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Influence of Organic Amendments and Metal Toxicants on Petroleum Hydrocarbon Degradation in Diesel Contaminated Soil

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Abstract:- The influence of organic amendments – poultry manure (PM), human sludge (HS), urea (UR) and metal toxicants (Cu, Pb, Zn) on total petroleum hydrocarbon (TPH) degradation in soil artificially contaminated with diesel was assessed in microcosms. A parent soil (sandy loam) was characterized and used for the rest of the study. TPH degradation in soil was monitored spectro photometrically (UV-visible) under the effect of diesel load, amendment dose and metal dose. TPH degradation rates decreased with increase in diesel load in soil treated with PM and HS, but remained somewhat constant under natural attenuation and UR amendment. Pseudo-first-order rate constant, k_1 (day^{-1}) and half-life, $\tau_{½}$ (day) for TPH degradation at the lowest diesel load were: natural attenuation ($k_1 = 3.0 \times 10^{-3}$, $\tau_{½} = 231.0$); PM ($k_1 = 2.6 \times 10^{-2}$, $\tau_{½} = 26.7$); HS ($k_1 = 1.9 \times 10^{-2}$, $\tau_{½} = 36.4$); UR ($k_1 = 1.7 \times 10^{-2}$, $\tau_{½} = 40.8$). TPH degradation efficiencies (%) increased with amendment dose in the order: UR (35 – 61) < HS (39 – 78) < PM (44 – 88) with plateaux observed beyond 30 %w/w. TPH degradation was reduced in the presence of metals in the sequence Zn > Pb > Cu, implying the vitiation of biodegradation process by the co-contaminating metals. These findings may become useful whenever bioremediation is the option for cleanup of diesel-contaminated soils.

Keywords:- *Biodegradation, diesel, total petroleum hydrocarbon, natural attenuation, poultry manure, human sludge.*

I. INTRODUCTION

Environmental pollution with petroleum and petrochemical products has attracted much attention in recent years. With the development of the economy and petroleum exploration, the environmental impact of exploration, production, refinery, storage, transport, distribution and final disposal of petroleum and their derivatives is a major concern in both developed and developing countries (Okieimen and Okieimen, 2005). This is rapidly increasing due to global increase in the usage of petroleum products (Mandri and Lin, 2007).

About forty percent of hazardous waste sites on the Environmental Protection Agency's (EPA) National Priority List (NPL) are co-contaminated with organic pollutants and heavy metals, and remediation of these sites pose a complex problem because of the mixed nature of contaminates. Co-contamination often causes a synergistic effect on cytotoxicity and two components often must be treated differently (Ademola *et al.*, 2013).

It is now generally recognized that land as a component of the environment deserves the same attention and protection as water and air. This recognition has risen perhaps because of increased incidents of land pollution on terrestrial and aquatic eco-systems and groundwater quality (Okieimen and Okieimen, 2005).The presence of different types of diesel engine automobiles and machinery has resulted in an increase in the use of diesel. Spillage of diesel or jet fuel contaminates our natural environment with hydrocarbon (Husaini *et al.*, 2008).

Hydrocarbon contamination of the air, soil, and freshwater especially by polycyclic aromatic hydrocarbons (PAHs) attracts public attention because many PAHs are toxic, mutagenic, and carcinogenic (Cerniglia and Sutherland, 2001). Contamination of the soil with petroleum hydrocarbon affects soil microflora and microfauna, underground water and plants, depending on the degree and magnitude of contamination, such soil may remain unsuitable for crop growth, (plates 1-3) for a very long time (Akpoveta *et al.*, 2011).

Diesel is natural product and as such is susceptible to degradation by naturally occurring microflora. However, diesel contamination is accompanied by depletion in both nutrient and oxygen levels of soil and this retards the ability of natural microflora to degrade diesel (Okieimen and Okieimen, 2005). Therefore, lack of the essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment. Consequently, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Hollender *et al.*, 2003;Walworth *et al.*, 2007).

Bioremediation exploits the ability of certain microorganisms, usually heterotrophic bacteria and fungi, to degrade organic materials to innocuous materials such as carbon dioxide, methane, water, inorganic salts, and biomass

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In the biological treatment of soils contaminated by petroleum, microorganisms being bacteria the most studied, use hydrocarbons, major components of petroleum, as source of carbon and alternative energy in the production of biomass. This process involves the transformation of hydrocarbons into smaller units and later incorporation as cellular material (biotransformation) or conversion to carbon dioxide (mineralization), resulting in the reduction of the concentration of the petroleum hydrocarbons (Raphael *et al.*, 2013).

In this study three amendments (poultry manure, human sludge and urea) have been used as sources of nitrogen (bio stimulation) and/or microorganisms (bio augmentation), as the case may be, in the degradation of total petroleum hydrocarbons (TPH) in diesel contaminated soil. The objectives of this study are to determine the potential of the amendments for enhanced biodegradation of diesel in soil. Specifically, a parent soil and the organic amendments were characterized in terms of physicochemical and bacteriological attributes. The study was also aimed at accessing effects of diesel load on rate of TPH degradation in unamended soil (natural attenuation) and in amended soil (bio stimulation), evaluated the influence of level of amendments on rate of TPH degradation in diesel contaminated soil and determine the influence of heavy metal toxicants (Cu, Pb and Zn) on TPH degradation in diesel contaminated soil.

II. MATERIALS AND METHODS

A. Materials

Diesel was sourced from a petroleum depot in Apir, Benue State Nigeria. Poultry manure was collected from the animal farm of the University, while human sludge was collected from domestic disposal sites in Makurdi and urea, a product of Notore Chemical Industries Onne, Nigeria, was obtained from a dealer in Makrudi Nigeria.

B. Soil sampling, pretreatment and characterization

Top-soil (0 – 20 cm) samples were collected at five different points (200 m apart) using a chrome-plated trowel from a fallow land at the University of Agriculture Makurdi. The soil samples were air-dried, ground and sieved to give < 2mm particle size, bulked up to get a composite sample, the parent soil (PS) and stored in polythene bags for further use. The PS was then characterized in terms of physicochemical properties – pH (Ajoy *et al*., (2011)), textural analysis (Bouyoucos, 1962), organic matter (Walkley and Black, 1934); cation exchange capacity (Rhoades, 1982), total nitrogen (Kjeldahl, 1883), total phosphorus (Olsen and Sommers, 1982) and pseudototal Cu, Pb and Zn (Baker and Amarcher, 1982).

The poultry manure (PM) and caked human sludge (HS) samples were air-dried for one week after which they were separately grinded, thoroughly mixed, also sieved through a 2mm sieve to achieve uniform particle size and stored separately in neat polythene bag for use.

Table 1. Physicochemical properties of parent soil and organic amendments

Property	Soil	Poultry manure	Human sludge
pH	6.85	6.87	5.74
Sand (%)	71.20	NA	NA
Silt (%)	13.10	NA	NA
Clay (%)	15.70	NA	NA
Cation exchange capacity ($cmolkg^{-1}$)	6.85	NA	NA
Organic carbon (%)	0.84	7.30	6.98
N ($mg\ kg^{-1}$)	0.09	2.35	1.49
P ($mg\ kg^{-1}$)	1.03	0.71	1.06
Cu ($mg\ kg^{-1}$)	0.0089	0.189	0.448
Pb ($mg\ kg^{-1}$)	0.199	0.226	0.214
Zn ($mg\ kg^{-1}$)	0.058	0.855	2.904

*NA implies not applicable

C. Soil microbial analysis

The enumeration and identification of microbial load for the un-polluted parent soil sample was done.The standard method of Agamuthu and Dadrasnia (2013) for Enumeration of Bacteria and Identification was adopted in microbial analysis. The method is described as follows; 0.1mL of serially diluted soil samples were plated out on nutrient agar medium (oxide) for isolation of aerobic, heterotrophic bacteria with addition of 50 $\mu g\ mL^{-1}$ fungazol to suppress the growth of fungi. Plates were incubated at 32 ^{o}C for 24, hours after which the colonies were counted. Hydrocarbon utilizing bacteria in the soil samples were enumerated using oil agar (Zajic and Supplisson, 1972). The bacterial isolates were characterized using Biolog Microstation method (Ruan et al., 2005).The result of soil bacterial characterization reveals that ten heterotrophic bacteria are present, out of which six are hydrocarbon utilisers. The identified heterotrophic bacteria include Norcardia spp, Cellulomonas spp, Bacillus spp, Serratia spp, Flavobacterium spp, Micrococcus spp, Alcaligen spp, Chromobacterium spp, Alcaligen spp and Pseudomonas

spp. Total heterotrophic microorganisms for the un-polluted parent soil was found to be $780x10^5$cfu/g, but decreased to $430x10^5$cfu/g on addition of diesel to the soil.

D. Experiments to monitor TPH degradation in soil

- *Effect of soil diesel concentration on TPH degradation without amendment*

Separate 100g subsamples of the parent soil (PS) each weighing 100g were weighed into a clean 1-L beaker. Next, increasing volumes of diesel; 2, 5, 10and 20mL (*ca* 2, 5, 9 and 17% (v/w)) were added to each of the beaker containing the PS. The spiking pattern was adopted to achieve severe contamination because beyond 3%(v/w) concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth (Akpoveta *et al.*, 2011).The individual mixtures were thoroughly mixed to achieve an artificial contamination and left undisturbed for 48 hours to allow the volatilization of toxic components of the oil. This also served as the control. Total petroleum hydrocarbon was determined in each of the samples periodically i.e. (5, 10, 20, 30 and 45 days).

