UMYU Scientifica, Vol. 2 NO. 3, September 2023, Pp 001 – 008
https://doi.org/10.56919/usci.2323.001
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Effect of Different Diesel Treatments on Growth of Single and Mixed Plant Communities and Petroleum Hydrocarbon Dissipation During Rhizoremediation

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ABSTRACT

The use of mixed plant communities has been proposed to address phytotoxicity while improving plant stress tolerance and contaminant degradation. However, there has been conflicting findings on the use of mixed plant community. This study assessed the impact of three diesel treatments on plant growth and TPH dissipation in single and mixed plant communities. This involved greenhouse experiment with *Medicago sativa, Festuca arundinacea*, and *Lolium perenne* and *Medicago sativa* + *Lolium perenne* with the diesel-spiked soils at 102,000, 151,000 and 320,000 µg kg^{-1} TPH represented as Treatments 1, 2 and 3 respectively. Plant growth was inhibited with root biomass yield greater compared to plant shoots especially for *F. arundinacea* and *L. perenne*. There was a significant decrease in the root biomass yield of *M. sativa*, *L. perenne*, *F. arundinacea* and *M. sativa* + *L. perenne*. The highest TPH dissipation of 81, 69 and 72 % was displayed by *L. perenne* in the Treatments 1, 2 and 3 respectively. However, TPH dissipation was generally comparable between the vegetated and unvegetated soil and were not significantly different ($p>0.05$) for the different plants and treatments. The impact of plant communities on the rhizoremediation of TPH-contaminated soils may dependent on factors such as plant species, TPH concentration, stress tolerance and benefits of individual plant if mixed plants are to be employed. Other strategies to improve stress tolerance, growth promotion and remediation efficiency may be considered based on the peculiarities of each contaminated site.

ARTICLE HISTORY
Received March 10, 2023
Accepted September 01, 2023
Published September 19, 2023

KEYWORDS
phytotoxicity, total petroleum hydrocarbon, phytoremediation.

INTRODUCTION

Global industrialization and high energy demands over the past two centuries have led to widespread contamination of the environment with organic and inorganic compounds (Gaskin *et al*., 2010; Borowik *et al*., 2019). As petroleum is the largest global energy source, the majority of global contamination with petroleum hydrocarbons is attributed to accidental spills, leaks from storage tanks and pipelines and illegal waste disposal (Afzal *et al*., 2011). The adverse health effects of contaminated land and increase in the number of brownfield sites emphasize the need for eco-friendly and cost-effective remediation strategies such as phytoremediation (Truu *et al*., 2015; Ali *et al*., 2021). Studies have shown that during rhizoremediation, plant roots enhance degradation of petroleum hydrocarbon by stimulating microbial metabolic activities, improving aeration and increasing water infiltration (Lin *et al*., 2008). As such efficient rhizoremediation depends on successful plant root growth and distribution in contaminated soils. Root growth however, may be affected by the presence of contaminants and soil factors such as temperature, moisture, nutrient content, porosity and oxygen levels (Hou *et al*., 2001). The total petroleum hydrocarbon (TPH) content is the most important factor that limits rhizoremediation of petroleum-contaminated soils as it affects soil properties, diversity, abundance and activity of soil microbes, plant growth and establishment, plant biomass yield, stress tolerance, plant-microbe interaction with an overall impact on degradation (Kechavarzi *et al*., 2007; Tang, *et al*., 2010a; Cui *et al*., 2020). Total petroleum hydrocarbon (TPH) content significantly affects hydrocarbon removal rate which varies amongst plant families down to species and genotype level (Lin *et al*., 2008). A few recent studies have reported the potential of mixed plant communities and biodegradative bacteria with plant growth promoting property instead of single plant communities to enhance plant tolerance and TPH removal during rhizoremediation (Kamath *et al*., 2004; Nedunuri *et al*., 2010). However, few studies on the rhizoremediation

