



Mycosensing of soil contaminants by *Ganoderma lucidum* and *Omphalotus subilludens* including the insights on growth media requirements



Chandrika Gupta^a, Raj Mohan Balakrishnan^{a,*}, Uddandarao Priyanka^a, Arivalagan Pugazhendhi^b

^a Department of Chemical Engineering, National Institute of Technology Karnataka, India

^b Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Keywords:

Mycoremediation

Biosorption

Ganoderma lucidum

Heavy metals

Omphalotus subilludens

ABSTRACT

Heavy metals are regarded as one of the major threats to environmental biota due to prolonged soil residence time. In this scenario, mycoremediation emerged as an effective tool for the removal of heavy metal contaminants. The present study reports the responses of two wild mushroom species *Ganoderma lucidum* and *Omphalotus subilludens* under metal stress conditions and the nutritional requirements of the mushroom species. The media was screened wherein the media containing glucose and sucrose as carbon source showed better growth for *Ganoderma lucidum* and *Omphalotus subilludens* respectively. In addition, peptone as a nitrogen source is required for the growth of both *Ganoderma lucidum* and *Omphalotus subilludens*. Further, it is observed that macronutrients play a crucial role in the stimulation of enzymes and the micronutrients are mandatory for intermediary metabolism of the fungi in both the species. Tolerance studies are carried out in-vitro and the results reveal that the *Ganoderma lucidum* showed tolerance towards Cr (VI), Ni (II), Pb (II) and Cd (II) at maximum tolerant concentrations of 1000 mg/kg, 500 mg/kg, 100 mg/kg and 10 mg/kg, in case of, *Omphalotus subilludens* showed tolerance towards Cr (VI), Ni (II) and Pb (II) at maximum tolerant concentration of 700 mg/kg, 700 mg/kg and 500 mg/kg respectively. Moreover, FTIR spectral analysis indicated the presence of components like oxalic acid and thiol compounds during metal stress conditions.

1. Introduction

Over the years, with the evident of industrialization and technological advancement large quantities of heavy metals are inflicting serious damage on the ecosystem causing serious global health concerns. The wastewaters containing heavy metals are often discharged into the environment without appropriate treatment, resulting in severe socio-environmental problems even at low concentrations (Alvarez et al., 2017; Ayangbenro and Babalola, 2017; Dangi et al., 2019). Heavy metals are one of the persistent pollutants in the environment and are retained in the soil as exchangeable metals, carbonates, hydroxides and oxides. In most cases, heavy metals are retained in the upper horizon of soils (< 0.5 m) depending on local environmental conditions. They cannot be eliminated completely as they have long half-lives and are resistant to degradation process inhibiting soil respiration, nitrogen mineralization and nitrification (Sobolev and Begonia, 2008). The propagation of heavy metals throughout the food chain has consequences on the environmental habitat and human health. Unlike organic contaminants, heavy metals are non-biodegradable and do not

undergo microbial or chemical degradation (Adriano, 2001). However, the oxidation states of heavy metals can be transformed or result in organic complex formation, becoming water-soluble and comparatively less toxic.

Extensive research revealed that clean technologies using biological agents such as algae, fungi and bacteria have emerged as low-cost promising technologies. Bioremediation is a sustainable technology for the restoration of heavy-metal-contaminated soils since it is eco-friendly and cost effective compared to the conventional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low (Ojuederie and Babalola, 2017; Sobariu et al., 2017; Song et al., 2017; San et al., 2018; Gu et al., 2018; Rai et al., 2019). Mycoremediation is one of the methods used for the remediation of polluted soils and aqueous effluents for the heavy metals removal (Stamets, 1999). Many reports have emphasized the role of mushroom in bioremediation of wastes by the process of biodegradation, biosorption and bioconversion.

Mushroom is a macro fungus with a distinctive fruiting body that consists of a cap (pileus) with spore forming part (sporophore), a stem

* Corresponding author.

E-mail address: rajmohanbala@gmail.com (R.M. Balakrishnan).

<https://doi.org/10.1016/j.bcab.2019.101239>

Received 22 April 2019; Received in revised form 7 June 2019; Accepted 7 July 2019

Available online 08 July 2019

1878-8181/ © 2019 Elsevier Ltd. All rights reserved.

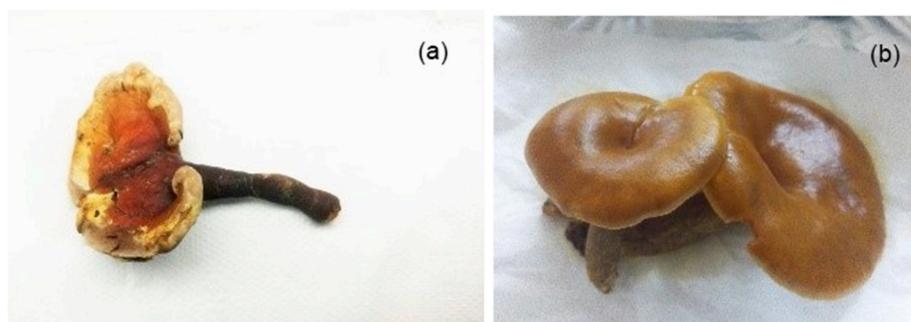


Fig. 1. Photographic pictures of (a) *G. lucidum* and (b) *O. subilludens*.

(stripe) and the vegetative part mycelium that is observed as fine white threads. The vegetative part of mushroom contains an underground network of branched, tubular filaments (hyphae) which can serve as biological filters (Volezky and Holan, 1995). Mushrooms have the capability to bind to heavy metals by several chemical processes like ion exchange processes, covalent binding and adsorption. They have the capability to produce several groups of enzyme complexes such as extracellular peroxidases, pectinases, ligninase, xylanases, cellulases and oxidases (Stamets, 2005). The polar groups of proteins, amino acids, lipids and polysaccharides contribute to biosorption process. The present work aims at the collection, identification of wild mushrooms and to determine nutrient requirements affecting the mycelium growth based on carbon source, nitrogen source, macronutrients and micronutrients. Further, deals with studying the tolerance and sensing ability of wild mushroom towards heavy metals Cd (II), Hg (II), Pb (II), Ni (II) and Cr (VI).

