JASEM ISSN 1119-8362

Full-text Available Online at www.ajol.infoand www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *Feb.* 2017
Vol. 21 (1) 18-28

Microcosm Trial of the use of detergent for Mitigation of Crude Oil Toxicity for Optimal Growth of Maize (*Zea mays*, L)

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ABSTRACT: Study on the use of detergent as an ameliorating agent in crude oil polluted soil on hydrocarbon reduction and optimal growth of maize was carried out at the Centre for Ecological Studies, University of Port Harcourt. Pollution level of 3% w/v was remediated with 20 g detergent and 40 g detergent alongside a control (polluted soil without amendment). The amended soil was allowed for 4 weeks before maize seeds were planted in them. Soil total hydrocarbon content (THC) was determined at initial and end of the experiment. Measurements of growth parameters of maize were done monthly for three (3) months. Significant reduction in THC was observed in the treatment soils in the order of 20 g detergent (44.2%)> control (28.0%) > 40 g detergent (19.9%). Maize parameters such as germination, plant height, root length, leaf number, leaf area, fresh and dry weights and chlorophyll content showed significant (P=0.05) increase in 20 g detergent treatment than 40 g detergent and control treatments, except in moisture content (%) and shoot/root ratio which recorded highest level in the 40 g detergent. Therefore, low concentration of detergent is effective for the remediation of crude oil polluted soil for optimal growth performance of maize. ©JASEM

http://dx.doi.org/10.4314/jasem.v21i1.3

Keywords*:* detergent, crude oil, toxicity, remediation, maize, hydrocarbon.

The wide spread contaminations of the Niger Delta environment with petroleum hydrocarbon has been on the increase due to increasing petroleum exploration in the area (Odokuma and Ibor, 2002; Ite *et al.*, 2013). This situation became worse with the incessant vandalism of oil pipelines by militants and oil thieves. Petroleum hydrocarbon contaminations pose serious environmental problems and remain the major cause of depletion of the Niger Delta ecosystems. Anoliefo *et al*. (2000) stated that acute or chronic crude oil pollution has deleterious effects on the ecosystem.

On terrestrial ecosystem, crude oil contamination affects soil chemical properties such as electrical conductivity, mineral and organic matter content; cation exchange capacity and pH (Gighi *et al*. 2012). The anaerobic and hydrophobic conditions in the soil created by crude oil may pose threat to plant growth because it hinders soil water infiltration and aeration. Crude oil spills on land have been reported to affect all aspect of plant performance such as germination and growth (Onwurah, *et al*., 2007, Essien and John, 2010); the yellowing (chlorosis) of impacted leaves (Raven, *et al*, 1999); biomass accumulation of arable crops such as rice, cassava, maize, etc (Tanee and Anyanwu, 2007, Onwurah, *et al*., 2007; Akujuru, 2014).This may indirectly have dire consequences on food production (Emmanuel and Gordon, 2006). It has been observed that the degree of impact on such crops depends on many factors such as the growth habit and age of the plant, type of crude oil, time of the year and quantity of spill (Pezeshki *et al*., 2000; Sarkar *et al*., 2005). For instance, Oyedeji *et al.*, (2012) observed poor seed germination in *Abelmoschus esculentus* while rye grass was not impacted in a crude oil pollution soil with reference to the control.

However, due to serious pressure on the available land for agriculture as a result of crude oil pollution coupled with urbanization, increase in human population, commercialization, road construction and other projects; hence the need to apply remedial measures on polluted lands. Due to the exigency of the pollution problem, several remediation technologies and trials have been propounded for a crude oil polluted environment. Although, some besides being more expensive and difficult to apply, have been reported to cause more harm to the environment than the pollutant. So the use of simple, economical, environmentally friendly and efficient technology for the remediation of crude oil contaminated site has become imperative.

Chemical remediation involves the use of chemical substances such as detergent/ degreasers as

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amendment in polluted environment. Detergent contains some active ingredients such as surfactant, sodium, chlorine and bleach. It is mostly used for domestic and industrial washing/cleaning. The use of these chemicals in crude oil contaminated soil is among the current progressive techniques available for decontaminating crude oil polluted soils or removal of hydrocarbon from the soil. These chemicals emulsify and weaken the hydrocarbon chains and thus, provide a good surface area for biodegradation (Couto *et al*., 2010). The use of these chemicals has been extensively reviewed by Lee and DeMora (1999) and; the fear of its long-term environmental effect and toxicity has been a major concern (USEPA, 1999).

The objective of this study is to evaluate the effectiveness of detergent for the reduction of crude oil toxicity in a microcosm experiment for optimal growth of maize. Result from this study will expand our scope of bioremediation technology of crude oil pollution and boost agricultural production especially in the Niger Delta where crude oil pollution is a problem.

