EFFECTS OF HYDROCARBON POLLUTED SOIL ON THE BIOMASS AND PROTEIN CONTENT OF COWPEA AND MAIZE SEEDLINGS

Achuba, F. I.
Department of Biochemistry, Delta State University, PMB 1, Abraka Nigeria.
achubabch@yahoo.com

ABSTRACT

Effect of hydrocarbon from crude oil released into the environment, including farmlands on production continues to cause both physical and metabolic derangements in crops. The objective of this study was to determine the effect of hydrocarbon tainted soil on the biomass and levels of protein in both cowpea (Vigna unguiculata L Walp) and maize (Zea mays L) seedlings. The soil was treated with hydrocarbons (kerosene, diesel, engine oil and petrol) at different concentrations (0.0% 0.1%, 0.25%, 0.5%, 1.0% and 2.0% (v/w). The experimental set up was completely randomized design and treatment was replicated five times. Three seeds were planted in each bag and watered daily. The biomass of the whole seedling and protein content were determine on each crop stating from four days after germination at interval of four days. The results revealed that the hydrocarbons caused significant ($P<0.01$) decrease in the biomass as well as the protein content in both cowpea and maize seedlings. The hydrocarbons exhibited differential effects on the biomass and the levels of protein in the crop seedlings. It is obvious that hydrocarbons toxicity is dependent on the type of product as well as crop under consideration

Keywords: Biomass, Cowpea, Maize, Protein, Petroleum product

INTRODUCTION

In recent times, the environmental impact of crude oil or related products have been a popular area of interest in experimental research (Nwaogu and Onyeze, 2010, 2014; Achuba and Ogwumu, 2014; Achuba and Nwokogba, 2015). Petroleum affects plants at different physiological stages which culminate in reduction in yield through its effects on morphological indices (Peretiemo-Clarke and Achuba, 2007).

The detrimental effect of Petroleum on plant growth is ubiquitous (Amadi *et al.,* 1996; Achuba, 2006; Peretiemo-Clarke and Achuba., 2007 Achuba and Okoh, 2015; Achuba and Asagba, 2015). This is because plant growth depends on minerals present in soil (Shanker et al., 2005). Therefore, petroleum in soil, which is toxic to plants, depresses seed germination and there are documents that various forms hydrocarbons subdued seed germination (Eriyamremu, *et. al.*, 1999; Akaninwor et al., 2007; Anoliefo and Edegbai, 2000; Njoku, *et. al*, 2008).

More so, it has been reported that hydrocarbons induced metabolic modifications in plants (Achuba, 2006; Peretiemo-Clarke and Achuba, 2007; Achuba and Okoh, 2015), including increased production of organic macromolecules in plants (Achuba, 2006). Achuba, (2010) and Nwaogu and Onyeze (2010) observed an

increase in lipid peroxidation product in plant exposed to petroleum. Various authors documented that cultivation of plant in hydrocarbon impacted soil and other forms of pollution inhibited starch metabolism as well as peroxidase activity in plant species (Eriyamremu *et. al.,* 1999; Achuba 2006; Aki *et. al,* 2009; Achuba and Okoh 2015).

Large quantities of crude oil are released into agricultural lands in oil-rich regions that can affect crops in so many ways (Ogri, 2001, Achuba and Okoh, 2015).This affects the survival of crops in such soil and culminate in threat to food security. The objective of the present research was to determine the effect of hydrocarbons on the level of protein and biomass of selected food crop (cowpea and maize) seedlings exposed to hydrocarbons treated soil.

MATERIALS AND METHODS

Materials used

Petroleum products (specific gravities: kerosene = 0.81; diesel = 0.85; engine oil=0.87; petrol = 0.75) were supplied by Warri Refining and Petrochemical Company, Warri, Nigeria. One batch grains of maize (Suwan 1 variety) were was supplied by Delta Agricultural Development Project (DTADP) Ibusa Delta State, Nigeria. Similarly, one batch of cowpea seeds (IT845-2246-4 accession) was acquired from International Institute of Tropical Agriculture IITA, Ibadan, Nigeria. The soil sample employed for the experiment was acquired from an uncultivated land in the premises of Delta State University, Abraka, Nigeria. The physico-chemical properties of test soil sample were previously analysed (Achuba, 2006)

