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**EFFECT OF LEAD (Pb) IN MYCELIA AND SPOROCARP OF *Lentinus tigrinus*  
MUSHROOM**

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**ABSTRACT**

The effect of Pb on mycelial growth, sporocarp production and the accumulation ability of *Lentinus tigrinus* were investigated. The presence of Pb in potato dextrose agar (PDA) showed inhibitory effect on the mycelial growth of *L. tigrinus*. After 4 days of incubation, 100 ppm of Pb had 64.66 mm mycelial diameter which is statistically comparable to the other two lower concentrations. With regards to sporocarp production, both treatments showed incubation period of 28 days while Pb-contaminated substrate significantly recorded shorter period (29 days) of primordial initiation than that of the Pb-free substrate (32 days). Pb-free substrate produced heavier sporocarp in all flushes than in Pb-contaminated substrate having total yields of 215.17 g (28.69% BE) and 202.03 g (26.94% BE), respectively. The pileus of sporocarp from Pb-contaminated substrate had narrower diameter and wrinkled. Pb accumulation significantly varied in three different flushes having 1.03 ppm, 3.57 ppm, and 9.21 ppm of Pb, respectively. Thus, fructification interval or flushing is an important factor in the accumulation ability of *L. tigrinus*.

**Keywords: *Lentinus tigrinus*, sporocarp, mycelia, lead (Pb), toxic metals**

## INTRODUCTION

Mushrooms are used in the bio-conversion of agro-industrial wastes into protein source. The different wastes including rice straw, rice hull, sawdust, banana leaves, coconut coir, rice bran, water lilies, corn cobs, corn stalks, tobacco midrib, leguminous leaves, paper waste and other cellulosic materials are formulated into mushroom substrates. In mushroom culture, minerals and some toxic metals from the substrate are being accumulated by the mycelia and translocated into the sporocarp. For instance, the *Laetiporus sulphureus* collected from Northern Thailand contained Fe, Zn, Mn, Cu, Na, Ca and Mg [1] while all eleven fruiting bodies of wild edible mushrooms growing in the East Black Sea Region of Turkey such as *Laccaria laccata*, *Leucopaxillus giganteus*, *Russula rosea*, *Cantharellus cibarius*, *Tricholoma saponaceum*, *Agaricus arvensis*, *Boletus edulis*, *Clavulina rugosa*, *Hydnum repandum*, *Cantharellus tubaeformis*, and *Lepista nuda* have considerable amounts of As, Cd and Pb [2].

Lead (Pb) is one of the trace metals which the level in the environment represent a reliable index of pollution. In human physiology, Pb has no beneficial role in the metabolic processes. According to Agency for Toxic Substances & Disease Registry [3],

acute exposure to very high levels of Pb may cause encephalopathy and other accompanying signs of ataxia, coma, convulsions, death, hyperirritability, and stupor in children. In adults, higher exposures to Pb increase the risk for hypertensive heart disease and cerebrovascular disease as latent effects.

*Lentinus tigrinus* is a wood rotting basidiomycetes that is commonly found growing on decaying logs in the forest. It is also called as *kabuteng tigre* because of its color and appearance. Previously, we have successfully established its production technology using rice straw sawdust based substrate formulation and elucidated its functional activities [4, 5]. With the aim to demonstrate other biopotentials of this wood-rotting mushroom, this present work evaluated the effect of Pb on the mycelial growth and sporocarp production of *L. tigrinus*. The ability of *L. tigrinus* sporocarp in three flushes to uptake lead from the lead contaminated substrate was also investigated.

## MATERIALS AND METHOD

### Source of mushroom and inoculant preparation

The wild fruiting bodies of *L. tigrinus* were collected from the forested area of Central Luzon State University, Science City of

Munoz, Nueva Ecija, Philippines. The collected samples were brought in the mushroom laboratory for tissue culture on potato dextrose agar plates. After 7 days of incubation, a flame sterile 10 mm diameter cork borer was used to prepare mycelial discs as culture inoculant in growth response evaluation.

