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PHYTOEXTRACTION OF ARSENIC FROM SOIL BY *LEERSIA ORYZOIDES*

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*The potential of *Leersia oryzoides* (rice-cut grass) to remediate arsenic-contaminated soil was studied in greenhouse pot experiments. *Leersia oryzoides* grown in soil amended with arsenic to a concentration of 110 mg kg⁻¹, extracted up to 305 mg kg⁻¹ and 272 mg kg⁻¹ arsenic into its shoots and roots, respectively, giving a shoot:root quotient of 1.12 and phytoextraction coefficients up to 2.8. Plants in the arsenic-amended soil showed visible signs of stress in the first 8 wk of growth, but then recovered. Based on the 132 plants that were grown in a surface area of approximately 180 cm², the calculated total arsenic taken up by shoots is 120, 130, and 130 g ha⁻¹ at 6, 10, and 16 wk, respectively, suggesting that additional arsenic could be removed by periodic mowing over a growing season. Extraction with a mixture of nitric acid and hydrogen peroxide indicated that the available arsenic was constant after the first 6 wk. Uptake is comparable to that reported for duckweed (*Lemna gibba* L.) and overlaps the low end of the values reported for Chinese brake fern (*Pteris Vittata* L.)*

KEY WORDS: *Leersia oryzoides*, arsenic extraction, shoot:root quotient (SRQ), phytoextraction coefficient (PEC)

INTRODUCTION

Arsenic has been used for industrial purposes for many years. Analyses of bronze originating from Near Eastern excavation sites show that, at the end of the fourth and at the beginning of the third millennium BC, alloys with an arsenic concentration up to 5% were in general use (De Ryck, Adriaens, and Adams, 2005). Arsenic is the 20th most abundant element in the earth's crust (National Research Council, 1977), making up about 5 mg kg⁻¹ of the earth's crust, with an average concentration of 2 mg kg⁻¹ in igneous and sedimentary rocks (Mandal and Suzuki, 2002). Its presence in many mineral deposits has resulted in its wide distribution in the environment (Murdoch and Clair, 1986). The most abundant forms of arsenic in the environment are the inorganic arsenites and

arsenates (Feng *et al.*, 2001). The trivalent compounds (arsenites) are more toxic than the pentavalent compounds (arsenates) (Cervantes *et al.*, 1994; Leonard, 1991), whereas most organic arsenic compounds generally are less toxic (Gochfeld, 1995). Some marine sediments could contain up to 3000 mg kg⁻¹ arsenic (Frankenberger, 2002) and soils can be contaminated with arsenic by mining activities, smelting of sulfide ores, application of pesticides, timber preservation, and arsenic-rich groundwater.

Contaminated soils typically have higher arsenic concentrations than is found in rocks (Peterson, Benson, and Zeive, 1981). Sandy soils and those derived from granites usually have the lowest arsenic concentrations; alluvial and organic soils have larger concentrations (Kabata-Pendias and Pendias, 1984). The concentrations that require notification and action within a state in the United States vary according to use (industrial, residential, agricultural, and recreational). The threshold concentrations for each use also vary widely (Association for the Environmental Health of Soils, 1998). However, in the state of Massachusetts, soil is considered to be contaminated when it contains ≥ 30 mg kg⁻¹ arsenic. A concentration of 300 mg kg⁻¹ arsenic in soil is the upper concentration limit allowed for building houses (Massachusetts Department of Environmental Protection, 2005). An estimated 300 metric tons of arsenic is present in soils, as well as in riverine and lacustrine sediments in the Aberjona watershed in eastern Massachusetts (Aurilio *et al.*, 1995). Soils in Barber Orchard, North Carolina, have been found to contain as much as 2460 mg kg⁻¹ arsenic (Salido *et al.*, 2003). Soils in some glacial tills in Canada have arsenic ranging from 1 to 6590 mg kg⁻¹ (Environment Canada, 1996).

