EFFECTS OF PETRODIESEL-CONTAMINATED SOIL ON EMERGENCE AND GROWTH OF SEEDLINGS OF PROSOPIS JULIFLORA (SWARTZ.) DC.

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ABSTRACT
The effects of diesel oil pollution on Prosopis juliflora Swartz DC. were investigated in a series of artificially and freshly polluted sandy soil with 0, 0.5, 0.75, 1.0, 2.5, 4.0, 5.0, 7.5 and 10 mL diesel oil per 100g soil. Emergence of seedlings was impeded and germination losses occurred at high diesel oil concentrations (> 1.0 mL diesel oil per 100 g Soil). Emergence of seedlings was completely inhibited in 7.5 and 10 mL diesel oil concentration. All the parameters of seedling growth viz. height, number of leaves, hypocotyl and epicotyl lengths, and cotyledon area per seedling, declined progressively with increase of diesel oil concentration in the rhizosphere. Some 66% of the seedlings died in 1% diesel oil concentration. Hundred percent seedling mortalities were observed in 4 and 5% diesel concentrations. Plant death was preceded by dehydration, wilting and defoliation. P. juliflora appeared to be a sensitive species to petrodiesel pollution.

Key Words: Prosopis juliflora Swartz DC., Diesel oil pollution, Seedling emergence and growth, cotyledon area.

INTRODUCTION
Several published reports have evaluated the toxic effects of oil spills or pollution of soil with petrochemicals on such biological phenomena as germination, seedling growth, recovery and establishment of vegetation, etc. (e.g., Green et. al., 1966; Baker, 1970; Udo and Fayemi, 1975; Walker et al., 1978; de Jong, 1980; Amakiri and Onofeghara, 1984; Holt, 1987; Analiefo and Vwioko, 1995; Wiltsie et. al., 1998; Campbell and Vavrek, 1999; Vavrek and Cambell, 1999; Kroening et. al., 2001; Adam and Duncan, 2002; Odjegba and Sadiq, 2002; Gill et. al., 2004; Smith et. al., 2006; Sharifi et al., 2007; Shahriari et. al., 2007; Ogbo et al., 2009a and b; Serrano et al., 2009; LIU et. al., 2009, Khan and Shaukat, 2009). Such studies are preliminary to the understanding of phyto-remediation potential of plants at population and community level. There is, however, a paucity of such information from the local flora

P. juliflora, one the dominant species in the coastal areas including salt marshes of Pakistan (Khan, 1987), is fast naturalizing in the tidal zones of the Indo-West pacific and is regarded as potential mangrove species by Mepham and Mepham (1985). The evaluation of phyto-remediation potential of some tropical species including P. juliflora against petroleum hydrocarbons have been advocated by Sun et. al, (2004). Since, for effective Phytoremediation, seeds must germinate and subsequently grow and establish in contaminated soil (Kroening et. al., 2001), the present paper investigates the emergence and seedling growth of Prosopis juliflora, a highly invasive species of wide ecological amplitude, in diesel-contaminated soil.

MATERIAS AND METHODS

The experiment was conducted in earthen pots containing 500g sandy garden soil (sand 85%), thoroughly mixed with diesel oil (fuel grade) in a series of concentration – 0, 0.5, 0.75, 1.0, 2.5, 4.0, 5.0, 7.5 and 10 mL of diesel oil per 100 g soil. The soil was incubated for 24h before sowing seeds. Ten P. juliflora seeds of similar sizes abraded with conc. H2SO4 for 20 minutes to enhance germination (Khan et. al., 1984) were sown at 1.5 cm depth in the soil in each pot and irrigated at alternate days with 50 ml of tap water. The pots were kept in open (ambient temperature fluctuating around 30°C). The seedling emergence was recorded for seven days and the emerging crop was grown up till 20 days of growth in July 2006. The crop was harvested after 20 days. There were at least three replicates to each treatment and control for morphometric analysis, drawn randomly. Cotyledon area was determined graphically by drawing outlines with all possible precision. The data were analyzed statistically.

