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Ecotoxicity Effect of Illegal Refineries on the Environment: A Case Study of Delta State, Nigeria

[1]Akpomrere, O. R. & [2*]Uguru, H.

[1]Surveying and Geoinformatics Unit, Delta State Polytechnic, Ozoro, Nigeria
[2]Department of Agricultural and Bio-environmental Engineering Technology, Delta State Polytechnic, Ozoro, Nigeria
***Corresponding author: erobo2011@gmail.com**

ABSTRACT
This study was carried to assess the impact of illegal refineries on the environment. A total of 21 soils samples were randomly collected from an illegal refineries site, located at Isoko South Local Government Area of Delta State, Nigeria. To have a good reference point, another batch of soil samples was collected from a non-polluted area, about 20 km from the study site. The heavy metals (Ni, Cd, Pb, Zn, and Cu) concentration of all the soil samples was analyzed by using the Atomic Absorption Spectrophotometry. While three crops species (maize, cucumber and bean) were used to determine the level of ecotoxicity of all the soils samples. The results showed that activities of the illegal refineries have negative impacts on the environment, as they significantly ($p \leq 0.05$) increased the heavy metals concentrations in the soil, and inhibited plants growth. Even though most of the heavy metals concentrations fall below WHO/FAO maximum permitted level; cadmium (Cd) was slightly above WHO/FAO maximum permitted level. Cadmium concentration in the soils samples from the site ranged between 0.87 and 4.32 mg/kg. Copper (Cu) concentration in the soil samples ranged between 3.63 and 11.76 mg/kg. Ni (Ni) concentrations ranged between 15.46 and 28.01mg/kg; while Lead (Pb) concentration ranged between 10.08 and 20.65 mg/kg. With regards to the ecotoxicological results, it was observed that the soil samples collected from the study site adversely affected the seed germination index, and the radicle growth. Out of the three crops used, it was observed that the cucumber seeds were greatly affected. Seed germination rate and radicle growth inhibition were highly influenced by the heavy metals concentrations in the soil. This study recommends Niger Delta stakeholders meeting, to stop the activities of these illegal refineries in order to save the already fragile environment from further degradation.
Keywords: Ecotoxicity, illegal refineries, oil theft, germination index, radicle inhibition

INTRODUCTION
Petroleum (crude oil) is a naturally mixture of hydrocarbons and non-hydrocarbon compounds (including heavy metals), which is highly toxic to the environment at high concentrations (Anderson and Labelle, 2000). Petroleum exploration, production, transportation, and utilization are in continuous transit globally, and had increased recently despite some of its negative effects on the environment. Petroleum exploration in Nigeria can be dated back to the pre-independence era, in the coastal region of southern Nigeria. During 2019, Nigeria produced about 2.4 million barrels of petroleum per day, and its petroleum reserve standing at about 37 billion barrels (OPEC, 2020). Sabotage of Nigeria petroleum production facilities and crude oil theft, had increased drastically science the recent militancy activities in the Niger Delta region of Nigeria. According to the Nigeria Extractive Industries Transparency Initiative (NEITI), Nigeria had lost 41.9 billion USD to petroleum and its refined products theft between 2009 and 2018. NEITI further stated that Nigeria loss between 150,000-250,000 barrels per day (bpd); implying that about 20% of Nigeria daily petroleum production is lost to oil thieves and pipeline vandals (NEITI, 2019). A fair proportion of the oil

stolen in the country is been sold to operators of illegal refineries across the southern region of Nigeria. These illegal refineries operators used local materials and methods in refining some petroleum products from the crude oil. The activities of the illegal refineries operators have become a menace in the region, and the Nigeria government is trying its best to curtail it.

