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Original Research

Comparative study of the biomass, water content and total protein content of white and yellow maize plant (*Zea mays*) grown in crude oil contaminated soil.

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Abstract
The effect of crude oil on microorganisms, animals, plants and the environment has become a major concern to the environmental scientist. Therefore this work is aimed at comparing the effect of various contamination of crude oil on the total wet weight, biomass, water content and total protein of two varieties of maize plants (Zea mays) i.e. the white and yellow Maize. Two kilograms (2Kg) of soil were contaminated with various concentration of crude oil (in percentages) using pots. The experiment was allowed to run for one week and the plants were harvested for the determination of wet weight, biomass, water content and protein content. The results reveal that there was a gradual decrease in the parameters determined as the concentration of crude oil was increasing. Also the result showed that the even though the protein concentration in white maize was higher than the yellow maize, the effect of the toxicity of crude oil was found to affect the yellow maize more than the white maize.

INTRODUCTION

Crude oil is a naturally-occurring substance found in certain rock formations in the earth. It is a dark, sticky liquid which, scientifically speaking, is classified as a hydrocarbon. Crude oil, a complex mixture of hydrocarbon, liquid in their natural state is classified into aliphatic, alicyclic and aromatic compounds [1]. Crude oil is highly flammable and can be burned to create energy. Along with its sister hydrocarbon, natural gas, derivatives from crude oil make an excellent fuel. Most of these components are known to be toxic in nature to different biomass [2], and this has raised considerable concern on the subject of crude oil pollution especially on aerable agricultural land. In Nigeria, a large amount of crude oil is spilled annually into the environment. There was about 2,000 oil spillage in Nigeria between 1976 and 1988 which involved about 2x106 barrels of crude oil into the environment. Oil spillages have been known to exhibit various deleterious effects on both plants and microorganisms. Crude oil spillage on soil generally retard plant growth [3, 4, 5, 6], reduces aeration by blocking air spaces between soil particles hence create condition of anaerobiosis [7] and causes root stress in plant which also reduces leaf growth [8]. The initial reaction of the micro organisms as it gets in contact with oil in the soil is a reduction of activity due to reduced air availability. This has been noted to arise from selective destruction of aerobic bacteria and fungi thus leaving the resistant and adaptive microbial strains to proliferate [9]. However microorganisms remain the only organ of effective natural removal of crude oil in a contaminated soil. This ability however depends on a

number of factors which include temperature, viscosity of the oil, coarseness of the soil and the level of oil in the environment. It has been reported that in tropical conditions, crude oil disappears with unprecedented rapidity in freely well-drained soils but degradation is slowed down by poor aeration [9]. Roscoe et al. [10] have also reported the increase in anaerobic microorganisms in crude oil polluted soil. Contamination of soil by crude oil spills is a wide spread environmental problem that often requires cleaning up of the contaminated sites [11]. Disposal of oil based wastes, oil spills from well blow outs and pipeline ruptures are the most common sources of petroleum contamination [12]. Crude oil spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. It has been recorded that oil contamination causes slow rate of germination in plants. Adam and Duncan [13] reported that this effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen. Some plants can render harmless, extract or stabilize a contaminant in soil, thus making it unavailable for other organisms and reducing environmental hazards in a process termed phytoremediation [14]. Current phytoremediation techniques require that plants live in the zone of contamination. Consequently plants viability is a critical issue in the successful application of phytoremediation. If the contaminant in its present concentration is not phytotoxic, cultivation of plants can be a valuable tool in soil remediation [15].

Contaminated soils pose a major environmental and human health problem. Microorganisms and plants can have complementary roles in phytoremediation of the polluted soil. Phytoremediation refers to the use of plants to clean contaminated soil [16]. Increased biodegradation of organic contaminants occurs in the rhizosphere, the zone of soil directly adjacent to and under the influence of plant roots [17]. Sometimes maize can be used in phytoremediation. The aim of this work is to determine the impact of crude oil contamination on the total protein content and total wet weight in maize plant grown in oil contaminated environment during phytoremediation using the maize plant.

MATERIALS AND METHODS

Soil Sample collection

Two kilograms (2kg) of soil was collected from the study area (Botanical Garden University of Nigeria, Nsukka), and was sieved with a 0.2mm mesh. The soil sample was contaminated with fresh crude oil obtained from Department of Petroleum Resources Port Harcourt and was transferred into a perforated poly-ethene bag.

