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Estimation of potential fungal species in a copper tolerance and bioaccumulation study

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ABSTRACT

Copper (Cu) contamination in soil is a considerable environmental concern as it is becoming common and widely present due to the high industrial activities. Cu is naturally found in soil, and it is known to be an important trace element for living organisms. However, industrial processes have contributed to the huge release of Cu into the environment causing Cu pollution in soil. Scientists have developed mycoremediation methods to remediate Cu, which are more efficient, safer and cost effective. Therefore, the objectives of this research are to screen the potential fungi and observe their tolerance of Cu in potato dextrose broth (PDB) based on the biomass produced, and to determine the ability of the fungi to accumulate Cu through metal level determination by using inductively coupled plasma mass spectrometry (ICP-MS). Three fungal species were used in this experiment, namely, *Pleurotus ostreatus*, *Aspergillus niger* and *Agaricus bisporus*. These fungus species were inoculated in potato dextrose broth (PDB) with different $CuSO_4$ concentrations (0 ppm / 50 ppm / 100 ppm / 200 ppm / 300 ppm) for two weeks. The biomasses of the fungi species were then measured. The results showed that all three fungal species have good growth rates at all concentrations and can tolerate Cu in the PDB media. For tolerance study, *A. bisporus* showed the highest growth rate while *A. niger* showed the highest biosorption of Cu after 14 days of incubation. Generally, *A. bisporus* and *A. niger* are potential fungi for Cu remediation.

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KEYWORDS: *Agaricus bisporus, Aspergillus niger*, copper pollution, mycoremediation, *Pleurotus ostreatus*.

INTRODUCTION

One of the major issues of the 20th century is environmental contamination. Heavy metal contamination is one of the most common types of soil contamination and is mainly caused by industrial activities [1]. The presence of heavy metals in soil is a major threat to the environment due to their persistence, toxicity, long half-lives and the potential of bioaccumulation [2, 3]. Fortunately, heavy metals' chemical properties and bioavailability could be modified to reduce their percentages in the soil [4].

Copper (Cu) is one of the major heavy metal contaminants in the environment. Cu can enter the environment through the discharges from Cu-utilizing factories and mining sites, landfill combustion, waste disposals, and even from the discharges originating from domestic residential areas, agricultural sites and urban areas [5]. Cu is normally present in the soil at concentrations between 2 to 50 ppm (parts per million). Contaminated soil, with more than 20 ppm of Cu, will affect the cultivation and growth of plants [6].

In addition to that, high level of Cu present in soil can threat humans' health mainly through the food chain [7, 8]. According to the Environmental Protection Agency (EPA), Cu is classified as a hazardous waste because once it enters the soil it will strongly bind to the components and organic materials present in the soil's top layers, and when water is introduced, water-soluble Cu can be dissolved and leak into the groundwater supply and contaminate the water [9]. Cu in soil does not break down in the environment and can be accumulated in plants and animals [9].

Mycoremediation is one of the most cost-effective bioremediation methods utilizing fungi to remediate polluted soils and effluents [10]. Fungi are known to be important natural decomposers in the ecosystem as they degrade complex molecules and long chain toxic compounds to less-toxic forms. Fungi degrade toxic materials by means of secreting digestive enzymes or by absorbing the contaminants from the soil and accumulating them in their fruiting bodies [11]. In general, the ability of fungi to adapt to the heavy metal toxic environment conditions and their ability to still develop resistance towards it and grow in the presence of heavy metals, makes fungi a favored species compared to others bioremediator species [12].

Some reported studies have shown that *Aspergillus niger*, a common fungus species, has the potential in Cu remediation [3]. Some other studies also reported that edible mushrooms have high accumulation properties for heavy metals [13]. Since there are a few potential fungi in Cu remediation, it will be interesting to compare their ability in Cu remediation. Hence, in this experiment we investigated the potentials of the selected fungi *Aspergillus niger*, *Agaricus bisporus* and *Pleurotus ostreatus* in mycoremediation of Cu. The aim of this study is to determine the ability of the fungi to remediate Cu by looking into the fungi's potential to tolerate and accumulate Cu at different concentrations.

METHODS

Aspergillus niger was screened with rose bengal agar (RBA) from a metal scrapping facility in Shah Alam, Klang, Selangor, Malaysia (geo-coordinate: 3.028138, 101.479779). The mushroom *Agaricus bisporus* (white button mushroom) and *Pleurotus ostreatus* (grey oyster mushroom) were purchased from a fresh market in Nilai, Malaysia. The inner part of fresh mushroom cap was picked by using sterilized forceps, transferred onto potato dextrose agar (PDA) and incubated at 25 °C for 1 week [14]. Colonies formed were sub-cultured onto potato dextrose agar (PDA) obtained from OXOID to obtain pure and young cultures. All the culturing work was conducted in aseptic conditions.

