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Protein and Chlorophyll Contents of *Solanum melongena* on Diesel Oil Polluted Soil Amended with Nutrient Supplements

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Abstract: The study investigated the remediation effect of nutrient amendments of diesel oil polluted soil on protein and chlorophyll contents of eggplant (*Solanum melongena*). Soil samples were polluted and amended separately with different weights of poultry waste, pig waste, cow dung and inorganic fertilizer. Soil samples were also polluted with diesel oil without amendment to achieve 2, 4, 6, 8 and 10% pollution. Samples were analyzed at two weeks interval for sixteen weeks. The plant protein and chlorophyll were affected adversely by the diesel oil pollution and the higher the level of pollution, the more the effect. The nutrient amendments were able to remedy the effect of the diesel oil pollution. The remediation effect was nutrient weight dependent and the best remediation effect was observed in poultry waste amended samples. This study has shown that diesel oil contaminated soil may have adverse effect on the protein and chlorophyll contents of plants, but this can be remedied by addition of organic nutrient supplements especially poultry waste.

Key words: Diesel oil, eggplant, nutrient amendments, remediation

INTRODUCTION

The emergence of crude oil industries has contributed immensely to changing the state of Nigerian economy and the environment. The oil industry is a major source of environmental pollution and its adverse ecological impacts have been reported (Ibia *et al*., 2002; Ekpo and Thomas, 2007).This is widely spread with specifically more serious damage on the oil producing areas. The most obvious area which has generated a lot of concern is spillage resulting from oil well blowout or pipeline leakages with each major spill incident increasing the vulnerability of our fragile environment (Ibe, 2000; Ekpo and Nwankpa, 2005). The impact of petroleum exploration could alter essential microbial biogeochemical cycling processes, resulting in altered productivity of affected ecosystem (Caravaca and Rodán, 2003). Pollution generally, can lead to a succession or total extinction of species in the affected habitat (Budny *et al*., 2002; Delille and Pelletier, 2002). This is because most spills are often toxic and generally cause deficiency in essential plant nutrients. Apart from phytotoxicity, nitrogen is the major element limiting plant growth in most spills and hydrocarbon contaminated sites (Wyszkowski *et al*., 2004).

Diesel oil is a hydrocarbon product of boiling point between approximately 150 and 400ºC with carbon chain lengths of C_5-C_{22} (Czes and Radolf, 2005). It is largely comprised of simple unbranched n-alkane with only around 4% polycyclic aromatic compounds (Czes and Radolf, 2005). When diesel oil is present in soil, it limits soil aeration and alters the general biological cycle/balance of soil in which it is present (Seklemora *et al*., 2001; Zhou *et al*., 2002). The increasing use of diesel oil in diesel engines of cars, industrial trucks and generators has led to an increased demand for diesel oil (Ogbo, 2009) and accidental spillage of diesel and pollution of agricultural lands. Diesel oil is one of the major products of crude oil and it constitutes a major source of pollution to the environment (Nwaogu *et al*., 2008). Diesel oil can enter into the environment through leakage from storage containers, refueling of vehicles, wrecks of oil tankers and warships carrying diesel oil and through improper disposal by mechanics when cleaning diesel tankers (Hill and Moxey, 1960). Soil pollution through such many small and common sources of these products poses large environmental threat (Wyszkowski and Ziolkowska, 2008). Diesel spills on agricultural land generally reduce plant growth (Nwaogu *et al*., 2008), it can also lead to a significant reduction of organic carbon content of the soil (Wyszkowski and Ziolkowska, 2008). Diesel oil is phytotoxic to plants at relatively low

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concentrations and had been shown to cause a reduction in the length of the radicles of *Arachis hypogea*, *Vigna unguiculata*, *Sorghum bicolor* and *Zea mays* (Ogbo, 2009).

