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A preliminary study on the effects of E-Safe Treatment of Crude Oil Impacted Soil on microbial load, seed germination and Morphological Indices in Cowpea Seedlings

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Abstract

Effectiveness of E-safe© product Treatment of Crude Oil Impacted Soil on seed germination and Morphological Indices in Cowpea Seedlings was investigated at the Teaching and Research screen house of the Department of Plant Science and Biotechnology, Hezekiah University, Umudi, Imo State. The study was conducted on soil sample collected from aged crude oil polluted site in Rivers State. E-safe product was artificially spiked in each of the 4kg soil at 0 ml, 6 ml, 12 ml, 24 ml, and 48 ml and arranged in a Completely Randomized Design. The percentage germination and morphological parameters were estimated for a period of two (2) weeks. Microbial load was determined using basic microbiological protocols. At 0ml/kg (polluted untreated) there was a drastic inhibition in germination inhibition (46%) compared to the treated soil group in which the germination percentage was estimated to be higher (79%). Morphological indices of Cowpea were negatively affected by crude oil impacted soil. Crude oil contamination of the soil led to significant decrease in contents of chlorophyll a, chlorophyll b and total chlorophylls in cowpea seedlings but treatment with E-Safe product obviously improved the chlorophyll contents in seedling grown on E-safe treated soil. The highest bacterial load was obtained from 48ml (3.29 x10^{6} CFUg^{-1}) soil samples while the least values were gotten from control (1.02 x10^{6} CFUg^{-1}). The highest fungal load was obtained from 24ml soil (0.91 x10^{6} CFUg^{-1}) while the least was recorded at the control soil (0.05 x10^{6} CFUg^{-1}). The mineral components of the E-safe product could have acted as effective substance in the total removal of oil contaminants in the contaminated soil. Based on the results of this study, treatment of crude oil impacted soil with E-safe improved germination and early seedling growth of cowpea and could be used in reclamation of crude oil impacted agricultural soils in the tropics.

***Keywords**: E-Safe Treatment, Crude Oil, Soil, seed germination, Morphological Indices, Cowpea*

Introduction

Soil contamination occasioned by indiscriminate spill of petroleum and its allied products is considered a serious challenge in Imo [1] and in Nigeria at [2-3]. Unguarded disposal of crude oil has been documented to be the most remarkable ecological contaminant in most parts of Nigeria today [4].

Soils are a rich ecological system comprising of both abiotic and biotic matter with varying levels of interaction [5] and provision of ecosystem services. Globally, human societies are interconnected economies that rely on services provided by the ecosystems which constitute the foundation upon which human existence is based. The resilience of socio-ecological systems solely depends on a sustainable

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management of these ecosystems, hence, the need for sustainable eco-friendly management strategy to curb the menace of environmental contamination [6].

Petroleum and their allied products are a critical part of modern life and a cornerstone of any modern industrial strategy [7]. Environmental problems posed by indiscriminate disposal of petroleum products have thus far contradicted their original beneficial use [8]. In recent years, there has been growing concern over environmental pollution by petroleum products, mounting public apprehension over the ecological and human health effects and the consequence of global warming [9]. However, since the commercial exploration and exploitation of petroleum products in Nigeria since 1958 [10] petroleum and its allied products have become the lynchpin of the Nigerian economy with the petroleum exploration, exploitation and distribution activities leading to the pollution of land and waterways [11]. A typical case in point is the Niger Delta region of the country where oil exploration and exploitation are carried out on regular basis [12]. The creeks and fishing water have become more or less dead [13] and the agricultural lands in these areas have become infertile and less productive.

In Nigeria, contamination of soil and water by crude petroleum and refinery products is a rising problem as oil mining and refining activities increase steadily. This problem results particularly in the loss of fertility of agricultural lands and death of plants, including crops, in the oil producing areas of Nigeria. Soil contamination by crude oil spills is a widespread environmental problem requiring cleaning up of the contaminated sites. The most common sources of petroleum contamination are the disposal of oil- based wastes, oil spills from well blowouts and pipeline ruptures [14]. Crude oil spills adversely affect plants by creating conditions which make essential nutrients like nitrogen and oxygen required for plant growth unavailable to them. It has been noted that oil contamination results in a slow rate of germination in plants. [15] stated that this effect might be due to the oil which acts as a physical barrier preventing or reducing the access of seeds to water and oxygen.

