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EFFECTS OF DIESEL OIL-POLLUTED SOIL ON EMERGENCE AND GROWTH OF SEEDLINGS OF *THESPESIA POPULNEA* (L.) SOL. Ex. CORR.

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ABSTRACT

The effects of diesel oil pollution on *Thespesia populnea* (L.) Sol. Ex. Corr. were investigated in a series of artificially and freshly polluted sandy soil with 0, 0.5, 0.75, 1.0, 2.5, 4.0 and 5.0 mL diesel oil per 100g soil. Emergence of seedlings was impeded and germination losses occurred at high diesel oil concentrations. All the parameters of seedling growth viz. height, number of leaves, stem diameter, hypocotyl and epicotyl lengths, cotyledon area per seedling, area of the largest leaf, total leaf area per seedling and dry biomass of shoot, root and seedling declined progressively with increase of diesel oil concentration in the rhizosphere. Chlorophyll – a, b and total chlorophyll contents in fresh leaves declined significantly. Against control where around 10 internodes were produced, under diesel oil pollution only six or at the most seven internodes were produced. Diesel oil shortened the internodes. Diesel oil reduced the number of days of retention of cotyledons and primary and secondary leaves with the seedlings i.e., diesel oil pollution not only enhanced the cotyledon abscission but also the abscission of primary and secondary leaves and even the tertiary leaves under very high concentrations. In spite of the extreme diesel toxicity to *T. populnea* under very high diesel oil contamination, the plant showed good potential of phytoremediation against diesel pollution in sandy soil contaminated up to 10, 000 ppm of petrodiesel.

Key Words: *Thespasia populnea* (L.) SOL. Ex. CORR., Diesel oil pollution, Seedling emergence, seedling growth, cotyledon abscission, Chlorophyll, pytoremediation.

INTRODUCTION

Pollution with petrochemical hydrocarbons is one of the serious problems all around the world. There are some published reports which 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 (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; Wiltse *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.*, 2009 a and b; Serrano *et.al.*, 2009; LIU *et. al.*, 2009). Such studies are preliminary to the understanding of phyto-remediation potential of plants at population and community level.

Thespesia populnea (L.) Sol. Ex. Corr. (Cork tree, Milo, Umbrella tree, Indian tulip tree – more than 60 vernacular names), is a coastal and moderately to highly salt tolerant plant in tropical countries. In Pakistan, it is confined to coastal region particularly Karachi – cultivated in parks or along roadside as ornamental or a shade plant (Abedin, 1979). It is preferably raised with seeds as then the timber is knot free, straight, even-grained and tough. It is a valuable plant and medicinal too (Chopra *et. al*., 1956; Varier, 1997).

The evaluation of phyto-remediation potential of *T. populnea* 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 this very useful plant in diesel-polluted soil.

MATERIAS AND METHODS

The experiment was conducted in earthen pots containing 500g sandy (sand 85%) garden soil (containing compost @ 2%), thoroughly mixed with diesel oil (fuel grade) in a series of concentration – 0, 0.5, 0.75. 1.0, 2.5, 4.0 and 5.0 mL of diesel oil per 100 g soil. This concentration roughly corresponded to 0, 4,000, 6,000, 8,000, 20,000, 32,000, and 40,000 ppm of diesel oil in soil, respectively, on the basis of specific density of the petro-diesel sample employed in the study to be 0.825 as per laboratory determination. The soil was incubated for 24h before sowing seeds. Ten seeds of similar sizes abraded with conc. H_2SO_4 for 20 minutes (to enhance germination) 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 around 30^o C). In last week before harvest, atmospheric temperature, however, rose to 40-41^o C for few days. The seedling emergence was recorded for seven days and the emerging crop was allowed to

grow up till 100 days from June 30, 2006 to October 09, 2006, when the crop was harvested and stored at 5 degree Celsius for analysis. There were three replicates to each treatment and control for morphometric analysis drawn randomly. Leaf and cotyledon area was determined graphically by drawing outlines of them with utmost precision. Chlorophyll determination was made with 0.5 g fresh leaves according to the method of Maclachlam and Zelik (1963). The data were analyzed statistically.

