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Phytoextraction of rhenium by lucerne (*Medicago sativa*) and erect milkvetch (*Astragalus adsurgens*) from alkaline soils amended with coal fly ash

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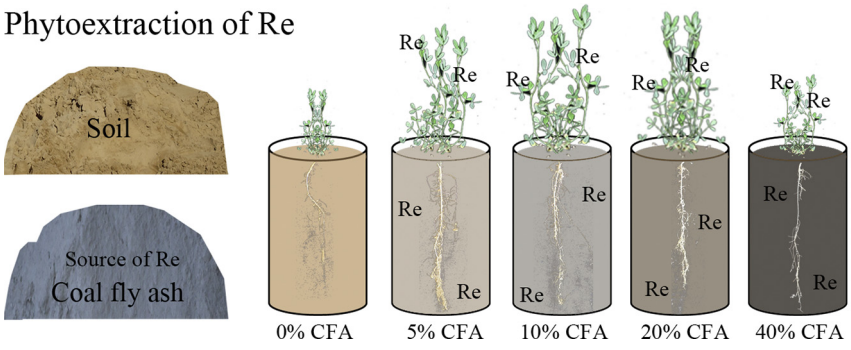
HIGHLIGHTS

- Coal fly ash was a source of rhenium.
- Biomass and Re concentration of plants were increased by applying CFA to the soil.
- Lucerne and erect milkvetch could hyperaccumulate Re when grown in CFA-amended soil.
- Phytoextraction of Re from alkaline soils amended with CFA is promising.

GRAPHICAL ABSTRACT



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ABSTRACT

Coal fly ash (CFA) is an industrial waste generated in huge amounts worldwide, and the management of CFA has become an environmental concern. Recovery of valuable metals from CFA is one of the beneficial reuse options of CFA. Rhenium (Re) is one of the rarest metals in the Earth's crust and one of the most expensive metals of strategic significance in the world market. A CFA at the Jungar Thermal Power Plant, Inner Mongolia, China, contains more Re than two alkaline soils in the surrounding region. Pot experiments were undertaken to grow lucerne (*Medicago sativa*) and erect milkvetch (*Astragalus adsurgens*) in a loessial soil and an aeolian sandy soil amended with different rates (5%, 10%, 20%, and 40%) of CFA. The results show that plant growth was considerably enhanced and Re concentration in plants was significantly increased when CFA was applied to the alkaline soils at rates of ≤20%; while in some cases plant growth was also markedly enhanced by the 40% CFA treatment, which increased plant Re concentration the most of all treatments. Both lucerne and erect milkvetch showed potential for phytoextracting Re from CFA-amended alkaline soils. Using CFA for soil amendment not only offers a potential solution for the waste disposal problem of CFA, but the phytoextraction of Re by both lucerne and erect milkvetch may also bring an economic profit in the future.

1. Introduction

Coal fly ash (CFA) is an industrial by-product derived from coal combustion in thermal power plants. It is one of the most complex and abundant anthropogenic materials. Due to the enormous demand for

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electricity, the amounts of coal consumed and CFA produced globally are huge (Shaheen et al., 2014; Yao et al., 2015). Substantial amounts of CFA end up in landfill every year. If not properly disposed of, CFA can cause air, soil, and water pollution. Therefore, CFA has been regarded as a problematic solid waste worldwide. Management of CFA has become an environmental concern, especially for China, which is the largest coal-consuming and CFA-producing country in the world (Dutta et al., 2009; Pandey et al., 2009b; Raja et al., 2015; Yao et al., 2015).

Beneficial reuse of CFA has received increasing attention over the past three decades as more sustainable solutions other than landfilling have been sought (Blissett and Rowson, 2012). Potential utilization of CFA includes its application as an ingredient in cement and other construction products, as an adsorbent for gases and waste water processes, as an agent for soil amelioration, and as catalysts and catalyst supports. Coal fly ash has also been used in the manufacture of glass and ceramics, in the synthesis of zeolites, and in the formation of mesoporous materials. In addition, it can be used for the recovery of valuable metals such as germanium (Ge), gallium (Ga), titanium (Ti), aluminum (Al), and vanadium (V) (Blissett and Rowson, 2012; Shaheen et al., 2014; Yao et al., 2015). However, only <30% of CFA produced globally is reused currently, the recycling and utilization of CFA is still a pressing issue (Blissett and Rowson, 2012; Yao et al., 2015).

Rhenium (Re) is one of the rarest and most widely dispersed elements on Earth, with an estimated concentration of 0.4–0.6 ng g^{-1} in the Earth's upper crust (Kabata-Pendias, 2011). Due to its unique physicochemical properties, Re is mainly used in high-temperature superalloy turbine blades in jet engines, land-based gas-powered turbines, aerospace industry, and for producing lead-free gasoline. These important applications, together with its scarcity, make Re one of the most expensive metals of strategic significance on the world market (Naumov, 2007; Polyak, 2014a; Novo et al., 2015). The demand for Re is expected to increase significantly with the development of aerospace industry, but the demand cannot be met by current global Re production (Polyak, 2014a; Polyak, 2014b). Rhenium primarily occurs in minerals of other elements, but the average concentration of Re in its main carrying minerals such as molybdenum-copper ores is often very low (Bozhkov et al., 2007; Kabata-Pendias, 2011). Currently, Re is obtained as a by-product from extraction and refinement of metals such as molybdenum, copper, and uranium, but the technologies for Re production are not effective enough to concentrate and extract Re. Therefore, there have been extensive efforts to search for new Resources and more effective technologies for its production (Bozhkov et al., 2012; Anderson et al., 2013; Hoai Thanh et al., 2017; Markovic et al., 2017). The extraction of Re from waste sources (e.g., CFA), could increase the availability of Re.

