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EFFECT AND ACCUMULATION OF LEAD (Pb) IN MYCELIA AND FRUITING BODY OF *Lentinus sajor-caju*

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ABSTRACT

This paper highlighted the effect of lead on the mycelial growth and fruiting body production of *Lentinus sajor-caju*, and its ability to accumulate this metal. Potato dextrose agar with varying concentrations of lead showed inhibitory effect on the mycelial growth. A 100 ppm of lead had 59.43 mm mycelial diameter which is significantly lower when compared to the lead free media (63.76 mm) after four days of incubation. Lead at low concentration has no significant effect in the cultivation phases but significantly induced the yield (67.50 g) and biological efficiency (13.5%) of fruiting bodies. The maximum accumulation of lead (7.37 ppm) was recorded in the second flushing of *L. sajor-caju* basidiocarp. Thus, *L. sajor-caju* is another species of mushroom with great potential in bioremediation.

Keywords: *Lentinus sajor-caju*, myco-accumulation, sporocarp, mycelia, lead.

INTRODUCTION

Wild growing mushrooms have become popular delicacies for Filipinos. They can be found growing on rotten logs, rice straw, sawdust, banana leaves, soil, termite mound, animal dung, grasses, and other agricultural wastes. However, several studies have published that many wild growing species of mushrooms contain high amounts of heavy

metals such as lead, copper, cadmium, chromium, iron, manganese, zinc, nickel, aluminum, and mercury **[1, 2]**. Some of these metals are considered representative of a reliable index of environmental pollution and could cause toxic effects to any life forms. However, some are essential metals because they have important roles in biologic functions.

Lead naturally occurs in the Earth's crust but only in trace amounts **[3]**. It can also be present in food, water and air which may cause human health problems. The presence of lead in the body may accumulate and affect the brain and nerve causing impaired development especially in children, and heart and blood causing high blood pressure, heart diseases in men and anemia in women. It can also damage gastrointestinal tract, kidney, bone, liver, thyroid gland and reproductive organs **[4, 5, 6]**.

Lentinus sajor-caju, also known as *kulat-kulat* because of leathery texture, is a wood rotting basidiomycetous fungus that is commonly found growing on the decaying logs. The fruiting body is tough with well-developed central stipe and gray to brown pileus. Santos et al. **[7]** optimized the spore germination and mycelial growth of *L. sajor-caju*. The basidiospores germinated rapidly on corn grit broth with a pH of 8.0 at air-conditioned room (23°C) and lighted conditions. Its mycelia grew luxuriantly on coconut water media with a pH of 8.0, and incubated in room temperature (32°C) and dark conditions. Basidiocarps were successfully grown using rice straw and sawdust based formulation as substrate in the fruiting bags. This mushroom contains important amino acid and exhibits functional activities such as anticoagulant and anti-inflammatory **[8]**. In this study, the effect of Pb on the mycelial growth and fruiting body production of *L. sajor-caju* and the ability of this mushroom to accumulate lead from the lead contaminated substrate were investigated.

MATERIALS AND METHOD

Source of mushroom and inoculant preparation

The pure culture of *L. sajor-caju* was obtained from the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The culture was revived on potato dextrose agar plates and incubated for 7 days. 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 fruiting body production and accumulation ability

The effect of Pb on the fruiting body production and ability of *L. sajor-caju* 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 fruiting 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 fruiting bodies 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. sajor-caju* mycelial growth

Mycelia are the vegetative structure of fungi particularly mushrooms. They accumulate nutrients, minerals and other elemental compositions from the substrates and transport to their fruiting bodies to produce biomass. The effect of varying concentrations of lead on the mycelial growth of *L. sajor-caju* was studied and the results were presented in Table 1. Apparently, in all observation periods, the mycelial diameters of *L. sajor-caju* were significantly lower when compared to the lead-free mycelia. After 4 days of incubation, media with 100 ppm of Pb had 59.43 mm mycelial diameter which statistically comparable with 60.12 mm obtained in media with 10 ppm. Similarly, these two concentrations only showed thick mycelial density compared to the very thick mycelial density observed at 1 ppm and lead-free media. These results clearly indicate that Pb at higher concentration has inhibitory effect on the growth rate of *L. sajor-caju*. But, its ability to grow at these concentrations dictates its strong resistance to lead.

Effect of Pb on the development and yield of fruiting body

The culivation phase and yield of fruting bodies of *L. sajor-caju* on the treated and lead free substrates were investigated. The effects of lead on the fruiting body development and yield of *L. sajor-caju* are shown in Table 2. Apparently, no significant difference was observed between lead-treated and lead-free substrates in terms of incubation period, initiation of primordia and fructification of *L. sajor-caju*. Contrastingly, the yield of fruiting body was significantly affected by the presence of lead in the substrate (Figure 1). Lead-free substrate produced higher yield in the first flush whereas lead-treated subtrate had higher yield in the second flush and third flush of fruiting bodies. In total, lead-treated substrate had higher total yield of 67.50 g compared to the total yield of 59.95 g in lead-free substrate. These results imply that the presence of lead at low concentration in the substrate could induce the yield of fruiting

bodies of *L. sajor-caju*, indicating its promising potential as lead accumulator.

