Determination Of Heavy Metal Concentrations In Transformer Oil Polluted Soil Inoculated With *Pleurotus tuber-regium* (Sing.) AND *Lentinus squarrosulus* (L.)

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Abstract: The main environmental challenge facing the world today is the contamination of air, soil and water by toxic compounds due to extensive use of pesticides in agriculture and industralization. Heavy metals are also introduced into the environment through the means mentioned above, although they are need in trace amount by living organisms but in high concentration they are toxic and result in serious health implications such as damage of central nervous function, gastrointestinal and cardiovascular and systems, kidneys, lungs, bones etc. Overtime, various methods have been employed to combat this challenge (incineration, thermal desorption and removal and disposal etc.), most of which are cost effective and unsafe and hence the need for biological method such as bioremediation using bacteria and fungi, phytoremediation and bioaccumulation etc. This study investigated the effect of *Pleurotus tuber-regium* (PT) and *Lentinus squarrosulus* (LS) on the concentrations of heavy metals in transformer oil polluted soil collected from three different sites in Power Holding Company of Nigeria over a period of 3 months. After incubation, the mycelia ramified substrate (rice straw) was carefully separated from the soil. The soil and the straw were air dried and oven dried respectively and acid digested and analyzed for the following heavy metals Fe, Cu, Zn, Mn, Mg and Pb using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Soil from Ibadan substation was most contaminated with heavy metals (type and concentration) and soil from Ughelli substation was the least contaminated. Fe values at the Ibadan substation were significantly (P>0.05) higher than values from other locations, the Fe concentration in all the soil samples ranged from 53.04–438 mg/kg, Mg concentration ranged from 0.18–10.11 mg/kg and Pb concentration ranged from 0.17–14.02 mg/kg while heavy metal concentrations in straw/fungi biomass ranged from 9.21-41.42 mg/kg for Fe, 1.19– 12.88 mg/kg for Mn and 5.79 -14.88 mg/kg for Mg. The Fe concentration in both soil and straw/fungal biomass was observed to be highest in the three contaminated sites treated/inoculated with both *P. tuber-regium* and *L. squarrosulus* while the least is Pb concentration. Soil inoculated with *L. squarrosulus* had significantly (P>0.05) lower heavy metal concentrations in all the soils which indicates that it was more effective in accumulating heavy metals in soil than *P. tuber-regium.*

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Key Words: Transformer oil; pollution; *Pleurotus tuber-regium*; *Lentinus squarrosulus*; heavy metals.

Introduction

The release of heavy metals in the environment have been reported to be associated with mining, oil spill, traffic emissions, petroleum prospecting, waste incineration, smelters emission, atmospheric deposition and emissions from leaded gasoline, paints, fertilizers, sewage sludge, and pesticides (Khan et al. 2008 and Zhang et al. 2010). Heavy metals such as Pb, Cd, Zn, Co, Mn, Ni and Fe concentration or content vary in soil depending on human activities, climate, soil origin and composition. Transformer oil which is also called insulating oil has been reported to contain heavy metals such as Zn, Al, Ag, C, Sn, Pb and Fe (Bentum et al. 2012). Although, some of these heavy metals in trace amounts are needed by humans and other living organisms, high concentration of these metals as a result of exposure to them could be toxic.

High amount of Fe, for instance, could result in microbial inhibition, acute heavy metal intoxications, damage of central nervous function, gastrointestinal and cardiovascular and systems, kidneys, lungs, bones, liver, and endocrine glands (Jang and Hoffman 2011). Contamination with heavy metals is undoubtedly a serious threat to health because of the toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006}.