Planting of seeds

Each polybag was filled with 1.60 kg of the soil sample and treated with different amounts of the hydrocarbons (0.0% 0.1%, 0.25%, 0.5%, 1.0%, and 2.0% v/w). Every concentration was prepared five times and this was carried out by adding to the bag, for example the 0.1%, was prepared by 1.6 ml one of the products to the soil sample and was mixed with hand to achieve a fine mixture. The same procedure was adopted to prepare other treatments

Potentially good seeds were determined by pouring sizable quantity into bowl containing water. Seeds that did not float on water were used for planting while those that floated were discarded. The seeds(three) were planted in every polybag to a depth of 2cm immediately after treatment of soil with the respective petroleum products and protected from direct sunlight by placing under shade. Each treatment was watered (80 cm^3) to each bag to wet the experiment daily and keep the soil moist for twelve days. The biomass the whole plant and levels of protein in the leaves of cowpea and maize seedlings were determined at every four days for up to twelve days after germination

Preparation of homogenate and assay for protein

The leaf homogenate was prepared by collecting leaves (1.0 g) from each treatment. The leaves were placed in a mortar containing 0.5 g acid washed sand. This was followed by grinding with a pestle. At the end of each homogenization 5.0 ml of distilled water was added and stirred with a glass rod. The homogenate was filtered using cheese cloth and the filtrate subjected to centrifugation at 1000 rpm for 30 minutes to

produce a supernatant that was employed for the determination of protein according to Lowry's method (1951) using bovine serum albumin as standard. The reagent (2.5 ml) and 1.0ml of leaf extract were added into a test tube and mixed thoroughly. The assay was allowed to stand for 10 minutes and 0.5 ml of Folins-Ciocalteau reagent added to each tube, mixed and allowed to stand for another 30 minutes. Absorbance was then read at 660 nm against reagent blank

Determination of dry weight

The dry weight of the seedlings was determined by drying whole plants of each treatment to constant weight in an electric oven at 105°C for 24 hour**.** Relationship between the various parts of the seedlings was obtained through the use of correlation coefficient analysis (SPSS, version 20)**.**

Statistical Analysis

Analyses of variance (ANOVA) as well as Fisher's least significant difference (LSD) were adopted for multiple comparisons using statistical package for social science (SPSS) (version 20) to determine statistical significant differences between means. P values <0.01 were taken as being significantly different. Relationship between the various parts of the seedlings were obtained through the use of regression analysis (SPSS, version 20)

Table 1: Concentration of protein in leaves of cowpea (*Vigna unguiculata*) seedlings measured at four days interval up to twelve days germination in soil treated with different concentrations of hydrocarbons.

levels of hydrocarbons (% v/w ml/g)	Concentration of protein (mg/gdw)											
	Kerosene			**Diesel**			**Engine Oil**			**Petrol**		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	22.5 ± 1.5^{a}	32.5 ± 1.8^{ac}	37.7 ± 2.5^{a}	22.5 ± 1.5^{a}	32.5 ± 1.8^{a}	37.7 ± 2.5^{a}	22.5 ± 1.5^{a}	32.5 ± 1.8^{a}	37.7 ± 2.5^{a}	22.5 ± 1.5^{a}	32.5 ± 1.8^{a}	37.7 ± 2.5^{a}
0.10	62.82±8.01^{b}	10.2±1.3^{b}	27.0± 3.6^{b}	79.71±6.64^{b}	11.58±9.55^{b}	30.3±2.5^{b}	76.81±4.4^{b}	25.17±6.84^{b}	67.7±1.72^{b}	71.01±6.06^{b}	39.58±9.64^{ab}	70.3±3.84^{b}
0.25	50.0 ± 10.2^{b}	31.25 ± 2.5^{ac}	21.7 ± 4.7^{c}	62.31 ± 10.94^{c}	29.58±15.73^{a}	23.7 ± 4.0^{c}	75.36 ± 10.94^{b}	31.33±2.73^{a}	28.7 ± 4.0^{a}	47.83 ± 8.10^{c}	32.25 ± 11.09^{a}	95.0 ± 3.12^{c}
0.50	29.49 ± 5.88^{ac}	37.92 ± 9.55^{c}	12.6 ± 2.5^{d}	44.92 ± 9.05^{d}	34.58±13.01^{a}	17.0 ± 3.6^{c}	57.97 ± 5.02^{c}	33.3 ± 7.22^{a}	44.3 ± 3.96^{c}	10.89 ± 2.27^{d}	45.92 ± 2.95^{b}	39.3 ± 6.0^{a}
1.00	23.46 ± 3.29^{ac}	38.13 ± 5.3^{c}	10.1 ± 1.6^{d}	28.12 ± 5.03^{a}	40.42 ± 7.24^{a}	10.7 ± 1.9^{d}	31.73 ± 4.58^{d}	45.63±6.59^{d}	31.8 ± 3.93^{a}	56.52 ± 4.35^{c}	51.25 ± 6.25^{b}	21.7 ± 1.5^{c}
1.50	17.82 ± 1.55^{ac}	28.95 ± 2.53^{d}	8.3 ± 1.5^{d}	23.04 ± 3.45^{a}	33.125±4.96^{a}	8.8 ± 1.3^{d}	44.49 ± 3.75^{c}	33.96±5.39^{a}	17.0 ± 1.0^{d}	56.82 ± 4.38^{c}	41.67 ± 6.29^{b}	21.7 ± 1.5^{c}
2.00	19.10 ± 3.45^{ac}	21.04 ± 5.60^{d}	8.3 ± 1.5^{d}	21.59 ± 3.89^{a}	21.042±5.60^{c}	8.2 ± 1.5^{d}	40.58 ± 6.64^{c}	29.33±9.55^{ab}	15.3 ± 2.5^{d}	81.16 ± 9.05^{b}	32.67 ± 1.01^{c}	31.0 ± 3.6^{a}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference

