CHAPTER 8

Bioremediation of toxic metal ions from coal washery effluent by *Pleurotus florida*

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8.1 Introduction

Coal is one the major source of energy in India as well as in other developing countries due to its low cost and easy availability. Cleaning of coal generates a lot of toxic chemicals, dust and other hazardous materials which pollute 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 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, neurological and nephrological disorder in humans [Ghose, 1999].

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, electro-dialysis, reverse osmosis and electrochemical precipitation have been used for the removal of toxic metal ions from the domestic and industrial wastewaters [Ali and Hashe, 2007]. These methods are not cost-effective at large scale, leads to generation of secondary chemical sludge [Ahalya, 2003] and are less effective when less concentration of heavy metal is present in the effluent [Wang and Chen, 2006].

On the contrary, bioremediation is an eco-friendly, specific and cost-effective technique which is involved in converting/removing of harmful organic/ inorganic pollutants into less toxic form [Igiri et al., 2018]. 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 [Sharma et al., 2018].

Among above mentioned techniques, fungal bioremediation is more advantageous compared to other methods [Sharma et al., 2018; Kapahi and Sachdeva, 2017]. The fungus can adopt easily in their surrounding environment and are capable to decompose organic/ inorganic materials under natural condition [Archana and Jaitly, 2015]. They can be cultivated under highly stressed condition such as extreme pH, temperature and salt concentration [Hamba and Tamiru, 2016]. Macro-fungi such white rot fungi have ability to uptake massive amount of toxic metal ions in their fruit bodies, this property of fungi (*Pleurotus* spp. of mushrooms) makes them appropriate for extraction of heavy metal ions from the contaminated sites [Ogbo and Okhuoya, 2011].

Pleurotus spp. of mushrooms (macro-fungi) is more advantageous in terms of heavy metal removal as compared to other mushroom species. Hence, *Pleurotus* spp. has been considered superior for treatment of contaminated water and soil [Zheng et al., 2007; Vaseem et al., 2017]. Additionally, *Pleurotus* spp. has a unique quality of effortless cultivation on various types of solid substrates (biomasses) under extreme environmental conditions.

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 physiochemical analysis

of CWE such as pH, temperature, BOD, electrical conductivity, dissolved oxygen and the concentration of heavy metals together with growth kinetic modelling and quantification of stress markers such as metallothionein, superoxide peroxidase (SOD), lipid peroxidase, reduced glutathione (GSH) and catalase activity has also been elucidated.

8.2.0 Physiochemical characterization of CWE

The physiochemical analysis showed that CWE was heavily polluted (as shown in Table 8.1) with toxic heavy metals.

Table 8.1 Characterization of CWE

Parameters	**CWE**	**WHO (2008)**	**USEPA (2003)**
Temperature (°C)	26.8°C ± 0.40	25	40
pH	5.1 ± 0.26	6 – 9	5 – 9
Total solids (mg/l)	5353 ± 419	1500	--
Total suspended solid (mg/l)	731 ± 159	--	--
Turbidity (NTU)	26.9 ± 1.51	--	--
Phosphate (mg/l)	36.1 ± 2.42	--	1
Nitrate (mg/l)	29.1 ± 3.78	50	10
NH_3 (mg/l)	58.1 ± 2.12	1.50	1
Electrical conductivity (µS/cm)	1.2 ± 0.31	--	--
Alkalinity (mg/l)	55.1 ± 4.05	--	--
Free CO_2 (mg/l)	25.1 ± 4.69	--	--
Oxygen (mg/l)	2.0 ± 0.29	--	--
BOD (mg/l)	66.3 ± 4.03	--	--
Ni (mg/l)	6.89 ± 0.34	0.07	0.10

Zn (mg/l)	4.89 ± 0.59	0.05	2
Mn (mg/l)	15.6 ± 3.8	--	0.20
Cd (mg/l)	38.1 ± 5.98	0.003	0.01
Pb (mg/l)	39.9 ± 4.12	0.01	0.10
Ti (mg/l)	0.59 ± 0.11	0.05	--
Cr (mg/l)	2.5 ± 0.12	0.05	0.05
As (mg/l)	0.62 ± 0.29	0.01	0.05

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 [Sharma and Gihar, 1991]. The concentration of suspended solid was very high; it was much higher than the permissible limit of the USEPA, 2003 and WHO, 2008. 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 increase in mortality rate in fishes [Garcia et al., 2005; Das et al., 2009; Ghose et al., 2001]. The nitrate and phosphate concentrations were much higher in the CWE. Higher concentration of nitrate and phosphate play a major role in 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 [Tiwary, 2001]. Heavy metals like nickel, zinc, magnesium, cadmium, lead, arsenic and titanium in the CWE were analyzed. These heavy metal ions in CWE were present in higher concentration than the permissible limit of USEPA, 2003 and WHO, 2008.

8.3.0 Bioaccumulation of heavy metals in the *P. florida* fruit bodies

Figure 8.1(a-h) shows the heavy metal accumulation efficiency of *P. florida* exposed to various dilution of CWE (25%, 50%, 75% and 100% CWE) at several time interval (20^{th}, 25^{th}, 30^{th}, 35^{th} and 40^{th} days).