Crops grown in arsenic-contaminated soils can take up considerable amounts of arsenic. English ryegrass (*Lolium perenne*), rape (*Brassica napus*), and sunflower (*Helianthus annuus*) grown in arsenic-contaminated soil for 4 mo were found to have arsenic concentrations in the leaves and grains that are above the Swiss tolerance limits for fodder (4 mg kg⁻¹) and food crops (0.2 mg kg⁻¹) (Gulz, Gupta, and Schulin, 2005). In West Bengal, India, vegetables contain up to 690 μ g kg⁻¹ of arsenic (Roychowdhury *et al.*, 2002); rice grown in the same area contains up to 605 μ g kg⁻¹ arsenic (Roychowdhury, Takunaga, and Ando, 2003). Bangladesh vegetables contain 70–3990 μ g kg⁻¹ arsenic (Das *et al.*, 2004).

The remediation of contaminated water bodies and soils typically involves the use of costly and laborious conventional engineering methods (*e.g.*, soil/water removal, soil blending) that are mainly designed for large-scale applications such as community water-treatment facilities (Garelick *et al.*, 2005). The efficient phytoextraction of metal and/or metalloid contaminants from soil can offer an alternative form of remediation for some applications. Ideally, a plant that is suitable for phytoremediation should have a high above-soil biomass that acts as a sink for the contaminant. Some plants, such as Chinese brake fern (*Pteris vittata*) (Tu, Ma, and Bondada, 2002) and duckweed (*Lemna gibba* L.) (Mkandawire and Dudel, 2005), are arsenic hyperaccumulators. Hyperaccumulating plants take up large amounts of a contaminant from soils and move them into their shoots (Brooks, 1998), without exhibiting any toxicity effects. Other plants that do not hyperaccumulate contaminants also have some potential applications in areas that are not ecologically suitable for the growth of a hyperaccumulating species. We have investigated the potential of *Leersia oryzoides* as an arsenic accumulator and its suitability for phytoremediation of arsenic-contaminated soils.

Leersia oryzoides (rice cut-grass) is a C3 grass in the family Poaceae. It is a perennial herb native to the United States with a wide ecological range, and flowers from June to October. *Leersia oryzoides* has characteristic rough, saw-toothed leaf edges, with

4–6 mm-long spikelets. It naturally grows in swamps, sloughs, wet meadows, roadside ditches and around ponds and lakes. Its stems are erect, branched, usually smooth, and can grow up to 150 cm tall. The leaves can grow up to 13 mm wide. *Leersia oryzoides* develops one flower per spikelet, with many spikelets arranged in a panicle up to 200 mm long; each spikelet is greenish-white, oblong, and 4–6 mm long. The grains, when they develop, are red-brown, ellipsoid, and 3 mm long (U.S. Department of Interior, United States Geological Services, 2005). *Leersia oryzoides* is widespread from southern Canada to the southern United States (Iowa State University, 2005), which makes it suitable for growth in all kinds of climates—temperate, arid, or tropical. *Leersia oryzoides* is an abundant early successional species in many wetlands (Farnsworth and Meyerson, 1999). *Leersia oryzoides* extracts metal contaminants from complex wetland sediments in the order $Zn > Cd > Cu > Pb$ (Lanza, 2002). An advantage of using a grass for phytoextraction is its aesthetic property, which makes it suitable for many locations. As a grass, regular mowing can facilitate quick removal of arsenic from contaminated soil.

