Establishment of Perennial Ryegrass in Soil with Simulated Petroleum-based Spills and Remediation

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SUMMARY. **Petroleum-based spills on turfgrass often occur during lawn care maintenance. The damages caused by hydrocarbons to turfgrass can be long lasting and difficult to correct because of the stable and toxic nature of hydrocarbons. The objective of this study was to compare the effectiveness of using detergent, nitrate nutrient, humic substance, and activated charcoal to enhance bioremediation and turf recover after gasoline, diesel, and hydraulic fluid spills. The turfgrass quality and reestablishment of perennial ryegrass (*Lolium perenne*) reseeded at 0, 1, and 2 weeks after spills were evaluated. The results showed that using a liquid humic substance to remediate soil and reseed immediately after a gasoline spill was a practical method to reestablish acceptable turfgrass quality in 5 weeks. The most significant injury to perennial ryegrass caused by gasoline was bleaching of green tissues. Gasoline caused negligible residual herbicidal effects under the remediation regime in this study. However, diesel or hydraulic fluid showed phytotoxicity and residual effects in the contaminated soil for more than 2 months. Seeds applied immediately after diesel and hydraulic fluid spills lost viability as a result of the herbicidal effect of these hydrocarbons. As a result, reseeding was only successful 4 months after diesel and hydraulic fluid spills. Therefore, the time span for reestablishing perennial ryegrass turf may be too long for practical purposes in the lawn care industry.**

Turfgrass management today involves the use of many sophisticated types of mechanical equipments that are usually powered by petroleum fuels and controlled by hydraulic fluids. Petroleum-based spills occur (Johns and Beard, 1979) primarily because of equipment failure, careless refueling over turfgrass, or improperly connected hoses. Hydrocarbons are a major component of petroleum-based fuels and hydraulic fluids and are hazardous to the environment and toxic to plants and animals. Damage caused by hydrocarbons to turfgrass can be long lasting and difficult to correct because of the stable nature of most hydrocarbons (Aislabie et al., 2006). Water-soluble nutrients often become unavailable to plants in contaminated soil because of the coating of soil particles by hydrophobic hydrocarbons (Everett, 1978). Also, the elevated carbon (C) content in hydrocarbon-contaminated soil causes an increased C to nitrogen (N) ratio resulting in decreased bioavailability of N and phosphorus (P) (Aislabie et al., 2006).

Johns and Beard (1979) evaluated the remediational effectiveness of an activated charcoal and a detergent on gasoline, motor oil, grease, and hydraulic fluid spills on 'Tifgreen' bermudagrass (*Cyodon dactylon*). The results showed that the charcoal and detergent can remediate motor oil damage in 3–4 weeks but not for gasoline. Elliott and Prevatte (1995) compared damages caused by hydraulic fluid that is petroleum-based or vegetable oil-based on 'Tifgreen' bermudagrass. They found that both products caused immediate damage to turfgrass, but the petroleum-based hydraulic fluid persisted much longer in the soil preventing turfgrass reestablishment. Powell (1981) tested the effectiveness of various detergents, activated charcoal, and calcined clay on a motor oil spill on a 'Penncross' creeping bentgrass (*Agrostis stolonifera*) putting green and found that dishwashing detergent was the most effective product allowing turfgrass recovery within 20 to 30 d. Greenwalt (2003) recommended flushing and scalping before reseeding to reestablish turfgrass on soil contaminated with hydraulic fluid because many commercial products recommended for soil remediation of fuel contamination do not work satisfactorily.

