THE EFFECT OF ABATTOIR WASTE WATER ON THE METABOLISM OF COWPEA SEEDLINGS GROWN IN DIESEL CONTAMINATED SOIL

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The effects of abattoir waste water on the macromolecules (total sugar, protein, amino acid, beta carotene and chlorophyll) of cowpea grown on diesel contaminated soil at various concentrations (0.1%, 0.25%, 0.5%, 1.0% and 2.0%) as well as the activities of alpha amylase, starch phosphorylase and oxidative stress markers (lipid peroxidation, superoxide dismutase activity, catalase activity and xanthine oxidase) in cowpea seedlings were investigated. The results showed that diesel imposed environmental stress in cowpea seedlings. This is indicated by the decrease in total sugar, total protein and amino acids and a decrease in the chlorophyll contents of the leaves of 12-day-old seedlings. The activities of alpha amylase and starch phosphorylase in the cotyledon of 4-day-old seedlings were inhibited by the various diesel concentrations in the control treatment, but abattoir waste water ameliorated the effect of diesel toxicity. Also, the results indicated that the petroleum product caused a significant increase in lipid peroxidation and a significant decrease in the activities of the antioxidant enzymes: Superoxide dismutase, catalase and xanthine oxidase activities in the control; but abattoir waste water ameliorated the effect of these stresses posed by the diesel contaminated soil. The following observations, therefore, suggest that abattoir wastewater is capable of remediating the undesirable effects of diesel contamination on cowpea seedlings.

Key words: Abattoir waste water, cowpeas seedlings, enzyme activities, contaminated soil

INTRODUCTION

Human activities such as extraction of crude oil, processing, distribution and use of petroleum products lead to contamination of the soil environment (Adam et al., 2002; Clark, 2003; Ayotamuno et al., 2006). Moreover, accidental spills caused by pipeline leakages and ruptures and accidents during transport have been reported (Ogbo, 2009).

Contamination of soil with petroleum hydrocarbon can limit its productive potentials; this can upset its metabolic activity, affect its chemical characteristics, and reduce its fertility and impact on plants' production (Gong et al., 1996; Torstenssen et al., 1998; Wyszkowska and Kucharski, 2000; Wyszkowski et al., 2004; Wyszkowski and Wyszkowska, 2005; Wyszkowski and Ziolkowska, 2008).

Previous studies have reported a decrease in seed germination in soil contaminated with petroleum hydrocarbon (Amakiri and Onofeghara, 1984; Adam et al., 2002; Vavrek and Campbell, 2002; Méndez-Natera et al., 2004; Achuba, 2006; Smith et al., 2006; Sharifi et al., 2007; Korade and Fulekar, 2009; Ogbo, 2009). Earlier study indicated that petroleum hydrocarbons may form a film on the seed, preventing the entry of oxygen and water (Adam and Duncan, 2002).

Similarly, earlier report indicated that crude oil inhibited the activities of starch degrading enzymes such as amylase and starch phosphorylase; this affected the assimilation of starch by the germinating cowpea seedlings (Achuba, 2006). This interference in metabolic activity as well as the anaerobic and hydrophobic conditions caused by diesel contamination has been reported to be the most important problem to seed germination and plants' growth (Racine, 1993).

Generally, plants require a large quantity of major nutrients for better growth and yield. The application of abattoir wastewater as a fertilizer and soil conditioner was earlier reported (Matheyarasu et al., 2016). Abattoir waste is rich in organic materials (Bustillo-

Lecompte, 2016), making it rich in nitrogen, phosphorous, potassium as well as micro-organisms involved in the degradation of excess carbon imposed by pollution. This study was conducted to investigate the effect of abattoir waste water on the growth and metabolism of cowpea seedlings grown in diesel contaminated soil.

MATERIALS AND METHODS

Dry cowpea seeds were purchased from an International Institute of Tropical Agriculture (IITA), Ibadan. The seeds' viability was tested by placing them in water. The non-viable seeds floated signifying the cotyledons were destroyed.

They were discarded. The seeds that did not float were collected and used for this research. The diesel used in this study was obtained from Foste Petrol Station, Abraka, and abattoir wastewater was collected from an abattoir at Igbo quarters, Abraka. The other chemicals used were of high quality and analytical grade. Loamy soil was collected from a land opposite the Department of Biochemistry, Delta State University. The loamy soil obtained was mixed with impurities such as stone, bones, dead leaves (undecomposed) etc; it was sieved using 2 mm mesh.

