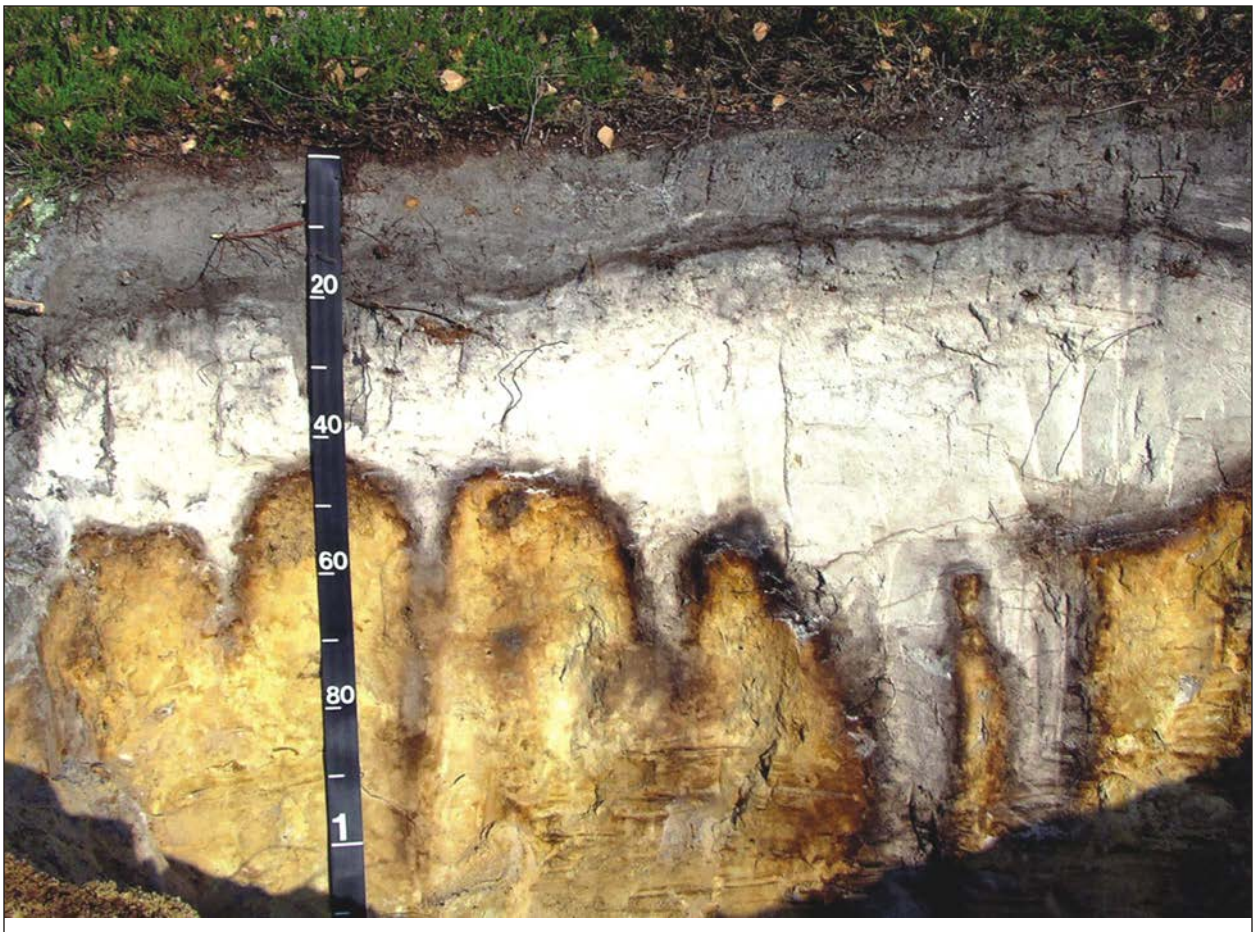

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Bio-Absorption of Some Heavy Metals by *Pleurotus tuber-regium* Fr. Singer (An Edible Mushroom) from Crude Oil Polluted Soils Amended with Fertilizers and Cellulosic Wastes

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ABSTRACT

In this study, the fate of *Pleurotus tuber-regium* harvested from crude oil contaminated substrates is investigated with regard to the bio-absorption of heavy metals specifically-Fe, Mn, Co, Ni, Zn, Cu, Pb, Cr, Cd, Hg and As. The fungus was grown in crude oil contaminated soils amended with poultry litter, NPK-fertilizer, sawdust and shredded banana leaf blades. Harvested fruit bodies were digested with acids and heavy metal content determined. The metallic element content of contaminated soil was low for elements analyzed for with reference to recommended limits for them in normal soils. The only metal that was relatively higher than permissible concentration in soil was chromium. The contaminated soil was deficient of essential metallic elements like Zn, Mn and Cu. There was further reduction of these metallic elements caused by their uptake by the fungus. Cadmium, mercury and arsenic were not detected in the soils and fruit bodies of the mushroom. The transfer of metals from the soils to the mushroom varied with type of metal, its concentration and substrate composition. The transfer factor of the various metals varied from 10.47 in zinc to 0.31 in iron. Fruit bodies from substrates with poultry litter accumulated Fe, Pb and Cr to toxic levels. Mushrooms harvested from crude oil contaminated sites should be analyzed before consumption and properly disposed by incineration or recycling if concentration of metals is too high. The fungus can be used as a bio-indicator of heavy metal pollution and fungal remediated sites can be augmented with micronutrients.

Key words: *Pleurotus tuber-regium*, bio-absorption, heavy metals, transfer factor, crude oil

INTRODUCTION

The presence of heavy metals in some environments has been attributed to petroleum prospecting and mining as well as oil spills (Nwadinigwe and Nworgu, 1999; Osuji and Onojake, 2004; Nduka *et al*., 2006). Crude oil spills can therefore release these metals in to the environment and their subsequent accumulation within the food chain is expected. 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). 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).

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. The process by which both filamentous and white rot fungi take up metals from the soil is not by bioaccumulation but by bio-absorption (Gadd, 1990; Blackwell *et al*., 1995; Gadd, 2001; Singh, 2006). The protons, organic acids and extracellular enzymes produced by fungi assist in solubility and complexing of metal cations and thus their uptake (Burford *et al*., 2003; Singh, 2006). The use of fertilizers for enhancement of biological components used in bioremediation is a common practice and is adopted here to improve the growth of the fungus in the contaminated substrates (Greer *et al*., 2003; Minai-Tehrani and Herfatmanesh, 2007). 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 by lowering soil pH and altering soil ion composition (Sistani and Jeffrey, 2006).

