Assessing Bioaccumulation of Heavy Metals in Sporocarp of Pleurotus ostreatus

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Abstract: The present investigation was aimed to characterize the concentration of heavy metals from selected industrial units to collect the basal concentration of toxic heavy metals and the assessment of potential bioaccumulation of some heavy metals (Pb, Cd and Ni) in mushroom Pleurotus ostreatus. P. ostreatus was grown on artificial compost medium containing 2 mg/L, 8 mg/L and 15 mg/L of Pb, Cd and Ni. The concentrations of Pb, Cd and Ni in the substrate and in the mushroom were evaluated by atomic absorption spectroscopy. The results showed that more accumulation occurred in the pileus as compared to the stipes. The maximum and minimum Cd content accumulated by cap of the tested edible mushroom fruiting bodies were 1.3 and 0.049 mg/L, respectively at 15 mg/L and 2 mg/L supplied Cd concentration. The increasing order of heavy metals accumulation in P. ostreatus could be arranged as Ni>Pb>Cd. The potential of rot fungi to accumulate various toxic heavy metals in aerial fruit bodies points out towards their three important aspects which can be exploited in environmental monitoring programmes. These include biomarkers of metal pollution, bioaccumulation and bioremediation. P. ostreatus has considerable potential of heavy metal bioaccumulation when grown on metal containing growth media.

Key words: Biomonitoring • Bioaccumulation • Metal pollution • Pleurotus ostreatus • Sporocarp

INTRODUCTION

Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure [1]. The heavy metals may bioaccumulate in food crops, vegetables and fruit plants, thus entering in food chain. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms. Once in the food chain, these heavy metals can profoundly disrupt biological processes and pose a serious threat to human health [2]. Heavy metal contamination of agricultural soils has become a serious issue in crop production and human health in many developed countries of the World. Heavy metals are among the conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are permanent additions to the environment. Subsequently, their concentrations often exceed the permissible levels normally found in soil, waterways and sediments.

Mushrooms are very important low-cost alternative of the expensive sea food throughout the world. The fungal sporocarps are rich source of proteins. It is well established that all cultivated mushrooms have the ability of metal bioaccumulation. Some heavy metals are essential for the fungal metabolism, whereas others have no known biological role. Both essential and nonessential heavy metals are toxic for fungi, when present in excess [3]. Whereas fungi have metabolic requirements for trace metals, the same metals are often toxic at concentrations only a few times greater than those required [4]. The metals necessary for fungal growth include copper, iron, manganese, molybdenum, zinc and nickel. Nonessential metals commonly encountered include chromium, cadmium, lead, mercury and silver [5].

Compared with higher plants, mushrooms can build up large concentrations of some heavy metals such as Pb, Cd and Hg. Several studies have dealt with the absorption of heavy metals from sawdust culture media by mushrooms such as Pleurotus ostreatus, Hypsizigus marmoreus, Pholiota nameko, Flammulina velutipes and Ganoderma lucidum [6]. It has been reported by Kalac and Svorcova, [7] that the metal accumulation is influenced by fungal species, substrate composition, age of mycelium and intervals between fructifications.
Moreover, there existed a correlation between fungal metal concentration and metal point sources [8]. The metal uptake by such edible mushrooms may cause dietary toxicity. However, the status of dietary toxicity caused by mushrooms grown on contaminated waters is unknown.

In Pakistan, there are a number of Industrial Estates which release toxic effluent in the ambient environment without any prior treatment. The wastewaters generated from Hattar Industrial Estate (HIE) is being used for irrigation of many crop plants by ignorant farmers living near HIE. The present study aimed to:

- Characterize the concentration of heavy metals from selected industrial units to collect the basal concentration of toxic heavy metals.
- Based on the background information, assessment of potential bioaccumulation of some heavy metals (Pb, Cd and Ni) in mushroom *Pleurotus ostreatus*.