Table 2: Concentration of protein in leaves of Maize (*Zee mays*) seedlings measured at four days interval up to twelve days after germination in soil treated with different concentrations of hydrocarbons

levels of hydrocarbons (% v/w ml/g)	Concentration of protein (mg/gdw)											
	Kerosene			Diesel			Engine Oil			Petrol		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	15.0 ± 1.0^{a}	35.0±1.4^{a}	30.0±2.0^{a}	15.0 ± 1.0^{a}	35.0±1.4^{a}	30.0±2.0^{a}	15.0 ± 1.0^{a}	35.0 ± 1.4^{a}	30.0 ± 2.0^{a}	15.0 ± 1.0^{a}	35.0 ± 1.4^{a}	30.0 ± 2.0^{a}
0.10	41.03 ± 5.88^{b}	66.67±9.55^{b}	21.3±3.1^{b}	52.17±4.35^{b}	17.75±4.75^{b}	24.9±2.0^{b}	17.39 ± 3.00^{ad}	17.75 ± 4.75^{b}	54.0 ± 14.2^{b}	79.71 ± 41.32^{bc}	26.33 ± 6.40^{b}	82.7 ± 1.90^{b}
0.25	33.21 ± 7.79^{c}	33.96±12.66^{a}	17.3±4.1^{b}	40.58±6.64^{c}	40.83±9.55^{ac}	16.0±7.0^{bc}	49.27 ± 6.64^{b}	40.83 ± 9.55^{a}	22.7 ± 3.1^{c}	65.22 ± 5.4^{b}	34.50 ± 8.06^{b}	76.0 ± 2.49^{b}
0.50	19.23 ± 3.85^{a}	41.25±6.25^{c}	10.0 ± 2^{c}	28.99±6.64^{d}	51.50±5.07^{c}	13.3±3.1^{c}	36.52 ± 3.35^{c}	51.50 ± 5.07^{c}	62.0 ± 7.79^{d}	68.12 ± 10.94^{b}	47.92 ± 15.73^{bc}	31.3 ± 5.0^{a}
1.00	15.26 ± 2.18^{a}	44.79±3.55^{c}	7.9 ± 1.0^{c}	18.69±3.39^{a}	49.38±2.87^{c}	8.6 ± 1.6^{c}	20.43 ± 1.99^{d}	49.38 ± 2.87^{c}	40.0 ± 5.36^{e}	37.25 ± 2.96^{c}	53.54 ± 4.25^{c}	17.3 ± 1.5^{c}
1.50	12.69 ± 2.40^{a}	30.63±3.90^{a}	6.6 ± 1.3^{c}	15.22±2.30^{a}	42.29±3.21^{c}	7.0 ± 1.1^{c}	29.42 ± 2.23^{e}	42.29 ± 3.21^{a}	13.5 ± 1.0^{f}	37.54 ± 2.47^{c}	53.96 ± 3.55^{c}	17.3 ± 1.1^{c}
2.00	12.69 ± 2.34^{a}	27.63±3.80^{a}	6.6 ± 1.3^{c}	14.34±2.65^{a}	32.50±6.25^{a}	6.6 ± 1.2^{c}	26.09 ± 4.35^{e}	32.50 ± 6.25^{a}	12.0 ± 2.0^{f}	53.62 ± 6.64^{b}	47.08 ± 9.55^{bc}	24.7 ± 3.1^{c}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

