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Temporal Profiles of Soil Properties and Performance of *Amaranthus* Grown on Farm Yard Manure-mediated Crude Oil Polluted Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AS and IR designed the study, wrote the protocol while authors IR, AP, HY and OK wrote the first draft of the manuscript. Authors IR and HY managed the literature searches, analyses of the results, performed the field and laboratory analysis and managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted in the plant house of the Department of Crop, Soil and Pest management, Federal University of Technology, Akure, Nigeria, to examine the efficacy of manuring and incubation periods on the amelioration of the toxic effects of crude oil contamination on soil properties and *Amaranthus* performance. The time dynamics of physical, chemical and microbial population of the manured mediated crude oil contamination were observed following 270 days (9 months) of incubation. Bonny light crude oil was applied at zero and 5% (0 and 325 ml per pot) to the containerized (pot) soil which were amended with Farm Yard Manure (FYM) at 0 and 40 g per pot. The manure-mediated crude oil polluted containerized soil were incubated for periods of 0, 2, 4, 6 and 9 months. Treatments were arranged using Completely Randomised Design (CRD) with three replications. The toxicity of the crude oil pollution and its capacity to support plant growth was assessed via germination, seedling survival and growth of *Amaranthus* at the various periods of incubation. Data were also collected on soil biological, physical and chemical properties at the various sampling dates (periods of incubation). Application of organic fertilizer to crude oil-

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contaminated soil produced significant influence on soil chemical properties, microflora populations, and on the growth characters of *Amaranthus*. Organic amendment of crude oil contamination at two rates of organic fertilizer produced increases in soil pH and its contents of organic matter, N, P and K, and the colony sizes of microflora populations, and improved root, shoot biomass and leaf development of *Amaranthus*. Soil application of of crude oil affected soil chemical properties via decreases in soil pH, organic matter, N, P and K) and the colony sizes of microflora populations (bacterium, yeast and fungi counts). Crude oil contamination inhibited seed germination and growth of *Amaranthus* from the commencement of experiment to 4 weeks after treatment application. The time changes in soil elemental concentrations and biological populations following contamination with crude oil was monitored. Data obtained on soil chemical constituents (pH, organic matter, CEC) biological populations were fitted to correlation and regression models. The observed trendlines in time-dependent elemental concentration and soil biological populations were described by power, logarithmic and polynomial relations with high R^2 values. The initial declining trends in values with time up to the forth months after crude oil application were followed by a little linear increases in soil chemical properties and microbial population with time. These trends were consistent for both soil chemical properties and microbial population. The nature of the trends (polynomial function as best fit) indicate that the responses were not sole function of a factor but suggest synergy among factors responsible for the decay of petroleum hydrocarbon compounds and hence its toxicity to soil chemical properties and biological activities. The study showed that soil microbiological population (diversity) may be useful tools for assessing the effect of petroleum hydrocarbon contamination on soil and environmental health.

Keywords: Petroleum hydrocarbon; soil; toxicity; growth; inhibition; organic; amendment.

1. INTRODUCTION

Crude oil and petroleum products such as gasoline, fuel oils and diesel fuels are complex mixtures of organic compounds, is a natural flammable viscous liquid consisting of various hydrocarbons of various molecular weights that are found in geologic formations beneath the earth surface. Crude oil is formed from the remains of marine algae and animals which died leaving their remains settled on the sea bed, covered by mud, silt and other sediments these remains become piled up over millions of years with their mass exerting a great pressure on the lower layer thereby changing them to hard sedimentary rocks.

Environmental degradation associated with oil exploration and exploitation is a major problem confronting oil producing countries. In Nigeria, a large amount of crude oil is spilled annually into the environment. Oil spillage is the release of a liquid petroleum hydrocarbon into the environment, especially marine areas due to human activity [1]. Oil spillage has been known to exhibit various deleterious effects on both plants and micro-organisms. The presence of petroleum hydrocarbon compounds in the soil generally retards plant growth [2,3] reduces aeration by blocking air spaces between soil particles hence creating a condition for anaerobiosis [3,4,5,6] and cause growth retardation in plants. Oil spills may occur for reasons ranging from equipment failure, disasters, deliberate acts, or human error [3,7,8]. In soils, petroleum hydrocarbons create conditions which lead to the availability of toxic heavy metals such as: Arsenic, Lead and Iron [4,7,8,9]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [1,7]. These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their natural environment [10,11].

Studies have been carried out on the effects of crude oil pollution on seed germination, plants and soil. For example, Ogboghodo et al. [12] reported that crude oil inhibited the germination and growth of maize at high pollution levels while the growth of Okra and Telfaria seedlings [13] were highly reduced with an increase in crude oil concentrations [3]. Soil and water represent the first lines of recipients of oil pollution. Surface and ground water contamination by crude oil therefore is becoming an increasing sensitive issue in Nigeria because most of the water supply is derived from streams, shallow and unconfined aquifers [1]. Furthermore, contamination of land is of paramount importance to man in that it is on this portion that man's existence depends. Heavy metal uptake occurs directly from the surrounding as a chemical compound in the atmosphere. Additionally, concentration of trace elements in sediment may render soils non-productive because of phytotoxicity. 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 [8].

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 bacterial species. This is particularly true in pollutants that are made up of many different compounds such as petroleum compounds and complete mineralization to CO_2 and H_2O is desired. Microorganisms are specific in their ability to metabolize petroleum hydrocarbon compounds, hence, species (strains) of microorganisms can only metabolize a limited range of hydrocarbon substrates. Therefore, assemblages of mixed populations of microorganisms with overall broad enzymatic capacities are required to increase the rate and extent of petroleum biodegradation [11,14]. 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 [11,14]. Microbes are the main degraders of petroleum hydrocarbons contaminated ecosystems [15,16]. The measurement of microbiological parameters, such as soil respiration, microbial population and diversity provides information on the presence and activity of viable microorganisms as well as on the intensity, kind and duration of the effects of hydrocarbon pollution on soil health [11,14,17]. Improved understanding of the effect of hydrocarbon contaminants on plant, and soil physical, chemical and microorganisms may be of help in assessing the recovery potential of a soil [15,16,18]. Bioremediation/ phytoremediation (which is the use of plants and/or microorganisms in the biological processes of the breaking down of petroleum hydrocarbon pollutants and their use as indicators of the toxicity of petroleum hydrocarbon compounds on soil and plants [19,20].

Amaranthus species (A. hybridus Linn.), a nutritious and leafy vegetable, is a member of the family Amaranthaceae. *Amaranthus* also has the ability to bioaccumulate hydrocarbons [21]. The aim of this study is to examine the efficacy of Farm Yard Manure (FYM) and organic fertilizers for amelioration of the toxicity of crude oil on soil and *Amaranthus* under field and laboratory conditions.

