Current Topics in Toxicology
Vol. 16, 2020

Original Article

Evaluation of the plastic degradation ability of edible mushroom species based on their growth and manganese peroxidase activity

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ABSTRACT

The accumulation of plastics in the environment has led to many environmental issues. Therefore, numerous approaches had been proposed in order to reduce the amount of plastic materials in the environment. One of the most effective and efficient way is the use of fungi to biodegrade plastics. The aim of this study is to determine the growth and degradation capability of four mushroom species, namely *Pleurotus ostreatus*, *Pleurotus eryngii*, *Lentinula edodes* and *Agaricus bisporus* on plastic components (Bisphenol-A (BPA) and di-(2-ethyhexyl) phthalate (DEHP)). This study was conducted through cultivation of these mushrooms in PDB medium, infused with different concentrations of BPA (0-10 µl/L) and DEHP (0-1000 µg/L). The potential of degradation for each mushroom was identified based on their manganese peroxidase (MnP) activity in the presence of the plastic components by using a UV-spectrophotometer. The results showed that all mushroom species in this study were not ideal bioremediation agents for BPA as no biomass growth was observed in the culture. The low MnP activities in the species throughout the study further indicate the intolerance of these fungi species towards BPA. However, all fungi in this study were able to tolerate and biodegrade DEHP. *P. eryngii* displayed the highest ability to biodegrade DEHP as it produced the highest MnP activity (54.11 U/L) and growth of biomass (0.283 g).

KEYWORDS: edible mushroom, plastic degradation, manganese peroxidase.

INTRODUCTION

Plastic is one of the most utilized materials in the industry. Due to its non-biodegradable nature, the accumulation of plastic wastes in the environment had become a serious problem that threatens all living beings in the ecosystem. It causes adverse health effects to human such as cancers, birth defects, impaired immunity and endocrine disruption [1]. According to North and Halden [2], the two main components in plastic that cause endocrine disruption in humans are Bisphenol-A (BPA) and di-(2-ethyhexyl) phthalate (DEHP).

In 2015, BPA was one of the highest produced chemicals worldwide, with a whopping amount of 4 million tons per year [3]. In the terrestrial environment, the highest amount of BPA (approximately 17 mg/L) was reported in landfill leachates and pulp mill while in the aquatic sediments, the BPA concentration ranges from 43 to 191 g/kg [4]. Long-term exposure to BPA could affect estrogen-responsive gene expression leading to developmental, reproductive, neurological and immune defects in both humans and wildlife [4]. DEHP is commonly added into polyvinyl chloride plastics (PVC) which are used in the

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production of many medical devices. DEHP has been identified as an endocrine disruptor and causes adverse health effects to organisms and their offspring [5, 6]. According to the European Union Risk Assessment Report on DEHP [7], the concentration of DEHP found in plastic production sites ranged from 1 to 220 µg/L on water surface and 7.5 to 2045 mg/kg in sediments.

To date, some of the most common methods used for disposing or degradation of plastics are biodegradation, land filling, incineration and recycling [8]. The methods of incineration and land filling require large amount of space and is detrimental to the environment. Polymers such as plastic can be recycled and be reused, which would be an effective method if public awareness is spread firmly and practiced consistently. As for biodegradation, fungi and bacteria are used as agents to degrade plastics, which is a more environmental-friendly approach, and possess different metabolic pathways with various enzyme machineries that degrade a diverse range of pollutants [9].

Edible mushroom are widely used in many cuisines nowadays [10]. Apart from having high nutritional values, edible mushrooms are also easily obtained and relatively cheap. On top of that, the feasibility of using by-products from agricultural sector for mushroom cultivation has made them a valuable commodity in waste management [11]. The most commonly cultivated edible mushrooms are *Agaricus bisporus, Lentinus edodes, Pleurotus* spp. *and Flammulina velutipes* [10]. These fungi are known as white-rot fungi that are able to produce many isoforms of extra-cellular oxidases including manganase peroxidase (MnP), which has been widely used in bio pulping, biobleaching and bioremediation [12].

