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Full Length Research Paper

Removal of Heavy Metals from an Atrazine Herbicide Polluted Soil, using *Pleurotus tuberrigium,* a white rot fungus.

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Heavy metals like Zinc (Zn), Lead (Pb), Copper (Cu), Nickel (Ni) and Cadmium (Cd) were removed from a plot of soil polluted with atrazine herbicide and a control without pollution, using the white rot fungus Pleurotus tuberrigium. Results showed that heavy metals were more in the atrazine polluted soil than in the control plot. At the end of 120 days, 11%, 72%, 94%, 100% reduction was achieved for Zn, Pb, Cu and Ni, in the control plots respectively. While 50%, 88%, 96%, 97%, and 78% reduction was achieved for Zn, Pb, Cu, Ni, and Cd in the atrazine herbicide polluted plot. Other physicochemical parameters like pH, EC, %OC, Na, K, Ca and Mg contents during these periods were also studied. Significant increase of essential nutrients like Na, K, Ca and Mg was achieved at P≤ 0.05 while pH reduced. Heavy metals could be very toxic to plants, animals and microorganisms if left unpremeditated.

Key words: Heavy metals, Removal, Atrazine herbicide, *Pleurotus tuberrigium*

INTRODUCTION

In recent years, heavy metals (lead, zinc, copper, mercury, iron, manganese, cadmium, vanadium, antimony, arsenic and cobalt) pollution has become one of the most serious environmental problems in both developed and developing nations of the world. This is due to metals processing industries, tannery, combustion of wood, traffic and plant protection (Sarkar, 2002).

Heavy metals in the soil can inhibit several cellular processes and their effects are often concentration dependent and also vary in their individual toxicity. (Talley, 2006 ; Shtangeeva, 2006). At higher concentrations these heavy metal ions form unspecific complex compounds within the cell, which leads to toxic effects, making them too dangerous for any physiological function. The quest for the most cost-effective method of removing heavy metals are directed towards bio-sorption. Fungi, algae, bacteria etc has demonstrated great potential as metal bio-sorbent due to their metal sequestering properties and this can decrease the concentrations of heavy metal ions in soil (Nilanjana *et al*., 2008).

Among these, Mushrooms has shown proven results in its use to evaluate the level of environmental pollution (Angeles *et al*., 2009; Borovička and Řanda, 2007; Alonso *et al*., 2003; Isikhuemhen *et al*., 2003; Garcia *et al.,* 1998; Fourest and Roux, 1992). *Pleurotus tuberrigium,* a white rot fungus have been reported in the bioaccumulation of heavy metals, degradation of total petroleum hydrocarbons (TPH, 1998), lignin and increased activity of polyphenol oxidase and peroxidase (Kari et al., 2001).

This is due to the fact that white rot fungi produce extracellular enzymes with low substrate specificity that enable degradation of a wide array of aromatic compounds including petroleum hydrocarbons (Ogbo and Okhuoya, 2009; Singh, 2006; Adenipekun and Fasadi, 2005; Isikhuemhen *et al*., 2003). *Pleurotus tuberrigium* is a tropical sclerotial mushroom. It is the only species of *Pleurotus* known to produce fruit bodies from a globose true sclerotium (Isikhuemhen, 1999).

This study has come with an alternative treatment process using the white rot fungus *Pleurotus tuberrigium* to biotreat heavy metals in soils.

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METHODS

Soil sample collection and preparation

The method of surface and sub-surface sampling at random was used in the collection of soil samples in clean and dry plastic buckets using a shovel. (Ibitoye, 2006). Collected soil samples were sieved through a 2mm sieve. About 20kg of the soil samples were poured on two different plots, 3x4 inches square each making a heap of about 15cm, on a porous sack (Ibitoye, 2006).

Soil Contamination

The atrazine herbicide (trade name; Atraforce) was applied in a 1:10 of pollutant/soil on one of the plot, while the other plot (control) was left unpolluted. Mixing was thoroughly done to attain homogeneity. (Christensen and Larsen, 1993).