Wild and cultivated edible mushrooms have been traditionally eaten by specific groups of people and are becoming more and more important diet components for their nutritional and pharmacological characteristics (Manzi *et al*., 2001). These same organisms also referred to as 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 enable degradation of a wide array of aromatic compounds including petroleum hydrocarbons (Singh, 2006). *Pleurotus tuber-regium* is able to grow in crude oil contaminated soils and degrade petroleum hydrocarbons present successfully (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). The fate of the mushrooms harvested from such sites that are also prone to heavy metal contamination is thus investigated. *Pleurotus tuber-regium* is a tropical sclerotial mushroom. It is the only species of *Pleurotus* known to produce fruit bodies from a globose true sclerotium. The geographical distribution of *P. tuber-regium* includes 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 is to find out if the remediation of crude oil polluted soils with *Pleurotus tuber-regium* is a two phased process that can take care of both petroleum hydrocarbons and also heavy metals associated. The edibility of the mushrooms harvested from such sites with regards to heavy metals concentration in them is also investigated.

MATERIALS AND METHODS

Pleurotus tuber-regium was cultivated using both sclerotium and spawn inocula in crude oil contaminated soils from 2005-2006. Sclerotia of *P. tuber-regium* were bought from a local market in Benin City, Nigeria and the spawn got after fruit bodies were got from sclerotial induction

following methods outlined by Ogbo and Okhuoya (2008). The crude oil contaminated soils used were collected from a spill site in Uvwiamughe near Ughelli Delta State Nigeria. The topsoil (0-10 cm) were collected from the spill site and supplemented with sawdust, shredded banana leaf blades, poultry litter and NPK fertilizer as also outlined in Ogbo and Okhuoya (2008).

Analysis of mushroom tissues and remediated soils: Some selected heavy metals were analyzed for and they were mercury, cadmium, lead, copper, arsenic, zinc, manganese, iron, nickel and chromium. Digestion of the mushroom was done using the oxi-acidic method of Demirbas (2000) with Trioxonitrate (VI) acid, tetraoxosulphate (VI) acid and hydrogen peroxide in the ratio of 4:1:1, respectively. All digestion was carried out in a fume cupboard. Air-dried ground soil samples (1g) from each treatment and control were wet-digested using concentrated HNO_3 and H_2SO_4 in a whole glass system which consisted of a round bottom flask, partial condenser and water cooler. Final measurements of heavy metal content of mushrooms and 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 transfer factor TF is the ratio of the metal content in plant tissue ($[M]_P$) to the total concentration in the soil ($[M]_T$).

$$TF = [M]_P/([M]_T$$

TF values indicate metal transferability from soil to plant (Knox and Adriano, 2002). This was adopted for mushroom tissues to get the transfer factor from the substrates or soils.

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

Heavy metal content of remediated soils and tissues of harvested fruit bodies: The cultivation of the mushroom *Pleurotus tuber-regium* in crude oil contaminated soils caused a significant reduction in the heavy metal concentration of the soils and accumulation of same to varying degrees. The concentration of the metals in the substrates in order of decreasing concentration was as follows Fe>Zn>Cu>Mn>Ni>Pb>Cr>Co (Table 1). The metals arsenic, cadmium and mercury were not detected in both soils and tissues of the mushroom. The heavy metal concentration of crude oil contaminated control soils were significantly higher than all contaminated treatments that had mushroom grown in them e.g., Fe concentration in crude oil contaminated soil not remediated with fungus was 4520.70 mg kg^{-1} and its concentration in contaminated soil only substrate remediated with the fungus was 3179.15 mg kg^{-1} (Table 1) for the sclerotium inoculum. The same result was recorded for the spawn inoculum, the contaminated soil only substrate recorded 3552.68 mg kg^{-1} which was significantly less than what was recorded for the control (Table 2). There was significant reduction of Fe (4520.70-3179.15 mg kg^{-1}), Zn (98.43-45.92 m g kg^{-1}), Cu (95.43-21.70 mg kg^{-1}), Mn (37.31-11.46 mg kg^{-1}), Ni (28.80-10.90 mg kg^{-1}), Pb (25.95-17.38 mg kg^{-1}), Cr (18.35-4.25 mg kg^{-1}) and Co (5.02-1.39 mg kg^{-1}) in contaminated substrates caused by the growth of the mushrooms in them using the sclerotia inoculum option

Table 1: Heavy metal concentration of crude oil contaminated soil remediated with *Pleurotus tuber-regium* using sclerotium inoculum (mg kg^{-1})

Substrate composition	Fe	Zn	Cu	Mn
Control (contaminated soil -no remediation)	4520.695±327.59^{a}	98.432±5.578^{a}	95.025±4.440^{a}	37.31±2.19^{a}
Contaminated soil only	3179.15±341.58^{b}	45.937±3.551^{b}	21.700±1.248^{b}	11.46±2.02^{b}
Poultry litter +Contaminated soil	3388.13±396.94^{b}	17.005±2.194^{c}	12.575±2.212^{b}	17.07±3.74^{b}
Sawdust+Poultry litter+Contaminated soil	3155.74±327.87^{b}	21.455±2.086^{c}	8.675±1.174^{d}	25.40±2.43^{c}
NPK+Contaminated soil	3480.53±228.65^{b}	69.965±10.472^{d}	76.230±5.179^{e}	21.35±2.29^{c}
Sawdust+NPK+Contaminated soil	3461.39±328.34^{b}	42.487±5.592^{b}	32.050±3.76^{f}	26.19±1.24^{d}
Sawdust+Contaminated soil	2624.26±293.68^{d}	23.923±4.578^{c}	10.500±2.784^{d}	18.02±3.15^{b}
Banana leaf blade+Contaminated soil	3474.27±421.71^{b}	66.710±4.700^{d}	63.928±4.801^{g}	23.14±2.89^{c}
Banana leaf blades+NPK+Contaminated soil	3512.65±280.39^{b}	62.020±6.75^{d}	80.084±4.179^{e}	24.71±1.47^{c}
Substrate composition	Ni	Pb	Cr	Co
Control (contaminated soil -no remediation)	28.80±1.66^{a}	25.92±2.06^{a}	18.35±0.79^{a}	5.02±0.53^{a}
Contaminated soil only	10.90±0.57^{b}	17.38±2.62^{b}	4.25±0.97^{b}	1.39±0.38^{b}
Poultry litter +Contaminated soil	11.03±0.74^{b}	13.92±0.69^{c}	7.03±1.21^{c}	1.56±0.37^{b}
Sawdust+Poultry litter+Contaminated soil	9.53±0.79^{b}	15.13±0.86^{c}	7.38±0.41^{c}	1.47±0.30^{b}
NPK+Contaminated soil	20.19±1.60^{c}	20.50±3.87^{a}	17.66±0.86^{a}	3.04±0.45^{d}
Sawdust+NPK+Contaminated soil	21.83±2.56^{c}	18.69±2.65^{a}	14.82±0.95^{a}	2.26±0.56^{b}
Sawdust+Contaminated soil	10.90±0.69^{b}	13.07±1.02^{c}	6.49±1.03^{b}	2.61±0.36^{b}
Banana leaf blade+Contaminated soil	19.63±2.72^{c}	17.68±2.73^{a}	16.62±1.51^{a}	3.13±0.34^{d}
Banana leaf blades+NPK+Contaminated soil	20.86±2.10^{c}	19.85±1.43^{a}	16.64±1.48^{a}	3.25±0.35^{d}