### **Evaluation of mycelial growth**

Potato dextrose agar with varying levels lead sulfate  $PbSO_4$  was 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, 1 ppm, 10 ppm, and 100 ppm). A Pb free medium served as the control, 0 ppm. 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 Pb. 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 (++), thick (+++), very thick or cottony (++++).

### **Preparation of grain spawn**

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 Pb.

### **Evaluation of sporocarp production and accumulation ability**

The effect of Pb on the sporocarp production and ability of *L. tigrinus* to accumulate lead from the rice straw - sawdust based substrate was also 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 Pb with 5 replicates for each mushroom species. Pb 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 and the initiation of primordia were recorded. Once completely colonized with mycelia, fruiting bags were transferred into the growing house with 80-90% RH to allow fruiting body development. The sporocarp were harvested, weighed and air-dried up to the third flush. The biological efficiency was computed. The accumulated amounts of Pb of the three flushes of sporocarps 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) and T-test. Duncan Multiple Range Test (DMRT) was used to determine the significant difference of the treatments at 5% level of significance.

### RESULTS AND DISCUSSION

#### Effect of Pb on *L. tigrinus* mycelial growth

The mycelial growth of *L. tigrinus* on PDA with varying concentration of Pb was evaluated (Table 1). The presence of Pb reduced the mycelial growth of *L. tigrinus*. It can be seen that in all observation periods, mycelial growth diameter was significantly lower in Pb-treated media. After 4 days of

incubation, 100 ppm of Pb had 64.66 mm mycelial diameter which statistically comparable with the other two lower concentrations. This result showed that Pb at higher concentration has inhibitory effect on the growth rate of *L. tigrinus* but this ability to grow at these concentrations of Pb strongly indicates its resistance. However, with regards to the mycelial density, no significant difference was observed in *L. tigrinus* grown on the media with varying concentrations of Pb, which produced very thick mycelia as shown in Figure 1.

In the previous study of Lee et al. [6], addition of Pb and Cr to PDA media of white color mutant of *Pleurotus ostreatus* resulted in the increase of the fungal growth rate while Cd and Cu strongly inhibited mycelial growth. Among the 21 species of mushrooms evaluated by Sanglimsuwan et al., [7], the *Pleurotus* species strains showed higher resistance to copper, cadmium, zinc, nickel, cobalt, mercury than the other species and *Pleurotus ostreatus* showed the highest resistance. They also reported that *Pholiota* species, *Flammulina veltipes*, *Lyophyllum ulmarium*, *Agaricus bisporus* and *Polyporus arcularius* were rather sensitive to all the heavy metals tested.

Lead (ppm)	Mycelial Growth Diameter (mm)				Mycelial Density
	Day 1	Day 2	Day 3	Day 4	
0.0	17.33 <sup>a</sup>	27.33 <sup>a</sup>	49.66 <sup>a</sup>	70.33 <sup>a</sup>	++++
1.0	14.33 <sup>b</sup>	24.33 <sup>b</sup>	45.66 <sup>b</sup>	66.66 <sup>b</sup>	++++
10.0	14.00 <sup>b</sup>	23.33 <sup>b</sup>	43.66 <sup>b</sup>	65.00 <sup>b</sup>	++++
100.0	13.66 <sup>b</sup>	23.00 <sup>b</sup>	43.33 <sup>b</sup>	64.66 <sup>b</sup>	++++

