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Full Length Research Paper

Determining the tolerance level of *Zea mays* (maize) to a crude oil polluted agricultural soil

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This research sought to investigate the tolerance level of *Zea mays* (maize) on a crude oil contaminated soil using indices of plant performance such as plant height, leaf area and fresh cob yield. In the experiments described, conditions of a major spill were simulated by pouring different amounts of crude oil on experimental pots containing agricultural soil. Maize seeds were then grown amidst adequate fertilizer application and irrigation. The results of the study revealed that maize can survive soil contamination of about 21% (similar to 177 000 mg/kg) and still produce fresh cob yield of about 60% than on normal soil. There was a stimulated increase in fresh cob yield, than that obtained on normal soil at 12.5% soil contamination (similar to 112240 mg/kg). Hence, contaminant concentrations of 112 240 mg/kg and 177 000 mg/kg are identified as the 'optimum yield limit' and 'lethal threshold' respectively for maize growing on crude oil polluted soils. These results highlight the fact that, while concerted efforts should be made to remedy petroleum-contaminated agricultural soils, certain crops like maize can still produce beneficial yield in the presence of good soil management practices.

Key words: Contaminant concentrations, crude oil contamination, fresh cob yield, lethal threshold, optimum yield limit, plant height.

INTRODUCTION

The increasing dependence of humanity on fossil fuels, especially petroleum- hydrocarbons has led to the pollution of agricultural lands, owing to spillage of crude oil during extraction and processing operations, in many oil-producing countries. In Nigeria, the story is unique as it is on record that between 1976 and 1980, a total of about 784 incidences of oil spills led to the release of about 56.1 million barrels of crude oil into aquatic and terrestrial ecosystems (Awobajo, 1981). Since then, crude oil contamination of farmlands has become common experience in the country, and many of these farmlands have been abandoned in the aftermath of pollution. Consequently, the present study was carried out to ascertain the growth potentials of a commonly grown crop in the country, *Zea mays* (maize), on a crude oil contaminated soil.

Studies have revealed that the occurrence of large amounts of hydrocarbons in the soil leads to a nitrogen deficiency and hence upsets the carbon-nitrogen ratio at the spill site thereby threatening the survival of soil biota (Jobson et al., 1974). Moreover, the infiltration of the contaminant into soil pores leads to the expulsion of air thus depleting oxygen reserves in the soil and impeding its diffusion to the deeper layers (Bossert and Bartha, 1984; Ayotamuno et al., 2006a). Thereafter, as microbial activities (involving the utilization of oxygen for biodegradation of the contaminant) increase, available oxygen diminishes in the soil environment (Ayotamuno et al., 2006b). This limits the survival of plants as they then lack essential elements for their growth. Furthermore, research on the remediation of crude oil contaminated soils using plants (phytoremediation) has shown that certain plants (including maize) could contain, translocate and/or volatilize petroleum-hydrocarbons as they grow on crude oil contaminated soils, although not without constraints like leaf burn, wilting and stunted growth (Cunningham et al., 1996; Wiltse et al., 1998; Ayotamuno et al., 2006c). However, the exact level(s) of contaminant con-

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centration(s) which they can tolerate and give benefi-cial yield remains not well understood.

The present study emanated from the lines of convergence of the above works. Its focus was to investigate the level(s) of toxicity within which the crop under study could survive and still produce beneficial yield to farmers operating in environmental conditions similar to those of the study area. This stems from the observation that when an oil spill occurs in a rural community in Nigeria, irrespective of the volume of spill, the local farmers usually abandon their farms in search of compensation from the powers that be. Their claims consist in the argument that their farms would no longer yield any useful returns. A similar notion is also held in other regions of the world as knowledge of the deleterious effects of petroleum pollution of soils has informed the conclusion that polluted soils are incapable of compasssing beneficial crop yield. This has contributed to low agricultural productivity in the short and long terms. In the light of the above, this study sought to evaluate the measure of justification in these claims. Its primary objective was to determine the level(s) of soil contamination that could be tolerated by maize crop growing on soil conditions similar to those of the study area, using parameters such as plant height, leaf area and fresh cob yield as the indices of evaluation. The study also sought to investigate the utility of increased levels of fertilizer application and irrigation in the enhancement of the tolerance level of the crop.