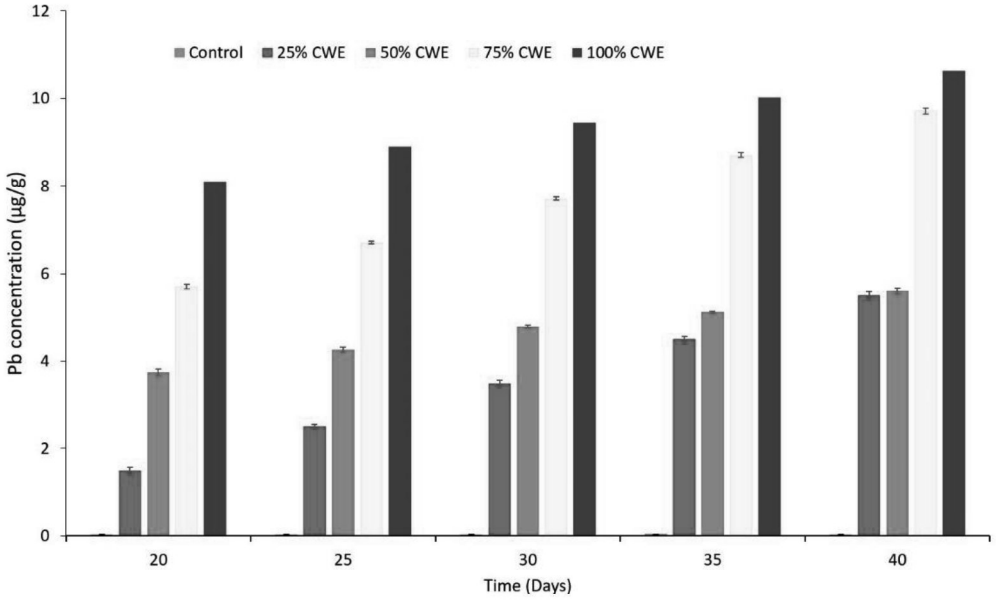

Figure 8.1a. Pb concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

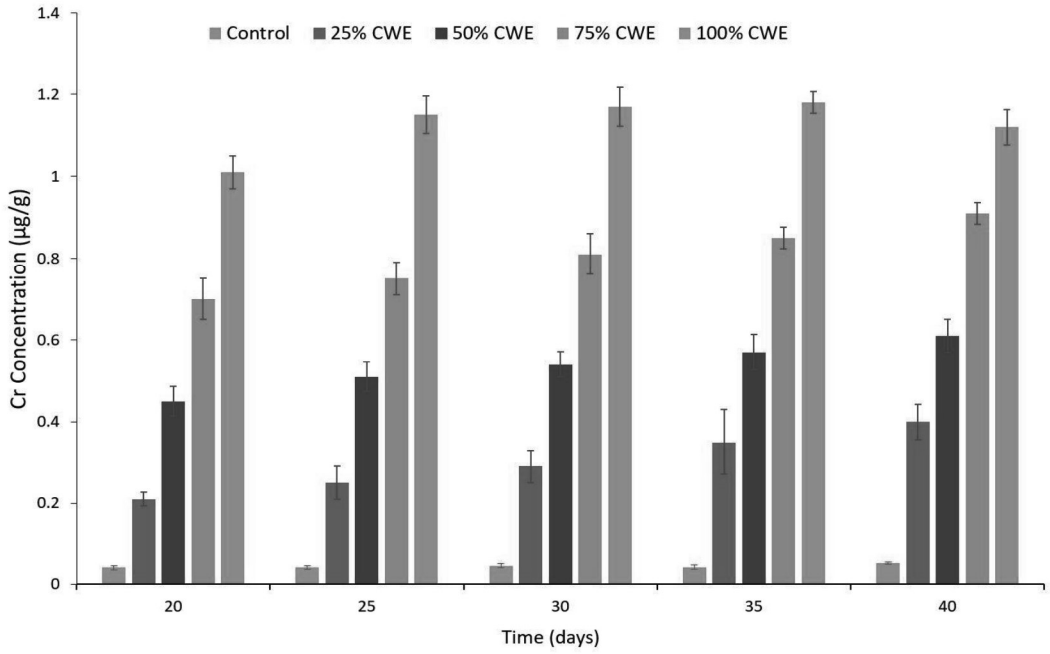

Figure 8.1b. Cr concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

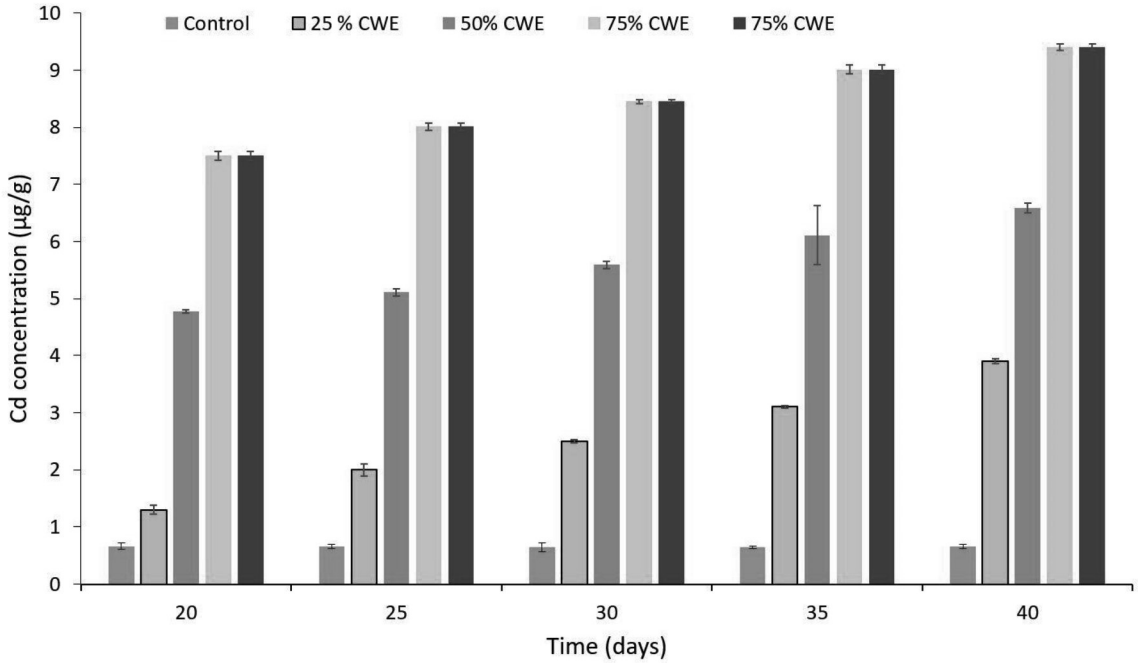

Figure 8.1c Cd concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

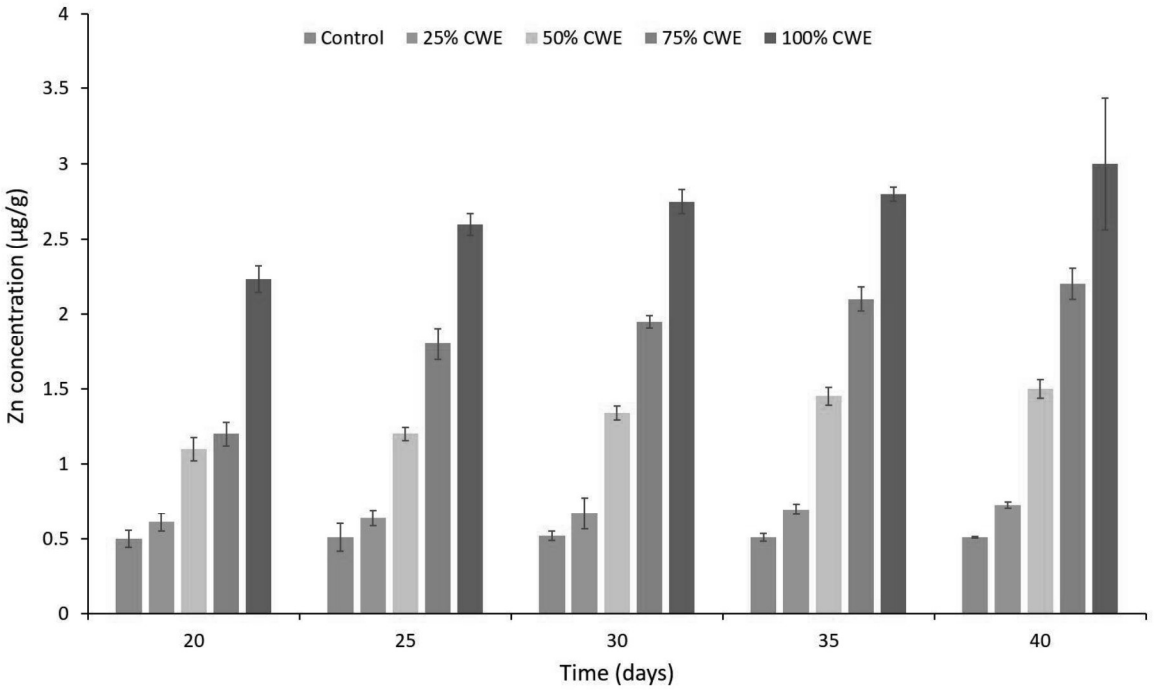

Figure 8.1d. Zn concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

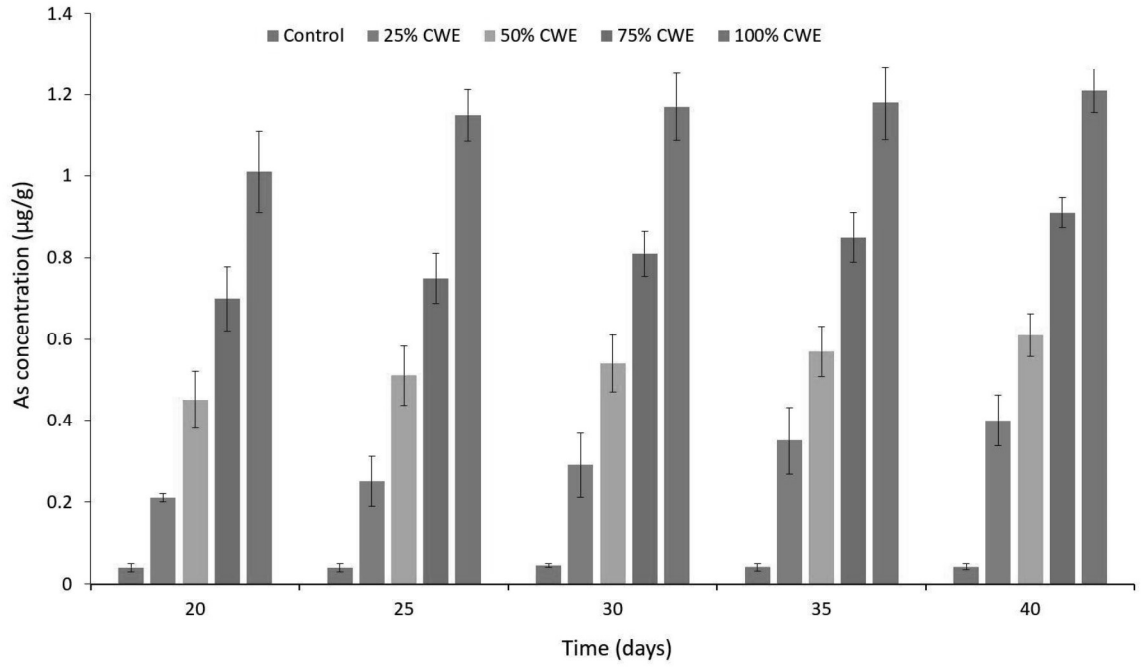

Figure 8.1e. As concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

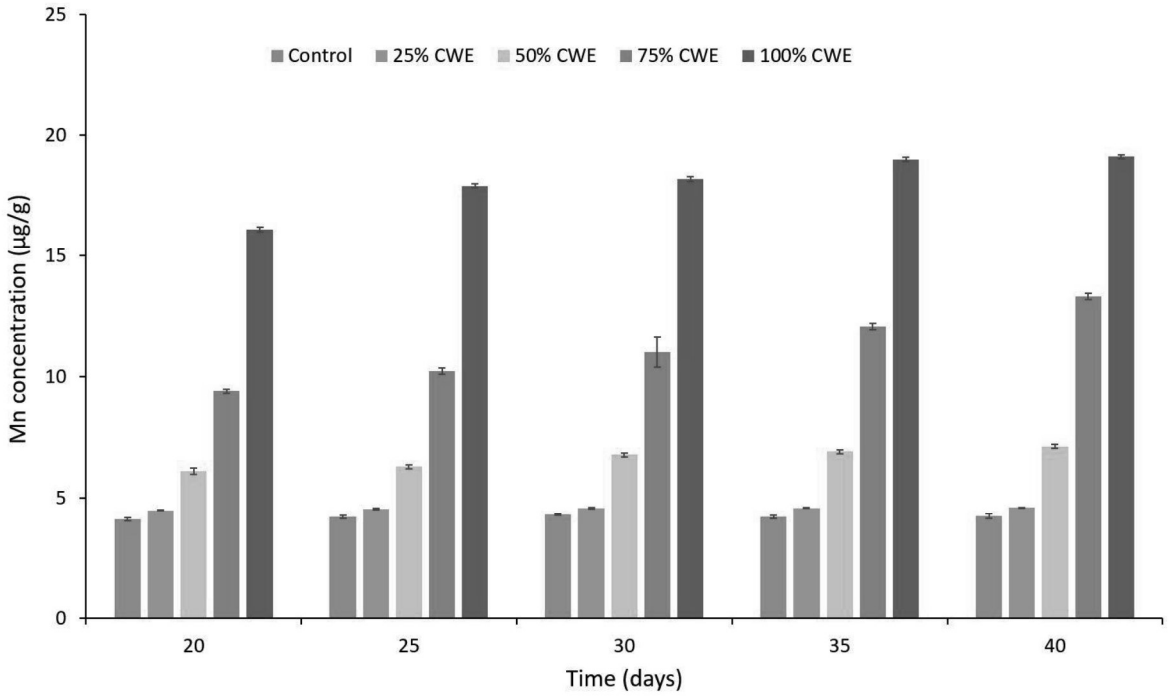

Figure 8.1f Mn concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

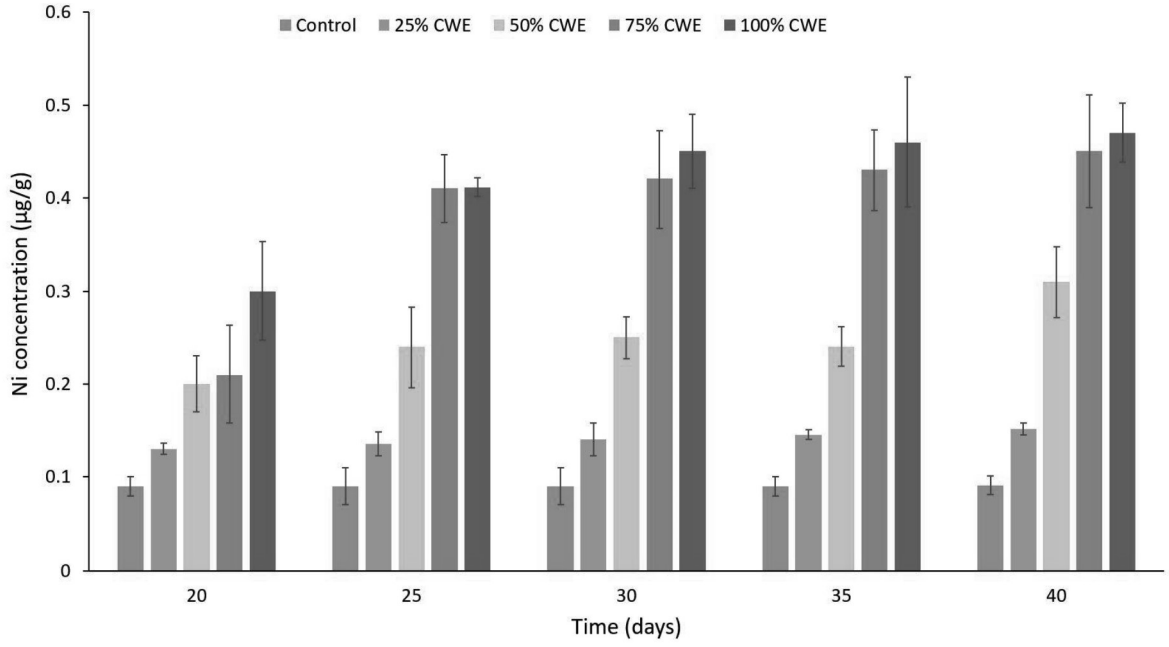

Figure 8.1g Ni concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

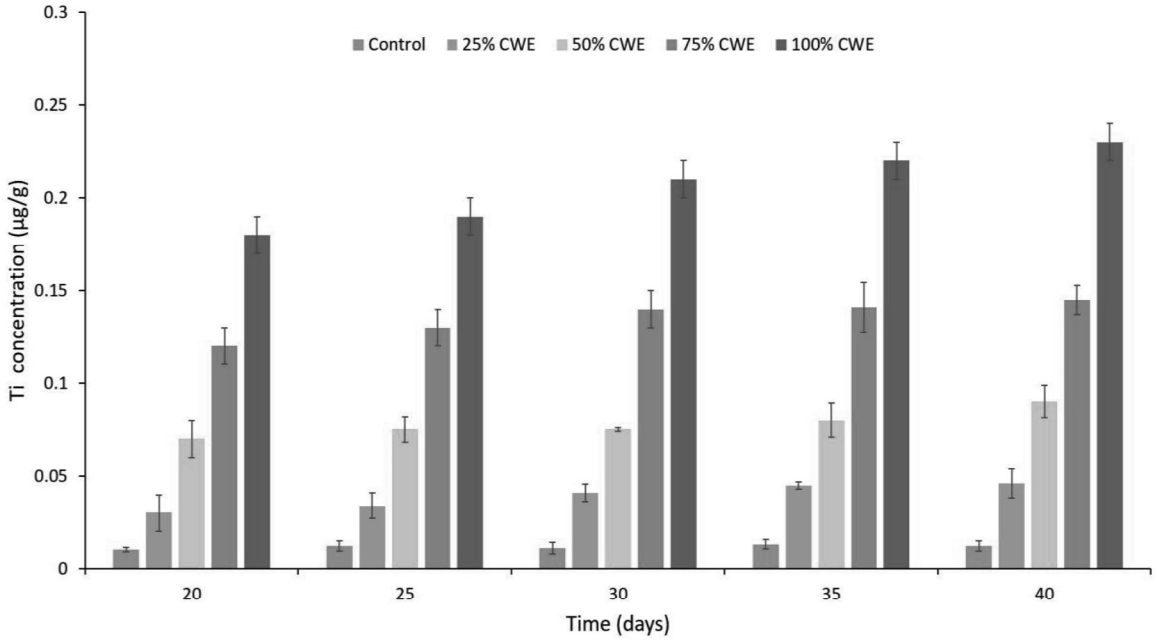

Figure 8.1h Ti concentration in the mushroom fruit body grown in paddy straw substrate containing different concentration of CWE at various interval of time

The heavy metal concentration in control was found less compared to CWE exposed mushroom. The concentration of heavy metals was observed constant time interval. The accumulation of Pb, Cd, Cr, Zn, Ni, Mn, Ti and As (Figure 8.1a-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 20th day in all CWE exposed medium.

The accumulation of Cd, Pb, Zn, Cr and Mn in the fruit body of mushroom was more than the others metals. The accumulation of Cd, Pb, Zn, Cr and Mn in *P. florida* grown in CWE medium was found up to 13.46, 10.65, 3.0, 1.2 and 19.11 µgg^{-1} respectively. The hyper accumulation of heavy metal was due to reason that higher concentration of these metals was 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, 2003 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. Rationale behind this hyper accumulation was the non-toxic nature of Zn ion [Ansar and Khad, 2005].

Vaseem et al., 2017 has also reported the similar kind of results while performing the bioextraction of heavy metal ions from CWE by *Pleurotus ostreatus.* 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 capability of accumulation of heavy metal ions in an organisms/plant depends upon the concentration of heavy metal ions present in its growth environment [Turkekul et al., 2004; Basile et al., 2008; Gonz-alez et al., 2004]. Faria et al., 2018 performed the bioaccumulation study of lithium in *P. ostreatus* at various concentration of initial metal ions. Authors reported that highest bioaccumulation of lithium was 1575.29 $\mu g g^{-1}$ in the intracellular space of mycelia. Almeida et al., 2015 investigated the iron bioaccumulation efficiency of *P. ostreatus.* Authors performed the bioremediation experiment in the presence of 150 mg/L iron in the growth medium inoculated with *P. ostreatus* and authors reported that the maximum iron uptake capacity of mushroom was 3500 $\mu g g^{-1}$. Li et al., 2017 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.