[16] reported that oil pollution thwarts crop growth and yield in those areas for changing periods of time. Crude oil adversely affects the germination, shoot growth and yield of most plant species including seashore plants and field grasses [4] Crude oil and its products are made up of aliphatic, oleic, naphthenic and aromatic hydrocarbons, which change the chemical and physical properties of soil and its structure. These compounds are mainly responsible for the altered fertility of soil. [17] noted that oil in soil has deleterious effects on the biological, chemical and physical properties of the soil depending on the dose, type of the oil and other factors.

Crude oil impedes proper soil aeration as an oil film on the soil surface acts as a physical barrier between air and soil thereby causing a breakdown of soil texture followed by soil dispersion. Crude oil alters the soil's redox potential ratio and also increases the soil's pH. Hence, as crude oil pollution levels increase, soil pH also increases. [18] found that the microbiological components of soil are usually adversely affected when oil is applied to soil. The alteration in the physical and chemical nature of the soil is one of the environmental challenges posed by oil pollution, which consequently affects the growth of plants. Petroleum hydrocarbon contamination may affect plants by retarding seed germination and reducing height, stem density, photosynthetic rate, and biomass or causing complete mortality.

Certain plants can render harmless, extract or stabilize a contaminant in soil, therefore making it unavailable for other organisms and reducing environmental hazards in a process termed as phytoremediation. Recent phytoremediation techniques require that plants live in the zone of contamination. Subsequently, plant viability is a critical issue in the successful application of phytoremediation. Cultivation of plants can be a valuable tool in soil remediation if the contaminant in its present concentration is not phytotoxic [19].

Although conventional methods, such as physical removal, often are the first response option, they rarely achieve complete cleanup of oil spills. Bioremediation is beginning to emerge as a promising technology, particularly as a secondary treatment option for oil cleanup. Bioremediation has been defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes". It is an evolving method with the advantages of cost effectiveness and noninvasiveness to the species that are adapted to the environment. This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature. Bioremediation has several potential advantages over conventional technologies, such as being less costly, less intrusive to the contaminated site, and more environmentally benign in terms of its end products. As opined by [11] the success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation [10].

Among the more soluble and toxic compounds found in crude oil polluted soil and water, aromatic compounds (e.g., benzene, toluene, ethylbenzene, xylene isomers, and phenols) are of great concern as the removal of these compounds is extremely strenuous. Furthermore, they are highly toxic, which hinders the direct use of biological treatments. To overcome this worrisome limitation, E-Safe has been introduced and applied in this research work for the reduction of clean-up costs and permanent elimination of hazardous wastes without the need for secondary clean-up.

Therefore, the prime objective of the current investigation was to investigate the effect of E-safe treatment of crude oil impacted soil on morphological indices of cowpea.

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Materials and methods

Study area

The study was carried out at the Teaching and Research Laboratory of the Department of Plant Science and Biotechnology, Hezekiah University, Umudi, Imo State located at Latitude 5.3866° N, and Longitude 6.9916° E.

Collection of soil samples

Soil samples used in this study was collected from an aged oil polluted site in Rivers State, Nigeria. The samples were collected by using improvised soil auger at from 0 cm to 30 cm. The auger was screwed to the desired depth and the sample was withdrawn. Soil samples were transferred to plastic bags and sent to the laboratory for further studies. The soil has been polluted with Petroleum Hydrocarbons due to accidental spills but more by third party interference on the crude oil pipelines.

Sample Preparation

The samples were air-dried after being transported to the laboratory, and any grass or other items were removed manually. After rolling the samples to break up big clumps of soil particles, sifting was performed using a mechanical sieving system with various mesh sizes. For further investigation, the sieved samples were placed in their respective cleaned and labeled plastic buckets. Preliminary analysis and remediation of crude oil impacted with E-Safe at 6 ml, 12 ml, 24 ml, 48 ml, and 0 ml (control) had been carried out as reported in our previous work (Iheagwam *et al.*, 2023)

Experimental Design and treatment combination

The experimental design used in this experiment 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. Approximately 4kg of crude oil polluted soil was measured and filled in plastic buckets and spaced 1.5 m apart. The buckets were perforated at the base to allow aeration. The E-safe product was thoroughly mixed and applied in each of the experimental soil at five treatment levels: 6 ml, 12 ml, 24 ml, 48 ml, and 0 ml (control) respectively.