MATERIALS AND METHODS

Experimental site description. The experiment was conducted at University of Port Harcourt, Choba, Rivers State of Nigeria. University of Port Harcourt is located between Latitude 4 ^{0}N and 5 ^{0}N, and Longitude 6 ^{0}E and 7 ^{0}E. The site of this research is the research field of the Centre for Ecological Studies of the Department of Plant Science and Biotechnology, behind Faculty of Science Multipurpose Building Complex at the main University Campus.

The mean precipitation (rainfall) for the area was about 2500 mm/year; while the minimum and maximum temperatures were 25 ^{0}C and 35 ^{0}C.

Soil collection and contamination. Soil was collected from an old agricultural farmland within the university environment for the experiment. Loamy soil was collected at the surface with a spade. Plant and other particles were removed and the soils thoroughly mix to homogeneity. Ten (10) kg of soil was weighed into each perforated experimental bag. A total of 54 bags filled with study soil were setup for the experiment. Each bag filled with soil was artificially polluted with 300 ml of Bonny light crude oil obtained from Port Harcourt Refinery Company, Eleme. This pollution treatment is equivalent to 3% v/w pollution. The set up was allowed for 14 days (2 weeks) for acclimatization of the oil with the soil under natural (ambient) environmental conditions.

Addition of Amendments: Detergent (brand name: Zip) obtained from Eleme market was applied as the amendment agent. The chemical ingredients of the detergent used are surfactant, enzymes, photobleach, optical brightening agent, builder, perfume and anti-redeposition agent. Two treatment (concentration) levels of detergent were used as amendment alongside the control as shown below:

T_a: 10 kg soil + 300 ml crude + 20 g detergent,
T_b: 10 kg soil + 300 ml crude + 40 g detergent),
T_c: 10 kg soil + 300ml crude oil (Control).

The amendments were thoroughly mixed with the contaminated soil to obtain a uniform mixture. The amendment set-up was allowed for one month before planting of the maize seeds.

Maize planting: Maize seeds *(Zea mays L)* (var: swan-1 yellow) obtained from the Centre for Ecological Studies, University of Port Harcourt were planted after one month of amendment treatment addition. The maize seeds were planted in the experimental bags at the rate of five seeds per bag at the depth of 4 cm. After two weeks of planting, the seedlings were thinned to three (3) per bag after germination. The growth performance of the test plant (maize) was monitored for three (3) month using some selected growth parameters as indices of treatment effects.

Determination of Total Hydrocarbon content (THC): Soxhlet Extraction Method was used for the determination of Total Hydrocarbon Content of the treatment soils (APHA, 1995). Ten (10) grams of the sample was homogenized with 10 grams of anhydrous sodium sulphate, and the sample was transferred to an extraction thimble and covered with glass wool. The soxhlet apparatus containing the extraction thimble and sample was set up with a 250 ml boiling flask containing 90 ml of n-hexane. At the end of 4 hours extraction period, the organic extract was filtered through grease-free cotton, into the pre-weighed boiling flask and the solvent was distilled. On complete distillation, the flask was then cooled in desiccators for 30 minutes and weighed. The concentration of Hexane Extractable Material (HEM) in the soil samples was calculated.

Evaluation of maize growth parameters: Selected plant growth parameters such as germination percentage, plant height, number of leaf, root length, total fresh weight, total dry weight, dry weights of shoot and root, shoot-root ratio were determined. The chlorophyll and percentage moisture contents were also analyzed. These parameters were determined at monthly interval for three months.

Germination percentage was determined by counting the number of seeds that germinated within 5days after planting. This was calculated as:

$$\% \text{ Germination} = \frac{number\ of\ seeds\ that\ germinated\ per\ pot}{number\ of\ seeds\ planted\ per\ pot}\ x\ \frac{100}{1}$$

Plant height (shoot length) determination was done by measuring the shoot lengths of the plants in the bags from the soil level to the terminal bud using a meter rule. Mean height of the plant was calculated.

The leaf number count was done by taking into consideration all leaves for each plant sampled.

Using the destructive method 3 replicates (i.e 3 bags planted with maize of each treatment) were harvested. The poly bags were shaken in order for the plants to lose grip, to make the removal of the plants easier, and to prevent root damage. The fresh weight of the harvested plants were taken using an electronic weighing balance (Model EK5350) immediately after harvest and the values obtained were recorded. Root length was measured with a metre rule calibrated in centimetre (cm). Lengths of three longest roots on each plant sampled/bag were measured (cm) and average values calculated were recorded

.

Total dry weight, dry weight of shoot and root were determined after oven-drying for 48 hrs at 75 ^{o}C. The dried plants were then weighed on an electronic weighing balance (Model EK5350) to obtain the respective weights. The formula: Leaf area (LA) = 0.89 × length of the leaf × width of the leaf (broadest part) and 0.89 is a constant (Asoegwu, 1988) was used for the calculation of leaf area and the total leaf area was calculated. Shoot-root ratio was calculated by dividing the above-ground (shoot) dry weight by the below-ground (root) dry weight.