RESULTS AND DISCUSSION

EMERGENCE OF SEEDLINGS

Although there was substantial seedling emergence after four days of incubation but the rate of emergence of seedlings was significantly impeded as a result of diesel oil contamination of soil (0.5 – 5 mL /100g soil) as compared to the control (Fig. 1). *T. populnea* seeds couldn't emerge at all in higher diesel oil concentrations of 7.5 and 10 mL diesel oil per 100g soil (not shown in the graph). 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. Kroening *et. al.* (2001) has reported losses of diesel oil from contaminated soil as high as 58% over around a year. Approximately 10.6% of diesel range organic compounds have been demonstrated to volatilize from soil by Pichtel and Liskanen (2001). The germination activity of the soil in case of *Lepidium sativum* L. is reported to recover after around 200 days of the diesel spill (Serrano *et. al*., 2009).

The concentration of 10,000ppm of diesel in soil may perhaps the critical concentration beyond which not only the seedling emergence of *T. populnea* was highly impeded but there was substantial loss of germination also as was well evident from the un-germinated seeds recovered from the soil on 7^{th} day of incubation. 1% diesel oil contamination is reported to have no significant effect on overall germination of *Sorghum bicolor* and *Zea mays* but at this level of contamination germination in *Vigna unguiculata* was significantly reduced. Higher level of diesel contamination (> 1%) affected all these species significantly (Ogbo *et. al.,* 2009 a)

The contamination of soil with 4 and 5% spent oil has been reported to consistently inhibit germination of *Capsicum annum* and *Lycopersicon esculentum* (Analiefo and Vwioko, 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.

SEEDLING GROWTH

All the parameters of seedling growth viz. height, number of leaves, stem diameter, hypocotylar and epicotylar lengths, cotyledonary area per seedling, total leaf area and dry biomass of shoot, root and seedling declined progressively with increase of diesel oil concentration in the rhizosphere (Table 2). Oil pollution is known to retard seedling growth (de Jong, 1980; Atuanya, 1987). Diesel fuel 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% (Odjgba 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 reportec 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 pea, *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).

The root growth of *T. populnea* seedlings, which was inhibited significantly with pollution (Fig. 2) related with the diesel concentration as follows:

Log_e root mass = -2.08208 – 0.258581 diesel oil concentration

t = -8.70 t = - 2.86

p < 0.001 p < 0.001

R^2= 0.3008; r = - 0.5484; F = 8.17 (p < 0.001)

The root growth not only declined abruptly but became more or less asymptotic from 1.0 to 5 mL diesel oil per 100g soil. The roots in control were creamy white in colour. They, however, turned dark brown to black in soil contaminated with higher petro-diesel contents. Crude oil treatment to *Chromolaena odorata* has been reported to result in distortion of cells of epidermal and cortical region of root, stem and petiole (Gill *et. al.*, 2004).

Figure 3 depicts the quanta of decline of various growth parameters. Parameters such height, number of leaves, hypocotylar and epicotylar lengths were never inhibited more than 50% over control from 0.5 to 5 ml / 100g soil. However, the parameters such as root, shoot and seedling biomass and on plant and total leaf area per seedling declined up to 60 - 80% in soil contaminated with more than 8000 ppm diesel (1.0 mL diesel / 100g soil).

The area of the largest leaf of the plant remained generally around 12 to 15 sq. cm in treated plants as compared to that around 22.5 sq. cm in control plants (Fig.4). Among treated plants, however, this parameter didn't vary significantly and remained more or less half in magnitude to that in control. The area of the largest leaf related with the oil concentration as follows.

Log_e Area of the largest leaf = 2.75548 – 0.092103 Oil Concentration

t = 33.14 t = - 2.933

p < 0.001 p < 0.009

R^2 = 0.3116, Adj R^2 = 0.2754; F = 8.60 (p < 0.001); SE = 0.2554

Against control where around 10 internodes were produced, under diesel oil pollution only six or at the most seven internodes were produced (Table2). The length of internode declined progressively from base to apex ((F = 162.8, p < 0.001) and also along the increasing diesel oil concentration (F = 6.12, p < 0.001). Internodal length and diesel oil concentration interacted significantly (F = 1.45, p < p.043). Retardation of growth under the influence of diesel in common but contrary to general belief Campbell and Vavrek (1999) have reported that *Bacopa monneiri* individuals surviving the application of oil had longer stems, more leaves but shortened internodes.