MATERIALS AND METHODS

Study area description

The study was conducted at the Rivers State University of Science and Technology, Port Harcourt between the months of February and May 2006. The experimental area is made up of flat plains with soil characterized as coastal plain sand. The soil type is Ultisol (USDA classification) and its texture is sandy loam. The climatic condition is that of a humid tropical climate with an average rainfall of about 2100 mm, of which 70% falls between May and August. The rest of the year is relatively dry with the mean air temperature varying between 24 and 30^{o}C (Ayotamuno et al., 1997). Port Harcourt is economically the most important city in the Niger delta region of Nigeria. From this region, more than 98% of Nigeria's current economic mainstay, crude oil, is derived. Hence this research became pertinent in the study area as frequent oil -spills arising from crude-oil exploration and development activities have devastated farmlands and other agricultural settlements over the years (Ayotamuno et al., 2003).

Experimental set up

Quantities of top soil was collected from the study area and put into planting pots each 0.21 m deep with a volume of 0.008 m^3. Spilled crude oil obtained from an acute spill site in K-Dere, Ogoniland in the Niger delta area of Nigeria was then used to contaminate the planting pots. The experimental set up consisted of seven (7) treatment options and a control, each option had three replications. The pots were treated with seven different volumes of crude oil varying from 1.0 to 3.0 liters (that is, 0.001 – 0.003 m^3), in exception of the control option which was left uncontaminated. Hence the percentage soil contamination varied between 12.5 and 37.5%. This was based on the formula; Percentage soil contamination = (Volume of crude oil applied/Volume of soil) x 100.

Maize seeds (JO – 195), which are an early-maturing variety were then planted in the pots seven days after crude oil contamination. The planting pots were perforated at the bottom and sides to allow for aeration and drainage of excess water. However, this was done after the interval of seven days in the aftermath of crude oil contamination when the contaminant had been fully adsorbed unto soil pores. The aim was to ensure adequate aeration necessary for seed germination, and also to simulate conditions of vertical and lateral movement of water that occurs in the soil.

Other field practices

A green house was constructed on the field sites to provide the much needed heat radiation, for the optimal growth of the plants. The black nylon used for this purpose also served to prevent the interference of rainfall and moisture from the ground. Pre-planting irrigation (first irrigation after seed bed preparation) was done for a period of five days after crude oil contamination prior to seed planting. The irrigation continued throughout the experimental period (thirteen weeks) and was applied using watering cans such that each treatment option received a daily irrigation depth of 3.5 mm. The choice of this application depth emanated from the findings of Ayotamuno et al. (2000) that such an application level could yield great returns for maize during the dry season in the study area. Fertilizer (15-15-15 NPK) was applied to all the options (including the control) to improve the nutrient content of the soils. The application rate was 100 g per pot (similar to 26 300 kg/ha); this was done on a weekly basis for a period of five weeks beginning from the third week after planting (3 WAP). Moreover, it is necessary to point out that prior to planting (after crude oil contamination) 200 g of the fertilizer was worked into the contaminated soils, so as to give way for seed germination, the same was done to the control option to ensure uniformity. Each of the pots originally contained five maize seeds which were later thinned to three stands, 2 WAP.

Analysis of soil and plant characteristics

Surface soil samples were collected from soils within the experimental site before crude oil contamination using a hand dug soil auger. This was done by way of auguring different random spots and pooling them together to form composite samples. This procedure was carried out three times to form three replicates. The samples were then put in polyethylene bags and transferred to the laboratory for analyses. Soil properties such as texture, bulk density, moisture content, field capacity, permanent wilting point, organic carbon and total nitrogen contents were analyzed using methods adapted from relevant literatures (Black, 1979; Juo, 1979).

After crude oil contamination the total hydrocarbon content (THC) of the soils in the various planting pots were determined, so as to establish the true concentration of the contaminant (in mg of oil per kg of soil). The analytical procedure involved the use of toluene to extract the hydrocarbon content, the absorbance of the extract was then determined at 420 nm in a Spectronic 70 spectrophotometer. Thereafter, the THC of the soils was determined from standard curves of known concentrations of petroleum fractions. Details of this method of analysis are contained in the work of Osuji et al. (2006). During the course of the experiments, indices of plant performance such as plant height, fresh cob yield and leaf area were determined in order to evaluate the tolerance level of the plant to the contaminant. Leaf area determination was based on the non

Table 1. Soil characteristics before crude oil contamination.

