African Journal of Biotechnology Vol. 6 (16), pp. 1922-1925, 20 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Performance of *Phaseolus vulgaris* L. in a soil contaminated with spent-engine oil

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Accepted 28 May, 2007

Phytoremediation is an alternative low cost approach for *in situ* treatment of polluted soils. This study evaluated growth and biochemical composition of *Phaseolus vulgaris* as influenced by spent engine oil contaminated soil. The experiment was conducted in a pot during the 2005 cropping season. The soil received (0% (control), 1, 5 and 10%) v/w of oil and was completely randomized with three replications per treatment. Significant treatment effects were observed in plant height, number of leaves and dry weight. However, plant height 42 days after planting (DAP) and dry weight 28 DAP were not significantly different (p<0.05). The control dry weight accounted for greater percentage of the total dry weight recorded in each of the sampling regimes relative to other treatment. There was about 1.3, 1.4 and 1.6-fold yield reduction as concentration of spent oil increased (1-10%) respectively. This study established that *P. vulgaris* was growing under stress as indicated by the dose-dependent significant increase in protein content relative to control and low chlorophyll content of the plant with treatment. There is evident that *P. vulgaris* can sustain growth in an oil polluted soil and a good candidate for phyto-remediation.

Key words: Phytoremediation, *Phaseolus vulgaris*, spent engine oil contamination.

INTRODUCTION

Crude oil is refined to yield products such as gasoline oil, fuel, liquefied gas and grease. Engine oils are used mainly to provide a film between the moving parts of auto machines and engines, which helps to reduce wear and tear and loss of power. It can also prevent corrosion of these auto machine parts. Disposal of spent engine oil into gutters, watercourses, open vacant plots and farm-land are common practice among automechine operators. This practice increases incidence of oil contamination of agricultural soils. Whisman et al. (1974) reported presence of heavy metals such as vanadium, lead, aluminum, nickel and iron in unused lubricating oils, with high values in used ones.

Oil contaminated soils are of environmental concern because they are unsuitable for agricultural and recreational use and are potential sources for surface and ground water contamination. Topsoiling and incineration are possible clean up methods (Schwab and Banks, 1999; Sung et al., 2004). These methods are expensive and do not give permanent solution to the problem.

Phytoremediation is an attractive low cost alternative to traditional remediation strategies. Plants can enhance bioremediation process by absorbing, translocating or sequestering the organic contaminant, which removes them from the soil compartment (Cunningham et al., 1995). Nye and Tinker (1977) observed enhanced aeration within root areas resulting from direct release of O_2 from roots and O_2 diffusion along old root channels. This phenomenon stimulates microbial activity within the region, which enhance bioremediation of hydrocarbons. Roots may also reduce movement of contaminant in soil by extracting excess water, thus reducing the downward flow of water (Topp et al., 1986; Nair et al., 1993; Viamis et al., 1985).

Phytoremediation has shown good performance in bioremediation of crude oil contaminated soil. Issoufi et al. (2006) reported significant percentage emergence and plant biomass production in *Zea mays* and *Glycine max*

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Table 1. Mean values of growth indicators on *P. vulgaris* grown on spent engine oil contaminated

Conc. (%)	14 DAP			28 DAP			42 DAP			66 DAP
	PH (cm)	LN	DW (g)	PH (cm)	LN	DW (g)	PH (cm)	LN	DW g)	H (g)
0	11.6	7.6	4.0	14.6	11.0	4.3	24.0	14.3	6.7	10.3
1	9.0	6.0	2.8	13.7	8.0	3.7	23.7	11.3	4.7	7.7
5	6.7	5.3	2.0	11.0	7.0	2.7	22.3	10.0	5.3	4.6
10	6.3	5.0	1.36	10.0	6.7	2.0	22.6	9.0	2.6	3.0
$LSD_{0.05}$	0.32	0.15	0.62	0.35	0.30	NS	NS	0.14	1.31	2.4

PH, Plant height; LN, number of leaves; DW, dry weight; H, harvest; DAP, days after Planting; NS, not significant.

when grown in crude oil contaminated soil. *Lolium perenne* L (perennial rye grass) significantly reduced the concentration of petroleum hydrocarbon compared with an unvegetated control in a laboratory study (Günther et al., 1996). However, petroleum can create unsatisfactory conditions for plant growth through a number of processes; (a) oil could displace air from soil pore spaces, (b) an increase in the demand for oxygen brought about by activity of oil-decomposing microorganisms (Gudin and Syratt, 1975), and (c) petroleum hydrocarbon creates hydrophobic environment, which limits water absorption to plant roots (Schnoor, 1997). In this present experiment, *P. vulgaris* was evaluated for growth and development in spent oil contaminated soil.