8.4.0 Removal of heavy metals from substrate (paddy straw) *using P. florida*

Table 8.2 shows the detailed analysis of heavy metal concentration before and after the innoculation of *P. florida* cells.

Table 8.2 Metals present in paddy straw (µg/g) 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

It became evident from Table 8.2 that there was a decrease in the concentration of heavy metal ions in the substrate after inoculation phase.

Before innoculation of *P. florida* the maximum concentration of heavy metals was obserbed 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 upto 99.53%, 70%, 77.77%, 76.23% and 52.10%, respectivily. Vaseem et. al., 2017 performed the bioremediation of CWE by *Pleurotus 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. Garcia et al., 2005 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 34^{th} and 50^{th}day. Li et al., 2017 investigated the role of *Pleurotus ostreatus* HAU-2 in the metal detoxification. The authors performed the Cr and Cd bioremediation study and observed that the maximum Cd and Cr removal from liquid medium was 44.85% to 80.36% and 14.49% to 45.55%, respectively. Anwar et al, 2018 isolated Cr resistant endophytes from the soil and identified as *Aspergillus fumigatus*, *Rhizopussp. Penicillium radicumand, Fusarium proliferatum*. 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., 2018 performed the bioremediation study of three metal ions (Cd, Cu and Pb) from liquid broth medium by using fungus *Absidia cylindrospora*. Authors reported that *Absidia cylindrospora* was capable to reduce 68% (Cd), 59% (Pb) and 14% (Cu) after 3 days of incubation at an initial metal ion concentration of 50 mg/L.

8.5.0 Growth modelling of *P. florida* in CWE

At the onset of growth phase in control (0% CWE with PDA), it was observed that the growth of mycelium sectors is time-consuming and smooth. On a later stage, 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 days. The compactly packed mycelial structure with a feathery look was observed at a distance of 31 mm after 15 days of incubation. In growth media containing

CWE from 25% to 100%, the similar pattern of growth was observed. The growth in mm of various sectors at various concentration of CWE has been shown in Table 8.3.

Table 8.3. Growth of *P. florida* in various concentration of CWE

