

Germination and growth of six plant species on contaminated soil with spent oil

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ABSTRACT: Effects of contaminated soil with spent oil on germination, above ground height and biomass of six herbaceous plant species were investigated by conducting a general phytotoxicity test and growth inhibition assessment. Six local plant species were used in order to investigate plant's ability to germinate and survive in a gradient of contaminated soil with spent oil. The species selected for this experiment include one species of Fabaceae (*Medicago truncatular*), four species of Gramineae (*Bromous mermis*, *Secal seral*, *Triticum sativa* and *Agropyron deserterum*) and one species of Linaceae (*Linum ussitasimum*). Inhibitory effect of contaminated soil on germination, height of young seedling and dry weight were measured. In this study an artificial soil with a light texture included 85% sand, 10% silt and 5% clay was used. The exposure to the contaminated soil carried out using four consecutive concentrations (25, 50, 75, 100 g/kg). Results obtained from the current investigation indicate that all species perform dose-dependent responses to the contaminated soils. Reduction in germination, above ground height and biomass for all species were significantly ($P < 0.05$) different when compared to their controls, however, *Medicago truncatular* performed the highest and *Linum ussitasimum* the lowest inhibitory effect for germination, above ground height and dry weight of seedling.

Key words: Spent oil, germination, phytotoxicity test, contaminated soil

INTRODUCTION

Various petroleum products are common soil contaminants and often contain potentially hazardous chemicals, particularly the polycyclic aromatic hydrocarbons (Huang, *et al.* 2004). The accumulations of PAHs in soil are due to many anthropogenic sources such as coking plants, solid fuel domestic heating, aircraft exhaust, car exhausts and forest fires (Smith, *et al.* 2006). Amongst petroleum pollutants spent oil contains heavy metals and polycyclic aromatic hydrocarbons and chemical additives including amines, phenols, benzenes, Ca, Zn, Pb, Ba, Mn, P and S which are dangerous to living organisms (Meinz, 1999). The spent lubricant, otherwise called waste engine oil, is usually obtained after servicing and subsequent draining from automobile and generator engines. Pollution from spent engine oil is one of the environmental problems and is more widespread than crude oil pollution (Odjegba and Sadiq, 2002). The concentration of PAHs in lubricating oil increases with time of usage and those with two and three rings

accumulate rapidly in used lubricating oil to very high levels (Vwioko and Fashemi, 2005). Phytoremediation is an innovative technology that uses plants to remove environmental contaminants such as heavy metals and organic compounds. Utilizing plants to absorb, accumulate and detoxify contaminants in the growth substrate through physical, chemical or biological processes is a wide spread practice (eg. White, *et al.*, 2006; Jilani and Khan, 2006). This technology has been applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Ghosh and Singh, 2005). In medium size cities such as Kermanshah with a population of approximately 600,000, the main sources of spent oil appears to be small unregulated enterprises. In order to explore more financially acceptable options for biological control of small contaminated sites polluted by spent oil phytoremediation potentials of selected local plant species have been considered as an effective measure. The main aim of this study was to find possible phytoremediation capacity of the local plants in reducing the extent of contaminated soils with spent oil.

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MATERIALS AND METHODS

Six plant species including *Medicago truncatular*, *Agropyron deserterum*, *Bromous mermis*, *Secal seral*, *Triticum sativa* and *Linum ussitasimum* were screened for their ability to germinate on artificial soil contaminated with spent oil in September 2005. The seeds of these plants were collected from the plants in the pervious growing season. The spent lubricating oil was obtained from an oil car workshop in Kermanshah. An artificial soil was prepared for this experiment containing 85 % sand, 10 % silt and 5 % clay. This light loamy soil provides a suitable substrate for plant growth in phytotoxicity test as has been used by Adam and Duncan (2002), Anoliefo and Vwioko (1995) and avoids anomalies normally associated with natural soils Ghosh and Singh (2005). In order to provide a relatively inert substrate, the artificial soils were placed into autoclave (121 °C) for 30 minutes in two consecutive days. To obtain an even distribution of spent oil in the soil a mixture of acetone: spent oil (1:1) for each concentration was prepared and mixed with the inert soil by a glass rod. The acetone allowed to evaporating by placing the soil in a laboratory hood. This procedure was used to prepare specimens of 25, 50, 75 and 100 g of spent oil per kg soil. Uncontaminated controls were prepared by adding only acetone to the soil. Fifty g of uncontaminated soil and soil specimens containing 25, 50, 75 and 100 g of spent oil/kg were prepared in small plastic pots. Each treatment consisted of a set of 20 seeds in three replicates. Seed surface was sterilized prior to use by soaking in a 5 % sodium hypochlorite solutions for five minutes then rinsed three times and soaked in distilled water for 5 minutes. Seeds of each species were planted in small plates and soil moistened with tap water. Plates were placed in dark incubator at 22±2° C until the majority of seeds had germinated then were maintained under controlled laboratory conditions. After one or two weeks percentage germination was recorded and the height and dry weight of the plants measured from the soil surface to the terminal bud. The variables measured in the contaminated soils and their controls were expressed as percentage of the control. Statistical significance was determined by single factor analysis of variance (ANOVA) and Tukey test. A probability of 0.05 or lower was considered as significant. In Table 1 to 6