2. Materials and methods

2.1. Collection and identification of isolates

Macro fungi belonging to *Basidiomycota* phylum were collected from National Institute of Technology Surathkal Campus in Dakshina Kannada District, Karnataka, India located at 12° 57', 13° 50' N latitude and 74°, 75° 50' E longitude. The selected mushrooms after surface sterilization were dissected for totipotent regions using fine sterile surgical blades without any tissue damage. The totipotent regions were inoculated onto PDA plates and incubated at room temperature (28 °C - 30 °C) for 10 days. Growth of the fungal species was observed for the formation of uniform mycelium mat (Chen et al., 2009) under in vitro cultivation of mushrooms. Further, mushrooms were sent for species identification at Yaarazah Xenomics, Madurai Tamil Nadu for Internal transcribed spacer (ITS) 18 rRNA fungal sequencing.

2.2. Optimization of the nutritional requirements

To determine the medium composition required for the optimum growth of the fungal species the culture was inoculated in a basal media. The flasks containing the basal media- MgSO₄ · 7H₂O 560 mg/L; CaSO₄ 550 mg/L; KH₂PO₄ 1000 mg/L; NaNO₃ 1000 mg/L; Thiamine-Hydrochloride 12000 mg/L; NH₄SO₄ 1000 mg/L; Glucose 20000 mg/L maintained at pH 8 with 1M NaOH was further amended with different carbon sources such as glucose, starch and sucrose, nitrogen sources such as ammonium sulphate and peptone yeast extract, macronutrients (Ca, K, Mg, Na by excluding one element at a time from the complete medium) and micronutrients (Cu, Mg and Zn in sulphate forms were added separately to the basal medium at concentration of 1–10 mg/L) which is incubated for 20 days at 30 °C on a rotary shaker. The mycelium of both the mushroom species was inoculated with 5 mm discs of a 5-day old pure culture in a basal media with different carbon, nitrogen sources, macro nutrients and micro nutrients. Distinct flasks

were kept for different number of days and the dry weight ((Dry weight) = (weight of filter paper + mycelium) - (weight of filter paper)) assessment was done by pouring the sample through a filter paper (Whatman filter paper grade 1, 11 μm) and collecting the mycelium on it.

2.3. Heavy metal tolerance and in-vitro establishment of the isolates

Stock solutions of heavy metals were prepared for Cd (II), Cr (VI), Hg (II), Ni (II) and Pb (II) using chemicals of the analytical grade of CdSO₄, HgCl₂, K₂Cr₂O₇, NiSO₄ and PbSO₄. PDA plates amended with the heavy metals individually at a concentration range of 10–1000 mg/L were inoculated with mushrooms (Bai, 2002). The lone effect of the heavy metals was studied and the mushroom species was incubated for 5 days at 26 ± 2 °C. The growth patterns and the adsorption of heavy metals of the fungal species were observed by Fourier-transform infrared spectroscopy (FTIR) analysis. Further, the tolerant mushroom species were then inoculated in 120 mL sterilized flasks, each containing soil amended with heavy metals individually at a concentration of 10–1000 mg/kg for a period of 20 days at 30 ± 2 °C at pH of 6.8.

G. lucidum was tested for the metal concentrations in the ranges 10–1000 mg/l for Cr (VI), 10–1000 mg/l for Ni (II), 10–50 mg/l for Cd (II) and 10–500 mg/l for Pb (II). *O. subilludens* has tolerance for heavy metals Cr (VI), Ni (II) and Pb (II) and Hg (II) at a concentration range of 10–1000 mg/l, 10–700 mg/l, 10–100 mg/l and 10 mg/l respectively.

3. Result and discussions

3.1. Identification of macro fungus

The isolated macro-fungi were subjected to morphological characterizations which are the important features of the macro fungi that help in species identification. Similar morphological criteria for identification of mushroom species was employed by Pegler (1973), Adaskaveg (1986) and Järup (2003). Figure: 1 (a) and Figure: 1 (b) illustrates the morphological description of the fungi considered for the study. The morphology of Figure: 1 (a) indicates pileus which is kidney shaped with zones and varnished appearance. The margin (edge) yellow/tan in colour with 2.0–5.0 cm broad, surface rough, reddish brown in colour and stipe of 4.5–5 cm long and 0.5–2.0 cm thickness. The spore is an oval shape and is brown in colour. According to the morphological identification the species may belong to *G. sp.* Figure: 1 (b) indicates the pileus of 10–12 cm broad not usually featuring a central bump, slightly greasy bright brownish orange colour and shape is convex with the margin slightly inrolled. The gills running down the stem, stipe 7–9 cm long; 3 cm thick; tapering to base, spore round in shape, and white in colour. The species might be *O. sp.* with respect to the characteristics. The ITS analysis results confirmed that the mushrooms had the standard genome of *G. lucidum* with accession number AH008113.2 and *O. subilludens* with accession number AY313284.1. Table S1 shows the ITS results with higher percentages of conformation

