INFLUENCE OF FISH POND WASTEWATER TREATMENT OF DIESEL TAINTED SOIL ON METABOLIC ACTIVITIES OF COWPEA (*Vigna unguiculata*) SEEDLINGS

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Abstract: The effect of fish pond spent water on the metabolic activities of cowpea seedlings cultivated in diesel tainted soil was examined in this study. The work employed the double placebo fold design. Both groups comprised of seven groups of A1-A7 were treated with diesel (0.0 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0%) and groups B1-B7 (same concentrations of diesel in each group treated with 80 ml of fish pond spent water daily for 12 days). Group in 0.0% served as the control without treatment except 80 ml of water. Results revealed that there was a significant ($P<0.05$) decrease in total sugar, total protein and total amino acid and ß carotene contents of the seedlings relative to control. Also, diesel treatment of soil elicited significant decrease ($P<0.05$) in chlorophyll content, α-amylase and starch phosphorylase activities of the cowpea seedlings compared to the control. While a significant increase in lipid peroxidation and decreases in the activities of xanthine oxidase, aldehyde oxidase and superoxide dismutase due to diesel exposure were noted. Treatment with fish pond wastewater significantly ($p<0.05$) contributed to a reversal of the observed negative trends in enzyme activities and levels of metabolic markers close to the values in seedlings not exposed to diesel tainted soil. The study concluded that fish pond spent water is a candidate for the remediation of diesel contaminated soils and at the same time is able to remediate the negative effects of diesel contamination on the biochemistry of cowpea seedlings cultivated in diesel tainted soil.

Keywords: Cowpea seedlings, diesel, macromolecules, oxidative stress, oxidase enzymes

Introduction

The world over where petroleum is produced is faced with contamination of cultivated land with hydrocarbons that are injurious to terrestrial environment (Adam *et al.*, 2002; Clark, 2003; Ayotamuno *et al.*, 2006; Ogbo, 2009; Nwaogu *et al.*, 2006; Das and Chandran, 2011). Among the mostly affected life forms are soil microbes and plants. Plants are affected in a myriads of ways such as depression of seed germination and negative modulation of plants' physiological processes (Adam and Duncan, 2002; Achuba and Peretiemo-Clark, 2008; Achuba and Okoh, 2014; Chikezie *et al.*, 2016; Achuba and Okoh 2015; Achuba and Asagba, 2016).

In a bid to make these petroleum degraded soil productive again, a number of approaches are adopted, which include nutrient amendments (Akujobi *et al.*, 2011; Okafor, 2016), addition of cow dung to polluted soil (Njoku *et al.*, 2008) and slaughterhouse wastewater treatment (Achuba and Erihijivwo, 2017; Achuba and Iserhienrhien, 2018; Achuba and Ja-anni, 2018). The use of fish pond wastewater as a soil management strategy has not been reported. Consequently, this study evaluated the influence of fish pond wastewater management of diesel tainted soil on the biochemical reactions of cowpea seedlings with the intent to determine the ability of fish pond wastewater to mitigate the deleterious effect of diesel on exposed plants.

Materials and Methods

Polybags (1178.3 cm^3, 15 cm deep) for planting were bought from a supermarket in Abraka, Nigeria. Cowpea (*Vigna unguiculata L*) seeds of Ozoro local were supplied by a local dealer at Eku, Nigeria and were identified by a competent staff of Department of Botany, Delta State University. Diesel (Automobile gas oil) was procured from a commercial petroleum station in Abraka, Nigeria. Fish pond wastewater (FPWW) was collected from Ugboroke commercial fish pond in Warri (5.5544° N, 5.7932° E), Delta State, Nigeria and soil was collected from a vacant plot of land opposite Faculty of Science, Delta State University, Abraka, Nigeria. Prior to the study, the physicochemical parameters of the soil and wastewater samples were determined using the methods of Association of Official Analytical Chemists (1990) and American Public Health Association (1998). The physicochemical properties of fish pond wastewater and soil sample used are presented in Tables 1A and 1B.

