Effect of some heavy metals on the growth and development of *Pleurotus tuber-regium*

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The effects of five heavy metals (cadmium, copper, mercury, lead and zinc) on the growth and fruit body production in *Pleurotus tuber-regium* was investigated. Lead sulphate, zinc sulphate, copper sulphate, cadmium nitrate and mercury chloride were added to garden soil at concentrations of 0, 0.125, 0.25, 0.5, 1.0, and 2.0 mmol per 3 kg of soil. Sclerotia of the test mushroom were used to inoculate the artificially contaminated soil. Mercury prevented growth and fruit body production in *P. tuber-regium*. Fungal morphometry was greatly affected by lead. The heavy metal content in the fungal biomass complex increased with increase of heavy metal concentration in the soil. The highest concentration (183.06 mg/kg) was found in zinc at 2 mmol/L.

Key words – bioaccumulation – heavy metals – morphology – white-rot fungi

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Introduction

Mushrooms are valuable health foods, low in calories, high in vegetable proteins, iron, zinc, chitin, fibre, vitamins and minerals. The consumption of edible mushrooms is increasing, even in the developed world. Mushrooms have been also reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, and cancer. These functional characteristics are mainly due to their chemical composition (Manzi et al. 2001). In recent years, considerable attention has been focused on the bioaccumulation of heavy metals in fruit bodies of some mushrooms (Zhu et al. 2011, Oghenekaro et al. 2008, Demirbas 2001). Compared to green plants, mushrooms can build up large concentrations of some heavy metals such as lead, cadmium and mercury. However, until now, there is only scattered information about the physiological and morphological effects of heavy metals on mushrooms.

Heavy metals present in the environment can directly interact with extracellular enzymes of fungi. However, to cause physiological and morphological response, heavy metals must be taken up by the fungus. Some heavy metals are important for fungal metabolism, where as others have no known biological role. The same metals are often toxic at concentrations only a few times greater than those required (Baldrian 2003)

Pleurotus tuber-regium (Fr.) Singer is a tuberous wild species of white-rot basidiomycetes that produces fruit bodies or sporophores from a globose sclerotium; it occurs in both tropical and sub-tropical regions of the world (Isikhuemhen & LeBauer 2004). Oghenekaro et al. (2008) showed that *P. tuber-regium* was able to bioaccumulate some heavy metals. The objective of the present study was to determine the physiological and morphological effects of

Table 1 Days to primordial emergence in *Pleurotus tuber-regium*

	Concentration (mmol/L)					
Metals	**0**	**0.125**	**0.25**	**0.5**	**1.0**	**2.0**
Cadmium	13.33±1.76*	16.33±0.33	12.00±0.58	16.00±2.65	17.33±0.30	12.33±0.33
Copper	13.33±1.76	17.33±0.33	11.00±2.08	11.33±0.33	12.00±1.73	13.00±2.08
Mercury	13.33±1.76	0.00	0.00	0.00	0.00	0.00
Lead	13.33±1.76	16.00±0.58	17.00±0.58	9.67±0.33	20.33±0.67	17.33±2.91
Zinc	13.33±1.76	16.00±0.58	20.67±0.33	14.33±0.33	19.00±1.53	10.33±2.03

*Mean of triplicate determination±S.E

five heavy metals on the vegetative growth of *P. tuber-regium.*

Materials and Methods

Sclerotia collection

Sclerotia of *P. tuber-regium* were obtained from Ekiuwa market, Benin City, Edo State, Nigeria.

Heavy metals

The salts of the heavy metals - lead sulphate ($PbSO_4$), zinc sulphate ($ZnSO_4.7H_2O$), copper sulphate ($CuSO_4.5H_2O$), cadmium nitrate ($Cd(NO_3)_2.4H_2O$) and mercury chloride ($HgCl_2$) came from the Department of Plant Biology and Biotechnology Laboratory, univer-sity of Benin.

Soil collection and preparation

Garden soil at the Faculty of Education, University of Benin, was collected using a hand trowel. The soil was sieved and 3 kg of soil was loaded into each of 78 perforated plastic bowls (7 × 16 cm).

Treatment of soil with heavy metals

Each salt was prepared at 0, 0.125, 0.25, 0.50, 1.0 and 2.0 mmol/ L of sterile distilled water and 500 ml of each solution was thoroughly mixed with the soil, and replicated three times. The control lacked any heavy metal.

Inoculation of contaminated soil

Seventy-eight sclerotia pieces, each weighing 20 g were soaked for 3 hours in water. The sclerotia were then soaked in dilute sodium hypochlorite solution for 15 minutes to remove contaminants. They were then sown 2 cm deep in the artificially contaminated soil contained in the plastic bowls. The bowls were incubated at ambient temperature (26 ± 2^{o}C) and periodically watered to ensure that the environment was kept humid. The fruit bodies were harvested 7 days after primordial emergence and dried in an oven at 100^{o}C for 48hours. The following parameters were determined:

time of primordial emergence,
stipe length,
stipe diameter,
cap diameter and
heavy metal analysis of fruit bodies.

