



HEAVY METAL RESISTANCE AND MYCOREMEDIATION POTENTIAL OF SPLIT-GILL BASIDIOMYCETES, *Schizophyllum commune* Fr.

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ABSTRACT

The effect of three heavy metals (lead, chromium and cadmium) on mycelial growth and fruiting body production and the mycoremediation potential of *Schizophyllum commune* were highlighted in this work. The performance of mushroom was evaluated in heavy metal contaminated culture media and formulated substrate. The heavy metal content of the mushroom was analyzed using atomic absorption spectrophotometer. Cadmium registered the lowest mycelial diameter while chromium showed the highest mycelial diameter. A 1000 ppm of lead, chromium, and cadmium showed percentage growth inhibitions of 44.92%, 6.30%, and 82.98%, respectively. Lead infused substrate produced 81.83 g of fruiting bodies while cadmium infused substrate yielded 67.31 g. Fruiting bodies contained 2.03 ppm lead and 4.18 ppm chromium. Cadmium was not detected in the sample. Based on these findings, *S. commune* can be considered as another mushroom species that possess heavy metal resistance and mycoaccumulation potential.

Keywords: *S. commune*, heavy metal, cadmium, lead, chromium

INTRODUCTION

Aside from the fact that mushrooms are sources of bioactive substances with various functionalities (e.g. antimicrobial, anti-

oxidant, anticancer, antitumor, anti-diabetic immunomodulator), they are also capable of accumulating heavy metals and other

minerals from the substrate into their cells. Among the wide variety of mushrooms in the Philippines, *Schizophyllum commune* is one of the widely distributed wood-decaying Basidiomycetes. This mushroom is commonly called as *kudit*, *kudupdop*, *kurakdeng*, and *kuditdit*. Spores of *S. commune* germinated best when submerged in rice bran decoction (pH 7.5) and incubated under illuminated condition at 30°C [1]. Coconut water is the most appropriate culture medium for its mycelial production while milled rice and palay seeds support the luxuriant mycelial growth as spawning materials or starters. Its fruiting bodies grow best in a combination of sawdust and 5% rice bran. Among the logs evaluated, mango yielded quality fruiting bodies as compared to ipil-ipil, rain tree and paper tree [2]. It is also a medicinal mushroom, as it exhibits anticoagulative and anti-inflammatory activities [3].

Mushroom has a potential to degrade a wide variety of environmentally persistent pollutants, and transform industrial and agro-industrial wastes into useful products [4]. In an earlier work, the ability of some Philippine native and exotic mushrooms to accumulate heavy metals from the substrates or media was already demonstrated. In particular, the five *Pleurotus* species namely;

P. djanour, *P. salmoneostramineus*, *P. florida*, *P. ostreatus*, and *P. cystidiosus* accumulated lead in the range of 1.63 - 2.58 ppm, and this abilities may even vary depending on the species type and fructification flushes [5]. Moreover, *Coprinus comatus* showed high tolerance and ability to accumulate heavy metals such as lead, copper, chromium and cadmium [6]. Indeed, mushroom species are very good accumulators of heavy metals.

Heavy metals in high amount may create serious environmental problems. Aside from mining activities, these chemicals are also introduced into the environment through cosmetic, paints, and other products, smelting activities and manufacturing processes. Generally, metals are emitted during their mining and processing activities and because of the improper waste management of this activities, it causes increase in heavy metal pollution. Heavy metal pollution and its biotoxic effects can cause medical problems to humans including gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, vomiting and convulsion, depression and pneumonia [7].

With the current information on the heavy metal accumulation abilities of mushrooms

and the environmental problems and potential human health hazards caused by heavy metal pollution, this study was conducted to explore the potential of *S. commune* as mycoaccumulator of heavy metals. In this present work, the mycelial growth response on the media contaminated with the varying concentrations of three heavy metals like lead, chromium, and cadmium and the ability of *S. commune* to accumulate these heavy metals were demonstrated.

MATERIALS AND METHODS

Source of mushroom species

The pure culture of *S. commune* was obtained from the culture collection of the Center for Tropical Mushroom Research and Development, Science City of Muñoz, Nueva Ecija, Philippines.

Preparation of culture inoculant

An agar block of approximately 10 mm² x 3 mm from the pure culture of *S. commune* was aseptically inoculated into sterilized potato dextrose agar (PDA) plates and incubated at 30°C to allow mycelia growth. 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.

