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Germination of grass species in soil affected by crude oil contamination

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ABSTRACT
Many grass species exist in the oil exploration areas of North Dakota. The objective of this study was to evaluate seed germination of 65 grass species affected by crude oil. Germination of all species was reduced by crude oil, ranging from 4.3 to 100%. Twenty-eight species were tolerant, 29 moderately tolerant, 6 moderately sensitive, and 2 sensitive. Based on the tolerance levels, the following were used to further test the dose response to crude oil: strong creeping red fescue (Festuca rubra L. ssp. rubra), perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), buffalograss [Buchloe dactyloides (Nutt.) Engelm.], little bluestem [Schizachyrium scoparium (Michx.) Nash], witchgrass (Panicum capillare L.), sand dropseed [Sporobolus cryptandrus (Torr.) Gray], Johnsongrass [Sorghum halepense (L.) Pers.], and smooth crabgrass [Digitaria ischaemum (Schreb.) Schreb. ex Muhl.]. The EC_{50} of germination and biomass was effective in ranking the 9 species. Buffalograss, sand dropseed, and orchardgrass were ranked as the most tolerant species with EC_{50} values of 0.1, > highest concentration tested, 0.05 $m^3\ m^{-3}$ ($P < 0.05$), respectively. Smooth crabgrass and little bluestem were ranked as most sensitive with EC_{50} values of 0.03 and 0.04 $m^3\ m^{-3}$ ($P < 0.05$), respectively. Buffalograss showed the lowest germination (10.4%) and biomass reduction (25%) ($P < 0.05$).

KEYWORDS
Hydrocarbon; petroleum; *Poaceae*; remediation; reclamation

Introduction

Phytoremediation is an effective way to alleviate soil contamination due to petroleum-based operations. The efficacy of phytoremediation is site-specific. The history of oil drilling in North Dakota dates back to the 1950s. More than 8,000 wells have been built in western North Dakota's rugged prairie since the most recent oil boom started in 2010. Despite its $4 billion tax contribution to the state (Kusnetz 2014), oil drilling may have a negative impact on the environment when spill accidents occur. One of the major components that causes damage to the environment is hydrocarbons from crude oil. Hydrocarbons are organic compounds consisting of hydrogen and carbon, which are detrimental to natural resources, vegetation, and wildlife (Van Epps 2006).

The success of phytoremediation and soil reclamation depends on many factors, including environmental conditions, soil type, plant species, and methods of plant establishment (Tara *et al.* 2014; Nwaichi *et al.* 2015; McIntosh *et al.* 2016). Direct seeding is the most economical way to reclaim soils contaminated by oil drilling operations. The advantages of direct seeding include rapid establishment of vegetation and the use of prescribed species. One of the major limitations in reclaiming soils contaminated by petroleum hydrocarbon using direct seeding is seed germination failure (Van Epps 2006).

The tolerance levels to crude oil during plant seed germination are different among different species and cultivars. Dicotyledonous species have shown a large variation in germination when affected by hydrocarbons (Kummerova and Kmentova 2004; Banks and Schultz 2005; Hong *et al.* 2009; Ertekin *et al.* 2011). Similarly, different grasses are differently affected by petroleum hydrocarbon (Besalatpour *et al.* 2008). The majority of species used for soil reclamation in the oil and gas exploration areas of North Dakota are grasses (Rinella *et al.* 2012). About 90 native and introduced grass species are commonly found in North Dakota. These species are used as field crops, forage crops, biofuel crops, conservation, and as natural habitat for wildlife, although some are considered weeds. These grassy weeds are not listed as noxious in North Dakota and can be mechanically or chemically controlled after remediation. Some of the grass species have proved good species for reclaiming contaminated soil (Sedivec *et al.* 2011). Prairie grasses have great potential to be used in phytoremediation of hydrocarbon-contaminated soil because they have a fibrous root system, which provides a large surface area for hydrocarbon-degrading microbes to colonize (Galal and Shehata 2016). Some of the root exudates also play an important role in hydrocarbon degradation (McIntosh *et al.* 2017). Aprill and Sims (1990) evaluated 80 prairie grasses used for phytoremediation of polycyclic aromatic hydrocarbons (PAH) in soil. Some of the grasses they included are found in North Dakota.