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

Statistical analysis

Data collected were presented in charts and tables and means were separated using Duncan Multiple Range Test (DMRT) at probability of < 0.05 level.

Results and discussion

As shown in Figure 1, germination was negatively and positively affected by E-Safe treated crude oil polluted soil. It is obvious that there was a significant increase in germination for concentrations above the second lowest, with a monotonic dose-response relationship. At 0ml/kg (polluted untreated) there was a drastic inhibition in germination inhibition (46%) comparatively to the treated soil group in which the germination percentage was estimated to be highest at 48ml treated soil (70.8%).

This implies that while crude oil treatment of soil inhibited the germination of cowpea seedlings, the addition of E-Safe product soil improved the germination of the seedlings compared to seedling grown in untreated soil.

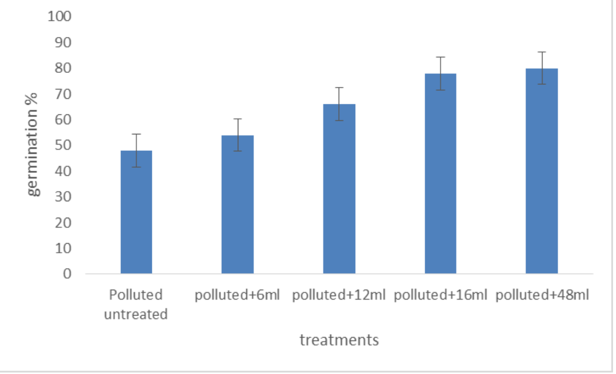

Figure 1: **percentage germination of cowpea seedlings grown on E-safe treated crude oil polluted soil.**

Morphological indices of Cowpea were negatively affected by crude oil and E-Safe treatment of crude oil polluted soil (Table 1). An evidential decrease in Leaf length (cm), Leaf width (cm), Plant Height (cm), Stem length (cm), and Root length (cm), was observed at 0ml/kg oil concentration in the soil, and an increase in growth parameters assayed was detected E-safe treated soil. The increase in morphological indices of cowpea seedlings growing in E-Safe treated crude oil polluted soil showed a potential ability of E-Safe to remediate toxic effects of crude oil as reported in our previous study [20]. This observation also gives credence to the postulation that organic materials reduce the impact of crude oil on plants [11]. The treatment of soil with E-safe product positively modulated the effect of exposure of cowpea seedlings to crude oil polluted soil.

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Table 1: Morphological indices of Cowpea grown on E-safe treated crude polluted soil

Groups (mg/kg)	Leaf length (cm)	Leaf width (cm)	Plant Height(cm)	Stem length(cm)	Root length(cm)
1	13±1.8^a	13.6±1.16^a	8.4±1.14^a	8.7±1.09^a	5.3±1.06^a
2	12±1.24^a	11.0±1.15^a	6.1±1.11^a	7.9±1.03^a	5.8± 0.84^a
3	7±1.13^b	9.5±1.13^b	5.6±1.10^{ab}	5.3±0.82	5.6±0.71^a
4	3± 0.67^c	7.4±1.07^b	5.3±1.06^b	4.9±0.72^b	4.3±0.70^b
5	3± 0.41^c	5.7±1.01^c	3.5± 0.43^c	3.3± 0.60^c	2.9±0.44^c
6	14± 1.24^a	15.5±1.12^a	7.8± 1.11^{ab}	6.6±1.08^b	5.1±0.69^a

Values are mean+ SD of triplicate samples; Mean along the column having different superscript of letters differ significantly at P≤ 0.05 level according to Duncan Multiple Range Test (DMRT). Key: Group 1 = Control (unpolluted); Group 2 = Polluted control; Group 3 = polluted with 6ml/kg of E-Safe; Group 4 = polluted with 12ml/kg of E-safe; Group 5 = Polluted with 24ml/kg of E-safe; Group 6 = Polluted with 48ml/kg of E-safe.