In each 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

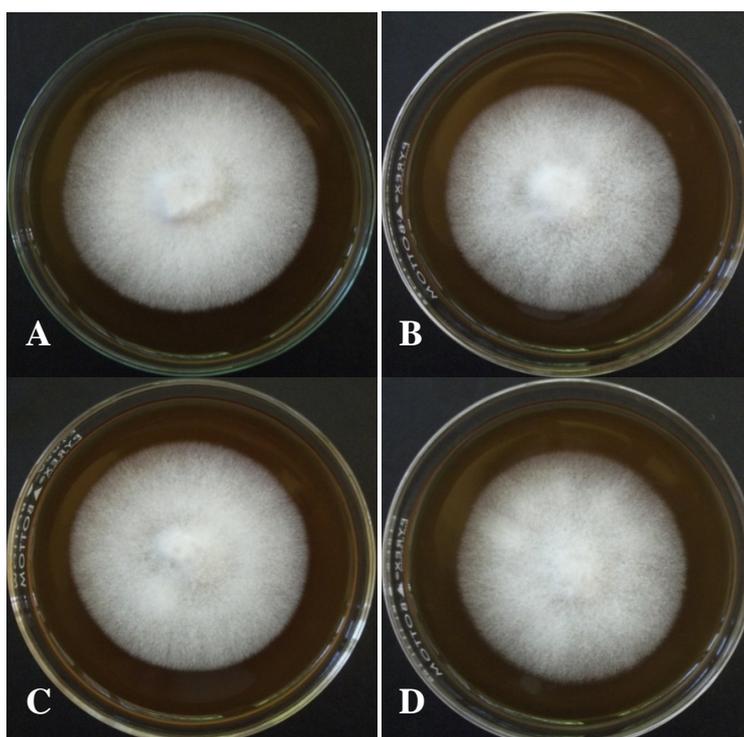


Figure 1: Plate cultures of *L. tigrinus* on Pb contaminated PDA at (A) 0 ppm, (B) 1 ppm, (C) 10 ppm and (D) 100 ppm after 4 days of incubation.

### Effect of Pb on the development and yield of sporocarp

Aside from the response of mycelia to PDA with Pb, the production performance of *L. tigrinus* as affected by Pb was also considered in the present study. Table 2 shows the effect of Pb on the sporocarp development and yield of *L. tigrinus*. Both

treatments showed incubation period of 28 days. In terms of the initiation of primordia, *L. tigrinus* in Pb-contaminated substrate significantly recorded shorter period (29 days) than that of the Pb-free substrate (32 days). In fructification, Pb-free had 35 days while Pb-contaminated had 37 days. The presence of Pb delayed the emergence of

sporocarp. With regards to the yield, Pb-free substrate produced heavier sporocarp in all flushes than in Pb-contaminated substrate, which are respectively having total yields of 215.17 g (28.69% BE) and 202.03 g (26.94% BE). These results strongly imply the significant effect of Pb on the different development phases and yield of *L. tigrinus* sporocarp. Interestingly, although Pb significantly affects the sporocarp development of *L. tigrinus*, still sporocarps emerged luxuriantly (Figure 2B), indicating its promising potential as heavy metal accumulator. Contrastingly, in the study of Oghenekaro et al., [8], the fruiting body

development of *Pleurotus tuberregium* was inhibited by the presence of Pb and Zn in the substrate. However, those grown in Cu-contaminated substrate produced fruiting bodies with very low biological efficiency. High Pb concentration in the substrate can cause maximum reduction in the production of fruiting body of mushrooms [9]. In terms of the sporocarp quality, the pileus of sporocarp from Pb-contaminated substrate had narrower diameter but longer stipe and some were found wrinkled. Therefore, aside from the yield and development, the quality of the sporocarp was also affected by this metal.

Parameters	Lead-Free	Lead-Contaminated
Incubation Period (day)	28 <sup>a</sup>	28 <sup>a</sup>
Initiation of Primordia (day)	32 <sup>a</sup>	29 <sup>b</sup>
Fructification (day)	35 <sup>b</sup>	37 <sup>a</sup>
Yield of 1 <sup>st</sup> flush (g)	89.37 <sup>a</sup>	83.21 <sup>b</sup>
Yield of 2 <sup>nd</sup> flush (g)	73.49 <sup>a</sup>	71.52 <sup>a</sup>
Yield of 3 <sup>rd</sup> flush (g)	52.31 <sup>a</sup>	47.30 <sup>b</sup>
Total yield (g)	215.17 <sup>a</sup>	202.03 <sup>b</sup>
Biological efficiency (BE) (%)	28.69 <sup>a</sup>	26.94 <sup>b</sup>

In each row, means with the same letter of superscript are not significantly different at 5% level of significance using T-test.



