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## Bioavailability of Some Heavy Metals in Crude Oil Contaminated Soils Remediated with *Pleurotus tuber-regium* Fr. Singer

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### ABSTRACT

The effect of *Pleurotus tuber-regium* on the bioavailability of metallic elements in crude oil contaminated soils was investigated. The fungus was grown in crude oil contaminated soils amended with sawdust, shredded banana leaf blades, NPK-nitrogen, phosphorus and potassium and poultry litter. The soils were analyzed for heavy metal content; total and exchangeable (Fe, Mn, Co, Ni, Zn, Cu, Pb, Cr, Cd, Hg and As). The mushroom caused dissolution of the metallic elements analyzed for, increasing concentration of heavy metals in their bio-available states. The addition of cellulosic wastes and fertilizers to crude oil contaminated soils affected the ability of the fungus to release metals from their bound states. The substrates with poultry litter recorded the highest bioavailability factor and control soils that had no fungal treatment recorded the least. Metals like nickel and iron recorded low bioavailability factors both in crude oil contaminated soils without and with fungal treatment. Other metals like manganese and cobalt recorded high bioavailability factors in soils with or without the mushroom. In conclusion the growth of the fungus *Pleurotus tuber-regium* in crude oil contaminated soil caused the release of metals into their bio-available states. The solubilization of metals by the fungus was affected by substrate composition with poultry litter having the highest impact on solubility of metals. The fungus can be used for heavy metal harvesting of some metals from contaminated sites. *Pleurotus tuber-regium* has the potential of increasing metal toxicity in soils by increasing the bioavailability of some metals needed in trace quantities.

**Key words:** Bioavailability, heavy metals, substrate composition, contamination, crude oil, biodegradation

### INTRODUCTION

Trace metals in crude oils and other bituminous substances have been recognized worldwide (Nwadinigwe and Nworgu, 1999; Nduka *et al.*, 2006). The presence of heavy metals in some environments has therefore been attributed to petroleum prospecting and mining as well as oil spills (Osuji and Onojake, 2004). It is estimated that more than four thousand incidents of crude oil spills have occurred in the Niger Delta region of Nigeria since 1960, releasing several million barrels of crude oil (sometimes containing heavy metals) into the surrounding areas (Nduka *et al.*, 2006). The concentrations of heavy metals in crude oil according to Stigter *et al.* (2000) are generally lower than reported. These metals however, can inhibit various cellular processes and their effects are

often concentration dependent and also vary in their individual toxicity (Talley, 2006). The list of elements commonly considered as pollutants is rather short and includes Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn and some radionuclides (Shtangeeva, 2006). *Pleurotus tuber-regium* is able to grow in crude oil contaminated soils and degrade petroleum hydrocarbons present (Isikhuemhen *et al.*, 2003; Adenipekun and Fasidi, 2005; Ogbo and Okhuoya, 2008). Other white rot fungi like *Pleurotus ostreatus* have also been cultivated successfully on petroleum based contaminated soils (Stamets, 2005). Thus white rot fungi have shown a lot of promise in their use in the clean-up of petroleum hydrocarbon contaminated soils (Lamar and Glaser, 1994; Eggen and Sveum, 1999; Stamets, 2005; Singh, 2006). This is because white rot fungi produce extracellular enzymes with low substrate specificity that enables degradation of a wide array of aromatic compounds including petroleum hydrocarbons (Singh, 2006). Edible wild and cultivated mushrooms have been shown to accumulate great concentrations of toxic metallic elements and metalloids such as cadmium and lead (Kalac and Svoboda, 2000; Falandayzs *et al.*, 2001, 2003; Fomina *et al.*, 2005). Studies on heavy metals of macro fungi have shown a correlation between mushroom heavy metal concentrations and sources of metal pollution such as smelters and road sides (Isildak *et al.*, 2007). Heavy metal concentrations of some species of wild edible mushrooms can be high, even if the degree of pollution in soil is low (Falandayzs and Chwir, 1997; Falandayzs *et al.*, 2003). The heavy metal concentrations are dependent on the physiology of species and particularly on its trophic pattern, collection site of the sample, mineral composition of soil, metal uptake in mushroom and distance from the source of pollution (Gadd, 1990, 2001; Blackwell *et al.*, 1995; Singh, 2006). The solubilisation of soil components for the successful uptake of metals is made possible by the ability of fungi to produce protons, phosphatases, organic acids and other metabolites. The organic acids and extracellular enzymes produced by fungi assist in solubility and complexing of metal cations (Burford *et al.*, 2003; Singh, 2006). The use of fertilizers for enhancement of biological components used in bioremediation is a common practice (Greer *et al.*, 2003; Minai-Tehrani and Herfatmanesh, 2007). However, fertilizers have been shown to be able to effectively and specifically increase solubility and therefore bioavailability of metals. This is because metal bioavailability may often be increased by lowering soil pH and altering soil ion composition. The application of organic materials such as animal manure, poultry litter and pig slurries to soil during agricultural practices may affect the level of metal concentrations in the soil solution. Organic materials may influence soil metal mobility through their influence on solubility or dissociation kinetics of metals and changes in the solid state equilibrium (Sistani and Jaffrey, 2006). Heavy metal accumulation in soils fertilized with animal wastes is thus possible through increased bioavailability (Sistani and Jeffrey, 2006). In this study, the contaminated soils are supplemented with NPK and poultry litter to enhance the growth of the fungus in the substrates. Literature show that this can affect the heavy metal availability and as a consequence the uptake as stated above.

