The Effects of Nitrogen Fixing Tree (*Leucaena leucocephala*) and Mushroom (*Pleurotus tuber-regium)* on Spent Engine Oil Polluted Soil

Adanikin B. A[*] and Kayode J.

Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Ekiti State. Nigeria

ABSTRACT: Greenhouse experiment was conducted for four months using *Leucaena leucocephala* and *Pleurotus tuber-regium* to determine their bioremediation potentials. *Leucaena leucocephala*, *Pleurotus tuber-regium* and *Leucaena leucocephala* combined with *Pleurotus tuber-regium* were tested for their ability to improve nutrient (N, P, K, total organic carbon) and reduce heavy metals (Zn, Ni, Pb, Cu) of soil polluted with spent engine oil [5% (v/w)] and soil without spent engine oil was used as control. Bioaccumulation of nutrients and heavy metals in *Leucaena leucocephala* and *Pleurotus tuber-regium* were also determined. The highest reduction in Zn, Ni, Pb and Cu (41%, 48.39%, 61.60 and 52.72% respectively) were recorded in soil remediated with *Leucaena leucocephala* alone, reduction of 30.40%, 26.53%, 48.07% and 39.60% respectively were recorded in soil remediated with *Pleurotus tuber-regium* alone while in soil remediated with combined *Pleurotus tuber-regium* and *Leucaena leucocephala,* reductions of 32.7%, 33.43%, 88.41% and 46.22% respectively were recorded. Bioaccumulation of Zn, Ni, Pb and Cu in *Leucaena leucocephala* increased by 73.41%, 85.46%, 3366.04% and 125.53% respectively, similarly in *Pleurotus tuber-regium* by 30.16%, 21.67%, 71.11% and 53.21% respectively. These studies have shown that *Pleurotus tuber-regium* and *Leucaena leucocephala* are capable of bioremediating spent engine oil polluted soil although, treatment with *Leucaena leucocephala* alone tends to be most effective of these treatments.

Keywords: Bioremediation, Heavy metals, Nutrients, *Leucaena*, *Pleurotus.*

INTRODUCTION

Soil contamination by spent engine oil from automobiles is a growing concern in many countries, especially in Asian and African continents. This is due to increase in quantity of engine oil and other engine fluids from servicing and subsequently draining spent oil from automobiles and generator engines due to the increase in population, roadway, railway, generators and vehicles (Baladincz *et al.*, 2008; Agamathu *et al.*, 2010). In some less developed countries, such as Nigeria, up to 80 million liters of spent engine oil are generated and discharged into the environment without any form of processing, this may pose a great threat to the environment and human being at large (Adelowo *et al.*, 2006).

Various studies have confirmed the deleterious effects of spent engine oil in our environment. Agbogidi & Ejemete (2005) noted that oil in soil has deleterious effects on biological, chemical and physical properties of the soil. Ekundayo *et. al.*, (2001) reported that the germination

---

[*] Corresponding Author, Email: adanikindele4dove@yahoo.com

of seeds planted in crude oil polluted soil area were delayed and percentage germination was significantly affected. Kayode *et al*., (2009) reported the inhibitions of radicle and plumule growths as well as the reduction in the number of leaves in seedlings of *Vigna unguiculta* and *Zea mays* in spent engine oil polluted soil.

Phytoremediation is a promising strategy for in situ removal of many contaminants (Greenberg, 2006; Pilon-Smits, 2005; Pulford & Watson, 2003). According to Palmroth *et al*. (2002), root exudates from plants do help to degrade toxic organic chemicals and acts as substrates for soil microorganisms in the soil which directly results in increased rate of biodegradation of the organic contaminants. Different types of plants have been found useful for phytotreatment of soil contaminated by hydrocarbons. *Mirabilis jalapa* was found to reduce concentration of total petroleum hydrocarbon (TPH) by 41.61 % - 63.2 % in 127 days (Peng, *et al*., 2009). Studies by researchers have shown that *Jatropha curcas* is a potential plant for remediation of heavy metals-contaminated soil due to its bioaccumulation potential (Jamil *et al*., 2009; Mangkoedihardjo & Surahmaida, 2008).

