



Comparative study of the Effects of Three Edible Crops on Complementary Biostimulated-Landfarming of petroleum Products Polluted Soils

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Abstract

Bioremediation has been documented as a cost effective and environment friendly approach to decontaminate hydrocarbons polluted soils, but not effective for heavy pollution levels. This study investigated the use of multi-facet bioremediation approach using edible crops that could serve a dual purpose of being phytoremediation plant and food provider. Soil samples were spiked with petroleum hydrocarbon products' mixture (PHPM), comprising of diesel, benzene, toluene, ethyl-benzene and xylene and naphthalene making a 5 or 10% levels of pollution. The samples were biostimulated with mineral and organic fertilizers at the rate of 0, 10 and 20% using NPK (15:15:15) fertilizer (NPKF), Chicken layer compost manure (CLCM) and Chicken layer manures digestate (CLMD). The polluted soils were landfarmed for 336 days; then, analyzed for the residual total petroleum hydrocarbon (RTPH) according to USEPA method 815B using GC-FID. Three edible crops, maize, okra and bean were then tested for their phytoremediation capacity using the landfarmed soils. The results showed that, at day 84, the soils' RTPH were reduced by the preceding phytoremediation from 6185.72 mg/kg to 49.49, 69.28 and 1779.01 mg/kg soil representing 99.20, 98.88 and 71.24% remediation when maize, bean and okra were used respectively for the phytoremediation as against the value of 3498.64 mg/kg for the control samples spiked with same 5% PHPM. Similar trend was observed in the 10% PHPM polluted samples. The study buttressed the use of maize and revealed brown bean to be another good option for phytoremediation of heavily petroleum polluted soils.

Keywords: Biostimulation, Landfarming, Germination Test, Phytoremediation, Petroleum Hydrocarbon Polluted Soils

Introduction

Phytoremediation is one of the domains of bioremediation technology (Murphy & Coats, 2011; 2005; Ali et al., 2023). In recent years, different bioremediation approaches have been applied in decontamination of polluted edaphic and aquatic environments with the effectiveness and efficacy of these applied approaches subjected to various scientific investigative procedures (Ali et al., 2023; Abatenhet al., 2017; Mmom&Deekor, 2010; Oghoje et al., 2023). This is because, the bioremediation technology has been well documented to be cost effective, environment friendly and compatible and can as well be localized (USEPA, 1994). Biostimulation, bio-augmentation and landfarming have been well researched and reported to be effective in the removal of various pollutants from soils (Ali et al., 2023; Feiziet al., 2020; Ifijen, 2018; Oghoje&Ukpebor, 2020; Oghoje et al., 2023). However, the active drivers of these bioremediation techniques are the microbial flora or fauna or both present in the polluted environment as indigenous or are intentionally introduced to the polluted environment as exogenous (Ali et al., 2023; Bing et al., 2019). But the tolerance of microorganisms to environmental

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pollutants is quite limited (Radwan et al., 2000; Rehman et al., 2020). Consequently, relatively high levels of environmental pollution would not be effectively remediated via biostimulation, bio-augmentation or landfarming without prior or post remediation treatments. For example, Mmom and Deekor (2010), reported that landfarming would not be effective for the remediation of petroleum polluted soils higher than five percent (5%) per 100g soil). This is because high level of petroleum pollution would destroy indigenous microorganisms and reduce the population of exogenous pollutants degraders or inoculants in hydrocarbon polluted soil and water (Akpovetaet al., 2011). The limitation of environmental pollutants' degrading microorganisms exposed to elevated levels of organic pollution, particularly with petroleum products and its allies, has necessitated further investigation into the usage of multi-remediation approaches, in which, one technique could complement another for better soil and water remediation results (Xiao-Dong et al., 2005).