Phytomining is the bioharvesting of metals from hyperaccumulator plant species or high-biomass crops grown in soil with metal concentrations that are too low to extract using conventional processes (Sheoran et al., 2009; Bozhkov et al., 2012). This phytoextraction-based technology allows further metal recovery through smelting, and has potential application in the mineral industry to return an economic profit by commercial production (Novo et al., 2017). Recycling and use of anthropogenic secondary sources such as CFA might be an alternative way of retrieving valuable metals. Previous studies have reported that plants such as lucerne (*Medicago sativa*) and white clover (*Trifolium repens*) can hyperaccumulate Re, suggesting that phytomining of Re may offer an environmentally sound and cheap technology to recover Re from secondary sources (Bozeikov and Tzvetkova, 2009; Novo et al., 2015; Rosenkranz et al., 2015). However, studies on the feasibility of Re phytomining are lacking.

Coal fly ash contains a series of essential elements for plant growth, and it is a cheap and widely available waste material; therefore, it is often used as a soil amendment to improve soil fertility. There are a number of studies demonstrating that plant growth and yield of crops such as lucerne are significantly increased when CFA is applied to the soil (Jala and Goyal, 2006; Shaheen et al., 2014; He et al., 2017). Coal fly ash is a good source of Re, as concentrations of Re in a number of coal samples are higher than that in the Earth's upper crust (Maksimova and Shmariovich, 1983; Novo et al., 2015).

In our previous work, we noticed that a CFA obtained from a power plant in Inner Mongolia, China, has a higher Re concentration than some alkaline soils in northwest China. We therefore hypothesized that this CFA is a good source of Re, and when the CFA is applied to alkaline soils, plants such as lucerne and erect milkvetch (*Astragalus adsurgens*) will accumulate more Re and show potential for phytoextraction of Re from the CFA-amended soils. To test this hypothesis, pot experiments were carried out to grow lucerne and erect milkvetch in two alkaline soils amended with different amounts of CFA. Plant growth and Re concentrations in different parts of the plants were analyzed, and bioaccumulation factors and translocation factors of Re were calculated to assess the feasibly of using lucerne and erect milkvetch to phytoextract Re from CFA.

2. Material and methods

2.1. Characterization of the coal fly ash and alkaline soils

In October 2013, fresh CFA was obtained from an ash collector at the Jungar Thermal Power Plant, which is located in the Jungar Banner, Inner Mongolia, China. Two alkaline soils, i.e. a loessial soil and an aeolian sandy soil were collected from the top 40-cm layers of two undisturbed sites close to the Jungar Thermal Power Plant. After collection, the CFA and the two alkaline soils were air-dried and passed through a 2-mm sieve for physicochemical characterization. Particle size distribution was analyzed using a Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments, Malvern, UK) (Lamorski et al., 2014), field capacity was determined using the indoor cutting-ring weighing method, and pH was measured in a 1:5 soil:water (w:v) suspension. Soil organic carbon (C) concentration was determined using the potassium dichromate titrimetric method (Mebius, 1960), and concentrations of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were analyzed using a segmented-flow analyzer after extraction by a 2 M potassium chloride (KCl) solution [soil:solution = 1:10 (w:v)] (Kachurina et al., 2000).

Sub-samples of the CFA, the loessial soil, and the aeolian sandy soil were ground and passed through a 0.149-mm mesh; about 0.5 g sample of each soil was taken and extracted following a modified aqua regia digestion procedure described by Reimann et al. (2015). After the extraction, concentrations of total phosphorus (P), potassium (K), and rhenium (Re) in the solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS), ELAN 9000 (PerkinElmer Instruments, Shelton, CT, USA). Certified soil reference material STD DS10 was processed following the same procedure at the same time for data quality control, with the recovery rates of P, K, and Re being 100%, 99.4%, and 87.7%, respectively.

2.2. Preparation of the substrates, cultivation and harvest of plants

2.2.1. Preparation of CFA-amended loessial soil, cultivation and harvest of lucerne and erect milkvetch

In late January 2014, air-dried CFA and loessial soil were passed through a 2-mm sieve. After sieving, the CFA and loessial soil were thoroughly mixed at a CFA-application rate equivalent to 5%, 10%, 20%, and 40% on a dry weight basis, respectively. Non-transparent PVC tubes of 11-cm diameter and 40-cm height with a sealed bottom were first filled with some gravels at the bottom and then with 3 kg of the thoroughly mixed substrates. Tubes filled with 3 kg loessial soil without adding CFA, i.e. CFA-application rate of 0%, served as the control. No additional fertilizer was supplied during the experiment. Twenty-seven pots per treatment (control, 5%, 10%, 20%, and 40% CFA) were prepared, giving a total of 135 pots. The substrates were watered weekly with deionized

water to 21% gravimetric water content, i.e. 60% of the field capacity of the loessial soil, and incubated at room temperature.

In early May 2014, all pots were transferred to a transparent rain shelter in the Institute of Soil and Water Conservation, Yangling, Shaanxi, China. Of the 27 pots per treatment, 21 pots (seven harvest schemes × three replicates) were used for the growth of lucerne, while the remaining six pots (two harvest schemes × three replicates) for the growth of erect milkvetch. One day prior to sowing, seeds of lucerne and erect milkvetch were sterilized in 30% (v/v) hydrogen peroxide (H_2O_2) solution for 5 min, rinsed with cold sterile water three times and then soaked in cold tap water overnight (Kereszt et al., 2007). Lucerne seeds were sown in early May 2014, with four seeds per pot. While erect milkvetch seeds were sown in late May 2014, with eight seeds per pot. Seedlings were thinned to one plant per pot 30 days after sowing (DAS). All pots were watered to 21% gravimetric water content with deionized water weekly or when necessary during the growing season. The experiment was carried out at ambient temperature from May 2014 to September 2015. For lucerne, the 21 pots within each treatment were allocated to seven harvest schemes (coded as H1, H2, H3, H4, H5, H6, and H7, and harvested according to the schemes presented in Table 1), with three replicates per treatment per harvest scheme. Lucerne was harvested at 100 DAS (August 2014, hereafter referred to as T1) and 160 DAS (October 2014, hereafter referred to as T2). Further harvests were at100 days (June 2015, hereafter referred to as T3) and 200 days (September 2015, hereafter referred to as T4) after >80% of the plants of which the roots were not harvested resprouted in late February 2015, respectively (He et al., 2017). Plants of erect milkvetch were harvested at 90 DAS (late August 2014) and 150 DAS (late October 2014), with the shoots and roots of plants grown in three pots harvested each time.