Table 3: Concentration of protein content in stem of cowpea (*Vigna unguiculata*) seedlings measured at four days interval up to twelve days after germination in soil treated with different concentrations of hydrocarbons

	Concentration of protein (mg/gdw)											
levels of hydrocarbons (% v/w ml/g)	Kerosene			Diesel			Engine Oil			Petrol		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	22.8 ± 4.0^{a}	28.5 ± 4.0^{a}	22.5 ± 1.5^{a}	22.8 ± 4.0^{a}	28.5± 4.0^{a}	22.5 ± 1.5^{a}	22.8 ± 4.0^{a}	28.5 ± 4.0^{a}	22.5 ± 1.5^{a}	22.8 ± 4.0^{a}	28.5± 4.0^{a}	22.5 ± 1.5^{a}
0.10	17.7± 3.5^{ab}	25.41±3.8^{ab}	16.3 ± 2.0^{ab}	19.9± 2.7^{ac}	28.54±2.19^{a}	18.3 ± 1.5^{a}	44.37±11.77^{b}	24.67±16.86^{ab}	40.7± 1.02^{b}	68.06±16.60^{b}	28.33±2.31^{a}	62.3± 1.45^{b}
0.25	14.2 ± 3.1^{b}	20.41±4.6^{ab}	13.0 ± 2.7^{b}	15.62±2.65^{ac}	22.08±3.82^{a}	14.3 ± 2.5^{b}	18.96±2.21^{a}	27.75±3.79^{a}	17.3 ± 2.5^{c}	63.54±20.00^{b}	29.08±2.96^{a}	57.0± 1.87^{b}
0.50	82.61± 2.7^{c}	18.75± 2.5^{b}	7.6 ± 1.5^{c}	11.01±2.47^{a}	15.83±3.55^{b}	10.3 ± 2.0^{c}	51.59±6.40^{b}	24.42±9.45^{a}	13.3 ± 1.2^{ce}	26.97± 4.78^{a}	37.83±6.06^{b}	23.7 ± 3.5^{a}
1.00	63.8±9.05^{cd}	16.7± 1.31^{b}	6.6 ± 0.85^{cd}	71.01±13.98^{bc}	10.21±2.09^{b}	6.5 ± 1.2^{d}	76.81±6.64^{c}	11.42±9.55^{b}	7.3 ± 0.5^{d}	14.48± 1.04^{c}	21.25±2.75^{ad}	13.0 ± 1.0^{c}
1.50	55.1 ± 9.1^{cd}	17.9± 1.31^{b}	4.6 ± 0.4^{d}	59.42±9.05^{bc}	18.52±13.01^{ab}	5.3 ± 0.79^{d}	11.59±6.64^{d}	16.42±3.55^{b}	10.2 ± 1.0^{ed}	13.78± 7.53^{c}	19.75±1.83^{d}	13.0 ± 1.0^{c}
2.00	53.6±10.04^{d}	7.08 ± 1.4^{d}	5.0 ± 0.9^{d}	53.62±10.04^{bc}	17.08±14.43^{ab}	4.9 ± 0.9^{d}	10.00±2.39^{d}	21.88±11.41^{ab}	9.3 ± 1.5^{d}	20.34± 2.21^{a}	21.75±3.79^{ad}	18.7 ± 2.1^{c}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

Table 4: Concentration of protein content in stem of Maize (*Zee mays*) seedlings measured at four days interval up to twelve days after germination in soil treated with different concentrations of hydrocarbons.