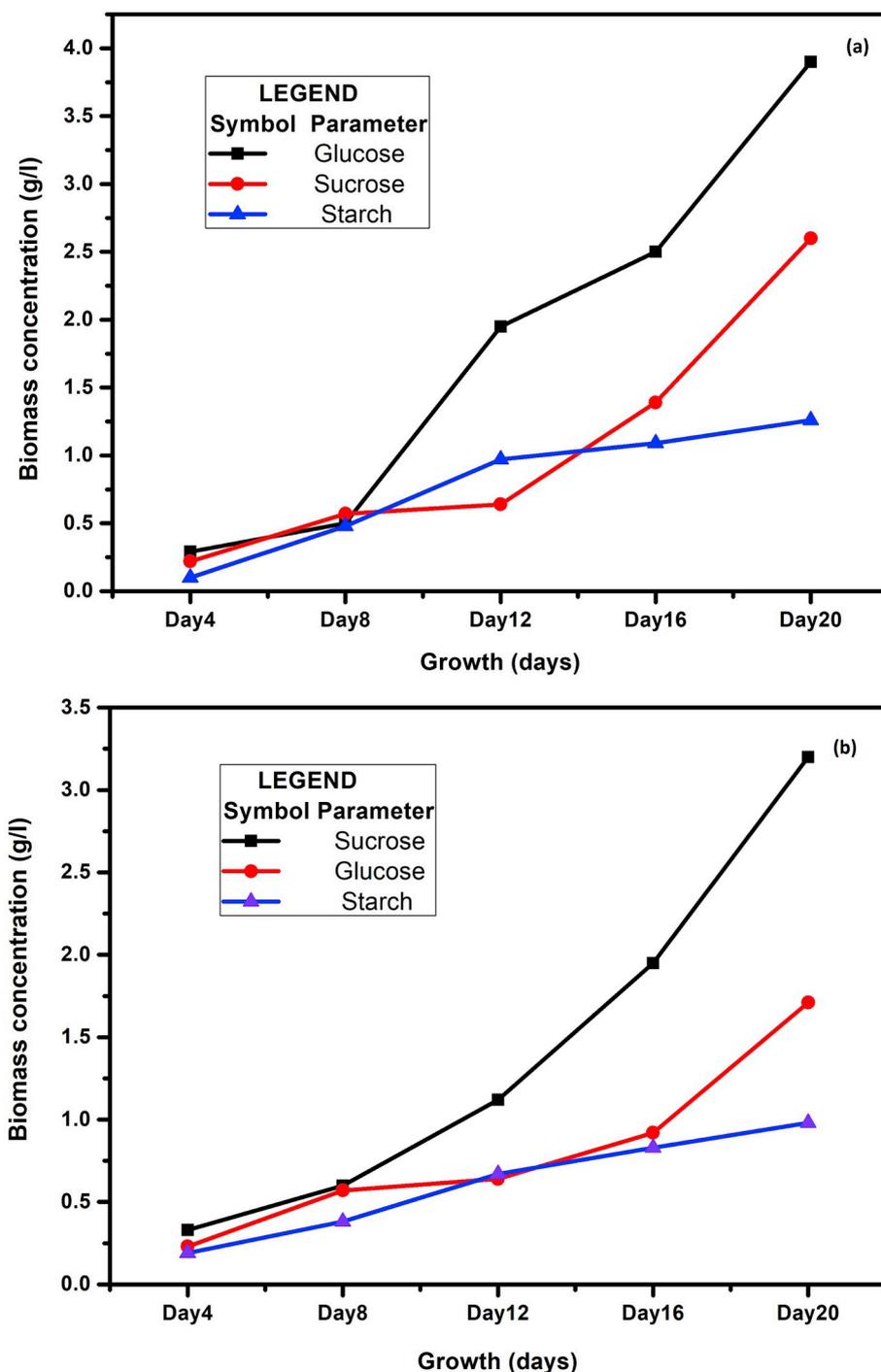


Fig. 2. Effect of different carbon sources on mycelium growth of (a) *G. lucidum* and (b) *O. subilludens*.

with respective species.

3.2. Studies on growth characteristics of the isolated macro fungi using different media

After 10 days of incubation at room temperature, the mycelium growth was observed with different media composition like Sabouraud's Dextrose Agar Medium (SDA), Glucose Peptone Agar Medium (GPA), Malt Extract Agar Medium (MEA) plates. Figure S1 (a) and Figure S2 (a) (Supplementary information) shows that the totipotent parts of both the organisms placed in the PDA medium found to have fine white threads like structures, hyphae, that grew uniformly across the petri-plate surface whereas, there were no significant growth of these fungi on the rest

of the plates containing the other two media. Thus, both the species showed better mycelium growth in PDA media compared to SDA (Figure S1 (b) and Figure S1 (c); Supplementary information) and MEA (Figure S2 (b) and Figure S2 (c); Supplementary information) media. The results are in par with the results obtained by Jayasinghe and Parkinson (2008).

3.3. Growth profile of mycelium with different substrates

3.3.1. Carbon source

Macro fungi being non-photosynthetic depend on an external supply of organic carbon compounds such as carbohydrates. From Fig. 2 (a) and Fig. 2 (b) effects of different carbon sources on mycelium growth of

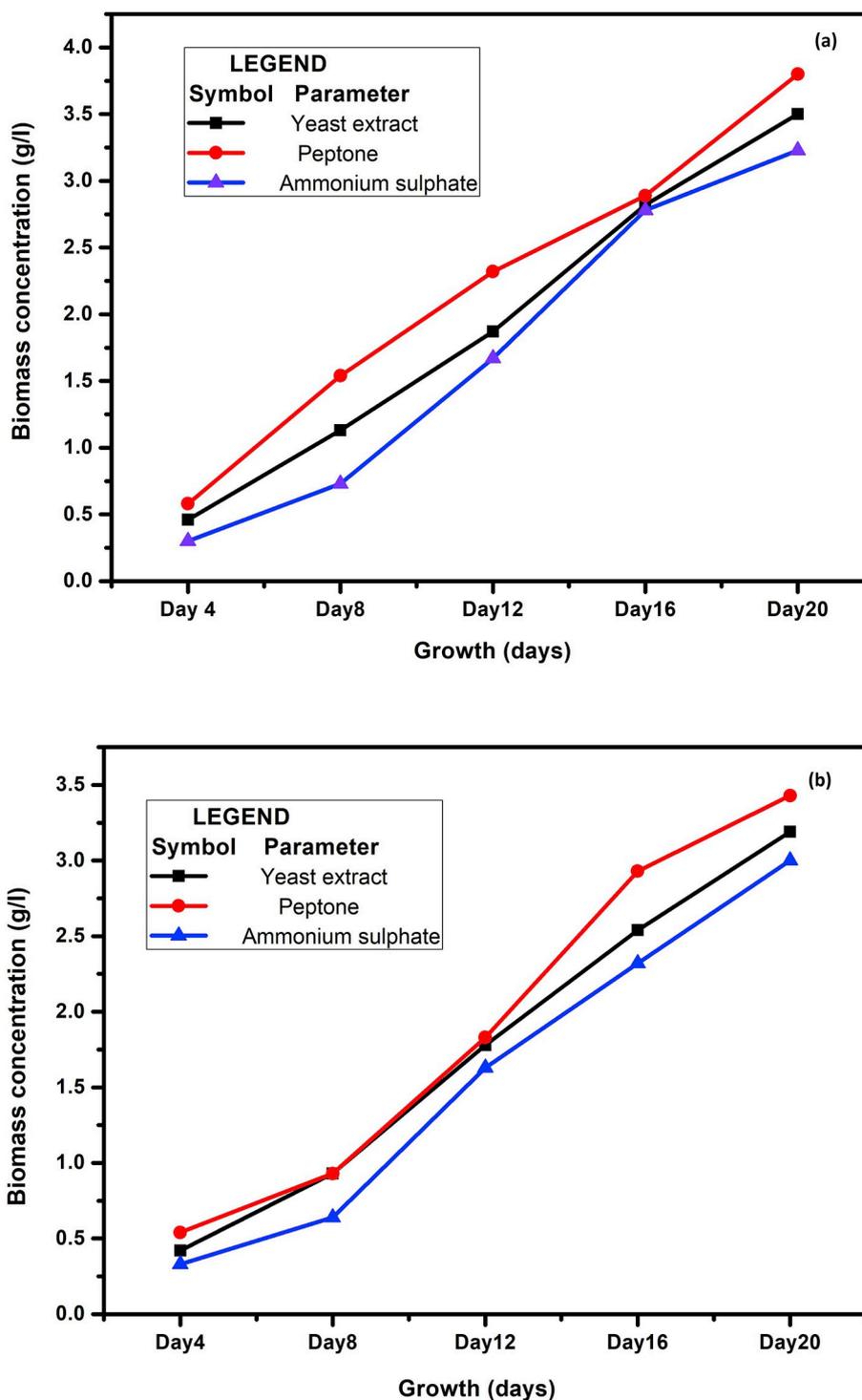


Fig. 3. Effect of different nitrogen sources on mycelium growth of (a) *G. lucidum* and (b) *O. subilludens*.

