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Dynamics of Chemical and Biological Properties of Organically Amended Petroleum Hydrocarbon Polluted Soil as Affected by Incubation Periods

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Abstract

A study was conducted in the Screen house of the Department of Crop, Soil and Pest Management, FUTA Akure to examine the time dynamic trends in soil chemical properties as affected by contamination with petroleum hydrocarbon compounds (PHC) in a 6 months incubation period as affected by organic amendments. The petroleum hydrocarbon compounds used were spent engine oil (SEO), bitumen (BIT) and crude oil (CRO). The organic amendments used were poultry manure and mycorrhizal fungi (Glomus mossae) spores. The respective petroleum hydrocarbon compounds (spent engine oil SEO, bitumen BIT and crude oil CRO) were applied at 2 rates of 0 and 455 ml per pot and 3 levels of organic amendments followed by incubation for six (6) months. Soil samples were collected from each treatment at 0, 2, 4 and 6 months after treatment application for the determination of some chemical properties (pH, organic carbon, N, P, K, Ca and Mg). The time changes in elemental concentrations of soil contamination with petroleum hydrocarbon compounds (SEO, BIT and CRO) showed declining trends in the values of pH, organic carbon and Mg while N-Total, Ca and available P and K rose with time. In general, the nature of the trends (polynomial function as best fit) indicated that the observed responses were not uni-function of a factor but suggest interaction among factors responsible for the breakdown of petroleum hydrocarbon compounds and hence its toxicity to soil chemical properties. The time course of changes in soil chemical properties as affected by petroleum hydrocarbon compounds (PHCs) contamination in soil differed among the PHCs evaluated. The values of the measured soil parameters recorded at the commencement of the experiment and at 2, 4, 6 months after the application of the petroleum hydrocarbon compounds showed declining trends in the values of pH, organic carbon and Mg were obtained. Increasing trends in N-total and available P with time up till the end of the experiment while SEO recording the highest values at 6 months of incubation. Trends of the mean values (highest and lowest values) with respect to treatment showed that CRO had the highest soil pH at 2 and SEO had the lowest at 6 month. SOC increased across treatments with SEO recording the highest value at 2 months of incubation, which was followed by general decline in values with time across the PHCs. The results indicated that PHCs adversely affect soil chemical properties and that addition of organic substances improved chemical properties of the soil following pollution with petroleum hydrocarbon compounds compared with the un-amended soils. Poultry manure in particular performed better compared with mycorrhizal inoculum with respect to improvement of soil chemical properties.

Keywords: Petroleum hydrocarbon compounds; Environment; Toxic; Remediation

Introduction

Contamination of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is a widespread environmental problem worldwide. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [1,2]. Environmental pollution by heavy metals (Lead, chromium, arsenic, nickel, cadmium etc.) which are released into the environment through various anthropogenic activities such as mining, energy and crude oil production and utilization, electroplating, wastewater sludge treatment and agriculture is one of the world's major environmental problem.

Oil Spillage is the release of a liquid petroleum hydrocarbon, its derivatives into the environment, especially soil, and water bodies due to human activities and is a form of pollution. Oil spillage has a major impact on the ecosystem. Large tracts of the mangrove forest, which are especially susceptible to oil (that is mainly because it is stored in the soil and re-released annually with inundation) have been destroyed. Spills take out crops and aquaculture through contamination of the groundwater and soils. Oil pollution prevents normal oxygen exchange between soil and the atmosphere due to hydrophobic properties of oil [3]. Drinking water is also frequently contaminated and sheen of oil is visible in many localised bodies of water. If the drinking water is contaminated, even if no immediate health effects are apparent, the numerous hydrocarbons and chemicals present in oil represent a carcinogenic risk. Some studies have been carried out on the effects of crude oil pollution on seed germination, plants and soil. For example, Ogboghodo et al. [4] reported that crude oil inhibited the germination and growth of maize at high pollution levels while the growth of okro and fluted pumpkin seedlings were highly reduced with an increase in crude oil concentrations [5]. The response of plants to oil contamination depends upon the level of contamination. While crop leaves wither gradually under slight contaminations, heavy contamination results in total shedding of leaves. In both conditions, total crop failure occurs. Soils polluted with crude oil may remain unsuitable for crop growth for months, even years depending on the level of pollution until the oil is degraded to a tolerable level [6].

Earlier studies carried out by Olajire et al. on qualitative and quantitative analyses of Polycyclic Aromatic Hydrocarbons (PAHs) in the river and soil in Agbabu, showed an elevated Aliphatic and PAHs levels in the water and soils. In Nigeria, although the full exploration of the bitumen is yet to take off, seepage of the material exist, especially during the dry season when temperature is above 37°C when it occurs as a free-flowing liquid.

Mechanical method to reduce hydrocarbon pollution is expensive and time consuming. The cheap, effective and safe method for reducing hydrocarbon pollution could possibly be done through microbial degradation. Biodegradation of complex hydrocarbon usually requires the cooperation of more than a single microorganism species. This is particularly true in pollutants that are made up of many different compounds such as petroleum compounds. Microbes are the main degraders of petroleum hydrocarbon contaminated ecosystems. Bioremediation has become an alternative way of remediation of oil polluted sites, where the addition of specific microorganisms (bacteria, cyanobacteria, algae, fungi, protozoa) or enhancement of microorganisms already present can improve biodegradation efficiency in both in situ or ex situ procedures [7].

Concentration of petroleum hydrocarbon determines to a great extent, the rate of their breakdown within the soil environment. High concentration of hydrocarbon inhibits microorganisms, and the concentration at which inhibition occurs varies with the compound. Report of Ijah and Antai [8] showed high degradation of hydrocarbons in soil contaminated with 10% and 20% crude oil compared to those contaminated with 30% and 40% crude oil, which experienced partial degradation of hydrocarbon within a period of 12 months. The contamination by petroleum hydrocarbon compound is generally expected to reduce the biodiversity of the soil micro biota [9]. A probable explanation for this is that the addition of large amounts of hydrocarbons selects for a limited number of fast-growing hydrocarbon degraders, which are enriched in the soil environment under these conditions. Processing and distribution of petroleum hydrocarbon as well as the use of petroleum products are the main cause of soil contamination [10]. Changes in soil properties due to contamination with petroleum derived substances e.g., diesel, petrol, lubricating oil can lead to water and oxygen deficit as well as shortage to available forms of nitrogen and phosphorous [11].

Amaranthus species from which (*A. hybridus*) a leafy and nutritious vegetable is a member of the family Amaranthaceae. Amaranthus has the ability to bio-accumulate hydrocarbons [12]. The existing mode of indiscriminate disposal of waste oil increases pollution incidents in the environment. Odjegba and Idowu [13] reported that germination of *Amaranthus hybridus* seeds was significantly affected in spent engine oil polluted soil, while seedlings growth, chlorophyll and protein contents were seriously affected [14]. The specific objectives of the study are to examine the effect of petroleum hydrocarbon compounds (crude oil, bitumen and spent engine oil) contamination and soil amendment with poultry manure and mycorrhizal inoculum the temporal trend of the toxicity of petroleum hydrocarbon compounds (crude oil, bitumen and spent engine oil) on soil properties.

