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The effect of continuous contamination of soil with heavy metals on growth of *Pleurotus tuber-regium*

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Abstract

The effect of continuous contamination of soil with Cu, Fe and Mg on growth of *Pleurotus tuber-regium* and its implication for bioremediation was studied. *Pleurotus tuber-regium* sclerotia were grown in soil contaminated with different concentrations (50, 55 & 60ppm) of $CuSO_4$, $FeSO_4$ and $MgSO_47H_2O$ respectively. Sporophore emergence and fresh weight were determined. Dried soils and sporophore were digested with acid and analyzed for the metals using atomic absorption spectrophotometer. Results showed that concentration of Cu and Fe in soil and sporophore increased with contamination level and ranged between 203.5-288.0 ppm and 47.0-57.5 ppm in Cu; 1019.0-1950.0 ppm and 132.5-328.5 ppm in Fe respectively. Sporophore emergence was delayed in Fe contaminated soil (33-35 days) compared to Cu (16-25 days) and no sporophore was produced at 60ppm $FeSO_4$. Fe was the most bio-accumulated metal, but adversely affected *P. tuber-regium* growth. Bioaccumulation factor for Mg, Fe and Cu ranged between 0.94-1.01, 0.11 - 0.17 and 0.17 - 0.23 respectively, which is an indication that *P. tuber-regium* may not be efficient in bioremediation of these metals in a continuously contaminated soil at the stated concentration.

Key words – Bioaccumulation – mushroom – pollution – Sclerotium – Sporophore,

Introduction

Heavy metals are highly persistent pollutants and one of the most serious environmental problems (Sarkar 2002) that alters soil ecosystem diversity, structure, functions and inhibits cellular processes depending on their concentration (Talley 2006). Metals are required by biological components in definite proportions and are subject to bioaccumulation (Jarup 2003). Fungi, algae, bacteria, plant and activated sludge have demonstrated great potential as metal bio-sorbent due to their metal sequestering properties and can decrease the concentrations of metal ions in soil (Congeevaram et al. 2006). Heavy metal accumulation in soils is of concern due to the adverse effects on food safety, marketability, crop growth, phytotoxicity, and environmental health of soil organisms. Edible and wild mushrooms have been shown to accumulate high concentrations of toxic metallic elements and have a comparatively remarkable ability in the remediation of environmental pollutants (Falandayzs et al. 2003, Isikhuemhen et al. 2003). This accumulation has been attributed to the physiology of the organism, and the ability of fungi to produce metabolites (Burford et al. 2003, Singh 2006). Fruiting bodies of many fungi have been reported to contain varying quantities of heavy metals (Kalac &

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Svoboda 2000, Soylak et al. 2005, Cocchi et al. 2006, Svoboda et al. 2006) and the reported values vary over a wide range (Alonso et al. 2003, Isildak et al. 2004, Singh 2006). The present study was therefore undertaken to investigate the effect of continuous Cu, Fe and Mg contamination of soil on growth and metal accumulation in *Pleurotus tuber-regium* and its implication for bioremediation.

Materials & Methods

Collection of materials

Sclerotia and soil used in the study were procured from a local market, Nwagu market in Agulu, Anambra State and Bells University of Technology, Ota, Ogun State Nigeria, respectively. Plastic bowls used for seeding were purchased locally. Metals used were $CuSO_4$, $FeSO_4$ and $MgSO_4$ $7H_2O$.

Substrate preparation and inoculation

The soil (1 kg) was sieved with 1mm mesh size and distributed in to perforated plastic bowls. Preparations of 50, 55 and 60 ppm $MgSO_4$ $7H_2O$, $CuSO_4$, and $FeSO_4$ were made. The sclerotia were soaked in water for 18hrs and cut into about 30g pieces while the soil was treated with respective metal ion solution to maintain a humid environment. Thereafter the sclerotia were seeded into the loamy soil. Soil without metal contamination served as the control. Subsequent watering was done with metal solutions at the rate of 100ml/48 hours. The plastic bowls were kept at room temperature ($28^oC \pm 2^oC$) on laboratory bench top. Growth of the mushroom was monitored, fresh weight of harvested mushroom sporophore and days for sporophore emergence were determined. Metal accumulation in the fruit body and in the soil was analyzed by atomic absorption spectrophotometer (BUCH 21) according to AOAC, (1990).

