

ASSESSMENT ON THE *Chromolaena Odorata* L. IMPACT ON CRUDE OIL POLLUTED SOIL AMELIORATED WITH COW DUNG

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ABSTRACT

This study was aimed at assessing the *Chromolaena odorata*. impact on crude oil polluted soil ameliorated with cow dung. 3600 grams of soil samples were polluted with crude oil at different concentrations ranging from 1 % to 3 % and 5 % while the cow dung levels remained constant. The plants were harvested by 12 weeks. Parameters such as these soils, cow dung and crude oil were analyzed for initial heavy metal properties while growth parameters data were collected and subjected to descriptive statistics to obtain the means and standard deviations. The differences in the heavy metal properties of soil, cow dung, crude oil, means of growth data and laboratory analysis were compared using analysis of variance. The result showed that the heavy metals in soil, cow dung and crude oil varied significantly ($P < 0.05$). The growth parameters studied generally varied considerably ($P < 0.05$), except SG 2 WAP which do not differ significantly. In addition, Pb and Cd, Cr, Hg heavy metal levels in the plant and soil samples were very low and almost non-existing. Finally, cow dung an agro waste can improve phytoremediation of crude oil polluted soil by acting as a remediating agent.

Keywords: Assessing, *Chromolaena odorata*, concentrations, polluted, remediating.

INTRODUCTION

Petroleum and other fossil fuels have played a significant part in the effort to find enough energy to satisfy the expanding global demand (Azubuike *et al.*, 2016). There has been a rise in the discharge of organic pollutants into the environment as a result of industrialization and the ever-increasing human population (Fatima *et al.*, 2017). Among these, petroleum spillage caused by either human error or machine malfunction—has harmed the environment for many years (Fatima *et al.*, 2017). The bulk of petroleum spills happen on land, with severe health and ecological repercussions, even though large-scale maritime spills frequently make headlines (Duffy *et al.*, -2000). To reduce the risks to humans and the environment, petroleum-contaminated locations must be cleaned up (Azubuike *et al.*, 2016). Ex situ and in situ are the two categories of remediation techniques (Azubuike *et al.*, 2016).

As a vital component of the biosphere that sustains life, soil benefits the environment in a variety of ways, including primary production, biogenic gas control, water cycling, life preservation, and biodiversity (Kuske *et al.*, 2002). Before, people used to think that our land and its resources were abundant and would last for millennia (Balba *et al.*, 1998). Unfortunately, half of this natural riches is either damaged or on the danger of depletion due to excessive usage and, more recently, misuse (Andreoni *et al.*, 2004). The use of chemical fertilizers, the discharge of other anthropogenic chemicals, and the disposal of home and industrial

waste into the environment are the causes of the ongoing exhaustion of a healthy soil ecosystem (Reda and Ashraf, 2010). Petroleum oil and its byproducts are a major component of the world's energy supplies, and during the next 20 years, there will be a sharp increase in global energy consumption (Aqeel and Butt, 2000). On the other hand, constant exposure to high oil concentrations might be harmful to everyone's health, including humans and other life forms (Janbandhu and Fulekar, 2011). Due to their high cost, environmental impact, lack of engineering expertise, labor administration issues, and operational management issues, conventional solutions based on physicochemical approaches (soil washing, chemical reduction or oxidation of contaminants, and incineration) are not practical (Pandey *et al.*, 2009). Given the limitations of current technology, a significantly more effective strategy is required to either eliminate the contaminants or convert them into benign compounds (Shabir *et al.*, 2013). This can be accomplished by using effective microorganisms along with the right plants, a process known as microbe-assisted phytoremediation (Weyens *et al.*, 2009). When the amount of pollutants exceeds the soil's capacity to act as a buffer, it results in long-lasting negative impacts on the environment (Weyens *et al.*, 2009).

To reduce the harmful impacts of pollutants, this method makes use of physical, biochemical, biological, chemical, and microbiological processes (USEPA, 2001). The process of phytoremediation involves uses plants to purge contaminated areas (Meagher *et al.*, 2001). Rhizoremediation stands out as an integrated plant-microbe attempt, even while different components of phytoremediation engage the plant and its microbiota (Correa-Garca *et al.*, 2018). Rhizoremediation is the microbial activity that breaks down contaminants in the rhizosphere, or the region around plant roots (Cheng *et al.*, 2019). This method is based on carefully chosen plants with fibrous roots that act as a natural habitat for bacteria that break down hydrocarbons (Cheng *et al.*, 2019). The broad roots system also functions as a natural venting mechanism, allowing organic pollutants to be degraded by aerobic bacteria (Cheng *et al.*, 2019). The plant roots exude exudates throughout the rhizoremediation process (Correa-Garca *et al.*, 2018).

