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Greenhouse Study on the Phytoremediation Potential of Vetiver Grass, *Chrysopogon zizanioides* L., in Arsenic-Contaminated Soils

Rupali Datta · Mario A. Quispe · Dibyendu Sarkar

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Abstract The purpose of this greenhouse study was to assess the capacity of vetiver grass to accumulate arsenic from pesticide-contaminated soils of varying physico-chemical properties. Results indicate that vetiver is capable of tolerating moderate levels of arsenic up to 225 mg/kg. Plant growth and arsenic removal efficiency was strongly influenced by soil properties. Arsenic removal was highest (10.6%) in Millhopper soil contaminated with 45 mg/kg arsenic, which decreased to 4.5 and 0.6% at 225 and 450 mg/kg, respectively. High biomass, widespread root system and environmental tolerance make this plant an attractive choice for the remediation of soils contaminated with moderate levels of arsenic.

Keywords Arsenic · Phytoremediation · Vetiver · Soil properties

Introduction

Inorganic arsenic (As)-based pesticides such as calcium arsenate, lead arsenate, sodium arsenite, etc., were used extensively to control insect pests in agriculture. Inorganic arsenical compounds have also been used as defoliants in cotton and potato fields, control ripening of grapefruit and oranges and as herbicides to control weeds (Mellanby 1967). It is estimated that approximately 15 million pounds of As was applied to New Jersey soils between 1900 and 1980 (Murphy and Aucott 1998). Since As is a group A human carcinogen, the USEPA banned usage of many inorganic As-based pesticides in the late eighties and early nineties (USEPA 1992; Southworth 1995). However, due to rapid expansion of suburbia in the US., pesticide-applied former agricultural soils are now routinely being converted to residential developments. Ingestion of As-contaminated soil due to incidental hand-to-mouth activity by children is now being increasingly taken into account in assessing human health risks associated with exposure to such soils (Datta et al. 2006).

Phytoremediation is a green and sustainable technology that has continued to generate considerable research and commercial interest worldwide (Byong-Gu et al. 2008). Chinese Brake fern *Pteris vittata* is a known As hyperaccumulator, but there is a risk that the plant could become an invasive weed, and hence, it is difficult to introduce this species for research or commercial activities in many countries (Byong-Gu et al. 2008). Vetiver grass (*Chrysopogon zizanioides*) is a tall (1–2 m), fast growing perennial grass with quick regeneration ability. It has a long (3–4 m), massive and complex root system which can penetrate to the deeper layers of the soil (Truong 2000; Pichai et al. 2001). Recent studies have shown that vetiver grass is a promising plant to use in phytoremediation of heavy metals (Chen et al. 2004). In Australia, vetiver has been used to stabilize excessive mining waste in highly saline, alkaline and acidic tailings of coal and gold mines (Truong 2000). Vetiver is well adapted to environments ranging from aquatic to desert conditions, and is tolerant to frost, heat, acidic and alkaline conditions (Dalton et al. 1996).

R. Datta (✉)
Department of Biological Sciences, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA
e-mail: rupdatta@mtu.edu

M. A. Quispe
Environmental Geochemistry Laboratory, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA

D. Sarkar
Department of Earth and Environmental Studies, Montclair State University, 1 Normal Avenue, Montclair, NJ 07043, USA

While a few studies have reported that vetiver grass is tolerant to low to moderate levels of As contamination, and can accumulate As in their tissues (Truong 2000), no reports are currently available on the suitability of this plant to remediate arsenical pesticide contaminated agricultural soils. This study was intended to provide baseline information on the capacity of vetiver grass to tolerate and accumulate As from pesticide-contaminated soils of varying physical and chemical properties.

