Soil Environ. 39(2): 184-193, 2020
DOI:10.25252/SE/2020/162236
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Goat droppings attenuates morphological and metabolic aberrations in cowpea seedlings grown in crude oil polluted soil

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[Received:** May 24, 2020 **Accepted:** October 09, 2020 **Published Online:** November 28, 2020**]

Abstract

The ameliorative potential of goat droppings (GD) for crude oil polluted soil was explored in this study. The aim of this study was to evaluate the possibility of using stored goat droppings to attenuate crude oil toxicity on crop plants. It comprised six study groups as follows; (group 1= control; group 2=unpolluted soil+75g GD; group 3=unpolluted soil +150g GD; group 4= polluted soil; group 5=polluted soil+75g GD; group 6=polluted soil +150g GD). There was crude petroleum mediated significant decreases in stem length, leave length and breadth, plant height and root length. Also observed were significant reduction in chlorophyll and beta-carotene contents of cowpea seedlings grown in polluted soils compared to those raised unpolluted soils. Antioxidant indices (glutathione, superoxide dismutase, catalase, glutathione peroxidase, vitamin C, and uric acid) were all significantly reduced cowpea seedlings grown in polluted soil, compared to control. GD amendment of polluted soil significantly elevated the antioxidants near control values. Also observed were significant rise in lipid peroxidation levels in cowpea seedlings grown in crude oil polluted soils while GD amendment significantly reversed the trend. Furthermore, the activities of drug metabolizing enzymes (glutathione-s-transferase, sulphite oxidase, xanthine oxidase and aldehyde oxidase) reduced concomitantly with lipid peroxidation in seedlings grown in crude oil polluted soils. All observed aberrations in the seedlings grown in polluted soil were significantly reversed to near control values in seedlings grown in soil samples amended with GD with and without petroleum treatments. The positive effect of GD on growth parameters, chlorophyll and beta-carotene, antioxidants and oxidative stress indices in cowpea seedling grown in polluted and unpolluted soils are indicative of the efficacy of GD to reduce crude oil toxicity and holds a potential as organic manure.

Keywords: Goat droppings, toxicity, pollution, crude oil, cowpea

Introduction

Food sustainability continues to be a challenge to most economies owing to continual increase in human populations world over. In addition, industrialization continues to pose great challenge to efforts made to cushion the monster of hunger before us as a result of waste generation from human activities. This is because the aftereffects of most industrial activities have continually left our supposed agricultural lands no longer arable (Tetteh, 2015).

Plants growing in soils contaminated with various hydrocarbons have been reported to exhibit several anomalies in their metabolism and growth indices. Some of the anomalies and symptoms according to earlier studies include reduced chlorophyll content, decreased protein content, reduced carbohydrates and ascorbic acid as well as inhibition of arrays of antioxidant enzymes, and thus, culminating in increased lipid peroxidation in most plants exposed to petroleum pollution (Achuba 2006; Peretiemo-Clarke and Achuba 2007; Agbogidi *et al.*, 2007; Al-Hawas *et al.* 2012; Achuba and Okoh, 2014; Achuba and Ja-anni, 2018, Achuba and Ohwofasa, 2019).

The militating effects of crude oil on food sufficiency have led to continuous research efforts in most oil rich nations to see for the remediation of most contaminated sites. Although most methods used for remediation remain elusive because of cost and further contributing to environmental contamination, there is a new trend of incorporating readily available and cost-effective agricultural wastes for crude oil clean up. Some of the earlier explored options include cow dung (Njoku *et al.*, 2008), abattoir effluent (Achuba and Erhijovwo, 2017; Achuba and Iserhienrhien, 2018), oil palm leaves (Achuba, 2019).

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Over the years, many goat droppings have been tried either as a source of organic manure or organic remediating agent of contaminated soil (Nwogu *et al*., 2015; Ukpaka and Amadi, 2016; Erenee *et al.*, 2017). One major drawback of the use of organic manure is seasonal availability or insufficient quantity at a time. In a bid to promote continuous availability of sufficient quantity at all time, this study focuses on the effect of treatment of crude oil polluted soil with stored goat droppings on the growth and metabolic features of cowpea seedlings.

