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# EFFECT OF TREATED REFINERY EFFLUENT ON PHYSICAL, CHEMICAL AND MICROBIAL (BACTERIA AND FUNGI) PROPERTIES OF SOIL

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## ABSTRACT

*This study investigates the effect of treated refinery effluent on some selected soil properties. The experiment involved the use of four different rates of refinery effluent 0, 50, 100, and 150 ml / 2 kg soil in twelve pots laid out in a Completely Randomized Design at a screen house. The soil samples were analyzed for physical, chemical, and microbial properties before and after the experiment, and changes in soil microbial properties were analyzed using simple descriptive statistics. Results showed that the soil physical properties (textually) remained unchanged but the exchangeable acidity (0.11 – 0.98 cmolkg$^{-1}$), ECEC (2.5 – 4.1 cmolkg$^{-1}$), exchangeable bases and O.C (0.17 – 0.94%) , Cr (0.27 – 0.95 mgkg$^{-1}$), Cd (2.4 – 3.6 mgkg$^{-1}$) and Ni (0.8 – 4.1mgkg$^{-1}$) contents of the treated soils were greatly enhanced with increase in the rate of treated refinery effluent applied and the quantity of the heavy metals were far above the permissible limit (0.3 mgkg$^{-1}$). The predominant bacteria species in the soil were Bacillus, Pseudomonas and Staphylococcus while Micrococcus was present only in the uncontaminated soil and Corynebacteria were found in soils with higher rates ($T_{150}$ and $T_{100}$) of effluent. The fungal species were Penicillum notatum, Trichoderma, Yeast, Aspergillus niger and Aspergillus flavus. The results from the study implied that, treated refinery effluent has the capacity of altering the microbial composition of soils.*

**Keywords**: *Bacteria, Fungi, Heavy metals, Refinery effluent, Soil.*

## INTRODUCTION

Rapid urbanization combined with industrialization has led to the generation and disposal of enormous amount of waste in our environment (Marathe, 2022). Petroleum based industries including oil refineries are part of the major industrial sectors that generate tremendous amount of pollutant (Otitolaiye, 2022). The increased petroleum exploration, refining and other allied industrial activities have led to the wide scale contamination of most land and water bodies. Optimum utilization and benefits from crude oil are derived by converting crude oil through processing in a refinery into a wide range of products such as petroleum-fuel, lubricants, waxes and bitumen. While petroleum refinery and petrochemicals industries are most desirable for national development and improvement in the quality of life, the unwholesome and environmentally unacceptable pollution effects from these industries have been reported world-wide (Ruiz *et al.,* 2001).

Anywhere refineries are sited, there are bound to be waste products and this waste (effluent) is suspected to have effects on population and morphology of soil biota. Disposal of wastewater from an industrial plant is a difficult and costly task and petro-chemical plants generate solid waste/sludge, some of which may be considered hazardous because of the presence of toxic organic and heavy metals. Heavy metals in general are not biodegradable, they have long biological half-lives with the potentials of accumulating in the different body organs leading to unwanted side effects (Sathawara *et al.*, 2004). Accidental discharge as a result of abnormal operation especially from polyethylene and ethylene oxide-glycol plants in a petro-chemical complex can be a major environmental hazard (Beg *et al.,* 2003). Most petroleum refineries and petro-chemical plants have on-site facilities to treat their wastewater so that the pollutant concentrations in the wastewater complies with the local and / or national regulation regarding disposal of wastewater into community treatment plants or into rivers, lakes and ocean (Ivan *et al.,* 2016). Most wastewater are treated in industrial scale wastewater treatment plants which may include physical, chemical and biological treatment processes (Comber *et al.,* 2015)

Contamination of soil by refinery sludge creates a distinct type of ecological habitat that invariably changes the physical and chemical properties of soils. This oily sludge may completely eliminate the vegetation of a place, hampering soil aeration, destroying the rhizosphere system including its microbial populations (Baruah and Sarma, 1996) which will hence bring about a series of disastrous changes in floral composition, leading to habitat loss. The study was carried out on the potential effects of treated refinery effluent on some physical and chemical properties of agricultural soils, as well as the population of soil bacteria and fungi.

