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Physiological parameters of *Phaseolus vulgaris* L. in Diesel Oil Polluted Soil and Amelioration Treatment

[1]*Etukudo Mbosowo M., [2] Roberts Eneni M.I. and [3]Okereke Ifeoma J.

[1,2]Department of Biological Sciences, Federal University Otuoke, Bayelsa State, Nigeria
[3]Department of Environmental Resource Management, Abia State University, Abia State, Nigeria

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Abstract

Physiological parameters of *Phaseolus vulgaris* L. in diesel oil polluted soil and amelioration treatment using palm bunch ash on the chemical properties of experimental soils were examined for 8 weeks. The pollution treatments were obtained by mixing thoroughly 100 ml of diesel oil with 2 kg of sandy-loam soil, while 0 ml (unpolluted soil) was used as control. Amelioration treatments were carried out by adding palm bunch ash at the rate of 0.5, 1.0, 1.5 and 2.0 kg to the 100 ml level of diesel oil polluted soil. Diesel oil contamination significantly ($p > 0.05$) reduced the available phosphorus and total nitrogen contents of the contaminated soil. The application of palm bunch ash increased the available phosphorus and total nitrogen contents of the amended soils. The values recorded for plant height, leaf number, root length and shoot/ root ratio in all treatments were lower than that of the control - 0 (unpolluted soil). In remediation treatment, the crop growth parameters increased with increase in the rate of application of palm bunch ash with values higher than those of the pollution treatments. These values were comparatively lower than that of the control (0) treatment. The calcium, magnesium, phosphorus, nitrogen, sodium, zinc, and copper contents in leaves of *Phaseolus vulgaris* increased significantly ($p > 0.05$) with increase in the level of palm bunch ash in amended soils. There were marked differences in nutrient element contents of experimental soils before and after harvest. Therefore, palm bunch ash is an effective organic supplement for remediation of diesel oil polluted soil.

****Corresponding author:*** Etukudo, M.M., mbosombosowo@yahoo.com

Introduction

Increasing petroleum exploration has led to wide-spread contamination of agricultural lands (Eisenberg and Laughlin, 2001). In the Niger Delta region of Nigeria, most of the terrestrial ecosystems and shore-lines where crude oil pollution occurs with its deleterious impacts, are important agricultural lands for cultivation (Pezeshski *et al.*, 2000; Etukudo, 2010). Petroleum oil pollution usually results in damage to the soil microorganisms and plants together with increase in soil organic carbon, and reduction in soil nitrates and phosphorus, thus imposing a condition that impairs oil degradation in the soil (Okolo *et al.*, 2005; Adedokun and Ataga, 2007). Diesel oil is one of the refined components of petroleum oil and a broad mixture of hydrocarbons produced either as distillate, or residual materials, or as blend of the two during the refining of crude petroleum (Moeller *et al.*, 2008). The use of organic supplements such as palm bunch ash to remediate petroleum oil pollution is one of the viable cost effective methods of soil recovery (Etukudo *et al.*, 2011, Etukudo *et al.*, 2014a). Amendments that provide limiting resources (oxygen, nutrients and moisture) at the oil spill sites may stimulate the performance and reproduction of hydrocarbonclastic degraders (Tabak *et al.*, 2003; Lei *et al.*, 2005). The addition of organic material to soil may accelerate remediation of contaminated oil sites by improving soil structure, water infiltration and percolation, and increasing soil biological activity.

Common Bean (*Phaseolus vulgaris L.*) belongs to the family Fabaceae. The crop has edible leaves often used in soups preparation as well as the high protein content of the seeds, which makes it a valuable food for domestic consumption and as export crop (Debouck, 1994, Gomez, 2004). Organic manure has over time been used to improve soil fertility, however, its efficacy in assessing specific physiological response of *Phaseolus vulgaris* L. in diesel oil polluted soil has not been extensively studied. Therefore, this study is aimed at investigating the potential of palm bunch ash on the soil properties, growth performance and mineral nutrient composition of *Phaseolus vulgaris* L. in diesel oil polluted soil.

Materials and methods

Remediation treatment

Remediation experiments were designed to investigate the effects of organic supplement (palm bunch ash) on the growth parameters and mineral nutrient composition of *Phaseolus vulgaris* L. grown in diesel oil polluted soil. Palm bunch ash was obtained from local farmers in Oyigbo, Rivers State, Nigeria. The Chemical Properties of palm bunch ash were analysed using standard procedures (A.O.A.C, 1999). The amelioration treatments were carried out by adding organic supplements at the rate of 0.5, 1.0, 1.5, and 2.0 kg to 100 ml level of diesel oil polluted soils. The soil samples with or without organic material were left undisturbed for another four weeks before being placed in perforated polythene bags (18 x 36 cm). The period was supposed to be enough to facilitate loosening of the oil constituent completely (Amadi *et al.*, 1992).