Phytoremediation has been described as the usage of plants to enhance the degradation and transformation of environmental contaminants (Xiao-Dong et al., 2005). Plants roots could absorb soil and water contaminants and bio-transform and or accumulate them in their roots or shoots (Shimpet al., 2009). Plant roots also produce oxidase/oxidants in the roots' rhizosphere which could enhance soil microbial population and diversity for biodegradation of various soil pollutants (Shrestha et al., 2019; Wuana and Okieimen 2010). Previous studies focused more on the usage of perennial plants whose parts are not edible to human and livestock. Few reports have been documented for the use of edible crops for phytoremediation (Wuana&Okieimen 2010). Even when such crops could play a dual role of being used as phytoremediation plants and also, as food for human and livestock (If found to be safe). Therefore, in this study, three local crops, maize (*Zea may L*), okra (*Abwemoschuseculentus*) and brown bean (*Phaseolus vulgaris*) which are commonly cultivated in the Niger Delta region of Nigeria, an area often exposed to petroleum hydrocarbon pollution, were investigated. These crops could relatively grow well in the poor nutrients and clay-loamy soil type of the region. Some previous reports on the utilization of maize for phytoremediation of petroleum hydrocarbon pollution soils have been encouraging (Wuana&Okieimen 2010). Hence, this study compared the phytoremediation potential of the local brown bean and okra to that of maize. Specifically, the study focused on the tolerance of the crops to petroleum products pollution and their ability to complement in biostimulated-landfarming (A technique that involves addition of nutrient supplements and tilling of the polluted soils) for effective remediation of heavily petroleum polluted soils. The concentrations of total hydrocarbon (TPH) in the polluted and remediated soils were utilized as indices of evaluation in the study.

Materials and Methods

Soil sampling and processing

Composite bulk soil was collected from University of Benin oil palm farm, Igue, Edo State, Nigeria at the depth of 0 – 30 cm with a stainless spade and taken to Chemistry department of the University in polyethylene bags for processing. The soil was air-dried in a greenhouse for days, ground and sieved through 2mm mesh and kept in plastic container for future use.

Soil spiking

Addition of the PHPM (Which comprises of various proportions of weathered diesel, benzene, toluene, ethyl-benzene xylene and naphthalene) to the soil was done sequentially using three rates, 0, 5 and 10% (Wrt the soil dry weight). Specifically, the soil samples were spiked with PHPM, comprising of diesel (4 or 8%) benzene, toluene, ethyl-benzene and xylene (0.2 or 0.4% each) and naphthalene (0.2 or 0.4%) making the 5 and 10% of the PHPM pollution. In other words, 0, 50 or 100g of the PHPM was added per kilogram of the processed soil. The zero (0) % PHPM spiked soils served as the control for the crops' seeds germination tests. The procurement, treatment of the diesel and preparation of PHPM and stabilization of the spiked soils was reported earlier (Oghoje&Ukpebor 2020; Oghojeet al., 2023).

Samples Biostimulation

Three forms of flora-nutrients supplement NPK (15:15:15), CLCM and CLMD were applied consecutively to the separated samples at rates of 0, 10 and 20% (Wrt the levels of PHPM pollution) per week up to four (4) weeks. Specifically, a total of 0, 40 and 80g of the fertilizers were added accordingly to the 5 and 10% PHPM polluted soils (Table 1). The PHPM spiked samples to which no nutrient supplements was added (I.e 0% biostimulation which are PSC5 and PSC10) served as the controls for comparing the percentage removal of TPH in the biostimulated samples. The procedures for the sequential nutrients supplements addition and the concentration of each Biostimulants to the PHPM spiked soil samples have been reported (Oghoje&Ukpebor 2020; Oghojeet al., 2023).

Proper labeling and coding of the PHPM spiked and un-spiked soil samples as well as the biostimulated and non-biostimulated samples were done. And the samples were properly arranged in sets according to their levels of PHPM pollution, rates and forms of biostimulation in a greenhouse used for the study.

Table 1: The characteristics of the treatments made on the samples

Samples labels/codes	Level of PHPM Used (%)	Rates (%) and forms of Biostimulation		
		NPK (15:15:15)	CLCM	CLDM
BTC	-	-	-	-
PSC5	5	-	-	-
NPKF510	5	10	-	-
NPKF520	5	20	-	-
CLCM510	5	-	10	-
CLCM520	5	-	20	-
CLDM510	5	-	-	10
CLDM520	5	-	-	20
PSC10	10	-	-	-
NPKF1010	10	10	-	-
NPKF1020	10	20	-	-
CLCM1010	10	-	10	-
CLCM1020	10	-	20	-
CLMD1010	10	-	-	10
CLMD1020	10	-	-	20