Material and Methods

An experiment was conducted in the screen house of the Department of Crop, Soil and Pest Management Federal University of Technology Akure. Buckets were filled with 4 kg top soil collected from the Teaching and Research farm of the Federal University of Technology Akure.

Experimental design and treatments application

The temporal trends in soil chemical properties as affected by contamination with petroleum hydrocarbon compounds (PHC) in a 6 (0, 2, 4, 6) months incubation period. For the respective petroleum hydrocarbon compounds (spent engine oil, bitumen and crude oil), treatments consisted of 2 rates of application 0 and 455 ml per pot, 3 levels of organic amendments and 6 months incubation period arranged in a CRD with 3 replications.

The factors are 2 rates of PHC application (0 ml and 455 ml) and 3 levels of organic amendments (unamended control, (ii) mycorrhizal inoculation (10 g/pot=2.5 t/ha), (iii) poultry manure application (40 g/pot=40 t/ha) and (iv) 0, 2, 4 and 6 months of incubation. The treatment combinations for the second experiment were:

$T_0P_0A_0$: O month+0 ml SEO+0 t/ha PM

$T_0P_0A_1$: O month+0 ml SEO+10 t/ha PM

$T_0P_0A_2$: O month+0 ml SEO+2.5 t/ha MI

$T_2P_1A_0$: 2 month+455 ml SEO+0 t/ha PM

$T_2P_1A_1$: 2 month+455 ml SEO+10 t/ha PM

$T_2P_1A_2$: 2 month+455 ml SEO+2.5 t/ha MI

$T_4P_1A_0$: 4 month+455 ml SEO+0 t/ha PM

$T_4P_1A_1$: 4 month+455 ml SEO+10 t/ha PM

$T_4P_1A_2$: 4month+455 ml SEO+2.5 t/ha MI

$T_6P_1A_0$: 6 month+455 ml SEO+0 t/ha PM

$T_6P_1A_1$: 6 month+455 ml SEO+10 t/ha PM

$T_6P_1A_2$: 6month+455 ml SEO+2.5 t/ha MI

$T_0P_0A_0$: O month+0 ml BIT+0 t/ha PM

$T_0P_0A_1$: O month+0 ml BIT+10 t/ha PM

$T_0P_0A_2$: O month+0 ml BIT+2.5 t/ha MI

$T_2P_1A_0$: 2 month+455 ml BIT+0 t/ha PM

$T_2P_1A_1$: 2 month+455 ml BIT+10 t/ha PM

$T_2P_1A_2$: 2 month+455 ml BIT+2.5 t/ha MI

$T_4P_1A_0$: 4 month+455 ml BIT+0 t/ha PM

$T_4P_1A_1$: 4 month+455 ml BIT+10 t/ha PM

$T_4P_1A_2$: 4month+455 ml BIT+2.5 t/ha MI

$T_6P_1A_0$: 6 month+455 ml BIT+0 t/ha PM

$T_6P_1A_1$: 6 month+455 ml BIT+10 t/ha PM

$T_6P_1A_2$: 6month+455 ml BIT+2.5 t/ha MI

$T_0P_0A_0$: O month+0 ml CRO+0 t/ha PM

$T_0P_0A_1$: O month+0 ml CRO+10 t/ha PM

$T_0P_0A_2$: O month+0 ml CRO+2.5 t/ha MI

$T_2P_1A_0$: 2 month+455 ml CRO+0 t/ha PM

$T_2P_1A_1$: 2 month+455 ml CRO+10 t/ha PM

$T_2P_1A_2$: 2 month+455 ml CRO+2.5 t/ha MI

$T_4P_1A_0$: 4 month+455 ml CRO+0 t/ha PM

$T_4P_1A_1$: 4 month+455 ml CRO+10 t/ha PM

$T_4P_1A_2$: 4month+455 ml CRO+2.5 t/ha MI

$T_6P_1A_0$: 6 month+455 ml CRO+0 t/ha PM

$T_6P_1A_1$: 6 month+455 ml CRO+10 t/ha PM

$T_6P_1A_2$: 6month+455 ml CRO+2.5 t/ha MI

(where P: PHC (Spent engine oil SEO, Bitumen BIT and crude oil CRO), T=0, 2, 4 and 6 periods (months) of incubation, A: Amendment: PM: Poultry Manure; MI: Mycorrhizal Inoculation)

The petroleum hydrocarbon compounds (PHC) were separately obtained from a mechanic workshop (spent engine oil), Ondo State Asphalt company Akure (Bitumen) and NNPC Headquarters, Port Harcourt, Rivers State (Crude oil). The partially decomposed poultry manure from the livestock unit of the Teaching and Research Farm, FUTA and the mycorrhiza culture (inoculum) from IITA, Ibadan, Nigeria. Soil samples were collected before treatment application and at crop harvest. The soil samples were analysed to determine physical (sand, silt and clay contents), chemical (pH, organic matter, N. P, K, Ca, Mg,) and the heavy metal content (Zinc, Copper, Chromium, Lead and Manganese) in soil and plant tissue. Data were also collected on agronomic characters of *Amaranthus hybridus* such as root weight, stem/leaf weight and head weight.

Determination of the selected soil physical properties (soil textural class)

The soil texture was determined using part of the soil samples collected for the experiment; this was set aside before the contamination of the remaining part. The soil was air-dried to reduce the moisture content after which it was taken to the laboratory where the soil texture class was determined as sandy-loam as defined according to FAO/USDA soil classification system. Soil pH was determined by using 1:1 water suspension by adding 10 ml distilled water to 10 g of soil. The suspension was stirred continuously for 30 minutes allowed to stand for 20 minutes. After calibrating the pH meter with buffer solutions of pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension. Organic carbon was determined by a modified Walkley–Black wet oxidation method. Soil organic matter of the soil sample was calculated by multiplying the percent organic carbon by a Van Bemmelen factor of 1.724. Potassium and sodium in the soil extract were determined by flame photometry. Standard solutions of 0, 2, 4, 6, 8 and 10 ppm K and Na were prepared by diluting appropriate volumes of 100 ppm K and Na solution to 100 ml in volumetric flask using distilled water. Flame photometer readings for the standard solutions were determined and a standard curve constructed. Potassium and sodium concentrations in the soil extract were read from the standard curve. Available P was determined using the Bray P1 method. The method is based on the production of a blue complex of molybdate and orthophosphate in an acid solution. A standard curve was constructed using the readings. The concentration of P in the extract was obtained by comparison of the results with a standard curve. For the determination of calcium, a 10 ml portion of the extract was transferred into an Erlenmeyer flask. To this, 10 ml of potassium hydroxide solution was added followed by 1 ml of triethanolamine. Few drops of potassium cyanide solution and few crystals of cal-red indicator were then added. The mixture was titrated with 0.01 M EDTA (ethylene diamine tetraacetic acid) solution from a red to a blue end point. The exchangeable magnesium alone was calculated by subtracting the value obtained from calcium alone from the calcium+magnesium value.

