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The Growth and Development of *Phaseolus vulgaris* L. Under Petrol and Diesel-Oil Polluted Water Irrigation

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Abstract: The growth and development of *Phaseolus vulgaris* L. var. Ife Brown under petrol and diesel oil-polluted water irrigation were investigated. The polluted water from petroleum tankers depot at Ijora-Lagos, Nigeria had significant high levels of copper, lead and cadmium ($p = 0.05$), which are metals prominent in oil spillage environment. The seeds were sown in soil irrigated with oil-polluted water and tap water (control). The seeds in both conditions germinated at the same time and rate. The heights of plants treated with the polluted water were significantly higher ($p = 0.05$) than those of the control plants but the former plants had little or no sprawl. There was significant ($p = 0.05$) increase in the growth of treated plants in terms of total area and dry weight during vegetative stage but the growth of the plants decreased significantly ($p = 0.05$) during flowering stage due to early leaf senescence. Copper, lead and cadmium accumulated at significant higher ($p = 0.05$) levels in the leaves of treated plants. Two of the treated plants that tried flowering shed the buds without opening unlike the control that flowered and produced pods. Aside from establishing the fact that the water is polluted by petrol and diesel oil spillage, an overflow of the polluted water on plant vegetation over time would endanger the growth and development of plants.

Key words: Polluted water, treated plants, heavy metals, growth, vegetative state, flowering state

INTRODUCTION

Crude oil pollutants are highly stable compounds persisting in the environment for a long time before they can be broken down (Lastmer *et al.*, 1996). They accumulate in tissues of most fauna and flora life, poisoning and causing a wide range of toxic effects on them. Oil pollutants include aviation fuel, engine oil, petrol and diesel. The extent to which the environment is polluted by these oils depends on the frequency and severity of the spills in a given area. Onshore and offshore operations including terminal operations and petroleum refineries aid oil pollution (Bragg, 1995).

The act of black-market sale of petrol and diesel in Nigeria in the last decade has resulted in the large spillage of these products at petroleum tanker depots in the urban area. Tanker drivers empty their tankers into barrels for the purpose of selling petrol and diesel at exorbitant prices during scarcity. Oil sand present at various points in Nigeria results from the indiscriminate discharge of petroleum products by industries, roadside mechanics and petroleum tanker drivers.

Toxicity of heavy metals such as cadmium, zinc, lead, chromium, nickel and manganese are associated with fuel pollution (Davies, 1991; Archambault and Winterhalder, 1995). Different heavy metals at supra optimal concentrations have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development (Bala and Setia, 1990; Davies, 1991). Plants may also be killed by oil pollution or suffer reduced growth and reproductive rates due to combination

of physical coating, altered soil chemistry and toxic effects of crude oil components (Leighton, 2000). Noticeable forms of stunted growth and chlorosis in plants and also marked anatomical changes in plant tissues are responses to heavy metal toxicity (Cox *et al.*, 1995). Oil spillage on land retards vegetative growth for a period of time and in extreme cases, leads to the destruction of vegetation. It also increases potential fire hazards and renders the soil unfit for cultivation. Any hydrocarbon solvent is liable to penetrate into plants through lipophilic surface, roots and once inside, it dissolves the cell membranes and cause loss of cell sap and in some cases, accumulates in the tissue of fruits of crops (Gallager, 1995).

The effect of oil pollution due to high level of sabotage going on at petroleum tanker depots in Nigeria necessitated the need to investigate the effect of the flow of petrol and diesel oil polluted water over vegetations, on the growth and development of *Phaseolus vulgaris* L. var. Ife Brown.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. var. Ife Brown were obtained from Institute of Agricultural Research and training (IAR and T), Ibadan, Oyo-State, Nigeria. Petrol and diesel oil-polluted water was gotten from Ijora, Lagos State, Nigeria where the process of emptying of tankers into smaller containers for black market sale of diesel and petrol have resulted in the flow of the polluted water to the surrounding regions. Planting experiments were carried out at the University of Lagos Botanical Garden between 2001 and 2002.

Chemical Analysis

The pH of the soil, tap and polluted water were measured using a pH meter (Jenway model). The concentrations of heavy metals (copper, lead, cadmium, iron and zinc) in the soil were determined according to the methods described by Anonymous (1992).

Planting Procedure

Two sets of three batches of 0.7×1.9 m farm beds were made about 10 km away from each other. Ordinary tap water was used to irrigate a set (control) and polluted water was used on the other (treated). Five seeds were placed in holes about 0.3 m apart in the beds. Irrigation was done on alternate days.

Growth Parameters

Five plants from each treatment group were randomly uprooted weekly from day 24 after sowing. The height and total leaf area of each plant were taken. The number of nodules was counted. Dry weight of the each plant and its nodules were determined after oven drying at 80°C for 72 h. Relative Growth Rate (RGR) was calculated according to the formula given by Noggle and Fritz (1976).