Materials and Methods

Soil sample

The soil sample employed for this study was collected from fallow land in site III, opposite Faculty of Science in Delta state University, Abraka, Nigeria. The soil was sieved with a 2 mm mesh and stored under laboratory conditions for use. The physiochemical properties of the soil sample are as published by Achuba and Okoh (2014).

Table 1: physicochemical properties of test soil

Parameters	Value
pH	6.09
Total organic carbon %	2.90
Phosphorus mg kg^{-1}	<0.01
Nitrogen mg kg^{-1}	8.47
Nitrate mg kg^{-1}	9.86
Cation exchange capacity c mol kg^{-1}	0.74
Sodium mg kg^{-1}	9.06
Potassium mg kg^{-1}	6.72
Calcium mg kg^{-1}	2.98
Magnesium mg kg^{-1}	0.31

Source: Achuba and Okoh (2014).

Goat droppings

Fresh goat droppings were collected from a goat farmer and dried to remove moisture; the dried goat dropping was then ground into fine powder and stored at room temperature for future use. The physicochemical properties of goat droppings are depicted in table 2.

Table 2: physicochemical properties of goat droppings

Parameter	Value
pH	6.4
Total organic Matter %	72.1
Phosphorus mg kg^{-1}	3.9
Nitrogen mg kg^{-1}	3.8
Magnesium mg kg^{-1}	0.8
Potassium mg kg^{-1}	1.8
Calcium mg kg^{-1}	0.8
Moisture (%)	16.8

Experimental design

The experiment was subdivided into a preliminary experiment and a major experiment. The preliminary experiment was undertaken to establish the quantity of stored goat droppings that enhanced the growth of cowpea seedlings. Five undamaged cowpeas, initially presumed viable by water floatation method (Achuba, 2006), were sown in each polybag and each treatment was watered with 100 mL of tap water to keep the soil moist. After 12 days, it was observed that seedlings treated with 75 g to 190 g enhanced the growth of the cowpea seedlings relative to those grown in other concentrations. This was the basis for treating the samples with the quantities of goat droppings.

Fifty kilograms of filtered loamy soil was mixed thoroughly with one litre of Bonny Light crude oil collected from Nigerian National Petroleum Commission (NNPC) Warri. From this bulk sample, 600 g of polluted soil was measured into nine different bowls. Another set of 600 g of filtered loamy soil was measured into nine different bowls. A weighing balance was used to measure 75 g (soil: goat droppings, 4:1) and 150 g (soil: goat dropping, 8:1), respectively. To three out of the nine polluted soils 75 g the ground goat droppings was added, and 150 g of the goat droppings was added to another three polluted soil samples and the other three polluted soil samples were left untreated with goat droppings. This same procedure was carried out for the unpolluted soil samples, as 75 g of ground goat dropping was added to three unpolluted soil samples, 150 g to another three sets of unpolluted soil and the last set of three soil samples were left untreated with goat droppings.

After thorough mixing of soil, crude oil, goat droppings and 100 mL of water in the respective groups, each soil mixture was put in polythene planting bags and labelled appropriately. This experiment had six groups as shown in the table below.

Table 2.1: Experimental design and treatment distribution

Groups	Soil Type	Goat dropping (600 g^{-1} soil)	Crude Oil
Group 1	Normal Soil	0	-
Group 2		75 g	-
Group 3		150 g	-
Group 4	Crude oil	0	+
Group 5	Polluted Soil	75 g	+
Group 6		150 g	+

