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Effect of Untreated Petroleum Refinery Effluent on Bacterial and Fungal Population of Soil and Seedling Growth of Maize (Zea mays L.) in an Ultisol.

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ABSTRACT

This trial was conducted to investigate the effect of applying untreated petroleum refinery effluent on bacterial and fungal population and seedling growth of maize in an utisol. 2 kg of soil from an uncultivated land was measured into 12 pots which were then polluted with untreated refinery effluent at the rate of; 0, 50, 100, 150 milliliters, these treatments were replicated three times and laid out in a Completely Randomized Design (CRD). Two weeks after pollution, the pots were seeded to maize. The variables measured were plant height, number of leaves, leaf area, leaf nutrient concentration, bacterial and fungal count in the soil. The agronomic data collected were subjected to Analysis of Variance (ANOVA) with the means separated using $LSD_{(0.05)}$ while the changes in the microbial population were analyzed using simple descriptive statistics. The results obtained at the end of the experiment showed that untreated petroleum effluent had impeding effluence on the growth of maize plant, population and growth of soil microorganisms (bacteria and fungi). The bacteria and fungi strain in the soil mostly affected by the untreated petroleum effluent were *Pseudomonas* spp and *Trichoderma* spp., respectively. Also, leaf area and leaf nutrient concentration were observed to increase with an increase in the quantity of effluent applied. The heavy metal content in the soil was discovered to increase with increasing rate ($T_{150} > T_{100} > T_{50} > T_0$) in effluent applied and the values obtained for Ni, Pb, Cd and Cr all exceeded the permissible limit of 0.3mgkg^{-1} as stipulated by World Health Organization (W.H.O).

1.0 Introduction

Advancement in science and technology since the industrial revolution has increasingly enabled humans to explore natural resources. Soil is one of the most vital natural resources considering the fact that it produces food for millions of people and supplies raw materials for a large number of industries on which the word economy is sustained. Obviously, progress of civilization and rapid industrialization brought with it the danger of soil pollution. Soil is the primary recipient of waste products resulting from anthropogenic activities of the modern society (Ndukwu *et al.,* 2015). Soils suffer from environmental pollution as a result of indiscriminate disposal of these waste, moreso, some major hazards to human health had been reported by several researchers to result from elements contained in petroleum products. For instance, cadmium had been investigated to be a rare metal but known to be present in refined petroleum products (Mashi and Alhassan, 2007) and regarded as an environmental pollutant in agricultural soils because of the potential adverse effects it poses to food quality, soil health and the general environment (Gray *et al.,* 2004).

The wide spread and use of petroleum had created serious environmental problems in some parts of Nigeria with the Niger Delta ecosystem highly subjected to degradation by petroleum product spillage from tankers, offshore wells and other effluents resulting from refinery operational activities (Adeniyi and Afolabi, 2002). More than 90 percent of petrochemical plants in Nigeria are situated in the Niger Delta region (Tawari and Abowei 2012., Oteh and Eze 2012.), with most of them having low or no surveillance technologies for neutralizing refinery effluents before disposal thus leading to high levels of pollutants to the environment, aquatic and terrestrial lives.

Untreated petroleum effluents are waste from the processing of crude oil into petroleum and other products containing aliphatic, aromatic, polycyclic hydrocarbons alongside with nitrogen, sulphur and oxygen containing mixtures. Although known to contain some plant nutrients such as N_2, and S, diverse and significant adverse impact on soil fertility/ health can be reached when the effluent is exposed to the soil in the untreated state and the impact of this environmental degradation calls for more concern (Benka-Coker and Ekundayo, 1995).

Owing to the fact that life in our planet is sustained in a fragile biological balance, microorganisms play a vital role in nutrition chains that are important in the biological balance. The disposal of untreated petroleum effluents on the environment had led to higher levels of pollution and is suspected to have effects on the soil beneficial microorganisms and ecological functioning.

2.0 Methodology.

Description of study site: This trial was conducted at the screen house of the Department of Soil Science, University of Benin, Benin City. Nigeria. This area lies within latitude 6° 23'59.4 N and longitude 5° 37'30.5 E with an elevation of 92 m in the rain forest ecological zone of Southern Nigeria. The zone is characterized by two distinct seasons; the wet season from April to October and the dry season from November to March. The mean annual rainfall ranges between 1800 and 2000 mm.

