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Original Research

Metal Uptake in Reeds from 'Flowback' Fluids

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Abstract

Flowback fluids from the hydraulic fracturing process that contain high levels of metals may pose environmental risks. This laboratory study investigated the remediation potential of *Phragmites australis* to sequester Ba and Sr from flowback liquids. The results indicated that reeds can uptake different concentrations of Ba and Sr from solutions. Roots were the main tissues for metal storage, with 12.26±0.58 mg/g Ba and 2.92±0.12 mg/g Sr sequestered in roots from solutions that contained 80 mg/L Ba and 20 mg/L Sr. The more metals in solutions, the more metals that entered the biomass. Reed, which possesses strong adaptability to different conditions and environments, is a good candidate to clean heavy metal-contaminated water or soil via phytoremediation. Field research on metal accumulation in reeds cultured in flowback liquids is needed to further prove its potential to *in situ* remediation of a heavy metal-contaminated environment.

Keywords: reeds, phytoremediation, barium, strontium

Introduction

Hydraulic fracturing is currently one of the primary techniques for extracting gas and oil for energy production across the United States. During the hydraulic fracturing process, large amounts of water, sands, and chemicals are injected underground to fracture rocks and release gas or oil. According to future energy forecasts, energy production from hydraulic fracturing will double by 2035 [1]. The process involves utilizing millions of gallons of fresh water each year and managing high concentrations of toxic elements released in flowback water [2]. "Flowback" fluids produced during the hydrofracturing process currently pose potential environmental risks [1, 3]. Flowback fluids are water that return to the surface when the hydraulic fracturing process is done [1]. High concentrations of toxic elements such as barium (50-9,000 ppm) and strontium (50-6,000 ppm) are reported in most flowback water [1, 4-5].

Currently, treating and removing metals in flowback fluids is mainly done by utilizing centralized wastewater treatment. Centralized wastewater treatment facilities include basic separation techniques such as precipitation for disposal of oil and metals to advanced treatments such as membrane bioreactor and reverse osmosis for removal of total dissolved solids (TDS), dissolved organics, and boron [2]. The effluents from centralized wastewater treatment facilities mainly are for reuse in fracturing activity, and/or direct discharge to surface water with a National Pollution Discharge Elimination System [2]. Although reuse minimizes the wastewater management procedures in the

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short term, it may result in the accumulation of dissolved solids [2]. Eventually, wastewater reused more than once will still need to be disposed of, either through centralized wastewater treatment or injection [2]. Injection wells are regulated under the Safe Drinking Water Act. They are designed and constructed to inject fluids to authorized zones that avoid contamination of underground sources of drinking water [6]. However, these treatment methods are costly, and have high energy and chemical demands. Additionally, centralized wastewater treatment cannot effectively remove all of the toxic chemicals. High levels of toxic metals and radioactivity have been reported in drinking water intake streams and in rivers near wastewater treatment outflows [7]. For example, high levels of toxic metals (248.6-1084 mg/L Sr, 95-140 mg/L Ba), and radioactive chemicals (benzene and toluene) have been reported in effluent discharge from treatment sites [7].

A promising method for removing metals from surface water contaminated by spilled flowback fluids or rivers near centralized wastewater treatment outflows is plant-based technologies known as phytoremediation. As a clean, simple, low-cost, non-environmentally disruptive green technology, phytoremediation has attracted more and more attention from the public [8]. Phytoremediation is the use of plants to restore a contaminated environment [9]. Plant species that can survive under atrocious conditions (i.e., low pH, low levels of nutrients, and high metal concentrations) such as common reed (*Phragmites australis*) and cattail (*Typha latifolia*) are commonly used in wetlands for wastewater treatment [10-11]. Phytoremediation has also been reported to be a potential way to remove metals from acid mine drainage (AMD)-contaminated water or soil [12]. However, most of the studies related to phytoremediation have focused on the removal of organic contaminants or single metals from water or soil. Very little is known about the effect of phytoremediation on removing multiple toxic metals from contaminated water. In addition, studies on phytoremediation of hydraulic fracturing fluid-contaminated water have not been widely reported. This project studied the potential of plants to uptake metals from hydraulic fracturing-contaminated solutions. Reed was used due to its high tolerance to metals and high reproduction ability. The main metals in consideration were strontium and barium because of their extreme harmful effects to human health and the ecological environment. For instance, barium compounds can cause effects ranging from diarrhea to tachycardia to ventricular fibrillation in the animal experiments [13]. Risks of cancer increase with increased exposure to strontium [14].

