**Sustainable Remediation Technique for Heavy Metals-Contaminated Soil using Agro-Waste**

**Abstract**

Investigating the potential of *Vernoni*a *Amygdalina* (bitter leaf) leaf extract to stimulate the removal of selected heavy metals (chromium, zinc and iron) in spent engine oil-contaminated soil, which would help in managing the agro waste. To achieve this, 4kg of sandy loam soil was artificially polluted with a fixed used engine oil of 0.5L to simulate conditions of a spill. The leaf extract was derived from the bitter leaf plant which were harvested, washed with distilled water, blended and filtered to get the juice. The experiment used a randomized complete block design (RCBD), the set-up consisted of three treatments (T1, T2 and T3) and one control (T4), each replicated twice. The stated treatments contain 250, 500 and 750 g of the leaf extract except for the control with no treatment. The set-up was tilled and irrigated twice weekly for a period of 40 days. The nutritional (nitrogen, phosphorus and potassium) value of the bitter leaf extract and selected heavy metals were determined following standard procedures. The heavy metals content were determined before and after application of treatment, till the end of the experiment. After 40 days of treatment, chromium content dropped to 0.023, 0.015, 0.0065 and 0.121 mg/kg (T1, T2 T3 and T4), accounting for 61.76, 42.31, 25.81 and 19.01% reduction, respectively. Zinc and iron followed same trend. However, C reclaimed the selected heavy metals in the spent engine oil-contaminated soil, better than other levels of treatments.

**Keywords:** *Vernoni*a *Amygdalina;* leaf; treatment; Biostimulation; Spent engine oil; Metals; Bioremediation.

**1 Introduction**

Engine oil is a bye-product of crude oil, the used engine oil is often referred to as spent engine oil and the chemical composition of engine oil varies widely and depends on the original crude oil, the techniques used in refining, the efficiency and kind of engine the oil is lubricating. Others are the gasoline combustion products, the additives added to the fuel and the original oil, and the amount of time that the oil resides in the engine (Obinna & Afiukwaa, 2013). The oil usually comprises 73-80% weight/weight aliphatic hydrocarbons (mainly alkanes and cycloalkanes with l-6 rings), 11-15% monoaromatic hydrocarbons, 2-5% diaromatic hydrocarbons, and 4-8% polyaromatic hydrocarbons (Vazquez-Duhalt, 1989). This oil is typically collected after servicing and then draining from car and generator engines (Anoliefo & Vwioko, 2001). In Nigeria, it is common to pour used engine oil (SEO) into gutters, water drains, abandoned lands, and farms. It is also, applicable in other developing countries particularly by motor mechanics. A large portion of this oil is then thrown straight into the ground. These days, petroleum product and grease pollution of exposed unoccupied spaces and farmlands are more common than crude oil spills, particularly in urban areas (Osaigboro et al., 2013; Nwite, 2013). For example, it was stated that Nigeria produces around 87 million liters of spent oil waste each year, and its ultimate fate has not received enough consideration (Anoliefo & Vwioko, 2001). Removal of SEO-polluted soil is a global problem, particularly in most underdeveloped and developing countries. Because of how SEO affects the soil, challenges of hydrocarbons in the surroundings have greatly increased. Heavy metals are of particular importance because these pollutants can accumulate in the soil and present a major risk to people and other living things through a variety of routes (Denys et al., 2006). Spent engine oil contains hazardous chemical contaminants that can induce cancer and change living cells (Udeani et al., 2009; Ajao et al., 2011). According to Adams et al. (2014a), these substances contaminate both surface and groundwater, seep into the water table, and then enter the human body through plant food chain. Several recent studies in Adams et al., (2014a); Agarry and Ogunleye (2012), stated that leftover engine oil floats as scum on the surface, blocking sunlight and oxygen from entering the water body and as a result harming aquatic life such as fish, frogs, crabs, and water plants. Soil-dependent industries like agriculture are severely impacted by SEO pollution. The impact from SEO causes soil nutrient immobilization, loss of water-retaining capacity, low pH, decreased soil catalase enzyme activity, and inhibition of plant nitrate reductase activity (Imam et al., 2011 & Muhammad et al., 2022). However, several cost-ineffective and environmentally unfriendly approaches such as the physicochemical technique including thermal desorption, incineration, soil washing, soil vapor, disposal in landfills, excavation, etc. have been applied at different levels.