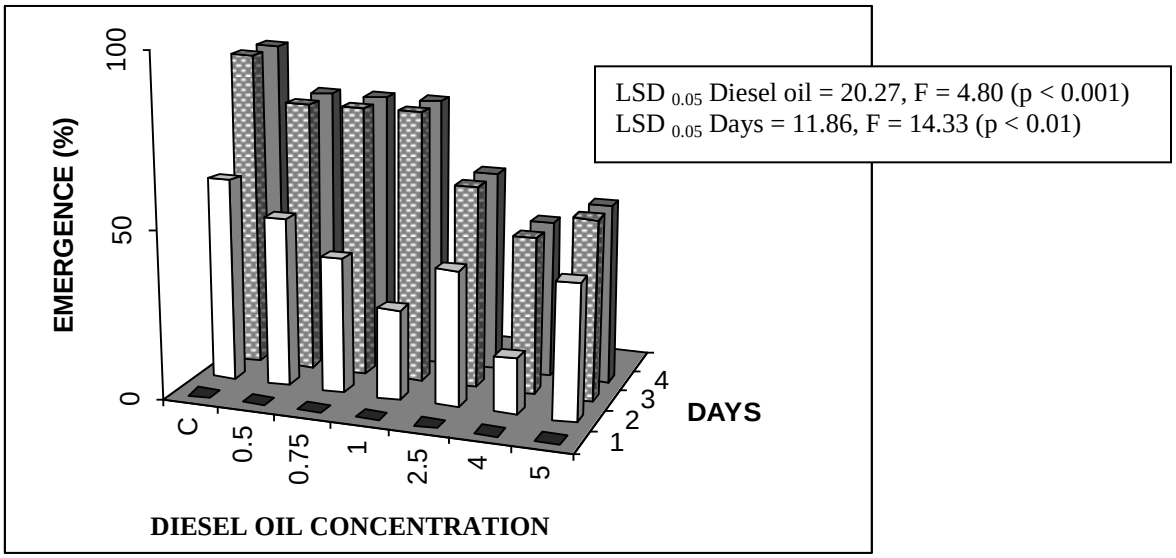

Fig. 1. Emergence of seedlings from abraded seeds of *Thespesia populnea* under diesel oil pollution in sandy soil (mL per 100g Soil).

CHLOROPHYLL CONTENT

The concentration of chlorophyll-a, chlorophyll-b, and total chlorophyll in fresh leaves of the plant declined substantially with the increase of diesel oil pollution (Fig. 5). The chlorophyll has already been reported to decline under spent oil pollution significantly (odjegba and Sadiq, 2002). Udo and Fayomi (1975) have also reported chlorosis of leaves resulting from the dehydration of plants under oil pollution.

SEEDLING MORTALITY

There were a few mortalities of seedlings in diesel oil contaminated soils. One seedling died in soil treated with 2.5 mL diesel per 100g soil on 40th day of growth, one seedling died in 4.0 mL diesel oil per 100g soil and one seedling died in 5 mL per 100 g soil on 50th day of growth.

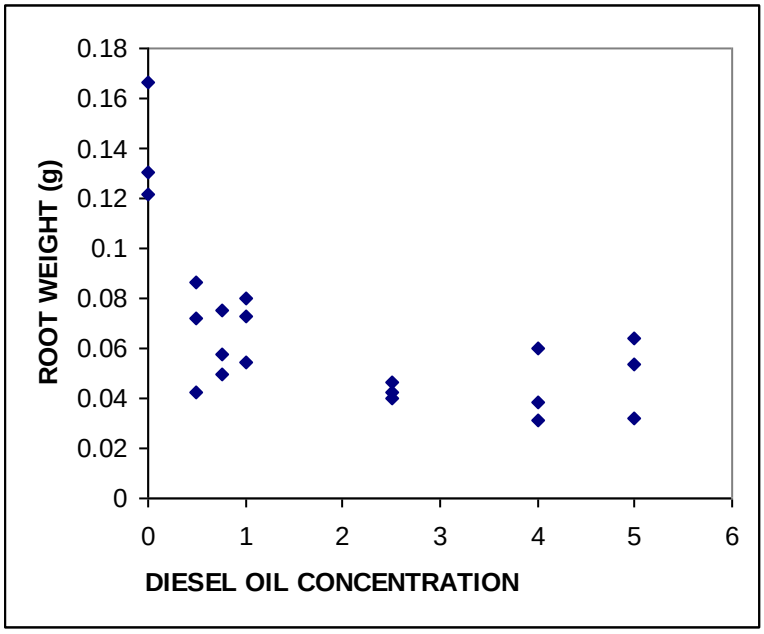

Fig. 2. Effect of diesel oil concentration (mL per 100g Soil) on root growth of *T. populnea* seedlings.

