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The Effect of Crude Oil Spill on the Ascorbic Acid Content of Some Selected Vegetable Species: *Spinacea oleraceae*, *Solanum melongena* and *Talinum triangulare* in an Oil Polluted Soil

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Abstract: The research work focused on the effect of oil spill on some certain soil physicochemical parameters and the concentrations of vitamin C (Ascorbic acid) of three leafy vegetable species at pre and post pollution periods. The vegetable species involved in the study included: *Spinacea oleraceae* (Spinach), *Solanum melongena* (Eggplant) and *Talinum triangulare* (Water leaf) in an oil polluted soil. The ascorbic acid contents of the species pre and post pollution periods expressed in mg/100 g of samples were as follows: *Spinacea oleraceae*, 1057.1 ± 0.1 and 635.8 ± 0.2 ; *Solanum melongena*, 880.0 ± 0.0 and 712.8 ± 0.1 while the values for *Talinum triangulare* were 550.0 ± 0.0 and 350.0 ± 0.0 respectively. The physicochemical parameters of the polluted and unpolluted soils were also determined and result indicated that pH (4.4-5.3), moisture content (4.23 ± 0.2 - $7.99 \pm 0.1\%$), bulk density (1.28 ± 0.1 - 1.36 ± 0.2 g/cm³), particle density (2.05 ± 0.1 - 2.35 ± 0.1 g/cm³) and porosity (37.56 ± 0.0 - 42.49 ± 0.1) were higher in unpolluted than in polluted soil, while sodium (Na, 94.1403 ± 0.01 - 31.5517 ± 0.01 mg/L), potassium (K, 188.0226 ± 0.0 - 74.1663 ± 0.01 mg/L), organic carbon content (1.93515 - 1.21695%) and percent organic matter (3.34587 ± 0.0 - 2.10410 ± 0.01), growth rate of the different vegetable species: *Spinach oleraceae* (0.24 ± 0.02 - 0.12 ± 0.0), *Solanum melongena* (0.30 ± 0.1 - 0.15 ± 0.0), *Talinum triangulare* (0.29 ± 0.11 - 0.12 ± 0.2) and plant biomass were highly decreased. Students t-test at 95% confidence level or at 5% level of significance ($p < 0.05$), showed significant difference between the ascorbic acid concentrations of the test samples before and after pollution periods. Results from the study indicated that oil pollution affected the growth rate, the vitamin C content of the vegetables, the nutrient content, the chemical composition of plants and the physicochemical parameters of the soil.

Key words: Vegetable species, oil spill, vitamin C, soil physico-chemical properties

INTRODUCTION

Petroleum, generally referred to as "crude oil", is a mixture of hydrocarbons, oils and chemicals obtained below the sub-surface of the earth. Crude oil contains a mixture of complex hydrocarbon molecules and also small quantities of sulphur (up to 10%), oxygen (up to 5%) and nitrogenous compounds (up to 1%) bound in complex organic molecules. Several metallic elements such as vanadium, nickel, iron, aluminium, sodium, potassium, copper and uranium are present in traces (Bremmer and Tabalabai, 1973). The hydrocarbons are classified into the following: Normal alkanes, branched alkanes, cycloalkanes and the aromatics (benzene, phenol, toluene, xylene and catechol (Njoku, 2004). Nigeria's crude is mainly of the light grade quality with high concentrations of naphthalenic hydrocarbons (Odu, 1981). Crude oil spill is the release of crude petroleum hydrocarbons into the environment due to human activities and are classified into two main types; the land (on-shore) and the marine (off-shore) oil spills. Land oil spill occurs when crude oil is released on land which affects soil ecosystem. The different ways by which crude oil enter the environment are from natural seeps (1%), atmospheric input (1%), off-shore production (1%), coastal and estuarine effluents (3%), non-refinery

