EFFECT OF CRUDE OIL AND BIOSTIMULANT (BIOREMEDIATION) ON GROWTH EXTRACT OF MAIZE (ZEA MAYS (L.) AND COWPEA (VIGNAUNGUICULATA (L.) WALP).

Iyagba, A.G.
Offor, U.S.
Department of Agricultural Science,
Ignatius Ajuru University of Education, Port Harcourt, Nigeria

Abstract

The phytotoxic effects of crude oil concentration in soil on growth extracts – Relative growth rate (RGR), Relative leaf growth rate (RLGR), Leaf Area ratio (LAR) and Net Assimilation rate (NAR) *of* maize *(Zea mays (L.)* and cowpea *(Vigna unguiculata (L.)* Walp*) was* examined. The beneficial effects of bioremediation with addition of Biostimulant – Sawdust *on the* growth maize and cowpea in an oil contaminated soil was also determined. It was observed that crude oil contaminated soil (400ml) per plot was phytotoxic to both crops. In contrast, obvious phytotoxicity was observed in soils planted with maize. In addition, bioremediation with Sawdust reduced phytotoxicity and increase *the* growth indices of crops during the study.

Keywords: Sawdust, crude oil, growth extract and bioremediation

Introduction

Crude oil is a mixture of naturally occurring hydrocarbons that is refined into diesel, gasoline, heating oil, jet fuel, kerosine, and literally thousands of other products called petrochemicals (Anon 2013a). It also contains heavy metals. During oil exploration and exploration, oil spillage occurs which have adverse effects on farming activities and soil fertility in the Niger Delta and such often requires cleaning up of the contaminated sites (Bundy*et al*., 2002). The toxicity of this compound viz- crude oil and refined products varies and is extremely difficult to assess due to limited knowledge on the additive, synergistic or antagonistic effects of mixtures involved (Saterbaca *et al*., 2000). In addition, the chemical composition of crude oil varies significantly and can have diverse effects on different organisms within the ecosystem and these differences are due to variation in concentration levels of the various constituents (Srerdrup *et al*., 2003).

Oil spills affect plants by creating conditions which make essential nutrients like nitrogen and oxygen needed for the plant growth unavailable to them (Adam and Duncan, 2002).Since land is becoming scarce, polluted lands can be ammeriolated or amended by adding materials to the soils. A soil amendment is any material added to a soil to improve its physical properties such as water retention, permeability, water infiltration, drainage, aeration and structure (Anon., 2012). The primary purpose of soil amendments is to provide a better environment for roots and nutrients for crops growth or to provide materials for soil improvements. Misuse of soil amendments can result not only in damage to crop but can also cause negative impacts on the receiving soil, water, air or habitat environment (Anon, 2013b). According to Mamiroli and McCutcheon (2003). phytoremediation is an alternative to more expensive remediation technologies because it is feasible, effective and non intrusive technology that utilizes natural plant growth processes to enhance degradation and removal of oil contaminations from the environment.

Several soil amendment materials exist and one of such is sawdust which is readily available. Economic disposal of saw dust and wood shaving is a problem of growing concern to the wood industries (Akowuah*et al*., 2012). Enormous quantity of sawdust are produced annually by sawmills and this can be diverted to soil conditioning/amendments. Sawdust is composed of about 40% of lignin and about 60% cellulose along with various waxes, resins and oils. (Roberts, 2010). This high lignin content makes sawdust potentially a good source of humus and thus good for soil amendments.Sawdust has been used for mulching in crop cultivation (Iyagba and Adesina, 2007). According to Shulga *et al*.(2007) addition of a lignosulphonate to sawdust will enhance its ability and the lignosulphonate/polymer complex, in which the macromolecules of the both components are linked together by physico-chemical bonds, has been applied as a new effective lignin-based soil conditioner. It has an adhesive affinity both for mineral soil particles and the organic surface of lignocellulosic mulch. The modification of the mulch particles with aqueous solutions of the developed conditioner by means of impregnation makes it possible not to anchor mulch to sandy soil and thereby to diminish significantly evaporation from the soil surface but also, due to mulch biodegration, to enrich soil with the main nutrients elements and to create favourable conditions for plant growth (Shulga *et al*., 2007).

