ASSESSMENT OF BIOSTIMULATION USING SOME ORGANIC WASTES IN BACTERIAL RECLAMATION OF CRUDE OIL CONTAMINATED AGRICULTURAL SOIL

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Abstract

Crude oil contamination of agricultural lands is a major problem in oil producing nations. Even the non oil producing nations that depend on supply through cross country underground and on high sea transportation are not spared due to accidental spillages. Apart from loss of farms, oil spills have led to shortened fallow periods, land use deterioration and led to a loss of soil fertility. The Effect of cow dung, sewage sludge and poultry droppings was tested in reclamation experiment. Nine of the twelve plots selected for this work were deliberately contaminated with Bonny light crude oil. The other three were uncontaminated (control). The Plots were left for seven days after which they were amended with the three organic wastes tested. The seeds of *Amaranthus* spp. were scattered in all the treatment plots and the control. Post planting irrigation was ensured for 10 weeks. Also cultures of the bacterial isolates were inoculated in all the plots. Seedling growth was monitored. Different parameters such as stem length, leaf length, leaf number and plant population were measured and counted respectively. Sewage sludge had shown better results than others, however, all the organic wastes showed appreciable effect in crude oil decontamination as indicated by growth and development of *Amaranthus* spp.

Introduction

Land contaminated by oil may be rendered unsuitable for plant growth by increasing the toxic contents in the soil (Nwankwo and Ifeadi, 1988). Crude oil shows a coagulatory effect on soil, it binds the soil particles and hence reduces aeration. Therefore, seed sown on such soils will fail to germinate (Ogboghodo *et al.*, 2004). Heavily contaminated soils may remain un-usable for months or years until the oil has been degraded to tolerable levels (Eboe, 1986).

Other adverse effects oil has on plant growth may range from root stress, morphological aberration and reduction in biomass.

Crude oil contamination of agricultural soils has dramatically affect food production particularly in oil producing areas. Also the use of cross country underground pipelines to convey crude oil and/or refined hydrocarbon products to different parts of Nigeria has led to more frequent instances of farmland contamination through pipe rupture and spillage. Oil contamination in soils result in imbalance in the carbon to nitrogen ratios. This causes a nitrogen deficiency which not only retards the growth of agriculturally relevant microorganisms but even plants grown on such soils (Chikere and Chijioke 2006).

Crude oil contamination affects biodiversity which is critical to agricultural productions. For example, extensive destruction of insects due to oil pollution can affect pollination and hence fruit formation in seed plants. Also the birds which may suffer reproductive problems through reduced egg productions are important in dispersal of fruits. This may limit distribution of plant species leading to extinction. Sea birds are particularly affected by spills as the oil penetrates and open up the structure of their plumage thereby, reducing the insulating ability of their feathers. This makes them more vulnerable to temperature fluctuations. The smothered feathers also impair flight abilities. The oil may also cause kidney damage, altered liver function and digestive tract irritation in birds.

Nigeria has the third largest mangrove forest in the world and the largest in Africa (9.730Km^2) occupying the lower stretches of the southern limit of the Niger Delta and covering between 5,400Km^2 and 6000Km^2 (Niger Delta Environmental Survey, 2000). There are three main mangrove families (Rhizophoraceae, Avicenniaceae, and Combretaceae) comprising six species namely *Rhizophora racemosa, Rhizophora, mangle, R. harrisonii, Languncularia racemose, Avizeania germinans and Conocarpus erectus*) spreading in the Niger Delta, Nigeria (Research Planning Institute, 1985; NDES, 1996, 2000,; Niger Delta Development Commission, 2004) Another important component of the mangrove vegetation is the exotic Nypa palm (*Nypa fruticans*) of the family palmae introduced from Singapore Botanical gardens to Calabar and Oron.

The Mangrove plants (*Rhizophora* spp.) are salt tolerant species that grow on sheltered shores in the tropics and subtropical estuaries (International Petroleum Industry Environmental Conservation Association, 1993). They provide ecosystem functions and human utility benefits especially for coastal communities of Niger Delta, Nigeria. Their halophytic nature and ability to compensate for low oxygen in the soil allows them to flourish in the environment. However, their complex breathing roots make them vulnerable to crude oil which can block the openings of the breathing roots. This has posed serious threats to mangrove plants. The interaction between crude oil and breathing roots and pores leads to asphyxiating of the subsurface of the roots that depends on the pores for oxygen transfer (Odu *et al.,* 1985). This in turn impairs the normal salt exclusion process resulting in accumulation of excess salt in the plant contributing to enhancing the stress condition of the plant and ultimately, to death. On account of this, mangrove plants are vulnerable and undergo steady unpalatable decline in quality and functions in the integrity of the ecosystem. This is why in this research the effect of Bonny light crude oil on growth and development of *Amaranthus* seedlings using growth attributes (such as shoot length, leaf length, number of leaves, *vis a vis* Soil reclamation efforts using organic wastes