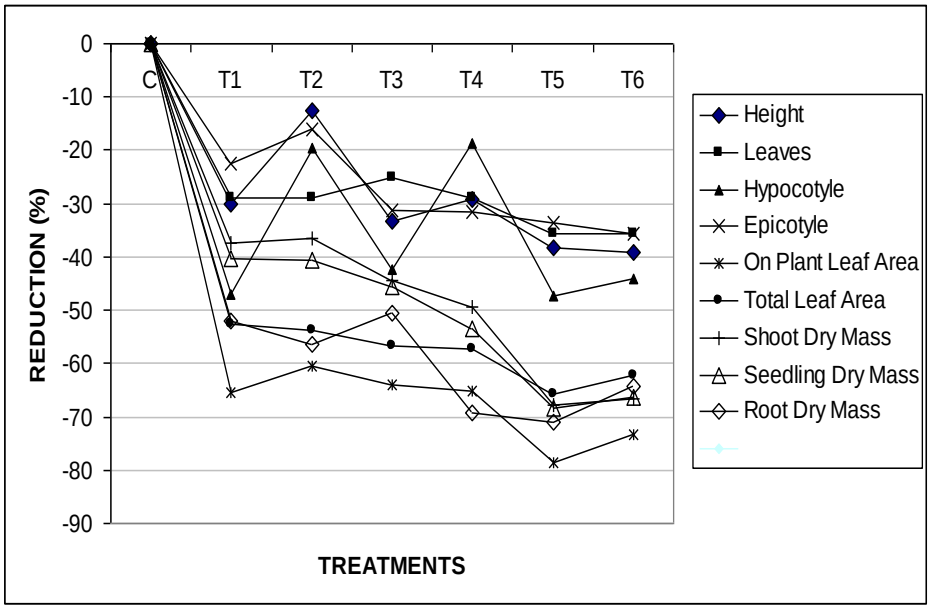

Fig. 3. Growth performance of *T. populnea* in terms of reduction over control of various growth parameters under diesel oil pollution. C, Control; T1, 0.5; T2, 0.75; T3, 1.0; T4, 2.5; T5, 4.0 and T6, 5.0 mL diesel per 100g soil.

COTYLEDONARY AND FOLIAR ABSCISSION

The abscission of plant parts in terms of proportion of area of cotyledons and leaves to total leaf area of the plant and their biomass proportion increased greatly with oil pollution as compared to the control (Fig. 6). The cotyledons were normally fleshy, thick and leathery and dark green in colour. In higher oil concentrations they slowly turned yellow, shrunk and abscised much earlier than the onset of cotyledonary abscission took place in

control plants (Table 3). Oil pollution not only enhanced the cotyledonary abscission but also the abscission of primary and secondary leaves and even the tertiary leaves under high diesel concentrations. During experimental period ambient temperature remained around 32 ^{o}C. However, during pre-harvest period of the experiment, the ambient temperature rose to 40-41^{o}C. Since no secondary or tertiary leaves abscised in control plants at this point of time, the abrupt rise in temperature at 85th to 87th day of experiment has probably been a factor to accelerate foliar abscission in the treated plants already under abiotic stress. After 100 days of growth at the time of harvest, one of the control plants had even stipules still attached with the petiole of the primary leaf, whereas fall of tertiary leaves have taken place in plants under very high pollution. High reduction percentages over control in total leaf area of the seedlings in oil polluted environment may be attributable to temperature interaction with the pollution effects in treated plants.