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How to cite: Afegbua, S.L, Batty, L.C and Renshaw, J.C. (2023). Effect of Different Diesel Treatments on Growth of Single and Mixed Plant Communities and Petroleum Hydrocarbon Dissipation During Rhizoremediation. *UMYU Scientifica*, 2(3), 001 – 008. https://doi.org/10.56919/usci.2323.001

of petroleum-contaminated soils have been carried out using mixed plant communities with conflicting findings on their impact on biomass yield and contaminant dissipation (Phillips *et al.*, 2006; Phillips *et al.*, 2009; Cheema *et al.*, 2010). These conflicting findings have resulted in the need for further study on the potential of mixed plant community to enhance plant tolerance and biomass yields during phytoremediation. This study was undertaken to assess the impact of diesel treatments on shoot height, plant biomass yields (root and shoot) during rhizoremediation by single and mixed plant communities. The selected plant candidates were *Festuca arundinacea* (Tall fescue), *Lolium perenne* (Ryegrass), *Medicago sativa* (Alfalfa) and a mixed plant community of *M. sativa* and *L. perenne* (Cheema *et al.*, 2009; Gurska *et al.*, 2009; Tang *et al.*, 2010b).

MATERIALS AND METHODS

Soil preparation and experimental design

Sandy loam soil (pH 7.5, organic matter; 61.9 g/kg, conductivity; 1450 mS cm^{-1} , moisture content; 0.80%) sourced from a commercial supplier (Travis Perkins, UK) was air-dried and sieved. Diesel was obtained commercially from a gas station in Birmingham, United Kingdom. About 250 g of air-dried and sieved soil (25% of soil for planting in each pot) was spiked with diesel at 0.5%, 1% and 2% w/w represented as Treatment groups 1, 2 and 3 respectively. The spiked soils were mixed thoroughly to achieve homogeneity in a fume hood, then mixed with about 750 g of unspiked soil to make about 1 kg of soil. Following the spiking, soils were homogenized by sieving through a 2 mm mesh (Gamalero *et al.*, 2010). The soils were packed in plastic bags and kept at room temperature for 4 weeks. Four-week old seedlings of *Medicago sativa, Festuca arundinacea*, and *Lolium perenne* (single plants) in perlites, and mixed plants (*Medicago sativa* and *Lolium perenne*) were transplanted into Desch plant plastic pots with spiked and control soils (1 kg dry weight soil pot^{-1}). Plants were watered as required and grown in a controlled environment growth chamber for 60 days (16 h, 25°C day:8 h, 20°C night). After 1 week, plant seedlings were thinned to 20 seedlings per pot. Soil samples were collected before transplantation of seedlings to assess the initial TPH concentration by gas chromatography flame ionization detector (GC-FID). For the final soil TPH concentration, plants were harvested and about 10 grams of soil collected from each pot after the 60-day greenhouse experiment. All soil samples were stored at 4°C prior to analysis. Plant shoot heights were measured at intervals of two weeks. TPH was extracted from soil by microwave extraction method as described by Afegbua and Batty (2018). Sample extracts were concentrated over a gentle stream of Nitrogen gas to 1 mL of sample and followed by sample clean up by solid phase extraction without fractionation. Concentrated samples were analysed using the New Jersey Department of Environmental Protection (NJDEP EPH 10/08) method on GC–FID Perkin-Elmer Autosystem XL at the School of Chemistry, University of Sheffield, United Kingdom. The initial TPH concentrations were Treatment 1; 102000±2870, Treatment 2; 151000±15900, Treatment 3; 320000±160000 μg kg^{-1}.