Presently, the biological treatment (bioremediation) techniques for the treatment of SEO-polluted soil have been demonstrated to be affordable and eco-friendly. To reduce (degrade, detoxify, mineralize, or change) the concentration of contaminants to a safe level; a technique known as "bioremediation" is used (Azubuike et al., 2016). Its affordability and environmental friendliness have led to its adoption over physical and chemical technologies (Muttalab & Ali, 2022). Some well-known biological treatment technologies include biostimulation, biosparging, bioventing, bioaugmentation, and phytoremediation.

In order to promote the growth and metabolic activity of naturally occurring bacteria that are friendly to humans, biostimulation entails adding nutrients or fertilizers, often known as amendments (Udume et al., 2023). Most polluted locations are often low in phosphorus and nitrogen, which makes it difficult for native microbes to get enough resources for their survival and degradation processes. The existing microbial population ability for degradation is increased when rate-limiting nutrients are added to the environment. The nutrients used in both organic and inorganic fertilizers are biostimulants (Okonwu et al., 2014; Udume et al., 2023). Biostimulation with either organic nutrients or a combination of organic and inorganic nutrients has been shown to be more productive for pollution cleanup. Hence, it enriches the soil in addition to giving bacteria nourishment (Jiang et al., 2016). Therefore, organic fertilizers could provide nutrients for the biological stimulation of soil contaminated by wasted engines in a way that is safer for the environment.A significant medicinal plant abundant throughout East and West Africa is known as *Vernonia amygdalina* (Burkill, 1985). It functions as an effective anti-bacterium, anti-malarial, anti-parasitic, and anti-cancer agent (Tadesse et al., 1993; Izeybigie, 2003).

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| **Figure 1:** *Vernonia amygdalina* (Bitter Leaf) Plant |

This plant has complex active ingredients with potential applications in pharmacology. In traditional medicine, the leaves and roots are used to cure kidney issues, fever, hiccups, and stomach pain (Hamowia & Saffaf, 1994). Chew sticks are made from the stem and root of the bark in various West African countries, such as Nigeria, Ghana, and Cameroon.

Despite the medicinal importance of the leaf, the extract which is often discarded as agro-waste may also be utilized for remediation of heavy metals in spent engine oil-contaminated sites. Therefore, the aim of this research was to examine the potential of the *Vernonia amygdalina* leaf extract to clean-up selected metals in soils polluted with SEO.

**2 Materials and Methods**

**2.1 Study Area**

The spent engine oil was obtained from a car mechanic garage in the Niger Delta region of Nigeria. Clean soil was collected from the Rivers State University (RSU) research farm in Port-Harcourt, Nigeria at 0.3 m diameter and 0.35 m depth, cylinder-shaped polyvinyl chloride (PVC) plastic containers were utilized to hold the soil and SEO combination used as contaminated media. To allow for direct control over the amount of moisture and nutrients, the PVC containers were placed in an open area but protected from rainfall (Kogbara et al., 2018). The aforementioned study area has tropical rainforest flora, with an average annual rainfall of 2000 to 2884 mm, 70% of which falls between May and August with an average temperature of 27oC (Ayotamuno et al., 2006; Fubara et al., 2021).

**2.2 Formulation of *Vernonia Amygdalina*** **Leaf Extract**

The *Vernonia Amygdalina* leaves were grown and collected at Rivers State Institute of Agricultural Research and Training (Figure 2a). The leaves underwent multiple water washing and a distilled water was used to rinse (Figure 2b). Then, the mixture was put into a handheld electric blender (Figure 2c). After mixing, whatman No. 1 filter paper was used to filter the resultant solution to remove impurities (Atata et al., 2003). For additional purification, the filtrate was run through a millipore membrane filter with a 0.45 µm pore size as shown in Figure 2d (Sule & Agbabiaka, 2008). Before being used, the extracts were kept in sterile, sealed bottles and chilled.