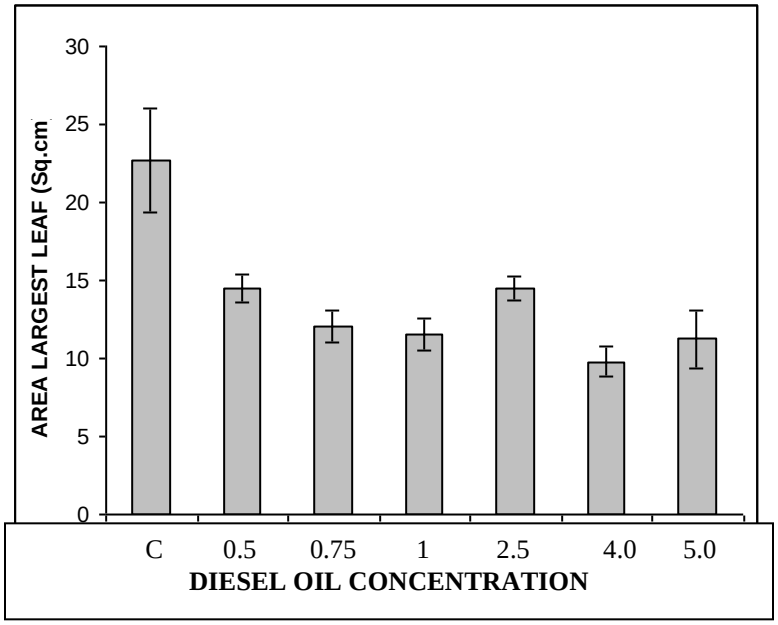

Fig. 4. Area of the largest leaf of *T. populnea* seedlings as a function of the diesel oil pollution in soil (mL per 100g soil).

The diesel oil pollution in the soil inhibited cotyledonary and foliar expansion, internodal elongation, height of the plants, leaf area and chlorophyll synthesis. The onset of abscission of cotyledons and leaves was significantly earlier in treated plants and dose dependent in magnitude and probably accelerated under high temperature regime. 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 truncatur* suffered the most phytotoxic effects and *Linum ussitatissimum* the least. Response of the plants to diesel oil is species-specific. Vavrek and Campbell (1999) have reported *Eleocharis* species and *Cyperus erythrorhizos* to be insensitive to oil application where as B*acopa monneiri* and *Rotala ramoseor* to be sensitive to oil.

The contamination of soil with Petroleum and refinery products (PRPs) causes degradation of soil (Sztompka, 1999) 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 part (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. Poultry manure has recently been reported to alleviates the effects of crude oil on maize (Onuh *et.al.*, 2008).

Table 1. Effect of petro-diesel pollution on seedling morphology of *T. populnea*.

Parameter	**CONCENTRATION OF DIESEL OIL IN SOIL (VOLUME / WEIGHT – mL / 100g)**						
	Control	0.5	0.75	1.0	2.5	4.0	5.0
Seedling Height (cm)	21.96 ± 1.32 a	15.33 ± 0.44 b	19.16 ± 0.12 a	14.67 ± 0.27 b	15.56 ± 1.86 b	13.53 ± 0.61 b	13.33 ± 1.30 b
Number of leaves	9.33 ± 0.44 a	6.63 ± 0.33 b	6.63 ± 0.33 b	7.00 ± 0.57 b	6.63 ± 0.33 b	6.00 ± 0.33 b	6.00 ± 0.33 b
Stem diameter (cm)	0.40 ± 0.0 a	0.316 ± 0.016 bc	0.40 ± 0.0 a	0.333 ± 0.033 ac	0.373 ± 0.037 bc	0.270 ± 0.033 c	0.316 ± 0.016 bc
Hypocotyl (cm)	7.53 ± 0.09 a	4.00 ± 0.46 b	6.06 ± 0.63 ab	4.33 ± 1.17 b	6.13 ± 0.59 ab	3.96 ± 0.67 b	4.20 ± 0.90 b
Epicotyl (cm)	14.40 ± 1.27 a	11.16 ± 0.58 bc	12.07 ± 0.98 ab	10.03 ± 0.64 bc	9.86 ± 1.44 c	9.56 ± 0.36 c	9.26 ± 0.59 c
CA * / seedling (sq.cm)	11.52 ± 0.20 a	9.74 ± 0.17 ab	10.03 ± 0.64 ab	7.87 ± 0.68 b	7.94 ± 0.79 b	7.96 ± 0.49 b	8.05 ± 0.74 b
Leaf area abscised (sq.cm)	2.25 ± 2.25 a	15.00 ± 0.99 b	8.39 ± 2.09 c	8.74 ± 0.92 c	9.29 ± 0.89 c	13.32 ± 3.69 b	12.88 ± 2.20 b
On-Plant Leaf Area (sq.cm)	106.42 ± 19.36 a	36.56 ± 6.30 b	41.94 ± 1.18 b	38.38 ± 3.99 b	37.19 ± 2.14 b	22.85 ± 2.71 c	28.32 ± 4.97 c
Total Leaf Area (sq.cm)	108.67 ± 19.28 a	51.53 ± 6.85 b	50.33 ± 0.95 b	47.13 ± 3.93 b	46.49 ± 3.03 b	36.20 ± 4.73 c	41.19 ± 3.50 c
Root Biomass (g.DW)	0.1397 ± 0.0138 a	0.0671 ± 0.0129 b	0.0610 ± 0.0074 b	0.0690 ± 0.0077 b	0.0429 ± 0.0019 c	0.0405 ± 0.0096 c	0.0497 ± 0.0094 c
Abscised Biomass (g.DW)	0.0629 ± 0.0034 a	0.0797 ± 0.0010 b	0.0661 ± 0.0036 a	0.0866 ± 0.0031 b	0.0900 ± 0.0010 b	0.0889 ± 0.0029 b	0.0741 ± 0.0063 b
Net Shoot Biomass (g.DW)	0.4832 ± 0.0584 a	0.2579 ± 0.0346 b	0.2802 ± 0.0379 b	0.2174 ± 0.0256 b	0.1850 ± 0.0382 c	0.0869 ± 0.0115 d	0.1076 ± 0.0295 d
Total Shoot Biomass (g.DW)	0.5460 ± 0.0584 a	0.3414 ± 0.0379 b	0.3463 ± 0.0365 b	0.3040 ± 0.0445 b	0.2770 ± 0.0368 c	0.1759 ± 0.0112 c	0.1816 ± 0.0342 c
Biomass Yield per seedling (g.DW)	0.6859 ± 0.0541 a	0.4085 ± 0.0454 b	0.4073 ± 0.0436 b	0.3730 ± 0.0182 b	0.3183 ± 0.0393 b	0.2164 ± 0.0203 c	0.2313 ± 0.0267 c