RESULTS

Although there no plant death was observed for any treatments throughout the growth period but other signs of phytotoxicity such as yellowing of leaves and stunted growth were observed compared to control plants for all plants in all treatments. The diesel treatments affected plant growth compared to those of control plants over the growth period irrespective of plant type. The impact of diesel on plant height was greatest for Treatment 3 compared to control plants. Figures 1, 2 and 3 show the impact of diesel treatments on *M. sativa*, *F. arundinacea* and *L. perenne* shoot height, respectively. The plant heights for treatments 1, 2 and 3 decreased in comparison to their control plants by 20, 39 and 46% for *M. sativa*, 24, 43 and 50% for *F. arundinacea* and 23, 29 and 38% for *L. perenne* respectively after 57 days. In general, there was an inverse relationship between diesel treatment concentration and plant height hence average plant shoot height (cm) was in this order; Treatment 3 (320000 μg kg^{-1}) < Treatment 2 (151000 μg kg^{-1}) < Treatment 1 (102000 μg kg^{-1}) < control. The impact of diesel treatment on the growth of *F. arundinacea* and *L. perenne* was similar to that of *M. sativa* except that it was observed that Treatment 1 may have had a stimulatory effect on *F. arundinacea* and *L. perenne* growth at the early stage compared to those of the control and other treatments. The result of two-way ANOVA showed diesel treatments and growth period significantly affected the shoot height of *M. sativa, L. perenne* and *F. arundinacea* ($p<0.05$).

Impact of diesel treatments on plant biomass

The diesel treatments also affected plant biomass yield (shoot and root dry weights) irrespective of plant species compared to those of controls after the growth period (Table 1). Also biomass yield was generally greater for plant roots compared to plant shoots especially for *F. arundinacea* and *L. perenne.* A decrease in biomass yield was observed across the treatments for all the plants. The biomass yield decrease was greatest in treatment 3 and lowest for the treatment 1 (Table 1). The greatest decrease in biomass yield amongst the plant monoculture was observed for *M. sativa* (shoot biomass; 44-73% and root biomass; 81-90%) while for the mixed plant community (*M. sativa* + *L. perenne*), the decrease in shoot and root biomass was 13-62% and 14-86% respectively. There was a decrease in the root/shoot ratio of the plants from the different treatments in comparison to their controls (Table 1). The impact of diesel treatments on *M. sativa* root biomass was statistically significant ($p<0.05$). Tukey test showed that biomass yield for treatment 2 was significantly different from those of the control group. There was a significant antagonistic effect and inverse relationship between *F. arundinacea* shoot and root biomass yield and increase in diesel concentration. Results from Tukey post-hoc test showed that *F. arundinacea* root and shoot biomass of treatments 2 and 3 were significantly different from those of the control group ($p < 0.05$). For

L. perenne, the impact of diesel treatments on biomass yield was statistically significant for shoot biomass ($p < 0.05$) for Treatments 2 and 3 but significantly different from those of the control based on the Tukey post-hoc test. The effect of diesel treatment was not significant for root biomass ($p > 0.05$) however, there was a substantial difference in mean root biomass. Also there was a statistically significant relationship between the treatments and mixed plant biomass yields ($p < 0.05$). Tukey post-hoc test revealed shoot and root biomass from treatments 2 and 3 were significantly different from those of the control and treatment 1. The biomass yield of the mixed plant was greater than those of the monoculture of *M. sativa* but less those of the monoculture of *L. perenne.*

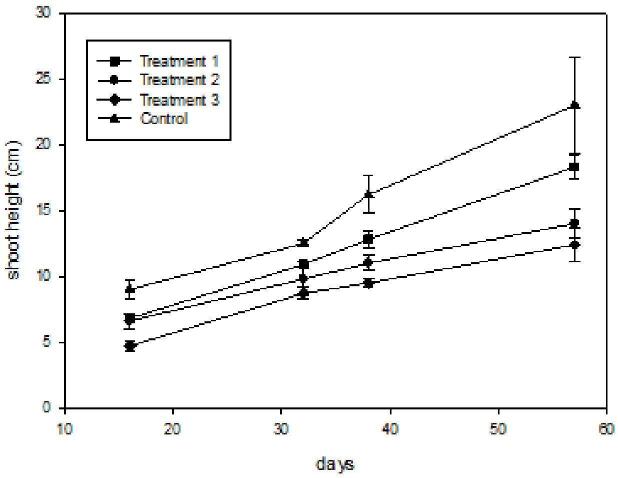

Figure 1: Effect of diesel treatments on *M. sativa* shoot height (Average values ± SE, n=3).