where results of statistical analysis are presented values at each column for control, 25, 50, 75 and 100 g/kg soil are designated by A, B, C and D. Values followed by capital or lower case were show non-significant or significant different respectively.

RESULTS

The effects of spent oil on germination rate, above ground height and biomass for *Medicago truncatular*, *Agropyron deserterum*, *Bromous mermis*, *Secal seral*, *Triticum sativa* and *Linum ussitasimum* are shown in Figs. 1 to 6. Generally the various responses of the plant species to the contaminant appear to be dose dependent. The pattern of responses to the contaminant in terms of per cent reduction in germination rate follows an exponential model in all species except *T. sativa* and *S. seral* judging on the basis of coefficient of variations obtained from various fitted curves. Although most plant species demonstrated an overall dose dependent response to the contaminated soil but an increase in germination rate was evident at some concentrations. For example *T. sativa* at 25 and 50 g/kg, *S. seral* at 25 g/kg and *M. truncatular* at 25, 50 and 75 g/kg contaminated soil showed an increase in germination rate relative to the control. Moreover, no seed germination was found at 100 g/kg spent oil in *T. sativa*. Statistical comparisons between germination rate, above ground height and biomass measured under various concentration of spent oil for six plant species using single factor analysis of variance (ANOVA) and Tukey test is shown in Tables 1 to 6. Generally, the average percentage of above ground heights responded to the contaminated soils follow an apparent dose dependent reduction most likely similar to an exponential model. However, other species such as *T. sativa* and *A. deserterum* demonstrate a linear reduction. Moreover, *S. seral* at 25 g/kg and *M. truncatular* at 25 and 50 g/kg soil showed an increase in seedling height. Similar pattern of exponential reduction in biomass was found in other plant species except *T. sativa*, *L. ussitasimum* and *S. seral* which performed a linear reduction. Inhibitory effect of spent oil contaminated soil on germination, height of seedling and above ground biomass in each plant species expressed as IC_{50} is shown in Fig. 7.

Table 1: Effect of spent oil on germination and growth of *Agropyron deserterum**

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 1.7A	9.4± 0.47A	0.02± 0.003A
25	72.5± 1.8AB	4.7± 0.57aB	0.009 ± 0.002aB
50	57.5± 1.2aBC	7.3± 0.59AbC	0.01 ± 0.0008aBC
75	26.9± 1.4abCD	7.3± 1.09ABCD	0.005 ± 0.0008aBCD
100	16.2± 0.33abcD	4.87± 1.1aBCD	0.002 ± 0.0006aBCD

Table 2: Effect of spent oil on germination and growth of *Bromous mermis* *

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 1.76A	5.3± 0.33A	0.009± 0.001A
25	80.2± 2.4AB	4.35± 0.43AB	0.006± 0.001AB
50	54.9± 0.3ABC	3± 0.38aBC	0.002± 0.001aBC
75	32.3± 1.2aBCD	2.05± 0.43abCD	0.002± 0.0005aBCD
100	15.04± 0.57abCD	2.5± 0.56aBCD	0.001± 0.0003aBCD

