Selection of Suitable Emergent Plants for Removal of Arsenic from Arsenic Contaminated Water

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Abstract: This study focused on the selection of suitable emergent plants for arsenic removal from water. The arsenic uptake abilities of *Typha* spp, *Canna* spp, *Colocasia esculenta*, *Heliconia psittacorum* and *Thalia dealbato* J. Fraser were determined for plants harvested at 14 and 28 days after being treated with 1 mg As L⁻¹. *Colocasia esculenta* was the best plant for removing arsenic from water for a 28 day exposure period. Arsenic accumulated mainly in the root (approximately 195 μ g As g⁻¹ dry weight of *Colocasia esculenta* at 28 days of exposure). The efficiency of the plants for arsenic accumulation depended on the arsenic content in the nutrient solution and exposure time. As the exposure period gets longer, the greater the amount of arsenic is uptaken by the test plants.

Keywords: emergent plants, arsenic, removal, contaminated water.

INTRODUCTION

In Thailand, contamination of domestic water supplies by arsenic causes serious health problem to people living in the Ron Phibun district, Nakhon Si Thammarat province. The disease is called "Kai-Dum" in Thailand, but in other parts of the world it is called "arsenic poisoning or arsenicosis" (Nriagu, 1994; Williams et al., 1996). Mine tailings have been proven to be the source of the problem (Williams et al., 1996). Arsenic is released from mine tailings, contaminated soil, surface water and ground water. The development of efficient, cost-effective and environmentally friendly methods for arsenic removal from water is important to safeguard the quality of drinking water, agricultural produce and the environment. Although toxic metal contaminants in water are usually removed by ionexchange, reverse osmosis, microfiltration, precipitation, or flocculation, these methods may require arduous maintenance and can be prohibitively expensive for treatment of large water volumes. Aquatic plants and emergent plants have been used to remove toxic metals from water. The floating aquatic plants commonly utilized for water remedial work include duckweed (Lemna minor L), water hyacinth (Eichhornia crassipes), water zinnia (Wedela trilobata

Hitchc.) and water lettuce (*Pistia stratiotes* L.) while commonly used emergent aquatic plants are bulrushes (*Scipus validus*), reeds (*Phragmites australis*), umbrella plants (*Cyperus alternfolius* L.), Mare's tails (*Hippuris vulgaris* L.), stripped rushes (*Baumia rubiginosa*), Cattails (*Typha* spp and *Typha latifolia*) and parrot's feathers (*Myriophyllum brasiliense* Camb). These plants have been reported to remove various toxic metals, such as Cu²⁺, Cd²⁺, Cr⁶⁺, Ni²⁺, Pb²⁺ and Zn²⁺, from the aquatic phase (Duchenkov *et al.*, 1995; Mitchell and Karathanasis, 1995; Ye *et al.*, 1997; Adel *et al.*, 1998; Qian et.al., 1999; Zhu *et al.*, 1999; Cardwell *et al.*, 2002; Manios *et al.*, 2003).

The ability of aquatic plants to remove toxic metals from water depends on (1) sediment geochemistry, (2) water physico-chemistry, (3) plant physiology and (4) plant genotype (Outridge and Nollor, 1991; Karathanasis and Thompson, 1993). The first two parameters control species of toxic metals in sediments and water, whereas the last two control the ability of plants to accumulate plant-available forms of the metals. Thus, it is important to acquire knowledge of the ability of different wetland plant species to absorb toxic metals under different conditions. This study focused on the selection of suitable emergent plants to remove arsenic from arsenic contaminated water.



Fig 1. The percentage fresh weight change of 5 emergent plants.

MATERIAL AND METHODS

Emergent plants were selected for the experiments based on the following characteristics: (i) adaptation to local climate and soils, (ii) tolerance to arsenic in wastewater, (iii) high biomass production, (iv) perennial species, (v) rapid growth and colonization, (vi) nonweedy, aesthetic habit and (vii) value as wildlife habitat. Four of the five chosen species are flowering plants that possess the above characteristics, especially on aesthetic habit, while Colocasia esculenta is the dominant wetland plant species in Ron Phibun district, the arsenic polluted area in southern Thailand. Fullstrength, modified Hoagland solution was used as the test medium since it could provide enough nutrients for plants throughout the experiment. The Hoagland solution was modified by omitting ferrous sulfate from the original recipe to prevent the precipitation of arsenic by iron. Nevertheless, arsenic uptake by plants may have been influenced by the high level of phosphorus in the Hoagland medium.