Pollution treatment

Sandy-loam soil obtained from the University of Port Harcourt Botanical Garden, Rivers State, Nigeria was analysed for physico-chemical properties using standard procedures of A.O.A.C. (1999). The pH values of the soil were measured in 1:2 soil to liquid suspension with electro pH meter. Organic carbon was determined by dictrometric wet oxidation method. Total nitrogen was determined by the Macro-kjeldal's method. Available phosphorus was estimated by the Bray P-I method. Exchangeable bases were extracted with 1M ammonium acetate solution. The potassium and sodium extracts were determined by flame photometry, while calcium and magnesium were determined by the EDTA filtration method (A.O.A.C., 1999).

Two kilograms (2 kg) of sandy-loam soil were weighed using a triangular weighing balance. Polluted soils were obtained by mixing thoroughly 2 kg of sandy-loam soil with 100 ml of diesel oil and left undisturbed for one week. 0 ml (unpolluted soil) was used as control (Etukudo *et al.*, 2010).

Germination studies

Seeds of *Phaseolus vulgaris* L. obtained from Akwa Ibom State Agricultural Development Project (AKADEP), Uyo, Akwa Ibom State were sterilized with approximately 0.01% mercuric chloride solution for 30 seconds, thoroughly washed several times with distilled water and air dried. Ten (10) seeds of the test crop were

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sown directly in each polythene bag containing the various level of palm bunch ash mixed with 2 kg sandy-loam soil (with or without diesel oil). The seedlings were thinned to three (3) per bag. Each level of treatment was replicated five times using randomized complete block design. The Experiment was maintained at a mean minimum temperature of 22.42°C and a mean maximum temperature of 33.06°C, under natural light condition for two (2) months.

Growth studies

Growth parameters such as plant height, root length, leaf number, and shoot/root ratio were determined after harvest.

Determination of mineral contents of plant material

Leaf material of the crop were harvested, rinsed with distilled water and dried. The dried plant material of each sample was macerated into powdered form using pestle and mortar. The powder was sieved through a 0.002 mm wire mesh to obtain fine powdered form. Each sample of the powdered plant material was kept in small bottles for analysis. The determination was made using standard methods described by A.O.A.C. (1999) and Hack (2000). Total nitrogen concentration in plant dry matter was estimated by standard Micro-Kjeldahl method. Phosphorus was assayed spectrophotometrically by ammonium-vanadate-molybdate method, potassium by using a frame photometer and other elements by atomic absorption spectrophotometer.

Statistical analysis

Standard errors of the mean values were calculated for the separate readings and data were subjected to analysis of variance (ANOVA) to compare the means at 0.05 confident interval (Obi, 2002)

Results

The palm bunch ash had a pH of 9.52 showing that it is an alkaline medium. The macro and micro nutrients contents of the palm bunch ash are presented (Table 1).

Table 1: Chemical properties of palm bunch ash

Properties	Contents
pH	9.52
Moisture content (%)	57.21
Potassium (ppm)	51.22
Calcium (ppm)	8.36
Magnesium (ppm)	6.32
Sodium (ppm)	6.47
Zinc (ppm)	1.02
Iron (ppm)	1.21
Copper (ppm)	0.92
Manganese (ppm)	0.72

Table 2: Chemical properties of experimental soil before harvest

Properties	Garden soil (control - 0)	Pollution treatment	Remediation treatment – Concentration of Palm bunch ash (kg)			
			0.5	1.0	1.5	2.0
pH	5.20 ± 0.21	5.60 ± 0.32	5.70 ± 0.73	5.80 ± 0.54	6.24 ±0.43	6.90 ± 0.34
Available-P (mg/100g)	4.42 ± 0.02	0.14 ± 0.02	0.92 ± 0.03	1.24 ± 0.21	2.72 ± 0.47	3.40 ± 0.83
Total- N (%)	0.62 ± 0.04	0.23 ± 0.06	0.26 ± 0.03	0.35 ± 0.02	0.40 ± 0.03	0.57 ± 0.32
Organic-C (%)	2.62 ± 0.12	3.71 ± 0.22	3.88 ± 0.24	3.94 ± 0.77	4.02 ± 0.51	4.11 ± 0.26
Ca (mg/100g)	0.56 ± 0.04	2.62 ± 0.53	2.75 ± 0.33	2.92 ± 0.56	3.17 ± 0.81	3.52 ±0.23
Mg (mg/100g)	2.83 ± 0.13	3.96 ± 0.46	4.02 ± 0.52	4.36 ± 0.34	4.47 ± 0.31	4.92 ± 0.42
Na (mg/100g)	4.11 ± 0.25	7.21 ± 0.67	7.43 ± 0.65	7.54 ±0.41	7.76 ± 0.46	7.96 ± 0.38
K (mg/100g)	0.36 ± 0.08	0.62 ± 0.02	0.99 ± 0.04	1.52 ± 0.05	2.33 ± 0.67	2.52 ± 0.43
Mean	2.59	3.01	3.24	3.46	3.88	4.24
LSD ($p < 0.05$)	1.27	1.09	1.02	1.52	1.46	1.35