levels of hydrocarbons (% v/w ml/g)	Concentration of protein (mg/gdw)											
	Kerosene			Diesel			Engine Oil			Petrol		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	32.50 ± 8.0^{a}	30.0 ± 8.0^{a}	15.0 ± 1.0^{a}	32.50 ± 8.0^{a}	30.0 ± 8.0^{a}	15.0 ± 1.0^{a}	32.50 ± 8.0^{a}	30.0 ± 8.0^{a}	15.0 ± 1.0^{a}	32.50 ± 8.0^{a}	30.0 ± 8.0^{a}	15.0 ± 1.0^{a}
0.10	13.9 ± 2.9^{b}	20.00 ± 2.80^{b}	10.7 ± 1.5^{b}	15.65 ± 1.04^{b}	22.50 ± 2.75^{ab}	12.0 ± 1.0^{ab}	35.17 ± 9.30^{a}	31.25 ± 13.25^{a}	27.0 ± 7.0^{b}	34.57 ± 2.64^{a}	49.13 ± 4.23^{b}	41.3 ± 9.5^{b}
0.25	11.26 ± 2.4^{b}	16.88 ± 3.78^{b}	8.6 ± 2.0^{a}	12.74 ± 2.92^{b}	17.50 ± 2.64^{b}	9.3 ± 1.5^{ab}	15.83 ± 2.92^{b}	31.5 ± 2.64^{a}	11.3 ± 1.50^{a}	49.65 ± 2.60^{b}	31.5 ± 2.19^{a}	38.0 ± 1.25^{b}
0.50	65.22 ± 13.04^{c}	23.75 ± 1.875^{b}	5.0 ± 1.0^{ab}	86.96 ± 19.92^{c}	12.50 ± 2.64^{b}	6.7 ± 1.5^{bc}	40.34 ± 5.00^{a}	38.25 ± 7.78^{a}	8.20 ± 0.72^{a}	20.35 ± 3.83^{c}	39.75 ± 5.19^{b}	15.7 ± 2.5^{a}
1.00	51.74 ± 7.42^{c}	24.38 ± 1.06^{b}	3.9 ± 0.6^{b}	56.09 ± 10.19^{d}	20.63± 14.64^{ab}	4.3 ± 0.8^{cd}	61.30 ± 5.98^{c}	38.13 ± 8.59^{a}	4.7 ± 0.50^{a}	11.74 ± 8.88^{d}	26.63 ± 1.76^{a}	8.6 ± 0.7^{c}
1.50	43.04 ± 8.15^{d}	21.88 ± 1.17^{b}	3.3 ± 0.6^{b}	16.43 ± 2.24^{b}	23.63 ± 3.65^{ab}	3.5 ± 0.5^{d}	88.26 ± 6.69`	33.88 ± 9.62^{a}	6.8 ± 0.50^{a}	11.61 ± 7.42^{d}	26.88 ± 1.66^{a}	8.6 ± 0.6^{c}
2.00	38.69 ± 13.07^{d}	25.63 ± 1.87^{ab}	3.3 ± 0.6^{b}	43.04 ± 7.93^{d}	21.88 ± 11.41^{a}	3.3 ± 0.6^{d}	78.26± 13.04^{cd}	31.50 ± 2.75^{a}	6.0 ± 1.00^{a}	16.87 ± 19.92^{c}	23.25 ± 3.64^{a}	12.3 ± 1.5^{ac}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

Table 5: Concentration of protein content in root of Cow pea (*Vigna unguiculata*) seedlings measured at four days interval up to twelve days after germination in soil treated with different concentrations of hydrocarbons.

	Concentration of protein (mg/gdw)											
levels of hydrocarbons (% v/w ml/g)	Kerosene			Diesel			Engine Oil			Petrol		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	33.33± 2.00^{a}	35.80± 2.00^{a}	90.0 ± 8.05^{a}	33.33±2.00^{a}	35.80±2.00^{a}	90.0 ± 8.05^{a}	33.33 ± 2.00^{a}	35.80 ± 2.00^{a}	90.0 ± 8.05^{a}	33.33± 2.00^{a}	35.80 ± 2.00^{a}	90.0 ± 8.05^{a}
0.10	19.7 ± 2.6^{b}	16.80± 2.50^{b}	40.7 ± 6.1^{b}	18.19±1.94^{b}	18.96± 2.57^{b}	45.7 ± 3.5^{b}	29.20 ± 7.85^{a}	42.92± 11.59^{b}	102.7± 26.9^{a}	31.79 ± 2.06^{a}	44.58 ± 2.14^{b}	157.3±3.60^{b}
0.25	14.20± 2.05^{b}	13.50±2.90^{bc}	32.6 ± 7.4^{b}	19.29±2.75^{b}	14.79±2.53^{bc}	35.3 ± 6.1^{c}	28.64 ± 2.57^{a}	17.17 ± 2.26^{c}	43.0 ± 5.5^{b}	41.04±13.76^{a}	19.75 ± 2.90^{c}	44.3 ± 4.75^{c}
0.50	15.07± 10.9^{b}	11.17± 1.57^{c}	19.0 ± 4.0^{c}	23.91±2.57^{b}	10.63±2.53^{c}	23.5 ± 5.7^{d}	29.75 ± 2.07^{a}	18.08 ± 2.36^{c}	117.7± 1.48^{a}	37.01± 3.21^{a}	24.83 ± 4.67^{c}	59.3 ± 9.6^{c}
1.00	23.91± 7.31^{a}	13.13± 1.05c	14.7 ± 2.1^{c}	26.81±8.43^{b}	17.29±1.2^{b}	16.3 ± 3.2^{de}	28.12 ± 2.77^{a}	19.54 ± 2.48^{c}	17.7 ± 1.5^{c}	41.20± 6.64^{a}	13.42 ± 2.55^{d}	33.0 ± 3.0^{d}
1.50	15.94± 6.75^{b}	11.67± 1.71^{c}	12.7 ± 2.1^{c}	18.26±5.75^{b}	15.00± 8.27^{b}	16.7 ± 2.0^{de}	33.91 ± 4.35^{a}	11.25 ± 6.25^{c}	25,7 ± 1.5^{c}	37.20± 5.64^{a}	13.42 ± 9.55^{cd}	31.0 ± 1.7^{d}
2.00	15.94± 6.54^{b}	10.67±9.40^{bc}	12.3 ± 2.3^{c}	19.94±6.54^{c}	11.67± 2.40^{bc}	12.3 ± 2.3^{e}	26.67±10.94^{a}	15.83 ± 15.73^{c}	23.0 ± 4.0^{c}	33.78± 2.67^{a}	19.75 ± 2.53^{c}	47.0±0.056^{d}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

