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RESEARCH ARTICLE

PHYTOREMEDIATION CAPACITY OF MAIZE (Zea mays) ON DIESEL OIL POLLUTED SOIL

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Abstract

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The potential of maize plants in the remediation of diesel oil-polluted soil was evaluated. Soil samples were polluted with diesel oil in concentrations of 100ml, 200ml, 300ml, and 400ml and amended separately with poultry droppings. Samples were analyzed at two weeks intervals for 9 weeks. Results show a significant reduction of diesel oil in polluted soil. The effect was concentration dependent. Agromorphological parameters that were observed were Petiole Length, Plant Height, Leaf Length, Leaf Width, and Number of Leaves. Results obtained showed that as the concentration increased the more it reduced the growth of the plant. TPH content of diesel oil showed a significant reduction through the growth period, reducing from a baseline data of 8502mg/kg to 2843mg/kg. Hence, the plants have a higher potential for phytoremediation of diesel-contaminated soils.

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Introduction: -

Oil industries in Nigeria are the single most important source of pollution in the country (USEPA, 2006). Nigeria is reported to be the sixth-largest oil producer in the world (USEPA, 2006). Contamination of soil and water by crude petroleum and other refinery products has become a threat to agricultural lands in Nigeria and around the globe as oil mining and refining activities increase steadily (Vidal, 2010). However, the threats include the loss of fertility of agricultural lands and the death of plants in the oil-producing areas of Nigeria (Vidal, 2010).

Pollution by crude oil occurs as a result of the transportation of its products from the point of production to that of processing has resulted in spillage with adverse consequences (Hewelkeet al., 2018). The method adopt includes the use of oceanic tankers and pipelines overland. These transportation methods sometimes pollute the environment by accidental oil spills and operational discharge resulting in the loss of very large quantities of crude oil into land and sea bodies. (Ugbomehet al., 2010).

Contamination of soil by oil spills is a widespread environmental problem that often requires cleaning up of the contaminated sites (Hewelkeet al., 2018). These petroleum hydrocarbons adversely affect the germination and

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growth of plants in soils (Samina and Adams 2002). Oil spills affect plants by creating conditions that make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them (Adam and Duncan, 2002).

The Chemical Content of Diesel Oil in the soil inhibits germination by physically impeding water and oxygen transfer between the seed and the surrounding soil environment; thus, hindering the germination response (Wyszkowskwa, et al., 2002). This stage of development is crucial since the plant has not yet got sufficient detoxication ability within this relatively short period (Adam and Duncan, 2002).

Materials and Methods:-

The soil sample was collected from agricultural soil at the Federal University of Technology, Owerri farmland using sterile containers. The soil was mixed thoroughly and air-dried for 7 days. The soil was also sieved with a 2mm mesh sieve.10kg of the soil sample was used for planting in polythene bags. These bags were perforated by the sides and at the base to enable adequate aeration of the soil and avoid water logging. Soil analysis was performed at the beginning of the experiment to also determine the initial and final levels of hydrocarbon parameters. The parameters measured include Total Petroleum Hydrocarbon (TPH), Totalsulphate, Total calcium, Total carbon, Total Nitrogen, Total Phosphorus, Total Potassium, pH, Temperature, Electrical Conductivity, Boron, and Cadmium analysis.