For both lucerne and erect milkvetch, shoots were severed at the base of the stem at each harvest. When roots were harvested, roots and nodules were first washed with tap water thoroughly to remove soil, and then rinsed with deionized water. Due to the small number of nodules for some plants, nodules and roots were combined and hereafter referred to as roots. Shoots and roots harvested were oven-dried at 65 °C for 72 h and weighed separately to obtain shoot and root dry mass. For lucerne when the harvest schemes included ≥two harvests, total shoot dry mass of each harvest scheme was calculated as the sum of shoot dry mass harvested at different time.

2.2.2. Preparation of CFA-amended aeolian sandy soil, cultivation and harvest of lucerne

In late January 2015, air-dried CFA and aeolian sandy soil were passed through a 2-mm sieve. After sieving, the CFA and aeolian sandy soil were thoroughly mixed at CFA application rate equivalent to 10%, 20%, and 40% on a dry weight basis, respectively. The same PVC tubes mentioned above were used, and the same substrate filling procedure described previously was followed, except that the weight of the substrate filled in each tube was 3.5 kg. Tubes filled with 3.5 kg aeolian sandy soil without adding CFA, i.e. CFA application rate equivalent to 0%, served as the control. No additional fertilizer was supplied during the experiment. For each treatment (control, 10%, 20% and 40%), nine pots were prepared, giving a total of 36 pots. The substrates were watered every three days with deionized water to 10% gravimetric water content, i.e. about 80% of the field capacity of the aeolian sandy soil, and incubated at room temperature.

In late April 2015, seeds of lucerne were treated in the same way described above. The following day after sterilization, seeds were sown in all prepared pots for each treatment, with ten seeds sown in each pot. Seedlings were thinned to keep three plants per pot at 30 DAS. All pots were placed randomly under a transparent rain shelter at ambient temperature, watered with deionized water to 10% gravimetric soil water content weekly or when necessary after sowing. All plants were let to grow till early November 2015, when the shoots were harvested. In 2016, shoots in all pots for each treatment were harvested for four times, i.e., in early May (H1), late June (H2), early August (H3), and late September (H4). At each time of harvest, shoots in each pot were severed at the stem base first and then separated into stems (including both main stems and small branches) and leaves. Stems and leaves were oven-dried at 65 °C for 72 h and weighed separately to obtain their dry mass.

2.3. Analysis of rhenium concentrations in plants

For the loessial soil experiment, all roots of both lucerne and erect milkvetch, shoots of lucerne plants which were harvested only once and all shoots of erect milkvetch harvested were analyzed for Re concentration; for those pots in which shoots of lucerne were harvested more than once, only shoots harvested in the last time were subjected to analysis of Re concentration. When the sample dry mass of one plant was <1.0 g, samples of different plants in the same treatment were pooled for the analysis. For the 40% CFA treatment of H1 of both lucerne and erect milkvetch, and for the control of H2 of erect milkvetch, root dry mass was very low, thus analysis was not performed for the roots. For the aeolian sandy soil experiment, only stems and leaves harvested in H1 and H4 were subjected to analysis of Re concentration, with samples of each type from three pots pooled to form one sample for the analysis.

For each sample to be analyzed for Re concentration, about 1.0 g finely ground plant sample was first cold leached with concentrated nitric acid (HNO_3) for 1 h; then the sample was digested in a hot water bath for another 1 h. After cooling, a modified aqua regia solution of equal parts of concentrated ACS grade hydrochloric acid (HCl), HNO_3, and deionized water was added to each sample to digest in a hot water bath (95 °C) for 2 h. After cooling, the solution was made up to a final volume of 20 ml with 5% HCl and then filtered (Reimann et al., 2015). The concentration of Re in the solution was determined using ICP-MS NexION 300 (PerkinElmer, Inc., Waltham, MA, USA).

2.4. Calculation of the bioaccumulation factors and translocation factors of rhenium

Because the concentration of Re in the loessial soil and the aeolian soil was below the detection limit of the analytical method, it is impossible to calculate the bioaccumulation factor of Re directly from the ratio

Table 1
Harvest schemes of the pot experiment of growing lucerne in coal fly ash-amended loessial soil.

Code of harvest scheme	Harvest time			
	August 2014 (T1)	October 2014 (T2)	June 2015 (T3)	September 2015 (T4)
H1	Shoots + roots			
H2		Shoots + roots		
H3	Shoots	Shoots + roots		
H4		Shoots	Shoots + roots	
H5	Shoots	Shoots	Shoots + roots	
H6		Shoots	Shoots	Shoots + roots
H7	Shoots	Shoots	Shoots	Shoots + roots

Note: The information was modified after He et al. (2017).

of Re concentration in plants to that in the substrate used in the present study. Instead, we assumed that the concentration of Re in both the loessial soil and the aeolian sandy soil was equal to the Clark of Re in the Earth's crust, which is 0.7 ng g^{-1} (Bozhkov et al., 2007), and then calculated the concentrations of Re in the prepared substrates according to the proportions of alkaline soils and CFA. We calculated the bioaccumulation factor of Re as the ratio of the mean Re concentration in the aboveground parts of plants ($C_{Re\ plant}$) in the same treatment of the same harvest to the calculated Re concentration in the substrate. For the loessial soil experiment, root-to-shoot translocation factor of Re was calculated as the ratio of the mean concentration of Re in shoots to that in roots in the same treatment of the same harvest (Ghosh et al., 2015). For the aeolian sandy soil experiment, the stem-to-leaf translocation factor of Re was calculated as the ratio of the mean concentration of Re in stems to that in leaves in the same treatment of the same harvest.