Kechavarzi et al. (2007) studied the rooting prospect of perennial ryegrass in soils with heterogeneously distributed petroleum hydrocarbons and found that the efficiency of rhizosphere biodegradation and the perennial ryegrass remediation potential were affected by the preferential growth of roots in uncontaminated zones. Bioremediation is a process of using microbes to degrade hydrocarbons. N and other nutrients are needed for bioremediation of hydrocarbon-contaminated soils by microorganisms (Norris and Dowd, 1993). In general, results from previous research indicate that removal of contaminated soil and leaching contaminants with detergent water are the most effective methods for reclaiming soils subjected to petroleum-based spills. There is a lack of information on the effectiveness of balancing the N nutrient in the contaminated soil by using nitrate and organic soil amendments to enhance bioremediation. The effect of placing an organic or inorganic absorbent in the seedbed on reseeding cool-season turfgrass after a spill is

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
254.0000	acre-inch/acre	$m^3 \cdot ha^{-1}$	0.0039
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
0.1242	gal/100 ft	$L \cdot m^{-1}$	8.0520
9.3540	gal/acre	$L \cdot ha^{-1}$	0.1069
2.54	inch(es)	cm	0.3937
1.1209	lb/acre	$kg \cdot ha^{-1}$	0.8922
1	mmho/cm	$dS \cdot m^{-1}$	1
28.3495	oz	g	0.0353
2.2417	ton/acre	$Mg \cdot ha^{-1}$	0.4461
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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unclear. The findings will benefit turfgrass managers as well as lawn owners. The objective of this study was to compare the effectiveness of perennial ryegrass recovery methods after a petroleum-based spill using different soil surface amendments with a goal to remediate turf after such spills.

Materials and methods

Plastic pots measuring 7.5 inches in diameter and 8 inches deep were filled with Fargo clay soil (Fargo series, fine, smectitic, frigid Typic Epiaquerts) and seeded with 'Pleasure Supreme' perennial ryegrass at 305 lb/acre on 22 Sept. 2011. A starter fertilizer was applied at seeding at an N rate of 50 lb/acre using 24N–10.5P–3.3K (Turf Builder; Scots, Marysville, OH). Starting on 22 Oct. 2011, the grass was fertilized once every 2 weeks at an N rate of 50 lb/acre using 29N–0.9P–2.5K (The Andersons, Maumee, OH) and micronutrients were applied at a rate of 28 oz/acre from 0N–0.4P–0.8K (The Andersons) containing 0.02% boron, 2.45% iron (Fe), 0.25% manganese(Mn), and 0.05% zinc (Zn). The grass was mowed at 2-cm height once weekly.

On 3 Nov. 2011, diesel, unleaded gasoline and hydraulic fluid (AW100; Fleet Whole Supply, Appleton, WI) were applied to the potted perennial ryegrass turf at a rate of 15 $L \cdot m^{-2}$. Untreated pots containing clean soil represented the control treatment. The spill treatment rate was based on Johns and Beard (1979). The following remediation treatments were applied immediately after the spill treatments at a volume rate of 8 $L \cdot m^{-2}$: activated flowable charcoal (D. TOX; Cleary Chemical Corp., Payton, NJ), humic amendment (Rev™; Dakota Inc., Grand Forks, ND), nitrate, dishwashing detergent (Ultra Concentrated Dawn®; Procter and Gamble, Cincinnati, OH), and tap water as a control. The humic amendment is derived from mined humic materials, pH = 6.2, 92 $mg \cdot kg^{-1}$ nitrate-N, 5.3 $mg \cdot kg^{-1}$ ammonical-N, 4 $mg \cdot kg^{-1}$ P, and 10 $mg \cdot kg^{-1}$ K. The material contains particle sizes smaller than 100 μm, 21.2% humic acid, and 0.8% fulvic acid based on dry weight. The activated flowable charcoal rate was based on Johns and Beard (1979), whereas humic amendment rate was used at a similar volumetric base. To avoid leaf burning, the nitrate total rate of 15 oz/acre was split into five applications in 2-d intervals using 4N–0P–0.8K (Cytozorb-S™, The Andersons), which contains 4.00% N from nitrate-N, 0.8% K from K-nitrate, 0.53% magnesium (Mg), 1% sulfur (S) from combined sulfur, 2% Fe, 0.25% Mn, and 0.20% Zn. The remediation treatments were washed in with 8.5 fl oz water immediately after the applications.

