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ORIGINAL PAPER

Plants to harvest rhenium: scientific and economic viability

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Abstract Rhenium (Re) is one of the rarest (7×10^{-8} %) and most widely dispersed elements on Earth's upper crust. As a consequence of its scarcity, Re is also one of the most expensive metals in the world market. Re is indeed highly demanded by the aerospace industry for the production of high-temperature superalloy turbine blades. There is a lack of study on the viability of Re phytomining. The occurrence of Re in vegetation surrounding natural and anthropogenic sources of Re suggests the ability of plants for Re accumulation and biogeochemical indication. Here we studied the aptitude of Indian mustard and scouring rush to uptake Re, in order to test the feasibility of Re phytomining. An organic substrate was spiked with $KReO_4$ to attain Re concentrations of 5, 10, 20, 40, and 80 mg kg^{-1}. The plants were grown for 45 and 75 days under controlled greenhouse conditions. Plant tissue samples from roots and shoots were collected in septuplicate at both harvests and analysed by atomic emission spectroscopy. Our results show high concentrations of Re in plants, ranging from 1553 to 22,617 mg kg^{-1} at 45 days and from 1348 to 23,396 mg kg^{-1} at 75 days for Indian mustard range. A profit of 3906 US$ ha^{-1} $harvest^{-1}$ is expected from the recovered Re. Our findings thus demonstrate for the first time the scientific and economic viability of Re phytomining.

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Introduction

Rhenium is one of the rarest elements on Earth, with an abundance fivefold lower than gold and an estimate concentration of 0.4–0.6 µg kg^{-1} in the upper crust (Tagami and Uchida 2010; Kabata-Pendias 2011). Due to its scarcity, Re was not discovered until 1925, making it the last stable element to be detected (Tagami and Uchida 2010). Because of its distinctive physicochemical properties, Re is also one of the most expensive metals in the world market (Naumov 2007; Polyak 2014a). It is highly sought after by the aerospace industry, because Re-containing turbine blades allow operation at higher temperatures, extending engine life, increasing fuel efficiency, and enhancing engine performance (Naumov 2007; Polyak 2014a). Additional applications of Re, principally as tungsten–rhenium and molybdenum–rhenium alloys, comprise electromagnets, electron tubes and targets, flashbulbs, heating elements, ionization gauges, mass spectrographs, metallic coatings, semiconductors, thermocouples, vacuum tubes, and X-ray tubes, to name a few (Polyak 2014a, b). Bimetallic platinum–rhenium catalysts are employed in petroleum reforming for the production of lead-free gasoline, and in a smaller scale, to make high-octane hydrocarbons like benzene, toluene, and xylenes (Naumov 2007; Polyak 2014a). With an ever-increasing demand of Re, current world production is 53 tonne per year, featuring Chile, USA, Poland, Uzbekistan, Kazakhstan, and Russia as the leading producers (Polyak 2014a, b).

Although widely distributed in the environment at ultratrace levels, high concentrations of Re can be found in molybdenite ore, copper sandstones, and black shale, as well as in sea sediments under anoxic conditions. Soils and natural waters usually present low Re concentrations. Anthropogenic sources of Re include motorways, coal-burning plants, non-ferrous metals smelters, scrap recycling units, copper processing factories, and specially, copper–molybdenum mines (Tagami and Uchida 2010; Bozhkov et al. 2012; Polyak 2014a; Zakrzewska-Koltuniewicz et al. 2014). Molybdenite (MoS_2), a by-product in copper mining, is the main host of Re, typically as rhenium disulphide (ReS_2). Conventionally, MoS_2 concentrates are roasted between 500 and 700 °C to obtain molybdenum trioxide (MoO_3), while volatile rhenium heptoxide (Re_2O_7) is released with the flue gases. Re_2O_7 is then scattered on the soil, where in the presence of water it is promptly transformed to perrhenate (ReO_4^-), the most stable form of Re (Askari Zamani et al. 2005; Tagami and Uchida 2010). Bioleaching of MoS_2 concentrates is, to a lesser extent, another pathway to the generation of ReO_4^- (Askari Zamani et al. 2005). Because of their great mobility and solubility, ReO_4^- ions can be broadly dispersed at significant concentrations in areas surrounding copper–molybdenum mines and copper processing factories (Askari Zamani et al. 2005; Bozhkov et al. 2007, 2012).

