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Full Length Research Paper

Growth of *Pleurotus tuberregium* (Fr) Singer on some heavy metal-supplemented substrates

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The effect of three heavy metals, that is, lead, zinc and copper, on the growth of Pleurotus tuberregium, was investigated. Lead carbonate, zinc carbonate and copper sulphate were added to the mushroom substrate at concentrations of 0.1, 0.5, 1.0 and 2.0 g/250 g of substrate. Two sets were prepared using spawn and sclerotia as inocula. On a mycelial density rating, ranging from 0 - 5, the spawn-inoculated treatment was higher than that of the sclerotia. The average mycelial density was highest in copper-contaminated substrate that was inoculated with spawn, with a mean value of 5.0, while the lowest was in copper-contaminated substrate that was inoculated with sclerotia, with a mean value of 2.0. Fruit bodies were formed only in the copper-contaminated substrate at concentrations of 1.0 and 2.0 g/ 250 g of substrate. Shrinkage of the mushroom fruit body occurred seven days after formation of primordia. Biological efficiency of the harvested sporophores was 0.01 and 0.02%, respectively. There was a general inhibition of fruit body development by the heavy metals, except in the two treatments with copper.

Key words: heavy metals, *Pleurotus tuberregium*, mycelial density, biological efficiency, bioaccumulation.

INTRODUCTION

The term heavy metal refers to any metallic chemical element that has a relatively high density and that is toxic or poisonous at low concentrations. Examples of heavy metals include lead, zinc, copper, mercury, iron, manganese, cadmium, vanadium, antimony, arsenic and cobalt (Jarup, 2003). Heavy metals are natural components of the earth's crust and they cannot be degraded or destroyed**.** A small portion can be inhaled or ingested through water and food, and heavy metal poisoning could result, for instance, from drinking contaminated water (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain (Woodbury, 1993).

As trace elements, some heavy metals (e.g. copper, selenium and zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Other heavy metals such as mercury, lead and cadmium (with one exception for the latter) are toxic metals. They have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of mammals can cause serious illness. In humans, exposure to lead can result in a wide range of biological effects, depending on the level and duration of exposure. Lead in the air contributes to lead levels in food through deposition of dust, and rain containing the metal on crops and the soil (Garcia et al., 1998). Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth (Gabriel et al., 1996). Even though zinc is an essential requirement for a healthy body, high concentrations of zinc can be harmful. Excessive absorption of zinc can also suppress copper and iron absorption (Cunningham, 2005).

Mushrooms have fruiting bodies that grow out of a

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mass of mycelium, a web of thread-like hyphae that grow underground or colonize a substrate, such as dead wood or cardboard. The mycelium excretes enzymes that break down complex substances into simpler molecules. They can also take up heavy metals into their fruiting bodies. Heavy metal concentrations in mushrooms are considerably higher than those in agricultural crop plants, vegetables and fruits. They tend to bioaccumulate more molecules because of their mycelia (Turkekul et al., 2004). This suggests that mushrooms possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem.

Pleurotus tuberregium (Fr) Singer, also popularly known as the king tuber oyster mushroom, is a tuberous white rot basidiomycete, that occurs in both tropical and sub-tropical regions of the world (Isikhuemhen and LeBauer, 2004). Anoliefo et al. (2003) and Isikhuemhen et al. (2003) have shown that *P. tuberregium* was able to mycoremediate crude oil-polluted soil when the soil was mixed with a fully colonized substrate containing the mycelium of the mushroom. The objective of this study was therefore to determine the effect of three heavy metals on the vegetative growth of *P. tuberregium*, using spawn and sclerotia as inocula.

MATERIALS AND METHODS

Spawn preparation

A pure culture of the mushroom was obtained from the mushroom Biology unit, department of plant biology and biotechnology, University of Benin, Benin City, and was used in the preparation of spawn. The spawn was prepared using sorghum (*Sorghum bicolor*) grains as substrate. The inoculated grains were incubated at room temperature. After the grains had been fully colonized, they were kept in the refrigerator (5°C) until use.

Sclerotia collection

Sclerotia of *Pleurotus tuberregium* were obtained from Ekiuwa market, Benin City, Edo State, Nigeria.