Preparation of heavy metal contaminated culture media

Coconut water gulaman with varying concentrations of three heavy metals namely; lead sulfate PbSO₄, chromium nitrate Cr(NO₃)₃, and cadmium nitrate CdNO₃, were used as culture media in the evaluation of mycelial growth. One liter of coconut water and 20 g of gulaman were combined and boiled until homogenized. Three hundred ml of the medium was prepared for each heavy metal and 100 ml was allotted for each concentration (10 ppm, 100 ppm, and 1000 ppm). A lead free medium served as the control, 0 ppm. Each prepared medium was dispensed in a flask, cotton plugged and properly labeled. These were sterilized in an autoclave at 121°C, 15 psi for 30 min.

Evaluation of mycelia growth response

The different media were pour-plated and aseptically inoculated with mycelia discs of mushrooms. Triplicate was done per concentration of heavy metals. 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 mycelia growth was also calculated as follows: Growth inhibition (%) = [(growth diameter of control – growth diameter of treated) / growth diameter of control] x 100.

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 heavy metals.

Evaluation of the mycoaccumulation ability

The ability of the fruiting bodies of *S. commune* to accumulate heavy metals from the rice straw - sawdust based substrate was also studied. 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 metals with 5 replicates for each mushroom species. Bags without heavy metals 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. 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 last flush. The accumulated amounts of heavy metal of the fruiting bodies were analyzed using atomic absorption spectrophotometer. Data were analyzed using analysis of variance (ANOVA) and LSD was used to determine the significant treatment comparison at 5% level of significance.

RESULTS AND DISCUSSION

Mycelial growth response of *S. commune*

Mycelium is the vegetative structure of mushroom and its morphology could be affected by several factors like nutritional and physical factors. In this present work, the mycelial growth response of *S. commune* on the media at varying concentrations of the three heavy metals was evaluated. Table 1 presents the mycelial growth diameter, growth inhibition and mycelia density of *S. commune* on coconut water gulaman with varying levels of lead, chromium and cadmium after 6 days of incubation. Interestingly, all heavy metal treated media significantly recorded lower mycelial growth

diameter compared to lead free media. It can also be noticed that the effect of the three heavy metals was concentration dependent, as lead concentration increased the mycelial growth diameter decreased. However, among the three heavy metals, cadmium registered the lowest mycelial diameter whereas chromium showed the highest mycelial diameter. In terms of growth inhibition, 1000 ppm of lead, chromium, and cadmium showed percentage growth inhibitions of 44.92%, 6.30%, and 82.98%, respectively. Therefore, *S. commune* showed high tolerance to chromium, moderate tolerance to lead and high sensitivity to cadmium. This strongly indicates that the heavy metal tolerance of *S. commune* may vary depending on the type of heavy metal. In similar study, copper and cadmium at 6 µg/ml respectively showed 41% and 93% mycelial growth

reduction of *Pleurotus sajor-caju*, whereas lead and mercury caused more than 85% reduction of growth at 15 and 6 µg/ml, respectively [8]. Among the 21 species of mushrooms including 7 *Pleurotus* species, 2 *Pycnoporus* and *Pholiota* species and one each of *Agrocybe*, *Cryptoporus*, *Coriolus*, *Inonotus*, *Lampteromyces*, *Grifola*, *Flammulina*, *Lyophyllum*, *Agaricus*, and *Polyporus* species tested for heavy metal resistance, *Pleurotus* species strains showed the highest resistance to copper, cadmium, zinc, nickel, cobalt, mercury and among *Pleurotus*, *Pleurotus ostreatus* exhibited the highest resistance. However, *Pholiota* species, *Flammulina veltipes*, *Lyophyllum ulmarium*, *Agaricus bisporus* and *Polyporus arcularius* were sensitive to these heavy metals tested [9].