2. MATERIALS AND METHODS

An experiment was conducted in the Screenhouse of the Department of Crop, Soil & Pest Management, Federal University of Technology, Akure, Nigeria to examine the efficacy of farm yard manure and organic fertilizer in the amelioration of the toxic effects of crude oil contamination on soil properties and *Amaranthus* performance for 270 days (9 months). The experiment was conduct between September, 2011 and June, 2012. Treatments consisted of 5 x 2 x 2 factorial combinations of incubation periods, and levels of crude oil and FYM applications arranged in a Completely Randomised Design (CRD) with three replications. Bonny light crude oil was applied at zero and 5% (0 and 325 ml) to the containerized (pot) soil which were amended with Farm Yard Manure (FYM) at 0 and 40 g per pot. The manure-mediated crude oil polluted containerized soil were incubated for periods of 0, 2, 4, 6 and 9 months. The toxicity of the crude oil pollution and its capacity to support plant growth was assessed via germination, seedling survival and growth of *Amaranthus* at the various periods of incubation. Soil chemical properties and microbial population were determined as monitoring tools for the degradation process (decay of petroleum hydrocarbon compounds and hence its toxicity to soil chemical properties and biological activities) was made via measurements of microbial diversity and their population counts. Crude oil (light crude) was obtained from the Nigerian National Petroleum Corporation (NNPC) refinery in Warri, Delta State, Nigeria. The temporal changes in soil elemental concentrations and biological populations following soil contamination with crude oil was monitored. 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, CEC) and microbiological (Fungi, Bacteria, Yeast and soil Nematode) properties using standard methods. Data were also collected on agronomic characters such as plant height, stem girth and number of leaves, root and shoot biomass, head weight of *Amaranthus*.

The treatment combinations were:

Table 1. Treatment combinations

Treatments	Incubation period (months)	Amount of crude oil (ml/pot)	Amount of FYM (t/ha)
$T_0P_0M_0$	0	0	0
$T_0P_0M_1$	0	0	10
$T_0P_1M_0$	0	325	0
$T_0P_1M_1$	0	325	10
$T_2P_0M_0$	2	0	0
$T_2P_0M_1$	2	0	10
$T_2P_1M_0$	2	325	0
$T_2P_1M_1$	2	325	10
$T_4P_0M_0$	4	0	0
$T_4P_0M_1$	4	0	10
$T_4P_1M_0$	4	325	0
$T_4P_1M_1$	4	325	10
$T_6P_0M_0$	6	0	0
$T_6P_0M_1$	6	0	10
$T_6P_1M_0$	6	325	0
$T_6P_1M_1$	6	325	10
$T_9P_0M_0$	9	0	0
$T_9P_0M_1$	9	0	10
$T_9P_1M_0$	9	325	0
$T_9P_1M_1$	9	325	10

2.1 Determination of Soil Chemical Properties

Soil samples collected were air-dried and sieved using a 2 mm sieve. The samples were subjected to a Particle size analysis by hydrometer method using 50 g soil sample dissolved in water in a 200 ml beaker. Readings were taken at 40 seconds and 2 hours in the sedimentation cylinder. Also, samples were analysed for $_{P}H$ using 1:1 water suspension by adding 10 ml distilled water to 10 g of soil and read on the $_{P}H$ meter. Organic matter content was determined by Dichromate Oxidation Method [22]. Nitrogen was determined by Micro-Kjeldahl apparatus [23]. Exchangeable K was extracted using Ammonium acetate [23] and was determined on a Flame Photometer. Available Phosphorus byBray-P-1 extraction [24] and read on a Spectrophotometer. Other soil chemical constituents especially K, Ca and Mg were extracted using the Mehlich III extractant and were determined by ICP [25].

2.2 Determination of Soil Micro Organisms

Nutrient agar (NA) and potato dextrose agar (PDA) are the media of choice used for this investigation. The media were prepared by dissolving 28 g of NA and 39 g of PDA in a litre of distilled water separately, allowed to soak for few minutes, mixed thoroughly, cotton plugged and sterilised in an autoclave at 121℃ for 15minutes and allowed to cool to about 45℃ before pour plated.

2.2.1 Sample preparation

Ten (10) gram of each of the soil samples were aseptically transferred into a separate beaker containing 90 ml of sterile distilled water and allowed to soak for about 30 minutes. The samples were granulated using a sterile glass rod and mixed thoroughly on a mechanical orbital shocker to dislodged the microbial propagules in water. About 1ml aliquot of the sample was pipette in a test tube followed by serial dilution (using dilution factor of 10^{-8}) into another sets of test tubes containing 9 ml of sterile distilled water. Exactly 1 ml portion of the diluant were aseptically pipetted into sterile petri dishes followed by addition of 20 ml of cooled molten agar media. The diluant was mixed properly for even distribution of the inoculum and was allowed to set. Nutrient agar plates for bacterial were incubated at 30℃ for 24 hours while P.D.A (for yeast and fungi determination) were incubated at about 27℃ for 48-72 hours. At the end of incubation period, the total visible bacterium, yeast and fungi colonies were counted and the main value were recorded per gram of the soil samples. Isolation and enumeration of nematodes from the soil samples was carried out using the Baerman funnel method. The funnel was placed on a stand and filled with water. A freshly collected soil samples of about 100-300cc was placed in the funnel on muslin cloth. The funnel should contain enough water to make contact with and saturate the soil samples. It was allowed to stand overnight. The live nematodes moves actively and migrate through the cloth or porous paper into the water and sink to the bottom of the rubber tubing just above the pinchcock. The water drawn from the rubber tubing was centrifuged at 3000 rotations per minute (RPM) for between 2 to 5 minutes. The supernatant was discarded while the sediments were placed on a shallow dish and examined under a microscope at $10\text{-}40^{x}$. Alternatively, the water drown from the rubber tubing was allowed 1hr for nematodes to settle to the bottom of the tubes, siphoned down to 10-20 ml, and pour contents carefully into a counting dish, allow a few minutes for nematodes to settle, and examine with a stereomicroscope ($10\text{-}70^{x}$).

