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BIOREMEDIATION USING THE SPECIES PLEUROTUS OSTREATUS

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ABSTRACT

In this article Mushrooms grown on substrates treated with different concentrations of manganese, copper, lead and zinc were studied. Mushrooms grown on substrates treated with different concentrations of manganese, copper and zinc fruited at about the same time (4 weeks after spawning). The mushrooms treated with lead took about 2 more weeks to fruit, This could be due to toxicity caused by lead.

The heavy metal concentration extracted from the mushroom fruiting bodies of Pleurotus ostreatus were 2000 mg/kg mushroom tissue, 3000 mg/kg mushroom tissue, 1000 mg/kg mushroom tissue and 500 mg/kg mushroom tissue for lead, copper, manganese and zinc respectively. The results shows that Pleurotus ostreatus cultivated mushrooms are able to uptake and bioconcentrate the above heavy metals.

INTRODUCTION

Mushrooms do not constitute a significant portion of the human diet, but the consumption of wild and cultivated mushrooms has become increasingly popular in recent years (Melgar et al., 2009). Sources of wild mushrooms provide thousands of species to choose from (García et al., 2009). Certain species have an extraordinarily great concentration of a particular element (Stijve et al., 1998; Melgar et al., 1998; Falandysz et al., 2001; Dogan et al., 2006; Borovicka and Randa, 2007; Chudzynski and Falandysz, 2008). The relationship between the abundance and the bioavailability of these elements from soil or other substrates that provide nourishment to the fruiting body is very complex. By measuring the concentrations of the elements in the soil substrate and in the mushroom tissues, it is possible to evaluate the suitability of higher mushrooms as possible bioindicators of soil pollution by heavy metals (Mejstrik and Lepsova, 1993; García et al., 1998; Falandysz and Gucia, 2008).

Normally, reference to bioremediation of toxic wastes implies the use of bacteria or lower fungi and rarely is the use of fleshy fungi (mushrooms) considered. However, higher fungi should not be forgotten. Saprophytic mushrooms are natural decomposers because they secrete enzymes and acids that degrade organic polymers into simpler moieties. The dominion of mushroom mycelium is enormous since each colony extends long chains of cells forming a network that can occupy many square meters (Marcia Pletsch et al., 1999). Even the most popular edible mushroom (*Agaricus bisporus*) is capable of accumulating silver as demonstrated by

Falandysz et al. 1994, who investigated the uptake of this element from artificially enriched substrates. Accumulations of up to 150 mg/kg (on a dry weight basis) of silver were found in the fruit bodies (caps and stalks) when the level of the metal in the substrate was more than 12 times lower, showing that *A. bisporus* is not only an extractor but is also an efficient concentrator for this element. Silver (as silver nitrate) at this concentration did not affect mycelia growth or the emergence of fruiting bodies. In normal terrestrial plants the accumulation of silver is typically of the order of 60 mg/kg. Weber and coworkers 1997, showed that the Basidiomycete *Boletus badius* is particularly efficient in accumulating gold and arsenic, which are stored in different parts of the mushrooms. Thus, gold is accumulated in caps and stalks, while arsenic accumulates in the hymenium.

Stijve 1995, investigated 300 species of mushroom and found that only six species showed abnormally high arsenic levels, but such levels ranged from 10 mg/kg up to 2.4 g/kg. This unusual accumulation is even higher than that found in some aquatic plants, such as *Ceratophyllum demersum and Lagarosiphon major*, which store up to 1.2 g/kg arsenic (Robinson et al., 1995).

Contamination of soils with radiocesium is still a major concern within global society (Peplow, 2006). Despite huge efforts towards safe storage of radioactive waste, there is a possibility of uncontrolled release of radiocesium to the natural environment. Although it is still a subject of debate, bioremediation might be regarded as an alternative solution for decontamination of soils polluted with cesium radioisotopes. It was found that *Pleurotus eryngii* is capable of significant mycoextraction of cesium from organic medium, the fruit bodies collected in that study removed more than 60% of radiocesium initially present in the growth medium (Bystrzejewska-Piotrowska et al., 2005).

According to Melgar ((Melgar et al., 2009), the levels of mercury found in mushroom samples varied with the species of the mushroom sample, the collection site of the sample and the mercury content in the soil at the collection sites. At least four factors could affect mercury concentrations of the edible mushrooms: species, ecology, morphology and physical properties of the soil (such as metal levels, pH and composition). These factors influence metal concentrations and thus bioconcentration factors (BCF). BCF measure the relationships between the metal concentrations in mushrooms and the metal concentrations in the underlying soils.

