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Sequestration of PAHs in a Phytoremediation Using Indian Mustard and Bambara Plants

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Abstract: The prospective use of Indian mustard and Bambara plants for phytoremediation of crude oil contaminated farmlands was investigated in a 60 - day greenhouse and laboratory experiments. Results obtained showed that hydrocarbon contaminants produced deleterious effects on both biomass and total organic carbon. For Indian mustard – vegetated regimes, most reliable growth index, produced biomass gave over 57% at 5% and 10% crude oil spiking in relation to control and showed up to 2.4 fold increases compared to Bambara plants. Irrespective of tested spiking level, over 89% PAHs phytodegradation efficiency was achieved with both plants. Metabolites (such as naphthalene and anthracene) detected in the present study may suggest that these compounds are less phytotoxic than their less distinct degradable counterparts. These results indicate that Indian mustard could successfully be used preferentially as potent phytoremediating agent at petroleum hydrocarbon contaminated farmlands.

Keywords: Crude oil pollution; Environmental stress; Phytoremediation; Clean – up technologies; Hydrocarbon contaminants.

1. Introduction

Search for energy has made crude oil that takes millions of years to form, to be used up more swiftly than it is made and its supply is projected to run out in approximately 30 years, except use is streamlined. The activities of multinational oil companies and recurring pipeline vandalization in recent times have led to recurrent environmental pollution especially in the host communities of the Niger delta area. Frequent crude-oil spillages on agricultural soils, and the consequent fouling effect on all forms of life, render the soil (especially the biologically active surface layer) toxic and unproductive [1]. Given myriads of environmental pollution incidences with this global search for energy due maybe to economic expansion, many researchers have continued to find cheap, sustainable and environmentally – friendly approaches to clean – up and mitigation of posed chronic problems without the need for 'excavate and dump' traditional practices especially for soil environmental components.

Biological technologies including phytoremediation for remediation of polluted sites, have received wide attention over the past few years in this regard to curb reported adverse effects, high cost and site disruptions associated with conventional clean – up technologies. Phytoremediation, a variant of bioremediation and also the use of plant – based technologies at clean – up of contaminated environments has been noted among researchers for reducing cross-site contamination and soil erosion [2], offering cheaper option [3], nature to clean nature technology [4], promotion of biodiversity [5], re – use possibility of recovered contaminants mostly metals, least harmful option [3] and ease of monitoring and management.

Efforts have been geared towards optimizing and enhancing phytoremediation technology by putting in place robust search for distribution, fate, physiological and molecular mechanisms of interest contaminants during phytoremediation and plant screening at laboratory and field scales.

Some plants have been identified to multi – task as they proved successful at remediation of more than a type of contaminant e.g. Indian mustard at Caesium–137 [6], Pb, As [7] and Cd [8]; Pine spp. at TCE-trichloroethylene and by-products [8], Organic solvents, Methyl tert-butyl ether and Hydrocarbons; Tall Fescue at Caesium–137 and Hydrocarbons [9]; and Willows at Potassium ferrocyanide [10], Cd [11] and hydrocarbons [12]. Most screened plants have heavy metals as target leaving a big gap in remediation of organics especially from crude oil.

Bambara plant, with origin in West Africa, is known scientifically as *Vigna subterranea* (L.) Verdc. and belongs to a family of Fabaceae. Mostly neglected and underutilised, Baryeh [13] reported their less demand of a soil while Yamaguchi [14] documented their ability to thrive in soils with undesirable characteristics, including temperature

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extremes. Nwaichi, *et al.* [15] reported that *Vigna subterranea* is a drought resistant crop that can grow in marginal, low-input, environments like those found in crude oil contaminated soils. Indian mustard (mustard greens or Amsoi Green) scientifically known as *Brassica juncea* (L.) is a herbaceous dicot of the family Brassicaceae. Besides the edibility of the leaves, seeds, and stem, its essential oil under the grandfather clause is accepted as 'generally recognized as safe (GRAS)' by the US FDA [16]. Reported successes at phytoremediation with Indian mustard [2, 17, 18] at heavy metal – laden sites, their survival in parched environments and possession of fibrous root system has elicited our choice in this study. It is therefore pertinent to increase existing knowledge on biologically friendly approach to responding to crude oil spill on agricultural soil while maximizing potentials in multi – tasking plants at phytoremediation design given inherent complexities.

Performance of Indian mustard (*Brassica juncea* (L.)) and Bambara plants (*Vigna subterranean*) at degradation, transformation and metabolism of polycyclic aromatic hydrocarbons was investigated. Distributions of individual EPA priority PAHs were also studied among others.

2. Materials and Methods

2.1. Sourcing of Materials

Seeds of Indian mustard and Bambara plants were procured from Chiltern seeds UK and Plant resources conservation unit of the University of Ilorin Nigeria respectively and were well characterized. Bonny light crude oil was sourced from Shell Petroleum Development Company Port Harcourt Nigeria and was shipped to Energy Technologies Building laboratory, Jubilee Campus, University of Nottingham UK and was characterized for source confirmation. A sandy loam agricultural soil was purchased from Rolawn Ltd Elvington York UK and reported to have the following characteristics: pH 6.7, organic matter 7.0 %, total N 0.23 %, exchangeable Na 1.2 %, extractable P, K and Mg 16, 307 and 129 mg l^{-1} respectively.

