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Influence of Spent Engine Oil Pollution and Organic Amendment on Soil Physicochemical Properties, Microbial Population and Growth of *Capsicum annuum* (L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MON and LAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JNA and MCG managed the analyses of the study. Authors CEI and RIAN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the impacts of spent engine oil on the physicochemical properties of soil, soil's microbial population and growth of *Capsicum annuum*. It covered assessment of different levels of contamination (0, 20, 40, 60 and 80%) in soil; which represents the degree of oil spillage concentration on the growth performance of *C. annuum* investigated. Percentage germination, seedling height, number of leaves and number of branches decreased as the concentrations of the spent engine oil in soil samples increased and affected soil physicochemical properties. The

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screening experiment conducted showed that poultry manure improved the physicochemical properties of sandy loam soils contaminated engine oil. The effects of poultry manure as an organic amendment was assessed using pepper (*C. annuum*) as test crop. All amendment made significant increase in soil organic carbon and calcium content over the polluted soils. Soil acidity increased, soil exchangeable ions decreased. N, P and K were altered in the polluted soils as compared to the controls. There were increased bacterial counts (2.21 – 2.85) and a decrease in fungi population (0.48 - 0.59) in the spent engine oil-contaminated soils compared with the control. The oil reduced germination percentage, depressed growth, reduction in leaf number and plant height of the *C. annuum.* Therefore the spent engine oil clearly had detrimental effects on soil's physicochemical and biological properties. The oil contributed largely to the extreme acidic nature of the polluted soils. However, maximum increase in plant height, germination percentage, number of leaves and branches were recorded with amendment of the polluted soils with poultry manure. Results show the considerable potential of remediation protocols with poultry manure as a remediating agent for oil spill remediation in the soil samples.

Keywords: Soil; amendment; manure; Capsicum annuum.

1. INTRODUCTION

Spent engine oil is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain. They are lubricants that have been used to operate an automobile machine and considered not fit for initial purpose [1]. Spent engine oil also referred to as used or waste motor oil is a mixture of different chemicals, including low and high molecular weight (C_{15} – C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they wear down [2,3]. Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment [4], but get into it when the motor oil is changed and disposed indiscriminately into the environment by motor and generator mechanics or artisans, and small scale engine oil sellers along the roads [5]. The waste oil also found its way into the environment during engine use and leaks released from the exhaust system [6]. The disposal of spent engine oil into gutters, water drains, open plots and farms is a common practice in Nigeria especially by motor mechanics. These oils, also called spent lubricating or waste engine oil, is usually obtained after servicing and subsequently drained from automobile and generator engines [7] and much of this oil is poured into the soil. This indiscriminate disposal of spent engine oil adversely affect plants, microbes and aquatic lives [8,9] because of the large amount of hydrocarbons and highly toxic polycyclic aromatic hydrocarbons contained in the oil [2]; [10].Heavy metals such as vanadium, lead, aluminium, nickel and iron which are found in large quantities in used engine oil may be retained in soil, in form of oxides, hydroxides, carbonates, exchangeable cation and/or bound to organic matters in the soil [11]. These heavy metals may lead to build up of essential organic (carbon, phosphorous, calcium, magnesium) and non-essential (magnesium, lead, zinc, iron, cobalt, copper) elements in soil which are eventually translocated into plant tissues [12]. Although heavy metals in low concentration are essential micronutrients for plants, but at high concentrations, they may cause metabolic disorder and growth inhibition for most of the plant species [13]. According to several authors [14,15] contamination of soil arising from oil spills affect the growth of plants and causes great negative impacts on food productivity. Spent engine oil contains complex mixtures of paraffinic, naphthalenic and aromatic petroleum hydrocarbons and various contaminants that may contain one or more of the following: carbon deposits, sludge, wear metals and metallic salt, aromatic and non aromatic solvents, water (as water-in-oil emulsion), glycols, silicon-based antifoaming compounds, fuel, polycyclic aromatic hydrocarbons [PAHs] and miscellaneous lubricating oil additive materials [16]. Engine oil becomes contaminated as a result of physical and chemical reactions. Metals from engine from time to time erode into the engine oil forming impurities. Oxidation of hydrocarbon chains bond together to form sludge due to high temperature. Incombustible gasoline up to about 5% wt often leak from fuel injector line, contaminating the oil [17]. Some additives such as multiple sulfur-based detergents which keep materials from depositing on the engine piston often begin to break down as sludge and accumulate in motor oil [17]. Used motor oils are also characterized by high concentrations of PAHs. According to Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) list of hazardous substances, PAHs ranked 7th in 2005 in the biennial ranking of chemicals deemed to pose the greatest possible risk to human health [18]. Some PAHs have been demonstrated to be mutagenic and carcinogenic in humans and those that have not been found to be carcinogenic may, however, synergistically increase the carcinogenicity of other PAHs [19]. The global emphasis on soil health and sustainable food security is persuading soil scientists to consider rehabilitation of degraded lands, especially where oil contamination limits the use of land. Thus information regarding the use of organic manure to improve physical, chemical and biological properties of soils contaminated with petroleum products, with a view of making them available for crop production is very important. The indiscriminate discharge of engine oil into water drain, open vacant plots of lands are becoming an acute environmental problem in Nigeria, particularly when large areas of agricultural land are contaminated. In most cases, the soil may remain unsuitable for crop growth for months or years, until oil is degraded to a tolerable level [7]. Depletion in nutrient status (Nitrogen and Phosphorus), inhibition of microbial activities and seed germination have been reported in spent engine oil contaminated soils [20]. The formation of waxy texture in soils contributes to reduction of oxygen content in such soils [7]. The formation of oily scum, which impedes oxygen and availability of water to biota as well as formation of hydrophobic micro-aggregates with clay surfaces, are associated with oil contaminated soils [21].