industrial wastes (5%), municipal wastes (5%), urban run-off (5%), rivers (26%) and oil waste discharge from oil industries (53%) (Okereke, 2006). There have been several cases of oil pollution in our environment posing serious threats to both terrestrial and aquatic habitats. It has been observed in aquatic habitats that free oil emulsions coat and destroy algae, zooplanktons, interfere with the photosynthetic process and kill different species of fish. Soluble poisonous fractions of the oil such as toluene, have been known to kill different fish samples, aquatic birds and oysters, while the accumulation of refinery effluents, prevent the germination and growth of plants (Bossert and Bartha, 1984). Oil may inhibit, retard growth or cause the death of the vegetables that were originally established (Cook and Westlake, 1977). Oil readily penetrate pore spaces of terrestrial vegetation. Bossert and Bartha (1984) reported that hydrocarbons penetrate plant tissues easily and prevent the effective translocation of vital mineral salts between the roots and shoot systems. This is usually demonstrated by the darkening of the leaf as its air spaces are filled with oil (Edwin-Wosu and Kinako, 2004). Amakiri and Onofeghara (1983), suggested that crude oil could enter the intercellular spaces in the plant and interfere with water and nutrient

uptake, gaseous exchange in cells these may result in a situation of physiological drought and gradual suffocation. Oil spill contamination of the top soil has rendered the soil unsuitable for plant growth by increasing the toxic contents of the soil (Odu, 1977; 1981). Studies by Taylor (1983) in Nigeria, indicated that the leaves from two species of Solanum: *Solanum melongena* and *Solanum macrocopon* provide all nutritionally important amino acids in adequate amounts. The egg plant fruits or seeds are fairly good sources of calcium, phosphorus, iron and vitamin B. The fruit contains 92% moisture, 6% carbohydrate, 1% proteins and 0.3% fat. It also contains some vitamins (Kochlar, 1981).

Spinach (*Spinacea oleraceae*) is a member of the chenopodiaceae, a family including the spinach beef and pigweed. It contains high level of vitamins and minerals especially vitamin A, calcium, iron, potassium and moderate level of proteins (Spedding, 1981). All constituents of spinach are of nutritional benefit especially as a source of phytochemicals (Whitney *et al.*, 2002). Water leaf (*Talinium triangulare*) is a perennial herb used in Nigeria for cooking soups and stews. It is also rich in vitamins, especially vitamin C, calcium and iron (Tindall, 1983). Plants are good sources of vitamin C, for example: grapes, oranges, spinach, pawpaw, mangoes, water-melons, cabbages, cucumber, lettuce, etc. Animal products such as milk and eggs are extensively rich in vitamin C. The vitamin plays a key role in a large number of biological systems as it is required for the synthesis of collagen; an important structural component of blood vessels, tendons, ligaments and bones (Vasudevan and Sreekumari, 2007). It is an important precursor in the synthesis of a neurotransmitter 'Norepinephrine' (Burtis *et al.*, 2006). It is required for the synthesis of carnithine, a small molecule required for the transport of fatty acids to mitochondria for oxidation and generation of ATP (Martin, 2006). It is a highly effective antioxidant and free radical scavenger. In small amounts, it can protect the body from damage by free radicals and Reactive Oxygen Species (ROS). This study aims to (a) examine the effect of oil spill on the vitamin C content of some popular vegetables (b) determine the vitamin C content of three selected vegetables in polluted and unpolluted soils (c) determine the effect of oil pollution or spill on some physicochemical parameters of the soil (d) determine the effect of oil spill on the growth rate of the vegetable species.

MATERIALS AND METHODS

Plant materials: Three vegetable species (*Spinacea oleraceae*) Spinach (*Talinium triangulare*) Water leaf and (*Solanum melongena*) egg-plant seeds were supplied by the personnel of the Crop Production and Technology Department of the Root Crop Research

Institute, Umudike in Abia State of Nigeria. After germination, the vegetables were identified by the technical crew of the Crop Science and Technology Department of Federal University of Technology, Owerri as being of high yielding variety.

Chemicals: Reagents used in the study included: 2,6-dichlorophenolindophenol (DCPIP), oxalic acid, 10% alum solution, ascorbic acid standard, distilled water, ferrous sulfate (0.5 N), potassium dichromate, concentrated sulfuric acid, phenolphthalein, 1 M ammonium oxalate solution. The crude oil was obtained from Shell Petroleum Development Company (SPDC) PortHarcourt, Nigeria by the Chief Technologist of the Department of Biochemistry, Federal University of Technology, Owerri.

Determination of soil chemistry: This was carried out in the Laboratory of the Department of Soil Science and Technology, School of Agriculture and Technology, Federal University of Technology, Owerri, in which the soil moisture content, porosity, pH and soil density were determined before and after planting the crops.

