)	26.9 ± 1.51	–	–
Phosphate (mg L^{-1})	36.1 ± 2.42	–	1
Nitrate (mg L^{-1})	29.1 ± 3.78	50	10
NH_3 (mg L^{-1})	58.1 ± 2.12	1.50	1
Electrical conductivity (μS cm^{-1})	1.2 ± 0.31	–	–
Alkalinity (mg L^{-1})	55.1 ± 4.05	–	–
Free CO_2 (mg L^{-1})	25.1 ± 4.69	–	–
Oxygen (mg L^{-1})	2.0 ± 0.29	–	–
BOD (mg L^{-1})	66.3 ± 4.03	–	–
Ni (mg L^{-1})	6.89 ± 0.34	0.07	0.10
Zn (mg L^{-1})	4.89 ± 0.59	0.05	2
Mn (mg L^{-1})	15.6 ± 3.8	–	0.20
Cd (mg L^{-1})	38.1 ± 5.98	0.003	0.01
Pb (mg L^{-1})	39.9 ± 4.12	0.01	0.10
Ti (mg L^{-1})	0.59 ± 0.11	0.05	–
Cr (mg L^{-1})	2.5 ± 0.12	0.05	0.05
As (mg L^{-1})	0.62 ± 0.29	0.01	0.05

Table 2
Metals present in paddy straw (μg g^{-1}) before and after bioremediation

Metal	Before bioremediation	After bioremediation	Percentage removal
Cr	1.75	0.51	70.85
Ni	0.90	0.20	77.77
Zn	2.15	0.51	76.23
Mn	62.16	35.66	42.63
Cd	19.25	0.09	99.53
Pb	25.48	12.20	52.10
Ti	0.30	0.11	49.07
As	0.31	0.15	51.66

zinc, magnesium, cadmium, lead, arsenic, and titanium in the CWE were analyzed. These heavy metal ions in CWE were present in higher concentrations than the permissible limit of USEPA [48] and WHO [49].

3.2. Bioaccumulation of heavy metals in the P. florida fruit bodies

Figs. 2a–h shows the heavy metal accumulation efficiency of *P. florida* exposed to various dilution of CWE (25%, 50%, 75%, and 100% CWE) at the time interval (20th, 25th, 30th, 35th, and 40th days). The heavy metal concentration in control was found less compared to CWE exposed mushroom. The concentration of heavy metals was observed as constant. The accumulation of Pb, Cd, Cr, Zn, Ni, Mn, Ti, and As (Figs. 2a–h) in the mushroom fruit bodies increased with the exposure time period and was found maximum at 40th days in all CWE concentration. The minimum concentration of heavy metals was found in the 20th day in all CWE exposed mediums.

The accumulation of Cd, Pb, Zn, Cr, and Mn in the fruit body of mushroom was more than the other metals. The accumulation of Cd, Pb, Zn, Cr, and Mn in *P. florida* grown in the CWE medium was found up to 13.46, 10.65, 3.0, 1.2, and 19.11 μg g^{-1} respectively. The hyperaccumulation of heavy metal was due to the reason that higher concentration of these metals was a higher concentration of metals in the growth medium was not too toxic for the growth of *P. florida* or these metal ions frequently bind with the intracellular metal-binding proteins and other biomolecules. Baldrian and Gabriel [54] mentioned that there was hyper-accumulation of Zn metal ions in the cells of *Ganoderma lucidum* in comparison to mercury, cadmium, copper, uranium, lead, and manganese. The rationale behind this hyperaccumulation was the non-toxic nature of Zn ion [55].