G. lucidum and *O. subilludens* can be observed. Glucose was found as a better carbon source for *G. lucidum* followed by sucrose whereas for *O. subilludens* sucrose showed better biomass growth. The utility of starch as carbon source resulted in the reduction of mycelium growth in both the organisms. The growth enhancement utilizing glucose over other carbon compounds may be due to the ease of sugar metabolization to produce cellular energy (Jandaik and Kapoor, 1976). Kadiri and Fasidi (1994) reported that the best carbon sources for *Lentinus subnudus* were fructose and glucose. Starch (polymer composed of amylose and amylopectin linked by glycoside bond) was the least used carbon source for mycelium growth. The decrease in the mycelium growth

may be due to the inability of this fungus to produce enzymes (amylase) to metabolise this carbon source.

3.3.2. Nitrogen source

Nitrogen is one of the essential nutrients that stimulate the mycelium growth and uptake of other nutrients. The effect of different nitrogen sources on mycelium growth of *G. lucidum* and *O. subilludens* is represented in Fig. 3 (a) and Fig. 3 (b) respectively. Out of the tested nitrogen sources, peptone showed a better yield. Peptone is a hydrolysed protein, consisting of free amino acids and short peptides enabling macro fungus to easily utilize it (Kim et al., 2013). This is in

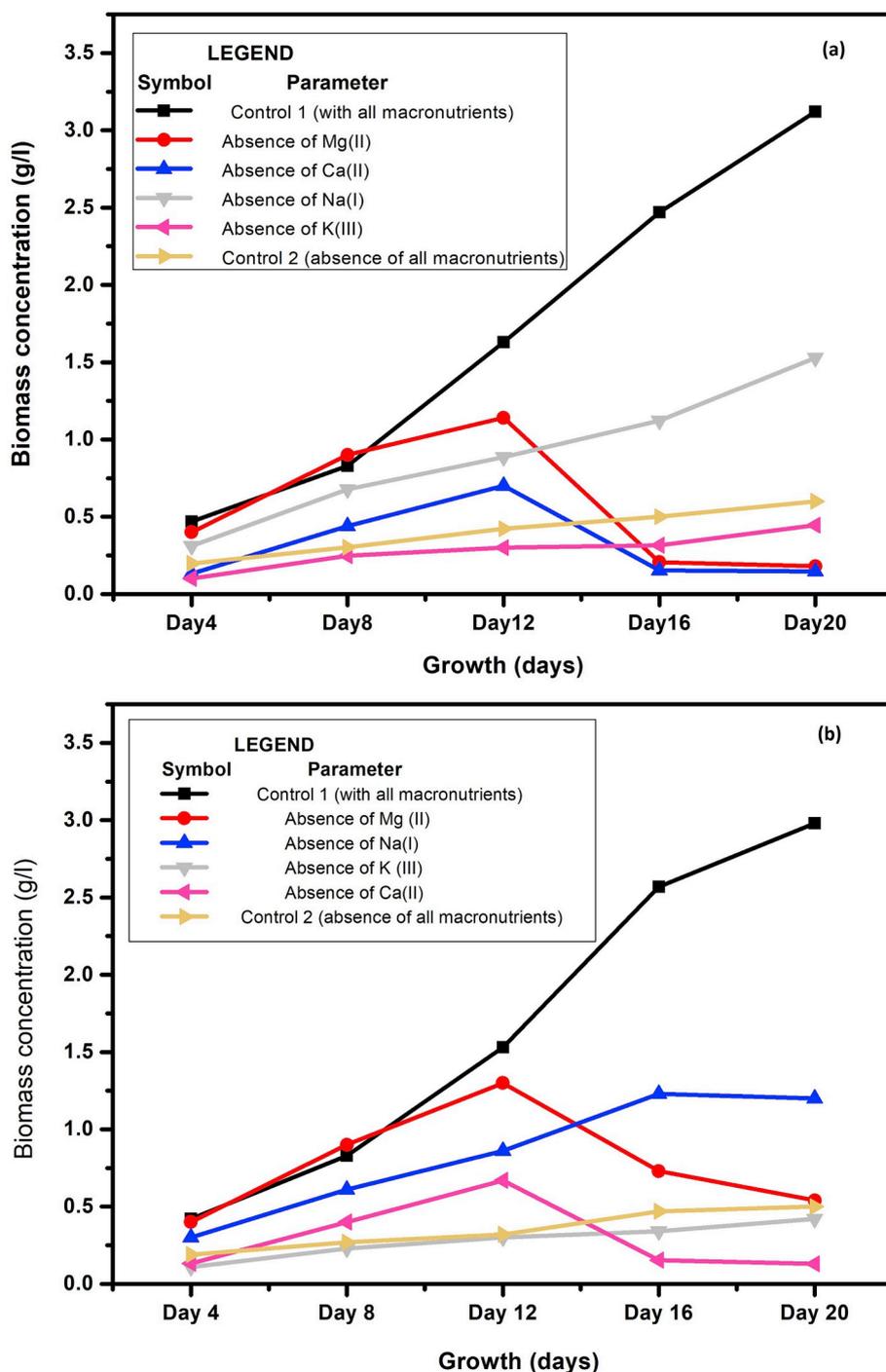


Fig. 4. Effect of macronutrients on mycelium growth of (a) *G. lucidum* and (b) *O. subilludens*.

concordance with the work reported by Kuforiji and Fasidi (2009) where they found that peptone significantly increased the growth of *Pleurotus ostreatus* (0.2 g of peptone in 100 ml of media) while inorganic nitrogen sources had no appreciable effect. Appreciable mycelium growth was also noted in the presence of yeast extract compared to ammonium sulphate as a nitrogen source.