Some heavy metals like copper, zinc, and manganese are essential for humans at certain concentrations, while others like lead, mercury and cadmium may pose health hazards at low levels. Copper is essential to humans as it is needed for the development of red blood cells, but may be toxic if in high levels. In high levels it may cause skin problems or even urinary tract infections. Three of the most toxic heavy metals are cadmium, mercury and lead. These are toxic even in low levels. Toxic metals like lead may increase in the environment due to lead smelting, refining, and lead mining. Some industries also discharge wastes that may contain heavy metals into the environment. Lead toxicity may cause

liver problems and it may also replace calcium in bones. In high levels, lead may cause arthritis or diseases of circulatory or nervous system. Zinc and manganese are nutrient elements, but may also be toxic in high levels. Manganese blocks calcium channels and may cause pneumonia, while zinc may cause anemia and gastrointestinal irritations. In general, all heavy metals target the nervous system, kidneys and respiratory system. However, they all have a major action of inhibiting enzymes in the body. Most enzymes have the SH-group with which these heavy metals interact leading to inhibition of the enzymes. In some cases toxic metals may also displace essential metals present in the specific enzyme. An example is the displacement of manganese that is a metal cofactor in some enzymes by lead.

In this study, the species *Pleurotus ostreatus* was cultivated on waste materials supplemented with copper, lead, zinc and manganese to examine the ability of *Pleurotus ostreatus* to uptake heavy metals. *Pleurotus ostreatus* (oyster mushroom) belongs to the family *Tricholomataceae*, it is edible and was selected in the study because it is easy to cultivate and is able to grow on many substrates.

MATERIALS AND METHOD

Culture preparation :

Culture preparation was done using PDA (Potato Dextrose Agar) which was autoclaved at 121^{o}C for 10 minutes, cooled and poured into sterile petri dishes. The petri dishes were inoculated with a tissue from a young fresh mushroom and kept at room temperature for 2 weeks.

Spawn making :

Spawn was prepared from the pure culture obtained as described above. Grains were soaked in water overnight. They were drained of excess water and mixed with lime and this was placed in clean bottles. The bottles were autoclaved at 121^{o}C for 15 minutes. The grains were allowed to cool down and were inoculated with the prepared culture in a sterile environment to avoid contamination.

Substrate preparation :

The substrate used was grass. About 16 kgs of grass were soaked for 24 hours in bags. After the given period of time, the substrate was placed in big bags for sterilization. The purpose of sterilization was to kill all microorganisms that may inhibit the growth of the mushroom. The substrate was sterilized by means of an autoclave, which generate high-pressure sterilization using steam. Before sterilization, each of the solutions of Copper, Zinc, Manganese and Lead were added separately to the soaked substrate and thoroughly mixed. The metal solutions were prepared by dissolving $CuSO_4.5H_2O$, $MnCl_2.4H_2O$, $ZnCl_2$ and $Pb(NO_3)_2$ separately in 250 ml distilled water in Erlenmeyer flasks. Two bags of each of the treatments were autoclaved at 121^{o}C for 15 minutes. The bags were left overnight to cool. The initial concentration of each metal ion was 200 mg/Kg substrate.

Substrate Inoculation :

The prepared spawn of *Pleurotus ostreatus* was used to inoculate the sterile substrate under sterile conditions (treatments were done in duplicates). Spawning was done in layers by first putting the substrate in a plastic bag and then inoculating it with spawn.

Another layer of the substrate was placed on top of that and was also inoculated with spawn. This procedure was followed until the plastic bag was full. The bags were sealed and were placed in a dark room, as mycelia growth requires no light.

Fruiting :

When the bags were fully invaded by the mycelia, they were taken to the fruiting room where temperature and humidity were controlled. Spraying the fruiting room with water raised the humidity. In addition to controlled temperature and humidity, light was also switched on to induce fruiting. Cuts were made on the bags where the mushrooms were to sprout. When the mushrooms were big enough, they were harvested and dried. The mushroom harvested from each treatments (Cu, Mn, Zn, and Pb) were grinded separately. Each of the samples was then placed in a labeled vial.

Analysis of the heavy metals in the mushroom tissue :

About 1g of each of the grinded sample was placed in a 100ml Erlenmeyer flask to which 10ml of concentrated nitric acid (HNO_3) was added. The solutions were heated, orange fumes were produced. The heating was done for about 30-40 minutes and the solutions were cooled to room temperature in the fume hood. As the solutions cooled down, about 30ml distilled water was added to the flasks and the solutions were filtered and transferred into 100ml volumetric flasks. The volumetric flasks were filled to the mark with distilled water. The control was also prepared in the same heating procedure. The concentration of Copper, Manganese, Zinc and Lead were determined using spectrophotometric techniques.