Superscripts with different letter(s) are significantly different ($p<0.05$) within the same column

Table 2: Heavy metal concentration of crude oil contaminated soil remediated with *Pleurotus tuber-regium* using spawn inoculum (mg kg^{-1})

Substrate composition	Fe	Zn	Cu	Mn
Control (contaminated soil-no remediation)	4520.700±327.59^{a}	98.432±5.59^{a}	95.025±4.440^{a}	37.305±2.188^{a}
Contaminated soil only	3552.678±496.16^{b}	34.817±7.95^{b}	20.878±2.135^{b}	13.775±2.172^{b}
Poultry ltter+Contaminated sil	3813.710±342.31^{b}	28.540±9.64^{c}	15.775±1.604^{b}	21.026±2.435^{c}
Sawdust+Poultry ltter+Contaminated soil	3311.490±319.55^{b}	9.800±2.69^{e}	3.900±0.631^{c}	22.147±1.053^{c}
NPK+Contaminated soil	4057.470±141.47^{c}	76.172±5.00^{d}	83.987±5.674^{e}	25.310±1.21^{c}
Sawdust NPK+Contaminated soil	3809.250±96.35^{b}	61.247±5.21^{d}	61.475±4.020^{g}	21.110±1.58^{c}
Sawdust+Contaminated soil	3284.930±379.07^{b}	23.350±2.34^{c}	14.950±2.935^{b}	18.680±2.19^{b}
Banana leaf blade+Contaminated soil	3348.320±233.09^{b}	54.177±10.74^{d}	72.975±5.324^{e}	24.160±3.54^{c}
Banana leaf blades+NPK+contaminated soil	3488.730±215.15^{b}	67.140±4.24^{d}	64.472±4.837^{g}	26.580±4.00^{d}
Substrate composition	Ni	Pb	Cr	Co
Control (contaminated soil-no remediation)	28.80±1.66^{a}	25.92±2.06^{a}	18.35±0.79^{a}	5.02±0.53^{a}
Contaminated soil only	11.41±0.95^{b}	12.94±1.75^{c}	7.20±1.14^{c}	2.13±0.65^{b}
Poultry ltter+Contaminated sil	11.77±0.91^{b}	12.56±0.41^{c}	7.01±0.85^{c}	0.91±0.12^{c}
Sawdust+Poultry ltter+Contaminated soil	10.30±2.38^{b}	15.14±1.43^{c}	7.15±0.70^{c}	1.98±0.35^{b}
NPK+Contaminated soil	19.41±1.89^{c}	19.64±2.16^{a}	15.21±0.69^{a}	3.29±0.36^{d}
Sawdust NPK+Contaminated soil	14.95±1.36^{b}	17.77±2.98^{a}	15.41±0.31^{a}	2.88±0.32^{b}
Sawdust+Contaminated soil	11.47±1.76^{b}	11.87±0.32^{c}	6.65±1.06^{b}	2.64±0.69^{b}
Banana leaf blade+Contaminated soil	20.34±2.01^{c}	16.94±1.81^{a}	6.21±0.39^{b}	3.67±0.94^{d}
Banana leaf blades+NPK+Contaminated soil	21.21±1.43^{c}	18.19±1.06^{a}	6.83±0.54^{b}	3.03±0.36^{d}

Superscripts with different letter(s) are significantly different ($p<0.05$) within the same column

(Table 1). The same trend was noticed for the spawn inoculum where there was significant reduction caused by the growth of the fungus in the contaminated substrates-Fe

(4520.70-3552.68 mg kg^{-1}), Zn (98.43-38.82 mg kg^{-1}), Cu (95.43-20.88 mg kg^{-1}), Mn (37.31-13.78 mg kg^{-1}), Ni (28.80-11.41 mg kg^{-1}), Pb (25.95-12.94 mg kg^{-1}), Cr (18.35-7.20 mg kg^{-1}) and Co (5.02-2.13 mg kg^{-1}) (Table 2).

The accumulation of heavy metals in fruit bodies varied with the type of metal and total concentration of metals in the substrates and substrate composition. The most abundant metal was iron and the least both in the tissues and the substrates was cobalt. Fruit bodies from substrates with poultry litter recorded the highest concentration of metals compared to other substrate combinations. The substrate composition affected the uptake of the metallic elements and caused the unusually high concentrations of metals like iron, chromium and lead to toxic levels in human diet. Substrates like sawdust+poultry litter+contaminated soils accumulated up to 16.90 as against the 2.30 mg kg^{-1} of chromium which is the permissible dose. The substrates sawdust+poultry litter+contaminated soil accumulated up to 1016.59 mg kg^{-1} of iron (Table 3) and 6.75 mg kg^{-1} of lead (Table 4).

The concentration of iron, zinc and chromium were highest in tissues harvested from the substrate sawdust+poultry litter+contaminated soils. The highest accumulation of copper in tissues was recorded in sawdust+poultry litter+contaminated soils (76.41 mg kg^{-1}) (Table 3). Manganese was highest in sawdust + contaminated soil (20.76 mg kg^{-1}). The concentrations of lead in tissues harvested from all treatments with crude oil contamination were significantly higher (5.25, 5.03, 5.11, 4.70 and 4.20 mg kg^{-1}) than those harvested from that of the control (0.35 mg kg^{-1}) without crude oil indicating that lead must have come from crude oil contamination using the sclerotia inoculum (Table 3). The spawn inoculum showed the same trend with the control without crude oil recording the least concentration (0.47 mg kg^{-1}) and t he other treatments being significantly

Table 3: Concentration of heavy metals in tissues of *Pleurotus tuber-regium* grown in crude oil contaminated soil (mg kg^{-1}) using sclerotium inoculum