Soil is the key component of natural ecosystem and environmental sustainability depends largely on sustainable ecosystem [9]. Spent engine oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and an induction of a limitation in soil Nitrogen and Phosphorus [22]. In Nigeria, most of the terrestrial ecosystem and shore lines in oil producing communities are important agricultural land under continuous cultivation. The adverse effect of spent oil on these aerable agricultural lands have given rise to various treatment options such as the use of surfactants, alternate carbon substrates, organic and inorganic manure and bioremediation strategies [23,24]. The goal of sustainable agricultural practices is to control pest (Weeds, parasites, and pathogens) and maintain soil fertility and crop yields, while minimizing or eliminating synthetic chemical inputs. The application of organic amendment to soils (eg cover crops, manures and plant biomass) is an integral tool for sustainable agriculture, as evidenced by the increasing interest in optimizing this strategy [25]. Organic amendment has been shown to enhance soil organic matter and fertility as well as prevent erosion [26]. The addition of organic amendments on soil contributes to organic matter and has great potential for influencing the structure and function of soil food web and possibly inducing nematode suppression [27]; [28]. Organic amendments are known to increase the abundance of various components of the soil food web, including the soil microbial community, protozoa and microbivorous nematodes [29]. Spent engine oil contains non-aromatic, mono-aromatic and an extensive suites of polycyclic aromatic hydrocarbons(PAHs) which can be toxic to organisms [30] and pose significant hazards to human health and the earth's ecology if split. An area of land contaminated with petroleum hydrocarbons may remain totally barren for about ten years after a spill incidence, depending on the quantity of oil in the soil, the soil remediation techniques adopted and many other interacting environmental factors [31]. The soil pH of most spill sites fail within the range, suitable for plant growth, although the nitrogen level of such soil is usually low [31]. There is considerable potential for increasing world food supply by reclaiming polluted lands with organic amendment in low-production countries [32]. Hence, this research was carried out to ascertain the influence of spent engine oil pollution and organic amendment on soil physicochemical properties, microbial population and growth of *C. annuum* (L.).