Materials and Methods

Five different soil types were used in this study. Surface soil from the Eufaula series was collected from Wilson County, Texas; Millhopper series soil from University of Florida campus at Gainesville, Florida; Orelia series soil from Corpus Christi, Texas; Orla series soil from Pecos County, Texas and Pahokee Muck series soil was collected from the Everglades Research and Education Center at Belleglade, Florida. The soils selected vary in their physico-chemical properties such as pH, clay content, Fe/Al and Ca/Mg concentrations and organic matter content to represent a range of properties that are important for As retention and/or plant-availability. Air-dried and sieved (2 mm) soils were used for the determination of pH, electrical conductivity (EC), soil moisture and organic matter content using standard protocols (Sparks 1996). Total P was extracted by the ignition method (Datta et al. 2006). Total recoverable Ca, Mg, Fe, Al, P and As was determined by acid digestion according to the USEPA Method 3050B (USEPA 2000). The concentrations of P and Fe in the extracts and digests were determined colorimetrically using the molybdate-ascorbic acid method and 1,10-phenanthroline-reagent method respectively as described in Datta et al. (2006). The concentrations of Ca, Mg and Al in the extracts/digests were analyzed using an atomic absorption spectrophotometer in the flame mode. The digests were analyzed for As using a graphite furnace atomic absorption spectrophotometer. The physical and chemical properties of the soils are presented in Table 1.

The soils were fortified with sodium arsenite ($Na_2HAsO_4 \cdot 7H2O$) at 3 different concentrations: 45,225 and 450 mg As/kg soil. Subsequently, 3 kg of the fortified soil treatments were placed in PVC columns (0.38 m high and 0.15 m in internal diameter). An outlet nozzle was placed at the bottom of each column and connected with a tube to collect the leachate in a clean, acid-washed 1-L plastic bottle. To prevent soil from washing down through the columns, a layer of fine nylon mesh (8 μm mesh size) was placed at the bottom of the column and above the outlet nozzle. Each column was filled with 0.18 m of bleached sand, followed by 0.15 m of soil. Prior to using the sand for the greenhouse experiments, three composite sand samples were acid-digested by the USEPA 3050B method (USEPA 2000) and analyzed for metals and metalloids by ICP-MS. The total As concentration of the bleached sand was below the detection limit of the instrument, with good spike recovery (95–105%). Sixty PVC columns were assembled in the greenhouse at the University of Texas at San Antonio [5 soils × 4 rates (3 As treatment rates +1 set of controls without As) ×3 replicates]. Eight sprouts of vetiver grass obtained from HIS plants, Parrish, Florida were transplanted into each column and allowed to grow for 4 months. The moisture content of the columns was maintained at 80% of the water holding capacity. The columns were overwatered with deionized water every month to facilitate leaching, and the leachates were analyzed for As. Vetiver plants were harvested after 4 months. Soils were collected and analyzed for As at the beginning of the experiment (initial) and after 4 months of vetiver growth (final).

After harvest, root and shoot lengths and biomass of vetiver grass were measured. The plants were washed thoroughly using tap water and 0.01 mol/L HNO_3, rinsed with deionized water, separated into root and shoot compartments, and analyzed for As uptake following the procedure by Carbonell et al. (1998). Total As concentration in soils was determined using the USEPA acid digestion method (3050B). Soil and plant samples after digestion were analyzed by atomic absorption (GFAAS; Perkin Elmer AAnalyst 700 spectrophotometer).

Table 1 Soil properties

Soil properties	Eufaula	Millhopper	Orelia	Orla	Pahokee Muck
pH	6.1	6.4	8.2	8.2	5.9
EC (ds/cm)	0.02	145	203	205	558
SOM (%)	2.0	4.38	2.39	2.29	85.4
P (mg/kg)	67	4,875	1,688	1,688	6,812
Ca–Mg (mg/kg)	64.6	3,155	13,125	59,200	40,800
Fe–Al (mg/kg)	847	4,745	6,060	17,550	6,010

EC Electrical conductivity, *SOM* Soil organic matter

Data collected for all the parameters were analyzed statistically using JMP software (Version 5.01). Two-way ANOVA test was performed followed by Tukey's HSD multiple comparison tests to determine significant differences at $p < 0.05$.