Phytoremediation of the biostimulated-landfarmed PHPM soils

At the end of the biostimulated-landfarming (Which involved the addition of the nutrients supplements and regular tilling and spreading of the polluted soils) of the PHPM polluted samples, the 0, 10 and 20% CLCM/CLMD biostimulated soils were pull together accordingly making three sets of the PHPM polluted soils. That is, 0, 10 and 20% CLCM/CLMD biostimulated-landfarmed soils. The soils were thoroughly harmonized and the residual TPH were evaluated. Then, 5 kg (n = 3) of each soil samples were weighed into separate plastic containers (bottom- perforated). And the three crops, bean (*Phaseolus vulgaris*), maize (*Zeamay L*) and okra (*Abwemoschusesculentus*, Figure 1) were plated separately to mimic field phytoremediation as a compliment remediation technique; while the 0% organic biostimulated-landfarmed PHPM polluted soils received no crop planted on them (They served as the control samples). The crops were watered daily after germination for the first two (2) weeks and twice weekly up to day 84. Sampling from the experimental samples was done at day 28, 56 and 84 and the chemical analyses for the residual TPH were carried out accordingly (USEPA Method 8015B, 1996), to ascertain the impact of the crops for soil phyto-remediation using the percentage removal of the RTPH from these previously biostimulation-landfarmed soils, as measurement. All the physical and chemical analyses were done according to standard methods and procedures as earlier reported (Oghoje&Ukpebor, 2020; Oghojeet al., 2021, 2023, 2024).



Figure 1: Three local crops used for the the Phytoremediation

Soil Physicochemical and hydrocarbons analyses

Prior to the soil spiking, the soil was characterized for its physicochemical and TPH content. And after the soil spiking with the PHPM, samples were collected at days 1, 14, 28, 56, 84, 164 and 336 for TPH analyses to ascertain the levels of the biostimulated-landfarming remediation of the PHPM polluted soils. This result has earlier been documented (Oghoje&Ukpebor 2020; Oghojeet al., 2021, 2023, 2024). The proceeding phytoremediation of the partially bioremediated soils followed similar chemical analysis for the RTPH.

Quality control and Assurance

All the chemicals including the reagents used in the chemical analyses were of analytical grade procured from Sigma Aldrich, UK. The sample handling apparatus were well cleaned and dried according to standard methods while the instruments used were carefully calibrated according to standards (Okalebo, 2002).Prior to their usage to ensure reliable, valid and verifiable data.

Results

The study results are as presented in table 2 and 3. Table 2 showcased the effects of the three named edible crops as phytoremediation plants on moderately petroleum products polluted soils while table 3 expressed the impact of using the same named crops as phytoremediation tools in complementary landfarming of heavily petroleum hydrocarbon polluted soils.

Table 2: The effects of the selected crops on the remediation of the 5% PHPM polluted soils

Crops	PHYTOREMEDIATION PERIODS (DAYS)							
	1	28		56		84		
	^a RTPH Conc. (mg/kg)	^b RTPH ^b Conc. (mg/kg)	^c PRem (%)	^b RTPH Conc. (mg/kg)	^c PRem (%)	^b RTPH ^b Conc. (mg/kg)	^c PRem (%)	
Control (No crop)	6186 ±307	5162 ±227	16.55 ±0.88	4226 ±203	31.68 ±0.75	3499 ±47.57	43.44 ±1.55	
Maize	6186 ±307	3546 ±117	42.68 ±0.55	2216 ±113	64.17 ± 0.59	49.49 ±2.51	99.20 ±2.99	
Bean	6186 ±307	3510 ±126	43.25 ±0.45	2086 ±102.35	66.28 ± 0.55	68.28 ±3.55	98.84 ±3.75	
Okra	6186 ±307	4261 ±227	31.10 ±0.51	3210 ±160.95	48.10 ± 0.37	1779.01 ±37.55	68.24 ±3.45	

Table 3: The effects of the selected crops on the remediation of the 10% PHPM polluted soils

Crops	PHYTOREMEDIATION PERIODS (DAYS)							
	1	28		56		84		
	^a RTPH Conc. (mg/kg)	^b RTPH ^b Conc. (mg/kg)	^c PRem (%)	^b RTPH Conc. (mg/kg)	^c PRem (%)	^b RTPH ^b Conc. (mg/kg)	^c PRem (%)	
Control (No crop)	14542 ±317	12748 ±257	16.55 ±0.88	11635 ±227	19.99 ±0.75	9180 ±417	36.87 ±1.75	
Maize	14542 ±317	10391 ±207.58	28.55 ±1.59	7313 ±237.58	49.71 ±0.55	4687 ±250.25	67.77 ±2.55	
Bean	14542 ±317	9982 ±212	31.36 ±0.99	6887 ±117	52.64 ±2.55	4380 ±147.55	69.88 ±2.75	
Okra	14542 ±317	11856 ±337	18.47 ±0.75	8708 ±207	40.12 ±2.05	7108 ±317	51.12 ±2.68	

