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Seed germination in tailings cake and cake-reclamation substrate mixtures with oil sands process water

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Abstract

We investigated the germination of 13 species commonly used in oil sands mining reclamation of boreal forest as influenced by substrate type (potting soil, tailings cake and mixtures of cake-sand, cake-peat and cake-forest floor mineral mix (FFMM)) and water quality (0, 50 and 100% oil sands process water). Germination responses clustered into three groups with trees and graminoids exhibiting the highest germination (84-99%), followed by shrubs and forbs with intermediate germination (46-69%), and the native forb species, *Chamerion angustifolium*, *Achillea millefolium* and *Galium boreale*, with the lowest germination (7-18%). Among substrates, potting soil supported the highest germination (69%), followed by cake mixed with peat (64%) or FFMM (63%), cake-sand (60%) and cake (57%). Concentrations of ions, e.g. sodium and chloride, were higher in cake and cake-sand than in cake-peat or cake-FFMM suggesting that mixing cake with FFMM or peat can alleviate salt stress and encourage germination. Process water had little or no effect on germination especially on cake and cake amendments possibly due to the high ionic content of these substrates. There were major differences in germination response among species. Trees and graminoids may be well suited for reclaiming oil sands tailings whereas native forbs may perform poorly when used for revegetating tailings.

Keywords: land reclamation, oil sands, process water, seed germination, tailings cake

Introduction

A major by-product of bitumen mining in the oil sands region of Alberta, Canada, is oil sands tailings, which is an aqueous suspension of coarse sand, fine solids, clays and residual bitumen that are fed to large ponds for storage and water recycling (Wang *et al.*, 2010). Tailings ponds are a significant environmental risk because of the potential for leaks, failure and migration of contaminants into the surrounding environment (Frank *et al.*, 2014). Reclamation of tailings is therefore considered to be a major environmental priority for the oil sands industry (Renault *et al.*, 2000).

Through tailing treatment technologies, such as addition of gypsum and dewatering techniques such as centrifugation and/or filtration, tailings can be separated into tailings cake, hereafter referred to as cake, and process water (Wang *et al.*, 2010; Alamgir *et al.*, 2012; Farkish and Fall, 2013). The cake typically comprises 55 to 60% solids by weight and the process water is alkaline, slightly brackish and has high concentrations of organic acids (MacKinnon and Sethi, 1993). As tailings are being reclaimed, it is important to know how plants will respond to dewatered tailings as a substrate and possibly to process water as groundwater seepage.

The impacts of oil sands effluents on seed germination have been studied by incubating seeds on filter papers moistened with distilled water and different wastewaters from an operational mine site (Crowe *et al.*, 2002). The oil sands effluents adversely affected the germination of most of the species tested suggesting that the negative effects of the effluents on seed germination may be responsible for the low species diversity on the oil sands impacted wetlands in the study area (Crowe *et al.*, 2002). Renault *et al.* (1998) however reported species variability in survival rates (25-100%) of different plants hydroponically grown in various dilutions of consolidated tailings water, which indicates that the choice of species is critical for tailings reclamation. Germination of dogwood (*Cornus stolonifera* Michx) and jack pine (*Pinus banksiana* Lamb) were delayed, and survival rates after six months were 100 and 55%, respectively after planting in peat moss:sand (3:1 by volume) containing 15% fine tailings (Renault *et al.*, 2000). These studies provide insights on how tailings or process water affects seed germination and survival but an important question that needs to be addressed is how combinations of cake or cake-soil mixtures and irrigation water quality (process water and process-pure water mixture) affect germination of common species found in the boreal forest region. Understanding the combined effect of cake and process water on seed germination and plant growth is important to know how current reclamation practices could be modified to incorporate tailings and process water in revegetation efforts, as well as how different plant functional groups will respond to such modifications. This will help identify species that would be suitable for consideration in reclaiming oil sands tailings.

Seed germination is essential for ecosystem restoration following land reclamation but there is little information on the effects of excess salts and hydrocarbons on seed germination processes in forest plants (Renault *et al.*, 2000). In this study, we investigated the effects of process water and substrate type (cake, cake-soil mixtures and potting soil) on the germination of 13 species commonly found in the boreal region or used in reclamation efforts. Our research questions were: (i) Do seed germination responses differ for cake, cake-reclamation substrate mixtures and potting soil? (ii) How does the quality of irrigation water affect seed germination? (iii) Does the germination response pattern differ for common boreal species germinated in cake, cake-reclamation substrate mixtures and potting soil?