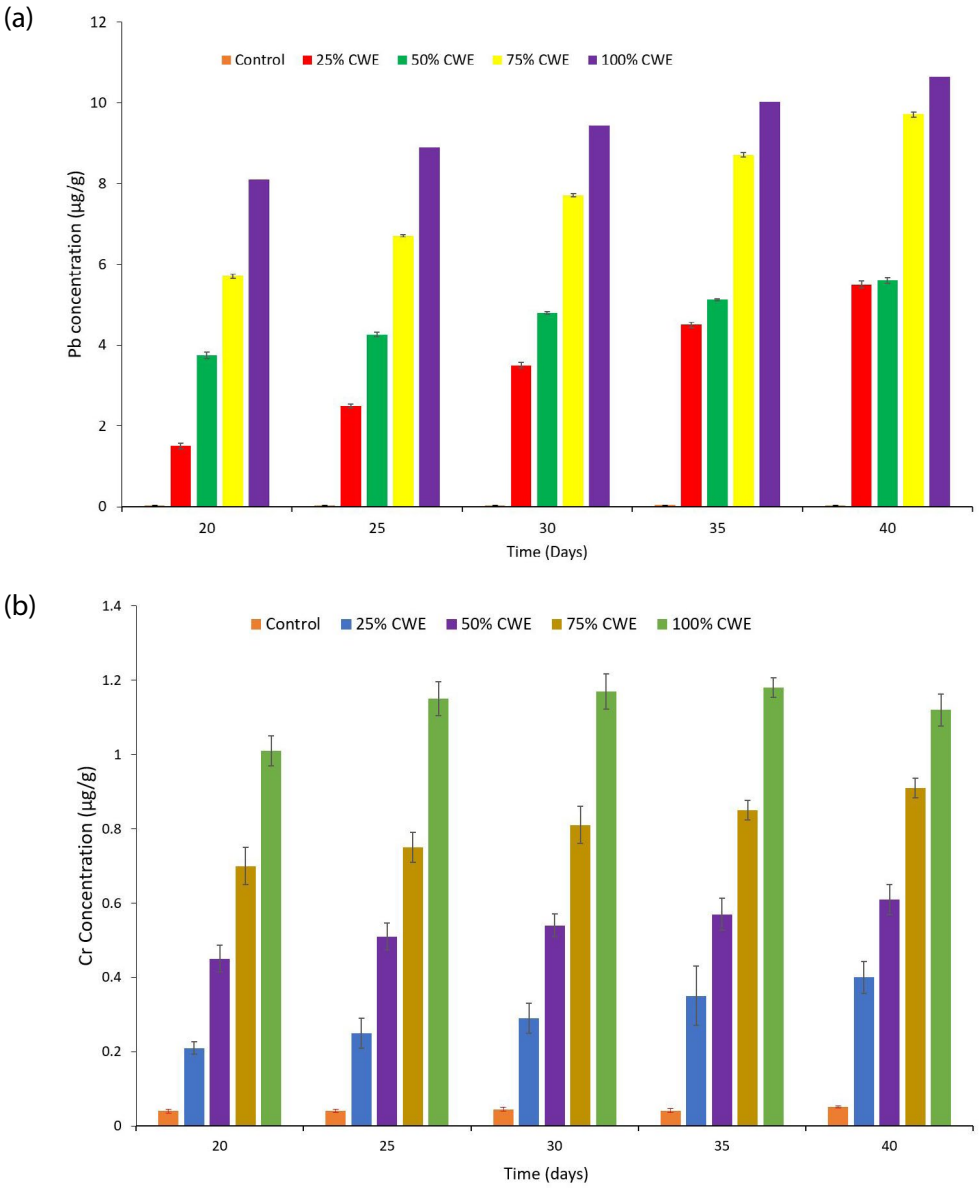
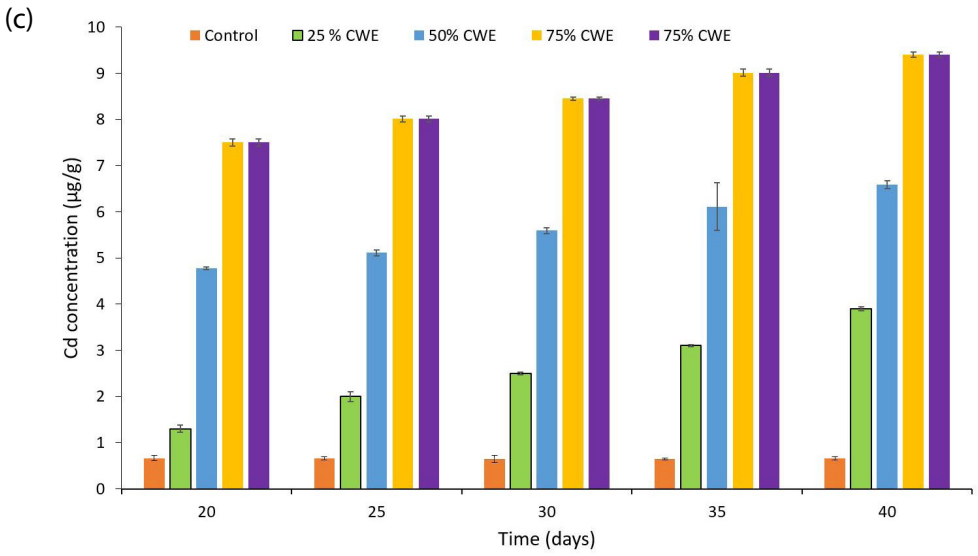
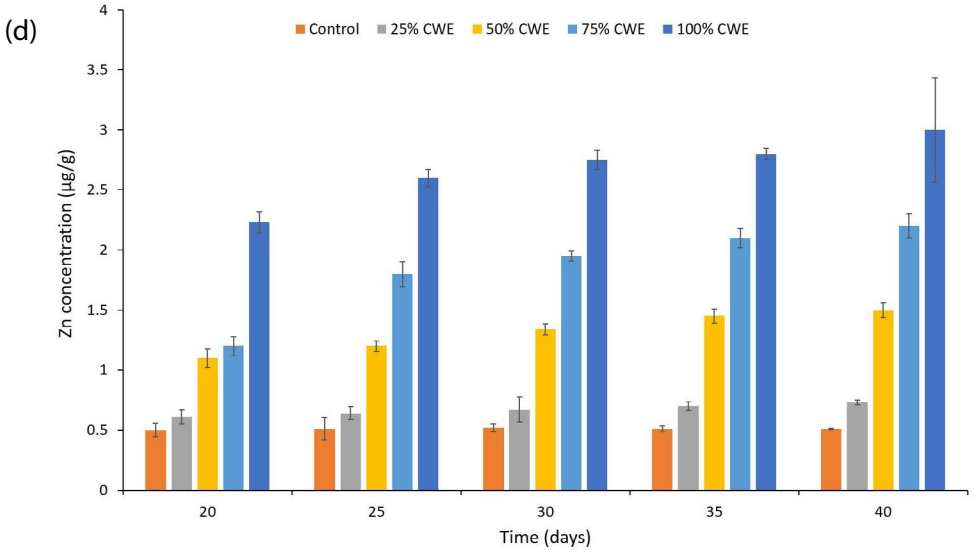