2.2. Design

Sourced seeds of Mustard and Bambara were sown in seedling tray cells with dimensions: width 22cm (8.6") x length 33cm (12.9") x height 5cm (1.9"). In the glasshouse (18^{o}C day and 20^{o}C night heating with 6 – 8 hours photoperiod lighting) at Sutton bonington Campus University of Nottingham, raised seedlings were transplanted to 20cm (8") plant pots two weeks after germination and were allowed a 2 – month growth trial. Sourced agricultural soil was spiked to 0, 5 and 10% (after a range test) with Nigerian Bonny light crude oil and experiment was performed in replicates of three. All soil handling was done when soil was visibly dry and in friable consistency. Unplanted regimes were instituted for all treatments to compare plant's performance to natural attenuation. Growth parameters were measured and included bi – weekly measurements of leaf length, leaf width, and plant height and produced biomass. Watering with regular nutrition was done every other day using sprinkler and ensuring there was no overflow. Soils were sampled by hand from the surface to the bottom of plant pots using a mini closed bucket soil auger. Harvested samples were sealed in whirl pack bags and transported to the Energy Technologies Building laboratory and analyses were done within 0 to 3 days while samples were stored at 4^{o}C.

2.3. pH and Conductivity Determination

Field scale HANNA HI 9033 pH and conductivity meter was used to measure pH and conductivity of study soils (one part soil to 3 part water) at days 0 and 60.

2.4. Growth parameters

For determination of root and shoot (dry biomass) growth in plants under study, measuring tape and weighing balance were employed.

2.5. Elemental Analysis

Soil samples were dried 48hours in the oven (60° C). Dried samples (2mm screen) were ground using mortar and pestle to obtain a homogenous and representative sub – sample and emptied into well – labelled flip - cap vials. In a calibrated and levelled microbalance, a Leco Tin Foil cup, introduced into the holder using a forceps, was tared and sample was loaded using a spatula. Weight (0.07g) was noted in the laboratory notebook and sample was compacted within the tin from the base to form a ball. Sample balls were placed in correct cell in 12 - well tray ensuring the number matches those in notebook and transferred accordingly to matching wells in the sample compartment for the Leco CHN elemental analyser. Kimwipes were used to wipe dry spatula between samples. Sample ID, position and mass were entered into the system's spreadsheet and the 'analyze' icon was clicked to start run. Two bypass and blank samples constituted the first four wells. This was followed by Leco standard (BBOT containing C% 72.50, N% 6.45, H% 6.08 and S% 7.51 for CHN and Coal Reference material containing S% 1.16 and Ash% 12.98 as well as BBOT for S) and standards were again run every 10th sample as well as the last run. For the S analysis however, supplied ceramic wells were used instead of tin to weigh in samples (0.3g) and placed accordingly in the S analysis sample compartment. Results were collected as displayed on the spreadsheet. Note that residues were all subjected to elemental analysis.

2.6. % Total Organic Carbon, TOC Removed

TOC serves as an indicator of the amount of biodegradable carbon in the soil and was calculated from the formula given below:

% TOC removed from soil = 100 – {((Sf/100 * EAf) / (Si/100 * EAi)) * 100 , where Si, EAi and EAf represent initial soil (g), initial soil C from elemental analysis and final soil C from elemental analysis respectively.

2.7. Multi-elemental Analysis by ICP-MS

ICP analysis was done in Agricultural and Environmental Science laboratory, Gateway Building Sutton Bonington Campus University of Nottingham. Soil samples were digested to obtain 2% HNO_3 solution matrix. Microwave (Multiwave PRO) digestion method was employed for both soil and tissue samples. Multi-element analysis of diluted solutions was undertaken by ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). The instrument was run employing three operational modes, including (i) a collision-cell (Q cell) using He with kinetic energy discrimination (He-cell) to remove polyatomic interferences, (ii) standard mode (STD) in which the collision cell is evacuated and (iii) hydrogen mode (H_2-cell) in which H_2 gas is used as the cell gas. Samples were introduced from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a PEEK nebulizer (Burgener Mira Mist). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L^{-1}), Rh (10 µg L^{-1}) and Ir (5 µg L^{-1}) in 2% trace analysis grade (Fisher Scientific, UK) HNO_3. External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) included Ag, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, U, V and Zn, in the range 0 – 100 µg L^{-1} (0, 20, 40, 100 µg L^{-1}). A bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, France) was used to create Ca, Mg, Na and K standards in the range 0-30 mg L^{-1}. Phosphorus, boron and sulphur calibration utilized in-house standard solutions (KH_2PO_4, K_2SO_4 and H_3BO_3). In-sample switching was used to measure B and P in STD mode, Se in H_2-cell mode and all other elements in He-cell mode. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required. Obtained screened data were subjected to statistical analysis as described under ‘statistical analysis.

2.8. PAHs Assay

Prior to analyses, 2 mm screen soils (oven - dried at 40 °C for 72 hours) were rid of visible organic matter, like the stems and roots. Approximately 20 g of screened soil sample was weighed into a clean thimble and loaded into the main chamber of the soxhlet extractor. Into a 500ml distillation flask (in a water – connected hood), an azeotropic mixture of dichloromethane (DCM) and methanol (93:7 % v/v) up to 250 ml was added and was placed on a heating element. The flask was connected to a soxhlet extractor linked to a reflux condenser. The heated solvent refluxed and the solvent vapour travelled up the distillation arm flooding into the chamber housing the thimble containing soil. The condenser provides cooling effect that cools the solvent vapour and causing them to drip back down into this chamber that slowly gets filled with warm solvent containing some of the dissolved desired compounds. When full, the soxhlet chamber was emptied using the siphon and the solvent returned to the distillation flask. Use of thimble here ensured no transport oil soil, given the rapid movement of the solvent, to the distillation flask. This cycle was allowed to repeat continuously for 24 hours to maximise extraction after which the solvent was removed using a rotary evaporator, yielding the extracted oil. The non-soluble portion of the extracted soil in the thimble was dried and weighed and the weight of organic extract is equivalent to the maximum solvent extractable organic matter of study soil.