Haimi (2000) and Marwood *et al*. (1998) posited that phytotoxicity tests have been suggested as useful tools in assessing the risk of contaminated soil or to evaluate the efficacy of a remediation process. Plant height and shoot biomass are good indicators of plant health and the sustenance of plant growth by the treated soil is an indicator of enhanced bioremediation (Banks *et al*., 2003). This gave rise to our using the growth indices of these crops in this study. Degradation of chlorophyll (Malallah *et al*., 1998), alterations in the stomotal mechanism and reduction in photosynthesis and respiration, accumulation of toxic substances or their by products in vegetal tissue (Baker 1970), increase in the production of stress-related phytohormones (Larcher, 2000); decrease in size and less production of biomass (Brandt et al., 2006, Daniel-Kalio and Pepple, 2006; Adenipekun *et al*., 2008) are commonly and important symptoms observed in plants contaminated with oil and its bye products. Henner *et al*.(1999) indicated that petroleum hydrocarbons consisting of small molecules and those that are water soluble are more phytotoxic for crop germination while Achuba (2006) stated that toxic hydrocarbon molecules could inhibit the activities of amylase and starch phosphorylase and thereby affecting the assimilation of starch.

Bioassays such as growth extracts – RLGR, LAR, RGR and NAR have been used to monitor treatment effects and restoration of oil contaminated soils (Sayles *et al*., 1999; Saterbaca, Toy and Dor, 2000). Dorn and Salanitro (2000) indicated that seed germination and plant growth using corn, wheat and oats differed from different soils and concentration before, during and after remediation. Luhach and Chaudry (2012) also recorded that concentration of diesel fuel beyond 7.5% significantly decreased the germination and reduced radicle and plumule growth of *Zea mays*, *Vigna radiata*, *Sorghum vulgare* and *Pennisetum glaucum* but noted a higher potential for phytoremediation of diesel contaminated soils of *S. vulgare* and *P. glaucum*. Sayles *et al*. (1997) showed that oil contaminated soil treated with aerobic biodegradation was less toxic to lettuce and oat roots enlongation. Stressing further, Offor, Akonye and Onuwugbuta-Enyi (2009) reported the ability of sawdust to enhance plant growth under normal condition. Invariably, the potentials of sawdust to promote plant growth under stress condition becomes a question hence this study.

The objectives of this study were:

a) evaluate the comparative phytotoxicity response of maize and cowpea in a crude oil contaminated soil and
b) investigate the reduction in phytotoxicity following bioremediation with sawdust.

Materials and Method:

Crude oil was obtained from the Nigeria Agip Oil Company, Ebocha Base- Port Harcourt, Nigeria (Bonny type) consisting of an API (American Petroleum Institute) gravity of 33.2% sulphur and nitrogen 1.9% (w/w) and

1.7% (w/w) respectively. Seeds of maizeand cowpea were procured from the Green River Project of the same Agip oil Company,Ebocha.

For biodegradation, a good garden soil weighting approximately 6600g was obtained from the Botanical garden of the University of Port Harcourt, Nigeria with no history of hydrocarbon contamination was used to fill black cellophane bags of diameter measuring about 50cm and height 4.5cm leaving a space of 7.00cm from the top end of the polythene bags to make allowance for crude oil and addition of sawdust. Four hundred miilitres of crude oil was added and thoroughly mixed with the soil using a hand trowel.

Biodegradation of crude oil

50g of sawdust was mixed to each of the cellophane bags which had already been polluted. A 1 x 1 x 2 factorial arrangement fitted into a randomized complete block design (RCBD) was used. The factors are:

the crops – maize and cowpea

biostimulant - Sawdust

crude oil – (400ml pollution level)

The various treatments were replicated four times within each block for each crop. The test crops used in the study were chosen because of their national acceptance as food and industrial raw materials as well as fodder crops.

