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Toxicological response and bioaccumulation of strontium in *Festuca rubra* L. (red fescue) and *Trifolium pratense* L. (red clover) in contaminated soil microcosms

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Abstract

Potentially toxic elements (PTE) from industrial activities remain a global concern for their environmental hazards. In particular, strontium is found in drinking water and food, primarily from contamination from the nuclear industry, petroleum extractions, fireworks, and electronics. Its carbonate form is bioavailable and closely resembles calcium; thus, it has become a health concern, and phytoremediation has often been considered for Sr^{2+}. We toxicologically determined Sr^{2+} tolerance in *Festuca rubra* (red fescue) and *Trifolium pratense* (red clover), and their ability to bio-accumulate strontium was compared to the sorption capacity of the soils. These plants were chosen for their ubiquity and as primary colonisers in soils. Experimentally uncontaminated farm soils from Lanarkshire, Scotland, were used, along with these two common plants. Further, seed-germination and plant-growth assays demonstrated that strontium chloride exposures impact both species (0–40mM; $p < 0.05$). Moreover, translocation factors suggest that *T. pratense* more efficiently accumulated strontium, and *F. rubra* has the potential to be the excluder species, which restricts strontium to the roots. This knowledge is relevant to how strontium contamination may be phytoremediated, and suggests using clover during the early stages of ecological succession to sequester strontium from soils.

Keywords Bioaccumulation, *Festuca rubra*, Produced water, *Trifolium pratense*, Strontium, Wastewater

Introduction

Strontium (Sr) is a common alkaline earth metal used extensively in industries, including fireworks and electronics (e.g., historically in cathode-ray televisions); it is also a contaminant resulting from nuclear accidents as in Chernobyl (1986) and Fukushima (2011) nuclear disasters (Wang et al. 2017). More recently, strontium concerns resurfaced as a naturally-occurring geochemical contaminant from tertiary petroleum and natural gas extractions (Gregory et al. 2011). Produced water from hydrofracture fluids, known as 'flowback', contains strontium and various chemicals spreading throughout the operation site and reservoir (Howarth et al. 2011; Mair et al. 2012). In addition, several studies reported its presence in natural gas developments (Haluszczak et al. 2013; Ferrar et al. 2013; Lester et al. 2015; Thacker et al. 2015; Vidic 2015; Wang et al. 2017).

Concerns for its toxicity and presence continue. Yost et al. (2016) reported chronic oral toxicity values from flowback waters that included Sr. In addition, strontium exposure in drinking water and food affects human health, including bone tumours, blood-cell reduction, and leukaemia (Kabata-Pendias and Mukherjee 2007). Furthermore, its ability to accumulate growing bones

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raises concerns about children's bone development and may induce skeletal abnormalities in humans with high doses (Marie et al. 2001).

Acidic soil conditions increase the mobility and concentrations of strontium (Kabata-Pendias and Mukherjee 2007; IAEA 1994; Savinkov et al. 2007), which can impact plants. Nevertheless, tolerances remain plant-specific, which often necessitates experimental trials. For example, it has been demonstrated that a high intake of Sr ions was often associated with decreased chlorophyll *a* and *b* contents on maize leaves (Moyen and Roblin 2010). However, minimal chlorophyll-related impacts have been found with grasses species (C_3 pathway) (Sivaram et al. 2018).

However, on the contrary, its common molecular form as strontium carbonate makes the element recognisable in biological activity as calcium, which makes it a candidate for phytoremediation (Bamberger and Oswald 2012). Numerous studies describe how the plant can uptake or absorb inorganic, organic, or radionuclide from various sources of pollution (e.g., Jadia and Fulekar 2009; Rai 2012; Mani and Kumar 2014; Pantola and Alam 2014). Sr uptakes vary depending on the plant and soil conditions.

Hence, the principal objective of this research was to investigate the efficiency of *F. rubra* and *T. pratense* in tolerating and possibly bioaccumulating strontium. While unlikely to directly phytoremediate full-strength wastewaters from petroleum industries, we aimed to examine how these primary-succession plants respond to dispersed concentrations likely from spills or runoff. The plants were selected for their omnipresence and the suggestion of *F. rubra* as a "marginally salt-tolerant representative of monocotyledonous species" (Hanslin and Eggen 2005). Among the dicotyledons, *Trifolium* spp. potentially perform better under environmental imbalances such as salinity (Ab-Shukor et al. 1988; Zhang et al. 2008) and *T. pratense* L. (red clover) grows rapidly and broadly in various habitats (including the UK), with a greater nitrogen-production yield, and is a beneficial cultivar for animal feed (Bowley et al. 1984). Furthermore, the plants' ability to sequester was compared with the soil's sorption capacity for strontium. The crucial insight is to evaluate whether the plants accumulate strontium ions from contaminated soils.

Materials and methods

Reagents and materials

Strontium solutions included 0 (no-treatment control), 5, 10, 20, and 40mM of strontium chloride hexahydrate ($SrCl_2.6H_2O$; Sigma-Aldrich). All solutions were prepared by dissolving weighed masses (267 mg/mmol) into one litre of deionised water (Barnstead™ Nanopure D3-Hollow Fibre Filter; Triple Red Limited, UK). Calcium chloride hexahydrate solutions were similarly prepared (with its molecular weight at 219 mg/mmol); calcium was used to make up differences in strontium additions to maintain equivalent electrical conductivity in soils.

Plant and soil samplings

The seeds of *F. rubra* L. and *T. pratense* L. were purchased from OMC Seeds® (Cartagena, Spain) and Sow Seeds® (South Cave, UK), respectively. Soils were collected from a South Lanarkshire farm (Pape et al. 2015) and air-dried at room temperature, sieved (< 2 mm mesh), and kept in polyethene bottles.

Experimental setup

Experiment 1: preliminary plant germination

Four seeds, in duplicate, were placed in each $SrCl_2$ solution to determine germination rates. The assays involved seeds placed on filter paper (Whatman No.1) and submersed in each solution in 150 x 15 mm plastic petri dishes. Water (10mL) was provided every 2 days for 4 weeks to account for evaporation. The emergence of radicles and plumules defined germination.

Experiment 2: soil physicochemical characteristics and batch test

The following physicochemical characteristics in soils were measured: pH, electrical conductivity (EC), total organic carbon (TOC), loss on ignition (LOI), and cation-exchange capacity (CEC). Soil texture was determined before and after soil tests (BSI 2011; 2012).