The chlorophyll content was determined by the colorimetric method at optical density of 660 and 645 nm according to the method of Comar and Zscheile (1942) while the percentage moisture content was calculated using the formula

$$\% \text{ moisture content} = \frac{fresh\ weight - dry\ weight}{dry\ weight}\ x\ \frac{100}{1}$$

*Statistical Analysis***:** Mean and standard error mean (SEM) were calculated. The data generated were analyzed using Analysis of variance (ANOVA) and Least Significant Difference (LSD) statistical tools according to SPSS data analysis package (2014 version).

RESULTS AND DISCUSSION

*Reduction in total hydrocarbon content (THC):*The total hydrocarbon content was recorded to reduce significantly (P=0.05) in the detergent remediated soil from the initial to the final as compared to the unamended soil. The reduction was more significant in the 20g detergent amended soil (44.2%) than the control soil (28.0%) while the least reduction was recorded in 40 g detergent amended soil (19.9%) (Fig.1)

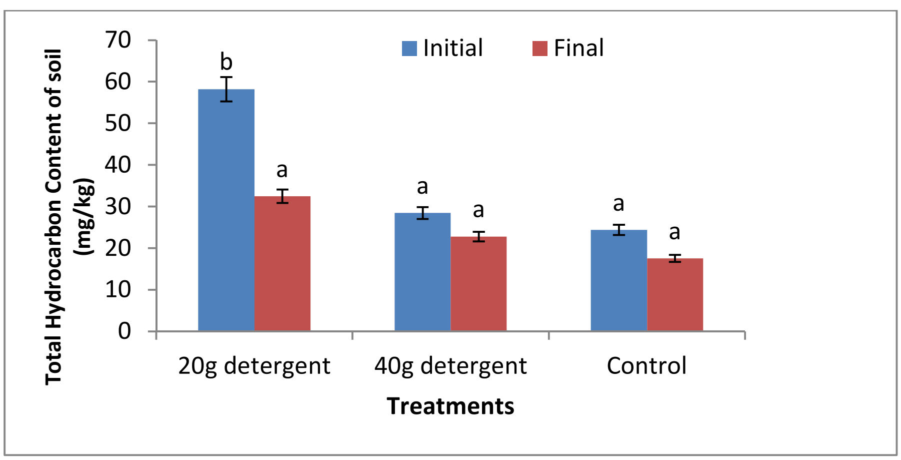

Fig. 1: Hydrocarbon content in the different remediation treatments.

Several materials have been identified with the potential to decontaminate polluted environment such as crude oil contaminated soil. Such materials can be organic or inorganic in nature.Since crude oils are hydrophobic in nature, their availability to microorganisms is limited and this leads to their slow degradation as recorded in the control. Adding detergent to the contaminated soil helps desorbs the petroleum product and hence boosts remediation process by hydrocarbon degrading microorganisms. Detergent removes oil attached strongly to soil particles for easy access and accelerated degradation by microorganisms (Couto *et al*., 2010). This is in line with Essien and John (2010) who observed that the molecule bonds in the oil that contributed to the blocking of soil pores are broken by the detergent solution and as a result, the media channel for transfer of water, soil aeration and ionic exchange are opened for enhanced bioremediation.

Results show higher reductions in THC in 20 g detergent remediated soil than the 40 g detergent remediated and control soil. This is an indication that detergent in higher concentration may be toxic to soil microorganisms and alters the soil chemistry such as pH, conductivity and ionic contents. This assertion is justifiable since detergent contains chemical substances such as bleach, surfactant, chlorine and sodium. These substances have been known to be toxic to soil microorganisms especially at high concentration (Chan, 2015) leading to death of crude oil degraders in the soil.

*Effect of amendment on growth parameters of maize***:** The result obtained for percentage germination of maize grown in crude oil polluted soil amended with detergent (Fig. 2) showed that treatments with 20 g detergent and 40 g detergent recorded high percentage germination while the least percentage germination was recorded in crude oil polluted soil without amendment.