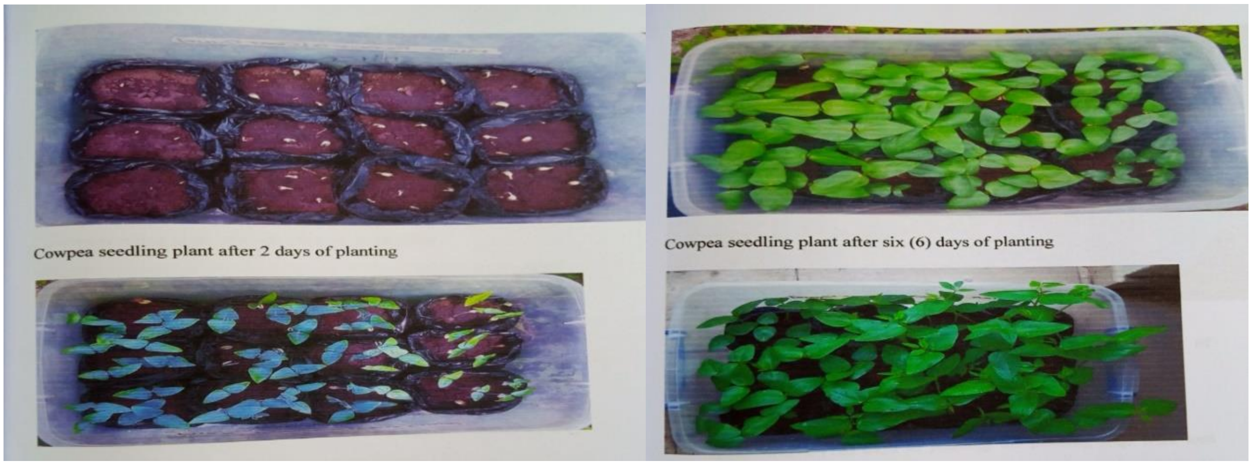
The – sign indicates absence of the crude oil while + indicates the presence of crude oil. The main experiment was done by the sowing of five seedlings in each of the treatments and watered daily with 100 mL of tap water. The seeds were allowed to germinate and grow for twelve days. The twelve-day-old seedlings were used for various morphological and biochemical analyses.

Determination of morphological parameters

The seedlings were carefully removed from the polypots and washed under slowly running tap to remove soil particles and other debris. The morphological indices (leaf length, leaf width, stem length, root length) of the 12-day -old cowpea seedlings were taken with measuring ruler.

Determination of photosynthetic pigments (chlorophyll and β-carotene)

Methanol (10 ml of 96%) was used to homogenize the leaves (1.0 g) with a mortar and pestle. The homogenate was



Cowpea seedling plant after 4 days of planting

Cowpea seedling plant after 9 days of planting

Cowpea seedling plant after 12 days of planting

Figure 1: Pictorial representation of seedlings 2-12 days post planting periods

Table 3: Effect of goat droppings treatment of crude oil polluted soil on morphological parameters of cowpea seedlings

Soil Type	Goat dropping (600 g^{-1} soil)	Stem length (cm)	Leaves Length (cm)	Leaves Breadth (cm)	Plant Height (cm)	Root Length (cm)
Normal Soil	0	16.73±0.59^a	6.13±0.23^a	3.70±0.20^a	30.10±1.64^a	13.03±0.60^a
	75g	22.17±0.76^b	6.50±0.50^a	4.30±0.46^b	45.87±1.21^b	21.50±0.50^b
	150g	19.33±0.76^c	6.57±0.40^a	3.83±0.15^a	36.50±2.18^c	15.00±2.00^a
Crude Oil Polluted Soil	0	8.63±2.58^d	4.17±0.53^b	2.93±0.29^c	19.92±1.30^d	7.37±1.46^c
	75g	16.33±1.55^a	6.07±0.40^a	3.60±1.00^a	27.67±5.51^a	12.33±1.53^a
	150g	13.00±1.00^e	5.88±0.91^a	3.57±0.32^a	21.67±2.80^d	8.33±0.76^c

All Values are expressed as Mean ± SD of five replicates. Values followed by different alphabet superscript in the same column indicate that there is a significant (p <0.05) difference.

centrifuged at 2500 rpm for 10 minutes. The supernatant produced was used to determine concentrations of chlorophylls and carotene following the methods of Lichtenthaler (1987) and Duxbury and Yentsch (1956), respectively.

Preparation of homogenate for biochemical assays

The leaves (0.5 g) were collected and washed with ice-cold water (4 °C). The leaves from each group were differently homogenized with 0.1g butylated hydroxyl toluene (BTH) in 10 mL of 0.05M Phosphate buffer, pH 7.4 at 4 °C . Each mixture was filtered with cheese cloth and the filtrates were centrifuged at 7000 g for 20 minutes (4 °C). The supernatants (S_1) generated were used for the assays of biochemical indices