Data on Re accumulation in plants, both in laboratory experiments as in field measurements in the vicinity of copper mines (Tagami and Uchida 2005, 2010; Kabata-Pendias 2011), open a window of opportunity for phytomining, a cost-effective and environment-friendly plant-based technique to extract valuable metals from low-grade surface ores or mineralized soils, and obtain an economic profit after their recovery (Sheoran et al. 2013). Nevertheless, plants should be able to hyperaccumulate Re or at least present sufficiently high biomass yield and shoot Re concentrations, to attain economic viability. Hyperaccumulation is defined as the capacity of a plant to accumulate a given metal to a concentration 10–1000 times greater than ‘normal’ plants growing in the same environment (Anderson et al. 2005; van der Ent et al. 2013). Moreover, hyperaccumulators should also present a bioconcentration factor (ratio of metal concentration in plant to soil) >1 and a translocation factor (ratio of metal concentration in shoots to roots) >1 (Sun et al. 2008; Ali et al. 2013). In fact, Bozhkov et al. (2012) have reported promising results on the practicability of Re phytomining; however, their most comprehensive study fails to provide critical methodological details, experimental consistency, and statistical analysis, preventing to duplicate their work. Our objective was to evaluate the feasibility of Re phytomining, using Indian mustard [*Brassica juncea* (L.) Czern], a species well known for its aptitude in the fields of phytoremediation and phytomining (Sheoran et al. 2009; Novo et al. 2013; Hunt et al. 2014), and scouring rush [*Equisetum hyemale* (L.)], a member of the ‘horsetail’ group whose capability to accumulate precious metals has been recognized since the early years of biogeochemistry (Warren and Delavault 1950; Cannon et al. 1968; Dunn 2007).

Materials and methods

Substrate preparation

Commercial organic substrate (Gärtner-substrat, Gramoflor GmbH & Co. KG, Vechta, Germany) was added to pots and amended with potassium perrhenate (KReO4) to consist 5 treatments with increasing Re concentration into the substrate: 5, 10, 20, 40, and 80 mg kg^{-1}. Some of the substrate physicochemical properties include 2.34 µS cm^{-1} (salinity), pH of 5.6, 180 mg L^{-1} of N, 200 mg L^{-1} of P_2O_5, 250 mg L^{-1} of K_2O, 150 mg L^{-1} of Mg, and 120 mg L^{-1} of S. Every pot, with a volume of 1.5 L, was manually mixed to ensure homogenization. Each treatment was prepared in septuplicate per plant species and harvest. One week before starting the plant experiment, ‘Rhizon’ soil pore water samplers (Eijkelkamp Agrisearch Equipment, The Netherlands) were inserted into the substrate of each treatment, through a pre-drilled hole in the corresponding pots at an angle of 45°. The holes were then sealed with silicone to avert water leakage. Vacuum tubes (10 mL) were attached through a Luer-lock system, and hypodermic needles were used to extract pore water (PW). ‘Rhizon’ samplers are an effective, simple, and low-cost method for collecting soil solution that most likely represents the fraction of soil water extracted by plants (Clemente et al. 2008).

Plant growth and development

Healthy Indian mustard seeds (Herbiseed, Berkshire, UK) were allowed to germinate and grow in organic substrate till two fully expanded leaves and then were transferred to pots containing the different Re treatments, at the rate of 3 seedlings per pot. After 14 days, plants were thinned to one per pot according to uniform criteria. Scouring rush was cultivated from identical rhizome fragments in organic substrate. Immediately after the emergence of the stem, one plant was moved to each experimental pot for development. Growth of both species occurred under controlled greenhouse conditions: photoperiod of 11:13 h (light/dark), temperature of 22 ± 2 °C and 65 ± 5 % relative air humidity. Soil moisture was maintained at 60 ± 5 % with Milli-Q deionized water (Milli-Q System, Millipore, Billerica, MA, USA), according to field capacity on a daily basis. Plants, 7 replicates per treatment, were harvested 45 and 75 days after sowing for posterior analysis.