The chemical form of arsenic selected for use in this study was arsenate (As V) because it is the dominant arsenic species in the water phase under oxidation condition. The total arsenic concentration in surface water in Ron Phibun district is about 1 mg L⁻¹ (Williams, 1996; Bunnag, 2000). Thus, this concentration was selected to use in the screening of the 5 emergent plant species for arsenic uptake.

Five species of emergent plant, namely Typha spp,

Colocasia esculenta, *Canna* spp, *Heliconia psittacorum* and *Thalia dealbato* J. Fraser, were collected from an uncontaminated area, washed and acclimatized in Hoagland solution for at least 7 days before use in the experiment. Each plant species was divided into 2 groups. The plants in each group, weighed approximately 50 g, were separately transferred into a 18 L glass container containing 4 L of full strength modified Hoagland solution. The arsenic treated plants were exposed to arsenate (Na₂HAsO₄,7H₂O) at 1 mg L⁻¹. The pH of the medium was adjusted to 5.5. Each treatment had 3 replicates. The total volume of the solution was kept constant by adding deionized water at weekly intervals to compensate for water lost through plant transpiration and evaporation.

Both plants and the test medium were sampled at 14 and 28 days after starting the experiment. Shoots and roots were separated, cleaned thoroughly with tap water and rinsed with 0.1 M HCl solution, followed by several rinses with distilled water and weighed for fresh weight. They were then dried at 75°C for 48 hr and weighed again for dry weight. The dried plant samples were ground in a stainless-steel blender and digested with concentrated HNO3 using a wet digestion technique (Carbonell-Barrachina et al., 1998). The digestion temperature was controlled at approximately 80-100 °C to avoid As volatilization as recommended by Jinadasa et al. (1997). Determination of total arsenic content in plants and water was done using Graphite Furnaces Atomic Absorption Spectrophotometry following the method of Visoottiviseth et al. (2002).

Species	Days of	Plant c	dry weight (g	t) Shu	oot/Root r	atio l	KG ³ A	rsenic concentra	tion [mg kg ⁻¹ (dry	wt.)]	RAI	ACR
	harvest	Shoot	Root	Total	control A	s treated		Shoot Root	Total			
Typha spp	14	5.97±1.09	4.29±1.12	10.3±2.12	1.50	1.43	0.91	2.85±1.34	33.38±6.55	15.35±1.14	33.38	0.09
	28	6.19±1.59	3.15 ± 0.3	9.34±1.84	2.15	1.96		5.19±2.12	79.94±10.54	30.73±6.16	79.94	0.07
Canna spp	14	4.87±0.89	1.22 ± 0.17	6.09±1.03	1.99	3.98	1.55	4.16 ± 4.8	26.68±5.32	8.76±3.19	26.68	0.17
	28	7.78±1.58	1.63 ± 0.17	9.41±1.73	4.49	4.75		11.49 ± 1.16	58.89±16.9	19.64 ± 2.38	58.89	0.21
Colocasia	14	3.33±0.64	1.18 ± 0.47	4.5 ± 1.01	2.58	3.10	2.49	1.89 ± 1.72	78.46±25.84	20.58 ± 3.03	78.46	0.03
sculenta	28	8.79±3.44	2.4±0.43	11.2 ± 3.85	4.59	3.50		3.33 ± 0.46	194.87 ± 115.3	43.9±18.31	194.87	0.02
Heliconia	14	6.10±2.73	2.86±1.42	8.96±1.38	2.05	3.13	1.35	4.43±1.4	27.34±9.74	10.95 ± 2.21	27.34	0.17
pittacorum	28	8.63±1.78	3.51 ± 1.23	12.1±1.73	3.59	2.81		7.20±6.01	82.81±6.65	28.5±11.7	82.81	0.09
Thalia	14	4.33±3.02	2.38 ± 0.54	6.71±3.03	1.28	1.89	1.67	4.34 ± 2.66	22.73 ± 22.35	9.35±5.52	22.73	0.19
lealbato	28	7.51±1.68	3.72±2.47	11.2 ± 1.67	2.41	2.71		7.03 ± 5.86	122.9 ± 27.32	32.28±11.44	122.9	0.06