Determination of lipid peroxidation and antioxidant parameters

The protocol of Gutteridge and Wilkins (1982) was adopted to determine lipid peroxidation using extinction coefficient of 1.56 x10^5 M/cm to calculate the concentration of malondialdehyde (MDA), an indicator of membrane lipid peroxidation. The protocol of Ellman (1959) was used to determine reduced glutathione, a procedure that develops a chromophore with maximum absorption at 412 nm. Ascorbic acid in the plant extract was determined by titrating with 2,6-dichlorophenol-indophenol (Achuba 2008). Diagnostic kit in line with Caraway and Hard, (1963) was adopted to determine uric acid in the plant extract. The method of Misra and Fridovich (1972) was used to determine superoxide dismutase (SOD) activity by measuring 50% inhibition of auto-oxidation of epinephrine to adrenochrome at 480 nm. To determine manganese dependent SOD, 1 mM NaCN was added to the reaction mixture to inhibit Cu-ZnSOD activity and its activity determined as the difference between total SOD and MnSOD according to Crapo *et al*. (1978). The disappearance of the purple color of potassium permanganate at 480 nm by oxygen produced from the breakdown of hydrogen peroxide due to catalase action was monitored at 30-60 intervals and was used to determine the enzyme activity according to Cohen *et al*. (1970). The reduction of hydrogen peroxide and organic peroxides by glutathione to the resultant stable alcohols and water produces chromophore that absorbs maximally at 412 nm which was adopted in measuring glutathione peroxidase activity in line with Rani *et al.*, (2004).

Determination of drug metabolizing enzymes

The methods of Macleod *et al*. (1961), Johns (1967) and Stirpe and Della Corte (1969) were adopted to determine sulphite oxidase, aldehyde oxidase and xanthine oxidase activities, respectively.

Statistical analysis

Statistical Package for Social Sciences (SPSS), version 22 was used to compare the experimental data through a two-way analysis of variance (ANOVA). Group cross comparison was done using the Bonferroni Post-hoc test. Significant differences were set at p<0.05 and all results expressed as mean + SD.

Results

Effect of goat droppings on morphological parameters of cowpea seedlings grown in crude oil polluted soils

Results in table 3 show a significantly increased stem length in soils treated with 75 g of goat droppings compared to control in normal soil. Untreated soil samples exposed to crude oil had significantly reduced stem length compared to all other groups. Treatment with 75 g of goat droppings improved the stem length to near normality. Leaves length showed a significant reduction in 75 g treated plants in normal soil to control while all other groups were unchanged. Leaves Breadth also indicates that 75 g of goat dropping treatment in normal soil increased leaves breadth compared to control while 150 g treatment had no significant influence. Seedlings grown in un-amended soils exposed to crude oil had significantly (p <0.05) reduced leave breadth while amendment of polluted soil with 75 g and 150 g of goat droppings reversed the reduction to control levels. Plant Heights were also observed to follow similar trends as 75g and 150 g Goat dropping amendment led to increased plant height relative to control while crude oil pollution significantly reduced it. Polluted soil amendment with 75 and 150g goat droppings improved the plant height with 75 g nearing normality. Root lengths were also significantly (p <0.05) reduced in seedlings grown in untreated crude oil polluted soils relative to control. Amendment with 75 g of goat droppings improved it to near normality compared to control. Likewise, soil amendment with only 75 g of goat droppings significantly (p <0.05) increased root length compared to control (group 1).

Effect of goat droppings on photosynthetic and metabolic pigments of cowpea seedlings grown in crude oil polluted soils

Table 4 presents effect of goat droppings on photosynthetic pigments in cowpea seedlings grown in crude oil polluted soils. Total chlorophyll was significantly (p <0.05) increased relative to control following treatment with 75 g and 150 g goat droppings (groups 2 and 3).

Crude oil pollution (group 4) significantly (p <0.05) reduced total chlorophyll content while polluted soil amendment with 75 g and 150 g goat droppings improved total chlorophyll content to near normality. Chlorophyll a content was also significantly increased relative to control by 75 g goat droppings but had no significant reduction change across all experimental groups except seedlings grown in un-amended crude oil polluted soils (4). Also, CuSOD and MnSOD activities were significantly reduced by crude oil pollution (group 4); it remained unchanged in groups 2 and 3 while treatment with goat droppings improved these activities in groups 5 ad 6 near control values.