Mushroom description

Pleurotus tuber-regium (common names: Tiger king mushroom**,** King tuber musroom), is a tropical sclerotial mushroom. The mushroom produces a sclerotium, or underground tuber, as well as a fruiting body . Both the sclerotium and the mushroom are edible. The mushroom looks like an oyster mushroom (*Pleurotus ostreatus*) except that, when mature, the cap curves upward to form a cup-like shape. The sclerotium is spherical to ovoid and can be quite large-up to 30cm (11.8inches) or larger in diameter (Oso, 1997).

It is dark brown on the outside and white on the inside. It occurs naturally in both subtropical and tropical climates. Temperature range is between 28-32^{o}C and sclerotia formation is in 3-5 months. The mushroom is predominant in Africa (Stamets, 2010).

Inoculation of mushroom spawns

Mushroom spawns were inoculated on substrates made from saw dust and wood chips , amended with wheat chaffs, both the polluted plot and control were inoculated (Stamets, 2005).

Heavy metals analysis in the soil

Heavy metals present in the soil sample were determined using the Atomic Absorption Spectrophotometer, (Buck Scientific model 210 VGP, USA). This was carried out by pipetting 0.5ml of the sample in solution into test tubes. Thereafter, 1ml of 5% salicylic acid solution was added to each test tube and mixed thoroughly. It was allowed to stand for 30mins after which 10m of 4M NaOH solution was added. Thereafter, it was allowed to stand for 1hr for colour development and 12hrs for color stabilization, after which the heavy metals levels were determined.

Statistical Analysis

One way Analysis of Variance was used to test for statistical differences in heavy metals reduction between the two plots at 0.05 significant level.

RESULTS ANALYSIS

Results for the level of heavy metals removal from both plots and effects on the physicochemical parameters within the 120 days of the study are shown in the figure 1.0- 6.0 respectively.