Table 1A: Physicochemical properties of fish pond wastewater

Parameters	Values
pH	5.4
Temperature (^{0}C)	23.5
Conductivity (ppm)	14
Colour (Unit)	Brownish
TDS (ppm)	32
Total hardness (mg/L)	4330
Akanility (mg/L)	94.0
Sulphate (mg/L)	0.35
Nitrogen (mg/L)	0.85
BOD (mg/L)	14.2
Chloride (mg/L)	94.25
COD (mg/L)	62.0
Dissolved Oxygen (mg/L)	12.0

Table 1B: Physicochemical properties of test soil

Parameters	Values
pH	6.98 ± 0.15
Sand	85.12± 0.33
Silt	4.87± 0.51
Clay	0.39± 0.44
Organic matter (%)	0.55 ± 0.22
Total Nitrogen (%)	0.18 ± 0.02
Phosphorus (ppm)	3.43 ± 0.22
Cation Exchange Capacity (Meq/100g)	5.74 ± 0.55

Determination of seed viability

Probable seed viability was carried out as described by Achuba (2006). The possible viable seeds were determined by placing the seeds on water. Seeds that floated were presumed to be damaged and were rejected while the seeds that did not float were used for this study.

Preliminary investigation

A pilot experiment was conducted to observe the amount of pond wastewater that will influence the growth of cowpea seedlings better. To achieve this 1600 g of soil was treated with different amounts of fish pond wastewater (0, 20, 40, 60, 80 and 100 ml). At twelve days post planting, seedlings treated with 80 - 100 ml of pond were detected to have good growth relative to the control and seedling grown in soil treated with other quantities of fish pond wastewater. This was the reason for treating the diesel tainted soils with 80 ml each of fish pond wastewater.

Planting procedure

The soil sample was sieved using 2 mm mesh to remove debris such decaying organic matters. Exactly one thousand six hundred grams (1,600 g) of soil samples were thoroughly mixed with various concentrations of diesel (A1- A7) (0.00, 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0%). The control (0.00%) was not mixed with diesel. Corresponding concentrations (B1- B7) were prepared but also treated with known amount of fish pond wastewater. Five bags were prepared for each concentration and five cowpea seeds were planted in each bag.

During the period of seedling development, the soil was watered twice a day (morning and evening) for twelve days. Water (80 ml) was provided to the control and soil treated with diesel groups (A1 – A7) to keep the soil wet, while the corresponding diesel tainted soils (B1- B7) were treated daily with 80 ml of fish pond wastewater. Seed germination score was taken every four days for a maximum of twelve days and seeds that did not sprout were regarded as being numb. At the end of experimental period, the seedlings were carefully removed and washed under running tap water.

Estimation of biochemical parameters

Determinations of total carbohydrate, total protein and total amino acids of the leaves

The procedure reported by Achuba (2006) was adopted to prepare leaf homogenate needed for the determination of total sugar, amino acid and protein. Total sugar and protein were estimated by the methods described by Tietz (1982) and Lowry *et al*. (1951), respectively. Total free amino acid was determined by ninhydrin method.

Estimation of total chlorophyll content of the leaves

The concentration of total chlorophyll was determined as described by Lichtenthaler (1987); using homogenate of the leaves that was prepared as reported by Achuba and Ja-anni (2018).

Assay of alpha amylase and starch phosphorylase activities of the cotyledons

Alpha-amylase assay was determined by the combined methods of Gupta *et al*. (2003) and Xiao *et al*. (2006) after heating the homogenate to a temperature of 55ºC to inactivate other enzymes. The method of Singh and Steinnes (1976) was used for the determination of starch phosphoryase activity.

Assay of oxidative stress parameters of the leaves

Homogenate for the determination of oxidative stress makers and oxidase enzymes was prepared as described earlier (Achuba and Ja-anni, 2018). Lipid peroxidation was determined by the method of Gutteridge and Wilkins (1982). The activity total superoxide dismutase was determined by Misra and Fredorich (1972) method and MnSOD and Cu/ZnSOD determined by Crapo *et al*. (1978) method. The activity of catalase was determined by Rani *et al*. (2004) method. The activities of sulphite oxidase, aldehyde oxidase and xanthine oxidase were determined by the methods of Johns (1967) and Stirpe and Della Corte (1969), respectively.

Statistical analysis

The results were analysed by means of one way analysis of variance (ANOVA) and post Hoc Fisher's test for multiple comparison with the aid of Statistical Package for social Science (SPSS), version 20. Level of significance was set at P values < 0.05.