Treatment option	Quantity of crude oil (m^3)	Moisture by weight (%)	Field Capacity (%)	Permanent Wilting Point (%)	Bulk density (Kg/m^3)	Organic carbon (%)	Total nitrogen (%)
S_1	0.0030	14 ± 1	15.30 ± 0.8	6.18 ± 0.9	1560 ± 10	1.25 ± 0.03	0.07 ± 0.008
S_2	0.0025	15 ± 2	15.16 ± 1.0	6.03 ± 0.6	1510 ± 10	1.02 ± 0.04	0.06 ± 0.009
S_3	0.0020	12 ± 1	15.86 ± 0.6	6.25 ± 0.6	1590 ± 20	1.26 ± 0.03	0.06 ± 0.01
S_4	0.0017	10 ± 2	15.03 ± 0.8	5.97 ± 0.8	1440 ± 30	1.19 ± 0.02	0.07 ± 0.007
S_5	0.0015	16 ± 1	14.78 ± 1.0	5.74 ± 0.5	1420 ± 30	1.18 ± 0.02	0.07 ± 0.008
S_6	0.0013	9 ± 3	14.94 ± 0.5	5.81 ± 0.6	1430 ± 18	1.19 ± 0.04	0.08 ± 0.01
S_7	0.0010	14 ± 2	15.08 ± 0.9	5.97 ± 0.5	1450 ± 26	1.21 ± 0.03	0.06 ± 0.009
S_8	0.0000	12 ± 2	15.22 ± 0.7	6.18 ± 0.7	1540 ± 20	1.22 ± 0.01	0.08 ± 0.007

Results represent means ± standard deviation of three replicates.

destructive length x width method described by Saxena and Singh (1985) . The method estimates the leaf area as equal to: 0.75 x length of leaf x width of leaf; where 0.75 is a constant.

A one-way analysis of variance (ANOVA) was performed on the data obtained from the various replications of the experiment in order to compare the mean values for the indices of plant performance, gotten from the various treatment options. The purpose of the analysis was to determine whether the differences in the mean values for the various indices of performance were real and due to the different treatment applications or due to error. The analysis followed the procedure described by Frank and Althoen (1994).

RESULTS AND DISCUSSION

The particle size analysis of soil samples from the various planting pots indicated that on the average the soils were made up of 78 sand, 10 silt and 12% clay. From the soil textural triangle, this corresponds to a sandy loam soil; a soil type characterized by moderate permeability and conducive for plant growth. Other physical characteristics of the soils are as shown in Table 1.

The results of the THC analysis indicated that the concentration of the contaminant in the various pots varied from 111 240 to 312 862 mg/kg (Table 2, column 4). These results in turn produced distinct variations on the indices of plant growth. It was observed during the study that the seeds in options S_1, S_2, and S_3 (which had 31.75, 31.25 and 25% soil contamination respectively) (Table 2) germinated two days after the ones in the other options with lesser contaminant levels; the seeds in the other options germinated four days after planting thus highlighting the fact that very high concentration(s) of the contaminant in the soil leads to delayed seed germination. Amakiri and Onofeghara (1983) have previously reported a similar observation. Moreover, when the plants in the said options eventually sprouted, they all died 3 WAP after attaining a maximum height of the order 10 – 15 cm (0.1 – 0.15 m) . During their growth period, the leaves of the plants in the three options had more pronounced traces of leaf burn (yellowing of leaves) than the ones in the other options, an indication that the plants were trying to accumulate and volatilize the contaminant. However, as the concentrations of the contaminant were so high the plants could not withstand the toxicity of the soil environment, hence they eventually wilted away and died.

The other options, S_4, S_5, S_6, S_7 and S_8 (which had 21.25, 18.75, 16.25, 12.5 and 0% soil contamination) produced plants that thrived to maturity with the indices of performance used in the study responding to the variations in contaminant concentrations. After a thirteen-week period it was observed that the plants in the control option (S_8) grew to an average plant height of 2.4 m which is in line with the range for maize plant height (1.5 – 3.5 m, depending on variety) reported by the International Institute of tropical Agriculture (I.I.T.A), Ibadan, Nigeria. Using the mean height of the plants in the control option as a standard (Table 2), column 5 that options S_4-S_7 had plant heights similar to that of the control, with option S_4 (THC of 177 289 mg/kg) having a height of 2.2 m and option S_7 (THC of 111 240 mg/kg) having a height of 2.32 m. These values buttress the position that maize has a high tolerance level to crude oil contaminated soils. This is further supported by a related study (Ayotamuno et al., 2006c) in which maize plants grown on a crude oil contaminated plot with a THC of about 22 000 mg/kg induced a reduction in the THC of the soil to about 6000 mg/kg after a six-week period.