Concentration of CWE in PDA (%)	Incubation (days)	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

The growth of *P. florida* followed a sharp decreasing order with subsequent increase in concentration of CWE in PDA (Table 8.3). The elevation in toxicity due to heavy metal ions present in CWE led to the decrease in growth of *P. florida*. Validation of toxicity due to metal ions has been shown in section 3.5 which is relevant to antioxidant activity (resistance mechanism of *P. florida* against metal ions).

8.5.1 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 has been shown in Table 8.4.

Table 8.4. 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

Linear and exponential growth kinetic functions were fitted in the growth curve models (Table 8.4). Primarily, data fitting showed that both exponential growth function and linear growth extension rate decreased with simultaneous increase in concentration of CWE in growth media. This trend of growth was dedicated to the enhancement in level of toxicity with increase in concentration of CWE. The disagreement between experimental and theoretical values of exponential growth function showed that growth of *P. florida* was not actually 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 bisporous* mycelium on solid substrate was performed by Straatsma et al., 1991. The authors reported that exponential growth model had less fit in growth kinetics compared to linear extension rate

model. Zervakis et al., 2001 studied the growth kinetics of seven high quality strains of fungus explicitly *Lentunula edodes, Pleurotus ostreatus, P. energy, P. plumonarius, Volvariella volvacea, Agrocybeaegerita* and *Auricularia auricular-judae*. Authors reported that growth kinetics of these fungal strains had better goodness of fit in linear extension growth rate model in the range of time period between inoculation of substrate and fructification. Carroad et al., 1977 and Zakaria et al., 2014 studied the growth kinetics of *Pleurotus ostreatus*and *Pleurotus sajor-caju* in complex culture media, PDA and potato dextrose broth (PDB). Carroad et al., 1977 reported that n^{th} power law had a better over the kinetic data. Precisely, 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 exponential model in the same environmental conditions which assumes that growth rate is proportional to cell mass. Similarly, Zakaria et al., 2104 observed the supremacy of exponential growth model over the linear function model. The results of present work were not analogous with the results reported by Carroad et al. 1977 and Zakaria et al. 2014. The differences in the results were due to varying growth conditions of fungal cells.