Results and Discussion

In this study, the effectiveness of fish pond wastewater (FPWW) in amelioration of the effect of petroleum on plant metabolism was detected (Table 2). Significant (P<0.05) increases in the concentrations of the biomolecules in seedlings grown on soil treated with FPWW compared to those grown on diesel treated soil was observed. In fact, management of the diesel tainted soil with fish pond wastewater modulated the negative changes in the macromolecules investigated. This result is supported by earlier works in which abattoir wastewater was used to reduce the influence of crude oil (Achuba and Ja-anni, 2018); diesel (Achuba and Erhijiwvo, 2017) and premium motor spirit (Achuba and Iserhienrhien, 2018) polluted soil on plant. Also, the current investigation is in line with other studies which use animal waste to improve nutrient in crude oil polluted soil that encouraged plant development and yield (Njoku *et al.,* 2008; Agarry, 2010; Njoku *et al.,* 2012; Obiakalaije *et al.,* 2015).

Table 2: Effect of fish pond wastewater treatment on the level of sugar, protein, amino acid and β-carotene in the leaves of 12 day old cowpea seedlings grown in diesel contaminated soil

Concentration of diesel in soil (% v/w ml/g)	Total Sugar (mg/g)		Total Protein (mg/g)		Total Amino Acid (mg/g)		Beta-Carotene (mg/g)	
	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW	Diesel	Crude oil +FPWW
0.00	34.07±1.11	35.75±1.50	20.20±0.76 [a]	22.60±1.47 [b]	3.83±0.17	3.98±0.29	22.85±0.91[a]	25.08±1.08
0.10	28.90±1.30 [a]	33.15±1.19 [b]	17.95±1.00[a]	19.20±0.69 [b]	3.10±0.22 [a]	3.60±0.08[b]	20.90±0.45 [a]	22.18±0.43[b]
0.25	25.53±1.32 [a]	31.18±1.06 [b]	16.73±1.08 [a]	18.40±0.67 [b]	2.75±0.17 [a]	3.35±0.06[b]	19.82±0.21[a]	20.70±0.34 [b]
0.50	22.35±1.58 [a]	30.13±0.68 [b]	15.43±0.40 [a]	18.60±0.41 [b]	2.40±0.08 [a]	3.13±1.00[b]	18.55±0.69[a]	19.80±0.37 [b]
1.00	19.28±0.91 [a]	28.13±1.04 [b]	14.27±0.51 [a]	17.35±0.76 [b]	2.28±1.00 [a]	2.95±1.29[b]	16.25±0.79[a]	18.93±0.37 [b]
1.50	18.05±0.79 [a]	28.13±1.04[b]	12.60±0.64 [a]	17.48±0.98 [b]	2.10±0.08 [a]	2.75±0.13[b]	14.13±0.51 [a]	17.78±0.78[b]
2.00	16.17±0.74 [a]	25.48±1.24[b]	11.50±0.61 [a]	16.43±1.95 [b]	1.99±0.08 [a]	2.60±0.14[b]	12.33±0.74 [f]	17.03±0.51 [b]

Results are expressed as mean ± SD of four determinations values with a superscript indicates a significantly reduced level compared to control; while values with b superscript indicates it is significantly higher compared to the correspondent diesel only treated group at P<0.05.

Table 3: Effect of fish pond wastewater treatment on the content of chlorophyll and the activities of α-amylase and starch phosphorylase in the leaves of 12 day old cowpea seedlings grown in diesel contaminated soil

Concentration of diesel in soil (% v/w ml/g)	Total chlorophyll (mg/g)		α-amylase activity (Unit/ml)		Starch phosphorylase activity (mg/min gfw)	
	Diesel	Diesel +FPWW	Diesel	Diesel + FPWW	Diesel	Diesel + FPWW
0.00	288.25±1.71[a]	323.75±10.34[a]	21.05±1.22[a]	24.98±1.20[a]	1.06±0.47[a]	1.18±0.04[a]
0.10	220.00±7.11[b]	288.00±10.95[b]	14.83±1.24[b]	19.18±0.84[b]	0.97±0.09[ab]	1.03±0.08[ab]
0.25	208.25±3.86[c]	266.00±4.32[c]	13.08±0.53[bc]	19.15±0.47[b]	0.85±0.06[b]	0.95±0.05[b]
0.50	201.50±1.29[d]	258.25±4.64[c]	12.03±0.26[c]	17.68±0.34[bc]	0.66±0.06[c]	0.90±0.05[c]
1.00	192.25±4.19[e]	248.50±4.80[c]	10.80±0.53[d]	16.50±0.58[cd]	0.55±0.05[c]	0.81±0.09[b]
1.50	189.00±1.83[ef]	238.75±2.22[d]	9.63±0.40[de]	15.93±1.02[d]	0.47±0.04[d]	0.68±0.05[d]
2.00	174.50±8.10[f]	230.00±2.94[d]	8.68±0.43[e]	15.58±0.56[d]	0.42±0.06[d]	0.65±0.07[d]