Table 6: Concentration of protein content in root of maize (*Zee mays*) seedlings measured at four days interval up to twelve days after germination in soil treated with different concentrations of hydrocarbons.

	Concentration of protein (mg/gdw)											
levels of hydrocarbons (% v/w ml/g)	Kerosene			Diesel			Engine Oil			Petrol		
	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12	Day 4	Day 8	Day 12
0.00	41.78 ± 2.52^{a}	43.00 ± 2.52^{a}	57.0 ± 4.0^{a}	41.78 ± 2.52^{a}	43.00 ± 2.52^{a}	57.0 ± 4.0^{a}	41.78 ± 2.52^{a}	43.00 ± 2.52^{a}	57.0 ± 4.0^{a}	41.78 ± 2.52^{a}	43.00 ± 2.52^{a}	57.0 ± 4.0^{a}
0.10	20.75 ± 1.32^{b}	13.33 ± 2.09^{b}	32.0 ± 4.6^{b}	21.35 ± 8.69^{b}	15.00 ± 12.50^{b}	36.0 ± 3.0^{b}	23.78 ± 6.87^{b}	34.5 ± 9.50a	81.0 ± 2.10^{b}	36.42 ± 8.65^{a}	52.67 ± 12.81^{a}	78.1 ± 5.60^{b}
0.25	15.07 ± 1.76^{b}	10.79 ± 2.52^{b}	25.9 ± 6.0^{c}	19.57 ± 31.35^{b}	10.00 ± 4.07^{b}	28.0 ± 4.6^{b}	29.55 ± 13.28^{b}	14.67 ± 2.09^{b}	34.0 ± 4.6^{c}	33.44 ± 10.61^{a}	18 ± 6.13^{b}	40.0 ± 3.75^{c}
0.50	23.48 ± 8.69^{b}	12.50 ± 12.50^{b}	15.0 ± 3.0^{d}	27.97 ± 13.28^{c}	13.33 ± 19.09^{b}	20.0 ± 4.5^{c}	27.56 ± 3.91^{b}	19.50 ± 4.18^{b}	93.0 ± 1.17^{b}	38.23 ± 2.88^{a}	19.83 ± 3.46^{b}	47.0 ± 7.5^{c}
1.00	24.20 ± 4.46^{b}	19.17 ± 6.41^{b}	11.9 ± 1.7^{d}	27.39 ± 6.79^{c}	13.75 ± 9.76^{b}	12.9 ± 2.3^{d}	30.91 ± 2.47^{b}	25.00 ± 3.61^{c}	14.1 ± 1.31^{d}	45.07 ± 6.29^{a}	11.92 ± 9.04^{b}	25.7 ± 2.0^{d}
1.50	18.69 ± 5.43^{b}	11.25 ± 7.81^{b}	9.9 ± 1.9^{d}	20.43 ± 4.60^{b}	11.75 ± 6.61^{b}	11.9 ± 4.85^{d}	35.84 ± 4.46^{ab}	14.58 ± 6.42^{b}	20.3 ± 1.5^{d}	45.07 ± 4.95^{a}	11.92 ± 7.11^{b}	25.9 ± 1.9^{d}
2.00	18.69 ± 5.29^{b}	11.25 ± 7.60^{b}	8.9 ± 3.0^{d}	22.69 ± 5.29^{bc}	11.25 ± 7.60^{b}	9.9 ± 1.8^{d}	30.17 ± 8.69^{ab}	17.00±12.50^{b}	18.0 ± 3.0^{d}	41.25 ± 1.28^{a}	15.17 ± 1.09^{b}	37.0 ± 4.6^{d}