8.6.0 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 [Ge et al., 2011]. Heavy metals are responsible for the generation of ROS which in turn leads to disruption of cell organelles [Joho et al., 1995]. Therefore, only those micro/ macro organisms 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, glutathione, superoxide dismutase (SOD), catalase, GSH and lipid peroxidase [Hall, 2002]. Mehra et al., 1988 and Munger et al., 1985 observed that macro fungi have better tolerance as well as bioaccumulation mechanism as compared to other micro fungi. Understanding the heavy metal uptake mechanism from solid substrate to the mushroom

fruiting bodies can help in the design of waste treatment plant. In the present study, heavy metal uptake mechanism was described through estimation of stress marker at various time interval and at various concentration of CWE exposed *P. florida*. The expression level of stress marker found enhanced (Figure 8.2 and 8.3 a-d) in the *P. florida* grown on CWE soaked paddy straw.

8.6.1 Metallothionein concentration in the *P. florida*

The metallothionein was estimated in the mushroom fruit bodies grown in different concentration of CWE and in control at various interval 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 [Cobbett and Goldsbrough, 2002]. Metallothionein concentration in the mushroom fruit bodies exposed to several concentration of CWE is shown in Figure 8.2.

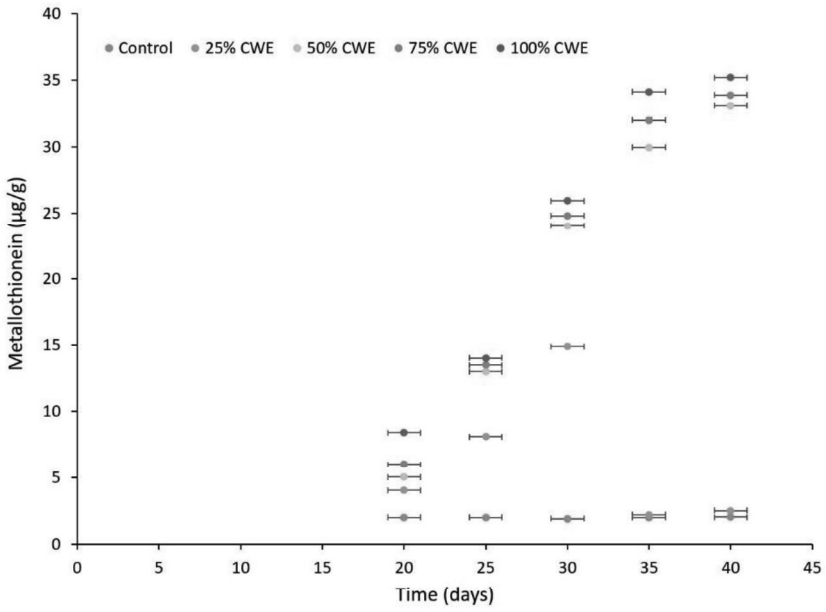

Figure 8.2. Metallothionein concentration in the mushroom grown in the various dilutions of CWE containing medium at several interval of time

It became evident from Figure 8.2 that concentration of metallothionein substantially increased in *P. florida* grown with increase in 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 at 40th day in 100% CWE containing medium. The *P. florida* grown in the double distilled water expressed low level of metallothionein concentration and not affected by exposure period. It remained constant till end of the 40th day. The enhanced concentration of metallothionein showed that immobilization of metal ions in the intracellular space is the key mechanism of bioremediation by *P. florida.* Ramesh et al. 2009 reported that the transcription level of metallothionein genes in *Hebeloma cylindrosporum* was induced in the presence of Cu and Cd. Kameo et al., 2000 reported similar kind 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 metallothioneins production in the bacteria. The increased metallothioneins expression was also found in the *Bacillus cereus* when exposed to Pb [Murthy, 2011].

8.6.2 Antioxidant enzymatic system of *P. florida*

Heavy metals show much toxic effect such as denaturation of nucleic acid and proteins. The production of ROS significantly increased with the increase in 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 [Lazarova et al., 2014]. The antioxidant enzymes such as GSH play an important role in the various types of stress situation. GSH react with ROS within cell and protect to cell components from oxidative damage [Pocsi et al., 2014]. The concentration of antioxidant enzymes such as SOD, catalase, GSH and lipid peroxidase increased with increase in the concentration CWE in growth medium (shown in Figure 8.3 a-d).

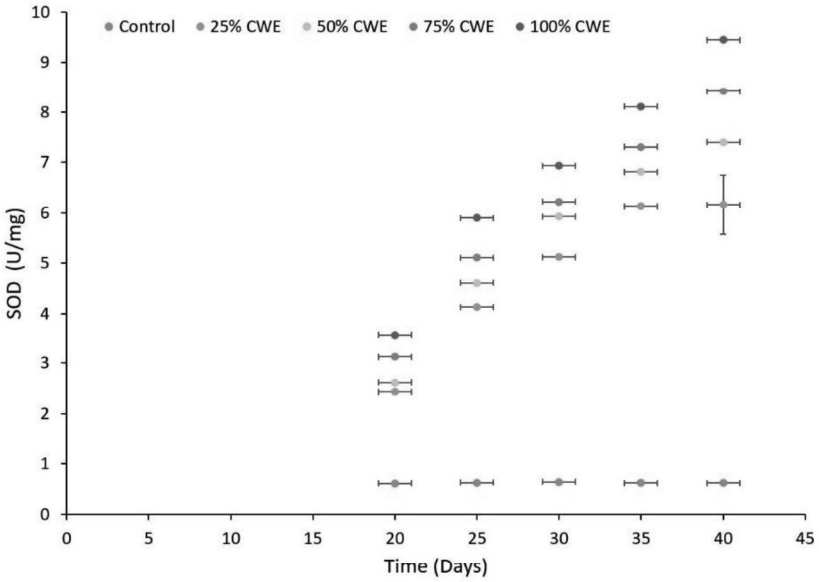

Figure 8.3a. Superoxide dismutase (SOD) concentration in the mushroom grown in the various dilutions of CWE containing medium at several interval of time

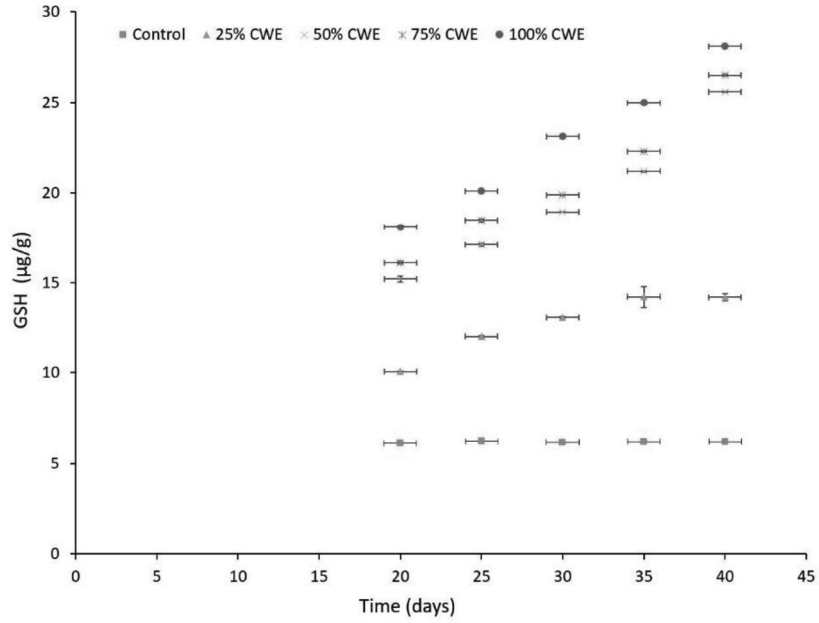

Figure 8.3b. Glutathione concentration in the mushroom grown in the various dilutions of CWE containing medium at several interval of time