## 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 which is located on latitude 6° 23'59.4 N and longitude 5° 37'30.5 E with an elevation of 92 m above sea level and falls within 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 (**NIFOR, 2013).**

### Sources of Materials

The Port Harcourt Refinery in Nigeria provided the treated refinery effluent used in the study. The surface soil (0-15 cm) was collected from the research farm site of the Faculty of Agriculture, University of Benin. Composite soil sample was collected by combining soil samples collected at this depth. The composite soil were weighed into twelve 2kg pots. Thereafter arranged in a Completely Randomized Design (CRD). The experiment included four treatment rates of 0, 50, 100, and 150 ml. the treatments were replicated three times to give a total of 12 treatments.

### Physical and Chemical Analysis of Soils and Effluent

These soils were first air dried and passed through 2mm sieved. Twenty grams (20 g) of each soil sample was weighed into a 100 ml beaker followed by the addition of 20 ml distilled water. The mixture was stirred intermittently for 30 minutes while the glass electrode pH meter was standardized with buffer 7.0 and 4.01. At the end of the 30 minutes, the suspension was read and recorded as pH (1:1) $H_20$. The particle size distribution of the soils were determined by the Hydrometer method (Gee and Or, 2002). Exchangeable bases (Ca, Mg, K and Na) were extracted with 1 N ammonium acetate ($NH_40Ac$). Exchangeable calcium and magnesium were determined by Ethylene Diamine-Tetraacetic Acid (EDTA) titration method while exchangeable potassium and sodium were estimated by flame photometry (Jackson, 1962). Exchangeable acidity was extracted with KCl (1 N) and -measured titrimetrically according to the procedure of Maclean (1982). Soil organic carbon (SOC) was determined by Walkley and Black digestion method as modified by Olsen and Sommers, (1982). Total Nitrogen of soils was estimated by micro-Kjeldahl digestion method (Bremner and Mulvaney, 1982) while available phosphorus was determined by Bray 1 Method. The trace metals; Fe, Mn and Cr were extracted using the Diethylenetriaminepentaacetic acid (DTPA) solution and determined by Atomic Absorption Spectrophotometer procedure as described in IITA (1979).

The physical and chemical analysis of the treated effluent was done using a Jenway 2030 pH meter for about 30 minutes at a temperature of 22°C to determine the pH. Its conductivity was done using a calibrated and stablilzed (0.0 $uScm^{-1}$) conductivity meter and the total suspended solid achieved using filtration method. Mara (1978) procedure was used to determine the biochemical Oxygen demand in the effluent whereas, the chemical Oxygen demand measurement was achieved by the APHA (1989) method. Nitrate content in the effluent was determined using phenol-disulphoric acid method and absorbance read at 4.15 nm and the amount of oil and grease determined after several extractions with hexane and measured gravimetrically while further extraction of oil with Xyline absorbance and read at 412 nm to give the total hydrocarbon (THC) in the effluent.

**Plate Culture and Identification of Organisms**

A gram of each soil sample was dissolved in 9 ml of sterile water to form the stock sample. 1 ml of each stock sample was transferred into separate test tubes containing 9 ml of sterile water to achieve the desired dilution rate of $10^{-3}$. Also, 1 ml of the serially diluted samples of $10^{-3}$ was transferred into petri dishes containing molten nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for fungi culture and were incubated for 24 hours at 37°C and 3- 5 days at 25°C respectively. The bacterial and fungal isolates were characterized and identified based on their cultural, morphological and biochemical characteristics as described by Cowan and Steel (1970).

**Statistical Analysis**

Soil samples were separately collected from each of the experimental pots and analyzed for changes in the microbial (bacteria and fungi) population at an interval of 2 weeks for a total period of 10 weeks and the data were thereafter analyzed using simple descriptive statistical method via Microsoft Excel Software.