During the early stages of growth, the plants were characterized by leaf burn, but as time progressed, especially with the application of the nitrogenous fertilizer the leaf burn decreased to a minimum across the various treatments. This corroborates with the findings of previous studies (Wiltse et al., 1998; Schwab et al., 1999) that the adaptive mechanisms of some plants to crude oil contaminated soils include: the uptake of hydrocarbons from contaminated soils by plants (phytoaccumulation), and the transfer of volatile fractions of the contaminant to the atmosphere through the leaves (phytovolatilization).

Table 2. Indices of assessment of *Zea mays* tolerance to crude oil polluted soils.

Treatment option	Quantity of crude oil (m^3)	soil Contamination (%)	THC (mg/kg)	Max. plant height (m)	Leaf area (m^2)	Fresh cob yield (Kg/pot)
S_1	0.0030	37.50	312 862 ±250	*	**	0.00
S_2	0.0025	31.25	260 750 ±180	*	**	0.00
S_3	0.0020	25.00	208 575 ±120	*	**	0.00
S_4	0.0017	21.25	177 289 ± 80	2.20 ± 0.03	0.090 ± 0.0009	0.60 ± 0.02
S_5	0.0015	18.75	156 431 ±150	2.29 ± 0.01	0.092 ± 0.0009	0.65 ± 0.01
S_6	0.0013	16.25	135 574 ±110	2.30 ± 0.005	0.088 ± 0.001	0.79 ± 0.02
S_7	0.0010	12.50	111 240 ±110	2.32 ± 0.01	0.094 ± 0.0008	1.00 ± 0.01
S_8	0.0000	0.00	25 ± 4	2.4 ± 0.02	0.098 ± 0.0008	0.93 ± 0.01

Results represent means ± standard deviation of three replicates.
*Negligible height of the order 10 – 15 cm.
**Negligible leaf area of the order 4 x 10^{-5} m^2.

Moreover, at a contaminant concentration of 177 289mg/kg the plants produced a mean leaf area of 0.0924 m^2 similar to the leaf area (0.098 m^2) obtained in the control (Table 2, column 6). The import of the above is that at contaminant concentrations or better put, THC of about 177 000 mg/kg, the plants can still adapt with adequate nutrient supplementation, while translocating volatile petroleum hydrocarbons through the leaves.

The weights of the fresh maize cobs at the end of the thirteen-week study period are shown in column 7 of Table 2. Of interest here is the observation that the overall weight of the fresh cobs (1.00 kg/pot) produced by plants grown on option S_7 which had an initial THC of 111 240 mg/kg exceeded that of the control option (which was not contaminated with crude oil but had a background THC of 25 mg/kg) whose fresh cob weight was 0.93 kg/pot. This implies that the presence of some amounts of hydrocarbons in the soil, that is tolerable to plants, probably has an effect on increased fresh cob yield since there was uniformity in fertilizer application across the treatments. Previous studies have documented related developments. Harper (1939) reported that there exists some enhancement in the growth of crops growing on crude oil polluted soils. Rowell (1977) also pointed out that nutrients could be more available to plants on complete decomposition of spilled crude oil, while Plice (1948) found out that a soil contaminated with crude oil to a depth of about 121.9 cm remained unproductive for several years but after seven years it became more fruitful than the surrounding normal soil. Similarly, Vwioko and Fashemi (2005) reported stimulation in growth of *Ricinus communis* (Castor oil) which affected parameters like fresh and dry weights, leaf area and root length, at 1% w/w contamination on soil polluted by lubricating oil. Reconciling these observations with the one in the present study, it could be pointed out that the fertilizer applied facilitated the degradation of the contaminant by aiding the activities of the large numbers of microbes usually associated with the plant root zone (rhizosphere) (Lee and Banks, 1993). The result of this was the remediation of the soil through the combined effects of bioremediation and phytoremediation alongside the availability of nutrients for plant utilization thus leading to an increased fresh cob yield at a more tolerable THC level (111 240 mg/kg). The other options with higher contaminant levels (S_1 - S_6) had fresh cob yields lower than that of the control, thus highlighting the position that these levels of contamination imposed a greater stress on the plants.