Leucaena leucocephala has numerous inherent characteristics that can be exploited to augment phytoremediation and lower the cost of soil regeneration. The species can survive in harsh environmental conditions with the exception of heavily frosted conditions and occurs in a wide range of ecological settings. It is fast growing, capable of reaching maturity in 6 to 7 months to produce a vast amount of seeds that can germinate into numerous seedlings to carry on further remediation of the polluted site. It can produce large quantities of phytomass that can accumulate heavy metals and can repeatedly be harvested to regenerate a polluted area through phytoextraction (Jamilu *et al*., 2017). Also, studies have shown that mushrooms have remarkable ability in the remediation of

environmental pollutants (Isikhuemhen *et al*., 2003; Oyetayo *et al*., 2012). Therefore the objective of this study is to investigate the potential of *Leucaena leucocephala* and *Pleurotus tuber-regium* (singly and combined) in enhancing the bioremediation of spent engine oil polluted soil.

MATERIALS AND METHODS

The study was carried out at the greenhouse of the Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria.

Collection of Soil Samples and Plant materials: Top soil samples were collected from the research farm of the Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria. Spent engine oil was sourced from mechanic workshops in Ado-Ekiti, Ekiti-State. *Leucaena lecocephala* (LL) were nursed from seeds at the greenhouse of the Department of Plant Science and Biotechnology of the University and *Pleurotus tuber-regium* (PR) sclerotium was procured from markets around Ado-Ekiti, Ekiti State, Nigeria.

Experimental Procedures: Soil samples (10 kg) were weighed into thirty (30) planting pots. These planting pots were divided into two groups (A and B). Each planting pot in group A was polluted with 500 ml spent engine oil while group B were without spent engine and served as the control. The polluted soil samples were left for seven (7) days without wetting to soak. Each group was sub divided into three groups (A_1, A_2, A_3 and B_1, B_2, B_3) and subjected to the following treatments:

Each A_1 planting pot was planted with a seedling of *Leucaena lecocephala*,

Each A_2 planting pot was planted with 30 g of *Pleurotus tuber-regium*, and,

Each A_3 planting pot was planted with a seedling of *Leucaena lecocephala* and 30 g of *Pleurotus tuber-regium*,

B_1, B_2 and B_3 were treated as above with unpolluted soils to serve as controls for treatments A_1, A_2 and A_3 respectively.

Each LL was transplanted at 3 weeks of growth into their respective pots and PR (30 g) was buried in respective pots deep enough to be covered by soil (Oyetayo *et al.*, 2012). Each pot was watered daily and the experiment was monitored for four months.

Sample collection and Analysis: The physicochemical properties of the soil and plants – total organic carbon (TOC), nitrogen (N), phosphorus (P) and potassium (K) and also the heavy metals contents, zinc (Zn), nickel (Ni), lead (Pb) and copper (Cu) - were determined before planting. Soil samples were collected in the first and last months of the study and their N, P, K, TOC, Zn, Ni, Pb, Cu contents were determined. At the end of the experiment, N, P, K, TOC, Zn, Ni, Pb, Cu in the LL and PR used in the treatments were determined.

Soil samples were collected, air-dried, crushed, and sieved through 2 mm sieve. Soil organic C and total N were determined by dichromate oxidation (Nelson & Sommers, 1982) and Kjeldahl digestion–

distillation procedure (Bremner, 1996), respectively. The total metal content was determined using an atomic absorption spectrophotometer (AAS, GBC Avanta Ver. 1.33, Australia) after digesting 0.5 g of dried soil samples with 15 ml of HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1 ratio at 80°C in a microwave digestion system (MDS 2000) and filtered through Whatman No. 42 filter paper followed by dilution up to 50mlwith triple distilled water (Baker & Amacher, 1982).

