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ASSESSMENT OF THE BIOSORPTION POTENTIAL OF HEAVY METALS BY *PLEUROTUS TUBER-REGIUM*

[a]Oyetayo, V.O., [b]Adebayo, A.O. and [a]Ibileye, A.
[a]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure (340001), Nigeria
[b]Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure (340001), Nigeria

ABSTRACT
The ability of *Pleurotus tuber-regium*, a white rot fungus, to absorb heavy metals from artificially contaminated soil was investigated. Four heavy metals viz: lead, zinc, manganese and copper were used to artificially contaminate the soil in the form of lead nitrate, zinc sulphate, manganese sulphate and copper sulphate at concentration of 0.05mol/kg of soil while uncontaminated soil serves as control. *Pleurotus tuber-regium* was grown on the heavy metal contaminated and uncontaminated soil to maturity in a period of 30 days. The heavy metals was determined using atomic absorption spectrophotometer on the soil before contamination with heavy metals, after contamination with heavy metals and in the mushroom sample before cultivation on soil and the matured mushroom fruiting body cultivated on the heavy metal contaminated soil. The experiment revealed the effect of each of the heavy metals on the pileus development of the mushroom. Moreover, it was observed that *Pleurotus tuber-regium* is efficient in absorbing heavy metals as the levels of remaining heavy metals in the contaminated soil are below the limit of detection (<0.01mg/100g) and having high bioconcentration factor for all the metals.

KEYWORDS: biosorption, *Pleurotus tuberrigium*, heavy metals, bioconcentration

INTRODUCTION
In recent years, heavy metal pollution has become one of the most serious environmental problems in both developed and developing nations of the world. Heavy metal contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil, traffic, and plant protection (Sarkar, 2002). The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes. In contrast to organic pollutants, metals are not mineralized by microorganisms but can be oxidized or reduced, transformed to different redox stages, or complexed by organic metabolites (Sarkar, 2002). Some metals are subject to bioaccumulation and may pose a risk to human health when transferred to the food chain (Jarup, 2003). The presence of heavy metals even in traces is toxic and detrimental to both flora and fauna (Talley, 2006). Examples of heavy metals include lead, zinc, copper, mercury, iron, manganese, cadmium, vanadium, antimony, arsenic and cobalt.

Besides excavation and deposition, a conventional treatment for decontamination of metal-polluted soil is extraction using mineral acids. The disadvantages of such a treatment are the destruction of soil, high costs of acids, and low acceptance. Alternative remediation strategies to reduce bioavailability of metals are: (1) immobilization with repeated addition of substances such as carbonate, phosphate, apatite, zeolite, clay minerals, peat, or humus: and (2) bioleaching with heterotrophic microorganisms, preferably fungi. The latter method represents a sustainable remediation treatment of metal-polluted soils (Bradford, 1976; Dong *et al*., 2000; Alam *et al*., 2001, Chatain *et al*., 2005; Vaxevanidou *et al*., 2008). The quests for the most cost-effective method of removing heavy metals are directed towards bio-sorption. Fungi, algae, bacteria, plant and activated sludge have has demonstrated great potential as metal bio-sorbent due to their metal sequestering properties and this can decrease the concentrations of heavy metal ions in soil (Nilanjana Das *et al*., 2008).

Mushrooms has been used to evaluate the level of environmental pollution (Alonso *et al*., 2003; Borovička and Řanda, 2007; Angeles *et al*., 2009; Garcia *et al.,* 1998) and to remediate the metal polluted soil. Also, many studies had been carried out to evaluate the possible danger to human health from the ingestion of mushrooms containing heavy metals (Cast *et al.,* 1998; Borovička and Řanda, 2007; Svoboda *et al*., 2006, Kalač and Svoboda, 2000). Numerous data on metals contents in fungal fruiting bodies were published previously (Kalac and Svoboda, 2000; Soylak *et al*., 2005; Svoboda *et al*., 2006; Alonso *et al*., 2003; Isildak *et al*., 2004; Cocchi *et al*., 2006;), and the reported metal concentrations vary over a wide range within the mushrooms species, due to many factors affecting the accumulation rate. The successful growth and reduction of total metals in industrial waste contaminated soil by fungal mycelial has been reported (Fourest and Roux, 1992; Stamet, 2000).