'Pleasure Supreme' perennial ryegrass was overseeded into the pots at 305 lb/acre at 0, 1, and 2 weeks after the spill and remediation treatments were applied. Irrigation was provided throughout the experiment to prevent drought stress in the untreated control. The experimental treatments were a factorial combination of spill, remediation, and reseeding. The experiment was arranged in a randomized complete block design with three replications and was conducted concurrently in two separate greenhouses at 25/15 °C (day/night), with a 14-h photoperiod, and a minimum midday PAR of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with supplemental lighting from metal halide lamps.

The seed was considered germinated when seedlings first appeared, and the days required for seed germination after overseeding was noted. Clippings were collected at each mowing. To determine dry clipping yield, clippings were dried in an oven at 68 °C for 48 h. Turfgrass visual quality was evaluated based on a 1 to 9 scale, where 1 is dead, 6 is minimum acceptable, and 9 is the best. A digital image was taken from each pot under natural light using a digital camera (Power Shot G3; Canon, Tokyo, Japan) with settings of F2.0 and 1/60 s. The digital images were then analyzed using the software package NIH Image (version 1.45i; National Institutes of Health, Bethesda, MD), and turfgrass green density was calculated following the methods of Richardson et al., (2001).

Two months after the spill and remediation treatments, the turfgrass in the diesel and hydraulic fluid treatments failed to show signs of recovery. Therefore, those pots were reseeded at 305 lb/acre. Germination and turfgrass quality were monitored weekly for 2 months, until 3 Mar. 2012. At this point, only some remediation in diesel treatments provided acceptable turf quality, whereas hydraulic fluid treatments had poor germination and unacceptable turf quality. Therefore, the pots that were treated with hydraulic fluid were reseeded again at 305 lb/acre. Germination and turfgrass quality were again monitored weekly for another 2 months.

The data were subjected to analysis of variance (ANOVA) using mixed procedures in SAS (version 9.2; SAS Institute, Cary, NC) with replication blocks treated as a random variable. Homogenity of means and variability between the studies in two greenhouses were tested with Hovtest procedure in SAS. Treatment means were separated using Fisher's protected least significant difference at the 0.05 *P* level.

Results and discussion

There were no differences in mean and variability between the two studies. Therefore, the data were combined for the ANOVA (Table 1). Also, no interactions were observed

Table 1. Analysis of variance for turfgrass quality of perennial ryegrass. Data were from 5 weeks after petroleum-based spill (diesel, gasoline, and hydraulic fluid) applied at 15 $L \cdot m^{-2}$ and remediation (nitrate, humic substance, activated flowable charcoal, detergent, and tap water) at 8 $L \cdot m^{-2}$[z].

		Reseeding time after fuel spill treatment								
		0 wk			1 wk			2 wk		
Source of variation	df	VQ[y]	CY[x]	GD[w]	VQ	CY	GD	VQ	CY	GD
Fuel spill (F)	2	***[v]	***	***	***	***	***	***	***	***
Remediation (R)	4	NS	NS	*	**	NS	NS	**	NS	NS
F × R	8	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)[u]		32.6	41.2	27.7	24.5	52.2	26.4	28.1	57.8	24.8

[z]1 $L \cdot m^{-2}$ = 3.1414 fl oz/ft².
[y]Visual quality based on a 1 to 9 scale, with 1 = dead, 6 = minimum acceptable, and 9 = best.
[x]Clipping yield with grass mowed at 2-cm (0.8 inch) height weekly.
[w]Green density is the percentage of green pixels in the total pixels of a digital image following the method of Richardson et al., (2001).
[v]***, **, *, represent significant at 0.001, 0.01, and 0.05 *P* levels, respectively; NS = not significant.
[u]Coefficient of variation.

between the spill type and remediation treatment. The spills caused significant differences in visual quality, clipping yield, and green density in all three reseedings (Table 1). Gasoline caused the quickest decrease in visual quality compared with the other petroleum products by bleaching and killing the shoots 1 week after treatment (WAT). The development of phytotoxicity was slower with the diesel compared with gasoline. Diesel killed the existing perennial ryegrass within 2 weeks leaving the dead leaf tissue with a greasy black appearance. The hydraulic fluid treatment was the slowest to cause any phytotoxicity and it took ≈5 weeks to kill the existing turfgrass. Reseeding improved visual quality in the gasoline treatments indicating no significant soil residue at the spill rate used in this test. The results were different from Johns and Beard (1979) on bermudagrass. This indicates different species may respond to gasoline spill differently and further research is needed to test more turfgrass species. The effects of reseeding on visual quality were significant at 1 and 2 WAT with the humic product and nitrate remediation yielding highest quality at 5 WAT (Table 1). The improvement of turf quality was from the new germination because the quality of nonreseeded treatments decreased continuously as time passed.