Table 4: Effect of goat droppings on chlorophyll and beta-carotene content in cowpea seedlings grown in crude oil-polluted soil

Soil Type	Goat dropping (600 g^{-1} soil)	Total chlorophyll (mg g^{-1})	Chlorophyll a(mg (mg g^{-1})	Chlorophyll b (mg g^{-1})	Beta-carotene (mg g^{-1})
Normal Soil	0	207.60±4.72a	125.00±2.24a	81.00±2.24a	23.00±2.24ab
	75 g	215.80±3.77bc	130.00±2.24b	68.00±2.24b	26.00±2.24a
	150 g	218.80±3.77c	121.00±2.24ae	83.00±2.24a	27.00±2.24a
Crude Oil-Polluted Soil	0	158.80±3.77d	99.00±2.24c	58.00±2.24c	20.00±2.24b
	75 g	187.80±3.77e	110.00±2.24d	72.00±2.24b	23.00±2.24ab
	150 g	209.80±3.77ab	120.00±2.24e	79.00±2.24a	23.00±2.24ab

All Values are expressed as Mean ± SD of five replicates. Values followed by different alphabet superscript in the same column indicate that there is a significant (p <0.05) difference.

Table 5: Effect of goat droppings on levels of non-enzymatic and enzymatic antioxidant activities in cowpea seedlings grown in crude oil-polluted soil

Soil Type	Goat dropping (600 g^{-1} soil)	Vit. C (ug g^{-1})	Uric acid (ug g^{-1})	GSH (ug g^{-1})	CAT (nMol min^{-1} gfw)	GPx (nMol min^{-1} gfw)
Normal Soil	0	3.71±0.49ad	3.70±0.24a	$0.43±0.05^{ac}$	$1.29±0.08^{a}$	$6.96±0.06^{a}$
	75g	3.85±0.37a	3.05±0.06b	$0.48±0.03^{ac}$	$1.33±0.07^{a}$	$7.79±0.41^{b}$
	150g	4.63±0.30b	3.03±0.03b	$0.51±0.06^{a}$	$1.39±0.07^{a}$	$8.63±0.42^{c}$
Crude Oil-Polluted Soil	0	2.39±0.38c	1.95±0.18c	$0.24±0.048^{b}$	$1.05±0.03^{b}$	$5.31±0.37^{d}$
	75g	3.16±0.16d	2.69±0.41b	$0.31±0.07^{bc}$	$1.10±0.02^{bc}$	$6.06±0.42^{e}$
	150g	3.28±0.13ad	2.99±0.12b	$0.37±0.06^{c}$	$1.17±0.03^{c}$	$6.19±0.31^{e}$

All Values are expressed as Mean ± SD of five replicates. Values followed by different alphabet superscript in the same column indicate that there is a significant (p <0.05) difference.

following 150g soil amendment relative to control. Crude oil pollution, however, significantly reduced chlorophyll a content while both weights of the goat droppings improved the chlorophyll a content significantly. Chlorophyll b content reduced significantly (p <0.05) relative to control, following soil amendment with 75g goat droppings but had no significant (p <0.05) change when amended with 200g. Following crude oil pollution (group 4), chlorophyll b reduced significantly (p <0.05) relative to control while treatment with both weights of goat droppings (5 and 6) improved the chlorophyll b content.

Effect of goat droppings on antioxidants indices of cowpea seedlings grown in crude oil polluted soils

Figure 2 shows the effect of goat droppings on the activities of SOD in cowpea seedlings grown in crude oil-polluted soil. Total SOD activities had no significant (p <0.05)

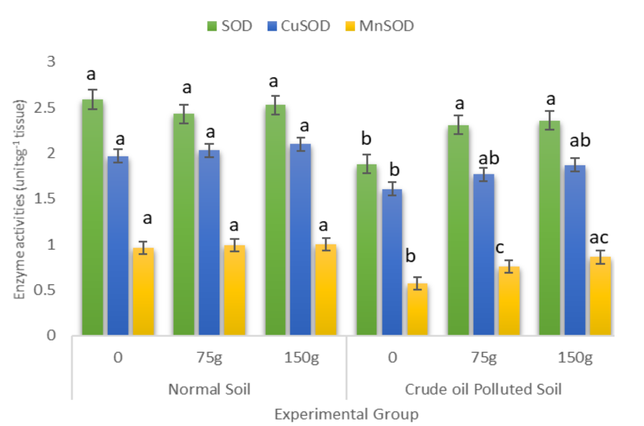


Figure 2: Effect of Goat Droppings on SOD activities of cowpea seedlings grown in crude oil-polluted soil. (Different letters on the bars depict significant (p <0.05) difference)