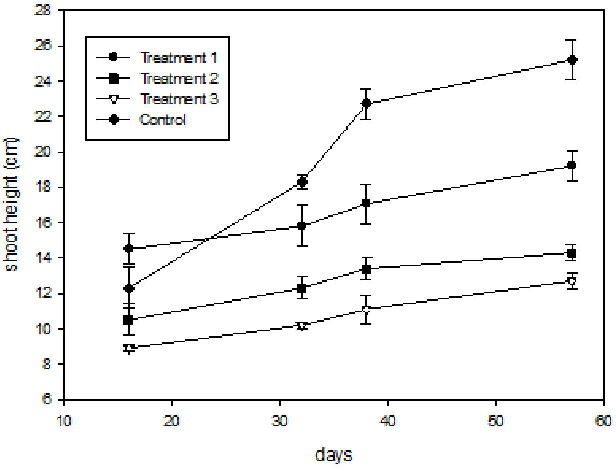

Figure 2: Effect of diesel treatments on *F. arundinacea* shoot heights. (Average values ± SE, n=3).

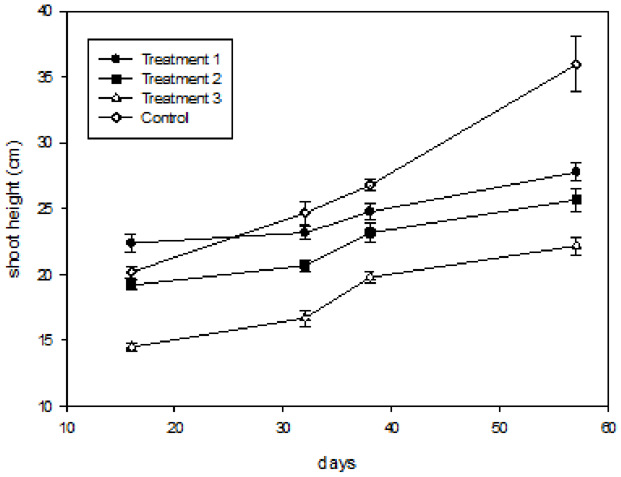

Figure 3: Effect of diesel treatments of *L. perenne* shoot height. (Average values ± SE, n=3).

Table 1: Shoot and root biomass of *M. sativa, L. perenne* and *F. arundinacea* following a 60 day growth period in different diesel treatments. (Average values ± SE, n=3). (Treatment 1; 102000±2870, Treatment 2; 151000±15900, Treatment 3; 320000±160000 μg kg^{-1}).

Plant	**Diesel Treatment**	**Shoot biomass (g)**	**Decrease in shoot biomass yield (%)**	**Root biomass (g)**	**Decrease in root biomass yield (%)**	**Root/ shoot ratio**
M. sativa	Control	1.97±0.72		6.73±4.60		3.41
	1	1.10±0.31	44	1.27±0.43	81	1.15
	2	0.67±0.09	66	0.57±0.13	92	0.85
	3	0.53±0.12	73	0.7±0.15	90	1.32
L. perenne	Control	4.37±0.38		16.77±4.96		3.84
	1	3.17±0.30	28	11.97±3.77	27	3.78
	2	2.77±0.24	37	5.87±0.58	65	2.12
	3	2.20±0.15	50	4.33±0.50	74	1.97
F. arundinacea	Control	3.97±0.58		8.20±0.42		2.07
	1	2.33±0.33	41	4.50±1.47	45	1.93
	2	1.57±0.32	61	2.30±0.44	72	1.46
	3	1.43±0.09	64	2.13±0.34	74	1.49
Mixed plants	Control	4.50±0.36		19.83± 2.16		4.41
	1	3.90±0.46	13	16.97± 2.95	14	4.35
	2	2.20±0.35	51	4.13±0.96	79	1.88
	3	1.73±0.13	62	2.80±0.66	86	1.62

Impact of plant choice and TPH concentration on dissipation

Among the selected plants, the highest TPH dissipation were displayed by *L. perenne* while the lowest were displayed by *M. sativa* and mixed plant (Table 2). Results from a two-way ANOVA revealed that TPH dissipation did not significantly differ across the treatments, plant species and unplanted controls ($p>0.05$). TPH dissipation for the mixed plants from treatments with 102000 μg kg^{-1}and 151000 μg kg^{-1} were comparable to that of the unplanted control but greater than those of *M. sativa* and *F. arundinacea*. As for treatment with 320000 μg kg^{-1}, dissipation for the mixed plant was comparable to that of *M. sativa* but less than those for *L. perenne*, *F. arundinacea* and the unplanted control (Table 2).

