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Lead accumulation in oyster mushroom, *Pleurotus tuber-regium* (Sing) from a continuously lead contaminated soil

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Abstract

The effect of continuous lead contamination on lead accumulation, growth of oyster mushroom *Pleurotus tuber-regium* and its implication for bioremediation is studied. *P. tuber-regium* sclerotium was grown in soil continuously contaminated with lead at 50, 55 and 60ppm respectively. Dried soil and mushrooms were acid digested and analyzed for lead using flame atomic absorption spectrophotometer. The results showed that lead in soil increased as the contamination level was increased. The highest lead accumulation in soil and mushroom were 500±0.41 ppm and 85±0.03 ppm respectively. Fruit body emergence was faster in contaminated soil except at 60ppm. Contaminated soil recorded higher mushroom fresh weight (9.2±1.79 - 15.9±1.55 g) and %ash (10.83±0.03 - 18.85±0.04 %) than the control (6.6±0.49 g and 8.48±0.02 %). Bioaccumulation factor was 0.12 to 0.48 indicating that *P tuber-regium* cannot be employed in bioremediation of a continuously lead contaminated soil.

Key words – Bioaccumulation – fruit body – growth – heavy metal – sclerotium

Introduction

Lead is a naturally occurring toxic heavy metal found in the environment. Heavy metal pollution poses a serious threat to the environment, public and soil health. The use of rudimentary mining methods for extraction of gold ore from rocks in Zamfara State, Northwest Nigeria, has resulted in an epidemic of lead poisoning (Fig. 1). Between March and June 2010, lead poisoning accounted for more than 163 deaths including 111 children in that community. This also has contributed to high rates of infertility and miscarriages among affected adults (Anonymous 2013). Fungi, algae, bacteria, plant and activated sludge have demonstrated great potential as metal bio-sorbent due to their metal sequestering properties which can decrease the concentrations of heavy metal ions in soil (Nilanjana Das et al. 2008). Accumulation of metals by biological species has been attributed to physiology of the organisms. Mushrooms have comparatively remarkable ability to accumulate high concentration of metallic elements and could be employed for a more sustainable and effective intervention in remediation of polluted environment (Fourest & Roux 1992, Falandayzs & Chwir 1997, Falandayzs et al. 2003, Isikhuemhen et al. 2003). The purpose of

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this study was to investigate the effect of continuous artificial lead contamination of soil on the growth and lead accumulation in *P tuber-regium* and its implication for bioremediation.

Materials & Methods

Collection of materials

Sclerotia of *Pleurotus tuber-regium* were procured from a local market, Nwagu market in Agulu, Anambra State, Nigeria while loamy soil was collected from Bells University of Technology, Ota, Ogun State. Plastic bowls used for seeding were bought from Ojuore market in Ota, Ogun State, Nigeria.

Fig. 1 – Anthropogenic activity in a gold mining site in Zamfara State, Nigeria (Anonymous 2013)

Substrate preparation and inoculation

Soil was sieved with 1mm mesh size and 1kg was distributed in to perforated plastic bowls measuring 20cm diameter. Preparations of 50, 55 and 60ppm PbO were constituted. Treatments were four: soil + sclerotia, soil + 50ppm Pb + sclerotia, soil + 55ppm Pb + sclerotia and soil + 60ppm + sclerotia. The sclerotia were soaked in water for 18hrs and cut into 30g sizes before seeding in to soil treated with approximately 500ml of respective lead ion concentration. The plastic bowls were kept at room temperature ($28^oC \pm 2^oC$) on laboratory bench top. Subsequent watering was done with the respective lead ion solutions at the rate of 100ml/48 hours. Oyster mushroom was harvested from all treatments; fresh weights were taken and dried at 50^oC for 24hrs. The time for fruit body emergence was noted and % ash determined according to AOAC (1990).