The mushroom-*Pleurotus tuber-regium* is an edible one grown in crude oil contaminated substrates. It is a tropical sclerotial mushroom found distributed around most of equatorial Africa, India and Sri Lanka, South East Asia and North Australia as well as the southern pacific (Isikhuemhen *et al.*, 1999).

In light of the fact that oil spills are associated with heavy metal contamination also, the aim of this study was to find out if the remediation of crude oil polluted soils with *Pleurotus tuber-regium* through mineralization or degradation of petroleum hydrocarbons can significantly release heavy metals associated into their bio-available state and to what extent.

## MATERIALS AND METHODS

The fungus *P. tuber-regium* was grown in crude oil contaminated substrates. The research was conducted in 2006 at the University of Benin, Benin City. The inoculation of contaminated substrates, spawn preparation, collection of crude oil contaminated soils, contaminated soils amendment with NPK, poultry litter, sawdust and shredded banana leaf blades, particle size analysis of soil, composition of the various substrates used are as stated in Ogbo and Okhuoya (2008).

**Analysis of remediated soils:** Some selected heavy metals were analyzed for and they were mercury, cadmium, lead, copper, arsenic, zinc, manganese, iron, nickel and chromium. All digestion was carried out in a fume cupboard. Air-dried ground soil samples (1 g) from each treatment and control were wet-digested using concentrated  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  in a whole glass system which consisted of a round bottom flask, partial condenser and water cooler. To get the concentrations of heavy metals in the exchangeable state, the solution was filtered after 1 h. The total concentration of heavy metals in the soil was determined by allowing all the soil particles to go into solution. Final measurements of heavy metal content of soil samples were performed using the Perkin Elmer Analyst 700 Atomic Absorption Spectrometer (AAS) determination of heavy metals was carried out in an air/acetylene flame.

The bioavailability factor was determined as a ratio of the metal content in the exchangeable state against the total concentration metals in the soil.

Bioavailability Factor (BF) of heavy metals is defined as the ratio of the metal content in the exchangeable phase ( $[\text{M}]_{\text{ex}}$ ) to the total metal concentration ( $[\text{M}]_{\text{T}}$ ) in the soil (Knox and Adriano, 2002);  $\text{BF} = ([\text{M}]_{\text{ex}}/[\text{M}]_{\text{T}}) 100\%$ .

**Statistical analysis:** Standard error values were calculated to get the confidence interval of six replicates. Duncan's multiple range tests and least significant difference were used to identify where there was significant difference between variables and the treatments using the software SPSS 11.00 for windows.