Materials and methods

Our experimental design consisted of 13 species × 20 seeds × 5 substrates × 3 watering treatments × 5 replicates for a total of 19,500 seed samples. The seeds were acquired from seed centres across Canada and the United States of America and consisted of: a) the forbs *Achillea millefolium* L., *Chamerion angustifolium* (L.) Holub, *Galium boreale* L., *Sonchus arvensis* L. and *Vicia american*a Muhl. ex Willd.; b) graminoids *Agropyron trachycaulum* (Link) Malte ex H.F. Lewis, *Agrostis scabra* Willd. and *Hordeum vulgare* L.; c) shrubs *Alnus viridis* (Chaix) DC. ssp. *crispa* (Ait.) Turrill and *Salix bebbiana* Sarg.; and d) trees *Populus tremuloides* Michx., *Pinus banksiana* Lamb. and *Picea glauca* (Moench) Voss. Twenty seeds of each species were placed in individual Petri dishes (90 mm-diameter × 10 mm height) filled with one of five substrates up to approximately half the depth of the Petri dish: (i) potting soil; (ii) tailings cake (55.7% solids); and mixtures (1:1 by volume) of (iii) cake-sand, (iv) cake-sphagnum peat moss and (v) cake-forest floor mineral mix (FFMM). The cake was produced at CanmetEnergy, Natural Resources Canada, Devon, AB, by mixing fluid fine tailings (FFT), obtained from an operational mine site in northern Alberta, with gypsum (~900 ppm). A high molecular weight anionic polymer, A3338 polymer (~1000 ppm), was added to the dosed FFT and the resulting mixture was centrifuged to obtain the cake. The potting soil, sand and sphagnum peat moss (hereafter referred to as peat) were commercially obtained while FFMM was obtained from an operational mine site in northern Alberta and consists of surface organic layers (forest floor) mixed with the underlying mineral soil.

Each substrate was subjected to three watering treatments with 0, 50 and 100% process water and each watering treatment was replicated five times. The 0% process water consisted of reverse osmosis water while the 100% process water was the centrate water produced from the centrifuge while producing the cake. The 50% process water was made up of equal proportions of reverse osmosis water and 100% process water. The watering treatments were applied three days per week for the duration of the experiment (31 days), and each Petri dish was watered until the substrate surface was fully moistened. Characterisation of the process water and substrates were done by CanmetEnergy, Natural Resources Canada, Devon, AB, Canada.

The Petri dishes were covered with a clear plastic lid to prevent moisture loss and kept in microprocessor controlled plant growth chambers (Thermo Fisher Scientific, OH, USA) with alternating light (04:00-20:00) and dark (20:00-04:00) conditions for 31 days. Illumination level inside the plant growth chambers (measured at chamber center point at 20°C) was 3,678 lux and temperature was 24 and 18°C for light and dark conditions, respectively. The Petri dishes were monitored for germination three days per week for the duration of the experiment, and seeds were recorded as germinated when either the radical was visible, or the seed coat had visibly broken open to allow the radicle through.

Data analysis

Multivariate cluster analysis, K-means clustering, was performed on the 13 species using mean germination percentage of each combination of soil and watering treatments. K-means clustering is a non-hierarchical clustering technique, which assigns objects into clusters typically by minimising the within-cluster variation or, equivalently, maximising the between-cluster variation (Magidson and Vermunt, 2002).

A plot of within-group sum of squares versus number of clusters was used to determine the appropriate number of clusters required for the K-means clustering, and silhouette analysis were used to validate the results of the cluster analysis (Rousseeuw, 1987). A large silhouette width of almost 1 indicates that observations are well clustered, a small value of around 0 indicates that observations lie between two clusters, and a negative value indicates that observations are probably placed in the wrong cluster (Rousseeuw, 1987).