Samples collection: The untreated petroleum refinery effluent used for this trial was obtained from the Warri petroleum refinery in Delta State, Nigeria. While the soil used for the experiment was obtained from the Faculty of Agricultural Research Farm, University of Benin, Benin City. The soil samples were collected at soil depth of 0 – 15 cm and 2kg each was measured into twelve (12) perforated plastic pots resulting from four (4) treatment rates of; 0, 50, 100 and 150 milliliters that were replicated thrice. The samples in the containers were thereafter contaminated with the untreated petroleum refinery effluent and each covered for two (2) weeks to create a partial anaerobic condition to allow for proper mineralization and the proper mixing of the soil with the effluent. After the two weeks period, each of the plastic pots was seeded to maize *(Zea mays L.),* three seeds were planted per pot and later thinned to two after two weeks of sowing. The plant parameters measured in the maize include; plant height, length and width of leaves (leaf area) and numbers of leaves.

Sample analysis: An initial test analysis was done to determine the physical, chemical and biological properties of the untreated petroleum refinery effluent alongside with the control soil sample (sample with 0 ml effluent). The contaminated samples were subsequently analyzed at two weeks interval after pollution for a total period of 10 weeks to determine the changes in the microbial (bacteria and fungi) population. The particle size distribution of the soils was analyzed using the hydrometer method of Bouyoucos (1951) and the soil pH determined at 1:1 soil to water while the organic carbon and exchangeable bases were determined using the method described by Black (1965). The total nitrogen extraction was done using the micro kjeldahl method for digestion and ammonium electrode determination (Brenner, 1996). Adams *et al*., (1980) method was followed in the determination of the heavy metal content in the soil samples and also proximate analysis was carried out to determine the nutrient status of the herbaceous part of the plant.

Statistical analysis: The data generated from the plant parameters measured were subjected to ANOVA using a Genstat package and treatment means were compared using LSD at 5% level of probability while the changes in the microbial properties were analyzed using simple descriptive statistics.

3.0 **Result and Discusion**

Table 1 shows the physical and chemical properties of the studied soils before pollution. The soil had high percentage (937.2g) sand, low percentage clay (47.8g), and silt (15.0g). The organic carbon content of the soil was also observed to be low including the percentage Nitrogen (0.11), phosphorus, potassium and magnesium. The soil pH (5.8) was slightly acidic thus; all of these characteristics aligned with the findings of Ogunkunle (1983) whose investigation classified the soils of the region to be predominately sandy acid soils characterized by deep reddish colour. While table 2 shows the physical and chemical properties of the untreated petroleum effluent used for this trial. The pH (7.8) at 24.7 °c represented a slightly alkaline medium, the potassium content (15.7 mg/L) was noticed to be higher than the nitrate (2.61 mg/L) in the effluent. However, the untreated petroleum effluent had a relative higher amount of nitrate ($N0_3$), potassium (K), iron (Fe) and lead (Pb) than contained in the initial results of the soil sample as presented in table 1.

Table 1: Study locations and coordinates

Parameter	Results
pH	7.8 at 24.8 °c
Colour	Brown
Total dissolved solid (mg/L)	157.4
Conductivity (US/CM)	308
Potassium (k) (mg/ L)	15.7
Nitrate (mg/L)	2.61
Chemical oxygen demand (mg/L)	112
Biological oxygen demand (mg/L)	30.8
Total suspended solid (mg/L)	12
Dissolved oxygen (mg/L)	1.78
Oil grease (mg / L)	4.04
Nickel (Ni) (mg/ L)	1.019
Iron. (Fe) (mg/ L)	5.065
Lead (Pb) (mg/ L)	0.862
Total hydrocarbon (mg/L)	2.95
Salinity	0.2 at 24.7 °c

The results of the physical and chemical properties of the experimental soil after ten (10) weeks of pollution are presented in table 3. The soil pH was not significantly affected over the period of the trial. However, the pH of the controlled soil and the soil polluted with 50 milliliters of effluent had same value of pH 5.7 while 5.6 was recorded for soils polluted with 100 and 150 milliliters of effluent. The organic carbon and phosphorus were observed to have increased with increase in the effluent rate. This increase in organic carbon and phosphorus following increase in effluent rate is traceable to the high suspended solids (12 mg/L) in the effluent which had mineralized in the soil to increase organic carbon and phosphorus content of the soil. Also, the nitrate content (2.61 mg/L) in the effluent is suspected to be responsible for the increase in the soil nitrogen since soils amended to petroleum effluent had higher amount (0.1 – 0.11 $cmolkg^{-1}$) of nitrogen compared with the nitrogen (0.09 $cmolkg^{-1}$) in the controlled soil after ten weeks of trial. The physical properties of the studied soil at the end of the experiment showed that sand content was higher in the polluted soils with a mean value of 948 gkg^{-1} and least in the controlled or unpolluted soil with a mean value of 937$gkg^{-1.}$. The high sandy nature of the polluted soil is attributed to the relatively higher sodium content (0.26 – 0.47 $cmolkg^{-1}$) of the polluted soils compared with the 0.14 $cmolkg^{-1}$ present in the unpolluted soil. The soil Mn, Cd, Ni, and Cr were also observed to increase with increase in effluent application, this heavy metal increase agrees with the findings of Ekundayo *et al.,* (2001) that the occurrence of crude oil spillage on land introduces heavy metals such as Cu, Pb and Zn to the soil. More so, cadmium retention as reported by Kuo *et al.,* (1985) is greater in fine textured soils with high CEC than in coarse-textured soils with low CEC while the increase in the iron (Fe) ranged between 80.1 – 88.1 $mgkg^{-1}$.