The green density rating was used to determine the speed of loss of chlorophyll after the spill then speed of green up after reseeding. The green density rating was similar to visual quality. The green density evaluations revealed a continuous reduction up to 5 WAT in the diesel and hydraulic fluid treatments. The effect of remediation on green density was only observed for the reseeding at 0 WAT because of different amelioration on leaf discoloration by activated charcoal, humic substance, and nitrate immediately after the spill (Table 1). Again, the green density recovery was due to the new germination, not the recovery of existing turfgrass. The changes of clipping yield as affected by spill type and reseeding showed a trend similar to that of visual quality. No significant effects on clipping yield were observed between remediation methods at 5 WAT, although some differences were observed at 1 WAT due perhaps to regrowth from the reserved carbohydrates in the plants.

It took 8 d for the perennial ryegrass to germinate in the gasoline treatment regardless of the reseeding time or remediation methods. All pots received gasoline treatment showed germination on reseeding, indicating that gasoline had no residue preemergence herbicidal effect at the spill rate used in this study (Table 2). The perennial ryegrass seed took longer to germinate in the diesel treatments than the gasoline treatment, indicating residual toxicity in the soil (Table 2). Germination time in the diesel treatment also was affected by the remediation method used with the humic substance providing the most pots showing germination at all three seeding dates and the earlier germination reseeded at 1 and 2 WAT (Table 2). No germination was observed in the hydraulic fluid treatments that were reseeded at the time of the spill. However, the hydraulic fluid treatments remediated with the humic substance germinated 42 and 34.5 d after seeding, when reseeded at 1 and 2 WAT, respectively (Table 2).

At 5 WAT, the pots that were subjected to the gasoline spill and humic substance treatment showed an acceptable turfgrass visual quality.

Table 2. Germination of perennial ryegrass reseeded at 0, 1, and 2 weeks after petroleum-based spill treatment and remediation.

		0 wk		1 wk		2 wk	
Fuel ($15\ L\cdot m^{-2}$)[z]	Remediation ($8\ L\cdot m^{-2}$)	Germination units (%)[y]	Time to germination (d)	Germination units (%)	Time to germination (d)	Germination units (%)	Time to germination (d)
Diesel	Activated charcoal	<1d[x]	38.3 a	50 b	30 b	83 b	19.7 b
Diesel	Humic substance	83 b	39.4 a	100 a	19 d	100 a	15.3 d
Diesel	Nitrate	50 c	38.3 a	50 b	24 c	83 b	17.8 c
Diesel	Detergent	<1 d	n/a[w]	<1 d	n/a	32 d	17.5 c
Diesel	Tap water	<1 d	n/a	<1 d	n/a	33 d	17.8 c
Gasoline	Activated charcoal	100 a	8 b	100 a	8 e	100 a	8 e
Gasoline	Humic substance	100 a	8 b	100 a	8 e	100 a	8 e
Gasoline	Nitrate	100 a	8 b	100 a	8 e	100 a	8 e
Gasoline	Detergent	100 a	8 b	100 a	8 e	100 a	8 e
Gasoline	Tap water	100 a	8 b	100 a	8 e	100 a	8 e
Hydraulic fluid	Activated charcoal	<1 d	n/a	<1 d	n/a	<1 e	n/a
Hydraulic fluid	Humic substance	<1 d	n/a	20 c	42 a	67 c	34.5 a
Hydraulic fluid	Nitrate	<1 d	n/a	<1 d	n/a	<1 e	n/a
Hydraulic fluid	Detergent	<1 d	n/a	<1 d	n/a	<1 e	n/a
Hydraulic fluid	Tap water	<1 d	n/a	<1 d	n/a	<1 e	n/a

[z]$1\ L\cdot m^{-2} = 3.1414\ fl\ oz/ft^2$.
[y]Germination units were percentage pots germinated in total number of pots per treatment. Germination was defined as first appearance of seedlings.
[x]Means within a column followed by same letter are not significantly different at 0.05 *P* level based on Fisher's least significant difference.
[w]No germination 1 mo. after fuel spill and remediation treatment.