**Results and Discussion**

The physical and chemical composition of the treated refinery effluent (Table 2) showed that its pH at 22°C was slightly alkaline (6.8) while the potassium and nitrate contents were 5.48 and 0.52 $mgL^{-1}$ respectively. This result however suggest the treated effluent to be higher in potassium than nitrate. The effluent also had 0.59 $mgL^{-1}$ of lead

(Pb) and <0.001 mgL$^{-1}$ of iron (Fe). Whereas, Table 1 shows the physical and chemical properties of the soil before effluent application. The pH at 22ºC in this case was slightly acidic (5.8) and the percentage nitrogen (0.14) was low while the available phosphorus (7.2 mgkg$^{-1}$) showed a higher value. The order of relative amount of the exchangeable bases were in the following order; Ca (5.9 cmolkg$^{-1}$) > Mg (2.8 cmolkg$^{-1}$) > Na (2.63 cmolkg$^{-1}$) > K (2.08 cmolkg$^{-1}$). The textural class of the soil was sandy as the sand content (942.2 gkg$^{-1}$) constituted higher fraction of the bulk soil. This report of low nutrient content in the soil coupled with acidic pH and sandy texture aligns with the findings of Ogunkunle (1993) whose reports classified the soils of the region (Edo State) to be predominately sandy acid soils characterized by deep reddish colour. The Lead (Pb), Cadmium (Cd) and Chromium (Cr) in the soil before experiment (Table 1) were also observed to be low, having the values of 0.02, 0.15 and 0.13 mgkg$^{-1}$ respectively. These values were quite below the permissible limit (0.3mgkg$^{-1}$) as defined by W.H.O, (2001) whereas, the Pb (0.59 mg/L) and Ni (0.31 mg/L) content in the treated petroleum effluent were relatively higher (Table 2).

**Table 1: Initial Properties of Soil**

| Parameters | Results |
|---|---|
| pH | 6.8 at 22° c |
| Total dissolved solids (mg/L) | 55.3 |
| Conductivity (uS/cm) | 109.2 |
| Potassium (mg/L) | 5.48 |
| Nitrate (mg/L) | 0.52 |
| Chemical oxygen demand (mg/L) | 51 |
| Biological oxygen demand (mg/L) | 2.7 |
| Total suspended solids (mg/L) | 0.018 |
| Dissolved oxygen (mg/L) | 8.04 |
| Oil grease | 0.212 |
| Nikel (mg/L) | 0.314 |
| Iron (mg/L) | <0.001 |
| Lead (mg/L) | 0.591 |
| Total hydrocarbon content | 0.51 |
| Salinity | 0.1 at 22°C |

**Table 2: Initial Properties of Treated Effluent**

| Parameters | Results |
|---|---|
| pH | 5.8 |
| Organic carbon (%) | 1.10 |
| Total nitrogen (%) | 0.14 |
| Available phosphorus (mg/kg) | 7.2 |
| Exchangeable acidity | 1.7 |
| Calcium (cmol/kg) | 5.9 |
| Magnesium (cmol/kg) | 2.8 |
| Potassium (cmol/kg) | 2.08 |
| Sodium (cmol/kg) | 2.63 |
| Clay (gkg$^{-1}$) | 47.8 |
| Silt (gkg$^{-1}$) | 10.0 |
| Sand (gkg$^{-1}$) | 942.2 |
| Lead (mg/kg) | 0.02 |
| Cadmium (mg/kg) | 0.15 |
| Chromium (mg/kg) | 0.13 |

Table 3 presents the findings on the physical and chemical properties of the soil following the experiment. The results indicate that the different treatment rates had minimal impact on the soil textural class. Despite the gradual reduction of clay content caused by the effluent, the soil remained classified as sandy after ten weeks of the experiment. The pH values ranged from 5.20 to 5.30, which suggests that the soil was acidic. Notably, the various treatment rates (T50, T100, and T150) did not significantly influence the soil pH. As the levels of effluent treatment increased, there was an increase in the amount of Total N (0.09 – 0.12%), exchangeable acidity (0.25 – 0.98 cmolkg$^{-1}$), and ECEC (3.3 – 4.1 cmolkg$^{-1}$). It was also observed that the exchangeable bases (including Ca, K, Mg, and Na) increased with higher effluent treatment levels, except for Ca, which showed a decrease with higher rates of effluent application. The increase in the soil chemical properties with increasing effluent treatment levels could be due to the addition of nutrients and organic matter present in the effluent, which can increase soil fertility and improve soil structure. However, the decrease in Ca could be due to its displacement by other cations, such as Mg and K, which were present in higher concentrations in the effluent. Micronutrient content of the soil recorded a general increase across all treatments and Fe (41.2 – 76.8 $mgkg^{-1}$) was found to be the most abundant. The organic carbon increased consistently with increasing effluent treatment. The increase in the soil organic carbon resulting from the addition of treated refinery effluent is in line with the findings of Ogboghodo *et al.,* (2006) who observed an increase also in