Thereafter, the mixture was carefully filtered into clean solvent - rinsed extraction bottle, using filter paper fitted onto a Buchner funnel. The extraction was concentrated to 2 mL and then transferred for clean- up/ separation. A 1cm of moderately packed glass wool was placed at the bottom of 10mm ID X 250mm long chromatographic column. Slurry of 2g activated silica in 10 mL methylene chloride was prepared and placed into the chromatographic column. Half mL of sodium sulphate was added to the top of the column. Additional 10ml of methylene chloride was used to rinse the column. The column was pre-eluted with 20mL of hexane; this was allowed to flow through the column at a rate of about 2mins until the liquid in the column was just above the sulphate layer. A 1 mL of the extracted sample was immediately transferred into the column. The extraction bottle was rinsed with 1ml of hexane and was added to the column. The column stop-clock was opened and the hydrocarbon was collected with 10mL graduated cylinder. Before the exposure of the sodium sulphate layer to air, hexane was added to the column in 1-2 mL increments. Accurately measured volume of 8-10 mL of the element was collected and was labelled aliphatic and subsequently aromatic and polar fractions. The aromatic fraction was dried in the hood and transferred to GC MS vials for PAHs determination after creating sequence on the computer.

Liquid chromatographic analysis was performed using Varian CP3800 Gas Chromatograph coupled with 1200 Quadrupole MS/MS Varian connected to a Dell computer. The standard used for the aromatic fraction characterization was 1,1'-Binaphthyl 98%, ACROS Organics. Thorough review for initial and continuing calibrations, holding times, tuning and system performance, blank analysis, surrogate recoveries, replication precision, and compound quantitation and detection limits were used to validate obtained data.

2.9. Statistical Analysis

Data obtained from three replications per treatment were documented as mean ± S.E. and significance was determined according to ANOVA using SPSS Vs 22. Significance levels were considered at $p < 0.05$.

3. Results and Discussion

From this study, crude oil spill simulated to 5 and 10% into the agricultural soil environment influenced the total organic carbon content in the soil by 3 - and 5 – fold decrease respectively. NRCS (2011) described soil organic carbon as one of the key soil components as it can alter plant growth in form of energy source and their role in activating nutrient availability via mineralization. Increased % TOC removal in planted regimes (Figure 1) may be attributed to improved breakdown and disruption of soil aggregates attendant with cultivation. This also exposes protected soil organic matter for microbial degradation in tandem with enhanced aeration. TOC removal were greater in Indian mustard - cultivated soils and was markedly higher at 5% (Figure 1). Mean TOC removal were significant between phytoremediation soils and those undergoing natural attenuation.

Undue growth restraints to plant growth (Figure 2) evident in produced biomass may have lowered organic inputs expressed in organic carbon fluxes as shown in Figure 1. However, there were no marked differences between observed restraints for Indian mustard cultivated soils at 5% and 10% spiking. Indian mustard performed as much as 64.7 % and 57 % while Bambara gave 58.2 % and 39.5 % in relation to control at 5 % and 10 % crude oil contamination respectively. This suggests higher ameliorative effect on hydrocarbon contaminants - posed toxicity by Indian mustard plants which also showed similar growth phenotype between varied contaminant concentrations. Observed leaf abscission in hydrocarbon – stressed plants may be connected with reduced biomass accumulation as reported by Nwaichi and Wegwu [19].

Figure 3 shows growth as expressed in root length. At all concentrations tested, obtained root length was higher for Indian mustard. Interestingly, there was no significant differences between treatments for each plant. Such comparable root length was reported by Azab, *et al.* [20] in their study with transgenic *A. Thaliana* suggesting improved root length in transgenic over the wild type. However, more secondary root development was observed for the contaminated regimes for both plants. Presence of noodles was noted among Bambara plants at 60 – day especially with contamination. This observation is indicative of detoxification and phytoremediation potentials of study plants.

Increases in pH values (Figure 4) were documented at the end of the 60 - day experiment from 6.65 to 7.14, 6.31 to 6.88, and 6.14 to 6.28 for Indian mustard – cultivated soils and from 6.65 to 6.71, 6.31 to 6.7 and 6.14 to 6.38 for Bambara – cultivated soils at 0%, 5% and 10% spiking respectively. Only marginal increases from 6.65 to 6.66, 6.31 to 6.40 and from 6.14 to 6.15 at 0%, 5% and 10% spiking for unplanted regimes. Corrected pH is indicative of soil stabilization as reported by Dietz and Schnoor [21].

In order to obtain more information on the subsurface soil properties, electrical conductivity was investigated. Results shown in Figure 5 revealed soil structural influences on conductivity. Mean conductivity values decreased with compaction (poor soil integrity) as oil spill simulated compacted study soils after watering. Bai, *et al.* [22] described similar decrease in compacted lateritic soil due to inundation of water content.

Polycyclic Aromatic Hydrocarbons are potent ubiquitous environmental pollutants having fused aromatic rings and without heteroatom nor attachments. The fate of crude oil, occurring as liquid both in natural belowground reservoirs and on the surface, the non – renewable source of energy, in terms of PAHs degradation was greatly influenced in the soil by potential plant – induced microbial activities. PAHs have been associated with birth flaws, mal - development in plants, variations in genetic materials, and cancer causing [23] tendencies. Although complete detoxification of toxic PAHs was not possible, study plants showed the ability to metabolize these xenobiotics. Figure 6 shows percentage degradation obtained after the 60 – day phytoremediation experiment. % degradation efficiencies. Indian mustard achieved up to 95% and 89% PAHs degradation at the 5% and 10% crude oil polluted agricultural soils respectively. These values compares favourably with natural attenuation that gave 38.72% and 43% for 5% and 10% counterparts in the unplanted regimes. Similarly, 92% and 92% (Figure 6), for the 5% and 10% crude oil polluted agricultural soils respectively, were achieved with Bambara plants. These values again compares favourably with simulated natural attenuation. Ighovie and Ikechukwu [24] reported 60% PAHs degradation efficiency in a phytoremediation trial with Carpet grass while Nwaichi, *et al.* [25] submitted only 45 % with Centrocema plants with no soil amendments.