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| **Figure 2:** Flowchart of the Formulation of Bitter Leaf Extract |

**2.3 Treatments Employed**

Four treatments were considered, and each carried two replicated plastic containers. The four treatments (T1, T2 T3 and T4) comprise only one nutrient with a varying quantity excluding the control (T4) with no treatment. The mixture of soil–spent engine oil mixtures in all treatments (T1 to T4) was allowed to stay for 3 days to enhance proper interaction between soil and spent engine soil to stimulate typical car mechanic garages in the region. Each plastic container (T1 –T4) including the control had 4 kg of uncontaminated soil and 0.5 kg of spent engine oil. These were amended with 250, 500, and 750 g for T1, T2 and T3, respectively, except for the control (T4) with 0g treatment (as showed in Table 1). Each treatment received 100 ml of water twice weekly throughout the 40 days.

**Table 1. Experimental Mix Proportion**

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| --- | --- | --- |
| **Reactor** | **Mix Proportion** | **Mix ratio** |
| T1 | 4000 g of soil + 500 g of crude oil + 250 g of leaf extract | 8:1:0.5 |
| T2 | 4000 g of soil + 500 g of crude oil + 500 g of leaf extract | 8:1:1.0 |
| T3 | 4000 g of soil + 500 g of crude oil + 750 g of leaf extract | 8:1:1.5 |
| T4 (Control) | 4000 g of soil + 500 g of crude oil + 0 g of leaf extract (Control) | 8:1:0.0 |

**2.4 Sampling**

Samples were randomly collected from the uncontaminated soil before it was mixed with spent-engine oil. Samples were also collected from soil mixed with spent engine oil, after 3 days and every 10 days till the end of the 40 days study period.

**2.5 Testing and Analytical**

The selected soil physical and chemical characteristics were considered; pH, particle size distribution, moisture content, organic matter and organic carbon. The selected metal concentration of the contaminated soil was used to evaluate the performance of various treatments.

To determine Fe, Cr and Zn in SEO and contaminated soil, the sample was digested by di-acid method and analyzed by using atomic absorption spectrophotometer (AAS) model Hitachi Z-8230 (APHA, 2005).The total nitrogen was determined by Kjeldahl method (Bremner & Mulvaney, 1982), pH and EC were measured using portable electronic device known as Hanna instrument with model H198331. The hydrometer method of Bouyoucos (1962) was used to determine the particles of various sizes such as sand, silt and clay, the results revealed the texture of the soil according to United States Department of Agriculture (USDA) textural classification of soil while soil moisture content was determined by the oven-drying method.

**2.6 Statistical Analysis**

To evaluate the significance of the difference in mean heavy metals due to the quantity of treatment added to the reactors, one-way analysis of variance (ANOVA) in a randomized complete block design (RCBD) experiment was carried out using the ANOVA data analysis toolbox in Microsoft ® Excel-365 Microsoft Inc USA. This study considered the determination of heavy metal levels in SEO used for sandy loam soil contamination simulation. This was done since there were initial concentration of heavy metals in the treatment process and equally identified in the contaminated soil.

**3 Results and Discussion**

**3.1 Characteristics of the uncontaminated soil & spent engine oil**

Table 2 shows the values of essential parameters of the uncontaminated soil used for the remediation work. The uncontaminated soil composed of 59.7% sand, 28% silt and 12.9% clay and it’s classified as sandy loam soil. The spent engine oil is composed of 83.61 mg/kg chromium, 97.40 mg/kg zinc and 2073 mg/kg iron (See Table 2). The results show that pH, moisture content, electrical conductivity, organic matter and organic carbon of the soil samples were 5.80, 22%, 24 µs/cm, 0.84% and 1.54%, respectively.

**Table 2: Heavy metals content of SEO-polluted soil and spent engine oil (SEO)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Chromium (mg/kg) | Zinc (mg/kg) | Iron (mg/kg) |
| SEO-Polluted Soil | 0.761 | 2.891 | 1.932 |
| SEO | 83.61 | 97.40 | 2073 |

**3.2 Nutritional Values of the Formulated Nutrient**

Table 3 shows the Nitrogen, Phosphorus, and Potassium (NPK) contents of the *Vernonia Amygdalina* leaf extract. The NPK characteristics of the bio-stimulants (*Vernonia Amygdalina* leaf extract) revealed 0.52 mg/kg nitrogen, 24.41 mg/kg phosphorus, and 37.46 mg/kg potassium. Therefore, this confirms that NPK contents of the *Vernonia Amygdalina* leaf extract can be utilized for reclaiming spent engine-contaminated soil.

