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EFFECT OF CRUDE OIL CONTAMINATION ON CHLOROPHYLL CONTENT IN *ZEA MAYS* L.

Ezeonu, Chukwuma S. and I.N.E. Onwurah

Pollution Control and Biotechnology Unit, Department of Biochemistry, University of Nigeria, Nsukka.

ABSTRACT

The Zea mays plant is a valued Ecosystem component. This biotic component was used as a marker to determine the impact the crude oil had on the soil where it was spilled. Chlorophyll plays an important role in photosynthesis. The results indicated oil spill had negative impact on the productivity of the soil and declined chlorophyll contents of the Zea mays. The higher the amount of crude oil spilled on soil, the lower the quantity of chlorophyll measured, showing that crude oil reduces the rate of chlorophyll production.

Keywords: chlorophyll, crude oil, *Zea mays.*

INTRODUCTION

Plants are known to take up numerous inorganic and organic contaminants and store them in various plant organs. With respect to specific contaminants in petroleum, limited research has been directed to assess the extent to which they are taken up by various plant species.

The levels of Polycyclic Aromatic Hydrocarbons (PAHs) in vegetation are usually less than concentrations found in the soil where they were grown. Levels of PAHs in plants ranged from 0.1 (Kolar, 1975) to 150µg/kg (Fritz, 1971) with common levels of 1 - 10µg/kg.

According to Edwards (1983), PAH concentrations are higher in oils extracted from plants than from plant tissues. The implications for certain crops growing near PAH sources is that most seeds might not germinate or plants might record stunted growth and it could also have health implications when consumed, since plant organs such as seeds are important in the human diet and can also contain relatively high concentrations of oil if grown in a contaminated soil.

Wang and Meresz (1981), assessed onions, beets, tomatoes, and soil for 17 PAHs including Barium Phosphate (BaP). They found most of the PAH contamination localized in the 'peels'. The rate of PAH uptake by plant species, the nature of the substrate that the plant is growing in, PAH solubility, PAH phase (vapor or particulate), and molecular weight (Edwards, 1983). These latter findings are of potential significance since in an oil spill the PAH compounds would be present along with the benzene.

Based on his impressive review of PAHs in the terrestrial environment, Edwards (1983) concluded with the following tentative observations about the uptake of PAHs in vegetation:

(a) Some terrestrial plants can take up PAHs through their roots and/or leaves and translocate them to various plant parts.
(b) Uptake is dependent on PAHs concentrations, solubility, phase (vapour or particulate), molecular size, support media anchoring plants and plant species.
(c) PAHs may concentrate in certain plant parts more than in others.
(d) Some PAHs can be catabolized by plants.

According to Backer (1970), spillage on land (soil) causes oil to enter into the leaves of plants and hampers the process of photosynthesis and evapo-transpiration. The pores of leaves are penetrated by films of oil, which is evidenced by the darkening of leaves as the pore becomes filled with oil. A patch of dark oil cuts sunlight from the leaves and where the shielding of sunlight becomes too much the leaves experience necrosis and the plant eventually dies (Nelson-Smith, 1979). The aims of this work are to specifically determine the effect of medium to high crude oil contamination in soil on a biochemical parameter (chlorophyll content) in *Zea mays.*

MATERIALS AND METHODS

Field Contaminated Soil Sample Preparations

The method which was adopted here is the procedures described by Onwurah, (2003). It is known as Simulated Contaminated Oil Polluted Soils (SCOPS) tests. The contaminated experimental soils were used for the pollution experiments. Three separately weighed experimental soil microcosms were simulated by thoroughly mixing with

given ratios of crude oil of calculated weight to obtain 5%, 10%, and 20% of crude oil w/w (weight of Crude Oil/Weight of Soil) to make up the quantity of the various soils to 8 kg each after which they were separated into duplicates of 4 kg each. These contaminated soil were kept in polythene planting pots perforated and submerged into dug out portions in the field to allow for leaching of excess oil for a week before planting of maize was carried out.

***Zea mays* Planting and Monitoring**

Bearing in mind that this experiment can best be carried out during the rainy season the planting was carried out at the beginning of April 2007. Maize being one of the fastest growing as well as a good phytoaccumulator was used.

Forty (40) viable maize seeds were obtained from the seed bank of the Department of Crop Science, University of Nigeria, Nsukka, and used for the research.

The seeds were soaked in distilled water and their viability determined by floatation (Seed Priming) for 48 hours before sowing. Planting was done within 3 cm depth of two seed per hole in triplicate per pot and 18 cm distance between planting depths for all the soil samples. Additional seeds were also cultivated on the field around the experimental soil and subsequently used as nurseries

Germination was observed specifically for the growth of the cotyledons above the soil surface for some days. Two controls Control A – (Normal Unspiked soil) and Control B- (Spiked soil, treated with K_2SO_4, $CaCl_2$ and $MgSO_4$ salts) had 80% germination and only 9% germination on 5% test soil sample. This is in support of the observation made by Dejong (1980), Udo and Fayemi (1975), that oil in soil makes the soil condition unsatisfactory for plant growth, due to the reduction in the levels of certain elements such as iron and zinc.