Table 2: Total petroleum hydrocarbon dissipation by plant. (Average values ± SE, n=3). Initial TPH concentrations (μg kg^{-1}): Treatment 1; 102000±2870, Treatment 2; 151000±15900, Treatment 3; 320000±160000 μg kg^{-1}.

Diesel Treatment	**Plant**	**Mean extractable TPH (μg kg^{-1})**	**Mean Dissipation (%)**
1	Unplanted control	23100±2870	77
	M. sativa	48400±9680	52
	L. perenne	19100±4770	81
	F. arundinacea	25100±13800	75
	Mixed plant	22000±9020	78
2	Unplanted control	57100±8840	62
	M. sativa	65500±11700	56
	L. perenne	46200±3470	69
	F. arundinacea	62900±1650	58
	Mixed plant	58400±3530	61
3	Unplanted control	77700±35000	83
	M. sativa	133000±13300	58
	L. perenne	90400±11900	72
	F. arundinacea	96300±13600	70
	Mixed plant	138000±52800	57

DISCUSSION

Although no plant death was recorded in this study, phytotoxic effects such as stunted growth and sign of chlorosis were displayed by the treatment plants compared to the control plants (Figures 1, 2 and 3). The phytotoxic effects was greater for *M. sativa* compared to *L. perenne* in the mono-and mixed- culture in this study. There was a significant decrease in the root biomass yield of *M. sativa*, *L. perenne*, *F. arundinacea* and *M. sativa* + *L. perenne* compared to the control. However, the root biomass yield was greater compared to plant shoots especially for *F. arundinacea* and *L. perenne*. Although, *L. perenne* exhibited the highest TPH dissipation of 81, 69 and 72 %in the Treatments 1, 2 and 3 respectively, TPH dissipation was generally comparable to those of the unvegetated soil and were not significantly different ($p>0.05$) for the different plants and treatments.

A previous study by Agamuthu *et al.* (2010) reported displayed yellowing, stunted growth and plant death as signs of phytotoxicity in *Jatropha curcas* grown in soil contaminated with 1 and 2.5 % w/w waste lubricating oil and organic wastes. The phytotoxic effect of diesel as reflected by the decrease in biomass of *M. sativa*, *F. arundinacea*, *L. perenne* and the mixed plant are similar to findings of Borowik *et al.* (2019) of a negative response of *F. arundinacea* and *Festuca rubra* (red fescue) biomass to diesel treatment (7 cm^3 kg^{-1} soil d.m) for 105 days. The lower growth inhibition for *F. arundinacea* and *L. perenne* compared to *M. sativa* may be due to differences in stress tolerance as supported by previous studies (Adam and Duncan 1999; Olson *et al.*, 2007). Furthermore, previous studies by Kaimi *et al.* (2007), Zhang *et al.* (2010), Barrutia *et al.* (2011) and Borowik *et al.* (2019) have also shown that *L. perenne* is highly tolerant to diesel contamination. Barrutia *et al.* (2011) reported that *L. perenne* was more tolerant than *T. repens* following a five-month greenhouse experiment with diesel-spiked soil (12,000 mg diesel kg^{-1}). A phytotoxicity study with pure PAHs, coking soil and gas work soils by Henner *et al.* (1999) on a range of native plant species showed *L. perenne* and maize to be most tolerant to hydrocarbons compared to other plant candidates including the legumes; *M. sativa* and *T. repens*. A study by Meng *et al.* (2011) reported *L. perenne* was more dominant than *T. repens* in a mixed plant culture.