It has been reported that plants and soil microbes compete for the little nutrient available in soils that are not rich like that polluted with crude oil thereby suppressing the growth of plants in such soils. However it is generally known that when soils not suitable for plant growth are augmented with manure, growth and performance of plants in such soil are enhanced. Merkl *et al*. (2004) reported that addition of inorganic fertilizer in a crude oil polluted soil enhances the growth and performance of *Brachiaria brizantha* in crude oil polluted soil. Although, the performance of plants as reported by Merkl and co–workers can be enhanced in crude oil polluted soil with fertilizer, it also increases the cost of crop production in crude oil polluted soil. It is therefore necessary to investigate the impact organic manure like poultry waste, pig waste and cow dung can make in the growth of crops in petroleum oil polluted soil. This is because such manure is cheaper and is more affordable to farmers than the inorganic fertilizers. This study was therefore carried out to investigate the effect of diesel oil pollution on the protein and chlorophyll contents of eggplant and whether addition of organic nutrients and inorganic fertilizer to the polluted soil will enhance the protein and chlorophyll contents of these plants.

MATERIALS AND METHODS

Sample collection and preparation: The soil samples used for this study were collected from a site in the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri (FUTO), Imo State, Nigeria. The samples were collected from 5-10 cm of the topsoil and transported to the preparation site in clean plastic buckets. The diesel oil was bought from Nigerian National Petroleum Corporation (NNPC) mega station, Owerri. The cow dung was collected from the Department of Animal Science Technology, FUTO. The pig and poultry wastes were obtained from Songhai Redemption farms, Nekede, Owerri, while the inorganic fertilizer (NPK 15:15:15) was bought from ADC Farms, Nekede, Owerri. Garden egg seedlings (*Solanum melongena*) were purchased from Relief market, Owerri, Imo State, Nigeria and identified by Dr Ferdinand Mbagwu, a plant taxonomist with the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State, Nigeria.

To prepare the soil samples for diesel oil pollution, 84 kg of soil was properly mixed with 20 and 10 L of diesel oil respectively, for heavy and moderate pollution samples, respectively. These were dispensed into plastic buckets in 6 kg weights each and mixed with various quantities of organic (200, 400 and 600 g) and inorganic (100, 200 and 300 g) supplements. The samples were exposed to rain and sunlight throughout the period of the study.

Soil samples were also obtained and dispensed in 6 kg weights in plastic buckets. The soil samples were mixed with diesel oil to achieve 2, 4, 6, 8 and 10% pollution. A control was set without diesel pollution (0%). Each of these samples was planted with 4 seedlings of garden egg plant. Samples were taken for analysis at 2 weeks interval for 16 weeks.

Chlorophyll content: About 5 g of leaves were ground in a mortar with 5 mL of 80% (v/v) acetone to a fine pulp. The mixture was filtered and re-extracted using 5 mL of 80% acetone. This was done continuously until almost all the chlorophyll was extracted. The volume of the extract was measured.

The absorbance of a portion of the combined extract from above was read at 647 and 665 nm. The concentration of total chlorophyll, chlorophyll a and chlorophyll b were calculated using the relationship (Inskeep and Bloom, 1985).

Chlorophyll b (mg/L) = $20.47A_{647} - 4.73A_{665}$
Chlorophyll a (mg/L) = $12.63A_{665} - 3.52A_{647}$
Total chlorophyll (a+b) (mg/L) = $17.95A_{647} - 7.90A_{665}$

Protein content: The kjeldahl method was employed. About 0.5 g of the plant leaf was put into the kjeldahl digestion flask. A blank was set up. One tablet of selenium catalyst was added into each flask, moistened with distilled water and mixed with 10ml of conc. H_2SO_4. The mixture was heated in a fume cupboard for 2 h until a clear solution was obtained (the digest). The digest was transferred to 100 mL volume flask and diluted with distilled water. About 10 mL of this digest was mixed with equal volume of 45% NaOH solution in a semi-microkjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 10 mL of 4% boric acid solution containing three drops of mixed indicators (methyl red and bromocresol green). About 50 mL of the distillate was titrated against 0.02N H_2SO_4 solution. The same process was carried out on the blank. The percentage nitrogen content was calculated as follows:

$$\%N = 14 \times Na\ (Vf/Va)\ (100/w) \times X$$

where,
X = sample titre - blank titre
Na = concentration of normal acid (0.02N)
Vf = Total volume (100 mL)
Va = volume of distillate
W = mass of sample

$$\%\ \text{Crude protein}\ (\%p) = \%N \times 6.25$$

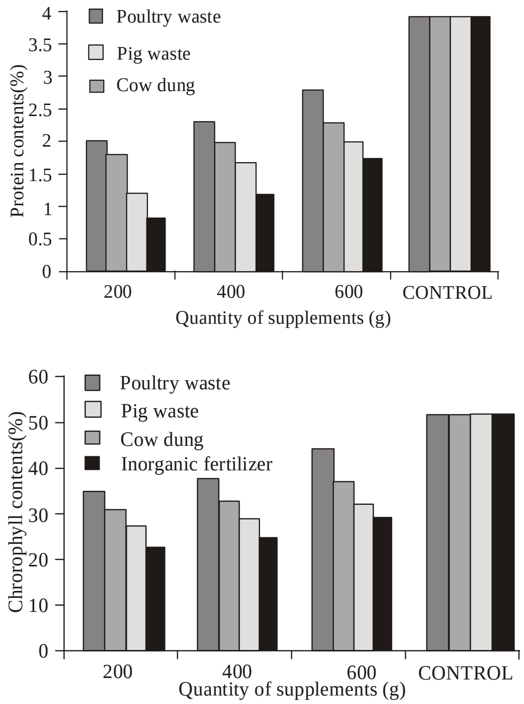

Fig. 1: Protein (A) and chlorophyll (B) content of eggplant in heavy diesel oil polluted soil

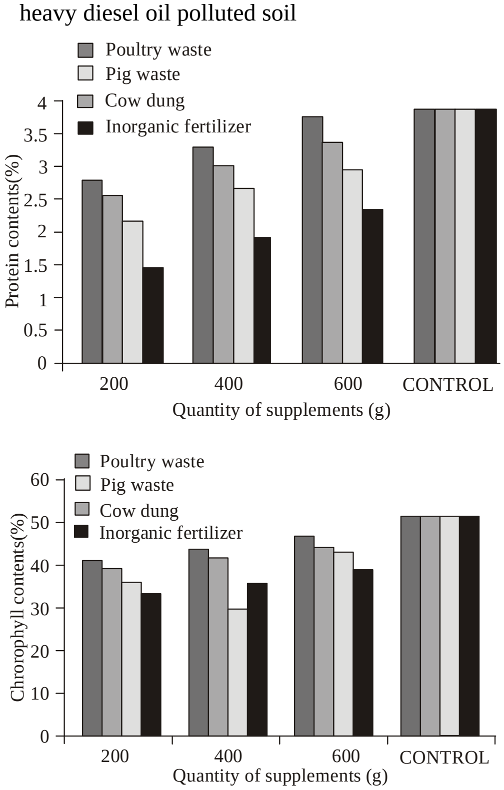

Fig. 2: Protein (A) and chlorophyll (B) contens of eggplant in moderatediesel oil polluted soil

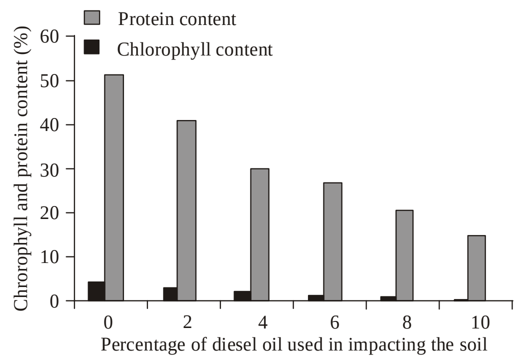

Fig. 3: Protein and chlorophyll contents of eggplant on soil s polluted with different concentration of diesel oil

