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Phytoremediation of petroleum hydrocarbon-contaminated soils with *Jatropha curcas* and *Vetiveria zizanioides* at Ghana Manganese Company Ltd.

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Abstract
The study investigated the growth performance and decontamination potential of two plant species on hydrocarbon concentration levels in petroleum polluted soils. A 2x2x3 factorial arrangement of treatments in a completely randomized design with 3 replications was adopted. Compost amended *Jatropha curcas* (JC) treatment caused 78.8 and 82.2% while *Vetiveria zizanioides* (VZ) caused 51.1 and 39.7% decline in soil TPH and TOG concentrations, respectively after 16 weeks. The reduction in TOG and TPH concentrations in both JC and VZ was significantly higher ($p<0.0001$) in compost amendments. Species effect on TOG and TPH concentrations were not statistically significant ($p = 0.081$). Contaminated soils repressed plant growth significantly in both species ($p < 0.05$). Phytoremediation with *Jatropha curcas* amended with compost is an alternate viable and rapid means of remediating hydrocarbon contaminated soils. Further research should examine type of compost and compare phytoremediation to other modes of hydrocarbon decontamination rates.

Introduction
Mining operations among the most crude-oil intensive sectors that contaminates soils and ecosystems with petrochemicals. Petroleum contamination of soil occurs through extraction, accidents, transportation, and leakage from tanks, pipeline ruptures, consumption and refining, land disposal of petroleum waste, and accidental or intentional spills (Robson, 2003; Scott, 2003). This results in soil hydrocarbon concentrations as high as 52,328 – 107,190 ug/g within the top 30 cm of soils near old wells, transport lines, and refueling areas (Sari *et al.*, 2018) and 90.72 - 121.79 mg kg^{-1} within/near workshops (Khan and Kathi, 2014). Such high concentrations exceed the maximum acceptable limits of TPH of 10,000 ug/g in soil (Agency for Toxic Substances and Disease Registry, 1999; Hamilton and Sewell, 1999).
Oil pollution lowers permeability and infiltration of water in soils, leading to accumulation of water on the soil surface and artificial droughts in the sub-surface soils. This restricts water and nutrient uptake by roots within the sub-soil. Furthermore, hydrocarbon toxicity can damage the function and stability of soils and lead to repressed plant growth and agricultural productivity (Xie *et al.*, 2017). To avert these hydrocarbon pollution related risks, it is recommended that crude oil polluted sites be cleaned up or remediated.
One promising environmentally benign, economically effective, non-sophisticated technology of remediating oil contaminated sites is phytoremediation. It entails the direct use of green plants for in-situ removal, degradation, and containment of contaminants in soils, sludge, sediments, surface water, and groundwater (Gerhardt *et al.*, 2009). Several plant species including maize, wheat,

brittle grass, alfalfa, *Jatropha curcas*, Vetiveria are known to significantly reduce oil contamination levels in soils with varying levels of efficiency and decontamination mechanism (Agamuthu *et al.*, 2010; Shahsavari *et al.*, 2013; Xie *et al.*, 2017). Despite these significant strides, phytoremediation as a means of hydrocarbon decontamination is of limited application within the oil producing and high consumption centers of West Africa. This study assesses the growth performance and decontamination potential of two plant species; *Vetiveria zizanioides* and *Jatropha curcas* on hydrocarbon contaminated soils at Ghana Manganese Company (GMC) Ltd.

Material and Methods

The experiment was conducted at GMC Ltd, Nsuta, located in the Tarkwa-Nsuaem Municipality (southwestern Ghana) between latitudes; 4°0' 0" N and 5°40'0" N and longitudes; 1°45'0" W and 2°1'0" W. A three factorial design, with "soil contamination level", "amendment" and "plant species", as treatment factors was adopted. Contaminated soils, obtained from the volatilization pad of the GMC, were sieved and the initial concentrations of total petroleum hydrocarbon (TPH) and total oil and grease (TOG) determined using the gravimetric technique (Weisman, 1998; Villalobos *et al.*, 2008). Non-contaminated soil was collected from a top soil heap site, located on the GMC mine site without any history of exposure to oil spills. Two plant species *Jatropha curcas* (JC) and *Vetiveria zizanioides* (VZ) were planted on equal weight of soil 1.5 kg placed in plastic containers of 25 cm diameter. Three amendments comprising of no amendment, compost and NPK fertilizer treatments were then applied in the respective containers. Each treatment combination was replicated three times. Equal lengths of 20 cm stem cuttings of JC and tillers of VZ were planted in September 2018. The sprouting, survival and growth of seedlings were monitored for 16 weeks. The height, collar diameter, and number of leaves of JC as well as the number of tillers and length of tillers of VZ were measured every other week. Soil hydrocarbon concentration was determined for each treatment at the end of the sixteenth week. The data was subjected to analysis of variance (ANOVA) using R version 3.5.3 and significant differences between treatments established via Tukey multiple range test ($p \leq 0.05$).