Chemical Analysis of Leaves

The method used was as described by Anonymous (1992). 0.5 g of dried leaves of the treated and control plants at day 54 were finely grounded separately. Digestion was carried out for each replicate. The round leaves were put into 250 mL beaker to which 50 mL of distilled water and 10 mL of analytical grade concentrated nitric acid (HNO₃) was added. The beaker was covered with a ribbed watch glass and heated on a hot plate till volume reduced to 10-15 mL without boiling. After this treatment, the sample was left to cool and later filtered to remove any insoluble material. The filtrate was poured into a 100 mL of standard flask and made up to 25 cm³ with distilled water. The reagent blank was also subjected to digestion. The digested filtrate was used for the determination of the various heavy metals by the use of the atomic absorption flame emission spectrophotometer (Unicam model 929).

Statistical Analysis

All analysis were carried out in three replicates and results recorded in averages with standard error. Using a one-way classification, analysis of variance (ANOVA) of the treated and control plants were done to test the level of significance between the plants at $p = 0.05$.

RESULTS

The pH and level of concentration of metals associated with oil pollution in polluted water from Ijora-Lagos, Nigeria, a site widely polluted by petroleum derivatives (petrol and diesel) spillages from conveying tanks are shown on Table 1, along with those of the tap water (control) and soil used for planting. The pH of the polluted water was not significantly different from that of the control water nor was the pH of the soil used for cultivating the 2 plant sets. The polluted water had significantly higher levels of copper, lead and cadmium than the control plants ($p = 0.05$).

Seeds of *Phaseolus vulgaris* L. cv. Ife Brown were sown in soil irrigated with polluted water (treated) and tap water (control). The seeds irrigated with either polluted or control water germinated at the same time and rate.

The treated plants grew significantly higher ($p = 0.05$) than the control plants but had little or no sprawl (Fig. 1).

Table 1: The pH and level of concentration of some heavy metals present in the tap water, polluted water and soil sample

Parameters	Tap water	Polluted water	Soil
pH	6.60±0.23 ^a	6.23±0.33 ^a	7.03±0.17 ^b
Concentration of metal (mg L⁻¹)			
Copper	ND	3.42±0.19 ^b	0.91±0.01 ^a
Lead	ND	2.26±0.02 ^b	0.77±0.55 ^a
Cadmium	ND	0.89±0.06 ^a	0.48±0.01 ^a
Iron	0.01±0.01 ^a	6.02±0.16 ^b	5.81±0.45 ^b
Zinc	0.07±0.002 ^a	1.37±0.05 ^b	2.14±0.59 ^c

Means followed by the same letter(s) are not significantly different at $p = 0.05$, based on the analysis of variance (ANOVA)

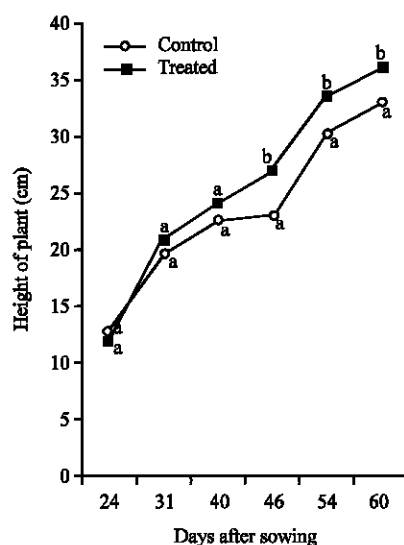


Fig. 1: Height of *Phaseolus vulgaris* plants in the course of growth under control and treated conditions

The treated plant had a significantly larger ($p = 0.05$) leaf area during vegetative growth than the control plant. However, there was no significant ($p = 0.05$) difference the leaf area of the two sets of plants during reproductive growth (Fig. 2).

There was a drop in the RGR at day 40 and an increase in subsequent weeks (day 46-54) in both sets of plants. However, the RGR of the control plants continued to increase during flowering stage (days 54-60) while that of the treated plants decreased. The differences observed were significant at $p = 0.05$ (Fig. 3).

