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Chlorophyll contents of oil palm (*Elaeis Guineensis)* leaves harvested from crude oil polluted soil: a shift in productivity dynamic

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ABSTRACT

The effect of crude oil pollution in the environment has an adverse effect on the chlorophyll contents of oil palm (Elaeis guineensis) leaves and also on the nitrogen fixing potential of the soil cores. This was demonstrated on the density and distribution of Azotobacter vinelandii within the polluted soil. This was observed in October 2006, for a parcel of land having more than 9,500 matured oil palm trees in Owaza, Nigeria where a massive oil spill occurred. The result showed that there was a significant reduction in nitrogen fixation in the crude oil-contaminated oil palm plantation as exemplified in the population and distribution of Azotobacter vinelandii. These diazotropic bacteria play important roles in the stability of the ecosystem, agriculture and marine productivity. There was a significant reduction (p<0.005) in the chlorophyll content of fresh palm fronts (leaves) samples collected from the polluted plantation when compared with control site. Chlorophyll, a vital pigment for photosynthesis plays its role of light trapping mechanism in the reaction center of photosynthetic plants. This suggests that any reduction in the chlorophyll content will proportionally affect the quantity and quality of food material produced by the green plants such as the oil palm trees.

Keywords: chlorophyll, nitrogen fixation, yield capacity, oil palm. *Azotobacter vinelandii*

INTRODUCTION

Nigeria is a major petroleum producing country. One drastic effect associated with its exploration and exploitation is the contamination of the immediate environment with petroleum hydrocarbons (Amadi *et al*, 1993). Most of the land in oil producing areas in Nigeria is under cultivated because the mainstay of people living in the region are farming and fishing (Onwurah *et al.*, 2007). This may result in contamination of agricultural produce and its concomitant shift in productivity dynamics.

Contamination of soil arising from spills is one of the most limiting factors to soil fertility and hence crop productivity.

Plant productivity is a unique process that depends greatly on the amount of chlorophyll present in the chloroplast. Chlorophyll is the pigment that gives plant their characteristic green colour, it plays a unique role in the physiology, productivity and economy of green plants including *Elaeis guineensis*. The quantity of chlorophyll per unit area is an indication of photosynthetic capacity and productivity of a plant. Therefore, the amount of chlorophyll in the leaf tissues may be influence by nutrient availability and environmental stresses such as drought, salinity, crude oil pollution of soil, heat etc. (Palta, 1990; Karacan, 2006 and Onwurah *et al*., 2007).

The oil palm is a monocotyledon belonging to the genus *Elaeis.* It is a perennial tree crop and the highest oil producing plant, yielding an average of 3.7 tons of oil per hectare per year in Malaysia. The crop is unique in that it produces two types of oil. The fleshy mesocarp produces palm oil, which is used mainly for its edible properties and the kernel produces palm kernel oil,

which has wide application in the oleo chemical industry (Afolabi, 2008).
Crude palm oil and palm kernel oil are adaptable vegetable oils and now have a wide range of markets in the food and oleo chemical industries. In addition, palm oil has been found as a very healthy component of the human diet. The oil palm remains a formidable competitor with other vegetable oil crops in terms of oil yield per hectare and resource use efficiency due to its unrivalled ability to transform solar energy into vegetable oil. For example, the oil yield from properly maintained oil palms is over six times larger than oil yields from commercially grown rapeseed. Additionally, the energy balance expressed by the ratio of energy output to input is wider for oil palm than other commercially grown oil crops (Abayeh et al., 1998; Fairhurst and Mutert, 1999). Hence the aim of this study was to determine the effect of crude oil pollution on the crop yield and nitrate fixing potential of the polluted soil.

Aim of This Study
This aim of this study was to determine the level of soil degradation caused by the massive oil spill that occurred at Owasa in October 2006. The work also tried to evaluate the extent of the damage caused on the productive capacity of the oil palm trees using chlorophyll contents as biochemical end pint parameter.

MATERIALS AND METHODS

Study Area.
The study area is a parcel of land located within latitudes 7^0 28’ E and 7^0 32’ E, and longitudes 6^0 26’ N and 6^0 29’ N and measuring 156.31 acres. It contains about nine thousand, five hundred (9,500) matured oil-palm trees. This parcel of land is at Umuololo, Owasa-Asa, Ukwa Local Government Area of Abia State, Nigeria. This area receives over 5000 mm of rainfall every year and is particularly good for oil palm plantation because of adequate rains and sufficient reserve of minerals. The crude oil spill incident occurred at a location very close to this parcel of land in October 2006, when rainfall was at its peak in the Niger Delta Area of Nigeria.