Before the major experiment, a minor experiment was conducted to observe the amount of abattoir waste water that would influence the growth of cowpea seedlings positively. About 1600 g of loamy soil was treated with different concentrations of abattoir waste water (0, 20, 40, 60, 80 and 100 ml). After 12 days, seedlings treated with 40 ml of abattoir were observed to enhance growth in the control and other concentrations. This was used a criterion for treating the samples with 40 ml of abattoir waste water.

Exactly one thousand six hundred grams (1,600 g) of soil samples was thoroughly mixed with various concentrations of diesel (0.1, 0.25, 0.5, 1.0, 1.5 and 2.0%). The control was mixed with water. Five bags were prepared for each concentration and five cowpea seeds were planted in each bag. The experimental soil was watered with 80 cm^3 twice a day (morning and evening) for twelve days. Seeds which failed to sprout after 12 days were regarded as non-germinable (Achuba, 2006).

At the end of the exposure period, leaf homogenate was prepared as earlier reported by Achuba (2006). Protein was estimated using the method of Lowry et al. (1951). The content of chlorophylls was estimated by the method of Lichtenthaler (1987) and that of carotenoid content by using the formula given by Duxbury and Yentsch (1956). α-Amylase assay was carried out by the method of Gupta et al. (2003) and the activity was calculated by using a formula proposed by Xiao et al (2006). Phosphorylase activity was evaluated according to the method of Singh and Steinnes (1976). The activity of catalase was determined by the method of Rani et al. (2004). Xanthine oxidase was determined by the method of McEwen (1971).

Total superoxide dismutase was estimated using the method of Misra and Fridovich (1972). Manganese dependent superoxide dismutase (MnSOD) was analyzed in the presence of 1 mM NaCN, to suppress Cu/Zn SOD activity. The cytosolic copper/zinc superoxide dismutase (Cu/Zn SOD) activity was determined, as the difference between total SOD and cyanide sensitive enzyme activity (Crapo et al, 1978).

RESULTS AND DISCUSSION

Changes in soil conditions caused by petroleum hydrocarbon can modify the content of biomolecules as well as biochemical processes in plants (Achuba, 2006; Peretiemo-Clarke and Achuba, 2007). Previous report indicated that diesel fuel exerted a negative effect on plants (Arellano et al., 2015). This negative effect was attributed to the inhibitory action of some of the polycyclic aromatic hydrocarbon components of diesel which are more soluble than the aliphatic hydrocarbons (Wang and Bertha, 1990; Trapp et al., 2001).

The result (Table 1) shows that diesel contaminated soil significantly ($P<0.05$) reduce the concentration of total sugar, total protein, total amino acid and β-carotene in the control. This observation is in line with the findings of Agbogidi et al. (2007), Ogbuehi et al. (2010) and Ekpo et al. (2014) who reported reduction in protein contents of maize, cassava and scent leaf

Table 1. The effect of diesel treated soil as well as diesel treated soil ameliorated with abattoir waste water treatment on the concentration of sugar, protein, amino acid and β-carotene in the leaves of 12 day old cowpea seedlings.

Concentration of petroleum products in soil (% v/w ml/g)	Total Sugar (mg/g) n=3		Total Protein(mg/g) n=3		Total Amino Acid(mg/g) n=3		**β**-Carotene (mg/g) n=3	
	Diesel	Diesel +AWW	Diesel	Diesel +AWW	Diesel	Diesel +AWW	Diesel	Diesel + AWW
0.00	33.27±0.82	35.87±1.81	19.60±0.25	22.07±1.25	3.77±0.15	3.57±0.25	22.57±0.87	25.00±0.55
0.10	28.63±1.45[a]	33.13±1.47[a, b]	18.10±1.18	19.37±0.75	3.03±0.21	3.60±0.10[b]	20.93±0.55[a]	22.10±0.50[b]
0.25	25.83±1.43[a]	30.80±0.9 [b]	16.33±0.91[a]	18.67±0.50[b]	2.77±0.06[a]	3.37±0.06[b]	19.83±0.25[a]	20.83±0.25[b]
0.50	22.17±1.89[a]	30.27±0.76[b]	15.43±0.49[a]	18.60±0.50[b]	2.37±0.06[a]	3.10±0.10[b]	18.57±0.85[a]	19.97±0.21[b]
1.00	19.50±0.94[a]	28.23±1.25[b]	14.33±0.61[a]	17.60±0.70[b]	2.23±0.06[a]	2.97±0.15[b]	16.20±0.95[a]	19.03±0.38[b]
1.50	18.20±0.90[a]	25.77±1.35[b]	12.77±0.67[a]	17.47±1.21[b]	2.13±0.06[a]	2.77±0.15[b]	14.30±0.46[a]	17.73±0.95 [b]
2.00	16.43±0.65[a]	22.03±1.16[b]	11.60±0.70[a]	16.77±2.24[b]	2.03±0.06[a]	2.63±0.15[b]	12.50±0.79[a]	17.23±0.35[b]