MATERIALS AND METHODS

Soil and Plants

A standard potting soil was selected, as we have observed variation in the uptake of contaminants by the same plant in different soils. To each of three pots, 500-g masses of potting soil (Fafard Professional Formula potting soil, Conrad Fafard Inc., Agawam, MA, USA) was added to an aqueous solution containing 0.1041 g of sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Fisher Scientific). The mixture, which contained 50 mg kg^{-1} of arsenic (wet weight), was dried overnight in an oven at 120°C , cooled, and transferred to the pot. To ensure healthy growth of the seedlings, 50 g of Espoma Kelp Meal 1-0-2 fertilizer (The Espoma Company, Millville, NJ, USA) was mixed with the soil. The moisture content of the soil was determined from the loss on drying (120°C for 36 h) of three 200-g samples of the wet material. Seedlings of *Leersia oryzoides* that were 2 wk old were grown in each of the three pots. The grass seedlings were planted in close proximity to each other, to form a mat, and each pot contained 132 plants in an area of 180 cm^2 . In a control experiment, *Leersia oryzoides* seedlings were grown in 500 g of potting soil that was not amended with arsenic. All plants were grown in a greenhouse under conditions of controlled temperature ($25 \pm 2^\circ\text{C}$) and ambient light and photoperiod. They were watered daily with tap water to maintain the initial moisture content of approximately 55%. A plastic dish was placed under each pot in order to collect any leaked water, which was returned to the pot.

Sample Preparation and Measurement

E-pure deionized water (Barnstead/Thermolyne, Dubuque, IA, USA) was used for all preparations and dilutions. At 6, 10, and 16 wk after the seedlings were transplanted, six plants from each pot were divided into shoots and roots, and then washed thoroughly with deionized water to remove contamination from the soil. The plant samples were then dried in an oven at 105°C for 2 d, and ground in a mortar and pestle until all the material passed through a 250-mesh sieve (maximum particle size $61 \mu\text{m}$). The biomass of the six plants from each pot was pooled. Soil samples from the same pots as the plants, collected at the same time as the plants, were treated similarly. A weighed amount of the soil (between 10

and 500 mg) or plant material (10–500 mg) was treated with 3 mL of concentrated nitric acid and 1 mL of 30% v/v hydrogen peroxide. The mixture was then subjected to microwave digestion using a Model MDS-2100 microwave system (CEM, Matthews, NC, USA). The digested samples were centrifuged, filtered, evaporated to near dryness, and made up to 10 mL with deionized water. The solutions were then analyzed for total arsenic content using a PerkinElmer 4100ZL atomic absorption spectrometer equipped with longitudinal Zeeman background correction and a transversely heated graphite atomizer tube, fitted with an integral L'vov platform (PerkinElmer part number BO504053). The arsenic electrodeless discharge lamp (EDL) from PerkinElmer was powered by a PerkinElmer system 2-EDL power supply and operated at 350 mA. A slit width of 0.7 nm and a wavelength of 193.7 nm were selected for the experiments. A PerkinElmer (Shelton, CT) AS-70 autosampler was used.

RESULTS AND DISCUSSION

The average moisture content of the three soil samples was 54.7%. Thus, the soil contained 110 mg kg⁻¹ of arsenic on a dry weight basis. After 6 wk, the extractable (nitric acid and hydrogen peroxide) arsenic content of the soil was 24 mg kg⁻¹ (dry mass) for the arsenic-contaminated soils and was 1.4 mg kg⁻¹ for the control soil. The values at 10 and 16 wk were similar, showing no systematic trend, though it is known that arsenic and other pollutants can become more tightly bound over time (Jacobs, Syers, and Keeney, 1970).