X-raying distribution of individual PAHs as shown in Table 1 for Indian mustard – cultivated soils, revealed 100 % degradation for all but Naphthalene, Pyrene, Benzo (a) anthracene and Chrysene at 5 % oil spill. Seeming differential resistance to biodegradation of Naphthalene may be due to degradation of larger aromatic compounds to yield Naphthalene. It was observed that PAHs such as Naphthalene, Acenaphthylene, Fluorene, Phenanthrene and Anthracene, all low – weight, had more distinct degradation than those with more than 3 aromatic rings. In comparison to un-vegetated regimes, significant degradation efficiencies were seen with phytoremediation with Indian mustard over natural attenuation for all studied PAHs. For example, low degradation percentages of 26.8 and 25.7 improved to 99.3 and 71.4 for Benzo (a) anthracene (Table 1) at 5 % and 10 % oil spill conditions respectively. Increased Naphthalene concentration (Table 2) in post – remediation soils planted with Bambara plant especially at 5 % oil spill, may be associated with Benz (a) anthracene breakdown products. Cajthaml, *et al.* [26] observed in their postulated degradative pathway for Benz (a) anthracene, that four Naphthalene – parent metabolites were released. Bambara plant – driven soil clean – up achieved no less than 84.2 % degradation efficiencies except for Naphthalene

irrespective of the studied degree of contamination. This value is incomparable to 25.7 % with un – vegetated counterpart for Benzo (a) anthracene. Average of 40% however was reported by Cofield, *et al.* [27] in a similar study with Switchgrass and Fescue.

Nutrients are very crucial for successful sequestration of hydrocarbon contaminants. Profiling of the nutrients in the soil prior to designed phytoremediation and at the end of the 60-day experiment. Results obtained are as shown in Tables 3 and 4 as well as Figures 6 and 7. Figure 7 shows the rapid decline of P level with increasing oil pollution. This agrees with the report by Das and Chandran [28] which stated that P and N became the limiting factor as C levels significantly increased when major oil spill occurred. Major dietary elements Atkinson, *et al.* [29] are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen present in common organic molecules. Studied calcium, phosphorus, potassium, sodium and magnesium (Figure 7) gave high concentrations (3942 to 7197, 514 to 1109, 4826 to 8167, 1164 to 1718 and 1004 to 1833 respectively) in mg kg-1 throughout although significant depletion was observed for the 10 % crude oil contaminated regimes. These sharp decline in major nutrients especially for P and K (Figure 7) may have contributed to stunted seedling growth and seeming poor water use for both plants. Woodleaf [30] underscored the roles played by P and K at root development, seedling growth, disease resistance and yield, and water use efficiency, photosynthesis, and crop quality respectively.

Potentially phytotoxic element, Zn gave levels ranging from 88 to 110 mg kg-1 (Table 3) and fell within <200 mg kg-1 recommended by BSI [31]. Abundance of Cu in both Mustard and Bambara plants are in line with several reports Woodleaf [30] that leafy greens and legumes are excellent and good (respectively) sources of Cu.

Uptake of P in both shoot and root tissues were greatly influenced by soil pH. Higher uptake of available P was observed for plants grown in more acidic soils (Figures 4, 8 and 9). Parvage, *et al.* [32] made similar observation in their study evaluating the effect of biochar addition on water – soluble P levels in different soils. They reported decreased P concentrations with increased pH. Figures 8 and 9 show the studied trace nutrients distribution in tissue samples. Evidence of significant depletion of phosphorus levels in shoot and root tissues of both leguminous plants were clear. This situation may have hampered nitrogen fixation potential of plant but was only marked at 10% petroleum contamination. Also decreased seedling growth rate evident in dwindled leaf area, leaf width, leaf length and plant height may be connected with reduced available phosphorus. Rosato [33] reported direct relationship between phosphorus availability and seedling growth, winter hardiness, disease resistance and yield. Similar trend was observed for potassium, a regulator of metabolic activities. While magnesium levels in the shoot tissues harvested from 5% petroleum contaminated reduced by 8% and 2% for Bambara and Mustard respectively, 27% and 25% were observed at 10% petroleum contamination after 60 days. These margins may have induced the chlorotic conditions observed at 10% contamination as Rosato [33] and Nwaichi and Wegwu [19] stated that Mg is an essential component in the chlorophyll of green plants. Calcium, a component of cell membranes and walls, decreased by 21% and 37% for Bambara at 5% and 10% contamination respectively. These values were 4% and 32% for Mustard plants. Although sodium, ubiquitous in nature has been reported not to be necessary in plant's growth and development Rosato [33], their levels in this study were higher in Mustard tissues and were not significant. Generally, higher levels were found in the shoot in comparison to the root tissues.

4. Conclusion

Phytoremediation using Mustard and Bambara has proven effective and non – disruptive in restoration of petroleum hydrocarbon contaminated soils from our study. Correction of pH was observed with applied technology and is indicative of soil stabilization. Mean conductivity values decreased with compaction (poor soil integrity) as oil spill simulated compacted study soils after watering. Improvement in soil structure and recovery however was observed after remediation and has implications for soil environmental quality and ecological health. The present study confirmed higher biodegradability of PAHs using Indian mustard (up to 95 %) over Bambara plants (up to 92 %). Marginal depletion of studied nutrients at 5% petroleum contamination suggests good performance of studied plants at crude oil contaminated soil within such bracket. Improved soil pH were consistent for both studied species and constitutes an advantage in terms of soil structure recovery. Metabolites detected in the present study may lead us to assume that these compounds are less phytotoxic than their less distinct degradable counterparts. Moreso, resulting non - extractable residues obtained here might be considered detoxification products. To measure up with the overwhelming pollution cases, region – available plant species need to be screened and validated to match favourably the more expensive, environmental - adverse and disruptive conventional methods. Being food crops with promising results, Indian mustard and Bambara plants could be deployed at remote phytoremediation sites with minimal humans and animal intervention in consideration of food safety. Data from this study could be useful in elucidating the fate, identity and transformation of individual PAHs. Also information on removal rates of individual PAHs traceable to plant is provided here.