The objective of this study was to evaluate grass seed germination affected by crude oil. The inclusion of grass species was based on their importance in oil production areas of North Dakota. These species are of different origins and uses, such as

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native vs. introduced, forage/crops vs. weeds, annual vs. perennial. A primary goal was to provide a list of grass species that are tolerant to crude oil contaminants and can potentially be used to establish vegetation for phytoremediation and soil reclamation.

Materials and methods

Preliminary screening of seed germination

Sixty-five grass species (including 5 cereal crops) that exist in oil production areas of North Dakota (46.1831 to 48.2325°N, -103.5258 to -100.7793°E) or are of great value in soil reclamation in other areas were included for preliminary screening of seed germination affected by crude oil. Seed sources are indicated in Table 1s, and species are described in Table 2s.

The soil used in this study was a sandy loam (Oye Hubert & Sons Construction, Fargo, North Dakota) with pH of 6.79, electric conductivity (EC) of 0.235 dS m^{-1}, and a bulk density of 1170 kg m^{-3}. The soil was air-dried and screened to pass through a 1-mm sieve before use. To simulate crude oil contamination, the uncontaminated soil was spiked with crude oil (Tesoro Refining & Marketing Co. San Antonio, TX) from the Bakken oil fields in western North Dakota. This oil contains 99% total petroleum hydrocarbon, which included 1% N-hexane, 1.5% benzene, 0.1% naphthalene, and 0.1% xylene. The FTIR spectrum of the crude oil is shown in Figure 1. One part of crude oil and 8.5 parts of soil were mixed on a volumetric basis and were allowed to incubate for 1 week on a bench shaker prior to use. Disposable sterile polystyrene Petri dishes measuring 100 mm in diameter and 15 mm in depth were used for the germination study. Thirty cubic centimeters of uncontaminated or contaminated soil were added to each Petri dish and pressed gently with a spatula. One hundred seeds of each grass species and 50 seeds of each crop species [wheat, barley, maize, and sweet corn (*Zea mays* var. saccharata)] were placed in individual Petri dishes, and 3 replicates were included. The seeds were either covered or pressed to a depth equivalent to their seed diameter. The soil surface was gently pressed again to make good seed-to-soil contact prior to adding 13 mL of distilled water, covering the Petri dishes with lids, and sealing them with Parafilm (Bemis Company, Inc., Oshkosh, Wisconsin).

Those species that require a chilling treatment (ISTA 1996) were seeded 1 week prior to other species and kept at 4 °C for the chilling treatment. After the chilling treatment, all species were put in a growth room at a temperature of 23 °C and with a 14-hour photoperiod. Each Petri dish was a treatment unit. The treatments were arranged in a randomized complete block design with 3 replicates.

Two weeks after all seeds were incubated in the growth room, germination was determined by counting the seedlings in each Petri dish. Only seedlings with essential structures (root system, shoot axis, and coleoptile) were counted (ISTA 1996).

The relative germination of each species was calculated using the germination in uncontaminated soil as 100%. Based on the germination reduction compared with the untreated control, the 65 species were grouped into sensitive (>75% reduction), moderately sensitive (50 to 75% reduction), moderately tolerant (25 to 50% reduction), and tolerant (<25% reduction) to crude oil. A similar grouping system was used by Hong *et al.* (2009).