We carried out a batch adsorption experiment according to Ghaemi et al. (2011) with samples routinely taken (0, 1, 24, 168, and 504 h) to determine the equilibrium of aqueous solutions (25mL) in 10 g of soil in triplicate. Samples were maintained in a closed system and continuously shaken at room temperature. After each pre-defined time, 1 g soil was removed and placed into 25mL $CaCl_2$ solution at the same molar concentration to wash the sample; this occurred while horizontally shaken for 15 min at room temperature. Next, the samples were twice washed and centrifuged (5 min, 1000×g). After washing, the soil was dried on filter paper (90 mm) at 50–55 °C for two days. For leaching, 1 g of soil was leached with 1% nitric acid (HNO_3) and filtered (Whatman No. 1). Leachates were topped to 50ml with 1% HNO_3 and then syringe-filtered (45-µm) before ICP-OES (Thermo Scientific iCAP 6000 series ICP Spectrometer) analysis.

Experiment 3: plant cultivation

In soils differentially contaminated with $SrCl_2$ (0, 5, 10, 20, and 40 mM $SrCl_2$), two seeds were sown, with the first emergent being kept (not all seeds germinate). Each treatment was monitored in triplicate. Besides solutions with different concentrations, the treatments included the timing as to when strontium was applied: (i) pre-germination exposure (Pre-GEx) where $SrCl_2$ solutions were mixed into the soil before sowing the seeds (but irrigated with deionised water), (ii) post-germination exposure (Post-GEx) where soils were irrigated with the $SrCl_2$ solutions after plants germinated (after week 6), and (iii) combined-germination exposure (Com-GEx) was implemented with $SrCl_2$ solutions used throughout the experiment. As such, we investigated whether plant responses to strontium doses were dependent on exposure timing.

Temperatures were 21 ± 3 °C, and the photoperiod was 16:8 h (day: night) with light supplied by fluorescent tubes (Sylvania GRO-LUX F58W/GRO, Germany) at 7,580 lx. Soils were irrigated twice weekly with 50mL of strontium solutions. The experiment lasted 10 weeks.

Leaves were harvested to determine chlorophyll content with an 80% acetone solution (Wellburn, 1994). For total metal content, dried materials were microwave digested (MARSXpress 240/50 CEM, Mathews, NC, USA) and then analysed on ICP-OES.

Collected data and statistical analysis

Determination of plant germination

The emergence of radicle and plumules defined germination; daily measurements included germinated-seed count and root and shoot length. From these measurements, the following metrics have been determined for comparison: final germination percentage (FGP; formula #1; adapted from Bae et al. (2016); mean daily germination (MDG; formula #2; (Kheloufi et al. 2017); mean germination time (MGT; formula #3), and vigour index (VI; formula #4). VI is a crucial assessment to indicate germination quality under stressful conditions, according to the Association of Official Seed Analysis (1983).

$$\%\mathrm{FGP} = \left(\frac{n\,\text{germinated seeds}}{\text{total } n \text{ seeds}}\right) \times 100 \tag{1}$$

$$\%\,\mathrm{MDG} = \frac{\mathrm{FGP}}{\mathrm{D}} \tag{2}$$

$$\mathrm{MGT} = \frac{\sum \mathrm{Dn}}{\sum \mathrm{n}} \tag{3}$$

$$\mathrm{VI} = \mathrm{FGP}\,(\%)\,\mathrm{x}\,\text{total seedling length (cm)} \tag{4}$$

where D is the number of days counted from the first day to final germination, and n is the number of seeds germinated on day D.

Determination of adsorption isotherms

A soil adsorption equilibrium model was determined for Sr^{2+}, with concentration (in triplicate) and agitation-time factored. First, equations were adapted from Ahmadpour et al. (2010) and Kaçan and Kütahyalı (2012) to calculate the amount of Sr^{2+} adsorbed at equilibrium as follows (5). Then, the efficiency of Sr^{2+} adsorption was determined by following an equation according to Dada et al. (2012) and Kaçan and Kütahyalı (2012) as (6) :

$$q_{e=}\frac{V\mathrm{x}(C_0 - C_e)}{W} \tag{5}$$

$$\%\,\text{Adsorption} = \frac{(\mathrm{C_0}\ -\ \mathrm{C_e})}{\mathrm{C_0}} \times 100 \tag{6}$$

Where C_e is the concentration of Sr^{2+} after adsorption at the equilibrium phase, C_0 is the concentration of Sr^{2+} in solution, q_e ($mg.g^{-1}$) is the amount of metal on the adsorbate at the equilibrium phase, V is the volume of solution in a litre. W is the adsorbent mass (g).

The consideration of adsorption isotherm for this study used Langmuir and Freundlich models according to Ahmadpour et al. (2010) and Dada et al. (2012) as follows:

Langmuir model Eqs. (7–8):

$$R_L = \frac{1}{(1 + K_L\mathrm{x}C_o)} \tag{7}$$

$$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{K_L\mathrm{x}Q_0\mathrm{x}C_e} \tag{8}$$

Freundlich model Eqs. (9–10):

$$Q_e = K_f\,\mathrm{x}\,(C_e)^{\frac{1}{n}} \tag{9}$$

$$\log Q_e = \log K_f + \frac{1}{n}\,\mathrm{x}\,(\log C_e) \tag{10}$$

Biomass production

Following the experiment, the plants were morphologically assessed: root and shoot lengths, fresh and dry weights, leaf number, and root nodules. While shoot lengths were estimated once the first plumule emerged, the most extended shoot and root lengths were measured using a vernier calliper (Mitutoyo Absolute®). Fresh material was weighed and then oven-dried at 105 °C for 24 h for dry weight.

Chlorophyll determinations

Chlorophyll *a* and *b* concentrations were calculated according to equations adapted from Porra et al. (1989) by the following Eqs. (11–12):

$$C_a = 12.21A_{663} - 2.81A_{646} \tag{11}$$

$$C_b = 20.13A_{646} - 5.03A_{663} \tag{12}$$

Where C_a is chlorophyll *a*, and C_b is chlorophyll *b*, A-values represent absorbances at a wavelength on a spectrophotometer.