organic carbon with increasing levels of crude oil in the soil. However, this report increase may be attributed to microbial mineralization of the effluent having adapted to the soil condition and able to utilize the carbon in the polluted or contaminated soils for metabolism. The heavy metal; Ni (0.8 – 4.1 $mgkg^{-1}$), Pb (0.70 – 4.80 $mgkg^{-1}$), Cd (2.40 – 3.60 $mgkg^{-1}$) and Cr (0.27 – 0.95 $mgkg^{-1}$) contents in the soil increased with increasing content of the effluent thus; the treated refinery effluent is capable of increasing the heavy metal content in soils. These results demonstrated the findings of Gupta and Ahmad (2012) and Daflon (2017) who reported high levels of heavy metal toxicity in refinery wastewater. Some heavy metals such as cadmium had been reported by several researchers to be present in several refined petroleum products, this results aligned with the earlier report of Mashi and Alhassan, (2007), Gupta and Ahmad (2012). Despite the treatment of petroleum effluent, the results of this experiment indicate that it still has the potential to increase the concentration of heavy metals in soil beyond the permissible limit of $0.3mgkg^{-1}$ defined by WHO (2001).

**Table 3: Physical and Chemical Properties of Soil after Experiment**

| | pH | Ec | Org.C | T.N | P | E.A | Na | K | Ca | Mg | ECEC | Sand | Silt | Clay | Fe | Mn | Cu | Zn | Ni | Pb | Cd | Cr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1:1) | uS/cm | % | | Mg $kg^{-1}$ | | | Cmol$kg^{-1}$ | | | | | $gkg^{-1}$ | | | | | $mgkg^{-1}$ | | | | |
| **$T_1$** | **5.2** | **315** | **0.17** | **0.18** | **0.09** | **0.11** | **0.12** | **0.07** | **1.98** | **2.5** | **0.25** | **947** | **12** | **41** | **41.2** | **34.1** | **1.0** | **4.03** | **0.4** | **0.01** | **0.3** | **0.10** |
| **$T_2$** | **5.3** | **629** | **0.33** | **0.49** | **0.09** | **0.25** | **0.24** | **0.17** | **2.30** | **3.3** | **0.37** | **961** | **15** | **24** | **82.6** | **70.3** | **1.5** | **4.40** | **0.8** | **0.7** | **2.4** | **0.27** |
| **$T_3$** | **5.3** | **679** | **0.55** | **0.74** | **0.10** | **0.55** | **0.37** | **0.16** | **1.88** | **3.3** | **0.36** | **951** | **19** | **30** | **82.1** | **62.3** | **1.9** | **4.30** | **3.0** | **1.6** | **2.9** | **0.44** |
| **$T_4$** | **5.3** | **249** | **0.94** | **1.20** | **0.12** | **0.98** | **0.13** | **0.96** | **1.74** | **4.1** | **0.24** | **941** | **22** | **37** | **76.8** | **54.8** | **1.5** | **4.50** | **4.1** | **4.8** | **3.6** | **0.95** |

$T_1$ (control- 0 ml effluent/2kg soil), $T_2$ (50 ml effluent/2kg soil ), $T_3$ (100 ml effluent/ 2kg soil), $T_4$ (150 ml effluent / 2kg soil)

**Effects of Effluent on Soil Bacterial and Fungal**

Table 4 presents the total number of heterotrophic bacteria and fungi species that were observed in the treated soils. The findings reveal that the initial bacteria species consisted of *Micrococcus spp*., *Bacillus spp*., *Pseudomonas spp*., and *Staphylococcus spp*. These soil bacteria species were part of the species reported by Delgado-Baquerizo (2018) on the global dominant bacteria found in soils. Additionally, the fungi species included *Trichoderma spp*., *Aspergillus niger*, *Penicillium notatum*, and yeast. Throughout the 10-week of this investigation, *Pseudomonas*, *Bacillus*, and *Staphylococcus* were the most dominant bacteria species, while *Micrococcus* were only present in the control soils. These observations suggest that *Micrococcus* does not thrive in soils polluted with treated refinery effluent. Furthermore, *Corynebacteria* could only survive in soil treated with higher rates ($T_3$ and $T_4$) of effluent.

