Original Research Article

INFLUENCE OF LEAD ON MORPHOLOGY AND GENETIC COMPOSITION OF *Pleurotus tuber-regium*

ABSTRACT

Aims: Lead is a heavy metal pollutant that persists in the environment, has no biological function, and is potentially toxic to microorganisms. The study examined the effect of lead on the morphology and genetic profile of *Pleurotus tuber-regium,* an edible mushroom.

Study design: Experimental study design.

Place and Duration of Study: Bells University of Technology between December 2017 and August 2018.

Methodology: *P. tuber-regium* sclerotium was planted in loamy soils polluted with PbO (50, 80, and 100 mg/kg). The effect of the lead was assessed by recording time for mushroom emergence, fresh fruitbody weight, stipe and pileus diameter, % protein, %ash, and molecular profile of mushrooms harvested from polluted soils against a control. Extracted DNA was amplified with ITS1 and ITS4 universal primers; amplicons were visualized with a UV Bio-Rad illuminator.

Results: Lead had varied influences on the morphometry. The fruitbody emergence was fastest in polluted soils and significantly different from the control. Fruiting occurred after 13.23±0.76, 5.41±0.88, 9.33±0.75 and 11.01±1.06 days in 0, 50, 80 and 100 mg/kg Pb polluted soils respectively. The fresh weight, stipe, and pileus diameter were significantly different at 50 mg/kg compared to the control. The range in values was 8.57±0.75 –

15.21±0.85g, 9.30±0.89 - 14.40±0.99mm, and 3.33±0.75 - 9.60±0.57cm respectively. The %protein and %ash contents were higher in mushrooms from polluted soils. Lead accumulated in polluted soils but the bioaccumulation coefficient was low. DNA profile showed variations in amplicon sizes.

Conclusion: The study revealed that continuous exposure of *Pleurotus tuber-regium* to lead caused varied morphological and genetic changes. It led to increased fresh weight, stipe diameter, pileus diameter, and variation in DNA amplicons. The implication is that lead could cause variations in the morphology and genetic composition of *P. tuber-regium* with implications for marketability and food safety.

Keywords: DNA profile, Lead, Morphology, Pleurotus tuber-regium, Pollution, sclerotium

1. INTRODUCTION

Environmental pollution by heavy metals is of concern due to their accumulation and persistence in soil which could be worsened by low mobility, even under heavy precipitations. Heavy metal contamination of soils is reported to have adverse effects on food safety, marketability, crop growth, and microorganisms [1, 2]. Although some heavy metals are required for life's physiological processes (components of metalloenzymes), excessive accumulation in living organisms is always detrimental. Heavy metals adversely influence microorganisms, affecting their growth, abundance, morphology, and activities [3]. [4] reported that the maximum specific growth rate of microorganisms (μ_m) changed significantly when the concentration of mobile forms of lead in the soil exceeded 170 mg Pb/kg. Generally, toxic metals cause enzyme inactivation, damage cells by acting as antimetabolites, or form precipitates or chelates with essential metabolites [5].

Lead is ranked second among all hazardous substances and is common in areas with high anthropogenic and processing pressure [6]. The mean lead in soil lies between 15-40 ppm

[7], but values exceeding 10,000 ppm could result from anthropogenic activities [8]. Lead is potentially toxic to living organisms and has no biological function. It does not biodegrade or disappear from the environment and is estimated to have a soil retention time of 150 to 5000 years [9]. Lead can indirectly influence the growth of white rot fungi by inhibiting fungal growth and reducing the release of enzymes. Edible cultivated and wild mushrooms have been shown to accumulate great concentrations of toxic metals. *Pleurotus tuber-regium,* an edible oyster mushroom is known to have high nutritional value and has been shown to have bio-accumulative potential [10]. [11] reported maximum accumulation of heavy metal after the third flush of fruitification while 90% removal of Pb, Zn, Cu, and Mn by *P. tuber-regium* from artificially contaminated soil had also been reported. Elevated levels of lead in soil could result in higher bioaccumulation above the action level for lead in food products [12]. Community analysis of the 16S and *nirK* gene markers showed that Pb has detectable effects on community diversity even at the lowest concentration tested [5]. There is an increasing interest in heavy metal absorption and its influence on the growth of microorganisms. Therefore, the study was to assess the effect of continuous lead pollution on the morphology and genetic composition of *Pleurotus tuber-regium*.

2. MATERIAL AND METHODS

2.1 Materials

Pleurotus tuber-regium sclerotium used was procured from a local market, Oye, Neni in Anambra State, Nigeria while loamy soil was collected from the premises of Bells University of Technology, Ota, Ogun State Nigeria.