2.5. Statistical analyses

Data of plant dry mass and Re concentrations in plants were analyzed by performing a One-sample *t*-test using the IBM SPSS Statistics 22.0 software package (IBM, Montauk, New York, USA), to obtain the means and standard deviations of the data of plants in the same treatment of the same harvest.

3. Results

3.1. Physiochemical properties of the coal fly ash and alkaline soils

The CFA had a higher silt content but lower clay content than the loessial soil; it had a lower sand content but higher silt content than the aeolian sandy soil (Table 2). Field capacity, pH, organic C concentration, NO_3^--N concentration, and total P concentration of the CFA were higher than those of both the loessial soil and aeolian sandy soil. The CFA had lower concentrations of NH_4^+-N and total K than both the loessial soil and aeolian sandy soil. Total Re concentration of the CFA was 7.3 ng g^{-1}, but those of both the loessial soil and aeolian sandy soil were below the detection limit of the method, which is 1 ng g^{-1}.

3.2. Plant biomass accumulation

When the CFA was added to the loessial soil, both root dry mass and shoot dry mass of lucerne in all harvest schemes were affected (Fig. 1). In H1, the 5%, 10%, and 20% CFA treatments increased lucerne root dry mass by 2.0–3.9 times, but the 40% CFA treatment reduced root dry mass by 56%. In H2, H3, H4, H5, H6, and H7, root dry mass was increased by CFA treatments by 1.0–4.0, 1.2–7.2, 3.0–5.4, 5.7–6.4, 2.4–4.0, and 2.0–3.0 times, respectively. In H1, the 5%, 10%, and 20% CFA treatments increased total shoot dry mass of lucerne by 1.1–2.4 times, but the 40% CFA treatment reduced total shoot dry mass by 45%. In H2, total shoot dry mass was increased by 1.3–4.3 times when CFA was added. In H3, total shoot dry mass in CFA treatments was 1.6–5.3 times as great as that in the control. In H4, H5, H6, and H7, total shoot dry mass in CFA treatments was 4.0–7.6, 4.0–7.4, 7.7–9.5, and 3.1–5.1 times greater than that in the control, respectively.

Table 2
Physicochemical properties of the coal fly ash, loessial soil, and aeolian sandy soil.

Parameter	Coal fly ash	Loessial soil	Aeolian sandy soil
Sand (%)	70	69	88
Silt (%)	22	18	5
Clay (%)	8	13	7
Field capacity (%)	54	35	12
pH (H_2O, 1:5)	9.2	8.7	8.5
Organic C (mg g^{-1})	17.1	4.2	1.7
NH_4^+-N (μg g^{-1})	1.2	5.0	7.5
NO_3^--N (μg g^{-1})	61	1.2	3.5
Total P (μg g^{-1})	420	340	290
Total K (mg g^{-1})	0.2	1.5	0.8
Total Re (ng g^{-1})	7.3	B.D.L.	B.D.L.

Note: B.D.L. means below the detection limit (i.e. 1 ng g^{-1}) of the method.

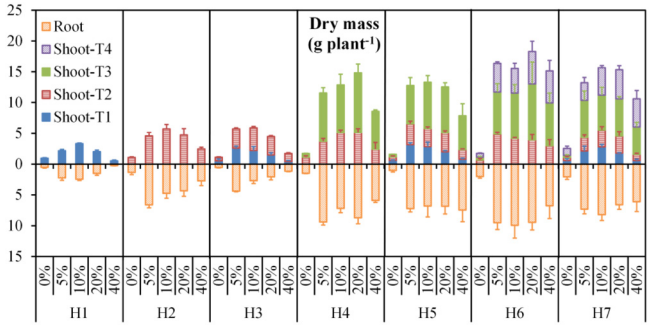

Fig. 1. Dry mass of lucerne grown in a loessial soil amended with different rates of coal fly ash in different harvest schemes. Data are presented as means + SD ($n = 3$). Shoot-T1, Shoot-T2, Shoot-T3, and Shoot-T4 represents shoots harvested at T1, T2, T3, and T4, respectively.

Applying CFA to the loessial soil also affected root dry mass and shoot dry mass of erect milkvetch (Fig. 2). In H1, root dry mass of erect milkvetch was increased by 32–213% under the 5%, 10%, and 20% CFA treatments, but was reduced by 78% under the 40% CFA treatment. In H2, root dry mass was increased by 71–130% under the 5%, 10%, and 20% CFA treatments, but was reduced by 18% under the 40% CFA treatment. Shoot dry mass of erect milkvetch in H1 was increased 1.6–4.4 times under the 5%, 10%, and 20% CFA treatments, but was reduced by 55% under the 40% CFA treatment. Shoot dry mass in H2 was increased 1.1–6.8 times by the CFA treatments.

Applying CFA to the aeolian sandy soil affected both stem and leaf dry mass of lucerne (Fig. 3). The 10% CFA treatment increased stem and leaf dry mass by 61–144% and 85–135% in all four harvests, respectively. The 20% treatment reduced stem dry mass by 19% in H1, but increased stem dry mass by 41–124% in H2, H3 and H4; while it increased leaf dry mass by 28–105% in all four harvests. The 40% CFA treatment reduced stem dry mass and leaf dry mass by 32–83% and 49–66% in all four harvests, respectively.