Procedure and Experimental Design

10kg of soil filled in 75 polythene bags with 15 bags per diesel treatment (100mls, 200mls, 300mls, and 400mls and control in which no diesel was added). These different concentrations of diesel oil were poured evenly on the surface of each soil sample (surface pollution) using a sterile measuring cylinder and thoroughly mixed for uniformity. The fifth bag was not being polluted and used as the control sample. The polluted soils were left for one week before planting to enable the volatile compounds present to escape into the atmosphere following the procedure of Frick et al. (1999). Samples of soils were taken before surface pollution and after surface pollution for physiochemical analysis. 5kg of poultry manure was added to each bag in each treatment. Poultry manure serves as an inorganic fertilizer to stimulate the growth of the plant in the contaminated soil. The maize seed was subjected to a viability test using the g floatation technique. Three seeds of maize were planted in each bag and reduced to two seeds after three weeks of establishment. The experimental area was hoe-weeded regularly before maturing to prevent the weeds from growing around the experimental area following the procedure of Awe (2008). Sampling and analysis were done every two weeks to monitor the decrease and increase in parameters of the plant and the soil. The experiment was a completely randomized design (CRD). The experiment was performed for a total of nine weeks and the Data collected was statisticaanalyzedysed using the Analysis of Variance (ANOVA) at a 5% probability level according to Agbogidi and Ofuoku (2005), significant means were subjected to least significant difference.

Plate 1:- Growth of maize plant at 8 weeks after pollution.
(Right to left: control, 100ml, 200ml, 300ml and 400ml respectively)

Physico-chemical Analysis

Sediment pH

The procedure for pH measurement of soil was carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005). The pH value of sediment is measured in a 1:1 sediment to a water (w/v) ratio.

Ten grams of air-dried sediment in 10 ml of deionized water was stirred vigorously to form a thin paste and stood for an hour before pH measurement. The pH will be measured using a standard pH meter (Thermo Orion 9103, USA). This is done to determine the level of acidity or alkalinity in the soil.

Electrical Conductivity (EC)
The procedure for measurement of E.C. of soil was carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005). The soil sample was saturated with distilled water and mixed to a paste consistency. After standing for 1 hour, the salts dissolved and the electrical conductivity of water extracted from the paste was measured using electrodes. Calibration of the electrodes with a standard solution will be necessary before measuring the conductivity of the sample to ensure that the results obtained were accurate. This was measured to determine the amount of dissolved materials present in the soil sample that will aid plant growth.

Total Nitrogen (TN) by Spectrophotometry Method
The procedure for Total nitrogen of soil was carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005).5gm of air-dried sediments was shaken with 1M $NaHCO_3$+0.005M HCl for about 30 minutes in a horizontal shaker. The extract was filtered through the Whatman No. 42 filter paper and then measured by Spectrophotometer at a wavelength of 420nm. This is measured to know the levels of nitrogen in the soil because nitrogen and its compounds act as fertilizers that plants require to grow.

Total phosphorus (TP) by Spectrophotometry Method
The procedure for the measurement of the Total phosphorus of soil was carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005).

5grams of air-dried sediments were shaken with 0.5 M NaHCO3 + 0.05M HCl for about 30 minutes in a horizontal shaker. The extract was filtered through the Whatman No. 42 filter paper and then measured by Spectrophotometer at a wavelength of 8800nm. This is measured to know the levels of phosphorus in the soil because phosphorus and its compounds act as fertilizers that plants require to grow.

Total Potassium by Spectrophotometry Method
The procedure for measurement of the Total Potassium of soil was carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005).5g of air-dried, ground, and sieved sample was placed in an Erlenmeyer flask, and 20ml of the extracting solution (0.05N HCl + 0.025 NH_2SO_4) is added to it. Then it will be placed in a magnetic stirrer and stirred for 20 minutes. The resulting solution will be filtered through a Whatman No. 42 filter paper into a 50ml polypropylene vial and diluted to 50ml with the extracting solution. The analytical reagent blanks are also prepared and these contained only the acid. The sample is now ready for analysis in the PerkinElmer Analyst 800 Atomic Absorption Spectrophotometer. This is measured to know the levels of potassium in the soil because potassium and its compounds act as fertilizers that plants require to grow.

Total Petroleum Hydrocarbon(TPH) of soil By Gas Chromatography (GC) Method.
The procedure for measurement of the TPH of soil is carried out by standard methods for the examination of water and wastewater (Andrew et al., 2005). 10g of air-dried and Ground soil is weighed into a 50ml extraction bottle, adding 5g sodium sulfate to absorb any water, then, adding an extracting solvent usually 20ml of Freon-113 to the mixture. This mixture is then kept in a sealed glass vial and placed in a sonic bath for about 30mins for assisting and hastening the extraction process. Silica gel is then added to the mixture to absorb any polar hydrocarbons (i.e. non-fuel related soil organic matter and fatty acids) and the mixture is mixed well. The filtered extract is then measured in a gas chromatograph. This is done to determine the concentration of diesel in the soil.

