CONCENTRATION OF HEAVY METALS AND DETERMINATION OF BIOREMEDIATION POTENTIAL OF SOME SPECIES OF OYSTER MUSHROOMS (PLEUROTUS SPP) IN THE SOIL OF GOLD MINING SITE

ABSTRACT

Intensive environmental exploitation by Man has led to the emergence of a key sector – mining sector which, to a very great extent has been undertaken for its economic benefits. Inspite of the beneficial aspect of the sector, it produces hazardous wastes, causing various degrees of damages and threats to plant and animals in an ecosystem. This research was aimed at evaluating bioremediation potential of 2 species of oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus polmonarius*) in the degradation of heavy metals contained in the soil of gold mining site located at Paiko, Niger State of Nigeria. Concentrations of the heavy metals detected in the soil samples were determined. Media were prepared with appropriate substrates and later inoculated with pure cultured spawns of the 2 species within 3, 6 and 9 weeks treatment intervals for determination of growth factors and concentrations of the heavy metals in the *Pleurutu*s species. The Bioaccumulation Factor (BAF) for each of the 2 species was determined and compared. Results obtained showed different mean concentrations for the metals detected (4.851mg/kg, 51.826mg/kg and 121mg/kg) for cadmium, chromium and zinc respectively. Also detected were nickel (23.245mg/kg) and lead (10.925mg/kg). Results of the growth parameters indicated that *Pleurotus ostreatus* was higher in height (7.42cm) than *Pleurotus polmonarius* (5.68cm). For the stipe length, *Pleurotus ostreatus* was longer (4.7cm) than *Pleurotus polmonarius* (3.4cm). In diameter of Pileus, *Pleurotus ostreatus* had higher fruiting bodies of 25 than *Pleurotus polmonarius* which has 22. The total yield measured after harvest were 42.31g and 39.56g for *Pleurotus ostreatus* and *Pleurotus polmonarius* respectively. The dry matter contents were 6.39g and 6.06g respectively for *Pleurotus ostreatus* and *Pleurotus polmonarius*. Also the biological efficiencies for the 2 species were 42.31% and 39.56% respectively. The results indicated higher concentrations of metals in the mycelia of the species than *Pleurotus ostreatus* which had 0.573mg/kg of cadmium, 0.813mg/kg of chromium and 0.231mg/kg lead all after 9 weeks of inoculation. While *Pleurotus polmonarius* had higher concentrations of 0.253mg/kg and 0.549mg/kg of nickel and zinc respectively after 9 weeks of inoculation. For the BAF of the 2 species, *Pleurotus ostreatus* had higher potential (0.150) for cadmium in 6 weeks treatment and 0.026 for lead after 9 weeks, while *Pleurotus polmonarius* had higher BAF of 0.08 for nickel and 0.006 for zinc after 9 weeks of treatment. Finally it was concluded that the species showed significant bioremediation potential.

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Introduction

Progress made in the discoveries and development of more scientific and technological techniques since the beginning of industrial revolution have increasingly enabled man in the course of proper exploitation of his natural environment. Mining is one of the key sectors that have benefitted from these advances (Girigisu *et al.,* 2014). As a global industry, mining is being undertaken as an enterprise due to its economic benefits of wealth generation and employment creation.

In Nigeria and most of the African countries. However, mining is chiefly done at the artisanal or small-scale level (Kogo *et al.,* 2009). Although, it is an important socio-economic sector for peasant people in rural areas, artisanal mining is a mining operation that is usually not officially licensed by any regulatory agencies, operated by independent miners or groups of miners through manually-intensive methods, using local or hand tools (Oduma *et al.,* 2011). More than 90% of Africa's mining work force are artesenal miners (Norgate and Nawshad, 2012).

According to Saidu *et al.,* (2016), artesenal mining activities pose numerous challenges, but the most serious ones are environmental pollution or hazards and negative health impact on miners and mining communities. The major artesenal mined metal in Nigeria is, usually mined in form of Gold ore in many places such as Paiko in Niger State; Mahuta in Kebbi State; Anka in Zamfara State etc (Baba *et al.,* 2011).

Artesenal mining as practicable in some places if not all, usually involves activities such as excavation, grinding, ore concentration and dispersal tailings (Ferrara da silva *et al.,* 2004). These activities constitute mining processes that can lead to deposition of hazardous run wastes in form of Gold ore dumps which are the major sources of heavy metals such as lead, chromium, zinc, gold,

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nickel etc. (New York, Fime, 2005). These metals can cause various degrees of damages and threats to plants and animals' lives (Boamposem *et al.,* 2010).

Environmental pollution by the accuralation of heavy metals as a result of activities of artesenal Gold mining sites has become a very serious concern in the recent times. This is mainly due to the persistence and accumulation of the metals in ecosystem and subsequently into food chains, Paiko gold mining site is one of the numerous site for gold mining in Niger State, Nigeria and currently under takes only surface mining on open-pit, producing large volumes of wastes that may contain heavy metals. (Baba *et al.,* 2011). These metals accumulate and persist in the ecosystem, flow into water bodies and farmlands, causing various economic and health challenges. Therefore, there is need for environmental friendly, cost effective and less energy intensive approach for the remediation of polluted environment such as the gold mine site located at Paiko. In the present study, mycorenediation techniques were applied using oyster mushroom species which indicated a relatively high remediation potentials in the mushrooms.