Methods and Materials

The rhizomes of common reed *Phragmites australis* were purchased from Lorenz's OK seeds, LLC (Okeene, Oklahoma). They were initially grown in commercial potting soil (Micracle-Gro lawn products, Inc.) in pans (45×25×7.5 cm). Two cm of potting soil was placed in the bottom of each pan. This was followed by the rhizomes and another 2.5 cm of potting soil. The rhizomes were cultured in a greenhouse under natural light conditions. The average temperature of the greenhouse was 22ºC and humidity was 50%. Five-hundred ml distilled (DI) water was sprayed into each pan every day to maintain soil moisture. After 30 days of growth in the potting soil, seedlings with similar biomass were transferred into artificial solutions to initiate experiments. Prior to being transferred, the rhizomes of the reeds were rinsed with DI water to remove the attached potting soil.

Artificial hydraulic fracturing-contaminated solutions were prepared by analytical grade metal salts: barium as $BaCl_2$ and strontium as $SrCl_2$. Based on the typical concentration of the hydrofracturing-contaminated stream and the tolerant limits of plants, the metals concentration were (5 mg/L, 20 mg/L, and 80 mg/L for Ba and 5 mg/L, 10 mg/L, and 20 mg/L for Sr) [7]. The pH of the solution was adjusted to 7. In order to support the growth of reeds, nutrients were added to the solution. The main nutrients were N (620 mg N/L, NH_4NO_3) and P (94 mg P/L, KH_2PO_4) [12].

At day 1, the solution was prepared. Then plants were transferred to the solutions and cultured in a greenhouse for one week. One reed was cultured in 1L solution that contained metals and nutrients. For each condition, three containers and reeds were used. At the end of the culturing period, reeds were harvested and then air dried. After drying, the tissues were separated into roots, rhizomes, and shoots, then weighed and crushed with mortar and pestle. The milled tissues were then digested following the methods described in Guo and Cutright [12]. Finally, the solutions were filtered by 0.45 µm filters and analyzed by atomic absorption spectroscopy 6300 (AAS).

Data on metal uptake in reeds were analyzed with one-way ANOVA using the Minitab statistical package (Minitab 16). Differences between specific metal levels were identified by Tukey's test at 5% probability.

Results and Discussion

Barium was found in the biomass of reeds cultured in solutions with different levels of metals. The translocation factors (TF) – the ratio of metal in plant shoots to that in roots – was listed in Table 1 [15]. The TF of Ba was less than 1, which also suggested that the translocation of Ba into the aboveground organs (shoots) of reeds was restricted. These findings were in accordance with previous studies. Ali et al. [16] indicated that large amounts of heavy metals were preferentially accumulated in the roots and rhizomes of reeds. According to Bonanno and Giudice [17], the concentration of Mn, Cu, and Cr in reeds grown in a wetland decreased in the order of root > rhizome > shoot. Baldantoni et al. [18] also found that metal levels were more than one order of magnitude lower in the shoots than those in the roots of reeds. Most of some other trace metals (e.g., Ag, Co, Be, Pd) were also accumulated in belowground organs rather than aboveground tissues of reeds [19]. However, Fawazy et al. [20] indicated that most Cu was stored in the roots while higher levels of Pb were found in the leaves of reeds. Similarly, according to the studies of Rzymski et al. [21], majorities of metals (e.g., Fe, Mn, Zn, Cr) were found in the roots, but higher concentrations of Cd and Pb were found in the leaves of reeds [21].