Heavy metal	Concentration (ppm)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial density
Lead	0	85.38 ± 0.94 ^a	0.00 ± 0.00 ^c	3+
	10	84.25 ± 1.09 ^a	1.31 ± 1.80 ^c	3+
	100	69.97 ± 2.40 ^b	18.06 ± 1.92 ^b	3+
	1000	47.01 ± 2.09 ^c	44.92 ± 3.04 ^a	3+
Chromium	0	85.38 ± 0.94 ^a	0.00 ± 0.00 ^c	3+
	10	84.90 ± 1.06 ^a	0.55 ± 2.27 ^c	3+
	100	83.69 ± 0.96 ^b	1.97 ± 2.07 ^b	3+
	1000	80.00 ± 0.81 ^c	6.30 ± 1.33 ^a	3+
Cadmium	0	85.38 ± 0.94 ^a	0.00 ± 0.00 ^c	3+
	10	79.45 ± 1.58 ^b	6.93 ± 2.70 ^b	3+
	100	77.13 ± 1.77 ^b	9.67 ± 1.97 ^b	3+
	1000	14.54 ± 0.99 ^d	82.98 ± 1.07 ^a	3+

Values are mean ± SD. Treatment means in each heavy metal with the same superscript are not significantly different from each other at 5% level of significance using LSD. In mycelial density column, very thin (+), thin (2+), thick (3+), very thick or cottony (4+)