Harvested plant samples were washed with water followed by $CaCl_2$ solution and distilled water, oven-dried at 70°C up to constant weight, were milled to pass through a 2-mm sieve. The milled plant material (0.5 g) was digested with a mixture of concentrated HCl/HNO_3 (4,1 v/v) and analyzed for metals using AAS (Allen *et al.*, 1986).

Accumulation Percentage (AP) was used to compare performance of various plants in accumulating heavy metal

$$AR = \frac{Level\ of\ heavy\ metals\ in\ treated\ plants\ at\ harvest}{Level\ of\ heavy\ metals\ in\ plants\ before\ planting}) \times 100 \qquad (1)$$

Bioaccumulation Ratios (BR) was calculated by using the procedure by Cai & Lena (2003) and expressed as:

$$BR = \frac{concentration\ in\ Plant}{concentration\ in\ soil} \qquad (2)$$

Percentage Reduction (PR) and Increase (PI) were also determined as follow:

$$PR = \frac{Metal\ level\ before\ transplanting - Metal\ level\ after\ harvest}{Metal\ before\ transplanting}) \times 100 \qquad (3)$$

$$PI = \frac{Metal\ level\ after\ harvest - Metal\ level\ before\ transplanting}{Metal\ before\ transplanting}) \times 100 \qquad (4)$$

RESULTS AND DISCUSSION
Table 1 shows that more nitrogen was recorded in LL (57.2 ± 0.3 mg/g) than in PR (27.3 ± 0.3 mg/g). N and TOC in soil increased by 19% and 336% respectively after pollution with spent engine oil. This

was in agreement with previous studies of Liu *et al.* (2007), and Okonokhua *et al.* (2007). These increases in N and TOC have been attributed to input arising from mineral elements in the spent engine oil (Liu *et al.*, 2007; Okonokhua *et al.*, 2007; Nwite & Alu,

2015). Values recorded for available P (0.33 ±0.003 mg/g) and K (0.21 ±0.005 mg/g) in non-polluted soil were lower than P (0.43 ±0.008 mg/g) and K (0.28 ±0.001 mg/g) recorded in polluted soil. Decrease in P and K in soil after pollution with spent engine oil has been reported in previous studies of Ogboghodo *et al*. (2004) and Nwite & Alu (2015). This is due to the inability of microbes to transform organic matter which led to low mineralization of the elements (Nwite & Alu, 2015). Values of metals, Zn (28.00 ± 0.2 mg/kg), Ni (14.63 ± 0.38 mg/kg), Pb (14.63 ± 0.38 mg/kg), Cu (12.25 ± 0.35 mg/kg) were lower compare to 73.17 ± 1.07 mg/kg, 26.30 ± 1.10 mg/kg, 57.35 ± 0.65 mg/kg and 15.10 ± 0.10 mg/kg recorded in polluted soil. According to Okonokhua *et. al.* (2007), the increase in metals in soil after pollution with spent engine oil is due to ability of soil to retain these metals even during drainage. Yong (2011) also attributed this to solute contamination in spent engine oil which attached to soil surface by a mechanism that satisfies the forces of attraction from the soil solid surfaces. Metals, Zn, Ni, Pb and Cu were also recorded in the LL and PR which were in amount lower than that recorded in the soil.

Values of nutrients and metals in the control and polluted soils in the first and fourth months of bioremediation are presented in Table 2. In the first month of bioremediation, values recorded for N; B_1 (1.8 mg/g), B_3 (2.0 mg/g), B_2 (1.9 mg/g) and TOC; B_1 (126.95 mg/g), B_3 (126.4 mg/g), B_2 (126.0 mg/g) in the control were

lower compare to the polluted soils, A_1 (2.8 mg/g), A_3 (2.55 mg/g), A_2 (2.55 mg/g) and A_1 (175.3 mg/g), A_3 (156.1 mg/g), A_2 (154.6 mg/g). The higher values of N and TOC recorded in the polluted soil may be due to the mineral elements added to the soil from the spent engine oil.