Determination of soil pH: Twenty grams (20 g) of dried soil sample collected from the pollution site was weighed into a 50 ml beaker. Twenty milliliters (20 ml) of distilled water was added, stirred and allowed to stand for 30 min. The electrode of the pH meter (Hanna pH 3 Meter) was inserted into the bottled suspension. The pH of the soil was noted and recorded at a temperature of 27°C. The electrode was rinsed with de-ionized water, cleaned with filter paper after each reading. The pH meter was calibrated at pH 7.0- 4.0 with buffer solution before use (Nwinuka *et al.*, 2003).

Determination of moisture content of the soil: The moisture content of the soil was determined shortly by gravimetric method. Ten grams (10 g) of soil sample was weighed into a dry moisture Can of known weight (W_1). The total weight of the soil sample + Can (W_2), was determined and recorded. The sample was dried at 105°C until constant weight was obtained. The soil sample was allowed to cool in a desiccator and re-weighed to obtain a constant weight (W_3) (Nwinuka *et al.*, 2003).

Determination of soil bulk density: The soil sample was transferred into a beaker and the volume taken by measuring the volume of beaker occupied by the sand sample. The soil sample was weighed and placed in an oven at 105°C until a constant weight was obtained. The bulk density is the ratio of mass of dried soil sample to the volume of the soil and this was determined by the methods of Nwinuka *et al.* (2003).

Determination of soil particle density: The dried pycnometer was weighed; 10 g of dried soil sample was added to the pycnometer. The neck and outside of the pycnometer were cleaned and after transferring the soil sample, the pycnometer and content were weighed. The moisture content of the duplicated soil sample was determined by drying at 105°C. The pycnometer was filled to one-half with water and washed into the flask. The entrapped air was removed by gentle agitation of the content to prevent loss of soil by foaming. More distilled water was added to the content of the pycnometer and sealed carefully with the stopper. The pycnometer and its content was reweighed. The soil sample was removed from the pycnometer and distilled water used to refill the pycnometer with its content reweighed again (Nwinuka *et al.*, 2003).

Determination of soil porosity: Soil porosity is the volume of all the open spaces between the solids of the soil. It has a relationship with bulk density and particle density and it's calculated thus: Porosity = $S-D/S$, where S = particle density and D = bulk density (Nwinuka *et al.*, 2003).

Determination of exchangeable cations (Na⁺) in the soil: 5 g soil sample and 30 ml of 1M Sodium acetate were mixed together and subjected to mechanical stirring or shaker for 2 h. The mixture was centrifuged and the clear supernatant carefully decanted into a 100 ml volumetric flask. Another 20 ml of Ammonium acetate solution were added to the mixture, stirred for 30 min. The mixture was centrifuged and the supernatant transferred into the same volumetric flask. The cations (Na⁺ and K⁺) were determined using a flame photometer (Pearl 2 ISE analyzer).

Determination of organic carbon content of the soil: The soil sample was weighed in duplicate and transferred to 250 ml flask. Five milliliters (5 ml) of 1 M K₂Cr₂O₇ solution was pipette into each flask and swirled gently to dispose the soil. Twenty milliliters (20 ml) of conc. H₂SO₄ was added using an automatic pipette, swirled until the soil and reagent were mixed thoroughly. The beaker was rotated and allowed to stand on a sheet of asbestos for 30 min. One hundred milliliters (100 ml) of distilled water was added to the mixture after standing for 30 min. Three drops of phenolphthalene were added and titrated with 0.5 M Ferrous sulfate solution. The solution changed from blue to red against a white background. A blank titration was also made in the same manner but without soil sample. Burette readings were recorded after each titration process.

Planting of vegetable species: Studies on the effect of crude oil pollution were carried on separate nursery beds at the farms of Federal University of Technology and Government Secondary School, all at Owerri, being

locations for the research work. The land space had a surface area of 500 m² for each species of plant, namely: *Spinacea oleraceae*, *Solanum melongena* and *Talinum triangulare* respectively. The plant species were planted in each locality with a controlled farm of the same dimension. Performance was monitored for 12 weeks, at the end of which biomass analysis was carried out on both the control and experimental plots.

Pollution of the farms with crude oil: The pollution was with 10 liters of crude oil equivalent to 10,000 ml were used to pollute 10 m² of each vegetable plot which equivalently gave 1 liter/1 m². The crude oil was supplied by the Shell Petroleum Corporation (SPDC), Port Harcourt, Nigeria, authenticated of being of high quality by the technical crew of the Petroleum Engineering Department of the Federal University of Technology, Owerri, Nigeria.