2.2 Cultivation of *P. tuber-regium* sclerotium in a loamy soil

Loamy soil was dried at 50^{o}C for 72 h, sieved with a 1 mm mesh sieve, and 1 kg measured into perforated plastic bowls measuring 27x12 cm. Different concentrations of lead oxide

solutions (50, 80, and 100 mg/kg) were prepared respectively. Each soil was moistened with the respective lead solution (200 ml) and soil without contamination served as the control. *Pleurotus tuber-regium* sclerotium (30 g) was soaked in distilled water for 18 h and planted in respective bowls. Watering was done with the respective lead solution (100 ml) every 24 h. The effect of lead was assessed by the time of fruitbody emergence, fresh weight, stipe length, stipe diameter, and pileus diameter. The accumulation of lead in the soil and the fruitbody of the mushroom was determined and the bioaccumulation coefficient was calculated. The bioaccumulation coefficient is the concentration of heavy metals in the mushroom fruit body divided by the heavy metal concentration in the soil [13] and indicates the ability of the mushroom to tolerate and accumulate heavy metals.

2.3 Lead Determination in the mushroom fruit body

Lead concentration was determined using the AAS (Atomic Absorption Spectrophotometer [14]. Dried ground mushroom samples (2g) in a crucible were placed into a muffle furnace at 550°C – 600°C for 18 h. Using safety tongs, the crucibles were quickly transferred to a desiccator, cooled, and weighed. Sulphuric acid (0.1M H_2SO_4) was added, stirred thoroughly, filtered through Whatman filter paper into a volumetric flask (100ml), and made up to the mark with deionized water and the Lead concentration determined.

2.4 Lead Determination in soil

Lead in soil was determined by weighing 2g of soil sample into a beaker containing 5 ml of perchloric acid and 5 ml nitric acid and boiled at 100^0C for 30 - 40 minutes. Filtration was done with Whatman filter paper into the 100ml volumetric flask and made up to the mark. The lead content was determined using the Atomic Absorption Spectrophotometer. The % Protein and ash compositions were determined according to [15].

2.5 Extraction of DNA

Mushroom DNA was extracted from the harvested mushrooms using a modified SDS DNA Extraction Protocol [16]. Using universal fungi primers ITS1 (forward) and ITS4 (backward), PCR amplification was performed in a total volume of 25 µl containing 2 µl of 50 ng/µl of genomic DNA, 1µl of 10X PCR buffer, 1.2 µl of 25mM $MgCl_2$ (Promega), 2µl of 2.5 mM dNTP, 0.3 µl Taq polymerase, and 14.5 µl of ultra-pure H_2O. Thermal cycling conditions were as follows; initial denaturation at 94 ^{0}C for 5 min, followed by 49 cycles of denaturation at 94 ^{O}C for 20 sec, annealing at 38 ^{o}C for 40 sec and extension at 72 ^{o}C for 1 min, and a final extension at 72 ^{o}C for 7 min. Amplicons were resolved on 2.0% agarose gel electrophoresis using 1X TBE buffer (45mM Tris-acetate, 5mM Boric acid, and 1mM EDTA, pH 8.0) at 100V for 2h. Gels were visualized by staining with ethidium bromide solution (0.5 µg/ml) and banding patterns were photographed over a bio rad UV mini dark room.

2.6 Statistical analysis

The data obtained were analyzed following standard statistical methods using SPSS. One-way ANOVA was conducted and means were compared by Duncan's Multiple range test.

3. RESULTS AND DISCUSSION

Continuous lead pollution induced significant morphological changes in the growth of the mushroom as revealed by One-way Analysis of Variance (ANOVA); it resulted in increased stipe diameter, higher fruit body weight, and pileus diameter, especially at 50 mg/kg pollution level. *P. tuber-regium* produced fruit bodies in all polluted soils but the emergence was fastest and significantly different at 50 mg/kg soil. The range was 5.41±0.88 days in 50 mg/kg lead-polluted soil followed by 80 mg/kg (9.33±0.75 days) and 100 mg/kg (11.01±1.06 days) while control emerged after 13.23±0.76 days.