In the fourth month of bioremediation, nutrients (N, P and K) were observed to have reduced in the control and polluted soils. According to previous studies, nodulation in nitrogen fixing trees may take up to 3 months after planting (Aganga & Tshwenyane, 2003; Schneider *et al*., 2013). Also, Saraswat & Rai (2011) also reported that the nodulation of LL in soil decreases as the level of pollution increases. The reduction in N recorded in the polluted soil may be due to delayed nodulation by LL. Study by Binkley & Menyailo (2005) has indicated that the ability of nitrogen fixing trees to improve TOC in the soil is associated with their nitrogen fixing capability. The reduction observed in these elements (P, K and TOC) may also be due to delayed nitrogen fixing ability in LL.

Metals (Zn, Ni, Pb, Cu) in the polluted soil were observed to have reduced in the months of bioremediation. In the first months the values recorded in A_1, Zn (64.67 mg/kg), Ni (23.52 mg/kg), Pb (49.45 mg/kg) and Cu (14.21 mg/kg) was significantly higher (P≤ 0.05) than values recorded in A_3, 51.74 mg/kg, 20.88 mg/kg, 46.78 mg/kg respectively and A_2, 58.21 mg/kg, 21.17 mg/kg, 42.71 mg/kg, 13.70 mg/kg respectively.

Table 1. Physicochemical properties of non-polluted soil (B), Polluted soil (A) L. *leucocephala* and P. *tuber-regium* used for the study

Parameters	LL	PR	B	A
N (mg/g)	57.2 ± 0.3	27.3 ± 0.3	2.1 ± 0.2	2.5 ± 0.4
P (mg/g)	0.58 ± 0.003	0.67 ± 0.010	0.43 ± 0.008	0.33 ± 0.004
K (mg/g)	3.91 ± 0.005	4.48 ± 0.025	0.28 ± 0.001	0.21 ± 0.005
TOC (mg/g)	414.8 ± 6.2	463.7 ± 3.7	22.5 ± 0.3	98.3 ± 0.5
Zn (mg/kg)	12.7 ± 0.1	13.25 ± 0.35	28.00 ± 0.2	73.17 ± 1.07
Ni (mg/kg)	5.53 ± 0.08	8.75 ± 0.11	14.63 ± 0.38	26.30 ± 1.10
Pb (mg/kg)	0.3 ± 0.09	1.06 ± 0.16	14.63 ± 0.38	57.35 ± 0.65
Cu (mg/kg)	4.09 ± 0.04	3.16 ± 0.06	12.25 ± 0.35	15.10 ± 0.10

Table 2. Nutrients (mg/g) and Heavy metal content (mg/kg) in Control and polluted Soil in the 1st and 4th months of Bioremediation.

Months	Treatments	Nutrients				Heavy metals			
		N	P	K	TOC	Zn	Ni	Pb	Cu
1st	B_1	1.80d	0.038a	0.241c	126.95d	24.42d	9.83c	9.21e	10.23e
	B_3	2.0bcd	0.037b	0.262a	126.4de	22.26e	9.49c	9.62d	11.31d
	B_2	1.90cd	0.035c	0.254b	126.0e	18.44f	9.60c	9.81d	11.90c
	A_1	2.8a	0.031e	0.198e	175.3a	64.67a	23.52a	49.45a	14.21a
	A_3	2.55ab	0.031e	0.199de	156.1b	51.74c	20.88b	46.78b	14.10ab
4th	A_2	2.40abc	0.032d	0.200d	154.6c	58.21b	21.17b	42.71c	13.70b
	B_1	1.25bc	0.033a	0.239a	29.25a	16.375d	12.19d	6.16f	7.225c
	B_3	1.6a	0.026c	0.210b	4.25e	14.56e	10.18e	8.21e	4.32d
	B_2	1.8a	0.030b	0.208b	2.90f	10.16f	12.17d	10.18d	3.26e
	A_1	1.4abc	0.020d	0.166c	9.05d	42.26c	14.22c	22.38c	7.195c
	A_3	1.05c	0.016f	0.151c	9.70c	48.585b	18.43b	24.14b	8.17b
	A_2	1.2bc	0.018e	0.130d	10.75b	50.36a	20.14a	30.38a	9.30a

Means with the same letter within columns are not significantly different at $p \leq 0.05$.