MATERIALS AND METHODS

STUDY AREA

The study area, Paiko Mining Site, is located at Paiko, 24km along Minna-Suleja highway. The site is between latitudes $09^0$45'76.21"N-$09^0$45'77.05N and longitudes $06^0$66'68.14"E-$06^0$66'69.06"E. It is part of Sheet 185 N.W. The vegetation cover of the study area is typical of the central Savannah, a transitional type between the forest zone of southern Nigeria and the Guinea Savannah types of the Northern Nigeria (Ajibade and Woakes, 1976).

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Fig 1: Map of Nigeria showing Niger State

Fig 2: Map of Paikoro Local Government Area showing Paiko town

Experimental Design

The experimental set-up adopted for this study was the method by Purnomo *et al.,* (2010) with some modifications. The set-up consisted of three sets each for the two oyster mushroom species (*P. ostreatus* and *P. pulmonarius*) and were labeled SET PO1, SET PO2 and SET PO3 for *P. ostreatus* and SET PP1, SET PP2, and SET PP3 for *P. pulmonarius* respectively. Each set was composed of duplicate treatments containing contaminated soil from the mining pits. Incubation period of three (3) weeks for SET PO1 and SET PP1, six (6) weeks for SET PO2 and SET PP2 and nine (9) weeks for SET PO3 and SET PP3 were observed respectively. After every subsequent treatment period was reached (3 weeks, 6 weeks and 9 weeks respectively), the mycelia and mushroom fruiting bodies were carefully separated from the soil samples and placed into clean basins before digestion and heavy metal analysis using Atomic Absorption Spectrophotometry (AAS) was carried out. Uninoculated mining site soil samples as well as the substrate mixture (sawdust and rice husk) were served as controls which were then digested and analyzed using AAS as well.

Sterilization of Glasswares

All the glasswares used in this research were sterilized in an autoclave at 121°C (at 15 psi) for 30 minutes while work benches, containers and consumables were disinfected with 99% ethanol to ensure that the research materials were free from contamination.

Sample Collection and Preparation

Collection of Soil Samples

The soil samples were randomly obtained at the site using a hand trowel to a depth of 5-10cm. At each sampling location, One kilogram (1kg) of the soil sample was randomly collected as composite sample and placed into labeled and sterile plastic bags then transported to the laboratory for analysis.

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Preparation of the Gold mine Soil samples

Exactly 250g of contaminated soil from the goldmine pits each was weighed into well-labelled glass jars for each treatment. The prepared substrate mixture (sawdust and rice husk in a ratio of 2:1) was added and sterilized for inoculation. After inoculating the various treatment Sets, they were kept in a cool dark room and moistened with deionized water daily to ensure proper growth of the *P. ostreatus* and *P. pulmonarius* (oyster mushrooms).

Digestion of Gold mine soil and determination of metal concentration (AAS).

Digestion of Goldmine Soil

For the soil samples, wet acid digestion method was employed as described by Addis (2017) in which (10g) each of the treated contaminated soil sample and the controls were mixed with 60cm3 of H_2S0_4/HNO_3HC1 acid mixtures (5:5:1) and refluxed for 6 hours. The soil samples were washed with 1M HNO_3. Exactly 100cm3 of deionized water was then added and subsequently filtered using Whatman filter paper. The filtrate was transferred into clean labeled sample bottles before analysis for chromium, nickel, copper, cadmium and lead using an atomic absorption spectrophotometer (AAS).

Collection of *P. ostreatus* and *P. pulmonarius* (Oyster Mushroom) Spawns

The *P. ostreatus* and *P: pulmonarius* mushroom spawns used in this study were obtained from Federal Institute of Industrial Research Oshodi, Lagos State. Fresh hard wood sawdust were collected from Panteka wood processors and the rice husks from Zamina Rice Mill in Mando, all in Kaduna Metropolis. All substrates, spawns and equipment were kept in clean sterile polyethylene bags.

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Digestion of *P. ostreatus* and *P. pulmonarius* Oyster Mushroom and Substrates

After the duration of time stipulated for each treatment group (3 weeks, 6 weeks and 9 weeks respectively) exactly5g of the mycelia and the fruiting body of each oyster mushroom species as well as the substrate (control) was transferred into a clean crucible and then placed into an electric oven to remove all the moisture from the samples. After drying the samples, they were ashed in a muffle furnace at a temperature of 5 50°c for five hours and later digested with 20cm^3 of nitric acid/hydrogen peroxide (2:1). The digested residues were then dissolved with 50cm3 of deionized water and filtered in clean labeled sample bottles.

Tissue Culture of *P. ostreatus* and *P. pulmonarius*

Preparation of Potato Dextrose Agar (PDA)

In preparation of the Potato Dextrose Agar (PDA), the method described Adenipekun *et al.,* (2011) was followed. Exactly 200ml of distilled water was placed in the flat bottom flask and 7.8 g of the potato dextrose agar was weighed using an electric weighing balance and poured into measured distilled water in the flat bottom flask. Exactly, 100mg of Streptomycin was added to the media. The flask was corked using a piece of cotton wool and foil paper which were placed into the water bath for steaming and proper mixing at 90°C. The flask was removed from the water bath and allowed to cool for 3mins and placed into the autoclave for sterilization at 121^{0}C for 15mins. After sterilization, the autoclave valve was allowed to cool completely before removing it from the autoclave. Up to ten (10) labeled Petri dishes were properly arranged on the work bench and a Bunsen burner was lit and the mouth of the flat bottom flask was flamed. Subsequently, the media were transferred into the Petri dishes with further flaming of the flask. The plates were covered