Determination of Re in the plant tissue

At the end of each harvesting period, plant shoots were harvested, washed once with tap water and twice with Milli-Q deionized water in order to remove any dust deposits, and oven-dried 48 h at 65 °C. The roots were carefully taken out of the substrate, washed once with tap water and twice with deionized water in order to remove any surface substrate, and oven-dried at 65 °C for 48 h. Dry weights (DW) of both plant parts were determined, and all samples were milled, air-dried, and stored until metal content determination. Dry plant tissue samples were ashed in a muffle furnace at 450 °C during 4 h. The resulting ash was dissolved through acid digestion using a mixture of HNO_3 and HCl (1:3 v/v) (Jones 2001). In addition, the soil pore water samples were stabilized with 10 % (v/v) 0.1 M HNO_3. Elemental analysis was processed by inductively coupled plasma atomic emission spectroscopy (ICP-AES; PerkinElmer Optima 4300 DV, PerkinElmer, Waltham, Massachusetts, USA).

Statistical analysis

All analytical results were obtained from 7 replicates. Paired samples *T* test was employed for the evaluation of differences between harvests of the same treatment. The Kolmogorov–Smirnov and Levene tests were used to confirm the normality assumption and assess the equality of variances, respectively. One-way analysis of variance (ANOVA) was carried out, followed by Tukey's test for homoscedastic data and the Jonckheere–Terpstra test in case of heteroscedasticity, for post hoc comparisons between treatments. All statistical analyses were computed using IBM SPSS Statistics, version 22.0 (IBM Corp., Armonk, New York, USA).

Results and discussion

With the objective of evaluating the feasibility of Re phytomining, five increasing substrate Re concentrations (5, 10, 20, 40, and 80 mg kg^{-1}) were employed to assess the capacity of Indian mustard and scouring rush to extract and accumulate this metal. Plant tissue from both species was analysed 45 and 75 days after sowing. Apart from the concentrations of Re in the roots, we observed striking differences between Indian mustard and scouring rush and thus decided to depict the results for each species individually.

The biomass yield and metal concentrations of aerial plant parts are vital for metal phytomining because they govern the quantity of metal to be harvested from each plant (hereinafter referred to as harvestable amount). The aboveground mass of scouring rush (Fig. 1a) did not show significant differences between treatments in both harvesting periods, whereas Indian mustard considerably reduced its biomass with increasing Re concentrations at 45 and 75 days (Fig. 1b). Nevertheless, comparisons between harvests show a pronounced increment in the biomass of the two plants, which suggests the aptitude of these species to endure Re during their growth. The concentration of Re in the shoots of scouring rush grew from 74 and 87.4 mg kg^{-1} to 925 and 714 mg kg^{-1} at 45 and 75 days, respectively, as the supply of this metal to the substrate increased from the minimum to the maximum concentration (Fig. 2a). The Indian mustard displayed an analogue pattern, but its Re levels were an order of magnitude higher than those of scouring rush (Fig. 2b). In fact, the Indian mustard exhibited a remarkable capacity to uptake Re, with concentrations within the different treatments spanning from 1553 to 22,617 mg kg^{-1} at 45 days,