Table 3: Effect of spent oil on germination and growth of *Linum ussitasimum* *

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 1A	7.75± 0.5A	0.02± 0.001A
25	66.7± 1aB	5± 0.6aB	0.006± 0.002aB
50	33.3± 1.2abC	4.2± 1.09aBC	0.001± 0.0006aBC
75	8.3± 0abCD	1.1± 0.5aBCD	0 abCD
100	2.7± 0.33abCD	0.7± 0aBCD	0 abCD

Table 4: Effect of spent oil on germination and growth of *Medicago truncatular* *

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 1.8A	2.1 ± 0.1A	0.02 ± 0.002A
25	143± 2AB	2.4 ± 0.08AB	0.03± 0.008AB
50	119± 2.3ABC	2.4 ± 0.09ABC	0.02± 0.05ABC
75	115± 0.3ABCD	2.1 ± 0.08ABCD	0.01 ± 0.06ABCD
100	63.5± 1.5AbCD	1.8 ± 0.11AbcD	0.01± 0.004ABCD

Table 5: Effect of spent oil on germination and growth of *Secal seral* *

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 0.58A	9.7 ± 0.93A	0.04 ± 0.002A
25	111± 0.88AB	10.3 ± 0.7AB	0.05± 0.066AB
50	71.7± 1.2ABC	9.2 ± 0.86ABC	0.03± 0.061ABC
75	69.2± 1.7ABCD	7 ± 0.78AbCD	0.03± 0.004ABCD
100	10.8± 0.88abcd	9.3 ± 2.1ABCD	0.006± 0.004abCD

Table 6: Effect of spent oil on germination and growth of *Triticum sativa* *

Treatment	Germination (%)	Seedling height (cm)	Dry biomass (g)
Control	100± 0.33A	10.5 ± 1.6A	0.06± 0.002A
25	184± 0.88aB	8.2± 0.56AB	0.087± 0.01AB
50	134± 2.08ABC	7.2± 0.68ABC	0.057± 0.01ABC
75	70± 0.33AbCD	3.7± 1.01abCD	0.018± 0.005abCD
100	0abcD	0abCD	0abcD

*Values followed for the all above tables by capital or lower cases were show non-significant or significant different respectively

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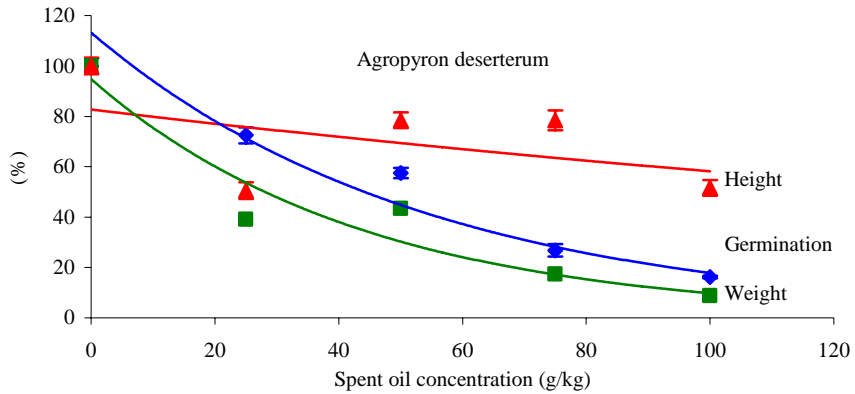