**Table 3:** *Vernonia Amygdalina* leaf extract NPK Values

|  |  |  |
| --- | --- | --- |
| Nitrogen (mg/kg) | Phosphorus (mg/kg) | Potassium (mg/kg) |
| 0.52 | 24.41 | 37.46 |

**3.3 Heavy Metals Content of Untreated Soil**

The preliminary investigation of the spent engine oil – contaminated soil recorded 0.761 mg/kg, 2.891 mg/kg and 1.932 mg/kg as the baseline value of chromium, zinc and iron, respectively (see Table 1), which were quite high but did not exceed local regulatory limit (See Table 4.) as prescribed by Nigerian Upstream Petroleum Regulatory Commission (NUPRC, 2018).

**Table 4** Nigerian Upstream Petroleum Regulatory Commission (NUPRC)

Regulatory Limits

|  |  |  |  |
| --- | --- | --- | --- |
|  | Chromium (mg/kg) | Zinc (mg/kg) | Iron (mg/kg) |
| Target Value | 100 | 140 | - |
| Intervention Value | 380 | 720 | - |

**3.4 Biostimulation of Spent Engine Oil-Contaminated Soil Over Time**

**3.4.1 Zinc**

The Zinc concentration of the untreated spent engine oil-contaminated soil was 2.053 mg/kg (See Figure 3). This shows that the zinc concentration in the contaminated soil was less than the 720 mg/kg intervention and the target value of 140 mg/kg spelt out by NUPRC (2018) as shown Table 3. However, there is a need to reclaim the soil. It is important to note that the zinc concentration was fixed in all the reactors (See Figure 3).

The extent of zinc concentration in the contaminated soil treated with *Vernonia Amygdalina* leaf extract for 40 days is shown in Figure 3. Also, the variation of zinc concentration in the SEO contaminated sandy loam soil at different quantities of *Vernonia Amygdalina* leaf extract (0, 250, 500 and 750 g). The reduction rate of zinc content at ten (10) days of remediation of sandy loam soil treated with different amounts of *Vernonia Amygdalina* leaf extract increased with time. Furthermore, reactor T1 reduced to 1.543 mg/kg, followed by T2, which reduced to 1.507 mg/kg, T3 also decreased to 1.401 mg/kg whereas T4 which is the control reduced slowly to 1.931 mg/kg (See Figure 3.). Figure 3, shows that the zinc content decreased with increase in the quantity of amendment (*Vernonia Amygdalina* leaf extract) administered to the contaminated soil and periods, respectively. Moreover, the lower the amendment, the lower the reduction of chromium content in the soil. However, there was a significant difference at 1 and 5% level of significance as shown in Table 5. The spent engine oil-contaminated soil at 20 days after applying amendments was observed that T3 dropped in zinc content compared to other amendment quantities (T1, T2 and T4) as shown in Figure 3. The reduction in percentage was still higher in T3 as shown in Figure 3, next was T2, followed by T1 and the least was the T4 (control). However, there were significant differences at 1 and 5% level of significance as shown in Table 5.

The trend continued up to the end of the remediation period of 40 days (as shown in Figure 3.) Although, Figure 3, shows that the remediation of spent engine oil-contaminated soil using *Vernonia Amygdalina* leaf extract was quite effective in reducing the level of zinc content to lesser value as the higher percentage reduction of 28.27% as observed in T3, followed by T2 (25.61%), next to T1 (19.31%) and the least was the control (T4) with percentage reduction of 3.42%. This therefore shows that T3 achieved the highest zinc reduction in the remediation of soil contaminated with SEO. However, it was still far below the NUPRC (2018) target and intervention values for soil and sediment (Table 3). At 40 days, there were highly significant differences at 1 and 5% level of significance as shown in Table 5. This suggests that the difference in treatment means may be determined with 95 and 99% confidence concerning the variations of *Vernonia Amygdalina* leaf extract applied. The contaminant dropped over time, first at a rapid pace and then at a slower pace. This is in agreement with the research conducted by Jiang et al. (2016) and Ntesat et al. (2023). It is evident, therefore, that heavy metals can change from one organic complex or oxidation state to another but cannot biodegrade during bioremediation. A shift in the oxidation state of heavy metals can cause them to become less bioavailable, less volatile, or less poisonous (Garbisu & Alkorta, 2003; Ntesat et al., 2023).