Soil Study

Soil sampling

The oil palm plantation was divided into four (4) transects (A, B, C and D) as shown in Fig 1 and the soil samples collected from all the transects in February 15, 2007 were taken to the Pollution Control and Biotechnology Unit (Laboratory), University of Nigeria, Nsukka. At each sampling location, a quantity of soil was taken at four (4) different depths (0 - 0.5; 0.5 - 1.0; 1.0 - 1.5 and 1.5 - 2.0 m), using an augur. A total of 25 locations were sampled, giving a total of 100 soil samples (4 × 25). Unpolluted soil samples were also obtained from an adjacent oil-palm plantation, about 100 metres away. All the soil samples were delivered to the laboratory in sterile labeled black polyethylene bags and subsequently dried under mild condition in the laboratory.

Soil Microbiology

When the soil becomes polluted by any type of oil, the effect on the indigenous microorganisms is in two-folds. On one hand , it might cause the inhibition of growth or death of certain microorganisms while on the other hand it may enhance the growth of certain chemotrophic microorganisms, especially those capable of metabolising the polluting oil. The microbiological study of the soil was directed at determining the average number distribution of *Azotobacter spp.* present in the crude oil-polluted palm plantation with the view of predicting the effect of the crude oil spill on the nitrogen fixing capacity of the soil cores when compared with control.

Azotobacter Isolation and Distribution

The numbers of *Azotobacter spp* in several soil cores taken from the four transects (A, B, C and D) were determined using *Azotobacter* medium which is selective for *Azotobacter spp* (Dicker and Smith, 1980). They were enumerated by the plate dilution frequency technique of Harris and Sumers (1968). The basic medium for the isolation was the modified Burk's nitrogen-free medium described by Newton *et al*. (1953).

Nitrogen Fixation in Soil Cores

This was determined by an indirect method based on the finding that *Azotobacter spp* occurring in numbers greater than 10^6 cells/g soil fix considerable amount of atmospheric nitrogen (Mulder, 1975). The calibration curve for the extrapolation was constructed by taking 5, 10, 15, 20 and 25ml fractions from primary culture medium of *Azotobacter* cells at a concentration of 10^8 cells/ml. These fractions were then centrifuged at 12,000 x g for 20 minutes and the harvested cells lysed by freezing and thawing technique in a phosphate buffer (0.025M, pH 7.4). The cell-free extracts were then incubated under nitrogen for 60 minutes after the addition of sodium pyruvate. The total nitrogen fixed as ammonia was assayed by the indophenol reagent method (Chaykin, 1969). A graph of cell numbers against ammonium ion concentrations ($\mu g[NH_4^+]$) was plotted as the calibration curve. From this, the nitrogen fixation potential of the soil cores was estimated based on the number of cells/g soil.

Chlorophyll determination

For analysis of chlorophyll content in oil palm (*Elaeis guineensis)* leaves, 3.0g of the leaves were collected and homogenized in 10ml acetone (90%) and was placed in refrigerator for 14 hours to allow complete extraction of chlorophyll. The content of brown bottle was centrifuged at 3000 rpm for about 15 minutes. The supernatant was transferred to a volumetric flask of 10 ml and the volume of contents was raised to 10ml by further adding 90% acetone. The optical density (OD) of the extract was recorded on Hitachi 220 Spectrophotometer at 662, 646 and 470 nm (Lichtenthaler and Wellburn1985).

RESULTS AND DISCUSSION

Crude oil pollution of the environment has very serious negative effects on agricultural soil productivity and this may occur in a number of ways. Records of the consequences of oil pollution on agriculture, aquatic life and human life are increasing. Crude oil spill on land, particularly agricultural soil can cause a serious damage by affecting both the biophysical and biochemical properties of the soil. Microbial population in the soil that provides adequate nutrients and stored energy for primary productivity (Aririatu and Iwuagwu, 2000; Kloepper *et al*., 1989) are equally affected by oil spill. According to Klokk (1984), soil contamination arising from oil spills is one of the most limiting factors to soil fertility and hence crop productivity. It was observed that non-agricultural occupation and migrations among some house-holds in Mkpanak area of Cross River State, Nigeria was due to the poor physical and nutrient condition of their farmlands after several oil spills (Adeniyi *et al*., 1983).