Table 1. Morphological data and fruit body emergence in lead polluted soil

Pollution (mg/kg)	F weight (g)	P diameter (cm)	Stipe length (cm)	F emergence (Days)	Stipe diameter (mm)
0	8.57 ± 0.75[a]	5.93 ± 0.81[b]	7.57 ± 1.17[a]	13.23 ± 0.76[a]	9.30±0.89[a]
50	15.21 ± 0.85[b]	9.60 ± 0.57[c]	8.60 ± 1.30[a]	5.41 ± 0.88[b]	14.40±0.99[c]
80	12.77 ± 0.67[a]	5.67 ± 0.76[b]	9.07 ± 0.47[a]	9.33 ± 0.75[c]	11.23±0.57[b]
100	8.70 ± 0.69[a]	3.33 ± 0.75[a]	7.57 ± 1.22[a]	11.01 ± 1.06[d]	13.73±1.29[c]

Means of triplicate ± standard deviation. Values followed by the same letters in each column are not significantly different. Legend: F Weight-Fresh weight; P diameter-Pileus diameter, F emergence-Fruitbody emergence

Lead contamination at 50 mg/kg produced a significantly higher fresh weight of fruit body (15.21±0.85g), and higher pileus and stipe diameter (9.60±0.57cm and 14.4±0.99mm) respectively compared to the control which recorded 8.57±0.75g, 5.93±0.81cm, and 9.30±0.89mm. *P. tuber-regium* seemed to tolerate 50 mg/kg lead pollution as morphological values at this pollution level were significantly higher. [17] reported that red clover plants were well developed when planted in 50 mg kg^{-1} Pb contaminated soil. Species of *Aspergillus niger* and *Aspergillus flavus* were reported to be tolerant to lead contamination in soil [18]. The morphometry of *Pleurotus* species was affected mostly at 100 ppm and 0.05 mmol/l heavy metal concentration [19, 11]. The study agrees with previous reports as mycelia ramification, density and emergence of primordia were higher and faster in polluted soil than in control soil [20, 19]. Although in another study, it was reported that lead had no effect on the length, dry weight, and production of rhizomorph by *Armillaria* species [21]. Heavy metals were reported to indirectly influence the growth of white rot fungi [22] by inhibiting fungal growth and reducing the release of enzymes. The different effects of lead

could be attributed to differences in organisms' metabolic function, the chemical form of metal, total metal ion concentration, its bioavailability, and interference with different physiological and morphological processes.

Lead accumulated in fruitbodies harvested from polluted soils (Figure 1). *Pleurotus tuber-regium* had been reported to have the potential for bioaccumulation of heavy metals [10, 20] but with varying degrees of biosorption abilities. Removal of 90% Pb, Zn, Cu, and Mn was reported from artificially contaminated soil; other studies supported the ability of *Pleurotus tuber-regium* to remediate soils contaminated with toxic hydrocarbons from crude oil and diesel fuel [23]. [24] and [25] reported that the accumulation of heavy metals in the fruit bodies tends to increase with an increase in the metals in the substrate. The result from the present study indicated that *P. tuber-regium* may not be efficient for bioremediation of lead continuously polluted soil as the bioaccumulation factor is below 1. Biosorption ability depends on the type of metal, concentration, and composition of the substrate.

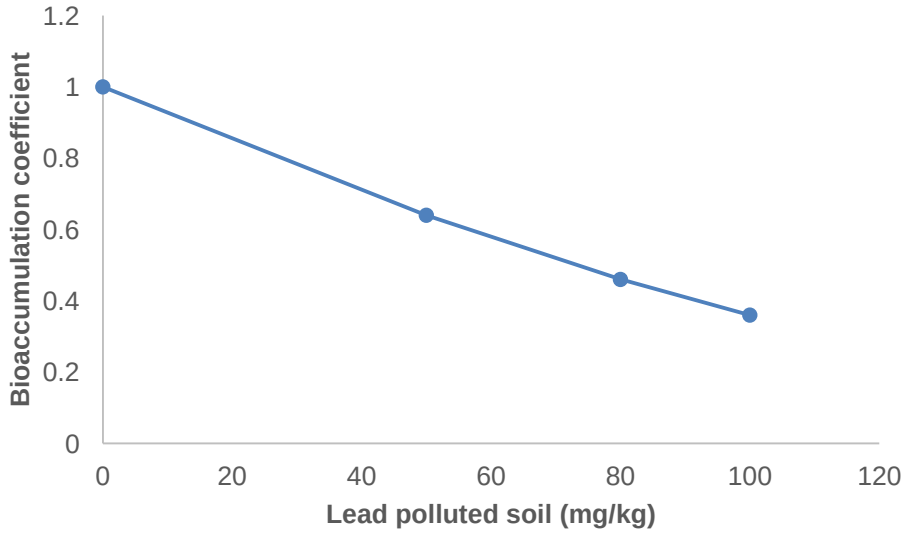

Figure 1: Bioaccumulation coefficient of *P. tuber-regium* in lead continuously polluted soil

Percentage ash and protein increased in fruitbodies harvested from polluted soils (Figure 2). The highest % ash was recorded at 50 mg/kg contamination while the highest % protein was at 50 and 100 mg/kg respectively.