Mushroom has shown a comparatively remarkable ability in the remediation of environmental pollutants (Isikhuemhen *et al*., 2003; Fourest and Roux, 1992). The present study was therefore undertaken to investigate the bio-sorption potential of *Pleurotus tuber-regium* in soil artificially contaminated with some heavy metals and the effect of such heavy metals on the pileus development of *Pleurotus tuber-regium*.

MATERIALS AND METHODS

Cultivation of Pleurotus tuber-regium on soil contaminated with heavy metals

Sclerotium (tuber) of *Pleurotus tuber-regium* was procured from the Odopetu Market, Akure, Ondo State Nigeria. Soil samples used was collected from the research farm of the Federal University of Technology, Akure, Nigeria. Two kilogram (2kg) was weighed into plastic bowl. Each of the heavy metals was dissolved in one litre of distilled water to make 0.1mol L^{-1} concentration as follows: 15.16g of $CuSO_4$; 16.16g of $ZnSO_4$; 33.12g of $Pb(NO_3)_2$ and 15.18g of $MnSO_4$. Three replicates were prepared for each of the soil samples and thoroughly mixed with the heavy metal solution artificially used to contaminate the soil except for the soil in the control which lacked the heavy metals. Thirty (30) gram of the sclerotium of *Pleurotus tuber-regium* was grown on each of the replicates. The mushroom sclerotium was buried in the soil just deep enough to be covered by soil.

Chemical characterisation and digestion of the soil

Determination of pH

The fraction of soil particles was air-dried at 40°C, grind and allowed to passed through a square-hole sieve with 2-mm mesh size. The soil pH was measured by mixing saturated $CaCl_2$ solution in 1:1 ratio and the supernatant used for the determination.

Determination of total organic carbon

Total organic carbon of the soil was determine by taking 5g of the sample which was previously oven dried at 105°C weighed and place it in cold muffle furnace. The furnace was heat gradually to 550 °C and maintains it for 4 h. The difference between sample before and after heating is a good approximation of the total organic carbon.

Determination of the metals

The total metals in the soil was determined by weighing 5g of 2-mm sample and treated with mixture of oxidizing agents containing 20 cm^3 concentrated nitric acid (70 % V/V)and 10 cm^3 hydrogen peroxide (40 % V/V) for complete digestion of the metals in the soil matrix.

Digestion of mushroom

One gram (1 g) of the dry mushroom samples was ashed at 550°C for three hours and then treated with nitric acid. The solution of obtained from the soil and mushroom sample was made up to 50 cm^3 with deionised water and analysed for Mn, Pb, Cu and Zn using atomic absorption spectrophotometer (Buck 201 VGP).

Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan's multiple range tests (SPSS 11.5 version). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Chemical characteristic of the soil

Some important characteristic of the soil was determined such as pH, Eh and total organic carbon. They are important in properties that affect fate of metals in the soil. The pH range of the sample soil is between 5.7- 6.7 while Eh is between -200 – -250mV. The total organic carbon of the soil sample was determine between 5.6 – 7.2 %.

Environmental problems associated with heavy metal contamination in the soil are very difficult to solve in contrast to organic matters that can be incinerated or biodegraded. Most heavy metals have toxic effects on living organisms at certain concentration. Some metals are subject to bioaccumulation and may pose a risk to human health when transferred through the food chain (Jarup, 2003). In this study, the potential of *Pleurotus tuber-regium* to absorb heavy metals used to artificially contaminate a soil is reported.

Table 1 shows the level of heavy metal in the soil before and after biosorption process. Lead (Pb) has the lowest concentration (2.0mg/100g) after artificial contamination of soil. The level of heavy metals in the soil reduced drastically after cultivating *Pleurotus tuber-regium* for a period of 30 days to bioabsorb the metals. More than 90% of the metals were removed according to this study. The sorption of the metals may be due to complexation of the metal ions by the *Pleurotus tuber-regium*.