a = Residual TPH after landfarming, b = Residual TPH after Phytoremediation

c = Percentage of Remediation

Discussion

Phytoremediation of the biostimulated-landfarmed 5% PHPM polluted soils

The effects of the selected crops on the remediation of the 5% PHPM polluted soils is presented in Table 2. The results showed that prior to the use of the crops on the polluted soils, that is, after using the biostimulated-landfarming, the residual concentration of TPH (RTPH) was 6185.72 ±30.94 mg/kg soils. This value is far above the targeted value of 5000mg/kg by Nigeria Federal Ministry of Environment (FMEvn) and Department of Petroleum Resources guidelines (DPR, 1991; FEPA, 1991). Hence, it is still necessary to either extend the biostimulated-landfarming period or to complement the process with the phytoremediation technique. This study has revealed that the usage of any of these plants; maize, bean or okra made significant reduction in the residual TPH concentrations in the samples within 84 days. Specifically, maize crops were able to remove 42.68, 64.17 and 99.20% of the RPHPM from the polluted soils at days 28, 56 and 84 respectively. Such that, at the end of 84 days, the TPH in the remediated soils was about 49.49 ±2.09 mg/kg soil which is about the acceptable limit of 50 mg/kg for residential and agricultural soils in Nigeria (DPR, 1991). Similarly, the bean crops were able to degrade 43.25, 66.28 and 98.84% of the RPHPM from the polluted soils at days 28, 56 and 84 respectively; such that, at day 84, the TPH remaining in the remediated soils became about 69.28 mg/kg soil. Again, this value is at a very close range to the acceptable limit of TPH for residential and agricultural soils and very far below the target value of 5000 mg/kg in Nigeria (DPR, 1991; FEPA, 1991). As for the okra crops, 31.10, 48.10 and 66.24% of the RPHPM were remediated at days 28, 56 and 84 respectively. In this case, the TPH in the remediated soils

was 1779.01 mg/kg. This value is very far higher than the acceptable value of 50 mg/kg soil but lower than the target value, which is an indication that okra is a less effective phytoremediation crop for petroleum hydrocarbon polluted soils. However, there were significant and arithmetical differences in the TPH degradation when the three aforementioned crops were used for the phytoremediation as against the control samples with no crop planted but the biostimulated-landfarming process was allowed to proceed for the 84 days (Table 2). These findings were in collaboration with previous studies on biostimulated-phytoremediation of organic and inorganic soil contaminants (Wuana&Okieimen, 2010; Asghar et al., 2017; Wojtowicz et al., 2023). Previously, maize has been reported as an effective plant for soil remediation purposes (Asghar et al., 2017; Wojtowicz et al., 2023). Specifically, Asghar et al. (2017) reported the decontamination of petroleum polluted soils by the use of maize up to 43% after 60 days of planting. The degradation was attributed to joint effect of the planted maize and bacteria inoculant as the percentage degradation was significantly higher with the presence of the maize crop. In a similar work by Baoune et al. (2019), the planting of maize in conjunction with a bacterium inoculant degraded a mixture of petroleum hydrocarbons from soil up to 70% during biostimulated phytoremediation protocol.

The present study has revealed that, brown bean and maize showed similar tolerance to the toxicity of petroleum pollution. Furthermore, it was observed that the brown bean could be an alternative to maize and a good phytoremediation crop for petroleum hydrocarbon polluted soils in the Niger Delta region of Nigeria. Furthermore, the study buttressed the potential utilization of phytoremediation as a compliment to stimulated-landfarming of petroleum hydrocarbon and by extension other organic and inorganic soil pollutants. Previous reports documented the successes of multi-soil remediation approach (Xiao-Dong et al., 2005; Wuana&Okieimen, 2010, Wojtowicz et al., 2023; Aparicio et al., 2022) to the decontamination of soils polluted with petroleum hydrocarbons particularly the recalcitrant hydrocarbons such as the PPHM used in this study. Also, Ochekwu et al. (2012), showed that Velvet bean (*Mucunapurens* L. DC) (which is of the same leguminous family with brown bean), has greater potential for the removal of heavy metals and petroleum products from soils.