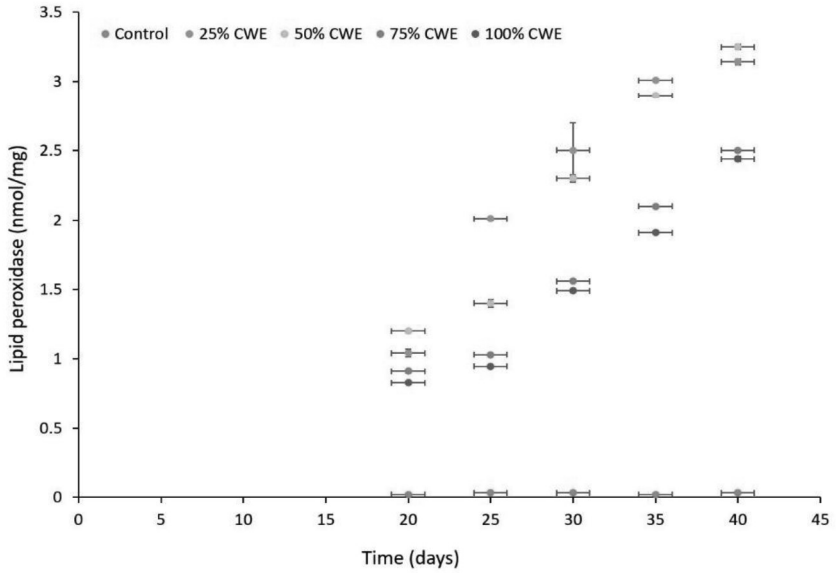

Figure 8.3c. Lipid Peroxidase concentration in the mushroom grown in the various dilutions of CWE containing medium at several interval of time

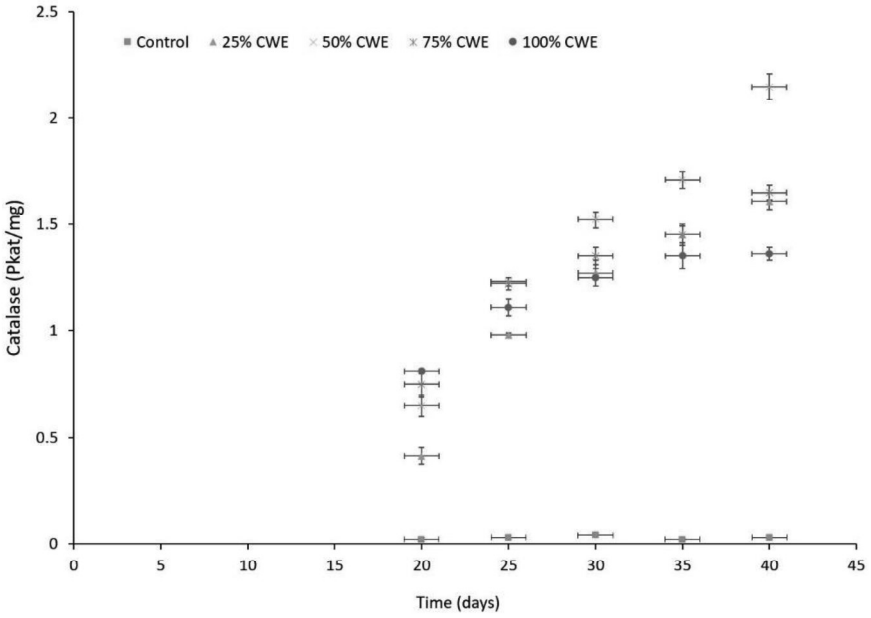

Figure 8.3d Catalase concentration in the mushroom grown in the various dilutions of CWE containing medium at several interval of time

The antioxidant enzymes concentration was measured in 25%, 50%, 75%, 100% CWE containing medium and control. The antioxidant enzymes concentrations were also measured

at various days like 20^{th}, 25^{th}, 30^{th}, 35^{th} and 40^{th}. Figure 8.3a 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 as 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 40^{th} day. Other two enzymes (Figure 8.3a-d) showed similar behaviour with respect to increasing concentration of CWE and time.

Lazarova et al., 2014 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 superoxide dismutase and catalase. Arojojoye and Adeosun, 2016 reported the antioxidant enzymes 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., 2004 reported the oxidative stress of Cd in the fungi *Paxillusin volutus.* 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 increase in production of GSH in the metal stress condition. Ye et al., 2018 performed the fungal bioremediation of Pb and investigated the mechanism of metal ion bioaccumulation in the intracellular space of *P. oxalicum* SL2. Authors reported that glutathione plays major role in the intracellular accumulation of heavy metals such as Pb. Authors estimated the concentration of glutathione in the control (without Pb) as 12.7 μmol L^{-1} and observed an increase of 49.3-fold in concentration of glutathione when *P. oxalicum* SL2 was exposed to Pb.

Wu et al., 2016 performed the mycoremediation of manganese by *Pleurotus eryngii* and estimated the expression of SOD in the Mn exposed and non-Mn exposed *Pleurotus*

eryngii. 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., 2016 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., 2015 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 1mM of Cu."

8.6.3 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 could be possibly explained by 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 bioaccumulation. The peaks generated in FTIR analysis and their respective functional groups shift has been explained in the Table 8.5.

Table 8.5 Functional groups present in the *P. florida* (before and after bioremediation)

Wave number (cm^{-1})		**Functional groups**
Before bioremediation	**After bioremediation**	
3404	3422	-NH and –OH stretching of amine and hydroxyl groups
2924	2925	Aliphatic C-H groups
---	2097	C≡C (stretching)

1645	1640	C=C groups of carboxylic, aldehyde, ketones and esters
---	1401	C-H deformation in alkaline
1374, 1312	---	C-H groups in aromatic compounds
1203	---	C-O groups in esters, C-N groups in amines
1150	---	C-O groups in alcohols and esters, C-N groups in amines
1043	1079	C-O groups in alcohols and esters, C-N groups in amines
890	---	Aromatic out-of-plane ring bends
---	538	Plane deformation

The changes in the FTIR spectra in the *P. florida* exposed to CWE have been shown in Figure 8.4a-b.

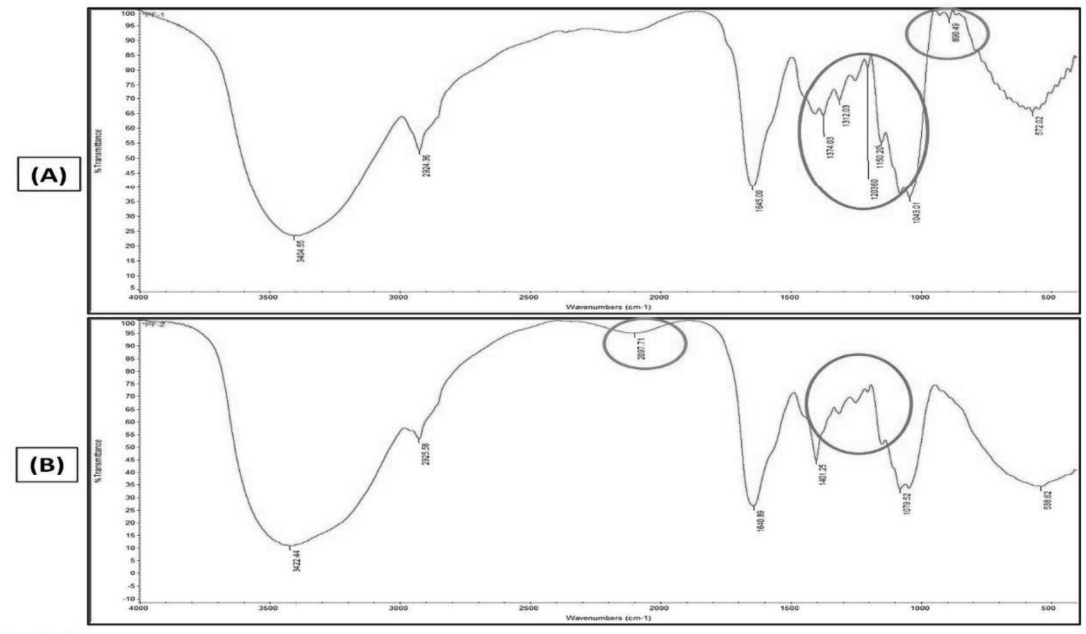

Figure 8.4. Schematic diagram representing the FTIR analysis of *P. florida* grown in control (Figure 8.4a) and CWE containing substrate (Figure 8.4b)