2. MATERIALS AND METHODS

2.1 Study Area

Investigations were carried out in the screen house at the Department of Biology, Federal University of Technology Owerri, Imo state located at latitude 5.3866° N, and longitude 6.9916° E.

2.2 Sample Collection

2.2.1 Soil

The sandy-loam soil used in the study was collected from a farm in the department of crop science, Federal University of Technology Owerri.

2.2.2 Spent engine oil

The spent engine oil was collected from the mechanic village at Orji, Owerri north Imo state.

2.2.3 *Capsicum annuum*

C. annuum was purchased from Umuchima market, Ihiagwa owerri west Imo state.

2.2.4 Poultry manure

Poultry manure was collected from the poultry farm of the department of Animal science, Federal University of Technology Owerri, Imo state.

2.3 Experimental Design

The experimental design used was Complete Randomized Design (CRD), with 5 treatments and 3 replicates.

The total number of experimental soil samples (n) as the product of the number of treatments (T) and the number of replications (r); that is, n = rT . n = 3 x 5= 15.

3 replicates were uncontaminated which served as the control and 12 replicates were the polluted soils. The spent engine oil was applied in different concentrations; 20, 40, 60 and 80%%? respectively to the total experimental soil samples to stimulate oil spill onto soil, apart from the 3 replicates of the control which were uncontaminated. The seeds of the *C. annuum* were planted in all the experimental soils.

2.4 Laboratory Analysis Procedures

2.4.1 Particle size procedures

The percentage sand, silt and clay were determined using the bouyoucos hydrometer method according to Nelson and Sommer [33]. The size of the solids in the suspension were estimated from the density of the solution measured using the hydrometer.

2.4.2 Soil pH determination

This was determined in a suspension of soil water at ratio of 1:2 soil to water and measured using a glass electrode pH meter as was described by Nelson and Sommer [33].

2.4.3 Available phosphorus determination

Available phosphorus was determined using the Bray method of Grossman and Ranches [34].

2.4.4 Exchangeable base

These were determined from ammonium acetate (NH_4OAC) leachate of soil [35]. Exchangeable calcium and magnesium were determined by the EDTA (Ethylene Diamine Tetra-Acetic Acid) Versanate titration method [35]. Exchangeable potassium was determined by the flame photometer method [33].

2.4.5 Total nitrogen determination

The total nitrogen was determined using the micro kjeldahl digestion method apparatus [35].

2.4.6 Organic carbon determination

The organic carbon content of the soil sample was determined by Walkey-Black method and organic matter [33]. The organic matter was determined by multiplying organic carbon with Van Bemmelen factor of 1.724.

2.4.7 Isolation of soil micro-organisms (Bacteria and fungi)

After the serial dilution, agar plating on the vials, following Warcups soil plate and Waksman direct inoculation methods, 0.5 g of the soil sample was added to the Erlenmeyer flask containing 50 ml of agar and shake well for one minute. 0.5 ml was placed in a vial containing 4.5 ml of 0.1% agar. 1 ml was pipetted into each of the petri dishes containing potato Dextrose Agar (PDA) and was spread over the entire surface using glass stirring rod. The petri dishes were sealed with the parafilm and were incubated at room temperature and observed after 24 and 48 hours. The number of colonies per petri dish was recorded and counted.

3. RESULTS

3.1 Physicochemical Analysis

The result gotten from the physicochemical analysis of the soil before and after soil amendment showed that the spent engine oil altered the physicochemical properties of the soil but the application of the poultry manure remediated the adverse effects as seen in Table 1.

3.2 Microbial Analysis

The result of the microbial analysis indicated an increase in the microbial population of bacteria and decrease in population of fungi in the polluted soils and a decrease in the microbial population of bacteria and fungi occurred with application of manure as seen in Table 2.