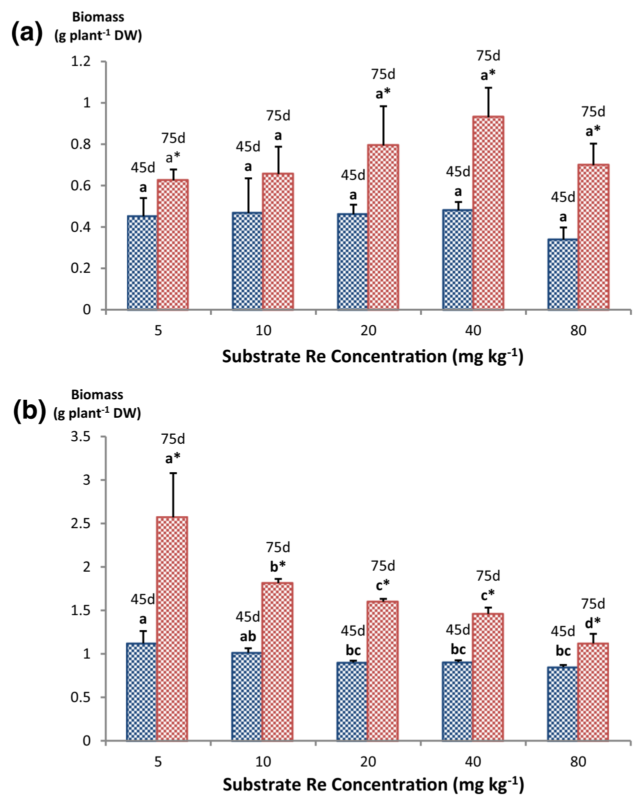

Fig. 1 Mean values for the dry weight of the aboveground parts of **a** scouring rush and **b** Indian mustard at 45 and 75 days (45 and 75 days). *Error bars* show the standard deviation. *Different letters* indicate significant differences between treatments of the same harvest at $p < 0.05$. An *asterisk* indicates significant differences between harvests for the same treatment at $p < 0.05$. All values are calculated from 7 replicates. *Note* that the biomass of scouring rush did not show significant differences between treatments, whereas Indian mustard considerably reduced its biomass with increasing Re concentrations. However, both species show a pronounced biomass increment for each treatment between harvests

