IJBPAS, November, 2016, 5(11): 3003-3012

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CADMIUM AND CHROMIUM TOLERANCE AND MYCOREMEDIATION ABILITY OF TIGER SAWGILL MUSHROOM, *Lentinus tigrinus*

DULAY RMR[1*] AND DE CASTRO MEG[2]

1:Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija 3120 Philippines

2:Department of Biology, College of Science, De La Salle University, Taft Avenue, Manila, Philippines

***Corresponding Author: E-mail:** richmiltondulay@clsu.edu.ph

Received 18th July 2016; Revised 29th Aug. 2016; Accepted 22nd Sept. 2016; Available online 1st Nov. 2016

ABSTRACT

This work investigated the heavy metal tolerance and mycoremediation ability of *Lentinus tigrinus* on cadmium and chromium treated culture media and cellulosic substrate. Coconut water media with heavy metal significantly recorded lower mycelial growth diameter and higher percentage growth inhibitions compared to heavy metal- free media. At 1000 ppm, cadmium registered the mycelial growth diameter of 23.17 mm (73.65% inhibition) whereas chromium had 74.15 mm (17.40% inhibition). Although inhibited, 1000 ppm of both metal showed very thick mycelial density. In terms of the fruiting body, chromium substrate produced 154.20 g (30.84% BE) while cadmium substrate yielded 133.40 g (26.68% BE), which are significantly lower than the heavy metal-free substrate (161.14 g with 32.23% BE).Chemical analysis revealed that cadmium was not detected in the first flush of fruiting body while the second and third flushing contained 0.94 ppm and 1.52 ppm, respectively. Chromium was accumulated in the first (2.17 ppm), second (2.02 ppm) and third (4.29 ppm) flushing of fruiting bodies of the mushroom. Therefore, *L. tigrinus* is a mycoremediator agent with high tolerance to cadmium and chromium and its accumulation ability is dependent on the type of heavy metal and fructification interval of fruiting body.

Keywords: ***Lentinus tigrinus*, cadmium, chromium, mycoremediation.**

INTRODUCTION

Mushrooms are basidiomycetous fungi commonly known as macrofungi. They are important sources of protein and other essential nutrients. They are also used in the conversion of lignocellulosic waste materials into useful and functional fruiting body. Mushrooms produce basidiocarps consisting stipe and pileus that exhibit good potential to accumulate heavy metalsfrom their substrates. A number of studies have proved the mycoaccumulation abilities of various mushroom species (e.g. *Boletus* sp, *Agaricus* sp, *Pleurotus* sp., *Armillaria* sp., *Polyporus* sp., *Russula* sp., *Termitomyces* sp., etc.) as enumerated in the review of Dilna Damodaran et al. **[1]**.Several factors contribute to the accumulation ability of mushrooms which include among others, species of mushroom, type and nature of heavy metals were it is exposed, pH of the substrates used, flushing of fruiting bodies, age of culture mycelia and even the immediate temperature and humidity.

Heavy metals are one of the major toxicants affecting different organisms. They are metallic elements that have a relatively high density and have been used in many different areas for thousands of years **[2]**. They can contaminate the soil, water and air through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition **[3]**.Exposure to different heavy metals may also pose adverse health problems. For example, cadmium exposure can cause kidney damage and bone fractures.

Lentinus tigrinus, tiger sawgill mushroom, is a white-rot fungus commonly found growing on decaying woods or logs. This is an edible mushroom species but not grown in commercial scale because of the lack of popularity among Filipinos. Its fruiting body can cultivate using rice straw sawdust based substrate in artificial logs **[4]**. In this work, the mycelial growth response and mycoremediation potential of *L. tigrinus* in cadmium and chromium treated media were investigated and quantified.

MATERIALS AND METHOD

Source of mushroom culture

The pure culture of *L. tigrinus* was obtained from the culture collections of the CTMRD, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Culture was revived by inoculating a mycelial block onto potato dextrose agar plate. After 7 days of incubation, mycelial discs were prepared using a flame sterile 10 mm diameter cork borer which served as inoculants in the evaluation.

