African Journal of Biotechnology Vol. 8 (2), pp. 250-253, 19 January, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effects of diesel fuel contamination on seed germination of four crop plants - *Arachis hypogaea, Vigna unguiculata, Sorghum bicolor* and *Zea mays*

Erute Magdalene Ogbo

Department of Botany, Delta State University, P.M.B 1 Abraka, Nigeria. E-mail: erute70@yahoo.com.

Accepted 8 December, 2008

The effects of different levels (%) of diesel fuel contamination on four crop plants *Arachis hypogaea, Vigna unguiculata, Sorghum bicolor* and *Zea mays* were studied. One percent level of contamination did not significantly reduce seed germination in *Z. mays* and *S. bicolor* but did in *A. hypogaea* and *V. unguiculata* (P<0.05). The 2.00% level of contamination reduced germination in *A. hypogaea*. Germination was reduced with increasing levels of contamination in the four test plants. There was total inhibition of germination in *S. bicolor* and *V. unguiculata* in 4.00% level of contamination. There was no significant difference in percentage seed germination between 2.00, 3.00, and 4.00% levels of contamination in *Z. mays*. The 4.00% level of contamination had more deleterious effect than 5.00% for *Sorghum* and *Vigna* species. Diesel fuel contamination caused a reduction in the length of the radicle for the four crop plants and this varied with the level of contamination. It was observed that 5.00% level contamination did not affect the radicle length of *V. unguiculata* significantly. Diesel fuel contamination also caused a reduction in the plumule length of *Z. mays* and *S. bicolor*. The shortest plumule was recorded in 5.00% level of contamination for *Z. mays* and 3.00% in *S. bicolor*. The phytotoxicity of the different levels of diesel fuel contamination on seed germination indices thus varied with the type of crop plant. The study indicates that *Z. mays* and *A. hypogaea* have more potential for use in phytoremediation of diesel fuel contaminated soils than *S. bicolor* and *V. unguiculata*.

Key words: Diesel fuel, contamination, germination, crop plants, phytotoxicity.

INTRODUCTION

The increasing use of diesel in diesel engines of cars, industrial trucks and generators has led to a marked increase in the demand for diesel fuel in Nigeria. Accidental spills caused by pipeline leakages and ruptures and accidents during transport have been reported. The environmental consequence of soil pollution cause adverse effect on the soil microflora, and reduces soil fertility (Torstenssen et al., 1998). Contamination of soil results in damage of crop growth; depending on the degree of contamination, the soil may remain unsuitable for plant growth for months or several years. Contamination of soil with petroleum products has been a major cause for concern. Damages due to soil contamination may be extensive and its effect may be long term. Diesel fuel is not a systemic killer; it kills plants cells on contact. Contamination by diesel fuel can kill the roots, and this prevents the plant from taking up water and other nutrients. It can also disrupt plant and water relationship in soil (McCown et al., 1972). Petroleum derived diesel consist of 75% saturated hydrocarbons primarily paraffin and 25% aromatic hydrocarbons. Regardless of this complexity, diesel fuel can be degraded by a number of soil microorganisms. Diesel fuel is phytotoxic to plants at relatively low concentrations. At levels below this phytotoxic level, the development of plants grown in diesel fuel contaminated soil differs greatly from plants grown in normal soil conditions (Adam and Duncan, 1999). Little is known about plants suitable for the phytoremediation of different pollutants in soils. Studies have shown that the plants that are suitable for remediation of a particular contaminant must be that that can tolerate the contaminant (Kirk et al., 2002). Often plants native to the site are considered for phytoremediation projects. However because different plant species respond to contaminants

Table 1. Percentage seed germination of four crop plants at different levels (%) of diesel oil contamination.

Crop plants	Control	1%	2%	3%	4%	5%
Z. mays	100±0.00[a]	89±11.35[a]	55±6.05[c]	44±5.92[c]	44±6.48[c]	33±6.15[d]
S. bicolor	100±0.00[a]	89±8.42[a]	55±3.22[c]	44±6.08[c]	00±0.00[f]	22±4.05[e]
A. hypogaea	100±0.00[a]	78±9.65[b]	98±4.25[a]	78±4.13[b]	22±4.65[e]	11±3.52[e]
V. unguiculata	100±0.00[a]	66±0.00[b]	55±3.87[c]	33±3.58[d]	00±0.00[f]	22±5.66[e]

Figures with the same letters (superscripts) are not significantly different (P<0.05).

differently, it may be beneficial to screen plants known to host a degrading rhizosphere community or that have shown past phytoremediation potential for survival/ tolerance at a specific site (Kirk et al., 2002). Grasses are sometimes used for phytoremediation because their root systems are extensive and fibrous. Grasses have been used to remediate chemically contaminated soils, including petroleum contaminated soils (Kirk et al., 2002). Legumes are also considered suitable for phytoremediation of organic contaminants because their root systems extend further than grasses into soils (Kirk et al., 2002). Toxicity tests have been suggested as useful tools in assessing the risk of contaminated soil or to evaluate the efficacy of a remediation process. Kirk et al. (2002) used these phytotoxicity assays to select plants that are potential candidates for phytoremediation of petroleum-contaminated soil.