Generalised linear models with binomial distribution were used to test for differences in germination percentage among substrate, water and their interaction for all species. Tests for significant differences among treatments for the binary logistic models were done with the Companion to Applied Regression package (Fox and Weisberg, 2019). Differences in ionic content were also compared among substrates with one-way ANOVA. Pairwise comparisons among treatments for the binary logistic and one-way ANOVA models were done with the estimated marginal means method in the package 'emmeans' with a Tukey adjustment of the probabilities (Lenth, 2019), and silhouette analysis was performed with cluster package (Maechler *et al.*, 2019). All statistical analyses were performed with *R* statistical software (*R* Core team, 2018).

Results

Mean germination was 63% across all species and treatments, but there was wide variation with germination ranging from 1 to 100% for specific species × substrate × water combinations. Average germination for species ranged from 7 to 99% (table 1). Germination percentage was highest on potting soil (69%), followed by cake mixed with FFMM (64%) or peat (63%), cake-sand (60%) and cake (57%), and was similar among the process water treatments, ranging from 62-64%. The germination responses clustered into three groups based on within-group sum of squares and silhouette widths (figure 1; table 1).

Cluster 1 – graminoids and trees

Cluster 1 consisted of all the graminoids (*Agropyron trachycaulum*, *Agrostis scabra* and *Hordeum vulgare*) and trees (*Picea glauca*, *Pinus banksina* and *Populus tremuloides*) and had the highest overall germination response: mean germination was 93%, ranging from 75 to 100% (figure 2). For the combined species in this cluster, using cake (90%) resulted in lower germination than potting soil (95%; $P < 0.001$) and cake mixed with FFMM (94%; $P = 0.001$) or peat (93%; $P = 0.005$). Using cake-sand also resulted in lower germination (92%) than potting soil ($P < 0.001$). Variations in germination percentage among treatments were not significantly affected by water or substrate × water interaction. However, for *Populus tremuloides*, germination in potting soil with 50 and 100% process water tended to decrease (8-13%) compared with germination in potting soil with 0% process water (mean = 100%) (figure 2F).

Table 1. Mean germination, functional group, cluster membership and silhouette widths (values ranging from −1 to 1, with values closer to 1 or −1 representing accurate or inaccurate cluster membership, respectively) for 13 species under five soil amendments and three watering treatments.

Species	Functional group	Germination (%)	Cluster	Silhouette width
Agropyron trachycaulum	Graminoid	84	1	0.579
Agrostis scabra	Graminoid	89	1	0.751
Hordeum vulgare	Graminoid	97	1	0.825
Picea glauca	Tree	99	1	0.812
Pinus banksiana	Tree	98	1	0.798
Populus tremuloides	Tree	92	1	0.778
Salix bebbiana	Shrub	46	2	0.377
Alnus viridis	Shrub	56	2	0.563
Vicia americana	Forb	69	2	0.105
Sonchus arvensis	Forb	50	2	0.533
Chamerion angustifolium	Forb	10	3	0.776
Achillea millefolium	Forb	18	3	0.65
Galium boreale	Forb	7	3	0.74

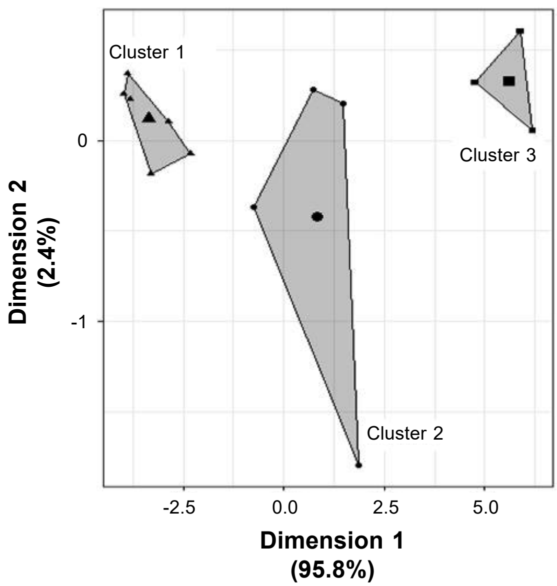

Figure 1. K-means clustering of the germination (%) of 13 species under five soil amendments and three watering treatments. The list of species in each cluster can be found in table 1.