- *Effect of diesel concentration on TPH degradation in amended soil*

This design is composed of three (3) sets of four numbers of 1-L beakers/set. The same diesel concentration levels of 2, 5, 10and 20mL was maintained for each set of four beakers with each also having same amount of soil (100g). However, unlike the un-amended control samples described above, the 3 different set were respectively amended with 10g of PM, HS and UR. Each beaker was mixed thoroughly to obtain homogeneity, and consistently sprinkled with distilled water to maintain the soil moistened. The total petroleum hydrocarbon content of the samples was determined periodically as mentioned above.

- *Effect of amendment dose on TPH degradation in soil*

Three sets (a set/amendment) of five numbers of 1-L beakers per set were filled with diesel simulated soil at a constant soil: diesel ratio of 10:1 maintained for each of the beakers. A varying amount of each of the amendments/set (PM, HS and UR) were added at increasing rates of 5%, 10%, 20%, 30% and 40%w/w, corresponding to 5.3, 11.1, 25.0, 42.8 and 66.6g (each representing a beaker in a set). The mixtures were left to equilibrate for two weeks with regular sprinkling with distill water to keep them moistened, thereafter the TPH was determined. The blank (control) samples were also prepared following the same course, however without the amendments.

- *Effect of heavy metal toxicants on TPH degradation in amended soil*

The effect of heavy metals on TPH degradation in amended soil was monitored in the experimental set up hitherto. Three sets with five numbers of 1-L beaker per set was filled with diesel simulated soil (at constant soil-diesel 10:1). Each set was respectively treated with 20%w/w of the three amendments. The set up was made in triplicate to represent the three metals which were added in form of their nitrates in an increasing order 50, 100, 200, 350and 500 mg/kg corresponding to the five beakers in the sets. Each was thoroughly mixed and was left to equilibrate for 2 weeks, thereafter the TPH was determined. The blank (control) samples were prepared following the same procedure, but without the metals spiking.

E. Extraction of TPH in soil

The TPH in soil samples was extracted following the solvent extraction method of Adesodun and Mbagwu (2008). The method is briefly described herein. Soil samples (approximately 10 g) was taken from each microcosm and put into a 50-mL flask and 20mL of n-hexane was added. The mixture was shakenvigorously for 30 minutes to allow the hexane extract the oil from the soil sample. The suspension was then filtered using a What man filter paper (No 41) and the liquid phase extract (filtrate) diluted by taking 1mL of the extract into 50mL of hexane. The absorbance of this solution was measured spectro photometrically at a wavelength of 420nm with HACH DR/5000 Spectrophotometer using n-hexane as blank. The TPH in the soil was estimated with reference to the standard curve derived from diesel diluted with n-hexane.

F. UV- spectro photometric determination of TPH in soil

In the entire experiment, all values were expressed as the mean of two replicates. The calibration (standard) curve in figure 1(with the mathematical relationship y = 1.1943x+0.0115) was prepared from the plot of diesel concentration (volume of diesel in liters) and absorbance. Standard solutions of known diesel concentration was prepared by dilution of various volumes of diesel (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, and 0.07)L in a constant volume (0.1L) of *n*-hexane. The absorbance of these solutions read at the same 420nm in the spectrophotometer are in agreement with Beer-Lambert's Law which governs the amount of radiation absorbed and indicates that absorbance is directly proportional to concentration. Thus it can be observed that as the concentration of diesel dissolved in the n-hexane increases, the absorbance of the solution increased in a proportion manner.

The percentage total petroleum hydrocarbons (TPH %) in the samples prepared from the extraction procedure described above were deduced from the equation (1) below. The concentration (mole per liter) in the equation was derived from solving for x in the standard curve equation, where y is the absorbance value of each extracts.

$$TPH(\%) = \frac{\text{Concentration x 50 (dilution factor)}}{\text{Weight of soil (10 g)}} x100 \quad (1)$$

The percentage degraded component was deduced from the arithmetic; (100-%TPH in absorbed component)

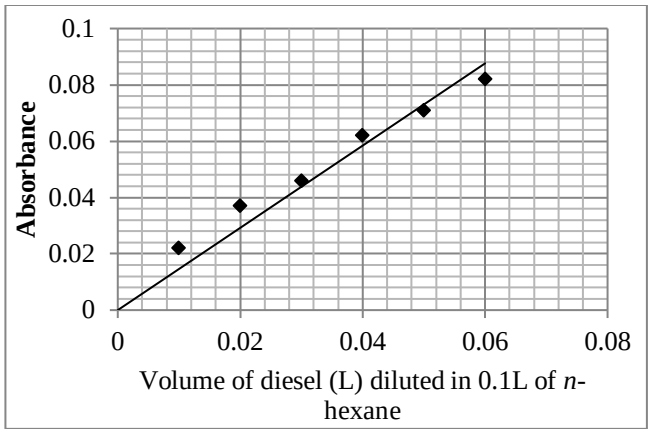

Fig 1:- Calibration curve for UV-visible spectrophotometric determination of total petroleum hydrocarbons using standard diesel-*n*-hexane solutions

III. RESULTS AND DISCUSSION

The result of physicochemical analysis of the parent soil sample and the amendments are presented in table 1. Table 2 and figures 2 present the result of influence of diesel load on TPH degradation under natural attenuation. Meanwhile, Pseudo-first order (ln TPH_t vs *t*) plots for natural attenuation of total petroleum hydrocarbons in soil contaminated with different diesel loadings is presented in figure 3. The results of the influence of increase in diesel loads under biostimulation with various organic amendments (human sludge, poultry manure and urea) are presented respectively in tables 3, 4 and 7 with graphical plots in figures 4, 7 and 10. Their degradation efficiencies are presented in figures 5, 8 and 11 respectively for human sludge, poultry manure and urea. The pseudo-first-order kinetic parameters for TPH degradation in diesel contaminated soil under natural attenuation and biostimulation is presented in table 6. The results generated from the investigation of effect of level of the various amendments are presented in table 7 and figure 13. The influences of the various heavy metals (Cu, Pb and Zn) concentrations on TPH degradation are generally presented in table 9 meanwhile, their graphical representations are presented in figures 14, 15 and 16 respectively.

Biodegradation kinetics: The biodegradation of total petroleum hydrocarbons is usually explained by pseudo-first order kinetics (Pala *et al*., 2006; Agarry *et al*., 2010b) typical model for which is given by:

$$TPH_t = TPH_o \exp^{-k_1 t} \quad (2)$$

where TPH_o is the initial TPH load in soil (%) and TPH_t is the residual TPH in soil (%) at time, *t* (day). k_1 is the biodegradation rate constant (day^{-1}). A semi-logarithmic plot of TPH_t versus *t* gives the slope as the rate constant.

Biodegradation Half-Life Times: The biodegradation half-life, $\tau_{½}$ (time taken for TPH to degrade by one-half of its initial concentration in soil) can be calculated using the equation 3 below:

$$\tau_{½} = \frac{0.693}{k_1} \quad (3)$$

Where *k* is the biodegradation rate constant (day^{-1}). The half life model is based on the assumption that the biodegradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil (Yeung *et a*l.,1997;Samuel and Lukuman, 2013).

Biodegradation half-lives are needed for many applications such as chemical screening, environmental fate modelling and describing the transformation of pollutants in environmental media.

TPH degradation efficiency

The TPH degrading efficiency, *DE* (%) of the amendment was calculated using the equation (Samuel and Lukuman, 2013).

$$DE(\%) = \left(\frac{TPH_u - TPH_a}{TPH_u}\right) x100 \quad (4)$$

Where$TPH_u$is the initial residual TPH (%) in the unamended soil and $TPH_a$is the residual TPH in the amended soil (%).