The use of white rot fungi (WRF) in treating and lowering the accumulation of heavy metals in various parts of Nigeria has been shown by earlier research works to have several positive effects (Adedokun and Ataga 2007; Adenipekun and Isikhuemhen 2008; Ogbo and Okhuoya 2008; Okparanma et al. 2011; Adenipekun et al. 2012). Mushrooms, a major type of

WRF, have been shown to be basidiomycetes which cause decomposition of wood as a result of their ability to degrade not only lignin but also several recalcitrant pollutants in the environment by producing lignolytic exoenzymes (lignin peroxidase, manganese peroxidase and laccase). Their highly oxidative lignin degrading systems enable them to degrade hydrophobic organic pollutants (Ogbo, 2006). Mushrooms have exploited in the evaluation of the level of environmental pollution and accumulation of heavy metals from metal polluted sites (Borovicka and Randa 2007; Angeles et al. 2009). Petroleum hydrocarbons improved the growth of white rot fungi in contaminated soils by increasing their size and yield indicating that the contaminants have a fertilizer effect (Ogbo and Okhuoya 2009). As these fungi grow in the soil, they carry out various functions like degradation, mineralization and accumulation of compounds in the soil. Adenipekun et al. 2012 exploited the use of *P. tuber-regium* in the accumulation of heavy metals in cutting fluids contaminated soil and reported a decrease in the heavy metal concentration with increase in incubation period. Ogbo et al. 2010 also reported that the use of *Pleurotus tuber-regium* in remediating diesel oil-polluted soil and that the treated soil supported seed germination and seedling growth of *Zea mays*. *Lentinus squarrosulus* (Mont.) was exploited in the mineralization of soil contaminated with different concentrations of crude oil resulting in high levels of carbon and organic matter after six months of incubation (Adenipekun and Fasidi 2005).

Although several studies have been carried out in recent times on the effects of used oil on economic crops and humans (Vwioko and Fashemi, 2006; Kayode *et al*., 2008, Adenikpekun *et al*., 2009; Stephen and Ijah, 2010; Adenikpekun *et al.,* 2012;), available literature on preliminary and detailed investigations on the level of heavy metal toxicity of soils contaminated with spent oil from transformers as it bothers on human health and land productivity in Nigeria is scarce. This study is therefore aimed at determining the heavy metal concentrations in soils and straw/fungal biomass after treating transformer oil-polluted soil with *P. tuber-regium* and *L. squarrosulus* in view of making such areas less harmful to workers and surrounding inhabitants.

Materials And Methods

Sampling

Soil samples were collected from three sites (Warri substation, Ughelli substation and Ibadan substation) around where electrical transformers are kept in Power Holding Company of Nigeria (PHCN) from a depth of 0 – 15 cm. The soils were collected into sterile plastic bags and taken to the laboratory. *Pleurotus tuber-regium* (PT) was collected from Plant Physiology Laboratory of the Department of Botany, University of Ibadan while *Lentinus squarrosulus* (LS) was collected from Bowen University, Ogun State, Nigeria. The rice straw used in this study was collected from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Tissue Culture/Sub-culturing

Tissue culture of the two mushrooms (WRF) and further subculturing were carried out on Potato Dextrose Agar for a period of 5-7 days. The procedure of was followed.

Experiment

A 2 X 3 X 4 Completely Randomized Design (CRD) was used for the experimental set-up. Transformer oil polluted soil (200 g) was weighed and placed in sterile bottles, 20 g of rice straw (moist) was laid on the soil in each bottle, separated by wire gauze and sealed with aluminum foil. The bottles were sterilized at 121^{o} C for 15 min. After the sterilization, the bottles were left to cool and each bottle was inoculated with 2 plugs of 5 mm cork borer of PT and LS separately. Thereafter, the bottles were incubated for 1, 2 and 3 months at 28 ± 2^{0} C. After incubation, the mycelia ramified substrate was separated from the soil carefully, the soil and the rice straw were then analyzed for heavy metals following the procedure of Adenipekun and Fasidi (2005). At the end of the experiment, 20 g of each soil sample was air-dried, grinded using mortar and pestle and sieved using a 200 µm mesh while the fungal ramified straws were oven dried, grinded and sieved to obtain fine powder of the straw/fungal biomass.

Digestion of samples/ determination of heavy metal concentrations

Samples were digested using the microwave digestion method; 2 ml HCl, 2 ml HNO_3, 1 ml H_2O_2 and 4 ml $HClO_4$ were added to 0.5 g of dried sieved soil and 0.3 g of the straw (which was dried, grinded and sieved) and heated slowly for 2 hours. The mixture was then allowed to cool and filtrate was collected into 50 ml volumetric flask by passing through a whatman filter paper and 25 ml of distilled water was added. Heavy metal concentrations in the filtrate were then determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). This analysis was carried out in Chemistry and Environmental Chemistry Department, Tshwane University of Technology, Acadia Campus.