Fig. 2. *(Continued)*





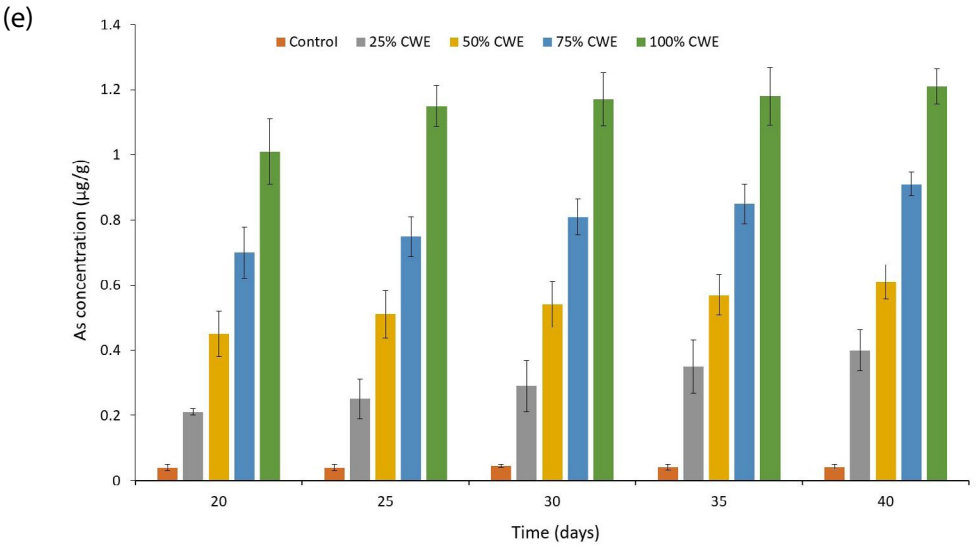


Fig. 2. *(Continued)*

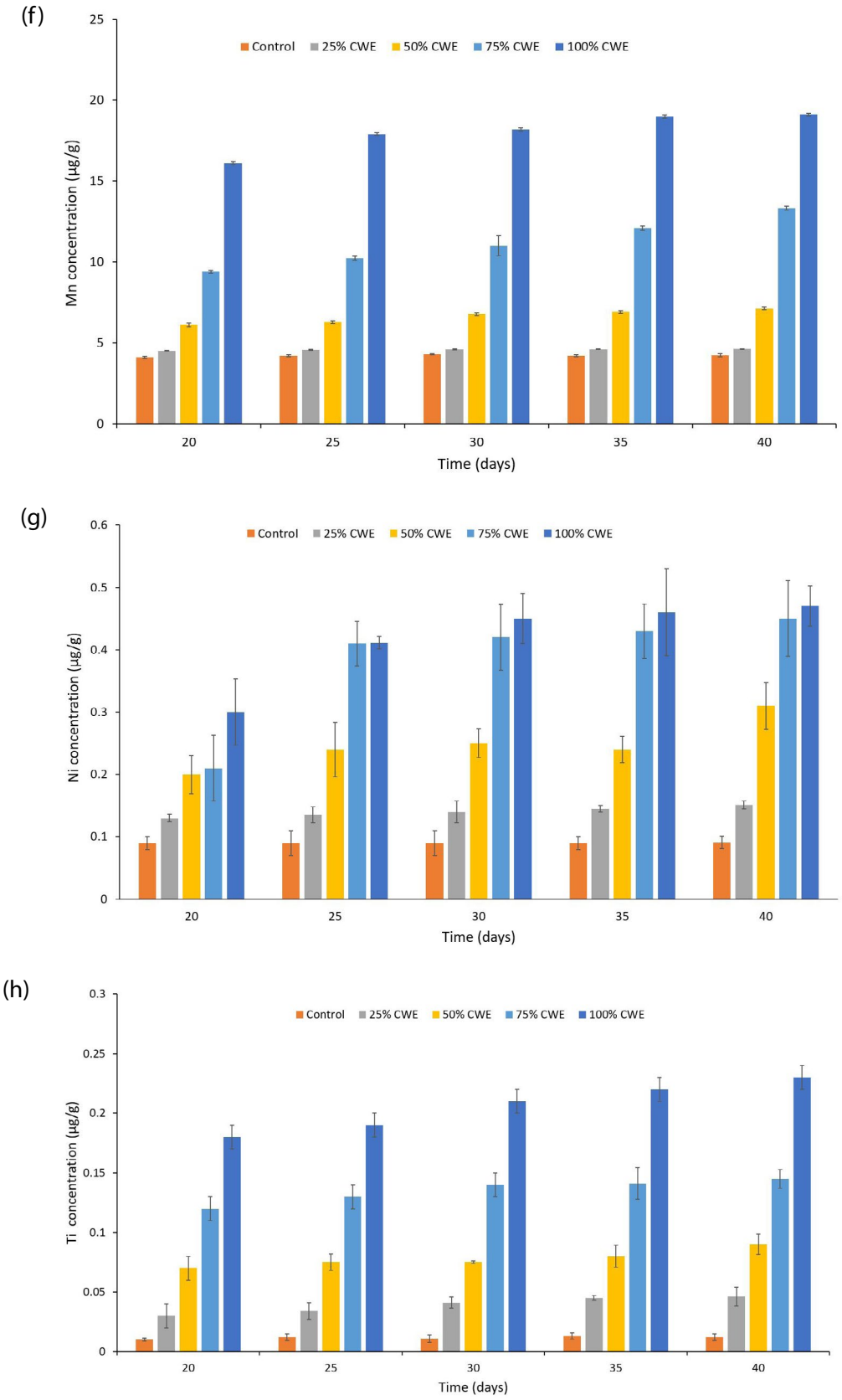


Fig. 2. (a) Pb, (b) Cr, (c) Cd, (d) Zn, (e) As, (f) Mn, (g) Ni, and (h) Cr concentrations in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at a various interval of time.

Vaseem et al. [12] have also reported a similar kind of results while performing the bio-extraction of heavy metal ions from CWE by *P. ostreatus*. An increase in the heavy metal concentration in the medium not only affected the mycelium efficiency for metal accumulation but also influenced the growth of mushroom and its metabolic activities. Many researchers have also reported that the capability of accumulation of heavy metal ions in an organisms/plant depends upon the concentration of heavy metal ions present in its growth environment [56–59]. Faria et al. [60] performed the bioaccumulation study of lithium in *P. ostreatus* at various concentrations of initial metal ions. The authors reported that the highest bioaccumulation of lithium was 1575.29 μg g^{-1} in the intracellular space of mycelia. Almeida et al. [61] investigated the iron bioaccumulation efficiency of *P. ostreatus*. The authors performed the bioremediation experiment in the presence of 150 mg L^{-1} iron in the growth medium inoculated with *P. ostreatus* and authors reported that the maximum iron uptake capacity of mushroom was 3,500 μg g^{-1}. Li et al. [62] reported the role of *P. ostreatus* HAU-2 in the removal of heavy metal ions such as Cd and Cr from synthetic simulated wastewater. The maximum uptake capacity of *P. ostreatus* HAU-2 was found 15.6 mg kg^{-1} (Cd) and 8.9 mg kg^{-1} for Cr.

3.3. Removal of heavy metals from the substrate (paddy straw) using P. florida

Table 2 shows the detailed analysis of heavy metal concentration before and after the inoculation of *P. florida* cells. It became evident from Table 2 that there was a decrease in the concentration of heavy metal ions in the substrate after the inoculation phase.

Before the inoculation of *P. florida* the maximum concentration of heavy metals was observed in the paddy straw but after inoculation and at the end of mycoremediation process lower concentration of heavy metal ions was observed. Among all the heavy metals, maximum removal Cd, Cr, Ni, Zn, and Pb was observed in the paddy straw. Cd, Cr, Ni, Zn, and Pb were remediated up to 99.53%, 70%, 77.77%, 76.23%, and 52.10%, respectively. Vaseem et al. [12] performed the bioremediation of CWE by *P. ostreatus*. Authors reported that at the end of the 20th day maximum heavy metals such as Ni, Zn Cr, and Pb were removed up to 98%, 82%, 99.1%, and 73%, respectively. García et al. [50] performed the bio-extraction of toxic metal ions such as Cd, Ni, Cu, Pb, Hg, and Zn using the viable mycelia of *Agaricus macrosporus* grown on wheat grains. The results showed that there was a substantial decrease in the metal ion concentrations at the end of 34th and 50th day. Li et al. [62] investigated the role of *P. ostreatus* HAU-2 in metal detoxification. The authors performed the Cr and Cd bioremediation study and observed that the maximum Cd and Cr removal from the liquid medium was 44.85% to 80.36% and 14.49% to 45.55%, respectively. Bibi et al. [63] isolated Cr resistant endophytes from the soil and identified as *Aspergillus fumigatus*, *Rhizopus* sp., *Penicillium radicum*, and *Fusarium proliferatum*. The authors reported that isolated fungal species play an important role in the removal of Cr(VI) from contaminated soil. 95% Cr(VI) contamination was reduced by using endophytic fungi. Albert et al. [64] performed the bioremediation study of three metal ions (Cd, Cu, and Pb) from liquid broth medium by using fungus *Absidia cylindrospora*. The authors reported that *Absidia cylindrospora* was capable to reduce 68% (Cd), 59% (Pb), and 14% (Cu) after 3 d of incubation at an initial metal ion concentration of 50 mg L^{-1}.

3.4. Growth modeling of P. florida in CWE

At the onset of the growth phase in control (0% CWE with PDA), it was observed that the growth of mycelium sectors is time-consuming and smooth. At a later stage, the rapid growth of sectors was observed. At the completion of growth, thick and feathery mycelia structure consisting of tightly packed hyphae were observed at the periphery of sectors.

After the inoculation in control, the first sector at a distance of 14 mm was observed after an incubation of 8 d. The compactly packed mycelial structure with a feathery look was observed at a distance of 31 mm after 15 d of incubation. In growth media containing CWE from 25% to 100%, a similar pattern of growth was observed. The growth in mm of various sectors at various concentrations of CWE has been shown in Table 3.

