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Evaluation of phytoremediation potential of *Peltophorum pterocarpum* (DC.) Heyne *Leucaena leucocephala* (Lam.) De Wit. and *Crotolaria retusa* Linn for waste oil contaminated soils.

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ABSTRACT: An ecological study was carried out to evaluate remediation potential of three hydrocarbon tolerant species (*Peltophorum pterocarpum* (DC.) Heyne*, Leucaena leucocephala* (Lam.) De Wit.*,* and *Crotolaria retusa* Linn) of Fabaceae plant family in relation to enzyme activity for cleaning up soils contaminated with waste oil hydrocarbon. Biochemical analyses were carried out using classical standard procedures to assess the level of enzyme expression in relation to hydrocarbon index assessment in remediation performance through a holistic test of significance using the PROC ANOVA and Duncan's New Multiple Range Test (DNMRT) procedures. Enzyme expression, oil removal and organic carbon sequestration of the species and the species treated soils showed that in pre-polluted soil foliar enzyme expression in the order *Cr>Ll>Pp* was high but reduction in post-polluted and post-phytoremediation soils in the order *Cr>Ll>Pp*. Generally, among species *Peroxidase* (POD) was higher in activity and expression than *Polyphenoloxidase* (PPO). The oil and grease recorded a lower content in the pre-pollution soil which increased in content in post-pollution with increase in pollution. However, the impact of phytoapplication has shown some significant ($p<0.05$) reduction in *L. leucocephala* soil in the order *Ll<Pp<Cr* but higher foliar content among the species in the order *Ll>Pp>Cr* at low enzyme expression in which *P. pterocarpum* had higher carbon content in the order *Pp>Cr> Ll*. The pre-pollution soil had a significantly lower carbon than post-polluted soils. The impact of phytoremediation has shown reduction in carbon content with *P. pterocarpum* treated soil significantly lower in content in the order *Pp<Cr< Ll* and higher foliar content in the order *Pp>Cr> Ll*. Thus by the forgoing trajectories and trend of indigenous enzymes, *P. pterocarpum* and *L. leucocephala* can thus be recommended as an integral component in any bioremediation technology package for waste oil polluted terrestrial environment.© JASEM

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KEY WORDS: Peroxidase, Polyphenoloxidase, Organic carbon, Oil & grease, Phytoapplication.

The use of biological material in phytoremediation or bioremediation offers promising techniques for sustainable waste management (Gerhardt *et al*., 2009). Such biological processes are gaining more importance over physicochemical process; hence they are more effective with the end product formed as non-toxic (Pradeep *et al*., 2011). Phytoremediation systems using plant species that destroy contaminants offer highly cost-effective alternative or complement to conventional remediation methods. Plants naturally produce enzymes for a variety of functions, including the degradation of various organic compounds. Some plant enzymes, which evolved to break down organic compound as part of a plant's natural processes, also completely destroy environmental contaminants.

Plants require physiological mechanisms in response to xenobiotics, like the exudation of phenols acting as chelates of heavy metals (Wang *et al*., 2009) or plant intracellular mechanisms involved in the specific scavenging of Reactive Oxygen Species (ROS) often generated in presence of xenobiotic compounds (Marquez-Garcia *et al*., 2009). The ROS transformation depends on the scavenging enzymes (Mittler *et al*., 2004); where their presence in roots gives high potential to plant species to metabolize xenobiotics and participate in de-pollution.

Plants have evolved capabilities to break down organic compounds and to extract such essential nutrients as nitrogen, phosphorus, and potassium. As with all living things, digestion of complex molecules occurs with enzyme. Enzymes serve as part of detection

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mechanism that alerts the plant to the presence of specific contaminants as a signal transducers (Bais *et al*., 2004; Holzapfel *et al*., 2010). Enzymatic degradation converts contaminants to less toxic forms, and in many cases, completely breaks them down to carbon dioxide, water, inert gases and other simple, non-toxic molecules.