Strontium translocation

In addition, the metal's translocation factor (TF) was calculated with the metal ratio in the aboveground dry-weight tissue to metal in the underground dry-weight tissue. The ratio, according to Sasmaz and Sasmaz (2009), indicates whether a plant is an accumulator ($TF > 1$) or an excluder ($TF < 1$) species. The equation was adapted as follows (13).

$$\text{Translocation Factor (TF)} = \frac{\text{metal in the shoot (mg.kg}^{-1})}{\text{metal in the root (mg.kg}^{-1})} \tag{13}$$

Statistical determinations

All data were expressed as means with standard errors (SE) and analysis of variance (one-way ANOVA) compared treatments. Further, Fisher's LSD post-hoc test ($p < 0.05$) was used to pairwise compare population differences among concentration treatments. All statistics were done using Minitab®17, and Origin® 2017 Graphing & Analysis produced the graphs.

Results and discussion

Preliminary growth response

Figure 1 illustrates the germination results of *F. rubra* and *T. pratense* over 4 weeks. The mean values of %FGP significantly differed among $SrCl_2$ concentrations for each species. Trends were similar between species, except relative differences were observed, with *F. rubra* > *T. pratense*, at 5 mM ($t = 2.23$, $p = 0.03$) and 10mM $SrCl_2$ ($t = 3.95$, $p < 0.001$); however, at 40mM *T. pratense* had greater %FGP. Both species' germination times (MGT) were not statistically different, although MGT was greater for both plants at 40mM. Moreover, the mean VI of both species significantly declined among concentrations. Furthermore, *F. rubra* showed greater vigour at 5, 10, and 20 mM $SrCl_2$ ($t = 5.01$, $t = 5.77$, and $t = 4.4$, respectively; all

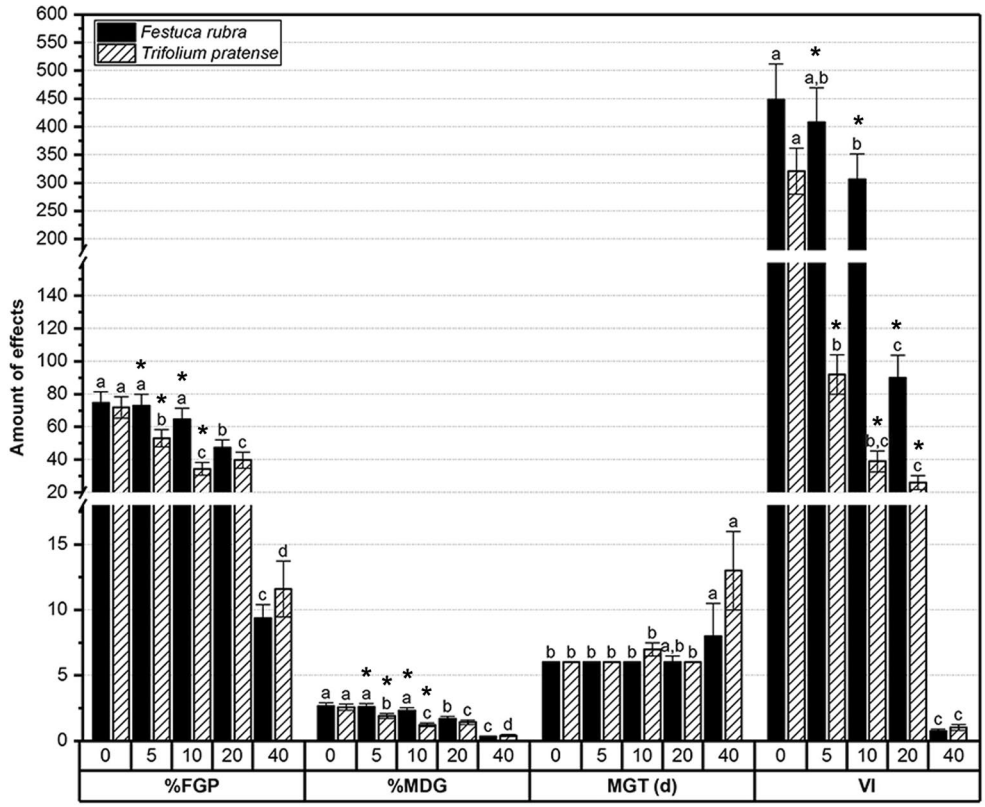

Fig. 1 Results of seed germination assays for *F. rubra* and *T. pratense* in different concentrations of $SrCl_2$ (mM) (FGP = final germination of percentage, MDG = means daily germination, MGT = mean germination time, and VI = vigour index)

Table 1 Physicochemical properties of soil used in this study ($n = 3$)

Parameters		Mean	SE.
pH	(H_2O)	6.98	±0.15
EC	($mS{\cdot}cm^{-1}$)	38.1	±1.1
CEC	($cMol^{+}{\cdot}kg^{-1}$)	11000	±2600
Exchangeable Ca^{2+}	($cMol{\cdot}kg^{-1}$)	137	±6
Exchangeable K^{+}	($cMol{\cdot}kg^{-1}$)	4.23	±0.16
Exchangeable Mg^{2+}	($cMol{\cdot}kg^{-1}$)	36.6	±1.4
Exchangeable Na^{+}	($cMol{\cdot}kg^{-1}$)	0.92	±0.05
Total carbon	(%)	5.02	±0.23
Loss on ignition	(%)	9.04	±0.42

$p < 0.001$). Even though the seeds showed vigour at these $SrCl_2$ exposures, they were confronted with environmental stress, especially at upper concentrations.

Soil characteristics

Physicochemical properties

Physicochemical measurements of the soils are presented in Table 1. The texture of this soil was slightly silty to coarse sand; pH was neutral, and EC results suggest low salinity. The soils had greater content of exchangeable $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$. A greater CEC of the soil sample suggests a soil structure with high clay and organic matter (e.g., total carbon and loss-on-ignition). The soil character is similar to those of Kabata-Pendias and Mukherjee (2007) in their Sr^{2+} mobility study. Therefore, it can be assumed that this experiment's chosen soil may be appropriate.

Batch adsorption test

Table 2 shows the adsorption efficiencies of the soils for each concentration of $SrCl_2$ after monitoring for 21 days (see supplemental information for trendlines). Adsorption efficiency values stabilised (>80%) within 7 days for the 20 and 40mM solutions; the 10mM solution remained around 60%. Table 3 presents the adsorption constants of the Freundlich and Langmuir models. Q_e values, the amount of the values adsorbed, were very close to the amount of equilibrium adsorption of the Freundlich parameters; a straight line across all concentrations provided best-fitted r^2. Moreover, the Langmuir model's RL values, and the adsorption isotherm's characteristics, were conducted by plotting at 10mM concentration; hence, the Langmuir model was not described by a single straight line. However, the values were $0 < R_L < 1$; therefore, favourable adsorption for this studied soil is possible. The best efficiency of retention time for adsorption was >24h. This result also agreed with numerous previous works by Ahmadpour et al. (2010); Li et al. (2010); Guan et al. (2011) that batch sorption experiment results were found to depend on Sr^{2+} aqueous concentration in studied soil. Further, Rediske and Selders (1953) suggested that Sr^{2+} will become adsorbed in these soils due to soil pH.