Determination of heavy metals in fruit bodies of mushroom

For elemental analysis, an atomic absorption spectrometer (Pye Unicam, Model 919) was used. Lead and cadmium in mushroom samples were determined using a GBC 3000 graphite furnace for atomic

Table 2 Heavy metal concentrations (mg/kg) in fruiting bodies of *Pleurotus tuber-regium*.

	Concentration of heavy metals in soil (mmol/L)					
Metals	**0**	**0.125**	**0.25**	**0.5**	**1.0**	**2.0**
Cadmium	0.430*	0.520	17.860	24.350	34.380	37.310
Copper	0.189	0.280	6.280	9.810	18.060	37.360
Lead	0.389	0.445	0.534	0.578	0.680	1.145
Zinc	52.304	73.223	83.686	104.608	130.760	183.064

*Unit= mg/kg dry weight of mushroom.

Table 3 Effect of heavy metals on stipe length (cm), stipe diameter (cm) and cap diameter (cm) of *Pleurotus tuber-regium*

Metals	Concentration (mmol/L)					
	0	**0.125**	**0.25**	**0.5**	**1.0**	**2.0**
			Stipe length			
Cadmium	7.70±0.66*	5.50±0.29	5.80±0.23	7.40±0.78	7.60±0.38	6.20±0.12
Copper	7.70±0.66	5.97±0.80	5.97±0.80	6.13±0.53	6.03±0.91	6.50±0.40
Mercury	7.70±0.66	0.00	0.00	0.00	0.00	0.00
Lead	7.70±0.66	4.63±0.27	5.73±0.58	7.47±0.58	4.83±0.38	4.43±0.23
Zinc	7.70±0.66	6.20±0.32	6.80±0.91	5.23±0.23	6.93±0.43	7.50±0.35
			Stipe diameter			
Cadmium	4.80±0.15*	4.70±0.25	4.50±0.12	4.50±0.20	4.50±0.54	4.50±0.23
Copper	4.80±0.15	4.13±0.19	4.73±0.40	4.60±0.27	4.40±0.31	4.40±0.31
Mercury	4.80±0.15	0.00	0.00	0.00	0.00	0.00
Lead	4.80±0.15	4.60±0.27	3.10±0.46	5.10±0.12	3.60±0.06	3.30±0.12
Zinc	4.80±0.15	3.80±0.21	4.17±0.24	3.80±0.21	4.60±0.27	4.87±0.38
			Cap diameter			
Cadmium	7.97±0.90*	6.10±0.46	9.87±0.41	8.43±0.38	6.27±0.66	6.73±0.33
Copper	7.97±0.90	6.90±1.03	10.27±0.12	10.13±0.26	9.83±0.99	9.03±1.18
Mercury	7.97±0.90	0.00	0.00	0.00	0.00	0.00
Lead	7.97±0.90	9.87±0.38	3.33±0.52	10.47±0.44	3.77±0.47	6.90±1.94
Zinc	7.97±0.90	6.57±0.27	3.43±0.12	7.17±0.54	8.90±0.44	9.90±0.38

*Mean of triplicate determination±S.E.

absorption spectrometer. Other measurements were carried out in air/ acetylene flame.

Statistical analysis

Results are expressed as mean ± S.E. of triplicate analysis. Data were evaluated using two-way analysis of variance (ANOVA).

Results and Discussion

The effect of the five heavy metals on primordial emergence is shown in Table 1. Mercury totally inhibited fruit body production in *P. tuber-regium* at all concentrations tested. The other heavy metals either delayed or slightly increased primordial emergence. Mercury is one of the most toxic metals for all white-rot fungi (Baldrian 2003). Baldrian & Gabriel (1997) showed that mercury and cadmium exhibited the highest toxicity in the fungus *Stereum hirsutum*.

P. tuber-regium was able to bioaccumulate the heavy metals (Table 2) with the content of heavy metal in fruiting bodies reflecting the metal concentrations in their environment. The highest concentration (183.06 mg/kg) was found with zinc at 2mmol/L. The higher up take of zinc may be due to the essential role that it plays in fungal growth and metabolism. The toxicity of cadmium is well known, and this study shows the ability of *P. tuber-regium* to bioaccumulate this metal.

Apart from mercury which completely inhibited growth and fruit body production in *P. tuber-regium,* the other heavy metals affected the morphometry of the mushroom (Table 3). Although *P. tuber-regium* was able to bioaccumulate the heavy metals, they affected both individual and complex metabolic process inducing morphological changes in the fungus as shown in difference between the stipe length stipe diameter and cap diameter of *P. tuber-regium* grown in heavy metal fortified soil and that grown in the control. Lead produced the greatest effect on stipe length, stipe diameter, and cap diameter followed by cadmium (Table 3).

The interference of heavy metals with physiological, morphological, enzymatic and reproductive processes of white-rot fungi has ecological consequences. The effect on growth or morphology directly affects the enzymatic activities, which leads to changes of community structure and influences the energy flux in the ecosystem. Further studies to understand the molecular mechanisms of heavy metal accumulation by *P. tuber-regium* are needed in order to enhance its use in heavy metal bioremediation.

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