Seed germination affected by different concentration of crude oil in soil

Nine species were selected based on tolerance for further evaluation of seed germination in response to different levels of crude oil contamination of the soil. Consideration was also given to different types of grass species when including them for further evaluation; such types include forage, turfgrass, erosion control, and weeds. There was also a balance between introduced and native species. Finally, species that had a germination of less than 30% in the treated soil were not included. The species used for the test were as follows: strong creeping red fescue (*Festuca rubra* L. ssp. rubra), perennial ryegrass

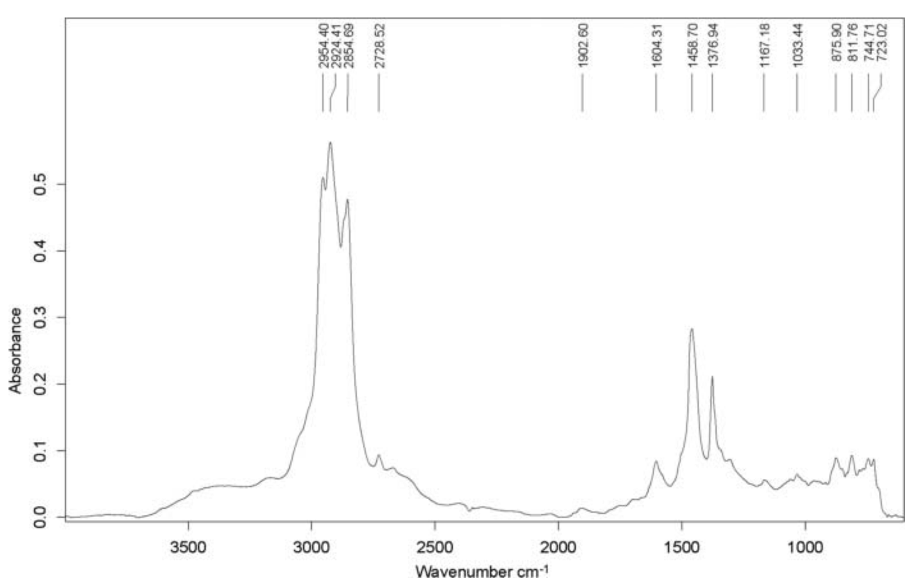

Figure 1. Fourier transform infrared absorbance spectrum of crude oil from Bakken oil fields in western North Dakota.

(*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.), buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], little bluestem [*Schizachyrium scoparium* (Michx.) Nash], switchgrass (*Panicum capillare* L.), sand dropseed [*Sporobolus cryptandrus* (Torr.) Gray], Johnsongrass [*Sorghum halepense* (L.) Pers.], and smooth crabgrass [*Digitaria ischaemum* (Schreb.) Schreb. ex Muhl.].

The same soil used for the preliminary screening was used for this experiment. The soil was screened to pass through a 1-mm sieve prior to use. Different content levels of crude oil in the soil mixture (0, 0.015, 0.030, 0.045, and 0.060 m^3 m^{-3}) were created by mixing crude oil thoroughly with the soil and incubating the mixture for 1 week on a bench shaker before use in the germination test. The germination test procedure was the same as is explained in the experiment above. Each Petri dish was a treatment unit. The treatments were arranged as randomized complete block design with 3 replicates, and the experiment was repeated once after 3 months.

Soil samples were taken from the mixtures with different concentrations of crude oil for the tests of pH and EC. The soil pH was tested in a 1:1 soil/ deionized water (V/V) suspension using a pH meter (HQ40d, Hach Company, Loveland, CO), and the EC was determined in a 1:5 soil/deionized water (V/V) extract using an EC meter (VWR Scientific, Radnor, PA).

Data analysis

At the end of the 2-week germination period, seed germination percentages were determined. The biomass of seedlings from each Petri dish was determined after harvesting by washing the soil off and oven-drying it at 80 °C for 48 hours (Mills and Jones 1996). The single-plant biomass was calculated by dividing the total biomass by the total number of seedlings in the Petri dish. Relative germination and biomass of each species were calculated using uncontaminated soil as 100% reference.

Plant germination and germination reduction data were tested with probability procedures in SAS® for the normality of distribution. Analysis of variance was conducted for the data of germination and relative germination using the general linear model in SAS® (SAS® Institute 2013). Mean separation was done using the F-protected Tukey test at a 0.05 significance level. Replicates were considered random effects, and treatments and species were considered fixed effects.