3.3.3. Macronutrients

The macronutrients play a major role in the completion of the life cycle of the mycelium as they stimulate the enzymes responsible for the growth of the fruiting body and cell rejuvenation. To study the effect of the presence of all macronutrients in the media, initially, the media containing all these macronutrients were considered. It was observed

that the mycelium growth enhanced in both *O. subilludens* and *G. lucidum* as indicated in Fig. 4 (a) and Fig. 4 (b). Further, the effect of the lack of individual macronutrients on mycelium growth was also studied. Medium lacking in K, Mg and Ca showed a significant decrease in growth in both organisms, indicating that Ca is an essential macroelement, followed by Mg and K. K plays essential roles in enzyme activation, protein synthesis, cation-anion balance and stress resistance (Agomuo, 2011). The significance of Ca in fungal growth was also reported by Fasidi and Jonathan, 1994 on *Volvariella esculenta*. Griffin (1994) reported that, Ca increases the enzyme activity thereby aiding fungal growth, while magnesium is important in ATP metabolism. Similar work has also been reported by Marschner (2012) in the case of *Cyathus stercoreus* where Ca was required for fruiting body formation.

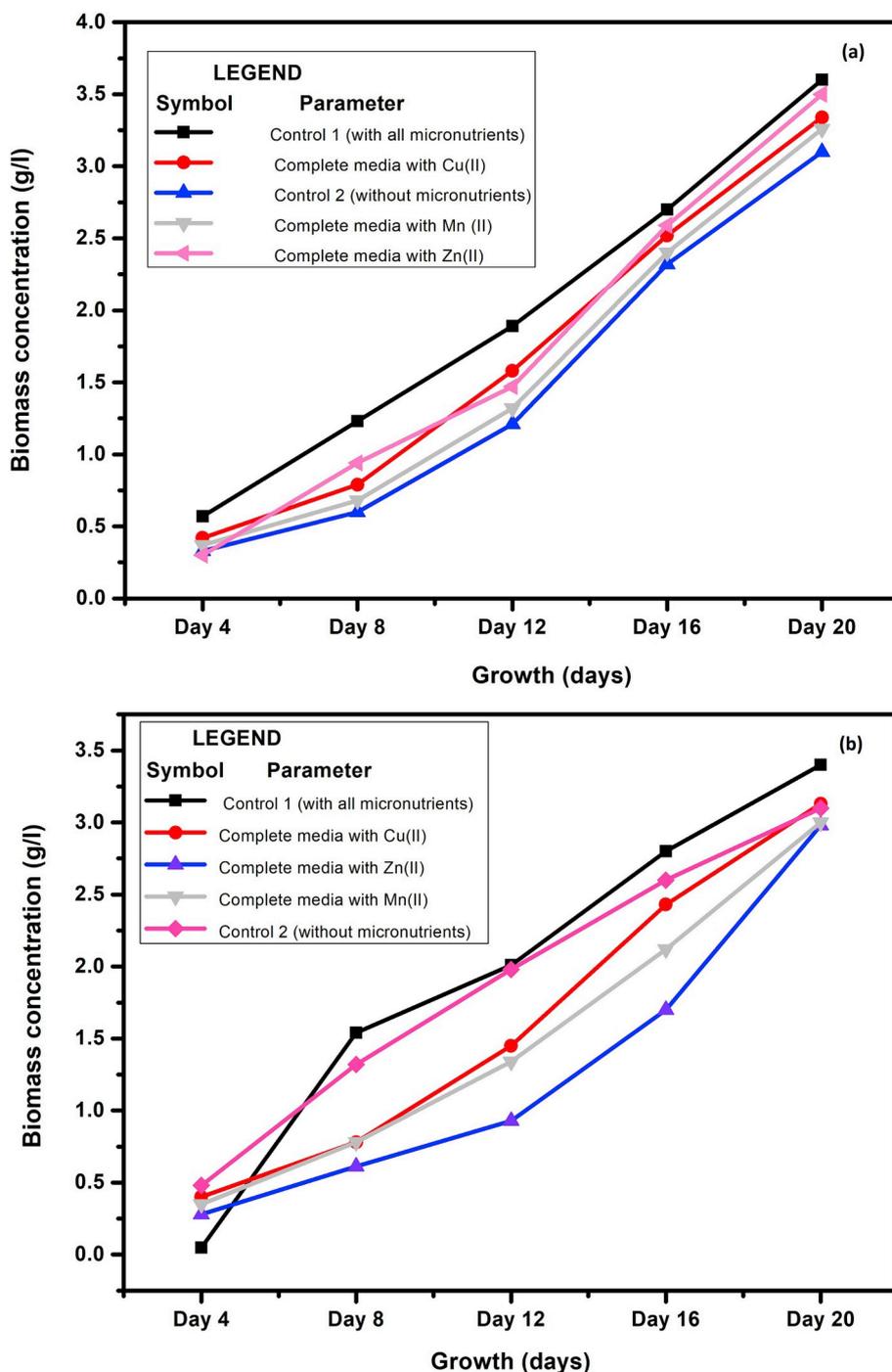


Fig. 5. Effect of micronutrients on mycelium growth of (a) *G. lucidum* and (b) *O. subilludens*.

However, the absence of a Na, stimulated a better mycelium growth in both organisms indicating the negative effect on the mycelium growth which is in accordance with the studies reported by Sykes and Porter (1973). Hence it can be inferred that Na did not have any significant effect on the mycelium growth.

3.3.4. Micronutrients

Zn is required for intermediary metabolism and is a functional component of a variety of fungal enzymes ranging from those involved in intermediary metabolism to the synthesis of DNA and RNA (Griffin, 1994) whereas Cu plays a key role as an enzyme activator, induces laccase expression in mycelium (Palmieri et al., 2000). Mg is also an important cofactor for the activation of manganese peroxidase activity

which is involved in delignification and is found in mushrooms required for degradation of the complex substrate as reported by Lamar (1992). Galhaup et al. (2002) have reported that the presence of Cu and Mg stimulated laccase and manganese peroxidase production which contributed the mycelium growth in *Trametes sp.*, *Pleurotus eryngii* and *Pleurotus ostreatus*. Laccases and manganese peroxidase play a major role in the degradation of lignin based substrate to simpler carbohydrate polymers that can be utilised by mushrooms are a major nutrient source (Faisidi and Jonathan, 1994). Singhal and Rathore (2001) found that the addition of low concentrations of Zn, Cu and Mg into the metal-free synthetic cultivation medium increased the activity of laccase and manganese peroxidase of *Phanerochate chrysosporium*. The effect of individual micronutrients was studied on *G. lucidum*, it was observed that