Plant samples

Two varieties of maize plant namely; white maize and yellow maize were bought from the local market in Nsukka. The seeds were soaked in distilled water to test for viability. The seeds that floated were removed as non – viable seeds while the viable seeds were used for the study.

Experimental Design

The experiment was separated into five groups; The seeds were germinated in a nursery bed for seven days after which the seeds where transplanted to the pots containing contaminated soil.

Group 1: Contains maize (Zea mays) seedlings transplanted in an uncontaminated soil.

Group 2: Group 5: contains maize transplanted to soil contaminated with crude oil spilled in around form, 1cm from each plant. The different contaminations were 0.00%, 0.1%, 0.15%, 0.2% and 0.25% for groups 1, 2, 3, 4 and 5 respectively. The experiment was allowed to run for a eight (8) days after which the plants were harvested for analysis. The experiments were set in three replicates.

Statistical Analysis

The result were analysed statistically for mean ± standard deviation and Analysis of Variance using SigmaPlot v 11. Differences in mean were considered significant when $p \leq 0.05$. The SigmaPlot v 11 was used.

Determination of wet weight of oil contaminated soil

The wet weight of the plant was carried out in an analysis to determine the level of water in the plant since the amount of water contained in the plant will depend on the amount of water in its environment.

Protein Analysis

Determination of Protein By Biuret Reaction

Ten clean test tubes were numbered and placed in a test tube rack, and 10 mg/ml solution of prepared bovine serum albumen of the order: 0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0ml were pipetted into the labelled test tubes. The total volume of liquid in each test tube was made up to 1.0 ml by adding an appropriate amount of glass-distilled water. About 4.0 ml biuret reagent was added to each tube and the mixture shaken for a few seconds for effective mixing of the solutions.

The tubes were incubated for 20 minutes at room temperature (37°C). The spectrophotometer was turned on and allowed to warm up for 15 minutes. The absorbance of each sample at a wavelength of 540nm was taken immediately after warming the spectrophotometer. The values of the samples were plotted and a standard curve prepared. The leaves were grounded with mortar and pestle. The tests (treatment) samples (ground leaf filtrates of Zea mays) were also assayed and the protein contents in mg/g extrapolated from the standard curve.

RESULTS

Determination of the effect of crude oil on the net weight of white maize

The result in figure 1 showed that the yellow maize has higher net wet that the white maize although both showed decreased net weight as the concentration of the contaminant increases.