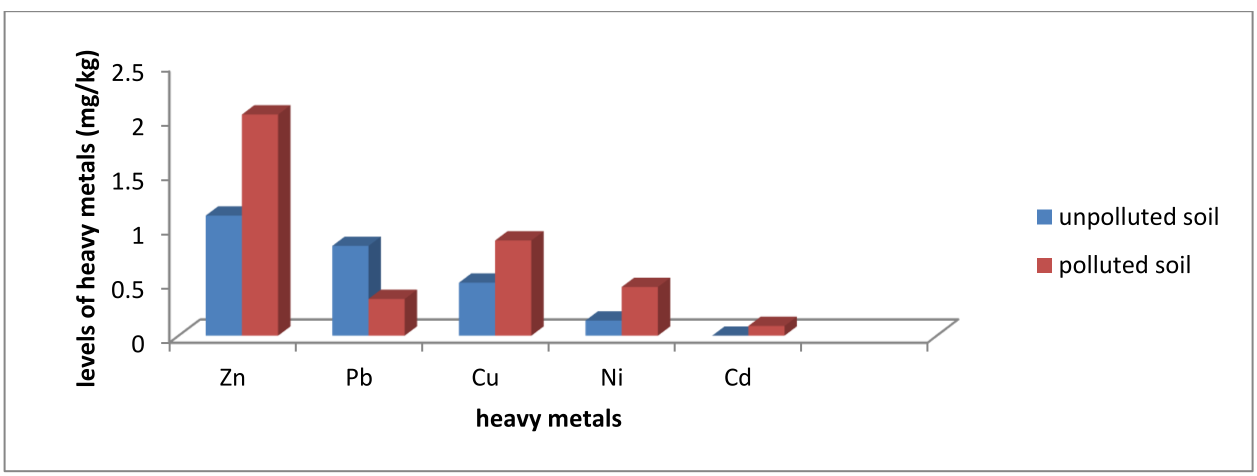

Figure 1. Levels of heavy metals at day 0 of experiment

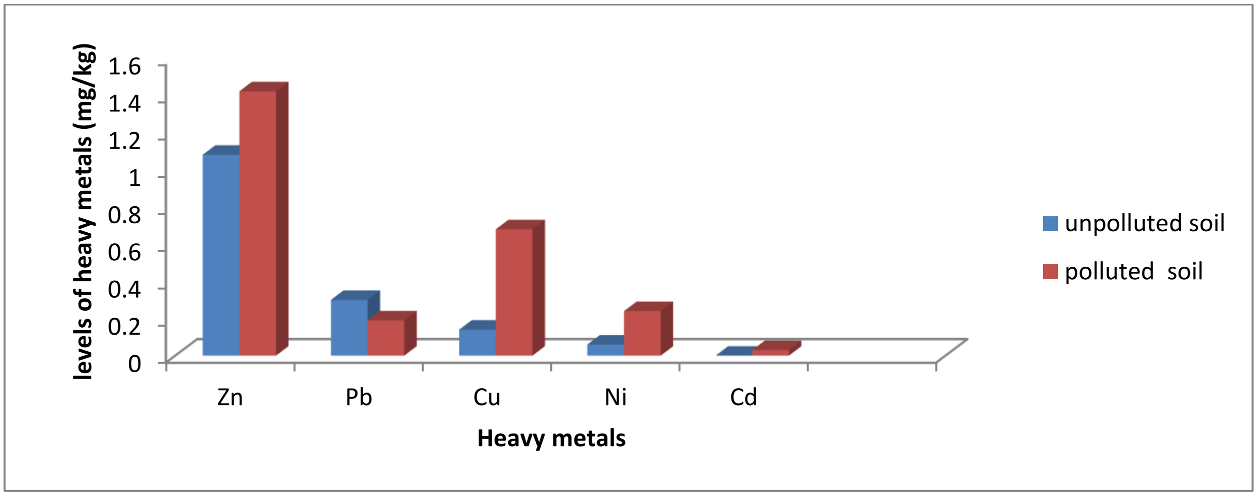

Figure 2. Levels of heavy metals after 60 days of experiment

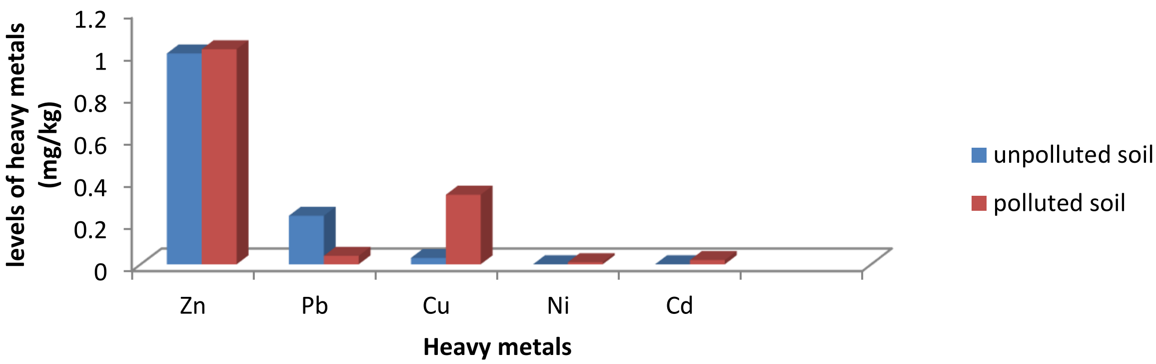

Figure. **3** Levels of heavy metals after 120 days of experiment

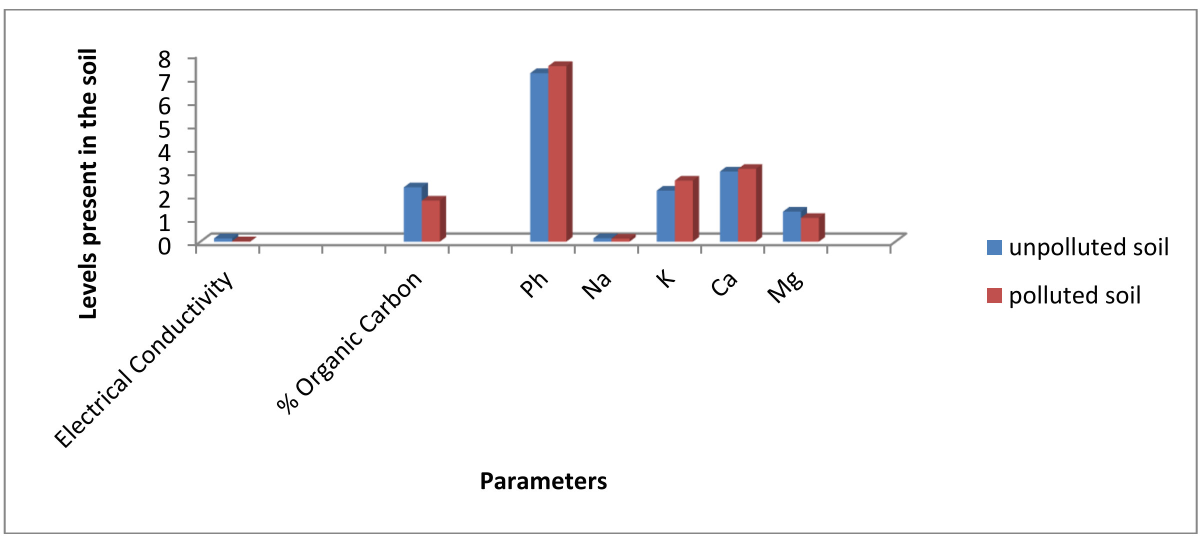

Figure 4. Physicochemical parameters at day 0 of experiment

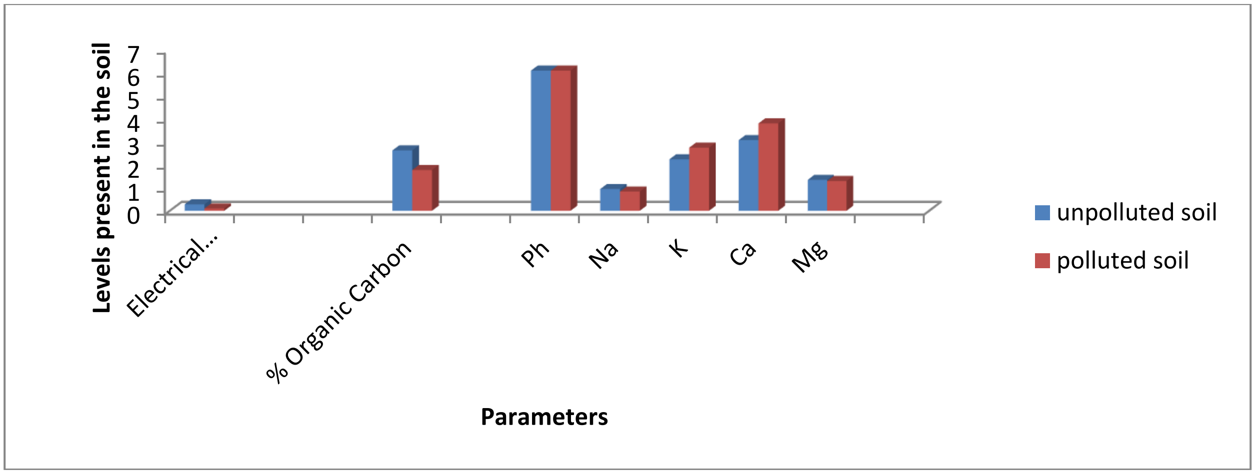

Figure 5. Physicochemical parameters after 60 days of experiment

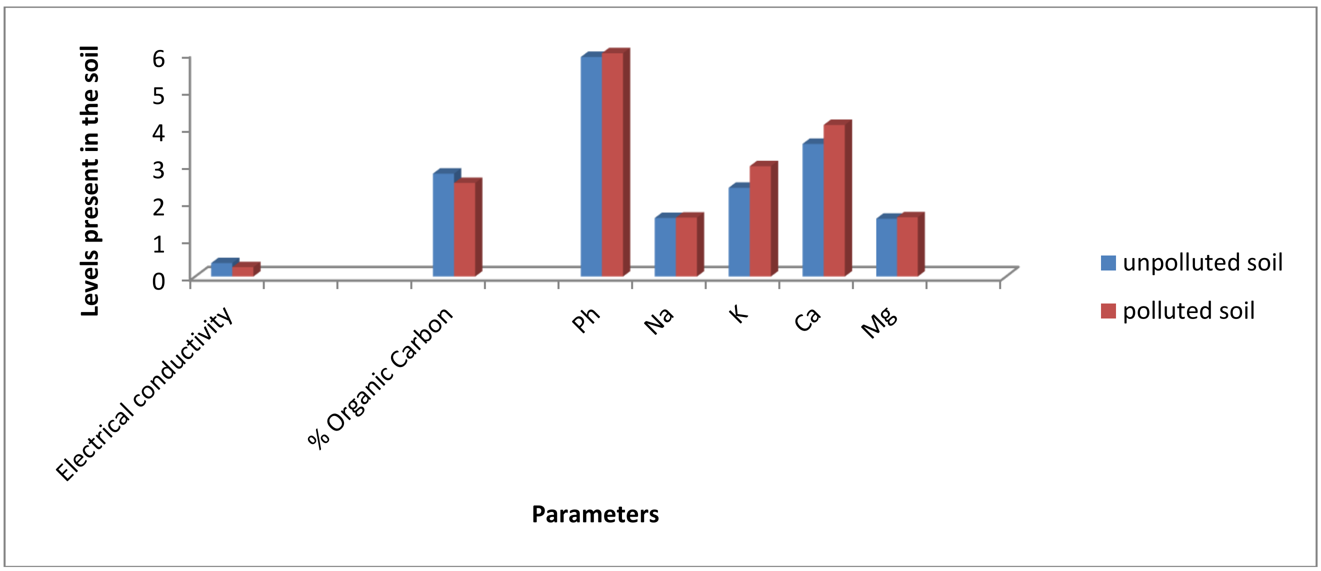

Figure 6. Physicochemical parameters after 120 days of experiment

DISCUSSION

Results showed that heavy metals were present in both the soil plot contaminated with atrazine and the uncontaminated plot. Sathawara, *et al*., (2004) reported that even at very low concentrations, heavy metals could still be carcinogenic and neurotoxigenic. Results also showed that the level of heavy metals in the atrazine herbicide contaminated plot was more than the non atrazine contaminated plot, especially for metals like