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immediately after the transfer of the media which were left to gel in the Petri dishes for 2 hours before they were stored in a dry place till the next day for inoculation (Adenipekun *et al.,* 2011);

Preparation of Pure Cultures of *P. ostreatus* and *P. pulmonarius*

The young and healthy fruity bodies of *Pleurotus ostreatus* and *P. pulmonarius* were prepared by breaking their caps and stems to expose the interior tissues, followed by excising and inoculating small tissue fragments using a sterile scalpel in petri dishes containing potato dextrose agar as described by Stamets (2000). The inoculated petri dishes were then incubated at 25-28ºC for 5 days. The Mycelia that appeared were sub-cultured until pure cultures were obtained for each oyster mushroom species. The pure cultures were preserved at 4ºC as stock cultures.

Preparation of Mother Spawn

The mother spawns of *P. ostreatus* and *P. pulmonarius* were prepared on millet grains as described by Herbert, (2010). Clean, healthy and well-sized millet grains were used as substrate for preparation of the mother spawn. The millet grains were washed and soaked in water for 24 hours, after which the water was decanted and grains were boiled for 10-15 minutes until they became soft while the seed coat remained intact. After which the excess water was decanted and then allowed to cool. The cold grains were mixed with 0.5% calcium carbonate solution and 2% calcium sulfate solution to avoid clumping and maintenance of pH respectively. In an empty sterile glass jars, the processed millet grains were filled up to half capacity and plugged with non-absorbent cotton then sterilized in autoclave at 121ºC for 2 hours. After sterilization, the jars were allowed to cool and then inoculated with small bits of the pure cultures of *P. ostreatus* and *P. pulmonarius* before incubation at 25°C for 5 days.

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Preparation of Substrates

The sawdust samples were rinsed in clean water to remove all chemicals added during wood processing. The rice husks were ground up with a miller to increase its surface area. The substrate was then prepared by mixing the sawdust and the rice husks samples in ratio of 2:1. The prepared substrates were then packed in clean sterile polyethylene bags (Herbert, 2010).

Bagging and Inoculation of Treatments

Exactly fifty grams (50g) of each contaminated soil sample was weighed and placed in clean polyethylene bags and 100g of the substrate mixture (sawdust and rice husk in a ratio of 2:1) was then added on top of the soil sample as described by Urunay (2015). This was done in duplicates for each sample in each treatment. The tops of the bags were then covered with cotton wool and pieces of polyvinyl chloride (PVC) pipes of 3cm long and 2 mm thick were used as bottle necks then secured with rubber bands. The bagged set-ups were sterilised in an autoclave for 1 hour at 121°c. The sterilized experimental set-ups were allowed to cool to ambient temperature (30°C). After cooling, mother spawns of *P. ostreatus* and *P. pulmonarius* were carefully added to the various treatment set-ups under an isolation hood. The various treatment groups were kept in a dark cool room with daily addition of deionized water throughout the duration of the study (Urunay, 2015).

Determination of Growth Parameters of *P. ostreatus* and *P. pulmonarius*

The growth parameters of the *P. ostreatus* and *P. pulmonarius* mushrooms after 9 weeks, were determined using the method described by Patar *et al.,* (2018). The growth parameters measured were height of fruiting body, length of the stipe and diameter of the pileus.

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Length of Stipe of *P. ostreatus* and *P. pulmonarius*

The length of the stalk was measured using the ruler. The fruiting bodies of the *P. ostreatus* and *P. pulmonarius* mushrooms were randomly selected using simple random technique and the lengths of the stipe were measured from the tip of the stalk to the base of the caps and then the average was calculated.

Height of Fruiting Body of *P. ostreatus* and *P. pulmonarius*

The height of the fruiting bodies were measured using ruler. Five fruiting bodies of the *P. ostreatus* and *P. pulmonarius* mushrooms were randomly selected using simple random technique and the lengths were measured from the tip of the caps of the mushrooms to the base and then the average calculated.

Diameter of the Pileus of *P. ostreatus* and *P. pulmonarius*

The diameters of the pileus of *P. ostreatus* and *P. pulmonarius* were measured using a measuring ruler. The ruler was placed across the circumference of the pileus of five randomly selected fruits and measured. The average of the length of the diameters was calculated and recorded.

Yield Parameters of Oyster Mushroom Species

The yield parameters of the *P. ostreatus* and *P. pulmonarius* mushrooms in the different treatment groups were measured and recorded at the end of the duration for each treatment. The yield of the mushrooms was determined in terms of dry matter estimation, yield and biological efficiency of each oyster mushroom species (Patar *et al.,* 2018).

Determination of the Dry Matter Content of *P. ostreatus* and *P. pulmonarius*

A cleaned crucible was oven dried at 100ºC and allowed to cool to a constant weight (W_1) in a desiccator. Five, grams of the *P. ostreatus* and *P. pulmonarius* fruiting bodies were placed into the crucible and the weight (W2) noted. The crucible and its content (W_2) were oven dried at 100°C for five hours and

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allowed to cool in a desiccator (AOAc, 2002). The drying procedure was repeated to a constant weight (W_3) and the percentage moisture content was calculated using formula below.