2.3 Bacteria and Fungi

Measurements of total microbial population were carried out using the standard plate count techniques. Bacterial counts were analysed using nutrient agar (NA) while fungi were analysed with potato dextrose agar (PDA). 1 g of each of the soil sample was aseptically transferred separately into the sterile sample bottles containing 20 ml of sterile distilled water and mixed to form homogeneous suspension. 1 ml of the sample suspension was pipette into sterile distilled water and serially diluted to ratio of 10^{-6}. The dilution factor was aseptically pipette separately into different sterile Petri-dishes and thoroughly mixed with 20 ml of the cool (45℃) molten agar media (NA & PDA), separately and the plates for bacterium were incubated at 37±2℃ for 24 hours while the PDA plates for fungi were incubated at 27±2℃ for 48 hours. The colonies formed from visible bacteria cells and that of the fungi spores were enumerated. Data obtained on soil chemical constituents (pH, organic matter, CEC) biological populations were fitted to correlation and regression models. In addition, data sets obtained on the soil chemical properties and growth parameters of *Amaranthus* were subjected to analysis of variance (ANOVA) and treatment means were separated using LSD at 5% level.

3. RESULTS

3.1 Effect of Crude Oil Contamination and Organic Amendment on Soil Nutrients

Soil application of 7% Bonny light crude oil produced affected chemical properties, for example, values of organic matter, N, P and K increased compared with the non-contaminated soil (Table 2). The values of soil organic carbon and P increased tremendously under 7% crude oil pollution and 10 t/ha FYM compared with the unamended control. The contents of soil organic matter declined whether there is an application of crude oil or not. The analysis showed the same trend for organic matter, N, P, K, Ca, Mg and Na. The population of the bacteria increases with time in all treatments (Table 3). About 8.8 x 10^{6} spore forming units (sfu/g) of yeast was recorded for the control which is the highest compared the contaminated pot at 2 months of incubation whose record is the lowest. Yeast population took a decreasing trend as the incubation period increases. Fungi also took to the same decreasing trend. Nematode population was high in the control and in the amended pots but reduced in the polluted plus amended pot while the pollution alone without amendment was the lowest during the incubation study. The pot with pollution and organic amendment at 9 months has the highest bacteria population. While pot with pollution and organic amendment at zero month has the lowest.

3.2 Effects of Incubation Period of Crude Oil Contaminated Soil and Organic Amendment on Growth Characters of *Amaranthus*

At four months after treatment application, 5% crude oil produced lower biomass yield compared with uncontaminated unamended control (Table 4). However, at 6 months, differences were found for biomass yields across the treatments (contamination alone and organically amended contaminated soil). At this sampling date, among the treatments, significant differences were found for number and weight of leaves per plant among the control pot, amended, polluted, pollution plus FYM. The number of leaves per plant increased unamended crude oil polluted soil and followed by decline in values until nine months of incubation. Application of 5% crude oil produced significant increases in total biomass yields of *Amaranthus* from the commencement of the experiment up until 4 months of incubation. Thereafter, there were gradual reduction in values of the measured agronomic characters up to the ninth months of incubation compared with the non-polluted soils.

There was no seed germination in the pots that were polluted with 5% crude oil alone until four months after application. No seed germination was also recorded at this period (4 months after crude oil application) for 5% (325 ml) crude oil pollution combined with 10 t/ha Farm Yard Manure. Among the treatments, crude oil alone or crude oil pollution amended FYM, there were no significant differences in leaf weight, however, at 6 months after treatment application, differences were obtained in leaf weight. The number of leaves took fluctuating trend as it slightly increases with the amended pots but with no significant difference until four months after pollution without manure. At six months after treatment, there was a significant decrease in the number of leaves. The number increased in the treated pot with 5% crude oil alone and then began to drop until the last treatment at nine month that was polluted and at the same time amended.

Table 2. Effects of period of incubation on soil chemical properties on manure-mediated crude oil contaminated soil

Treatments	pH	Organic carbon g/g	Organic matter g/g	N g/g	P mg/kg	K	Na	Ca	Mg
						(cmol/kg soil)			
$T_0P_0M_0$	5.74	1.47	2.54	0.13	7.08	0.56	0.69	5.5	3.0
$T_0P_0M_1$	5.97	2.15	3.70	0.18	56.50	1.03	1.01	5.5	4.0
$T_0P_1M_0$	6.44	2.47	4.26	0.21	15.10	0.21	0.35	4.5	0
$T_0P_1M_1$	6.70	3.05	5.25	0.46	37.80	0.79	0.77	5.5	0.5
$T_2P_0M_0$	5.87	2.68	4.62	0.38	10.50	0.42	0.57	4.5	2.5
$T_2P_0M_1$	6.65	3.52	6.08	0.78	46.90	0.10	1.07	5.0	4.0
$T_2P_1M_0$	6.27	2.91	5.02	0.55	12.50	0.60	0.61	6.5	3.5
$T_2P_1M_1$	6.58	2.99	5.15	0. 52	49.70	0.77	0.74	3.0	4.0
$T_4P_0M_0$	5.82	2.80	4.82	1.08	10.03	0.66	0.59	4.0	1.4
$T_4P_0M_1$	5.90	3.33	5.75	1.66	15.79	0.70	0.65	4.6	2.3
$T_4P_1M_0$	5.69	2.15	3.70	0.86	13.22	0.35	0.50	3.9	1.4
$T_4P_1M_1$	6.43	5.21	8.98	1.76	10.96	0.68	0.69	5.0	1.2
$T_6P_0M_0$	6.13	2.20	3.80	0.48	5.13	0.31	0.66	3.6	1.6
$T_6P_0M_1$	6.33	3.32	5.72	1.30	31.03	0.57	0.61	3.8	1.3
$T_6P_1M_0$	6.35	2.83	4.89	1.14	8.71	0.48	0.60	4.6	2.0
$T_6P_1M_1$	6.37	4.90	8.45	1.82	35.00	0.88	1.16	3.9	2.0
$T_9P_0M_0$	6.19	2.09	3.60	0.94	8.48	0.28	0.42	3.4	1.8
$T_9P_0M_1$	6.32	2.68	4.62	1.46	4.51	0.53	0.56	5.0	2.3
$T_9P_1M_0$	6.38	2.60	4.49	1.08	6.14	0.29	0.57	4.6	2.0
$T_9P_1M_1$	6.28	5.06	8.72	1.96	34.92	0.79	0.67	6.2	2.4

T is time in months (0, 2, 4, 6 and 9 months after treatment application); P_0 - Uncontaminated soil, M_o – Unmanured treatment;
M_1 is organic fertilizer applied at t/ha); P_1 -Mean of crude oil and organically amended crude oil contaminated soil

Table 3. Effects of period of incubation on soil microbial population on Farm Yard Manure - mediated crude oil polluted soil