Substrate composition	Fe	Zn	Cu	Mn
Control soil (no crude oil contamination)	49.66±6.38^{a}	65.43±9.29^{a}	14.76±1.47^{a}	12.26±1.94^{a}
Contaminated soil only	216.65±32.11^{b}	65.12±1.98^{a}	28.43±2.54^{a}	15.21±1.03^{a}
Poultry litter+Contaminated soil	706.81±36.38^{c}	72.04±6.98^{a}	46.96±10.16^{b}	18.00±2.46^{b}
Sawdust+Poultry litter +Contaminated soil	1016.59±35.65^{e}	83.58±8.92^{c}	76.41±3.08^{c}	15.56±3.17^{a}
NPK+Contaminated soil	-	-	-	-
Sawdust NPK+Contaminated soil	182.65±16.35^{f}	76.41±3.08^{a}	61.99±8.81^{d}	13.73±1.64^{a}
Sawdust+Contaminated soil	257.42±10.37^{g}	61.99±9.31^{a}	-	15.25±1.18^{a}
Banana leaf blade+Contaminated soil	-	-	-	-
Banana leaf blades+NPK contaminated soil	-	-	-	-
Substrate composition	**Ni**	**Pb**	**Cr**	**Co**
Control soil (no crude oil contamination)	8.87±0.75^{a}	0.35±0.19^{a}	1.50±0.23^{a}	0.00±0.00^{a}
Contaminated soil only	1.98±0.49^{b}	5.25±0.49^{b}	4.35±0.29^{b}	0.75±0.44^{a}
Poultry litter+Contaminated soil	8.47±1.14^{a}	5.03±1.06^{b}	6.05±1.63^{b}	0.50±0.29^{a}
Sawdust+Poultry litter +Contaminated soil	25.52±3.46^{c}	5.11±1.71^{b}	16.90±2.45^{d}	0.96±0.97^{a}
NPK+Contaminated soil	-	-	-	-
Sawdust NPK+Contaminated soil	5.48±0.91^{b}	4.70±0.94^{b}	5.48±1.44^{b}	0.41±0.41^{a}
Sawdust+Contaminated soil	11.95±1.74^{d}	4.20±0.83^{b}	11.90±3.97^{e}	1.21±0.82^{a}
Banana leaf blade+Contaminated soil	-	-	-	-
Banana leaf blades+NPK contaminated soil	-	-	-	-

Superscripts with different letter(s) are significantly different ($p<0.05$) within the same column. Substrates had no fructification so no data

Table 4: Concentration of heavy metals in tissues of *Pleurotus tuber-regium* grown in crude oil contaminated soil (mg kg^{-1}) using spawn inoculum

Substrate composition	Fe	Zn	Cu	Mn
Control soil (no crude oil contamination)	60.19±0.65^{a}	56.61±4.29^{b}	8.53±3.09^{a}	10.16±1.50^{a}
Contaminated soil only	200.13±53.59^{b}	66.12±8.91^{a}	19.97±2.76^{a}	15.44±2.87^{a}
Poultry litter+Contaminated soil	606.08±32.49^{d}	78.61±9.54^{a}	32.01±3.33^{b}	17.34±3.74^{b}
Sawdust Poultry litter+ontaminated soil	1018.97±42.73^{e}	65.84±7.38^{a}	40.84±4.12^{b}	14.72±1.78^{a}
NPK+Contaminated soil	-	-	-	-
Sawdust NPK+Contaminated soil	-	-	-	-
Sawdust+Contaminated soil	165.80±7.35^{f}	43.41±1.79^{b}	43.41±1.79^{b}	20.76±3.66^{b}
Banana leaf blade+Contaminated soil	-	-	-	-
Banana leaf blades+NPK+Contaminated soil	-	-	-	-
Substrate composition	**Ni**	**Pb**	**Cr**	**Co**
Control soil (no crude oil contamination)	2.43±0.29^{b}	0.47±0.08^{a}	2.27±0.38^{a}	0.00±0.00^{a}
Contaminated soil only	3.97±0.53^{b}	3.98±1.25^{b}	9.37±1.23^{c}	1.10±0.83 ^{a}
Poultry litter+Contaminated soil	4.95±0.60^{b}	6.75±0.25^{c}	7.86±1.29^{b}	1.15±0.51^{a}
Sawdust poultry litter+ontaminated soil	14.02±1.59^{d}	4.26±0.57^{b}	8.51±1.18^{b}	0.96±0.61^{a}
NPK+Contaminated soil	-	-	-	-
Sawdust NPK+Contaminated soil	-	-	-	-
Sawdust+Contaminated soil	17.80±2.94^{c}	5.83±0.26^{b}	8.51±1.55^{b}	1.50±0.89^{a}
Banana leaf blade+Contaminated soil	-	-	-	-
Banana leaf blades+NPK+Contaminated soil	-	-	-	-

Superscripts with different letter(s) are significantly different ($p<0.05$) within the same column. Substrates had no fructification so no data

higher (3.98, 6.75, 4.26 and 5.83 mg kg^{-1}) (Table 4). Cobalt was not detected (0.00 mg kg^{-1}) in the tissues of the mushrooms harvested from control soils that had no crude oil contamination and but in crude oil contaminated substrates where there was no significant difference among the treatments where it was detected also an indication that the metal came from crude oil sources i.e., 0.75, 0.50, 0.96, 0.41 and 1.21 mg kg^{-1} for the sclerotium option (Table 3) and 1.10, 1.15, 0.96 and 1.50 mg kg^{-1} (Table 4). There was little or no fructification in NPK+contaminated soil substrates, Banana leaf blades+contaminated soil and Banana leaf blades+NPK+ contaminated soils. Uptake was therefore not recorded for these substrates and the concentrations of metals in the substrates confirm this. The concentrations of metals were high for both sclerotia and spawn grown soils in the substrate NPK+contaminated soil, respectively; Fe (3480.53 mg kg^{-1}), Zn (69.97 mg kg^{-1}), Cu (76.23 mg kg^{-1}), Mn (21.35 mg kg^{-1}), Ni (20.19 mg kg^{-1}), Pb (20.50 mg kg^{-1}), Cr (17.66 mg kg^{-1}), Co (3.04 mg kg^{-1}) (Table 1). Fe (4057.47 mg kg^{-1}), Zn (76.12 mg kg^{-1}), Cu (83.99 mg kg^{-1}), Mn (25.31 mg kg^{-1}), Ni (19.41 mg kg^{-1}), Pb (19.64 mg kg^{-1}), Cr (15.21 mg kg^{-1}), Co (3.29 mg kg^{-1}) (Table 2).