Data analysis

Data collected on soil properties and Amaranthus growth were subjected to analysis of variance (ANOVA) test while the significant treatment means were separated using Duncan multiple range test at 5% probability level ($P<0.05$).

Result

Effect of application rates of petroleum hydrocarbon compounds (SEO, BIT, CRO), organic amendments and incubation periods on soil chemical properties

The results of the effect of application rates of spent engine oil, Bitumen and Crude oil on soil chemical properties are presented (Tables 1-4). Among the PHCs, significant differences which were observed for SOC and N-total were not found for soil pH, P, K, Ca and Mg. Spent engine oil recorded the highest pH, SOC, N-Total, K, Ca, and Mg among the petroleum hydrocarbon compounds considered (Table 1). The values of pH, SOC, N-Total, P, K, Ca and Mg were enhanced following organic amendments with 10 t/ha poultry manure compared with 2.5 t/ha MI and the control (Table 2). The values of pH kept on fluctuating up to the 6th month of incubation periods. At two months of incubation, SOC was at its peak and later decreased from the 4^{th} to the 6^{th} month of incubation. However, increasing trends in the values of N-Total, P and Ca from 0 to 6^{th} month of incubation was observed. Potassium (K) and Mg decreased at 2 month, followed by increases in their values from the 4th to 6th month of incubation (Table 3). Soil application of 455 ml produced higher values of pH (6.04 compared to the control with 5.28), soil organic carbon (1.36 compared to the control with 2.71), and N-Total (1.36 compared to the control with 0.45) while P, K and Mg were not significantly different (Table 4).

PHC	pH (H_2O)	OC (%)	N_{TOT} (%)	P(mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
SEO	5.93a	2.79a	0.62a	0.57a	0.76a	4.89a	2.51a
BIT	5.81b	2.11b	0.57a	0.59a	0.73a	4.29c	2.11b
CRO	6.10a	2.17b	0.58a	0.58a	0.73a	4.51b	2.17b

Table 1: Effects of contamination of petroleum hydrocarbon compounds on soil chemical properties. Means with the same letters along the column are not significantly different at $P<0.05$ according to Duncan Multiple Range Test.

Manure Type (t/ha)	pH (H_2O)	OC (%)	N_{TOT} (%)	P(mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)

0	5.58a	1.98b	0.54b	0.38b	0.78a	4.19b	1.90b
10:00 PM	6.04b	2.55a	0.63a	0.69a	0.82a	4.89a	2.52a
2.5 AMF	6.02b	2.54a	0.61a	0.68a	0.62b	4.61a	2.36a

Table 2: Effects of organic amendments on chemical properties of soil contaminated with PHC. Means with the same letters along the column are not significantly different (P<0.05: Duncan Multiple Range Test).

Incubation Period (mths)	pH (H_2O)	OC (%)	N_{TOT} (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
0	5.85a	2.54b	0.38d	0.44c	0.84b	4.15c	2.46a
2	5.94a	2.94a	0.51c	0.49c	0.66c	4.27c	2.42a
4	5.86a	2.16c	0.66b	0.57b	0.47d	4.64b	1.89c
6	5.87a	1.83d	0.84a	0.85a	1.04a	5.26a	

Table 3: Effects of incubation periods on chemical properties of soil contaminated with SEO, BIT and CRO. Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test

Application Rate (ml)	pH (H_2O)	OC (%)	N_{TOT} (%)	P(mg/kg)	K(cmol/kg)	Ca (cmol/kg)	Mg(cmol/kg)
0	5.28b	2.71b	0.45b	0.88a	0.86a	5.43a	2.91a
455	6.04a	4.62a	1.36a	0.89a	0.81a	4.41a	3.44a

Table 4: Effects of application rate of SEO, BIT and CRO on soil chemical properties. Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

Interactions between PHCs and incubation periods on soil chemical properties

Application Rate (ml)	Incubation Periods (months)	pH (H_2O)	OC (%)	N_{TOT} (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
0	0	5.89a	1.94bc	0.31b	0.47c	0.97a	4.36b	1.30d
	2	6.03a	1.91b	0.47b	0.49c	0.59c	4.64b	1.53c
	4	5.98a	2.01b	0.72a	0.57b	0.74b	5.17a	1.76b
	6	5.97a	2.09b	0.85a	0.63b	0.71b	5.56a	1.93a
455	0	5.82a	2.41a	0.45b	0.42c	0.70a	3.94c	1.63b
	2	5.86a	2.41a	0.56ab	0.48c	0.74b	3.89c	2.31a
	4	5.75a	1.42c	0.58ab	0.61b	0.96a	4.06c	2.08a
	6	5.77a	1.18d	0.80a	0.99a	1.13a	4.99a	2.57a

Table 5: Effects of rates of PHCs and incubation periods on soil chemical properties. Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

Table 5 presents the result of the time profile of the effect of application rates of petroleum hydrocarbons contamination and incubation periods on soil chemical properties. Increasing trends were obtained for soil pH with time of incubation. The results showed that application of the PHCs brought about significant changes in soil pH for Bitumen. Soil pH decreased following bitumen application while it increased for crude oil. The soil organic carbon (SOC) increased following application of 455 ml of SEO, BIT and CRO in the range 28, 40 and 36% respectively. Increases were also found for N, P and K while Ca appeared not affected. The value of SOC in the uncontaminated control at 0 month was 1.94, and 1.55 and 1.91% reduction in SOC were obtained between 2 and 6 months of

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incubation. The values increased at 4 and 6 months with 3.5 and 7.2% increment respectively. At 455 ml rate of application, the value of SOC was similar within the 1st 2 months of incubation followed by a 41.4 and 51% reduction between 4 and 6 months of incubation. N-Total, P, K, Ca and Mg increased with incubation periods at both levels of application rates.

Effect of SEO, BIT and CRO contamination and organic amendments on soil chemical properties

Tables 6 present the result of the effect of petroleum hydrocarbon contamination and organic amendments on the soil chemical properties. Application of poultry manure and AMF inoculation increased soil pH and SOC by 27 and 23%, 20 and 17%, 17 and 21% for the respective SEO, BIT and CRO over the control treatment. The values of N-Total, P, K, Ca and Mg increased with the application of organic amendments for all the PHCs examined (Table 6).

PHC	Manure Type (t/ha)	pH (H_2O)	OC(%)	N_{TOT}(%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
SEO	0	5.43a	2.24b	0.58a	0.35a	0.97a	4.74a	2.13a
	10:00 PM	5.90a	3.08a	0.66a	0.68a	0.70a	5.21a	2.70a
	2.5 AMF	5.87a	3.04a	0.64a	0.66a	0.59c	4.77a	2.70a
BIT	0	5.56a	1.84c	0.53b	0.42a	0.68a	3.92a	1.60b
	10:00 PM	5.94a	2.31b	0.60a	0.69a	0.86a	4.63a	2.38a
	2.5 AMF	5.93a	2.20b	0.59a	0.66a	0.62a	4.38a	2.32a
CRO	0	5.75a	1.88c	0.52b	0.38b	0.65b	4.00a	1.97b
	10:00 PM	5.95a	2.27b	0.63a	0.69a	0.88a	4.82a	2.47a
	2.5 AMF	6.00a	2.38b	0.60a	0.70a	0.64b	4.69a	2.07a

Table 6: Effects of SEO, BIT, CRO contamination and organic amendments on soil chemical properties. Means with the same letters along the column are not significantly different at $P<0.05$ according to Duncan Multiple Range Test.

