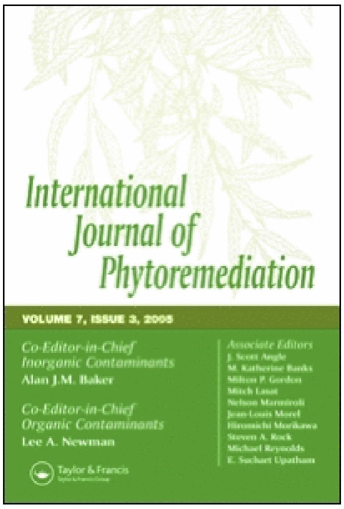

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Effects of Light Regime, Temperature, and Plant Age on Uptake of Arsenic by *Spartina Pectinata* and *Carex Stricta*

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EFFECTS OF LIGHT REGIME, TEMPERATURE, AND PLANT AGE ON UPTAKE OF ARSENIC BY *SPARTINA PECTINATA* AND *CAREX STRICTA*

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We report here on efforts to show that a combination of native wetland plant species might perform better than a monoculture in wetlands designed for arsenic remediation by supplementing weaknesses.* Carex stricta *and* Spartina pectinata *were used in hydroponic experiments. (i) Arsenic uptake was first assessed at two ages via exposure to control or arsenic-laden solutions (0 or 1.5 mg As L^{-1} as Na_2HAsO_4) for two weeks. Age had no significant effect on arsenic concentrations in roots, but translocation factors were greater in older plants of* C. stricta *and* S. pectinata *(0.45 and 0.07, respectively) than in younger plants (0.10 and 0.01, respectively). (ii) Seasonal effects were assessed by determining uptake kinetics for both species in conditions representative of spring temperatures (15/5°C) and light regimes (1050 μmol m^{-2} s^{-1}, 13 h day^{-1}) and summer temperatures (28/17°C) and light regimes (1300 μmol m^{-2} s^{-1}, 15 h day^{-1}). Both species had comparable rates of arsenic uptake into roots in summer conditions (44.0 and 46.5 mg As kg^{-1} dry wt. h^{-1} in* C. stricta *and* S. pectinata*, respectively), but* C. stricta *had a higher maximum net influx rate in spring conditions (24.5 versus 10.4 mg As kg^{-1} dry wt. h^{-1}).*

KEYWORDS: seasonal variation, kinetics, wetland plants, phytoremediation

1. INTRODUCTION

Arsenic is a ubiquitous element that naturally occurs in soil, plants, and water. Natural processes and anthropogenic activities have redistributed arsenic in the environment, elevating levels in drinking and surface waters in many areas of the world (Nordstrom, 2002; Mandal and Suzuki, 2002). The increased risk of human exposure and threats to natural ecosystems necessitate the development of cost-effective technologies for the remediation of arsenic-contaminated water. Engineered wetlands are one possible technology. They rely on naturally-occurring physical, chemical, and biological processes to remove arsenic from contaminated water, meaning they have relatively low energy and maintenance requirements.

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Many of the processes that contribute to arsenic removal have been studied in controlled environments. Primary removal mechanisms generally include adsorption to substrate and co-precipitation with iron, aluminum, or manganese oxides (Ye *et al.*, 2003; Buddhawong *et al.*, 2005). These processes are limited by the quantity of adsorption sites or reactants that are available within a wetland system. By way of contrast, biological processes, mediated by microorganisms (e.g., chemical transformation) and plants (e.g., uptake), might not be limited like other processes because they rely on living organisms. If the appropriate environmental conditions are maintained, then organisms' biological processes might be extended indefinitely, which by itself helps create a cost-effective process.

One possible biological removal mechanism relies on the ability of plants to take up arsenic, a process referred to as phytoextraction. The goal of our research is to select wetland plant species that will maximize phytoextraction at a site that produces leachate with 1.5 mg As L^{-1} in the temperate climate of northwest Ohio. Unfortunately, phytoextraction is often considered insignificant in the scope of an entire treatment wetland. For example, *Thalia* sp. can accumulate up to 80 mg As kg^{-1} when grown in hydroponics (Aksorn and Visoottiviseth, 2004). However, their arsenic accumulation combined with other wetland plant species was only 2–4% of the total quantity of arsenic removal in wetland microcosms (Ye *et al.*, 2003). The remaining 96–98% was adsorbed to soil or precipitated as insoluble compounds of arsenic. To increase the role of plants in engineered wetlands in temperate climates, we need to understand some of the factors, including plant age and seasonal variations that influence the ability of a plant to accumulate arsenic.