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**Fig. 3** Effects of different *Vernonia Amygdalina* leaf extract on zinc, iron and chromium concentrations in different Spent Engine oil-contamination sites (T1, 250g; T2, 500g; T3, 750g; T4, Control with no Treatment), Error Bar represents Standard Error.

**3.4.2 Iron**

It was obvious that the zinc content decreased with increase in the quantity of amendment (*Vernonia Amygdalina* leaf extract) administered to the contaminated soil and periods, respectively as shown in Figure 3. Moreso, the lower the amendment, the lower the reduction of zinc content in the soil. However, there was highly significant difference (P > 0.05) as shown in Table 5. The spent engine oil-contaminated soil at 20 days after applying amendments as was observed in Reactor C, dropped in iron content compared to other amendment quantities (T1, T2 T3 and T4) as shown in Figure 3). The reduction was still higher in T3 as shown in Figure 3, next was T2, followed by T1 and the least was T4 (control). However, there was significant difference at 1 and 5% level of significance as shown in Table 5.

This trend continued up to the end of the remediation period of 40 days as shown in Figure 3. Although, Figure 3, shows the remediation of spent engine oil-contaminated soil using *Vernonia Amygdalina* leaf extract was quite effective in reducing the level of iron content to lesser value as the higher percentage reduction of 29.77% (See Figure 4) was observed in T3, followed by T2 (12.67%), next to T1 (6.31%) and the least was the control (T4) with percentage reduction of 0.83%. This therefore shows that T3 achieved the highest iron reduction in the remediation of soil contaminated with spent engine oil. At 40-day, there was highly significant differences (P > 0.05) as shown in Table 5. On the other hand, the control container had the lowest Zn reduction (3.6% reduction). Therefore, it appears from the observed results that high amendment dosages can accelerate the removal of heavy metals (HMs) and shorten the time needed for soil contaminated with SEO to be cleaned up (Agarry, 2018). Similar findings have been documented in the works of Agarry and Ogunleye (2012); Chikere et al. (2012); Ntesat et al. (2023).

**3.4.3 Chromium**

Figure 3 shows that the chromium content decreased with increase in the quantity of amendment (*Vernonia Amygdalina* leaf extract) administered to the contaminated soil and periods, respectively. Moreso, the lower the amendment, the lower the reduction of chromium content in the soil. However, there was significant difference (P > 0.05) as shown in Table 5.

The spent engine oil-contaminated soil at 20 days after applying amendments with referenced to T3, dropped in chromium content compared to other amendment quantities (T1, T2 and T4) as shown in Figure 3. The reduction was still higher in T3 as shown in Figure 3, next was T2, followed by T1 and the least was the T4 (control). However, there was significant difference (P > 0.05) as shown in Table 5. This trend continued up to the end of the remediation period of 40 days as shown in Figure 3.

Although, Figure 3 shows the remediation of spent engine oil-contaminated soil using *Vernonia Amygdalina* leaf extract is quite effective in reducing the level of chromium content to lesser value as the higher percentage reduction of 61.76% as shown in Figure 4 was observed in T3, followed by T2 (42.31%), next to T1 (25.81%) and the least was the control (T4) with percentage reduction of 19.01%. This therefore shows that T3 achieved the highest chromium reduction in the remediation of soil contaminated with spent engine oil. However, it was still far below the NUPRC (2018) target and intervention values for soil and sediment (Table 2). At 40-day, there was highly significant differences (P > 0.05) as shown in Table 5. This suggests that with 95 and 99% confidence that the difference in treatment means was in respect to the variations of *Vernonia Amygdalina* leaf extract applied (See Table 4). Chromium exposure include occupational asthma, eye irritation and damage, perforated ear drums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, and respiratory cancer. Exposure can be through dermal or ingestion of contaminated water (Adams et al., 2014b). Bioremediation of chromium relies on the reduction of soluble and mobile hexavalent chromium, Cr (VI), to its reduced form Cr (III). Chromium reduction occurred due to the stimulation of microbes, enhancing the growth of microbes via the addition of a reduced carbon source such as lactate, acetate or molasses. The product, Cr (III) is often assumed to precipitate as relatively insoluble hydroxide solid (Adams et al., 2014b).

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**Figure 4:** Percentage reduction of zinc, iron and chromium concentration with different *Vernonia Amygdalina* leaf extract dosages on various spent Engine oil-contamination sites (T1 – 250 g; T2 – 500 g; T3 – 750 g; T4, Control with no Treatment), Error Bar represents Standard Error.