Ungerminated seeds in the pots from diesel and hydraulic fluid treatments were tested in a moisturized blot paper in petri dishes, which were placed in a growth chamber at daily alternating temperatures of 25 °C for 16 h and 20 °C for 8 h. Final results of germination rates at 2 weeks after the initiation showed the seeds lost viability and further reseeding was necessary (data not presented).

In the reseeding of diesel treatments 2 months after the initial spill, the average germination date was 7.5 d and the average turfgrass quality 8 weeks after reseeding was 5.2 (Table 3). Activated charcoal and humic substance provided the shortest germination time, highest turfgrass quality, and green density (Table 4). The only treatment combinations that resulted in an acceptable level of turfgrass quality, 6.0 and 6.2, respectively, for activated charcoal and humic substance in diesel spill (detailed data not shown because of lack of interaction). The results suggested that it took a total of 4 months to reach an acceptable turfgrass quality after the diesel spill and remediation and reseeded 2 months after the spill. None of the treatment in the reseeding of hydraulic fluid showed acceptable turfgrass quality.

In the reseeding of hydraulic fluid treatments 4 months after the spill, perennial ryegrass germinated in 1 week for all remediation treatments (Table 5). However, none of the treatments achieved an acceptable visual quality or green density (Table 5) indicating that further treatment or time was needed to reestablish turfgrass in the hydraulic fluid spill treatment.

Table 3. Germination, visual quality and green density of perennial ryegrass as affected by petroleum-based spill treatments. The grass was seeded 2 mo. after petroleum-based spill treatment. Data were collected 2 mo. after seeding (4 mo. after spill treatment) and were pooled across five remediation treatments (nitrate, humic substance, activated flowable charcoal, detergent, and tap water) at 8 L·m^{-2}.[z]

Spill treatment (15 L·m^{-2})[z]	Time to germination (d)	VQ (1–9 scale)[y]	GD (%)[x]
Diesel	7.5 b[w]	5.2 a	44.6 a
Hydraulic fluid	12.3 a	2.4 b	31.0 b

[z]1 L·m^{-2} = 3.1414 fl oz/ft^2.
[y]Visual quality based on a 1 to 9 scale, with 1 = dead, 6 = minimum acceptable, and 9 = best.
[x]Green density is the percentage of green pixels in the total pixels of a digital image following the method of Richardson et al., (2001).
[w]Means within a column followed by same letter are not significantly different at 0.05 *P* level based on Fisher's least significant difference.

Table 4. Germination, visual quality, and green density of perennial ryegrass as affected by remediation treatments. The grass was seeded 2 mo. after petroleum-based spill treatment. Data were collected 2 mo. after seeding (4 mo. after petroleum-based spill) and were pooled across two spill treatments (diesel and hydraulic fluid) at 15 L·m^{-2}.[z]

Remediation treatment (8 L·m^{-2})[z]	Time to germination (d)	VQ (1–9 scale)[y]	GD (%)[x]
Activated flowable charcoal	9.5 ab[w]	4.3 ab	39.2 ab
Humic substance	8.5 b	4.7 a	44.9 a
Nitrate	11.0 a	3.8 b	37.5 b
Detergent	10.5 ab	2.6 c	31.6 c
Tap water	10.5 ab	2.4 c	29.7 c

[z]1 L·m^{-2} = 3.1414 fl oz/ft^2.
[y]Visual quality based on a 1 to 9 scale, with 1 = dead, 6 = minimum acceptable, and 9 = best.
[x]Green density is the percentage of green pixels in the total pixels of a digital image measured following the method of Richardson et al., (2001).
[w]Means within a column followed by same letter are not significantly different at 0.05 *P* level based on Fisher's least significant difference.