Effect of SEO, BIT, CRO contamination and incubation periods on soil chemical properties

PHC	Incubation Periods (months)	pH (H_2O)	OC (%)	N_{TOT} (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
SEO	0	5.69a	3.10a	0.35a	0.50a	0.74b	4.57b	2.40a
	2	5.79a	3.32a	0.52a	0.53a	0.57b	4.57b	3.30a
	4	5.73a	2.63ab	0.66a	0.56a	0.64c	5.02a	2.15b
	6	5.72a	2.10b	0.97a	0.68a	1.07a	5.46a	2.22b
BIT	0	5.78a	2.22b	0.40a	0.40a	0.88a	3.91b	2.46a
	2	5.85a	2.66ab	0.52a	0.46a	0.77b	4.11b	2.15b
	4	5.79a	1.91c	0.61a	0.58a	0.41c	4.13b	1.73c
	6	5.82a	1.68c	0.78a	0.92a	0.83a	5.09a	2.07b
CRO	0	6.08a	2.32ab	0.40a	0.43a	0.88a	3.96b	2.53a
	2	6.17a	2.74a	0.51a	0.46a	0.65b	4.11b	1.83c
	4	6.08a	1.94c	0.68a	0.62a	0.50b	4.70b	1.86c

	6	6.08a	1.70c	0.73a	0.83a	0.87a	5.26a	2.46a

Table 7: Effects of SEO, BIT, CRO contamination and incubation periods on soil chemical properties Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

The interaction effect of petroleum hydrocarbons and incubation periods on soil chemical properties is presented in Table 7. At 2 months after treatment application for all PHCs, SOC increased and thereafter reduced between 4 and 6 months of incubation. The percentage reduction in SEO, BIT and CRO at 4 and 6 months over 2 months of the application are 20.8 and 36.8%, 28.2 and 36.8% and 29.2 and 38% respectively. Soil pH, N-Total, P and Ca increased as the incubation periods increased.

Effect of Incubation periods and organic amendments on soil chemical properties

Table 8 presents the interaction effect of the incubation periods and organic amendments on soil chemical properties. Values were average across all PHCs tested. Application of Poultry manure and AMF increased values of SOC from 0 to 2 months of incubation. Significant differences were observed in the values of SOC when 10 t/ha PM and 2.5 t/ha MI were added over the unamended control at 4 and 6 months of incubation. Similar trends of increases and decreases were observed in the values of pH, N-Total, P, K, Ca and Mg throughout periods of incubation.

Incubation Periods (months)	Manure Types (t/ha)	pH (H_2O)	OC (%)	N_{TOT}(%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
0	0	5.73a	1.57a	0.34b	0.32c	0.85b	3.89b	2.08a
	10:00 PM	5.92a	1.97a	0.42ab	0.52b	0.84b	4.29a	2.66a
	2.5 AMF	5.90a	1.94a	0.39b	0.49c	0.81b	4.25a	2.65a
2	0	5.65a	1.42b	0.46ab	0.36c	1.25a	3.91b	1.68b
	10:00 PM	6.10a	2.29a	0.54ab	0.54b	0.35d	4.53a	2.99a
	2.5 AMF	6.08a	2.30a	0.55ab	0.56b	0.39d	4.35a	2.60a
4	0	5.51a	1.31b	0.62a	0.39c	0.49c	4.24a	1.87a
	10:00 PM	6.06a	1.71ab	0.67a	0.67b	0.36d	4.95a	1.96a
	2.5 AMF	6.01a	1.71ab	0.66a	0.70b	0.70a	4.66a	1.92a
6	0	5.43a	1.26b	0.74a	0.47b	0.49c	4.85a	1.99a
	10:00 PM	6.06a	1.24b	0.88a	1.02a	1.71b	5.78a	2.47a
	2.5 AMF	6.09a	1.22b	0.85a	0.95a	0.58c	5.19a	2.28a

Table 8: Effect of incubation periods and organic amendments on chemical properties of PHC contaminated soil Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

Interaction between PHCs and organic amendment on soil chemical properties

Application Rate (ml)	PHC	Manure Types (t/ha)	pH (H_2O)	OC (%)	N_{TOT}(%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
455	**SEO**	0	5.22a	2.37b	0.64a	0.31b	1.34a	4.87a	2.19a
		10:00 PM	5.72a	3.24a	0.69a	0.74a	0.57b	5.32a	2.69a
		2.5 AMF	5.69a	3.22a	0.67a	0.76a	0.87a	4.45a	2.67a
	BIT	0	5.42a	1.48c	0.50b	0.43b	0.76b	3.18b	1.55b

		10:00 PM	5.76a	1.70b	0.58b	0.75a	0.89a	4.16a	2.05a
		2.5 AMF	5.73a	1.54c	0.59b	0.75a	0.94a	3.67a	1.91a
	CRO	0	5.74a	1.57c	0.51b	0.34b	0.70b	3.49b	2.12a
		10:00 PM	6.17a	1.63c	0.63a	0.75a	0.94a	4.54a	2.20a
		2.5 AMF	6.17a	1.90b	0.95a	0.81a	0.94a	4.29a	1.94a

Table 9: Interaction between PHCs and organic amendment on soil chemical properties. Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

The interaction effect of application rates and organic amendment of petroleum hydrocarbon on soil chemical properties is presented in Table 9. In the uncontaminated control (0 ml), soil pH increased following organic amendments. Soil organic carbon (SOC) increased significantly for all PHC tested when 10 t/ha PM and 2.5 t/ha AMF were applied. Similar trends were observed in N-Total, P, K, Ca and Mg when PHC contaminated soils were amended with PM and AMF inoculation.

Interaction between PHCs incubation periods and organic amendment on soil properties

The effect of application rates of SEO, BIT & CRO, incubation periods and organic amendment on soil chemical properties is presented in Table 10. In the uncontaminated control, soil pH increased from 2 to 6 months after the application of organic amendments (10 t/ha PM and 2.5 t/ha MI). Similar trends of increases in soil pH with time were observed with soils treated with 455 ml PHC. The percentage reduction at 4 and 6 months of incubation were higher compared with the control. There were noticeable increase in N-Total, P, K, Ca and Mg when contaminated soils were organically amended with 10 t/ha PM and 2.5 t/ha MI compared with the control.