Temperate climates are marked by seasonal variations, which create gradients in temperature, light intensity, and light regime. In these conditions, many native plant species have distinct growing seasons, which could prevent them from functioning within the context of a treatment wetland for a portion of the year. The amount of phosphorus accumulated by plants can fluctuate throughout a growing season (Jonasson and Chapin 1991; Tate *et al.*, 1991). The same might be expected for arsenic, because arsenate and phosphate are taken up by the same mechanisms in plants (Asher and Reay, 1979; Meharg and Macnair, 1990).

Another likely factor affecting arsenic accumulation is the age of the plants. Generally, young roots grow faster and have higher nutrient uptake rates than older roots (Yanai, 1994), but the effects of plant age on arsenic uptake have not been widely researched. In a previous study, eight week old *Pteris vittata* took up and translocated arsenic to shoots more rapidly than plants that were 16 months old (Silva Gonzaga *et al.*, 2007), suggesting that young plants would be most efficient for phytoextraction. *P. vittata* is not adapted to the environmental conditions found in wetlands in temperate climates. So, we would like to know whether native wetland plant species exhibit similar age-dependent variations in arsenic uptake. It would be prudent to utilize or harvest plants at an age that maximizes arsenic phytoextraction during a growing season.

We report here on the effects of (i) plant age and (ii) light and temperature conditions on uptake of arsenic from hydroponic solutions by a cool-season sedge (*Carex stricta*) and a warm-season grass (*Spartina pectinata*). Our findings suggest that (i) older plants transfer greater quantities of arsenic to shoots, so aboveground portions should only be harvested at the ends of growing seasons; and (ii) a mixture of warm-season and cool-season plant species could be used to maximize arsenic uptake in temperate climates. This information will be used in the design of a pilot-scale treatment wetland at a local, arsenic-contaminated site.

2. MATERIALS AND METHODS

2.1. Plants and Growth Conditions

Two wetland plant species (*C. stricta* and *S. pectinata*) were previously shown to accumulate and tolerate arsenic (Rofkar and Dwyer, *in review*) and have been recommended for use in wetland restoration projects in Ohio (OEPA, 2007). They were selected for this study as representatives of the diverse community of wetland species native to the climate of northwest Ohio. *C. stricta* (tussock sedge) is a C_3, cool-season, perennial sedge and *S. pectinata* (prairie cordgrass) is a C_4, warm-season, perennial grass.

Seeds of *C. stricta* and *S. pectinata* (Prairie Moon Nursery, Winona, MN) were sown in a fibrous medium for hydroponics (Oasis Horticubes, Smithers-Oasis, Kent, OH) and placed in a greenhouse (20–25°C, 12-h photoperiod, 30–70% humidity). When roots protruded from the bottoms of the cubes (4–8 weeks after sowing), seedlings were transferred to plastic containers filled with aerated nutrient solution (4 L; 1.0 mM $Ca(NO_3)_2$, 1.5 mM KNO_3, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4$, 0.1 mM EDTA-Fe, 0.5 μM $ZnSO_4$, 6.0 μM $MnCl_2$, 50 μM H_3BO_3, 2.0 μM $CuSO_4$, and 0.09 μM Na_2MoO_4; pH 5.5–6.0) and allowed to acclimate to the hydroponic environment (4–6 weeks). The solution was replenished as needed and completely replaced weekly.

2.2. Variations in Uptake Kinetics under Different Temperature and Light Regimes

Following the initial acclimation period, plastic containers filled with nutrient solution (4 L) and either *S. pectinata* or *C. stricta* were transferred to a growth chamber with conditions that simulated either spring or summer in northwest Ohio (Table 1). Plants were left to acclimate for one week. They were then transferred to individual amber HDPE bottles filled with nutrient solution amended with arsenic (0, 0.375, 0.75, 1.5, 3.75, 7.5, 15, or 75 mg L^{-1} as Na_2HAsO_4). Roots were harvested after 4 h (this time was selected based on preliminary experiments). The harvested tissues were rinsed first with tap water, then HCl (10%), with tap water again, then dried (55°C; 48 h), and weighed. Arsenic content of root tissues was then determined by ICP-OES. Uptake rates were used to

Table 1 Settings used to simulate spring and summer in growth chambers. Values for light intensity indicate average photosynthetically active radiation at canopy height. Values for light regime indicate the timing of simulated daylight during each 24 h period

	Spring	Summer
Temperature		
Day	15°C	28°C
Night	5°C	17°C
Light intensity		
Day	1050 μmol m^{-2} s^{-1}	1300 μmol m^{-2} s^{-1}
Night	dark	dark
Light regime		
Day	13 h	15 h
Night	11 h	9 h

calculate kinetic parameters, including the maximum net influx rate (I_{max}) and the affinity constant (K_m).