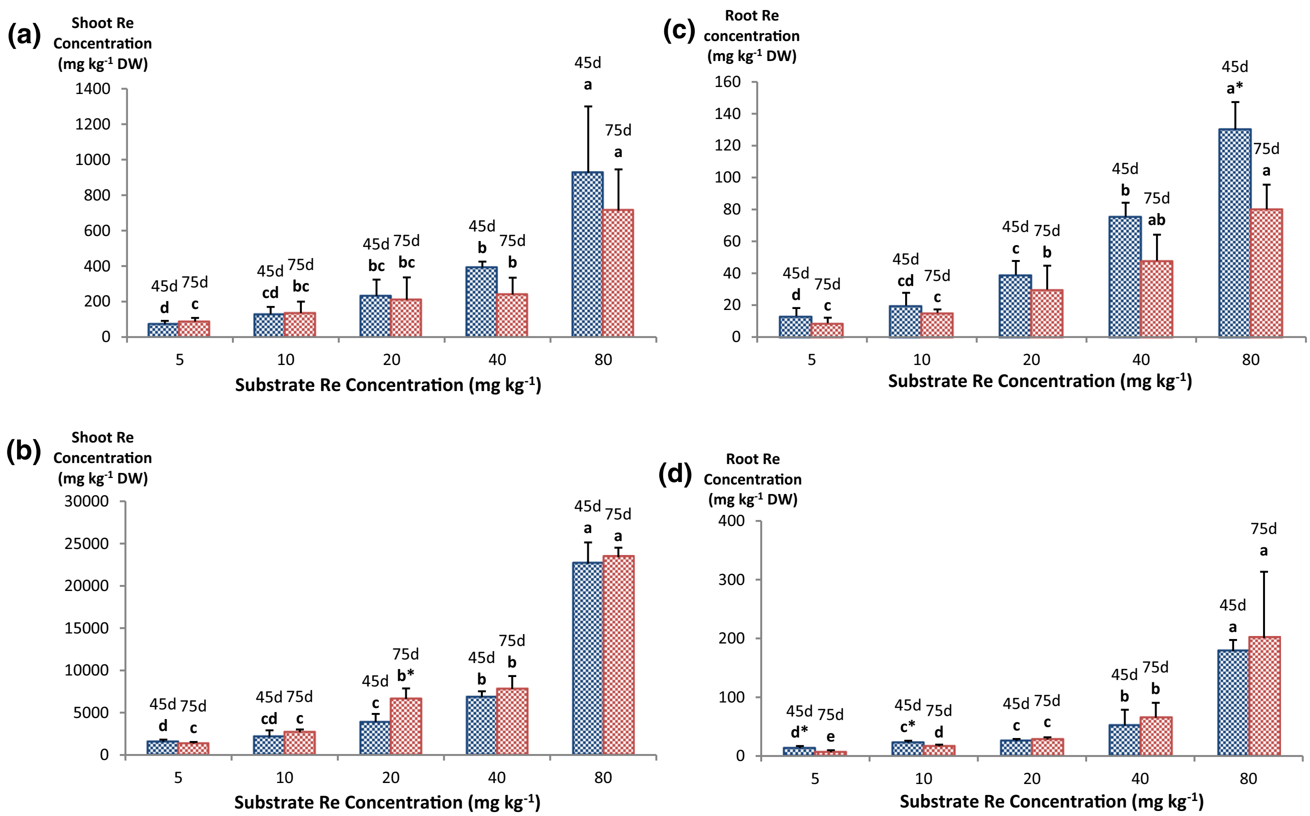

Fig. 2 Mean values for the concentration of Re in the shoots of **a** scouring rush and **b** Indian mustard, and the concentration of Re in the roots of **c** scouring rush and **d** Indian mustard at 45 and 75 days (45 and 75 days). *Error bars* show the standard deviation. *Different letters* indicate significant differences between treatments of the same harvest at $p < 0.05$. An *asterisk* indicates significant differences between harvests for the same treatment at $p < 0.05$. All values are calculated from 7 replicates. The concentration of Re in the shoots of scouring rush and Indian mustard grew as the amount of Re in the substrate increased. Yet, note that the levels of Re in the latter were an order of magnitude above those of scouring rush. Similarly, the roots of both species have also accumulated higher concentrations of Re with greater amounts of this metal in the substrate

and 1348 to 23,396 mg kg^{-1} at 75 days. These results imply an increment of 1356 and 1636 % at 45 and 75 days, respectively, between the treatments of 5 and 80 mg kg^{-1}. Although to a much smaller degree than the shoots, the roots of scouring rush and Indian mustard have also accumulated higher concentrations of Re with greater amounts of this metal in the substrate (Fig. 2c, d).

While the content of Re in the roots is not considered a critical result due to the impracticability of harvesting belowground plant parts in a commercial phytomining operation (reason why the root biomass has been disregarded in this study), it is useful to calculate the bioconcentration and translocation factors. Interestingly, the concentrations of Re in the roots of both species are identical on each treatment and harvest, but their relationship to the respective shoot concentrations differs by an order of magnitude. Thus, the translocation factor for scouring rush ranges from 5 to 7 and 5 to 11 at 45 and 75 days, respectively, whereas for Indian mustard it varies from 98 to 151 and 132 to 256 at 45 and 75 days, respectively (Table 1).

These values indicate the capacity of both species, but most especially Indian mustard, to translocate Re to their aerial parts. It has been suggested that since it is readily bioavailable to plants, ReO_4^- may use nutrient anion transporters on the root surface, and go together with the excess nutrient cation flow, acting as a substitute for Cl^-. Re would then accompany K^+ or other cations through the xylem and be translocated to the shoot (Tagami and Uchida 2005). For the accurate estimation of the bioconcentration factor, we have determined labile concentrations of Re through the analysis of pore water extracted from the substrate of each treatment (4.99 ± 0.23, 8.18 ± 0.86, 14.59 ± 1.58, 27.69 ± 3.01, and 47.08 ± 7.44 mg kg^{-1}, from the lowest to the highest Re substrate treatment, respectively; values are means ± the standard deviation, and all values are calculated from 7 replicates). The concentration of Re in pore water (mg L^{-1}) was converted to a soil weight basis (mg kg^{-1}) through the multiplication by the water-holding capacity of the soils (L kg^{-1}) (Clemente et al. 2010). The bioconcentration factor of scouring rush