The detoxifying effect is due to the presence of oxido-reductive enzymes in plants and particularly in roots, with a high production of them in response to chemical stress (Hirata *et al*., 2000). Enzymes are biological catalysts that increase the rate of chemical reactions taking place within living cells. The acceleration achieved by enzymatic catalysis is often tremendous (Bailey and Ollis, 1986). The use of enzyme based techniques to remove organic compounds from aqueous solution was first proposed by Klibanov *et al*., (1983) and has been continuously improved since then. The use of enzyme proteins may represent a good alternative for overcoming most disadvantages related to the use of microorganisms (Rao *et al*., 2010) and even to the conventional methods. The interaction between peroxidases, polyphenoloxidases and other extracellular enzymes in pollutant oxidation has not been studied extensively, but is presumed to be advantageous in terms of broader substrate range, decreased inactivation by free radicals and further mineralization of toxic compounds (Majeau *et al*., 2010).

Though particular attention has been focused on peroxidase activities that have a role in xenobiotic oxidation and organic pollutant sequestration by plant roots (Laure *et al*., 2010), also study has been carried out to evaluate enzyme expression activity in a macrophytic treated crude oil soil habitat (Edwin-Wosu; Nkang, 2015) but this present research focuses attention on two kinds of oxido-reductase enzymes: peroxidases and polyphenoloxidases regarding their importance and application as metabolic tools with the aim of understanding their implication in inducing and enhancing the degradation and transformation of waste oil contaminant to non-toxic forms and molecules in the soil.

MATERIALS AND METHODS

*Sources of materials***:** Replicates of top loam soil (20kg) were collected in bulk within the standardized 0-15cm soil layer (Stewarte *et al*., 1974) and (Song *et al.,*1990) from a fallowed garden land of the Faculty of Agriculture, University of Calabar, Cross River State, Nigeria. The seeds of *Peltophorum pterocarpum* (DC.) Heyne were obtained from one of the green belt formations of the University of Port Harcourt, Rivers State, Nigeria. The seeds of *Leucaena leucocephala* (Lam) De Wit, and *Crotolaria retusa* Linn were obtained from the wild in a dump site in Port Harcourt and authenticated in the University of Port Harcourt Herbarium. The waste lubricating oil used was obtained from the mechanic garage in Port Harcourt, Rivers State, Nigeria. All chemical reagents, glass wares and equipment used in this study were of analytical grade and were purchased and obtained from Welly International Company Nigeria (Scientific/Hospital and Chemical supplier) located in Port Harcourt and Department of Botany, University of Calabar.

Experimental design and pollution of the study site with waste oil: The "*nested design*" of Akindele (1996) was adopted in this study using a split-split plot design in which the nested analysis of variance (PROC. ANOVA) procedures (SAS, 2002) was carried out on series of experimental plots. Three different species of plants were employed in the remediation of different waste oil polluted sites. The waste oil pollutant was applied using a measuring cylinder. The pollution level was in four concentrations, by volume (ml) of 0, 75, 150 and 300 per 1,809 cm^2 of soil surface area. At each level of pollution treatments were performed and replicated five times. Data on enzyme expression of the plant species in relation to organic uptake and mineralization of waste oil in polluted sites were obtained. Differences in species performance and soil treatments were tested by treatment interaction and treatment by levels interaction as the error terms.

*Habitat reclamation treatments***:** Each of the three levels of pollution and the controls were subjected to habitat reclamation using three members of the Fabaceae family (*P. pterocarpum, L. leucocephala and C retusa*). Seedlings of the selected plants were raised in nurseries at the Faculty of Agriculture, University of Calabar. Following germination and seedling growth, healthy 14 day old seedlings were selected for analysis and represented the pre-pollution samples. Healthy 14 day old seedlings of the three species were also transplanted from the nurseries onto the post-pollution control and polluted soils. Some plants were harvested 7 days after transplantation and represented the post-pollution samples. Subsequently, plants were allowed to grow on the control and treated soils for 10 months before final harvesting, representing the post-phytoremediation samples. Foliar enzyme expressions of the plants were assessed at each harvest interval and used as a measure of their level of tolerance in the polluted environment. Analysis of enzyme, oil & grease, total organic carbon was done as described below.