Moisture content = $\frac{w2-w3}{w2\ w1}$ x 100

Where:

W_1 = Initial weight of dried crucible

W_2 = Weight of dried crucible and 5 grams of *P. ostreatus* and *P. pulmonarius* fruiting bodies

W_3 = Final weight of *P. ostreatus* and *P. pulmonarius* fruiting bodies after drying

Dry Matter content (%) = 100 — Moisture content

Total Weight of *P. ostreatus* and *P. pulmonarius*

The total weight of *P. ostreatus* and *P. pulmonarius* mushrooms harvested from the various treatments was measured using the electronic balance. The weights of the harvested mushrooms after 9 weeks were weighed and recorded and then calculated by simple addition. (AOAC, 2002).

Biological Efficiency

Total weight of the fruiting bodies harvested from the substrates within 30 days of fruiting was measured as total yield of the mushroom. The biological efficiency (yield of mushroom per kg substrate on dry weight basis) was calculated by the formula reported by Chang *et al.,* (1981).

Biological Efficiency (5) = $\frac{\text{Fresh Weight of Mushroom}}{\text{Dry Weight of Substrate}}$ x 100

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Bio-accumulation Factor of Heavy Metals from Contaminated Soil to *P. ostreatus* and *P. pulmonarius*

In order to determine the relationship between the concentrations of heavy metals in a soil to the level by which the heavy metals accumulate in the mycellia and fruiting bodies of *P. ostreatus* and *P. pulmonarius* mushrooms, the bio-accumalation factor was calculated based on the method described by Oyedele *et al.,* 1995 and Harrison and Chirgawi, 1989). That i.e:

Bio-accumulation Factor = Ps (μgg^{-1})/St (μgg^{-1})

Where Ps is the heavy metals' concentration in the mycellia and fruiting bodies of the *P. ostreatus* and *P. pulmonarius* mushrooms originating from the soil and St is the total heavy metal content in the soil.

Preparation of Stock Solution

Preparation of standards respectively for 5 metals (Ni, Cr, Pb, Cu and Cd was done following the procedures reported by Alpha-Awwa-WPCF, (2005).

Data Analysis

Data obtained from this study were subjected to descriptive statistics and one way analysis of variance (ANO VA) using SPSS 20.

RESULTS AND DISCUSSIONS

Heavy Metal Concentrations of Goldmine Soil Samples

The results of the heavy metal concentrations of the goldmine contaminated soil samples are presented in Table 1. The results showed that the mean concentration of cadmium was lowest (4.851mg/kg) while nickel had the highest (23.245mg/kg). Chromium had a mean concentration of 5 1.826 mg/kg while lead was 10.925mg/kg and zinc had the highest concentration of

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121.571mg/kg. Cadmium, chromium and zinc mean concentrations exceeded the WHO/FAO limit.

Growth Parameters of *P. ostreatus* and *P. pulmonarius* (Oyster Mushrooms)

Results of the growth parameters of *P. ostreatus* and *P. pulmonarius* after 9 weeks of propagation with substrates and contaminated gold mine soil samples are presented in table 2. The results showed that P. stratus was higher in terms of height with 7.42 cm while *P. pulmonarius* had 5.68 cm. In stipe length, P. stratus was still higher with 4.71 cm, while *P. pulmonarius* had 3.43cm. *P. stratus* also had a wide diameter of pileus of 5.13 cm than *P. pulmonarius's* (4.46cm).

Yield and the Biological efficiency of *P. ostreatus* and *P. pulmonarius*

The results of the yield and biological efficiency of *P. ostreatus* and *P. pulmonarius* cultivated with substrate and goldmine contaminated soil are presented in Table 3. In terms of fruiting bodies, the results showed that P. ostreatus had 25 which was higher than that of *P. pulmonarius* (22). The total yield after harvest were 44.10g for *P. stratus* and 37.42kg for *P. pulmonarius*. The dry matter content showed that *P. stratus* was 6.39g while that of *P. pulmonarius* was 6.06g. The biological efficiencies were found to be 42.3 1% and 39.56 % for *P. stratus* and *P. pulmonarius* respectively.

Heavy Metal Concentration of Goldmine Soil Remediated with *P. ostreatus* and *P. pulmonarius*

The results of the mean concentrations of the heavy metals in the goldmine soil samples that were inoculated with *P. ostreatus* and *P. pulmonarius* oyster mushrooms for 3 weeks, 6 weeks and 9 weeks respectively are presented in Table 4. It showed that the lowest heavy metal concentration of 3.785 mg/kg was observed for cadmium in the soil sample inoculated with *P. ostreatus* after 9 weeks. For Nickel however, the *P. pulmonarius* inoculated soil had the highest mean concentration of 22.653 mg/kg after 9 weeks. For chromium, the *P.*

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ostreatus treated samples showed the lowest concentration of 50.084 mg/kg after 9 weeks.

Heavy Metal Concentration of *P. ostreatus* and *P. pulmonarius* Mycelia

The results of the mean concentrations of the heavy metals in the mycelia of *P. ostreatus* and *P. pulmonarius* used in innoculating goldmine soil samples (Table 5) showed that *P. ostreatus* mycelia had higher mean concentrations of 0.573 mg/kg for Chromium after 9 weeks while that of *P. pulmonarius* was 0.185 mg/kg for the same period. For nickel, the mycelia concentration was higher in *P. pulmonarius* with 0.253 mg/kg over 9 weeks while that of *P. ostreatus* over the same period was 0.227 mg/kg. In chromium, the mycelia concentrations showed that *P. ostreatus* was higher with 0.813mg/kg while only 0.041 mg/kg was observed for P. pulmonarius. In lead, *P. ostreatus* had a higher mean concentration of 0.231 mg/kg over 9 weeks while that of *P. pulmonarius* was 0.198 mg/kg over the same period. However, for zinc, *P. pulmonarius* had a higher mean mycelia concentration of 0.546 mg/kg over 9 weeks while that of *P. ostreatus* was 0.373 mg/kg over the same period. The mean concentration of nickel, chromium, lead and zinc did not exceed the WHO limits after 9 weeks but mean concentration of cadmium after 6 weeks (0.63 1 mg/kg) and 9 weeks (0.573 mg/kg) both exceeded the WHO limit of 0.3 mg/kg.