Compared to other elements, there was not much research related to Ba accumulation in reeds. It was reported that Ba was mainly stored in the roots of reeds from a mining area [22]. Bonanno [23] also indicated that the trend of Ba contents in reeds was root > rhizome > leaf > stem. The accumulations of different metals in reeds are controlled by many factors such as specific physiological mechanisms, the concentrations and bioavailability of metals in the surrounding environment, and other physical/chemical environmental conditions [24-26]. One of the reasons that reeds can immobilize most toxic elements in the roots is to protect rhizomes, which are the only persistent part of the plant, thus less metals can be transferred from rhizomes to shoots [18]. The TF of Ba in this study was low (Table 1), which was similar to the value in the reports of Li et al. [22]. They found that the TF of Ba in reeds collected from uranium mill tailings was around 0.19 [22]. The low TF of Ba may be due to its low mobility as this metal was easy to be precipitated [23]. However, according to the study of Cicero-Fernández et al. [19], the TF of Ba in two populations of reeds transferred to the same contaminated sediment was totally different: one population initially grown in an uncontaminated area possessed a TF of 0.31 while the other, originally collected from contaminated sites, had a TF of 1.37. Guillaume et al. [27] also stated that TF can be highly different even for the same plant species. These differences of TF may reflect the fact that the mobility and translocation of metals in plants depends on such environmental factors as redox potential of soil or solution, and the concentration of a target element [28, 29]. The plant age and ecotype may also modify the translocation of metals to plant shoot from roots [30].

More Ba was accumulated in the roots and rhizomes of reeds cultured in solutions with high levels of Ba than those in reeds grown in solutions with low levels (Fig. 1). For instance, the roots of reeds grown in solutions with low levels of metals (LM) accumulated 6.81±1.47 mg/g Ba, while the roots in solutions with high level of metals (HM) sequestered 12.26±3.14 mg/g Ba after one week. This is not surprising as previous research also indicated that accumulation of metals in plants was correlated with concentrations in substrate [31]. Deng et al. [32] also stated that the concentration of metals in the underground tissues of wetland plants showed strong positive correlations with the levels of elements in sediment. Similarly, Ghassemzadehas et al. [33] also found that the uptake of metals by reeds was related to the amount of metals in the environment. However, the levels of Ba in the roots systems of reeds cultured in solutions with middle levels of metals (MM) was not significantly different from those in roots in LM solutions. This may be due to the fact that the Ba concentration in solutions was not high enough to affect Ba uptake in reeds. According to Coscione and Berton [34], the difference of Ba contents in sunflower grown in artificial Ba-contaminated soils was not significant, until up to the dose of 300 mg/ kg Ba. Different from the roots, the Ba level in shoots of reeds was not affected by the concentration of Ba in solutions at all (Fig. 1). Similar to the findings of Junior et al. [35], the absorption of Ba in the aboveground tissues of sunflower plants was not affected by the increase of Ba doses. Plants growing in metal-enriched soils may develop some tolerance mechanisms to avoid uptaking more metals to the aboveground tissues [36]. According to the view of ecotoxicology, the limit of transferring heavy metals into shoots may help to avoid the passing of pollutants into the food chain via herbivores [19].

The accumulation of Sr in reeds was similar to the trends of Ba (Fig. 2). Most of Sr was accumulated in the roots with less Sr in the rhizomes and shoots of reeds. For instance, the roots of reeds grown in LM solution accumulated 1.28±0.20 mg/g Sr, while the shoots uptake 0.10±0.06 mg/g Sr after one week. The study of Sarap et al. [37] also found that Sr was more concentrated in the roots of winter wheat rather than the aboveground tissues. High levels of Sr were also found in roots other than

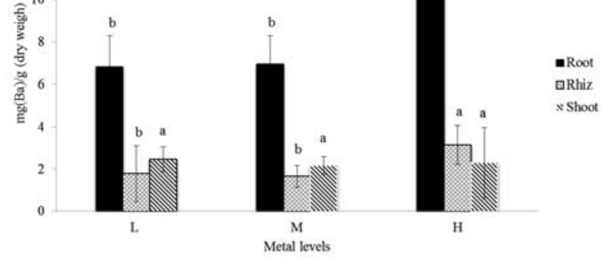

Fig. 1. Ba in the organs of reeds cultured in solution for four weeks. An error bar represented the standard deviation of triplicate samples. Different letters on the same plant organ indicated a significant difference at p<0.05; "Rhiz" is rhizome.