Since 2012, the Nigeria military Joint Task Force (JTF) had tried to curtail illegal oil mining activities across the region, destroying thousands of illegal crude oil refining centres and seizing over 500 boats conveying stolen crude oil. In 2012, over 2000 crude oil suspected thieves were arrested, and over 5,000 illegal refineries were destroyed. Despite the efforts by the Nigeria military in combatting illegal refineries, they continue to soar; and it seems almost impossible to be stamped out (Reliefweb, 2013). Illegal refineries use crude/local materials and methods to distil the crude oil into its various components (Balogun, 2015). During the illegal refining process of crude oil, only substandard kerosene, petrol and diesel are obtained by the refiners; while the remaining crude oil components are discarded untreated as waste products into the environment. In most cases, when these illegal refineries are destroyed by the Nigeria military, further harm is being done to the environment. This is because more petroleum products are discharged indiscriminately into the environment. This had been found to contaminate the environment (mostly swampy) with petroleum hydrocarbons and heavy metals. Research results have shown that petroleum and its refined products have the ability of increasing the soil heavy metals concentrations above the World Health Organization (WHO) recommended permissible maximum limits. Vwioko et al. (2006) and Akpokodje *et al.* (2019), observed drastic increase in some highly poisonous heavy metals (Ni, Cd, Co, etc.) concentrations, after soils samples were contaminated with petroleum products.

Heavy metals are those metals with high relatively density (greater than 5 g/cm^3), when compared with water (Fergusson, 1990; Akpomrere and Uguru, 2020). Heavy metals are persistence and non-biodegradable; therefore, they continue to accumulate in the ecosystems. This implies that plants and animals are continuously exposed to them throughout their life cycle, leading to in toxicity if accumulated in high dosage (Espin *et al.*, 2014; Ohanmu *et al.*, 2017). Heavy metals pollution has become a global environmental problem whose concentrations in the soils have increased recently due to anthropogenic activities. Many researches have been carried out on the effects of heavy metals in plants morphological and physiological development; due to their phytotoxic effects, which varies from plants' growth inhibition, chlorosis, wilting, and death in severe cases (Ikhajiagbe *et al.*, 2018; Akpokodje and Uguru, 2019). Wang and Zhou (2005) observed that rate of growth inhibition of Wheat (*Triticum aestivum*) seeds increased (3 to 35%) as the concentration of copper in the soil increases (1 to 10 mg/l). Nickel toxicity on plants were observed by Seregin and Kozhevnikova (2006) and Rahman and Mahmud (2010). According to Rahman and Mahmud (2010), there was declined in the germinated rate and growth performance of chickpea seeds planted in medium with high nickel and cobalt concentrations. Likewise, Jayakumar *et al.* (2008) reported that ragi and paddy germination rate, radicle growth and plumule length significantly reduced as the cobalt concentration in the increased from 5 µg/L to 100 µg/L. Scientific research results have shown that high concentrations of heavy metals in the soil can alter the soil's biochemical equilibrium; which will then reflect on the soil fertility, seeds germination, growth and performance (Cruz *et al.*, 2013; Khan and Khan, 2010; Jayakumar and Vijayarengan, 2006).