The growth of *P. florida* followed a sharp decreasing order with a subsequent increase in the concentration of CWE in PDA (Table 3). The elevation in toxicity due to heavy metal ions present in CWE led to a decrease in the growth of *P. florida*. Validation of toxicity due to metal ions has shown in section 3.5 which is relevant to antioxidant activity (resistance mechanism of *P. florida* against metal ions).

3.4.1. Growth kinetics of P. florida in control and at various concentrations of CWE

Linear and exponential growth kinetics models were evaluated to elucidate the growth kinetics of *P. florida*. The model constants and their derivatives have been shown in Table 4. Linear and exponential growth kinetic functions were fitted in the growth curve models (Table 4). Primarily, data fitting showed that both the exponential growth function and linear growth extension rate decreased with a simultaneous increase in the concentration of CWE in growth media. This trend of growth was dedicated to the enhancement in the level of toxicity with an increase in the concentration of CWE. The disagreement between experimental and theoretical values of the exponential growth function showed that the growth of *P. florida* was not exponential. Contrary to this, the theoretical and experimental values of linear growth rate function were observed in close range with each other which indicated the suitability of linear growth extension rate model in the present investigation. Growth kinetics of *Agaricus bisporus* mycelium on the solid substrate was performed by Straatsma et al. [65]. The authors reported that the exponential growth model had less fit in growth kinetics compared to the linear extension rate model. Zervakis et al. [66] studied the growth kinetics of seven high-quality strains of fungus explicitly *Lentinula edodes*, *P. ostreatus*, *P. energy*, *P. pulmonarius*, *Volvariella volvacea*, *Agrocybe aegerita*, and *Auricularia auricular-judae*. Authors reported that growth kinetics of these fungal strains had better goodness of fit in linear

Table 3
Growth of *P. florida* in various concentration of CWE

Concentration of CWE in PDA (%)	Incubation (d)	First-sector (mm)	Complete growth (feathery sectors) (mm)
25	08	11.5	–
Do	15	–	28.33
50	08	9.5	–
Do	15	–	22.14
75	08	6.5	–
Do	15	–	16.22
100	08	3.32	–
Do	15	–	5.44

Table 4
Study of exponential and linear growth models of *P. florida* in varying environmental conditions

Growth environment	Lag time (d)	Exponential growth function α (exp.) (d^{-1})	Exponential growth function α (Th.) (d^{-1})	Linear growth extension rate K_r (mm d^{-1}) (exp.)	Linear growth extension rate K_r (mm d^{-1}) (Th.)
0% CWE	6.28	1.81	2.94	2.21	2.23
25% CWE	7.36	1.54	2.83	1.89	1.92
50% CWE	9.42	1.49	2.76	1.74	1.79
75% CWE	10.33	1.38	2.62	1.92	1.89
100% CWE	11.21	1.33	2.58	1.36	1.41

extension growth rate model in the range of time period between inoculation of substrate and fructification. Carroad et al. [67] and Zakaria et al. [68] studied the growth kinetics of *P. ostreatus* and *Pleurotus sajor-caju* in complex culture media, PDA, and PDB. Carroad et al. [67] reported that n^{th} power law had a better over the kinetic data. Precisely, the authors observed that the growth rate of fungal cells was directly proportional to the two-thirds power of the cell biomass. Additionally, the authors further observed and reported the suitability of the exponential model in the same environmental conditions which assume that the growth rate is proportional to cell mass. Similarly, Zakaria et al. [68] observed the supremacy of the exponential growth model over the linear function model. The results of the present work were not analogous with the results reported by Carroad et al. [67] and Zakaria et al. [68]. The differences in the results were due to varying growth conditions of fungal cells.

3.5. Bioaccumulation mechanism of heavy metals in the P. florida

Heavy metals are the well know toxic agents in cellular growth, including denaturation of the DNA/RNA and protein [69]. Heavy metals are responsible for the generation of ROS which in turn leads to the disruption of cell organelles [70]. Therefore, only those micro/macroorganisms can be used for bioremediation of heavy metal ions which can tolerate heavy metal stress. The common stress makers expressed in the fungal cells are metallothionein, GSH, SOD, catalase, GSH, and lipid peroxidase [71]. Mehra et al. [72] and Muenger et al. [73] observed that macrofungi have better tolerance as well as bioaccumulation mechanism compared to other microfungi. Understanding the heavy metal uptake mechanism from the solid substrate to the mushroom fruiting bodies can help in the design of waste treatment plants. In the present study, a heavy metal uptake mechanism was described through the estimation of stress marker at various time intervals and various concentrations of CWE exposed *P. florida*. The expression level of stress marker found enhanced (Figs. 3 and 4a–d) in the *P. florida* grown on CWE soaked paddy straw.

3.5.1. Metallothionein concentration in the P. florida

The metallothionein was estimated in the mushroom fruit bodies grown in different concentrations of CWE and control at various intervals of time (20^{th}, 25^{th}, 30^{th}, 35^{th}, and 40^{th} days). Metallothionein is a cysteine-rich metal-binding protein and provides protection against heavy metal toxicity. Metallothionein expression increase when the heavy metal ions are present in excess [74].

It became evident from Fig. 3 that the concentration of metallothionein substantially increased in *P. florida* grown with an increase in the concentration of CWE containing substrate. The concentration of metal-binding protein was also affected by the exposure period of CWE because the maximum concentration of metallothionein was observed on the 40^{th} day in 100% CWE containing medium. The *P. florida* has grown in the double-distilled water expressed a low level of metallothionein concentration and not affected by the exposure period. It remained constant until the end of the 40^{th} day. The enhanced concentration of

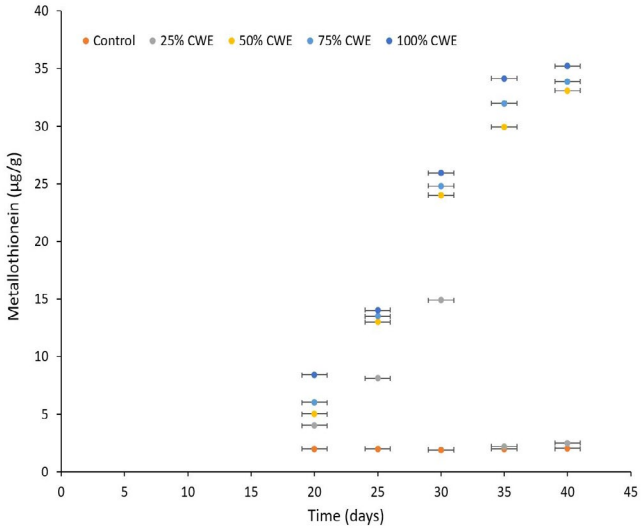


Fig. 3. Metallothionein concentration in the mushroom grown in the various dilutions of CWE containing medium at several intervals of time.

metallothionein showed that immobilization of metal ions in the intracellular space is the key mechanism of bioremediation by *P. florida*. Ramesh et al. [75] reported that the transcription level of metallothionein genes in *Hebeloma cylindrosporum* was induced in the presence of Cu and Cd. Kameo et al. [76] reported similar kinds of results in the fungus *Beauveria bassiana*. The authors reported that the metallothionein production was enhanced in the presence of Cd. Additionally, they also reported the effect of metal exposure mediated metallothionein production in the bacteria. The increased metallothionein expression was also found in the *Bacillus cereus* when exposed to Pb [77].