Table 2 The percentage of Sr adsorption under different contact times and aqueous concentration

Time	Conc. $SrCl_2$	Adsorption (Sr)	
(h)	(mM)	(%)	SE.
0	0	0	0
	10	58	±1.6
	20	60	±0.8
	40	91	±0.1
1	0	0	0
	10	41	±1.9
	20	71	±0.5
	40	92	±0.5
24	0	0	0
	10	63	±0.8
	20	73	±0.8
	40	95	±0.3
168	0	0	0
	10	53	±0.8
	20	83	±0.3
	40	94	±0.5
504	0	0	0
	10	73	±0.6
	20	91	±0.4
	40	96	±0.1

Plant productivity

F. rubra and *T. pratense* were sown in soils differentially treated with $SrCl_2$ at varying concentrations, but also the timing of exposure: (i) pre-germination exposure (Pre-GEx) with soils pre-contaminated with $SrCl_2$, (ii) post-germination exposure (Post-GEx) with $SrCl_2$-laden irrigation, and (iii) combined-germination exposure (Com-GEx) with $SrCl_2$ solutions used throughout the experiment. These approaches investigated whether plant responses to strontium doses depend on exposure timing. In addition, plant biometrics were monitored for 10 weeks.

Biomass production

Table 4 reports the total biomass of root and shoot, the number of leaves and nodules after 10 weeks of exposure. The results indicated a statistically significant difference in germination rate among strontium exposures. The significant differences among *F. rubra*

Table 3 Mean (±SE.) values of R_L for Langmuir and Q_e for Freundlich adsorption isotherm for Sr^{2+} in the studied soil

Time	Conc. Sr^{2+}	Initial conc.	Langmuir		Freundlich	
(h)	(mM)	($mg.L^{-1}$)	R_L	SE.	Q_e	SE.
0	0	0.000	0.000	0	0.000	0
	10	45.9	0.024	±0.00003	1.60	±0.01
	20	59.8	0.008	0	3.15	±0.01
	40	174	0.006	0	6.67	±0.02
1	0	0.000	0.000	0	0.000	0
	10	36.2	0.019	±0.00003	1.57	±0.01
	20	49.8	0.007	±0.00000	3.33	±0.01
	40	133	0.004	0	6.96	±0.09
24	0	0.000	0.000	0	0.000	0
	10	43.4	0.023	±0.00001	1.63	±0.01
	20	57.1	0.007	0	3.31	±0.02
	40	173	0.006	0	7.03	±0.08
168	0	0.000	0.000	0	0.000	0
	10	49.4	0.026	±0.00002	1.56	±0.01
	20	113	0.015	0	3.23	±0.01
	40	195	0.006	0	6.87	±0.11
504	0	0.000	0.000	0	0.000	0
	10	72.3	0.037	±0.00003	1.59	±0.01
	20	166	0.021	±0.00001	3.32	±0.03
	40	337	0.011	0	6.67	±0.04

treatments were found (*ANOVA*) in Pre-GEx treatment: root weight ($F_{(4,41)}=2.67$, $p=0.047$), shoot height ($F_{(4,41)}=3.12$, $p=0.026$), shoot weight ($F_{(4,41)}=6.12$, $p=0.001$), and the number of leaves ($F_{(4,41)}=3.94$, $p=0.009$). Adding Sr^{2+} to the soil before sowing did not require much Sr^{2+} before impacts were noticed (most commonly at 5 mM) in growing plants, although root lengths were not impacted. No statistical differences among biometrics were observed in the Post-GEx treatment (irrigated with Sr^{2+} water after germination). In the Com-GEx treatment, root weight ($F_{(4,41)}=3.22$, $p=0.023$) and shoot height ($F_{(4,41)}=6.64$, $p<0.001$) were impacted at 40mM concentration.

In contrast, *T. pratense* had statistical differences in the Com-GEx treatment (in terms of root length ($F_{(4,41)}=2.65$, $p=0.049$), shoot height ($F_{(4,41)}=4.48$, $p=0.005$), the number of leaves ($F_{(4,41)}=3.72$, $p=0.012$), and nodules ($F_{(4,41)}=3.73$, $p=0.012$) with effects being noticed starting at 5mM-concentration treatment. However, the Post-GEx illustrated a significant difference in shoot weight ($F_{(4,41)}=2.67$, $p=0.047$) and the number of leaves ($F_{(4,41)}=3.05$, $p=0.029$). Here, the biometrics were not trending with the concentration; somewhat improved performance was seen in the 10–20 mM concentration range. We have little explanation for the unexpected variability. The Pre-GEx treatment did not show any statistical differences across parameters.

Dose-response effects were evident, especially with elevated environmental concentrations (i.e., 40 mM Sr^{2+}), except for *F. rubra* leaf number. Some impacts were observed with the *T. pratense* related to the germination timing and Sr-exposure; pre-existing contamination appeared to have a more significant negative impact on plant performance. Less effect was seen with *F. rubra*.

Timing of exposure and seed planting

During the experimental planning, we questioned the timing of strontium addition versus seed planting. While it remains intuitive that earlier exposures would more likely have a more significant impact, it was unclear how much the impact would influence the biometrics.

Exposure timing had the most significant impact on *F. rubra*. Sowing seeds in pre-contaminated soils showed more detrimental effects on plant growth than irrigating the plants with contaminated water. The *T. pratense* showed a similar pattern until 40 mM concentration; the strontium showed greater toxicity to the clover with the irrigation water.