Mycelial growth evaluation

Coconut water agar with varying levels cadmium and chromium were used as culture media in the evaluation of mycelial growth. Four hundred ml of the medium was prepared and 100 ml was allotted for each concentration (0 ppm, 10 ppm, 100 ppm, and 1000 ppm) of each heavy metal. The 0 ppm serves as the control for the study. Each prepared medium was dispensed in a flask, plugged with cotton and properly labeled. These were sterilized in an autoclave at 121°C, 15 psi for 30 min. The different media were pour-plated and aseptically inoculated with mycelia discs from the pure culture. Triplicate plates were done per concentration of each heavy metal. The inoculated plates were incubated at 30 °C to allow mycelia growth. The daily mycelia growth was measured and the mycelia density was described as very thin (+), thin (2+), thick (3+), very thick or cottony (4+). The percentage inhibition of mycelial growth was also calculated as follows: Growth inhibition (%) = [(growth diameter of control – growth diameter of treated) / growth diameter of control] x 100.

Grain spawn preparation

Two hundred grams of rice seeds were boiled until swelling and slit opening of the husk attained. After which, seeds were air-dried until 65% moisture content was reached, and 40 g of boiled seeds were dispensed into polypropylene plastics plugged with cotton and wrapped with recycled paper. These were sterilized in an autoclave at 15 psi, 121°C for 30 min and aseptically inoculated with mycelia discs of mushroom. Grain spawn were incubated at 30°C for 10 days which served as the inoculant of the fruiting bags containing substrate contaminated with heavy metals.

Fruiting body production and evaluation of accumulation ability

The effect of the two heavy metals on the fruiting body production and ability of *L. tigrinus* to accumulate cadmium and chromium from the rice straw - sawdust based substrate were evaluated. Five hundred grams of formulated substrate (7 parts rice straw and 3 parts sawdust by volume) was compacted in a polypropylene plastic. Each bag was infused with 2 ml of 1000 ppm of heavy metal with 5 replicates for each treatment. Heavy metal free bags served as the control. These were individually provided with opening using cut pvc pipe, plugged with cotton, covered with recycled paper, and sterilized at 15 psi, 121 °C for 45 minutes. After cooling, each bag was inoculated with grain spawn and subsequently incubated at 30 °C in the incubation room. The incubation period was recorded. Once completely colonized with mycelia, fruiting bags were transferred into the growing house with 80-90% Rh to allow fruiting body development. The fruiting bodies were harvested, weighed and air-dried up to the third flush. The biological efficiency was computed and the the accumulated amounts of heavy metal of the three flushes of the fruiting body were analyzed using atomic absorption spectrophotometer.

Statistical analysis

Experiment was laid out in a Completely Randomized Design (CRD). Data were analyzed using analysis of variance (ANOVA) single factor. Duncan Multiple Range Test (DMRT) was used to determine the significant difference of the treatments at 5% level of significance.

RESULTS AND DISCUSSION

Mycelial growth of *L. tigrinus* on heavy metal treated media

Mycelium is the vegetative structure of mushroom. The effect of certain chemicals or compounds to mushroom can be directly identified and observe on their mycelial growth responses. In the present study, the mycelial growth response of *L. tigrinus* on coconut water agar at varying concentrations of cadmium and chromium was evaluated. The mycelial growth diameter, growth inhibition and mycelia density of *L. tigrinus* on solid media with varying levels of cadmium and chromium after 7 days of incubation are presented in Table 1.Apparently, heavy metal treated media significantly recorded lower mycelial growth diameter compared to heavy metal-free media. It can also be noticed that the effect of the heavy metals was concentration-dependent, that is, as concentration increased the mycelial growth diameter decreased. The medium with 1000 ppm of cadmium registered the mycelial growth diameter of 23.17 mm while medium with 1000 ppm of chromium had 74.15 mm. In terms of growth inhibition, 10 ppm, 100 ppm, and 1000 ppm of cadmium showed percentage growth inhibitions of 3.06%, 19.32%, and 73.65%, respectively. On the other hand, chromium-treated media respectively have 2.05%, 8.81%, and 17.40% growth inhibitions. However, although inhibited, the two highest concentrations (100 ppm and 1000 ppm) of both heavy metals showed very thick mycelial density (Figure 1). These results show that *L. tigrinus* has high tolerance to chromium but high sensitivity to cadmium.

The tolerance of *L. tigrinus* mycelia to heavy metals may vary depending on the concentration and type of heavy metals. The strong tolerance at high concentrations of heavy metal in the substrate of mushrooms through various mechanisms was also observed and reported by Bruins *et al.* **[5]** and Cobbett and Goldsbrough **[6]**. Moreover, *Pleurotus* species, particularly *P. ostreatus*, showed high resistance to heavy metals like copper, cadmium, zinc, nickel, cobalt, and mercury **[7]**.