RESULTS AND DISCUSSION

Emergence of seedlings

Although there was substantial seedling emergence after four days of incubation in control and lower diesel oil concentrations but the rate of emergence of seedlings was significantly impeded as a result of diesel oil contamination of soil (2.5 to 5.0 mL./100g soil) as compared to the control (Fig. 1). P. juliflora seedlings couldn’t emerge at all in diesel oil concentrations of 7.5 and 10 mL diesel oil per 100g soil. ANOVA of emergence data
suggested that days of incubation enhanced the emergence of seedlings (F = 102.7, p < 0.001) and petrodiesel concentration retarded or inhibited emergence significantly (F = 95.8, p < 0.001) and both these factors interacted significantly with each other (F = 9.8, p < 0.001). The substantial recovery of emergence in lower concentrations of petro-diesel may probably be attributed to the volatilization of diesel and partial loss of inhibitory principles from the soil or due to time lag phenomenon caused by retardation of germination. High diesel oil concentrations were obviously toxic to the process of germination.

![Graph showing seedling emergence (%)](image)

**Fig. 1.** Seedling emergence (%) from abraded seeds of *Prosopis juliflora* as function of time in soil contaminated with various petrodiesel concentrations (mL per 100 g Soil).

The concentration of 1 mL diesel oil per 100 g of soil is perhaps the critical concentration beyond which the seedling emergence of *P. juliflora* was not only moderately to severely impeded but there was also substantial loss of germination as was evident from the un-germinated seeds recovered from the soil on 7th day of incubation (Table 1). One percent diesel oil contamination is reported to have no significant effect on overall germination of *Thespesia populnea* (Khan and Shaukat, 2009), *Sorghum bicolor* and *Zea mays* (Ogbo et. al., 2009 a) but at this level of contamination germination in *Vigna unguiculata* is reported to be significantly reduced. Higher level of diesel contamination (> 1%) affected all these species significantly (Khan and Shaukat, 2009, Ogbo et. al., 2009 a). The contamination of soil with 4 and 5% spent oil has been reported to consistently inhibit germination of *Capsicum annuum* and *Lycopersicon esculentum* (Analiefo and Vwiko, 1995). Besalatpour et. al (2008) have reported that the presence of total petroleum Hydrocarbons (TPHs) in calcareous soils in equal proportion by weight had no effect on germination of *Agropyron*, and sunflower whereas Canola and white clover appeared to be sensitive to TPHs. Crude oil (Escravos light and Forcados light) inhibited growth of maize and also its seed germination. No germination could take place in Escravos light crude when applied in the soil @ 40 mL /Kg of soil (Ogboghodo et.al., 2004). Spiares et. al. (2001) investigated toxicity of crude oil to 19 plant species / varieties – of which 14 couldn’t emerge out of the polluted soil. *Hibiscus cannabinus* var. tainvng # 2 and *H. cannabinus* var. sf 459 were found to be quite
resistant to crude oil and showed considerable seedling emergence. Holt (1987) while studying effects of crude and diesel oils on plant communities at Mesters Vig, Northeast Greenland found diesel to be inhibitorier to plants germination than crude oil.

Table 1. Overall emergence / Germination status and hypocotylar and radicle growth of germinated seeds recovered from diesel oil contaminated soil after 10 days of incubation.

<table>
<thead>
<tr>
<th>Diesel oil Concentration (mL / 100g Soil)</th>
<th>Emergence / Germination status (%)</th>
<th>Growth based on germinated seeds that were recovered from the soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypocotyl Length (cm)</td>
<td>Radicle Length (cm)</td>
</tr>
<tr>
<td>Control</td>
<td>Emergence = 100 Germination = 100</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>Emergence = 100 Germination = 100</td>
<td>-</td>
</tr>
<tr>
<td>0.75</td>
<td>Emergence = 86.6 Germination = 86.6</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>Emergence = 100 Germination = 100</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>Emergence = 26.6 Germination = 50.0</td>
<td>0.39 ± 0.14                                                   1.04 ± 0.27</td>
</tr>
<tr>
<td>4</td>
<td>Emergence = 33.3 Germination = 43.3</td>
<td>-                                                             0.25 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>Emergence = 6.6 Germination = 6.6</td>
<td>-                                                             -</td>
</tr>
<tr>
<td>7.5</td>
<td>Emergence = Zero Germination = Zero</td>
<td>-                                                             -</td>
</tr>
<tr>
<td>10</td>
<td>Emergence = Zero Germination = Zero</td>
<td>-                                                             -</td>
</tr>
</tbody>
</table>