Chromolaena odorata possesses a number of growth characteristics that makes it attractive for phytoremediation studies, such as high germination rates of seeds, re-sprouting from stumps and stem cuttings, rapid growth in all kinds of soil, aggressive invasiveness, survival under relative stress conditions and low nutrient availability, high biomass accumulation and ability to dominate native vegetation in new environments (Atanaga, 2011). It makes economic sense to evaluate the potential of high biomass accumulating, fast growing and stress tolerant shrubs, such as *C. odorata* for phytoremediation purposes (Atanaga, 2011).

Concerns about soil contamination by petroleum hydrocarbons exist (Khan *et al.*, 2013; Ijaz *et al.*, 2015a). To ensure environmental safety and likely sustainability, best practices are usually adopted to minimize the release of these substances (Ahmed *et al.*, 2017). However, when such substances get into the environment, the urgent need is to remove them before accumulation becomes significant and uncontrollable (Chaudhry *et al.*, 2005). High levels of these elements can become harmful to organisms (Compant *et al.*, 2010). The method of phytoremediation offers a highly effective manner of treating contaminated land in-situ using natural biological activity (Glick, 2010; Sessitsch *et al.*, 2013; Ijaz *et al.* 2015). Metals display cytotoxicity and genotoxicity in both animal and plant levels (Ciriaková 2009). Hence, the need to under study the ameliorating capacity of cow dung on the growth performance and heavy metal properties of soil and plant cannot be over emphasized. The aim of the study was the assessment on the *C. odorata* impact on crude oil polluted soil ameliorated with cow dung.

MATERIALS AND METHODS

The screen house of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike was location of the study. Within Longitude 07° 34" E, Latitude 05°29" N and at an elevation of 122 m above sea level was the location of Umudike. (National Root Crop Research Institute, Umudike (NRCRI, 2022). Samples of used crude oil were collected from Ukwu West Local Government Area, Abia State, Nigeria. According to Ogedegbe *et al.* (2013), soil samples were collected at the depth of 0 – 15 cm in crop farms around Michael Okpara University of Agriculture, Umudike with the aid of a shovel. Plant samples were collected from the surroundings of Umuahia Township. Having only one factor (crude oil), the research design of this experiment was completely randomized design. Levels of cow dung were constant to amount of soil needed. While the crude oil was varied in 1 %, 3 % and 5 % to the soil and cow dung content. Control soil samples had zero levels of crude oil. Each soil sample were replicated three times.

Sample preparation of the soil: Soil samples were sieved with 2 mm sieve and was mixed in the following way; 0 % of 4000 grams of cow dung + 4000 grams of farm soil = Negative control. 10 % of 4000 grams of cow dung + 3600 grams of soil = Positive control. 10 % of 4000 grams of cow dung + 3600 grams of soil + 40 mills of crude oil = 1 %. 10 % of 4000 grams of cow dung + 3600 grams of soil + 120 mills of crude oil = 3 %. 10 % of 4000 grams of cow dung + 3600 grams of soil + 200 mills of crude oil = 5 %. The treated soil samples were permitted to stand for seven days. Subsequently, three plants samples uprooted with the roots and of same heights were transplanted into each sack bags filled with the treated soil samples. At the end of 12 weeks of transplanting, the plants samples were removed, the soil and plant samples were taken to the laboratory for physical, and chemical constituent evaluation.

Plant growth parameter measurements: Growth parameters comprising of sprouting number, leaf length, stem girth, number of leaves, and harvest parameters was determined.

Sprouting number (count per replicate): The number of germinations was done by daily observation of sprouting sample plant seeds per replicates.

Leaf length (cm plant⁻¹): The leaf length was measured using a ruler. A meter rule was used to measure upwards starting from base to the tip of plant leaf.

Stem girth (cm plant⁻¹): The girth was taken use mechanical Vernier Caliper. Girth was recorded in centimeter per plant.

Number of leaves (number plant⁻¹): The leaf number was collected by counting directly the number of leaves per plant.

Root length (cm plant⁻¹): The root length was taken by placing a ruler from the rooting point to the tip. Root length was recorded in centimeter per plant.

Root number (number plant⁻¹): The root number was collected by counting directly the number of roots per plant.

Biomass (gram plant⁻¹): The stem, root and leaf weight were taken by placing the various parts on a weighing balance. Records were taken in grams.

Analysis of heavy metals: Bio-available or soluble concentration of heavy metal was determined by Aqua Regia method (Chen and Ma, 2001). Conventional aqua regia digestion was performed in 250 mL glass beakers covered with watch glasses. A well-mixed sample of 0.5000 g was digested in 12 mL of aqua regia on a hotplate for 3 h at 100° C. After evaporation to near dryness, the sample was diluted with 20 ml of 2 % (v/v with H₂O) nitric acid and transferred into a 100 mL volumetric flask after filtering through Whatman no. 42 filter paper and diluted to 100 mL with DDW. The filtrates were analyzed for Zinc (Zn), Mercury (Hg), Manganese (Mn), Iron (Fe), Lead (Pb), Copper (Cu), Chromium (Cr) and Cadmium (Cd) using atomic absorption spectrophotometer. The values were compared with the widely used normal and critical levels of total concentration of heavy metal for the contaminant limit (c), p index was calculated as the ratio between the heavy metal content in the soil and the toxicity criteria (the tolerable levels).