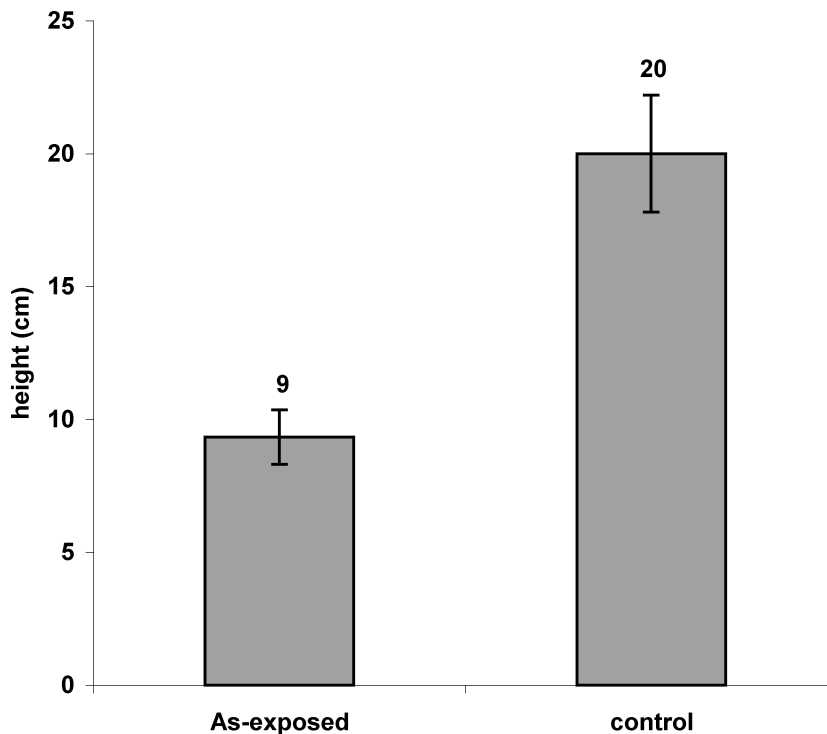


Figure 1 Heights of *Leersia oryzoides* plants 6 wk after planting. Values shown are average \pm 1 standard deviation ($n = 3$).

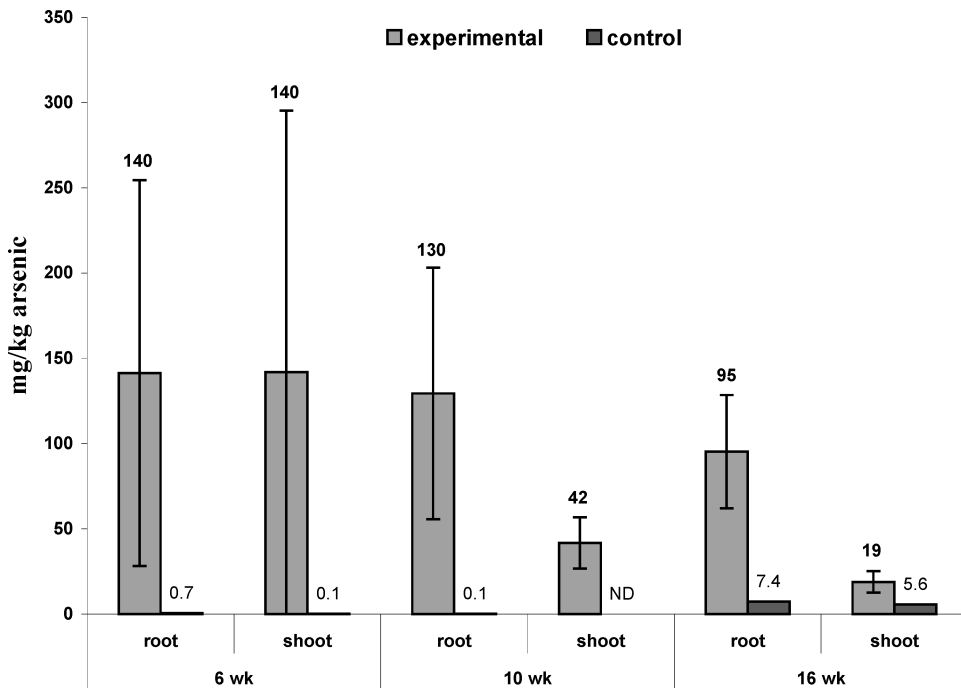


Figure 2 Uptake of arsenic from contaminated soil by *Leersia oryzoides* over a period of 16 wk. Values shown are concentrations in mg kg^{-1} expressed as average \pm 1 standard deviation ($n = 3$). ND stands for not detected.