Enzyme assay and hydrocarbon index analysis of the remediation species: An extraction buffer of 150 ml was prepared for use. The 20 mM mixed phosphate buffer consisted of a monobasic potassium phosphate salt (KH_2PO_4), and dibasic potassium phosphate salt (K_2HPO_4) containing 2% of the phenolic adsorbent polyvinyl polypyrollidone (PVPP). The pH was adjusted to 7.1 at room temperature (30^OC) using 1M KOH. This extraction buffer was stored at 4^oC. A known quantity of fresh leaf (0.5 g) sample was ground in 10 ml of the extraction buffer using a pestle and mortar. The ground tissue was filtered through cheese cloth and the filtrate centrifuged at 4000 rpm for 30 minutes to obtain a clear supernatant. The supernatant fractions were stored in refrigerator and used as crude enzyme source for assaying for peroxidase (POD) and polyphenoloxidase (PPO) activity.

Peroxidase assay: To 2.5 ml of assay buffer (20 mM mixed phosphate buffer, pH 7.1) in a cuvette was added 0.1 ml of the enzyme preparation and 0.1 ml of guaiacol (10 mM). The enzyme reaction was assayed at 30^oC and followed with the addition of 0.1 ml H_2O_2 preparation (10mM) to initiate reaction. The absorbance readings after 60 seconds were measured spectrophotometrically at 436 nm (Nkang, 1990) using the assay buffer as blank. Peroxidase activity was calculated using an extinction coefficient of 6.39 $mol^{-1}cm^{-1}$ for the guaiacol dehydrogenation product (Putter, 1974).

Polyphenoloxidase assay: To 2.5 ml of the same assay buffer in a cuvette was added 0.1 ml of Dihydroxyphenylalanine (20 mM DOPA) and 0.1 ml of enzyme preparation. The reaction was started with the addition of 0.1 ml of 20 mM H_2O_2. The absorbance of the mixture was measured spectrophotometrically at 470 nm (Kahn, 1983). The activity of PPO was calculated using an extinction coefficient of 1433 mol^{-1} cm^{-1} for the quinone oxidation product (Jimenez and Garcia – Camorra, 1995).

Determination of oil and grease content (O&G): The oil and grease content (OG) was determined spectrophotometrically according to the toluene extraction method adopted by Odu *et al.* [1989]. The content was calculated by reference to a calibration curve using toluene as standard.

Total organic carbon (TOC): This was estimated using the Walkley and Black (1934) method as modified by Nelson and Somners (1975) for both soil and plant samples, following a complete oxidation from the heat of solution and external heating of sulphuric acid (H_2SO_4) and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture. This was determined using classical formula.

Data analysis: The remediation performance was estimated using the Statistical Analysis System (SAS) PROC. NLIN procedure (SAS, 2002). Data were then analysed as a split-split (double-split) plot design with 10 replicates using the Analysis of Variance (PROC ANOVA) procedures (SAS, 2002). Where significant differences were observed, means were separated according to the procedures of the Duncan's New Multiple Range Test (DNMRT) at LSD ($p<0.05$).

RESULT AND DISCUSSION

Result of enzyme activity in the three plant species are shown in Table 1. In a pre-pollution phase expression of Polyphenoloxidase (PPO) and Peroxidase (POD) activities were significantly *($p<0.05$)* high in *C. retusa* while *P. pterocarpum* was significantly *($p<0.05$)* lower in POD expression level. In the post-pollution phase *Peltophorum pterocarpum* recorded decrease in POD as pollution level increased but with higher value than control. Also there was reduction in increased pollution in *L. leucocephala* and *C. retusa* foliar expression but greater expression than control, though non-significantly different within pollution levels. Generally, there was reduction in POD expressions in all species as pollution levels increased.