Limitations of the study may include the fact that we excluded Benzo (a) pyrene in the computation, as it was not detected on the obtained chromatograms for the standard used.

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Figure-1 Mean percentage TOC removed in 60 days. Values are mean ± SE (n = 3). Unp represents unplanted soil; 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

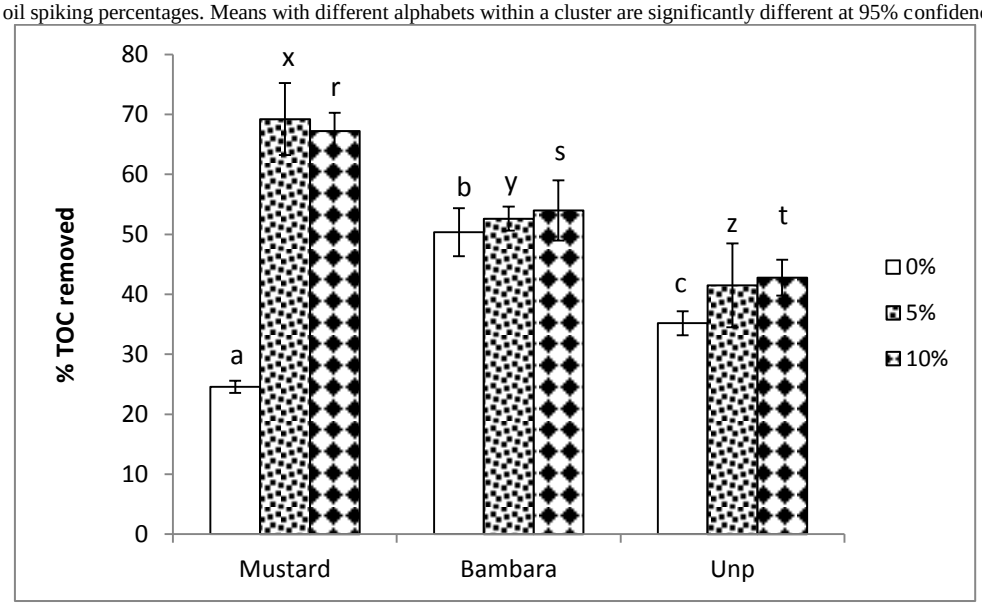

Figure-2. Produced biomass recorded for Indian mustard and Bambara plants. Values are means ±SE (n = 3); 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

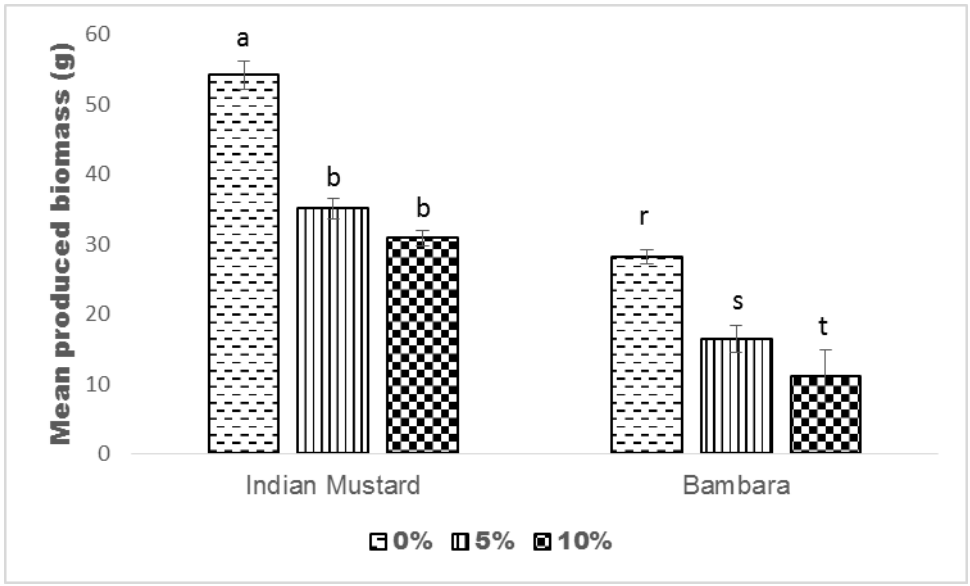

Figure-3. Recorded root length for Indian mustard and Bambara plants at 60 d. Values are means ±SE (n = 3); 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