[cdble 1. Plant dry weight (g) (mean \pm SD), Arsenic concentration (mg kg¹ DW) (mean \pm SD), RAl¹ and ACR² for each of 5 emergent plants. Numbers in bold TAG

RESULTS AND DISCUSSION

Plant Growth Rate

Plant growth rate is a crucial factor for showing tolerance of the test plants to exposure to arsenic. The results showed that all of the 5 emergent plants could survive well in the solution of 1 mg As L⁻¹ and the fresh weight of the plants increased as the exposure period increased. The percentage change of fresh weight was significantly affected by the plant species and the harvesting time (p < 0.01) (Fig 1). *Colocasia esculenta* had the highest fresh weight production which was significantly different from the others (p < 0.01). This shows that 1 mg L⁻¹ arsenate is not toxic to the plant.

Carbonell-Barrachina *et al.* (1998) reported that arsenate improved growth of *Spartina alterniflora*. The positive growth of plants was linked with phosphorus nutrition. Arsenate is not essential for plants but its structure is similar to phosphate, a major nutrient for plants. It has been reported that arsenate competes with phosphate as a substrate for the phosphate uptake system in a wide variety of plant species, although there is a higher affinity for phosphate than arsenate (Meharg and Macnair, 1990). Tongsawad (2002) reported that *Colocasia esculenta* was the best phosphate removal plant.

Shoot/root dry weight ratios revealed a change in response to arsenic toxicity. For the 14 day exposure period, the results showed that the shoot/root ratio of 4 emergent plants, except for Typha spp, was higher in the arsenate treated pots than in the control pots (Table 1). In addition, there were significant differences among emergent plant species (p < 0.01). At 28 day exposure, the shoot to root ratio of 3 test plant species namely, Typha spp, Colocasia esculenta and Heliconia psittacorum, was lower in the arsenate treated plants than in the controls. When comparison of shoot to root ratios between 14 and 28 days of exposure of the arsenate treated plants was made, it was found that the shoot to root ratios of the 4 plant species of the 28 day exposure, except for Heliconia psittacorum, were higher than that of the 14 day exposure.

The increase of shoot/root ratio implied that root growth was inhibited and/or shoot growth was promoted. This may be due to an effect of arsenate because many investigators have found that plants exposed to arsenic had significantly smaller root dry weights compared to controls (Carbonell-Barrachina *et al.*, 1997; Burlo *et al.*, 1999). This was due to root plasmolysis (Marin *et al.* 1992). In addition, Aira *et al.* (2001) reported that arsenate treatment positively stimulated shoot development. In this study, arsenate was found to be toxic to *Heliconia psittacorum* (Table 1). Therefore, the shoot to root ratio of *Heliconia psittacorum* after 28 day exposure was lower than at 14 day exposure.

Water Transpiration

Water transpiration was determined by measuring the volume of water added to bring up the volume of the test medium to the level as at the start of the experiment, to compensate for the water consumed by the plants. Interestingly, the water volumes transpired by the control and the arsenate treated plants were not significantly different. *Colocasia esculenta* was the highest water consuming plant (Fig 2). As the exposure to arsenate was lengthened, the greater the volume of water was consumed (p < 0.01). This was also true with the other three plant species tested namely, *Canna* spp, *Thalia dealbato* and *Heliconia psittacorum*.

Typha spp gave a result different to the other test plant species. The plants, both control and arsenate treated, grew best at the start of the experiment until 14 days. After that, the growth of the control plant was reduced and then remained stable until the last collection time (28 days), while a gradual reduction in water transpiration was observed in the arsenate treated plants. This may be the result of arsenic toxicity and is in accordance with other investigators (McFarlande and Pfleeger, 1987; Gadallah, 1995; Thompson *et al.*, 1998) who reported that growth and reduction of transpiration could be used to determine toxicity of test chemicals. Trapp *et al.* (2000) investigated the toxicity of cyanide to willow plants and concluded that the water transpiration rate of the cyanide treated

plants was reduced due to the toxicity of cyanide to the plant. Further more, it was reported that water transpiration was closely related to photosynthesis and growth of the test plant, which was also observed in this experiment. The test plants which exhibited the lowest to the greatest water transpiration were as follows: *Heliconia psittacorum* < *Thalia dealbato* < *Typha* spp < *Canna* spp < *Colocasia esculenta*, which corresponded to growth of the test plants as shown in Figure 1. Therefore, the arsenate was the most toxic to *Heliconia psittacorum*.