Potato dextrose broth (PDB) was used to test the fungi's ability to tolerate Cu and the biosorption ability. The pure fungi isolated from PDA were grown on potato dextrose broth (PDB) containing different concentrations of Cu (0 mg/L, 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L). The toxicity test was conducted in triplicates. The media bottles were then autoclaved for 15 minutes under 121 °C at a pressure of 1.05 kgcm^{-2}. The biomass from the fungi growth on PDB with Cu was filtered after 14 days. The biomass was then rinsed with deionized water to remove Cu residues on the surface. The culture was filtered using filter paper and dried in the oven at 60 °C to obtain constant dry weight. The dry biomass of fungi was recorded. The experiment was carried out in triplicates. The dried biomass was ground and homogenized prior to inductively coupled plasma mass spectrometry (ICP-MS) analysis at Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia.

RESULTS AND DISCUSSION

Copper tolerance study

Figure 1 shows that all fungi were able to grow in all concentrations, even in high concentrations (0.08 g biomass obtained from 50 ppm Cu concentration to 0.16 g from 300 ppm Cu concentration). Overall, *A. bisporus* (0.26 g) showed the highest growth rate followed by *A. niger* (0.18 g) and *P. ostreatus* (016 g) at 300 ppm after 14 days of incubation.

A. bisporus was able to produce biomass in all Cu concentrations. The biomass obtained showed decreasing trend at the beginning but increased after 100 ppm of Cu concentration. *A. bisporus* showed the highest growth rate of 0.26 g in copper concentration of 300 ppm, showing high

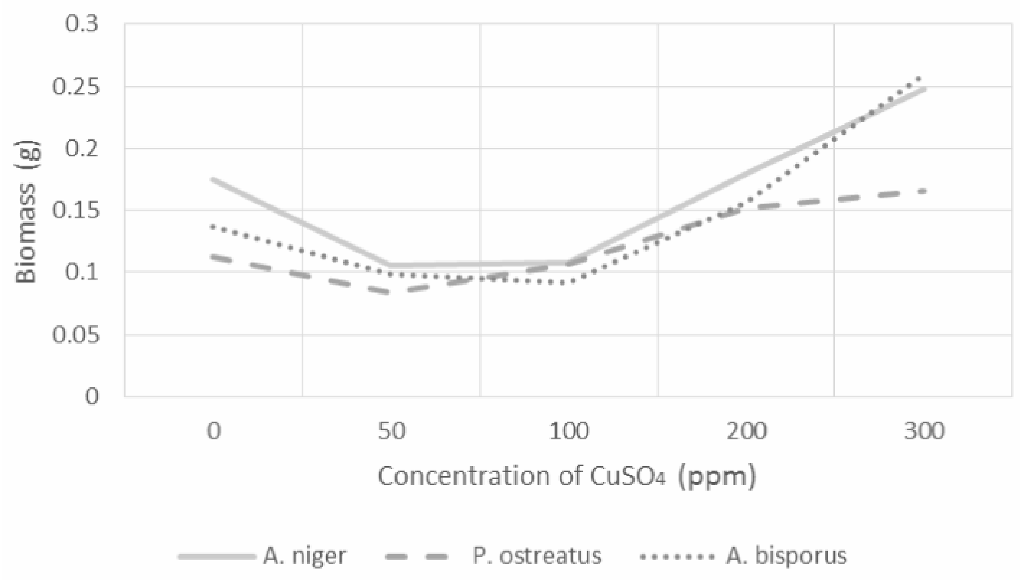

Figure 1. Biomass weight of *A. bisporus*, *A. niger* and *P. ostreatus* produced after 14 days of incubation in PDB.

resistance towards Cu. These results were in line with Xu *et al*. [15]'s study where they found that *A. bisporus* was able to tolerate a mixture of heavy metals including Cu, Zn and Cd in plastic pots full of the soil fortified with different concentrations.

A. niger showed a good growth rate for all Cu concentrations after 14 days of incubation. *A. niger* have lower biomass in 0 mg/L of Cu (0.15 g). It decreased at 50 ppm of Cu but started to increase again in the subsequent concentrations. The highest biomass produced by *A. niger* was at Cu concentration of 300 ppm (0.18 g). According to a reported study by Akhtar *et al*. [16], the highest Cu tolerance rate was recorded for *A. niger* among other species with the highest MIC (Minimum inhibitory concentrations) level of Cu (444 mg/L Cu). Another study stated that the Cu resistance ability of the *A. niger* is due to its inactive process involving Cu metallothionein synthesis [17].

P. ostreatus growth rate was consistent and the growth rate increased proportionally to the Cu concentration until it reached their highest biomass (0.16 g) at 300 ppm of Cu concentration. *P. ostreatus* is a white rot basidiomycete that produces several extracellular laccase isoenzymes [18]. These extracellular enzymes allowed them to withstand and survive in harsh environment condition. *P. ostreatus* is able to produce laccase, a multi-copper containing extracellular enzyme, which uses Cu as cofactor to activate itself and help to increase the rate of catalysis [19]. *P. ostreatus* was reported as a potential bioremediation agent in real applications to remove toxic heavy metals from landfill leachate [20].