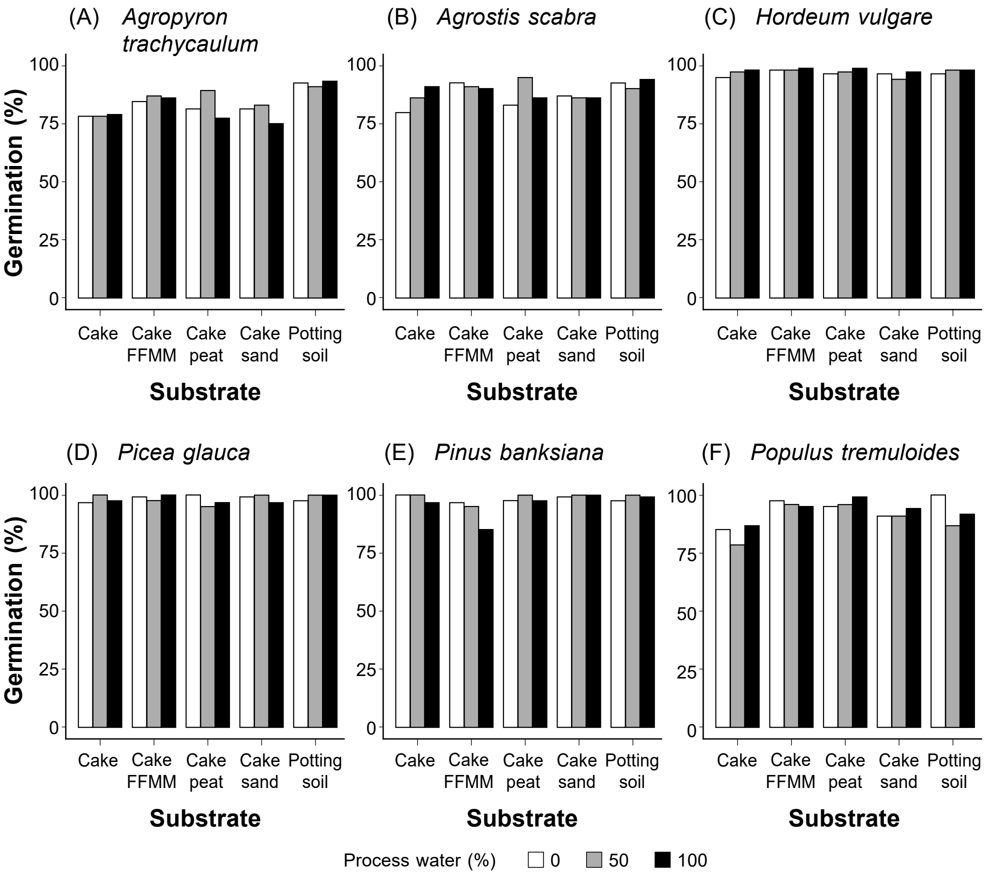

Figure 2. Group of species in cluster 1 based on K-means clustering of the germination (%) of 13 species under five soil amendments and three watering treatments.

Cluster 2 – shrubs and forbs

Cluster 2 consisted of the two shrubs, *Salix bebbiana* and *Alnus viridis*, and the forb species, *Vicia americana* and *Sonchus arvensis*. The species within this group were characterised by an intermediate germination response, with a mean germination of 55% and range of 12-100% (figure 3). For all the species combined, differences in germination across treatments were significantly affected by the substrate × water interaction ($P = 0.005$), with potting soil watered with 0% process water exhibiting 30-35% greater germination than all other treatment combinations. The greatest variation in germination percentage between treatments was found for *Salix bebbiana* (figure 3A). For this species, germination in potting soil with 0% percent process water (mean = 100%) was approximately double that of potting soil with 50 and 100% process water (figure 1A). Cake also had 6-16% lower germination than all the cake mixtures ($P < 0.001$ for all).