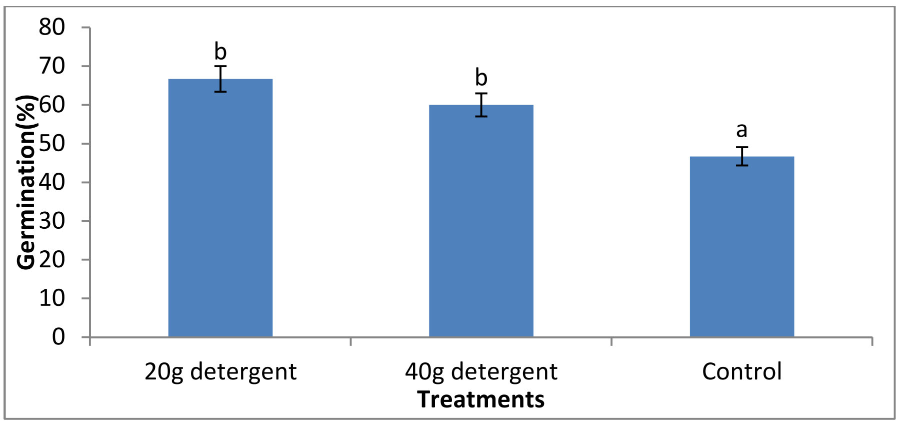

Fig. 2: Germination percentage in the different remediation treatments**.**

Germination was found to also occur in the polluted soil without amendment. This is in line with Gbadebo and Adenuga (2012) who observed germination of seeds especially cowpea in crude oil polluted soil. Least germination percentage recorded in the control soil than the remediated soil is an indication of the adverse effect of crude oil to seed germination (Onuh *et al*., 2008; Essien and John, 2010).This also shows that the conditions necessary for optimal germination of seeds such as oxygen concentration, nutrients, moderate temperature, pH, and moisture content were lacking in crude oil polluted soil. For instance, the hydrophobic nature of oil in soil may have reduced water availability to seed for germination. The high percentage germination recorded in the amended soil with detergent may be attributed to the fact that the amended agent (detergent) modifies the soil and provides a conducive ground for seed germination. Such modifications include reduction in soil THC toxicity, improvement in soil pH and other parameters. This is because crude oil pollution has been reported to increase the acidity of the soil; reduce soil water holding capacity, nutrient and aeration (Abosede, 2013; Oyem and Oyem, 2013) which may hinder germination. So amending the soil reduced these adverse effects.

The shoot length (height) of Maize at 1, 2 and 3 MAP (months after planting) showed significant increase in the 20g detergent remediated soil than the 40 g and unremediated soils (Fig. 3). However, 40 g detergent remediated soil recorded higher ($P=0.05$) shoot length at 1 and 2 MAP but no significant difference was recorded at 3 MAP between 40 g detergent remediated and the control soils.

The number of leaves (Fig. 4) and leaf area (Fig. 5) of maize grown on crude oil polluted soil amended with detergent showed a significant increase in the number of leaves and leaf area in the remediated soil than the control. Although, highest increase was observed in the 20 g detergent remediated soil followed by 40 g detergent remediated soil especially at 1 and 2 MAP. A decrease (P=0.05) in the leaf number and leaf area were recorded at 3 MAP in all the treatment options with the control having the least number of leaves.

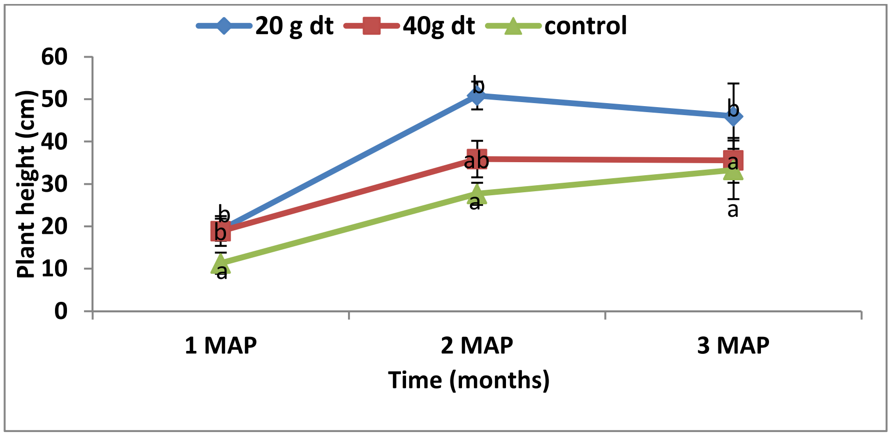

Fig. 3: Plant height of maize in the remediation treatments.

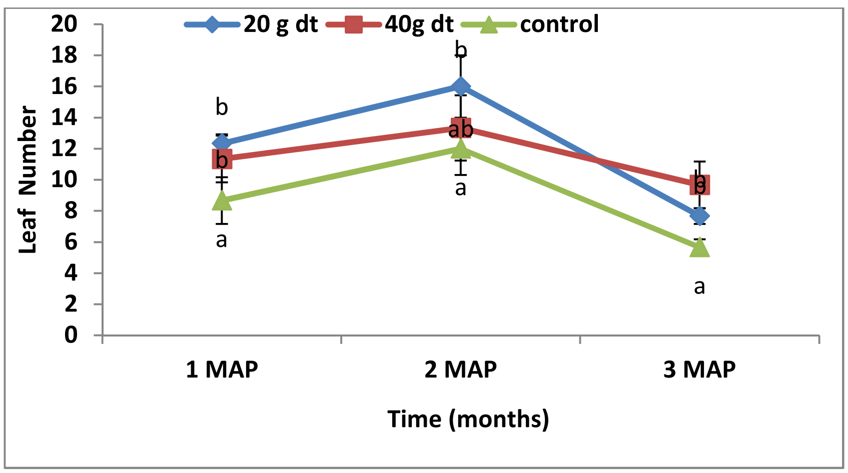

Fig. 4: Leaf number of maize in the remediation treatments.