TABLE 1: Total heavy metal concentration (mg/100g) in the contaminated soil before and after biosorption

Heavy metal	Heavy metal in the contaminated soil	Heavy metal concentration after biosorption
Cu	0.23 ± 0.19	0.011 ± 0.00
Pb	0.20 ± 0.16	0.010 ± 0.00
Mn	0.22 ± 0.07	0.012 ± 0.00
Zn	0.25 ± 0.14	0.010 ± 0.00

Each value is a mean of replicates (n=3).

TABLE 2: Heavy metal concentration (mg/100g) in mushroom Pileus before and after biosorption

Heavy metal	Heavy metal in pileus before biosorption	Heavy metal in pileus after biosorption
Cu	$0.55^a \pm 0.20$	$0.72^b \pm 0.22$
Pb	ND	$0.15^b \pm 0.09$
Mn	$3.51^a \pm 0.14$	$3.62^b \pm 0.15$
Zn	$0.75^a \pm 0.27$	$0.90^b \pm 0.28$

Each value is a mean of replicates (n=3). Values along rows with different superscript are significantly different ($P<0.05$).
ND: Not detected (below the limit of detection).

The level of the heavy metals in the pileus and stipe of the mushroom was compared before and after the biosorption process (Tables 2 and 3). There was a significant increase (P<0.05) in the level of heavy metals in the pileus of the mushroom after biosorption process (Table 2). The same trend was observed in the stipe of *Pleurotus tuber-regium.* The heavy metals were however more concentrated in the pileus than the stipe. Ayodele and Odogboli (2010) recently reported that more heavy metals are found in the pileus than the stipe.

TABLE 3: Heavy metal concentration (mg/100g) in mushroom Stipe before and after biosorption

Heavy metal	Heavy metal in stipe before biosorption	Heavy metal in stipe after biosorption
Cu	$0.49^{a} \pm 0.15$	$0.55^{b} \pm 0.18$
Pb	ND	0.05 ± 0.06
Mn	$3.28^{a} \pm 0.40$	$3.39^{b} \pm 0.04$
Zn	$0.73^{a} \pm 0.31$	$0.83^{b} \pm 0.15$

Each value is a mean of replicates (n=3). Values along rows with different superscript are significantly different (P<0.05). ND: Not detected (below the limit of detection).