Results and Discussion

The effect of As contamination on vetiver grass biomass yield is shown in Fig. 1. Vetiver biomass decreased with increase in soil As concentration. No external symptoms of toxicity were observed in the plants at the 45 mg/kg soil As concentration. At 225 mg/kg As concentration, vetiver showed minor yellowing of leaves and a small decrease in biomass. At the highest As concentration of 450 mg/kg, vetiver shoots showed distinct yellowing and necrosis at the tips, and major decline in biomass. Decrease in biomass was prominent in vetiver plants grown in Eufaula, Millhopper, Orelia and Orla soils. However, the effect of As was minimal in Pahokee Muck soil (Fig. 1). Maximum decline in biomass was seen in vetiver plants grown in Eufaula series soil. Statistical analysis of the effects of As concentration on vetiver biomass showed that there were significant differences in each of the soil types as the As concentration increased. The effect of As was most pronounced in Eufaula soil and least pronounced in Pahokee Muck soil. These results confirm the earlier reports that vetiver can successfully survive in soils moderately contaminated by arsenic, such as pesticide-contaminated soils (Truong 2000). However, it's capability of tolerating high concentrations of soil As is doubtful.

Total As accumulation in shoot and root tissues after 4 months are shown in Fig. 2. Arsenic uptake in plants growing in Pahokee Muck soil was the lowest, whereas uptake was the highest in Orelia and Orla soils. Vetiver accumulated substantial amounts of As in both shoot and root tissues; however, accumulation was higher in root tissue in all soils, and increased with increase in As concentration. These results are similar to those reported by Truong and Baker (1998).

Soil Properties had a major impact on growth and As accumulation in vetiver plants. According to Chen et al. (1999), the major factors controlling trace metal concentrations in soil include clay content, organic matter content, pH and Fe/Al content. Eufaula soil is a loamy fine sand, slightly acidic with low organic matter and low Fe, Al, Ca and Mg content. Lacking positively charged adsorptive surfaces, such as those contributed by Fe/Al oxides, this soil is likely to have minimum As retention capacity (Pierce and Moore 1980; Oscarson et al. 1981). Millhopper soil is acidic and sandy, but contains higher levels of Fe, Al, Ca and Mg, hence is likely to have higher As retention capacity, which was reflected in the increased biomass of plants grown in Millhopper soil. Previous studies in our laboratory showed that As strongly adsorbed to Fe/Al oxides, particularly in acidic soils, which become unavailable for plant uptake (Sarkar and Datta 2004; Datta et al. 2006). Orelia soil is a sandy clay loam, with an alkaline pH and higher levels of Fe, Al, Ca, and Mg content.

Arsenic accumulation in root tissue was much higher in Orelia compared to Millhopper (Fig. 2). This could be the result of higher levels of P in Millhopper soil, which is known to compete with As for uptake in plants. Also, As

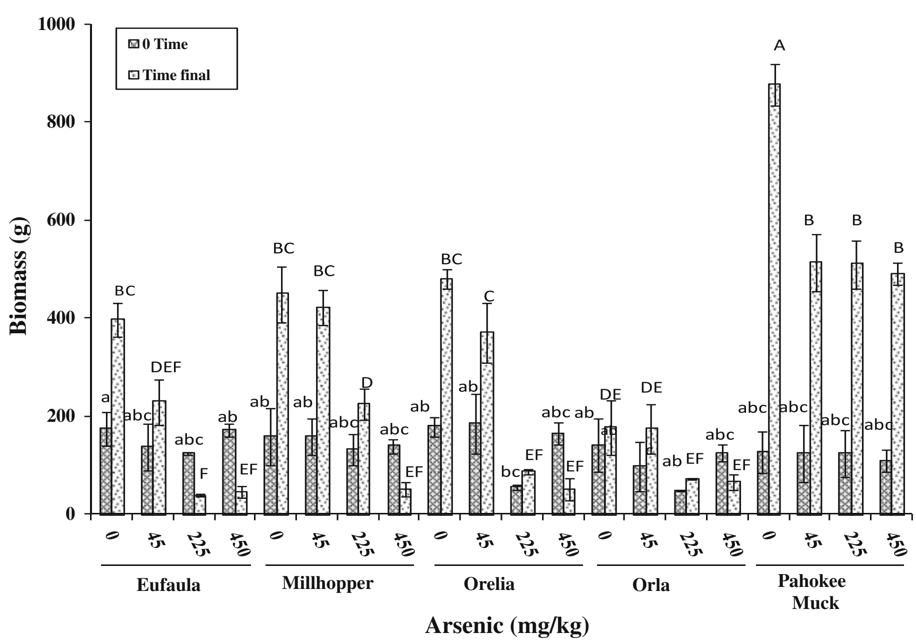

Fig. 1 Vetiver biomass production after 4 month of growth in As contaminated soils. Values represent mean (±SD) of 3 replicates. *Different letters* indicate significant difference at $p < 0.05$ which were determined using 2-way ANOVA followed by Tukey's HSD multiple comparison test