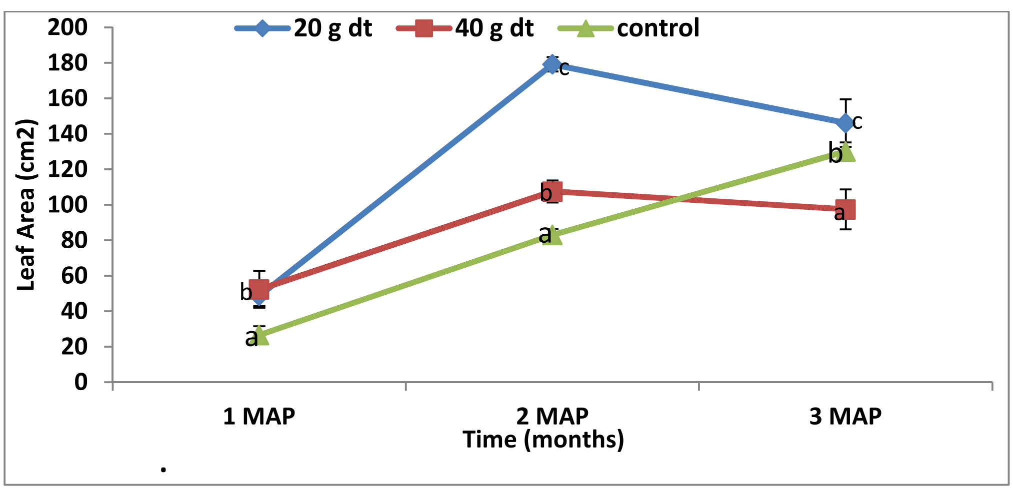

Fig. 5: Leaf area of maize in the remediation treatments

The mean root length of maize grown in crude oil polluted soil remediated with detergent showed a progressive increase from the first to second month after planting (MAP) but decrease at 3 MAP (months after planting) in all the treatments (Fig. 6). Highest root length (P=0.05) between treatments was observed in 40 g detergent treated soil from 1 to 3 MAP while the least was recorded in the control (unremediated soil).

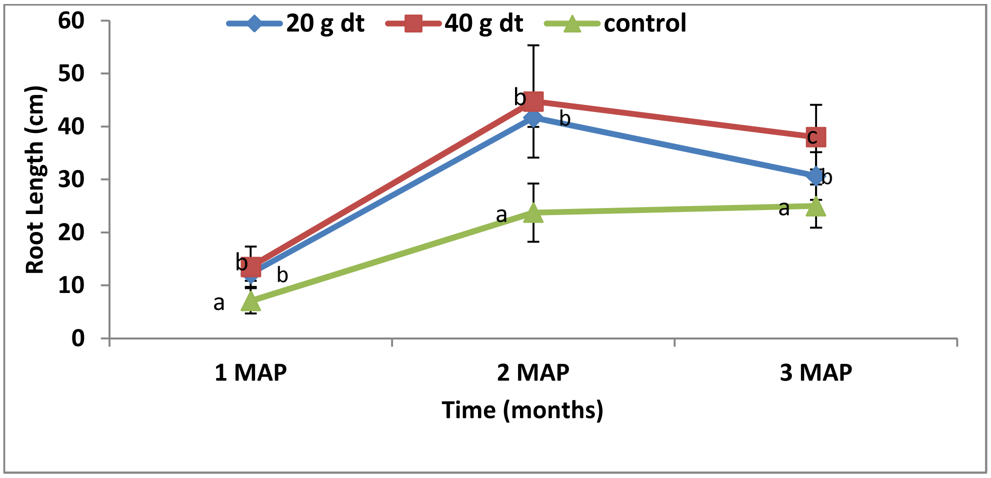

Fig. 6: Root Length of maize in the remediation treatments.

It was observed that the application of detergent as a remediating agent significantly increased the fresh and dry weights of maize as shown in Figs. 7-10. The mean fresh weight of maize showed progressive increase in the remediated soil from 1 to 3 MAP as compare with the control. Significant (P=0.05) highest fresh weight yield was recorded in the 20 g detergent remediated soil (Fig. 7). The mean shoot dry weight (Fig. 8), mean root dry weight (Fig. 9) and total dry weight (Fig. 10) showed similar response pattern in all the treatments. All the dry weight parameters showed progressive increase with time; with 20 g detergent treatment being the highest (P=0.05).

Observation showed that amending the crude oil contaminated soil with detergent also affected the shoot/root ratio (Fig. 11). Shoot/root ratio in the 20 g detergent peaked at 2 MAP and later decrease at 3 MAP. The other treatments (40 g detergent and control) showed steady increase with time.