Table 3. Effect of untreated petroleum effluent on soil properties

The untreated petroleum effluent had an impeding influence on the growth of the soil fungi. The fungi species in the control soil (UT_0) grew better than those in the polluted soils, this increased fungi growth

Treatment	pH (1:1)	Org.C	T.N	EA	Na	K	Ca	Mg	P mg/kg^{-1}	Clay	Silt	Sand	Fe	Mn	Cu	Zn	Pb	Cd	Ni	Cr	Ec us/cm
		→			cmolkg^{-1}	←				→	gkg^{-1}	←	→			mg/kg^{-1}	←				
UT_0	5.7	0.09	0.09	0.13	0.14	0.11	1.85	0.33	0.35	41.0	22.0	937	46.4	20.4	1.0	0.3	0.3	0.3	0.6	0.13	183
UT_{50}	5.7	0.49	0.10	0.29	0.26	0.11	2.36	0.46	0.52	27.0	12.0	960	82.4	57.7	1.4	4.3	2.4	2.4	0.9	0.22	374
UT_{100}	5.6	0.63	0.10	0.64	0.46	0.20	1.60	0.45	0.69	41.0	19.0	955	88.1	66.3	1.1	4.3	3.6	3.6	3.1	0.49	506
UT_{150}	5.6	0.88	0.11	0.95	0.17	0.09	1.70	0.28	0.91	34.0	22.0	945	80.1	80.1	0.9	4.9	5.9	5.9	4.6	0.97	742

UT_0 (control- 0 ml effluent), UT_{50} (50 ml effluent), UT_{100} (100 ml effluent), UT_{150} (150 ml effluent)

in the controlled soil is suspected to be due to the presence of the maize *(Zea mays L.)* planted on the soil during the experimental period considering the fact that, the roots of maize are easily colonized by mycorrhiza fungi but the growth finally decreased after the sixth week; an indication of reduced nutrient in the soil contained in the experimental pot which could not further support the growth of the plant and fungi. The effluent gradually reduced the *yeast* spp., eliminated *Trichodema* spp. from the soil while *Penicillum* and *Aspergillus niger* were not significantly affected.

The addition of the effluent to the soil introduced new organisms such as *Micrococcus* spp. and reduced the growth / presence of *Klebsiella* spp. while *Staphylococcus* spp. was not negatively affected by the effluent. However, most of the bacteria organisms grew better in the soil polluted with 100 milliliters but also observed to reduce after the sixth week of experimentation, this was probably due to reduction in the available soil nutrients. The results for the Fungal and Bacterial growth pattern are presented in figure1 and 2 respectively.