3.3 Growth of *Capsicum annuum*

The polluted soils with spent engine oil showed an adverse effect on the test crop, *C. annuum.* Reduction in germination percentage, height, no of leaves and number of branches were recorded but there was increase in these growth parameters with the application of the poultry manure as seen in Table 3.

4. DISCUSSION

The results on the polluted soils showed that the spent engine oil altered the physicochemical properties of the soils, affected soil microbial population and the growth of pepper as reported by Kayode et al. [36].

Table 1. Physicochemical properties of polluted and non-polluted soils before and after soil amendment

Treatments	pH	OC	TN	AP	Ca	K	Mg	TC	Sand	Silt	Clay
Before soil amendment											
T_0(0%)	5.69	0.76	0.040	26.69	0.42	0.13	0.22	SL	87.15	5.15	5.73
T_1(20%)	5.32	2.68	0.021	18.16	0.42	0.12	0.22	SL	82.96	5.00	5.02
T_2(40%)	5.02	3.25	0.022	16.79	0.37	0.13	0.22	SL	82.62	5.01	5.02
T_3(60%)	4.94	3.43	0.019	13.77	0.40	0.12	0.22	SL	81.81	5.01	5.01
T_4(80%)	4.86	3.53	0.017	6.47	0.36	0.12	0.21	SL	81.59	5.00	5.02
After soil amendment											
T_0(0%)	5.49	0.76	0.041	26.68	0.42	0.13	0.22	SL	87.15	5.25	5.66
T_1(20%)	5.47	2.68	0.024	18.16	0.423	0.14	0.22	SL	78.22	5.07	4.72
T_2(40%)	5.05	3.35	0.025	16.82	0.40	0.14	0.22	SL	82.96	5.09	5.06
T_3(60%)	4.31	3.53	0.023	13.83	0.38	0.16	0.22	SL	78.45	5.25	4.67
T_4(80%)	4.23	3.76	0.024	6.46	0.39	0.15	0.21	SL	78.27	5.10	4.68

OC = Organic Carbon; TN = Total Nitrogen; AP = Available Phosphorus; Ca = Calcium; TC = Textural class; K = Potassium; Mg = Magnesium; SL = Sandloam

Table 2. Microbial population beffore and after amendment with poultry manure

Treatments	Microbial count ($\times 10^6$ CFU g^{-1})	
	Bacteria	**Fungi**
Before soil amendment		
T_0	2.42	0.67
T_1	2.90	0.65
T_2	2.73	0.64
T_3	2.37	0.60
T_4	22.22	0.25
After soil amendment		
T_0	2.43	0.59
T_1	2.85	0.48
T_2	2.66	0.54
T_3	2.31	0.52
T_4	2.21	0.40

CFU = Colony forming unit

Table 3. Growth parameters on polluted and non-polluted soils amended with poultry manure

Treatment	Germination%	Mean height (cm)	Mean no of leaves	Mean no of branches
Before soil amendment				
T_0	77^a	$4.7^a \pm 1.6$	$18^a \pm 3.4$	$2.7^b \pm 0.8$
T_1	23^e	$3.5^b \pm 1.2$	$12^c \pm 2.1$	$2.7^b \pm 0.8$
T_2	23^e	$3.4^b \pm 1.2$	$12^c \pm 2.1$	$2.3^c \pm 0.5$
T_3	13^f	$2.6^c \pm 0.7$	$11.3^c \pm 1.8$	$1.3^d \pm 0.4$
T_4	10^f	$0.9^e \pm 0.3$	$4.0^d \pm 1.2$	$1.0^d \pm 0.3$
After soil amendment				
T_0	78^a	$4.7^a \pm 1.6$	$18^a \pm 3.4$	$2.6^b \pm 0.7$
T_1	57^b	$4.4^a \pm 1.3$	$17^b \pm 3.2$	$3.2^a \pm 1.1$
T_2	57^b	$4.6^a \pm 1.5$	$16^b \pm 3.1$	$3.1^a \pm 1.0$
T_3	45^c	$3.2^b \pm 1.1$	$15^b \pm 3.0$	$2.0^c \pm 0.9$
T_4	39^d	$1.4^d \pm 0.5$	$6^d \pm 1.7$	$1.6^d \pm 0.8$