Plants grown in arsenic-contaminated soil exhibited stunted growth beginning 10 d after being transplanted into the arsenic-contaminated soil, whereas plants grown in the control soil showed more lush growth. The height of the aboveground portion of the plants after 6 wk showed a marked difference between plants grown in contaminated and uncontaminated soil. Figure 1 provides average height measurements of three plants in the same pot. By the eighth week, plants grown in the contaminated soil had recovered. Visual inspection indicated that the growth of the plants in the arsenic-amended soil was the same as that in the control soil. No further quantitative comparisons of height and mass were made between the two groups. The arsenic contents of the roots and shoots over the study period are shown in Figure 2. The large variations in the initial period are considered to arise from the variation caused by the stress of the initial growth period.

At 6 wk, arsenic concentrations in the shoot were not significantly different from those in the roots. At 10 and 16 wk, the roots contained higher concentrations of arsenic than did the shoots. The concentration of arsenic in the plants decreased with time, as seen in Figure 2, indicating that the uptake of arsenic was not uniform over time. The decreases in concentration were accompanied by increases in the (dry) weight of the plants, as shown in Figure 3.

While the plant weight increased throughout the 16-wk period, the mass of arsenic taken up by the shoots remained practically constant after 10 wk. Figure 4 shows estimates of the potential phytoextraction of arsenic from a hectare ($10,000 \text{ m}^2$) of soil based on values observed in these studies.

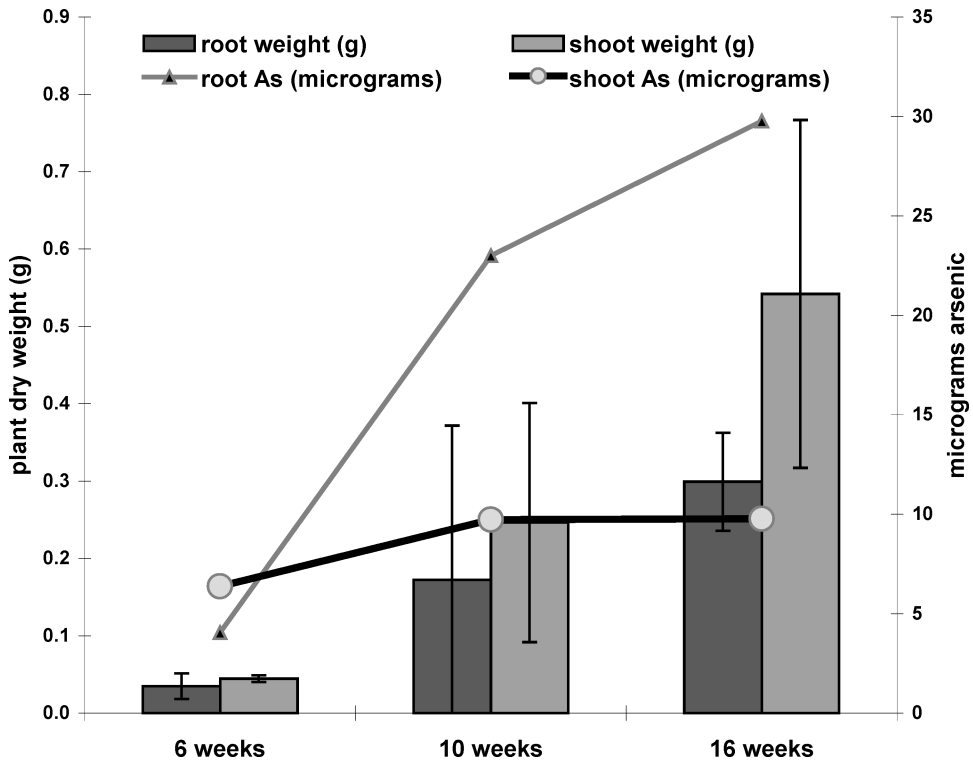


Figure 3 Average dry weight (g) of six plant roots and shoot harvested at 6, 10, and 16 wk for analysis (left scale) and the average mass of arsenic (μg) taken up by six plant roots and shoots (right scale). Values shown are average \pm 1 standard deviation ($n = 3$).