Table 5 presents the effect of goat droppings on selected enzymatic and non-enzymatic antioxidants in cowpea seedlings. Vitamin (Vit) C levels were not significantly affected by 75 g of goat droppings amendment of soil compared to control, but 150 g significantly (*p* <0.05) increased vitamin C content . Exposure to crude oil pollution significantly reduced Vit. C content but amendment of soil with 75 g and 150 g of goat droppings reversed the levels to control levels. Similarly, glutathione levels in seedlings grown in 75 g and 150 g goat dropping amended soils did not significantly increase compared to control. Uric acid contents were significantly (*p* <0.05) reduced by both weights of goat droppings (groups 2 & 3) relative to control. Likewise, crude oil pollution further significantly(*p* <0.05) reduced uric acid content in cowpea seedlings while treatment with both weights (groups 5 and 6) improved uric acid content near to those observed in groups 2 and 3 while it remained significantly (*p* <0.05) reduced compared to control (1). Crude oil significantly (*p* <0.05) depreciated GSH levels but amendment of polluted soils with both weights of goat droppings increased GSH levels to near control levels. On the activities of catalase enzyme, it was observed that goat dropping soil amendment had no effect at both treatment doses but crude oil pollution significantly (*p* <0.05) reduced catalase enzyme activities while treatment with both doses of goat droppings significantly(*p* <0.05) increased the activities relative to group 4 but reduced relative to control. Glutathione peroxidase enzyme activities increased in groups 2 and 3 relatives to control while those exposed to crude oil without amendments with goat droppings decreased significantly(*p* <0.05) . Although crude oil polluted soil amendment significantly improved enzyme activities compared to group 4, there was a significant (*p* <0.05) reduction relative to control.

Table 6 presents the effect of goat droppings on lipid peroxidation and oxidative enzymes in cowpea seedlings grown in crude oil polluted soil. There were no observed significant (*p* <0.05) differences on levels of lipid peroxidation in groups 2 and 3 (75 g and 150 g) goat droppings alone compared to control group 1. There was a significant increase in lipid peroxidation levels in seedlings exposed to crude oil pollution alone compared to control. Likewise, increased lipid peroxidation in seedlings exposed to crude oil and amended with 75 g goat droppings (5) was observed relative to control but no significant(*p* <0.05) difference compared to crude oil alone (group 4). Amendment with 150 g of goat droppings (group 6) reduced lipid peroxidation in cowpea seedlings relative to group 4 and was not significantly (*p* <0.05) different from group 1.

Glutathione-s-transferase enzyme activities increased in groups 2 and 3 relatives to control while those exposed to crude oil without amendments with goat droppings decreased significantly. Although crude oil polluted soil amendment significantly (*p* <0.05) improved enzyme activities compared to group 4, these reduced relative to control significantly for only group 6. Xanthine oxidase enzyme activities revealed no significant (*p* <0.05) increase between groups 2 and 1 however, there was significant (*p* <0.05) increase in group 2 relative to control. Crude oil exposure alone (group 4) reduced significantly XO activities relative to control while amendment with goat droppings in group 5 reduced significantly (*p* <0.05) compared to group 1, it had no difference compared to group 4. Amendment with 150 g of goat droppings reduced significantly (*p* <0.05) compared to group 1 but increased compared to group 4. Similarly, Aldehyde oxidase (AO) enzyme activities revealed no significant (*p* <0.05) difference between groups 1, 2 and 3, respectively. There was a significant (*p* <0.05) reduction in group 4 compared to all other experimental groups while goat droppings significantly (*p* <0.05) increased AO activities in group 5 compared to group 4, this was significantly (*p* <0.05) reduced compared to control group. Goat dropping amendment in group 6 increased AO activities relative to

Table 6: Effect of goat droppings on lipid peroxidation and drug metabolizing enzyme activities in cowpea seedlings grown in crude oil-polluted soil