Peroxidase (POD) expression level within species at the various pollution levels recorded no significant difference except in *C. retusa* with a low expression, but significantly different *($p<0.05$)* at high pollution level. However, *P. pterocarpum* increased with increasing pollution level except in medium pollution but were still greater than control foliar expression. Among species *C. retusa* had high expression at medium pollution though not significantly different, but had significant *($p<0.05$)* decrease at high pollution level. Generally, *P. pterocarpum* had a lower enzyme expression level. In the post- phytoapplication phase, a similar trend of enzyme expression was indicated in the species. In the remediated habitat within the various pollution levels Peroxidase (POD) expression was significantly different *($p<0.05$)* in *Peltophorum pterocarpum* at medium pollution intensity. Subsequently, POD expression in all the species at various pollution levels decreased at increased pollution intensity. However, in all pollution levels foliar POD expressions in *P. pterocarpum* and at low level of *C. retusa* were higher than control plants. Among the species there was no significant difference at medium pollution level but with *C. retusa* recording a higher POD expression level.

The foliar Polyphenoloxidase (PPO) expression in the remediating species showed no significant difference within pollution levels in all the species. Also PPO decreased as pollution increased though with low level pollution greater than control except in *C. retusa*. Among the species at various pollution levels there was no significant difference but with *C. retusa* recording the highest expression values. In post-phytoapplication, the Polyphenoloxidase (PPO) expression within pollution levels had no significant difference in all the species. There was reduction in PPO expression of all the species as pollution increased but was greater than control at low level pollution in *P. pterocarpum* and *L. leucocephala* while *C. retusa* was higher than control at low and medium pollution levels with no significant difference. Among species *C. retusa* was significantly *($p<0.05$)* higher at all pollution levels.

The oil and grease content of remediating species (Table 2) shows that within species at respective pollution levels there was no significant reduction in oil and grease uptake with increase in pollution. However, *L. leucocephala* recorded higher content than control at low pollution level. Among the species there was a significant difference *($p<0.05$)* in content with *C. retusa* recording a significantly lower content at medium and high pollution levels. However, *L. leucocephala* recorded a higher content with significant difference *($p<0.05$)* among the species at low pollution levels.

The carbon content of remediating plants following phytoapplication process in the waste oil polluted soil habitat (Table 2) showed a significantly *($p<0.05$)* lower content within species at high pollution in *P. pterocarpum* and *L. leucocephala* and low pollution level in *C. retusa.*. The waste oil species also experienced decrease in carbon content at increasing pollution levels but higher than the control in *L. leucocephala* at low and medium except in *C. retusa* that had increase at increasing pollution levels and higher than control at medium and high pollution levels. Among species *C. retusa* was significantly high *($p<0.05$)* at high pollution level
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Table 1: Enzyme expression and activity performance level of remediation species on waste oil soil habitat.

Pre-pollution

Enzyme	*Pp**	*Ll*	*Cr*	Mean	LSD*(p<0.05)*
PPO	0.46[b]	0.53[b]	0.89[a]	0.63	0.25
POD	7.02[b]	272.13[a]	316.83[a]	198.66	105.56