Regression response of seed germination and biomass to different concentrations of crude oil was developed using regress procedures in SAS® (SAS® institute, 2013). The effective median concentration (EC_{50}) is defined as the concentration at which a 50% reduction occurs (Pena-Castro *et al.* 2006). The EC_{50} for crude oil was calculated from the regression equation developed above. The data were subjected to ANOVA using a general linear model in SAS® with experiment and block as random variables and with species and concentrations of crude oil as fixed variables. Mean separation was done with the F-protected Tukey test at a 0.05 significance level.

Results and discussion

Preliminary seed germination test

The data for germination were normally distributed. The main effect of species was significant (Table. 1). The germination of all species was reduced by crude oil. When expressed as a percentage reduction of the germination, crude oil contamination caused significant germination reduction (Table. 1). There was a significant interaction between the species and treatment, but this interaction is not discussed because the important parameter was the percentage reduction. The significance in block effects indicated that the blocking was effective and variation within the block was reduced.

The preliminary screening of grass germination as affected by crude oil contamination is shown in Table 2. The reduction in germination ranged from 4.3 to 100%. Using the scale described above, 28 species were tolerant, 29 were moderately tolerant, 6 were moderately sensitive, and 2 were sensitive. Wheat, barley, maize, and sweet corn showed a relatively low reduction in germination (<20%). Compared to drill cuttings (Zhu *et al.* 2017), "Park" and "Bewitched" Kentucky bluegrass showed a lower germination reduction (17.6% and 40.3%) due to the crude oil treatment and were ranked tolerant and moderately tolerant, suggesting that the lack of salinity tolerance of this species played an important role in its lower tolerance to drill cuttings. Therefore, there was a better separation of Kentucky bluegrass varieties in response to crude oil treatment. Weeping alkaligrass had a 64.7% germination reduction (Table. 2) due to the crude oil treatment and a 95% germination reduction due to the drill cuttings treatment (Zhu *et al.* 2017), indicating salt and other factors added to the inhibition caused by hydrocarbon. Downy brome and foxtail barley had a more than 95% germination reduction due to both crude oil and drill cuttings treatments (Zhu *et al.* 2017), indicating that hydrocarbons were primarily responsible for the reduction. Slender wheatgrass and hybrid crested wheatgrass showed a more than 70% germination reduction due to a drill cuttings treatment (Zhu *et al.* 2017) but a less than 8% germination reduction due to the crude oil treatment (Table. 2), indicating

Table 1. ANOVA of seed germination as affected by crude oil in the soil for the preliminary screening.

		Germination				Germination reduction		
Source of variation	*df*	MS	*F*	*Pr* > *F*	*df*	MS	*F*	*Pr* > *F*
Block	2	0.126	31.9	<0.0001	2	0.017	1.51	0.2238
Species (S)	64	0.186	47.4	<0.0001	64	0.117	10.27	<0.0001
Oil (O)	1	1.886	479.8	<0.0001				
S × O	64	0.024	6.0	<0.0001				
Error	258	0.003			128	0.011		
Total	389				194			

Table 2. Plant species germination and relative germination reduction (Red.) as affected by crude oil.