**MATERIALS AND METHODS**

**Collection of the Mushroom (Pleurotus Ostreatus):**
For this research *Pleurotus ostreatus* mushrooms were collected from Agricultural University of Peshawar in November -December 2007. This edible fungus belongs to Club fungi (Basidiomycetes). This study was carried out to check the bioaccumulation level of heavy metals by Mushrooms. *Pleurotus ostreatus* was grown in controlled environment at Life Sciences Centre (LiCert), COMSATS Institute of Information Technology (CIIT) Abbottabad. Chemical analyses for heavy metal accumulation were conducted in Central Analytical Laboratory, Environmental Sciences, CIIT, Abbottabad.

**Collection of Wastewater:** The wastewater was collected from selected industrial units of HIE. These industrial units included Ghatta Paper mills, Volta Battery, Prime chemicals, Marble industries, Glass factory, plywood industries, Steel mill and Plastic mills. The wastewater samples were collected in sterilized 1 Liter plastic bottles and were preserved at 4°C in refrigerator before analysis of heavy metals. The wastewater samples were analyzed at LiCert laboratory CIIT, Abbottabad.

**Feed Composition and Heavy Metal Treatments for Mushroom:** The feed used for the growth of mushroom contained (g/kg of soil) chopped wheat straw (500g), rice bran (20 g), ammonia (30 g), urea (30 g), corn (20 g), wood saw dust (20 g) and gypsum (20 g). All the materials were thoroughly mixed with the soil taken from small ranch in nearby agricultural field. The pH of soil was kept at neutral using 1M NaOH solution. Based on the baseline data regarding the concentration of various heavy metals in industrial effluents, we decided the concentrations of these heavy metals to be used for the experiment. Synthetic waste water samples were prepared in deionized water with lab analytical grade salts of Lead, Nickle and Cadmium: Lead Nitrate Pb(NO₃)₂, Cadmium Chloride (CdCl₂) and Nickle sulphate (NiSO₄). The stock solution was diluted to get the test solution of 2 mg/L, 8 mg/L and 15 mg/L of Pb, Cd and Ni concentrations. The spawns of mushrooms which were kept in incubator in polythene transparent bags were acid washed for growth at 28°C, watered twice a day. The heavy metal solutions containing 2mg/L, 8mg/L and 15mg/L of Pb, Ni, Cd respectively were applied before the start of experiment (day 0). Three replicates were made of each concentration. The grown mushrooms were harvested after three weeks for further analysis.

**Heavy Metal Analysis from Industrial Wastewater, Growth Medium and Mature Mushrooms:** The concentrations of various heavy metals like Cu, Cd, Ni, Cr, Pb, Zn, As and Hg were determined in wastewater samples (data not shown). Wastewater samples collected from different industries were acidified with 5ml of HNO₃ and digested. After digestion flasks were then cooled at room temperature and the concentrations were determined in atomic absorption spectrophotometer.

Only mature specimens were used for analysis of metals. Fruiting bodies were sliced and dried. The fruiting bodies of the treated and control mushrooms were compared for the bioaccumulation of heavy metals. These samples were washed with deionized distilled water. In total, 27 mushroom samples of *Pleurotus ostreatus* species were analyzed. The samples were digested for recovery of metals. Atomic absorption spectroscopy (AAS) was used for determining the composition of heavy metals in *Pleurotus ostreatus*. Heavy metals concentrations were also determined in the soil samples.