was assessed. Apart from using anatomy in the systematic of plants, some other workers have also used anatomy of plant to monitor environmental pollution (Omosun *et al.*, 2009). Sharma *et al.,* (1980) have reported morphological and stomatal abnormalities as an effect of environmental pollution on plants. Also Gill *et al.*, (1992) reported that stomata in *Chromolaena odorata* were grossly affected by crude oil which manifest as distortion and reduction in the number of stomata per unit area of the leaf. Several workers have also reported the effects of crude oil on the growth and physiology of different plants (Terge, 1984, Gill *et al.,* 1992; Pezeshki and Delaune, 1992; Quinones – Aquilar *et al.,* 2003 ;)

Materials and Methods

Media Preparation and Culture

All the media used in this work (Trypticase Soy Agar, Trypticase Soy Broth, Nutrient Agar, Glucose Phosphate medium, Nitrate medium, Urea medium, and peptone water) were of analytical grade and prepared in accordance with standard procedures described in Barrow and Feltham (1993).

Isolation of Crude Oil Degrading Bacteria

Soil and water samples were collected from the effluents emanating from Kaduna Refining and Petrochemical Company (KRPC) and neighbouring farms and streams. Samples were also collected from motor mechanic workshops. The samples were placed in sterile containers. From each container 0.1g of the soil and 0.5ml of the water were inoculated into 9.9ml and 9.5ml of trypticase soy broth respectively. The mixture was thoroughly shaken and about 0.05ml of each mixture was used to inoculate a minimal medium. Each tube of this minimal medium was overlaid with 1ml of Bonny Light crude before being inoculated with the growth obtained in trypticase soy broth. The medium was then incubated at an average of 24°C (ambient temperature) for 48 hours. After this period of incubation, the bacteria from the crude oil – overlay – minimal medium were streaked on trypticase soy agar and allowed to stand on the bench for three days at 24°C. The colonies that developed were re-inoculated into a fresh crude oil – overlay - minimal medium in order to obtain pure cultures. These pure cultures were gram stained in accordance with standard procedure described in Barrow and Feltham (1993).

Biochemical Characterization of the Bacterial Isolates.

The following distinguishing biochemical characterization tests were carried out in order to identify the bacteria down to species level. Each test was carried out in accordance with standard procedure described in Barrow and Feltham (1993).

Catalase test

In this test the bacteria were sub-cultured into test tubes containing nutrient broth and incubated for 24 hours. Then 1mL of 5% hydrogen peroxide was added and the tubes were observed for evolution of gas.

Carbohydrate Utilization Test (acid from carbohydrates)

The media for this test was composed using standard procedure. In each case sterile solution of sugars used for the test (glucose, lactose, maltose, mannitol, sucrose, trehalose etc.) was added aseptically to give a final concentration of 1%. Then 10ml of the media was distributed into small test tubes and inoculated with the bacteria.

Voges Proskauer Test

In this test glucose phosphate medium was prepared and inoculated with the bacteria. The medium was incubated at 37°C for 48 hours. Then 2 drops methyl red solution was added. The mixture was shaken and examined. Then 0.6ml of 5% alpha Naphthol solution and 0.2ml of 40% KOH aqueous

solution were added into the mixture. The mixture was also shaken and the test tubes sloped. The reaction was examined after 15 minutes and subsequently after one hour.

Nitrate Reduction Test

In this test, nitrate broth was prepared, inoculated and dispensed into tubes, Durhams tubes were then inverted in it. The tubes were incubated for five days. In those tubes where evidence of gas production was noticed, 1ml of nitrite reagents each was added. The tubes were observed and results recorded.

Urease Test

In this test, a urea medium was prepared and sloped. The medium was inoculated and incubated at 37°C for 96 hours. Observations were made for 4 hours and daily on five days after incubation.

Citrate Utilization Test

In this test, Koser's citrate medium was prepared. A suspension of the culture was made in sterile distilled water. Then a straight wire was used to inoculate the medium. The tubes were examined for 7 days and observations recorded.