Heavy Metal Concentration of *P. Ostreatus* and *P. Pulmonarius* Fruiting Bodies

The results of the mean concentrations of the heavy metals in the fruiting bodies of *P. ostreatus* and *P. pulmonarius* used in inoculating goldmine soil samples for 3 weeks, 6 weeks and 9 weeks respectively are presented in table 6. The results for cadmium showed that the fruiting bodies of *P. ostreatus* had higher mean concentrations of 0.291mg/kg after 9 weeks while that of *P. pulmonarius* was 0.093mg/kg for the same period. For nickel, the fruiting body mean

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concentration was higher in *P. pulmonarius* with 0.185mg/kg over 9 weeks while that of *P. ostreatus* over the same period was 0.045mg/kg. For Chromium however, *P. ostreatus* had higher fruiting body mean concentration of 0.673 mg/kg while that of *P. pulmonarius* over the same period was 0.025mg/kg. For lead, *P. ostreatus* had a higher mean concentration of 0.05 8 mg/kg over 9 weeks while that of *P. pulmonarius* was 0.026 mg/kg over the same period. However, for zinc, *P. pulmonarius* had a higher fruiting body mean concentration of 0.254mg/kg over 9 weeks while that of *P. ostreatus* was 0.184mg/kg over the same period. There were no fruiting bodies produced as at 3 weeks by the two Pleurotus species. At the end of the 9 weeks, none of the heavy metal concentrations exceeded the WHO limits.

Bioaccumulation Factor of Heavy Metals on *P. Ostreatus* and *P. Pulmonarius*

The bioaccumulation factors (BAF) of the heavy metals in *P. ostreatus* and *P. pulmonarius* over 9 weeks are presented in table 7. For cadmium, the highest bioaccumulation factor of 0.178 was observed in *P. ostreatus* over 9 weeks while the least was observed in *P. pulmonarius* over 6 weeks which had a BAF for 0.033. For nickel, the treatment with the highest bioaccumulation factor was the 9-week *P. pulmonarius* which was 0.018 while the least were 3 weeks *P. ostreatus* which had 0.0006. For chromium, the 9-week *P. ostreatus* treatment had the highest BAF of 0.028 while the lowest was the 3 weeks *P. pulmonarius* (0.0002). For lead, the 9-week *P. ostreatus* treatment also had the highest BAF with 0.026 while the least was the 3-week *P. ostreatus* treatment which had a bioaccumulation factor of 0.0011. For zinc however the 9-week *P. pulmonarius* had the highest factor of 0.006 while the least was the 3 weeks *P. ostreatus* which had a factor of 0.0007.

On the heavy metal concentrations of the soil samples cadmium had the lowest concentration of 4.851mg/kg and the highest was zinc with 121.571mg/kg.

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Chromium had 51.826mg/kg, nickel was 23.245 mg/kg while lead was 10.925mg/kg. From these results, the mean concentrations of cadmium, chromium and zinc in the soil exceeded the WHO limits. Cadmium is a known carcinogen and can increase the risk of developing cancers such as lung and prostate cancer (Sanusi *et al.,* 2017). Excessive cadmium exposure can also lead to severe kidney damage, potentially resulting in kidney failure, lung problems, and bone diseases. High concentrations of cadmium in soil can lead to uptake by plants which can accumulate in their tissues and may retard plant growth and development. Cadmium can also leach into groundwater, surface water bodies and negatively affect aquatic organisms (Salisu *et al.,* 2016). Also, high levels of zinc in the body can cause gastrointestinal disturbances, including stomach cramps, nausea, and vomiting. Elevated concentrations of zinc can affect the soil microbial stability, leading to imbalances in nutrient cycles and reducing soil fertility (Boamponsem *et al.,* 2013).

The health hazards posed by chromium is dependent on its valence state where the hexavalent chromium (Cr(VI)) is a potent human carcinogen and can cause lung cancer, skin irritation, and damage to the respiratory system the trivalent chromium (Cr(III)) is considered less toxic (Lackay, 2017). Environmentally, high chromium concentrations in the soil can leach into aquifers, streams and rivers and pose serious risks to aquatic habitats.