Measurement of some growth parameters of the plants: The height, leaf length and leaf width were measured after the fourth week of germination. This was carried out before and after pollution by the methods of Akonye and Nwauzoma (2003). The height, leaf length and leaf width of each of the vegetable stands were measured for a total of 15 stands in each plot for each plant species from the 5th week of growth through the 12th week before and after pollution of the farms. The measurements were carried out for three consecutive weeks, before and after pollution and the mean values and standard deviations determined.

Height of the plant (cm/plant): This describes the length of the plant from the soil surface and is measured upwards starting from the base to the tip of the plant. Other growth parameters measured included: Leaf length, Width of leaf, Fresh weight, Dry weight and Gravimetric weight measurements, all were carried by the methods of Akonye and Nwauzoma (2003).

Relative growth (cm/cm): This term describes growth measured relative to the initial parameters (West *et al.*, 1920).

$$"RG" = H_f - H_i \div H_i$$

Where:

H_i = Initial height of the plant

H_f = Final height of the plant

Extraction of the crude aqueous extract of the vegetable samples: Fifty grams (50 g) samples of the leaves from the various plant species were collected at the 5th week of germination prior to pollution. The leaves were washed with distilled water, blended or homogenized. The expressed juices were treated with 10 ml of 10% Alum solution to precipitate proteins,

debris, chlorophyll and other contaminants. This was left in a refrigerator at 8°C overnight. The supernatants were filtered, centrifuged at 1500 x g for 30 min to precipitate more impurities and debris. The final volumes of the supernatants were measured, capped and kept in a refrigerator until used (Nwaoguikpe *et al.*, 1999).

Determination of the ascorbic acid concentration of the vegetable extracts: This was carried out by the methods of Lambert and Muir (1974). The oxidoreductive properties of ascorbic acid were explored in this test. Ascorbic acid reduces blue colored 2,6-Dichlorophenolindophenol (DCPIP). A pink coloration of the acidified solution at a slight excess of the DCPIP indicates complete oxidation of ascorbic acid (Amadi *et al.*, 2004).

Determination of plant biomass: The weight of the vegetables or plant species was determined in two phases (the wet weight WW and the dry weight, DW) at the end of the study by the methods of Edwin-Wosu and Kinako (2004). The wet weights were obtained using a weighing balance. The entire plants of known fresh weights were dried under the sun for 16 days to constant weight. The dried plants were reweighed to obtain the dry weight.

Statistical methods: Two statistical methods were adopted in comparing the results of the test plants with the control. The mean and standard deviation values (Mean±SD) were used. Apart from this, students t-test was equally used to compare results.

RESULTS AND DISCUSSION

The results of the analyses are presented in the Tables 1-8 below. Table 1 shows the physico-chemical parameters of the polluted and unpolluted soils. Table 2 depicts the chemical parameters of the polluted and unpolluted soils. Table 3 shows the total ascorbic acid concentration of the vegetable samples expressed in mg/100 g of sample before pollution. Table 4a shows the total Ascorbic acid concentration of the samples expressed in mg/100 g of samples after pollution. Table 4b shows the total vitamin C concentration of the controlled plots. Table 5 shows the relative growth of *Spinacea olereceae* after oil pollution. Table 6 shows the relative growth of *Solanum melongena* after oil pollution. Table 7 shows the relative growth of *Talinum triangulare* after oil pollution. Table 8 compares the change in the relative growth of the vegetable samples, statistically.

The values in the table are the Mean±SD of the relative growth of the control group (R_{GC}) and that of the test plants (R_{GT}). It can be seen that there is no significant difference in the change in relative growth of the different plant species as a result of oil pollution. The change in relative growth (ΔR_G) is an index of pollution and a measure of loss in biomass.