Nitrogen fixation by free-living bacteria in some ecosystems has generated some interest in terms of their capability in enhacing soil productivity (Kloepper *et al*., 1989). The diazotrophs as well as other multitudes of microbial species in the soil can be affected by crude oil pollution incidents. In environmental monitoring and assessment, data are generally collected and analyzed to provide information that is necessary for resource damage assessment. A variety of toxicity assays have been used in soil ecosystems and these include measuring rates of respiration, litter decomposition, nitrogen fixation and mineralization (Greaves et al., 1980). The choice of using *Azotobacter vinelandii* (a nitrogen fixing bacterium) as an indicator organism was based on the fact that it is widely distributed in the soil, relatively easy to identify and collect, and suitable for laboratory studies. As free-living aerobic nitrogen fixing diazotrophs, azotobacters can contribute significantly to the nitrogen economy of agricultural soil (Onwurah, 1996)) and hence an important Value Ecosystem Components (VEC) for studying pollution impact of oil spill. It also plays important roles in the stability of the ecosystem, in agriculture and marine productivity. The soil is essentially an energy-processing unit (Doran *et al*., 1984) and the effect of a pollutant on the usually overlooked microorganism such as *A. vinelandii* can cause important changes in energy flow.

Chlorophyll is vital for photosynthesis, the process by which plants manufacture food. The energy for this process is trapped from sunlight by this pigment. The function of the vast majority of chlorophyll (up to several hundred per photosystem) is to absorb light and transfer it by resonance to a specific chlorophyll pair in the reaction center of the photosystems (Karacan, 2006). The process of light harvesting is an essential one when one considers that a molecule of chlorophyll (or a mole of chlorophyll) can absorb one photon of light per second to complete its reaction in 10^{-15} seconds.

Therefore, it is clear that photosynthesis requires a considerable free energy input, and this is where energy from sunlight and the light-trapping capacity of chlorophyll play their roles. In the reaction centre, chlorophyll a absorbs light at about 675nm and the quantum at this wavelength is 1.84 electron volts. This suggests that any reduction in the chlorophyll content will proportionally affect the quantity and quality of food material produced by the green plants such as the oil palm trees.

The soil resources in Nigeria, provided they are conserved and managed wisely (properly) have the great potential for the production of food and other agricultural products which can support and sustain human and indusrial development. Some major component parts and parameters of the soil ecosystem that influence agricultural productivity include microorganisms, soil nutrients, water availability, water-holding capacity of soil, cationic exchange capacity of soil. The mineral soils in the study area are predominantly the Alfisol/Utisol, while the organic is of Histosol order and it has the capacity to support industrial crops such as rubber, kola, oil palm and other food crops, for example yam, cassava, maize, etc. When the equilibrium in the balance of these parameters is affected due to pollution incident, the result is noticed in the final product which is food production by the plants within that environment.

It is on few occasions that sample estimates of changes in population size with time were enough in detecting the effect of a pollutant in the environment. However, applying the concept of VECs and biochemical end point parameters as measures of impact enables a fair scientific evaluation of the impact of a pollutant such as an oil spill.

Considerable changes in soil properties usually occur when a soil is polluted by an oil spill. These changes include the water-holding capacity of the soil, loss of soil structure, introduction of anaerobic conditions and reduction in activities of aerobic microorganisms and fauna such as earthworms (Mutters *et al*., 2006). These changes affect plants growth and yield. In the oil spill environment, the petroleum hydrocarbons reduced the nutrients bioavalability as well as water availability to the oil palm trees. The altimate effect being on chlorophyll content (chlorosis), failure or reduction in photosynthetic processes and plant productivity.

Figure 1 also shows the concentration and distribution pattern of chlorophyll in randomly sampled newly formed leaves of palm trees in the polluted palm plantation between February and December 2008. When this result is compared with that from the control site, the impact of the spilled oil becomes obvious in that the levels of chlorophyll were significantly reduced; thus suggesting that photosynthetic apparatus of the plant has been jeopardized. Muedec and Poupart (2004) reported that Erika oil spill incident in 1999 resulted in chlorosis, drying and death of leaves, reduction of germination and growth or complete mortality of plants. An important elements in the biosynthetic apparatus of green plants is chlorophyll. It helps in the trapping of energy for photosynthesis. When the amount of this pigment is reduced, the amount of energy trapped is equally reduced, thus hampering the productive capacity of the plant. This gives rise to a very significant reduction in the quantity/ quality of the palm oil derivable from the oil palm trees. Damaging of the light absorption potential (chlorophyll) can limit the development of palm fruits.