Data obtained were analyzed using Least Significant Difference (LSD).

Results And Discussion

Results of the heavy metal concentrations of transformer oil polluted soil at the Ibadan substation treated with *Pleurotus tuber-regium* and *Lentinus squarrosulus* are shown in Table 1. Transformer oil polluted soil treated with LS had significantly lower heavy metal concentrations than the soil treated with PT after 1, 2 and 3 months of incubation. Fe concentration for soil treated with PT decreased significantly from 438.30 mg/kg (control) to 384.96 mg/kg after 1 month of incubation, then later increased to 408.99mg/kg after 2 months incubation and at the end of the 3rd month, a decrease was observed (295.72 mg/kg). Fe concentration of soil treated with LS followed a different trend, a steady reduction in the Fe concentration of the soil was observed from control (255.02 mg/kg) to 197.79 mg/kg after 2 months but at the end of the 3rd month incubation, there was an increase in the Fe concentration of the soil.

For soil treated with PT, the Mn, Mg and Pb concentration of the soil followed the same order with that of Fe but the Cu and Zn concentration of the soil followed a different order; an increase was observed from 10.25 mg/kg in control to 11.37 mg/kg after 1 month, further to 11.90 mg/kg after 2 months and a decrease to 8.46 mg/kg after 3 months incubation and an increase was recorded from 10.13 mg/kg in control to 11.07 mg/kg after 1 month and reduction in Zn concentration of the soil was observed at 2 months and 3 months incubation period respectively. In soil treated with LS, the same trend was observed for Mn, Cu, Zn, Mg and Pb concentrations as that of Fe concentration where there was steady reduction in heavy metal concentration from control to 2 months and then an increase after 3 months incubation. The various types of heavy metal as well as high concentrations of these metals in transformer oil polluted soil from Ibadan substation compare to other substation is an indication that there are other sources of the heavy metal contamination apart from the transformer oil. The Pb concentration was also observed to be high the soils inoculated by the two WRF and this could be harmful to both plants, humans and other living organisms in that environment. Shills and Young (1988) and Aremu et al. (2010) stated that Pb even at low concentration is toxic and does not play any functional role in biochemical process. Although LS was observed to be a better heavy metal accumulator in this study, PT was also reduced some of the heavy metals of the soil significantly with increase in incubation period and this is in line with the findings of Adenipekun et al. 2012 where *Pleurotus tuber-regium* was observed to reduce the following heavy metals; Fe, Cu, Mn, Pb, Zn and Ni with increase in incubation period which is an indication of its bioaccumulation ability.

The heavy metal concentration in straw/fungal biomass is shown in figure 1, the Fe concentration is also higher than the other heavy metals present in straw inoculated with Pt and Ls. For straw inoculated with Pt, a steady increase was observed from control to 3 months incubation period for Mn and Mg concentrations while Fe concentration decreased with increase in incubation period and Zn concentration increased from 0.04 mg/kg in control to 0.19 mg/kg after 1month and at 2months and 3months Zn was below detectable limit in the straw. Fe and Mn content in straw inoculated with Ls increased from 24.43 mg/kg and 5.32 mg/kg in control to 36.33 mg/kg and 9.73 mg/kg after 1month incubation respectively but their concentration decreased after 2 and 3months incubation. While the Mg concentration increased from 8.80mg/kg in control to 10.43 mg/kg after 1month, 10.78 mg/kg after 2months and then reduce to 10.52mg/kg after 3months incubation. Zn was below detectable limit in control but Zn concentration of 0.37 mg/kg was detected after 1month incubation which increased to 0.77 mg/kg after 2omths and then decrease to 0.29 mg/kg after 3 months incubation.