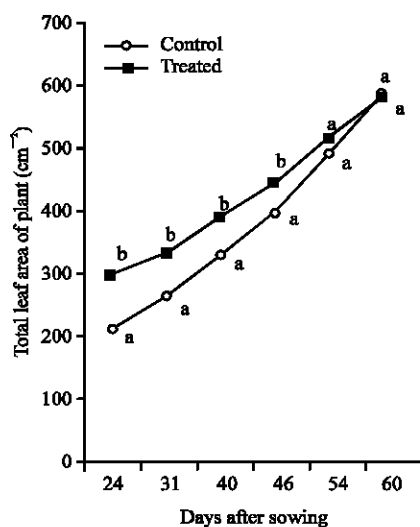


Fig. 2: Total leaf areas of *Phaseolus vulgaris* L. plants in the course of growth under control and treated conditions

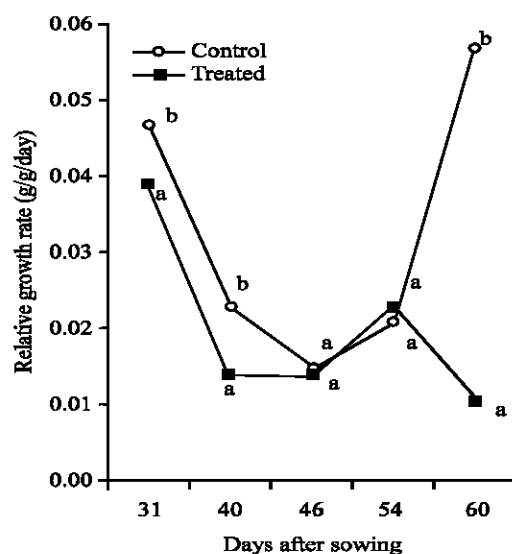


Fig. 3: Relative growth rates of *Phaseolus vulgaris* plants in the course of growth under control and treated conditions

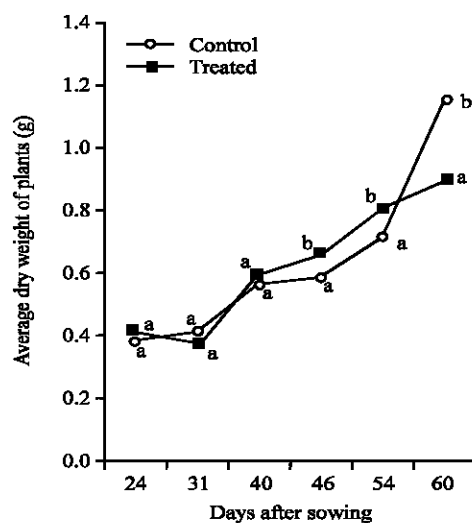


Fig. 4: Dry weights of *Phaseolus vulgaris* L. plants in the course of growth under control and treated conditions

Table 2: Maximum nodule weight and number of *Phaseolus vulgaris* L. under control and treated conditions

Parameters	Control	Treated
Nodule No.	26 ^a	27 ^a
Fresh weight of nodule (mg)	616 ^a	914 ^b
Dry weight of nodule (mg)	115 ^a	290 ^b

Means followed by the same letter(s) are not significantly different at $p = 0.05$, based on the analysis of variance (ANOVA)

Table 3: Level of heavy metal accumulation in leaves of *Phaseolus vulgaris* L. under control and treated conditions

Parameters	Level of accumulation (mg L^{-1})	
	Control	Treated
Copper	0.57 ± 0.08^a	0.89 ± 0.13^b
Lead	0.09 ± 0.01^a	0.27 ± 0.03^b
Cadmium	0.09 ± 0.01^a	0.24 ± 0.05^b
Iron	0.15 ± 0.08^a	0.17 ± 0.07^a
Zinc	0.18 ± 0.01^a	0.20 ± 0.03^a

Means followed by the same letter(s) are not significantly different at $p = 0.05$, based on the analysis of variance (ANOVA)

Plants from both control and treated populations had an increase in dry weight per time throughout the period assessed. The dry weights of the treated plant was significantly higher between days 46-54 but significantly lower ($p = 0.05$) by day 60 compared to the control plants (Fig. 4).

The treated plant had a significantly higher nodule weight than the control though the nodule number was not significantly different ($p = 0.05$, Table 2).

Table 3 shows the levels of accumulation of heavy metals in the leaves of the plants. The treated plants had significantly higher ($p = 0.05$) levels of copper, lead and cadmium than the control plants. The control plants sprawled extensively and produced flower buds by day 54, which later developed into flowers and then pods. On the contrary, the treated plants did not produce flower buds until day 70. The buds of the treated plants were shed without developing into flowers.

DISCUSSION

The pH of the polluted water was not significantly different from that of the soil at $p = 0.05$. The polluted water had a significantly higher level of copper, lead and cadmium at $p = 0.05$. According to

Alloway (1990), these metals are heavy metals prominent in oil spillage environment. This coupled with virtual evidence of oil films on top of the polluted water confirms the fact that the water is polluted by petroleum products from petroleum tanks at Ijora depot (Lagos, Nigeria).