Recently, oyster mushroom (*Pleurotus ostreatus*) has shown promising results in degrading plastics [13]. The ability of a fungi species to degrade plastic also depends on the plastic components [14]. Thus, the objective of this study is to determine the growth and MnP enzyme activity of four commonly found edible mushrooms (*Pleurotus ostreatus, Pleurotus eryngii, Lentinula edodes* and *Agaricus bisporus*) in the presence of plastic components (BPA and DEHP).

MATERIALS AND METHODS

Preparation of mushrooms culture

The mushroom, *Pleurotus ostreatus*, was purchased from NAS Agro farm, Sepang while *Agaricus bisporus, Lentinula edodes* and *Pleurotus eryngii* were purchased from a fresh market in Nilai. The inner part of fresh mushrooms' caps were picked by using sterilized forceps, transferred onto potato dextrose agar (PDA) and incubated at 25 °C for 1 week [15]. This process was repeated until pure cultures were obtained. All the culturing work was conducted in aseptic conditions.

Plastics tolerance study

Potato dextrose broth (PDB) was prepared together with addition of plastic components (BPA & DEHP from SIGMA-ALDRICH) at different concentrations. The concentrations of DEHP introduced into the PDB media were 0 (control), 250, 500, 750, 1000 µg/L, while BPA were 0, 2.5, 5.0, 7.5, 10.0 µl/L. The mushrooms from the PDA cultures were then transferred into the PDB media and were incubated for 20 days. After completing the incubation period, the PDB media were screened for MnP enzymatic activities and filtered through filter papers (Whatman No. 1) to obtain the biomass for growth determination. Mushrooms were grown in PDB without plastic materials as negative control.

Manganese peroxidase activity

The MnP activity was determined by the oxidation of phenol red when subjected to hydrogen peroxide [16]. The reaction media used contain 500 µL of supernatant, 50 µL of manganase sulfate (2.0 mM), 200 µL of bovine albumin (0.5% w/v), 50 µL of hydrogen peroxide (2 mM) in sodium succinate buffer (0.2 M, pH 4.5), 100 µL sodium lactate (0.25 M) and lastly 100 µL of phenol red (0.01% w/v). For each sample harvested, two sets of samples were prepared where one sample was boiled at 100 °C for 10 minutes, while the other sample was not boiled. The reaction of each sample was detected and recorded by using a UV-spectrophotometer at 610 nm. The difference of absorbance value between the boiled samples and un-boiled samples was used as the final absorbance value. The reaction time for each sample was 30 seconds and it was stopped with

the addition of 40 µL sodium hydroxide (2.0 M) [16].

The formula for MnP activity calculation is as follows:

$$\text{Enzymes activity (U / L)} = \frac{A_{610} \times 10^6}{0.5 \text{ mL (enzyme)} \times 0.5 \text{ minutes} \times e\ (4460\,\text{L, m}^{-1}\text{. cm}^{-1})}$$

Statistical analysis

The analysis of biomass and enzyme activity was carried out by using statistical package for the social sciences (SPSS). One-way analysis of variance (ANOVA) was performed at 95% confidence level to determine the significant differences among MnP activity and biomass weight for each species [17].

RESULTS AND DISCUSSION

Manganese peroxidase activity in DEHP

For the proposed enzymatic pathways, the enzyme MnP undergoes oxidation and oxidation-hydrolysis reaction which could yield precursors of ATP production in the Krebs such as phenyl methanol, benzoyl-CoA, 3-acetyl-CoA and acetic acid [17]. As by products, phthalic acid, benzaldehyde, hexanoic acid, hexanol, ethanol, hexanal, 5-hydroxyhexanoic acid, and pentan-2-ol are also produced in these enzymatic reactions [17].