3.3. Concentrations of rhenium in plants

In almost all cases, adding CFA to the loessial soil increased both shoot and root Re concentration of lucerne, and the Re concentration increased with increasing rates of CFA (Fig. 4). Concentration of Re in shoots of lucerne in the control of different harvest schemes was 2.0–7.0 ng g^{-1}, while that in the 5%, 10%, 20%, and 40% CFA treatment was 10–168, 25–256, 39–540, and 70–835 ng g^{-1}, respectively. In the 5%,

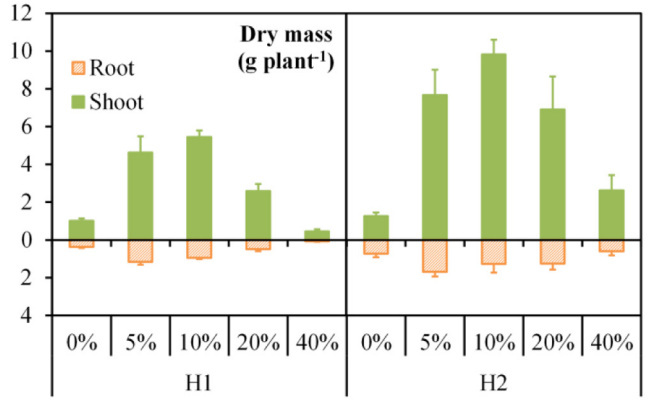

Fig. 2. Dry mass of erect milkvetch grown in a loessial soil amended with different rates of coal fly ash in different harvests. Data are presented as means + SD ($n = 3$).

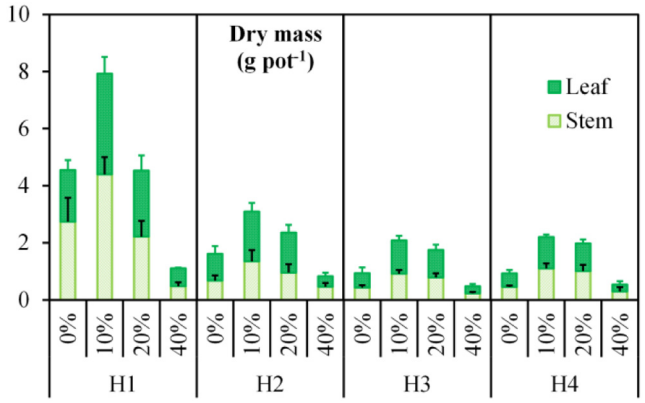

Fig. 3. Dry mass of lucerne grown in an aeolian sandy soil amended with different rates of coal fly ash in different harvests. Data are presented as means + SD ($n = 9$).

10%, 20%, and 40% CFA treatment the shoot Re concentration increased 2.5–36, 7.1–56, 17–119, and 31–185 times, respectively. The concentration of Re in roots of lucerne in the control of different harvest schemes was 1.0–5.3 ng g^{-1}, while that in the 5%, 10%, 20%, and 40% CFA treatment was 1.3–6.7, 2.0–16, 3.0–34, and 5.7–94 ng g^{-1}, respectively. In the 5%, 10%, 20%, and 40% CFA treatment the root Re concentration increased 0.3–5.7, 0.8–15, 1.3–19, and 2.1–93 times, respectively. Concentrations of Re in both roots and shoots of lucerne harvested in 2014 (H1–H3) were higher than in those of plants harvested in 2015 (H4–H7).

Adding CFA to the loessial soil increased both shoot and root Re concentration of erect milkvetch (Fig. 5). Concentrations of Re in shoots of erect milkvetch in H1 increased from 3.5 ng g^{-1} in the control to 389 ng g^{-1} in the 40% CFA treatment, and those in H2 increased from

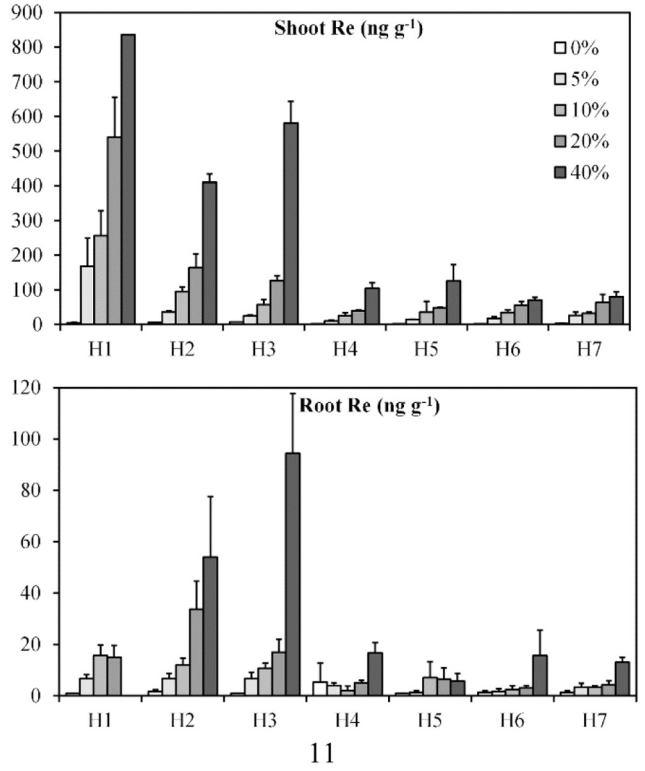

Fig. 4. Shoot and root rhenium (Re) concentration of lucerne grown in a loessial soil amended with different rates of coal fly ash (CFA) in different harvest schemes. Data are presented as means + SD ($n = 3$, except some pooled samples due to small sample weight for separate analysis). Note root Re concentration of the 40% CFA treatment in H1 was unavailable due to the very tiny root samples.