A. Effect of diesel concentration on TPH degradation in soil

Table 2. Residual and degraded total petroleum hydrocarbons, TPH (%) in unamended soil treated with different doses of diesel as a function of time

Diesel load	Residual TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	73.5	71.4	69.3	66.4	64.7
5	81.4	78.5	75.6	72.2	68.9
9	83.9	82.3	79.3	76.8	74.7
17	86.0	83.6	81.9	78.5	75.6
Diesel load	Degraded TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	26.5	28.6	30.7	33.6	35.3
5	18.6	21.5	24.4	27.8	31.1
9	16.1	17.7	20.7	23.2	25.3
17	14.0	16.5	18.1	21.5	24.4

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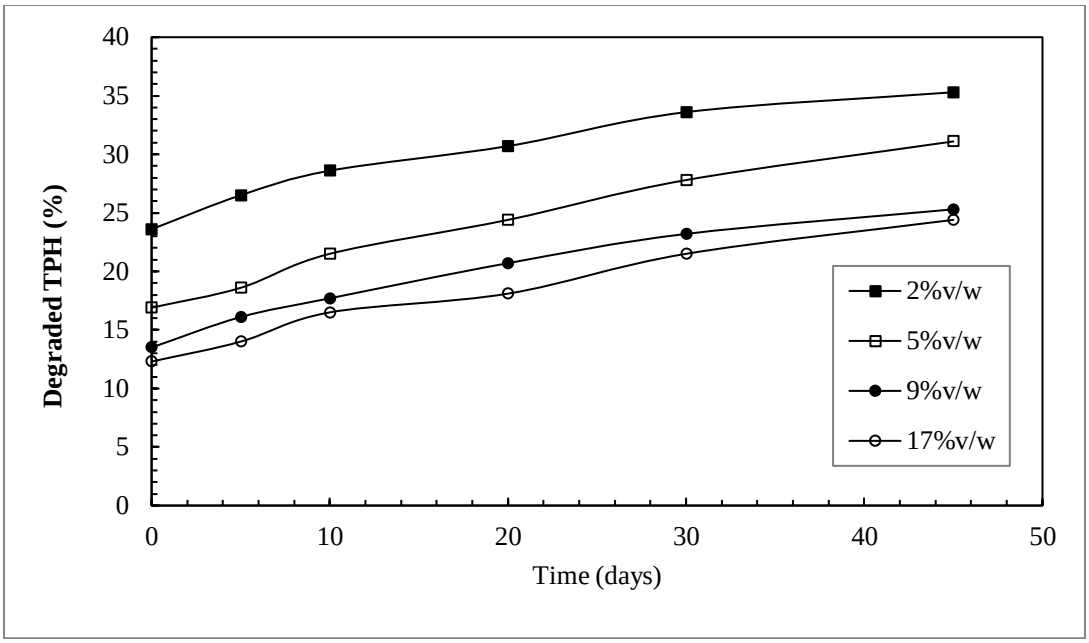

Fig 2:- Total petroleum hydrocarbons, TPH (%) degraded in soil contaminated with different diesel loadings under natural attenuation as a function of time.

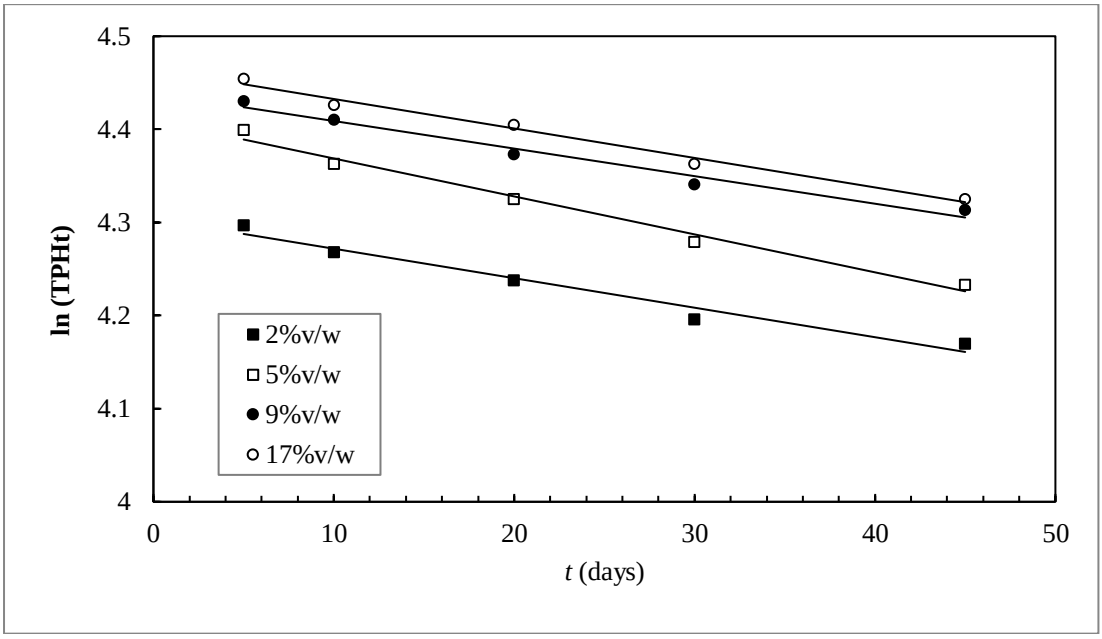

Fig 3:- Pseudo-first order (ln TPH_t vs t) plots for natural attenuation of total petroleum hydrocarbons in soil contaminated with different diesel loadings

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Table 3. Residual and degraded total petroleum hydrocarbons, TPH (%) in soil treated with different doses of diesel and 50%w/w sludge as a function of time

Diesel load	Residual TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	61.3	58.0	49.6	40.8	28.7
5	63.0	60.1	52.6	42.5	30.8
9	64.7	62.2	53.0	48.4	32.9
17	68.5	66.4	58.0	50.0	36.6
Diesel load	Degraded TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	38.7	42.0	50.4	59.3	71.3
5	37.0	39.9	47.4	57.5	69.2
9	35.3	37.8	47.0	51.6	67.1
17	31.5	33.6	42.0	50.0	63.4

Table 4. Residual and degraded total petroleum hydrocarbons, TPH (%) in soil treated with different doses of diesel and 50%w/w poultry manure as a function of time

Diesel load	Residual TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	52.5	47.9	40.8	25.3	19.5
5	54.6	49.6	43.8	29.1	23.2
9	57.6	52.5	46.3	32.9	25.3
17	60.5	55.9	48.4	35.4	29.1
Diesel load	Degraded TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	47.5	52.1	59.2	74.7	80.5
5	45.4	50.4	56.2	70.9	76.8
9	42.4	47.5	53.7	67.1	74.7
17	39.5	44.1	51.6	64.6	70.9

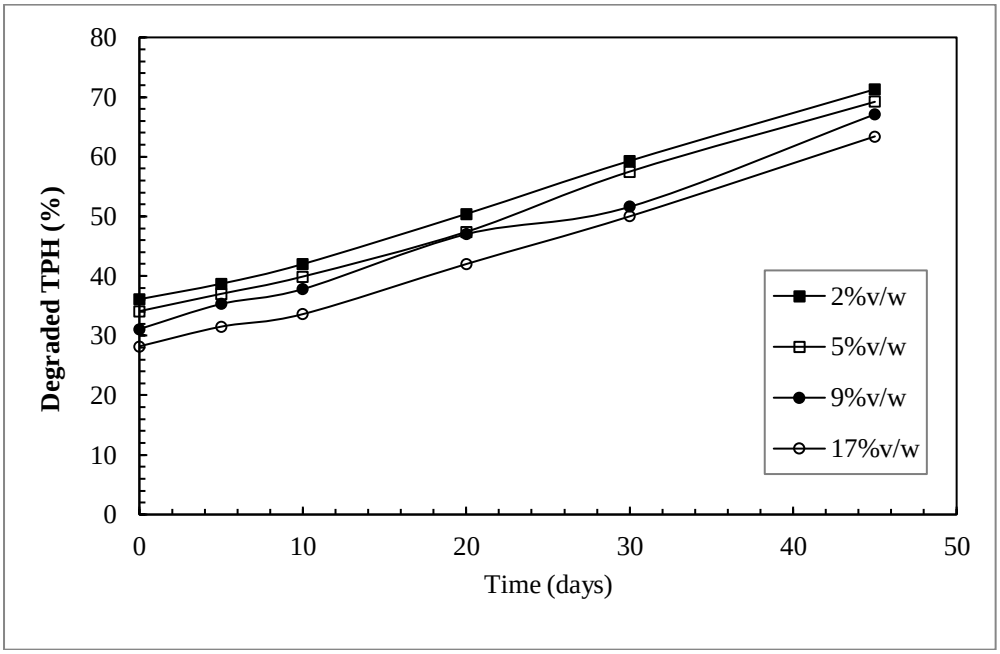

Fig 4:- Total petroleum hydrocarbons, TPH (%) degraded in soil contaminated with different diesel loadings and amended with 50% w/w human sludge

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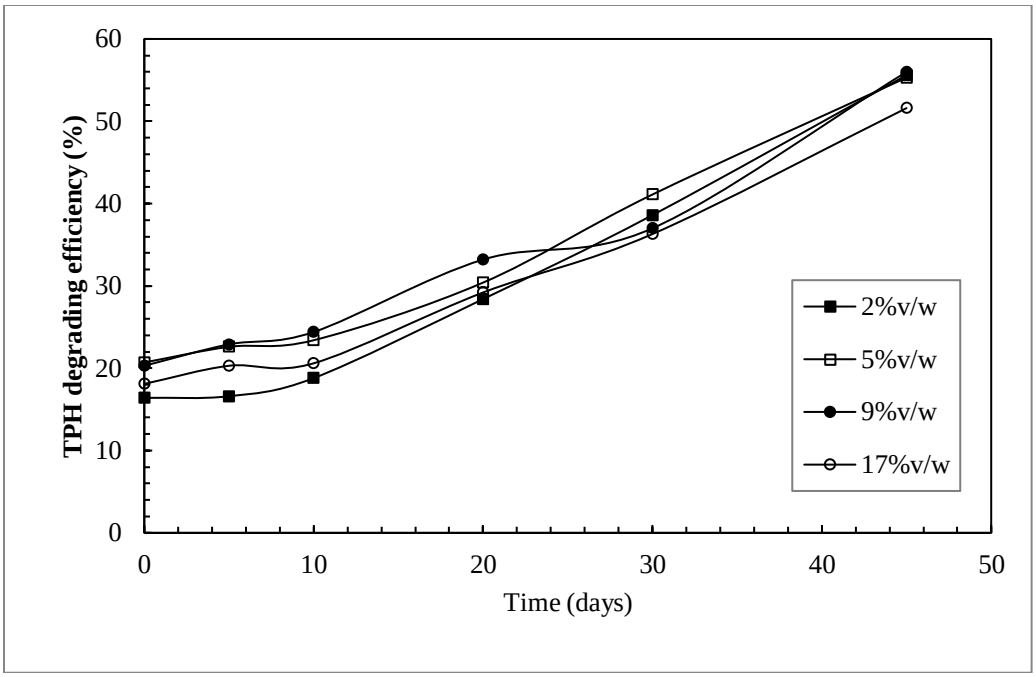