SEEDLING MORTALITY

There were significant mortalities of seedlings in diesel oil contaminated soils within 20 days of the treatment. Mortalities up to 0.75 mL diesel per 100 g soil were low (c 30%). Some 66% of the seedlings died in 1% diesel concentration. Seedling mortality was 100% in 4 and 5% diesel concentrations. Plant death was preceded by dehydration, wilting and defoliation. Seeds couldn’t germinate at all in higher diesel concentrations (7.5 and 10 mL diesel / 100g soil) (Fig. 2).

SEEDLING GROWTH

All the parameters of seedling growth viz. height, number of leaves, hypocotylar, epicotylar and radicular lengths and cotyledonary area per seedling, number of leaflets in primary and secondary leaves declined progressively with increase of diesel oil concentration in the rhizosphere (Table 2 and 3 ). Reduction in most of the growth parameters was generally not more than 30% except height which was inhibited by around 50% in 0.75 mL diesel oil per 100g soil. Epicotyl and number of leaflets per secondary leaf, however, were reduced more than 50% over the control under diesel oil pollution (Fig. 3). The reduction in number of leaves per seedling was quite irregular.

Diesel oil pollution is known to retard seedling growth (de Jong, 1980; Atuanya, 1987, Khan and Shaukat, 2009). It has been reported to be toxic to Tradescantia (Green et.al., 1996) and Secale cereale and Glycine max (Wang and Bartha, 1990). Seventy days old plants of Amaranthus hybridus in old polluted environment (5% spent oil in soil) were reported to exhibit decline in height by 79% and in leaf area by 67.5% (Odjegba and Sadiq, 2002). Ogbo et. al. (2009b) have reported 50.2% reduction in leaf area in Paspalum scrobiculatum L. under 15% crude oil
contamination. Crude oil (Escraval light and Farcados light) contaminated soil has been reported to reduce germination, plant height, leaf area and dry matter yield of maize significantly (Uzoho and Onweremadu, 2004). Serrano et al. (2009) have reported phytotoxicity of diesel oil to Lepidium sativum L. in polluted soil at the dose of 1L.m⁻² and Dubova et al., (2008) reported complete inhibition of growth of Pisum sativum, in sandy soil contaminated with 1% diesel oil. There are, however, some reports of growth promotion in lower diesel oil concentrations. Song et al. (1006) reported promotion in seedling growth of wheat in soil contaminated with 500 mg of diesel per Kg of soil. Stimulation of growth rate, biomass yield, chlorophyll-a and photosynthesis in estuarine alga Chlorella salina has been reported by Chan and Chiu, 1985). Sharifi et al. (2007) have recently reported the effects of spent oil on some grass and legume species. All species have shown dose-dependent reduction in germination, aboveground height and biomass. Medicago truncatula suffered the most phytotoxic effects and Linum usitatissimum the least. Vavrek and Campbell (1999) have reported Eleocharis species and Cyperus erythrorhizos to be insensitive to oil application where as Bacopa monneiri and Rotala ramoseor to be sensitive to oil. Response of the plants to diesel oil is thus species-specific, dose-dependent and differences to tolerate oil pollution even exist at subspecies level (Adam and Duncan, 1999, 2002).

Oil pollution not only reduced the cotyledonary expansion significantly but also their abscission under high diesel concentrations. During experimental period ambient temperature remained around 32 °C. The cotyledons, after around 20 days of growth, became shining blue green in colour (indicative of the presence of the oil film in epidermal and/or cortical region) and then after a day, they turned dark brown to black and died. It appears that during uptake of water and salts, some petrodiesel components have been absorbed by the roots. The presence of oil film below epidermis and cortical region of leaves has been reported in Chromolaena odorata growing under diesel oil pollution (Gill et al., 2004). The diesel oil has been suggested to move within plant via apoplastic pathway. The changes in colour observed in P. juliflora cotyledons could be due to similar reason. Further research may elucidate such a hypothesis.