Based on Figure 1(a), at the concentration of 250 µg/L, *P. ostreatus* has the highest ($P < 0.05$) MnP activity which is 26.61 U/L. It was reported that *P. ostreatus* expresses 5 short MnP genes which are capable of oxidizing phthalates such as DEHP [18]. *Pleurotus* sp. produces a denser spherical pellet of mycelia due to the production of hydrophobins. These hydrophobins play a role in making the fungal structure hydrophobic which facilitates absorption of hydrophobic phthalates such as DEHP by the fungi [19].

According to Figure 1 (b), *P. eryngii* showed the highest ($P < 0.05$) MnP activity of 54.11 U/L at 750 µg/L of DEHP. *P. eryngii* has the highest potential to degrade DEHP as it produced the highest MnP activity. A strong ligninolytic capability of *P. eryngii* has been reported, with MnP activity recorded up 570.82 U/L, in pomegranate cultures [20]. The enzymatic tolerance and stability of MnP produced by *P. eryngii* could be the reason of its higher ligninolytic capability in the presence of DEHP. During the degradation process of DEHP, various acids such as phthalic acid and benzene carboxylic acid were formed and thus, altered the pH of the medium [21].

For *Lentinula edodes*, no significant differences were seen in DEHP concentrations of 500, 750 and 1000 µg/L (Figure 1 (c)). The highest MnP activity (31.69 U/L) was observed in 750 µg/L of DEHP. In *L. edodes*, the MnP activity was recorded at 31.69 U/L, which was lower than *P. eryngii.* It had been reported that, in the presence of DEHP, the primary enzymes involved in the biodegradation of DEHP was laccase followed by esterase [22]. Hence, it can be assumed that MnP is not the primary enzyme secreted during the degradation of DEHP. *L. edodes* are able to utilize its glucan storage ability to accelerate the maturation process of its hyphal [22]. The increased amount of matured hyphal enhances the production of ligninolytic enzymes which will eventually lead to higher MnP activities.

In *A. bisporus*, the highest MnP activity (22.09 U/L) was recorded in the 250 µg/L of DEHP (Figure 1 (d)). Physiological enzyme regulators, such as MnPs, can increase the rate of dissociation of MnP to form Mn (III) ions, which has been reported in *A. bisporus* [23]. The results showed that *A. bisporus* has the lowest MnP activity among all the 4 species. In a study by Morin *et al*. [24], *A. bisporus* was found to only contain 2 MnP genes in its genome, in which only one of them was well characterized and had the potential to express ligninolytic abilities. This could be the reason why *A. bisporus* was unable to confer a higher MnP activity.

Growth of mushrooms in DEHP

As per Figure 2 (a), *P. ostreatus* produced the highest biomass (0.257 g) in control but showed