Data analysis: Data collected was subjected to descriptive statistics to obtain the means and standard deviations. Analysis of variance analysis was used to compare the difference in the heavy metal properties of soil, cow dung and Crude oil. Means of growth data and laboratory analysis were subjected to analysis of variance (ANOVA). Statistically significant means at 5 % probability were separated using Duncan multiple range test all the test was done using statistical package for social sciences (SPSS) version 26.

RESULTS

Initial heavy metal properties of soil, cow dung and crude oil (CRO) were presented in Table 1a. From the result, Zn ranged from 0.01 mg/kg (CRO) to 0.06 mg/kg (CD). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different (P<0.05). Ni ranged from 0.87 mg/kg (CD) to 3.46 mg/kg (CRO). Initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different (P<0.05). Mo ranged from 6.71 mg/kg (CD) to 22.61 mg/kg (CRO). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different (P<0.05). Hg values were 0.03 mg/kg (CD) and 0.03 mg/kg (Soil) while the metal was not detected in CRO. Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) did not vary significantly (P>0.05). Pb was not detected in all the

samples. Mn ranged from 0.44 mg/kg (CD) to 0.95 mg/kg (Soil). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). Fe ranged from

0.01 mg/kg (CD) to 0.53 mg/kg (Soil). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$).

Table 1a: Initial heavy metal properties of soil, cow dung (CD) and crude oil (CRO)

Sample	Zn mg/kg	Ni mg/kg	Mo mg/kg	Hg mg/kg	Pb mg/kg	Mn mg/kg	Fe mg/kg
Soil	0.01 ^a	1.62 ^b	11.05 ^b	0.03 ^a	ND	0.95 ^a	0.53 ^a
CD	0.06 ^b	0.87 ^c	6.71 ^c	0.03 ^a	ND	0.44 ^c	0.01 ^c
CRO	0.01 ^a	3.46 ^a	22.61 ^a	ND	ND	0.82 ^b	0.47 ^b

Mean with different superscript alphabet are significantly different ($P < 0.05$), CD = Cow dung, CRO = Crude oil, ND = Not Detected

Initial heavy metal properties of soil, cow dung (CD) and crude oil (CRO) were presented in Table 1b. From the result, Cu ranged from 0.08 mg/kg (CRO) to 0.24 mg/kg (Soil). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). Co ranged from 0.01 mg/kg (Soil) to 0.02 mg/kg (CRO). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). Cr ranged from 0.02 mg/kg (CD) to 0.09 mg/kg (Soil). Effect of initial heavy metal properties of soil, Cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). Cd values were 0.03 mg/kg (CRO) and 0.03 mg/kg (Soil) while it was not detected

in CD. Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). Ba ranged from 9.05 mg/kg (CD) to 45.75 mg/kg (CRO). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). B ranges from 0.35 mg/kg (CD) to 1.11 mg/kg (CRO). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$). K ranged from 1.14 mg/kg (CD) to 3.15 mg/kg (CRO). Effect of initial heavy metal properties of soil, cow dung and crude oil (CRO) varied significantly different ($P < 0.05$).

Table 1b: Initial heavy metal properties of soil, cow dung and crude oil (CRO)

Sample	Cu mg/kg	Co mg/kg	Cr mg/kg	Cd mg/kg	Ba mg/kg	B mg/kg	K mg/kg
Soil	0.24 ^a	0.01 ^c	0.09 ^a	0.03 ^a	21.74 ^b	0.74 ^b	1.72
CD	0.09 ^b	0.01 ^b	0.02 ^b	ND	9.05 ^c	0.35 ^c	1.14
CRO	0.08 ^c	0.02 ^a	0.06 ^b	0.03 ^b	45.79 ^a	1.11 ^a	3.15

Mean with different superscript alphabet are significantly different ($P < 0.05$), CD = Cow dung, CRO = Crude oil, ND = Not Detected

Sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 2. From the result, 5DAP ranges from 0.00 (5 %) to 5.33 (NC). Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 6DAP ranged from 0.00 (%) to 6.00 (NC). Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 7DAP ranged from 5.33 (NC, PC, 3 % and 5 %) to 5.67 (1%). Effect of Sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 8DAP ranges from 4.00 of 5 % to

6.00 of NC. Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 9DAP ranged from 4.33 (5 %) to 6.00 (NC). Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 10DAP ranged from 4.67 (5%) to 6.67 (NC). Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). 12DAP ranged from 5.00 (3%) to 7.00 (PC and 1%). Effect of sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$).