Table 1: Mycelial growth diameter, percentage inhibition, and mycelial density of *L. tigrinus* on solid media with different concentrations of cadmium and chromium after 7 days of incubation

Cadmium (ppm)	Mycelial Growth Diameter (mm)	Growth Inhibition (%)	Mycelial Density
0	89.77 ± 0.39 [a]	0.00	+++
10	87.09 ± 0.36 [b]	3.06	++
100	72.43 ± 0.05 [c]	19.32	++++
1000	23.17 ± 0.96 [d]	73.65	++++
Chromium (ppm)			
0	89.77 ± 0.39 [a]	0.00	+++
10	87.93 ± 0.35 [b]	2.05	++
100	81.86 ± 0.19 [c]	8.81	++++
1000	74.15 ± 0.58 [d]	17.40	++++

In mycelial diameter column, means with the same letter of superscript are not significantly different at 5% level of significance using DMRT. + very thin, ++ thin, +++ thick, ++++ very thick.

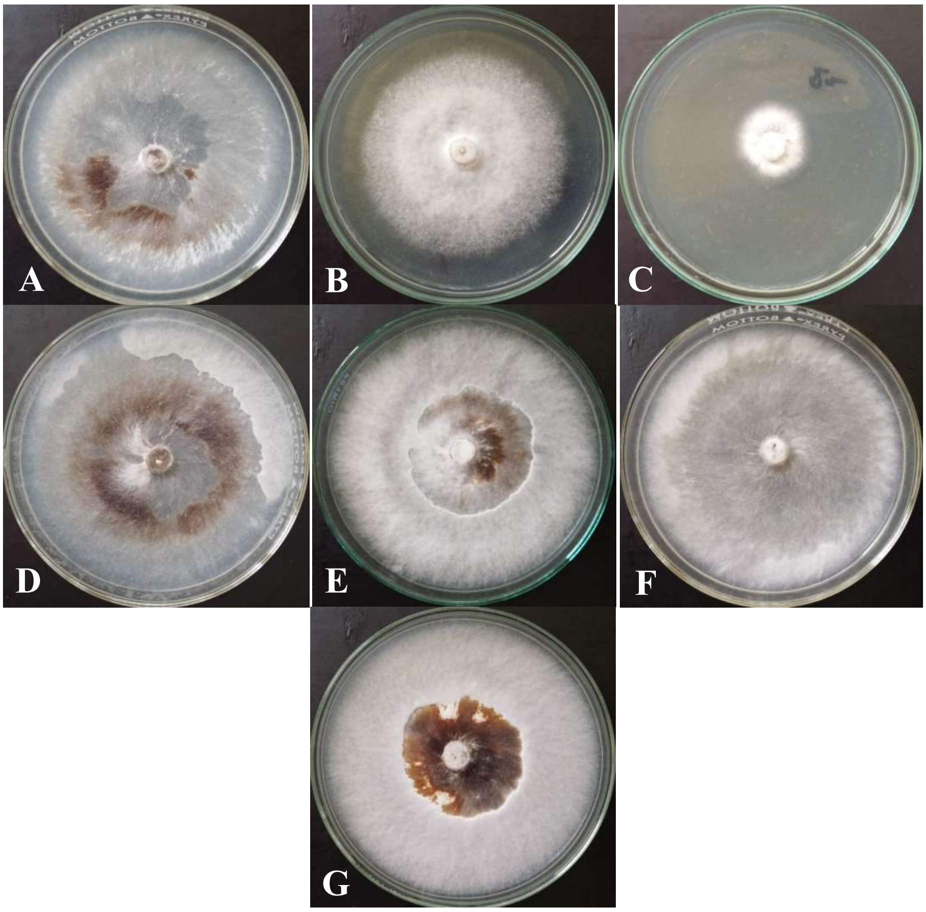

Figure 1: Plate cultures of *L. tigrinus* on coconut water agar with cadmium: (A) 10 ppm; (B) 100 ppm; and (C) 1000 ppm; chromium: (D) 10 ppm; (E) 100 ppm; and (F) 1000 ppm; and heavy metal free media (G) 0 ppm after 7 days of incubation.