Coagulase Test

In this test, a colony of the bacterial culture was picked and placed in a drop of saline on a glass slide. The colony was emulsified well using wire loop. A straight wire was dipped into plasma and used to stir the emulsified bacteria on the glass slide. Observations were made after 10 seconds.

Assessment of Biostimulation

A piece of land was obtained in the Botanical Garden of Usmanu Danfodiyo University Sokoto and divided into plots. Twelve plots of about 12 by 10 inches were earmarked for experimentation. The effect of Cow dung, Sewage sludge and Poultry droppings was tested in reclamation of soil contaminated with Bonny light crude oil. The three wastes served as treatments and the experiment was replicated three times. The three remaining plots served as control in which contamination with crude oil was made but they were not amended with organic wastes. In the experimental set up nine of the twelve plots were deliberately contaminated with one litre of Bonny light crude oil and the remaining three were uncontaminated. The plots were left for seven days after which they were amended with the three organic wastes accordingly.

Seeds of *Amaranthus* spp. were broadcasted in all the treatment plots including the control plots and watered constantly. Post planting irrigation was ensured throughout the experimental period (10 weeks). Also cultures of bacterial isolates were inoculated in all the plots. Germination and subsequent seedling growth were monitored. Different parameters such as stem length, leaf length, leaf number and plant number were measured accordingly using vernier caliper.

Results

Table 1: Biochemical Characterization of Crude Oil Degrading Bacterial Isolates

Morphological characteristics	Biochemical Test													Identity
	Catlase	Glucose	Lactose	Maltose	Manniol	Salicin	Sucrose	Trehelose	VP	Nitrate Reduction	Urease	Citrate	Coagulase	
Gram Positive Rods	+	-	-	-	-	-	-	-	-	-	+	-	-	*Corynebacterium pseudodiphtherticum*
Gram Positive Cocci in Clusters	+	+	+	+	+	+	+	+	-	-	+	-	-	*Staphylococcus saphrophyticus*
Gram Negative Rods	+	-	-	-	-	-	-	-	-	+	-	+	-	*Pseudomonas alcoligenes*
Gram Negative Rods	+	+	-	-	-	-	-	-	-	+	-	+	-	*Achromobacter xylosoxidans*

Table 2: Stem Height of *Amaranthus* spp. Exposed to Chronic Crude oil Contamination and amended with Different Organic Wastes

	Week 2	Week 4	Week 6	Week 8	Week 10	Total
Cowdung	2.0	5.2	4.2	4.0	3.2	18.6
Chicken-droppings	3.2	5.4	4.5	4.8	4.2	22.1
Sewage sludge	4.1	3.3	5.0	6.2	5.3	23.9
Control	2.4	2.6	3.2	3.6	3.4	15.4

Each value is a mean of three replicates.

Table 3: Leaf Length of *Amaranthus* spp Exposed to Chronic Crude oil Contamination and amended with Different Organic Wastes.

	Week 2	Week 4	Week 6	Week 8	Week 10	Total
Cowdung	1.8	3.2	3.8	3.9	4.0	16.7
Chicken-droppings	1.8	3.3	3.7	3.8	3.9	16.5
Sewage sludge	1.6	3.4	4.0	4.2	4.0	17.2
Control	2.0	2.1	3.9	3.7	3.8	15.5

Each value is a mean of three replicates.

Table 4: Leaf number of *Amaranthus* spp. Exposed to Chronic Crude oil Contamination and amended with Different Organic Wastes.

	Week 2	Week 4	Week 6	Week 8	Week 10	Total
Cowdung	4.0	5.0	7.0	10.0	12.0	38
Chicken-droppings	6.0	8.0	9.0	12.0	12.0	47
Sewage sludge	5.0	7.0	9.0	11.0	12.0	44
Control	8.0	11.0	15.0	13.0	14.0	28

Each value is a mean of three replicates

Table 5: Number of *Amaranthus* spp. Grown on Crude oil Contaminated and Organic Wastes amended Soil.

	Week 2	Week 4	Week 6	Week 8	Week 10	Total
Cow dung	10	15	17	18	20	80
Chicken-droppings	8	12	20	20	20	80

Sewage sludge	12	16	22	22	22	94
Control	8	7	5	3	3	26

Each value is a mean of replicates.