Fig 5:- TPH degrading efficiency of human sludge (50%w/w) in soil with different diesel loadings (2 – 17 %) as a function of time

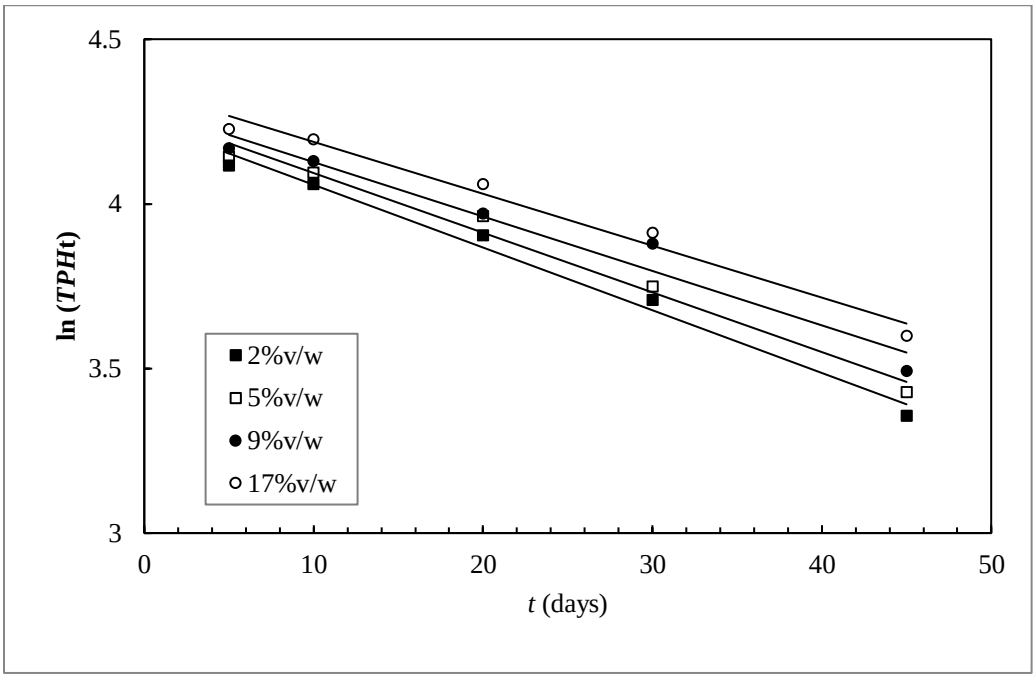

Fig 6:- Pseudo-first order ($\ln TPH_t$ vs t) plot for degradation of total petroleum hydrocarbonsin soil contaminated with different diesel loadings and amended with 50% w/w human sludge

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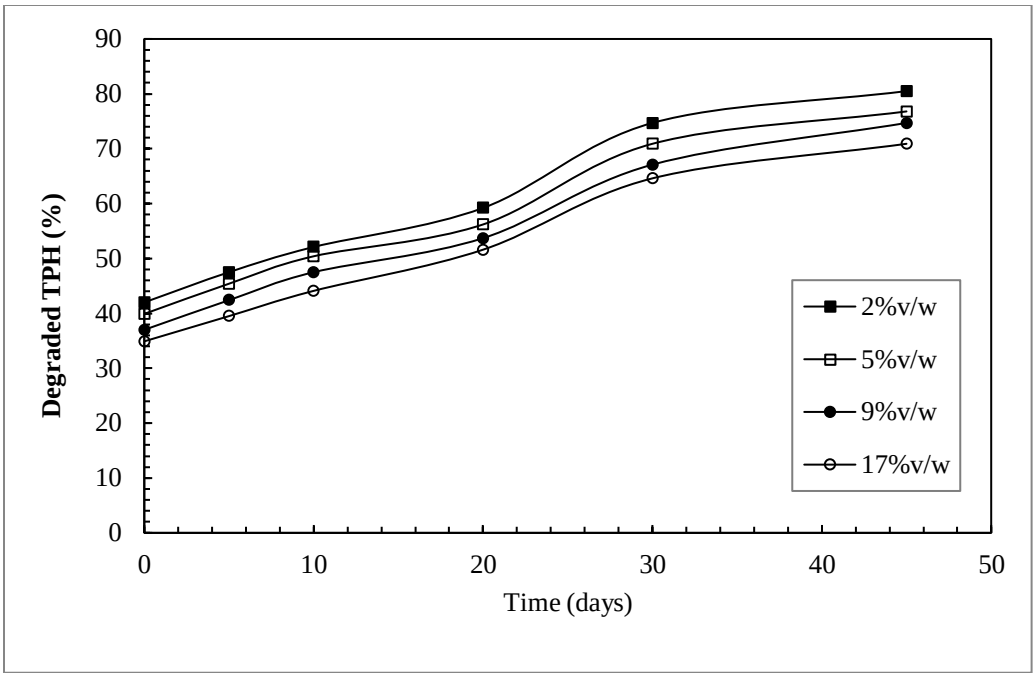

Fig 7:- Total petroleum hydrocarbons, TPH (%) degraded in soil contaminated with different diesel loadings and amended with 50% w/w poultry manure

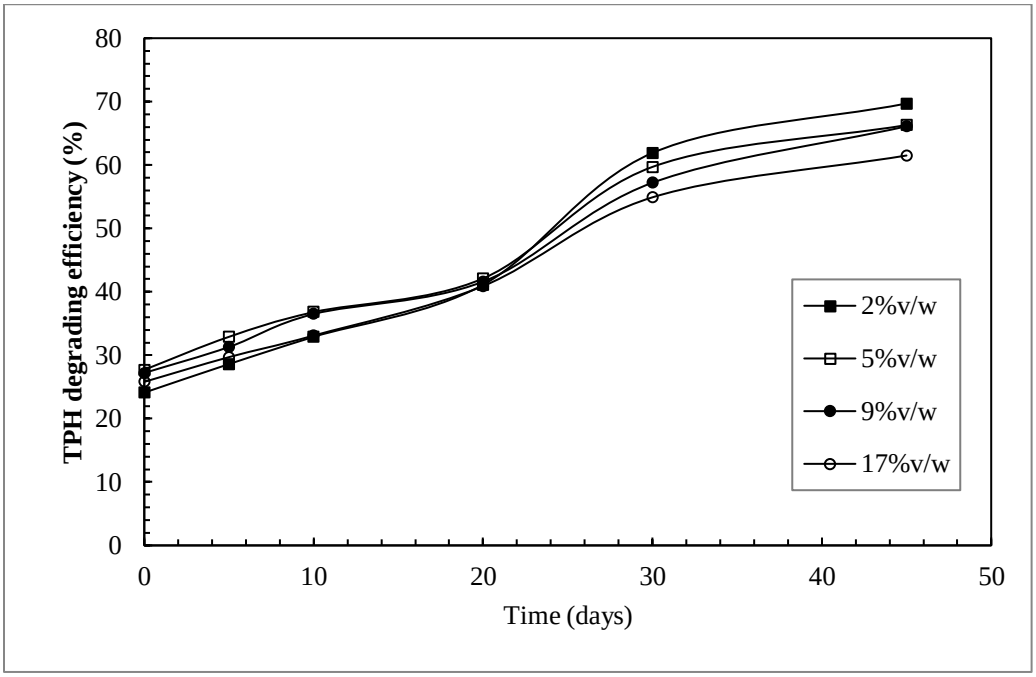

Fig 8:- TPH degrading efficiency of poultry manure (50%w/w) in soil with different diesel loadings (2 – 17 %) as a function of time.