Table 1: The physicochemical parameters of the polluted and unpolluted soils

Parameters	Unpolluted soil	Polluted soil
pH	5.30±0.1	4.40±0.1
Moisture content (%)	7.99±0.1	4.23±0.1
Bulk density (g/cm ³)	1.36±0.1	1.28±0.0
Particle density (g/cm ³)	2.35±0.0	2.05±0.0
Porosity	42.49±0.1	37.56±0.1

The values in the table are the Mean±SD from triplicate (n = 3) determinations

Table 2: The chemical parameters of the polluted and unpolluted soils

Parameters	Unpolluted soil	Polluted soil
Potassium (mg/L)	31.5517±0.1	94.1403±1.0
Sodium (mg/L)	74.1663±0.1	188.0226±1.0
Organic carbon content (%)	1.21700±0.1	1.93515±0.2
Organic matter (%)	2.10410±0.1	3.34587±0.2

The values in the table are the Mean±SD from triplicate (n = 3) determinations

Vegetables have become one of the most desirable foods because today's consumers perceive them as being healthy, tasty, convenient and fresh. Fresh vegetables as well as fresh fruits possess a number of nutritionally important compounds such as vitamins, proteins, essential minerals and carbohydrates, which cannot be synthesized by humans. Vitamin C is the most essential nutritive substance found mainly in fruits and vegetables (Weishmann, 1987). Table 1 shows the physicochemical parameters of the soil sample before and after pollution. It can be seen from the table that the parameters decreased in the polluted soil. The decrease in pH is an evidence of increased acidity which may not favor the growth of the vegetable species. The porosity of the soil is an indication of air spaces allowing the diffusion of water and air into the roots of the plant, which decreased significantly when compared with the unpolluted soil affecting the diffusion of plant nutrients. Many workers have reported the effect of crude or petroleum on soil physicochemistry as due to poor wetting and aeration of the soil which eventually led to depression in plant height, number of leaves and leaf area with concomitant reduction in plant growth. The result also compared favorably with the finding on *Amaranthus hybridus* on the decreased height of the plant (Omosun *et al.*, 2008; Rowell, 1977; McCown *et al.*, 1972). Table 2 equally showed the effect of pollution on the chemical parameters like organic carbon content, percent organic matter which were highly raised; other workers also reported the effect of crude oil including spent engine oil on plant species, which apart from increasing the carbon content of the soil, also contain the following additives: zinc, sulphur, barium, phosphorus and lead, which nonetheless created unfavorable conditions for plant growth (Odjegba and Sadiq, 2002; Obidike, 1985). These physicochemical parameters were highly increased including the level of potassium and sodium ions in the soil. Table 3 shows the total ascorbic acid concentrations of the vegetable

Table 3: Total ascorbic acid concentration of the samples expressed in mg/100 g of samples before pollution

Sample	Vol. of extract (ml)	Vit. C (mg/ml)	Vit. C (mg/200 g)	Vit. C (mg/100 g)
<i>Spinacea oleracea</i>	155.0	13.64±0.1	2114.2±0.0	1057.1±0.0
<i>Solanum melongena</i>	200.0	8.80±0.1	1760.0±0.0	880.0±0.0
<i>Talinum triangulare</i>	250.0	4.40±0.2	1100.0±0.0	550.0±0.0

The results in the table are the Mean±SD from triplicate (n = 3) determinations

Table 4a: Total ascorbic acid concentration of the samples expressed in mg/100 g of sample after pollution

Sample	Vol. of extract (ml)	Vit. C (mg/ml)	Vit. C (mg/200 g)	Vit. C (mg/100 g)
<i>Spinacea oleracea</i>	170.0	7.84±0.0	1271.6±0.0	635.8±0.0
<i>Solanum melongena</i>	180.0	7.92±0.0	1425.6±0.0	712.8±0.0
<i>Talinum triangulare</i>	200.0	3.52±0.0	704.0±0.0	352.0±0.0

The results in the table are the Mean±SD from triplicate (n = 3) determinations

Table 4b: Total ascorbic acid concentration of the controlled plot at the end of the growth expressed in mg/100 g of sample

Sample	Vol. of extract (ml)	Vit. C (mg/ml)	Vit. C (mg/200 g)	Vit. C (mg/100 g)
<i>Spinacea oleracea</i>	190.00	9.54±0.1	1812.6±0.1	906.30±0.0
<i>Solanum melongena</i>	205.00	10.22±0.0	2095.1±0.1	1047.55±0.1
<i>Talinum triangulare</i>	240.00	5.20±0.0	1248.0±0.0	624.00±0.0