Treatments	Bacteria (10^6 cfu*/g	Yeast (10^6 sfu**/g)	Fungi (10^6 sfu/g)	Nematode (10^{10}/ ml)
$T_0P_0M_0$	6.00	8.8	4.5	2.0
$T_0P_0M_1$	4.60	1.40	1.5	20.0
$T_0P_1M_0$	8.00	3.25	2.0	13.0
$T_0P_1M_1$	1.12	3.60	2.8	5.0
$T_2P_0M_0$	1.20	2.00	1.8	23.0
$T_2P_0M_1$	4.48	1.60	3.0	19.0
$T_2P_1M_0$	6.80	1.20	4.0	5.0
$T_2P_1M_1$	8.80	4.00	2.0	9.0
$T_4P_0M_0$	2.00	1.26	2.5	11.0
$T_4P_0M_1$	3.40	1.28	2.0	22.0
$T_4P_1M_0$	3.14	1.30	3.2	6.0
$T_4P_1M_1$	4.95	2.15	2.8	10.0
$T_6P_0M_0$	4.22	1.80	2.3	20.0
$T_6P_0M_1$	5.35	2.30	2.4	23.0
$T_6P_1M_0$	5.40	3.66	3.7	8.0
$T_6P_1M_1$	6.60	3.68	3.8	12.0
$T_9P_0M_0$	7.78	2.88	3.7	21
$T_9P_0M_1$	7.85	3.25	4.0	20.0
$T_9P_1M_0$	6.85	3.46	4.4	10.0
$T_9P_1M_1$	8.96	3.48	4.8	12.0

T is time in months (0, 2, 4, 6 and 9 months after treatment application); P_0 - Uncontaminated soil, M_o – Unmanured treatment;
*M_1 is organic fertilizer applied at t/ha); P_1 -Mean of crude oil and organically amended crude oil contaminated soil; *cfu (colony forming units); sfu (spore forming units) ***

Table 4. Effects of period of incubation on growth and biomass yield of *Amaranthus* grown on manure-mediated crude oil polluted soil

Treatments	Number of plants/Pot	Stem weight (g)	Leaf weight (g)	Root weight (g)	Total plant biomass (g)	Head weight (g)	Plant height (cm)	Stem girth (cm)	Number of leaves
$T_0P_0M_0$		78.90bc	75.67a	17.00ab	171.6	31.67ab	136.66a	1.33a	31.66ab
$T_0P_0M_1$		131.33a	78.67a	22.00a	232	44.00a	149.33a	1.26a	33.66ab
$T_0P_1M_0$	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
$T_0P_1M_1$	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
$T_2P_0M_0$		130.33a	73.67a	18.33ab	222.3	32.67ab	136.66a	1.33a	31.66a
$T_2P_0M_1$		131.33a	80.33a	22.00a	233.7	43.67a	149.33a	1.26a	33.66a
$T_2P_1M_0$	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
$T_2P_1M_1$	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
$T_4P_0M_0$		126.33ab	74.00a	18.33ab	218.3	31.67ab	135.00a	1.23a	32.66ab
$T_4P_0M_1$		126.33ab	74.00a	17.33ab	217.7	31.67ab	136.66a	1.30a	29.00abc
$T_4P_1M_0$		78.33bcd	73.33a	15.33ab	166.9	46.67a	128.50a	0.85b	20.50de
$T_4P_1M_1$	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
$T_6P_0M_0$		43.67cde	41.33b	16.67ab	101.7	16.67bc	80.00b	0.83b	26.00bcd
$T_6P_0M_1$	0	0.0	0.0	0.0	0	0	0	0	0
$T_6P_1M_0$		36.33cde	32.00b	15.33ab	83.7	14.00d	57.66b	0.76bc	25.67bcd
$T_6P_1M_1$		32.67cde	27.00b	14.00b	73.7	13.00d	27.66c	0.56c	22.33cde
$T_9P_0M_0$		42.67cde	28.67b	11.33b	82.6	12.33d	27.33c	0.67bc	12.67f
$T_9P_0M_1$		26.33de	28.67b	19.00ab	74	15.33cd	24.16c	0.73bc	16.67ef
$T_9P_1M_0$		26.00de	27.00b	14.00b	67	13.33d	24.83c	0.67bc	17.00ef
$T_9P_1M_1$		35.67cde	21.33bc	12.00	69	13.33d	14.6c	0.56c	12.00f

T is time in months (0, 2, 4, 6 and 9 months after treatment application); P_0 - Uncontaminated soil, M_o – Unmanured treatment; M_1 is organic fertilizer applied at t/ha) P_1 -Mean of crude oil and organically amended crude oil contaminated soil

Crude oil pollution at 5% level produced significant increases in total biomass yield in *Amaranthus* from the commencement of the experiment up until six months after crude oil application. This trend was followed by decline in values of agronomic characters till the ninth month (Table 4). The results of the soil chemical analysis (Table 5) showed that 10 t/ha FYM reduced the acidity of the soil (increase in pH), while an increase in acidity was recorded at10 t/ha of organic fertilizer in each of the treatments. There was no seed germination in the pots that were polluted with 5% crude oil alone until four months after application. At four months, germination was observed for 5% crude oil alone ($T_4P_1M_0$) while there was no germination for 5% crude oil plus 10 t/ha Farm Yard Manure ($T_4P_1M_1$).

Crude oil pollution-enhanced toxicity had resultant effects on growth and vigour of *Amaranthus*. Soil application of 5% crude oil concentration resulted in retarded growth of *Amaranthus*. The necrotic and chlorotic nature of *Amaranthus* plants grown in the crude oil contaminated soil could be traced to the chemical constituents in the soil following from crude oil application.

3.3 Temporal Profiles of Soil Properties on Manure-mediated Crude Oil Polluted Soil

The temporal trends of soil chemical properties and microbial population on manure-mediated crude oil polluted soil are presented in Tables 5 and 6 for uncontaminated soil and for mean of crude oil alone and organically amended crude oil contaminated soil .The values of the soil parameters were record for measurements taken at commencement of experiments and at 2, 4, 6 and 9 months after treatment application (To, T_2, T_4, T_6 and T_9). The Tables demonstrated declining trends in the values of the measured soil chemical properties and microbial diversity. In general, the initial declining trends in values with time up to the forth months after crude oil application were followed by increases in soil chemical properties and microbial population with time.