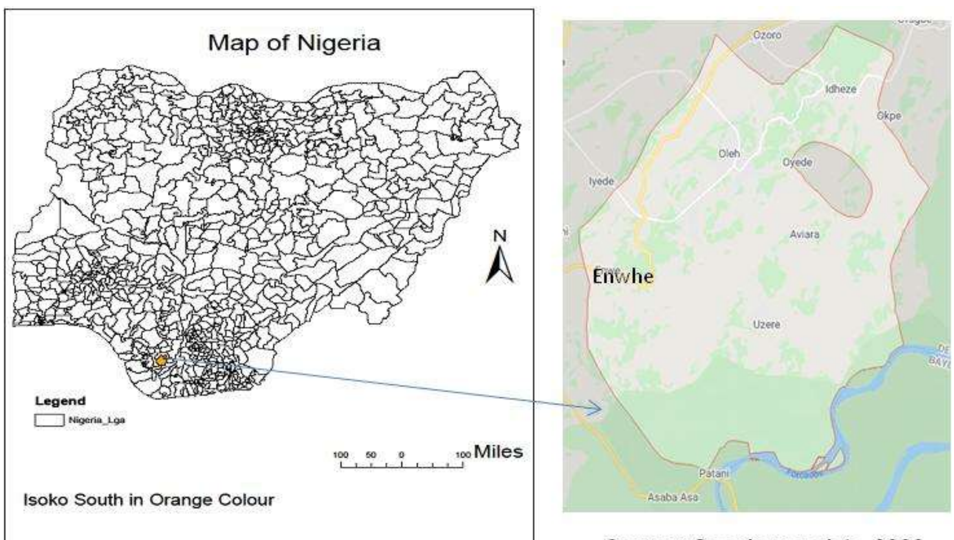
Earlier researches on ecotoxicology of pollutants in the soil were focused mostly on precious metals (Gold, silver) mining and oil spillage sites; nowadays, attention has been devoted to the issue of illegal crude oil refining sites. Ecotoxicology incorporate the synergistic and hostile effects of all pollutants within the ecosystem; thereby, providing vital information on the bioavailable fraction of the pollutants, which cannot be obtained through chemical analysis (Plaza *et al.*, 2005; Repetto *et al.*, 2001). With the rapid expansion in the number of the illegal refineries sites across the Niger Delta region of Nigeria, the contamination of the ecosystems would greatly increase. Therefore, studies of how this illegal crude oil refining activities affects the ecosystem have become inevitable. This is to avoid would be greater environmental pollution problems tomorrow. Therefore, the objective of this study is to evaluate the ecotoxicity of waste generated by illegal crude oil refineries on plants. The ecotoxicity of soils collected from all the spatial points will be assessed by testing the inhibition of the seedlings and the radicle growth inhibition of three selected crops (maize, cucumber and beans). Maize (*Zea mays*) cucumber (*Cucumis sativus*) and beans (*Phaseolus* spp.) are common crops planted in Isoko region of Nigeria.

MATERIALS AND METHODS

Study site description

The study area was a non-active illegal oil refinery sites located at Enwhe community, Isoko South Local Government Area of Delta State, Nigeria. Enwhe community has the following geographical coordinates Lat. 5°23'0" North and Long. 6°7'0" (Figure 1). The community experienced tropical rainforest climate conditions. Enwhe community is sparely populated with peasant farming as their major source of income. The community has a lot of swamp forest which experienced seasonal flooding (between May and November).

There were clusters of illegal oil refineries located within the swampy area of the community. After locating and identification all the illegal refineries within the community forest by the Nigeria military intelligence unit, in a surgical military operation, all of the illegal refineries were destroyed by the Nigeria military in March, 2020. When all the illegal refineries were in full operation (before their destruction), all the waste products they generated are discharged untreated into the environment. In most cases, the waste products find their ways into the water bodies, since they (illegal refineries) are located very close to natural surface water bodies (Figure 2).



Source: Google mapdata 2020

Figure 1: Nigeria map showing Enwhe community

Soil sampling and preparation

An area of 1 km x 1 km was demarcated within the area where the illegal refineries were sited before, by using the grid technique. Within the demarcated area, seven soil samples will be collected with the depth of 0 – 40 cm. Although the site was non-active during the time all soil samples were collected, visual assessment showed that the environment was heavily polluted with petroleum products (Figures 2 and 3).

Seven soil samples were randomly collected within the demarcated area at a depth ranging between 0 and 40 cm. This depth was considered as the effective root zone of most crops cultivated in the community. At each spatial point, the soil samples were collected in triplicate. All contaminated soil samples were collected manually, using of a calibrated soil auger.

In order to have good Reference point (control), another batch of soil samples were collected from a non-polluted area, about 20 km from the study site. The reference point has the following geographical coordinates (Lat. 5.409 N; Long. 6.188 E). The reference point does not have any history of oil spillage for the past 20 years, and it is currently under agricultural production (arable crop cultivation). The coordinates of locations were captured and registered by employing a hand-held global positioning system (GPS) receiver.