**RESULTS**

**Mycelium Morphology:** In ectomycorrhizal fungus *Pleurotus ostreatus* addition of Cd led to an increase in hyphal density caused by increased number of laterals per branch point and a decrease of the distance between branch points. Baldrian *et al.* [9] also observed the morphological changes in the ectomycorrhizal fungus *P. involutus* by the application of Cd.
Supplied Cadmium concentrations (mg/L)

Mean cadmium concentration (mg/L)

Cd conc. in Stipe
Cd conc. in Cap

Fig. 1: Mean Cd concentration in various parts of *Pleurotus ostreatus*

Supplied Pb concentration (mg/L)

Mean Pb concentration (mg/L)

Pb conc. in Stipe
Pb conc. in Cap

Fig. 2: Mean Pb concentration in various parts of *Pleurotus ostreatus*

Supplied nickel concentration (mg/L)

Mean nickel concentration (mg/L)

Ni conc. in Stipe
Ni conc. in Cap

Fig. 3: Mean Ni concentration in various parts of *Pleurotus ostreatus*
Bioaccumulation of Heavy Metals by Pleurotus Ostreatus:
The accumulation of heavy metals was indicated in Pleurotus ostreatus. There was greater accumulation in cap than in stipe in case of Cd\(^{2+}\). It is clear from the Figure 1 greater accumulation occurred in the cap of Pleurotus ostreatus than in the stipe when grown at 15mg/L of Cd concentration. The maximum and minimum Cd\(^{2+}\) content accumulated by cap of the tested edible mushroom fruiting bodies were 1.3 and 0.049 mg/L, respectively at 15 mg/L and 2 mg/L supplied Cd concentration as illustrated in Fig.1 while in the stipe of mushroom the values were 0.5 and 0.031 mg/L at 15 mg/L and 2 mg/L supplied Cd, respectively.

Similar trend was noted for Pb accumulation in the fruiting bodies of tested mushroom i.e. Pb accumulation was higher in cap as compared to stipes. However, the maximum Pb concentration in cap was noted for 8 mg/L supplied Pb concentration (Figure 2). A linear relationship was noted between Pb accumulation and supplied Pb concentration for stipe (Figure 2). The Pb accumulation in stipe increased with the increasing supplied Pb levels. Interestingly, Pb accumulation decreased in cap at 15mg/L supplied Pb concentration as compared with 8 mg/L supplied Pb (Figure 2).

The results indicated that nickel accumulation was relatively low as compared with Cd and Pb. An inverse relation was found between Ni accumulation and supplied Ni content (Figure 3). The accumulation in stipe and cap decreased with the increasing supplied Ni concentrations. The maximum and minimum Ni content accumulated by the caps of tested edible mushroom fruiting bodies were 0.082 and 0.066 mg/L, while in stipe these were 0.067 and 0.044 at 2mg/L supplied Ni concentration. Ni accumulation in both stipe and cap decreased with the increasing supplied Ni concentrations (Figure 3).

DISCUSSION

The results showed that more accumulation occurred in the pileus as compared to the stipe as reported by Anna [10]. Agaricus bisporus was found susceptible to increasing content of mercury and to a lesser extent to cadmium in substrate, while the yield of Pleurotus ostreatus (oyster mushroom) was not affected under these conditions [11]. Previously, it was observed that fruiting bodies of Pleurotus ostreatus immobilized in calcium alginate were capable of removing Pb (II) from solution efficiently [12]. The heavy metal concentrations in the mushroom are hardly affected by pH or organic matter content of the soil. The trace element contents of the species depend on the ability of the species to extract elements from the substrate and on the selective uptake and deposition of elements in tissues. The heavy metal ions uptake in mushrooms was higher than in plants. For this reason, the concentration variations of heavy metals could be considered due to mushrooms species and their surrounding habitats [3]. The Heavy metal (Hg,Pb,Cd) bioaccumulation levels of three mushrooms (Armillaria mellea, P.squamosus, P.sulphureus ) samples obtained from the East Black Sea region showed that the Hg\(^{1+}\) level in Amanita vaginata samples increased sharply with increasing Hg\(^{2+}\) concentration in soil samples [13].