The results obtained for the yield of oil palm shows beyond reasonable doubts that many metabolic pathway enzymes and soil quality were adversely affected by the oil spill and hence the productivity of the palm trees. Other factors affected by the spill include active transport of soil nutrients, CEC, sorbtive properties of the soil matrix. All these rendered the macronutrients unavailability and thus jeopardised productivity of the oil-palm trees.

Many factors, including soil organisms (oligochaetes and bacteria) that play very important roles in agricutural productivity are affected by oil spill events. It then becomes necessary to evaluate these valued ecosystem components (VECs) and their biochemical end point parameters that have direct or indirect consequences on the soil/crop productivity. The spill incident which took place during the rains resulted in a wide spread of petroleum hydrocarbons into a vast area of the oil-palm plantation. The surface slick of the oil was transported by the water current/ wind and the degree of spreading was proportional to the viscosity of the oil and the speed of the prevailing wind. Some surface oil slicks were visually detected on the scrubs/ plants in the palm plantation. The soil in the plantation contains 32% fine and 37-38% coarse sand and this porous property gives room for rapid ground water contamination.

Soil microbiological study was aimed at assessing the significant effect of the polluting crude oil on the aerable soil of the palm plantation. Diazotrophic bacteria such as *Azotobacter vinelandii* can be used to exemplify the effect of crude oil pollution on agricultural soil. It plays important roles in the stability of the ecosystem, in agriculture and marine productivity. The soil is essentially an energy-processing unit and the effect of a pollutant on the usually overlooked microorganism such as *A. vinelandii* can cause important changes in energy flow. Figure 2 shows the mean distributon of *Azotobacter spp* and hence the potential nitrogen fixation potential patterns in the four transects (A, B, C, D) and control site. In well cultivated soils (as the control site), *Azotobacter spp* will be found almost in all the soil samples taken but this was not the situation in the study area (oil-polluted plantation). The nitrogen fixation potential was based on the total ammonium ion ([NH_4^+]) synthesised by the crude protein extract corresponding to the cell density enumerated for each soil sample location (or cores). The low density of *Azotobacter* cells in the oil palm plantation soil environment was attributed to the toxicity of the crude oil spill, in that the control site had relatively significant numbers. It should be noted that the major function of *Azotobacter spp* in the soil is to enrich it with fixed nitrogen through biological nitrogen fixation.

The spilled crude oil has an EC_{50} value of (1.5g/100ml or 1.5%) with a toxicity index of 0.5 unit. Microbial nitrogen fixation in soil cores is mediated by diazotrophs and other nitrogen fixers in such plantations and such processes have been widely used in terrestrial impact assessments because of their sensitivity and agronomic importance. Stressors in the environment such as crude oil can affect the nitrogen balance of an ecosystem and in this case the productive capacity/ yield of the oil-palm trees.

Table 1: Physicochemical properties of soil sample taken from the crude oil polluted palm plantation six months after the spill and that of control (uncontaminated) site.

S/N	Parameters	Soil sample from crude oil polluted palm plantation	Soil sample from unpolluted Palm plantation
1	pH (H_2O) pH (KCl)	5.4 3.6	4.5 3.0
2.	Mechanical analysis Clay	22.4	22.4
	Silk	9.3	7.3
	Fine sand	32	32
	Coarse sand	37	38
3.	Nutrient composition (%) Carbon	1.21*	0.15
	Organic matter	2.09	0.26
	Nitrogen	0.04	0.04
4.	Exchangeable bases (Me/100g) Calcium	1.0	1.4
	Magnesium	0.6	0.4
	Sodium	0.20	0.10
	Potassium	0.04	0.08
5.	Cation exchange capacity (CEC).	12.8	11.2
6.	Phosphorus (ppm)	19.9	17.91

As at November 2007, significant nagative effects of the residual oil spill on the chlorophyll content of the palm trees were still observed. Such was the case at Prince Williams Sound after the Exxon Valdez Oil spill incident, which produced toxic effect / impact that lasted for over 13 years, in spite of the millions of dollars spent on clean-up operation. This goes to support the significant drop in the harvest yield (70 – 75%) of palm oil as reported by the farm manager of the oil palm plantation. The oil spill in Owasa was/is the most limiting factor to soil fertility and palm oil production. It was observed that this was due to the poor physical and nutrient condition of the plantation soil after the oil spill.

*It should be noted that the total carbon in the soil sample taken from "swampy" area of the polluted palm plantation was significantly higher than the value for the soil sample taken from unpolluted palm plantation. The significant difference is attributed to the petroleum hydrocarbons (PHCs) that persisted in the soil and this was made manifest by their bioaccumulation in exposed earthworms.