Agromorphological Parameters: Data was collected based on the following parameters:
Quantitative Estimation of Morphological Traits of Zea Mays
Petiole length (PL): Measured from the main stem to the leaf base with a meter rule (Agbogidi and Ofuoku, 2005).

Plant height(PH): Lenght from soil to the tip of the terminal flower head with a meter rule (Agbogidi and Ofuoku, 2005).

Number of leaves (NL): Visual counting of the number of leaves (Agbogidi and Ofuoku, 2005).

Leaf length (LL): Distance between the tip of the terminal flower head with a meter rule (Agbogidi and Ofuoku, 2005).

Leaf width (LW): Width of the broadest portion of the leaf from the tip with a meter rule (Agbogidi and Ofuoku, 2005).

Results:-

Agronomic Characters

The effect of the concentration of 100ml, 200ml, 300ml, and 400ml on the agronomic characters which includes the Plant Height, Petiole Length, Leaf Length, Leaf Width, and Number of Leaves are presented. Plant Height: There was a significant difference at ($p \leq 0.05$) in plant height which was observed in the varying heights of individual plants treated with different concentrations. This indicates that diesel added to the soil sample in different concentrations affected plant height significantly. Results also show as the diesel concentration increased, the plant height decreased and there was no noticeable growth in the 400ml concentration for the first 4 weeks. The highest value for plant height was noticed in the control with 32.17cm in 9 weeks after pollution and the lowest value recorded was for 400ml with 5.31 also at the end of 9 weeks.

Petiole length

The petiole length was significantly affected ($p \leq 0.05$) by different levels of concentrations of diesel oil in the soil. As the concentration of diesel oil increased, the petiole length decreased significantly. It was observed that the control had the highest value of 23.42cm after 9 weeks of pollution, followed by the 100ml treatment with 18.92cm after the period of 9 weeks. The lowest concentration was seen in 400ml which was 5.33cm at the end of 9 weeks.

Leaf Length

The leaf length had a high significant difference at ($p \leq 0.05$) which was observed every two weeks among the various concentrations of treatment which produced varying concentrations of leaf length. The concentration of the diesel oil affected the leaves and with increasing concentration, there was a decrease in leaf length. The highest leaf length was observed in the control at 50.58cm. The lowest value recorded was in 400ml with 11.92cm. The control however maintained a steady increase in leaf length value over the period of 9 weeks as indicated in Table 4.2.

Leaf Width

The leaf width varied with the different concentrations treated and it had a significant difference at ($p \leq 0.05$). The results observed showed that as the diesel concentration increased, the leaf width decreased minimally. This is because the control had the highest values recorded through the experiment. Soil polluted with 100ml showed little significant difference when compared with the control. The 100ml had values of 3.39 cm and the control 3.38cm respectively while the lowest value was observed in 400ml with 1.62cm as a mean value over the weeks of pollution.

Number of Leaves

The number of leaves was visually counted and recorded. It had a slow increase rate with the different concentrations used and also had significant differences at ($p \leq 0.05$). The increase varied minimally and the highest and lowest values were recorded. The control and 100ml maintained the highest values with little significant fluctuations in the numbers recorded. The control of the experiment was a 6.92 value for the number of leaves while the 100ml was 6.33 as shown in Table 1.

Table.1:- Change in agronomic characteristics of Zea mays in response to different hydrocarbon concentrations.