Statistical analysis (one-way ANOVA) based on the test hypothesis that the plant populations are not different in average fresh cob yield / leaf area, 13 WAP, indicated significance at 0.05% probability level (Table 3). This implies that the different levels of contaminant concentration at which the plants survived did not produce identical fresh cob yields / leaf area. Moreover, the significant differences in leaf area is expected since the leaf area is the major part of the plant involved in phytovolatilization (mentioned earlier in this paper), thus different contaminant concentrations would probably induce marked differences in the dimensions of leaves. Hence we conclude that so far as fresh cob yield / leaf area is relevant to plant performance, the results suggest that maize performance levels respond to variations in contaminant concentration. On the other hand, the average plant height was not significant at 5% probability level (Table 3). This forms the conclusion that at contaminant concentrations not lethal to plants, at which the plants can grow successfully, the populations produce identical heights.

Conclusions and recommendations

The results of this study has shown that *Z. mays* has a high tolerance level compared to many other crops and could be grown (or continue to grow) on crude oil contaminated soils with contaminant concentration of about 21% by volume of the soil. From spectrophotometer readings, this is similar to 177 289 mg/kg. However, the yield that would be obtained at that level is about 60% of the one on a normal sandy loam soil. At THC levels

Table 3: ANOVA summary table showingeffects of treatment applications on indices of plant performance

Parameter	Source of Variation	S.S	d.f	M.S	$F_{calculated}$	p-value	$F_{critical}$
Fresh cob weight (Kg/pot)	Among groups	0.3575	4	0.0894			
	Within groups	0.0035	10	0.00035	255*	0.9995	13.4
	Totals	0.361	14				
Maximum plant height (m)	Among groups	0.13	4	0.0325			
	Within groups	-0.041	10	-0.0041	-7.93^{ns}	0.95	3.48
	Totals	0.089	14				
Leaf area (m^2)	Among groups	0.00018	4	0.000045			
	Within groups	0.00001	10	0.000001	45*	0.9995	13.4
	Totals	0.000181	14				

* significant at 0.05% ; ^{ns} not significant at 5% probability levels

above this value, soil conditions become lethal to the plants. Thus 177 000 mg/kg (similar to 21% soil contamination) is identified as the 'lethal threshold' for maize crop growing on crude oil contaminated soil amidst adequate nutrient supplementation and irrigation.

From the experience of this study, it is worthy of mention that adequate fertilizer application and irrigation has to be resorted to, with immediate effect, in the aftermath of crude oil pollution of agricultural soils to forestall the adverse effects induced by the presence of the contaminant. Ayotamuno et al. (2006a) in a bioremediation study without plants on the experimental plots reported that increased concentrations of nutrients (through the application of fertilizers) lead to greater rates of biodegradation. Hence they established that an application rate of about 12.5 ton/ha will induce an accelerated biodegradation of the crude oil contaminant in a polluted agricultural soil. However, the experience of the present study has shown that application rates in the order of 26 ton/ha, with 15-15-15 NPK fertilizer, may be very effective in supporting plants grown on crude oil polluted agricultural soils, since in this case both the hydrocarbon degrading bacteria and the plants are competing for available nutrients. An evidence of this (higher fertilizer application rate) is the improvement in plant height and leaf area recorded in the present study, which is relatively higher than the ones reported by Egharevba and Mohammed (2005). The researchers used the same crop variety on a normal loamy sand soil and a fertilizer application rate of 250 kg/ha in two equal split dosages; and at the end of 16 weeks the maximum plant height and leaf area obtained were 1.95 m and 0.084 m^2 respectively, which is lower than the ranges (2.2 – 2.32 m and 0.09 – 0.094 m^2 for plant height and leaf area respectively) obtained in the present study (see Table 2, columns 5 and 6). These points to the utility of increased levels of fertilizer application in the enhancement of the tolerance level of the crop.

The results of this study further revealed that with adequate nutrient supplementation, the plants could even give a better fresh cob yield than normal soil conditions, at about 12.5% soil contamination (similar to 111 240 mg/kg). Hence 111 240 mg/kg is identified as the 'optimum yield limit'. This is probably due to the hypothesis that THC level stimulated microbes in the soil can still tolerate soil conditions and effect a reasonable biodegradation alongside the plants' containment of the contaminant thereby providing useful nutrients to the plants that favors their yield. However, the level(s) of contaminant attenuation during the study period was not investigated as this was outside the scope of the study. All in all, this study has shown that while concerted efforts should be made to remedy crude oil contaminated agricultural soils, certain crops like maize can still produce beneficial yield on such soils in the presence of good soil management practices.

We therefore recommend that future research may continue along the lines of investigating the maximum amount(s) of fertilizer that could be applied in similar situations, just until the plants can no longer survive it again. This will in turn provide knowledge on the optimum application rate that can support maize growing on crude oil contaminated soils.

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