The finding of a comparable dissipation in both vegetated and unvegetated treatments is supported in the literature by a comparable TPH degradation from highly contaminated petroleum sludge (TPH >35 g kg^{-1}) for planted treatments with *Cynodon dactylon* (68%) and *Festuca arundinacea* (62%) and unplanted treatment (57%) after one year (Hutchinson *et al.*, 2001). Some studies have reported that presence of plants may not necessarily enhance contaminant dissipation as a result of an inhibition of degrading microorganisms (Liste and Alexander, 2000) and catabolic repression by root extracts (Louvel *et al.*, 2011). On the contrary, some studies have reported a significantly higher TPH dissipation from vegetated soil compared to unvegetated soil (Gurska *et al.*, 2009; Gaskin and Bentham 2010; Pascale *et al.*, 2016; Khan *et al.*, 2018). The highest TPH dissipation although not significantly different displayed by *L. perenne* in all the treatments confirms previous findings of Tang *et al*, (2010b), which showed that *L. perenne* and *F. arundinacea* were better candidates for rhizoremediation of TPH-contaminated soil than *M. sativa* and *Gossypium hirsutum*. Kaimi *et al.*, (2006) also reported an enhanced biodegradation of diesel-contaminated soil (1.8% w/w) by *L. perenne*. The higher dissipation for the mixed plants in comparison to those for monocultures of *M. sativa* and *F. arundinacea* in treatments 1 and 2 were not significantly different but may be attributed to beneficial interaction between both plants and their microbial communities. The higher dissipation for the mixed plants in comparison to those for monocultures of *M. sativa* and *F. arundinacea* in treatments 1 and 2 is supported by studies by Cheema *et al.* (2009), Gurska *et al.* (2009), Meng *et al.* (2011) and Sun *et al.* (2011) which reported mixed plant communities enhanced hydrocarbon degradation. On the other hand, the greater TPH dissipation observed from Treatment 3 for the monoculture compared to that of the mixed culture is supported by the findings of Phillips *et al.* (2006) and Phillips *et al.* (2009). Also, Phillips *et al.* (2009) showed a monoculture had 54% TPH decrease compared to control and mixed plant culture with *L. perenne*, *M. sativa* and *Triticum aestivum*. Based on their findings, they concluded that the use of mixed plants may deter phytoremediation efficiency due to impact on factors including plant-plant interactions, plant-microbe interaction as well as other rhizosphere activities (Phillips *et al.*, 2006; Phillips *et al.* 2009).

The phytototoxic effects such as stunted growth, decreased biomass yield and decrease in root to shoot ratio are attributed to the uptake of small molecular weight volatile hydrocarbon and dissolved diesel causing early stress and inhibiting plant establishment, root elongation and viability over the growth period (Barrutia *et al.*, 2011; Zhu *et al.*, 2018; Ali *et al.*, 2021). Also petroleum hydrocarbons affect the pattern and quantity of plant growth regulators (PGRs) produced by plant roots. Consequently this affects plant development and senescence with plant relative growth found to be higher for plants in contaminated soils compared to those in uncontaminated soils (Ma *et al.*, 2018; Ali *et al.*, 2021). The difference in plant tolerance is attributed to the difference in the root systems and morphology between grasses and legumes (Adam and Duncan, 2002; Barrutia *et al.*, 2011; Hall *et al.*, 2011). Unlike, *M. sativa*, which has tap roots and less biomass, the extensive fibrous root systems of *F. arundinacea* and *L. perenne* are characterised by the presence of a sheath with an extensive surface area for microbial colonisation and increased penetration ability. These facilitate better soil aeration and bioavailability of contaminants for biodegradation and hence reduce phytotoxicity (Hall *et al.*, 2011). The dominance of *L. perenne* over *M. sativa* in the mixed plant treatment may also be attributed to their different root systems with an impact on the contaminant tolerance and biomass yield.