Treatment Phase	Treatment Level	POD					PPO				
		*Pp**	*Ll*	*Cr*	*Mean*	*LSD*	*Pp**	*Ll*	*Cr*	*Mean*	*LSD*
Post-pollution	Control	16.98 ±5.26[b]	81.64 ±5.26[b]	1056.10±178.87[a b]	*384.91*	*142.80*	1.37±0.45[b a]	1.60±0.50[bab]	2.67±0.86[a]	*1.90*	*0.87*
	Low	329.10±106.63[a b]	576.00±186.63[b a]	1334.00±225.94[a]	*746.37*	*248.10*	1.43±0.46[a b]	1.81±0.59[a ab]	2.55±0.82[a]	*1.93*	*0.88*
	Med	304.00±51.49[a c]	561.00±178.44[b a]	1180.00±199.86[aab]	*681.67*	*217.06*	1.33±0.43[b a]	1.47±0.47[bab]	2.48±0.80[a]	*1.76*	*0.82*
	High	364.50±209.62[a]	460.20±149.45[a]	102.13±25.07[b c]	*308.94*	*205.79*	1.13±0.37[b a]	1.13±0.37[b]	2.33±0.76[a]	*1.53*	*0.73*
	Mean	*256.94*	*419.71*	*918.06*			*1.32*	*1.52*	*2.51*		
	LSD	*161.53*	*200.78*	*235.69*			*0.57*	*0.67*	*1.09*		
Post-phytoapplication	Control	60.40±0.55[b d]	2419.20±1181.73[a]	3138.20±3.83[a]	*1872.20*	*939.74*	4.09±0.08[c a]	4.76±0.18[b]	7.81±0.27[a]	*5.55*	*0.27*
	Low	957.20±36.52[c a]	1696.60±41.37 [bab]	3971.40±178.01[a]	*2208.40*	*148.27*	4.29±0.30[c a]	5.42±0.16[b a]	7.97±0.11[a]	*5.90*	*0.29*
	Med	881.60±60.10[b]	1652.80±98.07[bab]	2858.40±1455.91[a]	*1797.60*	*1161.90*	3.60±0.13[c b]	4.35±0.32[b c]	7.84±0.71[a]	*5.26*	*0.63*
	High	618.00±8.63[a c]	1348.80±271.27[a b]	886.80±1464.84[a b]	*951.20*	*1185.20*	3.37±0.14[b]	3.36±0.14[b d]	6.68±0.59[a b]	*4.50*	*0.50*
	Mean	*629.30*	*1779.05*	*2713.70*			*3.84*	*4.74*	*7.58*		
	LSD	*47.48*	*815.57*	*1389.70*			*0.25*	*0.28*	*0.65*		

Note: ***Pp*** *= Peltophorum pterocarpum. Ll = Leucaena leucocephala. Cr = Crotolaria retusa, Enzyme levels are expressed in* µ/l.
* Means of five replicates and with the same superscript letter are not significantly different, using the Duncan's New Multiple Range Test (DNMRT). The superscripts are in two way analysis for within and among species with LSD and superscript in bold for horizontal test of significance

Table -2: Hydrocarbon indices of remediating plant on post waste oil polluted soils**.**

Hydrocarbon Index	Level	*Pp**	*Ll*	*Cr*	Mean	LSD (*p<0.05)*
Oil & Grease	Control	64.26±0.43[a]	64.06±0.72[a]	62.42±1.49[b]	63.58	1.36
	Low	63.33±0.88[b]	65.80±1.92[a]	62.06±0.66[b]	63.73	1.76
	Medium	62.79±1.23[a]	64.25±0.83[a]	60.58±2.00[b]	62.54	1.98
	High	63.08±1.58[a]	63.60±0.89[a]	60.36±1.37[b]	63.35	1.81
Organic Carbon	Control	2.61±0.31[a]	1.12±0.43[b]	1.45±0.42[b]	1.73	0.54
	Low	2.23±0.30[a]	2.09±0.33[a]	1.36±0.45[b]	1.89	0.50
	Medium	1.77±0.12[a]	1.67±0.44[a]	1.72±0.69[a]	1.72	0.66
	High	1.07±0.43[b]	1.01±0.24[b]	1.72±0.15[a]	1.27	0.41

Note: ***Pp*** *= Peltophorum pterocarpum. Ll = Leucaena leucocephala. Cr = Crotolaria retusa*

* Means of five replicates and with the same superscript letter are not significantly different, using the Duncan's New Multiple Range Test (DNMRT). a, b, c = degree of significance.