It became clear from Figure 8.4a-b that the arrangement of peaks changed in CWE exposed *P. florida*. The spectra showed in the Figure 8.4a 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 1374, 1312 represent C-H stretching, peak at 1645 cm^{-1} represents C=C groups, peaks at 1043 cm^{-1}, 1079 cm^{-1},1150 cm^{-1} and 1203 cm^{-1} represent C-O and C-N groups of aliphatic compounds [Kibami et al., 2017; Saha et al., 2013].

Figure 8.4b contain few different peaks from Figure 8.4a which were at 2097, 1401 and 538 cm^{-1}, which represent C≡C 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 accumulation process [Kibami et al., 2017; Saha et al., 2013; Shrestha, 2016].

On the basis of 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* [Vaseem et al., 2017]. 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* [Vaseem et al., 2017]. This study suggested that variety of functional groups present on the surface of *P. florida* were involved in the covalent binding with heavy metal ions [Saha et al., 2013; Coates, 1996].

8.7.0 SEM and EDX Analysis

Figure 8.5 shows the surface morphology of *P. florida* grown in double distilled water and CWE containing substrate. The Figure 8.5a-d represent the SEM micrograph of the control and Figure 8.5 e-h show the SEM micrograph of CWE exposed *P. florida.*

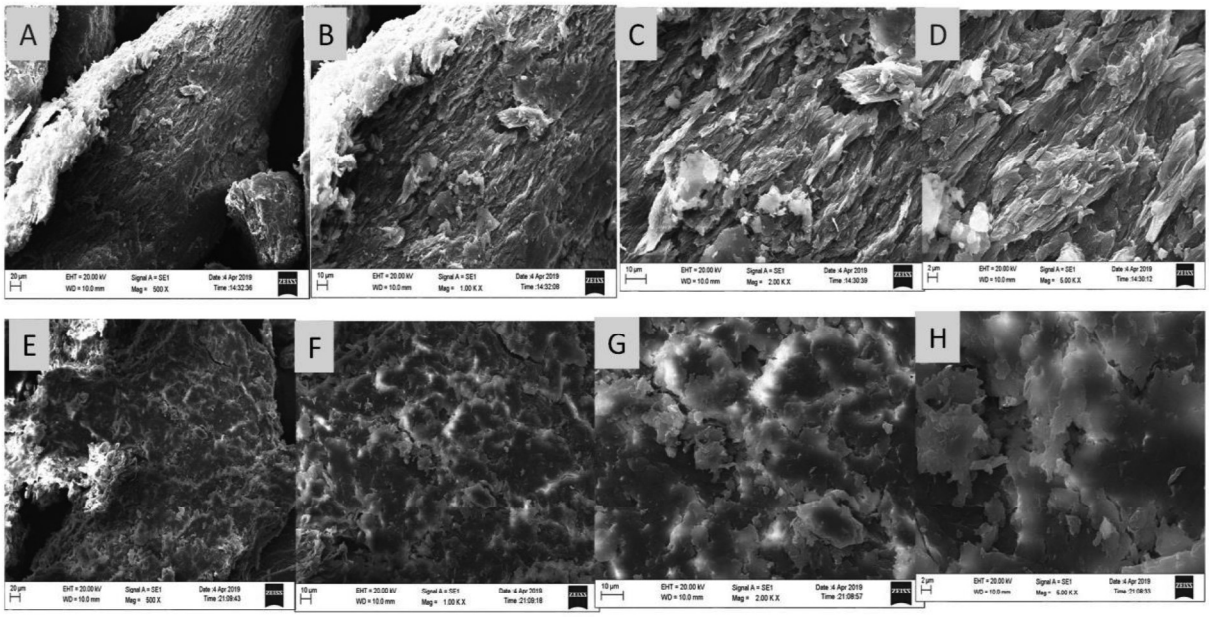

Figure 8.5 SEM micrograph cultivated in control and SEM micrograph (e-h) of *P. florida* (cultivated in CWE).

Figure 8.5a-d show that the surface of *P. florida* is mostly porous and irregular in shape. Figure 8.5e-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 adsorption of heavy metal ions occurred on the surface of *P. florida* [Kibami et al., 2017; Shrestha, 2016]. Saha et al., 2013 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, 2007 reported morphological changes in the *Acinetobacter* sp. after accumulation of chromium within the cell. The authors observed 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 bacterial surface. This was an evidence of adsorption of metal ions on the surface of *P. florida.*

EDX spectra (Figure 8.6) show the elemental contents in the sample. EDX analysis of *P. florida* is shown in Figure 8.6.

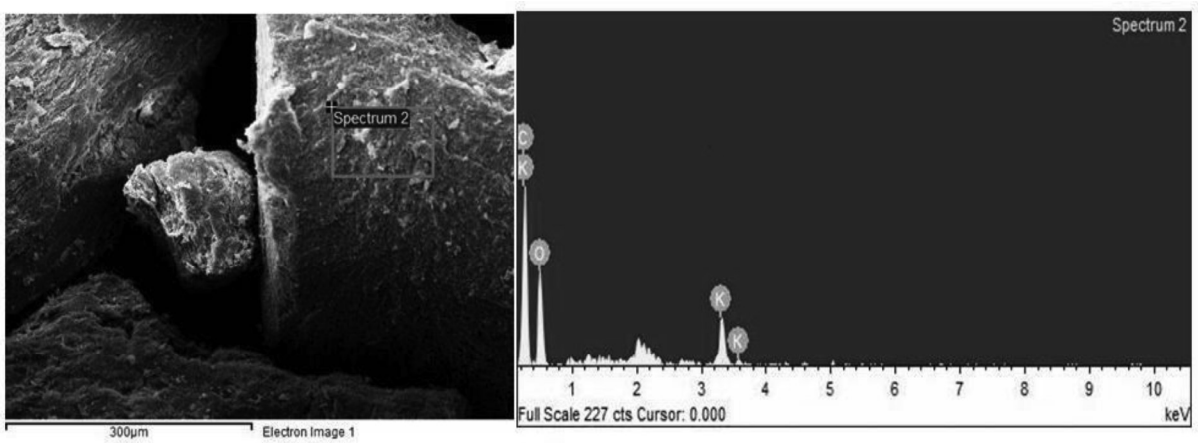

Figure 8.6 EDX spectra of *P. florida* mushroom.

The carbon, oxygen and potassium content were considerably higher in the *P. florid*a as compared to other elements. The EDX analysis of *P. florida* does not show the presence of phosphorous and nitrogen. The absence of nitrogen and phosphorous and presence of carbon and oxygen in the *P. florida* has been considered as a good quality in biosorbent for removal heavy metals from wastewater [Kibani et al., 2017]. Shrestha, 2016 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 sulphate (SDS). Michalak et al., 2018 performed the bioremediation of metal ions on the microalgae and suggested that the elemental components of 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.

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

Figure 8.7 (A-E) shows the growth of *P. florida* in control and CWE soaked paddy straw.