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

RESULTS AND DISCUSSION

The levels of protein in the leaves of cowpea seedlings were affected by the different hydrocarbons across four to twelve days after planting (Tables 2). These effects of the hydrocarbons were also exhibited on maize seedlings (Table 3). The hydrocarbons caused increases in the levels of protein in the leaves of the two seedlings at lower levels of soil treatment (0.1 – 0.5 %). These observations agree with that of several authors (Malallah *et. al.,* 1996; Achuba, 2006; Peretiemo – Clarke and Achuba, 2007), who reported similar rise in levels of protein in plants cultivated in crude oil tainted soil. The presence of sulphur and some other growth enhancing substances in petroleum was stated to be responsible for the rise in levels of protein in the leaves of seedlings cultivated in crude oil tainted soil. However, as the degree of soil contamination increased (1.0 % -2.0 %), the levels of protein in the leaves of exposed plants decreased comparative to the respective controls. Anoliefo and Edegbai (2000) reported that at low levels of oil pollution, hydrocarbons could be easily degraded by natural rehabilitation in soil. This will increase organic matter in soil as well as improve soil health and fertility. This may in part, explain why there was increase in protein at low levels of soil contamination as against the reduction at high levels of petroleum products in soil. On the other hand, increase in soil contamination with petroleum has been associated with deleterious soil condition and consequent decrease in crop yield (Achuba, 2006; Njoku et al., 2008; Achuba and Okoh, 2015).

Table 7: Correlation coefficients of protein in the leaves stem and root of cowpea and maize seedlings at four days interval up to twelve days after germination in hydrocarbons treated soil

Correlation is significant at p < 0.01 level

Physiological region of plant	Kerosene		Diesel		Engine oil		Petrol	
	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize
4 days after germination								
Leave/ Stem	-0.623	-0.385	-0.623	-0.286	-0.028	-0.265	0.155	0.218
Leave/ Root	-0.207	-0.415	-0.459	-0.425	-0.301	-0.269	-0.162	-0.656
Stem/ Root	-0.275	0.169	-0.038	0.317	-0.307	-0.90	-0.123	-0.713
8 days after germination								
Leave/ Stem	-0.034	-0.252	-0.626	-0.027	-0.732	0.546	-0.034	-0.656
Leave/ Root	0.017	-0.735	0.011	-0.153	-0.410	-.0607	0.017	-0.851
Stem/ Root	0.712	0.735	0.615	0.700	0.466	-0.231	0.712	0.680
12 days after germination								
Leave /Stem	0.997	0.997	0.998	0.989	0.851	0.421	0.941	0.990
Leave/ Root	0.957	0.981	0.938	0.975	0.794	0.818	0.400	0.666
Stem/ Root	0.954	0.983	0.932	0.976	0.643	0.615	0.644	0.680

The studied hydrocarbons also cause decreases in the levels of protein in both the stem and the root measured at four days interval up to twelve days after germination in the two seedlings (Tables 3-6). However, the effects on the two seedlings are not the same. No wonder an inverse relationship exists between the level of protein in the leaves, stem and root of cowpea and maize seedlings for up to twelve days of growth in petroleum products treated soil (Table 7).This may be due to hydrocarbon induced differences in the metabolic states of leaves, stem and root of a plant (Sadunishvili et al., 2009). In addition, growing plant tissues allocate sizeable amounts of shoot carbon and nitrogen to non-photosynthetic tissues like the stem (Gastal and Lemaire, 2002). It is documented that leaf and stem ratio decrease as crop biomass increases (Lemaire and Chartier, 1992; Belanger and McQueen, 1999; Belanger and Richards, 2000). Therefore, more carbons and nitrogen are assigned to the stem (Gastal and Lemaire, 2002). This is consistent with the result of the current study in which report more protein in the stem of maize seedlings compare to that in the leaves (Tables 5).This is not the same with cowpea seedlings, in which the level of protein in the leaf is same with that in the stem (Table 4). This observation is attributed to the photosynthetic activity of the stem of cowpea seedlings in early stage of seedling growth. On the whole, kerosene exhibited more noxious compared to the other hydrocarbons studied. And the manner of toxicity in cowpea and maize seedlings was plant specific.