Heavy metals

The heavy metals used were lead carbonate, zinc carbonate and copper sulphate.

Substrate collection and preparation

Sawdust from *Brachystegia nigerica*, locally known as okwhen among the bini-speaking people of Edo State, was used as compost. It was obtained from Uselu sawmill, Benin City, Edo State. Calcium carbonate (Labwaco Nigeria Ltd.), calcium sulphate (Labwaco Nigeria Ltd.), and wheat bran and sugar, bought at markets in the city of Benin, were used as nutrient supplement. The sawdust was sun-dried in order to achieve proper drying. After drying, the water content of the dried substrate was calculated and the oven dry weight of sawdust was then determined.

Composting

Compost for mushroom cultivation was prepared by fermenting the sawdust. For this, 77% of sawdust was mixed with 20% wheat bran, 1% calcium carbonate, 1% calcium sulphate and 1% sugar. The mixture was wetted thoroughly until the desired moisture content was attained, as determined by the method of Buswell (1984). The mixture was then piled up into a heap, and turning was done every 2 days during 7 days in order to produce a homogenous compost.

Treatment with heavy metals

Lead carbonate, zinc carbonate and copper sulphate were added to the substrate. Each salt was prepared at 0, 0.1, 0.5, 1.0 and 2.0 g per 750 ml of water, and replicates were made for each solution. The heavy metals were thoroughly mixed with the substrates, except in the control, which lacked the heavy metals.

Bagging and pasteurization

Two hundred and fifty grams (250 g) oven dry weight equivalent of wet (70 - 75%) moisture content of substrates was packed into 15 x 30 cm polypropylene bags. Five (5) replicate bags were prepared for each treatment. Polyvinyl chloride (PVC) pipes, 3 cm long and 5 cm wide, was fitted around the mouth of the bags. The opening was then plugged with cotton wool. The bagged substrates were then loaded into a steamer. Pasteurization was done for 4 h after which the pasteurized substrate was cooled to ambient temperature (30°C).

Inoculation, incubation and fruit body induction

The bags were inoculated with spawn and sclerotia at 3% (w/w). The sclerotia was soaked overnight in tap water to allow for maximum accumulation of water. After this, the sclerotia were cut into small pieces of 30 g each and then soaked in a sodium hypochlorite solution for 15 min to remove contaminants. They were then sown 4 cm deep in the substrate. After full mycelia colonization of the substrates and subsequent appearance of mushroom primordia, the bags were opened. This was followed by periodic watering of the bags and ensuring that the environment was humid. The following parameters were measured:

i.) The mycelia density, which was rated as described by Kadiri (1998). The following figures represented the extent of mycelia growth: 0 = No growth; 1 = Very sparse; 2 = Sparse; 3 = Moderate; 4 = Dense; 5 = Very dense.
ii.) Time of primordia emergence.
iii.) Fresh weight of mushroom.
iv.) Dry weight of mushroom.
v.) Biological efficiency, which was calculated as follows:
Biological efficiency (B.E %) = Fresh weight of mushroom/Dry weight of substrate x 100/1

RESULTS AND DISCUSSION

The fastest rate of colonization was observed with the substrate contaminated with copper at 2 g/250 g of substrate and inoculated with spawn. Mycelial density was taken after the bags were opened 21 days after inoculation and incubation. The lowest was observed in the copper-contaminated substrate inoculated with sclerotia

Table 1. Effects of three heavy metals on the mycelial density of *Pleurotus tuberregium*

Mycelial density						
	Spawn			Sclerotia		
Concentration (g/250 g substrate)	Lead	Zinc	Copper	Lead	Zinc	Copper
0	4.0(5)	4.0(5)	4.0(5)	3.0(4)	3.0(4)	3.0(4)
0.1	4.8(4)	4.2(5)	5.0(5)	4.2(4)	2.8(4)	3.2(5)
0.5	3.8(5)	4.8(4)	5.0(5)	3.2(4)	3.2(5)	3.6(5)
1.0	4.0(5)	5.0(5)	4.0(5)	3.0(5)	3.2(4)	3.6(5)
2.0	4.5(5)	4.2(4)	5.0(5)	3.2(5)	2.4(3)	2.0(3)

Table 2. Sporophore yield and morphometric measurement of fruit bodies.