The results obtained differed significantly from those reported by Salisu *et al.,* (2016) on the analysis of the distribution of heavy metals in the soils of Bagega mining area in Zamfara State. However, results obtained by Lackay (2017) from iron ore mining site showed much higher levels of heavy metal with 162mg/kg, 1173mg/kg and 751mg/kg for chromium, lead and nickel respectively. The concentration of zinc observed in this study was also lower than 237.96 mg/kg reported in an earlier study by Okunola *et al.,* (2007). Sanusi *et al.,* (2017) also obtained significantly higher concentrations of heavy metals with 658.80mg/kg

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for lead, 330.50mg/kg for zinc and 20.70mg/kg for cadmium. These differences in the concentrations of heavy metals can be attributed to the differences in geological characteristics and soil composition. The geology of an area plays a significant role in determining the presence and distribution of heavy metals because different types of rocks and soils contain varying levels of heavy metals. Areas with naturally occurring heavy metal-rich rocks or mineral deposits are more likely to have elevated concentrations of these metals in the soil (Chen *et al.,* 2019). The climate and precipitation patterns may also influence the movement of heavy metals through the environment. Higher levels of rainfall can leach heavy metals soil and rocks, thereby affecting the concentration of the heavy metals (Roullon *et al.,* (2013).

The growth parameters of *P. stratus* and *P. pulmonarius* after 9 weeks of propagation with substrate and goldmine contaminated soil showed that *P. stratus* had a mean height of 7.42 cm which was longer than the 5.68 cm observed for *P. pulmonarius.* These results slightly differed with the findings of Oladipo *et al.,* (2020) in which they observed a range of 7.10 cm to 9.4 cm as the height of P. stratus grown over different substrates. Similarly, these results were significantly lower than those obtained by Patel *et al.,* (2019) who carried out a comparative study on growth parameters and yield potential of five species of oyster mushroom and found a range of 9.35cm-12.02cm as the height of the oyster mushrooms. In terms of stipe length, *P. stratus* was found to be longer with 4.71 cm while *P. pulmonarius* was 3.43 cm which was slightly lower than the range of 5.05cm-6.43cm obtained by Patel *et al.,* (2019) and the range of 5.70cm-6.60cm observed by Oladipo *et al.,* (2020) as well. In the diameters of the pileus, *P. stratus* had 5.13cm while *P. pulmonarius* was lower with 4.46cm and both are lower than the range of 5.90cm-7.20cm obtained by Oladipo *et al.,* (2020) and the range of 6cm- 7.2cm obtained by Patar *et al.,*

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(2018) when they investigated the comparative growth parameters and yield potential of two species of Pleurotus mushroom.

In terms of the yield parameters, *P. ostreatus* slightly had the higher number of 25 fruiting bodies after 9 weeks while *P. pulmonarius* had a slightly lower number of 22 fruiting bodies. These results were similar to the findings of Patar *et al.,* (2018) where they obtained a range of 21-49 fruiting bodies but significantly lower than the number gotten from the research conducted by Patel et al. (2019) where the range was between 82.67 to 101.33. The total yields obtained in this study showed that *P. ostreatus* was higher with 42.31g while *P. pulmonarius* only had 39.56 g but both were much lower than the range of 47.33 to 48.87 observed by Patel *et al.,* (2019). The biological efficiencies (BE) in this study were found to be 42.31% and 39.56% for *P. stratus* and *P. pulmonarius* oysters mushrooms respectively. These were considerably lower than most studies in oyster mushrooms such as Buah *et al.,* (2010) with a range of 68.40% to 91.21%, Oladipo *et al.,* (2020) which had a range of 57.34% to 72.64%, the range of 94.0% to 136.3% obtained by Patel et al. (2019) and the range of 82.33% to 98.23% obtained by Patar *et al.,* (2018).

These significantly low growth and yield parameters observed in this study can be attributed to the uptake of heavy metals especially lead, cadmium, nickel and zinc as was reported by Baldrian *et al.,* (2000) who they demonstrated inhibition of oyster mushroom mycelia development and soil penetration in the presence of high concentrations of cadmium. Stamets, (2005) also reported that mushrooms absorb and mineralize heavy metals in the soil by extracellular digestion of these heavy metals. Lead has been shown to reduce biological efficiency of sporocarp production in Plearotus species as well as inhibit the growth and fruiting body production in *P. tuberregium*, while stipe length, stipe diameter and cap diameter were affected by cadmium (Akpaja *et al.,* 2012).

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The results of the remediated goldmine soil samples indicated by the concentrations of the metals in 2 mushroom species showed that *P. stratus* had the most significant reduction rate in cadmium concentration, with values decreasing from 4.473mg/kg to 3.785mg/kg over 9 weeks. *P. pulmonarius*, on the other hand, had a greater reduction rate in nickel concentration, decreasing from 23.088 mg/kg to 22.653mg/kg over the same period. With regards to chromium, *P. ostreatus* exhibited higher reduction rates compared to *P. pulmonarius*, with concentrations decreasing from 51.627mg/kg to 50.084mg/kg over 9 weeks. P. stratus also showed better results in reducing lead concentrations, which decreased from 10.894mg/kg to 10.618mg/kg over the treatment period. However, in terms of zinc *P. pulmonarius* exhibited better concentration reduction, decreasing from 121.091mg/kg to 120.538mg/kg over the 9-week period. The findings of this study are in conformity with those obtained by Adenipekun *et al.,* (2011) where *P. ostreatus* and *P. pulmonarius* oyster mushrooms where incubated over crude oil contaminated soil for 0, 1 and 2 months but however the *P. pulmonarius* accumulated more of the heavy metals than the *P. ostreatus*.