The differences in plant growth performance highlight the significance of exposure timing. Therefore, considerations must be made when designing experiments. It must

Table 4 Biometric measurements of freshly harvested *F. rubra* and *T. pratense* grown in different concentrations of $SrCl_2$ and exposure timings at week 10

Treated Soil	Conc Sr^{2+} (mM)	Root length (cm.plant^{-1})	Root weight (mg.plant^{-1})	Shoot height (cm.plant^{-1})	Shoot weight (mg.plant^{-1})	# Leaves (plant^{-1})	# Nodules
F. rubra							
Pre-GEx	0	$10.7^{a} \pm 0.8$	$0.21^{a^*} \pm 0.03$	$36.4^{a^*} \pm 1.6$	$0.54^{a^*} \pm 0.06$	$20.7^{a^*} \pm 1.6$	NA
	5	$10.7^{a} \pm 1.9$	$0.11^{b^*} \pm 0.04$	$31.3^{a,b^*} \pm 2.6$	$0.23^{b^*} \pm 0.05$	$11.0^{b^*} \pm 1.4$	NA
	10	$12.4^{a} \pm 1.0$	$0.17^{a,b^*} \pm 0.03$	$29.4^{b^*} \pm 1.6$	$0.30^{b^*} \pm 0.05$	$15.3^{a,b^*} \pm 3.2$	NA
	20	$10.6^{a} \pm 1.1$	$0.12^{b^*} \pm 0.02$	$31.3^{a,b^*} \pm 1.4$	$0.26^{b^*} \pm 0.03$	$12.8^{b^*} \pm 1.1$	NA
	40	$8.60^{a} \pm 1.5$	$0.09^{b^*} \pm 0.02$	$26.2^{b^*} \pm 4.4$	$0.21^{b^*} \pm 0.04$	$14.3^{b^*} \pm 3.0$	NA
Post-GEx	0	$10.7^{a,b} \pm 0.8$	$0.21^{b} \pm 0.03$	$36.4^{a} \pm 1.6$	$0.54^{a} \pm 0.06$	$20.7^{a,b} \pm 1.6$	NA
	5	$8.55^{b} \pm 0.9$	$0.31^{a} \pm 0.05$	$31.8^{a} \pm 0.8$	$0.48^{a} \pm 0.07$	$21.7^{a,b} \pm 2.0$	NA
	10	$8.25^{b} \pm 0.4$	$0.22^{a,b} \pm 0.03$	$31.3^{a} \pm 1.5$	$0.42^{a} \pm 0.07$	$19.3^{a,b} \pm 2.3$	NA
	20	$12.2^{a} \pm 1.3$	$0.23^{a,b} \pm 0.02$	$31.7^{a} \pm 2.6$	$0.51^{a} \pm 0.03$	$24.3^{a} \pm 3.8$	NA
	40	$9.03^{a,b} \pm 0.8$	$0.16^{b} \pm 0.02$	$37.0^{a} \pm 1.3$	$0.50^{a} \pm 0.05$	$15.7^{b} \pm 2.0$	NA
Com-GEx	0	$10.7^{a} \pm 0.8$	$0.21^{a^*} \pm 0.03$	$36.4^{a^*} \pm 1.6$	$0.54^{a} \pm 0.06$	$20.7^{a,b} \pm 1.6$	NA
	5	$9.75^{a,b} \pm 0.9$	$0.30^{a^*} \pm 0.10$	$35.3^{a^*} \pm 1.8$	$0.57^{a} \pm 0.11$	$23.7^{a,b} \pm 5.5$	NA
	10	$7.17^{b} \pm 0.8$	$0.19^{a,b^*} \pm 0.05$	$31.2^{a^*} \pm 1.5$	$0.46^{a} \pm 0.13$	$23.7^{a,b} \pm 5.7$	NA
	20	$8.67^{a,b} \pm 1.4$	$0.38^{a^*} \pm 0.13$	$32.2^{a^*} \pm 0.6$	$0.72^{a} \pm 0.27$	$28.2^{a} \pm 9.7$	NA
	40	$7.70^{a,b} \pm 2.5$	$0.03^{b^*} \pm 0.01$	$20.4^{b^*} \pm 4.8$	$0.13^{b} \pm 0.04$	$9.83^{b} \pm 2.1$	NA
T. pratense							
Pre-GEx	0	$12.0^{a} \pm 1.2$	$0.40^{a} \pm 0.06$	$24.1^{a} \pm 1.5$	$1.99^{b^*} \pm 0.17$	$9.67^{b^*} \pm 0.4$	$50.0^{a} \pm 7.6$
	5	$11.0^{a} \pm 0.8$	$0.53^{a} \pm 0.07$	$21.5^{a} \pm 0.8$	$2.90^{a^*} \pm 0.21$	$12.0^{a^*} \pm 0.7$	$49.3^{a} \pm 4.9$
	10	$11.8^{a} \pm 1.3$	$0.57^{a} \pm 0.09$	$22.2^{a} \pm 1.3$	$2.48^{a,b^*} \pm 0.38$	$11.7^{a,b^*} \pm 1.4$	$49.3^{a} \pm 4.4$
	20	$10.0^{a} \pm 0.6$	$0.45^{a} \pm 0.09$	$22.3^{a} \pm 1.7$	$2.78^{a^*} \pm 0.27$	$12.0^{a^*} \pm 1.0$	$38.2^{a} \pm 4.4$
	40	$10.3^{a} \pm 1.2$	$0.36^{a} \pm 0.10$	$22.7^{a} \pm 1.4$	$2.11^{a,b^*} \pm 0.34$	$9.33^{b^*} \pm 0.8$	$37.8^{a} \pm 7.8$
Post-GEx	0	$12.0^{a} \pm 1.2$	$0.40^{c^*} \pm 0.06$	$24.1^{a} \pm 1.5$	$1.99^{b} \pm 0.17$	$9.67^{a,b} \pm 0.4$	$50.0^{a} \pm 7.6$
	5	$8.25^{a} \pm 1.8$	$0.42^{b,c^*} \pm 0.09$	$24.2^{a} \pm 5.1$	$2.34^{a,b} \pm 0.57$	$7.67^{b} \pm 1.6$	$47.8^{a,b} \pm 11.6$
	10	$12.9^{a} \pm 1.2$	$0.79^{a^*} \pm 0.15$	$25.1^{a} \pm 3.2$	$3.11^{a} \pm 0.37$	$12.0^{a} \pm 1.7$	$41.2^{a,b} \pm 5.6$
	20	$12.9^{a} \pm 1.7$	$0.70^{a,b^*} \pm 0.07$	$23.2^{a,b} \pm 1.5$	$3.02^{a} \pm 0.16$	$10.7^{a,b} \pm 1.0$	$46.2^{a,b} \pm 5.1$
	40	$9.17^{a} \pm 3.4$	$0.42^{b,c^*} \pm 0.18$	$14.4^{b} \pm 4.7$	$1.94^{a,b} \pm 0.77$	$7.33^{b} \pm 2.9$	$23.5^{b} \pm 8.8$
Com-GEx	0	$12.0^{a^*} \pm 1.2$	$0.40^{a} \pm 0.06$	$24.1^{a^*} \pm 1.5$	$1.99^{a,b} \pm 0.17$	$9.67^{a,b^*} \pm 0.4$	$50.0^{a^*} \pm 7.6$
	5	$9.92^{a,b^*} \pm 1.1$	$0.42^{a} \pm 0.17$	$19.9^{a,b^*} \pm 1.3$	$2.14^{a,b} \pm 0.57$	$11.3^{a^*} \pm 1.9$	$39.8^{a,b^*} \pm 10.5$
	10	$9.50^{a,b^*} \pm 0.6$	$0.55^{a} \pm 0.17$	$20.3^{a,b^*} \pm 3.6$	$2.57^{a} \pm 0.33$	$12.7^{a^*} \pm 1.8$	$34.8^{a,b^*} \pm 8.2$
	20	$6.58^{b^*} \pm 2.3$	$0.30^{a} \pm 0.12$	$13.3^{b^*} \pm 4.6$	$1.49^{a,b} \pm 0.63$	$6.33^{c^*} \pm 2.3$	$13.5^{b^*} \pm 7.0$
	40	$7.00^{b^*} \pm 0.4$	$0.46^{a} \pm 0.11$	$12.7^{b^*} \pm 1.8$	$1.06^{b} \pm 0.24$	$7.0^{b,c^*} \pm 1.1$	$12.2^{b^*} \pm 4.1$