Concentration	Leaf Length	Leaf width	Num. leaves	Plant height	Petiole length
100	38.42a	3.39a	6.33a	26.25a	18.92a
200	30.65b	2.52b	5.42b	20.46b	14.57b
300	16.75c	1.79c	3.67c	13.00c	7.10c
400	11.92d	1.62d	3.00d	5.31d	5.33d
Control	50.58e	3.38e	6.92e	32.17e	23.42e
LSD(0.05)	3.59	0.28	0.67	2.49	1.77

Physico-Chemical Parameters

Nitrogen

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The unpolluted soil which is the control had a value of 0.13% while the polluted soil sample had decreased value of 0.09%. This shows that the level of nitrogen decreased after pollution making the available nitrogen present for the plant to be in smaller quantity.

Sulphate.

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The level of sulphate increased with an increase in the level of pollution which makes the mean to be greater than that of the control. The control had a value of 3.88 ppm while that of the polluted sample was 5.88 ppm.

Phosphorus

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The value of phosphorus in polluted soil increased to a value of 8.63 ppm/g while the control had a value of 6.16ppm/g

Potassium:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The increase in potassium was of little significance. The control was 0.04cmol/kg while the polluted soil had a value of 0.09cmol/kg.

Calcium

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The calcium content present in the soil increased significantly with the control having a value of 1.30cmol/kg and the polluted soil having a value of 2.08cmol/kg

Carbon:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The carbon content in the soil increased significantly and this can be attributed to some forms of carbon deposited in the soil with the introduction of diesel as a pollutant. The value increased from 1.63% in the control to 3.00% in the polluted sample.

pH:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The difference was little and it results in the soil being a little acidic. The control had a pH of 5.48 and the polluted soil had a pH of 4.96.

Electrical conductivity:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The electrical conductivity of the soil increased from 76µS/cm in the control to 79µS/cm in polluted soil and this causes an increase in electrical conductivity.

Organic matter:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. Organic matter present in the soil increased from a value of 2.85% to a level of 4.81% in the polluted soil. It can be concluded that the difference was significant.

Cadmium:

There was a significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The cadmium content of the soil polluted with diesel oil increased. This increase in the heavy metal can be attributed to the introduction of the pollutant hydrocarbon. The value increased from 0.09 to 0.16 in the polluted sample.

Boron:

There was significant difference ($p \leq 0.05$) in the level of this parameter when compared with the control. The boron content of the soil polluted with diesel oil increased. This increase in the heavy metal can be attributed to the introduction of the pollutant hydrocarbon. The value increased from 0.16mg/100g to 0.19mg/100g in the polluted sample.

Table 2:- Physicochemical parameters of soil polluted with Diesel oil.

Treatment	N (%)	SO (ppm)	Ph (ppm/g)	K (cmol/kg)	Ca (cmol/kg)	C (%)	E.C (μS/cm)	Cd (mg/100g)	B (mg/100g)	pH	Organic matter (%)
Unpolluted Soil	0.13	3.88	6.16	0.04	1.30	1.63	76.00	0.09	0.16	5.48	2.85
polluted Soil	0.09	5.88	8.63	0.09	2.08	3.00	79.00	0.16	0.19	4.96	4.81
LSD(0.05)	0.02	0.29	0.92	0.06	0.21	0.15	2.27	0.07	0.05	0.21	0.46