The oil and grease and organic carbon content of the pre-polluted, post-polluted and post-phytoremediated soil are shown in Tables 3a, b. The oil and grease content in the pre-polluted soil was 0.64 ± 0.10 and this was significantly different *(p<0.05)* and lower than in the polluted soils. The oil and grease values increased in post-pollution soils which recorded 0.96 ± 0.20, 1.40 ± 0.20 and 2.24 ± 1.00 at 75, 100 and 300ml pollution levels, respectively with significant difference *(p<0.05)* at 1.5% pollution level (Table 3a). In post phytoremediated waste oil soil, the oil and grease content within the species treated soil recorded increase relative to the various levels of post pollution increase. The increase in oil and grease was in increasing order of pollution levels and higher than control with significant difference *(p<0.05)* at low and medium species treated soil of *Peltophorum pterocarpum* and *C. retusa*. Among the species treated soil there was no significant difference in remediation; however *Leucaena leucocephala* recorded a lower oil and grease content with no significant difference (Table 3b).

The total organic carbon (TOC) in the pre-polluted soil was 0.85 ± 0.13 significantly *(p<0.05),* lower than the polluted soil. There was marked increase in post-pollution which recorded 1.40 ± 0.40, 1.96 ± 0.44 and 2.10 ± 0.20 TOC, at 75, 100 and 300 ml pollution levels respectively, but not significantly different between medium and high polluted soils (Table 3a). In post-phytoremediated soil within pollution levels there was increase in organic carbon higher than the control in all species treated soil. The decrease in remediation pollutant as compared to post pollution levels, though in increasing order of pollution levels was not significantly different in all species treated soils except at the high pollution level in *P. pterocarpum* treated soil. Among species treated soil there was no significant difference in organic carbon sequestration but with *P. pterocarpum* recording lower carbon content (Table 3b).

Table -3: Hydrocarbon indices of pre- and post-polluted waste oil contaminated soil

Hydrocarbon index	Pre-pollution	Post-pollution			Mean	*LSD (p<0.05)*
		Low	Medium	High		
TOC	0.85[c]	1.39[b]	1.96[a]	2.10[a]	1.58	0.37
O/G	0.64[c]	0.96[bc]	1.36[b]	2.24[a]	1.30	0.50

Table -4: Hydrocarbon indices of post-remediated waste oil contaminated soil

Hydrocarbon Index	Level	*Pp**	*Ll*	*Cr*	Mean	LSD (*p<0.05)*
Organic Carbon	Control	1.20 ± 0.17[a]	0.95 ± 0.02[a]	1.00 ± 0.53[a]	1.05	0.48
	Low	1.37 ± 0.30[a]	1.44 ± 0.35[a]	1.39 ± 0.26[a]	1.40	0.42
	Medium	1.49 ± 0.20[a]	1.51 ± 1.00[a]	1.57 ± 0.30[a]	1.52	0.46
	High	1.74 ± 0.36[b]	2.03 ± 0.40[a]	1.92 ± 1.00[ab]	2.01	0.56
Oil & Grease	Control	0.38±0.10[b]	0.46±0.08[ab]	0.56±0.14[a]	0.47	0.15
	Low	0.83±0.10[a]	0.79 ± 0.02[a]	0.94 ± 0.22[a]	0.86	0.18
	Medium	0.89±0.15[a]	0.89 ± 0.10[a]	0.97 ± 0.10[a]	0.92	0.14
	High	1.30±0.56[b]	1.27 ± 0.09[b]	1.64 ± 0.22[b]	1.40	0.49

Note: ***Pp*** *= Peltophorum pterocarpum. Ll = Leucaena leucocephala. Cr = Crotolaria retusa*

* Means of five replicates and with the same superscript letter are not significantly different, using the Duncan's New Multiple Range Test (DNMRT). a, b, c = degree of significance.