A total of 21 soils samples were collected from the non-active illegal refineries site. All collected soil samples were dried in the laboratory under ambient temperature of 30±5°C.

Soil chemical Analysis

The air-dried soil samples were sieved with a 2 mm stainless steel sieve, to remove all debris from the soil samples. 10 g of each soil sample was poured into a round-bottomed conical flask and was added a mixture of concentrated HCl and HNO_3 (ratio of 3: 1). The content of the conical flask was digested at a temperature of 80^0C, under a clear solution appeared above the soil in the flask. Then the digested mixture was poured into a volumetric flask, and diluted with distilled water up to the 100 ml mark. Afterwards, the diluted mixture was filtered with a Whatman filter paper (No. 1). An Atomic Absorption Spectrophotometer (AAS, Perkin Elmer 2380) was used to analyze some heavy metals (Ni, Cu, Cd, Pb, and Zn) concentration of the filtrated digested soils samples.

Ecotoxicity test

Seed germination rate test

The seed germination rate test of the soil samples collected from the polluted area was carried out in accordance with United States Environmental Protection Agency (US EPA 712-C-008) standard procedures. This test helps to determine if the concentration of the pollutants (heavy metals) in any polluted soil sample, is strong enough to adversely affect the growth performance of the crops growing in it (U. S. EPA, 2012). Three crops (maize, beans and cucumber) were for the ecotoxicity test.

Four hundred (400) g of thoroughly mixed soil from each soil sample was poured into a glass dish. Ten healthy seeds were then arranged on top of the soil in each dish; and they were covered with 50 g of unpolluted fertile top soil. All the dishes were placed under a shade, and were closely monitored for ten days (the experimental period). On a daily basis, all the dishes were watered with 60 mL of clean borehole water. The number of seed(s) that germinated per dish was counted daily and recorded till the 10^{th} day. The seed germination rate (SR) and Germination Index (GI) of the soils samples were calculated using Equation 1 and 2.

$$SR = \frac{\textit{number of seeds that germinated on contaminated soil}}{\textit{number of seeds that germinated on uncontaminated soil}} \times 100 \quad (1)$$

$$GI = (10\times n1) + (9\times n2) + \cdot\cdot\cdot + (1\times n10) \quad (2)$$

Where:

n1, n2 . . . n10 = No. of seeds that germinated on the 1^{st}, 2^{nd}, 3^{rd}, until the 10^{th} day

10, 9 . . .1 = weights assigned to the number of seeds germinated on the 1^{st}, 2^{nd}, 3^{rd}, until the 10^{th} day (kader, 2005)

Radicle growth inhibition test

At the end of the experimental period, all the germinated seedlings were carefully uprooted. Then the visible radicle of each germinated seedling was measured with a flexible measuring tape. The radicle growth inhibition of the seedlings was calculated using equations 3 and 4.

$$GR = \frac{\text{Length of radicle on contaminated soil}}{\text{Length of radicle on uncontaminated soil}} \times 100 \quad (3)$$

$$RI = 100 - GR \quad (4)$$

Where:
GR = Radicle growth rate
RI = Radicle growth inhibition

Ten experimental was chosen for this test, because if the experimental period is prolonged, there is high probability that secondary roots of the seedlings will start to grow. Therefore, the radicle length of the seed cannot precisely reflect the toxicity of the soil sample. According to Luo *et al.*, (2017) the germination period of each seed is highly dependent on the soil conditions and the seed species.
All the laboratory tests were carried out under ambient laboratory temperature of 30±5°C, at the Soil and Water Laboratory of Delta State Polytechnic, Ozoro, Nigeria. All tests were repeated three times and the mean value recorded

Statistical Analysis

The data of this study were statistically analyzed using the Statistical Package for Social Statistics (SPSS version 20.0) software, and the means were separated using The Duncan's New Multiple Range (DNMR) Test ($p \leq 0.05$). The mean results were plotted using Microsoft Excel 2015; while the correlation (r) relationship was determined using the MS Excel 2015.