Mean value ± Standard error of 5 replicates

Table 3: Growth parameters of *Phaseolus vulgaris* L. grown in diesel oil polluted soil amended with varying levels of palm bunch ash (PBA)

Treatment	Plant height (cm)	Leaf number	Root length (cm)	Shoot/Root ratio
0 (control)	63.23 ± 0.58	24.33 ± 0.41	23.47 ± 0.49	2.69
Pollution treatment	39.23 ± 1.20	13.00 ± 0.18	15.63 ± 0.25	2.51
0.5kg PBA	42.30 ± 1.03	17.33 ± 0.61	16.43 ± 0.80	2.57
1.0kg PBA	46.23 ±1.20	16.00 ± 0.37	17.70 ± 0.23	2.61
1.5kg PBA	53.63 ± 1.31	20.00 ± 0.43	20.23 ± 0.18	2.65
2.0kg PBA	54.23 ±1.62	20.23 ± 0.60	20.40 ± 0.49	2.66
Mean	49.81	18.48	18.98	2.62
LSD ($p > 0.05$)	2.72	2.06	1.97	0.36

Mean value ± Standard error of 5 replicates

Table 4: Nutrients content of *Phaseolus vulgaris* L. grown in diesel oil polluted soil amended with varying levels of palm bunch ash (PBA)

Nutrient element (mg/100g)	Garden soil (control - 0)	Pollution treatment	Remediation treatment – Concentration of Palm bunch ash (kg)			
			0.5	1.0	1.5	2.0
Calcium	1.37 ± 0.30	0.82 ± 0.02	0.86 ± 0.05	0.93 ± 0.03	1.06 ± 0.21	1.29 ± 0.21
Potassium	1.34 ± 0.16	1.26 ± 0.78	1.17 ± 0.16	1.08 ± 0.32	0.83 ± 0.02	0.79 ± 0.22
Magnesium	3.13 ± 0.36	1.03 ± 0.17	1.07 ± 0.33	1.28 ± 0.41	2.64 ± 1.02	2.95 ± 1.03
Phosphorus	10.94 ± 1.21	5.67 ± 1.07	7.70 ± 1.30	8.68 ± 0.07	9.68 ± 0.19	10.38 ± 1.27
Nitrogen (%)	4.44 ± 1.35	1.36 ± 0.16	2.04 ± 0.63	2.31 ± 0.46	3.02 ± 1.68	3.36 ± 0.77
Sodium	5.37 ± 1.82	4.69 ± 1.75	4.93 ± 1.22	5.27 ± 1.54	5.58 ± 1.20	5.89 ± 1.28
Zinc	0.20 ± 0.06	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.12 ± 0.04	0.17 ± 0.06
Iron	2.12 ± 1.20	0.30 ± 0.04	1.72 ± 0.06	1.34 ± 0.05	1.04 ± 0.24	0.87 ± 0.16
Copper	0.63 ± 0.02	0.43 ± 0.06	0.47 ± 0.02	0.05 ± 0.01	0.52 ± 0.04	0.60 ± 0.02
Mean	3.28	1.74	2.23	2.34	2.72	2.92
LSD ($p > 0.05$)	0.92	0.61	0.41	1.07	0.56	0.75

Mean value ± Standard error of 5 replicates

The pH of the soil increased with increase in the rate of application of palm bunch ash from 5.70 at 0.5 kg PBA to 6.90 at 2.0 kg PBA compared to the value of 5.20 and 5.60 recorded at the control - 0 (unpolluted soil) and diesel oil contaminated soil, respectively (Table 2). Diesel oil contamination significantly ($p > 0.05$) reduced the available phosphorus and total nitrogen contents as well as other components of the contaminated soil. Although, the application of PBA increased the available phosphorus and total nitrogen contents of the amended soils, the values recorded in all treatments were lower than that of the control-0 (unpolluted soil) (Table 2). The plant height, leaf number, root length and shoot/ root ratio of the crop decreased significantly ($p > 0.05$) in pollution treatment relative to the control - 0 (unpolluted soil). In remediation treatment, the crop growth parameters increased with increase in the rate of application of PBA, with values higher than those of the pollution treatments. These values were comparatively lower than that of the control - 0 (unpolluted soil) (Table 3).