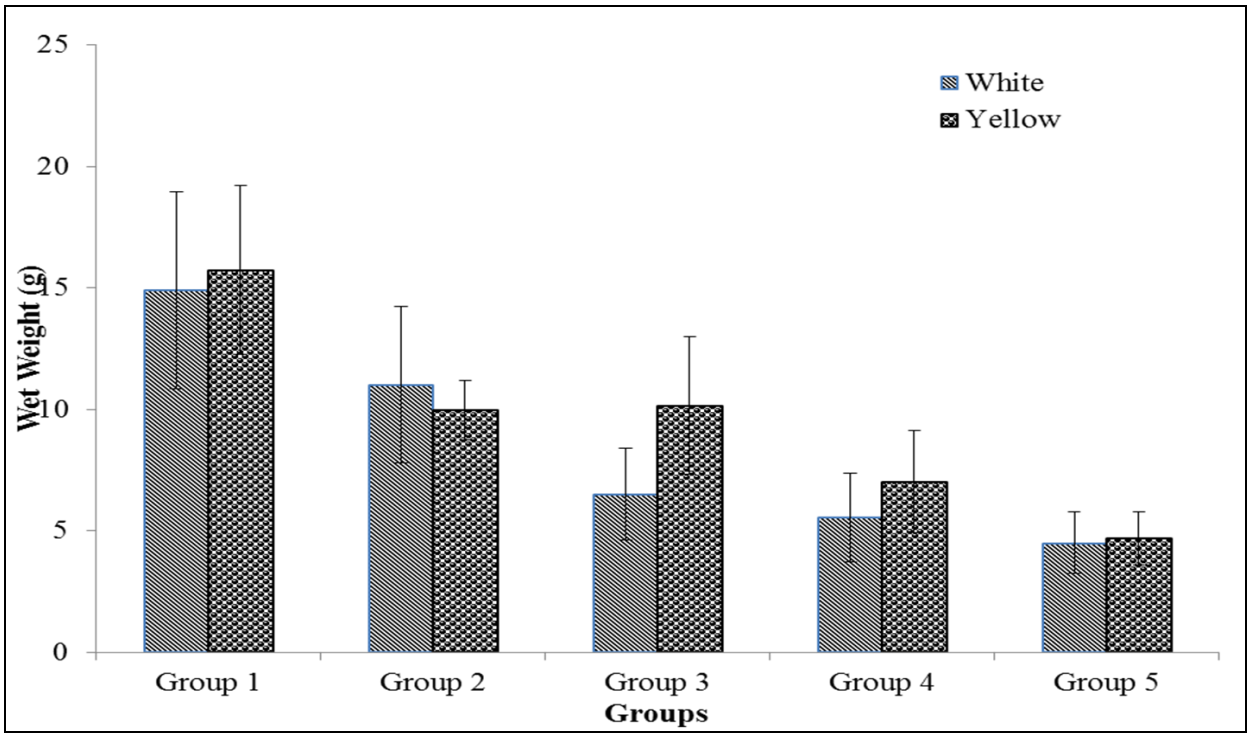

Figure1. The effect of varying concentration of crude oil on the total wet weight of white and yellow maize

Determination of the biomass of the white and yellow maize

The result revealed that the was a gradual decrease in the biomass of both maize as the concentration of crude oil was increasing as seen in table 1. The table also showed that the white maize has higher biomass than the yellow species.

Determination of water content

The result of the water content analysis showed that the yellow specie of the maize contents higher water content when compared with the white variety. The water content of both species where found to increase as the concentration of crude oil contamination is increasing. The differences in the mean values were found to be significant ($P<0.05$) (Table 2.).

Effect of the varing crude oil concentration on the protein concentration of in white and yellow maize

Figure 2. compares the protein concentration in both white and yellow maize planted on the contaminated soil. The result reveals that the white maize has higher protein concentration in the entire groups even though they were gradual decrease in protein concentration from on group to another. And the differences in protein concentration where found to be statistically significant ($P<0.05$).

Table 1. Effect of varying concentration of crude oil on the Biomass (dry mass) of white and yellow maize

Samples	White Maize	Yellow Maize
Group 1	8.398 ± 1.05	7.906 ± 2.17
Group 2	6.864 ± 0.44	5.119 ± 1.02
Group 3	2.656 ± 0.67	5.048 ± 1.44
Group 4	2.494 ± 1.02	3.029 ± 1
Group 5	1.718 ± 0.76	1.953 ± 0.82

Table 2. The effect of varying concentration of crude oil on the water content of the white and yellow maize.

	Samples	Water Content (g)	% water content
Group 1	White	6.516 ± 1.05	43.690
	Yellow	7.823 ±2.17	49.736
Group 2	White	4.152 ± 0.44	37.691
	Yellow	4.841 ± 1.02	48.604
Group 3	White	3.850 ± 0.67	59.176
	Yellow	5.100 ± 1.44	50.256
Group 4	White	3.063 ± 1.02	55.120
	Yellow	3.998 ± 1.00	56.895
Group 5	White	2.785 ± 0.76	61.848
	Yellow	2.735 ± 0.82	58.340