Results are expressed as mean ± SD of four determinations All values in the same column having different alphabet superscripts indicates a significant difference at P<0.05

Previous studies had showed that the quantities of plant pigments (chlorophyll and carotene) and α-amylase and starch phosphorylase activities in plants are reduced during hydrocarbon tension (Achuba, 2006; Achuba and Okoh, 2015). Similar to previous observations, the current study (Table 3) indicates that FPWW treated diesel contaminated soil significantly increased the concentrations of chlorophyll and carotene as well as the activities of α-amylase and starch phosphorylase in the cowpea seedlings. This result agrees with the report by Achuba and Ekute (2017) who hinted that the addition of abattoir wastewater to soil improved seedlings performance. The observed snag in the actions of these enzymes and chlorophyll content may be traceable to nutrient inherent in fish pond wastewater (FPWW). This assertion is buttressed by the work of Udoh *et al.* (2012) who reported enhancement in the fertility of the soil by FPWW that improved garden egg yield compared to those augmented by animal manures and inorganic fertilizer. Furthermore, fish pond wastewater has the ability to stabilize soil physiochemical properties (Oyem and Oyem 2013).

The rise in lipid peroxidation observed in seedlings grown in diesel tainted soil is indicative of the fact that diesel has the ability to induce oxidative stress in plant and it is in agreement with the studies of Olubodun and Eriyamremu (2013); Achuba (2014) and Ekpo *et al.* (2012) but this was significantly (P <0.05) upturned by the addition of fish pond wastewater to the diesel tainted soils (Table 4). The restoration of metabolic status in plant cultivated in polluted soil by the addition of organic waste materials had been reported (Njoku *et al.*, 2008; Agarry 2010). In addition, agro waste as fertilizer was also reported (Okafor, 2016; Matheyarasu, 2016).

In addition, the presence of diesel in plant environment decreased the activities the oxidase enzymes (xanthine oxidase and aldehyde oxidase) Asagba (2010) reported that the production of endogenous heterocyclic compounds due to the presence of a stress inducer will contribute to the eventual depletion of aldehyde, xanthine and sulphite oxidases. However, the activities of oxidative enzymes in the seedlings were significantly (P <0.05) increased in seedlings exposed to fish pond wastewater treated soil compared to seedlings exposed to only diesel treated soil. Therefore, it can be inferred that the increased activity of xanthine and aldehyde oxidases may have risen from the protective contributions of the nutrient constituents of the fish pond wastewater. Fish pond wastewater had been previously reported as a potential fertilizer and fertilizers that are organic in nature are antidote for crude oil polluted soil (Udoh *et al.*, 2012; Oyem and Oyem, 2013; Okafor, 2016; Matheyarasu, 2016).

Table 4: Effect of fish pond wastewater treatment on the level of lipid peroxidation and activities of xanthine oxidase, catalase and aldehyde oxidase in the leaves of 12 day old cowpea seedlings grown in diesel contaminated soil

Concentration of diesel in soil (% v/w ml/g)	Lipid peroxidation (nMol/cm³)		Xanthine oxidase activity (Unit/cm³)		Catalase activity (nmol/min gfw)		Aldehyde oxidase Activity (Unit/cm³)	
	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW
0.00	0.99±0.10[a]	0.89±0.07[a]	3.79±0.46[a]	4.57±0.27[ab]	1.14±0.16[a]	1.14±0.14[a]	1.33±0.15[a]	1.67±0.07[a]
0.10	1.14±0.01[a]	0.99±0.09[a]	4.76±0.24[b]	4.67±0.07[a]	1.13±0.02[a]	1.04±0.11[ab]	1.48±0.08[a]	1.90±0.03[b]
0.25	1.88±0.03[b]	1.52±0.08[b]	4.88±0.22[b]	4.74±0.10[a]	1.16±0.05[a]	1.13±0.10[a]	1.62±0.04[b]	2.06±0.08[c]
0.50	1.86±0.04[b]	1.59±0.09[bc]	4.28±0.31[b]	4.46±0.43[ab]	1.06±0.04[a]	1.15±0.15[a]	1.61±0.08[b]	1.74±0.06[a]
1.00	2.33±0.09[c]	1.64±0.02[bc]	3.86±0.13[c]	4.22±0.19[ab]	0.74±0.11[b]	1.03±0.07[ab]	1.39±0.07[a]	1.51±0.04[d]
1.50	2.62±0.07[d]	1.72±0.08[bc]	3.54±0.10[cd]	4.30±0.22[ab]	0.58±0.08[bc]	0.92±0.04[ab]	1.28±0.06[a]	1.38±0.04[de]
2.00	2.87±0.11[e]	1.80±0.21[c]	3.21±0.15[d]	4.07±0.07[b]	0.44±0.04[c]	0.81±0.06[b]	1.21±0.08[a]	1.34±0.03[e]