Maize (*Z. mays*) and Guinea corn (*S. bicolor*) (Poaceae) are coarse annual grasses. *Arachis hypogaea* and *Vigna unguiculata* (Leguminoceae) are important crop legumes. This study aims at investigating the effect diesel oil contamination would have on the seedling growth of the four crop plants; *Z. mays*, *S. bicolor, V. unguiculata* and *A. hypogaea.*

Germination and root elongation are two critical stages in plant development that are sensitive to environmental contaminants (Baud-Grasset et al., 1993). Plants that are able to germinate successfully amidst the contaminant and show root elongation are tolerant plants. The study therefore also aims to find out which of these crop plants is most tolerant to the contaminant diesel fuel with the aim of using it for phytoremediation of contaminated soils.

MATERIALS AND METHODS

The experiment was conducted in Petri dishes in the laboratory. Seeds of *A. hypogaea, S. bicolor, Z. mays* and *V. unguiculata* were purchased from the local market at Eku, Delta State, Nigeria. Sets of ten seeds per Petri dish were used for each crop plant and level of contamination. Five different levels of contamination were prepared with distilled water. 1, 2, 3, 4, and 5% were prepared by adding 0.8, 1.62, 2.43, 3.24 and 4.05 ml of diesel fuel to 10 ml of distilled water, respectively. Contaminated water was used to moisten filter paper in the Petri dishes. The seeds were then introduced into the different levels of contamination. The control had only distilled water. The seeds were allowed to grow for three days. The percentage germination, radicle and plumule length were recorded at the end of three days.

The experiment was a completely randomized design with five treatments each with five replicates. Data from the toxicity tests were analyzed using analysis of variance (ANOVA) to detect differences between treatment and control followed by Duncan's multiple range test (P<0.05) using the SPSS 13.00 for windows software.

RESULTS

The highest percentage germination was recorded in control (no contamination) for the four crop plants. All the test plants tolerated diesel fuel contamination at 1.00-3.00% levels of contamination as seed germination was between 89.00-33.00%. There were no significant differences between percentage germination of the seeds in the control of the four crop plants and 1.00% level of contamination in *Z. mays* and *S. bicolor* and the 2.00% level of contamination in *A. hypogaea*. There was significant reduction (P<0.05) in percentage germination recorded for *A. hypogaea* and *V. unguiculata* at 1.00% level of contamination (Table 1). As the level of contamination increased there was a marked reduction in the percentage seed germination. However there were exceptions; while there was no germination at all in 4.00% level of contamination for *S. bicolor* and *V. unguiculata*, there was germination at 5.00% level of contamination for these two crop plants. The 4.00% level was therefore the lethal level of contamination for the two crop plants. Seed germination at 5.00% level of contamination for the four crop plants were significantly lower than all other levels of contamination with the exceptions mentioned above. In *A. hypogaea*, 2.00% level of contamination had significantly higher percentage seed germination than 1.00% level of contamination. There was no significant difference between 2.00, 3.00 and 4.00% levels of contamination for *Z. mays* with regards to seed germination and also 2.00 and 3.00% for *S. bicolor* (Table 1). Diesel fuel contamination caused a reduction in the length of the radicle of the four crop plants studied to varying degrees. The longest radicle (3.00 cm) in *A. hypogaea* was recorded in 1.00% level of contamination and the shortest (0.97 cm) was recorded in 4% level of contamination (Table 2). In *S. bicolor*, the longest radicle

Table 2. Length of radicle of four crop plants in response to levels (%) of diesel oil contamination.

Level of contamination	*Arachis hypogaea*	*Sorghum bicolor*	*Zea mays*	*Vignia unguiculata*
Control	2.20±0.17[a]	4.10±0.04[a]	3.20± 0.26[a]	2.60±0.15[a]
1%	3.00±0.00[a]	1.73±0.05[a]	1.37±0.13[a]	1.60±0.04[a]
2%	1.40±0.15[a]	0.90±0.02[b]	0.97±0.18[b]	1.57±0.02[a]
3%	1.37±0.16[a]	1.07±0.09[b]	1.27±0.09[b]	1.67±0.06[a]
4%	0.97±0.13[b]	-	1.15±0.14[b]	-
5%	1.13±0.18[b]	0.80±0.07[b]	0.16±0.05[b]	2.30±0.09[a]

Figures with the same letters (superscripts) are not significantly different (P<0.05).

Table 3. Length of plumule of two crop plants in response to levels (%) of diesel oil contamination.