3.5.2. Antioxidant enzymatic system of P. florida

Heavy metals show many toxic effects such as denaturation of nucleic acid and proteins. The production of ROS significantly increased with increasing the heavy concentration in the growth medium. The production of ROS in the cell causes the generation of oxidative stress and cause dysfunction and tissue damage [78]. The antioxidant enzymes such as GSH play an important role in the various types of stressful situations. GSH reacts with ROS within the cell and protects cell components from oxidative damage [79].

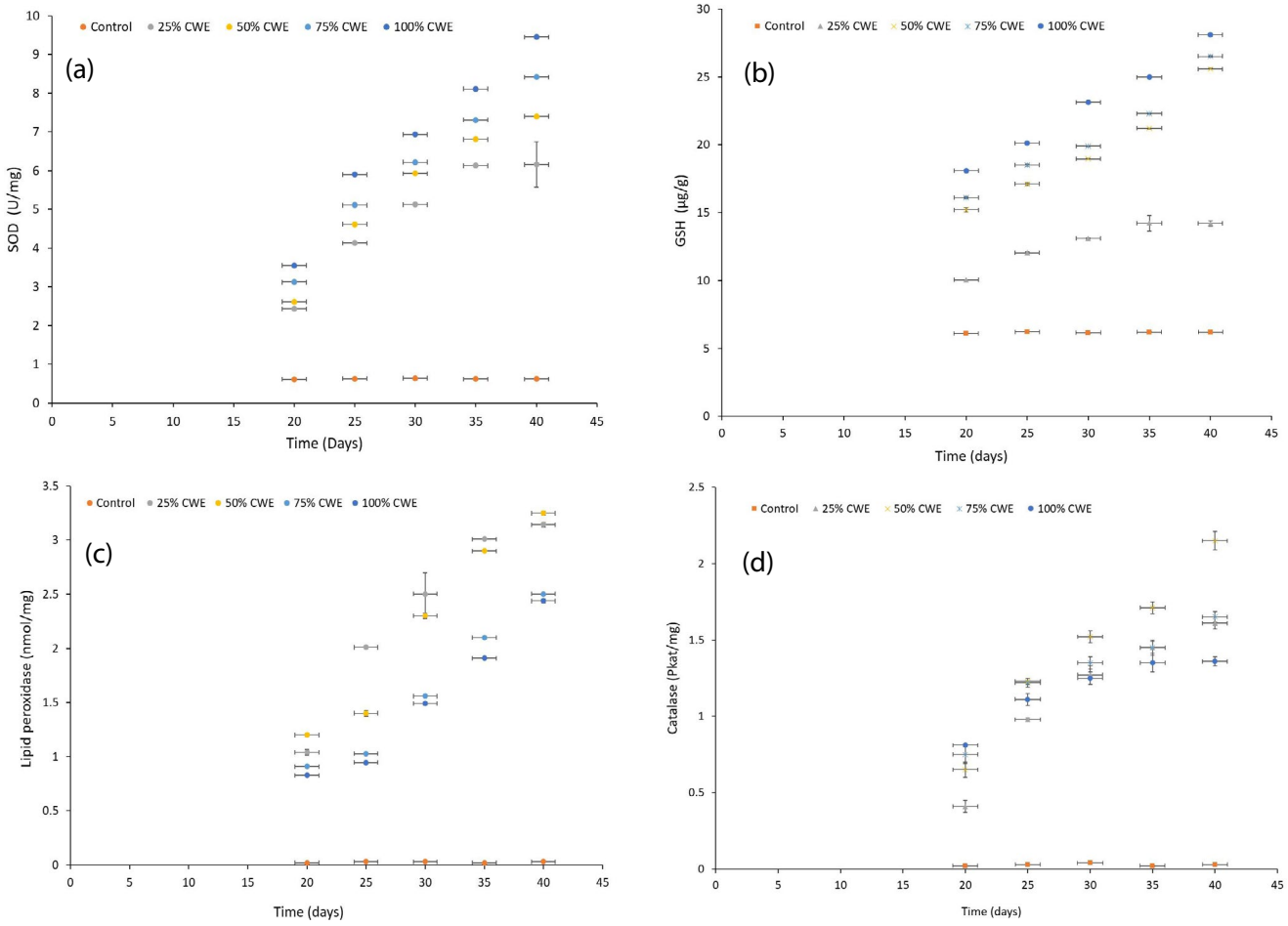


Fig. 4. (a) Superoxide dismutase (SOD), (b) glutathione, (c) lipid peroxidase, and (d) catalase concentration in the mushroom grown in the various dilutions of CWE containing medium at several intervals of time.

The concentration of antioxidant enzymes such as SOD, catalase, GSH, and lipid peroxidase increased with an increase in the concentration CWE in the growth medium (Figs. 4a–d).

The antioxidant enzyme concentration was measured in 25%, 50%, 75%, 100% CWE containing medium, and control. The antioxidant enzymes concentrations were also measured at various days like 20th days, 25th days, 30th days, 35th days, and 40th days. Fig. 4a shows the SOD activity in the CWE containing medium and control. The control shows the constant concentration of SOD for several days. The 100% CWE showed more SOD activity compared to other concentrations of CWE. The catalase activity was also increased with the increase in the concentration of CWE from 25%–100% CWE and time from 20 to 40th day. The other two enzymes (Figs. 4a–d) showed similar behavior with respect to the increasing concentration of CWE and time.

Lazarova et al. [78] explained the antioxidant mechanism in the *Trichosporon cutaneum* R57. The authors reported that the increase in Cd, Cr(VI) and Cu concentration in the growth medium was responsible for more expression of SOD and catalase. Arojojoye and Adeosun [80] reported the antioxidant enzyme production in the fishes. The authors reported that the GSH, SOD, catalase, and lipid peroxidase concentration was significantly increased with the increase in the concentration of heavy metals. Courbot et al. [81] reported the oxidative stress of Cd in the fungi *Paxillus involutus*. The authors observed that heavy metal exposure was responsible for the production of antioxidant enzymes such as GSH. It was further observed that the intracellular detoxification mechanism for Cd in the fungal cell was due to an increase in the production of GSH in the metal stress condition. Ye et al. [82] performed the fungal bioremediation of Pb and investigated the mechanism of metal ion bioaccumulation in the intracellular space of *Penicillium oxalicum* SL2. The authors reported that GSH plays a major role in the intracellular accumulation of heavy metals such as Pb. The authors estimated the concentration of GSH in the control (without Pb) as 12.7 μmol L^{-1} and observed an increase of 49.3-fold in the concentration of GSH when *Penicillium oxalicum* SL2 was exposed to Pb.