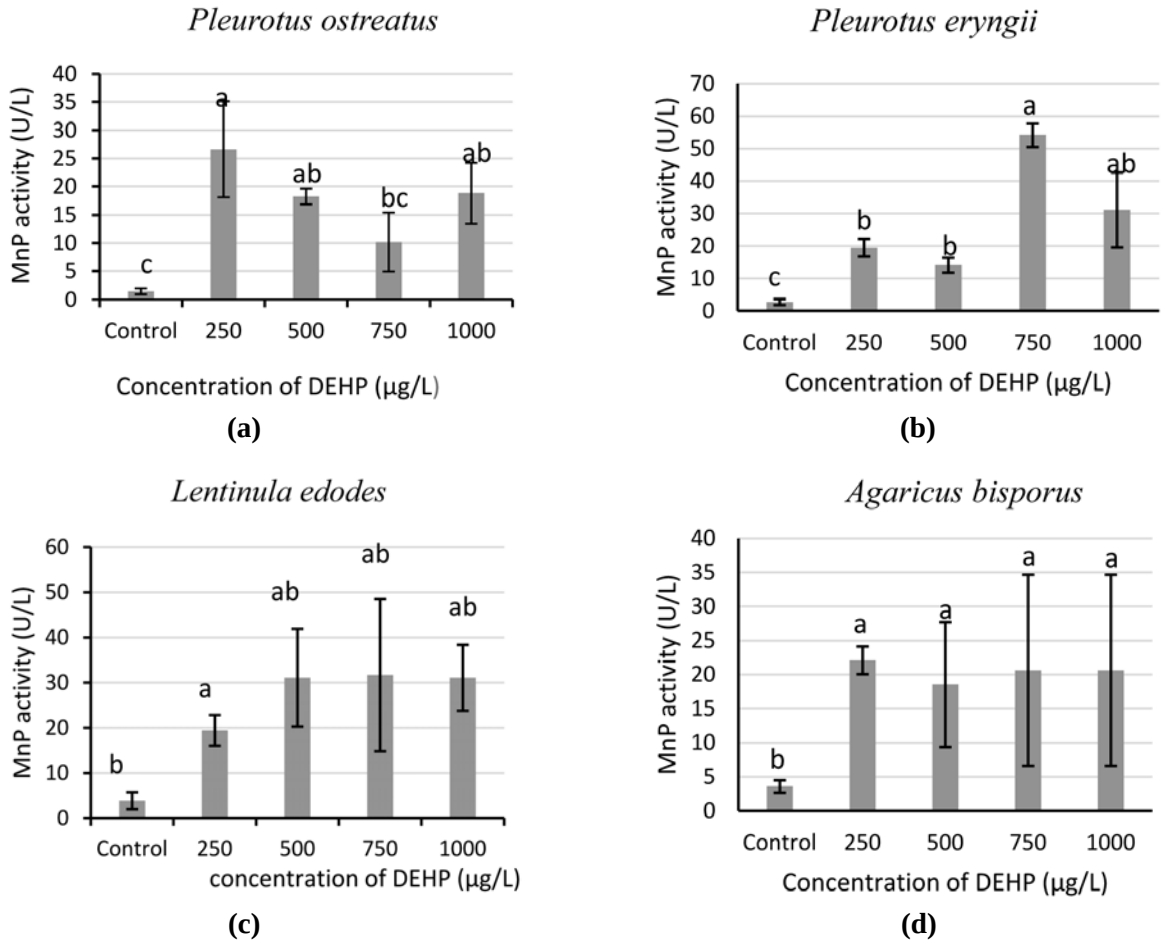

Figure 1. MnP activity ((mean ± standard deviation), U/L) for (a) *Pleurotus ostreatus* (b) *Pleurotus eryngii* (c) *Lentinula edodes* and (d) *Agaricus bisporus* at different concentrations of DEHP (µg/L) after 20 days.
Note: a,b,c: different alphabets in each column show different significant means (LSD test, $p < 0.05$).

no significant differences as compared to other samples at concentrations of 500, 750 and 1000 µg/L, denoting consistent growth in the presence of high concentrations of DEHP. With MnP activity of up to 26.6 U/L, it showed that *P. ostreatus* was able to utilize DEHP as a carbon source and in turn, contributed to its growth in biomass. It had been reported that *P. ostreatus* grown in glucose media containing 1000 mg of DEHP/L were able to produce mycelial growth at a rate of 0.060 mm per day [25].

According to Figure 2 (b), *P. eryngii* produced the highest ($P < 0.05$) growth of biomass (0.283 g) at 1000 µg/L DEHP. The MnP activity signifies that degradation of DEHP had occurred, producing products such as 3-acetyl-CoA and benzoyl-CoA [16]. These products are precursors of energy production involved in the Krebs cycle which in turn enhance the growth of the mushroom's biomass [26]. Comparing to the other species in this study, *P. eryngii* showed the highest tolerance and degradation for DEHP with the highest biomass weight (0.283 g) and MnP activity.

In terms of biomass growth (Figure 3 (b)), *L. edodes* showed the highest biomass growth (0.030 g) in 250 µg/L DEHP. *L. edodes* is able to produce higher amount of insoluble glucan in its media compared to *Pleurotus* sp. in the presence of DEHP and the glucan stored resulted in better maturation of the hyphal supporting its ability to grow in DEHP [21]. However, this ability seems futile in giving *L. edodes* the ability to grow in elevated concentration of DHEP [22].