The results of chlorophyll contents in the three treatments are as presented in Fig. 12. There was decrease in chlorophyll content from 1 to 2 MAP which later increase at the 3 MAP. Also at 1 and 2 MAP, 20 g detergent treatment recorded the highest (P=0.05) chlorophyll content while 40 g detergent treatment recorded the highest at 3 MAP.

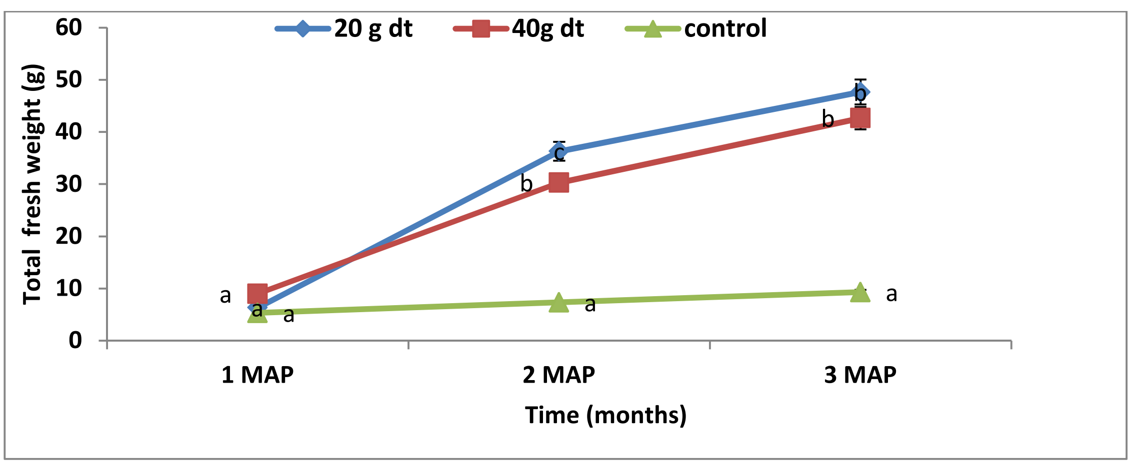

Fig. 7: Total fresh weight of maize in the remediation treatments.

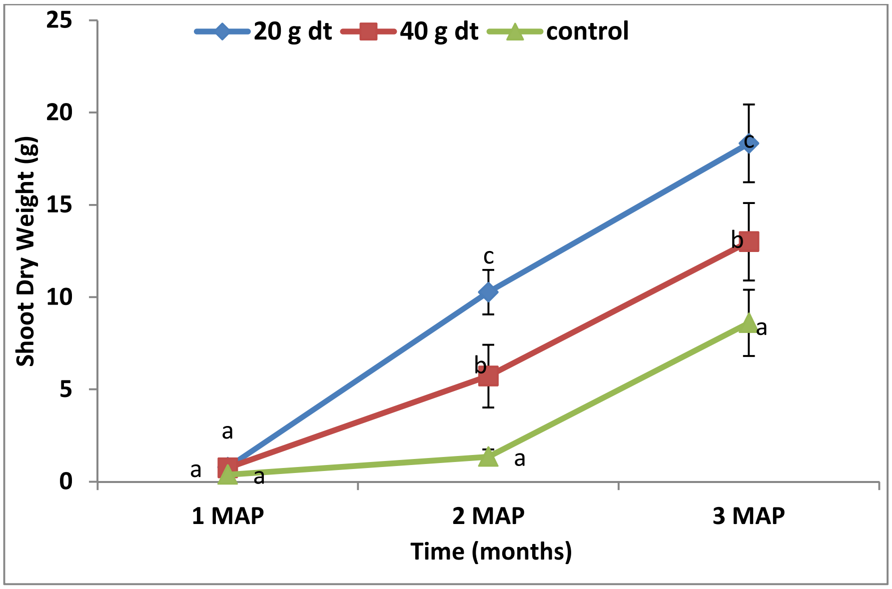

Fig. 8: Shoot dry weight of maize in the remediation treatments.

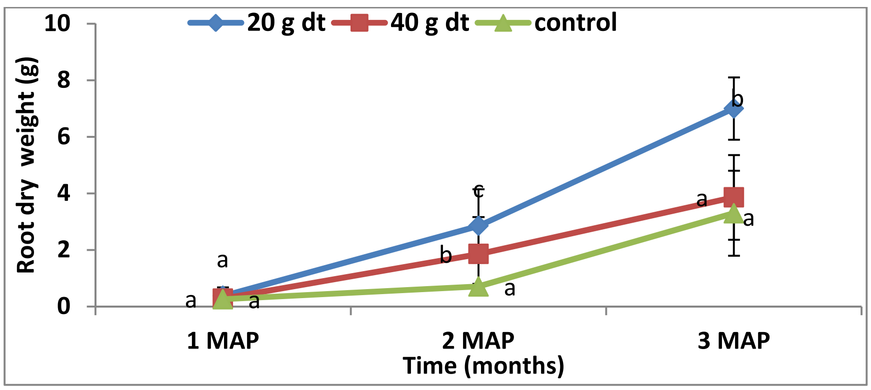

Fig. 9: Root dry weight of maize in the remediation treatments.