Table 1 Translocation factor (Re concentration ratio of shoot to root) and bioconcentration factor (Re concentration ratio of shoot to substrate) in scouring rush and Indian mustard at 45 and 75 days

Plant	Re (mg kg^{-1})	Translocation factor		Bioconcentration factor	
		45 days	75 days	45 days	75 days
Scouring rush	5	6.5 ± 3.5^a	11.2 ± 4.0^a	14.8 ± 3.6^a	17.5 ± 4.2^a
	10	7.3 ± 3.9^a	9.1 ± 4.4^a	15.7 ± 5.2^a	16.5 ± 7.9^a
	20	5.9 ± 1.6^a	8.7 ± 6.6^a	15.9 ± 6.3^{a*}	14.4 ± 8.6^a
	40	5.2 ± 0.5^a	5.1 ± 1.0^a	14.2 ± 1.2^a	8.7 ± 3.4^a
	80	7.4 ± 3.5^a	9.4 ± 4.0^a	19.7 ± 8.0^a	15.2 ± 4.9^a
Indian mustard	5	128.7 ± 43.9^a	256.0 ± 131.7^a	311.5 ± 37.6^b	270.3 ± 52.7^b
	10	97.9 ± 19.8^a	170.8 ± 70.0^a	264.8 ± 40.4^b	328.7 ± 92.0^b
	20	151.0 ± 46.3^a	241.6 ± 56.2^a	265.2 ± 85.3^b	453.7 ± 67.1^a
	40	151.6 ± 54.0^a	131.6 ± 38.5^a	246.6 ± 55.9^b	281.3 ± 25.6^b
	80	128.1 ± 14.2^a	144.5 ± 72.1^a	480.4 ± 23.6^a	497.0 ± 53.5^a

Values are means ± the standard deviation. *Different letters* indicate significant differences between treatments of the same harvest at $p < 0.05$. An *asterisk* indicates significant differences between harvests for the same treatment at $p < 0.05$. All values are calculated from 7 replicates

fluctuates between 14 and 20 at 45 days, and 9 and 18 at 75 days, denoting the plant's fitness to extract Re from the substrate (Table 1). Consequently, given the widespread presence of this species across the globe (Balbuena et al. 2012), it becomes a potential tool for biogeochemical prospecting of Re. Still, the values obtained for the Indian mustard were 15- to 33-fold higher, oscillating from 247 to 480 at 45 days, and 270 to 497 at 75 days (Table 1). These results highlight the extraordinary ability of this species to absorb Re from the substrate. Moreover, the absence of significant differences in most of the comparisons between harvests for these factors reflects the competence of the two plants to extract and translocate Re, even when subjected to concentrations unlikely to be found in natural environments (20, 40, and 80 mg kg^{-1}). Lastly, the harvestable amount of Re in scouring rush (Fig. 3a) was solely driven by the concentrations of Re in the shoots (Fig. 2a), since there were no significant differences on the biomass yield across the treatments (Fig. 1a). Conversely, the upsurge of the harvestable amount on Indian mustard (Fig. 3b) was also boosted by the increasing concentrations of Re in the shoots, whose effect largely surpassed that of the lessening of the aboveground mass (Fig. 1b). Once more, the Indian mustard expressively bested the scouring rush, achieving harvestable amounts of Re two orders of magnitude higher. Furthermore, the significant differences noted between the harvestable amounts of Re on Indian mustard at 45 and 75 days advise that a later harvest may be more profitable.