## RESULTS

The substrate composition affected the solubility of the metals into their exchangeable states. Poultry litter had the effect of increasing the bioavailability of most of the metals analyzed for. The substrate sawdust + poultry litter + contaminated soil released over 50% of the copper present into its bio-available state. The control which had no fungus had the lowest bio-available copper (Fig. 1). Substrates like NPK + contaminated soil, sawdust + NPK + contaminated soil, banana leaf blades + contaminated soil and banana leaf blades + NPK + contaminated soils had low bioavailability rates because the growth of the fungus in them was minimal (Fig. 1). The combination of sawdust + poultry litter encouraged the release of metals into their exchangeable state as the concentration of metals in their bio-available state was highest in them.

Poultry litter + contaminated soil recorded the highest concentration of bio-available lead which was less than 25% comparable to that recorded in the substrate banana leaf blades + NPK + contaminated soil. This indicates that the presence of poultry litter and NPK encouraged the release of the metal lead (Fig. 2). The bioavailability of lead in the control was up to 5% which was low compared with the other substrates except banana leaf blades + contaminated soil substrate (Fig. 2).

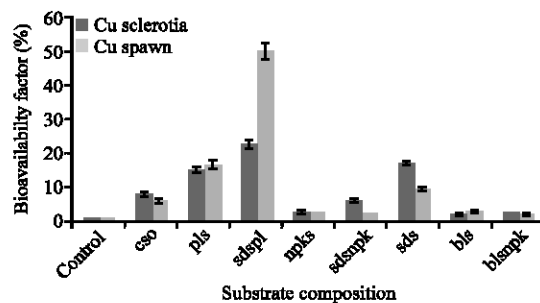


Fig. 1: Bioavailability factor of copper in crude contaminated substrates remediated with *Pleurotus tuber-regium*

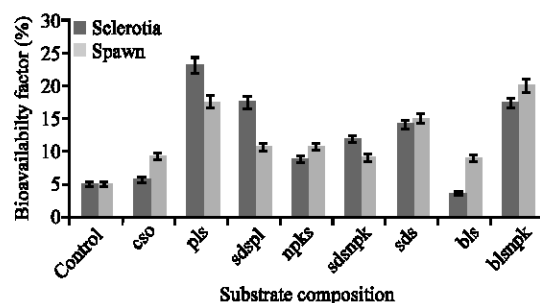


Fig. 2: Bioavailability factor of lead in crude contaminated substrates remediated with *Pleurotus tuber-regium*

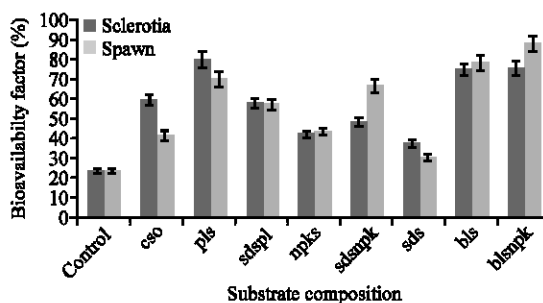


Fig. 3: Bioavailability factor of manganese in crude contaminated substrates remediated with *Pleurotus tuber-regium*

The contaminated control soils that had no mushroom in them had over 20% of the manganese present in the bio-available state. On the other hand substrates like poultry litter + contaminated soil and banana leaf blades + NPK + contaminated soil had over 70% of the manganese in them in its bio-available state (Fig. 3). The substrate sawdust + contaminated soil had less concentration of manganese in the exchangeable state because uptake in it was high. The substrates banana leaf blades recorded high values for bioavailability indicating that it encourages the release of the metal into the exchangeable state (Fig. 3).