Microbial remediation of heavy metals contamination of the soil has unique properties such as soil structure maintenance and low-cost effects. Notably, several microbial species such as bacteria and other strains have significant reduction ability (Jin et al., 2018). The formation of complexes with organic ligands and biomineralization (involved in the mobilization of heavy metals ions from insoluble ores by complexation and bio-oxidation) may enhanced plant extract and remediation properties (Henao & Ghneim- Herrera, 2021). Presently, a variety of bacteria may absorb soil heavy metals. Among these bacteria include Escherichia coli K-12 may absorb the widest variety of metal ions. Remarkably, the outer membrane of these bacteria strain can absorb over 30 diverse types of metal ions (Dasola et al., 2014). According to studies from Ali et al. (2019) and Degu et al. (2024), bitter leaf extract aqueous extract contain 27 mg/g of saponins, 46 mg/g of alkaloids, 122 mg/g of flavonoids, 17 mg/g of terpenoids, 12 mg/g of tannins, 48 mg/g of steroids, 36 mg/g of phenols and others. These numerous chemical chains, structure and properties of bitter leaf may be responsible for the effective remediation of soil contaminated by petroleum hydrocarbon. Furthermore, the high NPK values inherent in bitter leaf extract as seen in Table 3 further support the treatment potential.

**Table 5**:ANOVA for heavy metals concentration in spent Engine Oil contaminated soil treated with four (4) different dosages of Bitter Leaf Extract form an RCB experiment with two replications after 40 days

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Heavy Metals |  | Sources of Variation | Degree of Freedom  (df) | Sum of Square (SS) | Mean Square (MS) | Computed  F value | Tabular  F value  1% 5% | |
|  |  | Zinc |  | Replication | 1 | 0.0079 | 0.007938 |  |  |  |
|  |  |  |  | Treatment | 3 | 0.8820 | 0.293995 | 60.35\*\* | 29.46 | 9.28 |
|  |  |  |  | Error | 3 | 0.0146 | 0.00487 |  |  |  |
|  |  |  |  | Total | 7 | 0.9045 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  | Iron |  | Replication | 1 | 7.2E-05 | 7.2E-05 |  |  |  |
|  |  |  |  | Treatment | 3 | 1.361094 | 0.453698 | 2539.35\*\* | 29.46 | 9.28 |
|  |  |  |  | Error | 3 | 0.000536 | 0.000179 |  |  |  |
|  |  |  |  | Total | 7 | 1.361702 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  | Chromium |  | Replication | 1 | 9.2457005 | 0.005 |  |  |  |
|  |  |  |  | Treatment | 3 | 10.275722 | 0.345 | 63.89\*\* | 29.46 | 9.28 |
|  |  |  |  | Error | 3 | 0.0162 | 0.0054 |  |  |  |
|  |  |  |  | Total | 7 | 1.0562 |  |  |  |  |

\*\* Highly Significant

**4 Conclusion**

Biostimulation is a known ecofriendly and cost-efficient methods that transforms organic material into biofertilizer with a potential to remove spent engine oil-contaminated sandy loam soil. The *Vernonia Amygdalina* leaf extract was found to possess nutrient sufficient to stimulate microbial activity. The different amount of treatment T1, T2, T3 including the control (T4) revealed the following order biostimulatory effect for the removal of heavy metals after 40 days of remediation works: Zinc; T1 (19.3% ), T2 (25.6% ), T3 (28.3% ) and T4 (3.42%)., Iron; T1 (6.31%), T2 ( 12.7%), T3 ( 29.8%) and T4 (0.83% ), and Chromium; T1 ( 25.8%), T2 (42.31%), T3 (61.8% ) and T4 (19% ), 750g of the *Vernonia Amygdalina* leaf extract (T3) exerted greater biostimulation of spent engine oil-contaminated soil. These results proved the effectiveness of the leaf extract for the remediation of heavy metals polluted soil. Metal ions and ligands are bonded to the surface of the adsorbent by covalent or ionic bonding. Based on the results achieved by bitter leaf extract, more studies is required over the molecular mechanisms with respect to the interaction of bitter leaf extract and other soil petroleum hydrocarbon based contamination.

**5** Statements and Declarations

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

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