2.3. Effects of Plant Age on Arsenic Uptake and Translocation

Plants of two different ages were used for this experiment. Seeds were germinated and the seedlings were cultured as described above until 12 weeks of age (younger plants). The older plants (approximately 6 months in age) were purchased from a nursery (JFNew, Walkerton, IN), washed to remove adhered soil, and transferred to plastic containers filled with nutrient solution (4 L). The plants were acclimated to the hydroponic environment for two weeks, during which the nutrient solution was replaced every five days. Following acclimation, the plants ($n = 4$) were weighed to determine fresh biomass. The younger plants had significantly lower fresh biomass than older plants in each species.

After being weighed, the plants were returned to the containers, and the acclimation solutions were replaced with nutrient solutions amended with arsenic (either 0 or 1.5 mg As L^{-1}). The plants were grown for two more weeks with the solutions refreshed every five days. Plant tissues were treated as above to obtain dry weights. Arsenic concentrations in roots and shoots were determined by ICP-OES; relative growth rates (RGRs) were calculated from fresh biomass before and after treatment.

2.4. Chemical Analyses

Samples of dried plant tissues (0.15 g) were digested in a microwave according to a modification of USEPA Method 3051. Briefly, each sample was combined with HNO_3 (5 mL; Fisher Scientific, Pittsburgh, PA) in a 120-mL Teflon® digestion vessel and heated for 45 min (15 min ramp time, 200°C). Then, H_2O_2 (30%; 1.5 mL) was added to each vessel and reheated (15 min ramp time, 200°C, 45 min hold time). After cooling, deionized water (12 mL; 18 mΩ) was added to each vessel and the resulting solutions were filtered (No. 2 Whatman filter paper). To maintain a consistent sample matrix, an aliquot of the solutions (1.3 mL) were further diluted with deionized water (8.7 mL) prior to analysis by ICP-OES. Standardization of the ICP-OES was previously described (Rofkar *et al.*, 2007). The method detection limit for the ICP-OES was 10 μg As L^{-1} in solution, which corresponded to13.7 mg As kg^{-1} in dry plant tissues; values below that were set at one-half of the method detection limit (6.85 mg As kg^{-1}) for statistical analyses (Hornung and Reed, 1990). Quality control measures for the ICP-OES included analyzing (i) analytical standards following every tenth sample, (ii) certified reference materials after every twentieth sample (NIST No. 1547 and 1570a), (iii) a multi-element standard after every sixtieth sample (LPC Standard 1, SPEX Certiprep, Metuchen, NJ), and (iv) procedural blanks in each batch of digested samples.

2.5. Statistical Analyses

Uptake of arsenic by plants of different ages were compared using the Student's t-test ($p = 0.05$; Prism 5, GraphPad Software, Inc.). Kinetic parameters (I_{max} and K_m) were calculated using non-linear regression of root concentrations fitted to a model for Michaelis-Menten kinetics (Prism 5, GraphPad Software, Inc.).

3. RESULTS AND DISCUSSION

3.1. Variations in Uptake Kinetics under Different Temperature and Light Regimes

When designing wetlands for arsenic phytoremediation, it is possible to select a variety of plant species that maximize uptake throughout the growing season. Several observations suggested that *C. stricta* and *S. pectinata* would be most effective for uptake of arsenic during summer (Figure 1). The highest values of I_{max} were observed for both species when growing in the summer treatment (Table 2) and these values were not considerably different between the species. In the spring treatment, however, I_{max} values were 1.8 and 4.5 times less than in the summer treatment for *C. stricta* and *S. pectinata*, respectively. There was also no notable change in the affinity (K_m) of *C. stricta* for arsenic between the spring and summer treatments. In contrast, *S. pectinata* had a much greater K_m value in the summer treatment (2.8 times the K_m value in the spring treatment), indicating a greater affinity for arsenic in summer.

Seasonal growth patterns and photosynthetic mechanisms might explain some of the effects of seasonality observed in *C. stricta* and *S. pectinata*. *C. stricta* actively grows during the spring and summer months, amid a range of temperature and light conditions.