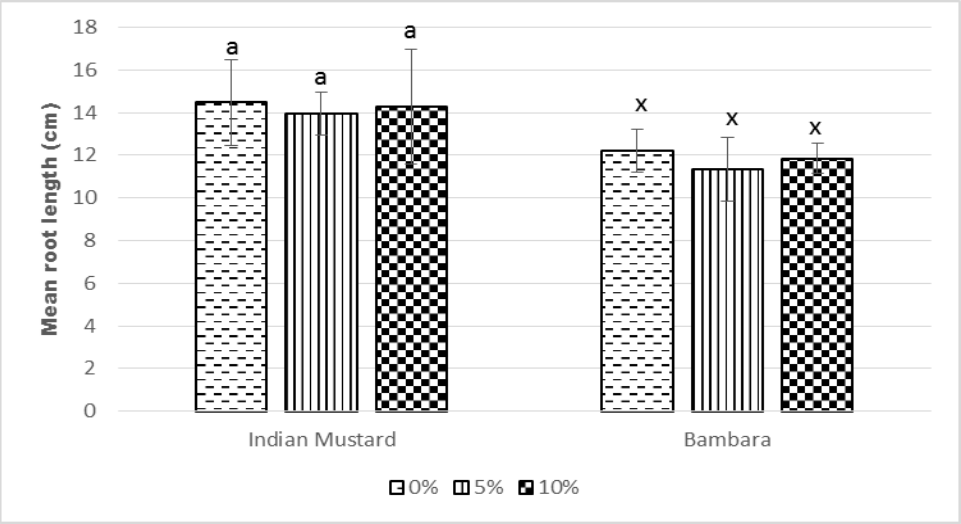

Figure-4. Recorded soil pH in Indian mustard and Bambara – vegetated treatments. Values are means ±SE (n = 3); 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

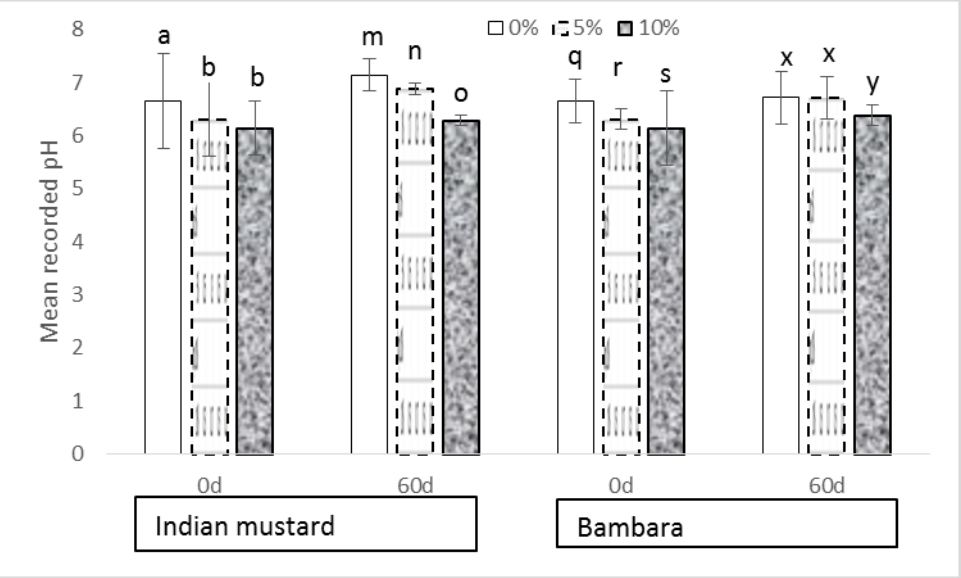

Figure-5. Recorded soil conductivity in Indian mustard and Bambara – vegetated treatments. Values are means ±SE (n = 3); 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

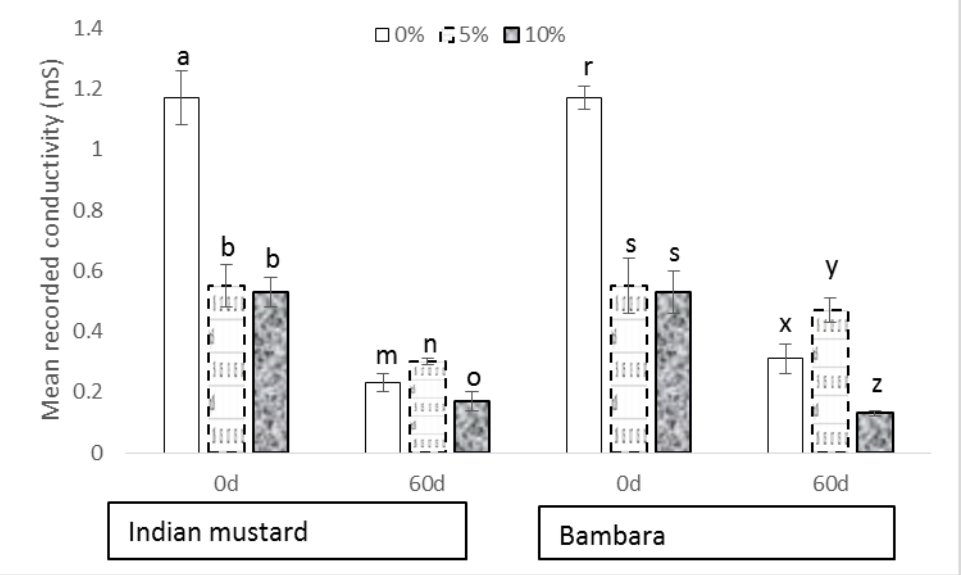

Figure-6. % PAHs degradation in Indian mustard and Bambara - cultivated soils. Values are means ±SE (n = 3); Unp represents unplanted soil; 0%, 5% and 10% represents different crude oil spiking percentages. Means with different alphabets within a cluster are significantly different at 95% confidence level.