Many studies have demonstrated the efficacy of most Pleurotus species to sequester a wide range of environmental contaminants such as heavy metals through biosorption. The accumulation of heavy metals in the mycelia and fruiting bodies of these oyster mushrooms tends to increase with an increase of the metals in the substrate as well as contact time (Ogbo and Okhuoya, 2011). Oyster mushrooms grown in heavily polluted areas like vicinities of smelters and forges have been reported to accumulate as much as 1000 times more than the background level of nickel and (Barcan *et al.,* 1998). The bioaccumulation potential of *P. ostreatus* from metal scrap sites has also been evaluated for Cu, Fe, Zn and Mn (Boamponsem *et al.,* 2013). However, the accumulation potential of Plearotus species varies with the type of metals.

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The heavy metal concentrations of *P. ostreatus* and *P. pulmonarius* mycelia used for treating goldmine soil samples as presented in Table 5 showed *P. stratus* exhibited a higher mean concentration of cadmium with 0.573mg/kg compared to the 0.185mg/kg obtained for *P. pulmonarius* over the 9 weeks of the study. For chromium, *P. ostreatus* showed a higher mycelia concentration of 0.8 13 mg/kg while *P. pulmonarius* had a much lower concentration of 0.041mg/kg. In the same vein, lead concentration of mycelia from *P. ostreatus* was 0.23 1 mg/kg which was higher than the 0.198mg/kg of *P. pulmonarius*. However, mycelia of *P. pulmonarius* had zinc and nickel concentrations with 0.546 mg/kg and 0.253mg/kg respectively.

The heavy metal concentrations of the fruiting bodies of Plearotus species obtained in this study showed that *P. ostreatus* exhibited a higher concentration of cadmium with 0.291mg/kg compared to the 0.093mg/kg of *P. pulmonarius*. For chromium, *P. ostreatus* showed a higher concentration of 0.673 mg/kg while *P. pulmonarius* had a significantly lower concentration of 0.025 mg/kg. In for lead, *P. ostreatus* also had a higher mean concentration of 0.058 mg/kg to the 0.026 mg/kg obtained from P. *pulmonarius*. However, *P. pulmonarius* had a higher zinc and nickel concentrations of 0.254mg/kg and 0.1 85mg/kg compared to the 0.1 84mg/kg and 0.045 from P. stratus respectively.

From these results, the comparison of heavy metal concentrations between *P. ostreatus* and *P. pulmonarius* in mycelia and fruiting bodies revealed some noteworthy distinctions. In mycelia, *P. ostreatus* had higher cadmium and chromium concentrations, while *P. pulmonarius* exhibited higher nickel concentrations. Moreover, *P. ostreatus* had higher lead concentrations in mycelia, but *P. pulmonarius* had higher zinc concentrations. Similarly, in the fruiting bodies, *P. ostreatus* showed higher cadmium and chromium concentrations, while *P. pulmonarius* had higher nickel concentrations. *P. ostreatus* also had higher lead concentrations, but *P. pulmonarius* had higher

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zinc concentrations. These results emphasize the varying heavy metal accumulation capabilities of the two species over different durations, particularly over 9 weeks.

The findings of this research also showed that the mycelia concentrations of the heavy metals were higher than those of the fruiting bodies. This is at variance with most researches on the mycoremediation of Plearotus species such as Boamponsem *et al.,* (2013) in which the fruiting bodies were found to be the site of most of the heavy metal sequestration. This anomaly can be attributed to the low yields of the fruiting bodies in both Plearotus species and only one flush (harvest) the fruiting bodies was observed when at least two or more flushes were observed in similar researches.

The bioaccumulation factor is the ratio of the concentration of an element in an organism to its concentration in soil provides the degree of accumulation of an element over a period of time. *P. ostreatus* was shown to have an overall increase of its bioaccumulation factor across all the heavy metals throughout the period of the study, especially in the case of cadmium and therefore a better candidate for mycoremediation of the heavy metals analysed in this study.

P. pulmonarius showed similar ability but after the firsts 3 weeks, a drop in its bioaccumulation factor was observed in the 6 weeks period before it showed increase in the 9 weeks period. This may be due to deleterious effects of the accumulation of the heavy metals on the species in the course of its growth and development.

Furthermore, bioaccumulation of metallic elements, metalloids and non-metals in fruiting bodies is highly species specific. Some macro-fungi are able to accumulate elements much more effectively than other species and the ability to accumulate a particular element is a characteristic feature of a particular fungal species (Falandysz and Borovika, 2013).

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As reported by Akpaja *et al.,* (2012), mercury can prevent overall growth and fruiting body production in *P. tuberregium*, while stipe length, stipe diameter and cap diameter can be negatively affect1 by lead and cadmium. In the same vein, heavy metals have also been reported to affect enzyme regulation among white rot mushrooms. Baidrian and Gabriel (2000) reported that the addition of mercury has been found to decrease the activity of laccase immediately and reduce the stability of the enzyme thereby affecting the growth and yield of the oyster mushroom.

Extracellular ligninolytic and cellulolytic enzymes can be regulated by heavy metals on the transcription level and these activities influences the energy flux within cellular metabolism (Boamponsem *et al.,* 2013).