The treated plants had higher shoot height but did not really sprawl unlike the control that sprawled extensively. Stimulation of higher shoot height due to crude oil treatment has been noticed in the salt marsh grasses, *Festuca rubra* L. and *Puccinellia maritima* (Baker, 1981). Also, Osibeluwo (1997) noticed that *Celosia* plants subjected to 15 ppm crude oil had significantly longer shoot lengths than the untreated plants. The reason for growth stimulation of plants by crude oil according to Osibanjo (1983) could be that the plants utilized some available macro and micro-nutrients present in the crude oil such as cadmium, copper and iron.

A significantly increase in vegetative growth during early stage of growth was observed in the treated plants. This was made obvious by the significantly higher ($p = 0.05$) total leaf area and dry weight increase. Agbogidi *et al.* (2007) have shown that the effect of crude oil pollution on plants is dependent on the level of pollution and that small amounts of mineral oils and oil products may actually be beneficial to plants. Oil pollution at high concentrations has an adverse effect on plant growth (Osibeluwo, 1997; Ogboghodo *et al.*, 2004; Agbogidi *et al.*, 2007). These reports support those of this result. The dry weight of the treated plant was significantly lower ($p = 0.05$) during later stage of growth compared to that of the control plant. Also, there was a significant drop in the relative growth rate of the treated plant as against a significant increase ($p = 0.05$) in the control plant. The early senescing of leaves of treated plants compared to those of the control plants may account for the fall in the growth rate of the plants at the latter stage of the plants' lives. The concentration of petrol and diesel oil in the soil may have increased by the latter stage of the treated plant's lives due to their accumulation in the soil over a long period of continuous irrigation, compared to the level of accumulation at the vegetative stage of the plants. Oil films according to the report of Anoliefo (1991) find their way into the epidermal and cortical regions of the root, stem and leaves of treated plants causing a disruption in the cells of these organs. This could account for the early senescing of the leaves during the late stage of growth.

A higher nodule development in terms of nodule weight and number was observed in the treated plants. Oil polluted soils have been shown to have a greater nitrogen fixation capability than normal polluted soil (Bidwell, 1999; Epstein, 1972). Oil sprayed plots have also been shown by Baker (1981) to have a high increase in nitrogen fixing activity. The continuous irrigation of the soil with the polluted water may account for the effects observed on the plant. This may have caused an increase in the concentrations of petrol and diesel oil accumulated in the soil with time. The growths of plants under petroleum oil treatment have been shown to be dependent on the concentration of oil in the treatment. According to Carr (1919), the growth of soy beans (*Glycine max*) improved in certain percentage concentration of crude oil. He found that 0.75% of crude oil in soil improved the growth and root nodule development of soy beans. 12% crude oil have also been observed by Galsoff and Smith (1935) to stimulate the growth of cultures of *Nitzshia* and *Closterium*. The stimulation of the shoot length and leaf area of the treated plant could be due to their stimulation by low concentration of accumulated petrol and diesel oil. A significantly higher ($p = 0.05$) level of dry matter accumulated during late vegetative stage could be due to crude oil accumulation in the chloroplast of plants as pointed out by van Overbeek and Blondeau (1954). This according to them leads to an increase in the dry weight of plants as the concentration of oil increased in the treated plants. This reason supports the results of these experiments. The treated plants had a significantly higher ($p = 0.05$) dry weight at late vegetative stage probably due to accumulation of petrol and diesel oil in the chloroplast of the leaves overtime. However, the dry weight of the treated plant was significantly lower ($p = 0.05$) than those of the control plants at later stage of growth.

The accumulation of copper, lead and cadmium in the leaves of the plant at significantly higher ($p=0.05$) values by day 54 maybe due to their significantly higher ($p=0.05$) concentrations in the

polluted water and accumulation in the soil over time as pointed out above. Lead causes low growth in plants (Adam and Duncan, 2001) and it has been implicated along with copper to cause chlorosis and wilting in plants in cases of excessive oil spillage on farmlands (Adam and Duncan, 2000). Thus, early chlorosis and wilting of leaves during the late stage of growth could be associated with the accumulation of copper and lead in the leaves during this period.

The adverse effect of petrol and diesel oil on the treated plants resulted in the non-flowering of the treated plants despite the high number of nodules on the roots. The buds produced by two of the treated plants were shed off without developing into flower. Irrespective of the element(s) behind the observations, petrol and diesel oil spillage has an adverse effect on the growth and development of *Phaseolus vulgaris*. Despite the fact that the petroleum tanker drivers deny any sabotage going on at the Ijora depot, their practices within the area (either emptying of tankers into drums or washing of tankers) is endangering the vegetation around Ijora. An overflow of the polluted water onto plant vegetations would result in the weeding off of the vegetation. The results of Akpofere *et al.* (1999) support this assertion. Also continuous petrol and diesel oil spillage on lands around Ijora would render the soils unfit for cultivation after some years as crops like *Phaseolus vulgaris* will not flower nor fruit when sown on the land.

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