The time dynamics and changes in elemental concentrations and soil biological populations with time following soil contamination with crude oil was monitored and the results are presented in Figs. 1 to 7. The figures demonstrated declining trends in the values of the measured soil chemical properties and microbial diversity (Figs. 1 to 7). However, data obtained on soil chemical constituents (pH, organic matter, CEC) biological populations were fitted to correlation and regression models and the summary of the regression relationships are depicted in Table 7. The observed trendlines in time-dependent elemental concentration and soil biological populations were described by power, logarithmic and polynomial relations (Table 7). These relationships gave a regression coefficient (R^2) which shows that the observed trends can be explained by a combination of factors. The initial declining trends in values with time up to 4 months after crude oil application were followed

Table 5. Temporal trends of soil chemical properties on manure-mediated crude oil polluted soil

Treatments	pH	Organic carbon g/g	Organic matter g/g	N g/g	P mg/kg	K	Na	Ca	Mg
						(cmol/kg soil)			
T_0P_0	5.74	1.47	2.54	0.13	7.08	0.56	0.69	5.5	3.0
T_0 P_1	6.22	2.29	3.94	0.25	29.12	0.65	0.71	5.25	1.75
T_2P_0	5.87	2.68	4.62	0.38	10.50	0.42	0.57	4.5	2.5
T_2 P_1	6.34	3.03	5.22	0.56	29.9	0.48	0.75	4.75	3.50
T_4P_0	5.82	2.80	4.82	1.08	10.03	0.66	0.59	4.0	1.4
T_4 P_1	6.0	3.37	5.82	1.34	12.5	0.59	0.61	4.38	0.9
T_6P_0	6.13	2.20	3.80	0.48	5.13	0.31	0.66	3.6	1.6
T_6 P_1	6.29	3.32	5.72	1.19	20.0	0.56	0.76	3.98	1.73
T_9P_0	6.19	2.09	3.60	0.94	8.48	0.28	0.42	3.4	1.8
T_9 P_1	6.30	3.10	5.36	1.36	36.29	0.48	0.56	4.80	2.13

P_0 - Uncontaminated soil; P_1 -Mean of crude oil alone and organically amended crude oil contaminated soil To, T_2, T_4, T_6 and T_9 were measurements taken at commencement of experiments and at 2, 4, 6 and 9 months after treatment application

Means followed by the same letters along column are not significantly different at 5% level of probability by linear increases in soil chemical properties and microbial population. The nature of the trends (polynomial function as best fit) indicated that the responses were not sole (uni-function) of a factor but suggest multiple contributions of the measured soil chemical constituents and activities of soil microbes for the decay of petroleum hydrocarbon compounds and hence its toxicity to soil chemical properties and biological activities.

Table 6. Temporal trends in soil microbial population on manure-mediated crude oil polluted soil

Treatments	Bacteria (10^6 cfu*/g)	Yeast (10^6 sfu**/g)	Fungi (10^6 sfu**/g)	Nematode (10^{10}/ ml)
T_0P_0	6.0	8.8	4.5	2
T_0 P_1	4.9	4.3	2.7	10
T_2P_0	1.2	2.0	1.8	23
T_2 P_1	5.3	2.3	2.7	14
T_4P_0	2.0	1.3	2.5	11
T_4 P_1	3.4	1.5	2.6	12
T_6P_0	4.2	1.8	2.3	20
T_6 P_1	5.4	2.9	3.1	16
T_9P_0	7.8	2.9	3.7	21
T_9 P_1	7.9	3.3	4.2	16

P_0 - Uncontaminated soil; P_1 -Mean of crude oil alone and organically amended crude oil contaminated soil; To, T_2, T_4, T_6 and T_9 were measurements taken at commencement of experiments and at 2, 4, 6 and 9 months after treatment application.

cfu (colony forming units); sfu (spore forming units) *

Table 7. Summary of regression relationships of time profiles of soil chemical and microbiological properties as affected by crude soil contamination and organic amendment

Soil properties (Means of crude oil and crude oil contamination plus organic amendment)	Regression equation	R^2
Soil pH	$Y = 0.028x^2 - 0.163x + 0.163$	0.13
Organic matter	$Y = 0.97Ln(X) + 4.26$	0.73
Nitrogen	$Y = 0.271x^{1.11}$	0.94
Cation exchange capacity (CEC)	$Y = -0.92Ln(X) + 2.81$	0.95
Bacteria count	$Y = 0.58x^2 - 2.86x + 7.6$	0.80
Free living soil nematodes	$Y = -1.04x^2 + 6.46x + 4.56$	0.58
Fungi count	$Y = 0.23x^2 - 21.1x + 3.72$	0.55
Yeast	$Y = 0.51x^2 - 3.17x + 6.78$	0.83