The concentration of nickel in the bio-available form was low indicating that the total concentration of metal was low and that the fungus had little influence on the solubility of the

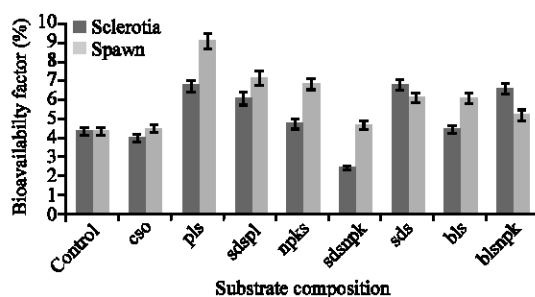


Fig. 4: Bioavailability factor of nickel in crude contaminated substrates remediated with *Pleurotus tuber-regium*

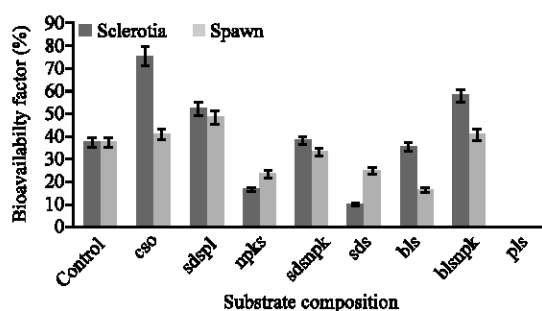


Fig. 5: Bioavailability factor of cobalt in crude contaminated substrates remediated with *Pleurotus tuber-regium*

metal nickel. Substrates with NPK and poultry litter also encouraged the release of the metal in into its exchangeable state (Fig. 4). Substrates like sawdust + NPK + contaminated soil recorded lower bioavailability factor than the control soils. The highest percentage of nickel in the available state in the substrates was less than 8% recorded in the poultry litter + contaminated soil substrate (Fig. 4).

The total concentration of cobalt was low but bioavailability was high. Even the control had over 35% of the cobalt present in the exchangeable state. Ironically, contaminated soil only had the highest concentration of cobalt in the bio-available state (Fig. 5). The concentration of cobalt in the bio-available state was affected by the uptake of the metal as substrates that had less yield recorded higher bio-available cobalt. There was no cobalt detected in the substrate poultry litter + contaminated soil after remediation (Fig. 5).

The bioavailability of iron was low for most substrates as the high concentrations were less than 14%. The least were recorded in control and the substrate sawdust + contaminated soil. Iron had the highest total concentration of metal but was one of the least bio-available metals (Fig. 6).

The bioavailability of chromium was comparable to that recorded in iron. The substrate sawdust + NPK + contaminated soil had the highest concentration of chromium 15.34% in the bio-available form. The least was recorded in the control. The spawn inocula recorded considerably higher concentrations of bio-available chromium than the sclerotia inocula in substrates like, banana leaf blades + contaminated soil banana leaf blades + NPK + contaminated soil and sawdust + NPK + contaminated soil (Fig. 7).

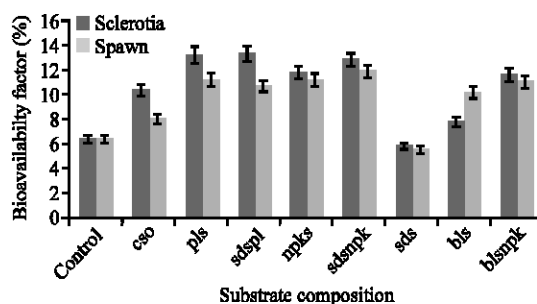


Fig. 6: Bioavailability factor of iron in crude contaminated substrates remediated with *Pleurotus tuber-regium*

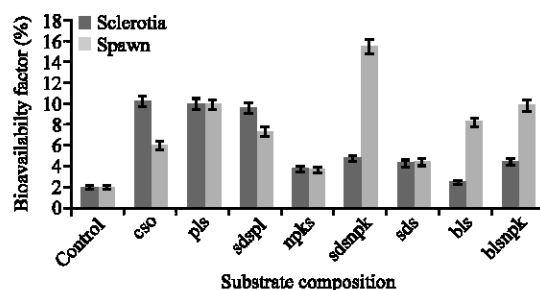


Fig. 7: Bioavailability factor of chromium in crude contaminated substrates remediated with *Pleurotus tuber-regium*