We used a total of 132 plants grown in a surface area of 180 cm^2 . If we assume the same average concentration of arsenic removal in mg kg^{-1} dry weight plant biomass at the same plant density and a surface area of 1 hectare, the total arsenic taken up by shoots would be 120, 130, and 130 g per hectare at 6, 10, and 16 wk, respectively. In natural fields, *Leersia oryzoides* can grow at a higher plant density than was achieved in this bench-top greenhouse experiment, and arsenic removal would be higher. It is recognized, however, that the extent of extraction from natural soil may be different from that from potting soil.

Values in Figure 4 indicate that the total amounts of arsenic extracted for 10 and 16 wk were very similar, differed from the uptake at 6 wk by only 8.3%. Our results suggest that if the shoots are completely harvested using periodic mowing at 6-wk intervals, additional arsenic may be taken up by the grass over a growing season, as has been reported for cadmium (McGrath, Dunham, and Correll, 2000) and zinc and cadmium (Vandenhove *et al.*, 2004). At 6 wk, the average ratio of arsenic concentration in the shoot to that in the root [the shoot:root quotient (SRQ); Baker and Whiting, 2002] was approximately 1. The SRQ changed to 0.32 by 10 wk, and about 0.20 by 16 wk (Figure 5).

A SRQ greater than unity shows a preferential partitioning of heavy metals to the shoot, indicating the suitability of the plant for potential phytoremediation applications. The concentration of arsenic in the roots decreased throughout the 16 wk (Figure 2). At the same time, the concentrations of arsenic found in the shoots decreased by a larger factor.

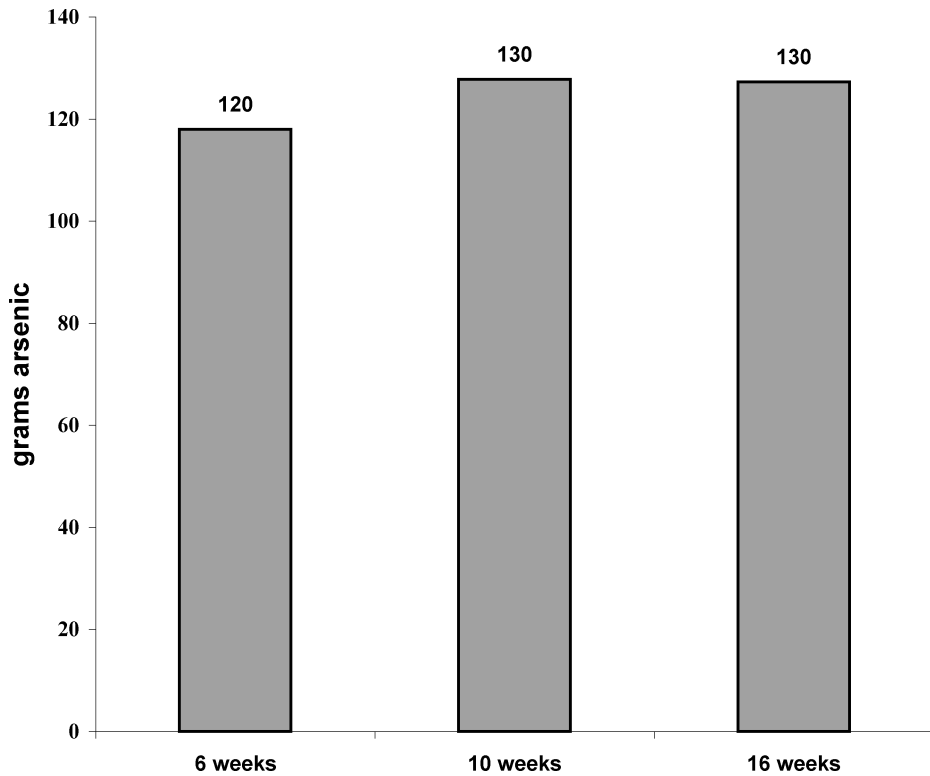


Figure 4 Potential arsenic removal (g) by *Leersia oryzoides* shoots from 1-hectare field after 6, 10, and 16 wk of growth.