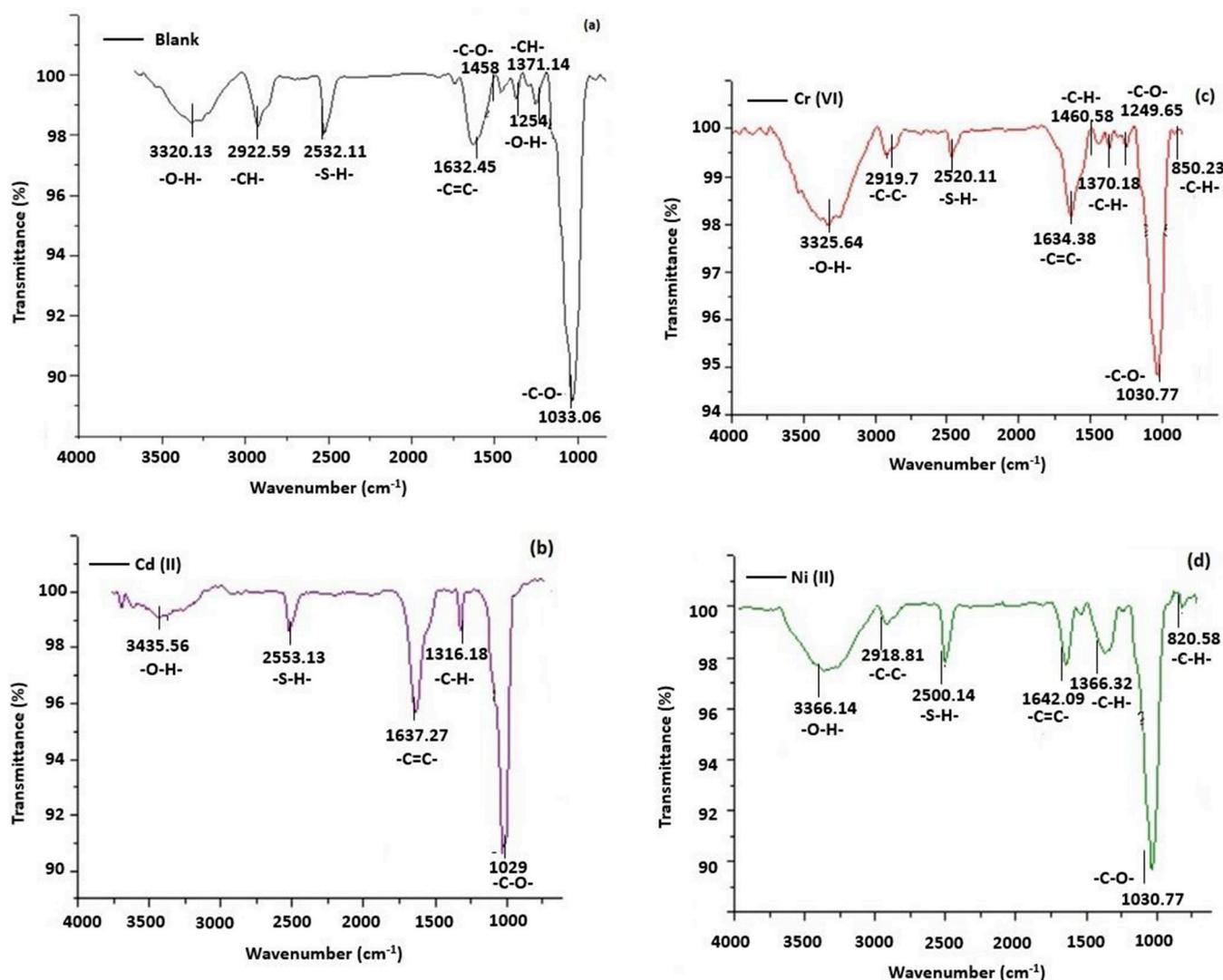


Fig. 6. FTIR analysis spectra of *G. lucidum* from (a) metal free environment (control), (b) Cd (II), (c) Cr (VI) and (d) Ni (II) laden soil system.

Zn showed better biomass growth followed by Cu and Mg (Fig. 5 (a)) whereas for *O. subilludens* better growth was observed in media containing Cu followed by Mg and Zn (Fig. 5 (b)). Further when the basal medium was monitored without any micronutrients and it was observed that the mycelium growth had comparatively reduced in both the species.

3.4. Optimized growth conditions for mycelium growth

These optimized concentrations of all the favourable nutrients were further utilized to study the mycelium growth as represented in Table S1; Supplementary information. To enhance the growth of *G. lucidum* the basal media was amended with 20000 mg/L of glucose, 10000 mg/L of peptone, 560 mg/L of magnesium sulphate, 1000 mg/L of potassium hydrogen phosphate, 10000 mg/L of copper sulphate, 5 mg/L of zinc sulphate and manganese sulphate. In *O. subilludens* the basal media was amended with 20000 mg/L of sucrose, 10000 mg/L of peptone, 560 mg/L of magnesium sulphate, 1000 mg/L of potassium hydrogen phosphate, 7000 mg/L of copper sulphate, 5 mg/L of zinc sulphate and 2 mg/L of manganese sulphate.

3.5. Metal tolerance and sensing of metals

Tolerance studies conducted on mushroom isolates revealed that the

concentration of the heavy metals amended and ceased growth of the mushroom mycelium was identified as the maximum tolerant concentration of heavy metals for a particular species. For the various tested metal concentrations *G. lucidum* exhibited a maximum tolerant concentration of 1000 mg/kg for Cr (VI), 500 mg/kg for Ni (II), 10 mg/kg for Cd (II), and 100 mg/kg for Pb (II), showing tolerance in the order Cr (VI) > Ni (II) > Pb (II) > Cd (II) and did not show tolerance to Hg (II). The maximum tolerant concentrations of heavy metals for Cr (II), Ni (II) and Pb (II) were 700 mg/kg, 500 mg/kg and 700 mg/kg respectively (Table S2; Supplementary information). Hence, this organism showed excellent tolerance in the order Cr (VI) > Ni (II) > Pb (II) and did not show tolerance to Hg (II) and Cd (II) (Table S2; Supplementary information).