![Fig. 2. Seedling mortality of P. juliflora in petrodiesel contaminated soil. * no seedling emergence or germination took place in 7.5 and 10.0 mL petrodiesel /100g soil treatments.](image)

The contamination of soil with Petroleum and refinery products (PRPs) causes degradation of soil by initiating a series of processes affecting soil’s biotic and abiotic elements. PRPs are composed of a number of aliphatic, oleic, naphthenic and aromatic hydrocarbons (Chi Yuan and Krishnamurthy, 1995) which modify soil’s physical and chemical properties and its structure – resulting in change in fertility of soil. Contamination with diesel has a strong negative effect on bio- and physico-chemical properties of the soil (Wyszkowska et al., 2002), which may be limiting to the growth and development of plants. Diesel oil is toxic to seedling emergence being detrimental to germination via mechanisms including direct toxicity to the embryo (Amakiri and Onofeghara, 1984), formation of...
anaerobic conditions (Udo and Fayomi, 1975) and hydrophobic soil conditions (Amakiri and Onofeghara, 1984). Diesel oil component hydrocarbons may exert direct toxicity and/or suffocation due to slow rate of diffusion of oxygen between soil and the atmosphere (Uzoho and Onweremadu, 2004) as a result of blocking of air spaces. Some plants can take up polycyclic aromatic hydrocarbons (PAHs) from the soil through their roots. The amount of PAHs absorbed being dependent on PAH’s concentration, its solubility, its nature—vaporous or particulate and the nature of the plant species and the plant parts (Edwards, 1983). Some PAHs may be metabolized by the plants. The seriousness of oil pollution is thus determined by the type of soil, amount of oil, local physiography, climate, season, biological and physical characteristics of the area, sensitivity of the species, etc, (Dick, 1999; Wyszkowski et al., 2004). Naidu (2001) has reported that oil refinery effluents causes four types of symptoms in plants which are typical of nutrient deficiencies – yellowing of foliage, chloronecrosis, wilting and defoliation. Our studies suggest that P. juliflora is sensitive to petrodiesel pollution—not surviving > 1.0% diesel oil in the rhizosphere. In comparison, Thespasia populnea is more tolerant to oil pollution (Khan and Shaukat, 2009).

Table 2. Effect of petrodiesel pollution on growth of 20-day old P. juliflora seedlings.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diesel oil concentration (mL/100g Soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Seedling Height (cm)</td>
<td>6.93 ± 0.43 a</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>2.55 ± 0.17 a</td>
</tr>
<tr>
<td>Radicle Length (cm)</td>
<td>5.47 ± 0.25 a</td>
</tr>
<tr>
<td>Hypocotyl (cm)</td>
<td>3.80 ± 0.28 a</td>
</tr>
<tr>
<td>Epicotyl (cm)</td>
<td>3.03 ± 0.25 a</td>
</tr>
<tr>
<td>Number of leaflets</td>
<td>11.20 ± 0.44 a</td>
</tr>
<tr>
<td>Primary leaf</td>
<td>38.22 ± 4.73 a</td>
</tr>
</tbody>
</table>

Significance at p < 0.05 by t-test.

Table 3. Effect of diesel oil contamination on area of cotyledon and cotyledonary area per seedling of Prosopis juliflora after 20 days of treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diesel oil concentration (mL/100g Soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Area of a cotyledon (cm)²</td>
<td>165.6 ± 6.16 a</td>
</tr>
<tr>
<td>Cotyledonary Area per Seedling (cm)³</td>
<td>331.2 ± 16.93 a</td>
</tr>
</tbody>
</table>

*< range; **, Coefficient of variability (%). significance at p < 0.05 by t-test.
Fig. 3. Growth performance of P. juliflora in terms of reduction over control in various growth parameters under diesel oil pollution. C, Control; T1, 0.5; T2, 0.75, and T3, 1 mL diesel oil concentration in 100g soil.

REFERENCES

EFFECTS OF DIESEL OIL CONTAMINATION IN SOIL ON PROSOPIS JULIFLORA.


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