Wu et al. [26] performed the mycoremediation of manganese by *Pleurotus eryngii* and estimated the expression of SOD in the Mn exposed and non-Mn exposed *Pleurotus eryngii*. The authors reported that the SOD concentration reached 27.6 U mg^{-1} in the 3 mM of Mn exposed *Pleurotus eryngii*. The SOD concentration in the control was found very less (0.4 U mg^{-1}) compared to Mn exposed *Pleurotus eryngii*. Hashem et al. [83] reported that the expression of antioxidant enzyme lipid peroxidase increased in the Cd exposed *Cassia italica*. The enhanced expression of lipid peroxidase represented the bioaccumulation of Cd in the intracellular space of *Cassia italica*. Luna et al. [84] performed work on the Cu induced adaptation and antioxidant activity *Aspergillus niger*. The authors reported that about 50% catalase activity increased in *Aspergillus niger* grown in 1 mM of Cu.

3.5.3. FTIR analysis of P. florida

During the process of bioremediation, many chemical bonds break and form on the surface of fungi. This bond formation and disruption process is possibly explained by the FTIR analysis of CWE exposed *P. florida*. The FTIR peaks of *P. florida* grown in control and CWE treated substrate were observed. The obtained FTIR spectra explained the interactions of the heavy metals with the cell biomolecules of *P. florida* during the bioaccumulation process. The peaks generated in FTIR analysis and their respective functional group's shift has been clearly explained in Table 5. The changes in the FTIR spectra in the *P. florida* exposed to CWE have been shown in Figs. 5a and b. It became clear from Figs. 5a and b that the arrangement of peaks changed in CWE exposed *P. florida*.

The spectra showed in Fig. 4a contain many peaks specifically, broad peak at 3,404 cm^{-1} represents –NH and –OH stretching of amine and hydroxyl groups, peak at 2,924 cm^{-1} represents aliphatic C–H groups, peaks at 1,374 and 1,312 represent C–H stretching, peak at 1,645 cm^{-1} represents C=C groups, peaks at 1,043; 1,079; 1,150 and 1,203 cm^{-1} represent C–O and C–N groups of aliphatic compounds [85,86].

Fig. 5b contains a few different peaks from Fig. 5a which were at 2,097; 1,401 and 538 cm^{-1}, which represent C≡C

Table 5
Functional groups present in the *P. florida* (before and after bioremediation)

Wave number (cm^{-1})		Functional groups
Before bioremediation	After bioremediation	
3,404	3,422	–NH and –OH stretching of amine and hydroxyl groups
2,924	2,925	Aliphatic C–H groups
–	2,097	C≡C (stretching)
1,645	1,640	C≡C groups of carboxylic, aldehyde, ketones and esters
–	1,401	C–H deformation in alkaline
1,374 and 1,312	–	C–H groups in aromatic compounds
1,203	–	C–O groups in esters, C–N groups in amines
1,150	–	C–O groups in alcohols and esters, C–N groups in amines
1,043	1,079	C–O groups in alcohols and esters, C–N groups in amines
890	–	Aromatic out-of-plane ring bends
–	538	Plane deformation

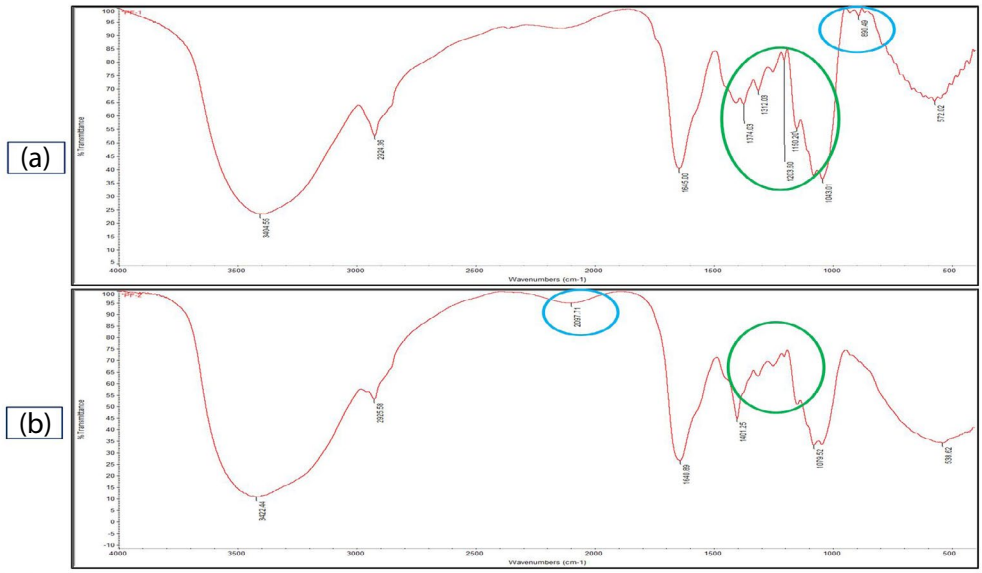


Fig. 5. (a) Schematic diagram represents the FTIR analysis of *P. florida* grown in control and (b) CWE containing substrate.

groups of aromatic ring bends, C–H deformation in alkaline, and plane deformation, respectively. The comparison between FTIR spectra of before and after bioremediation of *P. florida* suggested that functional groups of *P. florida* were involved in covalent interaction with heavy metal ions during the accumulation process [85–87].

On the basis of the FTIR shift in the spectra, it became evident that the metal ions interacted with surface functional groups of *P. florida*. The FTIR also suggested that various types of pretentious and non-pretentious factors were produced in the CWE exposed *P. florida* [12]. These factors played an important role in the binding of heavy metals and protect cells from metal toxicity. These factors also promote the accumulation of metal ions within *P. florida* [12]. This study suggested that a variety of functional groups present on the surface of *P. florida* were involved in the covalent binding with heavy metal ions [86,88].

3.5.4. SEM and EDX analysis

Fig. 6 shows the surface morphology of *P. florida* grown in double-distilled water and CWE containing substrate. Figs. 6a–d represents the SEM micrograph of the control and Figs. 6e–h show the SEM micrograph of CWE exposed *P. florida*. Figs. 6a–d show that the surface of *P. florida* is mostly porous and irregular in shape. Figs. 6e–h show that the surface of *P. florida* was smooth and non-porous after bioremediation. The surface of CWE exposed *P. florida* was found smooth and nonporous because the adsorption of heavy metal ions occurred on the surface of *P. florida* [85,87]. Saha et al. [86] performed the hexavalent chromium removal by *Citrus limetta* peel powder and reported that the porous and rough biosorbent surface became smooth and non-porous after adsorption of metal. Srivastava and Thakur [89] reported morphological changes in the *Acinetobacter* sp. after the accumulation of chromium within the cell. The authors observed the smooth surface of bacteria after chromium exposure. They suggested that the chromium uniformly bound on the bacterial surface which was responsible for morphological changes in the bacterial surface. This was evidence of the adsorption of metal ions on the surface of *P. florida*.