Application Rate (ml)	Incubation Period (mths)	Manure Types (t/ha)	pH (H2O)	OC (%)	NTOT (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
455	**0**	0	5.72a	1.59c	0.40c	0.31c	0.93a	3.71b	1.30c
		10:00 PM	5.86a	1.75b	0.49c	0.45b	0.57b	4.06a	1.80b
		2.5 AMF	5.86a	1.80b	0.47c	0.48b	0.61b	4.03a	1.80b
	2	0	5.52a	1.77c	0.54b	0.33c	0.90a	3.69b	2.19b
		10:00 PM	6.03a	2.06b	0.58b	0.49c	0.62b	4.13a	2.44b
		2.5 AMF	6.02a	2.09b	0.57b	0.63b	0.69b	3.84b	2.31b
	4	0	5.34a	1.92c	0.53b	0.34b	0.91a	3.60b	2.04b
		10:00 PM	5.98a	1.60b	0.61b	0.72b	0.65b	4.67a	2.13b
		2.5 AMF	5.95a	1.53b	0.61b	0.76b	1.31a	3.91b	2.06b
	6	0	5.25a	1.96a	0.74b	0.46c	0.99a	4.38a	2.29b
		10:00 PM	6.05a	1.25d	0.85a	1.32a	1.36a	5.83a	2.90a
		2.5 AMF	6.02a	1.21d	0.82a	1.22a	1.05a	4.75a	2.52a

Table 10: Interaction between incubation periods of PHCs and organic amendment on soil chemical properties. Means with the same letters along the column are not significantly different at P<0.05 according to Duncan Multiple Range Test.

Interaction between PHCs, incubation period and organic amendment on soil properties

Table 11 presents the interaction effects of PHCs application rate, incubation periods and organic amendment on soil chemical properties. Uncontaminated control produced non-significant increases in soil pH from 0 to 6 months of incubation when 10 t/ha PM and 2.5 t/ha MI was added. At 2 months, there was an increase in soil organic carbon (SOC) to 6 months of incubation though the trends were similar for PHC alone (unamended) for soil pH,N-Total, N, P, Ca and Mg except for K. Similar but significant results were obtained when the soil was amended with 10 t/ha PM and 2.5 t/ha MI. At 2 months, the SOC increased significantly. However, at 4 and 6 months of incubation, there was a significant reduction in the value of

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SOC across the PHC contaminated soils. There were significant increases in the values of N-Total, P, K, Ca and Mg when the organic materials (10 t/ha PM and 2.5 t/ha MI) were added to the contaminated soil at 455 ml PHC.

Application Rate (ml)	PHC	Incubation Period (months)	Manure Types (t/ha)	pH (H_2O)	OC (%)	N_{TOT} (%)	P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
455	**SEO**	**0**	0	5.44a	2.11b	0.36b	0.37c	1.45a	4.62b	1.13c
			10:00 PM	5.50a	2.32b	0.40b	0.65b	0.07d	4.87b	1.83c
			2.5 AMF	5.50a	2.33b	0.40b	0.59b	0.07d	4.88b	1.80c
		2	0	5.24a	2.55b	0.56n	0.33c	1.45a	4.52b	3.10a
			10:00 PM	5.79a	3.08a	0.58b	0.68b	0.06d	4.80b	3.52a
			2.5 AMF	5.72a	3.10a	0.57b	0.72b	0.22d	4.17b	3.52a
		4	0	5.12a	2.81b	0.57b	0.28c	1.45a	4.87b	2.48b
			10:00 PM	5.76a	2.52b	0.64b	0.65b	0.09d	5.60a	2.62b
			2.5 AMF	5.72a	2.48b	0.61b	0.72b	2.05a	4.13b	2.62b
		6	0	5.08a	2.11b	1.08a	0.25b	1.11a	5.47a	2.03b
			10:00 PM	5.81a	2.02b	1.12a	0.98a	2.05a	6.01a	2.80b
			2.5 AMF	5.80a	2.07b	1.11a	1.01a	1.14a	4.60b	2.73b
	BIT	**0**	0	5.69a	2.30b	0.41b	0.25c	0.65b	3.32c	1.15c
			10:00 PM	5.65a	2.47b	0.53b	0.31c	0.91a	3.61c	1.81c
			2.5 AMF	5.62a	2.47b	0.50b	0.39c	0.85b	3.45c	1.79c
		2	0	5.53a	2.35b	0.56b	0.33c	0.78b	3.27c	1.63c
			10:00 PM	5.71a	2.55a	0.57b	0.40c	1.11a	3.80b	1.90b
			2.5 AMF	5.71a	2.53a	0.58b	0.58b	0.97b	3.67c	1.83b
		4	0	5.20a	2.39a	0.46b	0.40c	0.68b	2.33d	1.52c
			10:00 PM	5.78a	1.69c	0.52b	0.72b	0.74b	3.62c	1.70b
			2.5 AMF	5.79a	1.55c	0.59b	0.64b	0.54c	3.41c	1.97b
		6	0	5.25a	1.90b	0.58b	0.75b	0.93b	3.80b	1.90b
			10:00 PM	5.88a	1.09d	0.71a	1.55a	0.80b	5.60a	2.80a
			2.5 AMF	5.80a	1.61c	0.69b	1.37a	1.10a	4.13b	2.06b
	CRO	**0**	0	6.04a	1.35d	0.42b	0.30c	0.68b	3.20c	1.60b
			10:00 PM	6.44a	1.45d	0.54b	0.40c	0.72b	3.70c	1.76b
			2.5 AMF	6.46a	1.60c	0.52b	0.47c	0.92b	3.77c	1.81b
		2	0	5.80a	1.40c	0.49b	0.33c	0.58b	3.27c	1.83b
			10:00 PM	6.58a	1.55c	0.58b	0.50c	0.69b	3.80c	1.90b
			2.5 AMF	6.62a	1.65c	0.57b	0.58b	0.87b	3.67c	1.58c
		4	0	5.69a	1.57c	0.55b	0.35c	0.60b	3.61c	2.11b
			10:00 PM	6.40a	1.68c	0.68a	0.78b	1.12a	4.80b	2.06b
			2.5 AMF	6.33a	1.97b	0.63b	0.92a	1.05a	4.20b	1.60b

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		6	0	5.42a	1.97b	0.56b	0.37c	0.93b	3.87c	2.93a
			10:00 PM	6.47a	1.84b	0.71a	1.42a	1.24a	5.87a	3.10a
			2.5 AMF	6.47a	1.38d	0.66a	1.29a	0.91b	5.53a	2.76a

Table 11: Interaction between PHCs application, incubation period and organic amendment on soil chemical properties. Means with the same letters along the column are not significantly different at $P<0.05$ according to Duncan Multiple Range Test.

Time-course of the effects of petroleum hydrocarbon compounds on soil chemical properties

The time–course of changes in soil chemical properties as affected by petroleum hydrocarbon compounds (PHCs) contamination in soil are presented in Figures 1-7. The values of the measured soil parameters recorded at the commencement of the experiment and at 2, 4, 6 months after the application of the petroleum hydrocarbon compounds showed declining trends in the values of pH, organic carbon and Mg were obtained. However, N-total and available P increased with time. The observed trend lines in time-dependent on elemental concentration were best described by polynomial relationships (Figures 1-7). Increasing trends in N-total with time up till the end of the experiment after the application of petroleum hydrocarbon compounds was observed. Trends of the mean values (highest and lowest values) with respect to treatment showed that CRO had the highest soil pH at 2 months incubation period with SEO recording the lowest at 6 month (Figure 1). SOC increased across treatments with SEO recording the highest value at 2 months of incubation (Figure 2) which was followed by general decline in values with time across the PHCs. The values of N-total and available P increased with time among the PHCs with SEO recording the highest values at 6 months of incubation while there were non-significant difference in Ca contents across the treatments (Figures 3 and 4). SEO recorded highest Mg value at 2 months, then a decline till the end of the incubation period while SEO increased towards the end of the incubation period (Figure 7).