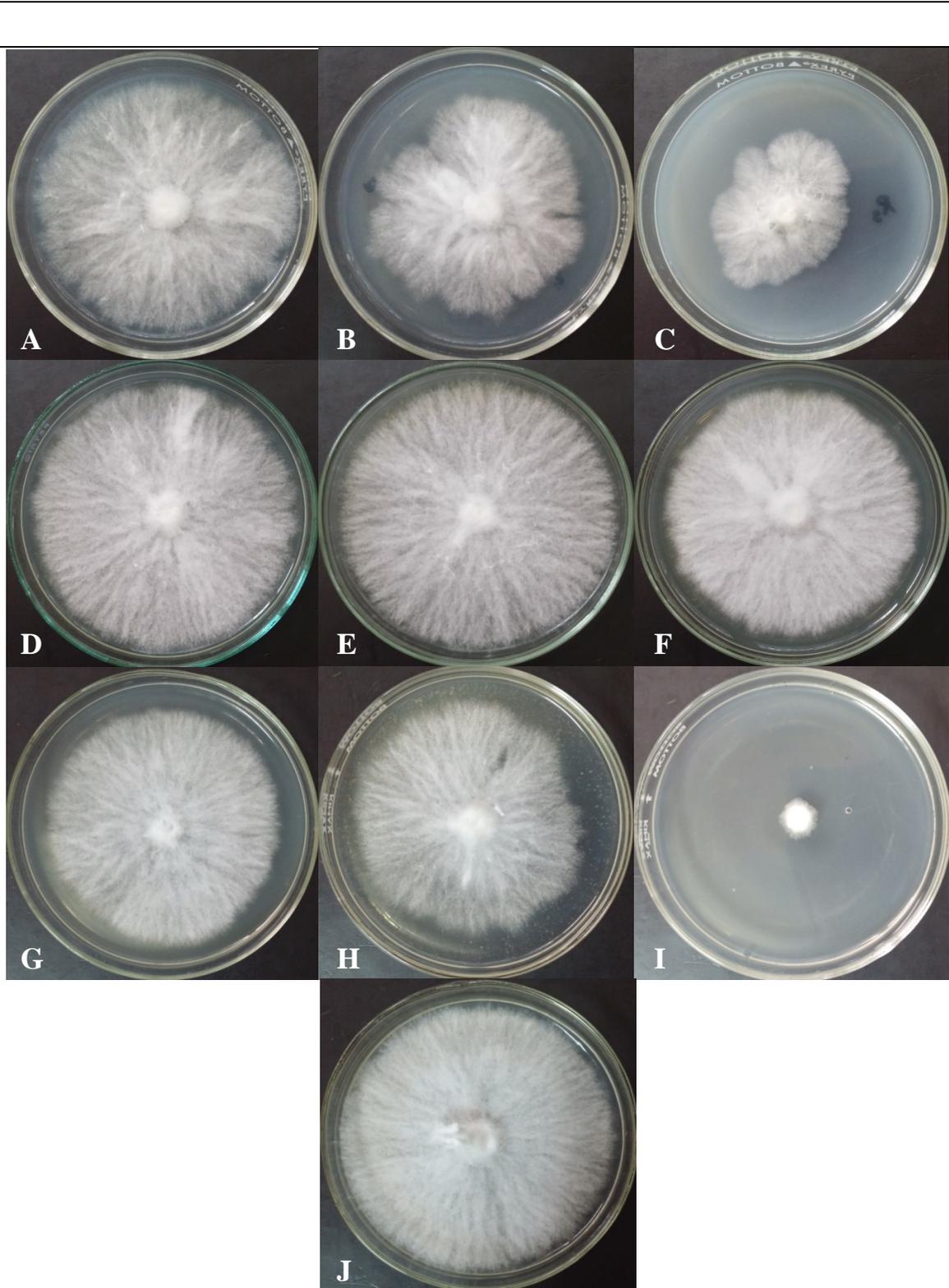


Figure 1. Culture plates of *S. commune* on coconut water gulaman media at varying concentrations (10 ppm, 100 ppm, and 1000 ppm, from left to right) of heavy metals, namely: lead (ABC); chromium (DEF); cadmium (GHI) and 0 ppm, heavy metal free (J) after 6 days of incubation.

However, with regards to the mycelial density, no significant difference was observed in *S. commune* grown on the three heavy metal media, which produced thick mycelia as shown in Figure 1. Although *S. commune* showed sensitivity and inhibition caused by the three heavy metals as demonstrated in the present study, it is still reasonable to assume that this mushroom has wide variety of resistance mechanisms against these pollutants. Thus, evaluation of the ability of *S. commune* to accumulate the three heavy metals is needed for further studied.

Yield and mycoremediation ability of *S. commune* fruiting body

The effect of heavy metals in the fruiting body production and its accumulation ability were studied. Apparently, the presence of heavy metal in the substrate significantly affects the yield of fruiting bodies of *S. commune* (Table 2). Lead infused substrate produced 81.83 g, cadmium infused substrate

yielded 67.31 g, and chromium infused substrate had 79.29 g. These values were significantly lower when compared to the heavy metal free substrate. The fruiting bodies of *S. commune* grown on the substrate infused heavy metals are shown in Figure 2. This clearly dictates that the production of fruiting body of *S. commune* could be reduced in the presence of heavy metal in the substrate. However, although yield reduction was observed, the accumulation of heavy metals in the fruiting body was still investigated. The accumulated amounts of heavy metals in *S. commune* fruiting bodies are presented in Table 2. Lead and chromium was detected while cadmium was not detected. Fruiting body samples grown on heavy metal infused substrate contained 2.03 ppm lead and 4.18 ppm chromium. Therefore, it very interesting to note that *S. commune* indeed has a great potential in mycoaccumulation of heavy metals particularly lead and chromium.

Heavy metal	Total yield (g)	Accumulated heavy metal (ppm)
Lead	81.83 ± 2.47*	2.03
Chromium	79.29 ± 1.59*	4.18
Cadmium	67.31 ± 3.27*	ND
Heavy metal free	86.45 ± 2.19	ND

Values are mean ± SD. Asterisk represents significant difference with the heavy metal free using T-test. ND means not detected