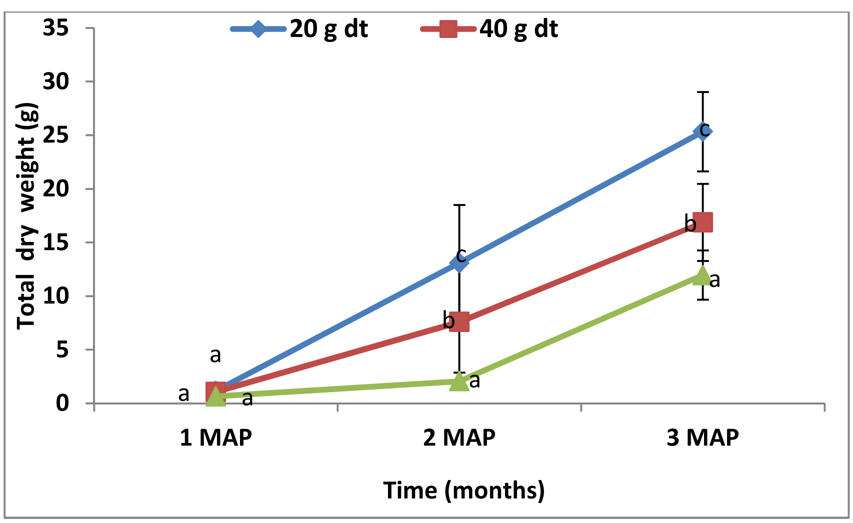

Fig. 10: Total dry weight of maize in the remediation treatments.

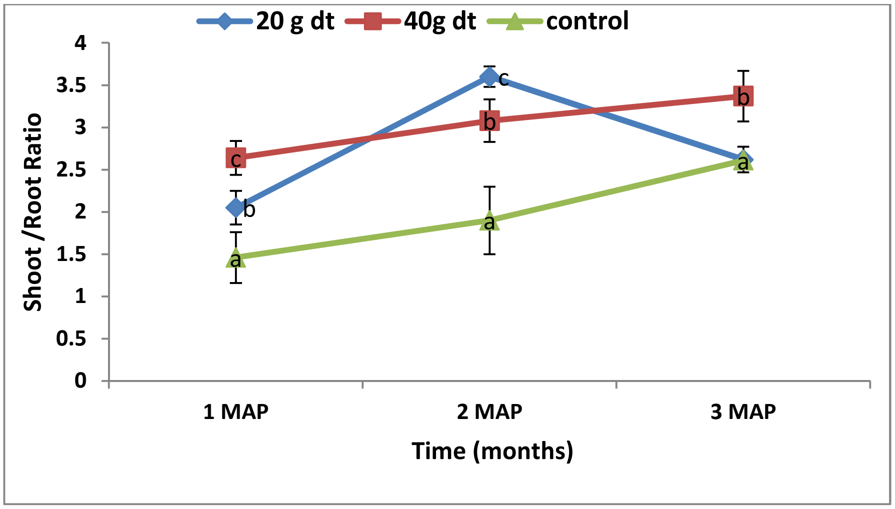

Fig. 11: Shoot/Root Ratio of maize in the remediation treatments.

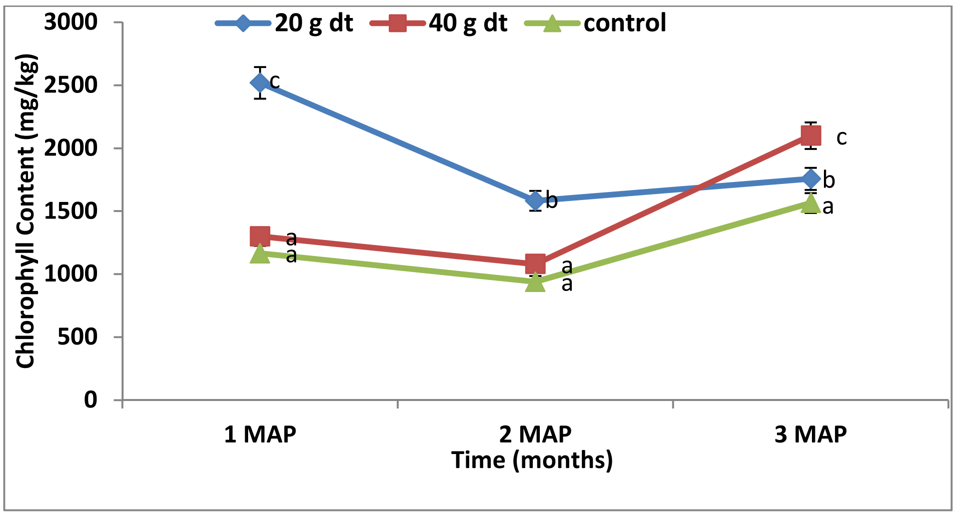

Fig. 12: Chlorophyll content of maize in the remediation treatments.