Zn, Cu, Ni and Cd. There was also a significant reduction in the level of heavy metals present in the soil at P≤0.05 for both the contaminated and uncontaminated soil using the mushroom. At the end of 120 days, 11%, 72%, 94%, 100% reduction was achieved for Zn, Pb, Cu and Ni, in the control plots respectively Cadmium was not detected in the plot at all stages of the study. Moreso, 50%, 88%, 96%, 97%, and 78% reduction was achieved for Zn, Pb, Cu, Ni ,and Cd respectively in the atrazine herbicide polluted plot. Oyetayo *et al*., (2012) also reported a drastic reduction after cultivating *Pleurotus tuberrigium* for a period of 30 days to bioabsorb these metals. More than 90% of the metals were removed according to his study. The sorption of the metals may be due to complexation of the metal ions by the *Pleurotus tuberrigium*. Isikhuemhen *et al.,* (2003) achieved an increase in nutrients contents like organic matter, organic carbon and available potassium and considerable removal of Zn and Ni from polluted soils using *P. tuberrigium* as a treatment agent. Damodaran *et al.,* (2013) also reported the removal of heavy metals like Cu, Cd, Cr, Pb and Zn from contaminated soils using the mushroom *Galerina viltiformis*, and achieved significant results.

Level of pH for the unpolluted soil decreased by 18% while the polluted soil decreased by 25% during the period of the experiment. This could be attributed to the fact that mushrooms secretes acids from their mycelium which increases soil acidity. Stamets, (2005) also confirmed this reports. Electrical conductivity increased significantly during the period of study from 0.14 to .036 in the control plot and 0.02mS/cm to 0.25mS/cm in the polluted plot

Levels of exchangeable bases like Na, K, Ca and Mg also increased generally during the period of the experiment (Figures 4-6). Toxicity of Heavy metals occur through the displacement of essential metals like Na, K, and Ca, from their native binding sites or through ligand interactions. Hence the removal of heavy metals will result in an increase in essential metals.

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