Table 2: Sprouting number of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	5DAP	6DAP	7DAP	8DAP	9DAP	10DAP	12DAP
NC	5.33	6.00	5.33	6.00	6.00	5.67	5.67
PC	4.33	4.33	5.33	5.33	5.67	6.67	7.00
1 %	2.67	2.67	5.67	4.67	5.33	5.67	7.00
3 %	1.00	1.00	5.33	4.67	5.00	5.00	5.00
5 %	0.00	0.00	5.33	4.00	4.33	4.67	5.33

NC = Negative control, PO = Positive control, CRO = Crude oil, DAP = Days after planting

Leaf length (LL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 3. From the result, LL2WAP ranged from 2.00 cm (5%) to 9.33 cm (PC). Effect of the leaf length (LL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). LL4WAP ranged from 3.67 cm (5%) to 11.50 cm (PC). Effect of the leaf length (LL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow

dung varied significantly different ($P < 0.05$). LL6WAP ranged from 3.17 cm (5%) to 14.13 cm (PC). Effect of the leaf length (LL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). LL8WAP ranged from 3.17 cm (5%) to 14.13 cm (PC). Effect of the leaf length (LL) of *C. odorata* treated with varying levels of crude oil - (CRO) enhanced with cow dung varied significantly different ($P < 0.05$).

Table 3: Leaf length (LL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	LL 2WAP cm	LL 4WAP cm	LL6WAP cm	LL8WAP cm
NC	5.40 ^b	7.13 ^b	7.57 ^b	7.57 ^b
PC	9.33 ^a	11.50 ^a	14.13 ^a	14.13 ^a
1 %	3.50 ^{bc}	4.63 ^{bc}	6.70 ^b	6.70 ^b
3 %	3.97 ^{bc}	5.10 ^{bc}	5.00 ^b	5.00 ^b
5 %	2.00 ^c	3.67 ^c	3.17 ^b	3.17 ^b

NC = Negative control, PO = Positive control, CRO = Crude oil, LL = Leaf length, WAP = Weeks after planting

Stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 4. From the result, SG 2WAP ranged from 1.00 cm (NC) to 1.83 cm (1%). Effect of stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). SG 4WAP ranged from 1.07 cm (NC) to 1.97 cm (1%). Effect of stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied

significantly different ($P < 0.05$). SG 6WAP ranged from 1.77 cm (NC) to 3.17 cm (3%). Effect of stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). SG 8WAP ranged from 1.77 cm (NC and 5%) to 3.23 cm (3%). Effect of stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$).

Table 4: Stem girth (SG) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	SG 2WAP cm	SG 4WAP cm	SG 6WAP cm	SG 8WAP cm
NC	1.00	1.07	1.77	1.77
PC	1.23	1.53	2.30	2.30
1 %	1.83	1.97	2.37	2.37
3 %	1.27	1.83	3.17	3.23
5 %	1.27	2.07	1.87	1.77

NC = Negative control, PO = Positive control, CRO = Crude oil, SG = Stem Girth, WAP = Weeks after planting.

Number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 5. From the result, NL 2WAP ranged from 18.00 (3%) to 36.67 (PC). Effect of number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). NL 4WAP ranged from 21.33 (3%) to 43.00 (PC). Effect of number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

varied significantly different ($P < 0.05$). NL 6WAP ranged from 19.00 (5%) to 108.33 (PC). Effect of number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$). NL 8WAP ranged from 19.00 (5%) to 108.33 (PC). Effect of number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P < 0.05$).

Table 5: Number of leaves (NL) of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	NL 2WAP	NL 4WAP	NL6WAP	NL8WAP
NC	33.67	39.67	49.33 ^c	49.33 ^{bc}
PC	36.67	43.00	108.33 ^a	108.33 ^a
1 %	22.67	26.33	63.67 ^b	57.00 ^b
3 %	18.00	21.33	21.67 ^c	23.33 ^{bc}
5 %	20.33	24.67	19.00 ^c	19.00 ^c

NC = Negative control, PO = Positive control, CRO = Crude oil, NL = Number of leaves, WAP = Weeks after planting

Harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 6. From the result, Root weight ranged from 6.30 mg (5 %) to 15.61 mg (PC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Leaf weight ranged from 0.96 mg (5 %) to 28.39 mg (PC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Stem weight ranged from 4.62 mg (5 %) to 34.59 mg (PC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$).

enhanced with cow dung varied significantly different ($P<0.05$). Biomass ranged from 11.89 mg (5 %) to 78.59 (PC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Number of roots ranged from 11.67 (1 %) to 20.67 (PC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Length of the longest root ranged from 6.00 cm (1 %) to 19.00 cm (NC). Effect of harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$).