Yield and biological efficiency of *L. tigrinus*

Fruiting bodies are the reproductive phase and are visible part of the mushroom which can be picked by hands. Nutrients and other elemental compositions of the substrate are accumulated by the mycelia and transported to their fruiting bodies. The effects of cadmium and chromium in the fruiting body development and heavy metal accumulation ability of *L. tigrinus* were investigated. The yield and biological efficiency of *L. tigrinus* grown on heavy metal-free and heavy metal-treated substrates are presented in Table 2. It can be seen that incubation period of mushroom was not significantly affected the presence of heavy metal in the substrate. However, the presence of heavy metal in the substrate significantly affects the total yield of fruiting bodies of *L. tigrinus*. Chromium substrate produced 154.20 g (30.84% BE) while cadmium substrate yielded 133.40 g (26.68% BE), which are significantly lower when compared to the heavy metal free substrate. The fruiting bodies of *L. tigrinus* grown on heavy metal free and treated substrates are shown in Figure 2.

Table 2. Yield and bio-efficiency of *L. tigrinus* grown on heavy metal-free and heavy metal-treated substrates

Parameters	Heavy metal free	Cadmium treated	Chromium treated
Incubation Period (day)	28.67	28.83	29.00
Yield of 1st flush (g)	78.34	66.41	73.60
Yield of 2nd flush (g)	48.57	39.25	46.29
Yield of 3rd flush (g)	34.23	27.74	34.31
Total yield (g)	161.14	133.40*	154.20*
Biological efficiency (BE) (%)	32.23	26.68*	30.84*

In total yield and BE, asterisk (*) indicates significant difference with the control (heavy metal free) at 5% level of significance using T-test.

Figure 2: Fruiting body of *L. tigrinus* grown on (A) heavy metal-free, (B) cadmium treated, and (C) chromium treated substrates.

Mycoremediation ability of *L. tigrinus*

Mushrooms can act as an effective biosorbent of toxic metals. In order to determine the ability of *L. tigrinus* to accumulate cadmium and chromium from the substrate with heavy metals, the fruiting bodies were subjected to cadmium and chromium detection analysis using atomic absorption spectrophotometer. The results of the analyses are presented in Table 3. These results proved the mycoremediation capability of *L. tigrinus* although the accumulated amount of heavy metals varied in three different flushes. An increasing amount of heavy metals was observed in every flushing of the fruiting bodies. In cadmium-treated substrate, accumulation was not noticeable during the first flush while in the second and third flushes have 0.94 ppm and 1.52 ppm, respectively. On the other hand, the first, second and third flushes of fruiting bodies harvested from chromium treated substrate have accumulated 2.17 ppm, 2.02 ppm, and 4.29 ppm, respectively. This suggests that the accumulation ability of *L. tigrinus* depends on the type of heavy metals and fructification interval. This finding is in conformity with the previous observations of Doĝan *et al.* **[8]** and Dilna Damodaran *et al.* **[1]** who reported that heavy metal concentrations vary depending on types of heavy metal, species of mushroom, and interval between fructification (formation of fruiting body).

Table 3: Heavy metal content of fruiting bodies of *L. tigrinus* grown on heavy metal-free and heavy metal-treated substrates

Parameters	Amount of heavy metals		
	1st flush	2nd flush	3rd flush
Heavy metal free	ND	ND	ND
Cadmium treated	ND	0.94	1.52
Chromium treated	2.17	2.02	4.29

ND means not detected

Several researches have been done on the biosorption and bioaccumulation abilities of wide spectra of edible and wild mushrooms. For instance, wild *Lactarius piperatus* was proven to have higher biosorption efficiency of cadmium ions than the cultured *Agaricus bisporus* **[9]**. In another study, *Pleurotus platypus*, *Agaricus bisporus*, *Calocybe indica* efficiently absorb copper, zinc, iron, cadmium, lead, nickel from the aqueous solution **[10]**. Akin et al. **[11]** studied the amount of five heavy metals (Cd, Cr, Cu, Pb, and Zn) in three edible mushroom species namely *Lactarius deliciosus*, *Russula delica*, and *Hizopogon roseolus* collected from the sampling sites of Canakkale province, Turkey. They found out that the highest amounts of Cd, Cu, Pb, and Zn were in *R. delica*, while the highest level of Cr was observed in *L. deliciosus*.