pH of the Medium

A change in pH of the medium was observed in this experiment. The pH of each emergent plant species showed some fluctuation with a trend for slight decreases. A decreased of pH of the medium to pH <4 was found for *Colocasia esculenta* and the *Canna* spp (Fig 3). Thursby (1984) reported that some aquatic plants such as rice and submerged macrophytes were able to transport oxygen, a product of photosynthesis, to the roots and release it at the rhizophere. However, this explanation may not apply to this experiment. Thus, further study should be performed in order to get a clearer understanding of the reduction of pH of the growth medium by *Colocasia esculenta* and *Canna* spp

A pH change may affect the bioavailability of arsenic. Mok and Wai (1990) reported that an acid pH (pH < 4) would enhance the mobility of arsenic in the water phase. Decreasing pH levels are thus indirectly correlated with arsenic availability for plant uptake.



species

Fig 2. Water transpiration by each emergent plant species for control (0 mg L^{-1}) and arsenic treated (1 mg L^{-1}) plants.



Fig 3. pH level of nutrient solution in each emergent plant treatment from the initial day (0D), 7 days (7D), 14 days (14D), 21 days (21D) and 28 days (28D) exposure.

Other investigators have reported that there was a better correlation between plant growth and available arsenic than between plant growth and total arsenic (Woolson *et al.*, 1971; Walsh and Keeney, 1975). Meharg and Macnair (1990) observed a decrease in arsenate uptake with an increase of pH value. Arsenate uptake was optimal at pH 5 where $H_2AsO_4^-$ was the dominant anion in solution. As the pH increased to pH 8 in which $HAsO_4^{2-}$ was the dominant form, arsenate uptake decreased. *Colocasia esculenta* decreased the pH to less than 4 and it seemed to be the best emergent plant

for total arsenic accumulation.

Arsenic Accumulation

The uptake and distribution of arsenate in each plant part is summarized in Fig 4. The 1 mg L⁻¹ arsenate treated plants showed differences in their abilities to remove arsenic. The arsenic uptake by the plants was significantly influenced by the plant species and the time of harvest (p < 0.05). This finding corresponds with Walsh and Keeney (1975). The total arsenic accumulation of all emergent plant species exposed to



Fig 4. Arsenic concentration in each plant parts for plants harvested at 14 days and 28 days exposure.

1 mg As L⁻¹ at 14 days was significantly lower than at the 28 day exposure (p < 0.05). At 14 days of exposure to arsenate, the growth of the test plants was slow, thus less arsenic accumulated. At 28 days, the growth rate was increased and more arsenic was uptaken. This finding corresponds to that of Meharg and Macnair (1991) who reported that the rate of arsenic uptake correlated with the time of arsenic exposure. In this study, arsenic uptake at 28 days of exposure was 2.0, 2.2, 2.2, 2.6 and 3.5 fold higher than at 14 days of exposure for Typha spp, Colocasia esculenta, Canna spp, Heliconia psittacorum and Thalia dealbato, respectively.

In this study, Colocasia esculenta was found to be the best plant for arsenic accumulation as compared to the other test plant species. At 28 days, Colocasia esculenta could accumulate arsenic at 194.87 mg/kg⁻¹ dry weight in the root. The ability of emergent plants in removing metals from the aquatic environment has been of interest to many researchers. Rai et al. (1995) investigated the potential of aquatic macrophytes for removal of metals (such as Cu, Cr, Fe, Mn, Cd and Pb) and concluded that plants differed in the extent of metal accumulation. However, metals present in relatively high concentrations were accumulated. They also suggested the use of aquatic macrophytes for metal abatement in dilute wastewater. In the Tulenovo oil deposit, Bulgaria, water contaminated with crude oil and toxic metals (such as Cd, Cu, Pb, Mn and Fe) was passed through a constructed wetland containing Typha latifolia, T. angustifolia, Phragmites communis, Scirpus lacustris and Juncus spp. The metal contents were reduced from 2-8 times the permissible level 5 to below the relevant permissible level (Groudeva et al., 2001).