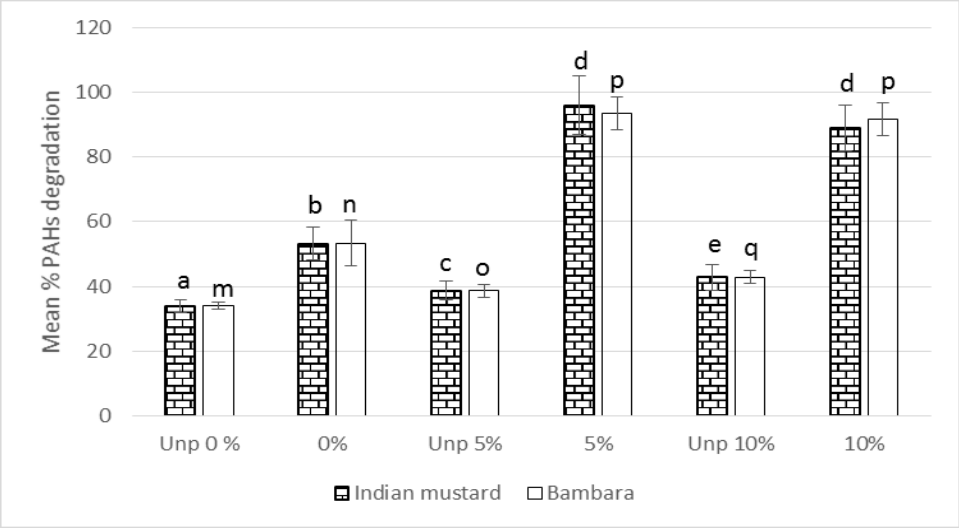

Table-1. Mean distribution of individual PAHs (ng g^{-1}) in Indian mustard – cultivated soils at 60 days

	0 %	Unp 0 %	0 % M	5%	Unp 5%	5% M	10%	Unp 10%	10% M
Naphthalene	8.501	4.587	4.789	7.416	6.818	4.958	7.645	4.694	5.252
Acenaphthylene	1.995	0.396	0.284	2.815	0.100	0.000	3.447	0.184	0.000
Acenaphthene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fluorene	0.000	0.000	0.000	2.592	0.190	0.000	4.600	0.000	0.000
Phenanthrene	4.585	0.000	0.000	10.374	0.300	0.000	14.745	0.000	0.000
Anthracene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.497
Fluoranthene	5.576	2.708	1.787	21.001	10.020	0.000	22.008	17.011	0.000
Pyrene	4.011	2.076	1.119	4.010	4.000	0.062	5.606	3.120	0.497
Benz(a)anthracene	6.076	5.828	3.739	30.101	22.031	0.210	61.252	45.520	13.027
Chrysene	6.076	5.828	3.739	31.566	26.126	0.043	70.001	47.439	13.027
Benzo(b)fluoranthene	4.604	4.599	3.091	20.020	10.011	0.000	54.681	20.031	0.000
Benzo(k)fluoranthene	5.003	4.599	3.091	10.000	6.000	0.000	50.081	30.005	0.000
Indeno(1,2,3-cd)pyrene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values are means for n = 3; Unp represents unplanted soil; 0%, 5% and 10% represents different crude oil spiking percentages; M represents Mustard – planted soil.

Table-2. Mean distribution of individual PAHs (ng g^{-1}) in Bambara – cultivated soils at 60 days

	0 %	Unp 0 %	0 % V	5%	Unp 5%	5% V	10%	Unp 10%	10% V
Naphthalene	8.501	4.587	4.346	7.416	7.308	7.004	7.645	4.694	4.098
Acenaphthylene	1.995	0.396	0.260	2.815	0.100	0.540	3.447	0.184	0.000
Acenaphthene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fluorene	0.000	0.000	0.000	2.592	0.000	0.000	4.600	0.000	0.000
Phenanthrene	4.585	0.000	0.000	10.374	0.000	0.000	14.745	0.000	0.000
Anthracene	0.000	0.000	0.226	0.000	0.000	0.000	0.000	0.000	0.000
Fluoranthene	5.576	2.708	1.714	21.001	10.020	0.544	22.008	17.011	0.546
Pyrene	4.011	2.076	2.003	4.010	4.000	0.000	5.606	3.120	0.000
Benz(a)anthracene	6.076	5.828	5.046	30.101	22.031	0.000	61.252	45.520	9.700
Chrysene	6.076	5.828	5.092	31.566	26.126	0.000	70.001	47.439	10.328
Benzo(b)fluoranthene	4.604	4.599	0.000	20.020	10.011	0.460	54.681	20.031	0.000
Benzo(k)fluoranthene	5.003	4.599	2.960	10.000	6.000	0.000	50.081	30.005	0.000
Indeno(1,2,3-cd)pyrene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dibenz(a,h)anthracene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i)perylene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total	46.428	30.622	21.646	129.895	79.595	8.548	294.068	168.004	24.471

Values are means for n = 3; Unp represents unplanted soil; 0%, 5% and 10% represents different crude oil spiking percentages; V represents Bambara – planted soil.

Table-3. Profile of trace nutrient in soil samples

	Co	Cu	Zn	Mn	Mo	Se	Fe
0%	10.514	12.518	92.387	444.470	1.311	0.617	54159.800
5%	10.032	12.895	94.471	427.055	1.559	0.671	54631.990
10%	8.971	11.541	88.151	371.104	1.458	0.585	54811.810
0% V	9.711	13.761	106.248	416.930	2.349	0.638	46881.050
5% V	9.873	13.307	107.134	406.857	2.569	0.697	48997.440
10% V	8.574	13.266	94.382	359.905	2.372	0.620	52021.920
0% M	10.519	14.154	110.749	480.651	2.540	0.711	43186.830
5% M	10.424	13.458	108.673	416.551	2.362	0.664	46457.980
10% M	9.726	13.649	98.153	399.452	2.239	0.624	50999.420
0% unp	10.402	12.448	97.469	401.599	2.470	0.675	54740.450
5% unp	9.502	13.346	90.252	402.587	2.310	0.641	54785.320
10% unp	8.838	12.666	88.387	309.846	2.224	0.536	56913.990

Table-4. Profile of trace nutrient in tissue samples (mg kg^{-1})