Fig. 1: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *M. truncatular*

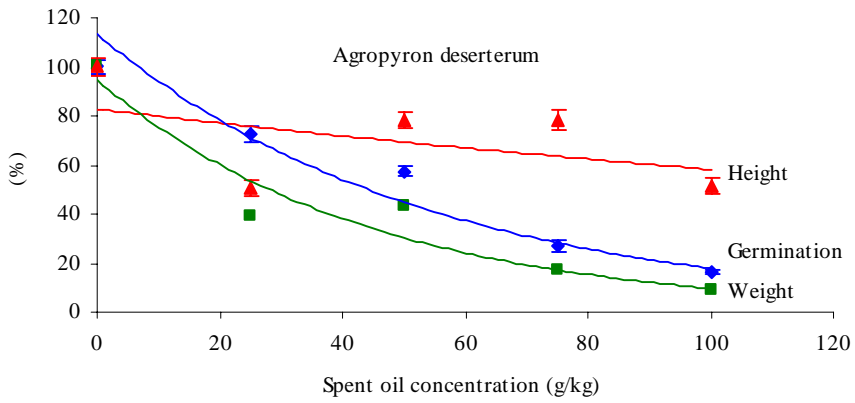


Fig. 2: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *A. deserterum*

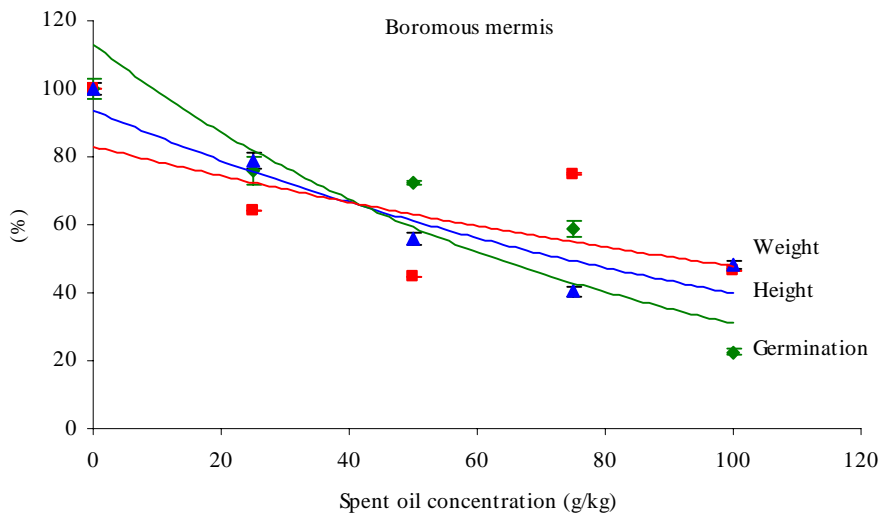


Fig. 3: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *B. mermis*

DISCUSSION AND CONCLUSION

Results obtained from current experiment are similar to those obtained by Anoliefo and Vwioko (1995). These authors have carried out an experiment using spent lubricating oil in soils supplemented with various concentrations of spent oil ranging from 1-5% w/w and two plant species *Capsicum annum* and *Lycopersicon esculentum*. Anoliefo and Vwioko (1995) reported that treatment of soil with 4 and 5 percent spent engine oil consistently inhibited germination of both plants. Also, they considered the mean height and leaf surface area after eighty-four days from sowing reported lowest values at 3% treatment. Odjegba and Sadiq (2002) also examined effects of spent engine oil on the growth of *Amaranthus hybridus* and reported significant differences between the control plants and those grown in the oil treated soil. In their experiment the mean height of the control plants was significantly ($p < 0.05$) greater than those for plants grown in soil treated with 1-5 percent spent engine oil.

Vwioko and Fashemi (2005) have investigated growth response of five different plant species in soil supplemented with spent lubricating oil at 1-6 % w/w strengths. The result of this experiment which conducted at six consecutive concentrations level showed that various growth parameters demonstrate an overall dose dependent response although at lower concentration some plant species showed a positive response to the contaminant. For parameters such as plant height, stem girth, leaf surface area, fresh and dry weights, and root length, the mean values obtained were higher for 1 % w/w than control. Plants in higher concentrations (2 – 6 % w/w) exhibited depression in growth. Plants grown in 1 % w/w spent lubricating oil in soil flowered earlier than those grown in control.

In a similar experiment Adam and Duncan, (2002) have also demonstrated a mixed species specific response to gas oil contamination. In their experiment diesel fuel oil was used as a contaminant in a series of artificial soil with 89 % sand, 4.9 % silt and 8.4 % clay, and two concentration levels of 25 and 50 g/kg soil. Plant response was evaluated by percent germination after 14 days exposure. In their experiment *Trifolium album*, *Vicia sativa*, *Medicago lupulina* and *Trifolium pratense* in 25 g/kg of contaminated soil showed seed germination nearly equal to control with no fuel oil. Another study Huang, *et al.*, (2004) demonstrated that the germination frequencies of the grass species were all affected by creosote in a concentration dependent

manner. Germination of *Festuca arundinacea* was not affected by 1 g/kg creosote in soil but *Poa pratensis* was able to germinate about 80%. The most sensitive species *Elymus canadiensis* germinated only 10% at 1g/kg and 40% at 0.5 g/kg creosote and at the higher concentration (2 and 3 g/kg) less than 50% germination was achieved for all grass species.