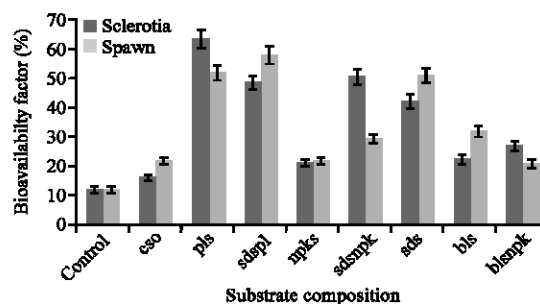


Fig. 8: Bioavailability factor of zinc in crude contaminated substrates remediated with *Pleurotus tuber-regium*

Zinc is a widely distributed element that occurs in small but adequate amounts in most soils and plants. The concentration of zinc in the bioavailable form was over 50% in substrates with sawdust and poultry litter. The least concentration of zinc in the bioavailable state was also recorded in control. The addition of poultry litter, NPK and sawdust to crude oil contaminated soils greatly affected the solubility of the metal zinc as over 50% of the zinc was in the bioavailable form in the substrates with these supplements (Fig. 8).

## DISCUSSION

Literature shows that some microbes can transform metals from toxic to non toxic species or alter their solubility or availability (Tobin, 2001). In this study, it is shown that the metals solubility

was affected by the fungus *Pleurotus tuber-regium*. This study agrees with Massaccesi *et al.* (2002) who concluded that the application of fungi as metallic bio-sorbents for polluted sites is possible. Previous researches have also shown that filamentous fungi are able to bio-absorb high levels of metals from the soil after releasing them from their bound states. The high bioavailability of metals in substrates with poultry litter confirms the suspicion of Sistani and Jeffrey (2006), that organic fertilizers increase metal bioavailability. There was also increased heavy metal mobility caused by the process of degradation as the substrates that did not have poultry litter but other additives and even contaminated soil only substrates recorded high bioavailability factors for some metals. This research therefore also agrees with the study of Du Plessis *et al.* (1995) who stated that degradation activities can cause increase in the concentration of elements in the soil.

The total concentration of manganese in normal soil is about 6000 ppm (Troeh and Thompson, 2005) indicating that the soil from the crude oil contaminated site was deficient in Manganese. The exchangeable manganese in soil is small and is usually between 10-100 ppm, an increase in the concentration of bio-available manganese can lead to increase in its toxicity. The activity of the mushroom therefore released most of the manganese into its available form and thus readily released and taken up. The growth of the fungus in contaminated soil can increase the toxicity of the soil by increasing concentration of bio-available manganese as is the case with substrates that had over 70% of the manganese in them biologically available (Fig. 3).

Nickel is the most recent addition to the list of essential elements. Nickel is also required for iron absorption. Seeds need at least ten parts of nickel per billion in order to germinate. So far as is known, all soils provide enough nickel to exceed this requirement by a considerable margin and the contaminated soils used were no exception (Troeh and Thompson, 2005).

The low bioavailability factor recorded for iron is expected because iron has low solubility and although abundant in soil than most elements it is the least available (Troeh and Thompson, 2005). It is also one of the most commonly deficient micronutrients. The problem is the extremely insoluble nature of certain compounds of ferric ( $\text{Fe}^{3+}$ ) iron. The increase in the concentration of bio-available is therefore desirable.

The concentration of zinc in the substrates is therefore within the normal range for plant growth. Zinc contents of 20 ppm produced normal growth and contents less than 15 ppm causes delayed maturity in field beans (Troeh and Thompson, 2005). Care should be taken in remediation as some of the metals present may fall below the basic requirements for plant growth after the remediation exercise.

Increasing the  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  supplies can help to avert manganese toxicity because, these ions reduce plant uptake of  $\text{Mn}^{2+}$  (Troeh and Thompson, 2005). The problem of manganese toxicity can be checked by the increase in the bioavailability Fe and Zn ions in the substrates.

## CONCLUSION

The use of fungi for the bioremediation of crude oil polluted sites can release metals/elements into the environment increasing the bioavailability of such metals and elements. The draw back in this process is that metals that are required in trace quantities that are naturally in nature unavailable biologically could be made biologically available thus increasing the toxicity of such soils as is seen here in the case of manganese. The solution here is to add chelating agents that will them make some of these metals unavailable biologically.



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