Crude oil contamination of the soil led to significant decrease in contents of chlorophyll *a*, chlorophyll *b* and total chlorophylls in cowpea seedlings but treatment with E-Safe product obviously improved the chlorophyll contents in seedling grown on E-safe treated soil as evidenced in Figure 2. The decrease in contents of chlorophyll *a* and total chlorophylls in conditions of crude oil was more significant in the untreated plants than in treated plants. According to [20] as the concentration of crude oil increases, the content of chlorophyll a , chlorophyll b , carotenoids, and total pigments decreases significantly. The level of reduction in chlorophyll content in plants has been an indication of environmental contamination [13]. Parallel observation was also observed by [9]. Decrease in the total chlorophyll content in the leaves is perhaps due to the alkaline condition created by dissolution of chemicals present in the oil in the cell sap which was responsible for chlorophyll degradation.

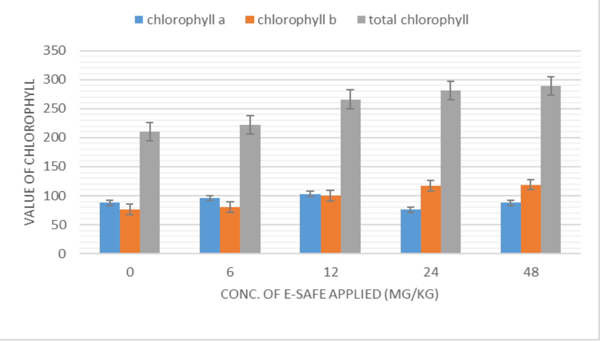

Figure 2: Average concentrations of chlorophyll a, chlorophyll b and total chlorophyll of cowpea grown on E-safe treated crude oil impacted soil

Microbial Population before and after application of E-Safe

Results of bacteria and Fungi Population before and after application of E-safe product is depicted in Table 2. The results showed that there was an increase in microbial load of the soil samples with the application of E-Safe. The highest bacterial load was obtained from 48ml (3.29 x10^6 CFUg^{-1}) soil samples while the least values were gotten from control (1.03 x10^6 CFUg^{-1}). The highest fungal load was obtained from 24ml soil (0.91 x10^6 CFUg^{-1}) while the least was recorded at the control soil (0.05 x10^6 CFUg^{-1}).

Table 2: Mean Bacteria and Fungi Population before and after application of Poultry dropping (PD).

Treatments	Microbial count (x10^6 CFUg^{-1})	
	Bacterial	**Fungi**
Before application of E-Safe		
Control	1.03	0.05
After application of E-Safe		
Control	1.02	0.03
6ml	1.68	0.18
12ml	2.69	0.23
24ml	3.0	0.91
48ml	3.29	0.79

CFU = Colony forming Unit.

Conclusion

Based on the results of this study, treatment of crude oil impacted soil with E-safe improved germination and early seedling growth of cowpea and could be used in reclamation crude oil agricultural soil in the tropics. In general, a weaker decrease in growth parameters and in contents of chlorophyll *a* and total chlorophylls were observed in treated polluted soil compared to the untreated soil oil. The results of the current study reveal that cowpea plants are able to grow on E-safe oil-polluted soils. Based on this, cowpea variety is considered a promising crop for cultivation on moderately oil-polluted soils with the aim of their decontamination. Biosurfactant enhances microbial degradation of hydrocarbon substrate by increasing the bioavailability of the substrate through emulsification and by facilitating association of hydrophobic substrates with bacterial cells through reduction of cell surface hydrophobicity of the bacterial cell. E-safe enhances the rate of hydrocarbon biodegradation.

Conclusively, this research suggests that E- safe serves as the best remedy for the bioremediation of crude oil contaminated soils for crop production as its cost implication is minimal and the product yields expected result within 8 weeks of treatment. At the same time, further studies should be undertaken in order to investigate the effects of E-safe treated soil on biochemical indices of crops.

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