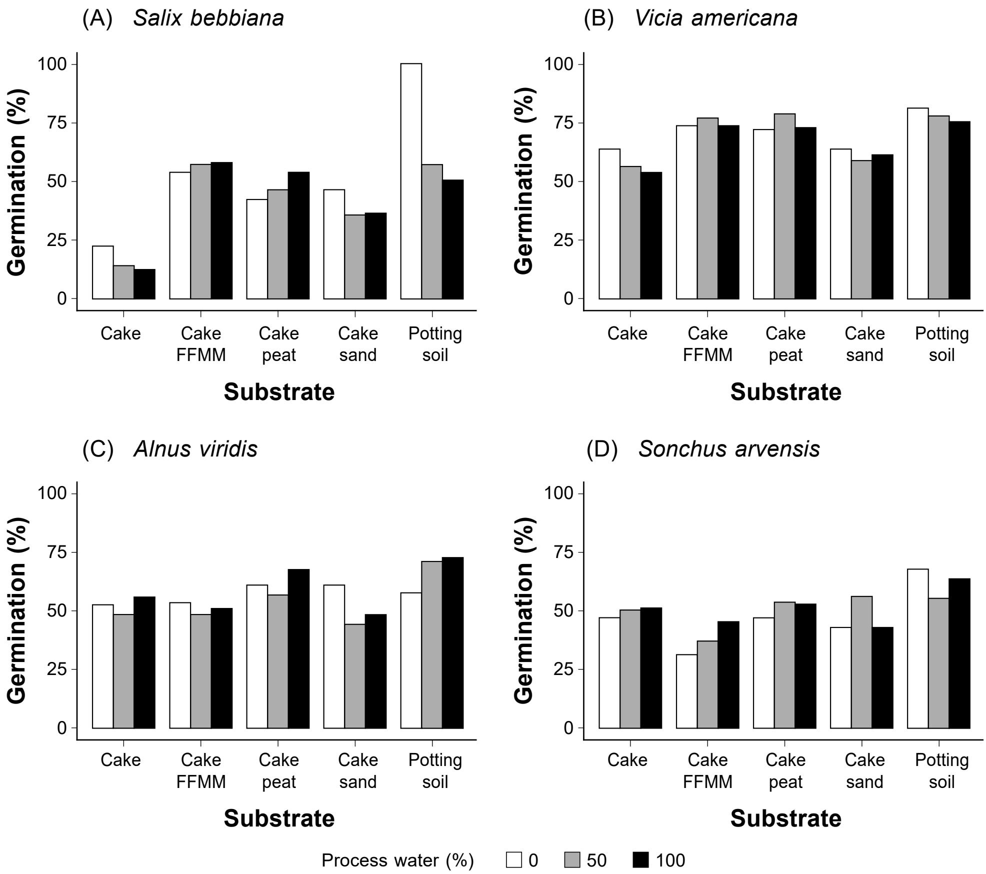

Figure 3. Group of species in cluster 2 based on K-means clustering of the germination (%) of 13 species under five soil amendments and three watering treatments.

Cluster 3 – forbs

Cluster 3 consisted of the native forb species *Chamerion angustifolium*, *Galium boreale* and *Achillea millefolium* and had the lowest germination, with a mean of 12% and range of 1-37% (figure 4). For all the species combined, cake had 4% lower germination than cake-sand ($P = 0.048$) and 8% lower germination than potting soil and cake-FFMM ($P < 0.001$ for both). Cake-peat also exhibited 6 and 7% lower germination than cake-FFMM ($P = 0.001$) and potting soil ($P < 0.001$), respectively. The 0% process water also had 3 and 5% greater germination than the 50% ($P = 0.020$) and 100% ($P = 0.001$) process water, respectively. The substrate × water interaction term was not significant for the combined species in this cluster. However, for *Galium boreale* (figure 4C), germination in potting soil with 0% process water (37%) was significantly higher than the other treatment combinations (1-8%; $P < 0.001$%).