Discussion

The crude oil degrading bacteria shown in Table 1, fall within those reported by Kajasheikh *et al.*, (2002) and Okoh (2006). It can be seen from their biochemical reactions that they lack the necessary enzymes to metabolize carbohydrates. This probably could be the reason why they use hydrocarbon as an alternative carbon source. The results of crop yield parameters (stem height, leaf size, leaf number and plant population indicated in Tables 2-5 shows that sewage sludge is better than chicken droppings and cow dung. It can thus, be seen as a good resource in agricultural soil conditioning practice particularly, in pollution prone areas. The trend observed here may be attributable to enhancement of porosity of the soil thereby, making movement of nutrients and gases much easier. Thus, popularizing the use of treated sewage sludge in vegetable farming is an endeavour that has a potential in boosting agricultural productions while achieving sustainable environmental sanitation.

References

Anonymous (1996). Niger Delta Environment Survey (NDES). Preliminary Report 1st phase vol. 1:1 – 96.

Anonymous (2004). Biodiversity of the Niger Delta environment. Niger Delta Development Commission (NDDC) Master Plan Project, Final Report.

Anonymous (1993). Biological impacts of Oil Pollution: International Petroleum Industry Environmental Conservation Association (IPIECA). Report Series Vol. 4. 24p.

Anonymous (1985). Environmental Baseline Studies for Establishment of control criterion and Standards Against Petroleum Related Pollution in Nigeria. Research Planning Institute (RPI): RPI/84/4/15-7.

Barrow, G.I. and Feltham, R.K.A. (1993). Cowan and Steel's *Manual for the Identification of Medical Bacteria.* Fifth edition – Cambridge University Press. Pp. 7 – 14, 219 – 238.

Chikere, B.O and Chijioke – Osiyi, C.C. (2006). Mircobial Diversity and Physico-chemical Properties of a crude oil Polluted soil. *Nigerian Journal of Microbiology*. 20 (2): 1039-1046.

Eboe, H. (1986). Oil and Environmental Pollution in Nigeria In: Claude, A.K. (ed.) The *political economy of Nigeria. Longman*, London Pp. 183-188.

Gill, L.S.; Nyawuame, H.G.K and Ehikhametalor, A.O. (1992). Effect of Crude oil on the growth and Anatomical features chromolaena odorata (L). *Chromolaena odorata Newsletter* No 6: 1-6.

Kajasheikh, M.; Thahira, R., Perumalsamy, L. and Ibrahim, M.B. (2002). Occurrence of Crude Oil Degrading Bacteria in Gasoline and Diesel Station Soils. *Journal of Basic Microbiology 42* (4): 284-291.

Nwankwo, W. and Ifeadi, C.N. (1998). Case Studies on the Environmental

impacts of Oil Production and Marketing in Nigeria In: Sada, P.O. and Odemerho, P. (eds.) *Environmentallissues and Management in Nigeria Development,* Evans Brothers, Ibadan Pp. 170-171.

Odu, C.T.I., Esuruoso, O.F., Nwoboshi, L.C. and Ogunwale, J.A. (1985). Environmental Study of the Nigerian Agip oil company, Operational Area. Soil and Fresh Water Vegetation Union Graft Publs. Milan pp. 21-25.

Ogboghodo, I.A.; Iruaga, E.K.; Osemwota, I.O. and Chokor, J.U. (2004). An Assessment of the Effects of Crude Oil Pollution on Soil Properties, Germination and Growth of maize (*Zea mays*) using two Crude types – Forcados light and Escravos light. *Journal of Environmental Monitoring and Assessment 96* (1-2): 143-152.

Okoh, A.I. (2006). Biodegradation Alternative in the Clean up of Petroleum Hydrocarbon Pollutants. African Journal of Biotechnology 1 (2): 38-50.

Omosun, G.; Edeoga, H.O.; Markson, A.A. (2009). Anatomical Changes Due to Crude Oil Pollution and its Heavy Metals Components in three Mucuna species. Recent Research in Science and Technology 1(6): 264-269.

Pezeshki, S.R. and Delaune, R.D. (1992). Effect of crude oil on gas exchange functions of *Juncus roemerianus and Spartina alterniflora*. Water, Air and Soil Pollution 68:461 – 468.

Quinones-Aquilar, E.E., ; Ferra – Cerrato, R., Gavi, R.F; Fernandez, L.; Rodriguez, V.R. and Alareom, A. (2003) Emergence and Growth of Merze in a crude oil polluted soil. *Agro-ciencia* 37: 585-594.

Sharma, G.K.; Chandler, C. and Salami, (1980). Environmental Pollution and leaf inticular variation in Puerreria Lobata. Annals of Botany 45:77-50.

Terge, K. (1984). Effect of oil Pollution in the Gemination and Vegetative Growth of Five Species of Vascular Plants. *Oil and Petroleum Journal* 2:25-30.