Table 5. Effects of remediation on germination, visual quality, and green density of perennial ryegrass seeded 4 mo. after hydraulic fluid spill treatment at 15 L·m^{-2}.[z] Data were collected 2 mo. after seeding (6 mo. after spill treatment).

Remediation (8 L·m^{-2})[z]	Time to germination (d)	VQ (1–9 scale)[y]	GD (%)[x]
Activated flowable charcoal	6.5 b[w]	2.9 b	59.0 a
Humic substance	6.1 b	3.9 a	69.8 a
Nitrate	6.4 b	3.4 ab	68.9 a
Detergent	14.0 a	1.8 c	30.4 b
Tap water	14.0 a	1.6 c	29.5 b

[z]1 L·m^{-2} = 3.1414 fl oz/ft^2.
[y]Visual quality based on a 1 to 9 scale, with 1 = dead, 6 = minimum acceptable, and 9 = best.
[x]Green density is the percentage of green pixels in the total pixels of a digital image measured following the method of Richardson et al., (2001).
[w]Means within a column followed by same letter are not significantly different at 0.05 *P* level based on Fisher's least significant difference.

Conclusions

In conclusion, using a liquid humic substance as a remediation method for a gasoline spill in a perennial ryegrass turf, it was possible to reestablish acceptable turfgrass quality in 5 weeks if seeded immediately after the spill. Bleaching of green tissues was the primary type of injury to perennial ryegrass caused by gasoline. Gasoline showed negligible residual herbicidal effects under the remediation regimes used in this study. Diesel and hydraulic fluid spills showed phytotoxicity and residual effects in the contaminated soil for more than 2 months. Seed applied immediately after these spills lost viability because of the herbicidal effect of these hydrocarbons. Therefore, reseeding was required a few months after the spills. However, the time needed for reestablishing perennial ryegrass turf maybe too long to be a practical method in the lawn care industry. Further research is necessary to develop cultural methods for remediation of diesel and hydraulic fluid contamination in perennial ryegrass turf. Although hydraulic fluid may have less opportunity to spill on home lawns, the findings from this research should be useful for general turfgrass managers as well as home owners. Based on previous research, the rates used in the spill treatment were high enough to transfer the results to actual turfgrass remediation after such spills.

Literature cited

Aislabie, J.M., M.R. Balks, J.M. Foght, and E.J. Waterhouse. 2006. Hydrogen spills on Antarctic soils: Effects and management. Environ. Sci. Technol. 38:1265–1274.

Elliott, M.L. and M. Prevatte. 1995. Comparison of damage to 'Tifgreen' bermudagrass by petroleum and vegetable oil hydraulic fluids. HortTechnology 5: 50–51.

Everett, K.R. 1978. Some effects of oil on the physical and chemical characteristics of wet tundra soils. Arctics 31:260–276.

Greenwalt, B. 2003. Preparing for hydraulic leaks. Golf Course Mgt. 71(8):73–80.

Johns, D. and J.B. Beard. 1979. Effects and treatments of petroleum spills on bermudagrass turf. Agron. J. 71:945–947.

Kechavarzi, C., K. Pettersson, P. Leeds-Harrison, L. Ritchie, and S. Ledin. 2007. Root establishment of perennial ryegrass (*L. perenne*) in diesel contaminated subsurface soil layers. Environ. Pollut. 145: 68–74.

Norris, R.D. and K.D. Dowd. 1993. In-situ bioremediation of petroleum hydrocarbon-contaminated soil and groundwater in a low-permeability aquifer, p. 457–476. In: P.E. Flathman, D.E. Jerger, and J.H. Exner (eds.). Bioremediation field experience. CRC Press, Boca Raton, FL.

Powell, A.J., Jr. 1981. Recovery of creeping bentgrass after hydraulic oil spill. Kentucky Turfgrass Res. 37–46.

Richardson, M.D., E.E. Karcher, and L.C. Purcell. 2001. Quantifying turfgrass cover using digital image analysis. Crop Sci. 41:1884–1888.