This assay revealed that maize and brown bean could serve as alternatively edaphic remediation crops. These two crops were able to degrade the TPH in the soil to a range of 4380 – 4587 mg/kg soil at day 84 (Table 2). These values were below the target limit for TPH in Nigeria soil environment (DPR, 1991; FEPA, 1991). However, these values were still higher than the acceptable limit for residential and agricultural soils, Therefore, in order to reduction the TPH concentration to the accept limit, a second routine of phytoremediation using either maize or bean would be required. The okra also showed good responses to the removal of TPH (When compared to the control) but a weaker polluted soil remediation plant (PSRP) when compared to maize and bean in the study. However, okra could be used as PSRP in a polluted soil when the topography or soil texture would not support maize or bean growth. Previous reports have implicated maize crop for remediation of petroleum hydrocarbon soils (Wojtowicz et al., 2023; Asghar et al., 2017 Ochekwu et al., 2012). This fact has been buttressed by this assay and further revealed the use of brown bean as an alternative crop to maize in a complementary phytoremediation remediation of heavily petroleum hydrocarbon polluted soils. These findings is in collaboration with previous documentations on multi-bio-remedial approaches to polluted soils remediation particularly those soil environment containing high concentrations of recalcitrant petroleum hydrocarbon pollutants. The efficacy and reliability of the phytoremediation and biostimulated landfarming have been well reported. Several plants have been investigated for PSRP and their efficiency have also been document (Anikwe, 2017; Wojtowicz et al., 2023;Asghar et al., 2017). Yet, more researches are being sought to enlist PSRP in harsh soil topography and that could provide short throughput in the phytoremediation protocols. This investigation has revealed that maize, brown bean and even okra could be used as optional PSRP to complement biostimulated-landfarming techniques.

Phytoremediation of the biostimulated-landfarmed 10% PPHM polluted soils

Table 3, presents the extent to which complimentary landfarming (with phytoremediation) could have on the ultimate remediation of heavily petroleum hydrocarbon polluted soil (particularly with recalcitrant compounds) could be. Persistent petroleum hydrocarbons including the BETX and PAHs are difficult to remove from soils particularly when their concentrations are high (Ali et al., 2023; Lee et al., 2019; Bacosa et al., 2021). Since the PPHM contained such high concentration of BTEX, Naphthalene and resident PAHs in the diesel used for the soil spiking, resistance to biodegradation was expected. However, the study revealed that complimentary the biostimulated-landfarming of the soils with phytoremediation could reduce the concentrations of TPH in the soil drastically even from very high pollution levels. Specifically, the results showed that the maize, bean and okra reduced the TPH in the PPHM by about 29, 31, and 18% respectively at day 28 of the phytoremediation protocol. And by day 56, the level of TPH degradation increased to about 50, 53 and 40% by the remediating crops, maize, bean and okra respectively. The impact of the phytoremediation was very significant when compared to the level

of the TPH degradation in the control soils (where no crop was planted) which was about 12 and 18% at days 28 and 56 respectively. Interestingly, as at day 84, the level of TPH degradation by the three aforementioned crops (Maize, bean and okra) had increased to about 68, 70 and 51% respectively such that the RTPH in soils reduced to about 4687, 4380 and 7108 mg/kg soil respectively (Using the mentioned crops) as against the value of RTPH of the control, about 9160 mg/kg soil (Table 3). The levels of removal of the PHPM pollutants from the soils by the brown bean were in a very close range with the 70% reported for the use of maize in the works of (Baoune et al., 2019). This biodegradation achievement may be attributed to the joint effect of the bean or maize and the indigenous soil microbes (Asghar et al., 2017).

Conclusion

The quest for bioremediation of heavily petroleum hydrocarbons polluted soils particularly in the Niger Delta region, Nigeria, necessitated this study. Complimentary phytoremediation of biostimulated-landfarming has been investigated as a multi-purposed approach to remediation of highly petroleum polluted environment. The tolerance to heavily petroleum products polluted soils and phytoremediation potency of maize, bean and okra were investigated. Maize and bean were observed to have similar tolerance to petroleum hydrocarbon polluted environment. The study buttressed the use of maize as PSRP and revealed brown bean as an alternative crop to maize as PSRP particularly in areas and soil types where the growth of maize would be poor. Furthermore, Okra could be used as PSRP plant in certain environment where the soil texture and topography would not favor the usage of neither maize nor bean. Based on the findings, two routine of complimentary phytoremediation is recommended for biostimulated-landfarmed petroleum polluted soils (with over 5 - 10% hydrocarbon with respect to weight of the soil) in order to reduce the concentration of the TPH in the environment to acceptable limit.

Recommendation

The following recommendations were made based on the findings of the study:

1. Similar study under field environment (i.e. Field scale) which could give room for comparison of these results to that of field is recommended.
2. The hydrocarbons and heavy metals content of the edible parts of the crops including the leaves, the fruits and seeds are need to be ascertained in order to establish the relevance and safety of these produces for human and livestock consumption.

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