Determination of Lead in fruit body and soil

Lead accumulation in fruit body and the soil was analyzed by atomic absorption spectrophotometer (BUCH Scientific Model 210) according to the method of Crosby (1977) and AOAC (1990). Dried samples were homogenized in a precleaned mortar, sieved and 2g sample were ashed in a muffle furnace at 550^oC-600^oC for 12-18hrs. The ash was digested with 10 ml nitric acid (HNO_3) (65 %) and 5ml perchloric acid ($HClO_4$) (70 %) on a hot plate for 40mins, filtered with whatman filter paper and diluted to 100ml with deionized water before lead determination.

Results and Discussion

Fruit body emergence

Fruit body emergence was faster in lead contaminated soil which could be an indication of tolerance of the mushroom to continuous lead contamination (Fig. 2). Similar effect of lead tolerance by *Pleurotus tuber-regium* was observed by Akpaja et al (2012) at 0.5 mmol lead contamination. Lead is reported to be tolerated by species of *Aspergillus* (Valix et al. 2000, Ezzouhri et al. 2009) and *Armillaria* (Rigling et al. 2006). Red clover plants cultivated in 50 mg Pb kg-1 soil was also reported to be well developed with numerous nodules (Stan et al (2011). These

reports are contrary to the work of Wargo & Carey (2001) and Majer et al. (2002) who reported that rhizomorph production by *Armillaria ostoyae* was inhibited in natural soils containing high concentrations of heavy metals, particularly lead. They concluded that lead contaminated soils reduced soil fertility, directly affected changes of physiological indices which caused a decline in mushroom yield. These contradictory reports could be due to the use of different species and experimental conditions.

Fresh weight

Fresh weight and %ash of all harvested mushrooms from lead contaminated soil were higher than the control (Figs. 2 & 3). This is also an indication that *P. tuber-regium* could grow in continuous lead contaminated soil. Highest and lowest weights in contaminated soil were recorded at 50 and 60ppm contamination respectively. The increase in weight and ash content could be associated with lead accumulation in the mushroom. The result agreed with the finding of Kalac & Svoboda (2000), Ita et al. (2006) and Kalac (2010) who reported that increase in mass of fruit body of wild and edible mushroom was associated with the concentration of metals. Although Akpaja et al. (2012) reported that lead greatly affected *P. tuber-regium* morphometry. The contradiction could be associated with the concentration and type of lead used. While 50-60ppm PbO was used in this work, the former used 0.125-2.0 mmol $PbSO_4$.

Lead Accumulation in soil and mushroom

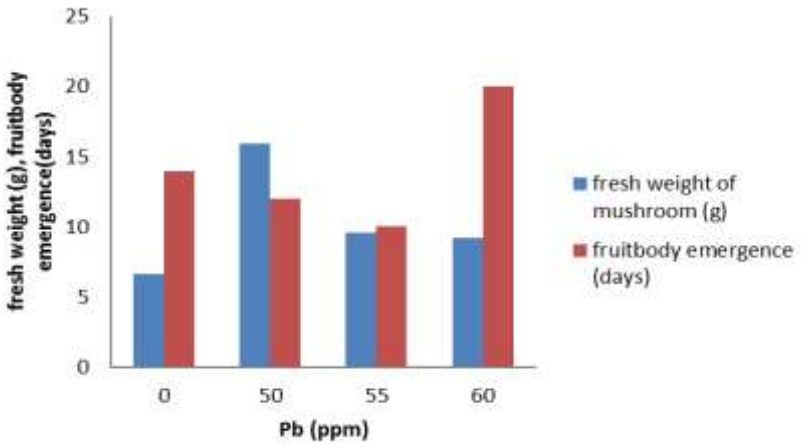

Fig. 2 – Fresh weight of mushroom and fruit body emergence in lead contaminated soil