However, in the fourth month, A_1 has recorded significantly lower (P≤ 0.05) Zn (42.26 mg/kg), Ni (14.22 mg/kg), Pb (22.38 mg/kg), Cu (7.20 mg/kg) than A_3, 48.59 mg/kg, 18.43 mg/kg, 24.14 mg/kg, 8.17 mg/kg respectively and A_2, 50.36 mg/kg, 20.14 mg/kg, 30.38 mg/kg, 9.30 mg/kg respectively. Previous studies have also reported the ability of LL to sequestrate meats from polluted soils (Ma *et al*., 2006; Tawfix, 2008). The significant reduction in metals in A_1, may have suggested that LL alone tends to be more efficient in the bioremediation of metals on spent engine oil polluted soil than when combined with PR and PR alone.

Figure 1 shows that Pb was the highest bioaccumulated metal in LL bomass. Pb increased by 3366.04% in LL biomass after harvest. Juson *et al*. (2016) have reported the ability of LL to bioacculate Pb in its tissues from polluted soils. This also agrees with Yitao *et al*. (2014) that high concentration of Pb can be bioaccumulated by LL in its above ground parts. Cu, Ni, Zn also increased in plant biomass after harvest (125.53%, 85.46% and 73.41% respectively). Studies have reported the ability of LL to accumulate Zn and Cu in its root and shoots (Gupta *et al*., 2000; Schneider *et al*., 2013).

Figure 2 shows that the metals (Zn, Ni, Pb, Cu) in PR after harvest have increased compared to before planting. Pb recorded the highest increase in PR. The value of Pb in PR before planting was 1.055 mg/kg which increased by 71.11% after harvest (2.89 mg/kg). Similarly, an increase of 30.16%, 21.67%, 53.21% in Zn, Ni and Cu respectively were recorded in PR after harvest. This agrees with previous studies on the ability of PR to uptake metals form polluted soil (Adongbele & Okhuoya, 2011; Oyetayo *et al*., 2012).