Soil Type	Goat dropping (600 g^{-1} soil)	MDA (nMol cm^{-3})	GST Units g^{-1} tissue	XO Units g^{-1} tissue	AO Units g^{-1} tissue	SO Units g^{-1} tissue
Normal Soil	0	1.14±0.08[a]	1.85±0.14[a]	3.26±0.25[a]	1.61±0.08[ad]	2.48±0.12[a]
	75 g	1.08±0.12[a]	2.09±0.098[b]	3.53±0.27[a]	1.68±0.07[a]	3.64±0.13[b]
	150 g	1.02±0.09[a]	2.21±0.04[b]	3.99±0.05[b]	1.73±0.04[a]	3.68±0.15[b]
Crude Oil-Polluted Soil	0	1.37±0.06[b]	1.43±0.07[c]	1.96±0.06[c]	1.08±0.03[b]	2.02±0.12[c]
	75g	1.29±0.06[b]	1.64±0.04[d]	2.18±0.09[c]	1.40±0.07[c]	2.59±0.05[a]
	150 g	1.23±0.07[a]	1.69±0.058[ad]	2.60±0.23[d]	1.49±0.09[cd]	2.83±0.44[a]

All Values are expressed as Mean ± SD of five replicates. Values followed by different alphabet superscript in the same column indicate that there is a significant (*p* <0.05) difference. MDA: Malondialdehyde, GST: Glutathione-S-transferase, XO: Xanthine Oxidase, AO: Aldehyde Oxidase, SO: Sulphite Oxidase group 4 but was not significantly(p <0.05) reduced compared to group1. Sulphite oxidase (SO) enzyme activities were significantly increased by both amendment doses of goat droppings (groups 2 & 3) compared to group 1. AO enzyme activities reduced significantly (p <0.05) compared to control levels in group 4 seedlings while amendment with goat droppings at both doses (75 g and 150 g) in groups 5 and 6 reversed the trend to control levels.

Discussion

A lot of research evidence exists on the negative impacts of crude oil pollution on plants (Achuba 2006; Achuba, 2014; Achuba and Ekute, 2017). Likewise, studies have continually employed the use of agricultural wastes for improving the yield of plants and as ameliorative agents against the negative impacts of crude oil polluted soils on plant metabolic parameters (Njoku *et al*., 2008; Achuba and Ja-anni, 2018; Achuba and Oshiokpu, 2019; Achuba and Ohwofasa, 2019). Findings from the present study revealed that goat droppings were able to improve the stem length, leaves breadth and plant heights and root lengths in cowpea seedlings grown in unpolluted soils and reversed the growth deficiencies reported for seedlings grown in crude oil-polluted soils. These findings corroborate earlier reports that crude oil pollution obstructs plant growth (Agbogidi *et al.,* 2006; 2007; Achuba and Okoh, 2014). It also agrees with the ability of cow dungs to improve the growth potentials reported for *Glycine Max* L. grown in crude oil polluted soils following amendment (Njoku *et al.,* 2008).

Crude oil mediated reduction in photosynthetic pigments; total chlorophyll, chlorophyll a, chlorophyll b and beta-carotene observed in this study is in consonance with earlier studies of Achuba (2006) and Achuba (2019) that observed vast reductions in carotenoids and chlorophyll contents in cowpea grown in crude oil polluted soils. Plant chlorophyll content is a significant macromolecule for the sustenance of photosynthesis. Thus, a reduction in chlorophyll and carotenoids is an established indicator of environmental stress (Hille and Nishino, 1995; Majid *et al.,* 2011; Taibi *et al.,* 2016). Chlorophyll content and synthesis have also been linked to the leaf breadth of a plant which also is a determinant of the rate of nutrient conversion from the sun because of leaf surface area contact with the sun (Tiezen, 1970; Li *et al*., 2018). The improvement in chlorophyll and beta carotene content of the cowpea seedlings amended with goat droppings with and without crude oil contamination may also be linked to improvement in soil nutrient composition. This observation is similar to those reported by Njoku *et al.* (2008) that used cow dungs for soil amendments following crude oil contamination. It is also in line with the trends of results reported by Achuba (2019) following amendment of crude oil polluted soil samples with oil palm leaf. The observed ability of goat droppings to significantly improve growth parameters such as plant height, stem length, leaf breadth and height and root length as well as chlorophyll and beta-carotene is indicative that the goat droppings could be used as a source of organic fertilizer for cowpea cultivation. The use of organic fertilizers for plant cultivation has been previously encouraged and has been reported to contribute significantly to increased plant yield and performance (Ekundayo *et al.,* 2001; Achuba and Ekute, 2017; Achuba and Oshiokpu , 2019; Achuba and Ohwofasa, 2019)