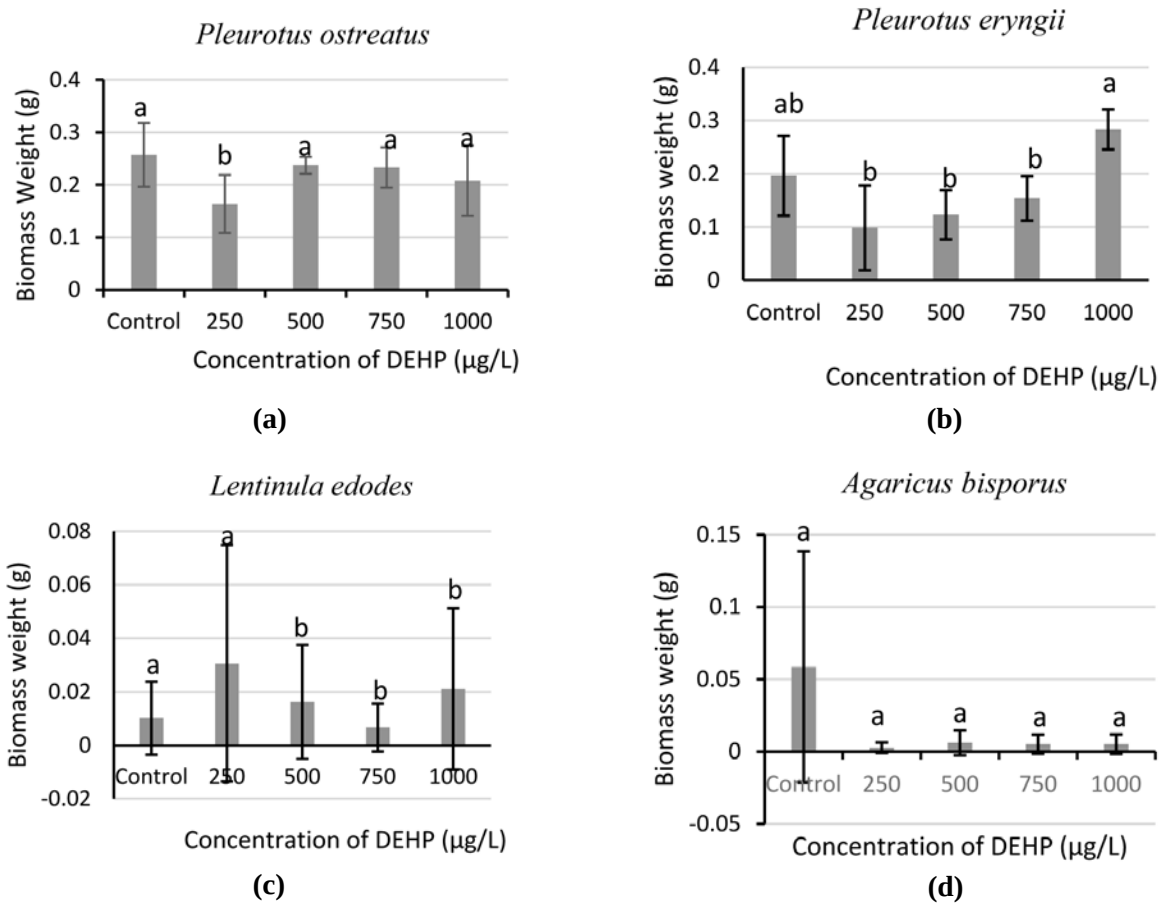

Figure 2. Biomass growth (mean ± standard deviation, g) for (a) *Pleurotus ostreatus* (b) *Pleurotus eryngii* (c) *Lentinula edodes* and (d) *Agaricus bisporus* at different concentrations of DEHP (µg/L) after 20 days.
Note: a,b: different alphabets in each column show different significant means (LSD test, $p < 0.05$).