Results and Discussion

Sprouting and growth performance of the two plant species

Two weeks after establishment, 100% sprouting success was recorded in both species. The average number of leaves at this time in JC was 3.4±0.7 and 2.4±0.3 in the contaminated and non-contaminated soils, respectively. By the 16th week, JC on non-contaminated soils had 3.39±0.4 more leaves than the JC plants on contaminated soils while the number of tillers of VZ on non-contaminated and contaminated soils were respectively, 26.5±1.1 and 23.8±1.4. Number of leaves ($p = 0.009$), collar diameter ($p = 0.010$) of JC and number of tillers ($p=0.048$) of VZ were significantly higher in non-contaminated soils than in contaminated soils.

The effect of soil amendment on number of leaves, increase in height and diameter of 16 week old JC plants was statistically significant ($p \leq 0.003$). Soil contamination level and amendment interaction was not significantly different. Amending both contaminated and non-contaminated soils with compost yielded significantly higher number of leaves (33.55±1.6), height (12.34±0.9) and collar diameter (8.63±0.3) compared to the control and fertilizer treatments (Table 1).

Plant species and amendment effects on soil total petroleum hydrocarbon and total oil and grease concentration levels

The initial total oil and grease (TOG) and total petroleum hydrocarbon (TPH) concentrations in soils at the start of the experiment were 31,333.33 mg kg^{-1} and 22,666.67 mg kg^{-1}, respectively. After sixteen weeks of experimentation, *Jatropha curcas* treated soils had 46.7 % and 46.4 % reduction in TOG and TPH, respectively while *Vetiveria zizanioides* caused 31.6 % and 23.5 %

reduction in TOG and TPH, respectively. The soil TPH concentrations remaining at 112 days of experimentation was significantly higher in JC than in VZ (Figure 1). The mechanism of degradation and the influence of plant species on degrader populations may account for these differences. While JC is a rhizodegrader of petroleum at a rather rapid rate (Agamuthu *et al.*, 2010), vetiver tends to accumulate contaminants in shoot and may degrade hydrocarbon slowly through rhizofiltration (Brandt *et al.*, 2006). Furthermore, populations of petroleum degraders can differ widely between soils under the influence of different species (Xie *et al.*, 2017).

Table 1. Comparison of growth responses of 16 week old *Jatropha curcas* and *Vetiveria zizanioides* plants to contaminated soils amended with compost and fertilizer. Numbers in the same column followed by different letters indicate significant difference between amendments at $\alpha < 0.05$.

Amendment	*Jatropha curcus*			*Vetiveria zizanioides*	
	Number of Leaves	Height (cm)	Collar diameter (cm)	Number of tillers	Height (cm)
Compost	33.55±1.6a	12.34±0.9a	8.63±0.3a	28.72±1.2a	124.73±3.1a
Control	24.17±1.2b	5.70±0.6b	7.70±0.2b	22.12±1.6b	118.82±17.5a
Fertilizer	27.30±1.4b	8.32±0.9b	7.58±0.1b	24.52±0.5b	116.12±4.1a