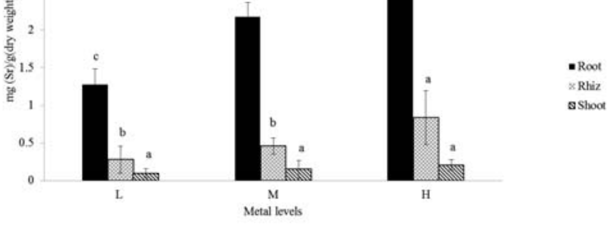

Fig. 2. Sr in the organs of reeds cultured in solution for four weeks. An error bar represents the standard deviation of triplicate samples. Different letters on the same plant organ indicate a significant difference at p<0.05; "Rhiz" is rhizome.

Table 1. Translocation factor of metals in reeds for four weeks.

Treatment	TF of Ba	TF of Sr
LM	0.38±0.09 a	0.07±0.04 a
MM	0.30±0.08 a	0.06±0.05 a
HM	0.19±0.13 b	0.07±0.03 a

LM is low level of metals, MM is middle level of metals, HM is high level of metals; results were reported as average±standard deviation, n = 3; different letters in the same column indicated a significant difference at $p<0.05$

shoots in reeds grown in an area affected by urbanization agriculture in Italy [23]. However, a higher accumulation of Sr was detected in shoots rather than roots of other plants, such as sunflower [38]. These may be due to the fact that different plants had different tolerant mechanisms and uptake models to metals [39]. Other researchers also indicated that the accumulation of metals (e.g., Sr) in plants was affected by site-specific and plant-specific parameters such as the capacity of plant species, the availability of metals, and other competing elements [27, 40, 41]. For reeds, root was the main pathway for trace metals to enter tissues [42]. Thus it is not surprising that root is the main organ for storing Sr. With the increase of Sr in solution, more Sr entered roots and rhizomes. According to previous research, Sr concentration in the belowground organs of plants was proportional to the Sr level in solutions [43]. Bonanno [23] also indicated that Sr amounts in roots of reeds were significantly correlated with Sr levels in water.

According to Kartosentono et al. [44], the concentrations of Sr in shoots of *Solanum laciniatum* increased with the Sr levels in media. However, in our studies the amount of Sr in shoots was only slightly increased ($p>0.05$) with the increase of Sr levels in solutions, which reflected the low mobility of Sr in reeds. Similarly, Tsialtas et al. [40] found that the contents of Sr in shoots of *Trifolium* species were not influenced by the amounts of Sr in soil. Most other studies also reported the low mobility and the low translocation of Sr in plants [22, 45]. The translocation of Sr from roots to shoots in some plants was even reduced with the increase of Sr, which was a mechanism used to protect plants [46]. The calculated TF of Sr was very low (Table 1), which further proved that the translocation of Sr to shoots of reeds barely happened. Bonanno [23] also stated that the TF of Sr in reeds (about 0.05) was the lowest compared to other elements (e.g. Ba, Al, Fe, Co, V). Many studies found that most trace metals were stored in roots of reeds while the translocation to shoots was limited [21, 47, 48]. Thus, reeds that possess large biomass and an extensive root system are a good candidate for rhizofiltration, sequestering toxic metals from aquatic solutions with fewer contaminants remaining in the environment [49].

Conclusions

Traditional ways used to treat flowback fluids, such as centralized wastewater treatment facilities, are expensive and may not effectively remove some toxic metals. This study conducted laboratory experiments to investigate the potential of *Phragmites australis* to sequester Ba and Sr from flowback liquids. The results indicated that reeds have the ability to uptake Ba and Sr from flowback liquids. According to the measurement, 12.26±0.58 mg/g Ba and 2.92±0.12 mg/g Sr were sequestered in roots from solutions that contained 80 mg/L Ba and 20 mg/L Sr. The more metals in solutions, the more metals entered biomass.

Phragmites australis is known to be widely distributed in the world, survive in both terrestrial and hydroponic habitats, and possesses high adaptability to different conditions is a good candidate for phytoremediation technique to clean heavy metal-contaminated water or soil. Underground tissues were the main tissues for metal storage in reeds. Chelator may be used to increase metal accumulation and metal translocation to aboveground organs. Studies on the interactions between rhizosphere microorganisms and plants may be conducted to find the ways to enhance plant growth and increase the efficiency of phytoremediation. More research on the physiology alternation in reeds caused by heavy metals is recommended. Further studies on the metal accumulation in reeds cultured in real flowback liquids are also needed. This will assist with any further research on *in-situ* remediation of heavy metal-contaminated sites.

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