RESULTS AND DISCUSSION

Mushrooms treated with manganese, copper and zinc fruited at about the same time (4 weeks after spawning). The mushrooms treated with lead took about 2 more weeks to fruit, This could be due to toxicity caused by lead. A maximum of three harvests were done in case of manganese, copper and zinc. Mushrooms treated with lead were harvested once and at a more mature stage than the mushrooms treated with the other three heavy metals.

The heavy metal concentration extracted from the mushroom fruiting bodies of *Pleurotus ostreatus* were 2000 mg/kg substrate, 3000 mg/kg substrate, 1000 mg/kg substrate and 500 mg/kg substrate for lead, copper, manganese and zinc respectively (Table 1). The ability of mushrooms to bio-concentrate

Metal ion concentrations in mushroom tissues are shown in Table 1.

Metal ion	Initial concentration in the substrat mg/kg	Concentration in mushroom tissue mg/kg
Lead	200	2000
Copper	200	3000
Manganese	200	1000
Zinc	200	500

some heavy metals more than others is depending on the species used provided that all other variables are fixed.

These results shows that *Pleurotus ostreatus* cultivated mushrooms are able to uptake and bioconcentrate the above heavy metals. Keeping in mind that the initial concentration of each of the metal ions was 200 mg/kg substrate then *Pleurotus ostreatus* mushrooms were able to bioconcentrate lead 10 times higher, copper 15 times higher, manganese 5 times higher and zinc 2.5 times higher than their concentrations in the substrate itself. The above results are showing that *Pleurotus ostreatus* mushrooms are not only extractors but efficient concentrators. The mechanism by which some heavy metals are accumulated is somewhat obscure. There is speculation that it involves a chelation reaction with the sulfhydryl groups of the methionine in mushroom tissues (Zurera-Cosano et al., 1988).

CONCLUSION

There is clearly a possibility of utilizing *Pleurotus ostreatus* as bioindicators and in the area of bioprospecting if not in biomining itself in case of trace metal ions.

Wild growing mushrooms are a popular delicacy in many countries, but some species accumulate high levels of toxic heavy metals, e.g., mercury, Lead and others, careful analysis of any waste material has to be made before it is used to cultivate mushrooms otherwise the consumption may lead to health hazards.

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الملخص العربى

إزالة بعض الملوثات باستخدام فطر المشروم بليتريتيس أوستراتيس

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يتناول البحث إستخدام المشروم بليتريتيس أوستراتيس لتنقية بعض الأوساط من بعض العناصر الثقيلة، وجد أن المشروم الذى تم إنباته على وسط ملوث بعناصر المنجنيز والنحاس والزنك قد أثمر بعد أربع أسابيع، أما المشروم الذى تم إنباته على وسط ملوث بعنصر الرصاص قد أثمر بعد ٦ أسابيع ويرجع ذلك لسمية الرصاص، وتم حصاد المشروم الذى تم إنباته على وسط ملوث بعناصر المنجنيز والنحاس والزنك ثلاث مرات أما المشروم الذى تم إنباته على وسط ملوث بعنصر الرصاص فكان أبطىء كثيراً فى النمو ولم نتمكن من حصاده سوى مرة واحد فقط فى نفس الفترة الزمنية، هذا وقد وجد بعد الحصاد والتجفيف أن تركيز العناصر تحت الدراسة كالتالى : ٢٠٠٠ملجم / كجم مشروم من العناصر التالية : الرصاص، النحاس، المنجنيز والزنك على الترتيب، بالأخذ فى الاعتبار أن تركيز كل من العناصر سالفة الذكر فى الوسط الملوث كان ٢٠٠ملجم/كجم، نجد أن المشروم بليتريتيس أوستراتيس لم يقم فقط بامتصاص العناصر السامة بل قام أيضاً بتركيزها فى أنسجته إلى ١٠ أضعاف التركيز فى الوسط فى حالة الرصاص و١٥ ضعفاً فى حالة النحاس و٥ أضعاف فى حالة المنجنيز و٤ر٥ ضعفاً فى حالة الزنك، النتائج السابقة توضح أن المشروم المستخدم قادر على تخزين العناصر السابقة بكفاءة، آلية تخزين الشروم للعناصر السابقة غير معروفة على وجه الدقة ولكن هناك توقعات بأنها ناتجة عن تفاعل العناصر الثقيلة مع عنصر الكبريت الموجود بخلايا المشروم.

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