RESULTS AND DISCUSSION

This study showed that wastes products discharged by the illegal refineries adversely affects the biochemical properties of the soil. Critical observation of the Figure 2 showed wide spread water and land pollution, as the water and soil are covered with thick film of crude oil. This has the capability of causing further degradation of the already fragile environment of the region. The aquatic organisms thriving in these areas were these illegal refineries are sited are not safe, due to the toxic effluent discharge into the water bodies. Heavy metals concentrations of soils samples collected from the refineries site was significantly higher than, the results obtained from the reference point (Table 1). The concentrations of most heavy metals in all the soils samples, collected from the study site were above the World Health Organization recommended permissible maximum limits. Spatial points D and E were observed to have the highest heavy metals concentrations among all the seven points from where soil samples were collected. This could be attributed to the volume of petroleum products received by these points during the crude oil refining process. Similar results on the effects of petroleum products on the heavy metals concentrations in soils samples were reported by Iwegbue (2011) and Akpomrere and Uguru (2020). According to the Iwegbue (2011), the Ni, Cd and Zn concentration of crude oil impacted soils increased from 7.0 to 31 mg/kg, 0.02 to 1.12 mg/kg and 23 to 29.3 mg/kg respectively. High heavy metals concentrations in the soil have a lot of adverse effects on living things; therefore, it has become a great concern to agricultural production and environmental health (Ferguson, 1990; Goyer, 1997).

Table 1: Mean of heavy metal concentration of soils (0–40cm depth) collected from study site

Spatial point	Heavy metals				
	Ni	**Cu**	**Cd**	**Pb**	**Zn**
Point A	19.11^{e}	8.45^{d}	1.95^{d}	15.11^{d}	12.88^{c}
Point B	16.73^{c}	3.63^{b}	1.10^{c}	18.53^{e}	15.18^{d}
Point C	15.46^{b}	6.01^{c}	2.62^{e}	14.67^{c}	16.06^{e}
Point D	26.78^{f}	10.63^{e}	4.32^{f}	20.65^{f}	24.44^{f}
Point E	28.01^{g}	11.76^{f}	2.92^{e}	23.11^{g}	25.09^{f}
Point F	15.96^{b}	4.1^{b}	0.87^{b}	10.08^{b}	10.44^{b}
Point G	17.55^{d}	6.89^{c}	1.88^{d}	15.46^{d}	15.12^{d}
Control	3.18^{a}	2.11^{a}	0.07^{a}	2.08^{a}	2.16^{a}
WHO standard	75	140	3	300	300

Means followed by a different letter(s) in the same column differ significantly ($p \leq 0.05$) according to Duncan's Multiple Range Test.

Figure 2a: Destroyed illegal refineries in Delta State, Nigeria

Figure 2b: Illegal refineries in Delta State, Nigeria

Results further clarified that the activities of the illegal refineries have adverse ecotoxicity effects on the three crops investigated. The Ecotoxicological observation of the polluted soils sample, compared to the reference point, showed that heavy metals concentrations in the soils samples highly suppressed the seeds germination rates and radicle growth rates (Table 2 and Figures 3 to 4). These indicate a strong correlation ($r \geq 0.90$) between the Ecotoxicological changes in germinating seeds, in all the three crops used in this study, and the concentrations of heavy metals in the soils samples. The seed germination rate, seed germination index and radicle growth inhibition rate were negatively affected in the populated soil samples; compared to the results obtained from the control soil sample.