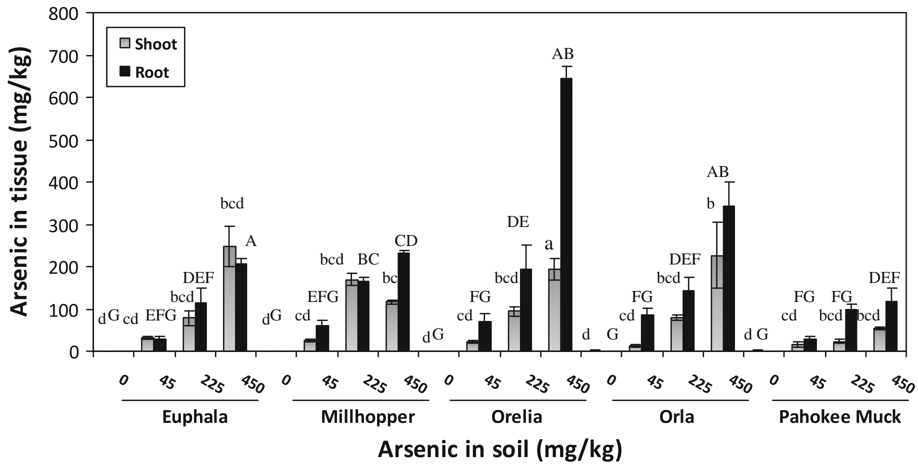

Fig. 2 Arsenic accumulation in plant tissue after 4 months of growth in As-contaminated soils. Values represent mean (±SD) of 3 replicates. *Different letters* indicate significant difference at $p < 0.05$ which were determined using 2-way ANOVA followed by Tukey's HSD multiple comparison test

adsorption decreases with increasing soil pH, thereby resulting in more plant-available As in Orelia. Orla series soil is an alkaline, gypsic, fine clay loam, with very high Fe, Al, Ca and Mg content. Soil Ca/Mg compounds play a major role in As sequestration of alkaline soils, providing retention sites for soluble As. Coprecipitation of As with Ca in soil solution and As incorporation into apatite or poorly crystalline apatite are some of the speculated mechanisms responsible for As sequestration in alkaline soils (Bothe and Brown 1999). Arsenic sorption to Ca/Mg compounds is significant in alkaline soils, contrary to those of acidic soils where majority of the As is retained by Fe/Al oxides (Sarkar et al. 2005; Datta et al. 2007). Pahokee Muck soil has high organic matter, Fe, Al, Ca and Mg content, which resulted in higher levels of As being bound to the soil, ensuing in lower uptake and high biomass in vetiver plants. Arsenic uptake is also influenced by the amount of As leached out of the soil during the 4 leaching events (Fig. 4).

Figure 3 shows the initial As concentration in the soil and the residual As after the vetiver plants were harvested after 4 months. The concentration of As decreased in all 5 types of soils, both as a result of leaching and plant uptake. Significant differences were found in the As concentrations in soils between the initial and final time periods. Figure 4 shows the amount of As in the leachates collected throughout the study period. As expected, the highest amount of As was lost in case of the Eufaula soil which had the least As retention capacity, followed by the Millhopper soil whereas the least amount was lost in case of Pahokee Muck soil.

Arsenic removal efficiency of vetiver grass was calculated as follows:

$$\text{Efficiency of As removal}\,(\%) = \frac{\text{Total As in vetiver tissue (mg)} \times 100}{\text{Total As in column (mg)}}$$

Efficiency of As removal of the various soil types is listed in Table 2. Arsenic removal efficiency decreased with increase in arsenic concentration in the soil. Removal efficiency was greatest in the Millhopper soil at 45 mg/kg As treatment, followed by Orelia and Pahokee Muck soils, and was clearly a function of soil As retention capacity.