The uptake of heavy metals by mushrooms involves two important mechanisms, (*a*) bioaccumulation (active metabolism-dependent) that includes heavy metal transport into the cell and partitioning into intracellular components, and (*b*) biosorption (active metabolism-independent) that includes heavy metal covalent binding and ion exchange **[12]**. Blaudez *et al.***[13]** reported that the accumulation of cadmium in the mushroom is through sub-cellular compartmentalization. Mushrooms uptake heavy metals in the form of organic or inorganic compounds and may get associated with lipids and proteins. These complexes form the integral part of a cell wall or cytoplasm or vacuole over the period of time based on the type of mechanisms involved during the uptake.

Aside from the biosorption and bioaccumulation abilities, mushrooms can also degrade pollutants by producing complex compounds. They can produce extracellular peroxidases, cellulases, pectinases, xylanases, and oxidases **[14]** which found to degrade non-polymeric, recalcitrant pollutants such as nitrotoluenes, PAHs, organic and synthetic dyes, and pentachlorophenol in vitro **[12]**. These capabilities of mushrooms and their role as agents of bioremediation could help solve problems on heavy metal pollution and contamination. However, on the other hand, consumption of the protein-rich fruiting body grown on substrates with heavy metal could cause human health risks. The toxicity and genotoxicity of mushrooms species grown on the different lignocellulosic wastes should be assessed and the non-toxic mushrooms can be used for consumption. In addition, the active biochemical present in the edible fruiting bodies should likewise be determined in detail.

CONCLUSION

L. tigrinus mycelia have high tolerance to chromium but high sensitivity to cadmium. The yield and bioefficiency of fruiting body of *L. tigrinus* are significantly affected by the presence of cadmium and chromium in the substrate. The accumulated amount of heavy metal increased in the interval of fructification or flushing of fruiting bodies. Accumulation is also dependent on the type of heavy metals present in the substrate. These important capabilities of *L. tigrinus* indicate its potential as bioremediation tool in solving problems on heavy metal contamination or pollution.

REFERENCES

[1] Dilna Damodaran Raj, Mohan B, Vidya Shetty BM. Mushrooms in the remediation of heavy metals from soil. International Journal of Environmental Pollution Control & Management,2011, 3(1), 89-101.

[2] Jarup L. Hazards of heavy metal contamination. British Medical Bulletin, 2003,68, 167-82.

[3] Wuana RA, Okieimen FE. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. 2014,5, 15.

[4] Dulay RMR, Kalaw SP, Reyes RG, Cabrera EC, Alfonso NF. Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., a new record of domesticated wild edible mushroom in the Philippines. Philip Agric Scientist, 2012, 95(3), 278-285.

[5] Bruins MR, Kapils S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety, 2000, 45, 198-207.

[6] Cobbett C, Goldsbrough P. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology, 2002, 53, 159-182.

[7] Sanglimsuwan S, Naoto Y, Tsutomu M, Yoshikatsu M.resistance to and uptake of heavy metals in mushrooms. Journal of Fermentation and Bioengineering, 1993, 75(2), 112-114.

[8] Doĝan HH, Sanda MA, Uyanöz R, Öztürk C, Cetin U. Contents of metals in some wild mushrooms. Biological Trace Element Research, 2006, 110(1), 79-94.

[9] Nagy B, Maicaneanu A, Indolean C, Manzatu Z, Silaghi-Dumitrescu MC. Comparative study of Cd (II) biosorption on cultivated *Agaricus bisporus* and wild *Lactarius piperatus* based biocomposites. J Taiwan Inst Chem., 2013, doi:10.1016/j.jtice.2013.08.013

[10] Lamrood PY, Ralegankar SD. Biosorption of Cu, Zn, Fe, Cd, Pb and Ni by non-treated biomass of some edible mushrooms. Asian J Exp Biol Sci., 2013, 4, 190-195.

[11] Akin C, Munevver C, Mahmut C. The heavy metal content of wild edible mushroom samples collected in Canakkale Province, Turkey. Biology of Trace Elements, 2010, 134, 212-219.

[12] Kulshreshtha S, Mathur N, Bhatnagar P. Mushroom as product and their role in mycoremediation. AMB Express, 2014, 4, 29.

[13] Blaudez D, Bernard B,Michel C. Cadmium uptake and subcellular compartumentalization in the ectomycorrhizal fungus *Paxillus involutus*. Journal of Microbiology, 2000, 146, 1109-1117.

[14] Nyanhongo GS, Gubitz G, Sukyai P, Leitner C, Haltrich D, Ludwig R. Oxidoreductases from *Trametes* spp. in biotechnology: a wealth of catalytic activity. Food Technol Biotechnol., 2007, 45, 250-268.