It was observed that the growth of soil fungal species was inhibited by the presence of treated petroleum effluent. The fungal species exhibited better growth in the control soils ($T_1$; soils without treated petroleum effluent) compared to their growth in the polluted soils. The effluent might have resulted in changes in the microbial community structure due to competition for resources, which could have further impacted fungal growth. This result is similar to the findings of Ogbemudia *et al.*, (2016) whose investigation showed that high concentrations of crude oil in soil reduced the amount of certain soil organisms. Thereby inhibiting their activities towards soil fertility improvement. The alteration in the native microbial composition of soils had recently been documented by Bastida *et al.*, (2021) to have a significant negative implications on soil health, plant growth, and wider environmental health.

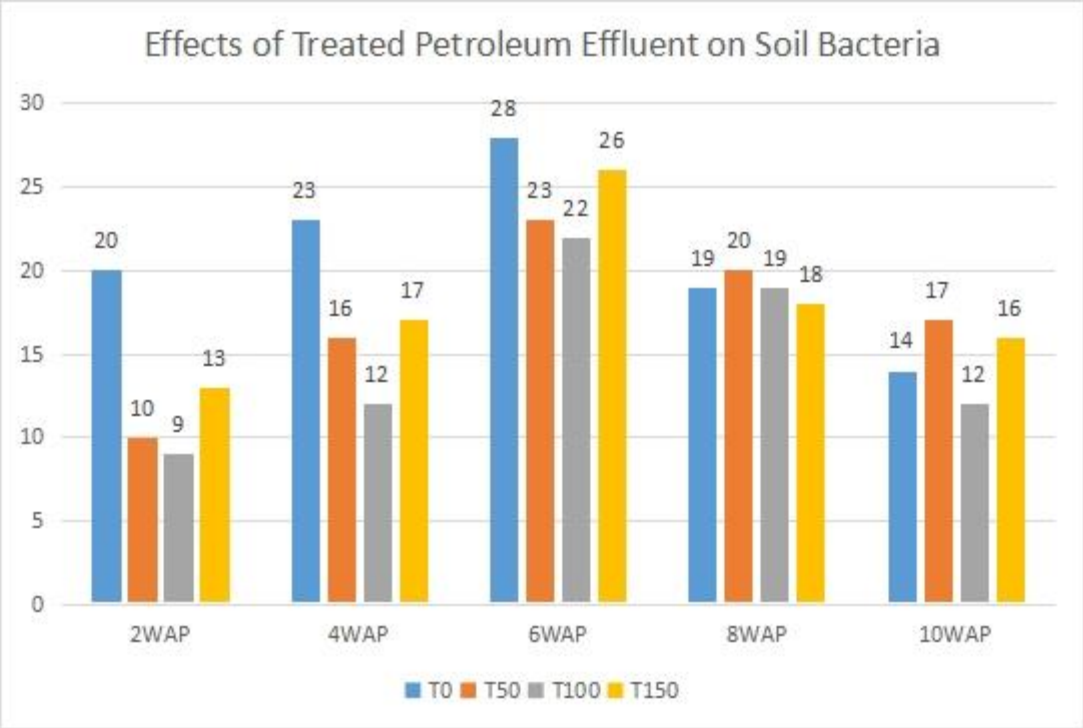
As the experiment progressed (between weeks 8 - 10), there was a general reduction in the growth of bacteria and fungi across all treatments (Figure 1 and 2). However, the control soil ($T_1$) still recorded the highest growth overall. The decrease in the growth of bacteria and fungi from the sixth week to the tenth week in all treatments suggests that the soil nutrients in the experimental pots had become depleted, which hindered further growth of soil organisms. The initial soil test revealed that the experimental soil had 1.10 % organic carbon, 0.14% total nitrogen, 7.2mgkg$^{-1}$ phosphorus and 2.08 cmolkg$^{-1}$ potassium while at the tenth week, these values reduced to between; 0.17 – 0.94 % O.C, 0.09 – 0.12 % N, 0.18 – 1.20mgkg$^{-1}$ P, and 0.07 – 0.96cmolkg$^{-1}$ respectively.