The use of biological material through phytoremediation or bioremediation offers promising techniques for sustainable waste management of organic and inorganic pollution. Plant species have been known for their capacity to release range of compounds from their root. Such compound known as phytoremediation *explanta* (Guo-Dang *et al.,* 2004), or *botanical explanta* (Edwin-Wosu, 2007) differs in type and rate considerably among such species and are capable of degrading chemical compounds in their immediate vicinity. Such enzyme exudates among other *explanta* include *laccases, dehalogenases, nitroductases, nitrilases, peroxidases, phosphatase, aromatic dehalogenase* and *O-demethylase.*

Enzyme activities of the species in pre-pollution, post-pollution and post-phytoremediation process showed significant differences within and among the species with *C. retusa* having a greater expression and in the order *Cr>Ll>Pp.* In pre-polluted soil, activity expression of PPO and POD in the order *Cr>Ll>Pp* was observed. *Peroxidase* (POD) and *Polyphenoloxidase* (PPO) enzyme activity were also shown to be expressed in the species, though with varying levels of expression in waste oil polluted soils. There was reduction in POD and PPO enzymes expression as pollution increased within species; this corroborates Edwin-Wosu and Nkang, (2015). However, *Crotolaria retusa* among species was high in enzyme level in the order *Cr>Ll>Pp.* Though with no significant difference within and among species as reduction occurred with increasing pollution, *Peroxidase* was higher in activity expression than PPO.

The soil condition might have been stressful for these plants or roots, thus reducing their activity and enzyme concentration at the root surface; this can be thought of as an enzymatic activation and inactivation process as hydrocarbon increased. This agrees with the assertion put up by Klumpp *et al*. (2000). However, similar study has shown that plant roots exude high concentrations of peroxidases into soil, particularly in response to chemical stress (Hirata, *et al*., 2000; Fediuc and Erdei, 2010; Angelica and Leonor, 2012).

In post-phytoremediation, similar foliar reduction in both enzymes was observed within species in increased polluted habitat but expression order of *Cr>Ll>Pp* was observed with the POD generally high among the species growing on the remediated soils. Considering the various stages of remediation, the polluted soil species had more POD activity expression, which further increased in post-phytoapplication while PPO in post-pollution and post-phytoremediation had decline in enzyme expression when compared to pre-pollution.

The comparative analysis of enzyme expression in the species studied showed a general pattern with considerable increase in activity after the time of exposition to pollutants. This might be related to plant age and time of pollutant exposure which might have also promoted enzyme activation or inducible enzymes under prevailing soil conditions. An activation of peroxidase occurred simultaneously with an inactivation of polyphenoloxidase. Enzymes up-regulation is generally transient; these enzymes are often strongly induced at the beginning of pollution and then slowly decrease with time. This type of response has indeed been shown in chemical stress, where peroxidase expression was only significantly increased in acute stress (Klumpp *et al*., 2000). This transient induction is however, not common to all peroxidase; there is always a basal level of peroxidase expression in plants, probably to perform "house keeping" functions, such as growth by elongation and lignifications (Passardi *et al*., 2005).

It has been shown that peroxidase up-regulation as a response of plant to pollutants can be used for the phytomonitoring of contaminated soils and of industrial or densely urbanized areas (Wu and von Tiedmann, 2002). These enzymes have been shown to be quite sensitive to atmospheric pollution, with a response that can be stronger than other classical biomakers (Wu and von Tiedmann, 2002); similar observations have been made with heavy metals present in soil (Geebelen *et al*., 2002); although the response may be lower than that observed with atmospheric stress (Klumpp *et al*., 2000).