Tang *et al*, (2010b) explained that the presence of plants roots had less impact on microbial degradation in TPH-contaminated soils than soil properties. The comparable dissipation in vegetated and unvegetated soil may question successful establishment of plant-microbe interactions in the rhizosphere and impacts of other factors on degradation (Abdel-Shafy and Mansour 2016; Varjani *et al.*, 2017). Previous reports explain that plant-microbe synergy was significantly affected by the diesel concentrations (0-5000 mg kg^{-1}) (Lin *et al.*, 2008). Hence the impact of diesel hydrocarbon on microbial diversity, plant health and plant-microbial interaction cannot be excluded in this study (Jia *et al.*, 2016). Also such plant-induced stimulation and changes in microbial population and diversity are usually plant-specific (Abdel-Shafy and Mansour 2016).

Noteworthy of mention is the possible difference in the concentration and composition of hydrocarbon groups (aliphatics and aromatics) in the residual TPH despite the comparable dissipation from the different plants and treatments at the end of the greenhouse experiment (Escalante-Espinosa *et al.*, 2005). The concentration of residual hydrocarbon fraction may have an impact on the recovery of soil health (Barrutia *et al.*, 2011). The physiological status of a plant and its tolerance determines the adverse impact of diesel contamination on rhizosphere microbial populations and the recovery of soil health. Consequently, the comparable dissipation displayed by the selected plants in the different diesel treatments may not indicate equal tolerance to diesel contamination. A high dissipation by *L. perenne* as previously discussed, this is attributed to high tolerance, extensive rooting systems and high biomass of *L. perenne* and *F. arundinacea* which facilitate aeration, microbial proliferation and biodegradation (Hall *et al.*, 2011). Also rooting intensity (mg root kg^{-1} soil) and root development are known to be crucial for high TPH loss and phytoremediation potential respectively (Hou *et al.*, 2001; Tang *et al.*, 2010a). Phillips *et al.* (2006) reported that the use of mixed plant community with legumes such as *M. sativa* may inhibit contaminant degradation due to its selective stimulation and proliferation of degraders which may not immediately result in an increased degradation.

Our findings indicate that the single plant (*L. perenne*) was more efficient in TPH dissipation than the mixed plant community (*M. sativa* + *L. perenne*). However, the insignificant difference of the TPH dissipation between the vegetated and unvegetated treatment raises the question about the impact of plants in the phytoremediation of contaminated sites. As this study was conducted in a greenhouse set up, various factors such as the soil quality and contaminant levels, environmental factors, indigenous microbial community, plant and microbial diversity in the field affect interactions (plant-plant and plant-microbe) and the remediation outcome. The phytotoxic effects on the selected plant candidates indicates that the plant stress tolerance and its beneficial interactions with other plants should be important criteria for selecting plant candidates. Apart from the use of mixed plant communities for phytoremediation, other strategies such as the use of plant growth promoting rhizobacteria, biochar assisted phytoremediation and electrokinetics may be considered to improve growth promotion, stress tolerance and the remediation outcome (Cui *et al.*, 2020; Ali *et al.*, 2021).

CONCLUSION

M. sativa, *F. arundinacea* and *L. perenne* grown on the different diesel treatments exhibited phytotoxicity signs including yellowing of leaves, stunted growth and decrease in plant biomass compared to the control groups. Plant stress and phytotoxicity from petroleum significantly affected plant growth and biomass yields. For the single plants, *L. perenne* was found to be most tolerant to the diesel treatments while *M. sativa* was the least tolerant with significant inhibitory effects on plant growth and biomass yield. The highest TPH dissipation was displayed by *L. perenne* in all the treatments; however, TPH dissipation was generally comparable and not significantly different for all the vegetated and unvegetated soil. Various factors including plant choice, TPH concentration and rhizosphere activities amongst others may affect the phytoremediation outcome in greenhouse experiments and to a larger extent on the field.

RECOMMENDATION

Further studies are required to understand individual site variables and conditions to exploit the benefits of the use of plants in single or mixed plant communities during rhizoremediation. Additional information such as the soil quality index may be useful in comparing plant tolerance and capacity for the maintenance of the rhizosphere conditions and interactions.

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