**Table 4: Identified Bacteria and Fungi Strains in Experimental Soils**

| TREATMENT. | ORGANISM | 2WAP | 4WAP | 6WAP | 8WAP | 10WAP |
|---|---|---|---|---|---|---|
| $T_0$ | Bacteria | *Micrococcus*<br>*Bacillus*<br>*Pseudomonas*<br>*Staphylococcus* | *Staphylococcus*<br>*Pseudomonas*<br>*Micrococcus*<br>*Bacillus* | *Staphylococcus*<br>*Pseudomonas*<br>*Micrococcus*<br>*Bacillus* | *Staphylococcus*<br>*Pseudomonas*<br>*Micrococcus*<br>*Bacillus* | *Staphylococcus*<br>*Pseudomonas*<br>*Micrococcus*<br>*Bacillus* |
| | Fungi | *Trichoderma*<br>*Aspergillus niger*<br>*Penicillum notatum*<br>*Yeast* | *Trichoderma*<br>*Aspergillus niger*<br>*Penicillum notatum*<br>*Yeast* | *Trichoderma*<br>*Aspergillus niger*<br>*Penicillum notatum*<br>*Yeast* | *Trichoderma*<br>*Aspergillus niger*<br>*Penicillum notatum*<br>*Yeast* | *Trichoderma*<br>*Aspergillus niger*<br>*Penicillum notatum* |
| $T_{50}$ | Bacteria | *Staphylococcus*<br>*Pseudomonas*<br>*Bacillus*<br>*Corynebacteria* | *Staphylococcus*<br>*Pseudomonas*<br>*Bacillus*<br>*Corynebacteria* | *Staphylococcus*<br>*Pseudomonas*<br>*Bacillus*<br>*Corynebacteria* | *Staphylococcus*<br>*Pseudomonas*<br>*Bacillus*<br>*Corynebacteria* | *Corynebacteria*<br>*Staphylococcus*<br>*Bacillus*<br>*Pseudomonas* |
| | Fungi | *Penicillum notatum*<br>*Aspergillus flavus*<br>*Yeast* | *Penicillum notatum*<br>*Aspergillus flavus*<br>*Yeast* | *Penicillum notatum*<br>*Yeast*<br>*Aspergillus* | *Penicillum notatum*<br>*Yeast*<br>*Aspergillus* | *Penicillum notatum*<br>*Aspergillus flavus*<br>*Yeast* |