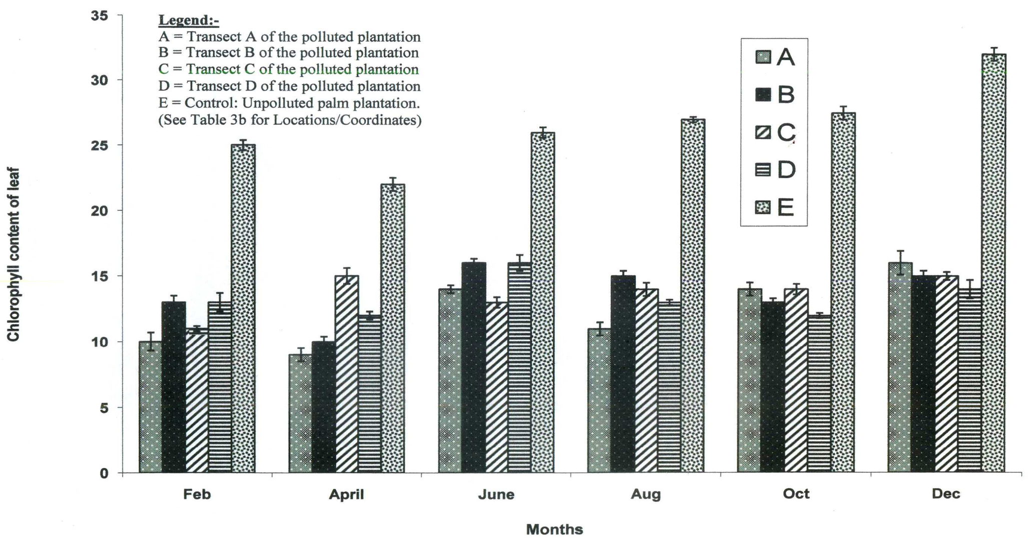

Fig. 1: Chlorophyll content of leaves from oil palm trees in the polluted and unpolluted plantation.

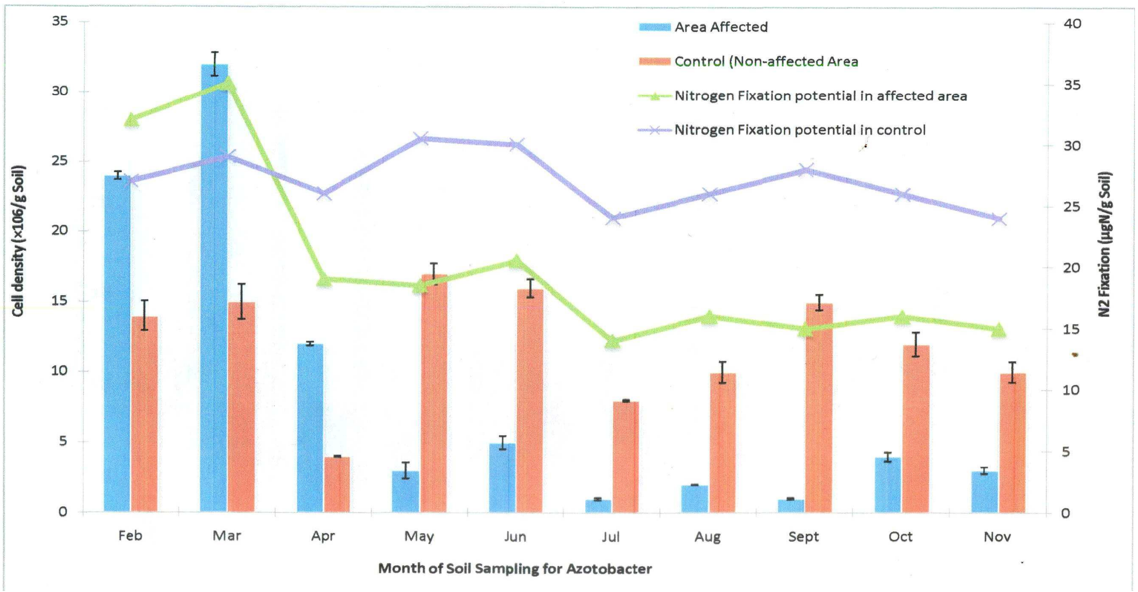

Fig. 2: Mean Azotobacter distribution and nitrogen fixation potential of soil cores at the four (4) transects (location) of the crude oil polluted palm plantation and control.

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