Biosorption study

Figure 2 shows that all fungi have the biosorption ability for all concentrations, even in high concentrations ranging from 8.57 ppm of biosorption in 0 ppm Cu concentration culture to 4148.30 ppm in 300 ppm Cu concentration culture). Overall, *A. niger* (4148.30 ppm) showed the highest biosorption of Cu followed by *A. bisporus* (1274.23 ppm) and *P. ostreatus* (835.57 ppm) at 300 ppm after 14 days of incubation.

Cu uptake by *A. bisporus* showed an average biosorption with 1274.23 ppm at 300 ppm concentration. According to a reported study by Ertugay and Bayhan [21], more Cu was absorbed at the higher Cu concentration in the media ranging from 30 to 100 mg/L. However, there was a decrease in the removal efficiency with increasing concentrations of Cu in the surrounding because of the saturation of the sorption sites on the adsorbent. Corral-Bobadilla *et al*. [22] reported that the Spent Mushroom Substrate (SMS) of

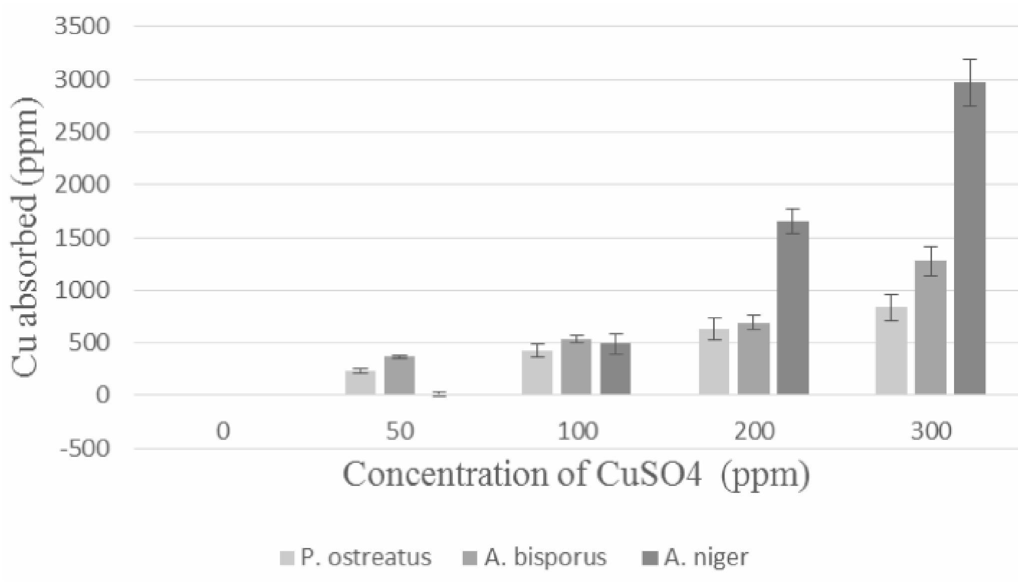

Figure 2. Cu uptake by fungi at different concentrations of Cu in medium after 14 days of incubation.

A. bisporus cultivation successfully treated wastewater that has been contaminated with heavy metals from industrial waters. Based on the reported study by Nagy *et al*. [23], *A. bisporus* showed adsorption capacities up to 3.49 and 2.39 mg/g for Cd (II) and Zn (II), respectively showing efficient removal of Cd (II) and Zn (II) from aqueous synthetic solutions.

A. niger showed an increase in Cu uptake when Cu concentration was increased up to 300 ppm. The highest biosorption (4148.30 ppm) was found in *A. niger* at Cu concentration of 300 ppm. The bioaccumulation ability of *A. niger* had been extensively studied to examine their ability to absorb heavy metals [24]. *A. niger* has the ability to accumulate large amount of Cu ions without negatively affecting their biological activity at low pH [25]. The capability *of A. niger* biomass in removing Cu is equal to or greater than other studied biomasses [26].

P. ostreatus shows a consistent increase in Cu uptake when there is an increase in Cu concentration. For example, at a 300 ppm concentration, the fungi can uptake up to 835.57 ppm of Cu. However, it showed the lowest uptake of Cu among all species. Akgul & Akgul [11] reported that *P. ostreatus* has potential bioremediation values for a diverse group of heavy metals due to their enzyme production and high biosorption rates related to their extensive biomass. Their heavy metal accumulation mechanism was observed and this mechanism indicates that there is a direct relationship between the uptake levels of heavy metals into the mycelia of *P. ostreatus* and the increase in the metal's concentrations [24]. Studies have found *Pleurotus* species to have an effective biosorption potential for Cu as they can accumulate Cu at high concentrations above the maximum permissible concentrations in their fruiting bodies, due to their extensive biomass [27].

CONCLUSION

All species in this study showed an increase in biomass and Cu accumulation when exposed to different concentrations of Cu. In the tolerance study, *A. bisporus* showed the highest growth rate followed by *A. niger* and *P. ostreatus* at 300 ppm after 14 days of incubation. However, *A. niger* showed the highest biosorption of Cu followed by *A. bisporus* and *P. ostreatus* at 300 ppm after 14 days of incubation. In general, *A. bisporus* and *A. niger* can be considered as potential fungi for Cu remediation as both species showed good tolerance and high biosorption abilities.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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