The calcium, magnesium, phosphorus, nitrogen, sodium, zinc, and copper contents in leaves of *Phaseolus vulgaris* L. increased significantly ($p > 0.05$) with increase in the level of palm bunch ash in amended soils. These values were comparatively higher than those of the pollution treatment, and relatively lower than those of the control - 0 (unpolluted soil). Conversely, the potassium and iron contents in leaves of the test crop decreased significantly ($p > 0.05$) with increase in the level of palm bunch ash in amended soils. Comparatively, these values were not higher than those of the control treatment (Table 4). There were marked differences in nutrient element contents of experimental soils before and after harvest. Considerable proportions of mineral elements were recorded in experimental soils after harvest. There were marked increases in organic carbon contents in experimental soil in all treatments after harvest (Table 5).

Discussion

The chemical properties of palm bunch ash as revealed in this study correspond with the physico-chemical analysis conducted by Omoti *et al.* (1991) and Etukudo *et al.* (2014a). Palm bunch ash has been shown to contain rich nutrients such as nitrogen, phosphorus, potassium, calcium and magnesium. Therefore, the alkaline properties and rich nutrient contents of the palm bunch ash used in this study could be a better source of remediation practice in diesel oil polluted soil with an acidic pH and nutrient depleted characteristics (Awodun *et al.*, 2007). The reduction in growth of the test crop in diesel oil pollution treatment could be as a result of depression of growth of leguminous plants usually observed in acidic soils as evidenced in petroleum oil polluted soils (Adedokun and Ataga, 2007).

Table 5: Chemical properties of experimental soil after harvest

Property	Garden soil (control - 0)	Pollution treatment	Remediation treatment – Concentration of Palm bunch ash (kg)			
			0.5	1.0	1.5	2.0
pH	5.10 ± 0.26	4.72 ± 0.22	5.40 ± 0.44	5.40 ± 0.48	6.10 ± 0.36	6.80 ± 0.35
Available-P (mg/100g)	0.21 ± 0.01	0.07 ± 0.03	0.41 ± 0.02	0.46 ± 0.09	0.52 ± 0.03	0.61 ± 0.02
Total- N (%)	0.30 ± 0.02	0.12 ± 0.01	0.14 ± 0.04	0.18 ± 0.06	0.20 ± 0.02	0.26 ± 0.01
Organic-C (%)	0.36 ± 0.02	6.27 ± 0.43	3.92 ± 0.39	4.03 ± 0.65	4.92 ± 0.17	4.97 ± 0.68
Ca (mg/100g)	0.21 ± 0.03	2.07 ± 0.21	1.64 ± 0.17	1.72 ± 0.64	2.16 ± 0.25	2.21 ± 0.36
Mg (mg/100g)	1.21 ± 0.40	2.12 ± 0.72	2.07 ± 0.26	2.32 ± 0.44	2.42 ± 0.57	2.69 ± 0.27
Na (mg/100g)	2.07 ± 0.26	5.27 ± 0.36	4.26 ± 0.19	4.53 ± 0.16	4.74 ± 0.63	4.21 ± 0.35
K (mg/100g)	0.12 ± 0.05	0.43 ± 0.03	0.18 ± 0.07	0.36 ± 0.04	0.42 ± 0.03	0.57 ± 0.06
Mean	1.20	2.63	2.25	2.38	2.69	2.79
LSD ($p < 0.05$)	0.73	0.44	0.61	0.58	0.47	0.36

Mean value ± Standard error of 5 replicates

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Thus, plant nutrition is affected with the promotion of aluminium toxicity, and certain physiological, morphological and biochemical problems set in due to increased osmotic potential, as well as suppression of rhizobial survival due to acidity (Rengasamy and Rathjen, 2003; Al-azab *et al.*, 2005). The enhanced plant growth in diesel oil polluted soil supplemented with palm bunch ash as observed in this study may be attributed to the important role played by the organic supplement in supplying the readily plant minerals and in providing better soil conditions (Belay *et al.*, 2001, Kapanen and Havaara, 2001). Palm bunch ash has been reported to contain high amount of calcium, potassium and magnesium with a considerable proportion of organic matter, nitrogen and phosphorus (Omoti *et al.*, 1991) as shown in this study. The enhanced nutrient uptake of *Phaseolus vulgaris* in diesel oil polluted soil amended with palm bunch ash could be attributed to the effectiveness of palm bunch ash as a liming material in raising the pH of the polluted soil to enhance nutrients availability. Soil nutrients status have been shown to increase due to the application of palm bunch ash as a result of improved pH of the soil and increased microbial activities leading to the production of organic matter and nutrient availability (Omoti *et al.*, 1999; Odedina *et al.*, 2003; Etukudo *et al.*, 2014b).

Conclusion

Palm bunch ash proves effective as a liming material for increasing soil fertility in petroleum polluted soil, as evidenced in this study. Therefore, this study reveals that palm bunch ash can be utilized as an organic amendment for the improvement of growth and mineral nutrients status of *Phaseolus vulgaris* grown in diesel oil polluted soil.

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