RESULTS AND DISCUSSION

There were adverse effects of the diesel oil pollution on the chlorophyll and protein contents of the eggplants This agrees with the work of Seklemora *et al*. (2001) which shows that diesel oil inhibited the growth of cereals causing chlorosis and dehydration. It also shows that diesel oil inhibited the metabolic and physiological processes including photosynthesis and transportation. The photosynthetic pigments are the most likely to be damaged by pollution. Chlorophyll pigments exist in highly organized state, and under stress they may undergo several photochemical reactions such as oxidation, reduction, pheophytinisation and reversible bleaching Puckett *et al*., 2003). Hence any alteration in chlorophyll concentration may change the morphological, physiological and biochemical behaviour of the plant.Pollution-induced degradation in photosynthetic pigments was also observed by a number of workers (Bansal, 1988; Singh *et al*., 1990; Sandelius *et al*., 1995).

The effect was however ameliorated by the addition of nutrient supplements. The amended samples recorded an increase in protein and chlorophyll contents with a corresponding increase in the quantity of supplements. The remediation effect of animal waste on pollution-induced damage to plants was observed by Mut *et al*. (2010) who recorded that addition of animal waste to soil increased the protein contents of plants. Some other authors also observed significant increases in protein content of plants with increasing nitrogen application (Behren *et al*., 2001; Rathke *et al*., 2005; Rathke *et al*., 2006). The remediation effect was also dependent on the type of nutrient supplement. The highest value in the amended samples with heavy diesel oil pollution was 2.8% protein and 43.8 mg/L chlorophyll obtained in the 600 g poultry waste sample, followed by 600 g pig waste (2.3% protein and 37.0 mg/L chlorophyll). The least values were obtained in the sample amended with 100g of inorganic fertilizer (0.8% protein and 22.3 mg/L chlorophyll). The control had significantly higher protein (3.9%) and chlorophyll contents (51.2 mg/L) than those of the amended samples ($p<0.05$) as presented in Fig. 1.

In the moderately polluted samples (Fig. 2), the protein and chlorophyll contents of the eggplants also increased with increase in the quantity of supplements. The protein and chlorophyll contents of the egg plants according to the supplements appeared in the following order: poultry waste > pig waste > cow dung > inorganic fertilizer. The highest values of protein and chlorophyll in the supplemented samples were obtained in the 600g poultry waste sample (3.8% protein and 46.5 mg/L chlorophyll), while the least were obtained in the 100g inorganic fertilizer sample (1.4% protein and 32.6 mg/L chlorophyll). The control samples also had significantly higher protein and chlorophyll contents than the test samples ($p<0.05$).

Protein and chlorophyll contents of the eggplant in soil samples amended with different percentage concentrations of diesel oil are presented in Fig. 3. There were progressive decreases in the protein and chlorophyll contents as the concentrations of diesel oil increases. The highest protein and chlorophyll contents were obtained in the 0% diesel oil sample (3.9% protein and 51.2 mg/L chlorophyll), followed by the 2% diesel oil sample (2.5% protein and 40.4 mg/L chlorophyll). The least protein and chlorophyll contents were obtained in the 10% diesel oil sample (0.2% protein and 14.6 mg/L chlorophyll). The decrease in chlorophyll level with increase in pollution concentrations was similar to the findings of Odjegba and Sadiq (2003) where reduction in chlorophyll and protein levels were reported in *Amaranthus hybridus* grown in soil contaminated with engine oil. Bayram *et al*. (2009) showed that the presence of oil in the soil significantly decreased the available forms of phosphorus and potassium to plants. These nutrients (nitrogen, phosphorus, potassium and oxygen) are essential to plant growth and development hence reduction in their bioavailability will lead to reduced plant growth and adverse effects on the protein and chlorophyll contents of plants. From the study, it can be concluded that diesel oil contaminated soil may result in reduced protein and chlorophyll contents of plants. But this can be remedied by the addition of organic nutrient supplements especially poultry waste and the quantity of supplement added has significant effect on the remediation process. The study underscores the need for the use of cheap and available, and environmental friendly organic nutrients as supplements as a remedy for the deleterious effects of petroleum contaminants in the soil.

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