*, CA = Cotyledonary area (sq.cm) per seedling. Each mean is based on three replicates. The figures followed by similar letter (s) are not significantly different from each other as given by DMR test.

Table 2. Internodal elongation (length in cm) of *T. populnea* seedlings under diesel oil pollution.

Internode # (Base to apex)	**CONCENTRATION OF DIESEL OIL IN SOIL (VOLUME / WEIGHT – (mL / 100g SOIL)**						
	Control	0.5	0.75	1.0	2.5	4.0	5.0
1	3.7 ± 0.44	3.03 ± 0.42	3.46 ± 0.15	2.93 ± 0.87	3.3 ± 0.60	3.13 ± 0.09	2.93 ± 0.32
2	1.63 ± 0.71	2.43 ± 0.29	2.60 ± 0.15	2.60 ± 0.47	2.26 ± 0.43	2.67± 0.09	2.23 ± 0.12
3	2.20 ± 0.0	2.23 ± 0.07	2.43 ± 0.32	1.93 ± 1.90	1.90 ± 0.10	2.53± 0.37	1.67 ± 0.09
4	1.40 ± 0.40	1.43 ± 0.15	2.10 ± 0.06	1.43 ± 0.21	1.50 ± 0.29	1.03± 0.09	1.13 ± 0.07
5	1.43 ± 0.07	1.13 ± 0.13	1.46 ± 0.03	1.07 ± 0.13	0.73± 0.13	0.73± 0.12	0.80 ± 0.06
6	1.13 ± 0.18	0.60 ± 0.26	0.80 ± 0.15	0.53± 0.15	0.13± 0.13	0.23± 0.12	0.40 ± 0.06
7	1.33 ± 0.29	-	-	0.17± 0.08	0.06± 0.06	-	-
8	1.07± 0.26	-	-	-	-	-	-
9	0.30 ± 0.3	-	-	-	-	-	-
10	0.20 ± 0.20	-	-	-	-	-	-

LSD $_{0.05}$ (internode length) = 0.2469, F = 162.8 ($p < 0.001$); LSD $_{0.05}$ (diesel oil concentration) = 0.2066, F = 6.12 ($p < 0.001$); Interaction (internode length x Oil: F = 1.45 ($p < 0.043$).