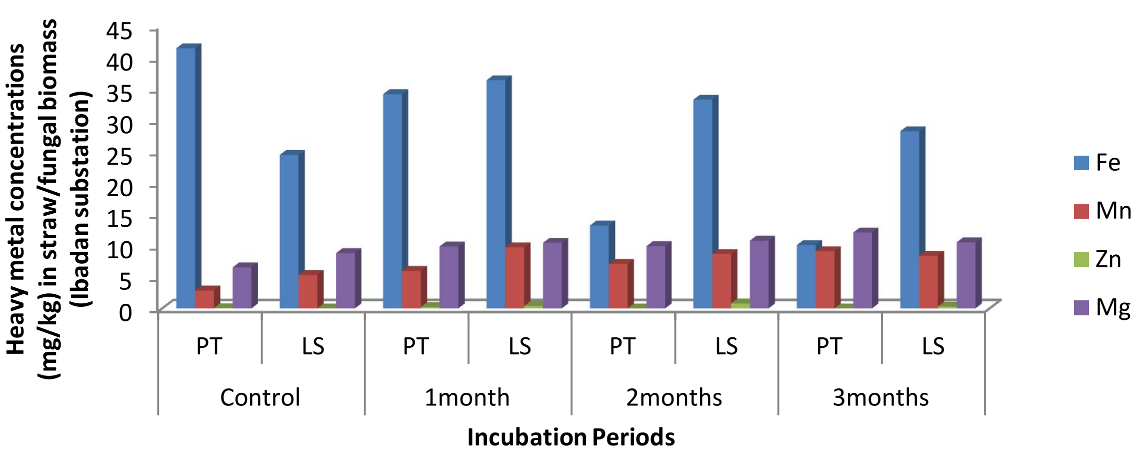

Figure 1: Heavy metal concentrations (mg/kg) in straw/fungal biomass (Ibadan substation)

Table 1: Heavy metal concentrations (mg/kg) in transformer oil polluted soil (Ibadan substation) treated with Pt and Ls

Incubation period		Fe	Mn	Cu	Zn	Mg	Pb
Control	**PT**	438.30^{a}	11.21^{a}	10.25^{a}	10.13^{b}	10.02^{a}	14.02^{a}
	LS	255.02^{d}	6.26^{c}	6.08^{c}	5.55^{d}	6.41^{c}	6.36^{d}
1Month	**PT**	384.96^{b}	9.55^{b}	11.37^{a}	11.07^{a}	9.57^{a}	10.49^{b}
	LS	226.53^{d}	5.16^{d}	5.63^{c}	4.88^{d}	5.68^{d}	6.22^{d}
2Months	**PT**	408.99^{a}	10.15^{a}	11.90^{a}	10.27^{a}	10.11^{a}	11.92^{b}
	LS	197.79^{e}	4.60^{e}	4.14^{d}	4.30^{e}	5.11^{d}	4.28^{e}
3Months	**PT**	295.72^{d}	9.23^{b}	8.46^{b}	9.22^{c}	8.74^{b}	8.41^{c}
	LS	254.88^{c}	5.76^{c}	4.74^{d}	5.38^{d}	6.72^{c}	5.66^{d}

Means with same superscript along column are not significantly different ($P<0.05$) (With the significance levels placed on this table, you can write more about it and discuss it further)

Table 2 shows the heavy metal concentrations in transformer oil polluted soil inoculated with *P. tuber-regium* and *L. squarrosulus*. The four heavy metals (Fe, Zn, Mg and Pb) that were detected were higher in soil inoculated with Pt than the soil inoculated with Ls. The Fe concentration in soil inoculated with Pt increased from 137.10mg/kg in control to 149.38mg/kg after 1month, then reduced to 144.51 mg/kg after 2 months and further reduction was observed at 3 months (72.52 mg/kg). The Zn concentration increased with increase in incubation period from control (0.57 mg/kg) to 2months (0.82 mg/kg) and at 3months the Zn content was below detectable limit while the Mg concentration decreased from 9.78mg/kg in control to 2.85mg/kg after 1months, increased to 2.86 mg/kg after 2 months and then reduce to 0.84mg/kg after 3months. The concentration of Pb increased from 0.81mg/kg in control to 1.17mg/kg after 1month which decreased to 0.89mg/kg after 2months and at 3months the Pb concentration was below detectable limit.