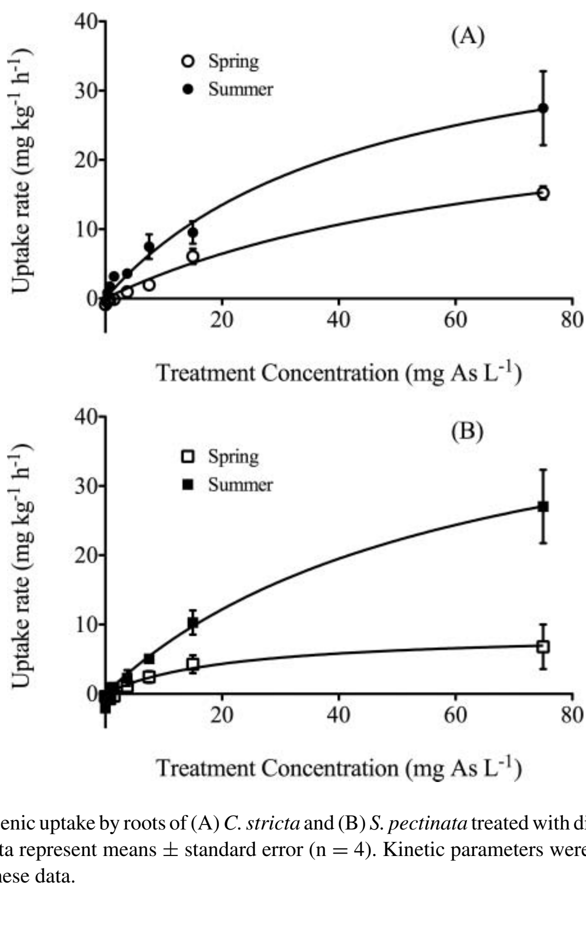

Figure 1 Rates of arsenic uptake by roots of (A) *C. stricta* and (B) *S. pectinata* treated with different concentrations of arsenic for 4 h. Data represent means ± standard error (n = 4). Kinetic parameters were calculated from non-linear regression of these data.

Table 2 Concentrations of arsenic in roots of *C. stricta* and *S. pectinata* after 4 h of exposure to 75 mg As L^{-1}, and kinetic parameters (I_{max} and K_m) determined from non-linear regression

	Root concentration (mg As kg^{-1} dry wt)	I_{max} (mg As kg^{-1} dry wt h^{-1})	K_m (mg As L^{-1})	r^2
C. stricta				
Spring	61.0 ± 3.8 a	24.5 ± 4.5	74.4 ± 23.8	0.9421
Summer	109.9 ± 21.4 a	44.0 ± 11.2	44.3 ± 16.7	0.8779
S. pectinata				
Spring	37.5 ± 10.7 a	10.4 ± 2.0	21.3 ± 15.2	0.5225
Summer	108.1 ± 21.2 b	46.5 ± 14.3	60.0 ± 27.0	0.8574

Values represent means ± standard error. Letters indicate statistically significant differences within each species ($p < 0.05$); kinetic parameters were not compared statistically.

The lengthy growing season of *C. stricta* might be reflected in its ability to maintain uptake through both the spring and summer treatments. In addition, organisms like *C. stricta* that perform C_3 photosynthesis typically experience maximum photosynthetic efficiency at temperatures below 20°C (Sage and Kubien, 2007, and references therein). *S. pectinata*, on the other hand, performs C_4 photosynthesis, and like other C_4 species might be most efficient at temperatures above 20°C (Sage and Kubien, 2007, and references therein). Therefore, *S. pectinata* tends to be most active during the summer months in temperate climates, which might explain the higher uptake rate and affinity observed in the summer treatment. Long-term studies, particularly in environmental conditions, would be useful for determining the effect of seasonal variations in plants on arsenic removal over the duration of a growing season.

Understanding seasonal variations in arsenic uptake is important for design and implementation of phytoremediation systems, particularly in temperate regions where phytoextraction is minimal during winter months. Each plant species likely has a specific set of conditions—a "sweet spot"—during which they extract arsenic most efficiently. Engineering biologically diverse wetlands might foster overlap of those "sweet spots," thereby maximizing phytoextraction during the course of a growing season. In the setting of a constructed wetland, plant species could be selected to insure this redundancy within the system. Selecting a diverse group of warm- and cool-season plant species could be one strategy to maximize arsenic uptake throughout the growing season of a temperate climate.