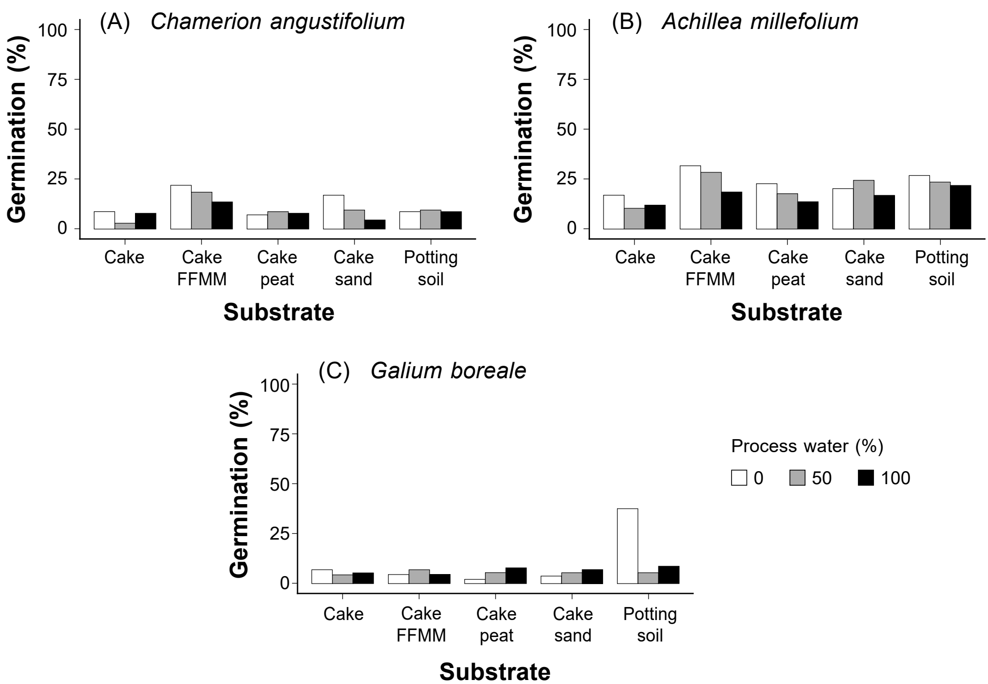

Figure 4. Group of species in cluster 3 based on K-means clustering of the germination (%) of 13 species under five soil amendments and three watering treatments.

Substrate and water chemistry

Tailings cake and cake mixtures had substantially higher ionic concentrations than potting soil (table 2). Concentration of sodium in cake was 867 mg kg^{-1}, which was 20-times higher than for potting soil ($P < 0.001$). Mixing cake with peat or FFMM reduced sodium concentration by 35 ($P = 0.001$) or 13% ($P = 0.667$), respectively, relative to pure cake, while mixing with sand increased sodium concentration by 5% ($P = 0.841$). Similarly, chloride concentration in cake (215 mg kg^{-1}) was reduced by 12% ($P = 0.019$), 6% ($P = 0.412$) and 2% ($P = 0.990$) for mixtures of cake with peat, FFMM and sand, respectively, and were all significantly higher ($P < 0.001$) than for potting soil (21.72 mg kg^{-1}). The carbonates (HCO_3^- and CO_3^{2-}) were absent from potting soil, but concentration of HCO_3^- for cake, cake-sand and cake-FFMM (1266-1678 mg kg^{-1}) were higher than for cake-peat (471 mg kg^{-1}; $P < 0.001$). Potting soil was acidic (pH of 4.9) and cake and cake mixtures were slightly alkaline to alkaline (pH of 7.2-8.2).

Concentrations of ions in 100% process water were approximately double that of 50% process water and were both substantially greater than process water (table 2). For example, sodium concentration was 495 mg kg^{-1} versus 285 mg kg^{-1} for 100 and 50% process water, respectively ($P < 0.001$), compared with 4 mg kg^{-1} for 0% process water. The 0% process was slightly acidic (pH of 6.7) and 50 and 100% process waters were alkaline (pH of 8.2).

Table 2. Basic chemical characteristics of substrates and watering treatments for seed germination.

Element	Substrate concentration (mg kg^{-1})					Water concentration (mg L^{-1})		
	Potting soil	Cake	Sand + cake	FFMM + cake	Peat + cake	0% Process water	50% Process water	100% Process water
Ca	716.75	85.14	367.82	294.21	86.15	0.33	11.38	21.38
K	462.00	42.01	56.25	78.34	44.32	0.00	7.73	13.09
Mg	196.64	34.62	96.9	115.73	53.1	0.37	7.73	12.4
Na	43.22	866.5	909.53	753.7	558.99	4.08	285.25	494.55
Cl^-	21.72	215.25	211.79	201.73	190.11	1.11	133.5	237.75
CO_3^{2-}	0.00	5.47	5.47	0.00	0.00	0.00	5.89	12.8
HCO_3^-	0.00	1265.83	1566.91	1677.71	470.58	12.71	465.69	858.00
$SO4^{2-}$	740.42	837.18	1766.01	1213.86	1017.16	2.3	94.38	151.46
S	280.38	319.01	614.44	434.71	383.22	0.88	35.53	58.89
pH	4.91	7.90	8.21	7.87	7.17	6.71	8.24	8.26