Results showed high performance of maize especially in their vegetative growth and chlorophyll contents in the remediated soil than the control. The improved growth performance observed in maize in remediation treatments could also be as a result of enhancement of the soil physico-chemical properties by the amendment material (detergent) addition. Similar results of high biomass yields in crude oil amended soil have been reported by other authors such as Jonathan *et al*. (2013), Ayolagha and Peter (2013) and Okon and Mbong (2013). Highest (p=0.05) growth of maize was observed in the 20 g detergent remediated soil than the other treatments. The high reduction in THC as observed in the test soil may have reduced the toxicity of hydrocarbon for optimal plant growth. Low THC in soil has been reported to stimulate growth of plant ((Li *et al.*, 1990). Also, low concentration of detergent in soil has been reported to be beneficial to plants due to the presence of phosphorus contained in it (Anonymous, 2016). The reduced growth performance in the 40 g detergent remediated and control soils may result from toxicity of high THC in the soil of these treatments. High hydrocarbon content in soil has been reported to be toxic to oil degraders and plants (Adam and Duncan, 1999; Molina-Baharoma *et al.* 2005; Ogbo, 2009). This could also be attributed to the fact that crude oil alters the fertility status of the soil by reducing the available soil nutrients especially nitrogen, thereby reducing the ability of the soil to support proper crop growth and development (Wyszkowski and Ziolkowska, 2008; Akujobi *et al*., 2011). This is in line with Oyedeji *et al.* (2012) and Eze *et al.* (2013) who in their separate studies observed reduction in growth performance of crops in soil polluted with Bonny-light crude oil. The alkaline nature of detergent (Anonymous, 2016) couples with it toxic ingredients especially at high concentration (Chan,

2015) may have altered the amended soil pH to alkalinity leading to poor growth of maize at 40 g detergent treatment since maize is reported to perform best at pH close to neutrality (Purseglove, 1985).

Fig. 13 showed that percentage moisture contents in all the treatments were high with progressive decrease with time. Highest percentage moisture content was recorded in the 40 g detergent remediated soil than the other treatment options. The high water moisture accumulation in the test crop especially in 40g detergent and control (no amendment) might be an adaptive mechanism against stress (Shukla and Chandel, 2006). Since crude oil has been known to create artificial water scarcity in soil as a result of their hydrophobic nature; thus causing low water infiltration into the soil (Tanee and Albert, 2016). The plants in the 40g detergent and control soils with high THC may have accumulated the available water into their tissues in order to withstand the artificial water scarcity created by the crude oil.

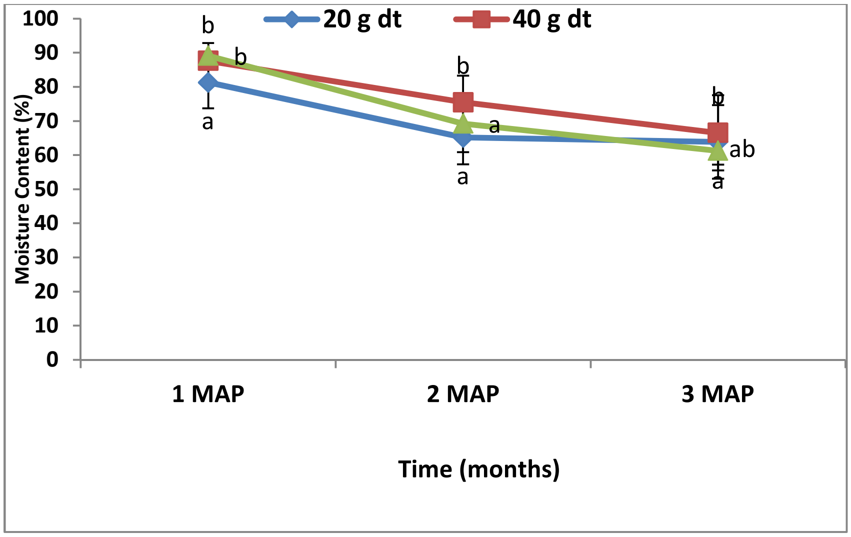

Fig. 13: Moisture Content maize in the remediation treatments.

*Conclusion:*The study has demonstrated the efficacy of detergent as an ameliorating agent in crude oil contamination for the reduction of hydrocarbon and phytotoxicity for optimal growth of crops especially maize. Optimal result can best be achieved at low concentration of detergent application. Therefore, it is imperative that cautions should be applied in the use of chemical remediating agents such as detergent to avoid toxicity effect on both soil and living organisms since they have been proven to be toxic at high concentration.

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