Bioaccumulation factor is used to evaluate the efficiency of a biota to efficiently remediate a polluted soil. A bioaccumulation factor higher than 1 has been considered most efficient in bioremediation of polluted soils (Garcia *et al*., 2009; Kalie & Svoboda, 2000).Table 3 shows that the bioaccumulation factor for Zn, Ni, Pb and Cu were 0.51, 1.53, 0.46 and 1.46 respectively in LL and 0.34, 0.53, 0.10 and 0.49 respetively in PR. Bioaccumulation factor (> 1) recorded for Ni and Cu in LL may suggest that this plant tend to be more prolific in the uptake of these metals from spent engine oil polluted soil. Previous studies have reported a bioaccumulation factor > 1for metals (Zn, Pb, Cu) in mushroom used in metal uptake from polluted soil (Adongbede & Okhuoya, 2011; Oyetayo *et al*., 2012). Lower bioaccumulation factor (< 1) recorded in PR in this study may be due to higher concentration of these elements in the polluted soil.
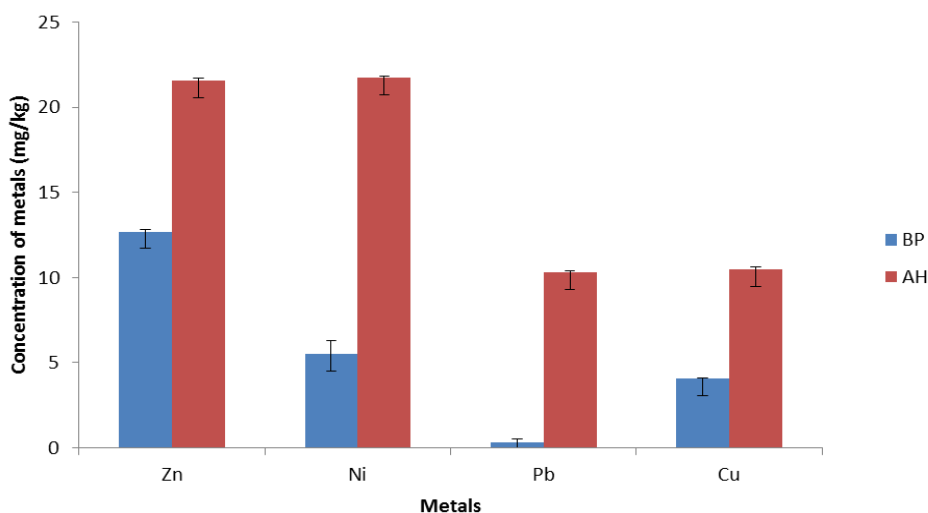
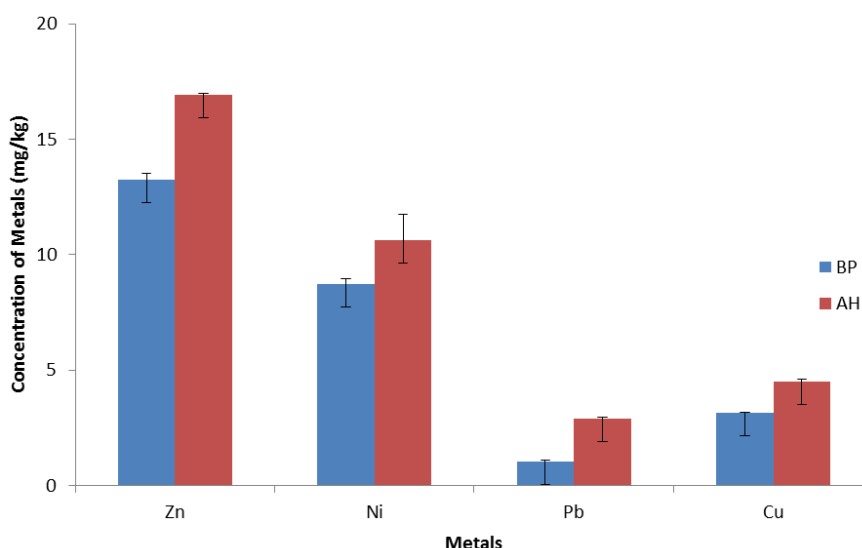**Fig. 1. Metals in L. *leucocephala* Biomass in Polluted soil; Before Planting (BP); After Havest (AH)**

Fig. 2. Metals in P. *tuber-regium* in polluted soil; Before Planting (BP); After Havest (AH)

Table 3. Bioaccumulation Ratio of L.*leucocephala* and P.*tuber-regium*

Samples	Zn	Ni	Pb	Cu
LL	0.51	1.53	0.46	1.46
PL	0.34	0.53	0.1	0.49

CONCLUSIONS

The study shows that LL alone, PR alone and LL combined with PR have the potential to bioremediate spent engine oil polluted soil. However, LL alone tends to be more efficient than PR alone and LL combined with PR. The reduction observe in N in both control and polluted soil indicates longer period of time (more than three months) may be needed for the nitrogen fixing ability of LL to attain its full potential. The bioaccumulation factor > 1 recorded for Ni and Cu in LL suggests that this tree is most efficient in remediating these metals from polluted soil. Low bioaccumulation factor recorded in PR compared to LL indicates that LL is better in the bioremediation of these metals, Zn, Ni, Pb and Cu in spent engine oil polluted soil.

REFERENCES

Adelowo, O. O., Alagbe, S. O. and Ayandele, A. A. (2006) Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi* and *Achromobacter aerogenes*. Afr J Biotechnol. 5(24), 2476-2479.

Adongbede, E. and Okhuoya, J. A. (2011) Bio-absorption of some heavy metals by *Pleurotus tuber-regium* Fr. Singer (An edible mushroom) from crude oil polluted soil amended with fertilizer and cellulosic wastes. Int J soil Sci. 6(1), 34-48.