Results

Emergence of sporophore was delayed in all contaminated soils and fruiting occurred at 16-25 days, 15-39 days and 33-35 days in Cu, Mg and Fe contaminated soils respectively (Fig. 1). Fresh weight of sporophore relatively increased with increase in metal concentration except in Fe contaminated soil where a reduction was observed. Fresh weight increased from 6.5g to 12.3g; 6g to 8.8g in Mg and Cu soils respectively while there was a reduction from 6g-3.4g in Fe soil (Fig. 2). Continuous Fe contamination adversely affected mushroom fresh weight and no sporophore was produced at 60ppm Fe.

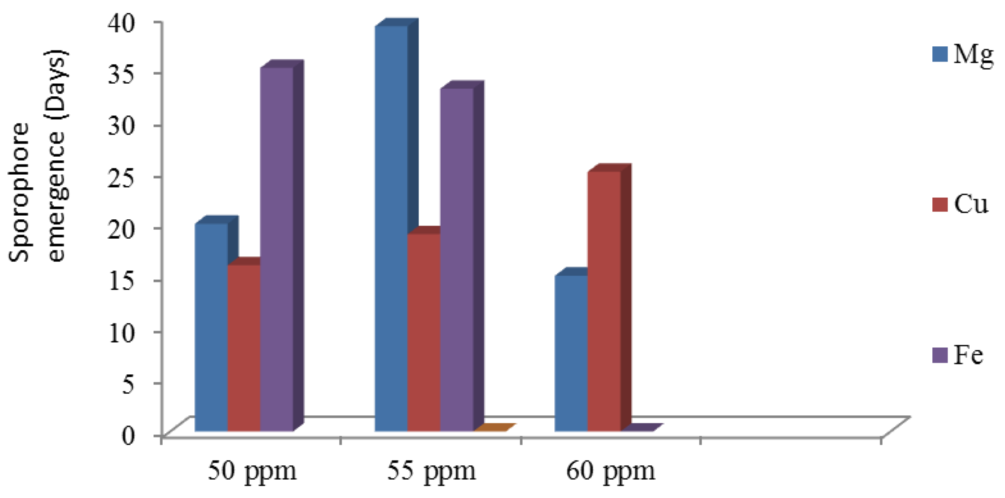

Fig. 1 – Sporophore emergence in heavy metal contaminated soil

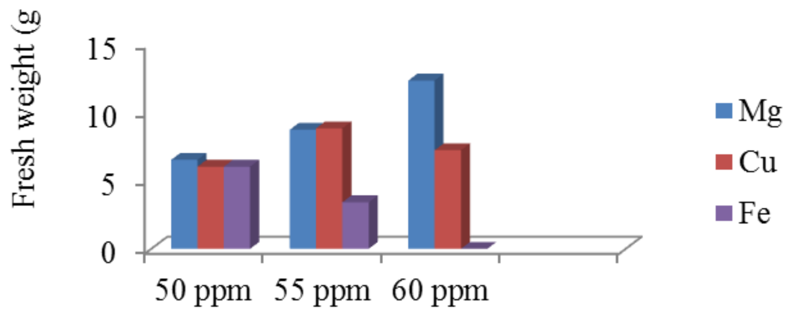

Fig. 2 – Sporophore fresh weight from heavy metal contaminated soil

Heavy metal concentration in mushroom and soil

Of the three metals studied, Fe was the most bio-accumulated and most abundant in the soil recording 132.5-328.5ppm and 1019.0 -1950 ppm in sporophore and soil respectively. The range in values for Cu was 203.5-288.0 ppm and 47-57.5ppm in soil and sporophore respectively (Figs. 3, 4). There was not much difference in quantity of magnesium in the soil and sporophore; the values for magnesium in the soil and sporophore were 97.5-100ppm and 96-98ppm respectively.

The bioaccumulation factor of metals in fruiting bodies ranged between 0.94-1.01, 0.17-0.23 and 0.11-0.17 in Mg, Cu and Fe contaminated soils respectively. The bioaccumulation factor obtained for all the metals was not important for bioremediation which could be an indication that *P. tuber-regium* may not be efficient in bioremediation of continuously contaminated soil with the respective metals at the stated concentrations.