Species	%			Species	%		
	Control	T[†]	Red.		Control	T	Red.
Sand dropseed[‡]var.1	57.7	55.0	4.3	Buffalograss	56.7	38.7	31.9
Basin wildrye	49.3	46.7	5.4	RS hybrid wheatgrass	44.7	30.3	32.5
Slender wheatgrass	74.7	70.3	6.2	Sweet corn	20.7	14.0	32.6
Pubescent intermediate wheatgrass	80.3	75.0	6.7	Smooth crabgrass	36.0	24.0	33.5
Hybrid crested wheatgrass	60.0	55.3	7.7	Proso millet	20.0	12.7	33.6
Oat	42.0	38.3	9.3	Colonial bentgrass	29.7	19.7	33.7
Hard red spring wheat	44.7	40.3	9.6	Large crabgrass	15.7	10.3	34.0
Japanese brome	50.0	45.0	9.9	Switchgrass	22.7	15.0	34.6
Sheep fescue	53.0	47.7	10.4	Sand dropseed var.2	26.3	17.0	35.7
Witchgrass	53.0	47.3	10.4	Strong creeping red fescue	85.7	55.0	35.8
Maize	52.0	44.7	14.0	Orchardgrass	64.7	40.7	37.1
Creeping bentgrass	47.7	40.7	14.8	Intermediate wheatgrass	76.7	48.0	37.2
Thickspike wheatgrass	67.7	57.7	14.9	Perennial ryegrass	66.7	41.7	37.7
Hard red winter wheat	41.7	35.3	15.2	Siberian wheatgrass	71.3	43.7	38.5
Durum wheat	33.0	27.3	17.4	Bluebunch wheatgrass	11.7	7.0	39.1
Kentucky bluegrass[§] var.1	64.3	53.0	17.6	Timothy	53.7	32.7	39.8
Barnyardgrass	47.7	38.7	17.6	Kentucky bluegrass[§] var.2	38.7	23.0	40.3
Little bluestem[¶] var.1	48.7	39.7	18.9	Prairie sandreed	35.7	21.7	41.1
Barley	38.7	31.3	18.9	Little bluestem[¶] var.2	24.3	14.0	42.2
Johnsongrass	36.3	29.7	19.4	Thickspike wheatgrass	76.0	43.3	42.9
Sand bluestem	35.3	28.0	20.7	Tall fescue	45.7	26.7	43.0
Creeping meadow foxtail	48.7	39.0	21.1	Tall wheatgrass	30.7	17.0	43.8
Yellow foxtail	24.7	19.3	21.5	Canada bluegrass[††] var.2	39.0	22.0	44.0
Quackgrass	79.7	63.0	22.0	Idaho bentgrass	26.7	14.7	45.0
Indiangrass	55.3	43.0	22.1	Russian wildrye	25.0	12.3	51.5
Sideoats grama	22.0	17.0	22.4	Beardless wheatgrass	27.3	13.0	52.1
Annual ryegrass	42.0	32.7	22.7	Weeping alkaligrass	70.0	24.0	64.7
Meadow brome	87.3	67.3	23.1	Fairway crested wheatgrass	12.3	4.3	66.0
Mammoth wildrye	42.0	31.0	25.7	Canada wildrye	22.3	7.0	68.3
Western wheatgrass	33.3	24.7	27.3	Desert wheatgrass	19.7	5.0	74.9
Big bluestem	51.0	36.3	29.0	Foxtail barley	79.7	3.7	95.5
Fowl bluegrass VNS	33.0	24.3	29.2	Downy brome	54.7	0.0	100.0
Canada bluegrass[††] var.1	75.3	51.7	31.0				
$HSD_{0.05}$[‡‡]	21.3	21.3	36.9	$HSD_{0.05}$	21.3	21.3	36.9

[†]Treatment.
[‡]Sand dropseed var.1was "SD native"; Sand dropseed var.2 was "Borden County."
[§]Kentucky bluegrass var.1 was "Park"; Kentucky bluegrass var.2 was "Bewitched."
[¶]Little bluestem var.1 is "Itasca"; Little bluestem var.2 is "Bad land ecotype."
[††]Canada bluegrass var. 1 was "Foothills"; Canada bluegrass var. 2 was "Cannon."
[‡‡]Tukey's Studentized Range (HSD) at the 0.05 probability level.

that these 2 species are tolerant to petroleum hydrocarbon but not to the added salinity in drill cuttings.