3.6. Optical characterization

FTIR analysis of mycelium extracts of *G. lucidum* and *O. subilludens* was used to determine the various changes in functional groups after absorption of heavy metals. Researchers like Yang et al. (1999) and Shi et al. (2002) have used FTIR to detect the presence of both primary and secondary stress factors in mushrooms. The spectra of *G. lucidum* and *O. subilludens* from metal free environment (control), Cd (II), Cr (VI), Ni (II) and Pb (II) laden soil system (Figure: 6 (a), (b), (c) and (d)). Presence of alkane, alkene, alcohol, amide and ether groups were observed

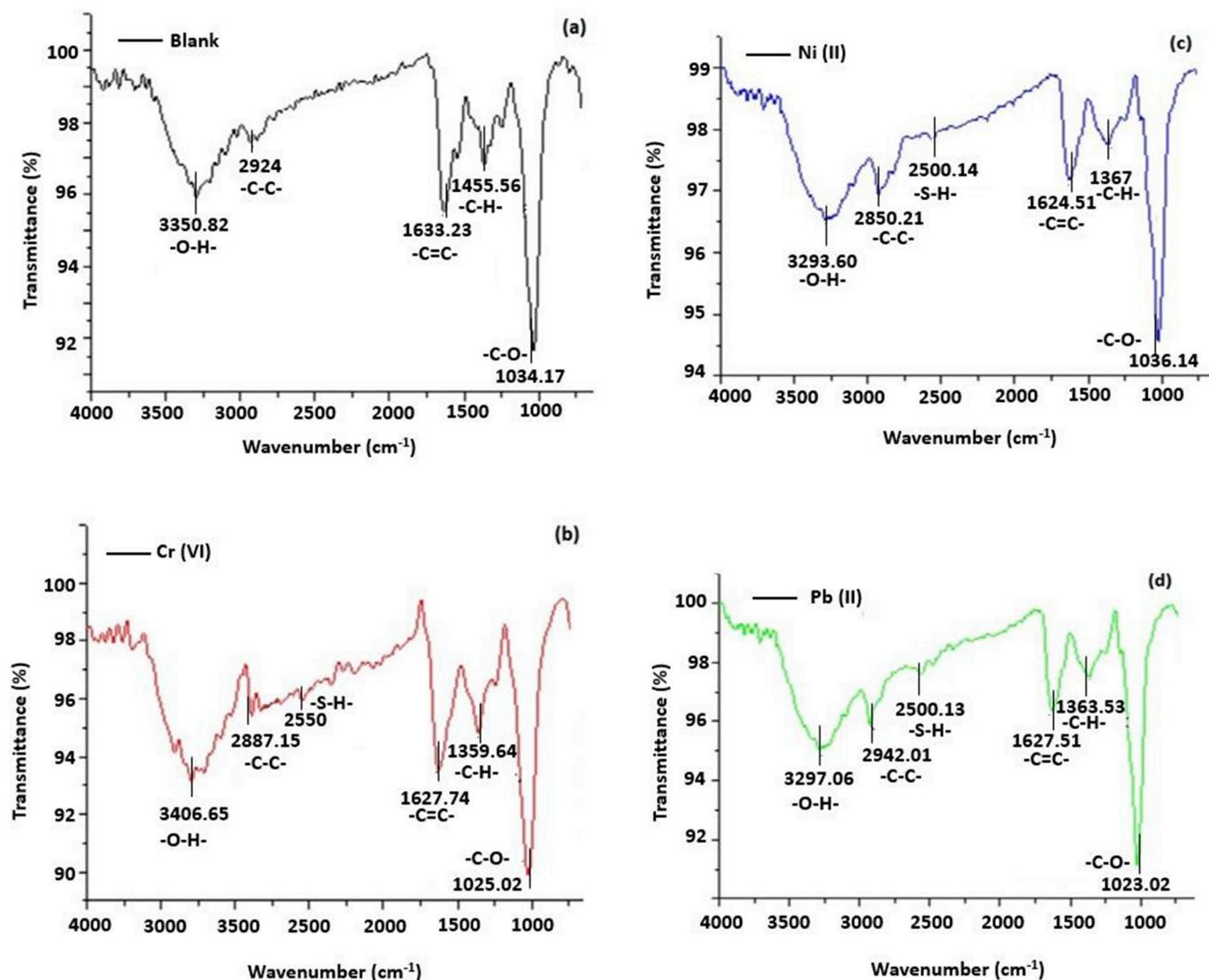


Fig. 7. FTIR analysis spectra of *O. subilludens* from (a) metal free environment (control), (b) Cr (VI), (c) Ni (II) and (d) Pb (II) laden soil system.

in all the samples of *G. lucidum* and *O. subilludens*. It also can be observed that Cr (VI), Ni (II) and Pb (II) FTIR shows peak range of 2500–2550 cm⁻¹ in both the species indicating the presence of thiol group (Fig. 7: (a), (b), (c) and (d)). Characteristic peak at a range of 1620–1650 cm⁻¹ and 1360–1380 cm⁻¹ were observed in all the samples excluding the control in both the organisms, indicates the presence of oxalic acid (Casliskan, 2000). Espejo and Agosin (1980) have studied the production of oxalic acid to counteract the metal stress in brown rot fungi; Sayer and Gadd (1997) also reported the production of oxalic acid by white rot mushrooms which provides a means of immobilizing soluble metal ions or complexes as insoluble oxalates, thus decreasing bioavailability and increasing metal tolerance and Akar and Tunali (2005) suggested that the main functional groups responsible for heavy metals biosorption are carboxylic, hydroxyl and amino groups.

4. Conclusions

In the present study, two macrofungi belonging to *Basidiomycota* phylum were collected which were identified as *G. lucidum* and *O. subilludens*. The media requirements were optimized in the study. The mushrooms showed maximum tolerant concentration towards heavy metal salts – Cd (II), Cr (VI), Ni (II) and Pb (II). The in-vitro cultivation of the mushrooms in the presence of modified media amended with

heavy metals showed a maximum tolerance level of Cr (VI) > Ni (II) > Pb (II) > Cd (II) in the case of *G. lucidum* whereas, *O. subilludens* expressed maximum tolerant concentrations of heavy metals Cr (VI) > Ni (II) > Pb (II). However, both the species showed no tolerance to media containing Hg (II). The change in physical and biological characteristics of two macro fungi identified as *G. lucidum* and *O. subilludens* under the influence of heavy metal toxicity and heavy metal removal potential. This work can be further extended to large-scale field studies and the future scope includes genetic engineering studies to find the gene responsible for heavy metal stress production to serve this technique as a better tool.

Acknowledgements

We sincerely thank Yaartzah Xenomics, Madurai Tamil Nadu for providing the 18 rRNA fungal sequencing. We also thank Dr. Anandhan Srinivasan, Associate Professor Metallurgy department NITK Surathkal, for providing FTIR facility.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101239>.