There is not adequate information regarding the precise mechanisms affecting the reduction in germination rate, above ground biomass and shoot height, however, there are several possible mechanisms suggested by various authors. According to Henner, *et al.* (1999) some volatile fraction with less than 3 rings are found in spent oil. These compounds are known to have severe inhibitory impact on germination of several plant species. Another group of chemical compounds which are found abundantly in spent oil are polycyclic aromatic compound (PAHs) and has shown to have indirect secondary affects including disruption on plant – water – air relationships (Renault, *et al.* 2000) and effects on soil microorganisms such as mycorrhizal fungi (Nicolotti and Egli, 1998). Vwioko and Fashemi (2005) have suggested that in experiments exposing plant species to contaminated soil with various petroleum products a reduction in germination rate could be resulted from coating of oil on seed surface, thereby affecting physiological functions within the seed. Various studies have shown that the dynamics of solubilization and ionic exchange in soils have been negatively affected under the influence of spent oil (Odjegba and Sadiq, 2002).

Inhibitory effect of contaminated soil with spent oil as measured by IC_{50} varies greatly within the plant species examined. The highest IC_{50} values for germination rate, above ground height and biomass were performed by *Medicago truncatular* approximating 10 times greater than *Linum ussitasimum* which showed to be the most sensitive species to the contaminant. All species belonging to Graminae (*Agropyron deserterum*, *Bromous mermis*, *Secal seral*, *Triticum sativa*) have IC_{50} values closer to each other. This study demonstrates that *M. truncatular* is the most tolerant plant species amongst the species examined. Although the experiment appears to trigger species specific responses all species showed significant differences when compared to their controls. Similar species specific responses to pollutant stress have also been reported by Lin, *et al.* (2002). The differential sensitivity of plants to spent oil toxicity

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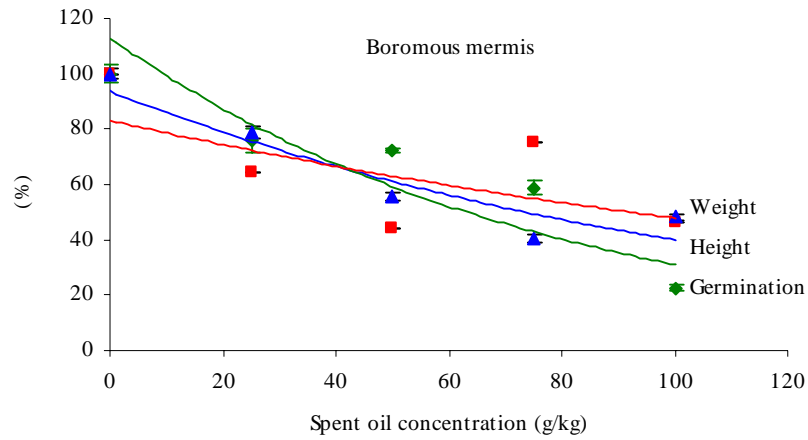