Crude oil contamination affected the transfer of heavy metals from the soil to the mushrooms. The mobility of the various heavy metals varied with the type and substrates composition as shown by the different transfer factors recorded. The least transfer factor for all metals analyzed for was recorded in control soils that had no crude oil; Zn spawn-1.10 (Fig. 1), Ni spawn recorded 0.14 (Fig. 2), Co was not detected in both spawn and sclerotia grown substrates (Fig. 3), Cu spawn-0.24 (Fig. 4), Pb sclerotia-0.06 (Fig. 5),Cr 0.29 for sclerotia (Fig. 6), Mn spawn -0.39 (Fig. 7), Fe sclerotia-0.04 (Fig. 8). The highest transfer factor was recorded in copper (10.47) (Fig. 4). The poultry litter treatments had the highest transfer factors for all the metals in the various treatments

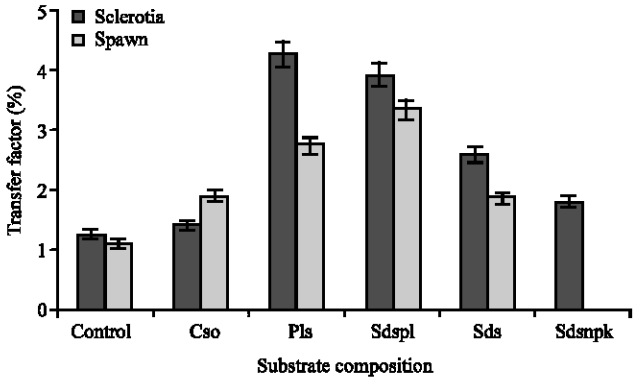

Fig. 1: Effect of different substrate composition on transfer factor of zinc in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

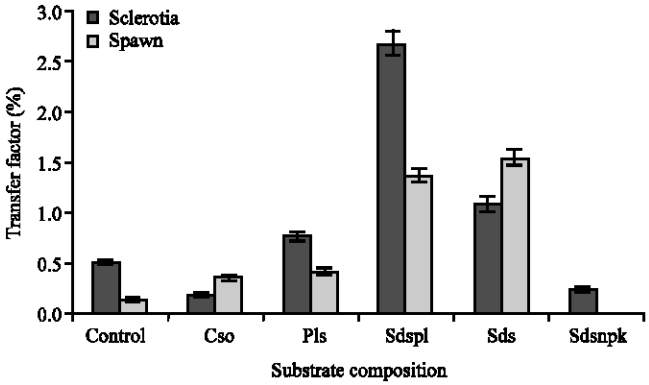

Fig. 2: Effect of different substrate composition on transfer factor of nickel in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

applied but one. The only exception was in manganese where the highest transfer factor was recorded in the treatment; contaminated soil only (Fig. 6). The transfer factors followed this trend from the highest to the least Cu>Zn>Ni>Cr>Mn>Co>Pb>Fe i.e., 10.47, 4.24, 2.68, 2.29, 1.33, 1.26, 0.54 and 0.34, respectively (Fig. 1-8). The accumulations of metals like Pb, Ni, Cr and Co were high and exceeded the acceptable limit for them in vegetables. This indicates that the mushroom accumulated these metals to toxic levels for food. The transfer factor for zinc varied from 1.27 in control soils to 1.90 in contaminated soil only treatments. The addition of sawdust, shredded banana leaf blades, poultry litter and NPK affected the transfer factor further increasing their mobility. The substrate with the highest transfer factor for zinc was poultry litter+contaminated soil (Fig. 1).

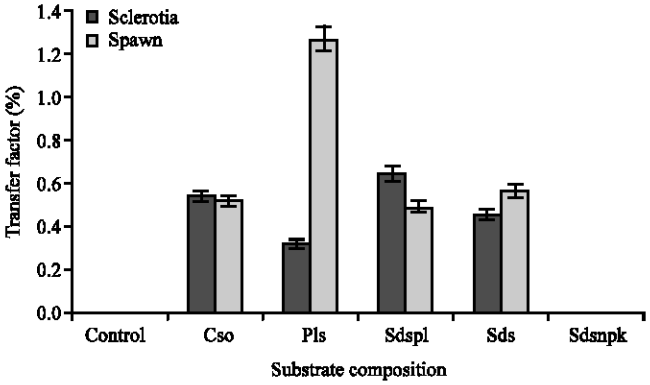

Fig. 3: Effect of different substrate composition on transfer factor of cobalt in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

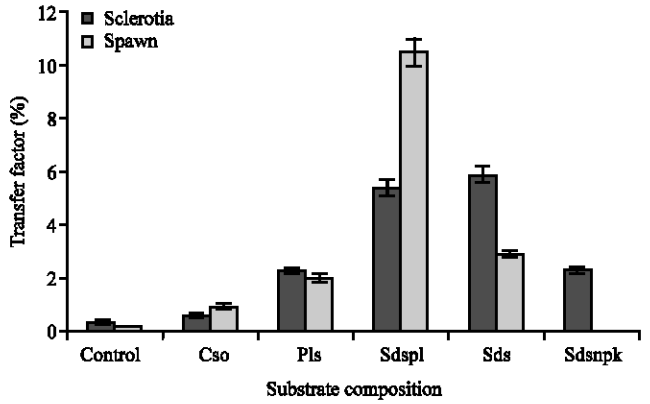

Fig. 4: Effect of different substrate composition on transfer factor of copper in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

The transfer factor for nickel also varied with the least (0.14) been recorded in control soils and the highest (2.68) in the substrate sawdust+poultry litter+contaminated soil in the sclerotia option (Fig. 2). There was no cobalt detected in control soils and the substrate sawdust+NPK+contaminated soil. The substrate sawdust+NPK+contaminated soil showed little or no fructification which explains this. There was however adequate fructification in control soils and the only explanation for the absence of cobalt is that it must have come with crude oil contamination. The highest mobility of cobalt was in the substrate poultry litter+ contaminated soil as it had the highest transfer factor of 1.26 (Fig. 3). The lowest transfer factor for copper was alos recorded in control (0.40) and the highest in 10.47 in sawdust+ poultry litter+contaminated soil substrate (Fig. 4).

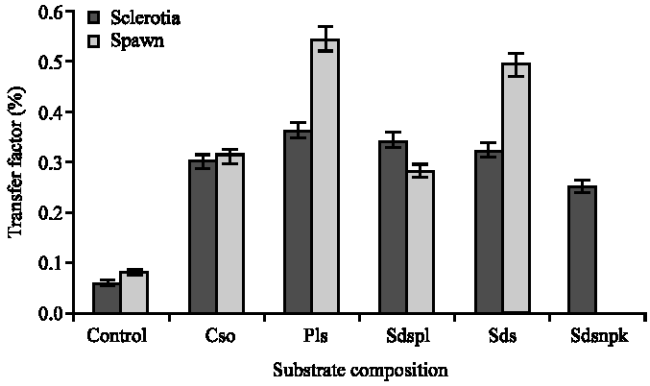

Fig. 5: Effect of different substrate composition on transfer factor of lead in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

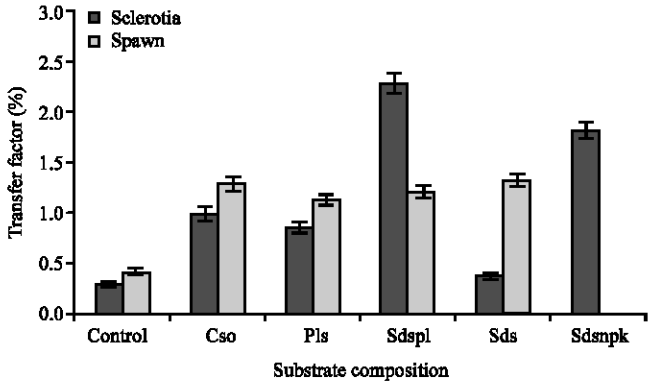

Fig. 6: Effect of different substrate composition on transfer factor of chromium in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

Transfer factor for lead was very low, less than 1 and the highest mobility was recorded in poultry litter+contaminated soil substrate (Fig. 5). The highest transfer factor for the metal chromium was recorded in the substrate sawdust+poultry litter+contaminated soil in the sclerotia option (Fig. 6). Crude oil contamination had more impact on the mobility of manganese than other metals analyzed for. The addition of supplements did not enhance its mobility as the highest transfer factor was recorded in contaminated soil only substrate (Fig. 7). The transfer or mobility of iron was the lowest among all the metals analyzed for. Transfer factor for iron in all substrates was less than 0.4 and the highest mobility were recorded in the substrate-sawdust+poultry litter+contaminated soil (Fig. 8).