Table 6: Harvest parameters of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	Root weight Mg	Leaf weight mg	Stem weight mg	Biomass mg	Number of roots	length of the longest root cm
NC	11.79	6.12 ^b	9.66 ^b	27.40 ^b	19.00	19.00 ^a
PC	15.61	28.39 ^a	34.59 ^a	78.59 ^a	20.67	15.67 ^{ab}
1 %	11.02	4.94 ^b	7.84 ^b	23.79 ^b	11.67	6.00 ^c
3 %	7.36	1.28 ^b	6.14 ^b	14.61 ^b	16.67	8.17 ^{bc}
5 %	6.30	0.96 ^b	4.62 ^b	11.89 ^b	19.00	6.33 ^c

NC = Negative control, PO = Positive control

Heavy metals composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung is presented in Table 7. From the result, Cd ranged from 0.07 mg/kg (NC) to 0.24 mg/kg (1 %). Effect of heavy composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Cr ranged from 0.05 mg/kg (5 %) to 0.14 mg/kg (1 %). Effect of heavy composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$).

enhanced with cow dung varied significantly different ($P<0.05$). Pb was not detected in all samples. Hg ranged from 0.05 mg/kg (NC) to 0.13 mg/kg (1 %). Effect of heavy composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$). Zn ranged from 0.08 mg/kg (NC) to 0.24 mg/kg (5 %). Effect of heavy composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung varied significantly different ($P<0.05$).

Table 7: Heavy composition of soil after 8 weeks growth of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung

Treatment	Cd mg/kg	Cr mg/kg	Pb mg/kg	Hg mg/kg	Zn mg/kg
NC	0.07 ^e	0.07 ^b	ND	0.05 ^e	0.08 ^e
PC	0.12 ^d	0.06 ^d	ND	0.06 ^d	0.13 ^d
1 %	0.24 ^a	0.14 ^a	ND	0.13 ^a	0.15 ^c
3 %	0.22 ^b	0.07 ^c	ND	0.12 ^b	0.20 ^b
5 %	0.16 ^c	0.05 ^e	ND	0.09 ^c	0.24 ^a

NC = Negative control, PO = Positive control, ND = Not Detected

Heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period is presented in Table 8. From the result, Cd ranged from 0.00 mg/kg (PC, 1 % and 5 %) to 0.01 mg/kg (3 %). Effect of heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period varied significantly different ($P<0.05$). Cr ranged from 0.00 mg/kg (NC, 1 %, 3 % and 5 %) to 0.01 mg/kg (PC). Effect of heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period varied significantly different ($P<0.05$).

significantly different ($P<0.05$). Pb was not detected in all samples. Hg ranged from 0.00 mg/kg (NC, 1 % and 5 %) to 0.02 mg/kg (3 %). Effect of heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period varied significantly different ($P<0.05$). Zn ranged from 0.02 mg/kg (1 % and 5 %) to 0.05 mg/kg (3 %). Effect of heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period varied significantly different ($P<0.05$).

Table 8: Heavy metal composition of *C. odorata* treated with varying levels of crude oil (CRO) enhanced with cow dung after 8 weeks growth period.

Treatment	Cd mg/kg	Cr mg/kg	Pb mg/kg	Hg mg/kg	Zn mg/kg
NC	ND	0.00 ^c	ND	0.00 ^c	0.03
PC	0.00 ^c	0.01 ^a	ND	0.01 ^b	0.04 ^b
1 %	0.00 ^c	0.00 ^b	ND	0.00 ^c	0.02 ^d
3 %	0.01 ^a	0.00 ^c	ND	0.02 ^a	0.05 ^a
5 %	0.00 ^b	0.00 ^b	ND	0.00 ^c	0.02 ^e

NC = Negative control, PO = Positive control, ND = Not Detected

DISCUSSION

The *C. odorata* remediation capabilities grown in varying crude oil concentrations was assessed. The results of this study indicated that, among the three-growth media, mater soil had the highest levels of Cu, Co, and Cr. This could be due to anthropogenic activities or other human activities occurring in the vicinity of the collecting location. Kogbara *et al.* (2019) confirmed this by reporting the presence of Ar, Cd, Copper, Zinc, Pb, Ni, V in varying quantities in drill cuttings and soil used in the planting media before mixing. Ifediora *et al.* (2023) also found that most heavy metals were present in soil and hydrocarbons like oil when they studied the changes in the physicochemical and heavy metal properties of soil treated with used motor oil and poultry manure after 12 weeks of growing *Phyllanthus urinaria*. The findings of Merkl *et al.* (2012), is consistent with this who investigated the effects of crude oil on tropical plant growth and phytoremediation in the tropics. They found that soil had greater chemical characteristics before contamination than did the crude oil. The findings demonstrated considerable variation ($P < 0.05$) in the levels of heavy metals in soil, cow dung, and crude oil. In comparison to soil and crude oil, cow dung had a less effect on the amounts of heavy metals in the growth media.