Results are expressed as mean + SD of three determinations. a=values significantly lower than those of control at P<0.05; b = values significantly higher than the corresponding diesel treatment; n=number of seedlings used per assay.

respectively when these plants were exposed to crude oil contaminated soil. On the other hand, soil treated with abattoir waste water enhanced total sugar level compared to seedlings grown on diesel soil. Abattoir waste water treatment used for the diesel contaminated soil restored the level of protein in low level soil. This is in agreement with the finding of Akujobi et al. (2011) who reported that the addition of animal waste to soil increased the protein contents of plants.

Previous reports documented increases in protein content of plants occasioned by the application of animal materials (Ojeniyi et al., 2007; Nogalska and Zalewska 2013). Similarly, using abattoir waste water treatment for the diesel contaminated soil restored the level of total amino acid and β-carotene at low level contaminated soil. Diesel contaminated soil significantly (P<0.05) reduced the level of chlorophyll in the control. This is consistent with the reports of previous studies (Odjegba and Sadiq, 2002; Achuba, 2006; Peretiemo- Clarke and Achuba, 2007). However, using abattoir waste water treatment for diesel contaminated soil restored the level of chlorophyll at low level contaminated soil, close to control values. Similar result was reported on the effect of cow dung on petroleum contaminated soil (Njoku et al., 2008).

Petroleum hydrocarbon induced changes in the activities of starch degrading enzymes (Achuba, 2006; Achuba and Okoh, 2015). The action of starch degrading enzyme, starch phosphorylase starts by attacking the amylopectin chain from the non-reducing end to give glucose-1-phosphate. After subsequent conversion to glucose-6-phosphate, it could enter the tricarboxylic acid cycle via glycolytic pathway for the production of ATP needed by germinating plants (Achuba and Okoh, 2015).

Therefore, inhibition of starch phosphorylase could disturb respiratory and metabolic activities of germinating cowpea (Achuba and Okoh, 2015). However, treating of diesel contaminated soil with abattoir waste water restored the activity of α-amylase and starch phosphorylase at low level contaminated soil, close to control values. This is consistent with the report of previous study (Ekpo and Nya, 2012). Moreover, the treatment of petroleum hydrocarbon contaminated soil with sawdust and waste cotton has been reported to improve the performance of plants (Adedokun et al., 2007).

The formation of lipid peroxidation products in plants exposed to adverse environmental conditions is an indication of free radical formation in tissue and it may be used as index of oxidative stress in biological systems (Achuba and Otuya, 2006; Achuba, 2010; Achuba 2014; Nwaogu and Onyeze, 2014). This study shows that diesel induced lipid peroxidation in exposed plants (Table 4). This study is in concordance with the findings

Table 2. The effect of diesel treated soil as well as diesel treated soil ameliorated with abattoir waste water treatment on the level of chlorophyll in the leaves of 12 day old cowpea seedlings.