Figure 1. Effect of untreated petroleum effluent on soil Fungi

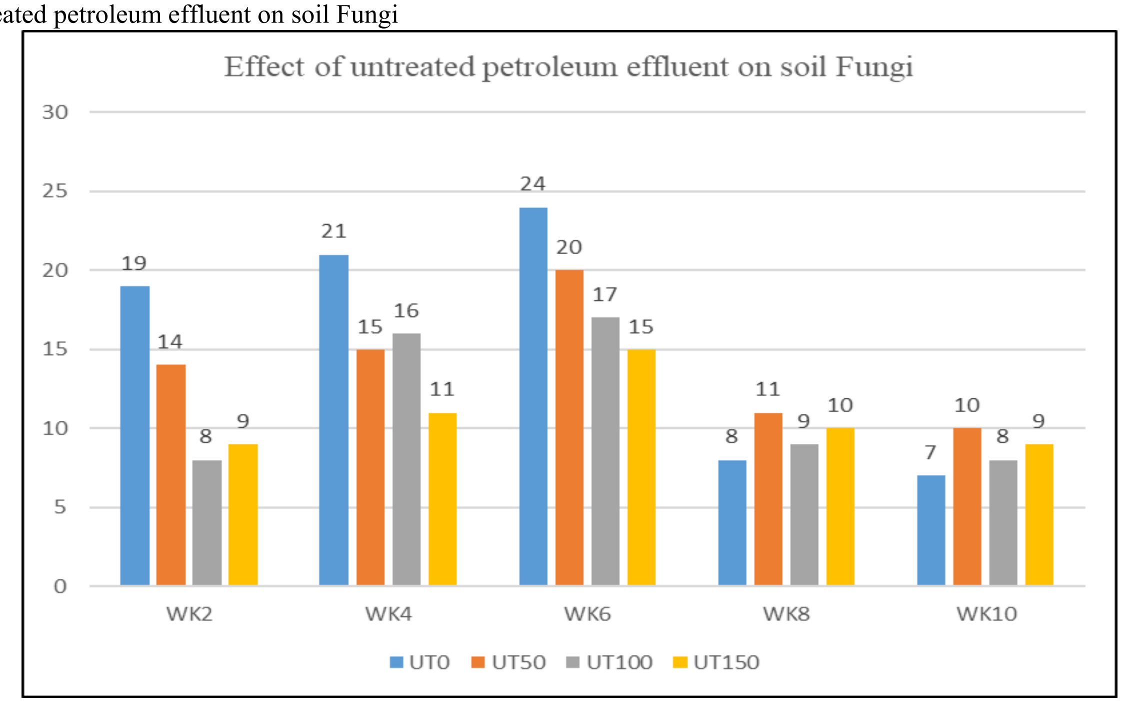
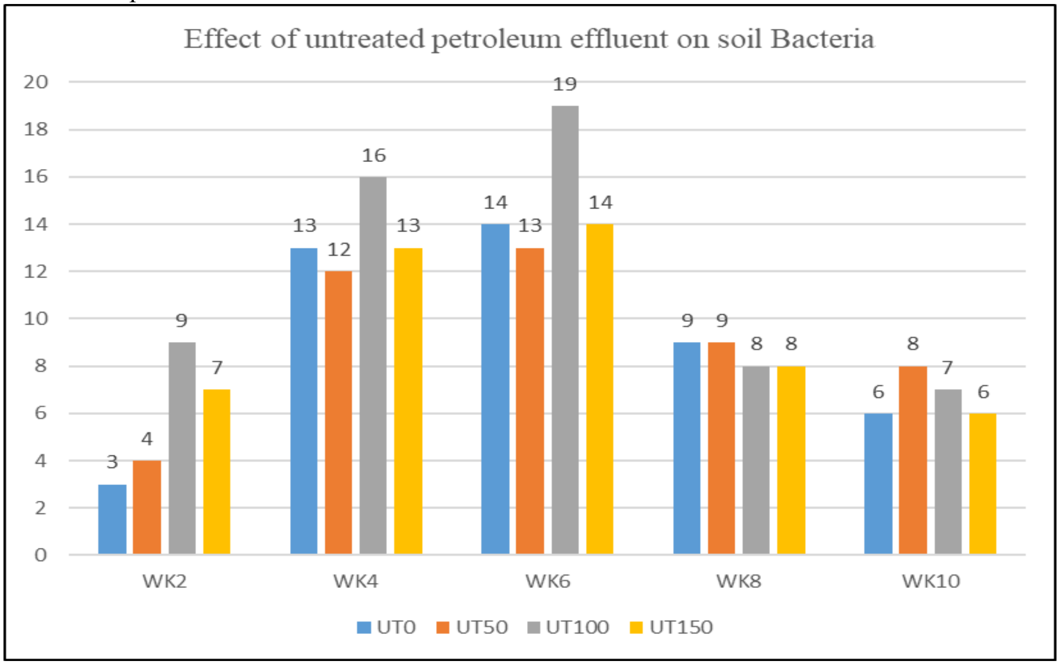


Figure 2. Effect of untreated petroleum effluent on soil Bacteria



Wk- weeks after pollution, UT_0 (control- 0 ml effluent), UT_{50} (50 ml effluent), UT_{100} (100 ml effluent), UT_{150} (150 ml effluent

All the agronomical data measured (plant height, leaf area, and leaf number) were not significantly (p>0.05) affected by the petroleum effluent. However, data collected for the plant height showed that petroleum effluent had an impeding influence on the plant height as higher values (16.2 – 50.7 cm) were obtained in the control plants than with the polluted soils. The plant heights were observed to reduce with increase in the effluent application and the least plant heights (14.5 – 46.8 cm) were recorded in plants grown on soils polluted with 150 milliliters of effluent. On the other hand, although not statistically different at LSD<0.05, higher values in leaf area (9.0 – 60.4) were observed in maize plants grown on the polluted soils compared to those grown on the unpolluted soil (9.2 – 49.9).