Means having different superscript along the column differ significantly at P = 0.05

At high concentrations of the spent engine oil in the soil, most species suffered remarkably decreased growth rates as accounted by Anoliefo and Okoloko [37]. High oil concentrations in soil decreased seedlings height and this was in accord [37,38,39]. Experimental results showed that seedling height of all species were significantly different at the various levels of contamination from those grown in non-contaminated soil. The control (non-contaminated soil had a mean height of 4.7 cm, whereas the contaminated soils with 20, 40, 60, and 80 concentrations had mean heights of 3.5 cm, 3.4 cm, 2.6 cm and 0.9 cm respectively indicating reduction in height with increased concentration of the spent engine oil in accord with the findings of Bamidele and Igiri [40]. The presence of spent engine oil pollution decreased the number of leaves produced by the *C. annuum* seedlings. Seedlings grown in non-contaminated soils produced significantly more leaves than those grown in oil-contaminated soils. Leaf production was strongly correlated with contamination. Similarly, leaf production as seen in Table 3. The number of leaves decreased as the concentration of spent engine oil in soil samples increased, this accords with Ezeala [41], who observed effects of oil pollution on leaf production in *Pistia stratoites*. There was equally decrease in the percentage germination and the number of branches with increased contamination.

The soil pH at all levels of oil-contamination tends to be acidic and the soil acidity was due primarily to the presence of spent engine oil in the soil samples. However, introduction of poultry manure into the oil-contaminated soil significantly decreased soil acidity as seen in Table 1, this observation accords with Osuji and Adesiyan [42], that crude oil-contamination increases soil acidity in the Niger Delta.

OC contents of soil increased due to spent engine oil contamination, which however is as the result of the carbon supplement from the hydrocarbons in the spent engine oil. OC in this study agrees with Thoma et al. [43], who observed a similar trend of OC in a soil sample contaminated with 3% by weight weathered crude oil that was phytoremediated with *Aeschynomene americana*. There was increase of OC with increase in spent engine oil contamination.

N and P are usually limiting in spent engine oil-contaminated soil [44] and similar observations were made in this study. However, a slight change in the values of these nutrients was noticed when amended with poultry manure. Similar trends were observed in K. Results of selected macronutrients indicated that N, P and K were altered in the polluted soils as compared to controls, but there was no significant difference.

The results obtained in this study showed that there were increased microbial counts in the spent engine oil-contaminated soil planted with *C. annuum*. Contamination of soil with hydrocarbons might increase hydrocarbon degrading micro-organisms in soils [45], this was demonstrated in this study.

The microbial populations increased in the spent engine oil contaminated soil at low (20) concentration, moderate at (60) concentration more than in the non-contaminated soils as seen in Table 2.

5. CONCLUSION

In conclusion, Oil-contamination affects the germination and growth of crops as seen in *C. annuum* and the physicochemical properties of the soil. The effects can be decreased by applying organic manure as seen in the application of poultry manure which can be used as an amendment agent of oil-contamination and is known to be an effective and environmentally-friendly remediation option for oil contaminated soils, which utilizes simple and inexpensive method of using 'nature to cleanse nature'.

6. RECOMMENDATIONS

In recommendation, the use of poultry manure should be implemented in the remediation of oil polluted areas especially in cropped areas for maximum crop production. Industries especially mechanic workshops should avoid the indiscriminate disposal of used engine oil, suitable means such as digging holes in non-agricultural areas for disposal should be devised, regeneration of used engine oil should be employed. Awareness should be created on the hazards accompanied by indiscriminate disposal of spent engine oil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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