Concentration of petroleum products in soil (% v/w)	Total Chlorophyll (mg/g)		Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
	Diesel	Diesel +AWW	Diesel	Diesel +AWW	Diesel	Diesel +AWW
0.00	288.33±2.08	320.33±9.50	173.00±1.25	192.20±5.70	115.33±0.83	128.13±3.80
0.10	221.33±8.08[a]	284.00±9.16[b]	132.80±4.85[a]	170.40±5.50[b]	88.53±3.23[a]	113.60±3.67[b]
0.25	207.33±4.16[a]	264.67±4.16[b]	124.40±2.50[a]	158.80±2.50[b]	82.93±1.67[a]	105.87±1.67[b]
0.50	201.00±1.00[a]	256.67±4.16[b]	120.60±0.60[a]	154.00±2.50[b]	80.40±0.40[a]	102.67±1.67[b]
1.00	192.67±5.03[a]	247.67±5.51[b]	115.60±3.01[a]	148.60±3.31[b]	77.07±2.01[a]	99.07±2.20[b]
1.50	189.33±2.08[a]	238.33±2.52[b]	113.60±1.25[a]	143.00±1.51[b]	75.73±0.83[a]	95.33±1.01[b]
2.00	175.00±9.85[a]	229.67±3.51 [b]	105.00±5.91[a]	137.8±2.11[b]	70.00±3.94[a]	91.87±1.40[b]

Results are expressed as mean + SD of three determinations. a=Values significantly lower than those of control at P<0.05; b = values significantly higher than the corresponding diesel treatment.

Table 3. The effect of diesel treated soil as well as diesel soil ameliorated with abattoir waste water treatment on the activities of α-amylase and starch phosphorylase in the seed of 3 day old cowpea seedlings.

Concentration of petroleum products in soil (% v/w ml/g)	α-Amylase activity (Unit/ ml)		Starch phosphorylase activity (mg/min gfw)	
	Diesel	Diesel + AWW	Diesel	Diesel + AWW
0.00	20.87±1.44	24.83±1.43	1.07±0.05	1.17±0.04
0.10	14.87±1.51[a]	18.87±0.70[b]	0.98±0.10[a]	1.03±0.10[b]
0.25	13.17±0.60[a]	18.37±0.21[b]	0.86±0.06[a]	0.93±0.05[b]
0.50	12.10±0.27[a]	17.67±0.42[b]	0.66±0.07[a]	0.91±0.04[b]
1.00	10.97±0.49[a]	16.47±0.70[b]	0.55±0.07[a]	0.83±0.09[b]
1.50	9.70±0.46[a]	16.20±1.05[b]	0.47±0.05[a]	0.67±0.06[b]
2.00	8.70±0.53[a]	15.50±0.66[b]	0.67±0.06[a]	0.65±0.09[b]

Results are expressed as mean + SD of three determinations. a= Values significantly lower than those of control at P<0.05; b = values significantly higher than the corresponding diesel treatment; n= number of seedlings used per assay.

Table 4. The effect of diesel treated soil as well as diesel soil ameliorated with abattoir waste water treatment on the level of lipid peroxidation and activities of xanthine oxidase and catalase in the leaves of 12 day old cowpea seedlings.

Concentration of petroleum products in soil (% v/w ml/g)	Lipid peroxidation (nMol/cm^3)		Xanthine oxidase activity (Unit/cm^3)		Catalase activity (nmol/min gfw)	
	Diesel	Diesel +AWW	Diesel	Diesel +AWW	Diesel	Diesel +AWW
0.00	0.98±0.12	0.91±0.08	3.78 ±0.57	4.58 ±0.33	1.08±0.14	1.14±0.16
0.10	1.14 ±0.02[c]	1.01±0.09	4.76±0.30[c]	4.69±0.07	1.13±0.02[c]	1.06±0.13[d]
0.25	1.81±0.13[c]	1.61±0.10[d]	4.91±0.25[c]	4.77±0.10	1.18±0.01[c]	1.09±0.10[d]
0.50	1.84±0.02[c]	1.61±0.10[d]	4.27±0.38[c]	4.32±0.40	1.06±0.04	1.09±0.13
1.00	2.32±0.10[c]	1.63±0.02[d]	3.83±0.1 [c]	4.18±0.21[d]	0.73±0.13[a]	1.02±0.08[b]
1.50	2.63±0.08[c]	1.68±0.03[d]	3.57±0.10[a]	4.37±0.22[d]	0.56±0.08[a]	0.91±0.04[b]
2.00	2.84±0.11[c]	1.70±0.02[d]	3.21±0.18[a]	4.09±0.07[d]	0.43±0.05[a]	0.80±0.07[b]

Results are expressed as mean + SD of three determinations. a = Values significantly lower than those of control at P<0.05; b = values significantly higher than the corresponding diesel treatment; c = values significantly higher than those of control at P<0.05; d = values significantly lower than the corresponding diesel treatment at P<0.05.