Creeping bentgrass and quackgrass were ranked as more tolerant than strong *F. rubra* L. ssp. rubra in this study (Table. 2), while they were more sensitive than strong *F. rubra* L. ssp. rubra in the study by Adam and Duncan (2002). Using a diesel-as-hydrocarbon treatment, Adam and Duncan (2002) found that annual *L. perenne* L. and sheep fescue were more tolerant than *D.glomerata* L. at 50 g kg^{-1}, which is in agreement with the findings of this study. Using only PAH to treat the soil, Hong *et al.* (2009) ranked downy brome as "highly susceptible" and yellow foxtail as "moderately susceptible." Similar results were observed in this study. However, Japanese brome was ranked as highly susceptible to PAH by Hong *et al.* (2009), while it was ranked tolerant to crude oil in this study. Tall fescue was ranked highly tolerant to PAH by Hong *et al.* (2009) but moderately sensitive to crude oil in this study. In addition to variety differences, different hydrocarbon effects will need evaluation.

Table 3. ANOVA of the reduction of seed germination and seedling biomass of 9 grass species affected by crude oil concentrations in the soil.

Source of variation	df	Germination			Biomass		
		MS	F	Pr > F	MS	F	Pr > F
Exp	1	0.203	3.4	0.1069	0.362	1.4	0.2735
Block within Exp	4	0.038	4.7	0.0013	0.179	14.6	<0.0001
Species (S)	8	2.086	69.9	<0.0001	0.575	8.8	0.0030
Concentration (C)	4	2.549	317.6	<0.0001	4.015	101.5	0.0030
S × C	32	0.119	15.6	<0.0001	0.073	6.3	<0.0001
Exp × S	8	0.030	3.9	0.0025	0.066	5.7	0.0002
Exp × C	4	0.008	1.1	0.3957	0.040	3.4	0.0192
Exp × S × C	32	0.008	0.9	0.5722	0.012	0.9	0.5592
Error	176	0.008			0.012		

Table 4. Germination and biomass of 9 grass species affected by different concentrations of crude oil in soil.

	Germination %			EC_{50}[†] $m^3\ m^{-3}$	
Species	Control	Treated	Reduction	Germination	Biomass
Witchgrass	53.0	48.3	8.9	0.03	0.04
Buffalograss	55.0	49.3	10.4	0.10	0.08
Little bluestem[‡]	48.7	31.0	36.3	0.04	0.03
Johnsongrass	35.7	21.7	39.2	0.05	0.03
Strong creeping red fescue	85.7	43.3	49.5	0.04	0.04
Sand dropseed[§]	57.7	28.3	51.0	N/A[¶]	0.07
Perennial ryegrass	66.7	33.0	50.5	0.04	0.04
Smooth crabgrass	35.7	17.0	52.4	0.03	0.03
Orchardgrass	68.0	24.0	64.7	0.05	0.08
$HSD_{0.05}$[††]			9.6	0.02	0.03

[†]EC_{50} is the effective concentration at which 50% of reduction in germination occurred.
[‡]Little bluestem "Itasca."
[§]Sand dropseed "Borden county germplasm."
[¶]N/A, less than 50% reduction at the highest concentration in this study was observed.
[††]Tukey's Studentized Range (HSD) at the 0.05 probability level.

Effects of crude oil amounts in soil

Seed germination of the grass species was significantly affected by crude oil concentrations in the soils (Table. 3). This effect also significantly differed among grass species. There was an interaction effect on the germination between species and crude oil concentrations, indicating that the sensitivity of those species may differ at different concentrations of crude oil levels. Similar results were found for the seedling biomass at the end of the germination test (Table. 3).

The EC_{50} of germination and biomass ranked 9 species in a similar order (Table. 4). *B. dactyloides* (Nutt.) Engelm., *S. cryptandrus* (Torr.) Gray, and *D. glomerata* L. ranked at the top as more tolerant species, whereas *D. ischaemum* (Schreb.) Schreb. ex Muhl. and *S. scoparium* (Michx.) Nash were ranked as more sensitive. The slightly different ranking based on germination and biomass may be caused by different sensitivity of the physiological process during seed germination and seedling development to the components in the oil. Those include components of different volatility and conversion of hydrocarbon into different chemicals in the soil (Chaineau *et al.* 1996). Similar phenomena were reported by Hong *et al.* (2009) in their study of germination and growth responses to PAH.