As per Figure 4 (b), the control sample showed the highest biomass of 0.058 g for *A. bisporus* but no significant differences were observed in the growth for all concentrations. By comparing to Figure 1 (d), *A. Bisporus* produced lower MnP activity as compared to other species as it only contains 2 MnP genes in its genome [24]. This indicated that the species was not able to tolerate DEHP and therefore had the lowest growth rate.

Manganese peroxidase activity in BPA

The degradation mechanism of BPA by MnP is proposed as the withdrawal of one electron by Mn(III) from BPA to form phenoxy radicals. This radical is produced through the oxidation of Mn(II) by MnP in the presence of H_2O_2. The radical of BPA formed will be cleaved randomly at its aromatic rings and the C-C linkages forming phenol, 4-isopropylphenol and hexestrol [27].

Based on Figure 3 (a) – (d), all species in this study showed the highest MnP activity in 7.5 µl/L of BPA, where the MnP activity values for each species were 2.69 U/L for *P. ostreatus*, 2.88 U/L for *P. eryngii*, 5.6 U/L for *L. edodes*, and 5.4 U/L for *A. bisporus*. In addition to that, there were no significant differences in MnP activities among *A. bisporus* grown in different concentrations of BPA (Figure 3 (d)). However, the enzymatic activities produced by all mushroom species were relatively low. Although many white-rot fungi are able to degrade BPA through ligninolytic enzymes, such as MnP and laccase, fungi with high degradability is very limited in which only 4 strains of fungi (*Fusarium sporotrichioides, Fusarium moniliforme,*