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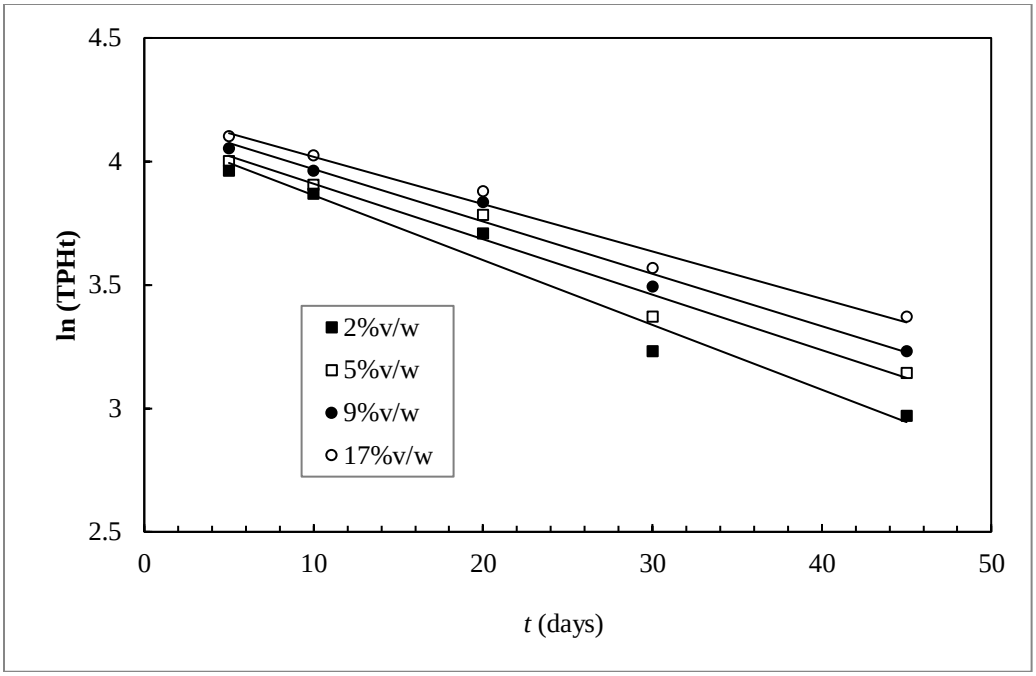

Fig 9:- Pseudo-first order (ln (TPH_t vs *t*) plot for degradation of total petroleum hydrocarbonsin soil contaminated with different diesel loadings and amended with 50% w/w poultry manure

Table 5. Residual and degraded total petroleum hydrocarbons, TPH (%) in soil treated with different doses of diesel and 50%w/w urea as a function of time

Diesel load	Residual TPH (%) after				
(%v/w)	5 days	10 days	20 days	30 days	45 days
2	62.6	60.1	49.6	41.7	31.6
5	63.0	61.8	50.9	44.2	32.5
9	67.2	63.0	53.4	47.1	34.1
17	69.0	65.9	56.3	49.6	36.2
Diesel load	Degraded TPH (%) after				
(%v/w)	0 day	5 days	10 days	20 days	30 days	45 days
2	37.4	39.9	50.4	55.8	67.6
5	37.0	38.2	49.1	58.3	68.4
9	31.0	37.0	46.6	52.9	65.9
17	32.8	34.1	43.7	50.4	63.8

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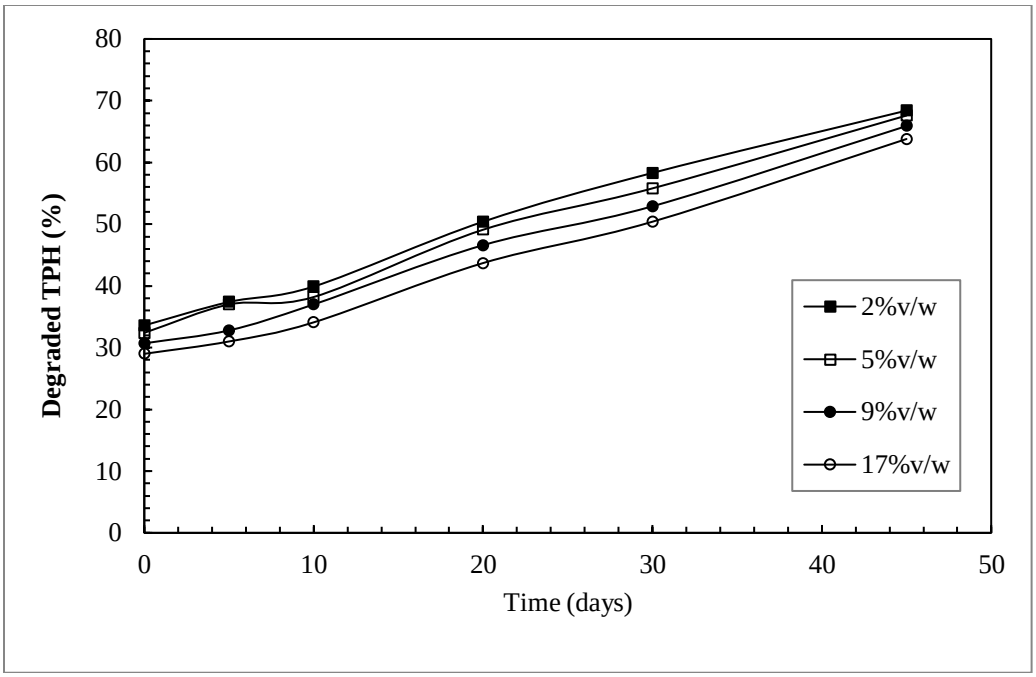

Fig 10:- Total petroleum hydrocarbons, TPH (%) degraded in soil contaminated with different diesel loadings and amended with 50% w/w urea

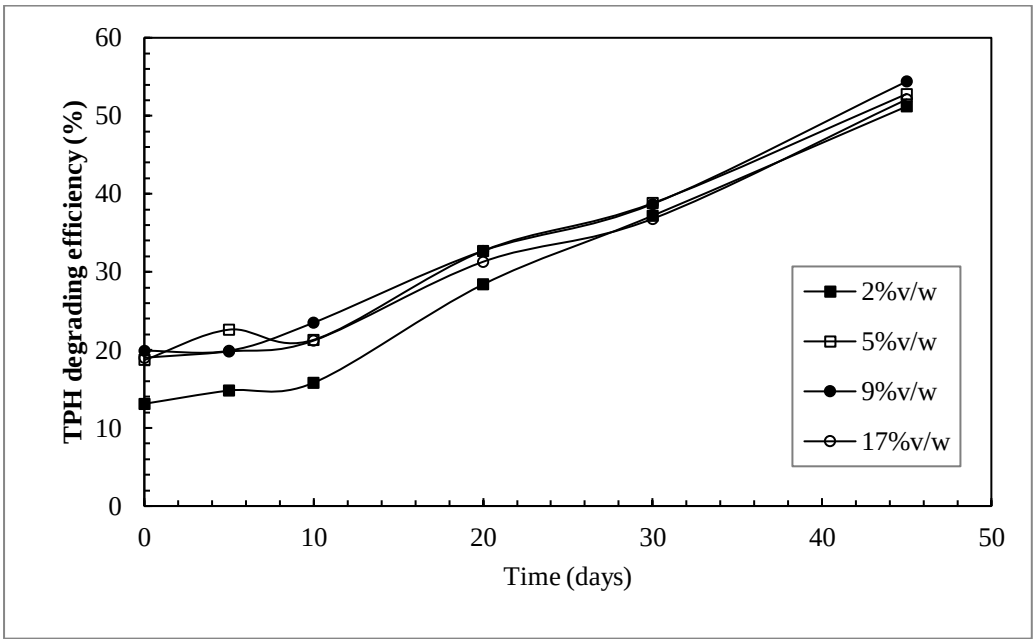

Fig 11:- TPH degrading efficiency of urea (50%w/w) in soil with different diesel loadings (2 – 17 %) as a function of time

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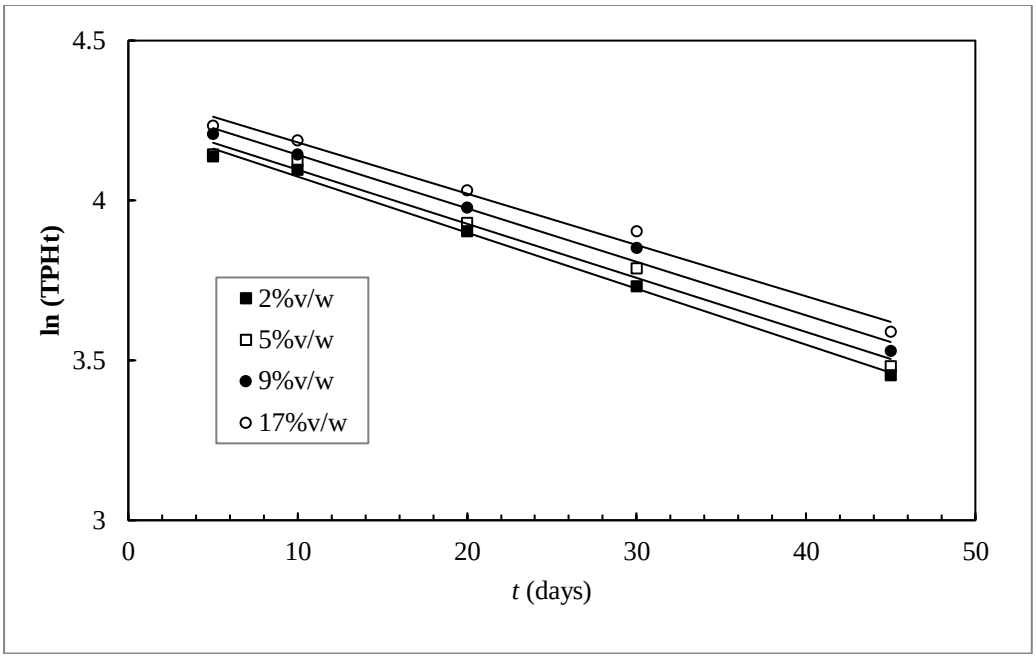