Results are expressed as mean ± SD of four determinations All values in the same column having different alphabet superscripts indicates a significant difference at P<0.05

Table 5: Effect of fish pond wastewater treatment on superoxide dismutase activities in the leaves of 12 day old cowpea seedlings grown in diesel contaminated soil

Concentration of diesel in soil (% v/w ml/g)	Total SOD (Unit/gfw)		CuZnSOD (Unit/gfw)		MnSOD (Unit/gfw)	
	Crude Oil	Diesel + FPWW	Diesel	Diesel +FPWW	Diesel	Diesel +FPWW
0.00	2.26±0.13[a]	2.27±0.18[a]	2.11±0.07[a]	2.04±0.02[a]	1.01±0.13[a]	0.83±0.09[a]
0.10	1.99±0.04[b]	2.16±0.16[a]	1.99±0.09[ab]	2.05±0.19[a]	0.66±0.09[b]	0.85±0.04[a]
0.25	1.82±0.09[c]	2.02±0.04[a]	1.91±0.06[b]	1.98±0.11[a]	0.43±0.08[c]	0.69±0.06[b]
0.50	1.63±0.04[d]	2.11±0.13[a]	1.56±0.06[c]	1.94±0.20[ab]	0.36±0.10[d]	0.57±0.07[bc]
1.00	1.51±0.05[de]	1.89±0.10[b]	1.49±0.05[cd]	1.73±0.26[ab]	0.25±0.07[de]	0.43±0.05[c]
1.50	1.36±0.07[e]	1.77±0.04[b]	1.35±0.06[de]	1.72±0.08[ab]	0.17±0.02[de]	0.24±0.04[d]
2.00	1.27±0.03[e]	1.71±0.06[b]	1.23±0.02[e]	1.58±0.06[b]	0.13±0.01[e]	0.23±0.05[d]

Results are expressed as mean ± SD of four determinations All values in the same column having different alphabet superscripts indicates a significant difference at P<0.05

This study observed no significant change in the activities of catalase in cowpea seedlings in the diesel exposed groups at low level of soil contamination (0.1 - 0.5%) but showed significantly reduced activities at higher contaminations (1.0, - 2.0%) relative to control (Table 5). This observation is in agreement with results of Olubodun and Eriyamremu (2013), Achuba and Okoh (2015), Okolo *et al*. (2005) and Njoku *et al*. (2012) who reported decreased activities of catalase in seedlings grown on hydrocarbon tainted soils. Also, increase in activities of catalase in seedlings grown on FPWW treated diesel contaminated soil compared to their corresponding diesel treated soil was observed. This is similar to the reports by Achuba and Okunbor (2018) who used abattoir waste water for the treatment of kerosene tainted soil. Same trend was observed in the total SOD activities which is an enzyme responsible for the dismutation of superoxide anions and has been acknowledged to initiate the first line of defense against reactive oxygen species (Ascher *et al.,* 2002; Choi *et al.,* 2002) thus increase in their activities are indicative of the presence of metabolic stress. The observed reduction in SOD activity at high concentrations of the contaminants in soil can be indications of the serious impact of the contaminants on the enzyme (Olubodun and Eriyamremu, 2013). Diesel treated soil in all concentrations had a dose dependent significant reduction of CuZnSOD and MnSOD activities in the cowpea seedlings. A similar dose related decrease except at 0.1% exposure occurred in seedlings cultivated in soil treated with FPWW. Moreover, a significant ($P < 0.05$) increases in CuZnSOD and MnSOD in seedlings grown on FPWW treated soil relative to their corresponding diesel exposed seedlings (Table 5). This result is in agreement with those of Akujobi (2011) and Njoku (2012) who reported improvements in CuZnSOD and MnSOD activities after treatment of soil with cow dung and organic waste, respectively.

Conclusion

The current study revealed that fish pond wastewater is able to remediate the negative effects of diesel contamination on the metabolic and antioxidant indices of cowpea seedlings cultivated in diesel tainted soil.

Conflict of Interest

The authors declare that there is no conflict of interest.

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