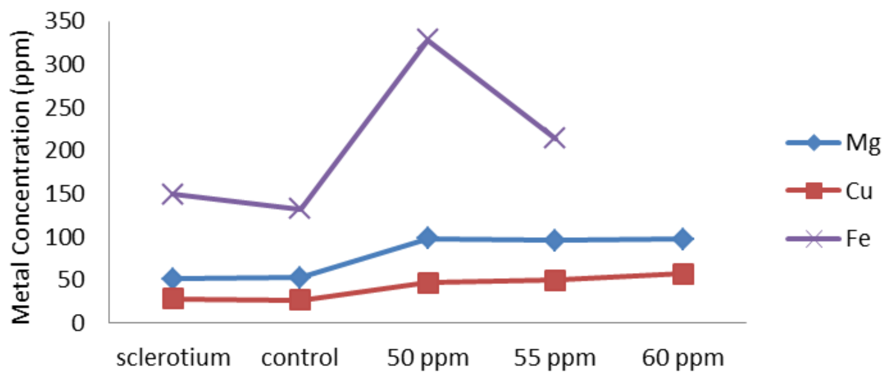

Fig 3 – Metal accumulation in mushroom

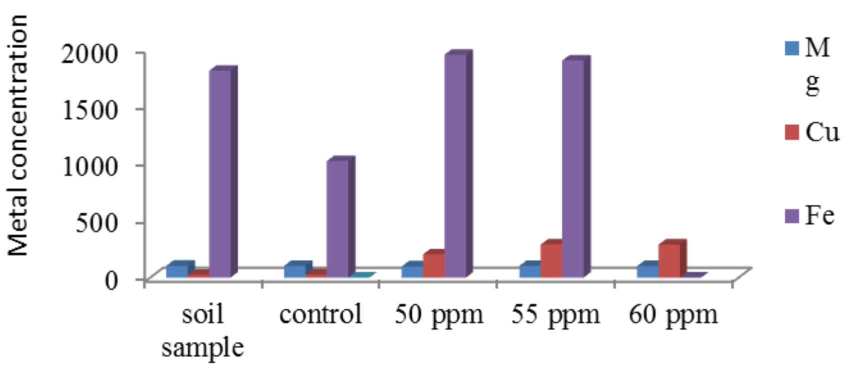

Fig. 4 – Metal accumulation in soil

Discussion

The results of the present study demonstrated that *Pleurotus tuber-regium* was tolerant to different concentrations of Cu and Mg, but most sensitive to Fe. *Aspergillus terreus* and *Alternaria alternata* were reported to tolerate to Cu metal ion in growth medium with increased growth at 100ppm (Al Abboud & Alawlaqi 2011).The most important inhibitory effects on the overall mushroom morphology was found in the case of Fe contamination. Although sporophore emergence was delayed in all contaminated soils, no sporophore was observed at 60ppm Fe. In addition, fresh weight of sporophore increased in all contaminated soil except in Fe contaminated soil. These could be indications of intolerance of *Pleurotus tuber-regium* to continuous exposure to Fe contamination.

Heavy metal concentration in soil and sporophore increased steadily with increased contamination except for magnesium. Similar observation was made in the rhizomorphs produced by *Armillaria* species in Cu contaminated soil where Cu was significantly higher in soils with added heavy metals and the mean concentration of Cu in the rhizomorphs was 228 mg/kg (Rigling et al. 2006). Yilmaz et al (2002) reported very high levels of Fe and Cu in edible, inedible and poisonous mushrooms isolated from road side and a background area of Pakistan. Although Cu was the least bio-accumulated in the mushroom the concentration increased steadily with increased contamination level. There was not much difference in quantity of magnesium in the soil and sporophore throughout the period of cultivation despite continuous soil contamination. Magnesium is an essential macro element needed by most enzymes for metabolism and could have been metabolized by the fungus. Of the three metals studied, Fe was the most bio-accumulated and most abundant in the soil. The bio-accumulation factor is an indication of rate of accumulation of metal by mushroom. For a biological specie to be used in bioremediation it must have a bioaccumulation factor of 1 and above. Bioaccumulation factor showed that *Pleurotus tuber-regium* may not be useful in bioremediation of continuously contaminated soil with respective metals at the stated concentration. This is contrary to earlier reports as fungal biosorbents were reported to have high affinity for Cu (Bayramoglu et al. 2003). Javaid and Bajwa (2008) and Busuioc et al (2010) reported biosorption efficiencies of 18-20.51% for Cu and 555-686%, 1532-3510% for Fe from electroplating effluents. The differences could be attributed to variable adsorption capacity due to differential innate properties of the organisms, nature of the medium (soil and effluent) coupled with continuous contamination rate carried out in this study.

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