3.2. Effects of Plant Age and Growth Rate on Arsenic Uptake and Translocation

Within each species, RGRs were highest in the younger plants that were not exposed to arsenic, followed by younger plants with arsenic, older plants without arsenic, and older plants with arsenic (Table 3). Based on these observations, the two factors controlling growth rates of *C. stricta* and *S. pectinata* during this study were plant age and arsenic availability. Typically, young plants have higher growth rates than older plants of the same species (Poorter, 1989, and references therein), so this observation was expected. Interestingly, arsenic inhibited growth of the younger *C. stricta*, but did not affect growth in the older *C. stricta* or either age of *S. pectinata*. This result was unexpected because arsenic inhibits growth of other non-hyperaccumulating species including *Oryza sativa* (Azizur Rahman *et al.*, 2007), mesquite (Mokgalaka-Matlala *et al.*, 2008), and spinach and tomato

Table 3 Fresh biomass, relative growth rates (RGR) and translocation factors of *C. stricta* and *S. pectinata* exposed to arsenic at different ages

	Initial fresh biomass (g)	RGR (mg g^{-1} d^{-1})	TF
C. stricta			
Young −As	4.28 ± 1.2 a	55.16 ± 2.3 a	N/A
Young +As	4.22 ± 0.8 a	34.54 ± 7.0 b	0.10 ± 0.05 a
Old −As	14.3 ± 2.3 b	17.38 ± 2.9 c	N/A
Old +As	13.0 ± 1.7 b	8.15 ± 3.2 c	0.45 ± 0.12 b
S. pectinata			
Young −As	5.62 ± 2.8 a	45.94 ± 7.1 a	N/A
Young +As	4.86 ± 1.6 a	41.33 ± 5.4 ab	0.01 ± 0.01 a
Old −As	26.7 ± 2.4 b	18.44 ± 5.3 bc	N/A
Old +As	29.7 ± 3.9 b	17.82 ± 3.8 c	0.07 ± 0.02 b

Values represent means ± standard error. Letters indicate statistically significant differences within each species ($p < 0.05$).

(Hartley and Lepp, 2008). In contrast, the hyperaccumulating species *Pteris vittata* (Caille *et al.*, 2005) tolerates higher levels of arsenic and maintains or even increases growth. Neither *C. stricta* nor *S. pectinata* could be considered a hyperaccumulator, but because they maintained growth, both species apparently have relatively high tolerance for arsenic.

The only significant effect of age on uptake of arsenic was observed in shoots of *S. pectinata*—older shoots contained 10.9 mg As kg^{-1} while the concentration was below the

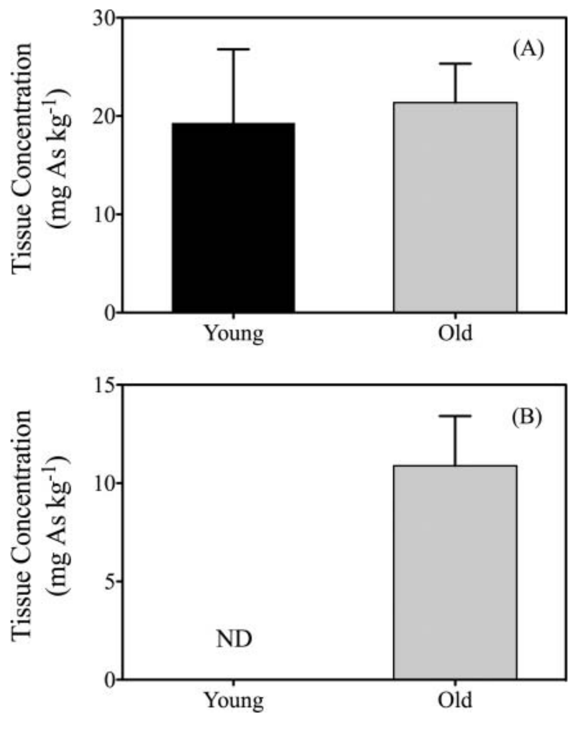

Figure 2 Concentrations of arsenic in shoots of (A) *C. stricta* and (B) *S. pectinata*. Data represent means ± standard error (n = 3 or 4). Differences were not statistically significant ($p < 0.05$).