Table 1: Heavy metal concentrations of goldmine soil

Heavy metal	Mean Concentration (mg/kg)	WHO/FAO Limit (mg/kg)
Cadmium	4.851	0.8
Nickel	23.245	68
Chromium	51.826	20
Lead	10.925	100
Zinc	121.571	50

Table 2: Growth parameters of *P. ostreatus* and *P. pulmonarius*

Mushroom species	Growth Parameters		
	Height (cm)	Strip length (cm)	Diameter of pileus (cm)
P. ostreatus	7.42	4.71	5.13
P. pulmonarius	5.68	3.43	4.46

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Table 3: Yield and biological efficiency of *P. ostreatus* and *P. pulmonarius*

Mushroom species	Growth Parameters			
	Number of Fruiting bodies	Total yield (g)	Dry matter (g)	Biological efficiency (%)
P. ostreatus	25	44.10	6.39	42.31
P. pulmonarius	22	37.42	6.06	39.56

Table 4: Heavy Metal Concentration of Goldmine Soil Remdiated with P. ostreatus and P. pulmonarius

Heavy Metal	***P. ostreatus* Remediated Soil Mean Concentration (mg/kg) (±SD)**			***P. pulmonarius* Remediated Soil Mean Concentration (mg/kg) (±SD)**		
	PO1	**PO2**	**PO3**	**PP1**	**PP2**	**PP3**
Cadmium	4.273±0.14[a]	3.812±0.11[a]	3.785±0.19[a]	4.781±0.13[a]	4.629±0.14[a]	4.534±0.13[a]
Nickel	23.197±0.021[a]	23.161±0.14[a]	23.135±0.21[a]	23.088±0.01[a]	22.876±0.12[a]	22.653±0.10[a]
Chromium	51.627±0.035[a]	51.179±0.04[a]	50.084±0.11[a]	51.793±0.10[a]	51.761±0.03[a]	51.752±0.11[a]
Lead	10.894±0.025[a]	10.687±0.01[a]	10.618±0.13[a]	10.875±0.17[a]	10.713±0.04[a]	10.682±0.14[a]
Zinc	121.138±0.05[a]	121.045±0.10[a]	120.851±0.14[a]	121.091±0.04[a]	120.816±0.12[a]	120.538±0.34[a]

Table 5: Heavy metal concentration of *P. ostreatus* and *P. pulmonarius* mycelia

Heavy Metal	***P. ostreatus* Mycelia Mean Concentration (mg/kg) (±SD)**			***P. pulmonarius* Mycelia Mean Concentration (mg/kg) (±SD)**			
	PO1	**PO2**	**PO3**	**PP1**	**PP2**	**PP3**	**WHO Limit**
Cadmium	0.215±0.14[a]	0.63±0.04[b]	0.573±0.19[b]	0.042±0.11[a]	0.162±0.14[b]	0.185±0.11[b]	**0.3**
Nickel	0.014±0.01[a]	0.018±0.14[a]	0.227±0.21[b]	0.227±0.11[a]	0.096±0.01[a]	0.253±0.14[b]	**1.5**
Chromium	0.086±0.14[a]	0.261±0.02[a]	0.813±0.11[c]	0.813±0.16[a]	0.037±0.10[b]	0.041±0.01[b]	**13**
Lead	0.0126±0.14[a]	0.189±0.19[b]	0.231±0.13[b]	0.231±0.19[a]	0.183±0.04[b]	0.198±0.01[b]	**2.0**
Zinc	0.088±0.11[a]	0.257±0.1[a]	0.373±0.14[b]	0.237±0.01[a]	0.383±0.11[a]	0.546±0.23[b]	**40**

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Table 6: Heavy metal concentration of fruiting bodies of *P. ostreatus* and *P. pulmonarius*

Heavy Metal	*P. ostreatus* Fruiting Bodies Mean Concentration (mg/kg) (±SD)			*P. pulmonarius* Fruiting Bodies Mean Concentration (mg/kg) (±SD)			
	PO1	**PO2**	**PO3**	**PP1**	**PP2**	**PP3**	**WHO Limit**
Cadmium	NP	0.097±0.02[a]	0.573±0.14[a]	NP	0.002±0.10[a]	0.093±0.19[a]	**0.3**
Nickel	NP	ND	0.045±0.09[a]	NP	ND	0.185±0.14[a]	**1.5**
Chromium	NP	0.186±0.22[a]	0.673±0.12[b]	NP	ND	0.025±0.02[a]	**13**
Lead	NP	0.029±0.14[a]	0.058±0.10[a]	NP	0.014±0.01[a]	0.026±0.01[a]	**2.0**
Zinc	NP	ND	0.184±0.10[a]	NP	ND	0.254±0.01[a]	**40**

* NP = Fruiting bodies Not Produced

* ND = Not Detected

Table 7: Bioaccumulation factor (BF) of heavy metals on from *P. ostreatus* and *P. pulmonarius*

	Bio-accumulation Factor (±SD)					
Heavy Metal	***P. ostreatus***			***P. pulmonarius***		
	PO1	**PO2**	**PO3**	**PP1**	**PP2**	**PP3**
Cadmium	0.044±0.01[a]	0.150±0.03[b]	0.178±0.09[b]	0.086±0.03[a]	0.033±0.04[b]	0.057±0.01[a]
Nickel	0.0006±0.00[a]	0.0007±0.0[a]	0.011±0.00[a]	0.002±0.01[a]	0.004±0.03[a]	0.018±0.04[a]
Chromium	0.001±0.00[a]	0.008±0.00[a]	0.028±0.01[a]	0.0002±0.00[a]	0.0007±0.00[a]	0.001±0.00[a]
Lead	0.0011±0.00[a]	0.019±0.01[a]	0.026±0.01[a]	0.002±0.00[a]	0.020±0.01[b]	0.020±0.01[b]
Zinc	0.0007±0.00[a]	0.002±0.00[a]	0.004±0.00[a]	0.001±0.00[a]	0.003±0.00[a]	0.006±0.00[a]

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