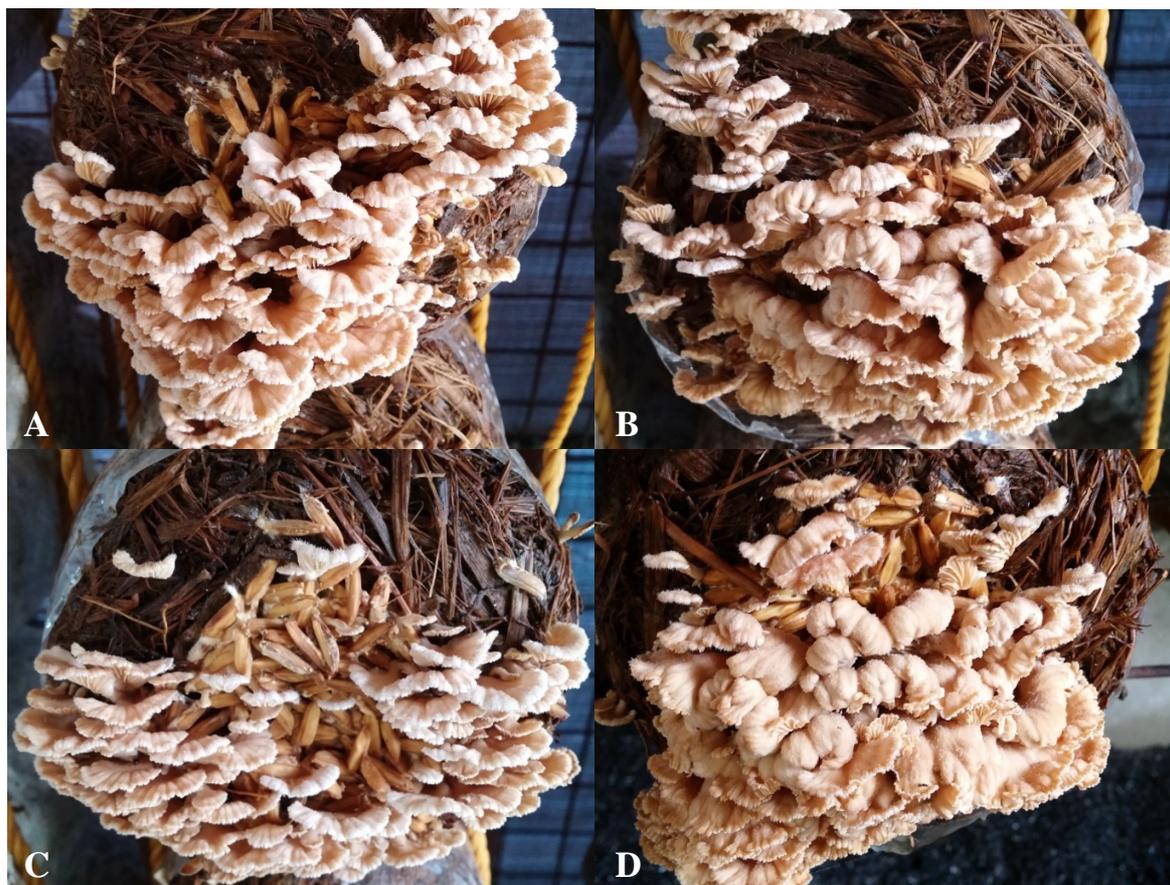


Figure 2: Fruiting bodies of *S. commune* grown on rice straw and sawdust based substrate infused with 2 ml of 1000 ppm of lead (A), chromium (B), cadmium (C) and heavy metal free (D).

Mushrooms especially the wild species have been reported to accumulate high amounts of heavy metals including lead, iron, manganese, chromium, mercury, cadmium, copper, aluminum, nickel, and zinc [10, 11, 12, 13, 14]. It was also reported that among the different wild mushrooms, some species are highly accumulating of certain heavy metal. For instance, *Agaricus* species are highly accumulator of cadmium as high as 100-300 ppm dry matter [15] while *Lycoperdon perlatum*, *Macrolepiota rhacodes* and *Lepista nuda* are

hyperaccumulator of lead [16]. Aside from the basidiocarp or sporocarp, mushroom mycelia are also known to be a good accumulator of heavy metals. Mycelia of *P. sajor-caju* demonstrated the highest uptake of Cu^{2+} ($182 \mu\text{g ml}^{-1}$) and Cd^{2+} ($178 \mu\text{g ml}^{-1}$) but lowest uptake of Co^{2+} ($11 \mu\text{g ml}^{-1}$) and Hg^{2+} ($12 \mu\text{g ml}^{-1}$) [8]. Moreover, *P. ostreatus* mycelia accumulated 8.9 ppm chromium, 6.4 ppm copper, 2.1 ppm cadmium, and 6.4 ppm cadmium whereas *P. florida* showed maximum accumulation of 8.1 ppm copper,

6.3 ppm chromium, 3.0 ppm lead, and 3.9 ppm cadmium [17].

Since *S. commune* is known for its edibility and medicinal attributes, this mushroom is widely collected in the wild by farmers and native people. However, based on the results obtained in the present study, it is safe to assume that its fruiting bodies collected from the wild are not always safe for human consumption because of the possible accumulation of heavy metals. Cadmium and lead have no beneficial role in human metabolism. Lead accumulates in the bones while cadmium accumulates mainly in kidneys, spleen and liver which may give rise to more serious diseases.

CONCLUSION

In general, the mycelia growth and fruiting body production of *S. commune* were significantly affected by the presence of heavy metals in the media and substrate as evident by the reduction of the mycelial growth and biomass yield. Its fruiting bodies accumulated certain amount of lead and chromium. However, cadmium was not detected in the sample. Thus, this present work demonstrated the heavy metal resistance and remarkable mycoaccumulation ability of *S. commune*.

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