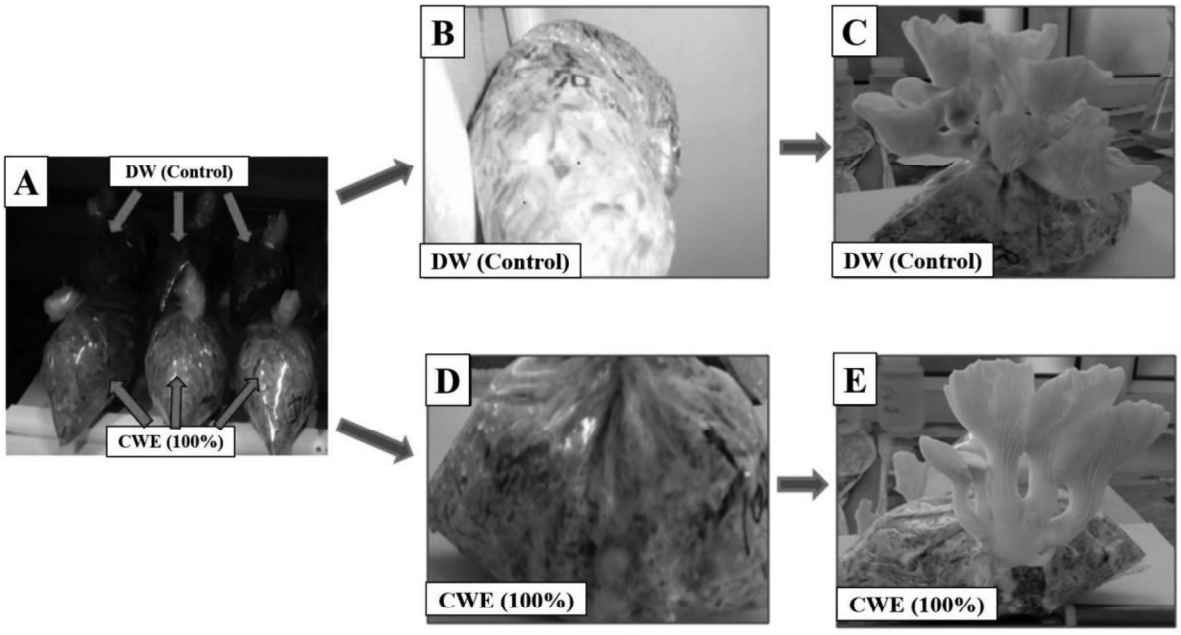

Figure 8.7 Growth of *P. florida* (A-E) in the control and 100% CWE containing paddy straw

P. florid 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 solid, total solid, 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 control study. Figure 8.7A shows polyethylene bags containing spawn inoculated in control and in 100% CWE soaked paddy straw at 0^{th} day. Figure 8.7B and 8.8C shows the growth of *P. florida* in control and Figure 8.7D and 8.8E shows the growth *P. florida* in the 100% CWE at end of the 40^{th} day. It became evident from Figure 8.7 (C and E) that these 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 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 (Figure 8.1a-h). This outcome indicated that the bioaccumulation of heavy metal in the intracellular space depends on the presence initial concentration of metal ion in the growth medium. The presence of other wastewater components such as suspended solid, dissolved solid, nitrate and phosphate did not significantly affect the bioremediation efficiency of *P. florida*.

8.9.0 Comparison of removal efficiency in terms of heavy metal ions by *Pleurotus florida* and other fungal species

Comparative study of various fungal cells in the terms of their bioremediation efficiency has been shown in Table 8.6.

Table 8.6 Heavy metal removal efficiency of *P. florida* and other fungal species

Microorganism	Metal	Initial metal ion conc.	Removal efficiency (%)	References
Absidia cylindrospora	Pb	50 mg L^{-1}	59.00	[Albert et al., 2018]
Agaricus macrospores	Pb	10 mg kg^{-1}	2.30	[Garcia et al., 2005]
Pleurotus Ostreatus	Pb	45.4 mg L^{-1}	35.60	[Vaseem et al., 2017]

Pseudochlorococcum typicum	Pb	20 mg L^{-1}	86.00	[Shanab et al., 2012]
Porphyra leucosticte	Pb	10 mg L^{-1}	90.00	[Ye et al., 2015]
Drechslera hawaiiensis	Pb	90 mg L^{-1}	99.26	[El-Gendy et al., 2017]
Pleurotus florida	Pb	25.48 µg g^{-1}	52.10	*(This study)*
Absidia cylindrospora	Cd	50 mg L^{-1}	68.00	[Albert et al., 2018]
Agaricus macrospores	Cd	10 mg kg^{-1}	13.00	[Garcia et al., 2005]
Pseudochlorococcum typicum	Cd	20 mg L^{-1}	70.00	[Ye et al., 2015]
Porphyra leucosticte	Cd	10 mg L^{-1}	70.00	[Ye et al., 2015]
Drechslera hawaiiensis	Cd	30 mg L^{-1}	99.26	[El-Gendy et al., 2017]
Pleurotus florida	Cd	19.25 µg g^{-1}	99.84	*(This study)*
Pleurotus Ostreatus	Cr	1.12 mg L^{-1}	99.10	[Vaseem et al., 2017]

Fusarium oxysporum	Cr	350 mg L^{-1}	95.00	[Khurshid et al., 2016]
Aspergillus flavus	Cr	25 mg L^{-1}	95.80	[Abubacker and Kirthiga, 2013]
Aspergillus niger	Cr	50 mg L^{-1}	48.70	[Shugaba et al., 2013]
Pleurotus florida	Cr	1.75 µg g^{-1}	70.85	*[This study]*
Pleurotus Ostreatus	Ni	1.79 mg L^{-1}	99.90	[Vaseem et al., 2017]
Rhizopus arrhizus	Ni	100 mg L^{-1}	40.50	[Silah and Gul, 2017]
Trichoderma harzianum	Ni	50 mg L^{-1}	90.20	[Sarkar et al., 2010]
Pleurotus florida	Ni	0.98 µg g^{-1}	77.77	*[This study]*
Pleurotus Ostreatus	Mn	14.6 mg L^{-1}	57.20	[Vaseem et al., 2017]
Pleurotus florida	Mn	62.16 µg g^{-1}	42.63	*[This study]*
Pleurotus Ostreatus	Zn	4.46 mg L^{-1}	82.60	[Vaseem et al., 2017]

Trichoderma viride	Zn	30 mg L^{-1}	54.33	[Ali et al., 2007]
Beauveria bassiana	Zn	200 µg g^{-1}	0.64	[Purchase et al., 2009]
Rhodotorula mucilaginosa	Zn	200 µg g^{-1}	2.05	[Purchase et al., 2009]
Agaricus macrosporus	Zn	20 µg g^{-1}	13	[Garcia et al., 2005]
Pleurotus florida	Zn	2.15 µg g^{-1}	76.23	*[This study]*

It became evident from Table 8.6 that viable mycelia cells of *P. florida* have tremendous and extensive potential of metal ion removal specially 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 metal contaminated environment.

8.10 Conclusion

The bioremediation of toxic metal ions present in CWE was done by the *P. florida* species. Based on experiments, it was observed that the concentration of toxic heavy metals in the paddy straw was high when *P. florida* was allowed to grow on the substrate. The study of growth kinetics of *P. florida* in CWE suggested that linear growth model had a 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 stress markers namely metallothionein**,** SOD**,** GSH**,** catalase**,** and lipid peroxidase were present in higher concentration in the fruit bodies of *P. florida* grown in CWE as 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 ability to remediate toxic metal ions from the contaminated sites. In the present investigation, bioremediation of toxic metal ions was through intracellular accumulation of metal ions in *P. florida.*