Table 4. Effects of Untreated Refinery Effluent on Plant Height

	Time duration		
Treatment (ml)	Week2	Week4	Week6
UT_0	16.2a	43.4a	50.7a
UT_{50}	16.2a	44.4a	49.5a
UT_{100}	16.9a	35.0a	48.3a
UT_{150}	14.5a	36.8a	46.8a
LSD(0.05)	8.74	12.76	13.17

Mean values with the same alphabets in a column are not significantly different.

Table 5. Effects of Untreated Refinery Effluent on Leaf Area

	Time duration		
Treatment (ml)	Week2	Week2	Week2
UT_0	9.20a	30.60a	49.90a
UT_{50}	9.00a	43.50a	60.40a
UT_{100}	9.50a	31.70a	48.80a
UT_{150}	12.20a	46.20a	55.80a
LSD(0.05)	7.45	21.96	23.71

Mean values with the same alphabets in a column are not significantly different.

Table 6. Effects of Untreated Refinery Effluent on Leaf Number

	Time duration		
Treatment (ml)	Week2	Week2	Week2
UT_0	4.00a	4.00a	4.33a
UT_{50}	3.67a	4.00a	4.67a
UT_{100}	3.67a	4.00a	4.67a
UT_{150}	3.67a	4.00a	5.33a
LSD(0.05)	0.941	0.941	1.087

Mean values with the same alphabets in a column are not significantly different.

On the basis of the nutrient concentration in the plant leaves as shown in table 4 above, an increase in N, P, K, Ca, Mg, Na, and Fe were observed following increase in the petroleum effluent application. Heavy metals concentrations were also observed to have increased with increasing rates of effluent which means that critical levels of these metals (Pb, Cd, Ni, and Cr) can be reached upon further increase in effluent rates that enters the soil. This report is in agreement with the findings of Oluyemi *et al.,* (2008), who earlier reported that critical levels in As, Cd, Cr, Ni and Pb can be reached in leaves of plants cultivated on petroleum polluted soils.

Table 4. Percentage Leaf Nutrient Concentration of Maize Seedlings after Three Weeks of Sowing

Treatment rates (ml)	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Cu** %	**Zn**	**Mn**	**Pb**	**Cd**	**Ni**	**Cr**
UT_0	0.11	0.08	0.22	0.88	0.04	0.07	1.04	0.003	0.15	0.31	ND	ND	ND	ND
UT_{50}	0.12	0.11	0.26	0.92	0.05	0.05	1.05	0.001	0.15	0.36	0.003	0.002	0.001	0.001
UT_{100}	0.28	0.20	1.88	0.92	0.09	0.09	1.15	0.004	0.18	0.48	0.003	0.009	0.004	0.001
UT_{150}	0.34	0.31	2.75	0.99	0.11	0.17	1.23	0.005	0.28	0.72	0.005	0.011	0.012	0.002

UT_0 (control- 0 ml effluent), UT_{50} (50 ml effluent), UT_{100} (100 ml effluent), UT_{150} (150 ml effluent.)

4.0 Conclusion

The processing of crude oil into petroleum and other products results in the generation of high amount of effluent as waste products and the soil is usually the primary receiver of this waste or effluent. The results obtained from this study generally revealed that, untreated petroleum effluent upon disposal into the soil can alter the soil health (the balance in the amount of air, water, mineral and microbial diversity in the soil.) The untreated petroleum effluent from this investigation was observed to change the naturally occurring balance between microbes and soil minerals. The bacteria strains isolated from the experimented soil include *Klebsilla* spp., *Pseudomonas* spp., *Staphyloccocus, Bacillus* and *Micrococcus* spp. while the fungi organisms were; *Aspergillus niger, Aspergillus flavus, Yeast, Penicillium and Trichoderma* spp. Pb, Cd, Ni and Cr were observed in the leaves of maize cultivated on soils polluted with untreated petroleum effluent and their concentrations were seen to increase with increasing rates of effluent application and these elements were observed to exceed the permissible limit earlier defined by WHO (2001). Since these heavy metals can easily find its way into the food chain as observed in their high concentrations in the leaves of maize plant (*Zea mays* L.) grown on the polluted soils, humans and animals fed with such crops are most likely to be predisposed to conditions that results in ill health.

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