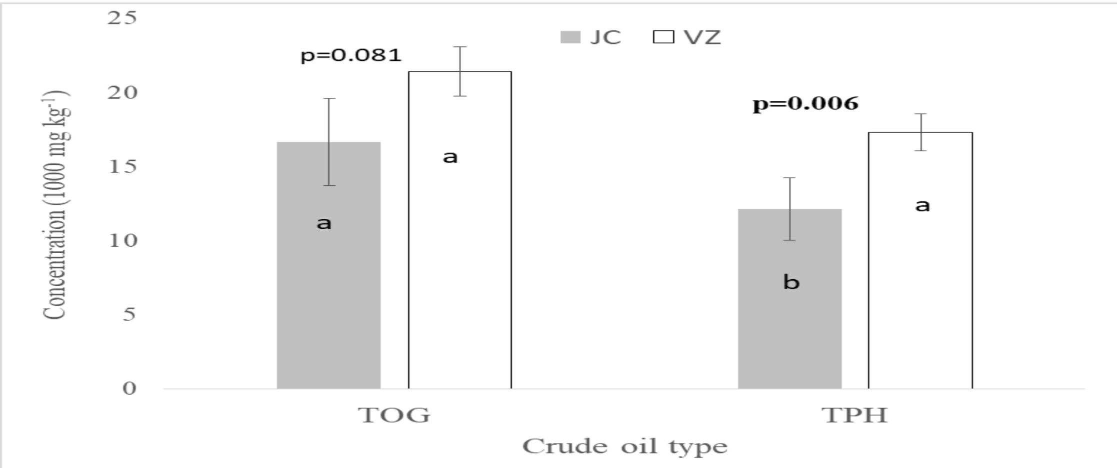

Figure 1. Soil total petroleum hydrocarbon (TPH) and total oil and grease (TOG) concentrations remaining in soils planted with two species: *Jatropha curcas* (JC) and *Vetiveria zizanoides* (VZ) for a period of 16 weeks. Bars followed by the same letter are not significantly different.

The quantity of TOG and TPH loss from the soil after 112 days of experimentation differed significantly among soil amendment ($p<0.0001$) (Figure 2). Compost amendment had the highest significant reduction in TOG (20950±2406 mg kg^{-1}) and TPH (13433±2129 mg kg^{-1}) compared to fertilizer amendment and the control. Compost enriches the soil with organic carbon and other nutrients particularly nitrogen and phosphorous which enhances the proliferation of hydrocarbon utilizing bacteria. This expedited the degradation of petroleum hydrocarbons in soils (Lawson and Nartey, 2012). In addition, the inherently high microbial populations in compost media readily metabolized the contaminant hydrocarbons in the soils (Atagana, 2008).

Conclusions and Outlook

Hydrocarbon contamination of soils significantly inhibits plant growth. On the other hand decontamination of these contaminants depends on the kind of plant species and amendments in the soil medium. *Jatropha* curcas decontaminated the soils of pollutants rather rapidly and faster than *V. zizanioides*. Amending the soils with compost resulted in even higher decontamination rates in both species but much higher for *Jatropha curcas*. We conclude *Jatropha curcas* grown on petroleum contaminated soils amended with compost offers an alternative more efficient means to decontaminate petroleum contaminated soils. Phytoremediation technology with *Jatropha curcas* with compost amendments is hence recommended as a viable means to address oil contamination challenges in the oil producing areas of West Africa.

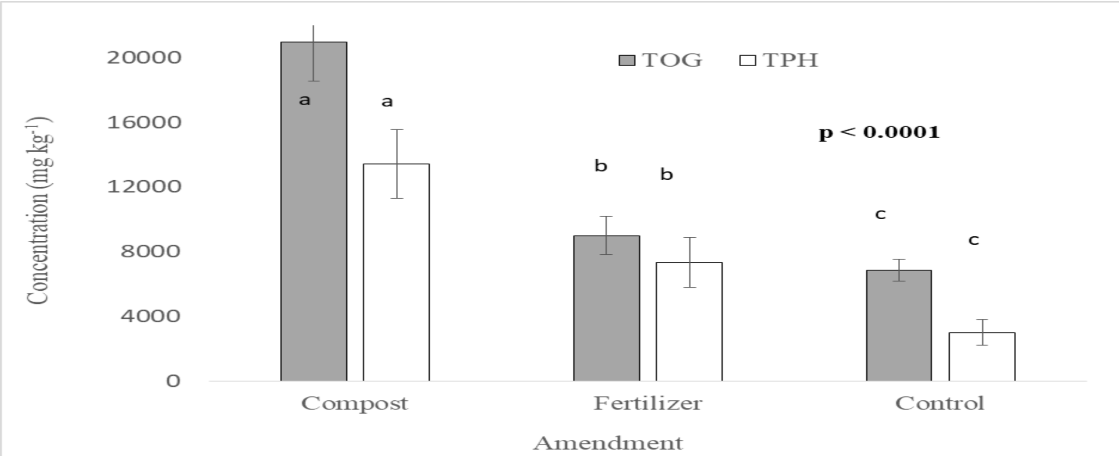

Figure 2. Soil amendment effect on the mean quantity of total petroleum hydrocarbon (TPH) and total oil and gas (TOG) loss from the soil after 16 weeks of planting with *Jatropha curcas* and *Vetiveria zizanioides.* Bars followed by different letters indicate statistically significant difference between amendments at $\alpha \leq 0.05$.

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