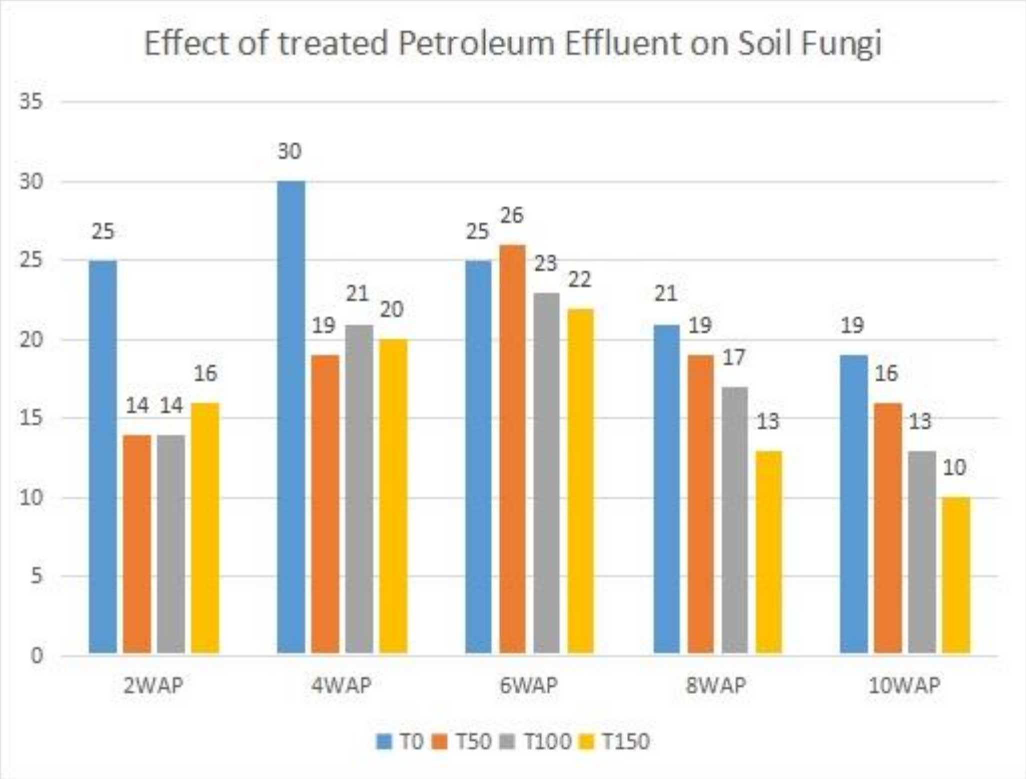
| | | | | | | |
|---|---|---|---|---|---|---|
| | | *Aspergillus niger* | *Aspergillus niger* | *niger* | *niger* | *Trichoderma* |
| $T_{100}$ | Bacteria | *Pseudomonas Staphylococcus Bacillus Corynebacteria* | *Pseudomonas Staphylococcus Bacillus Corynebacteria* | *Pseudomonas Staphylococcus Bacillus* | *Staphylococcus Bacillus Pseudomonas* | *Staphylococcus Bacillus Pseudomonas* |
| | Fungi | *Aspergillus flavus Penicillum notatum Yeast* | *Aspergillus flavus Penicillum notatum Yeast* | *Penicillum notatum Aspergillus flavus Yeast* | *Penicillum notatum Aspergillus flavus Yeast* | *Penicillum notatum Aspergillus flavus Trichoderma Yeast* |
| $T_{150}$ | Bacteria | *Pseudomonas Staphylococcus Bacillus* | *Staphylococcus Bacillus Pseudomonas* | *Staphylococcus Bacillus Pseudomonas* | *Bacillus Pseudomonas Staphylococcus* | *Bacillus Pseudomonas Staphylococcus* |
| | Fungi | *Aspergillus niger Penicillum notatum Yeast* | *Aspergillus niger Penicillum notatum Yeast* | *Aspergillus niger Penicillum notatum Yeast* | *Aspergillus niger Penicillum notatum Yeast* | *Aspergillus niger Penicillum notatum yeast* |

WAP: Weeks After Pollution




WAP- weeks after pollution, $T_0$ (control- 0 ml effluent/2kg soil), $T_{50}$ (50 ml effluent /2kg soil), $T_{100}$ (100 ml effluent/2kg soil), $T_{150}$ (150 ml effluent/2kg soil)

**Fig 1:** Effect of treated petroleum effluent on soil Bacterial Populations (cfu/g x $10^6$)




WAP- weeks after pollution, $T_0$ (control- 0 ml effluent/2kg soil), $T_{50}$ (50 ml effluent /2kg soil), $T_{100}$ (100 ml effluent/2kg soil), $T_{150}$ (150 ml effluent/2kg soil)

**Fig 2:** Effect of treated petroleum effluent on soil Fungal Populations (cfu/g x $10^6$)

## Conclusion

The investigation has revealed that the disposal of treated refinery effluent on soils can have a negative impact on the growth and population of soil organisms, including bacteria and fungi. It is important to note that the reduction in the population of these essential microorganisms in the soil could have adverse effects on soil fertility since there is a direct relationship between the presence and activities of microorganisms in the soil and soil fertility. The dominant bacterial species observed in the experimented soils were *Bacillus*, *Pseudomonas*, and *Staphylococcus*, while *Micrococcus* was only present in the uncontaminated soil, and *Corynebacteria* could only thrive in soils with T150 and T100 treatments. The fungal strains identified included *Penicillum notatum*, *Trichoderma*, Yeast, and two species of *Aspergillus* (*Aspergillus niger* and *Aspergillus flavus*), which were found to be present at the onset of the investigation but decreased with increasing effluent application. As the amount of treated refinery effluent applied to the soil increased, there was also an increase in the concentration of heavy metals (Cr, Cd, and Ni) in the soil, which exceeded the permissible limit. These heavy metals can easily enter the food chain, posing a risk to human and animal health. Therefore, it is important to prioritize efforts towards developing and implementing more environmentally friendly methods for treating refinery effluent that can safeguard soil health before disposal. This will help to minimize the negative impact of treated refinery effluent on the soil ecosystem and prevent the potential health risks associated with the accumulation of heavy metals in the food chain.

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