MATERIALS AND METHODS

The study was carried out in the Erosion and Environment Research Institute, Federal University of Technology. Owerri, Nigeria (lat.5°29 N, long.7°33 E). A sandy-loam soil collected from a nearby field in the Institute was analyzed for initial chemical properties. Results of initial soil chemical analysis were pH 5.6, total N (determined using micro kjeldahl's method followed by spectrophotometric procedure) of 0.723%, organic carbon (determined by oxidation with $K_2Cr_20_7$; Yeomans and Bremner, 1989) of 0.43%, available phosphorus (determined using method of Murphy and Riley, 1972) of 32 mg/kg, potassium (determined using flame photometry) 125 mg/kg. The soils was air-dried and grind to pass through 2 mm sieve. Polythene bags (15 cm height and diameter 10 cm) were filled with about 4.5 kg sandy-loam soil treated with spent engine oil (SAE 40) to achieve concentration of (0, 1, 5 and 10%) v/w (oil/soil), respectively. These treatments were replicated 3 times and were laid out in a completely randomized design.

The test crop, African beans, *P. vulgaris* L, is a member of leguminosae, subfamily papilionoideae. It is widely grown in Africa especially in Nigeria where the seed is cooked and consumed as source of protein or used for livestock feed supplement. The seeds used in this study were obtained from National seeds Umudike, Umuahia, Nigeria. Each pot received 2 seeds and later thinned to one after emergence. Parameters such as plant height (PH), number of leaves, dry weight (DW) were collected at 14 days after planting (DAP), 28 DAP, 42 DAP and at harvest (62 DAP). The following parameters were collected: pod yield, pod weight plant^{-1}, pod number plant^{-1}, number of seed plant^{-1}, seed weight plant^{-1} and number of root nodules plant^{-1}. Plant height was measured using a simple meter rule, placed at the soil level to the terminal bud. Number of leaves was counted as the growth of plant progresses. Dry weight was measured by carefully oven drying at 80°C for 72 h. At harvest only the yield, dry weight and number of root nodules were collected. Root nodules were counted with the aid of a hand lens after washing carefully under free flowing tap water.

The biochemical determination was done by collecting four leaves from each of the 42 DAP old plant of the treatments including control, weighed and homogenized in 80% acetone. The extract was centrifuged at 3500 rpm at a temperature 0°C for 20 min. The solution was diluted to 10 ml and chlorophyll content determined following the method of Lichtenthaler (1987). Plants from 42 DAP were also analyzed for total protein and sugar. The plant was homogenized with chloroform/methanol (2:1, /v/v) filtered and the residue was re-extracted, sugar was estimated by the method of Tietze (1982). The total protein was determined using the method of Lowry et al. (1951).

An analysis of variance was conducted at ($p = 0.05$) to determine if factors were significant. Significant means were separated using $LSD_{0.05}$.

RESULTS

The initial chemical analysis revealed the presence of appreciable level of macronutrients P and K but requires average supplemental nitrogen. Since the test crop is legume, this may not be essential assuming symbiotic nitrogen fixation is not inhibited.

Growth as influenced by spent oil concentration

The effect of spent oil on various growth parameters of *P. vulgaris* is presented in Table 1. The mean plant height of the control was significantly ($p<0.05$) greater than those means for plants grown in soil treated with 1 - 10% spent oil except in 42 DAP. There was progressive reduction in plant height as the concentration of spent oil increased from 1 - 10%. In this study, there was no case of phytotoxicity resulting from treatment application rather experimental units experienced appreciable growth patterns. Number of leaves was significantly different in all observations. The control plant had greater number of leaves than those with treatment. Plant dry weight was significantly different ($p<0.05$) in all the sampling regimes except in 28 DAP. Dry weight of the control plant was greater than the plants that received treatment. In 28 DAP, increasing the treatment concentration reduced plant dry weight but showed no significant different. The control dry

Table 2. Influence of spent oil on yield of *P. vulgaris*.

Conc. (%)	Pod yield $Plant^{-1}$	Pod wt $plant^{-1}$	Pod no. $plant^{-1}$	Seed no. $plant^{-1}$
0	4.6	9.0	6.3	60.3
1	3.5	7.6	3.3	50.2
5	3.2	2.3	1.7	43.6
10	2.8	2.7	2.3	25.4
$LSD_{0.05}$	0.28	0.08	0.26	5.21

Table 3. Mean values of sugar, total protein and chlorophyll of *P. vulgaris* at 42 DAP as influenced by varying concentration of spent engine oil.

% Conc.	Sugar (mg/g)	Total protein (mg/g)	Chlorophyll (mg/g)
0	34.6	17.6	315.0
1	46.7	30.2	250.2
5	48.6	36.2	230.2
10	52.2	37.2	220.0
$LSD_{0.05}$	4.0	6.2	15.2

Table 4. Influence of spent engine oil on *P. vulgaris* nodulation.

% conc.	14 DAP	28 DAP	42DAP	Harvest
0	12.0	15.2	22.1	24.2
1	8.2	10.6	18.2	20.2
5	5.8	10.0	15.4	17.3
10	5.2	8.9	14.2	16.2
$LSD_{0.05}$	2.4	1.6	2.2	2.3

weight accounted for appropriately 39, 34, 34.5 and 40.2% of the total dry weight recorded in 14, 28 and 42 DAP and harvest, respectively, relative to plants that received treatment.