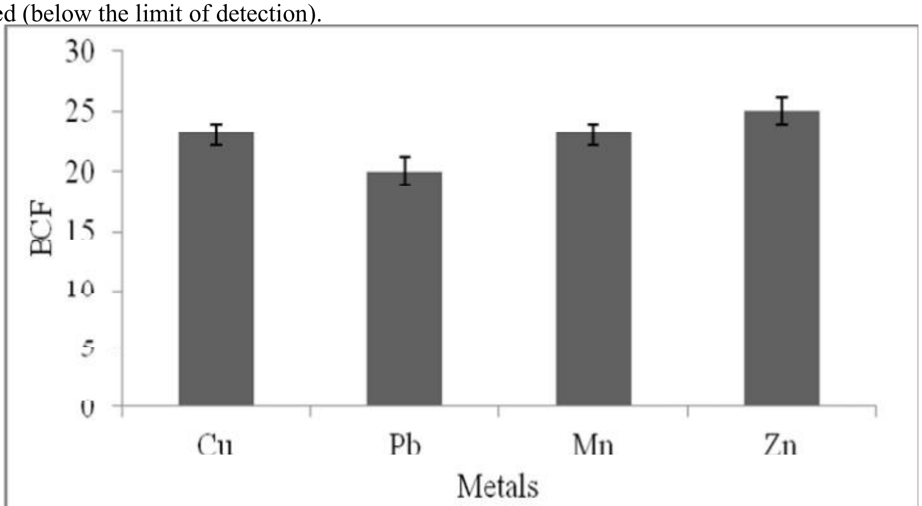

Fig. 1: Bioconcentration factor of the heavy metals

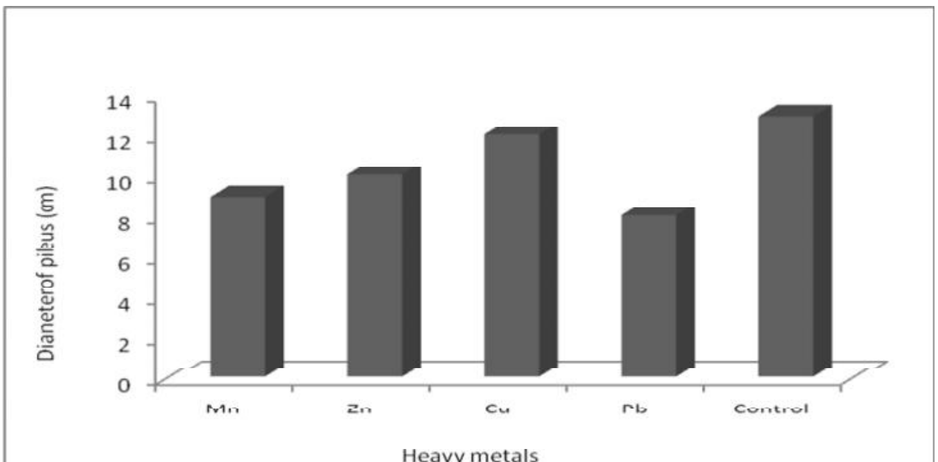

Fig.2: Effect of heavy metals on Pileus development of Pleurotus tuber-regium cultivated on soil artificially contaminated with heavy metals.

Bioconcentration factor (BCF) is an important criterion to evaluate the usefulness of biota to remediate metals in the environment and is calculated as a ratio between the metal concentration in the fruiting body and the content of metal in the under-stipe soil. For a biosystem to be efficient in the remediation of polluted soil, the bioconversion factor must have values higher than 1. The value of the indicator predicts the efficiency of the biosystem in the technology of "cleaning up" (Franke, 1996; Kalac and Svoboda, 2000; Angeles Garcia *et al*., 2009). The bioconcentration factor for *Pleurotus tuber-regium* is shown in Figure 1. This shows that *Pleurotus tuber-regium* has affinity for the heavy metals. The tendency of a chemical species to bioconcentrated depends on their physical and chemical properties, including solubility in water and in fat, molecular weight, and ease of metabolism (transformation of the chemical in the body) or degradation (transformation in the environment), environmental conditions, including soil quality organic carbon content, pH, electrode potential, etc). The nature of the organism, including its ability to metabolise the chemical and its fat (lipid) content (Gast *et al*., 1998; Collin- Hansen *et al*., 2003; Svoboda *et al*., 2006). The present study revealed that *Pleurotus tuber-regium* has the potential to bio-absorb heavy metals. It can thus be used as a mycoremediator in heavy metal polluted environment.

There were observable differences in the pileus development of *Pleurotus tuber-regium* cultivated on soil artificially contaminated with different heavy metals (Fig. 2). The inhibiting effect of Pb on the pileus diameter was more pronounced (8cm) when compared to the control (13cm). Lead (Pb) contamination in soils not only aroused the changes of soil microorganisms and its activities and resulted in soil fertility deterioration but also directly affect the changes of physiological indices and decline in yield (Majer *et al*., 2002). Accumulation of Pb in the body of humans through the food chain is known to endanger human health (Liu *et al*., 2003). Soil contaminated with Cu has less effect on the pileus radius (12cm).

CONCLUSION

Studies were conducted on removal of Pb, Zn, Cu and Mn from artificially contaminated soil using *Pleurotus tuber-regium*. The level of heavy metals in the soil reduced drastically after cultivating *Pleurotus tuber-regium* for a period of 30 days to bioabsorb the metals. More than 90% of the metals were removed. There was a significant increase ($P<0.05$) in the level of heavy metals in the pileus of the mushroom after biosorption process. The bioconcentration factors for the metals were very high. It can thus be used as a remediation of heavy metal polluted environment. The experiment revealed the effect of each of the heavy metals on the pileus development of the mushroom. The inhibiting effect of Pb on the pileus diameter was more pronounced when compared to the control.

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