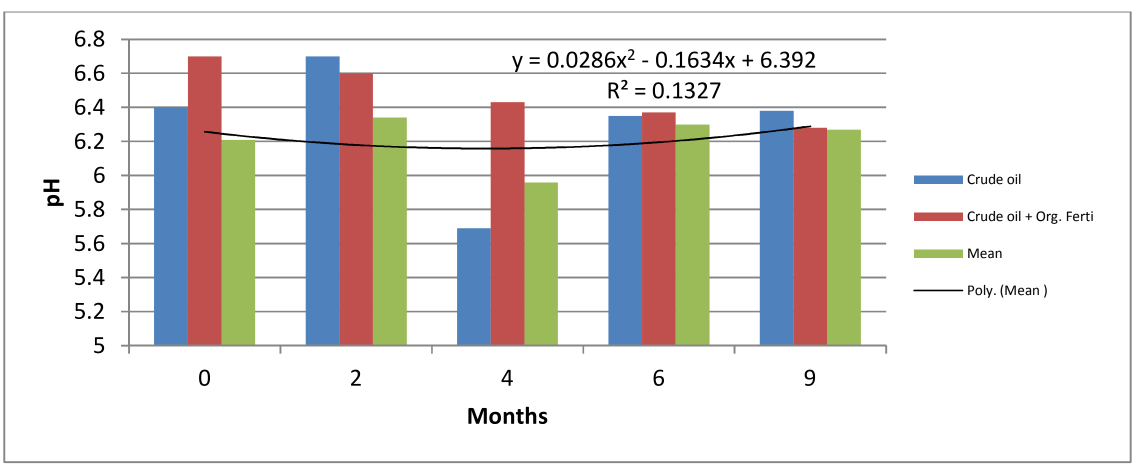

Fig. 1. Temporal profile of soil pH of manure-mediated crude oil polluted soil

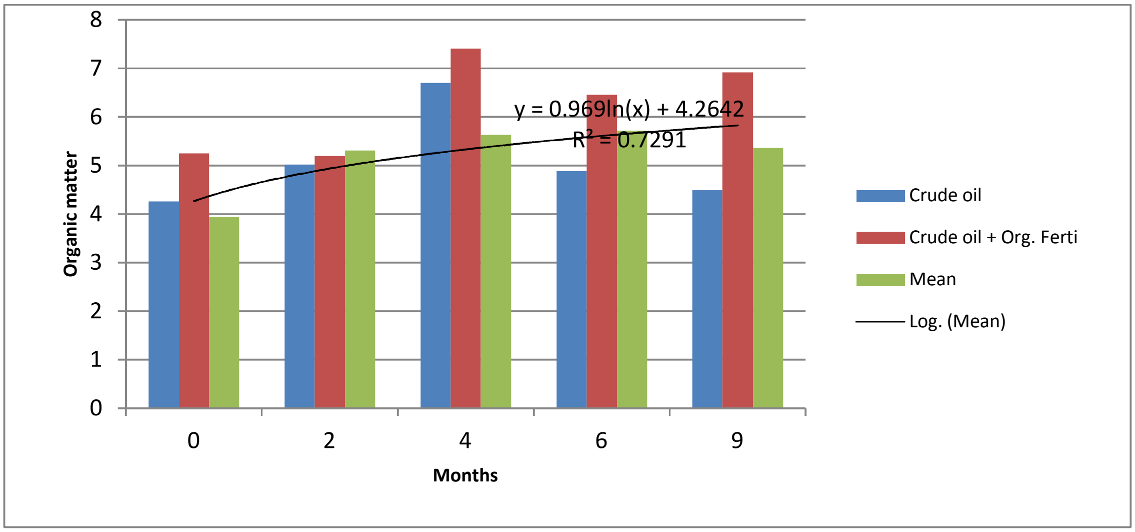

Fig. 2. Temporal profile of soil organic matter in manure-mediated crude oil polluted soil

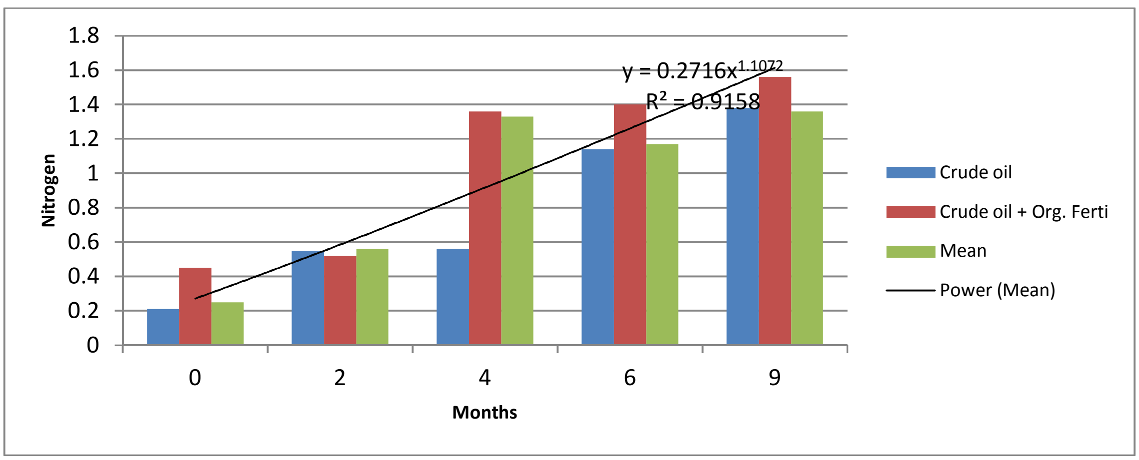

Fig. 3. Temporal profile of total nitrogen of manure-mediated crude oil polluted soil

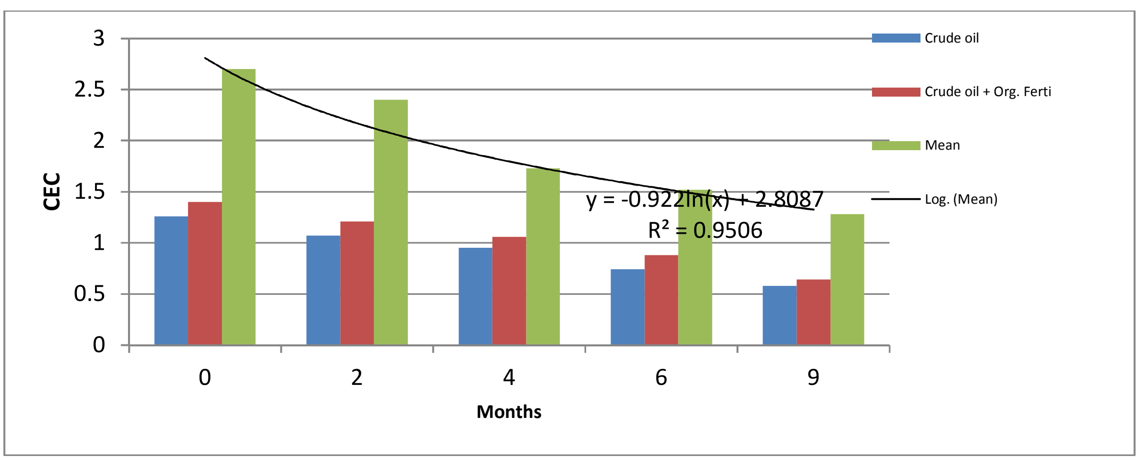

Fig. 4. Temporal profile of CEC of manure-mediated crude oil polluted soil

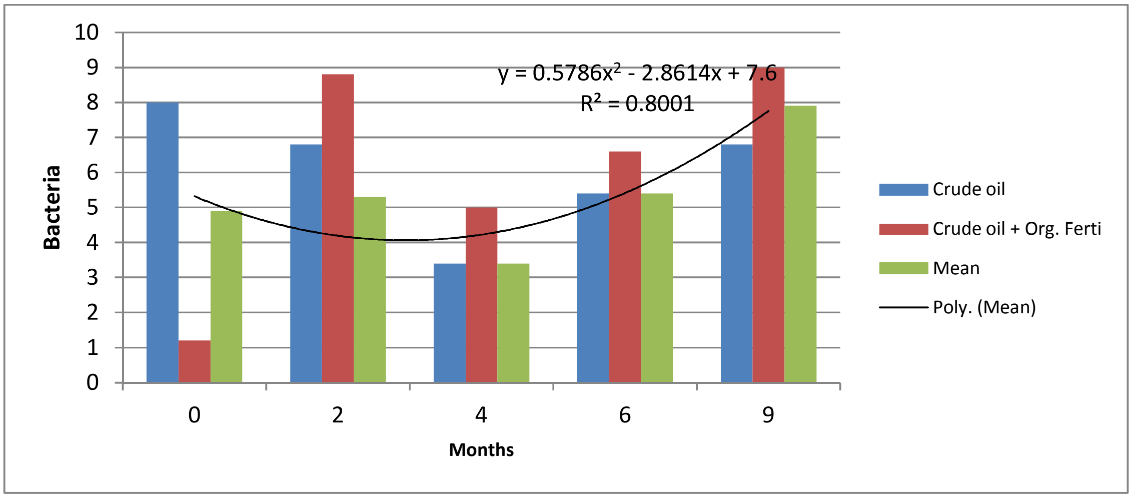

Fig. 5. Temporal profile of soil bacterial count in manure-mediated crude oil polluted soil