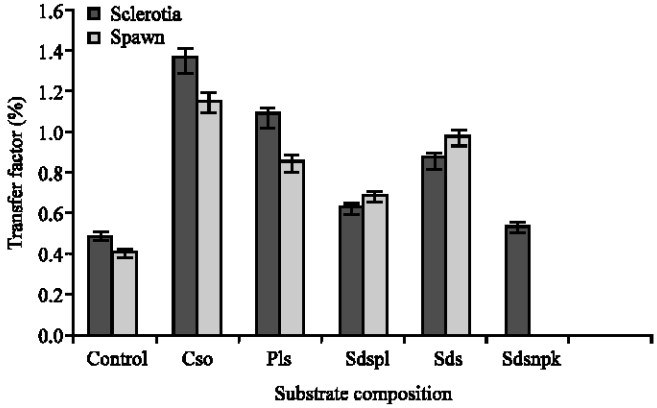

Fig. 7: Effect of different substrate composition on transfer factor of manganese in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

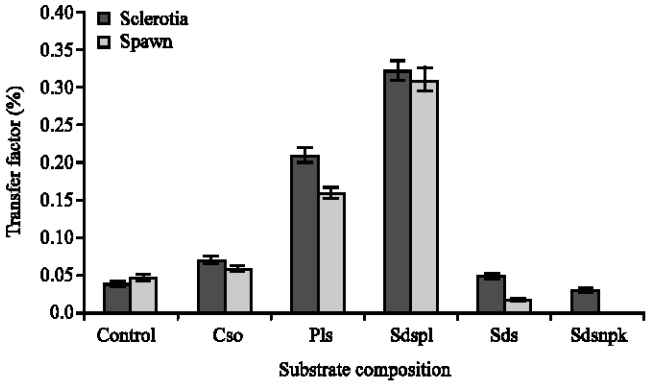

Fig. 8: Effect of different substrate composition on transfer factor of iron in crude oil contaminated soil remediated with *P. tuber-regium*. NPK: Nitrogen, phosphorus and potassium; cso: Contaminated soil only; pls: Poultry litter+contaminated soil; sdspl: Sawdust+poultry litter+contaminated soil; sds: Sawdust+contaminated soil; sdsnpk: Sawdust+NPK+ contaminated soil

DISCUSSION

The presence of crude oil in the soil did not cause serious heavy metal contamination. The total concentration of heavy metals analyzed for did not give any cause for concern. This agrees with the Stigter *et al.* (2000), who concluded in their work that concentrations of heavy metals in crude oil are generally lower than reported. The concentration of metals in the crude oil contaminated soil for metals like zinc, copper, lead, nickel and manganese did not exceed the limit but fell below the limits in normal soils (Table 1, 2). The accepted limits for these metals are (i.e., maximum acceptable limit) are 300, 100, 300, 100 and 2000 mg kg^{-1}, respectively (Elinex *et al.*, 1990). The highest concentrations of the metals analyzed for were recorded in crude contaminated control soils that had no remediation (no mushroom) as follows; Zinc (98.43 mg kg^{-1}), copper (95.03 mg kg^{-1}), lead

(25.92 mg kg^{-1}), nickel (28.80 mg kg^{-1}), manganese (37.31 mg kg^{-1}), chromium (18.35 mg kg^{-1}) (Table 1, 2). Chromium however, exceeded the acceptable limits for it in normal soil in all the substrates used. The acceptable limit for chromium in normal soil is 3.50 mg kg^{-1} (Elinex *et al.*, 1990) and the highest recorded for chromium in this study was 18.35 mg kg^{-1} in the crude oil contaminated control. The contaminated soils here after the growth of the fungus in them, recorded concentrations of heavy metals that were less than the than the maximum permissible limits for metals like zinc, copper, lead, nickel and manganese in soil even with the highest recorded concentrations (Elinex *et al.*, 1990). There was however increased heavy metal mobility caused by the process of degradation as shown by the transfer factors. This agrees with the work of Du Plessis *et al.* (1995), who concluded that the process of degradation increases metal or elemental mobility. The bio-absorption of metals by *Pleurotus tuber-regium* was dependent on the concentration of the metals in the various substrates applied. This agrees with the opinion of Talley (2006), who said that the bioabsorption of the metals from soil is dependent on the concentrations of metals in the soil. According to Tobin (2001), the uptake of metals by filamentous fungi is really not metabolism dependent. Previous researches have shown that filamentous fungi are able to bio-absorb high levels of metals from soil. Studies of the potential of *Rhizopus arrhizus* demonstrated that uptake exceeding those of commercial ion-exchange resins for uranium and thorium was attainable (Tsezos and Volesky, 1981). Studies have also shown that filamentous fungi like *Rhizopus*, *Aspergilus* and *Mucor* species are able to bio-absorb various concentrations of metals like cadmium, lead, copper, zinc and chromium in concentrations ranging from 0.18 to 1.15 mmol g^{-1} (Tobin, 2001). The high uptake of metals in substrates with poultry litter confirms the suspicion of Sistani and Jeffrey (2006), that organic fertilizers increase metal bioavailability and thus uptake (Table 3, 4).

The low transfer 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). The concentration of iron recorded in all the substrates ranged 2624.26-4520.70 mg kg^{-1} (Table 1). This is less than the iron concentration recorded for soils in America that ranged from 7000-500,000 mg kg^{-1}, but it is also one of the most commonly deficient micronutrients. The problem is the extremely insoluble nature of certain compounds of ferric (Fe^{3+}) iron. A small amount of manganese is essential but a large amount is toxic to plants. The total concentration of manganese in normal soil is about 6000 mg kg^{-1} (Troeh and Thompson, 2005) indicating that the soil from the contaminated site used in this study is highly deficient in manganese. Nickel is the most recent addition to the list of essential elements and is 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.