of Achuba (2014). However, treatment of diesel contaminated soil with abattoir waste water significantly (P<0.05) reduced the level of lipid peroxidation at low level contaminated soil in the control. Moreover, there was significant (P<0.05) increase in the activity of xanthine oxidase at all level of soil contamination in the control. The increase in the activity of this enzyme indicates induction of oxidative stress. Catalase is an important enzyme that protects living system from oxidative stress. It is able to scavenge hydrogen peroxide, a byproduct of superoxide dismutase activity (Asada, 1992). The result shows that diesel contaminated soil significantly (P<0.05) increases the activity of catalase at low concentration, but significantly

Table 5. The effect of diesel treated soil as well as diesel soil ameliorated with abattoir waste water treatment on the level of superoxide dismutase activities in the leaves of 12 day old cowpea seedlings.

Concentration of petroleum products in soil (% v/w ml/g)	Total SOD (Unit/gfw)		CuZnSOD (Unit/gfw)		MnSOD (Unit/gfw)	
	Diesel	Diesel + AWW	Diesel	Diesel + AWW	Diesel	Diesel + AWW
0.00	2.28±0.15	2.21±0.17	2.10±0.09	2.06±0.02	0.97±0.13	0.84±0.11
0.10	1.98±0.05 [a]	2.11±0.16 [b]	2.00±0.11	2 .07±0.22	0.62±0.03 [a]	0.84±0.05 [b]
0.25	1.84±0.10 [a]	2.02±0.05 [b]	1.92±0.07 [a]	2.03±0.05 [b]	0.43±0.10 [a]	0.70±0.07 [b]
0.50	1.64±0.03 [a]	2.14±0.14 [b]	1.53±0.13 [a]	2.03±0.089 [b]	0.37±0.12 [a]	0.57±0.09 [b]
1.00	1.52±0.05 [a]	1.93±0.08 [b]	1.47±0.05 [a]	1.85±0.06 [b]	0.22±0.01 [a]	0.42±0.05 [b]
1.50	1.37±0.08 [a]	1.77±0.05 [b]	1.36±0.07 [a]	1.37±0.09 [b]	0.16±0.02 [a]	0.24±0.05 [b]
2.00	1.28±0.03 [a]	1.73±0.06 [b]	1.23±0.03 [a]	1.73±0.06 [b]	0.13±0.01 [a]	0.24±0.05 [b]

Results are expressed as mean + SD of five determinations. a=Values significantly lower than those of control at P<0.05; n=number of seedlings used per assay.

(P<0.05) reduces it at a higher level of soil contamination; no significant difference was found at 0.5% v/w ml/g contamination. The inhibition of catalase activity as concentration of petroleum product increases is similar with the effect of petroleum hydrocarbon on superoxide dismutase activity in the leaves of cowpea seedlings.

However, after twelve days of exposure to diesel treated soil the activity of total SOD decreased in the control. Decrease in the activity of the enzyme indicates reduction in the capacity of the exposed plant to handle reactive oxygen species. This is because increase in the activity of the antioxidant enzyme may have a role in imparting tolerance against any type of environmental stress (Achuba, 2010).

Moreover, diesel contamination caused a reduction in Cu/Zn-dependent superoxide dismutase and Mn-containing superoxide dismutase (MnSOD) after twelve days of growth in contaminated soil. These enzymes are involved in the general defense system against petroleum induced free radical toxicity (Achuba and Osakwe 2003). Earlier report indicated that a decrease in the activity of these enzymes can lead to cell damage due to imbalance in the production and removal of toxic oxygen radicals. However, increase in the activity could contribute to cell protection from toxicants inherent in the environment (Achuba, 2010; Achuba, 2014).

However, the treatment of diesel contaminated soil with abattoir waste water restored the activity of superoxide dismutase, copper/zinc superoxide dismutase and manganese superoxide dismutase at low level of soil contamination in the control. Akujobi et al (2011) reported an improvement in plant growth after amendment with both organic and inorganic waste.

Conclusively, this study has indicated that exposure of plant to diesel in soil could impose metabolic and oxidative stresses on exposed plants and abattoir waste water can ameliorate the effect of diesel mediated metabolic and oxidative stresses in cowpea seedlings. So, abattoir waste water could be used for bioremediation of diesel polluted sites.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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