The result of the sprout count of *C. odorata* showed that sprouting occurred after five days of initiation. Although, in the first six days sprouting count for 5 % and 3 % crude oil sample was very low, no significant difference was observed throughout the twelve days of observation. Germination pattern was largely not affected by the crude oil contamination over time. Hence, *C. odorata* was able to easily utilize soil nutrient needed for germination irrespective of the presence of crude oil contamination. This may be as result the presence of the organic amendment. Among some tropical plants, Arthur *et al.* (2005) reported the concept of certain plants with heavy metal phytoremediation potential, and with specific characteristics: (1) the ability to accumulate metals, preferably in the aboveground parts; (2) tolerance to metal concentrations accumulated; (3) fast growth and high biomass; (4) a widespread, highly branched root system, (5) easy harvest ability; and (6) inability to be consumed by humans and animals. In a similar study on the potential of *C. odorata* (L) to decontaminate used engine oil impacted soil under greenhouse conditions, Atanaga (2011) reported that application of oil to soil has been reported to have different effects on germination of seeds. This was visible to the fact that 0 to 5 kg soil contamination had 80 % germination compared to 30 – 40 kg oil contamination which had less than 60 % germination rate.

The positive control samples varied significantly ($P < 0.05$) with all other treatment samples in the eight weeks of observation.

However, no significant variation ($P > 0.05$) was observed in among the negative control, 1.0 %, and 3.0 % samples. This may be a result the presence of the organic amendment and an indication that cow dung amplified the nutrient available in the soil and thereby aided the good growth performance of *C. odorata*. The 5.0% samples had the least leaf length performance across the time of observation. This indicated that at a higher crude oil percentage growth performance is lowered irrespective of the organic material added to ameliorate the soil. This may be as a result of physiological adaptations. This findings was supported by Uku and Dumkhana (2023) who reported that crude oil degraded more quickly in reactors that contained a mixture of yeast, NPK, and powdered swamp soil (*Moringa oleifera*) from moringa seeds in the study on mathematical models for the determination of best practice or method for remediation of crude oil contaminated clay and swampy soil environment. Nwokeji and Osaro-Itota (2017) was also in agreement in their work on the comparative evaluation of the physiochemical parameters of crude oil polluted soil remediated with mushroom (*Pleurotus tuberregium*). They deduced that the results provided evidence that showed that mushroom supplements modify the physical, chemical and biological properties of crude oil polluted soils and improve their nutritional status thereby restoring the fertility of the soil for agricultural purposes.

The stem girths did not vary along the treatment sample in all the eight weeks of observation. However, lower crude oil level (1.0 %, 2.0% and 3.0 %) and the positive control sample had greater stem girth than the negative control and 5 % crude oil sample. Hence, the ameliorating potentials of cow dung is active at lower percentage contamination. Similar trend was observed in the number of leaves produced by *C. odorata* in the eight weeks observation. The root weight, the leaf weight, stems weight, biomass, number of roots, and length of longest root followed the same trend. Hence, presence of heavy metal affected the general growth response. Although the ability of the *C. odorata* to sprout and grow in all the treatment can be seen as good remediation potential. Plant biomass has been widely used to evaluate metal tolerance (Hu *et al.*, 2012). The hydroponic experiment revealed that *C. odorata*, *I. patula*, and *G. pseudochina* had a strong tolerance to Cd stress, with no significant differences in growth among any of the treatments (Jampasri *et al.*, 2021).

Observably metal in soil were much lesser than they were prior to initiation of plant. This may be as a result of the ability of *C. odorata* to take up and utilize heavy metals in soil. Ngozi *et al.* (2009) supported in their study on the phytoremediation potential of *C. odorata* in heavy metal polluted environments. In their findings they

reported higher heavy metals in composite samples than the control sample. Ram *et al.* (2019) used two Napier grass (*Pennisetum americanus* L. × *Pennisetum purpureum* Schumacher) to study uptake of Cr and reported that even at highest Cr (VI) concentration, plant exhibited strong resistance, as evidenced by increase in superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities. The heavy metal (Cd, Cr, Hg, Zn) was lesser in the control samples, hence crude oil contamination was responsible for higher heavy metal residue in the soil. Toxic metals like lead was not observed in all the soil samples. Nevertheless, higher metals observed in crude oil contaminated soil showed that crude oil reduces the remediation capacity of the plant.