Lead accumulation ability of *L. sajor-caju* fruiting body

The accumulation ability of lead in the fruiting bodies of *L. sajor-caju* was also investigated. The harvested fruiting bodies in every flush were air-dried and subjected to lead detection analysis using atomic spectrophotometer. The lead content of the fruiting bodies and spent of *L. sajor-caju* is presented in Table 3. It can be seen that the accumulation of lead in the fruiting body was significantly varied in the three different flushes. Lead was not detected in the first flush while the second flush recorded the maximum accumulation of 7.37 ppm. The third flush had 3.51 ppm. The non-detection of lead in the first flush might be due to mechanism of uptake and translocation of lead by the mycelia, which indicate a time-dependent translocation. After three flushes of fruiting, the lead content of the substrate was reduced to 92.73 ppm. These significant results clearly dictate that *L. sajor-caju* could accumulate lead from the substrate, thus, it has a great potential in bioremediation of heavy metal pollution **(Table 3).**

Table 1: Diameter and density of *L. sajor-caju* mycelia on potato dextrose agar with varying concentrations of lead in 4 days of incubation

Lead sulfate (ppm)	Mycelial Diameter (mm)				Mycelial Density
	Day 1	Day 2	Day 3	Day 4	
0.0	12.19[a]	20.45[a]	41.57[a]	63.76[a]	++++
1.0	11.84[a]	19.31[b]	40.08[a]	62.94[b]	++++
10.0	11.23[b]	18.78[b]	38.13[b]	60.12[c]	+++
100.0	11.17[b]	17.26[c]	37.90[b]	59.43[c]	+++

Means in each column with the same letter of superscript are not significantly different at 5% level of significance using DMRT. + very thin, ++ thin, +++ thick, ++++ very thick.

Table 2. Effect of lead on the fruiting body development and yield of *L. sajor-caju*

Parameters	Lead-Free	Lead-Treated
Incubation Period (day)	30[a]	30[a]
Initiation of Primordia (day)	32[a]	33[a]
Fructification (day)	36[a]	36[a]
Yield of 1st flush (g)	29.54[a]	22.59[b]
Yield of 2nd flush (g)	17.13[b]	25.39[a]
Yield of 3rd flush (g)	13.28[b]	19.52[a]
Total yield (g)	59.95[b]	67.50[a]
Biological efficiency (BE) (%)	11.99[b]	13.5[a]

Means in each row (parameter) with the same letter of superscript are not significantly different at 5% level of significance using T-test.

Table 3: Lead content of fruiting body and spent of *L. sajor-caju*

Substrate	Amount of heavy metals (ppm)			
	Fruiting body			Spent
	1st flush	2nd flush	3rd flush	
Lead-free	ND	ND	ND	ND
Lead-treated	ND	7.37	3.51	92.73

ND means not detected in the sample.

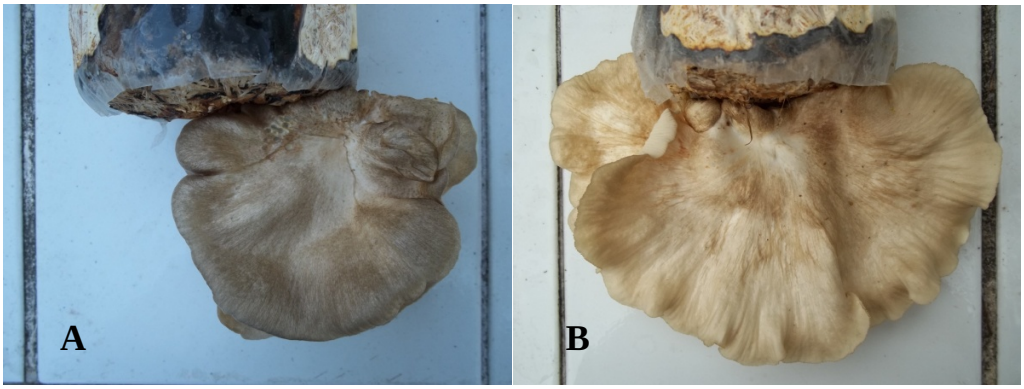

Figure 1: Fruiting body of *L. sajor-caju* on (A) lead-free and (B) lead-treated substrates

CONCLUSION

Collectively, lead at certain concentration inhibits the mycelial growth but induces the fruiting body production of *L. sajor-caju*. The maximum accumulated lead was recorded in the second flushing of fruiting bodies. The amount of lead in the substrate was reduced. Thus, *L. sajor-caju* is one of the useful basidiomycetes in bioremediation of lead pollution.

REFERENCES

[1] Kalac P, Svoboda L. Heavy metal in edible mushrooms. Czech Journal of Food Sciences, 1998, 16, 110-116.

[2] Kalac P, Svoboda L. A review of trace element concentrations in edible mushrooms. Food Chemistry, 2000, 69, 273-281.

[3] Khara K.. Uses of lead. 2000. http://www.buzzle.com/article/uses-of-lead.html.

[4] Asio VB. Heavy metals in the environment and their health effects. 2009. http://soilenvironment.blogspot.com/2009/07/heavy-metals-and-their-health-effects.html.

[5] Ona LF. Lead (Pb) contamination of dust from schools in an urbanized city in the Philippines. 2010. http://www.ijesd.org/papers/59-A10008.pdf.

[6] Zhang X, Yang L, Li Y, Li H, Wang W, Ye B. Impacts of lead/zinc mining and smelting on the environment and human health in China. 2011. http://www.ncbi.nlm.nih.gov/pubmed/21573711.

[7] Santos KK., Dulay RMR, Kalaw SP, Reyes RG. Morphogenesis of Lentinus sajor-caju Fr. In Transactions of the National Academy of Science and Technology (Philippines), 2012, 34(1), 79.

[8] Reyes RG, Kalaw SP, Dulay RMR, Yoshimoto H, Miyazawa N, Seyama T, Eguchi F. Philippine native and exotic species of edible mushrooms grown on rice straw-sawdust based formulation exhibit nutraceutical properties. Philipp. Agric Scientist, 2013, 96, 198-204.