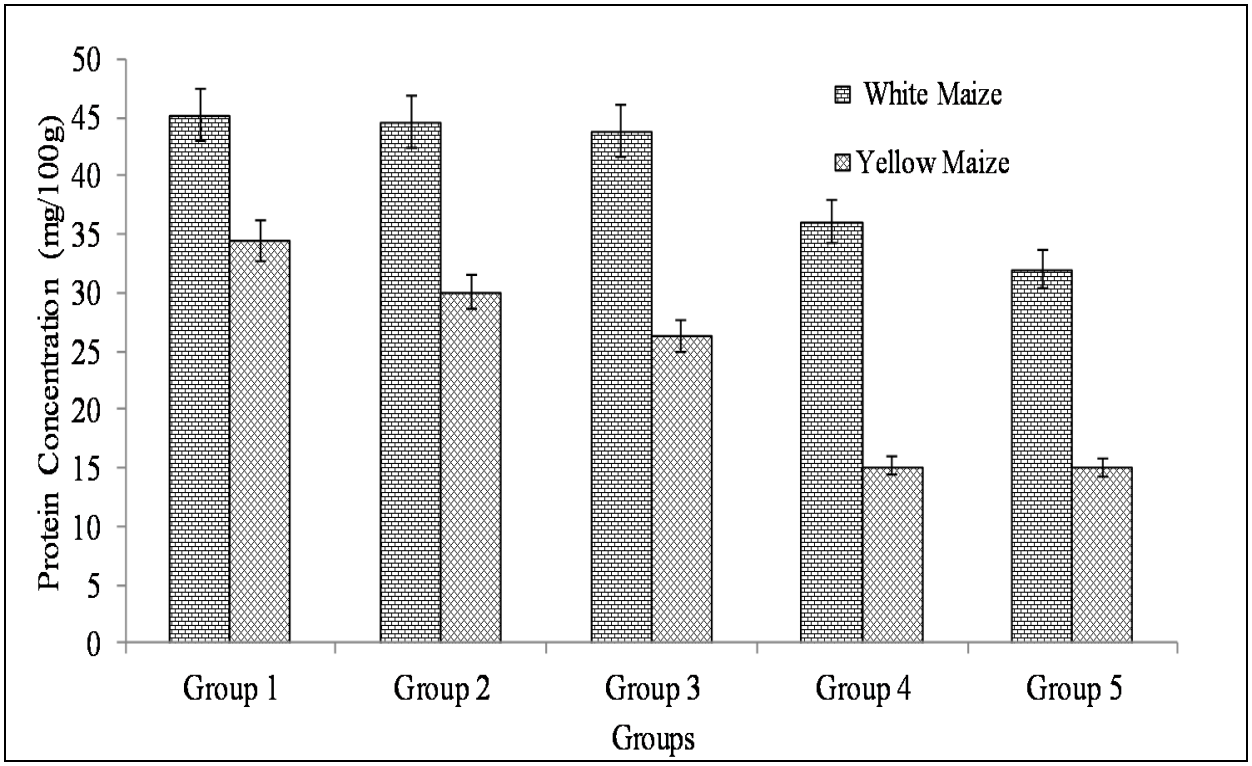

Figure 2. The protein concentration of in white and yellow maize grown on contaminated soil

DISCUSSION

Crude oil has been known to have adverse effect on both plants and animal, when found in soil, it makes the soil condition unsatisfactory for plant growth [18], due to the reduction in the level of available plant nutrient [19]. Contamination of soil with crude oil tends to upset the biological balance of soil [11, 20]. It usually alters the succession of microorganisms [21], which is directly associated with the activity of soil enzymes [22]. Maize has been known to be a good accumulator of heavy metals and crude oil. In this study, two varieties of maize (the white and the yellow) are used in both the laboratory experiment and the onsite experiment. The of the laboratory experiment results however showed in figure 1 and table 1 revealed a general loss in net weight of both corns. This could be as a result of the adverse effect of the crude oil on the maize plant. It was observed that the higher the concentration of the contaminants (crude oil), the lower the net weight. But it was also observed that the white maize lost more weight than the yellow maize. This loss in net weight could be as a result of the inhibition of growth and development

that might be induced by the crude oil [23]. The protein content was also determined and the result showed a gradual decrease in protein concentration as the concentration of the contaminant increase. But it was also observed that the protein concentration of in the white maize is higher than that of the yellow maize. Even though the net loss of weight was higher in the white maize when compared with the yellow maize. This decrease in protein concentration could be as a result of the effect of crude oil on the chlorophyll concentration [24]. It is known that crude oil contamination decrease the chlorophyll concentration thereby affecting plant growth and development. The occurrence of large amounts of hydrocarbons in the soil leads to a nitrogen deficiency and hence upsets the carbon-nitrogen ratio at the spill site thereby threatening the survival of soil biota [25]. This deficiency in nitrogen and phosphorus could be the factors responsible for the low protein content. It is obvious that the contamination of crude oil not only affects the environment but also affects the food chain.