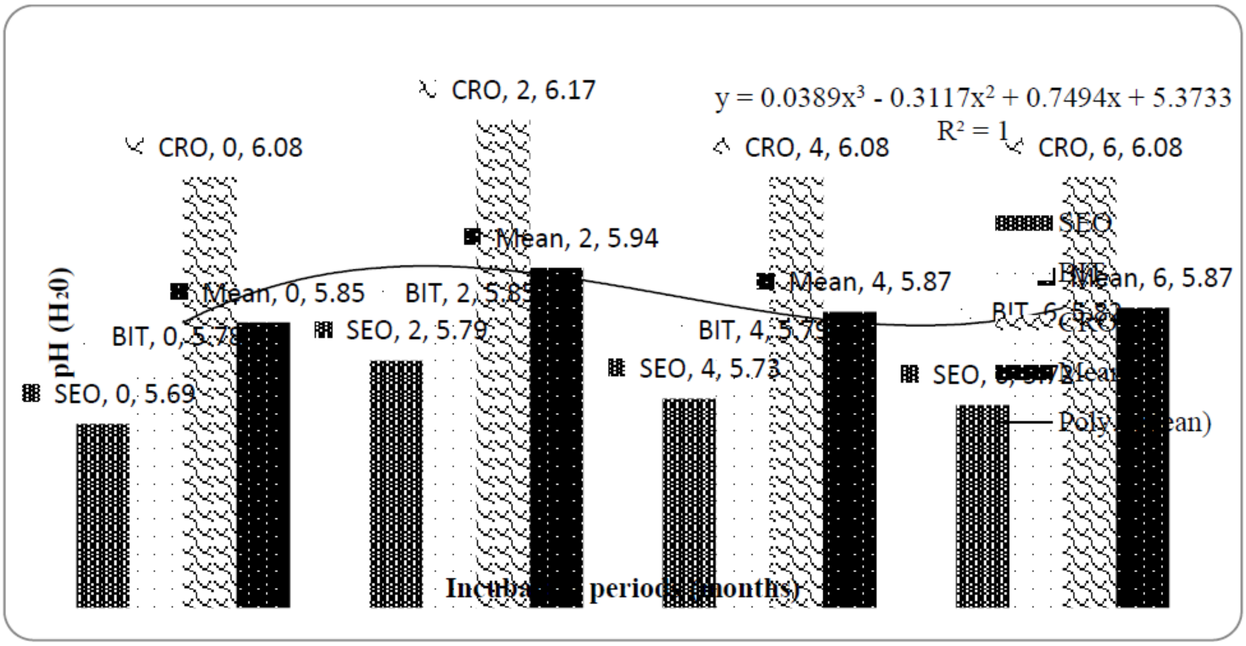

Figure 1: Effects of petroleum hydrocarbon compounds and organic amendment on soil pH SEO – SPENT ENGINE OIL; BIT - - BITUMEN; CRO – CRUDE OIL.

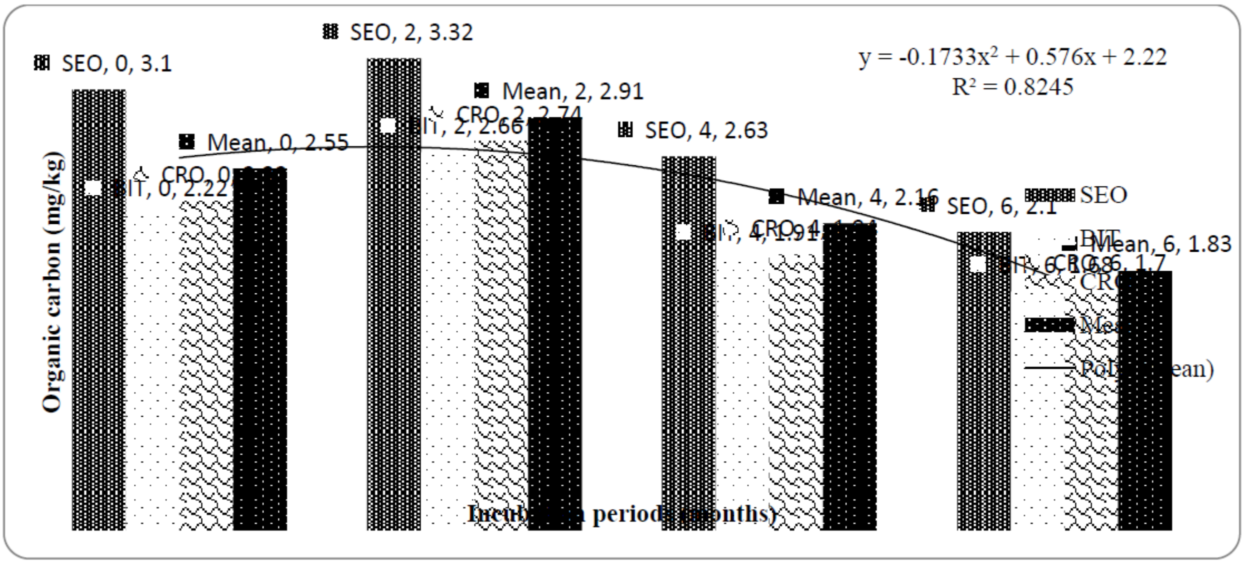

Figure 2: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil organic carbon.

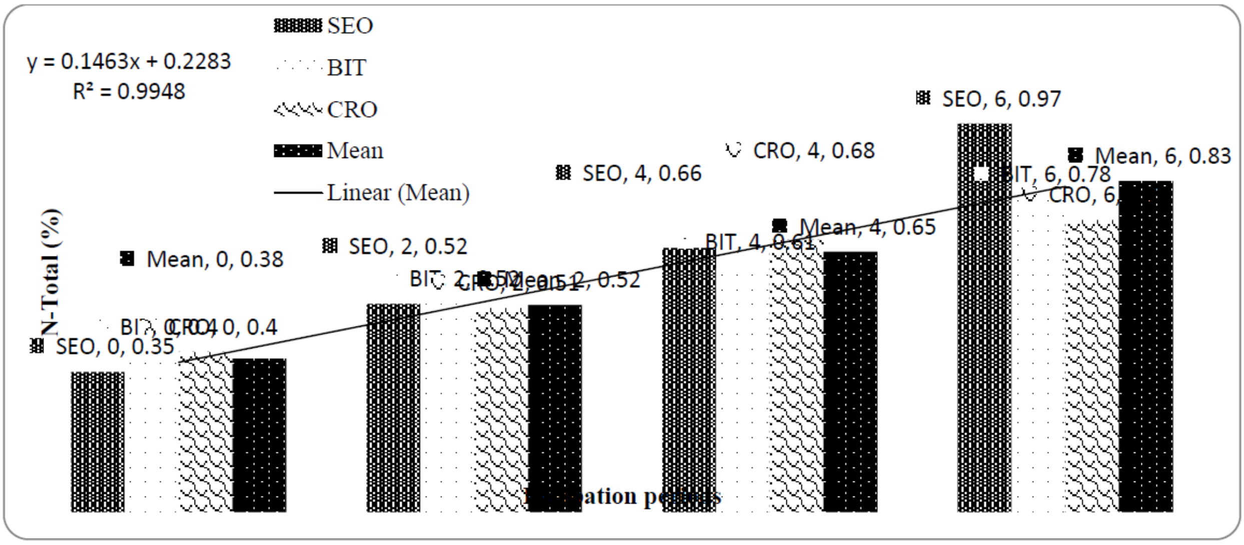

Figure 3: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil total nitrogen.