Ecotoxicity effect of the soils samples on seeds germination varied across the soil sample location and the crop species (Table 2). Table 2 revealed that spatial Points D and E can be considered as the epicenter of the pollution, since they had the highest seed toxicity effects, compared to the other spatial points. The highest sensitivity was observed at Point E, for which maize and cucumber seeds germination were inhibited by 100 %; while in the case of bean seeds, the seed germination was inhibited by 90 % (Table 2). Generally, bean seeds showed more resistant to the toxicity of the polluted soils samples, when compared with the results obtained from the Cucumber and maize seeds (Figure 3). In all the soil samples, bean seeds had the highest germination index. At point E, Bean seeds had GI of 36; while

cucumber and maize seeds recorded 0 GI respectively. Likewise, at spatial point F, which had mild pollution (compared to heavy metals concentrations at other spatial points), bean seeds recorded 136 GI; while the cucumber and maize seeds recorded GI of 58 and 68 respectively. Cucumber was highly sensitive to the soil toxicity, as it recorded the highest seed germination inhibition in all the soil samples. This affirmed earlier research observations that that *sativus* is highly sensitive to the presence of toxic substances (including heavy metals) in the soil. Therefore, this makes it a vital crop in the evaluation of seed toxicity if soils samples (Heemsbergen *et al.*, 2009; Oleszczuk, 2010, Walter *et al.*, 2006).
Furthermore, the ecotoxicity of waste products discharged by the illegal crude oil refiners was more noticeable in the radicle growth inhibition, than in the seed germination inhibition. This confirmed previous research result, which reported that seed radicle, is a more ecotoxicity sensitive indicator than the seed plumule (Fuentes *et al.*, 2006). As shown in Figure 4, the seed radicle inhibitory rate increased with general increase in the heavy metals concentrations in the soil. This attested to earlier research stating that, heavy metals had antagonistic effects on seeds radicle growth and development. At spatial point E, cucumber and maize seeds had 100 % radicle growth inhibition, while bean seeds recorded 90.11% radicle growth inhibition. Likewise, at spatial point D, the cucumber seeds still maintained 100% radicle growth inhibition; while the maize seeds radicle growth was inhibited by 89.17%, and bean seeds had 82.44% radicle growth inhibition (Figure 4). Furthermore, at spatial point F that had the lowest heavy metals pollution, the maize, cucumber and bean seeds had 68.17%, 74.76%, and 63.78% radicle growth inhibition respectively. It was observed from the results that seed germination and radicle growth inhibition were determined by the concentration of pollutants in the soil. According to Wang and Zhou (2005), seeds radicle and shoot will have normal morphological structure under very low heavy metals concentrations, because seed the seed germination and development is not sensitive to low pollutants concentrations. This study further confirms earlier findings Khan and Khan (2010), who reported that high Ni concentration in the soil can suppressed root growth and nodules development in plant roots. Rotkittikhun *et al.* (2006) observed plants growth inhibition in soil with high Ni and Co concentrations. According to Khan and Khan (2010), high concentration of Ni in the soil could antagonize Ca^{2+} and Fe^{2+}, causing their artificial deficiencies within the soil. Cadmium which among the metals present in the polluted soils, is highly poisonous to plants, even in small quantity; as it can impede plants growth and even modified their metabolism (Van-Assehe and Clijsters, 1990). Ahsan *et al.* (2007) reported that high dosage of copper inhibited rice seeds germination, radicle growth rate; and decreased the plant’s biomass. This study does not only detect poisonous substances (heavy metals) in the illegal refining site, but also the biological effects of petroleum products on the environment. Thus, calling for the need by the stakeholders of the Niger Delta region to stop the activities of the illegal crude oil refiners has become necessary, in order to safeguard the environment from further degradation.