Fig. 4: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *S. seral*

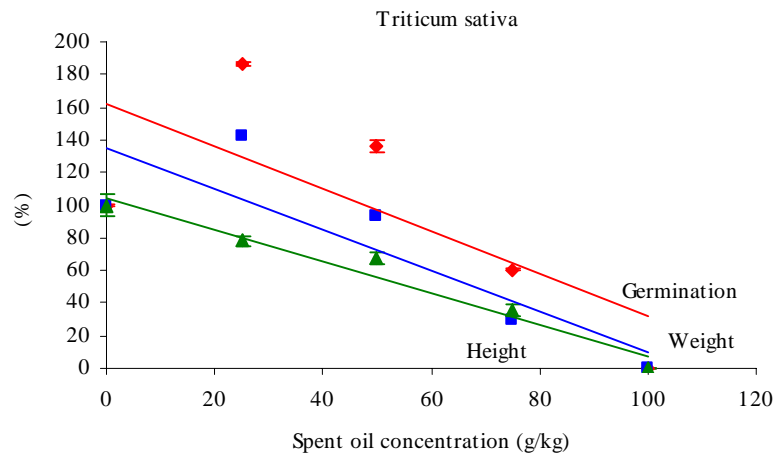


Fig. 5: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *T. sativa*

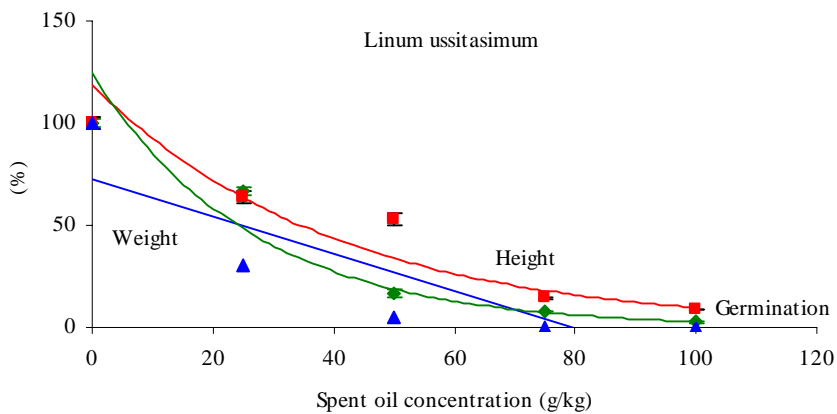


Fig. 6: Effect of spent oil contaminated soil on germination rate, above ground height and biomass of seedling in *L. ussitasimum*

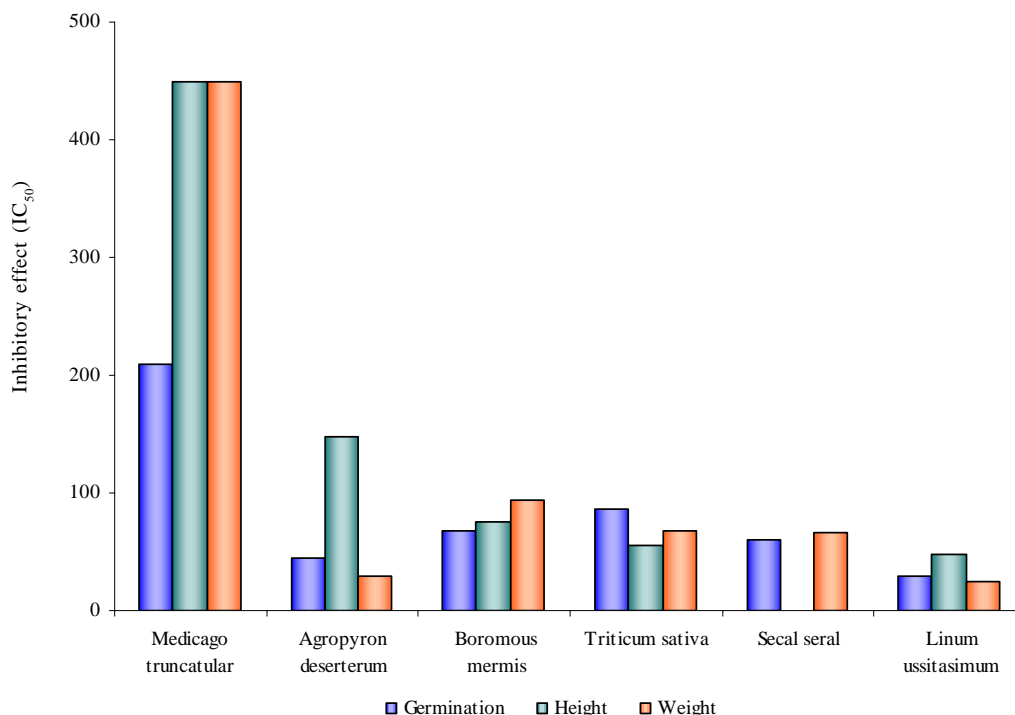


Fig. 7: Inhibitory effect (IC₅₀) of spent oil contaminated soil on germination rate, height of seedling and above ground biomass in each plant species.

can be exploited in phytoremediation practice by choosing species that are well tolerant to the contaminant. This is based on their ability to uptake, extract and transfer the pollutant out of contaminated soil. It is possible that the better response of *M. truncatular* compared with the plant species of Gramineae family is due to the ability of the former species in nitrogen fixation. Although it has been shown that in phytoremediation research grasses are usually more efficient than similar size forbs for their fibrous root system with extensive root surface area for microbial colonization and dense rhizosphere (Glick, 2003).

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