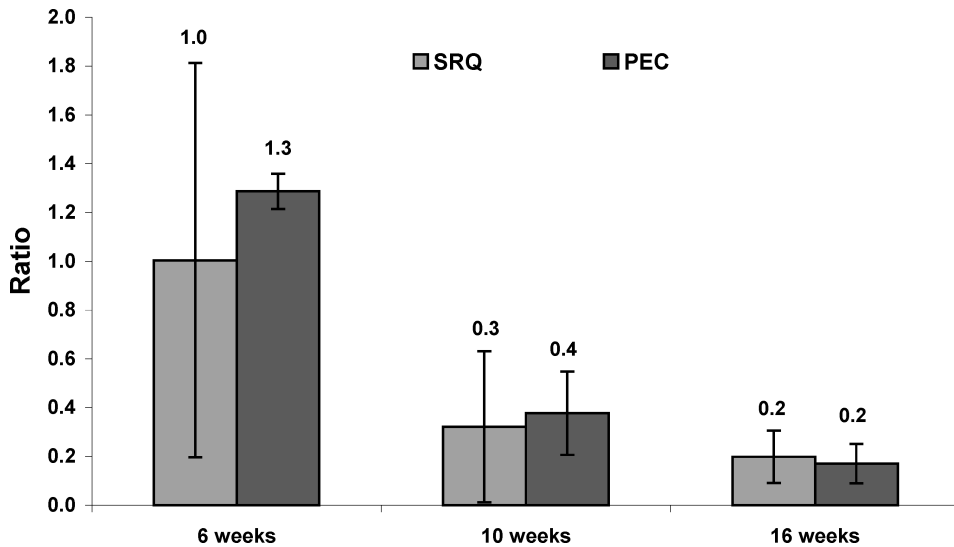


Figure 5 Shoot:root quotients (SRQ) for arsenic uptake from contaminated soil and shoot phytoextraction coefficients (PEC) using *Leersia oryzoides* at 6, 10, and 16 wk. Values shown are average \pm 1 standard deviation ($n = 3$).

This resulted in the steady decrease in the SRQ observed in Figure 5. The phytoextraction coefficients (PEC) calculated using the concentration of arsenic in the shoots and the initial concentration of arsenic in the soil over the period of the experiment are shown in Figure 5. A PEC value between 0.5 and 60 is considered to be desirable (Nanda Kumar *et al.*, 1995). A maximum value of 2.8 was observed. The large standard deviation in the value at 6 wk is attributed to the variation in uptake when the plants were stressed in the initial growth period. The PEC value at 6 wk was 1.3. This reduced considerably over the next 10 wk falling below 0.5 by 10 wk. Compared with other arsenic-accumulating plants, the performance of *Leersia oryzoides* is similar to that of *Lemna Gibba* L. in terms of the accumulation within the plant. Concentrations ranging from 60–2000 mg kg⁻¹ have been reported (Mkandawire and Dudel, 2005) for the removal of arsenic from mine-tailing waters. *Leersia oryzoides* did not accumulate as much arsenic as *Pteris vittata* L., for which concentrations as high as 7200 mg kg⁻¹ (after 20 wk) have been reported (Tu *et al.*, 2002). However, it should be noted that concentrations as low as 84 mg kg⁻¹ (for Cornwall soils) have also been found (Caille *et al.*, 2004)

CONCLUSION

It is observed that, with increasing time, the increase in plant size is matched by a decrease in shoot arsenic concentration, resulting in an approximately constant amount of arsenic taken up into the shoots. The data in Figure 4 show that 12, 13, and 13 mg m⁻² of arsenic was absorbed by the shoots at 6, 10, and 16 wk, respectively. This gives an average shoot uptake of 13 mg m⁻² arsenic. Since the SRQ and PECs all exhibit the same downward trend after 6 wk, it is suggested that periodic mowing of *Leersia oryzoides* grown for phytoextraction purposes on contaminated land could maintain the high arsenic uptake at 6 wk.

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