The nurseries were then transplanted into the various contaminated soil samples (5%, 10%, 15%, and 20% w/w (weight of Crude Oil/Weight of Soil). The transplanted plant had about 60% survival (germination and stunted growth). The maize seeds were planted under field conditions of (32 ± 4^oC), 12 hrs Light: Dark Photo periods.

Chlorophyll Estimation (J. B. Harborne, 1973)

After a period of 5 weeks, Maize leaves of the controls and test experiment were harvested 2 hours after the beginning of light period. The leaves were deribbed and 0.6g of the leaves from each sample was homogenized in a mortar in the presence of excess acetone (80%) until all the color was released from the tissue. About 2 ml of excess Acetone/$CaCO_3$ was added to aid extraction. The above were carried out at 0-5^OC. The homogenized samples were then washed by adding excess Acetone/$CaCO_3$ and made up to a total volume of 15 ml (Addition of 1 part (1% $CaCO_3$) to 9 parts (80% Acetone) reduces the formation of pheophytin). The samples were then centrifuged for 10 minutes at 4500 x g (to eliminate precipitated protein). The filtrate of each group was then divided in triplicate and readings of each homogenized sample taken at 645 and 663 nm. The above step was performed in all five sample groups. The final filtrate was placed in the refrigerator until the spectrophotometric reading was taken to avoid decomposition of the chlorophyll by intense light. Chlorophyll Concentrations in leaves were calculated as follows:

Total Chlorophyll (mg/l) = $20.2A_{645} + 8.02A_{663}$

Therefore Chlorophyll (mg/g of Maize leaves) = $\frac{20.2A_{645} + 8.02A_{663}}{\alpha \times 1000 \times \omega} \times V$

Where V = Volume of the final filtrate read in ml.

A_{645} and A_{663} = absorbance at 645 and 663 nm.

α = Length of light path in the cell (i.e. 1 cm).

ω = Weight of Fresh leave macerated/homogenized.

RESULTS AND DISCUSSION

The highest value (0.440 ± 0.030mg/g) as seen in table 1, is from the Unspiked control. This is followed by (0.398 ± 0.040mg/g) belonging to the Spiked Control. The two controls recorded high chlorophyll content compared with the contaminated soils respectively. The histogram also shows that there was a reduction in chlorophyll level in the contaminated soil which reflected most in 20% (PHC) w/w contaminated soil.

The result of the experiment showed that contamination of by soil crude oil negatively affected the chlorophyll content present in the *Zea mays* leaf (Table 1). It is obvious from the histogram that the controls (Control A) unspiked soil and (Control B) spiked soil had the plant with the highest value of chlorophyll content, with the Normal (Unspiked) soil supporting plant (*Zea mays*) with the highest quantity of mean chlorophyll content (0.440 ± 0.030 mg/g).

Table 1. Chlorophyll amount FW in mg/g of Maize Biomass.

	Group A	Group B	Group C	Mean Chlorophyll Concentration (mg/g) of leaf
Control A	0.402	0.471	0.447	0.440 ± 0.030
Control B	0.442	0.408	0.343	0.398 ± 0.040
5% Contamination.	0.301	0.312	0.334	0.316 ± 0.010
10% Contamination.	0.290	0.289	0.298	0.292 ± 0.004
20% Contamination.	0.095	0.096	0.103	0.098 ± 0.004

Furthermore, the crude oil contaminated soil samples (5%, 10% and 20% w/w) expressed plants (*Zea mays*) with low chlorophyll contents. Among these contaminated soils, 5% w/w crude oil contaminated soil yielded about 0.316 ± 0.010 mg of chlorophyll per g of Zea mays leaves, which is the highest quantity of chlorophyll present in *Zea mays* of the crude oil polluted soil. The lowest value (0.098 ± 0.004 mg/g) of chlorophyll quantity was present in *Zea mays* cultivated on 20% w/w of crude oil contaminated soil see table A. An experiment was carried out on gas flaring and its effect on chlorophyll contents of crops (maize and cassava) cultivated in the vicinity of the flare. It was discovered that the closer the crops are to the vicinity of the gas flare the lower the quantity of chlorophyll found in their leaves (Ukegbu and Okeke, 1987). The above experiment could be correlated with the result of this experiment. It can be adduced that crude oil pollution from the result of this experiment, has a negative effect on chlorophyll production in *Zea mays* and perhaps other crops. According to Backer, (1970), spillage on land (soil) causes oil to enter into the leaves of plants and other economic trees through their pores and hampers the process of photosynthesis and evapo-transpiration.

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