The Fe concentration of soil inoculated with Ls reduced from 91.88 mg/kg in control to 79.88 mg/kg after 1 month, at 2 months an increase (88.74 mg/kg) was observed which then reduce to 73.41 mg/kg at the end of the 3rd month. Zn was and Pb were below detectable limit in soil inoculated with Ls while the Mg concentration in the soil decreased as the incubation period increases from 1.14 mg/kg in control to 0.79 mg/kg after 3 months.

Table 2: Heavy metal concentrations (mg/kg) in transformer oil polluted soil (Warri substation) treated with Pt and Ls

Incubation period		Fe	Zn	Mg	Pb
Control	**PT**	137.10^{c}	0.57^{b}	9.78^{a}	0.81^{b}
	LS	91.88^{d}	ND	1.14^{c}	ND
1 Month	**PT**	149.38^{a}	0.65^{b}	2.85^{b}	1.17^{a}
	LS	79.88^{e}	ND	0.96^{d}	ND
2 Months	**PT**	144.51^{b}	0.82^{a}	2.86^{b}	0.89^{b}
	LS	88.74^{d}	ND	0.93^{d}	ND
3 Months	**PT**	72.52^{f}	ND	0.84^{e}	ND
	LS	73.41^{f}	ND	0.79^{e}	ND

Means with same superscript along column are not significantly different ($P<0.05$) (With the significance levels placed on this table, you can write more about it and discuss it further)

Figure 2 shows the heavy metal concentrations in straw/fungal biomass, for both inoculums, Fe concentration was highest followed by Mg and Mn was the lowest (Fe > Mg > Mn). The Mn and Mg concentration in straw/fungal biomass inoculated with Pt increased from 1.19mg/kg and 5.79mg/kg in control to 7.74mg/kg and 10.67mg/kg respectively after 3months while the Fe concentration decreased from 16.00mg/kg in control to 13.59mg/kg after 2months and then increase to 28.22mg/kg after 3months incubation.

The Mn and Mg concentration in straw/fungal biomass inoculated with Ls followed the same trend, an increase was observed from 1.56 mg/kg and 6.83 mg/kg in control to 9.06mg/kg and 11.46 mg/kg after 2months which then reduce to 7.91 mg/kg and 8.06mg/kg after 3months respectively. For Fe concentration, a steady decrease was observed from control (16.32mg/kg) to 2 months (12.37 mg/kg) and then an increase after 3months (17.72 mg/kg). In this table, it can be observed that the polluted soil treated with PT had high Fe and Mg which are important trace elements that can support the growth of plant. Heavy metals such as Fe, Cu and Zn etc. which are accumulated by these fungi are micronutrients which are needed for their growth. Metals like Cu, Zn and Fe are generally known to be of importance in plant nutrition, however many other heavy metals do not play any vital role in the physiology of plants (Oti Wilberforce and Nwabue 2013). The growth of Plants in polluted environment can result in the accumulation of toxic metals at high concentration which could cause serious health risk to humans when consumed (Vahter and Ljung 2007).