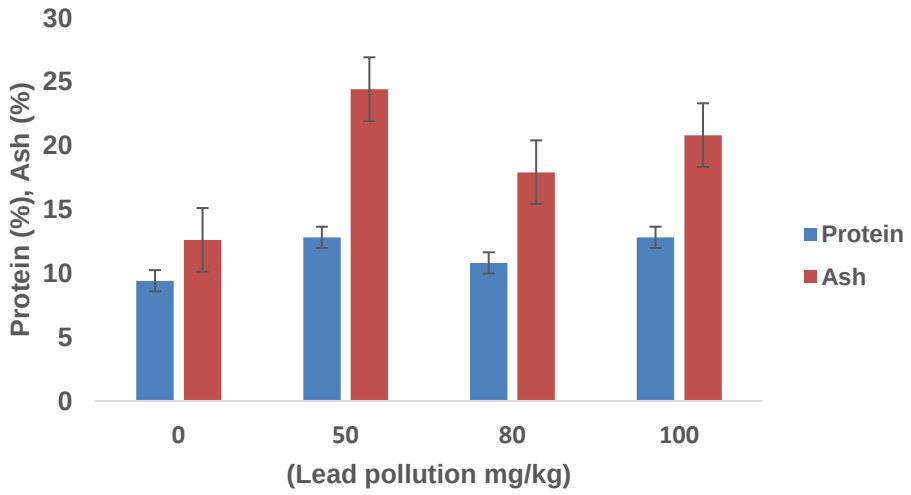

Figure 2: Percentage protein and ash in mushrooms harvested from lead polluted soil

The DNA profile of amplicons using 100 molecular base pair markers and ITS 1 (forward) and ITS 4 primers (backward) revealed variations in the sizes of DNA of *Pleurotus tuber-regium* (Plate 1).

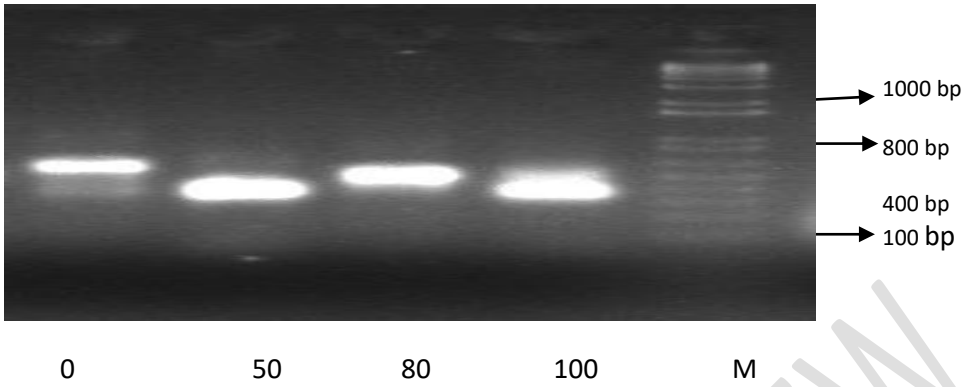

Plate 1: DNA profile of *Pleurotus tuber-regium* grown in lead-polluted soil (0, 50, 80, 100 mg/kg) using ITS 1/ITS 4 universal primers. Line M- 100 base pair marker

Gene expression in microorganisms was reported to be affected by heavy metals. There existed some level of genetic polymorphism in the sizes of extracted DNA using ITS 1 and ITS 4 primers. Expression of nod genes in rhizobium species varied in presence of an increasing concentration of heavy metals such as Pb, Cu, and Zn, and amplicons were different from the results obtained with similar strains isolated from unpolluted soil [17]. Community analysis by PCR/DGGE using 16S and *nirK* markers had shown that Pb has detectable effects on the community diversity even at the lowest concentration tested [5]. [26] observed significant species-specific differences in bacterial composition between natural and laboratory populations of drosophila and diversity increased under prolonged exposure to the lead-polluted substrate. These results suggest that the morphology and genetic composition of *P. tuber-regium* were affected by continuous lead pollution of soil.

Conclusion

The study revealed that continuous exposure of *Pleurotus tuber-regium* to lead caused varied morphological and genetic changes in the mushroom. It led to increased fresh weight, stipe, pileus diameter, and varying sizes of DNA amplicons. *P. tuber-regium* was not efficient in the bioremediation of continuous lead-polluted soil. However, further work is needed as the mechanism of such influence is unknown.

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