Mean values (± S.E.) (Samples n = 6, Control n = 18); significant differences were denoted by a different letter based on LSD post-hoc test similarities, *NA* = not available, *Pre-GEx* = pre-germination exposure, *Post-GEx* = post-germination exposure and *Com-GEx* = combined-germination exposure, * = statistically different at $p \leq 0.05$ (one-way ANOVA)

align with the real-world scenario, e.g. adding plants to already contaminated soils or soils that continue (or are likely) to receive contamination (e.g., runoff).

Chlorophyll contents

Figure 2 demonstrates the impacts of strontium exposure on chlorophyll content as a surrogate indicator of plant health. In addition, *F. rubra* demonstrated a brief inverse relationship between chlorophyll (*a* and *b*) and strontium concentration; however, at the highest Sr^{2+} concentrations, chlorophyll content increased—presumably as a plant stress response (Sivaram et al. 2018). Additionally, the plant had greater chlorophyll content when soils were pre-exposed with strontium rather than exposed during vegetative growth. The *T. pratense*, on the other hand, appeared to be minimally impacted by strontium treatment—by concentration and exposure timing. However, surprisingly this study's results disagree with Moyen and Roblin (2010), who suggested a direct relationship between Sr^{2+} chlorophyll concentrations.

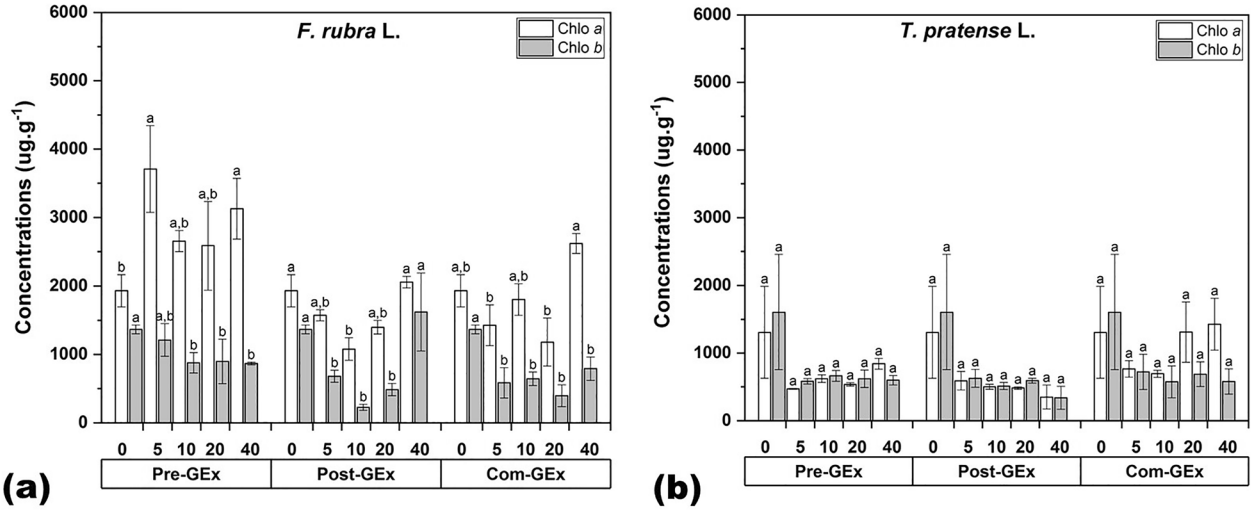

Fig. 2 The concentration of chlorophyll a and b of **a** *F. rubra*, and **b** *T. pratense* that grew under differentconcentrations of $SrCl_2$ solution at different conditional treatments (Pre-GEx = pre-germination exposure, Post-GEx = post-germination exposure, and Com-GEx = combined-germination exposure)

Instead, overall results by Srikhumsuk (2020) indicate that plants may be differentially affected by strontium, even from the same family or species. Thus, trial experiments are needed to verify anticipated outcomes.

Sr concentration in plant tissues

Table 5 illustrates Sr concentrations in the plant tissues following exposure to contaminated soil at different exposure strategies of $SrCl_2$ solution (Pre-GEx, Post-GEx, and Com-GEx). Aerial and root tissues were investigated to uptake Sr concentration into their tissues. Both plants had an average translocation factor > 1 (present mean and SE of TF here for each plant). However, the *T. pratense* demonstrated a greater propensity to translocate the strontium to its aerial tissues, as evidenced by higher concentrations and TF. Further, the *T. pratense* had higher tissue strontium concentrations than the *F. rubra*. The results of this study were consistent with Rediske and Selders (1953), which suggested that high strontium accumulation in the root part of the plant is usually found under slightly acidic soils. As a result, this experiment confirms that strontium concentration increases in aerial and root structures when pH declines.

In particular, the evidence suggests that *T. pratense* was the better accumulator species—meaning the plant translocates more strontium to aerial tissues. On the other hand, an excluder would have more in the root tissues. In addition, *F. rubra* appeared to maintain a more uniform distribution throughout the plant.