Root Absorption Index (RAI)

The RAI (the ratio of root As concentration (mg kg-¹) to nutrient solution As concentration (mg L⁻¹)) represents the translocation of arsenic in nutrient solution into the root. An increase in the RAI value implies that arsenic has accumulated in the root system. Carbonell-Barrachina et al. (1998) concluded that plants mainly accumulated arsenic in the root and only minor amounts of arsenic was translocated to the shoot. The results of this experiment showed that Colocasia esculenta had the highest RAI (p < 0.01) and, therefore, was the best arsenic accumulator among the emergent plants tested. Cardwell et al. (2002) determined the extent of metals accumulation in aquatic macrophytes from southeast Queensland, Australia, and found that roots consistently had higher metal concentrations than either the stems or leaves. However, they found no consistent trend of stems accumulating more metals than the leaves. For Typha spp, metal concentrations followed the order of roots> rhizomes> leaves, while for Persicaria spp, the order was roots> leaves> stems. In the present study, we determined arsenic contents of the shoot (stem and leaves) and root and found that arsenic consistently accumulated more in the roots than in the shoots for all 5 emergent plants tested (Table 1). Among them, Colocasia esculenta was found to be the highest arsenic accumulating species. Arsenic concentrations in the roots of Colocasia esculenta were 78.46 and 194.87 mg kg⁻¹ dry weight for 14 days and 28 days exposure time, respectively.

The Arsenic Concentration Ratio (ACR)

The ACR [the ratio of shoot As concentration (mg kg⁻¹) to root As concentration (mg kg⁻¹)] indicates the transport and movement of arsenic within the plant or the mobility of arsenic from root to shoot (Burlo et al., 1999). In this experiment, the ACR values were not significantly different among the emergent plant species and the exposure time tested (Table 1). The ACR values of Colocasia esculenta at 14 days and 28 days of exposure

ab	le	2	Arsenic	uptake	: by	each	emergent	plant	species	per m	2.
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Species	Days of 9 harvest	% arsenic shoot	accum root	ulation* total	Total accumulation rate**	Dry weight of 16 plants m ⁻² (g m ⁻²)	Plant uptake (mg day-1 m-2)
Typha. spp.	14	0.35	2.74	3.09	0.94±0.27	134.36±9.36	0.13±0.04
	28	0.59	1.06	1.65	0.79±0.25	105.96±24.6	0.08±0.02
Canna spp.	14	0.40	0.65	1.05	0.64±0.21	100.45±12.2	0.06±0.02
	28	1.74	1.93	3.67	0.72±0.05	155.21±21.8	0.11±0.01
Colocasia	14	0.14	1.67	1.81	1.48±0.32	79.54±10.07	0.13±0.01
esculenta	28	0.58	9.89	9.77	1.56±0.65	178.99±61.7	0.3±0.22
Heliconia	14	0.56	1.37	2.73	0.78±0.16	143.41±22.1	0.11±0.02
spittacorum	28	1.12	5.65	6.77	0.9±0.38	170.82±15.8	0.15±0.05
Thalia	14	0.41	1.03	1.44	0.73±0.17	120.71±22.8	0.09±0.03
dealbato. J Fraser	28	1.11	7.97	9.08	0.89±0.31	106.81±44.3	0.12±0.08

* % arsenic accumulation by shoot = [Shoot dry wt (g) × Shoot As (μ g g⁻¹)] × 100 / [As in nutrient solution (μ g mL⁻¹) × 4 L]

% arsenic accumulation by root = [Root dry wt (g) × Root As (μ g g⁻¹)] × 100 / [As in nutrient solution (μ g mL⁻¹) × 4 L] ** Total accumulation rate = [Shoot dry wt (g) × Shoot As (μ g g⁻¹)] + [Root dry wt (g) × Root As (μ g g⁻¹)]

[(Shoot dry wt (g) + Root dry wt (g)) × (day of harvest)]

owest ACR values among the 5 plant species. For *Canna*

were 0.03 and 0.02, respectively, and these were the lowest ACR values among the 5 plant species. For *Canna* spp, the ACR value was 0.17 at 14 days of exposure and 0.21 at 28 days of exposure. This is different from the other test plant species because the ACR value had increased after a longer exposure time. However, the difference was not significant. The strategy developed by emergent plants to tolerate arsenic is avoidance by limiting upward transport and thus, resulting in accumulation primarily in the root system (Meharg and Macnair, 1991; Burlo *et al.*, 1999).