EDX spectra (Fig. 7) show the elemental contents in the sample. The carbon, oxygen, and potassium content were considerably higher in the *P. florida* in comparison to other elements. The EDX analysis of *P. florida* does not show the presence of phosphorus and nitrogen. The absence of nitrogen and phosphorus and the presence of carbon and oxygen in the *P. florida* has been considered as a good quality in biosorbent for removing heavy metals from wastewater [87]. Shrestha [87] also reported that the higher concentration of carbon and oxygen components in the *Pinus densiflora* pine cones powder provided a good adsorption capacity for sodium dodecyl sulfate. Michalak et al. [90] performed the bioremediation of metal ions on the microalgae and suggested that the elemental components of the cell wall play an important role in the bioremediation of heavy metal ions. Above mentioned facts supported that *P. florida* may be considered as a good option of bioremediation of heavy metal ions from CWE.

3.6. Effect of initial heavy metal concentration including other wastewater components on the bioremediation efficiency and growth of P. florida

Figs. 8a–e shows the growth of *P. florida* in control and CWE soaked paddy straw. *P. florida* was grown on the paddy straw waste containing various dilutions (0%, 25%, 50%, 75%, and 100%) of CWE. CWE contained various components such as suspended solids, total solids, nitrate, phosphate, and heavy metal ions such as Pb, Cd, Ni, Zn, Mn, As, Ni, and Cr. Separately, the *P. florida* was also grown in fresh double distilled water as a controlled study. Fig. 8a shows polyethylene bags containing spawn inoculated in

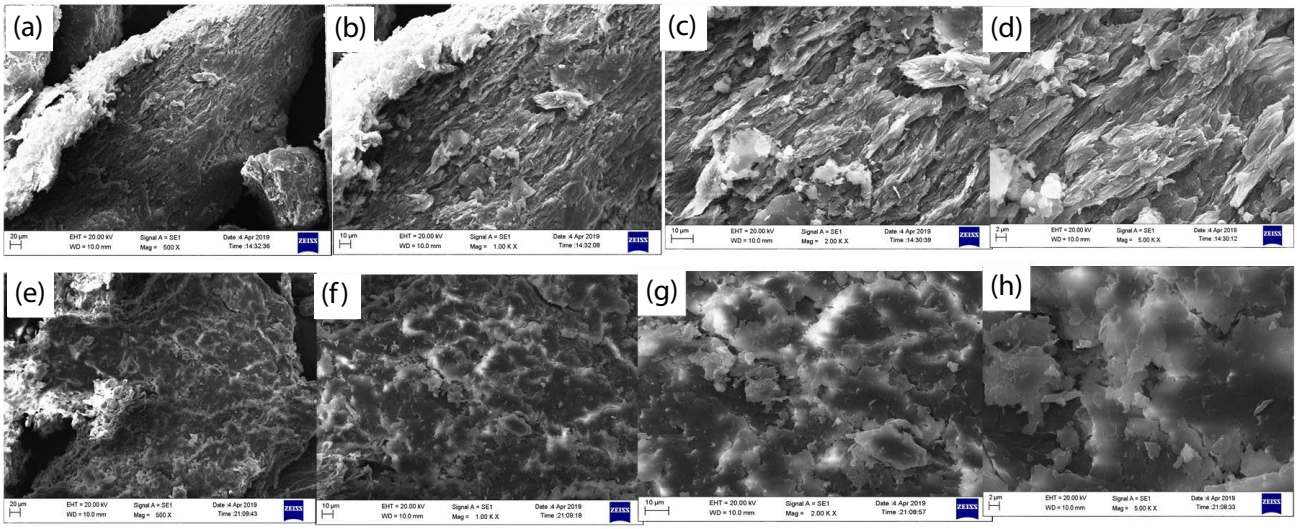


Fig. 6. (a-d) shows SEM micrograph cultivated in control and SEM micrograph (e-h) of *P. florida* (cultivated in CWE).

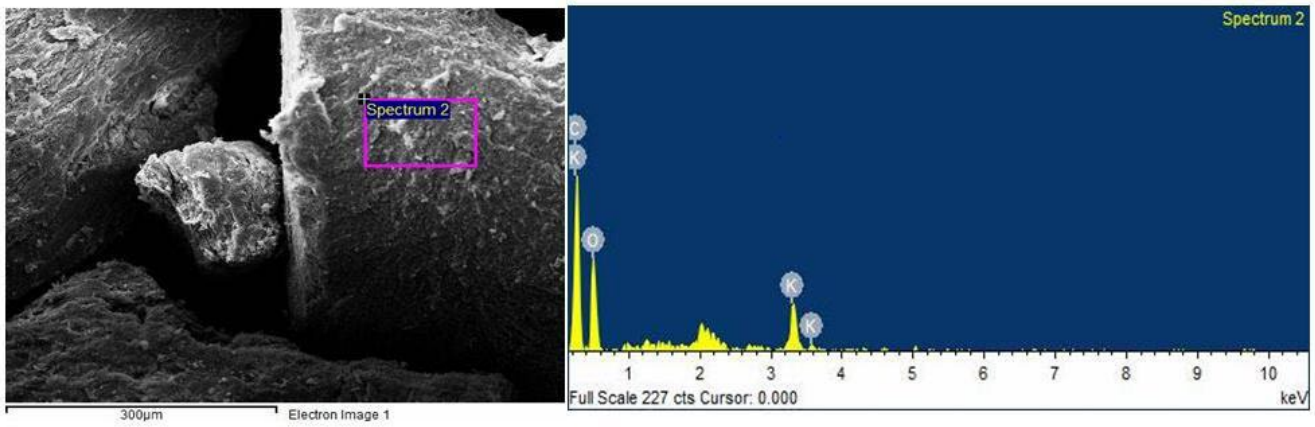


Fig. 7. EDX spectra of *P. florida* mushroom.

control and 100% CWE soaked paddy straw on the 0th day. Figs. 8b and c show the growth of *P. florida* in control and Figs. 8d and e show the growth of *P. florida* in the 100% CWE at the end of the 40th day. It became evident from Figs. 8c and e that there was no apparent variation in the growth of *P. florida* grown in CWE socked paddy straw or in control.

The bioremediation efficiency of the *P. florida* in the term of heavy metal uptake from paddy straw was also studied. The heavy metal concentration such as Pb, Cd, Ni, Zn, Mn, As, Ni, and Cr increased with the increase in the concentration of CWE from 0% to 100%. The maximum uptake capacity was observed when *P. florida* was grown on paddy straw soaked in 100% CWE (Figs. 2a–h). This outcome indicated that the bioaccumulation of heavy metal in the intracellular space depends on the presence of the initial concentration of a metal ion in the growth medium. The presence of other wastewater components such as suspended solid dissolved solids, nitrate, and phosphate did not significantly affect the bioremediation efficiency of *P. florida*.

3.7. Comparison of removal efficiency in terms of heavy metal ions by P. florida and other fungal species

A comparative study of various fungal cells in terms of their bioremediation efficiency has been shown in Table 6. It became evident from Table 6 that viable mycelia cells of *P. florida* have the tremendous and extensive potential of metal ion removal especially cadmium from CWE compared to the other fungal species. The extraordinary metal removal efficiency of *P. florida* is due to its surface morphological structure, functional groups present on the cell surface, and production of intracellular metal-binding proteins such as metallothionein in a metal-contaminated environment.