The values in the table are the Mean±SD from triplicate determinations

Table 5: Relative growth of *Spinacea oleracea* before and after soil pollution

No. of plant	Initial height (H _{ic}) cm	Final height (H _{fc})	Relative growth of control (R _{GC})	Final height of test plants (H _{FT})	Relative growth of test plants (R _{GT})
1	21.40	27.20	0.27	26.50	0.24
2	24.10	28.60	0.19	-	-
3	27.30	36.00	0.36	34.50	0.26
4	20.00	22.40	0.12	21.90	0.10
5	25.30	31.00	0.25	30.00	0.19
6	19.90	22.50	0.01	-	-
7	15.10	18.10	0.25	17.10	0.13
8	11.70	15.00	0.28	13.00	0.11
9	16.10	18.60	0.16	17.40	0.07
10	16.50	19.00	0.15	18.40	0.10
11	10.50	14.60	0.39	13.50	0.31
12	19.20	27.20	0.42	25.10	0.31
13	19.70	27.50	0.40	-	-
14	17.00	19.20	0.13	-	-
15	23.00	26.20	0.14	-	-
Mean±SD	19.12±0.02	24.20±4.14	0.24±0.02	14.49±4.83	0.12±0.0

From statistical analysis of students t-test at 95% confidence level or 5% level of significance (p<0.05), t = 5.11; t_α = 1.70. There is significant difference in the relative growth of the plants as a result of oil pollution as could be seen that most of the plants wilted away H_{FC} = Final height of control group; R_{GC} = Relative growth of the control group; H_{FT} = Final height of test plants; R_{GT} = Relative growth of test plants

species before pollution and when compared with the corresponding values in Table 4, there was a significant difference in the values of ascorbic acid in Tables 3 and 4 at p<0.05 showing that oil spillage affected the ascorbic acid concentrations of the test samples drastically resulting in pronounced decrease.

These vegetable species were found to be rich in vitamin C, with *Solanum melongena* having the highest concentration among the samples. The sample, *Spinacea oleracea*, appear to be more affected in terms of loss of vitamin C (39.35%), while *Solanum melongena* depreciated by 19%. Table 5 shows the relative growth (R_G) of *Spinacea oleracea*, the mean and standard deviation values for the initial and final heights of plants and the relative growths were determined with the following values: 19.12±0.02,

14.49±4.83 and 0.12±0.0. This decrease in relative growth emanated from oil pollution which resulted in the death of some plants due to physiological drought as reported by many workers on *Amaranthus hybridus* and cereal species (Udo and Fayemi, 1975). Table 6 shows the relative growth of *Solanum melongena*. The mean values and standard deviations for the parameters (H_{ic}, H_{fc}, R_{GC}, H_{FT} and R_{GT}) were 16.82±0.0, 13.853±4.55 and 0.15±0.005. Table 7 equally showed the same parameters for *Talinum triangulare*: 21.85±2.08, 16.24±5.56 and 0.128±0.02. For all vegetable species, the decrease in growth rate after pollution is an indication of the effect of pollution in retarding the growth of the plant species (Cook and Westlake, 1977; Omosun *et al.*, 2008). Bossert and Bartha (1984), reported that hydrocarbons penetrate the plant tissues easily and

Table 6: Relative growth of *Solanum melongena* before and after pollution

No. of plant	Initial height of plant (H _{IC}) cm	Final height of control (H _{FC}) cm	Relative growth of control (R _{CC}) cm	Final height of test plant (H _{FT}) cm	Relative growth of test plants (R _{CT}) cm
1	14.50	19.00	0.28	18.00	0.24
2	15.00	21.50	0.43	18.00	0.20
3	15.50	21.20	0.37	19.50	0.25
4	15.60	20.50	0.31	18.30	0.17
5	14.50	20.60	0.42	19.60	0.35
6	21.60	26.50	0.23	24.20	0.12
7	20.50	25.30	0.23	25.00	0.22
8	17.50	23.40	0.34	22.70	0.30
9	17.00	24.00	0.41	23.30	0.37
10	18.30	20.30	0.11	19.20	0.05
11	15.30	22.50	0.47	-	-
12	17.40	23.20	0.33	-	-
13	18.10	20.20	0.12	-	-
14	15.30	19.40	0.27	-	-
15	16.20	19.00	0.17	-	-
Mean±SD	16.82±0.0	23.10±3.75	0.30±0.07	13.85±4.55	0.15±0.0