Fig 12:- Pseudo-first order (ln (TPH_t vs t) plots for degradation of total petroleum hydrocarbonsin soil contaminated with different diesel loadings and amended with 50% w/w urea

Table 6. The biodegradation rate constants (k_1) and half-life ($\tau_{½}$) time of diesel biodegradation in the various treatments under (natural attenuation and bio stimulation)

Amendment*	Parameter	Diesel load (%v/w)			
		2	5	9	17
Natural attenuation	$k_1x10^{-3}(day^{-1})$	3.0	4.0	3.0	3.0
	$\tau_{½}$ (day)	231.0	173.3	231.0	231.0
Poultry manure	$k_1x10^{-2}(day^{-1})$	2.6	2.2	2.1	1.9
	$\tau_{½}$ (day)	26.7	31.5	33.0	36.4
Human sludge	$k_1x10^{-2}(day^{-1})$	1.9	1.8	1.6	1.5
	$\tau_{½}$ (day)	36.4	38.5	43.3	46.2
Urea	$k_1x10^{-2}(day^{-1})$	1.7	1.6	1.6	1.6
	$\tau_{½}$ (day)	40.8	43.3	43.3	43.3

*Amendments added at 20%w/w of diesel contaminated soil and incubated for 0 – 45 day

First-order kinetics was used to determine the rate of biodegradation of diesel in the various treatments as shown in figures 3, 6, 9 and 12.The first-order degradation kinetics has been reported in many studies on petroleum degradation (Jørgensen *et al.*,2000;Kristin *et. al.*, 2003).

The biodegradation half-life, $\tau_{½}$ (day)and the biodegradation rate constant $k_1x10^{-3}(day^{-1})$ for the natural attenuation (unamended control) and the amended samples are presented in table 6. The rate constant for the unamended sample shows a slight deviation of 1 unit with the value of 4 at the 5th day of incubation against the value of 3 obtained for the rest periods of incubation. Similar deviations were observed for the amended samples. The rate constants $k_1x10^{-3}(day^{-1})$ for poultry manure amended at 2v/w contamination is 2.6, while the rate constant of 2.2 was obtained for 5%v/w diesel contamination, and at 9%v/w diesel contamination the rate constant of 2.1 was recorded, at 17%v/w diesel contamination the rate constant of 1.9 was obtained. The sample amended with human sludge had the rate constants of 1.9, 1.8, 1.6 and 1.5 at 2%, 5%, 9% and 17% v/w diesel contamination respectively. The rate constants for the sample amended with urea are 1.7 at 2%v/w diesel contamination, 1.6 at 5% contamination, 1.6 at 9% contamination, also 1.6 at 17% diesel contamination.

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The value of biodegradation half life ($\tau_{½}$ day) for the unamended soil; (231.0, 173.3, 321.0 and 231.0)which respectively obtained for the samples treated with 2v/w, 5%v/w, 9%v/w and 17%v/w of diesel were extremely high compare to those obtained from the amended soil samples. Soil sample amended with poultry manure at similar diesel concentration gave the biodegradation half life $\tau_{½}$ (day) of 26.7, 31.5, 33.0 and 36.4. Soil sample amended with human sludge gave biodegradation half life $\tau_{½}$ (day) of 36.1, 38.5, 43.3 and 46.2, whereas the sample treated with urea recorded the half life $\tau_{½}$ (day) of 40.8, 43.3, 43.3 and 43.3 respectively for same levels of diesel contamination (2v/w, 5%v/w, 9%v/w and 17%v/w).

The kinetic parameters (half life $\tau_{½}$ (day) showed that the reduction rate in the poultry amended sample was 7.5 times higher than in the unamended sample. The reduction rate in sample amended with human sludge was about 5.8 times higher than in the unamended, while the sample treated with urea was about 5.8 times higher.The high reduction rate observed in the sample treated with poultry manure could be attributed to biodegradation factors (nutrients, organic matter, organic carbon, abundance of microorganisms) impacted by the amendment. The bio waste (poultry manure) had organic matter content of 12.63, nitrogen content of 2.35%, phosphorus 0.71%, organic carbon content of 7.30, favourable pH of 6.87 and rich in microorganism (although not determined.

The increase in TPH (%) reduction in oil contaminated soil as a result of amendment with various organic wastes has been reported by various authors. (Gupta and Tao, 1996, Choi *et al.*, 2002; Delille *et al.*, 2004; Angarry *et al.*, 2010b; Abioye *et al.*, 2012). The period of incubation is also noticed to play a vital role in the remediation process. The highest TPH (%) reduction observed for the various amendments was recorded at the last date of incubation. Poultry manure with the highest THP (%) reduction recorded 80.5% after 45 day of incubation for 2%v/w diesel contamination. This is followed by the sample amended with human sludge which recorded 71.3%, then 68.4% for the sample treated with urea on the same incubation date and diesel load.

However, with increase in diesel load, reduction in TPH (%) degradation was notice with least value of 63.4% recorded at 17%v/w for the sample amended with human sludge at the 45th day of incubation which supports the conclusion of Schaefer and Juliane (2007) who stated that bioremediation is a useful method of soil remediation if pollutant concentrations are moderate. This was also explained using the TPH degrading efficiency charts for the various amendments. Poultry manure recorded the highest degrading efficiency value of 61.5% at highest diesel contamination of 17%v/w and had a record of 69.7% at lowest diesel contamination of 2%v/w. The degradation efficiency of human sludge at 17%v/w and 2%v/w diesel load were 51.6% and 55.6% respectively, whereas urea recorded 52.1% and 51.2%.This suggests that poultry manure must have provided a more bio-available alternate carbon substrate that ensured a vibrant microbial population that led to the increased diesel degradation, even though urea has more Nitrogen content. The result proves the efficiency of poultry manure in handling biodegradation process of site contaminated with diesel which has been demonstrated by various legends of bioremediation (Gupta and Toa, 1996, Williams *et al.*, 1986; Okolo *et al*,.2005; Akpoveta *et al.*, 2011).

The enhanced diesel oil degradation observed withthe addition of poultry manure, human sludge or urea indicates that the soil amendments interacted in enhancing diesel oil degradation. This is an indication that adequate time of incubation with the organic wastes is required for complete degradation of fields contaminated with petroleum products.

B. Effect of amendment level on TPH degradation in diesel contaminated soil

Table 7. Residual and degraded total petroleum hydrocarbons, TPH (%) in soil treated with 10%v/w diesel and different levels of amendments

Amendment	Level of amendment (%w/w)				
	5	10	20	30	40
	.....Residual TPH (%).....				
Human sludge	56.3	48.4	35.0	21.1	20.7
Poultry manure	51.7	40.8	24.9	11.9	11.1
Urea	60.1	55.1	44.6	37.1	35.8
	.....Degraded TPH (%).....				
Human sludge	43.7	51.6	65.0	78.9	79.3
Poultry manure	48.3	59.2	75.1	88.1	88.9
Urea	39.9	44.9	55.4	62.9	64.2

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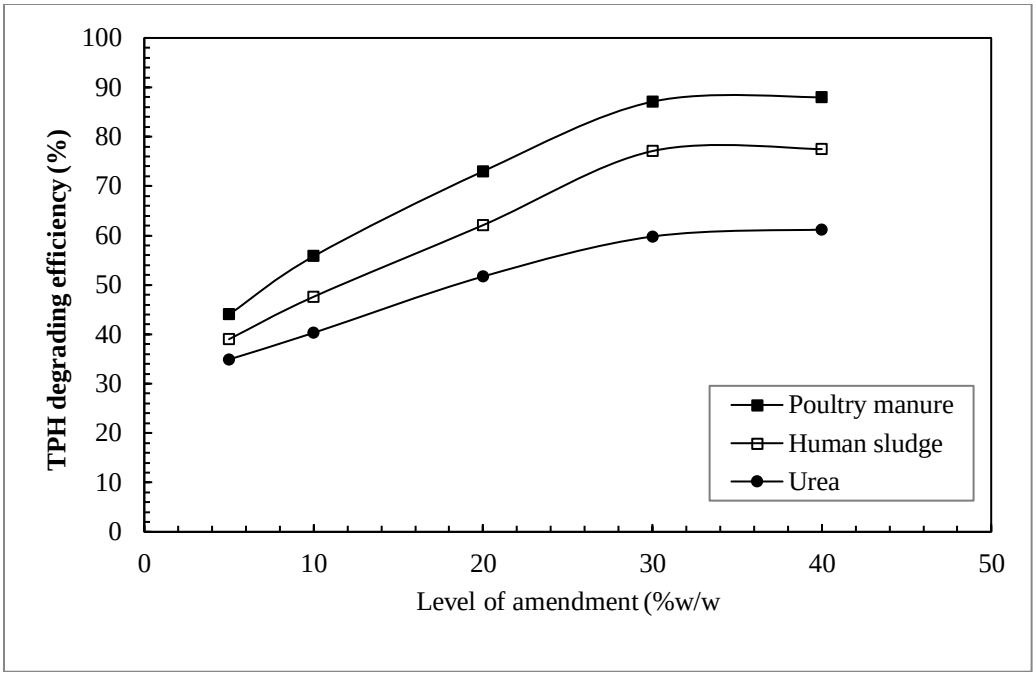