Pod yield and yield components as influenced by spent oil concentration.

The results of the pod yield and yield components of *P. vulgaris* are presented in Table 2. Pod yield and yield components were significantly different ($p<0.05$) in the entire sampling regimes. The pod yield ranged (2.8 - 4.6 g $plant^{-1}$) and the control recorded greater yield than plant with treatment. Increasing the spent oil from 1 - 5% showed no significant difference in pod yield. There was about 1.3, 1.4 and 1.6-fold in yield reduction as spent oil concentration increased (1, 5 and 10%, respectively). Pod weight per plant ranged from (2.67 - 9.0 g). And the control also had greater pod weight than plants that received treatment. From the results, there was 1.2, 3.9 and 3.3-fold pod weight reduction as spent oil increased (1, 5 and 10%, respectively). However, 10% had higher pod weight than 5%. There was appreciable increase in pod number per plant, the control recording the highest (6.3) and 5% the lowest (1.7).

Nodulation as influenced by spent oil concentration

The mean nodule recorded in the control was highest and significantly different relative to values from plants with treatment (Table 4). There was progressive decrease in nodulation as percentage spent oil increased.

Influence of spent oil on the chlorophyll, sugar and protein content of the tissue

The results of the biochemical analysis of the plant tissues are presented in Table 3. *P. vulgaris* was growing under environmental stress. This is indicated by the dose-dependent significant increase in sugar and protein contents relative to control. Sugar content ranged (34.6 - 52.2 mg/g), total protein ranged between (17.6 - 37.2 mg/g) and the chlorophyll content ranged (220 - 315 mg/g). The chlorophyll content of control was highest (315 mg/g) and significantly different relative to plants that received treatment.

DISCUSSION

Soil provides the essential macronutrients required for plant growth and development. These minerals are ionized or solubilized from solid phase of the soil to be available for plant uptake (Epstein, 1972). This pathway of nutrient release to plant is very vital. Thus any disruption of this normal pathway obviously has a negative influence in the normal growth and development of plants. The presence of spent oil in the soil-plant microenvironment appears to have affected normal soil chemistry wherein nutrient release and uptake as well as amount of water have been reduced. In this study, we have noted remarkable decrease in plant growth in treated soil relative to control. This result is consistent with works of previous authors (Kinghorn, 1983; De Song, 1980; Odjegba and Sadiq, 2002). Anoliefo and Vwioko (1995) noted poor germination of *Capsicum annum* and *Lycopersicon esculentum* when treated with 4 and 5% of spent oil.

Plant growth and reproduction are correlated with survival and this has been used to evaluate the potential of plants to grow in contaminated soil (Spiares et al., 2001). The survival of plants on oil-contaminated soils may be attributed to the presence of microorganisms in the root zones of plants (rhizosphere). Pierzynski et al. (2005) noted over 90 times population of microorganisms in rhizosphere than that of non-rhizosphere soil. The population of hydrocarbon degrading bacteria was five times more abundant in the rhizosphere of hybrid poplar trees relative to an unplanted reference site (Jordahl et al., 1997). Plants have capacity to withstand relative high concentrations of organic chemicals, such as oil, without toxic effect and they can uptake and convert this contaminates quickly to less toxic metabolites in some cases by rhizodegradation. This is achieved by the release of root exudates, enzymes that stimulate mineralization of the oil contaminant. This process builds up organic carbon in the immediate soil environment. Leguminous plants such as *P. vulgaris* capable of fixing nitrogen may have had advantage in the remediation of oil contaminated soil because of their ability to provide their own nitrogen fertilizer via nitrogen fixation to correct imbalance in C:N ratio. However, we observed less number of nodules in plants that received treatment relative to control plant. Rosa et al. (2005) reported reduction in acetylene within 4 and 8 weeks after planting of soya bean.

Higher sugar levels in plants under stress have been reported to result from changes in amylopast membrane and electrolyte leakage (Hayashi et al, 1992: Achuba, 2006). Increased protein concentration is reported in plants under SO_2 stress (Murray et al., 1992). Plants can detoxify oxides of nitrogen in leaves by synthesis of protein and amino acids (Kemble and McPherson, 1954; Murray et al., 1992; Piorreck et al., 1984). Therefore, the increase in protein in this study could be predicated on the bases of presence of oxides of sulphur and nitrogen in the spent oil (Achuba, 2006). This factor could be responsible for the low pod yield and yield components recorded in the plants that received treatment relative to control. The chlorophyll content is always an indication of the level of physiological condition of a plant species (Agrawal, 1992). We observed a reduction in chlorophyll content of the contaminated plant, indicative of the fact that our test crop grows under stress.

In conclusion, *P. vulgaris* in this experiment exhibited growth and produced yield. Thus it can be useful in remediation of spent oil spills. We recommend field scale trial to evaluate full season effect. Also, environmental consciousness should be instilled into automobile mechanics to avoid indiscriminate disposal. We also encourage research into recycling alternatives in spent engine oil.

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