Agamuthua, P., Abioyea, O. P., Abdul Aziz A. (2010) Phytoremediation of soil contaminated with used lubricating oil using Jatropha curcas. J Hazard Mater. 179, 891–894.

Aganga, A.A, Tshwenyane, S.O. (2003) Lucerne, Lablab and Leucaena leucocephala forages, Production and utilization for livestock production. Pak J Nutr. 2,46–53.

Agbogidi, O. M. and Ejemete, O. R. (2005) An assessment of the effects of crude oil pollution on soil properties, germination and growth of *Gambaya albida* (L.), UNISWA Res J Agric Sci Tech. 8(2),148 – 155.

Allen, S.E., Grimshaw, H.M. and Rowland, A.P. (1986) Chemical Analysis. In, Methods in Plant Ecology, Moore, P.D. and S.B. Chapman (Eds.). 2nd Edn., Blackwell Scientific Publication, Oxford, London, pp, 285-344.

Baker, D. E. and Amacher, M. C. (1982) "Nickel, Copper, Zinc and Cadmium," Methods of Soil Analysis, No. 9, Part II, Madison, pp. 331-333.

Baladincz, J. Szabo, L. Nagy, G. Hancsok,J. (2008) Possibilities for processing of usedlubricating oils – part 1", MOL Scientific Magazine, 3, 81-86.

Bremner, J.M., (1996) Nitrogen-total. In, Sparks DL (ed.), Methods of Soil Analysis. Part 3 Chemical Methods. Madison, WI, USA, SSSA Inc., ASA Inc, 1085122 Chandra, G., Ghosh, A., Biswas, D. and Chatterjee, S., 2006. Host plant preference of Mansonia mosquitoes. J Aquatic Plant Manage 44, 142–144.

Ekundayo, E. O., Emede, T. O. and Osayande, D. I. (2001). Effects of Crude oil spillage on growth and yield of maize (*Zea mays* L.) in soils of Mid-western Nigeria. Plant Foods Hum Nutr. 56, 313-324.

Fourest, E. and Roux, J. C. (1992) Heavy metals biosorption by fungal mycelia by products, mechanism and influence of pH. Appl Microbiol biot. 37, 399-403.

Fourest, E. and Roux, J.C. (1992). Heavy metal biosorption by fungal mycelial by products, mechanism and influence of pH. Appl Microbiol Biot. **37**, 399 – 403.

GarcíaI, Diez M, Martín, F., Simón, M., Dorronsoro, C. (2009) Mobility of arsenic and heavy metals in a sandy-loam textured and carbonated soil. Pedosphere 19, 166–175

Greenberg, B. M. (2006) Development and field test of multi-process phytoremediation system decontamination of soil. Canadian reclamation. Spring/summer (issue). 27-29.

Gupta, M., Kumari, A., Yunus, M. (2000) Effect of fly-ash on metal composition and physiological responses in *Leucaena leucocephala* (lamk.) de. wit. Environ Monit Assess. 61, 399–406

Isikhuemhen, O. S., Anoliefo, G. O. and Oghale, O. i. (2003) Bioremediation of crude oil polluted soil by while rot fungus, *Pleurotus tuber-regium* (Fr.) Sing. Environ Sci pollut R. 10, 108-112.

Jamil, S., Abhilash, P. C., Singh, N., Sharma, P. N. (2009) Jatropha curcas, a potential crop for phytoremediation of coal fly ash. J Hazard Mater. 172, 269–27.

Jamilu, E. S., Muhammed, N., Ingvar, B., Oryem-Origa (2017) Phytoremediation potential of *Leucaena leucocephala* (Lam.) de wit. For heavy metals polluted and heavy metals degraded environments. Phytoremediation potentials of bioenergy plants. 159-209.