Fig 13:- TPH degrading efficiency of poultry manure, human sludge and urea in 10%v/w diesel contaminated soil at different levels of amendments after 14 days incubation

The result of the effect of amendment level on TPH degradation in diesel contaminated soil at 10%v/w diesel: soil ratio is presented in table 7 and the degradation efficiency of the various amendments presented in figure 13. There is a general increase in the value of TPH degraded in the samples amended with increasing value (5%w/w, 10%w/w, 20%w/w, 30%w/w and 40%w/w) of the various amendments. The highest value of 88.9% diesel reduction was observed in the sample amended with poultry manure at 40%w/w amendment, whereas the least value of 64.2% was recorded for the sample treated with urea at the same amendment level. More so, the degradation efficiency recorded 88% efficiency for poultry manure, 77.5% efficiency for human sludge and 61.2% for urea at the 40%w/w amendment level. The progressive degradation of diesel with increase in the various amendments could be attributed to the increase in the supply of nutrients to the microbial population in the contaminated soil, and this indicates that the soil amendments interacted in enhancing oil degradation (Okolo *et al.*,2005). Literature reveals that microbial degradation of organic matter added to soil depends on the interaction between the chemicals in the soil (Knaebel *et al.*, 1994).Therefore urea to an extent presumably inhibits the enzymes responsible for petroleum degradation in the soil samples collected from the back of student's hostel of University of Agriculture Makurdi. Urea may not be a good nutrient source for bioremediation of petroleum contaminants in soils with similar chemical and physical properties. Results from this study suggest that applications of urea in enhanced biodegradation may be site specific.

C. Effect of heavy metal toxicants on TPH degradation in amended soil

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Table 8. Residual and degraded total petroleum hydrocarbons, TPH (%)† in soil treated with 10 %v/w diesel, 20%w/w amendments and different doses of heavy metals

Amendment	Cu concentration (mg/kg)				
	50	100	200	350	500
	.....Residual TPH (%).....				
Human sludge	43.8	46.3	48.4	57.6	68.9
Poultry manure	41.7	44.6	48.4	52.5	65.1
Urea	45.4	47.9	50.0	57.6	70.5
	.....Degraded TPH (%).....				
Human sludge	56.2	53.7	51.6	42.4	31.4
Poultry manure	58.3	55.4	51.6	47.5	34.9
Urea	54.6	53.1	50.0	42.4	29.5
	Pb concentration (mg/kg)				
	50	100	200	350	500
	.....Residual TPH (%).....				
Human sludge	42.1	44.6	46.7	58.4	66.4
Poultry manure	35.4	36.6	45.0	55.5	61.3
Urea	32.9	33.7	42.5	53.0	58.0
	.....Degraded TPH (%).....				
Human sludge	57.9	55.4	53.3	41.6	33.6
Poultry manure	64.6	63.4	55.0	44.5	38.7
Urea	67.1	66.3	57.5	47.0	42.0

Table 9. Residual and degraded total petroleum hydrocarbons, TPH (%)† in soil treated with 10 %v/w diesel, 20%w/w amendments and different doses of heavy metals continue

Amendment	Zn concentration (mg/kg)				
	50	100	200	350	500
	.....Residual TPH (%).....				
Human sludge	40.0	41.7	45.9	49.2	54.6
Poultry manure	42.5	45.4	48.4	52.1	55.9
Urea	33.7	36.2	42.5	46.3	53.4
	.....Degraded TPH (%).....				
Human sludge	60.0	58.3	54.1	50.8	45.4
Poultry manure	57.5	54.6	51.6	47.9	44.1
Urea	66.3	63.8	57.5	53.7	46.6

†Determined after 2 weeks of equilibration

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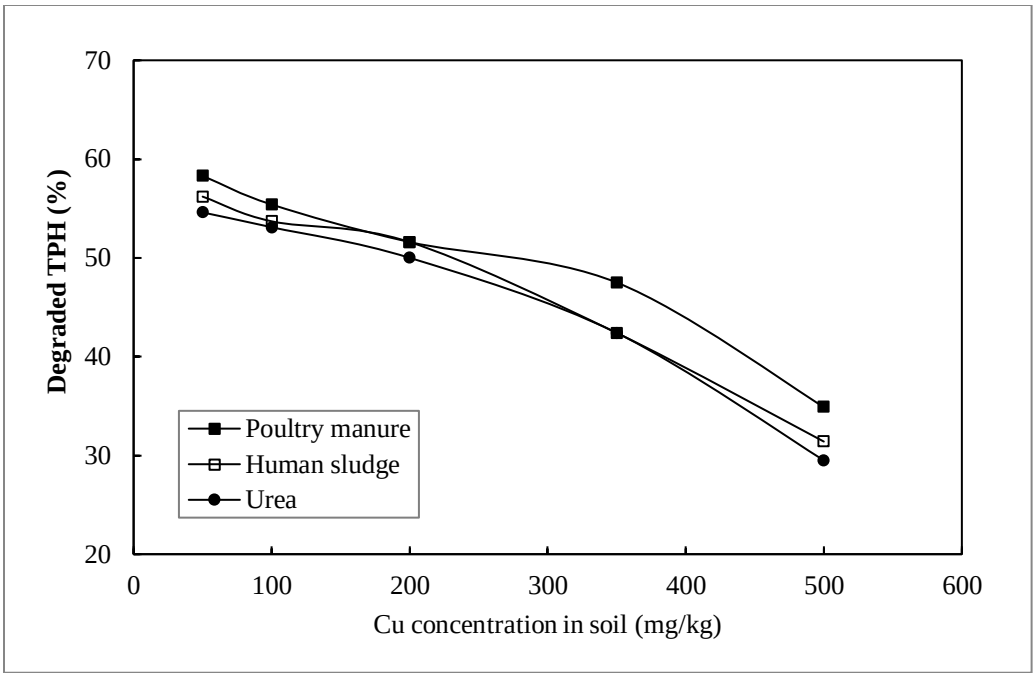

Fig 14:- TPH degraded by 20%w/w poultry manure, sewage sludge and urea in 10%v/w soil co-contaminated with 10%v/w diesel and different Cu concentrations after 14 days incubation

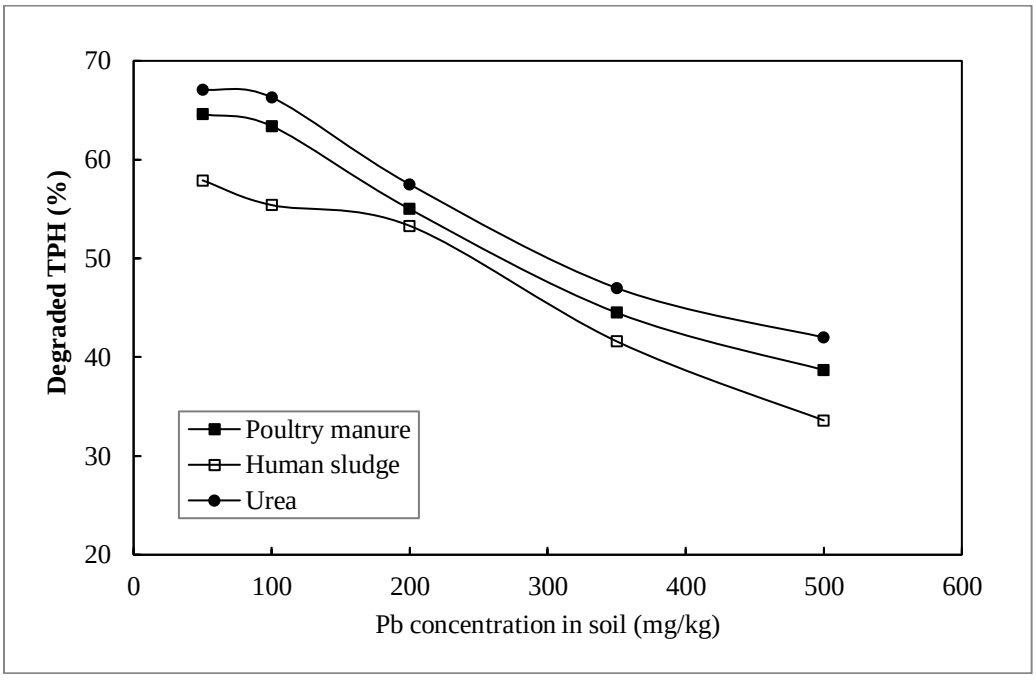

Fig 15:- TPH degraded by 20%w/w poultry manure, sewage sludge and urea in 10%v/w soil co-contaminated with 10%v/w diesel and different Pb concentrations after 14 days incubation