Zinc is a widely distributed element that occurs in small but adequate amounts in most soils and plants. The concentration of zinc in the substrates is therefore within the normal range for plant growth. Zinc contents of 20 mg kg^{-1} produced normal growth and contents less than 15 mg kg^{-1} causes delayed maturity in field beans (Troeh and Thompson, 2005). The study therefore shows that during remediation some of the metals or essential elements like zinc in the soils may fall below the basic requirements for plant growth.

Pleurotus tuber-regium is not the only mushroom that is able to bio-absorb heavy metals. Other mushrooms have been shown to accumulate manganese in their tissues- *Boletus badinus*, *Tricholoma equestry* and *Lepiota procera*, *Agaricus* sp. to varying degrees (Kalac and Svoboda,

2000; Carvalho *et al*., 2005). The mushrooms *Laccaria amethystine* and *Leccinum* sp. have also been shown to bio-absorb the metal nickel at low concentrations. Cobalt has also been noticed in the tissues of *Agaricus arvensis* and chromium in the tissues *Agaricus* sp., *Macrolepiota procera, Lactarius deliciosus* (Kalac and Svoboda, 2000).

The concentration of zinc recorded by previous works was between 30-150 mg kg^{-1} and the mushrooms that accumulated zinc within that range were *Suillus* variegates, *Suilus luteus*, *Lycoperdon perlatum* (Kalac and Svoboda, 2000). *Pleurotus tuber-regium* accumulated zinc within the range of 43.41-83.58 mg kg^{-1} from different substrate combinations (Table 1, 2). This shows that the accumulations of metals are species dependent as stated by Kalac and Svoboda (2000). The concentration of copper bio-absorbed by *P. tuberregium* falls within the range recorded for the mushrooms; *Agaricus* sp., *Macrolepiota procera* and *M. rhacodes* (Kalac and Svoboda, 2000).

The accumulation of metals in the tissues of the mushroom affects its edibility. Some of the metals like iron are essential for normal or healthy growth of plants and animals. The mushroom *Pleurotus tuber-regium* is an edible one and chances are they will find their way into the human diet. The mushroom is treated as a vegetable and there are maximum permissible limits for these metals or elements in vegetables. The maximum permitted concentrations of Fe, Mn, Cr, Cu, Ni, Zn and Pb in vegetables are 425.50, 500, 2.30, 73.30, 67.90, 99.40 and 0.30 mg kg^{-1}, respectively (Ewers, 1991; Kabata-Pendias and Pendias, 1992). Mn, Zn, Ni and Cu were therefore not accumulated to toxic limits by the mushroom (Table 3, 4). The accumulation of Fe in fruit bodies from poultry litter treatments however got to toxic levels (Table 3, 4). Chromium and lead were accumulated to toxic levels in all substrate combinations applied as concentrations exceeded the maximum acceptable limits for vegetables (Table 3, 4). These metals like lead can affect the nervous system and also cause a lot of damage to the entire metabolic system. In the absence of metals like these or in concentrations not as high as these the mushrooms can be eaten. The mushrooms used for the remediation of crude oil contaminated soils should be properly discarded. They can be incinerated and the ash not deposited in soils but recycled to get back the metals or removed to dump sites that are not used for agricultural purposes.

Iron, chromium and lead were accumulated to toxic levels. Iron over load in ones system can be caused by massive doses of dietary iron and long term over consumption of iron may cause a condition called hemosiderosis characterized by large deposits of the iron storage in the liver and other tissues. Iron over load can cause infections from bacteria that thrive on iron rich blood. Serious symptoms of iron overload include enlarged liver, skin pigmentation, lethargy, joint diseases, loss of body hair, amenorrhea and impotence. Untreated hemochromatosis aggravates the risk of diabetes, liver cancer, heart disease and arthritis (Kadiiska *et al*., 1995).

CONCLUSION

This study agrees with Massaccesi *et al*. (2002), who concluded that the application of fungi as metallic bio-sorbents for polluted sites is possible. There was removal of heavy metals from the crude oil contaminated soils by the fungus reducing the concentrations of the some of the metals or elements below accepted limits for normal soils. It is therefore suggested that soils remediated with white rot fungi be augmented with micronutrients for plant's sustenance. Fruit bodies from crude oil contaminated soils can accumulate heavy metals to toxic levels and this can affect their edibility. The fungus can also be applied to heavy metal contaminated sites to harvest heavy metals from such sites as it's been shown to bio-absorb heavy metals and such fruit bodies should be discarded responsibly and not allowed to go back into the soil.

REFERENCES

Adenipekun, C.O. and I.O. Fasidi, 2005. Bioremediation of oil-polluted soil by *Lentinus subnudus*, a Nigerian white-rot fungus. Afr. J. Biotechnol., 4: 796-798.

Blackwell, K.J., I. Singleton and J.M. Tobin, 1995. Metal cation uptake by yeast: A review. Applied Microbiol. Biotechnol., 43: 579-584.

Burford, E.P., M. Fomina and G.M. Gadd, 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineral. Magazine, 67: 1127-1155.

Carvalho, M.L., A.C. Pimentel and B. Fernanades, 2005. Study of heavy metals in wild mushrooms under different pollution conditions by X-ray flourescence spectrometry. Anal. Sci., 21: 747-750.

Demirbas, A., 2000. Accumulation of heavy metals in some edible mushrooms from Turkey. Food Chem., 68: 415-419.

Du Plessis, C.A., C.B. Phaal and E. Senior, 1995. Bioremediation of Soil Contaminated with Hydrocarbons and Heavy Metals. In: Applied Bioremediation of Petroleum Hydrocarbons, Hinchee, R.E., J.A. Kittel and H.J. Reisinger (Eds.). Battelle Press, Columbus, Ohio, pp: 107-113.

Eggen, T. and P. Sveum, 1999. Decontamination of aged creosote polluted soil: The influence of temperature, white rot fungus *Pleurotus ostreatus* and pre-treatment. Int. Biodeterioration Biodegradat., 43: 125-133.

Elinex, J., K. Ostwald and K. Danzer, 1990. Analytical investigations and chemometrical characterization of polluted soil. Fresenius J. Anal. Chem., 336: 394-399.

Ewers, U., 1991. Standards, Guidelines and Legislative Regulations Concerning Metals and Their Compounds. In: Metals and Their Compounds in the Environment, Merian, E. (Eds.), VCH, Weinheim, New York, Basel, Cambridge, pp: 687-711.

Falandayzs, J. and A. Chwir, 1997. The concentrations and bioaccumulation factors of mercury in mushrooms from the Mierzeja Wislana sand-bar, Northern Poland. Sci. Total Environ. 203: 221-228.

Falandayzs, J., K. Szymczyk, H. Ichihashi, L. Bielawski, M. Gucia, A. Frankowska and S. Yamasaki, 2001. ICP/MS and ICPAES elemental analysis (38 elements) of edible wild mushrooms growing in Poland. Food Addit. Contamin., 18: 503-513.