REFERENCES

1. Atlas RM, Bartha R. Fate and effects of polluting petroleum in the marine environment. Residue Reviews, 1973; 49: 49 – 85
2. Walker JD, Colwell RR. Measuring the potential activity of hydrocarbon-degrading bacteria. Applied and Environmental Microbiology, 1976; 31(2): 189–197.
3. Gill LS, Sandota RMA. Effect of foliarly applied ccc on the growth of Phaseolus aureus Roxb. (mung or green gram). Bangladesh Joum. Biol. Sci. 1976; 15:35-40.
4. Ghouse AKM, Zaidi SH. Atique, A. Effect of pollution on the foliar organs of Calistemon citrinus Stap-f. J. Sci. Res. 1980; 2:207-209.
5. Atuanya EI. Effects of waste engine oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated Delta soil in Bendel State. Nigerian. J. Appl. Sci. 1987; 5:155- 176.
6. Ekpo MA, Nwaankpa I L. Effect of crude oil on microorganisms and growth of ginger (Zingiber officinale) in the tropics. J. Sustainable Trop. Agric. Res. 2005; 16: 67-71.
7. Rowell MJ. The Effect of Crude Oil Spills on Soils. A Review of Literature. In: The Reclamation of Agricultural Soils after Oil Spills, Toogood, J.A. (Ed.). University of Alberta, Edmonton, 1977; pp: 1-33.
8. Smith TM, Miller, JR, Russell, GL. Seasonal oceanic heat transports computed from an atmospheric model and ocean temperature climatology. Dynam. Atmos. Oceans, 1989; 14, 77-92
9. Odu CTI. Degradation and weatherinf of crude oil under tropical condition. In: Proceeding of an International Seminaron the Petroleum Industry and the Nigerian Environment. November 1981, Petroleum Training Institute, Warri, Nigeria
10. Roscoe YL, Mcgill WE, Nbory MP, Toogood JA Method of accelerating oil degradation in soil. In:Proceeding of workshop on reclamation of disturbed Northern forest Research Center, Alberta. Information Edmonton 1989; pp. 462-470.
11. Bundy JG, Paton GI, Campbell CD. Microbial communities in different soil types do not converge after diesel contamination. J. Appl. Microbiol. 2002; 92:276-288.
12. Reis JC. Environmental Control in Petroleum Engineering, Gulf Publishing Company, Houston, TX. 1996
13. Adam GI, Duncan H. Influence of diesel on seed germination. Environ. Pollut. 2002; 120:363-370.
14. Cunningham SD. Owal DW. Promises and prospect of phytoremediation. Plant Physiol. 1996; 110:715-719.
15. Merkl N, Schultze-Kraft R. Infante, C. Assessment of tropical grasses and legumes for phytoremediation of petroleum contaminated soils. Water Air Soil Pollut. 2004;. 165:195-209.
16. Joner AJ, Hirmann, D, Szoler, OHJ, Todorovic D, Leyval C, Liobner AP. Priming Effects On PAH Degradation and Ecotoxicity during Phytoremediation Experiment. Environ. Pollut. 2004; 128: 429-435.
17. Frick CM, Farrell RE, Germida JJ. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites, Pet. Tech. All. Can., Calgry, AB. 1999.
18. De Jong E. Effect of a crude oil spill on cereals. Environmental Pollution, 1980; 22: 187-307.
19. Udo EJ, Fayemi A.A.A. The effect of oil pollution on soil germination, growth and nutrient uptake of corn. Journal of Environmental Quality, 1995; 4: 537-540.
20. Caravaca F, Rodán A. Assessing changes in physical and biological properties in a soil contaminated by oil sludges under semiarid Mediterranean conditions. Geoderma, 2003; 117, 53 – 56.
21. Kaplan CW. Kits CL. Bacterial succession in a petroleum land treatment unit. Appl. Environ. Microbiol. 2004; 70(3), 1777-1779
22. Wyszkowska J, Kucharski, J. The biochemical properties of soil contaminated by diesel oil and the yield of yellow lupin. Rocz. Glebozn. 2004; 50, 299, (in Polish).
23. Akaninwor JO, Ayeleso AO, Monago CC. Effect of

different concentrations of crude oil (Bonny light) on major food reserves in guinea corn during germination and growth. Scientific Research and Essay. 2007; 2(4); 127-131

24. Akujobi CO, Onyeagba RA, Nwaugo VO, Odu NN. Protein and Chlorophyll Contents of Solanum melongena on Diesel Oil Polluted Soil Amended with Nutrient Supplements. Current Research Journal of Biological Sciences, 2011; 3(5): 516-520
25. Jobson A, Mclauglin M, Cook FD. Westlake DWS. Effect of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 1974; 27: 166–171.