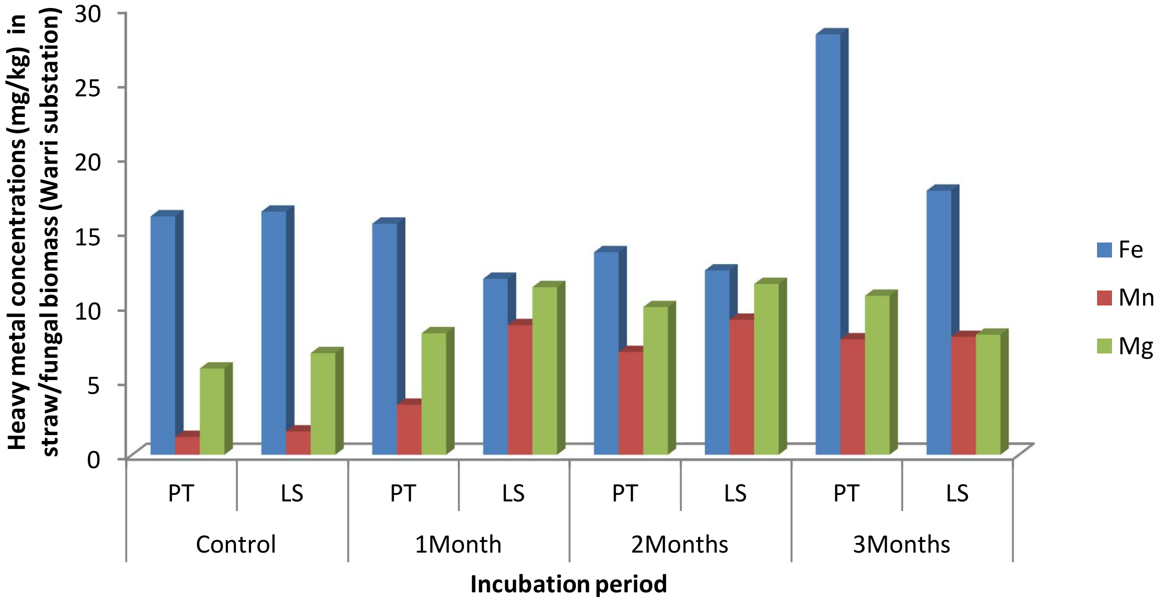

Figure 2: Heavy metal concentrations (mg/kg) in straw/fungal biomass (Warri substation)

The heavy metal concentrations in transformer oil polluted soil from Ughelli inoculated with *P. tuber-regium* and *L. squarrosulus* is shown in table 3, here for both inoculums Fe >Mg > Pb. For soil treated with Pt, the Fe concentration decreased from 105.09mg/kg in control to 76.13mg/kg after 1month which increased to 86.93mg/kg and 98.63mg/kg after 2 and 3months respectively. The Mg concentration in this soil decreased from 0.60mg/kg in control to 0.18mg/kg after 2months and then increased after 3months to 0.54mg/kg while Pb was detected only in control and after 2months having the same value 0.17mg/kg which is not significant ($p<0.05$).

The Fe content of soil inoculated with Ls decreased from 70.94mg/kg in control to 53.04mg/kg after 2months and then increased after 3months incubation to 69.56mg/kg while Mg and Pb were below detectable limit in this soil.

Figure 3 shows the heavy metal concentrations in straw/fungal biomass, the Fe concentration in straw inoculated with Pt increased from 9.38mg/kg in control to 14.55mg/kg after 1month which dropped to 13.01mg/kg and then increased to 23.84mg/kg after 3months incubation. The Mn concentration decreased from 7.40mg/kg in control to 3.66mg/kg after 1month, 9.66mg/kg was recorded at 2 and 3months incubation period while the highest Mg concentration (14.88mg/kg) was recorded at 1month and 2months incubation period. The Fe concentration in straw/fungal biomass inoculated with Ls increased from 9.21mg/kg in control to 13.52mg/kg after 1months which decreased to 12.36mg/kg and then increased to 14.07mg/kg while the Mn concentration increased steadily with increase in incubation period from 5.60mg/kg in control to 12.88mg/kg after 3months. The Mg concentration also followed the same order as the Fe concentration in straw/fungal biomass inoculated with Ls.

For all the heavy metals analyzed, transformer oil polluted soil treated with LS had lower heavy metal concentrations than the soil treated with PT indicating that LS is a better accumulator of heavy metals than PT because as a result of this WRF absorbing the heavy metals, reduction in the heavy metal concentrations in the soil was recorded. The fact that the availability of heavy metals in soil was influenced by the inoculation or introduction of the two WRF was observed in this study. This is in agreement with the report of Massaccesi et al. 2002 whose conclusion was that there is a possibility of fungi to be applied to polluted soils as metallic biosorbents. Ganguly (2013) also reported the use of dead cells of the fungi

Aspergillus niger X_{300} in the biosorption of As (III) from broth. Adenikpekun and Isikhuemhen 2008 also stated the capability of *L. squarrosulus* in accumulating some heavy metals like Ni, Cu, Pb and Fe from engine oil polluted site. The accumulation of metals such as Pb, Al, Ca and Cd by wood-rotting fungi from liquid medium supplemented with appropriate amounts of metal salt was reported also by Gabriel *et al.* (2005). Chary et al., 2008 reported an increment in the risk of public exposure to heavy metals due to the consumption of food grown in sewage wastewater. They are stated that the demerit of the entrance of heavy metals into the food chain demands systematic assessments to ensure timely decisions are made to prevent severe health effects as a result of the invisible mode of heavy metal toxicity.