Discussion

There were major differences in germination percentage among species. Trees and graminoids exhibited high germination irrespective of the substrate or irrigation water quality, whereas shrubs and forbs had low to high germination percentages. This is in agreement with other studies, which showed that different species respond differently to contaminated soils (Renault *et al.*, 2000; Shen *et al.*, 2016). These differences may be due to differences in species tolerance to osmotic stress as well as different sensitivities of enzymes and growth regulators to toxic ions (Ungar, 1978; Al-Niemi *et al.*, 1992). For species which showed very low sensitivity to tailings or process water, the defense mechanism of seeds, which ensures self-protection in adverse conditions (Heredia and Ellstrand, 2014) as well as nourishment of seeds from the endosperm with no contaminants (Chen *et al.*, 2013) may account for their high germination.

Among substrates tested, potting soil had the overall highest germination while cake had the lowest germination. Cake and cake mixtures were also highly saline. High soil salinity decreases the osmotic potential of soil solution, which retards imbibition of water and adversely affects seed germination (Ungar, 1978; Al-Niemi *et al.*, 1992). Salt ions can also cause cellular toxicity and cell death (Taiz and Zeiger, 2006; Quinn *et al.*, 2015) and the presence of sodium and chloride ions in cells may interfere with protein activity (Waisel, 1972). Ionic toxicity was found to inhibit germination in *Triticum aestivum* (Begum *et al.*, 1992) while Ungar (1996) reported that seed germination in 2% NaCl solution was 83% lower than in 0% NaCl due to osmotically enforced seed dormancy in the saline solution. In the present study, specific ion toxicity and/or osmotic effects may account for the generally lower germination in cake and cake mixtures compared with potting soil.

To improve plant establishment and performance, contaminants such as excess salts can be diluted by mixing sediments with soil or sand (Hanslin and Eggen, 2005). This was partly supported by our study. Cake mixed with FFMM or peat generally had better germination than cake-sand mixtures. The greater reduction in the concentration of specific ions may account for the better overall germination response in cake mixed with peat or FFMM than cake-sand mixtures. Carbonate salts have been reported to reduce seed germination and seedling growth (Patil and Karadge, 2014). In addition to sodium and chlorine, cake-peat mixtures showed a lower concentration of carbonates (HCO_3^- and CO_3^{2-}) than all the cake amendment substrates. This suggests that mixing cake with peat can alleviate salt stress and promote seed germination and plant growth more so than mixing with FFMM or sand.

Process water (50 or 100%) had little or no effect on variation in germination response except germination in potting soil for *Salix bebbiana* and *Galium boreale* where process water substantially reduced germination. This is contrary to findings by Crowe *et al.* (2002) who found that exposure to oil sands effluents inhibited the germination of several plant species. However, their study employed wastewaters without the addition of soil media. In the current study, concentrations of the process water was mostly very low compared with cake and cake amendments. This suggests that in highly saline soils, the effect of process water, with lesser ionic content, on seed germination is generally less detrimental. In non-saline substrates, germination rate of species with no or moderate salt tolerance, such as *Galium boreale* and *Salix bebbiana*, may be adversely affected by process water.

Our study showed that the tree species *Picea glauca*, *Pinus banksiana* and *Populus tremuloides*, and the graminoids, *Agropyron trachycaulum*, *Agrostis scabra* and *Hordeum vulgare* may be well suited for reclaiming oil sands tailings whereas the native forbs *Chamerion angustifolium*, *Achillea millefolium* and *Galium boreale* may perform poorly in revegetating tailings. The very low germination percentage of these native forbs in potting soil with 0% process water may probably be due to inherent seed characteristics originating from the cultivars used in this study. It would therefore be beneficial to test the germination response of native forbs from different cultivars to further ascertain their tolerance to cake and cake mixtures. Additionally, because the stage of plant development could affect response to fine tailings (Renault *et al.*, 2000), studies on survival and growth of common boreal species in tailings and process water should be conducted to further ascertain their suitability in reclamation efforts.

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