Table 2: Seed germination inhibited rate

Spatial point	**Seed germination inhibited rate (%)**		
	Maize	**Cucumber**	**Beans**
Point A	66.67	75	70
Point B	66.67	75	70
Point C	55.56	62.5	60
Point D	88.89	100	80
Point E	100	100	90
Point F	55.56	62.5	60
Point G	66.67	87.5	50

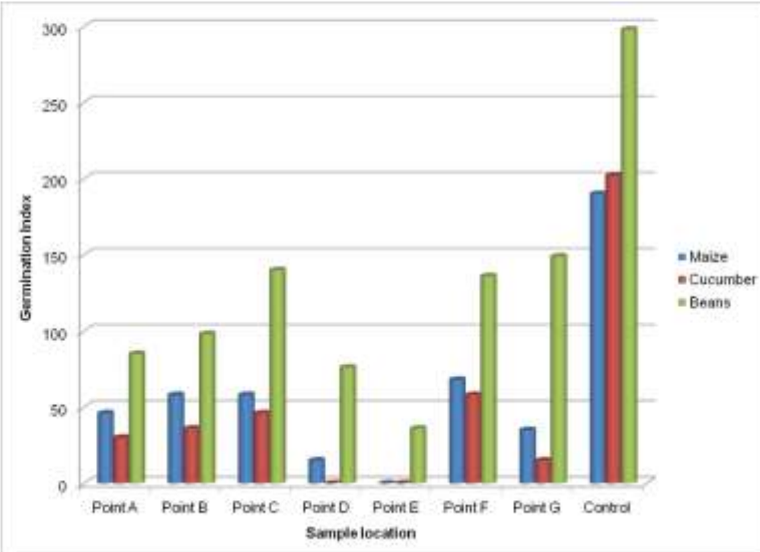


Figure 3: Seed germination index

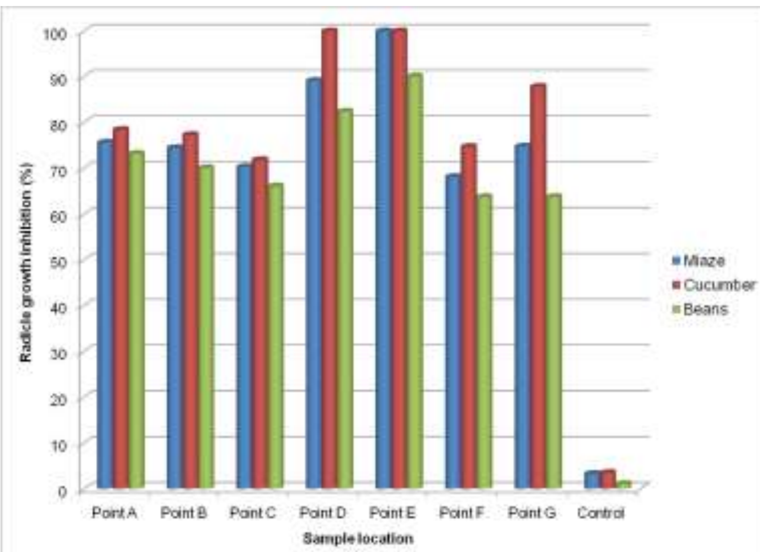


Figure 4: Radicle growth inhibition rate

CONCLUSION

The environmental impact assessment of illegal refineries Delta State, Nigeria, was carried out in this study. Soil analyses carried out on soils samples randomly collected from some illegal refineries sites, showed that the soils were highly polluted with heavy metals. Almost all the heavy metals concentrations in all the soils samples collected from the study site were above the World Health Organization recommended permissible maximum limits. Nickel concentration ranged between 15.46 and 28.01 mg/kg, Copper ranged between 3.63 and 11.76 mg/kg, Cadmium ranged between 0.87 and 4.32 mg/kg, Lead ranged between 10.08 and 23.11 mg/kg, while Zinc ranged between 10.44 and 23.44 mg/kg. In addition, ecotoxicological results obtained from this study showed that waste products produced by these illegal refineries and discharged indiscriminately into the environment, severely inhibited crops seeds germination and radicle growth. This will greatly affects crops production, which is a major source livelihood, within the region. Results obtained from study showed while the stoppage of the activities of illegal crude oil refiners becomes inevitable. This is to safeguard the already fragile environment from further degradation.

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