Table 3: Heavy metal concentrations (mg/kg) in transformer oil polluted soil (Ughelli substation) treated with Pt and Ls

Incubation period		Fe	Mg	Pb
Control	**PT**	105.09^{a}	0.60^{a}	0.17^{a}
	LS	70.94^{e}	ND	ND
1Month	**PT**	76.13^{d}	0.30^{b}	ND
	LS	68.03^{e}	ND	ND
2Months	**PT**	86.93^{c}	0.18^{c}	0.17^{a}
	LS	53.04^{f}	ND	ND
3Months	**PT**	98.63^{b}	0.54^{a}	ND
	LS	69.56^{e}	ND	ND

Means with same superscript along column are not significantly different ($P<0.05$) (With the significance levels placed on this table, you can write more about it and discuss it further)

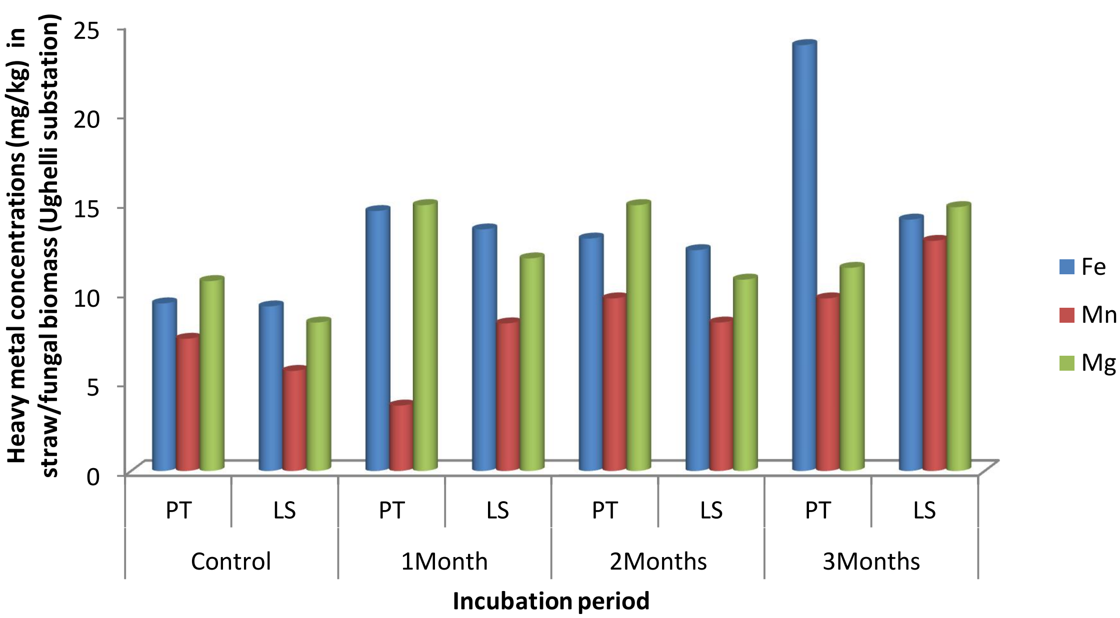

Figure 2: Heavy metal concentrations (mg/kg) in straw/fungal biomass

Conclusion

This study revealed that transformer oil polluted soil from Ibadan had more heavy metals in terms of type and concentration than soil from warri and Ughelli. Soils treated or inoculated with LS had lower heavy metal concentrations in all the soils which indicate that it may be more effective in accumulating heavy metals in soil than PT.

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