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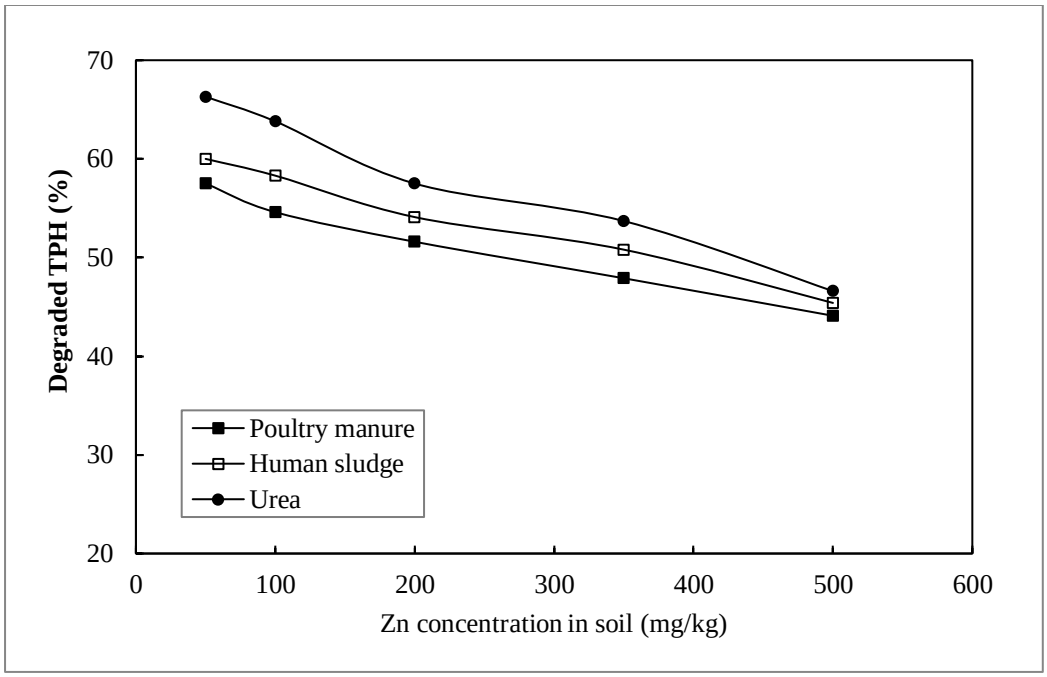

Fig 16:- TPH degraded by 20%w/w poultry manure, sewage sludge and urea in 10%v/w soil co-contaminated with 10%v/w diesel and different Zn concentrations after 14 days incubation

The influence of the various heavy metals (copper, lead and zinc) toxicants on TPH degradation on amendment with 20%w/w of each bio-wastes in 10%v/w diesel contaminated soil co-contaminated with different metal concentration ($50mgkg^{-1}$, $100mgkg^{-1}$, $200mgkg^{-1}$, $350mgkg^{-1}$ and $500mgkg^{-1}$) after 14 days incubation is presented in tables 9 and figures 14-16. The result reveals a general decrease in diesel reduction with increase in metal concentration. Amendment with the various organic wastes plays a vital role in ameliorating the negative influence of the metals on biodegradation of the contaminated soil. Meanwhile the efficacy of these organic amendments varies in the different metal phase.

The influence of copper in its nitrate ($Cu(NO_3)_2.5H_2O$) is presented in table 9 and figure 14. Copper is observed to exert the highest inhibitory influence on the biodegradation of diesel in soil. The maximum value of 58.3% was recorded for the sample amended with poultry manure at 50% copper load. At this same value of copper concentration, 56.2% diesel reduction was recorded for the sample amended with human sludge while 54.6% diesel reduction was recorded for the sample treated with urea. These values (above 50%) of TPH(%) degradation was obtained at lowest heavy metal (Cu) concentration of 50mg. With the increase in the concentration this metal, the value of TPH(%) degraded further depreciate. At the maximum Cu load of 500mg, 34.9% diesel reduction was recorded for the sample amended with poultry manure, 31.4% reduction obtained from the sample with human sludge biowaste, while 29.5% was recorded for urea amended sample.

The samples treated with the nitrate of lead $Pb(NO_3)_2$experienced similar decrease in diesel reduction after 14 days of incubation (Table 9 refers). The various organic amendments also show different degree of amelioration on the influence of Lead on diesel reduction in soil. 64.6% diesel reduction was recorded for the sample amended with 20%w/w poultry manure at 50mg Pb concentration. At this metal (Pb) concentration of 50mg, 57.9% degradation was recorded for the sample amended with 20%w/w human sludge, while the sample treated with 20%w/w urea gave 67.1% diesel reduction. The increase in Pb concentration also produced further depreciation of TPH (%) degraded as seen in figure 15. At the highest Pb concentration of 500mg, 38.7% diesel reduction was recorded for the sample amended with poultry manure, 33.6% recorded for the sample containing human sludge, while 42% diesel reduction was obtained for the sample amended with urea. In this situation, urea is noticed to provide a better condition for amendment which could be attributed to its high Nitrogen (nutrient) content and interaction with Pb.

The results of the samples spiked with various load of zinc nitrate ($Zn(NO_3)_2$) is presented in the table 9 and figure 16. Similar variations existed between the metal (Zn) concentration and the value of TPH (%) degraded, as seen in the figure. 57.5% diesel reduction was recorded for the 10% diesel contaminated sample amended with 20%w/w poultry manure at the lowest ($50mgkg^{-1}$) Zn concentration. 60% reduction was obtained for the sample treated with human sludge amendment at this level of Zn contamination and 66.3% reduction recorded at for urea amended sub-sample.

The highest value (46.6%) of TPH (%) degraded recorded at the highest Zn contamination was observed for the sample amended with urea, showing that urea provided better condition for biodegradation of diesel contaminated soil co-contaminated with Zn. Meanwhile 45.4% diesel reduction was recorded for the sample amended with human sludge at the 500mg Zn concentration and 44.1% reduction obtained for the sample treated with poultry manure at this level of Zn contamination.

The general decrease in the TPH(%) degraded with increase in metal concentration could be attributed to the toxic impacts of the heavy metals on the indigenous microorganisms. The finding is in agreement with the study of Ademola *et al*. (2013), who reported that at higher concentrations, these heavy metal ions form unspecific complex compounds within the cell of the microorganisms, which leads to toxic effects, making them too dangerous for any physiological function. In addition, at high levels of heavy metals, both essential (Cu, Zn) and nonessential (Pb) metals produces serious toxic effects on the microorganism; damage of cell membranes, alter enzyme specificity, disrupt cellular functions, and damage the structure of DNA (Ruins *et al*., 2000;Volker *et al*., 2002) investigated the degradation of diesel fuel by a microbial community from a soil polluted by heavy metals in the presence of Cu, Ni, Zn, Pb, Cd, Hg and Cr. From their study, the degradation of TPH was increasingly inhibited by higher metal concentration with the order of toxicity Hg>Cr(VI)>Cu>Cd>Ni>Pb>Zn. The result of this present study however shows that the samples treated with copper salt recorded lower TPH degradation than those treated with the salts of lead and zinc. Therefore this is in agreement with the findings of Volker and his colleagues.

IV. CONCLUSION

The enhancing of total petroleum hydrocarbon degradation in diesel contaminated soil is achieved with the addition of organic amendments (poultry manure, human sludge and urea). The extent of this depended on the degree of diesel oil contamination and the level of amendments added at time enough for adequate incubation. The results of the experiments on soil (100g) contaminated with 2%v/w, 5%v/w, 10%v/w and 20%v/w of diesel, amended with constant dose (50%) of each of poultry manure, human sludge and urea showed varying degree of increase in TPH (%) degradation with time of incubation compared to the un-amended soil samples. However, there is a general reduction in the value of TPH (%) degraded with increase in diesel load, but with increase in the level of amendment and time of incubation, the situation is ameliorated. This indicates that, the more a site is polluted, the longer the time it would require, and increased amendment dose in order to return the soil back to its original state.

The presence of heavy metals in the diesel contaminated soil distorts the bioremediation process by their toxic effects (imposing oxidative stress) on the microorganisms which influenced the biodegradation process.

The heavy metals may inhibit diesel pollutant biodegradation through the interaction with enzymes directly involved in biodegradation or those involved in general metabolism. Most commonly, reports on metal inhibition of bioremediation have been related to the total concentration of a metal in the system (Ademola *et al.*,2013). It has therefore been established that with increase in heavy metal concentration in diesel contaminated soil, the extent of diesel degradation is drastically reduced. Therefore, to achieve optimum degradation, thereby solving the problem of diesel contamination of soil co-contaminated with the heavy metals copper, lead and zinc or other metals, treatment with certain pH buffers which are able to complex and precipitate metals is suggested, though not experimented. Examples of such buffers are; phosphate (the most commonly used buffer) - well known for its ability to precipitate metals and reduces their bioavailability (Hugea and Poole, 1991), via formation of insoluble metal phosphate species, even at neutral and mild acidity pH values. Other buffers which are also used in microbiological media include zwitterionic buffers (examples; 4-2-hydroxyethyl-1-piperazine-ethanesulfonic acid, 2-4-morpholino-ethanesulfonic acid) and many more (Ademola *et al.*,2013).

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