Conclusions

This study aimed to determine the plants' tolerance and their indications of pollutant stress with excess Sr ions. Unsurprisingly, Sr^{2+} concentrations impact seedling germination rates and plants' growth performances. More significant impacts were noted in the *T. pratense* regarding growth performance, e.g., vigour and other biometric indicators.

The timing of planting (e.g., germination) versus exposure had some adverse effects if soils were pre-contaminated with strontium. This result suggests that established plants (post-germination) had a greater chance of survival. More importantly, exposure timing should be considered in toxicological studies. Not often considered in many ecotoxicological studies, but the timing of seed planting versus contamination contributes significantly to the results. These timings should reflect the contamination scenarios they aim to represent for the best results.

Additionally, trial experiments are needed to verify anticipated results. While general toxicological trends were observed, translocation factors could be more accurate. These depend specifically on the types of plants but also soil types. Further, chlorophyll responses to Sr^{2+} differed; their inverse relationship suggests that elevated chlorophyll signifies physiological stress rather than health.

These are both early-succession plant species ecologically favourable for colonising disturbed landscapes. While both could be used as toxicological indicators,

Table 5 Mean values (± S.E.) of Sr concentration in the aerial (aboveground) and root (belowground) tissues and translocation factors (TF) for *F. rubra* and *T. pratense*

Plant/soil treatment	$[Sr^{2+}]_{aq}$ (mM)	Tissue strontium concentrations ($g \cdot kg^{-1}$)		Translocation factor
		Aerial	Roots	
F. rubra				
Controls	0	$0.024^{c*} \pm 0.005$	$0.028^{e*} \pm 0.003$	1.28 A
Pre-GEx	5	$0.123^{c*} \pm 0.027$	$0.066^{d*} \pm 0.009$	1.80 A
	10	$0.187^{c*} \pm 0.020$	$0.112^{c*} \pm 0.006$	1.66 A
	20	$0.449^{b*} \pm 0.070$	$0.182^{b*} \pm 0.015$	2.55 A
	40	$1.08^{a*} \pm 0.132$	$0.376^{a*} \pm 0.009$	2.87 A
Post-GEx	5	$0.049^{c*} \pm 0.008$	$0.055^{b,c*} \pm 0.002$	0.89 E
	10	$0.057^{c*} \pm 0.003$	$0.089^{b*} \pm 0.005$	0.64 E
	20	$0.126^{b*} \pm 0.012$	$0.121^{b*} \pm 0.008$	1.08 A
	40	$0.944^{a*} \pm 0.031$	$0.275^{a*} \pm 0.043$	3.68 A
Com-GEx	5	$0.432^{c*} \pm 0.048$	$0.278^{b,c*} \pm 0.004$	1.55 A
	10	$1.05^{b,c*} \pm 0.059$	$0.630^{b,c*} \pm 0.082$	1.78 A
	20	$2.61^{b*} \pm 0.521$	$0.963^{b*} \pm 0.113$	2.79 A
	40	$12.0^{a*} \pm 1.77$	$4.00^{a*} \pm 0.568$	2.99 A
T. pratense				
Controls	0	$0.036^{d*} \pm 0.003$	$0.012^{c*} \pm 0.002$	2.65 A^{*}
Pre-GEx	5	$0.506^{c,d*} \pm 0.038$	$0.065^{b,c*} \pm 0.001$	7.81 A^{*}
	10	$0.930^{c*} \pm 0.148$	$0.087^{b,c*} \pm 0.009$	11.2 A^{*}
	20	$1.94^{b*} \pm 0.086$	$0.148^{b*} \pm 0.021$	14.0 A^{*}
	40	$6.05^{a*} \pm 0.513$	$0.281^{a*} \pm 0.059$	24.6 A^{*}
Post-GEx	5	$0.265^{b*} \pm 0.022$	$0.037^{b,c*} \pm 0.002$	7.04 A^{*}
	10	$0.530^{b*} \pm 0.032$	$0.063^{a,b,c*} \pm 0.003$	8.58 A^{*}
	20	$1.00^{b*} \pm 0.044$	$0.101^{a,b*} \pm 0.003$	9.99 A^{*}
	40	$2.99^{a*} \pm 1.06$	$0.120^{a*} \pm 0.044$	17.0 A^{*}
Com-GEx	5	$2.08^{c,d*} \pm 0.067$	$0.213^{c*} \pm 0.016$	10.0 A^{*}
	10	$8.72^{b,c*} \pm 1.38$	$0.356^{b*} \pm 0.019$	24.1 A^{*}
	20	$11.3^{a,b*} \pm 0.84$	$0.660^{a*} \pm 0.058$	17.5 A^{*}
	40	$17.4^{a*} \pm 5.31$	$0.726^{a*} \pm 0.036$	25.3 A^{*}

Mean values (Samples n = 3, Control n = 9) with standard errors; significant differences were denoted by different letters based on LSD post-hoc test similarities; *Pre-GEx* = pre-germination exposure, *Post-GEx* = post-germination exposure and *Com-GEx* = combined-germination exposure, *A* = Accumulator, *E* = Excluder, * = statistically different at $p \leq 0.05$ (one-way ANOVA)

the *T. pratense* shows more potential to phytoremediate contaminated soils with a stronger ability to translocate the strontium into the plant tissues – thus removing the element from the soils. Based on this study, they could represent a rapid bio-indicator and phytoremediator of dilute-contaminated landscapes.

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Author contributions
PS wrote the manuscript, conceptualised the experiments, and performed the bulk of the experiments and analyses; TP provided training and contributed to the analyses of potentially toxic elements; JR conceptualised the experiments; CK helped to conceptualise the experiments and write the manuscript. All authors have read and approved the final manuscript.