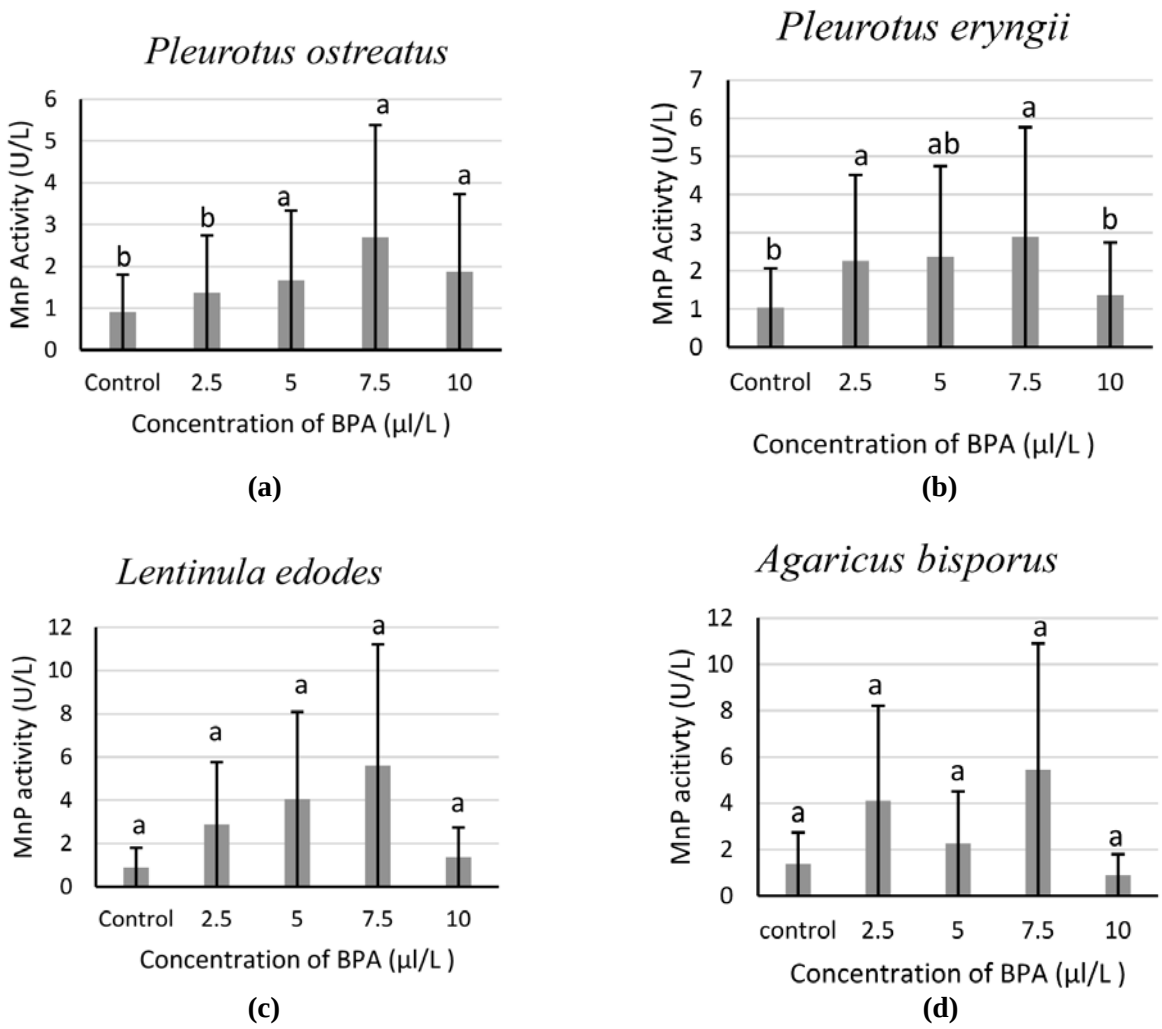

Figure 3. MnP activity (mean ± standard deviation), U/L) for (a) *Pleurotus ostreatus* (b) *Pleurotus eryngii* (c) *Lentinula edodes* and (d) *Agaricus bisporus* at different concentrations of BPA (µg/L) after 20 days.
Note: a,b: different alphabets in each column show different significant means (LSD test, $p < 0.05$).

Aspergillus terreus, and *Emericella nidulans*) had been found to degrade BPA by over 50% [28].

In another study, it was reported that white rot fungi, in the presence of BPA, always produce higher laccase activity than MnP indicating that laccase is the primary ligninolytic enzyme in the biodegradation of BPA [29]. This could be the possible reason of low MnP activity in this experiment. The poor MnP activity is also likely caused by the toxicity of BPA towards these mushrooms [30]. The core toxicity of BPA is due to the presence of two phenolic rings, where the higher number of aromatic rings leads to higher toxicity [31].

Growth of mushrooms in DEHP

In BPA, no growth of biomass was observed in all species of mushroom tested in the presence of BPA (Figure 4), despite the presence of MnP activities. This indicates poor tolerance level of these mushroom species in the presence of BPA. The possible reason for this poor tolerance could be the strains used. The strains in this study were obtained from large-scale commercial farming and not from industrial origins where they are exposed to toxic waste and possess ideal ligninolytic capability [32]. In addition to that, the concentration of BPA implemented in this experiment was derived from actual reports of landfill leachate