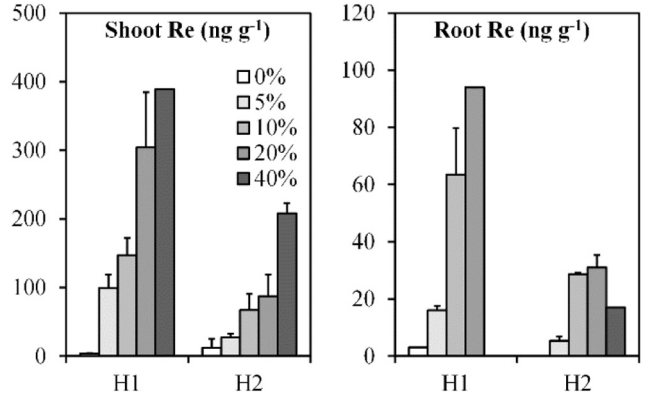

Fig. 5. Shoot and root rhenium (Re) concentration of erect milkvetch grown in a loessial soil amended with different rates of coal fly ash (CFA) in different harvests. Data are presented as means + SD ($n = 3$, except some pooled samples due to small sample weight for separate analysis). Note root Re concentration of the 40% CFA treatment in H1 and the control in H2 was unavailable due to the very tiny root samples.

12 ng g^{-1} in the control to 208 ng g^{-1} in the 40% CFA treatment. Shoot Re concentration in the 5%, 10%, 20%, and 40% CFA treatment of H1 was 27, 41, 86, and 110 times greater than that in the control, respectively, while that of H2 was 1.3, 4.6, 6.3, and 16 times greater than that in the control, respectively. Concentration of Re in roots of erect milkvetch in H1 increased from 3.0 ng g^{-1} in the control to 94 ng g^{-1} in the 40% CFA treatment, while root Re concentration in the 5%, 10%, 20%, and 40% CFA treatment of H2 was 5.3, 29, 31, and 17 ng g^{-1}, respectively. Root Re concentration in the 5%, 10%, and 20% CFA treatment of H1 was 4.0, 20, and 30 times greater than that in the control, respectively. Concentrations of Re in both roots and shoots of erect milkvetch in H1 were greater than those in H2.

Adding CFA to the aeolian sandy soil increased both stem and leaf Re concentration of lucerne (Fig. 6). The concentration of Re in stems of lucerne in H1 increased from 1.0 ng g^{-1} in the control to 57 ng g^{-1} in the 40% CFA treatment, while that in H4 increased from 4.0 ng g^{-1} in the control to 47 ng g^{-1} in the 40% CFA treatment. Compared with the control, stem Re concentration in H1 increased 2.0, 6.7, and 56 times under the 10%, 20%, and 40% CFA treatment, respectively, while that in H4 increased 1.4, 2.2, and 11 times under the 10%, 20%, and 40% CFA treatment, respectively. The concentration of Re in leaves of lucerne in H1 increased from 11 ng g^{-1} in the control to 531 ng g^{-1} in the 40% CFA treatment, while that in H4 increased from 7.5 ng g^{-1} in the control to 362 ng g^{-1} in the 40% CFA treatment. The leaf Re concentration in the 10%, 20%, and 40% CFA treatment of H1 was 4.1, 7.1, and 46 times greater

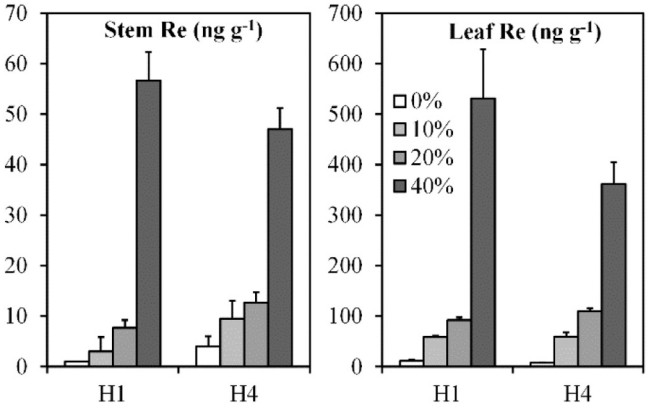

Fig. 6. Stem and leaf rhenium (Re) concentration of lucerne grown in an aeolian sandy soil amended with different rates of coal fly ash in two harvests, H1 and H4. Data are presented as means + SD ($n = 3$, except some pooled samples due to small sample weight for separate analysis).

than that in the control, respectively, while that of H2 was 6.9, 14, and 47 times greater than that in the control, respectively.

3.4. Bioaccumulation factors of rhenium

In the present study, bioaccumulation factors of Re for the aboveground parts of plants were always ≥2.9, with the highest bioaccumulation factors of Re in the 5%, 10%, 20%, and 40% CFA treatments being 163, 192, 267, and 250, respectively (Table 3). For both the loessial soil experiment and the aeolian sandy soil experiment, the bioaccumulation factor of Re was increased in all CFA treatments, with the shoots of the 20% CFA treatment in H1 of lucerne in the loessial soil experiment showing the highest bioaccumulation factor of Re. For all CFA treatments of lucerne in the loessial soil experiment, H1 showed the highest bioaccumulation factors of Re among all harvests. For all CFA treatments of erect milkvetch in the loessial soil experiment, bioaccumulation factors of Re in H1 were higher than those in H2. Among all CFA treatments in the aeolian sandy soil experiment, only leaf samples under the 40% CFA treatment showed a higher bioaccumulation factor of Re in H1 than in H4.

3.5. Translocation factors of rhenium

For the loessial soil experiment, root-to-shoot translocation factors of Re were always >1, except in the control of lucerne in H4 (Table 4). For lucerne in the loessial soil experiment, root-to-shoot translocation factors of Re in the 5%, 10%, and 20% CFA treatments were all the highest in H1; in all harvests except H3, root-to-shoot translocation factors of Re in all CFA treatments were higher than those in the control. For the aeolian sandy soil experiment, stem-to-leaf translocation factors of lucerne were all >1, with the translocation factors under the control and all CFA treatments in H1 being greater than in H4.