Table 5. Germination (y) (%) of 9 grass species responses to crude oil concentration (x) ($m^3\ m^{-3}$) in the soil.

Species	Equation	r^2[†]
Sand dropseed	y = 85.5 – 66.7x	0.03
Buffalograss	y = 50.6 – 243.3x	0.34
Johnsongrass	y = 46.0 – 443.3x	0.71
Smooth crabgrass	y = 31.5 – 587.8x	0.83
Little bluestem	y = 50.5 – 662.2x	0.86
Witchgrass	y = 61.4 – 1042.2x	0.94
Orchardgrass	y = 98.5 – 1095.6x	0.75
Strong creeping red fescue	y = 84.6 – 1160x	0.80
Perennial ryegrass	y = 77.2 – 1171.1x	0.87

[†]Coefficient of determination (r^2) of the germination affected by the crude oil concentration in the soil.

Table 6. Biomass (y) (mg $plant^{-1}$) of 9 grass species responses to crude oil concentration (x) ($m^3\ m^{-3}$) in the soil.

Species	Equation	r^2[†]
Sand dropseed	y = 0.27 – 2.4x	0.49
Witchgrass	y = 0.33 – 3.3x	0.42
Orchardgrass	y = 0.67 – 4.7x	0.50
Strong creeping red fescue	y = 0.55 – 7.3x	0.82
Buffalograss	y = 1.05 – 7.6x	0.65
Perennial ryegrass	y = 0.79 – 10.2x	0.82
Smooth crabgrass	y = 0.75 – 14.0x	0.82
Johnsongrass	y = 1.60 – 21.2x	0.83
Little bluestem	y = 1.51 – 21.3x	0.79

[†]Coefficient of determination (r^2) of the biomass affected by the crude oil concentration in the soil.

The slopes of the simple linear regression equations provide useful information (Tables. 5 and 6). The negative sign of the slope indicates a reduction of germination and biomass as the crude oil concentration increases. The absolute value of the slope indicates sensibility. The ranking of species based on the slopes of the simple linear regression equations (Tables. 5 and 6) is similar to the ranking in the preliminary experiment with crude oil. Unlike the drill cuttings experiment (Zhu *et al.* 2017), soil pH and EC did not change significantly as crude oil concentration increased (Figure. 2). This led to the conclusion that the crude oil was mainly responsible for the effects. At the highest crude oil levels, *B. dactyloides* (Nutt.) Engelm. and *S. halepense* (L.) Pers. demonstrated lower reduction in germination and biomass compared with other species (Figures. 3 and 4). Therefore, these 2 species are potentially useful for phytoremediation and reclamation of soil contaminated by crude oil. However, *S. halepense* (L.) Pers. is listed as a noxious invasive weed in many states (Gordon *et al.* 2011), but it does not survive winters in North Dakota. *S. cryptandrus* (Torr.) Gray showed the lowest reduction in germination when affected by crude oil, but the biomass at the end of germination was much lower than that of *B. dactyloides* (Nutt.) Engelm. and *S. halepense* (L.) Pers. Further evaluation of the growth of *S. cryptandrus* (Torr.) Gray in crude oil contaminated soils is necessary.