Analysis of Total hydrocarbon (Crude oil) TPH

The oil content was estimated using the method of USEPA (1986). For the extraction of hydrocarbon, one gram of soil sample was delivered into 10ml chloroform in an extraction flask. The mixture was shaken vigorously for 2 minutes and allowed to stand for the soil particle to settle. The oil was extracted and determined by the absorbances of the extract at 420nm in an SP 6 Pyeunican spectrophotometer. A standard curve of the absorbance of different known concentration of equal amount of crude oil in the extract was first drawn after taken reading from the spectrophotometer. The standard curve was used to estimate the oil concentration after multiplying by an appropriate dilution factor.

Plant Assay

1. **Relative Growth Rate (RGR)**: Relative growth rate was calculated at two weeks interval according to the formulaof West, Briggs and Kid (1920) as follows:

$$RGR = \frac{In\ W_2 - InW_1}{t_2 - t_1}$$

Where W_1 = Initial dry weight at time t_1

W_2 = Subsequent dry weight at time t_2

2. **Leaf Area ratio (LAR) -** Using the formula of West, Briggs and Kid (1920)

$$LAR = \frac{I_1 + I_2}{W_1 + W_2}$$

where LAR was computed by obtaining the leaf Area and dry weights, L_1 and L_2 respectively and subsequent L_2 and W_2at two weeks interval.

3. **Net Assimilation rate (NAR) from the formula of William (1969)**

$$NAR = \frac{W_2 - W_1}{L_2 - L_1} (\ln I_2 - \ln I_1)$$

where W_1 and W_2 were initial and subsequent dry weight. In I_1 and In I_2 = natural logarithm of initial and subsequent leaf area of whole plant.

4. **Relative leaf growth rate (RLGR)**

RLGR was determined using the formular of West, Briggs and kid (1920) as follows:-

$$RLGR = \ln I_2 - \ln I_1$$

where In I_1 and In I_2 are the natural logarithm of initial and subsequent whole plant leaf area.

Analysis of data

Data collected were subjected to the analysis of variance (ANOVA) and means seperated by Duncan MultipleRange Test (DMRT) according to the statistical analysis system at $P<0.05$. Phytotoxicity responses were also correlated through physical observation on plants.

Results and Discussion

From physical observation, the phytotoxicity of crude oil as tested on the two crops showed that the growth of maize was more sensitive to crude oil than that of cowpea especially in leaf colouration and abscission at the initial week of study. This might be due to the toxic hydrocarbon molecules causing a greater inhibition of the activities of amylase and starch phosphorylase more in maize than cowpea thereby affecting the assimilation of starch. The work of Luhach and Chaudry (2012) also indicated that *Z. mays* and *V. radiate* have lower potential for phytoremediaton of diesel contaminated soils when compared with *S. vulgare* and *P. glaucum*. A speedy recovery was observed three weeks after planting. Treatment with sawdust showed slight abscission than in control and uncontrolled plots. This is consistent with previous reports of interspecies differences in sensitivity to petroleum hydrocarbon and may be related to differences in systematic uptake of oil compounds, nutrient availability and cell wall structural differences (Lo and Ch, 1997).

Results on growth extract of bioremediated soil shows that LAR for cowpea in bioremediated and non-remediated soil did not significantly differ but was higher than the control. (Table 1). However, NAR and RGR of bioremediated soil gave significant increase than untreated soil. This is consistent with previous reports of improved germination and plant growth after bioremediation (Srerdrup *et al*.,2003; USEPA, 1986). In maize, LAR, RLGR and NAR of bioremediated soil (treatment with sawdust) were significantly higher than control and untreated soil at $P < 0.05$. In contrast to cowpea, the LAR of maize was higher and this may most likely be due to genetic constitution, plant biomass and differences in transpiration rates. However, the RGR and NAR of cowpea in untreated and bioremediated soil were significantly higher than in maize. The significant increase observed in cowpea were comparable as it is possible that cowpea has root exudates that affect rhizosphere. It might also be possible that cowpea during germination had developed higher plumule and radicle length than maize thereby absorbing more nutrients and consequently accumulating greater crop biomass. The process of phytoremediation is a complex one. Salanitro *et al.* (1997) indicated that the toxicity noticed in contaminated soils might not be just due to the contaminant concentration but also due to soil type and properties, hydrocarbon type, microbial community composition and plant species.