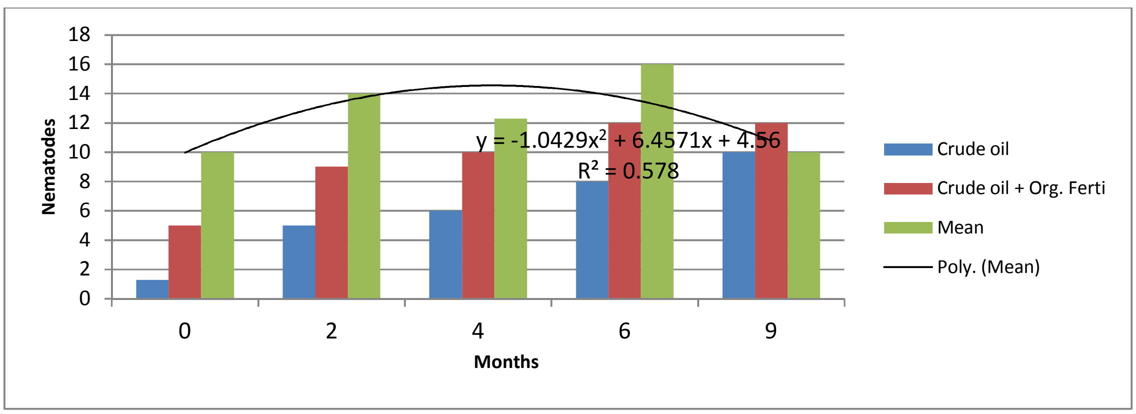

Fig. 6. Temporal profile of soil nemtaode counts in manure-mediated crude oil pollution

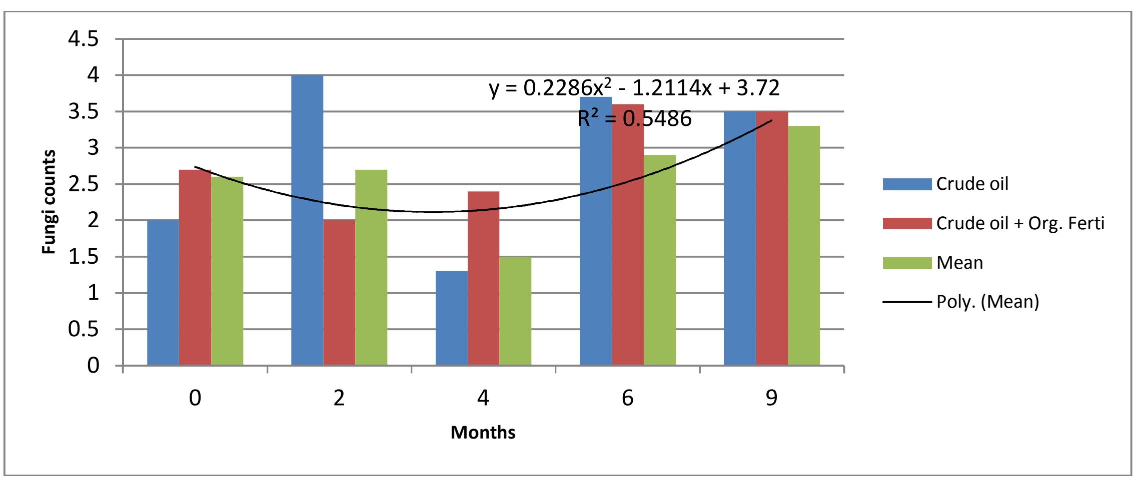

Fig. 7. Temporal profile of soil fungal in manure-mediated crude oil polluted soil

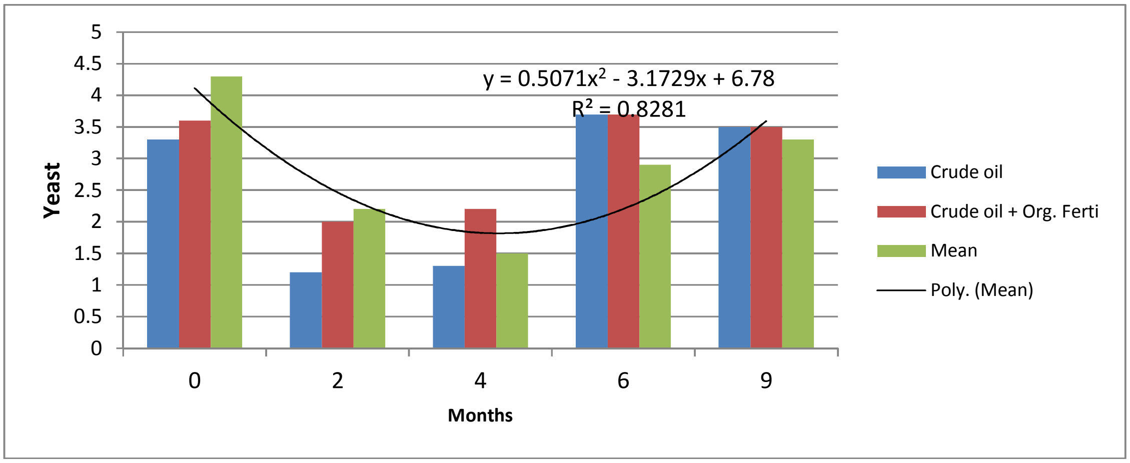

Fig. 8. Temporal profile of yeast in manure-mediated crude oil polluted soil

3.4 Effects of Periods of Incubation on the Profiles of Soil Properties on Manure-mediated Crude Oil Polluted Soil

At 4 months after treatment application (MAT), no germination of *Amaranthus* seeds was observed in the pots treated with 5% crude oil alone ($T_4P_1M_0$) or 5% crude oil amended with FYM compared with the uncontaminated control. However, between 6 and 9 MAT, there were significant differences among treatments (the control pot, crude oil contamination and organically amended crude oil contaminated soils) for most of the agronomic characters measured. These results were consistent with previous on the adverse effects of crude oil pollution on the germination of seeds, plant growth and vigor [1,3,12,26]. Crude oil contamination at 5% had significant effect on the total biomass of *Amaranthus* from the initiation of the experiment up until 4 months after treatment. The potted plants that germinated was consistent with previous reports on the effects of crude oil pollution on plant growth and leaf chlorosis and necrotic symptoms [27,28].