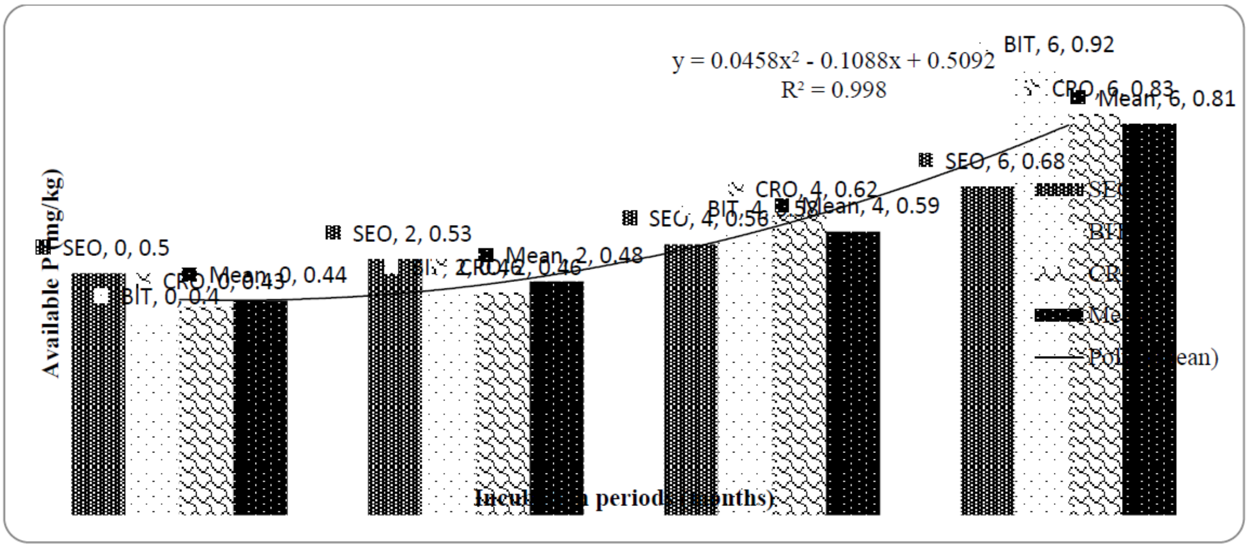

Figure 4: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil available phosphorus.

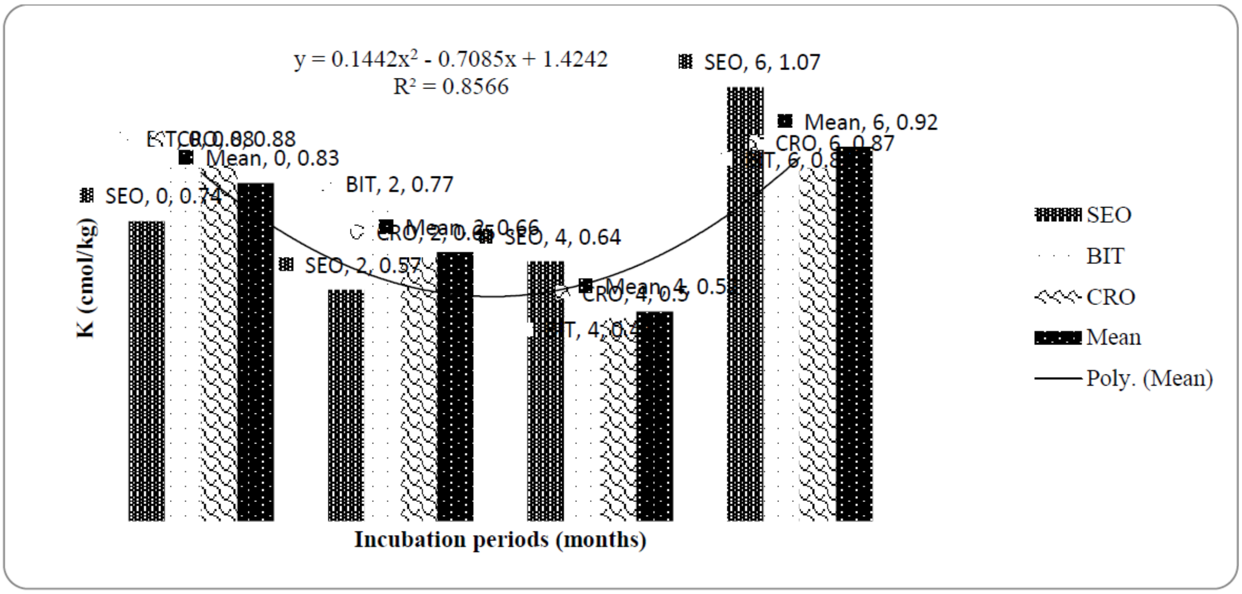

Figure 5: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil exchangeable potassium.

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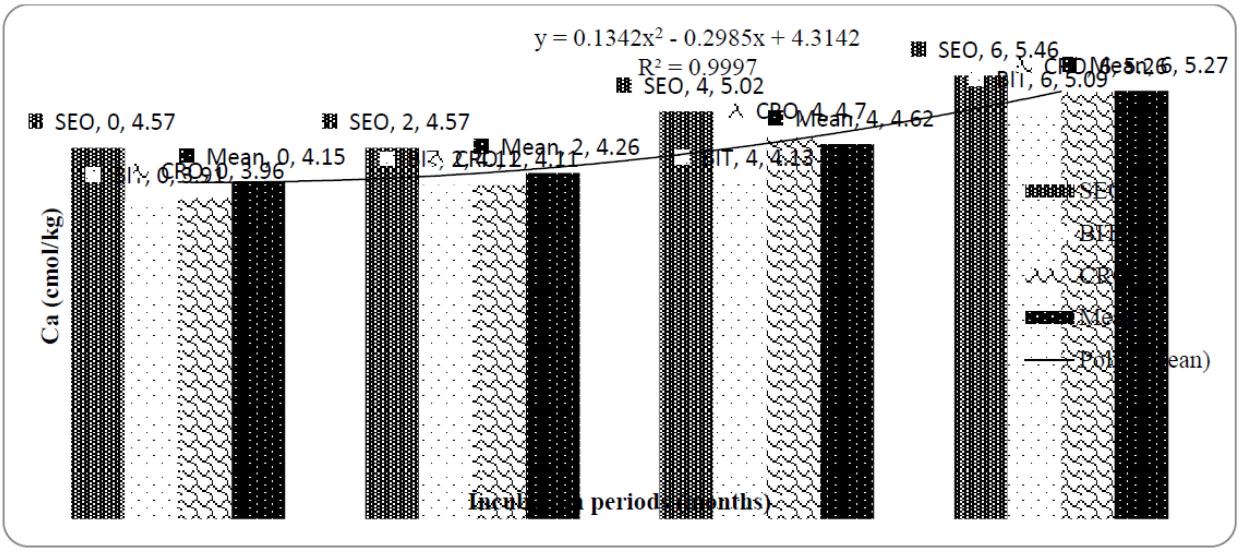

Figure 6: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil exchangeable calcium.