Falandayzs, J., M. Kawano, A. Swieczkowski, A. Brzostowski and M. Dadej, 2003. Total mercury in wild-grown higher mushrooms and underlying soil from Wdzydze landscape parc, Northern poland. Food Chem., 81: 21-26.

Fomina, M., S. Hiller, J.M. Charnock, K. Melville, I.J. Alexander and G.M. Gadd, 2005. Role of oxalic acid over secretion in transformation of toxic metal minerals by *Beauveria caledonica*. Applied Environ. Microbiol., 71: 371-381.

Gadd, G.M., 1990. Heavy metal accumulation by bacteria and other microorganisms. Experientia, 46: 834-840.

Gadd, G.M., 2001. Metal Transformation. In: Fungi in Bioremediation, Gadd, G.M. (Eds.). Cambridge University Press, Cambridge, pp: 359-383.

Greer, C.W., N. Fortin, R. Roy, L.G. Whyte and K. Lee, 2003. Indigenous sediment microbial activity in response to nutrient enrichment and plant growth following a controlled oil spill on a freshwater wetland. Bioremediation J., 7: 69-80.

Isikhuemhen, O.S., J.A. Okhuoya, E.M. Ogbo and E.O. Akpaja, 1999. Effect of substrate supplementation with Nitrogen, Phosphorus, Potassium (NPK) fertilizer on sporophore yield in *Pleurotus tuberregium*. Micol. Neotropol. Applied, 12: 19-21.

Isikhuemhen, O.S., G. Anoliefo and O. Oghale, 2003. Bioremediation of crude oil polluted soil by the white-rot fungus, *Pleurotus tu berregium* (Fr.) Sing. Environ. Sci. Pollut. Res., 10: 108-112.

Isildak, O., I. Turkekul, M. Elmastas and H.Y. Aboul-Ennein, 2007. Bioaccumulation of heavy metals in some wild-grown edible mushrooms. Anal. Lett., 40: 1099-1116.

Kabata-Pendias, A. and H. Pendias, 1992. Trace Elements in Soils and Plants. 2nd Edn., CRC Press, Boca Raton, Florida, pp: 365.

Kadiiska, M.B., M.J. Burkitt, Q.H. Xiang and R.P. Mason, 1995. Iron supplementation generates hydroxyl radical *in vivo* : An ESR spin-trapping investigation. J. Clin. Invest., 96: 1653-1657.

Kalac, P. and L. Svoboda, 2000. A review of trace elements concentration in edible Mushrooms. Food Chem., 69: 273-281.

Knox, S.A. and D.C. Adriano, 2002. Evaluation of Sequestering Agents for Cadmium Contaminated Soils. In: Environmental Science and Technology, Uzouchukwu, G.A., K. Schimnel, G.B. Reddy S. Chang and V. Kabadi (Eds). Battelle Press, Richland, pp: 205-213.

Lamar, R.T. and J.A. Glaser, 1994. Field Evaluation of the Remediation of Soils Contaminated with Wood-Preserving Chemicals Using Lignin-Degrading Fungi. In: Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds, Hunchee, R.E., A. Leeson, L. Semprini and S.K. Ong (Eds.). CRC, Press, Florida, pp: 239-247.

Manzi, P., A. Aguzzi and L. Pizzoferrato, 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chem., 73: 321-325.

Massaccesi, G., C.M. Romero, M.C. Cazau and A.M. Bucsinszky, 2002. Cadmium removal capacity of filamentous soil fungi isolated from industrially polluted sediments in, La Plata (Argentina). World J. Microbiol. Biotechnol., 18: 817-820.

Minai-Tehrani D. and A. Herfatmanesh, 2007. Biodegradation of aliphatic and aromatic fractions of heavy crude oil-contaminated soil: A pilot study. Bioremediation J., 11: 71-76.

Nduka, J.K.C., E. Constance and E. Obiakor, 2006. Selective bioaccumulation of metals by different parts of some fish species from crude oil polluted water. Bul. Environ. Contam. Toxicol., 77: 846-853.

Nwadinigwe, C.A. and O.N. Nworgu, 1999. Metal contaminants in some nigerian wellhead crudes comparative analysis. J. Chem. Soc. Nigeria, 24: 118-121.

Ogbo, E.M. and J.A. Okhuoya, 2008. Bioremediation of aliphatic, aromatic, resinic and asphaltic fractions of crude oil contaminated soils by *Pleurotus tuber-regium* Fr. Singer- a white rot fungus. Afr. J. Biotechnol., 7: 4291-4297.

Osuji, L.C. and C.M. Onojake, 2004. Trace heavy metals associated with crude oil: A case study of Ebocha-8 oil-spill-polluted site in Niger Delta, Nigeria. Chem. Biodiversity, 1: 1708-1715.

Shtangeeva, I., 2006. Phytoremediation of Trace Element Contaminated Soil with Cereal Crops: Role of Fertilizers and Bacteria on Bioavailability. In: Trace Elements in the Environment. Biogeochemistry, Biotechnology and Bioremediation, Prasad, M.N.V., K.S. Sajwan and R. Naidu (Eds.). CRC Press, Taylor and Francis Group, Boca Raton, pp: 549-581.

Singh, H., 2006. Mycoremediation; Fungal Bioremediation. John Wiley and Sons Inc., New Jersey, PP: 592.

Sistani, K.S. and M.N. Jeffrey, 2006. Trace Metal Accumulation, Movement and Remediation in Soils Receiving Animal Manure. In: Trace Elements in the Environment Biogeochemistry, Biotechnology and Bioremediation, Prasad, M.N.V., K.S. Sajwan and R. Naidu (Eds.). CRC Press, Taylor and Francis Group, Boca Raton, pp: 689-706.

Stamets, P., 2005. Mycelium Running: How Mushroom Can Help Save the World. Tenspeed Press, California.

Stigter, J.B., H.P.M. de Haan, R. Guicherit, C.P.A. Dekkers and M.L. Daane, 2000. Determination of cadmium, zinc, copper, chromium and arsenic in crude oil cargoes. Environ. Pollut., 107: 451-464.

Talley, J.W., 2006. Road Blocks to the Implementation of Bio-Treatment Strategies. In: Bioremediation of Recalcitrant Compounds, Talley, J.W. (Ed.). CRC Press, Taylor and Francis Group, Boca Raton.

Tobin, J.M., 2001. Fungal Metal Bio-Absorption. In: Fungi in Bioremediation, Gadd, G.M. (Eds.). Cambridge University Press, Cambridge, pp: 424-444.

Troeh, F.R. and L.M. Thompson, 2005. Soils and Soil Fertility. 6th Edn., Ames, Iowa, USA Blackwell Publishing, USA., ISBN: 978-0-8138-0955-7.

Tsezos, M. and B. Volesky, 1981. Bio-absorption of uranium and thorium. Biotechnol. Bioengineer., 23: 583-604.