The lowest colony size (population) of soil microbes were low for all treatments at the commencement of experiment (zero time period). The population of the bacteria increases with time in all treatments. For example, about 9 million sfu/g of yeast was recorded for the control which is the highest compared the contaminated pots at 2 months of incubation. At 9 months of incubation, crude oil contamination alone and with organic amendment had highest soil microbial population. The population of yeast and fungi took a decreasing trend as the incubation period increases. Nematode population was high in the control and in the organically amended contaminated soils but reduced in the polluted plus amended during the incubation study. Soils with high clay contents had been shown to offer greater capacity for better physicochemical quality under petroleum hydrocarbon contamination via surface adsorption and microbial metabolism [11]. The metabolic activities of microorganisms had been attributed to have attenuated toxicity petroleum hydrocarbon compounds on soil [14,15,28].

Low concentration (5%) of petroleum hydrocarbon compounds produced relatively low toxicity on soil and *Amaranthus* plants. However, organic amendment appeared to have enhanced biodegradation in the soil. This level of petroleum hydrocarbon contamination does not pose serious challenge to the metabolic activities of soil microorganisms. The mixture of species of microbial consortium ensures synergy among microbial strains, when such is found in petroleum hydrocarbon contaminated soil are reported to enhance degradation [19]. High concentration of crude oil exhibited toxicity to soil microbial flora and thus the high concentration of oil which might likely had negative effects on the biodegradative activities of the microbial population in the contaminated soil [28]. The result is in agreement with the findings of Adeyemo et al. [9] who reported decrease in the rate of biodegradation of crude oil, as the concentration of oil increased. Abioye et al. [29] carried out a comparative bioremediation study in Malaysia on soil contaminated with 5% and 15%

of used motor oil. They reported that 92, 84 and 79% degradation were observed in soil contaminated with 5%, while 55, 49 and 36 % degradation in soil contaminated with 15 % in soil amended with 10% Brewery Spent Grain (BSG), Banana Skin (BS), and Spent Mushroom Compost (SMC) respectively in 84 days under laboratory condition. Higher biodegradation rates were recorded in soil contaminated with 5% and this has a significant relationship between the rate of biodegradation and concentration of oil in the contaminated soil. However, in an earlier study Abioye et al. [30] reported that soil contamination with 10 % used motor oil, 95, 93 and 92 % degradation was observed in soil amended with 10 % brewery spent grain, banana skin and spent mushroom compost respectively. The results of this study showed that best soil quality in terms of its physical, chemical and microbiological properties were recorded in the non-contaminated. Application of FYM to the crude oil contaminated soil appeared to have enhanced rate of biodegradation which can be attributed to increase in the activity of soil microbes in the crude oil polluted soils [30]. This might be due to the activities of petroleum hydrocarbon-utilizing soil micro flora in the organically (FYM) amended crude oil contaminated soil.

The results show inverse relationships between the concentration of crude oil applied and soil quality in terms of its physical, chemical and microbiological properties and *Amaranthus* growth and yield characters. The observation might be attributed to the toxicity of the oil on the soil microbial flora, high concentration of the oil retarded biodegradative activities of the microbial population in the contaminated soil [29]. Many microbes can metabolized a wide range of petroleum hydrocarbon compounds. The sensitivity of soil microflora to petroleum hydrocarbons is a factor of quantity and quality of oil spilled and pervious exposure of the native soil microbes to oil [18,27,28]. Schaefer and Juliane [28] also concluded that bioremediation is a useful method of soil remediation if pollutant concentrations are moderate, and that beyond 3.0% concentration, crude oil becomes increasingly deleterious to soil biota and crop growth. The toxic effects of crude oil pollution on seed germination was tested using *Amaranthus* seeds sown on the oil polluted soils. Other studied used wheat, an important agricultural crops, and its sensitivity to toxic chemicals (mostly petroleum contaminants), has led to its wide use for toxicity tests [3].

In the unamended contaminated soil, phytotoxicity was higher, perhaps due to a lower degree of hydrocarbon degradation as a consequence of the low microbial biomass content and microbial activity of this soil. The negative effect of hydrocarbons on soil microflora may be attributed to their inherent toxicity and/or to the perturbations they cause in soil and plants due to their hydrophobic properties [11]. Hydrocarbons may coat roots, preventing or reducing gas and water exchange and nutrient absorption; they may also enter the seeds and alter the metabolic reactions and/or kill the embryo by direct, acute. toxicity; after penetrating the plant tissues, hydrocarbons damage cell membranes and reduce the metabolic transport and respiration rate Petroleum hydrocarbon compounds as contaminant could have opposite effects on the plant and microorganism growth. Various studies had indicated that the higher the concentration of unsaturated compounds, aromatic, and acids, the more toxic the hydrocarbons are to soil microorganisms (population and activities) [8,9,27,28]. Adeyemo et al. [11] reported that soil and plant parameters were significantly associated with the residual soil hydrocarbon content following contamination with 3.0% and 4.5% spent oil during the 90 days period of study. Evaluation of soil chemical properties and biological diversity and population trends (activities) as monitoring tools for the degradation process (decay of petroleum hydrocarbon compounds and hence its toxicity to soil chemical properties and biological activities) was made via measurements of microbial diversity and their population counts.

4. CONCLUSION

The results of this study proved that crude oil (petroleum hydrocarbon compound) is capable of affecting some physio-chemical properties of soils and hence, makes the soil unfavourable for plant growth. The toxic effects of hydrocarbons on soil quality and health appeared to have been buffered by organic amendment confer higher organic matter and higher pH and CEC. Application of farm Yard Manure to the petroleum hydrocarbon contaminated soil offers an environmentally friendly technical option that if properly and thoroughly explored can lead to the attainment of a healthy and sustainable environment for both plant and animals. Soil chemical properties and microbial population were evaluated as monitoring tools for the degradation process (decay of petroleum hydrocarbon compounds) and hence its toxicity to soil chemical properties and biological activities. The results showed that soil microbiological population (microbial diversity and their population counts) may be useful tools for assessing the effect of petroleum hydrocarbon contamination on soil and environmental health. Organic manuring of crude oil-contaminated appeared to have provided soil conditions for activation of aerobic activity required in the biodegradation of petroleum hydrocarbon contents of crude oil. The results confirmed the potential and efficacy of organic amendment with farm yard manure and organic fertilizer against the toxic effect of crude oil contamination on soil physical and chemical properties, biological populations and *Amaranthus* performance. It is concluded that contamination of soil with petroleum hydrocarbon has a negative effect on soil health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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