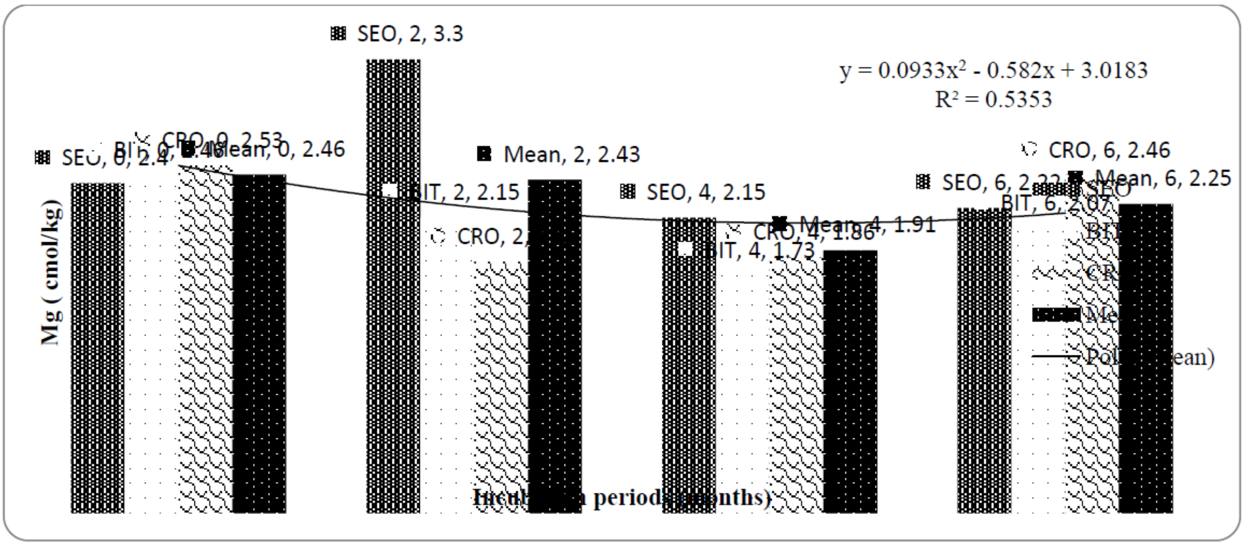

Figure 7: Time-course of the effect of petroleum hydrocarbon compounds and organic amendment on soil exchangeable magnesium.

Discussion

Application of petroleum hydrocarbon compounds and organic amendment of the contaminated soil affected trends of its chemical properties during the course (six months) of incubation. The trends in the time dynamics of the soil chemical properties differed among the petroleum hydrocarbon compounds applied for the six (6) months of incubation. Decreases in the values of pH were observed as at 455 ml per pot of PHCs pollution. This agrees with the report of Amadi et al. [15] who observed increase in soil acidity following increases in concentration of crude oil. The observed pH decreases recorded in our study compared favourably with pH values (4.7 to 5.4) reported by Osuji and Adesiyan [16] and Agele et al. [17]. The decreases in soil pH observed in this study contradicts the reports of Onuh et al. [18,19] who obtained increases in soil pH as the level of crude oil applied increased. The pH level of a soil which explains the degree of acidity and alkalinity of soil affects physicochemical soil properties as well as the flora and fauna of soil. Thus, the availability of many soil nutrients for plant growth and development is determined by soil pH. The percentage soil organic carbon content of the soil samples increased with increase in the concentration of the PHCs (SEO, BIT and CRO) applied at two months of incubation. The increase in the SOC content recorded in this experiment is consistent with earlier findings [4,15,18,19] and may be as a result of the microbial mineralization of the petroleum hydrocarbon compounds. Available N and P of the soil increased due to the contamination with petroleum hydrocarbon compounds, which contradicted the report of Onuh et al. [18] who observed decreases as the level of crude oil pollution increased. Exchangeable bases (Ca, K and Mg) were observed to decrease with increases in rates of PHCs (SEO, BIT and CRO). The high N and P contents may be attributed to the use of these exchangeable bases by the microbes present in the soil samples. Addition of poultry manure and mycorrhizal inoculum to the PHCs polluted soils slightly raised the soil pH, though without significant differences observed. The pH values of the soils amended with 10 t/ha PM and 2.5 t/ha MI were higher than unamended control. The results obtained are in agreement with those obtained by Ijah et al. [20] and the findings of Ijah and Antai [21] that organic manures (poultry droppings) have buffering effect on crude oil polluted soil. This rise in the pH of the amended soils may favour oil degradation by microorganisms as observed in similar studies that higher pH range (6 to 9) provides better conditions for mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH conditions close to neutrality [22]. The results also showed that percentage organic carbon increases as the PHCs was amended with poultry manure and mychorrhyzal inoculum. McGill [23] reported that organic carbon when present in sufficient quality have beneficial effect on soil chemical and physical properties. Total nitrogen and phosphorus content of the amended unpolluted and PHCs polluted soils were higher but not significantly different from those of the unamended soils. The increase in the percentage nitrogen and phosphorus may be as a result of anthropogenic inputs of these nutrients from the organic manures because organic manures have been reported as being capable of increasing soil nutrients by supplementing the limiting nutrients [24-27]. The organic materials had significant effect in the remediation process for PHC contaminated soil. The organic materials added to the PHC polluted soils would have enhanced the autochthonous microbiota, which naturally attenuates petroleum hydrocarbon [7].

Conclusions and Recommendations

It can also be asserted that the extent of toxicity and duration of PHC pollutants will depend on the nature of the hydrocarbon contaminant and the soil characteristics. The results of this study showed that PHC contamination of the soil affected its chemical properties. However, the addition of organic substances (poultry manure and AMF) ameliorated soil toxicity and on amaranthus. The findings also showed that amaranthus performance was not significantly affected when grown in PHC (spent engine oil) polluted soil while amendment of such soil with organic materials especially poultry manure and mycorrhizal inoculum further improved soil functional properties and amaranthus performance. The relevance of organic substances evaluated is given credence by the findings of this study. The organic materials played significant role in the remediation process for PHC contaminated soil. The results indicated that PHCs adversely affect soil chemical properties and that addition of organic substances improved chemical properties of the soil following pollution with petroleum hydrocarbon compounds compared with the un-amended soils. Poultry manure in particular performed better compared with mycorrhizal inoculum with respect to improvement of soil chemical properties.

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