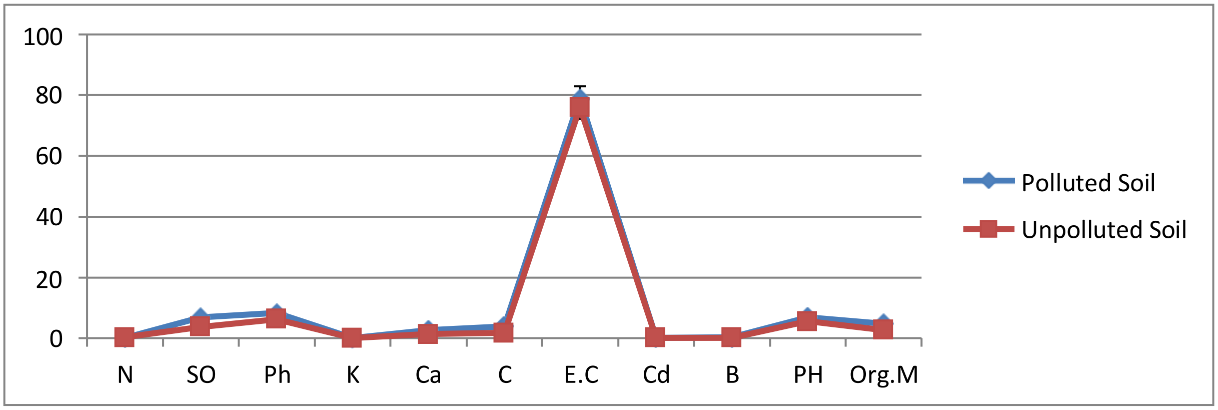

Figure 2:- Comparisms of Physicochemical properties of polluted and unpolluted soil.

Total Petroleum Hydrocarbon Content of Diesel-Polluted Soil

There was a highly significant difference in the total petroleum hydrocarbon content of the soil polluted with diesel in different concentrations. The 100ml concentration had the least concentration of 3041mg/kg while the highest concentration was recorded in the 400ml treatment which had a value of 7281mg/kg at the end of the study period. The value of diesel oil still present in the soil is relative to the concentrations used in polluting the soil. Therefore, the higher the concentrations of diesel oil, the more the levels of hydrocarbon present increase.

Table 3:- Mean concentration of hydrocarbon in the soil after pollution with different concentrations of petroleum.

Concentration of Petroleum Hydrocarbon	Petroleum Hydrocarbon
100ml	3041a
200ml	4123b
300ml	6595c
400ml	7281d
LSD(0.05)	401.8

Key: Different alphabets indicate significant differences; the same alphabet indicates no difference.

Table 4:- Concentration of hydrocarbons in the soil weeks after pollution.

Weeks	Petroleum Hydrocarbon
Week0(baseline)	8502^{a}
Week1	6471^{b}
Week2	3226^{c}
Week3	2842^{d}
LSD(0.05)	401.8

Discussion: -

Diesel oil like other petroleum products adversely affects the growth and performance of plants. (Akujobi, 2011). The results obtained over the duration of the study show that the potency of Zea mays as a phytoremediation tool is very effective. The efficiency of the uptake of petroleum hydrocarbon varies with the level of concentration of pollution. In lower concentrations of 100ml and 200ml, the plants show active growth physically. The addition of poultry manure to the soil to augment nutrients also helped to improve the soil properties. Davies and Wilson (2005) reported that soil amendments with manure improve the physical properties of the soil such as water retention, water permeability, aeration, and structure of the soil which results in increased degradation of polluted soil. Therefore, augmenting polluted soil can be concluded to have enhanced plant growth, allowing the uptake of hydrocarbon into the plant. In this study, it was observed that the presence of diesel oil significantly affected the germination of the Zea mays. The result of the cumulative percentage germination of the seeds clearly showed that the percentage of total germination exhibited differences. The control and 100ml concentration treatment had a100% germination while the higher concentrations were slowed down and impeded.

The addition of diesel oil or spent engine oil significantly delayed the period of germination and reduced the percentage of germination, plant height, leaf production, and biomass of plants (Adedokun and Ataga, 2007). This is because diesel oil in the soil inhibits germination by physically impeding water and oxygen transfer between the seed and the surrounding soil environment. This stage of development is crucial since the plant has not yet gotten sufficient detoxication ability within a short period (Ziad et al., 2005). After germination of Zea mays the plants showed symptoms of abnormalities at the early stage of growth. As it grew over weeks, the level of the hydrocarbon in the soil reduced while its content in the tissues of the plant increased. This can be seen by the physical appearance of the plants. The stems and the leaves had yellow spots and decolouration from green to grey. This decolouration is a phenomenon known as chlorosis. It can be deduced that the loss of the green colour of the plant is from the accumulation of heavy metals such as cadmium and manganese. Also, deficiency of nitrogen also contributes to this deformation. The adverse effects could be due to disruption of the absorption and uptake of nutrients by-petroleum products (Njoku et al., 2008).