Juson, A. E., Maria-Kariza. M. M., Johnny, A. C. (2016) Accumulation and distribution of heavy metals in *Leucaena leucocephala* (Lam.) and *Bougamvillea Spectabilis* Willd. Plant systems. J of Exp Biol Agric Sci. ISSN No. 2320-8694.

Kalac, P, Svoboda, L. (2000) A review of trace element concentrations in edible mushrooms. Food Chem. 69, 273–281.

Karthikeyan, R., Davis, L.C., Mankin, K.R., Erickson, L.E., Kulakow, P.A., (1999) Biodegradation of jet fuel (JP-8) in the presence of vegetation, Proceedings of the 1999 Conference on Hazardous Waste Research, St. Louis, MO. pp. 243–256.

Kayode, j., Olowoyo, O. and Oyedeji, A. (2009). The Effects of Used Engine Oil Pollution on the Growth of Early Seedling Performance of *Vigna unguiculta* and *Zea mays*. Res J Soil Biol. 1 (1), 15 – 19.

Li, M. S., Luo, Y. P., Su, Z. Y. (2007) Heavy metal concentrations in soils and plant accumulation in a restored manganese mine land in Guangxi, South, China. Environ Pollut. 147, 168–175.

Ma, Y., Dickinson, N. M., Wong, M. H. (2006) Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous shrubs on Pb/Zn mine tailings. Soil Biol Biochem 38, 1403–1412.

Mangkoedihardjo, S., Surahmaida, (2008) Jatropha curcas L. for phytoremediation of lead and cadmium polluted soil, World Appl Sci. J. 4 (4), 519–522.

Nelson, D. W. and Sommers, L. E. (1982) Total carbon, organic carbon and organic matter. pp. 539-579. In, A. L. Page *et al*. (ed.) Methods of soil analysis, Part 2. Chemical and microbiological properties. ASA Monograph Number 9.

Nwite, J. N. and Alu, M. O (2015) Effect of different levels of spent engine oil on soil porperties, grain yield of maize and its heavy metal uptake in Abakaliki, Southeastern Nigeria. J Soil Sci Environ Mgt. 5(4), 4451.

Ogboghodo, A. E., Inaga, E. K., Osemwota, O., Chokor, J. U. (2004). An assessment of the effects of crude oil pollution on soil properties, germination and Growth of maize (Zea mays L.) using two types Forcades light and Escravos light. J Environ Monitor Asses. 96, 142-152.

Okonokhua, B. O., Ikhajiagbe, B., Anoliefo, G. O., Emede, J. O. (2007). The effect of spent engine oil on soil properties and growth of maize (Zea mays L.). J Appl Sci Environ Mgt 11(3), 147-152.

Oyetayo, V.O., Adebayo, A.O., and Ibileye, A. (2012). Assessment of the biosorption potential of heavy metals by Pleurotus tuber-regium. Int J Advanced Biol Res.; 4, 293-297.

Peng, S., Zhou, Q., Cai, Z., Zhang, Z. 2009. Phytoremediation of petroleum contaminated soils by Mirabilis jalapa L. in a greenhouse plot experiment. J Hazard Mater. 168(2-3), 1490 – 1496.

Pilon-Smith, E. (2005) phytoremediation, Annual review of plant biology. 56, 15-39.

Pulford, I. D., Watson, C. (2003) Phytoremediation of heavy metal contaminated land by trees-a review. Environ Int 4,529–540.

Saraswat, S., Rai, J. P. N. (2011) Prospective application of *Leucaena leucocephala* for phytoextraction of Cd and Zn and nitrogen fixation in metal polluted soils. Int J Phytoremediat. 13(3), 271–288.

Tawfik, K. M. (2008) A monitory field study at El Saaf-Helwan faba bean farms irrigated by industrial waste water and polluted water with sewage. J Appl Sci Res. 4, 492–499.

Yitao, X.U., Qixian, F., Jixiu, W., Mingrui, L. I., Fangdong, Z., Xiao, Y. (2014) Heavy metal uptake characteristics of three plants grown in lead/zinc mine tailings. Environ Sci Technol. 4(2), 118–138.