4. Discussion

There are a number of studies demonstrating that CFA promotes plant growth when applied to the soil at low rates, but inhibits plant growth at high rates (Singh and Siddiqui, 2003; Gupta et al., 2007; Pandey et al., 2009a). In the present study, CFA application rates at 5%, 10%, and 20% enhanced plant growth of lucerne and erect milkvetch at almost all harvests. However, in some cases, growth of lucerne was inhibited when the CFA was added to either the aeolian sandy soil or the loessial soil at a rate of 40%, and similar growth inhibition was observed for erect milkvetch when grown under the loessial soil amended with 40% CFA. When soil is amended with CFA at appropriate rates, the resultant soil can have a more balanced nutrient supply, thus enhancing plant growth (Shaheen et al., 2014). In this study, it is very likely that the increased supply of macronutrients such as NO_3^--N and P in the alkaline soils amended with CFA benefited plant growth at lower CFA application rates. Other factors contributing to enhanced plant growth might include improved soil physical properties such as soil texture and water-holding capacity. The adverse effects of higher CFA-application rates on plant growth were probably due to increased phytotoxicity caused by high soil salinity, and high levels of Mo and B, as well as some potentially toxic trace elements such as Se and Hg (Pandey and Singh, 2010; Shaheen et al., 2014; Yao et al., 2015; He et al., 2017). For plants grown in the CFA-amended loessial soil, it is possibly because the growth period for the earlier harvests was not long enough for the plants to become adapted to and benefit from the 40% CFA treatment, or because the substrate became less hostile to the plants as time went on.

In general, all CFA treatments increased concentrations of Re in plants of the present study, with the highest Re concentration (835 ng g^{-1}) observed in shoots of lucerne in H1 in the loessial soil experiment. The concentration of Re in dry mass of lucerne and white clover growing in a soil spiked with 128.7 μg g^{-1} Re reached 46,586 and 35,090 μg g^{-1}, respectively, with the Re bioaccumulation factor being 362 and 273 for lucerne and white clover, respectively (Bozeikov and Tzvetkova, 2009). When scouring rush (*Equisetum hyemale*) and Indian mustard (*Brassica juncea*) were grown in a commercial organic substrate spiked with 5–80 μg g^{-1} Re, Re concentrations in shoots of scouring rush increased from 74 and 87 μg g^{-1} to 925 and 714 μg g^{-1} after 45 and 75 days, respectively, and those of Indian mustard increased from 1553 and 1347 μg g^{-1} to 22,617 and 23,396 μg g^{-1} at 45 and 75 days, respectively, with the Re concentration in the 80 μg g^{-1} Re treatment being 13.6 and 16.4 times greater than that in the 5 μg g^{-1} Re treatment, respectively (Novo et al., 2015). When green geranium (*Pelargonium* sp.) was grown in a soil spiked with 1 μg g^{-1} Re, the leaf Re concentration was 20 and 100 μg g^{-1} after one week and three weeks, respectively, and did not increase further with time after three weeks, while leaf Re concentration of plants grown in the unspiked soil was 1 μg g^{-1} (Tzvetkova et al., 2007).

Concentrations of Re in lucerne and erect milkvetch in our experiments were much lower than those in Re hyperaccumulators mentioned above, very likely because the Re concentrations in the CFA-amended alkaline soils were very low, when compared with those soils spiked with Re (Tzvetkova et al., 2007; Bozeikov and Tzvetkova, 2009; Novo et al., 2015). Concentrations of Re in plants, especially those under the 40% CFA treatment, declined with time, very likely because the soil Re concentration decreased when more Re was taken up by plants, and possibly also because loss of Re from the soil via volatilization (Bozhkov et al., 2012). The results suggest that using fresh CFA

Table 3
Bioaccumulation factors of rhenium (Re) for the aboveground parts of lucerne and erect milkvetch grown in alkaline soils amended with different rates of coal fly ash under different harvest schemes.

Soil type	Plant	Plant part	Harvest	Bioaccumulation factor				
				0%	5%	10%	20%	40%
Loessial soil	Lucerne	Shoot	H1	6.4	163	192	267	250
			H2	8.6	35	71	81	123
			H3	10.0	24	43	63	174
			H4	2.9	9.9	19	19	31
			H5	2.9	14	26	24	38
			H6	2.9	17	26	27	21
			H7	3.6	26	24	31	24
	Erect milkvetch	Shoot	H1	5.0	98	111	151	116
			H2	17	27	50	43	62
Aeolian sandy soil	Lucerne	Stem	H1	1.4	N.A.	2.3	3.8	17
			H4	5.7	N.A.	7.1	6.3	14
		Leaf	H1	16	N.A.	44	46	159
			H4	11	N.A.	44	54	108

Note: N.A. means data not available.

Table 4
Translocation factors of rhenium (Re) within plants of lucerne and erect milkvetch grown in alkaline soils amended with different rates of coal fly ash in different harvests.

Soil type	Plant	Harvest	Translocation factor				
			0%	5%	10%	20%	40%
Loessial soil	Lucerne	H1	4.5	25.2	16.4	36.0	N.A.
		H2	3.6	5.4	7.9	4.9	7.6
		H3	7.0	3.7	5.3	7.5	6.2
		H4	0.4	2.5	12.7	7.9	6.2
		H5	2.0	10.8	5.0	7.6	22.2
		H6	1.5	10.4	14.7	18.4	4.5
		H7	1.9	7.8	9.6	14.6	6.2
	Erect milkvetch	H1	1.2	6.2	2.3	3.2	N.A.
		H2	N.A.	5.1	2.3	2.8	12.2
Aeolian sandy soil	Lucerne	H1	11.3	N.A.	19.4	12.0	9.4
		H4	1.9	N.A.	6.2	8.6	7.7

Note: For the loessial soil experiment, the data were root-to-shoot translocation factors. For the aeolian sandy soil experiment, the data were stem-to-leaf translocation factors. N.A. means data not available.

would be more effective for Re phytoextraction than using weathered CFA.