Very interesting results were noted for the bioaccumulation of various heavy metals. The increasing order of heavy metals accumulation in Pleurotus ostreatus could be arranged as Ni>Pb>Cd. It seems that P. ostreatus lacks some carrier proteins essential for Ni uptake. The ability to accumulate Cd\(^{2+}\) is characteristic of mushrooms [14] and is closely related with the presence of a binding compound (cadmium-mycophosphatin) which is a genetically coded feature [7]. Two other essential cadmium-detoxification mechanisms were observed in the inedible mycorrhizal mushroom Paxillus involutus - cadmium bound onto cell walls and accumulated in the vacuolar compartments [15].

The heavy metals like Pb\(^{2+}\) and Cd\(^{2+}\) are of toxicological importance. Kalac and Svoboda, [7] reported that the heavy metal contents are related to mushroom species, composition of substrate, age of fruiting bodies and mycelium and distance from the source of pollution. According to Demirba [13] the variation in trace element content among various species is dependent upon the ability of the species to extract elements from the substrate. The Pb concentrations were slightly higher in the caps than in the stems of the fungal fruiting bodies. The present results showed that higher accumulation occurred in stipe for 15 mg/L supplied Pb concentration, while in cap more accumulation occurred at 8 mg/L concentration.

The potential of rot fungi to accumulate various toxic heavy metals in aerial fruit bodies points out towards their three important aspects which can be exploited in environmental monitoring programmes. These include bioindicators of metal pollution, bioaccumulation and bioremediation. The wood-rotting fungi have a good potential to accumulate heavy metals from their environment. Since there are only very low concentrations of heavy metals in wood (except Zn), the main source for heavy metals in fruit bodies is the atmosphere. This has led to the use of wood-rotting fungi for the biomonitoring of atmosphere pollution. The results of such studies confirmed, that there is a clear relationship between air
pollution and metal contents in fungal fruit bodies [16,17]. The biomonitoring is applicable for a wide range of heavy metals [9] and it provides a useful tool for environmental analysis.

The fact that heavy metals are toxic to fungi has been widely used in the fight against fungal deterioration of materials including timber. Due to its high toxicity, the early preparations of biocides were based mostly on mercury. However, the toxicity of mercury to living organisms was also the case for ending the use of mercuric antifungals. The much less environmentally problematic copper was also found to exhibit good biocidal activity [18], but the major requirement of any formulation of copper-based wood preservative is efficacy against copper-tolerant fungi.

A variety of methods have been tried for the treatment of heavy metals in water and soils including chemical-treatment, ion-exchange, electrolysis, adsorption, reverse-osmosis, evaporating concentration and biological treatment, etc. [19]. Many low cost adsorbents such as algae, fungi bacteria and mushroom by-products have also been investigated recently for their biosorption capacity towards heavy metals. Under warm and moist habitat conditions, the mushrooms can also be applied for phyto remediation of metal contaminated soils or wastewater as they have greater potential for bioaccumulation of heavy metals as compared with higher plants.

Further research is required to understand heavy metal tolerance in these mushrooms achieved either by detoxification through chelation by organic acids or proteins, compartmentation in the vacoule or by evolution of metal tolerance enzymes. The effects on the food chain should be studied further. Moreover, the hyper accumulating ability of mushroom should be enhanced using metal chelating agents or through the use of genetic engineering technology.

CONCLUSION

The wood-rotting fungi Pleurotus ostreatus has a promising potential to accumulate heavy metals from their environment. The potential of rot fungi to accumulate various toxic heavy metals in aerial fruit bodies points out towards their three important aspects which can be exploited in environmental monitoring programmes. These include biocindicators of metal pollution, bioaccumulation and bioremediation. Pleurotus ostreatus has considerable potential of heavy metal bioaccumulation when grown on metal containing growth media. The increasing order of heavy metals accumulation in P. ostreatus could be arranged as Ni>Pb>Cd. P. ostreatus can effectively extract Cd, Pb and Ni from the contaminated substrates and can be used for bioremediation for metal contaminated substrates as compared with higher plants.

REFERENCES