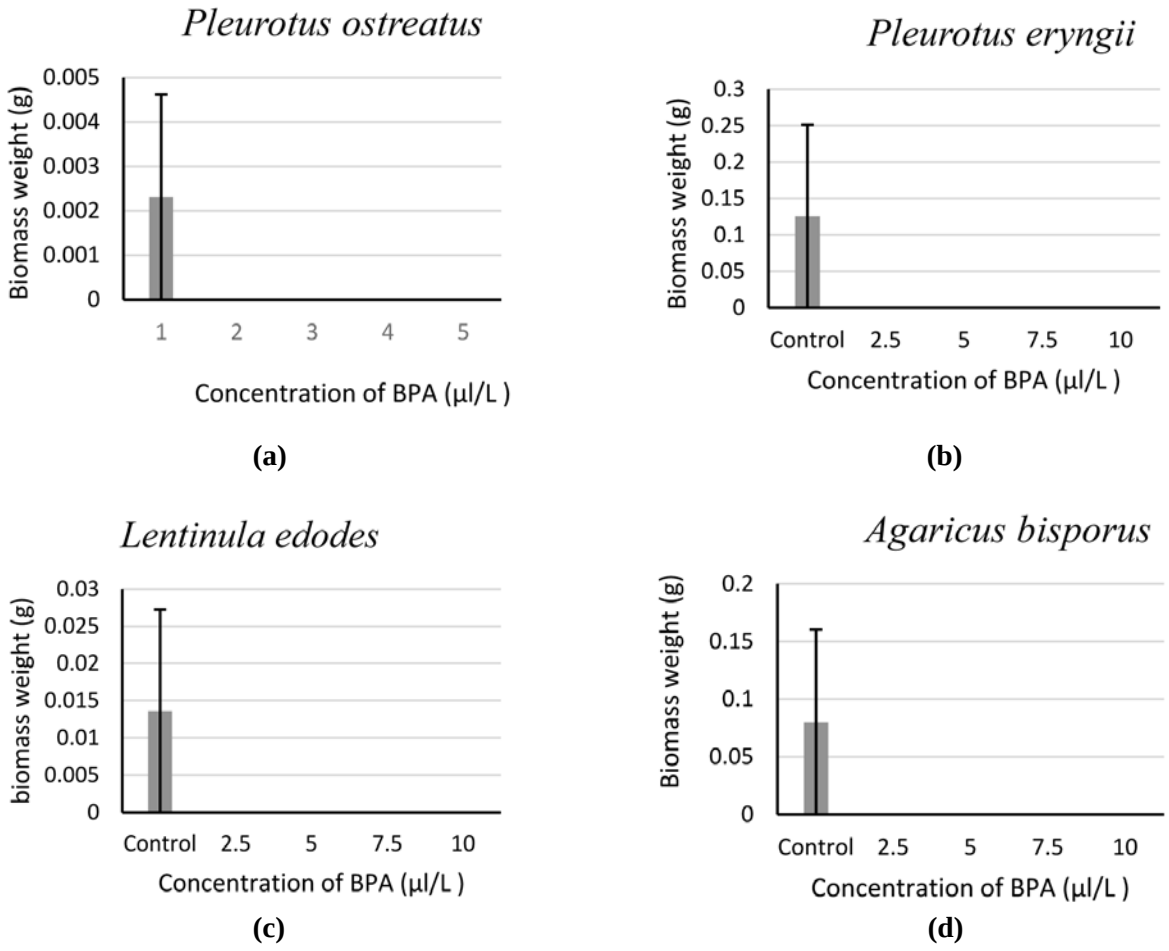

Figure 4. Biomass growth (mean ± standard deviation, g) for (a) *Pleurotus ostreatus* (b) *Pleurotus eryngii* (c) *Lentinula edodes* and (d) *Agaricus bisporus* at different concentrations of BPA (µg/L) after 20 days.
Note: a,b: different alphabets in each column show significant means (LSD test, $p < 0.05$).

and pulp mill effluents of up to 17 mg/L of BPA [4] which was not accounted for when introduced to strains of non-industrial origins.

Another reason for the absence of growth of the mushroom is that the phenol and hexestrol formed from the degradation of BPA [27] could not be utilized in Krebs cycle to produce ATP [26]. This had, in turn, caused metabolic exhaustion to the mushroom while trying to produce enzymes to degrade BPA [26].

CONCLUSION

In conclusion, this research has shown that *P. ostreatus*, *P. eryngii*, *L. edodes* and *A. bisporus* were able to tolerate and trigger MnP activity in DEHP indicating the occurrence of biodegradation. *P. eryngii* has the highest potential to biodegrade DEHP as it produced the highest growth of biomass and MnP activity in this study. On the contrary, *P. ostreatus*, *P. eryngii*, *L. edodes* and *A. bisporus* are not ideal bioremediating agents for BPA, because the mushrooms were not able to tolerate and grow in the presence of BPA and the MnP activities of these species were too low.

ACKNOWLEDGEMENTS

This project was supported by INTI International University Research Grant Scheme (INTI-FHLS-01-03-2018) and funding Biotechnology Programme.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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