References

- Adaskaveg, J.E., Gilbertson, R.L., 1986. Cultural studies and genetics of *G. lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* complex. *Mycologia* 78 (6), 694–704.
- Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, second ed. Springer, New York, USA.
- Agomuo, E.N., 2011. Proximate, phytochemical, and mineral element analysis of the sclerotium of *Pleurotus tuber-regium*. *Interface Sci. J.* 3, 104–107.
- Akar, T., Tunali, S., 2005. Biosorption performance of *Botrytis cinerea* fungal by-products for removal of Cd(II) and Cu(II) ions from aqueous solutions. *Miner. Eng.* 18 (11), 1099–1109.
- Alvarez, A., Saez, J.M., Costa, J.S.D., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C.S., Polti, M.A., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere* 166, 41–62.
- Ayangbenro, A., Babalola, O., 2017. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int. J. Environ. Res. Public Health* 14 (1), 94.
- Bai, S.R., Abraham, E.T., 2002. Studies on chromium (VI) adsorption-desorption using immobilized fungal biomass. *Bioresour. Technol.* 85 (3), 17–26.
- Çalışkan, M., 2000. The metabolism of oxalic acid. *Turk. J. Zool.* 24 (1), 103–106.
- Chen, X.H., Hong, Bo, 2009. Analysis of several heavy metals in wild edible mushrooms from regions of China. *Bull. Environ. Contam. Toxicol.* 83, 280–285.
- Dangi, A.K., Sharma, B., Hill, R.T., Shukla, P., 2019. Bioremediation through microbes: systems biology and metabolic engineering approach. *Crit. Rev. Biotechnol.* 39 (1), 79–98.
- Espejo, E., Agosin, E., 1980. Production and degradation of oxalic acid by brown rot fungi. *Appl. Environ. Microbiol.* 57, 80–86.
- Fasidi, I.O., Jonathan, S.G., 1994. Growth requirements of *Volvariella esculenta* (Mass) Singer, a Nigerian edible mushroom. *Chem. Microbiol. Technol.* 16 (56), 151–155.
- Galhaup, C., Goller, S., Peterbauer, C.K., Strauss, J., Haltrich, D., 2002. Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. *An. Microbiol.* 148 (7), 2159–2169.
- Griffin, D.H., 1994. *Fungal Physiology* (Eds-2). Wiley Liss, New York.
- Gu, T., Rastegar, S.O., Mousavi, S.M., Li, M., Zhou, M., 2018. Advances in bioleaching for recovery of metals and bioremediation of fuel ash and sewage sludge. *Bioresour. Technol.* 261, 428–440.
- Jandaik, C.L., Kapoor, J.N., 1976. Effect of carbon and nitrogen nutrition on growth of *Pleurotus sajor-caju*. *Indian Phytopathol.* 29, 326–327.
- Järup, L., 2003. Hazards of heavy metal contamination. *Brux. Med.* 68, 167–182.
- Jayasinghe, B.D., Parkinson, D., 2008. Actinomycetes as antagonists of litter decomposer fungi. *Appl. Soil Ecol.* 38 (2), 109–118.
- Kadiiri, M., Fasidi, I.O., 1994. Growth requirements of *Lentinus subnudus* Berk, a Nigerian edible mushroom. *Chem. Mikrobiol. Technol. Lebensm.* 16 (3–4), 80–84.
- Kim, M.K., Ryu, J., Lee, Y., Kim, H., 2013. Breeding of a long shelf-life strain for commercial cultivation by mono-mono crossing in *Pleurotus eryngii*. *Sci. Hortic.* 162, 265–270.
- Kufuriji, O.O., Fasidi, I.O., 2009. Biodegradation of agro-industrial wastes by an edible mushroom *Pleurotus tuber-regium*. *J. Environ. Biol.* 30, 355–358.
- Lamar, R.T., 1992. The role of fungal lignin-degrading enzymes in xenobiotic degradation. *Curr. Opin. Biotechnol.* 3, 261–266.
- Marschner, P., 2012. *Marschner's Mineral Nutrition of Higher Plants*, vol. 3. Academic Press, London, UK, pp. 178–189.
- Ojuederie, O., Babalola, O., 2017. Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. *Int. J. Environ. Res. Public Health* 14 (12), 1504.
- Palmieri, G., Giardina, P., Bianco, C., Sanna, G., 2000. Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. *Appl. Environ. Microbiol.* 66 (3), 920–924.
- Pegler, D.N., Young, T.W.K., 1973. Basidiospore form in the British species of *G. Kars.* *Biores.* 28 (1), 241–252.
- Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F., Kim, K.H., 2019. Heavy metals in food crops: health risks, fate, mechanisms, and management. *Environ. Int.* 125, 365–385.
- San Keskin, N.O., Celebioglu, A., Sarioglu, O.F., Uyar, T., Tekinay, T., 2018. Encapsulation of living bacteria in electrospun cyclodextrin ultrathin fibers for bioremediation of heavy metals and reactive dye from wastewater. *Colloids Surfaces B Biointerfaces* 161, 169–176.
- Sayer, J., Gadd, G.M., 1997. Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycol. Res.* 106, 653–661.
- Shi, Y.B., Fang, J.L., Liu, X.H., Du, L., Tang, W.X., 2002. Fourier transform IR and Fourier Transform Raman spectroscopy studies of metallothionein-III: amide I band assignments and secondary structural comparison with metallothioneins-I and -II. *Biopolymers* 65 (2), 81–88.
- Singhal, V., Rathore, V.S., 2001. Effects of Zn²⁺ and Cu²⁺ on growth, lignin degradation and ligninolytic enzymes in *Phanerochaete chrysosporium*. *World J. Microbiol. Biotechnol.* 17 (3), 235–240.
- Sobariu, D.L., Fertu, D.I.T., Diaconu, M., Pavel, L.V., Hlihor, R.M., Drăgoi, E.N., Curteanu, S., Lenz, M., Corvini, P.F.X., Gavrilescu, M., 2017. Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *Biotechnology* 39, 125–134.
- Sobolev, D., Begonia, M.F.T., 2008. Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *Int. J. Environ. Res. Public Health* 5 (5), 450–456.
- Song, B., Zeng, G., Gong, J., Liang, J., Xu, P., Liu, Z., Zhang, Y., Zhang, C., Cheng, M., Liu, Y., Ye, S., 2017. Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. *Environ. Int.* 105, 43–55.
- Stamets, P., 1999. *Earth's Natural Internet*. Whole Earth magazine, United States.
- Stamets, P., 2005. *Mycelium Running. How Mushroom Can Help Save the World*, first ed.s. Ten speed Press, Berkeley, Toronto.
- Sykes, E.E., Porter, D., 1973. Nutritional studies of *Labyrinthula spp.* *Mycologia* 65, 1303–1311.
- Volesky, Z., Holan, S., 1995. Biosorption of heavy metals. *J. Biotechnol.* 3, 11235–11250.
- Yang, T.H., Dong, A., Meyer, J., Cleland, J.L., Carpenter, J.F., 1999. Use of infrared spectroscopy to assess secondary structure of human growth hormone within biodegradable microspheres. *J. Pharm. Sci.* 88 (2), 161–165.