	Co	Cu	Zn	Mn	Mo	Se	Fe
Shoot							
0% V	0.464	9.916	199.297	139.428	2.709	0.207	965.055
5% V	0.744	9.193	191.397	108.292	3.365	0.257	966.340
10% V	1.498	10.471	203.593	120.745	3.526	0.287	3318.829
0% M	0.295	5.949	130.584	150.706	3.071	0.436	488.034
5% M	0.961	5.887	133.152	144.475	3.368	0.503	519.544
10% M	2.094	4.337	277.975	114.243	6.404	0.528	2188.330
Root							
0% V	2.057	11.753	68.819	90.609	6.725	0.730	7606.755
5% V	5.169	33.812	106.826	131.003	15.184	0.908	7521.630
10% V	5.027	19.877	140.760	173.612	15.166	0.647	7641.722
0% M	3.707	19.010	108.585	134.917	10.960	0.896	2237.550
5% M	10.226	20.617	101.104	1043.101	16.891	0.947	8833.107
10% M	25.284	112.937	657.399	1341.730	30.321	4.386	8834.520

Figure-7. Levels of major dietary elements in soil samples. Means of triplicate data per parameter across soil type was compared at $p < 0.05$. V and M denote Bambara and Mustard plants. Means with different alphabets or asterisks within a group denote significant difference.

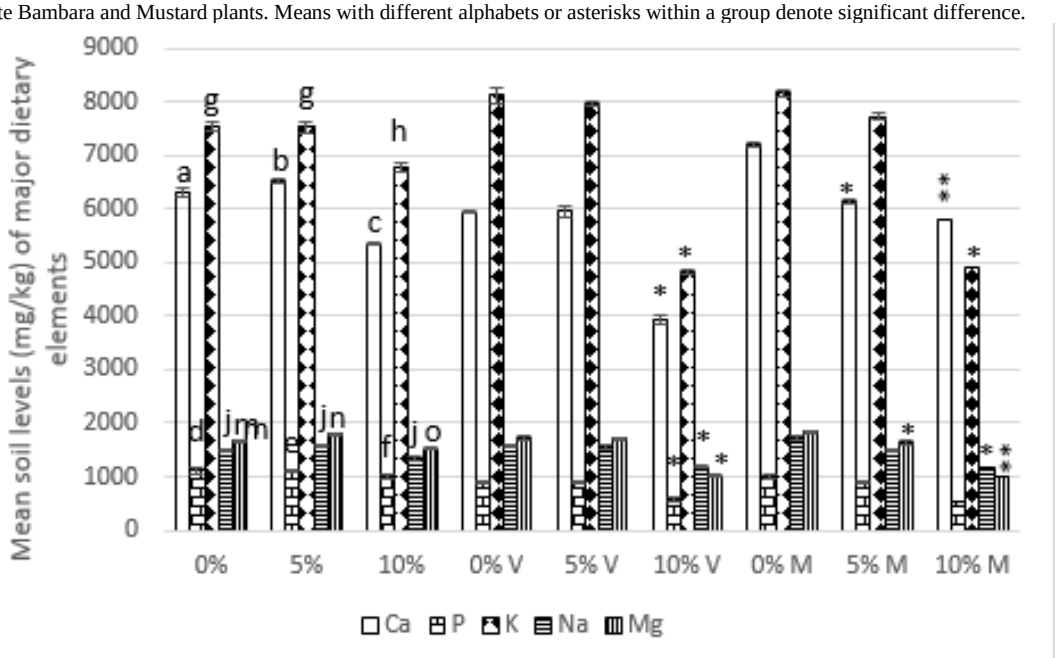

Figure-8. Levels of major dietary elements in shoot samples. Means of triplicate data per parameter across plant type was compared at $p < 0.05$. V and M denote Bambara and Mustard plants. Means with different alphabets within a group denote significant difference.

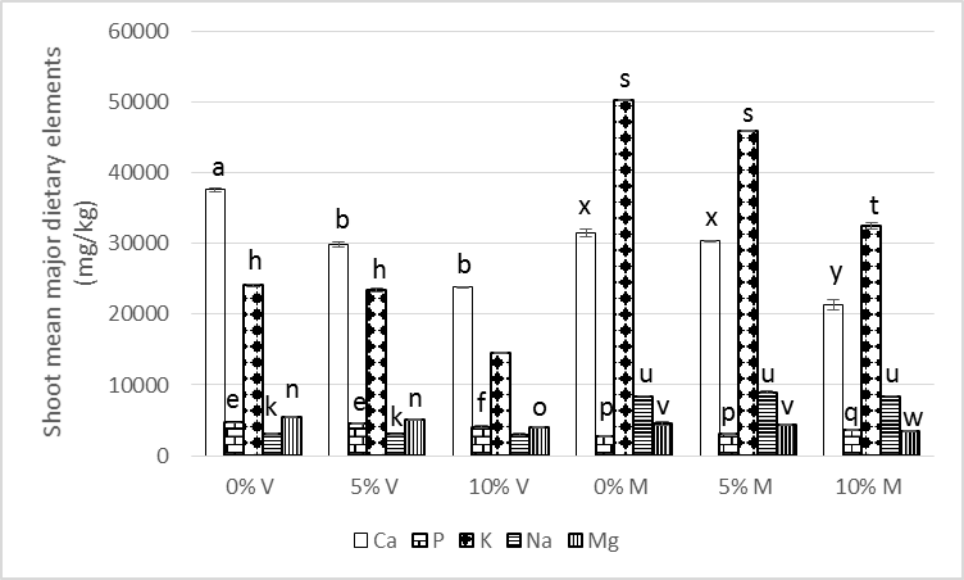

Figure-9. Levels of major dietary elements in root samples. Means of triplicate data per parameter across plant type was compared at $p < 0.05$. V and M denote Bambara and Mustard plants. Means with different alphabets within a group denote significant difference.