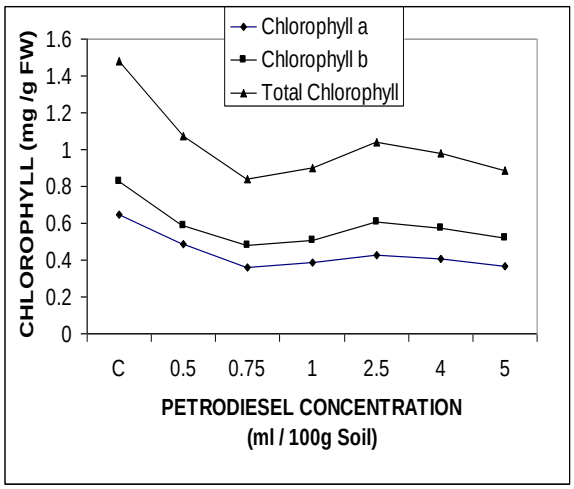

Fig. 5. Effect of petro-diesel polluted soil on chlorophyll contents in leaves of *T. populnea*.

Responses of plants to diesel oil pollution are species–specific and differences to tolerate oil pollution even exist at subspecies level (Adam and Duncan, 1999, 2002). As regards to *Thespasia populnea*, our results are in close agreement with Sun *et. al.*(2004). They have investigated the Phytoremediation potential of this species at two dose levels – 5,000 and 10,000ppm diesel oil concentrations. Our results are based on broader range of treatments - 4000 to 40,000 ppm diesel oil in soil. Higher concentration of diesel oil were, of course, very much phytotoxic but it may judiciously be concluded that *T. populnea* may be cultivated with success in diesel oil contaminated soils containing

up to around 1% (10,000 ppm) diesel oil in coastal desert terrestrial environment of Pakistan and on the expense of some reduction in growth and some probable mortalities.

Table 3. The number of days of retention of cotyledons, and primary, secondary and tertiary leaves by *T. populnea* seedlings under diesel oil pollution.

S. No.	Treatment Diesel oil Concentration (mL per 100g Soil)	Number of days of retention			
		Cotyledons	Primary Leaf	Secondary Leaf	Tertiary Leaf
1	Control	84.38 ± 3.84	94.20 ± 2.0	*	*
2	0.5	61.00 ± 2.04	81.7 ± 1.1	88.0 ± 4.0	*
3	0.75	56.50 ± 2.87	*	93.5 ± 0.5	*
4	1.0	59.25 ± 3.34	72.6 ± 4.4	92.0 ± 1.5	*
5	2.5	49.38 ± 1.53	79.0 ± 1.6	85.0 ± 1.0	*
6	4.0	55.00 ± 3.81	74.6 ± 2.7	92.5 ± 1.0	96.0 ± 1.00
7	5.0	52.67 ± 2.66	76.6 ± 1.5	88.0 ± 4.0	95.5 ± 1.50

*, No leaf abscission took place.

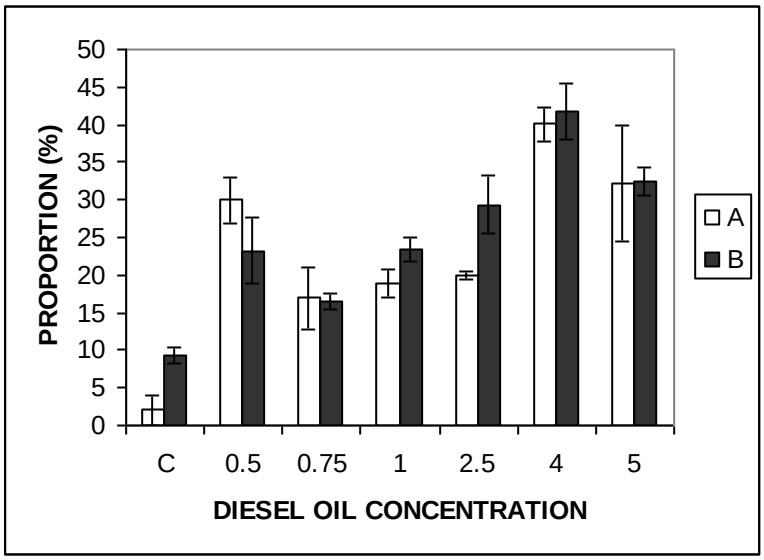

Fig. 6. Percent proportion of abscised part of the seedling of *T. populnea* under diesel oil pollution in soil (mL per 100g soil). A, based on leaf and cotyledon area abscised in relation to leaf area of the seedling , and B, based on the biomass of the abscised components (cotyledon and or leaves) in relation to total shoot biomass of the seedlings.

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