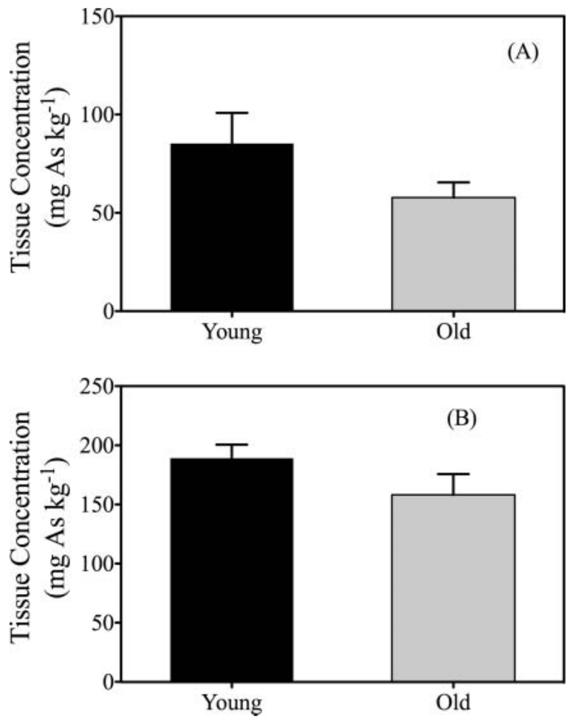

Figure 3 Concentrations of arsenic in roots of (A) *C. stricta* and (B) *S. pectinata*. Data represent means ± standard error (n = 3 or 4). Differences were not statistically significant ($p < 0.05$).

limit of detection in younger shoots (Figure 2). Plant age did not affect concentrations of arsenic in roots of *S. pectinata*, or roots or shoots of *C. stricta* (Figure 3). Concentrations of arsenic were below the method detection limit in roots and shoots of all control plants. In general, the average rate of uptake per unit of root is highest while plants are young, and decreases with age (Barber, 1995), but this pattern was not observed for uptake of arsenic by roots of *C. stricta* and *S. pectinata*. If our observations translate to the field, then *C. stricta* and *S. pectinata* might accumulate arsenic in root tissues throughout the growing season, regardless of their age.

Plant age did, however, affect transfer of arsenic from roots to shoots. Translocation factors (TF; the ratio of arsenic concentrations in shoots to roots), were highest in the older plants of each species (Table 3). In addition, we observed a general decline in TF as RGR increased for each species (Figure 4), although the TF value was relatively constant for young *S. pectinata*. This decline might indicate a greater tendency for *C. stricta* and *S. pectinata* to translocate arsenic as they age and their growth rate declines. Previously, phytoremediation using young P. vittata was recommended because TFs were generally lower in older plants (Silva Gonzaga *et al.*, 2007). The most efficient time for harvesting *C. stricta* or *S. pectinata*, however, might not be when they are young. Rather, harvest should occur when plants are older (*i.e.* at the end of a growing season) and growth rates are low.

Long-term effects of plant age on phytoremediation might be more significant than the results of this short-term study suggest. The primary mechanism for removal of arsenic in wetlands usually is adsorption to substrate (Buddhawong *et al.*, 2005) and in the long-term would likely be limited by the quantity of available adsorption sites. A community

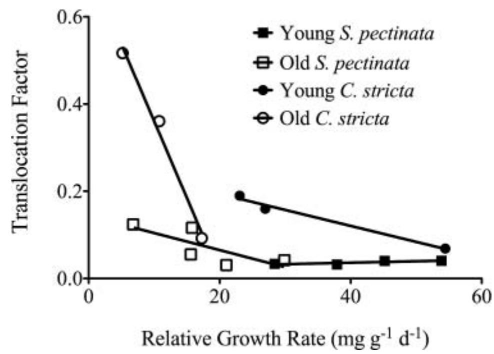

Figure 4 Translocation factors (TFs) of young and old *C. stricta* and *S. pectinata* at different relative growth rates (RGRs). Each point represents a single replicate.

of diverse wetland species could extract arsenic throughout the growing seasons, but the effects of plant age beyond the first year, have not been studied in depth. One focus of future research will be the long-term effects of plant age on arsenic removal in pilot-scale wetlands that will be constructed in the field.

4. CONCLUSIONS

1. A mixture of cool- and warm-season plant species could maximize arsenic removal by supplementing uptake during seasonal variations. In this study, we observed that *S. pectinata* had a much greater rate of arsenic uptake in summer conditions than in spring conditions, while *C. stricta* was more consistent across both treatments.
2. To maximize arsenic removal, it might be best to allow plants to accumulate arsenic for the duration of a growing season, and then harvest older plants that have had time to transfer a significant portion of the accumulated arsenic to shoots. As *S. pectinata* and *C. stricta* aged and their growth rates declined, they began to transfer greater quantities of arsenic to aboveground tissues.

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