Conclusion

This work has so far shown that addition of sawdust enhanced oil degradation and bioremediation while the amelioration agent (biostimulant) reduced oil phytotoxicity in soil and increased crop growth.

References:

Achuba, F.I. (2006). The effect of sublethal concentrations of crude oil on the growth and metabolism of cowpea (*Vignaunguiculata*) seedlings. *Environmentalist* 26:17-20.

Adams, G. and Duncan, H. J. (2002). Influence of Diesel fuel on seed germination. *Environ. Pollut*., 120:363-370.

Adenipekun, C. O., Oyetunji, O. J. and Kassim, L. S. (2008). Effect of spent engine oil on the growth parameters and chlorophyll content of *Corchorus olitorius* Linn. *Environmentalist* 28: 446-450.

Akowuah, J. O., Kemasour, F. and Mitchual, S. (2012). Physico-chemical characteristics and market potential of sawdust charcoal briquette.*International Journal of Energy and Environmental Engineering* 3:20-26.

Anon. (2013a). definition and meaning of crude oil. Retrieved June 20, 2013. Available in www.businessdictionary.com/definition/....

Anon. (2013b). Glossary of Soil Science Terms.Soil Science Society of America.Retrieved May 10, 2012. Available in http://en.m.wikipedia.org/wiki/.

Anon. (2012).Choosing a soil amendment.Retrieved on October 30, 2013. Available on www.extcolostate.edu/pubs/garden....

Baker, J. M. (1970). The effects of oils on plants.*Environ. Pollution* 1:27-44.

Banks, M. K., Kulakow, P., Schwab, A. P., Chen, Z., Rathbone, K. (2003). Degradation of crude oil in the rhizosphere of *Sorghum biocolor*. *International Journal of phytoremediation*. 5(3): 225-234.

Brandt, R., Merki, N., Schultze – Kraft, R., Infante, C. and Broll, G. (2006). Potential of vertiver (*Vetiveria zizaniodes*(L.) Wash) for phytoremediation of petroleum hydrocarbon – contaminated soil in Venezuela. *Int. J. Phytoremed*., 8:273-284.

Bundy, J.C., Paton, G.I., Campbell, C. D. (2002).Microbial communities in different soil types do not converge after diesel contamination. *Journal of Applied Microbiology* 92:276-288.

Daniel-Kalio, L.A. and Pepple, S. F. (2006). Effect of Bonny light crude oil pollution of soil on the growth of day flower (*Commelina benghalensis* L.) in the Niger Delta. *Nigeria J. Appli. Sci. Environ Mgt*. 10: 111-114.

Dorn, P. B. and Salanitro,S.P.(2000).Temporal ecological assessment of oil contaminated soil before and after bioremediation. *Chemosphere* 40: 419-426.

Haimi, J. (2000). Decomposer animals and bioremediation of soils. *Environmental Pollution* 107:233-238.

Henner, P., Schiavon, M., Druelle, V. and Lichfouse, E. (1999). Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. *Organic Geochemistry* 30:963-969.

Iyagba, A.G. and Adesina, O. L. (2007). Evaluating the effects of mulching materials on weed control and fluted pumpkin (*Telfairia occidentalis* Hooker) growth. *Journal of Vocational Education and Technology* 4(1,2):33-38.

Larcher, W. (2000).Ecofisiologia Vegetal Rima, Soa Carlos 365.