The post-phytoremediation variation in foliar concentration of oil and grease, among the species in the waste oil soil has shown increase in accumulation for oil and grease with a decrease in pollution level possibly in relation to an enhanced root development and enhanced moisture content in a low to medium pollution levels, this corroborates Edwin-Wosu and Nkang, (2015). There was significant difference among the species with *L. leucocephala* having a higher oil and grease content in the order *Ll>Pp>Cr* in waste oil soil with a decline in enzyme activity expression. This observation is explained by the fact that plants have the ability to take up and metabolise a range of environmentally problematic organic pollutant (Hugues *et al.,* 1997). Also reported was an enhanced mineralization and accelerated removal of organics by crested wheat grass (*Agropyron desertorum*) and prairie grasses (Foth, 1990). In a similar metabolic studies, the ability of plants to take

up and metabolize a range of environmentally problematic organic pollutant, including ammonia waste (TNT and GTN), polychlorinated byphynols (PCBs) and trichloroethylene (TCE) have been established through enzyme secretion (Schnoor *et al.,* 1995; Goel *et al.,* 1997; Hughes *et al.,* 1997).
Values of foliar organic carbon levels showed no significant differences in content among the species in waste oil remediated soil. However the foliar content showed increased accumulation at 0.4 & 0.8% pollution levels with *P. pterocarpum* having a higher content and in the order *Pp>Cr>Ll* with low level enzyme activity expression. Possibly this could be a result of higher root formation and enhanced moisture content at these pollution levels thus increase in absorption by the plant root.

The oil and grease recorded a lower content in the pre-pollution soil which increased in content in post-pollution with increase in pollution. The waste oil polluted soil at various levels of pollution recorded significant difference at high level pollution.

The impact of phytoapplication in the polluted soil habitat has shown some level of oil and grease reduction. In contrast to post-polluted habitat *P. pterocarpum, L. leucocephala* and *C. retusa* waste oil soils had recorded variable reduction in the order of *Ll<Pp<Cr*. The reduction in low pollution level could be attributed to the enhanced root formation and moisture content at such pollution level, which decreased as pollution increased. The lower content of oil and grease in waste oil soil could be attributed to the increased moisture content and this possibly could have contributed to the dissolution and emulsification of adhered oil films and hydrophobic portion of the oil on soil particle into soil solution, coupled with enhanced root formation which could have enhanced greater uptake of the content to the foliar portion of the species.

Values of organic carbon content in the soil showed a considerable difference. There was a gradual increase in carbon content as pollution increased in the post-polluted soil habitat. The pre-pollution soil was significantly lower in carbon than the waste oil polluted habitat. This could be due to lack of exogenous source of carbon from the hydrocarbon. The waste oil polluted soil had increase in organic carbon as pollution increased. The impact of phytoremediation has shown a reduction in carbon content of species treated soil than post-pollution though in an increasing order of increased pollution among the species and also higher than the controlled phytoapplication soil due to exogenous source of carbon from the waste oil. *Peltophorum pterocarpum, L. leucocephala* and *C. retusa* waste oil soils had TOC level in the order *Pp<Cr<Ll* with *P. pterocarpum* having a lower content with significant difference. The carbon in the various pollution levels however their reduction seem to be higher than post-phytoapplication control due to steady increase in hydrocarbon following the exogenous source of oil mineralization. The higher reduction in carbon content in *P. pterocarpum* treated soil is an indication of the species level of hydrocarbon mineralization than other species. This may represent their utilization by enhanced phytomicrobial association in the rhizosphere. The detoxifying effect is due to the presence of oxido-reductive enzymes in plants and particularly in roots, with a high production of the enzyme in response to chemical stress (Hirata *et al*., 2000), localized mainly in cell walls and vacuoles (Coniglio *et al*., 2008). This enhanced hydrocarbon degradation, producing carbon, water and carbondioxide in presence of phosphorus, nitrogen nutrient and other environmental variables. The carbon becomes incorporated into the plant biomass, thus depleting the carbon and organic matter content of the soil.

*Conclusion***:** The detection of an in-situ enzyme activity in the foliar component of the species studied might reveal some of the mechanisms that allow plants to become adapted to the polluted areas where they are currently growing. These kinds of plant species could be proposed as suitable candidates for the removal and transformation of organic compounds through the action of their enzymatic systems. Enzyme activation and inactivation process occurred with the increase of contaminants in soils. However, further in-depth research is recommended for several other concentrations of pollutant and other species of plant.

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