Mass Balance and Environmental Perspective

In this experiment, the total arsenic uptakes by *Typha* spp, *Canna* spp, *Colocasia esculenta*, *Heliconia psittacorum* and *Thalia dealbato* were approximately 3.09%, 1.05%, 1.81%, 1.93% and 1.44%, respectively, after the 14 day exposure period and 1.65%, 3.67%, 10.47%, 6.77% and 9.08%, respectively after the 28 day period. All the emergent plants, except for *Typha* spp, showed positive increases in arsenic uptake with a longer exposure period. *Typha* spp removed arsenic at 0.94 mg/ kg⁻¹d⁻¹ after 14 days of exposure but the accumulation rate of arsenic by *Typha* spp at 28 day exposure was 0.79 mg/ kg⁻¹d⁻¹ (Table 2). This may be the result of arsenic toxicity to *Typha* spp. *Colocasia esculenta* showed the highest arsenic accumulation rate at 28 days of exposure.

Assuming there are 16 plants m⁻² (the distance between each plant is 20 cm). Total arsenic accumulation rate ($\mu g g^{-1} d^{-1}$) by each plant species can be calculated as follows:

 $\frac{[\text{Shoot dry weight (g) x Shoot As conc. } (\mu g g^{-1})] + [\text{Root dry weight (g) x Root As conc. } (\mu g g^{-1})]}{[(\text{Shoot dry weight (g) + Root dry weight (g)}) x days of harvest]}$

The total accumulation rate along with the calculated dry weight of plants are presented in Table 2. It can be seen that *Colocasia esculenta* was the best plant species for arsenic accumulation among the 5 emergent plants tested. It could accumulate As at the rate of 0.3 g d⁻¹ m⁻². For 14 days of arsenic exposure, *Colocasia esculenta* was the best in arsenic accumulation i.e., 20.58 mg kg⁻¹ in this study. Zhu *et al.* (1999) did an experiment on arsenate accumulation by water hyacinth. They found that water hyacinth could accumulate arsenic at 0.69 mg kg⁻¹ at 14 day exposure. Therefore, each test plant species in this experiment showed better arsenic accumulation than the water hyacinth. Arsenic may be more toxic to water hyacinth than to the emergent plants used in this study. However, environmental factors may interfere with the removal efficiency of the emergent plants such as the presence of competing ions, temperature, salinity, competition with other emergent plants, pH conditions, etc.

The arsenic mass balance

The arsenic mass balance was calculated from the initial arsenic concentration, 1 mg L⁻¹, the arsenic uptake



Fig 5. Mass balance of arsenic uptake of each plant species at 14 days (14D) and 28 days (28D) of exposure.

by plants, dry biomass and arsenic content in the plant and is presented in Figure 5. Arsenic at 1 mg L⁻¹ with no plant was used as a control for the percentage of arsenic lost under the experimental conditions. The percentage arsenic lost for the control and the plant treatment system were approximately 20% and 40%, respectively. One possible explanation in this difference is that metabolites secreted from the emergent plants may have stimulated growth of microorganisms and thus changed the solubility of arsenic in the system.

CONCLUSION

All 5 emergent plants that were tested in this study had different growth rates and efficiencies for arsenic removal. Among the plant species tested, *Colocasia esculenta* had the highest fresh weight production and the highest ability to remove arsenic when exposed to 1 mg L⁻¹ arsenate. Arsenic accumulation by the emergent plants increased over the exposure period. Arsenic was accumulated mainly in the roots of the plants. Translocation of arsenic to the shoot was very low. From this study, *Colocasia esculenta* has good potential to be used for the removal of metals from polluted water. However, these results were obtained from hydroponic experiments. Field experiments are now needed.

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