Level of contamination	*Zea mays*	*Sorghum bicolor*
Control	5.2±0.22[a]	4.94±0.37[a]
1%	3.83±0. 09[a]	3.10±0.31[a]
2%	3.47±0.48[a]	2.89 ±0.29[b]
3%	1.56±0.18[b]	2.21±0.12[b]
4%	1.19±0.12[b]	-
5%	0.67±0.07[b]	2.81±0.44[b]

Figures with the same letters (superscripts) are not significantly different (P<0.05).

was recorded in the control (4.10 cm) and the shortest (0.80 cm) was recorded in 5.00% level of contamination. Diesel fuel contamination affected the radicle length of *Z. mays.* It reduced the length of the radicle from 3.20 cm in control to 0.16 cm in 5.00% level of contamination. Diesel fuel contamination at 5.00% level of contamination did not affect the radicle length of *V. unguiculata* negatively. There was no significant difference between the radicle length in this level of contamination and the control (Table 2). Diesel fuel contamination also affected the plumule of *Z. mays* and *S. bicolor*. It caused a reduction in the length of the plumule to varying degrees. In *Z. mays* the longest plumule (5.20 cm) was recorded in the control treatment. The shortest plumule (0.67 cm) was recorded in 5.00% level of contamination. In *S. bicolor* the longest plumule was also recorded in control 4.94 cm and the shortest (2.21 cm) in 3.00% level of contamination (Table 3). For *V. unguiculata* and *A. hypogaea* however, the plumules remained within the cotyledons such that the lengths were not recorded during the experimental period.

DISCUSSION

The results clearly showed that diesel oil contamination affects the germination of crop plants. The results also indicate that the four crop plants can tolerate the contaminant by germinating successfully in it. Kirk et al. (2002) also recorded that four species of grasses germinated successfully in different levels of petroleum hydrocarbon contamination and recommended some for phytoremediation of petroleum hydrocarbon contaminated soils on the basis of that. They also recorded a decrease in the percentage germination as the level of contamination increased. A similar effect was also observed by Adam and Duncan (2002) who reported reduction in germination rate in several plant species, mainly in commercial crops caused by petroleum contaminants. The process of phytoremediation is a complex one. The toxicity effect noticed in contaminated soils might not be just due to the contaminant concentration but also due to soil type and properties, hydrocarbon type, microbial community composition and plant species (Salanitro, et al., 1997). According to Kirk et al. (2002) onlyrye grass showed a 50% inhibition of germination and thus quite tolerant of the contaminant. Percentage germination of the four test plants in this study at the different levels of contaminations was also quite high and can thus be recommended for the remediation of diesel fuel contaminated soils.

The level of contamination determines the extent of damage and also inhibition. At high levels of contamination (in this case 4 and 5%) although there was germination in some crop plants, there was reduction in the seed germination of these crop plants and this affected the length of the radicle and plumule. This trend was also recorded for six agronomic plants by Issoufi et al. (2006). They recorded reduced seed germination as the level of contamination increased for plants like corn, perennial rye grass, wheat, alfalfa, hairy vetch and soy bean. The results in this study also agree with the works of Anoliefo and Vwioko (1995) and Anoliefo and Edegbai (2000) that petroleum hydrocarbons can inhibit the growth of plants. In their study, crop plants like *Abelmoschus esculentus* (okro), *Lycopersicon esculentum* (tomato), *Capsicum annuum* (pepper), *Solanum melongena* (egg plant) and *Solanum incanum* failed to germinate at 6% level of contamination, while in this study 4.00% was lethal to the germination of *V. unguiculata* and *S. bicolor*. This indicates that the lethal concentrations of petroleum contaminants to different plants vary. The presence of oil and level of contamination in the soil affects germination and subsequently growth of plants in such soils (Terge, 1984). Isirimah et al. (1989) attributed the poor performance of plant caused by oil contamination to poor wetness and aeration of the soil. The effect could also be as a result of formation of polar compounds dissolved in water that could penetrate the seed coat, exerting polar narcosis (Wang et al., 2001; Adam and Duncan, 2002).

The results also showed that with increasing levels of contamination, there was reduction in the length of the radicle. Amadi (1992) observed that increasing the concentration of oil beyond 3% in the soil reduced the percentage germination and growth, thereby affecting the physiological function of the plants. In the study by Molina-Barahona et al. (2005), diesel fuel exerted a negative effect on the tree species of plants use. According to them this effect may have been due to an inhibitory effect of some of the diesel's polycyclic aromatic hydrocarbon components which are more soluble than the aliphatic hydrocarbons (Wang and Bertha, 1990; Trapp et al., 2001; Molina-Barahoma et al., 2005). In this study there was reduction in the length of the plumule caused by the contaminant as length of plumule in control was higher than that in contaminated treatments. Baud-Grasset et al. (1993) reported that reduction in root length is a sensitive plant response to exposure to chemical substances. The roots by their direct contact with the polluting agent are the main targets of hydrocarbons in soils and their characteristics play a fundamental role in protection (Molina-Barahona et al., 2005). Issoufi et al. (2006) also concluded in their study that *Z. mays* can be used for phytoremediation of petroleum contaminated soils after studying seedling growth of six agronomic crops because germination rates was higher in *Z. mays* than other plants studied.

The four crop plants studied were sensitive to diesel fuel contamination, despite that the plants were also able to withstand the contamination to varying degrees. *Z. mays* and *A. hypogaea* are the plants of choice for phytoremediation of diesel fuel contaminated soils because they were able to withstand all levels of contamination investigated.

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