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Declarations

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References

Ab-shukor NA, Kay QON, Stevens DP, Skibinski DOF (1988) Salt tolerance in natural populations of *Trifolium repens* L. New Phytol 109:483–490
Ahmadpour A, Zabihi M, Tahmasbi M, Bastami TR (2010) Effect of adsorbents and chemical treatments on the removal of strontium from aqueous solutions. J Hazard Mater 182:552–556
Association of official seed analysis (1983) Seed vigor testing handbook. Contribution No. 32 to the handbook on seed testing. Association of official seed analysis, Washington
Bae J, Benoit DL, Watson AK (2016) Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes. Environ Pollut 213:112–118
Bamberger M, Oswald RE (2012) Impacts of gas drilling on human and animal health. New Solut 22:51–77
Bowley SR, Taylor NL, Dougherty CT (1984) Physiology and morphology of red clover. In: Brady NC (ed) Advances in agronomy. Academic Press, Cambridge
BSI (2011) Soil quality: determination of effective cation exchange capacity and base saturation level using barium chloride solution, BS EN 11260:2011. British Standard Institution, London
BSI (2012) Sludge, treated biowaste, soil and waste-determination of total organic carbon (TOC) by dry combustion, vol BS EN 15936:2012. British Standard Institution, London
Dada AO, Olalekan AP, Olatunya AM, Dada O (2012) Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherms studies of equilibrium sorption of Zn2 + unto phosphoric acid modified rice husk. IOSR J Appl Chem 3:38–45
Ferrar KJ, Michanowicz DR, Christen CL, Mulcahy N, Malone SL, Sharma RK (2013) Assessment of effluent contaminants from three facilities discharging Marcellus Shale wastewater to surface waters in Pennsylvania. Environ Sci Technol 47:3472–3481
Ghaemi A, Torab-Mostaedi M, Ghannadi-Maragheh M (2011) Characterisations of strontium(II) and barium(II) adsorption from aqueous solutions using dolomite powder. J Hazard Mater 190:916–921
Gregory KB, Dzombak DA (2011) Water management challenges associated with the production of shale gas by hydraulic fracturing. Elements 7:181–186
Guan W, Pan J, Ou H, Wang X, Zou X, Hu W, Li C, Wu X (2011) Removal of strontium (II) ions by potassium tetratitanate whisker and sodium trititanate whisker from aqueous solution: equilibrium, kinetics and thermodynamics. Chem Eng J 167:215–222
Haluszczak LO, Kump LR (2013) Geochemical evaluation of flowback brine from Marcellus gas wells in Pennsylvania, USA. Appl Geochem 28:55–61
Hanslin HM, Eggen T (2005) Salinity tolerance during germination of seashore halophytes and salt-tolerant grass cultivars. Seed Sci Res 15:43–50
Howarth RW, Engelder T (2011) Natural gas: should fracking stop? Nature 477:271–275
IAEA (1994) Handbook of parameter values for the prediction of radionuclide transfer in temperate environments. IAEA, Vienna
Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8: 921–928
Kabata-Pendias A, Mukherjee AB (2007) Trace elements from soil to human. Springer Science & Business Media, Berlin
Kaçan E, Kütahyalı C (2012) Adsorption of strontium from aqueous solution using activated carbon produced from textile sewage sludges. J Anal Appl Pyrol 97:149–157
Kheloufi A, Chorfi A, Mansouri L-M (2017) Germination kinetics in two acacia karroo hayne ecotypes under salinity conditions. Open Access Libr J 4:1
Lester Y, Ferrer I, Thurman EM, Sitterley KA, Korak JA, Aiken G, Linden KG (2015) Characterisation of hydraulic fracturing flowback water in Colorado: implications for water treatment. Sci Total Environ 512:637–644
Li Q, Liu H, Liu T, Guo M, Qing B, Ye X, Wu Z (2010) Strontium and calcium ion adsorption by molecularly imprinted hybrid gel. Chem Eng J 157:401–407
Mair R, Bickle M, Goodman D, Koppelman B, Selley R, Roberts J, Shipton Z, Thomas H, Woods E (2012) Shale gas extraction in the UK: a review of hydraulic fracturing.
Mani D, Kumar C (2014) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int J Environ Sci Technol 11:843–872
Marie P, Ammann P, Boivin G, Rey C (2001) Mechanisms of action and therapeutic potential of strontium in bone. Calcif Tissue Int 69:121
Moyen C, Roblin G (2010) Uptake and translocation of strontium in hydroponically grown maise plants, and subsequent effects on tissue ion content, growth and chlorophyll a/b ratio: comparison with ca effects. Environ Exp Bot 68:247–257
Pantola RC, Alam A (2014) Potential of *Brassicaceae burnett* (Mustard family; Angiosperms) in phytoremediation of heavy metals. Int J Sci Res Environ Sci 2:120
Porra RJ, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim et Biophys Acta (BBA) - Bioenergetics 975:384–394
Rai PK (2012) An eco-sustainable green approach for heavy metals management: two case studies of developing industrial region. Environ Monit Assess 184:421–448
Rediske JH, Selders AA (1953) The absorption and translocation of strontium by plants. Plant Physiol 28:594
Sasmaz A, Sasmaz M (2009) The phytoremediation potential for strontium of indigenous plants growing in a mining area. Environ Exp Bot 67:139–144
Savinkov A, Semioshkina N, Voigt G (2007) Radiostrontium uptake by plants from different soil types in Kazakhstan. Sci Total Environ 373:324–333
Sivaram AK, Logeshwaran P, Subashchandrabose SR, Lockington R, Naidu R, Megharaj M (2018) Comparison of plants with C3 and C4 carbon fixation pathways for remediation of polycyclic aromatic hydrocarbon contaminated soils. Sci Rep 8:1–10
Srikhumsuk P. Plant responses to toxic elements and salt stress of contamination from the petroleum industry. Doctor of Philosophy (PhD), University of Strathclyde, Glasgow, UK. 2020.
Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. https://doi.org/10.1155/2011/939161
Thacker JB, Carlton DD, Hildenbrand ZL, Schug KA (2015) Chemical analysis of wastewater from unconventional drilling operations. Water 7:1568–1579
Vidic R (2015) Sustainable management of flowback water during hydraulic fracturing of marcellus shale for natural gas production. The University Of Pittsburgh, Pittsburgh
Wang X, Chen C, Wang J (2017) Phytoremediation of strontium contaminated soil by Sorghum bicolor (L.) Moench and soil microbial community-level physiological profiles (CLPPs). Environ Sci Pollut Res 24:7668–7678
Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313
Yost EE, Burgoon LD (2016) Overview of chronic oral toxicity values for chemicals present in hydraulic fracturing fluids, flowback, and produced waters. Environ Sci Technol 50:4788–4797
Zhang J, Cheng G, Yu F, Kräuchi N, Li MH (2008) Intensity and importance of competition for a grass (Festuca rubra) and a legume (Trifolium pratense) vary with environmental changes. J Integr Plant Biol 50:1570–1579
Pape A, Switzer C, McCosh N, Knapp CW (2015) Impacts of thermal and smouldering remediation on plant growth and soil ecology. Geoderma 243-244:1–9. https://doi.org/10.1016/j.geoderma.2014.12.004

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