Crude oil has been established to significantly induce plant oxidative stress and also contribute significantly to depreciation in antioxidants and further inhibition of plant macromolecules (Achuba, 2006; Achuba and Ja-anni, 2018). Observed decrease in superoxide dismutase, catalase and glutathione peroxidase activities as well as reduction in the levels of vitamin C, uric acid and GSH in crude petroleum only exposed cowpea seedlings are indicative of response to an overwhelming effect of petroleum mediated free radical generation. This trend is corroborated by a side by side rise in lipid peroxidation levels in the seedlings exposed to crude oil only. Although treatment with goat droppings further reversed this trend, treatment with goat droppings only reportedly improved antioxidant potentials while reducing to a very minimal level lipid peroxidation levels in the seedlings even better than the control seedlings.

Drug metabolizing enzymes such as the glutathione-s-transerase, the oxidative enzymes (xanthine oxidase, aldehyde oxidase and sulphite oxidase are known as second messenger responders to the militating effects of oxidative stress in animals (Ichipi-Ifukor *et al.* 2019; Asagba, 2019). For example, the glutathione-s-transferase enzyme has been identified to significantly assist in the regulation of oxidative stress and also contributes to the control of light signaling and different aspects of plant development which also includes hormonal synthesis and transport (Chen *et al.,* 2012; Kuma and Trivedi, 2018). From the foregoing, there is no doubt, that the decrease in plant pigments (chlorophyll and beta-carotene) in crude oil exposed plants is related to decline in the GST activity. This is because the seedlings may have lost the efficiency and catalyst needed for the development of certain growth factors and hormonal synthesis. The oxidative enzyme xanthine oxidase, on the other hand, is majorly responsible for purine degradation which leads eventually to the synthesis of uric acid, a much more stable byproduct that can be easily released or absorbed for synthesis of other metabolites (Urarte *et al.,* 2015; Kostic *et al.,* 2015). A careful observation of the result in this study shows that reduction in xanthine oxidase activity negatively impacted on uric acid content in the cowpea seedlings implying that crude petroleum exposure to the cowpea seedlings significantly inhibited purine conversion to uric acid and follows a similar trend in the study of Achuba (2019). Treatment with goat droppings as in palm oil leaf by Achuba (2019), significantly up regulated xanthine oxidase activity implying that the goat dropping has the potential for inducing xenobiotic responders by improving on the degradation of crude petroleum through microbial stimulation or by preventing the absorption of crude petroleum by plants.

The aldehyde oxidase and sulphite oxidase enzymes are noted for the degradation of aldehydes and N-sulfoxides rising from the effect of lipid peroxidation (Kadam and Iyer, 2008). In the presence of stress indices, they are also known to be gradually overwhelmed and depleted if the trend continues uncontrolled (Asagba, 2010; Ezedom and Asagba, 2016; Asagba *et al.,* 2017). The observed reduction of AO and SO in this study is no doubt traceable to the overpowering efficiencies of the effects of crude oil which necessitated the rise in lipid peroxidation. This is in agreement with the results reported by Achuba and Ohwofasa (2019) and Achuba and Ja-anni (2018) for aldehyde oxidase when fish pond waste water and abattoir waste water were used, respectively, for amendment of a diesel and crude oil polluted soil. It also agrees with Achuba (2019) for the three oxidative enzymes assayed for in this study when oil palm leaf was used for mitigation of crude oil exposure in cowpea seedlings. The eventual rise in these enzyme activities is said to be from the protect ability of goat droppings from the continual accumulation of endogenous heterocyclic compounds because of increased nutrient factors in the plant. This further gives credence on the ability of organic manures to successfully mitigate metabolic aberrations mediated by crude petroleum (Oyem and Oyem, 2013; Achuba and Okunbor, 2015; Okafor *et al.* 2016; Arif *et al*., 2018).

Conclusion

This study further showed evidence supporting earlier submissions on the negative impacts of crude oil on plants. In addition to this, it revealed that goat dropping amendment of polluted soils holds promises for the reversal of these negative effects. In particular, the effects of goat droppings, like other organic manures previously investigated have the ability to inhibit lipid peroxidation, improve antioxidant enzyme activities, improve the activities of drug metabolizing enzymes, improve the content of several metabolic plant pigments. It further contributed to certain plant growth factors and indices such as plant height, stem length, leaf breadth, and root length.

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