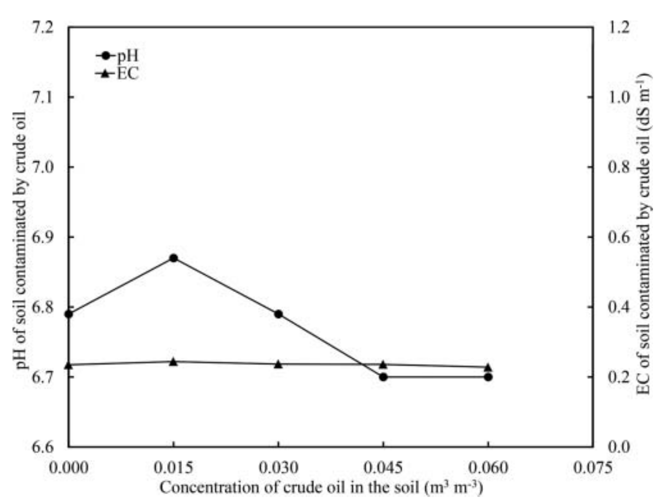

Figure 2. Changes of pH and electric conductivity (EC) of the soil contaminated by crude oil from Bakken oil fields in western North Dakota.

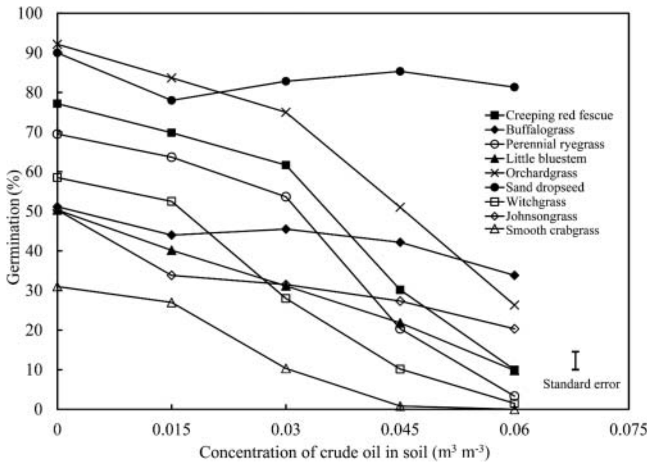

Figure 3. Germination of 9 plant species in soil contaminated with different concentrations of crude oil from Bakken oil fields in western North Dakota.

D. glomerata L. showed a great reduction in germination, but the final germination and biomass at the highest crude oil concentration were perhaps still high enough to be used in phytoremediation or reclamation of crude-oil-contaminated soils. However, because of its borderline winter hardiness (Van Santen and Sleper 1996), its application in North Dakota may be limited. *D. glomerata* L. can also be useful as an indicator of the levels of crude oil contamination in soil because an indicator requires sensitivity in germination and a sufficient amount of biomass for quantification (Banks and Schultz 2005).

Germination and seedling biomass of grass species are reduced by the presence of crude oil. There is a large variation of germination between species and genotypes within a species. Among the possible factors are hydrophobicity, toxic volatile components, salinity, and toxic metals contributed by crude oil. However, more research is needed to confirm a specific factor or combinations of factors in the role of germination inhibition and toxicity to seedlings.

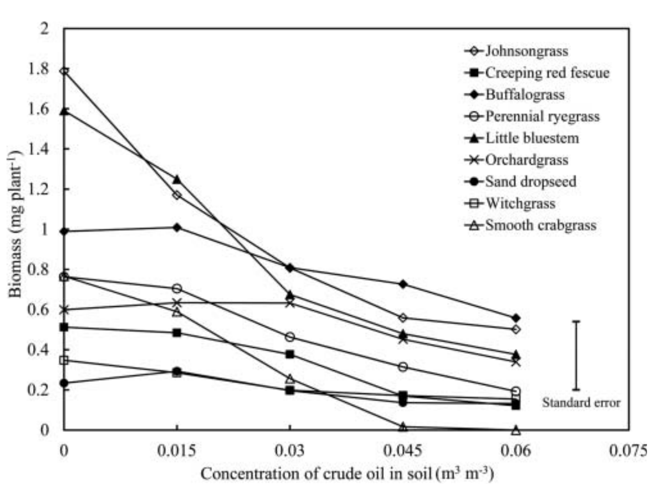

Figure 4. Biomass of 9 plant species in soil contaminated with different concentrations of crude oil from Bakken oil fields in western North Dakota.

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