4. Conclusion

The objective of the present investigation was to study the bioremediation of toxic metal ions present in

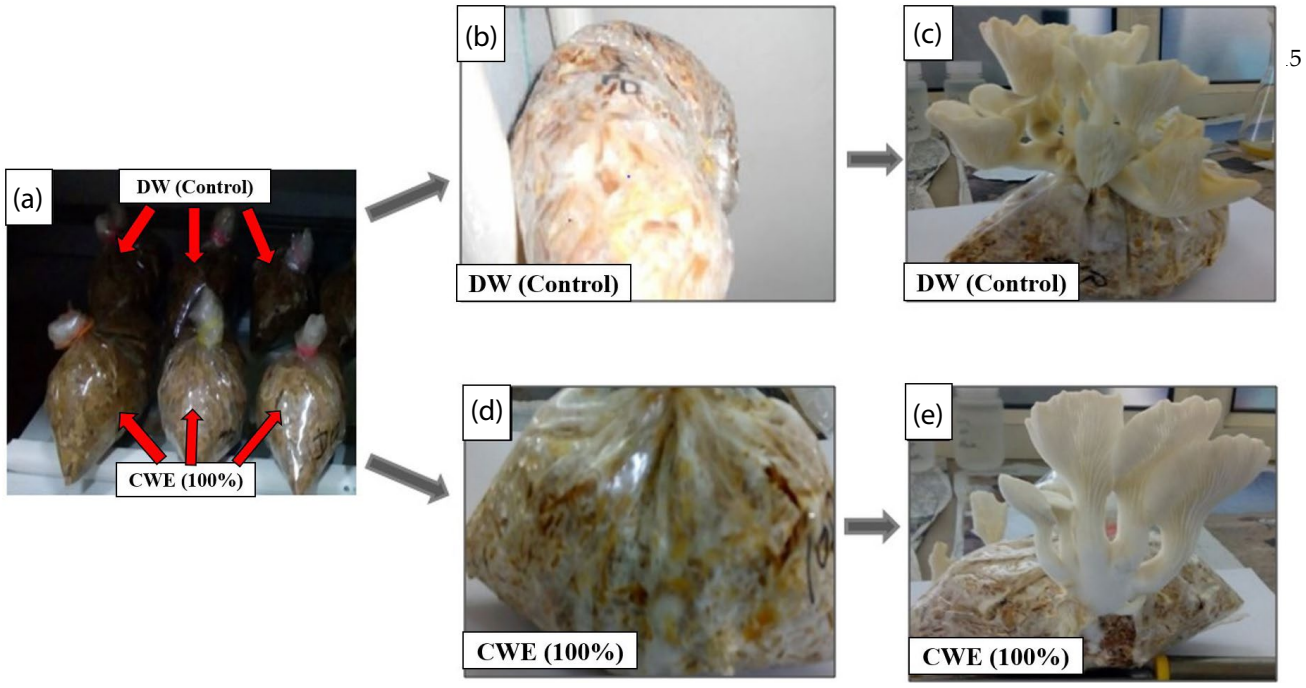


Fig. 8. Growth of *P. florida* in the control and 100% CWE containing paddy straw.

Table 6
The heavy metal removal efficiency of *P. florida* and other fungal species

Microorganism	Metal	Initial metal ion concentration	Removal efficiency (%)	References
Absidia cylindrospora	Pb	50 mg L^{-1}	59.00	[64]
Agaricus macrospores	Pb	10 mg kg^{-1}	2.30	[50]
Pleurotus ostreatus	Pb	45.4 mg L^{-1}	35.60	[12]
Pseudochlorococcum typicum	Pb	20 mg L^{-1}	86.00	[91]
Porphyra leucosticta	Pb	10 mg L^{-1}	90.00	[92]
Drechslera hawaiiensis	Pb	90 mg L^{-1}	99.26	[93]
Pleurotus florida	Pb	25.48 μg g^{-1}	52.10	This study
Absidia cylindrospora	Cd	50 mg L^{-1}	68.00	[64]
Agaricus macrospores	Cd	10 mg kg^{-1}	13.00	[50]
Pseudochlorococcum typicum	Cd	20 mg L^{-1}	70.00	[92]
Porphyra leucosticta	Cd	10 mg L^{-1}	70.00	[92]
Drechslera hawaiiensis	Cd	30 mg L^{-1}	99.26	[93]
Pleurotus florida	Cd	19.25 μg g^{-1}	99.84	This study
Pleurotus ostreatus	Cr	1.12 mg L^{-1}	99.10	[12]
Fusarium oxysporum	Cr	350 mg L^{-1}	95.00	[94]
Aspergillus flavus	Cr	25 mg L^{-1}	95.80	[95]
Aspergillus niger	Cr	50 mg L^{-1}	48.70	[96]
Pleurotus florida	Cr	1.75 μg g^{-1}	70.85	This study
Pleurotus ostreatus	Ni	1.79 mg L^{-1}	99.90	[12]
Rhizopus arrhizus	Ni	100 mg L^{-1}	40.50	[97]
Trichoderma harzianum	Ni	50 mg L^{-1}	90.20	[98]
Pleurotus florida	Ni	0.98 μg g^{-1}	77.77	This study
Pleurotus ostreatus	Mn	14.6 mg L^{-1}	57.20	[12]
Pleurotus florida	Mn	62.16 μg g^{-1}	42.63	This study
Pleurotus ostreatus	Zn	4.46 mg L^{-1}	82.60	[12]
Trichoderma viride	Zn	30 mg L^{-1}	54.33	[2]
Beauveria bassiana	Zn	200 μg g^{-1}	0.64	[99]
Rhodotorula mucilaginosa	Zn	200 μg g^{-1}	2.05	[99]
Agaricus macrosporus	Zn	20 μg g^{-1}	13	[50]
Pleurotus florida	Zn	2.15 μg g^{-1}	76.23	This study

CWE by the *P. florida* species. Based on experiments, it was observed that the concentration of toxic heavy metals in the paddy straw when *P. florida* was allowed to grow on the substrate. The study of growth kinetics of *P. florida* in CWE suggested that the linear growth model had better goodness of fit over the exponential growth model. The surface characterization of *P. florida* revealed that the surface of *P. florida* was rough and heterogeneous together with negatively charged functional groups like hydroxyl, esters, ketones, and carboxylic groups. Metal toxicity stresses markers namely metallothionein, SOD, GSH, catalase, and lipid peroxidase were present in higher concentrations in the fruit bodies of *P. florida* grown in CWE compared to the control. The enhanced concentration of metal toxicity stress markers revealed the intracellular accumulation of heavy metal ions from CWE in the fruit bodies of *P. florida*. Viable cells of *P. florida* has the ability to remediate toxic metal ions from the contaminated sites. In the present investigation, bioremediation of toxic metal ions was through the intracellular accumulation of metal ions in *P. florida*.

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