Copper contaminated with spawn as inocula (g/250 g substrate)	Fresh weight (g)	Dry weight (g)	Cap diameter (cm)	Stipe length (cm)	Stipe diameter (cm)	Biological efficiency (%)
1.0	2.77	1.06	2.60	2.60	1.60	0.01
2.0	4.86	2.60	2.50	4.10	1.40	0.02

(Table 1). The spawn-inoculated control had a higher mycelial density than the sclerotia-inoculated control (Table 1). For the spawn-inoculated substrate, mycelial density was highest in the zinc-and copper-contaminated substrate. The mycelial density did not increase with an increasing concentration of the heavy metals. Mycelial density was the same for copper-contaminated substrate at 1.0 g and 2.0 g/250 g of substrate. The average mycelial density of the heavy metal treatment was higher than that of the control. The lead-contaminated substrate at 0.1 g had the highest mycelial density among the lead-polluted substrates. 0.5 g was the highest among the zinc-contaminated substrate, while for copper 0.1, 0.5 and 2.0 g had the highest (Table 1).

For the sclerotia-inoculated substrate, mycelial density was highest in the lead- and copper-contaminated substrate. The mycelial density did not increase with increasing concentration of the heavy metals. Mycelial density was the same for copper-contaminated substrate at 0.5 g and 1.0 g/250 g of substrate. The lead-contaminated substrate at 0.1 g had the highest mycelial density among the lead-polluted substrates. 0.5 and 0.1 g were the highest among the zinc-contaminated, while for copper 0.5 and 1.0 g had the highest (Table 1).

Primordial emergence was first observed in copper-contaminated substrate at 2.0 g inoculated with spawn after 26 days. Substrate contaminated with copper at 1.0 g formed primordia after 31 days. Fruit bodies were only formed in these treatments five days after primordial emergence. Three days later, shrinkage was observed in the fruit bodies just before harvesting.

In this study, *P. tuberregium* was able to form mycelia in the substrate contaminated with lead (Table 1). It thus has potential for bioaccumulation of this heavy metal (Garcia et al., 1998). Most mushrooms do not actively concentrate lead above the concentration in the environment. Lead is made less soluble, and hence less extractable, in soils where the pH is near neutral. The addition of lime (calcium carbonate) significantly reduces the solubility of lead and other heavy metals, thus locking them up and reducing into water and/or living organisms. Fruiting was observed in the treatment with copper. The effect of copper was noticed much later as shrinking of the fruit bodies became visible. This was probably due to the fact that the fruit bodies had a threshold concentration that they can tolerate (Baldrian, 2003). Thus, *P. tuberregium* has a high potential for bioaccumulation of this heavy metal (Anoliefo et al., 2003; Baldrian, 2003).

P. tuberregium has more bioaccumulative properties when grown from spawn rather than from sclerotia. This is due to the fact that when sclerotia is used for inocula=-ion, the infection rate will be quite high as the sclerotia is laden with microbial contaminants (Isikhuemhen and Okhuoya, 1996). The mushroom thus thrives better when grown from spawn than from sclerotia.

The effect of the heavy metals on enzymatic activities influences the energy flux in the ecosystem. It is therefore not surprising that the degree of heavy metal tolerance by *P. tuberregium* is different for different heavy metals (Sanglinsuwan et al., 1993) (Table 1).

Fruit bodies were only formed in the two treatments with copper (Table 2). This shows that *P. tuberregium* can bioaccumulate this metal to an appreciable level due to the formation of fruit bodies (Isikhuemhen et al., 2003). However, the effect of the heavy metal is noticed in the shrinkage, probably due to the fact that *P. tuberregium* has a limit it can tolerate.

When heavy metals are present in soil or fungal sub-

strate, they indirectly influence the growth of white rot fungi (Gabriel et al., 1996). They inhibit fungal growth and thus significantly reduces releases of enzymes that biodegrade xenobiotics.

This study shows that *P. tuberregium*, a white rot fungus has the ability to accumulate heavy metals. It can thus be used as mycoremediators in polluted environments. Researches should be further encouraged into the use of white rot fungi in bioaccumulation of heavy metals in polluted soil. Because mycoremediation is an infant technology, many experiments and proof-of-concept trials need to be conducted before commercialization.

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