Table 8: Dry weight of cowpea (*Vignaunguiculata*) seedlings after twelve days of germination in soil treated with different concentrations of hydrocarbons.

	Dry weight (g)			
levels of hydrocarbons(% v/w ml/g)	**Kerosene**	**Diesel**	**Engine oil**	**Petrol**
0.00	1.32 ± 0.61[a]	1.32 ± 0.61[a]	1.32 ± 0.61[a]	1.32 ± 0.61[a]
0.10	1.24 ± 0.52[a]	1.22 ± 0.72[a]	1.31 ± 0.92[a]	1.30 ± 0.92[a]
0.25	1.22 ± 0.44[a]	1.22 ± 0.56[a]	1.28 ±0.81 [a]	1.26 ± 0.74[a]
0.50	1.15 ± 0.87 [a]	1.20 ± 0.41[a]	1.21 ± 0.36 [a]	1.31 ± 0.88[a]
1.00	1.13 ± 0.76[a]	1.17 ± 0.63[a]	1.18 ± 0.47 [a]	1.14 ± 0.49 [a]
1.50	1.11 ± 0.68 [a]	1.14 ± 0.47[a]	1.14 ± 0.54 [a]	1.12 ± 0.56 [a]
2.00	1.03 ± 0.11 [a]	1.11 ± 0.31[a]	1.11 ± 0.61 [a]	1.11 ± 0.42 [a]

TABLE 9: Dry weight of maize (Zea mays) seedlings after twelve days of germination in soil treated with different concentrations of hydrocarbons.

	Dry weight (g)			
levels of hydrocarbons(% v/w ml/g)	**Kerosene**	**Diesel**	**Engine oil**	**Petrol**
0.00	1.21 ± 0.82[a]	1.21 ± 0.82[a]	1.21 ± 0.82[a]	1.21 ± 0.82[a]
0.10	1.12 ± 0.15[a]	1.16 ± 0.76 [a]	1.26 ± 0.52[a]	1.23 ± 0.66[a]
0.25	1.12 ± 0.51[a]	1.14 ± 0.43 [a]	1.16 ± 0.41 [a]	1.18 ± 0.92 [a]
0.50	1.10 ± 0.49 [a]	1.11 ± 0.31 [a]	1.18 ± 0.33 [a]	1.22 ± 0.47 [a]
1.00	1.06 ± 0.12 [a]	1.08 ± 0.73 [a]	1.13 ± 0.67 [a]	1.17 ± 0.77
1.50	1.01 ± 0.16 [a]	1.02 ± 0.88 [a]	1.11 ± 0.52 [a]	1.13 ± 0.84 [a]
2.00	1.00 ± 0.21 [a]	1.01 ± 0.46 [a]	1.08 ± 0.31 [a]	1.12 ± 0.92 [a]

All values are expressed as Mean ± SD, values followed by different alphabet superscript in the same column indicates a significant difference.

Previous studies have reported petroleum products mediated reduction in dry weight of exposed plants (Omosun et al., 2008; Njoku et al 2009). This agrees with the present study in which increase in concentration of hydrocarbons in soil caused successive decrease in dry weight of cowpea and maize seedlings (Tables 8 and 9). According to Wyszkowski and Zoikowska (2008) growth of plant is dependent on the content of soil nutrient. Therefore, the reduction in dry matter in both cowpea and maize seedlings may be predicated on the effect of petroleum products on soil. The adverse effect could be due to the disruption of the absorption and uptake of nutrients by plants (Njoku *et. al.*, 2008). Dimitrow and Markow (2000) reported that hydrocarbons decrease the availability of phosphorous and potassium in the soil to plant. These nutrients are essential for plant growth and development; hence reduction in the bioavailability will lead to reduced plant growth (Njoku *et. al.*, 2009). The biomass was lower in kerosene exposed seedlings than the seedlings exposed to the other petroleum products, just like its effect on the level of protein.

In conclusion, different refined petroleum products have different toxic effects on cowpea and maize seedlings. This is indicated by the petroleum induced reduction in protein and biomass of the studied food crops. This will affect crops growth and development in petroleum impacted areas and consequently impinge on food security.

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