Figure 2: Sporocarp of *L. tigrinus* on (A) Pb-free and (B) Pb-contaminated substrate

### Accumulation ability of *L. tigrinus* sporocarp

In order to determine the ability of *L. tigrinus* to accumulate Pb from the substrate added with Pb, the harvested sporocarps were subjected to lead detection analysis using atomic absorption spectrophotometer. Table 3 shows the lead content of sporocarp and spent of *L. tigrinus*. It can be noticed that the accumulation of Pb significantly varied in three different flushes. The first, second and third flushes have accumulated 1.03 ppm, 3.57 ppm, and 9.21 ppm of Pb, respectively.

Apparently, there was an increasing Pb concentration in every flush of the sporocarp. Based on this observation, it is therefore important to determine the flush of sporocarp that would accumulate the maximum amount of Pb. It was not determined in the present because the Pb-contaminated fruiting bag only produced sporocarp up to three flushes. The low accumulation of Pb in the first flush could probably due to mechanism of uptake and translocation of Pb by the mycelia, which clearly indicate a time-dependent translocation.

Substrate	Amount of heavy metals (ppm)			
	Sporocarp			Spent
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	
Lead-free	ND	ND	ND	ND
Lead-contaminated	1.03	3.57	9.21	67.34

ND means not detected in the sample.

The results are in agreement with the previous results of Busuioc and Elekes [10] who reported that after lead acetate treatment in soil, *Russula vesca*, *R. atropurpurea* and *R. integra* showed an increased Pb bioaccumulation capacity compared to the control. They also added that Pb ions are being absorbed during the absorption of other essential elements from the substrate. The concentrations of the different mushroom species on various reports were found between 0.1 and 40 mg/kg [11]. In a review

on the Pb concentrations in 25 mushroom species by Kalac and Avoboda [12], *Lycoperdon perlatum*, *Macrolepiota rhacodes*, and *Lepista nuda* are the highly accumulating species while *Xeroconomus badius*, *Suillus variegatus* and *Lactarius volemus* are the low Pb accumulators. In addition, some wild mushrooms collected from Yunnan Province in China also contain Pb ranging from 0.67 to 12.9 mg/kg. These mushrooms include *Coprinus comatus* (1.61 mg/kg), *Volvariella volvacea* (1.70 mg/kg),

*Pleurotus nebrodensis* (2.49 mg/kg), *Hypsizigus marmoreus* (2.62 mg/kg), *Hericium erinaceus* (3.04 mg/kg), *Agrocybe aegerita* (4.43 mg/kg), *Lentinus edodes* (0.92 mg/kg), *Collybia velutipes* (0.99 mg/kg), *Agaricus bisporus* (2.21 mg/kg), *Russula albida* (3.28 mg/kg), *Clitocybe conglobata* (2.42 mg/kg), *Pleurotus eryngii* (12.9 mg/kg), *Lepista sordida* (3.08 mg/kg), *Pleurotus ostreatus* (0.67 mg/kg) [13]. Thus, the abilities of mushrooms to accumulate Pb from the substrate may vary depending on the species, fructification interval, and amount of Pb present in their substrates.

### CONCLUSION

Conclusively, the mycelial growth and sporocarp production of *L. tigrinus* are significantly affected by the presence of Pb in the solid media and substrate. The accumulated concentrations of Pb increased in the interval of fructification or flushes, therefore, considered as one of the important factors of Pb accumulation. This important biopotential of *L. tigrinus* denotes the usefulness of this wood-rotting basidiomycete for the mycoremediation technologies that can be used in addressing Pb contamination or pollution.

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