The heavy metal levels in the plant was very non-existing in some toxic metals like Pb, Cd, Cr, and Hg. The available metals in the plant is as a result of the its ability to take up metals. Phytoremediation of contaminated sites were found to be dependent upon the soil amendments used as they rendered contaminants unavailable for uptake. Similar findings were made by Ifediora *et al.* (2023) that reported that higher heavy metal was observed to be in spent engine oil than those of the farm soil; confirming hydrocarbon oil as soil contaminant. Given the reduction in heavy metal originally in the soil. Reduction in heavy metals in the soil treated with hydrocarbons over time of planting have been reported by Udom and Nuga (2015) in the study of the biodegradation of petroleum hydrocarbons in a tropical ultisol using legume plants and organic manure.

Conclusion

This study was done to observe the remediation potentials of *C. odorata* grown on different levels of crude oil enhance with cow dung. The findings this study indicated that soil, crude oil and cow dung are natural sources of heavy metal. The sprout response of *C. odorata* was observed not to significantly differ ($P > 0.05$) along the treatment. The least performed sample in sprouting was the 5 % crude oil sample. The *C. odorata* had better growth response in cow dung treated samples. Specifically, positive control and 1 % crude oil treated sample had bigger stem girth, longer leave and root numbers. The biomass was observed to be higher in cow dung treated samples, although they didn't significantly differ ($P > 0.05$) along the treatment. Higher crude oil treatment samples negatively affected the root length of *C. odorata*. The available heavy in the plant samples were generally low and at a negligible level. Lesser levels of heavy metals in the soil after growth period and availability of heavy metal in the plant tissue indicates the phytoremediation potentials of *P. purpureum*.

REFERENCES

- Ahmed, M. M. M., Mazen, M. B. D., Nafady, N. A. and Monsef, O. A. (2017). Bioavailability of cadmium and nickel to *Daucus carota* L. and *Corchorus olitorius* L. treated by compost and microorganisms. *Soil and Environment*, 36: 1-12.
- Andreoni, V., Cavalca, L., Rao, M., Nocerino, G., Bernasconi, S., Dell Amico, E., Colombo, M., and Gianfreda, L. (2004). Bacterial communities and enzyme activities of PAHs polluted soils. *Chemosphere*, 57: 401-412.
- Aqeel, A. and Butt, M. S. (2001). The relationship between energy consumption and economic growth in Pakistan. *Asia-Pacific Development Journal*, 8: 101-110.
- Arthur, E. L., Rice, P. J., Rice, P. J., Anderson, T. A., Baladi, S. M., Henderson, K. L. and Coats, J. R. (2005). Phytoremediation—an overview. *Critical Reviews in Plant Sciences*, 24(2): 109-122.
- Atagana, H. I. (2011). The potential of *Chromolaena odorata* (L.) to decontaminate used engine oil impacted soil under greenhouse conditions. *International Journal of phytoremediation*, 13(7): 627-641.
- Azubuike, C. C., Chikere, C. B. and Okpokwasili, G. C. (2016). Bioremediation techniques—classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, 32: 180.
- Balba, M., Al-Awadhi, N. and Al-Daher, R. (1998). Bioremediation of oil-contaminated soil: Microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods*, 32: 155-164.
- Chandra, S., Sharma, R., Singh, K. and Sharma, A. (2013). Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Annals of Microbiology*, 63: 417-431.
- Chen, M. and L. Q. Ma (2001) Comparison of three aqua regia digestion methods for twenty Florida soils. *Soil Science Society of American Journal*, 65(2):491-9.
- Cheng, L., Zhou, Q. and Yu, B. (2019). Responses and roles of roots, microbes, and degrading genes in rhizosphere during phytoremediation of petroleum hydrocarbons contaminated soil. *International Journal of Phytoremediation*, 21: 1161–1169
- Ciriaková, A (2009). Heavy metals in the vascular plants of Tatra Mountains. *Oecologia Montana*, 18: 23-26.
- Compant, S., Saikkonen, K., Mitter, B., Campisano, A. and Mercado-Blanco, J. (2016). Editorial special issue: soil, plants and endophytes. *Plant Soil*, 405: 1-11.
- Correa-Garcia, S., Pande, P., Séguin, A., St-Arnaud, M. and Yergeau, E. (2018). Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. *Microbiology and Biotechnology*, 11: 819–832.
- Duffy, J. J., Peake, E. and Mohtadi, M. F. (2000). Oil spills on land as potential sources of groundwater contamination. *Environmental International*, 3: 107–120
- Fatima, K., Imran, A., Amin, I., Khan, Q. M. and Afzal, M. (2017). Successful phytoremediation of crude-oil contaminated soil at an oil exploration and production company by plants-bacterial synergism. *International Journal of Phytoremediation*, 4: 432 – 440.
- Glick, B. R. (2010). Using soil bacteria to facilitate phytoremediation. *Biotechnological Advances*, 28: 367-374.
- Hu, P., Yuan-Yuan, G. A. N., Ye-Tao, T. A. N. G., Zhang, Q. F., Jiang, D., Nan, Y. A. O. and Rong-Liang, Q. I. U. (2012). Cellular tolerance, accumulation and distribution of cadmium in leaves of hyperaccumulator *Picris divaricata*. *Pedosphere*, 22(4): 497-507.
- Ifediora, N., Oti, V., and Adaji, A. (2023). Changes in physicochemical and heavy metal properties of soil treated with spent engine oil and poultry manure after 12 weeks of growing *Phyllanthus urinaria*. *BIU Journal of Basic and Applied Sciences*, 8(1): 36-48.
- Ijaz, A., Imran, A., Haq, M. A., Khan, Q. M. and Afzal, M. (2015). Phytoremediation: Recent advances in plant endophytic synergistic interactions. *Plant and Soil*, 12: 1-17.
- Jampasri, K., Saeng-Ngam, S., Larperkarn, P., Jantasorn, A., and Kruatrachue, M. (2021). Phytoremediation potential of *Chromolaena odorata*, *Impatiens patula*, and *Gynura*