From statistical analysis of students t-test at 95% confidence level or 5% level of significance (p<0.05), t = 12.86; t_α = 1.70. There is significant difference in the relative growth of the plants as a result of oil pollution as could be seen that most of the plants wilted away

Table 7: Relative growth of *Talinum triangulare* before and after pollution

No. of plants	Initial height of plants (H _{IC})	Final height of plants control (H _{FC})	Relative growth of plants control (R _{CC})	Final height of test plants (H _{FT})	Relative growth of test plants (R _{CT})
1	32.10	36.60	0.14	34.50	0.07
2	22.20	28.10	0.27	27.00	0.22
3	45.30	52.30	0.15	49.90	0.10
4	13.60	17.50	0.27	16.90	0.18
5	14.10	17.30	0.23	16.80	0.19
6	25.00	40.70	0.63	33.70	0.35
7	13.20	17.40	0.32	16.50	0.11
8	18.90	22.40	0.19	20.90	0.28
9	12.00	17.40	0.45	15.30	0.42
10	09.00	17.30	0.92	12.90	-
11	42.40	45.60	0.08	-	-
12	13.90	16.50	0.19	-	-
13	40.90	43.70	0.07	-	-
14	12.00	15.70	0.31	-	-
15	13.30	15.60	0.17	-	-
Mean±SD	21.85±2.08	26.94±5.22	0.29±0.07	16.24±5.56	0.13±0.2

From statistical analysis of students t-test at 95% confidence level or 5% level of significance (p<0.05), t = 11.89; t_α = 1.70. There is significant difference in the relative growth of the plants as a result of oil pollution as could be seen that most of the plants wilted away

Table 8: Effect of pollution or change in relative growth of the samples

Samples	χ (R _{CC})	χ (R _{CT})	ΔR _G (R _{CC} -R _{CT})
<i>Spinaciae olereceae</i>	0.24±0.02	0.12±0.00	0.12±0.02
<i>Solanum melongena</i>	0.30±0.07	0.15±0.00	0.15±0.07
<i>Talinum triangulare</i>	0.29±0.07	0.13±0.02	0.16±0.05

prevent the effective translocation of vital minerals salts between the root and shoot systems. This is usually demonstrated or seen by the darkening of the leaves as their air spaces or stomatal pores were filled with oil. Many workers have equally found the same effects of crude oil pollution on vegetables and plant species with spent engine oils on soil physicochemical parameters and plant anatomy and physiology (Anoliefo *et al.*, 2003; Anoliefo and Edegbai, 2001; Udo and Fayemi, 1975). The results on relative growth among the species of plants used in the study did not show any significant difference among the species as can be seen in Table

8, showing that the vegetable species were equally affected by the pollutant. Some workers equally explained that the wilting of some of the plants may be due to the influx of oil into the intercellular spaces resulting in stressful situation and gradual suffocation (Amakiri and Onofeghara, 1983; Gill *et al.*, 1992). The decrease in ascorbic acid content can be explained by the fact that ascorbic acid is an antioxidant vitamin and free radical scavenger and may have been used in the plants to mop up the reactive oxygen species and free radicals. The Oxygen Radical Absorbing Capacity (ORAC) of *Solanum melongena* has been found to be

400 units. The vitamin C content of these vegetables have been found to be equivalent to those of some commonly consumed leafy vegetables in Nigeria such as *Ocimum gratissimum* and *Hibiscus esculentus*, but however lower than the reported values for *Talinum triangulare* (Akindahunsi and Salawu, 2005). The polluted soil had lower pH value, low moisture content and more organic carbon than the unpolluted soil. The results obtained in this study also revealed that oil spillage/pollution had an adverse effect on the soil chemistry, plants species, vegetables, water (aquatic medium) and more especially on the ascorbic acid content of the test vegetable plants. The change in relative growth (ΔR_G) found for the plant species is an index of pollution. It is also a measure of loss in biomass of the vegetable samples. The different vegetable species used in this study exhibited the same level of change in relative growth as a result of oil pollution with the following values: *Spinacea oleracea* (0.12±0.02); *Solanum melongena* (0.15±0.07) and *Talinum triangulare* (0.16±0.05) respectively. Petroleum pollution inflicts anatomical, physicochemical and ecological changes on the soil, plants, animals and other inhabitants of both aquatic and terrestrial environments.

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