Lead accumulation was more in soil than in mushroom fruit body (Fig. 4) and increased with increased contamination levels. The range in lead values in soil and mushroom was 145.0±0.57, 165.0±1.15, 463.0±0.82, 500.0 ppm±1.41 and 25.0±0.13, 80.0±0.16, 85.0±0,03, 60.0±0.35 ppm respectively. Edible and wild mushrooms have been shown to accumulate high concentrations of toxic metallic elements even when the concentration of such metal is low in the soil (Falandayzs & Chwir 1997, Falandayzs et al. 2003). *Cantharellus cibarius*, a mushroom widely used in European cuisine was reported to accumulate 4.86 g/g lead in its tissue. The values of lead in the studied mushroom exceeded recommended EU acceptable standard of 3 mg Kg^{-1} so mushrooms collected from such environment should not be consumed. The bioaccumulation factor of metals is a ratio of the metal concentration in the fruit body to the metal in the soil and 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 of lead in this work has values between 0.12 and 0.48 which are not important for bioremediation. This is similar to the finding of Ringling et al. (2006) who obtained a bioaccumulation factor of

0.009-0.27 mgKg^{-1} in *Armillaria*. This could be an indication that *P. tuber-regium* may not be efficient as a lead bio-sorbent in a continuously lead contaminated soil at the studied concentration.

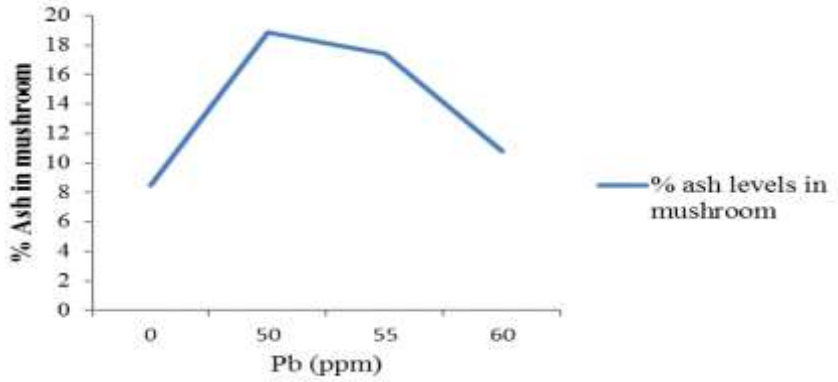

Fig. 3 – % Ash of mushroom in lead contaminated soil

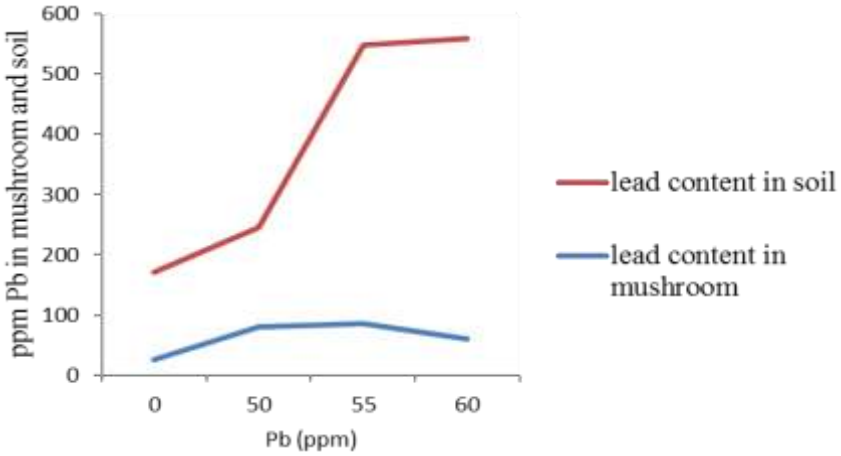

Fig. 4 – Lead accumulation in mushroom and soil

Table 1 Fruit body emergence, fresh weight of mushroom, %ash in mushroom, lead in soil and lead in mushroom at 0, 50 55 and 60 ppm respectively

	Concentration (ppm)			
Paramaters	**0**	**50**	**55**	**60**
Fruitbody emergence	14±1.41	12±0.82	10±0.82	20±1.63
Fresh weight	6.6±0.49	15.9±1.55	9.6±0.44	9.2±1.79
%Ash	8.48±0.02	18.85± 0.04	17.37±0.07	10.83±0.03
Lead in soil	145±0.57	165±1.15	463±0.82	500±1.41
Lead in mushroom	25±0.13	80±0.16	85±0.03	60±0.35

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