- pseudochina* grown in cadmium-polluted soils. *International Journal of Phytoremediation*, 23(10): 1061-1066.
- Janbandhu, A. and M. Fulekar. (2011). Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. *Journal of Hazardous Materials*, 187: 333-340.
- Khan, S., M. Afzal, S. Iqbal and Q.M. Khan. (2013). Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. *Chemosphere* 90: 1317-1332.
- Kogbara, R. B., Badom, B. K. and Ayotamuno, J. M. (2019). Tolerance and phytoremediation potential of four tropical grass species to land-applied drill cuttings. *International Journal of Phytoremediation*, 20: 1-10.
- Kuske, C. R., Ticknor, L. O., Miller, M. E., Dunbar, J.M. Davis, J.A. Barns, T. and Belnap, J. (2002). Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68: 1854 - 1863.
- Meagher, R. B. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biology*, 3: 153-162.
- Merkel, N., Schultze-Kraft, R. and Infante, C. (2004). Phytoremediation in the tropics: The effect of crude oil on the growth of tropical plants. *Bioremediation Journal*, 8:177-184.
- National Root Crops Research Institute, Umudike (NRCRI): Geographical data. (2022).
- Ngozi, I. M., Jude, I. C. and Catherine, I. C. (2009). Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. *Pakistan Journal of Nutrition*, 8(5): 521-524.
- Nwokeji, P. A. and Osaro-Itota, O. (2017) Comparative evaluation of the physiochemical parameters of crude oil polluted soil remediated with mushroom (*Pleurotus tuberregium*) *WorldWide Journal of Multidisciplinary Research and Development*, 3(7): 363- 367.
- Ogedegbe, A., Uwaila, Ikhajiagbe, B. and Anoliefo, G. O. (2013). Growth response of *Alternanthera brasiliana* (L.) Kuntze in a waste engine oil-polluted soil. *Journal of Emerging Trends in Engineering and Applied Sciences*, 4(2): 322-327.
- Pandey, P. H. Pathak and Dave, S. (2016). Microbial ecology of hydrocarbon degradation in the soil: A Review. *Research Journal of Environmental Toxicology*, 10: 1-12.
- Ram, B. K., Han, Y., Yang, G., Ling, Q. and Dong, F. (2019). Effect of Hexavalent Chromium [Cr(VI)] on phytoremediation potential and biochemical response of hybrid Napier grass with and without EDTA application. *Plants*, 8(11): 515 - 535.s
- Reda, A. B. and Ashraf, T. (2010). Optimization of bacterial biodegradation of toluene and phenol under different nutritional and environmental conditions. *Journal of Applied Sciences Research*, 34: 1086-1095.
- Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W. W., Fallmann, J. and Puschenreiter, M. (2013). The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biochemistry*, 60: 182-194.
- Shabir, G., Arslan, M. Fatima, K., Amin, I., Khan, Q. M. and Afzal, M. (2016). Effects of inoculum density on plant growth and hydrocarbon degradation. *Pedosphere*, 26: 774-778.
- Udom, B. E. and Nuga, B. O. (2015). Biodegradation of petroleum hydrocarbons in a tropical ultisol using legume plants and organic manure. *Journal of Agricultural Science*, 7(4): 174 178.
- Uku, E. P. and Dumkhana, B. B. (2023). Comparison of Different Models for the Remediation of Crude Oil Contaminated Clay and Swampy Soil Environment. *International Journal of Pollution: Prevention and Control*, 1(1): 1-8.
- USEPA (2001). Brownfields technology primer: selecting and using phytoremediation for site cleanup. EPA 542-R-01-006 (United States Environmental Protection Agency, 2001).
- Weyens, N., Van der Lelie, D., Taghavi, S. and Vangronsveld, J. (2009). Phytoremediation: Plant- endophyte partnerships take the challenge. *Current Opinion in Biotechnology*, 20: 248-254.