Considering the results obtained for Indian mustard plants developed on the 5 mg kg^{-1} treatment [concentration akin to areas neighbouring copper mines (Bozhkov et al. 2012)], the current market price of Re (3400 US$ kg^{-1}) (Polyak

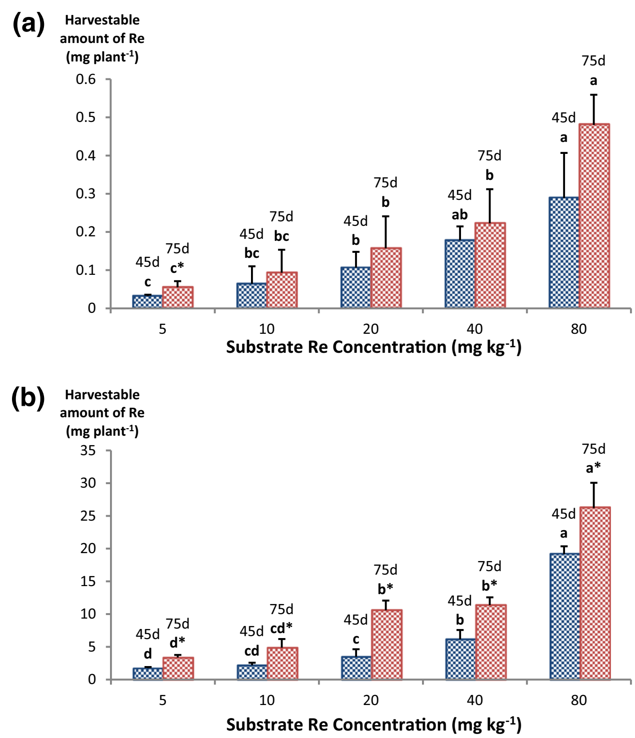

Fig. 3 Mean values for the harvestable amount of Re per plant of **a** scouring rush and **b** Indian mustard at 45 and 75 days (45 and 75 days). *Error bars* show the standard deviation. *Different letters* indicate significant differences between treatments of the same harvest at $p < 0.05$. An *asterisk* indicates significant differences between harvests for the same treatment at $p < 0.05$. All values are calculated from 7 replicates. The harvestable amount of Re in both species increased with greater concentrations of Re in the substrate. However, note that the results of the Indian mustard were two orders of magnitude above those of scouring rush. In addition, the harvestable amount of the Indian mustard increased significantly from 45 to 75 days

2014b), a 98 % efficiency on the Re extraction process from plant mass (Bozhkov et al. 2012), a moderate plant density (50 plant m^{-2}) (Liu et al. 2012), the energy production income from biomass incineration (215 US$ $tonne^{-1}$) (Harris et al. 2009), the typical agricultural costs (1000 US$ ha^{-1}, including fertilizers, irrigation, and seed costs) (Sheoran et al. 2013), and a projected cost of 1000 US$ ha^{-1} for the extraction and purification process (Abisheva and Zagorodnyaya 2002; Harris et al. 2009; Tagami and Uchida 2010; Bozhkov et al. 2012; Wilson-Corral et al. 2012), then a profit of 3906 US$ ha^{-1} $harvest^{-1}$ would be expected from the recovered Re. In the light of this result, the profitability of Re phytomining could be used to finance the phytoremediation of soils with coexisting and often toxic metals (As, Cd, Cr, Cu, Mn, Pb, Se, and Zn) (Stankovic et al. 2014) that can be concurrently removed by the Indian mustard (Vamerali et al. 2009) and promote carbon dioxide abatement (Witters et al. 2012; Yang et al. 2012). Moreover, the earnings from Re phytomining may be of particular interest for developing countries, not only because of its simplicity, low cost, and potential economic impact, but also due to the vast areas of tailings from commercial and artisanal mining found in these nations (Wilson-Corral et al. 2012; Anderson 2013; Anderson et al. 2014; Krisnayanti and Anderson 2014).

Conclusion

The findings of this study demonstrate that scouring rush and Indian mustard are Re hyperacccumulators, for both species accumulate this metal to a concentration 100 times greater than plants found in environments with similar levels of Re (Kabata-Pendias 2011; Bozhkov et al. 2012), and their translocation and bioconcentration factors noticeably surpass 1 within a comprehensive array of Re treatments. More importantly, contingent to the transferability of these results to a real soil, this work suggests the feasibility of obtaining an economic revenue from the accumulation of Re on Indian mustard.

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