Results obtained from this study demonstrate the ability of vetiver grass to establish themselves tolerate and accumulate arsenic in moderately contaminated soils. No significant toxicity symptoms were detected in plants grown in soils contaminated with 225 mg/kg of arsenic. Hence,

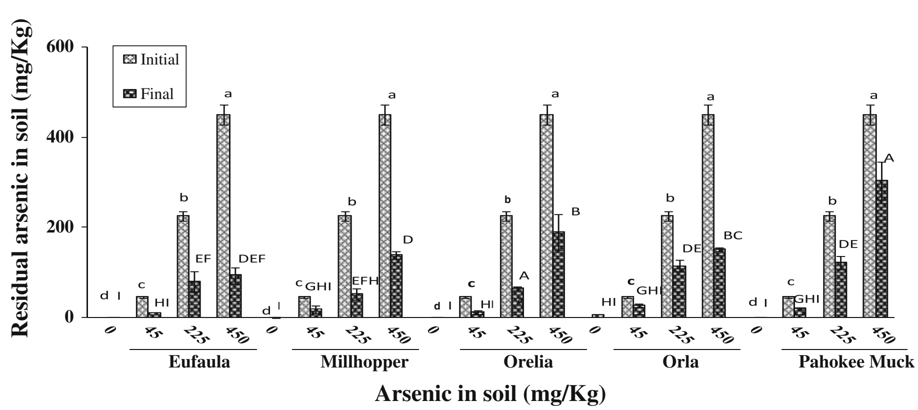

Fig. 3 Arsenic concentration in soils at the beginning (initial) and after 4 months of vetiver growth (final). Values represent mean (±SD) of 3 replicates. *Different letters* indicate significant difference at $p < 0.05$ which were determined using 2-way ANOVA followed by Tukey's HSD multiple comparison test

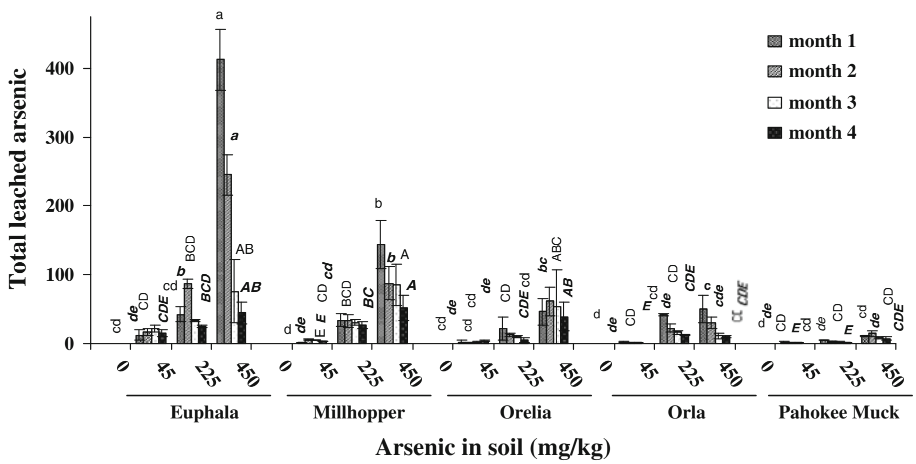

Fig. 4 Arsenic concentration in leachates collected at monthly intervals. Values represent mean (±SD) of 3 replicates

Table 2 Efficiency of arsenic removal by vetiver grass

Soil series	Arsenic removal efficiency (%)		
	45 mg/kg rate	225 mg/kg rate	450 mg/kg rate
Eufaula	4.19	0.65	0.59
Millhopper	10.60	4.51	0.61
Orelia	9.22	1.13	1.62
Orla	3.41	0.82	1.23
Pahokee Muck	7.36	3.95	2.89

vetiver plants would be suitable for phytoextraction of As in pesticide contaminated agricultural soils, but possibly not for highly contaminated Superfund soils. The As removal efficiency of vetiver grass will depend on the soil physico-chemical properties.

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