The degrading effect of petroleum-derived compounds on soil leads to severe nitrogen and phosphorus depletion, depletion of water balance, and biological equilibrium (Baran et al., 2002). Dimitrov and Markow (2000) showed that these nutrients (nitrogen, phosphorus, potassium, and oxygen) are essential to plant growth and development hence reduction in their bioavailability will lead reduce plant growth. From the study, it can be concluded that diesel oil-contaminated soil may result in low soil fertility. But this can be remedied by the addition of organic supplements, especially poultry waste and the quantity of supplements added has a significant effect on the bioremediation process.

The plant heights were reduced as the level of concentration of diesel increased. Nutrients are also needed to maintain stability in the growth of plants as well as the physiological processes involved in plant growth. This corresponds with the results of Ogbuehi and Ezeibekwe (2010) that the presence of TPH in the soil causes a deficiency of available nutrients required to maintain plant growth. Molina Adedokunet al., (2007) recorded similar effects which were negative concluded that it was due to the impermeability effect of petroleum hydrocarbon or immobilization of nutrients mainly nitrogen, or the inhibitory effect of some polycyclic aromatic compounds present. Poor growth can be attributed to higher concentrations will be due to the assimilation of heavy metals that were present and the inability of the plants to absorb nutrients from the soil due to poor functioning of vascular bundles (phloem and xylem). Edemet al., 2009.

The number of leaves produced decreased significantly as the concentration increased and this can be as a result of the decrease of micro and macronutrients responsible for the production of leaves (Jung, 2008). There was also a reduction in leaf width and leaf length. The leaf width and length amount to the total surface area of the leaf where photosynthetic activities would occur (Smith et al., 1989). The reduction in its surface area leads to a decrease in photosynthesis and little amount of food molecules would be produced which will affect the whole plant.

The physicochemical parameters also showed varying results in the analysis done. The soil analysis on the sample soil before it was polluted showed that it had amounts of Nitrogen, Phosphorus, Potassium, Sulphate as well as heavy metals. Since petroleum products are known to reduce nitrogen availability (Agbogidi et al., 2007) it had an adverse effect on the growth parameters. There was a decrease in the level of nitrogen in the polluted soil analyses after 9 weeks and this is due to the presence of diesel and can also be attributed to the fact that Zea mays is not a leguminous crop but a cereal crop and therefore lacks the ability to fix nitrogen in the soil. The pH of the soil was increased significantly and this is a result of the presence of diesel also and the rainfall that was prevalent during the period for which the study was conducted which caused leaching of the cations from the soil through perforations made in the planting polythene bags.

Conclusion: -

Phytoremediation is undoubtedly a biotechnological process that cannot be overlooked in developing and developed countries as a tool for restoring soil fertility due to contamination by petroleum hydrocarbons. This is because it is cost-effective, it uses natural vegetation resources which are plants and also it is an environmentally friendly technique that does not leave accumulation of toxic materials in the environment. The result showed that Zea mays are an effective tool for phytoremediation of diesel-polluted soil. This is seen in the plant's ability to reduce the level of pollution in the soil gradually. Augmentation of the soil with poultry manure was also beneficial in creating the optimum conditions for the plants to grow, thereby making the phytoremediation a success. There was significant degradation of the hydrocarbon-contaminated soil with the addition of manure.

However, Zea mays was unable to tolerate the high concentrations of diesel pollution at 400ml. The plants grown in 400ml treatment did not germinate until the 5^{th} week and even after germination at the 5^{th} week, the plant died off. The 300ml treatment performed poorly as well as it was seen that the degradation of hydrocarbon was very minimal. Therefore, it can be concluded that the activity of Zea mays in phytoremediation is altered with minimal activity at high concentrations of pollution.

Notwithstanding, it is a good staple cereal plant with promising attributes of a heavy metal accumulator and which is tolerant to low levels of pollution and available for environmental restoration.

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