Lo, S.I. and Ch, P.T. (1997). Site alternative and assessment for soil contaminated by heavy metals in Tawian.*Taiwan Roc.*19-46.

Luhach, J. and Chaudry, S. (2012). Effect of Diesel Fuel contamination on seed germination and growth of four agricultural crops. *Universal Journal of Environmental Research and Technology*. 2(4):311-317.

Malallah, G. Afzal, M., Kuriam, M. Gulshan, S. and Dhami, M.S.I. (1998). Impact of oil pollution on some desert plants. *Environ. Int*. 24:919-924.

Marmiroli, N. and McCutcheon, S.C. (2003). Making Phytoremediation Transformation and control of contaminants. *John Wiley*, Hoboken.

Marwood, T. M., Knoke, K., Yau, K., Lee, H., Trevors, J. T. Suchorski Tremblay, A., Fleming, C.A., Hoglge, V., Liu, D. L., Seech, A. G. (1998). Comparison of Toxicity Detected by Five Assays during Bioremediation of Diesel Fuel spiked soils. *Environ. Toxicol.Water Qual*., 13:117-126.

Offor, U.S., Akonye, L. A. and Onwugbuta-Enyi, J. (2009). Plant species responses to oil degradation and toxicity reduction in soil. *African Journal of Biotech*. 8(1) 211-220.

Roberts, A. N. (2010). Sawdust as a mulch and soil amendment for Rhododendrons and Azeleas. Available on URL:http://scholar.lib.vt.edu /ejournals/JAR/v5n2-roberts.htms.

Saterbaca, A. Toy, R.J., andDor, P.B., 2000 – Entomological and analytical assessment of effect of bioremediation on hydrocarbon contaminated soils. *Environ-ToxicolChem* 19:2643-2652.

Sayles, G. D. Acheson, C. M., Kupfeala, M. J. Shan, Y. Zhon, Q, Meer, J.R., Change L. and Brenner, R. C. (1999). Land treatment of PAH-contaminated soil, performance measured by chemical and toxicity assays. *Environ Sc. Tech.* 33: 4310-4317.

Srerdrup, L. G. Krogh and P. H. Nielsen, J. 2003. Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pretense*) and ryegrass. *Chemosphere* 53.

USEPA.United States Environmental protection Agency. (1986). *Agency of water regulation and Standards*, *Washington DC Eds*. 44.

West, C. Briggs G.R. and Kid F. (1920). Method and significant relations in the quantitative analysis of plant growth. *New phyto* 19:200-207.

William, F.R. (1964). The physiology of plants growth with special references to the concept of NAR.*Ann. Bot.* 10:41-72.

Table 1: Effects of crude oil pollution and bioremediation on growth extracts of crops

Treatments	Cowpea				Maize			
	LAR	RLGR	RGR	NAR	LAR	RLGR	RGR	NAR
Crude oil pollution	160.34 ± 25.66^{d}	0.13 ± 0.11^{g}	0.030 ± 0.006^{bd}	0.000 ± 0.0002^{c}	151.96 ± 20.96^{g}	0.34 ± 0.13b	$0.023 \pm 0.32^{c+}$	0.0028 ± 0.0013^{b}
Crude oil + sawdust application (bioremediation)	162.21 ± 25.66^{d}	0.18 ± 0.07^{d}	0.05 ± 0.03b^{d}	0.0017 ± 0.025^{g}	328.13 ± 94.16^{c}	0.36 ± 0.13^{ab}	0.012 ± 0.031^{f}	0.0057 ± 0.013^{g}
Control	108.77 ± 20.95^{f}	0.26 ± 0.10^{ba}	0.048 ± 0.010^{bc}	0.0118 ± 0.002^{h}	233.88 ± 28.01^{b}	0.27 ± 0.1^{b}	0.052 ± 0.005^{b}	0.0023 ± 0.0012^{bc}

Within columns mean ± SEM with different superscripts are significantly different at $P < 0.05$.