A basic feature of Re is that it is predominantly accumulated in the aboveground plant parts (Bozhkov et al., 2007). The most stable chemical form of Re in the soil is perrhenate (ReO_4^-), which is readily available to plants. Perrhenate ions in soil may use anion transporters on the root surface to enter root cells; they act like Cl^- and enter together with excess nutrient cations, and are transported through the xylem to the stem, branches, and finally to leaves (Bozhkov et al., 2007; Tagami and Uchida, 2011). A mechanism that explains the gradual accumulation of Re in green leaves has been described by Bozhkov et al. (2007). For scouring rush and Indian mustard grown in a commercial organic substrate spiked with 5–80 μg g^{-1} Re, the root-to-shoot translocation factor of Re was 5–7 and 98–151 at 45 days, respectively, and 5–11 and 132–256 at 75 days, respectively, indicating a high capacity of both species, especially Indian mustard, to translocate Re from roots to their aboveground parts (Novo et al., 2015). In the present study, concentrations of Re were higher in shoots than in roots in both lucerne and erect milkvetch, and higher in leaves than in stems of lucerne, the highest root-to-shoot and stem-to-leaf translocation factor was 36 and 19, respectively. The bioaccumulation and translocation factors of Re for lucerne and erect milkvetch were high, and increased with increasing soil Re level, suggesting that both species exhibit potential to hyperaccumulate Re in a soil presenting higher levels of Re (>1 μg g^{-1}) Theymay be important candidates for Re phytomining (Bozhkov et al., 2012; Novo et al., 2015). Based on the results of the pot experiments, assuming that shoot dry mass production of lucerne is 7.5 t per harvest per hectare, shoot Re concentration is 0.8 μg g^{-1} when CFA application rate is 40% under field conditions, and lucerne is harvested every 30 days, 6 kg Re could be accumulated by lucerne per hectare within 30 days.

After harvesting, the aboveground parts of plants that hyperaccumulate Re can be treated in a couple of ways to preconcentrate Re. For example, plant dry mass can be incinerated directly, then Re is leached from the ash by alkaline solutions. Rhenium in plant dry mass can also be extracted using ethanol; after evaporation of ethanol and ashing of dry residue, Re can be leached from the ash by alkaline solutions. The preconcentrating procedures can result in tens of thousands of times of increase in Re concentration compared with the initial Re concentration in soil, and can be used for the development of a cheap, simple, and environment-friendly biotechnology for phytoextraction of Re from soils (Bozhkov et al., 2007; Tzvetkova et al., 2007).

Tzvetkova et al. (2011) suggest that lucerne has the potential to phytomine Re from soils of ore dressing regions which will not only return an economic profit but also lead to remediation of exhausted soils in mine regions. Bozhkov et al. (2012) have also reported promising results on the practicability of Re phytomining. In the present study, by amending alkaline soils such as the loessial soil and the aeolian sandy soil with CFA, plant growth was markedly enhanced in most cases, and accumulation of Re by plants was always significantly increased. For the loessial soil experiment, although the 40% CFA treatment inhibited plant growth in the first harvest for both lucerne and erect milkvetch, it increased Re accumulation in shoots of lucerne 101 times and that in shoots of erect milkvetch 49 times. For the aeolian sandy soil experiment, although the 40% CFA treatment inhibited the growth of lucerne in all harvests, the dry mass reduction rates (49–66% for leaf dry mass, and 32–83% for stem dry mass) were relatively small, when compared with the increased Re concentrations in plants (46 and 47 times for leaf in H1 and H4, and 56 and 11 times for stem in H1 and H4, respectively). Therefore, there were net increases (14 and 18 times in H1 and H4, respectively) in Re accumulation in shoots of plants in the 40% CFA treatment compared with the control. Our results suggest that it is promising to phytoextract Re from CFA by growing lucerne and erect milkvetch in CFA-amended alkaline soils.

As CFA often contains higher levels of heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr), and volatile metalloids such as arsenic (As), selenium (Se), and mercury (Hg) than soils, applying CFA to soils may cause risk of contamination of soils with the above-mentioned elements, which may also be leached by rainfall to the groundwater and pose a risk of water contamination. Soil and water contaminated by these elements can cause toxicity to plants and animals, and pose a health risk for humans (Pandey and Singh, 2010; Blissett and Rowson, 2012; Shaheen et al., 2014; Yao et al., 2015). Therefore, application of CFA to farmland for the purpose of Re phytoextraction in the field should be monitored carefully to avoid the potential risks mentioned above. Studies on the mobility and leaching of potentially toxic elements should be conducted to assess the potential risk of environmental contamination, and effective measures such as permeabilization control should be taken to contain potential hazards. To further assess the feasibility of using CFA for Re phytoextraction, field trials should be carried out to confirm the results of the pot experiment, effects of CFA application rate on the accumulation of plant biomass and Re in the aboveground parts of plants warrant further investigation under field conditions, and the cost and benefit should be calculated based on the results of field trials.

5. Conclusions

To our knowledge, this is the first study on phytoextraction of Re by plants from CFA-amended soil. For lucerne and erect milkvetch, plant growth was considerably promoted, and Re concentration in plants was significantly increased when CFA was applied to the alkaline soils at appropriate rates. Both lucerne and erect milkvetch showed potential for phytoextracting Re from CFA-amended alkaline soils. Using CFA for soil amendment and phytoextraction of Re from the CFA-amended alkaline soils is expected to return an economic profit and offer a solution for the waste disposal problem of CFA as well. However, the feasibility of using CFA for Re phytoextraction should be assessed through further experiments under field conditions, and the development of a cost-effective and environment-friendly biotechnology to phytoextract Re from CFA warrants thorough multi-disciplinary studies.

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