Efficiency of Arsenic Removal from Soil by *Vetiveria zizanioides* and *Vetiveria nemoralis*

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Abstract: Phytoremediation is an alternative technology to remove heavy metals in contaminated soil. *Vetiveria zizanioides* (Linn.) Nash (Surat Thani ecotype) and *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) were chosen for arsenic removal experiments. Both were studied after nursing for one month. They were then grown in the experimental pots, which contained soil with sodium arsenate ($Na_2HAsO_4.7H_2O$) at different concentrations (control, 50, 75, 100, 125, and 150 mgAs/kg soil). Three experiments were conducted for each concentration. Plants were harvested, their growth observed and analyzed for arsenic accumulation in their roots, stems and leaves every 15 days up to 90 days. From the results, all the plants grew well in every concentration of arsenic, with 100% survival. In addition, *V. zizanioides* had plant per clump numbers and diameter of clump higher than *V. nemoralis*. In contrast, the height and dry weight of *V. nemoralis* was higher than of *V. zizanioides*. Accumulation of arsenic in the roots of both species was higher than in the leaf. The amount of arsenic accumulation in *V. zizanioides* was more than in *V. nemoralis*. In addition, arsenic removal efficiency of both species increased with increasing exposure time. The highest efficiency of *V. zizanioides* was very low, for the 90 day experiment at a concentration of 75 mgAs/kg soil dry weight, and the highest efficiency of *V. nemoralis* was very low at 0.04%, for the 90 day experiment at a concentration of 125 mgAs/kg soil dry weight.

Key Word vetiver, arsenic removal, phytoremediation
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1 INTRODUCTION

In Thailand, human health problems caused by the exposure to arsenic was first recognized in 1987. People living in 12 villages at Ron Phibun District and near by at Nakhon Sri Thammarat Province, southern Thailand showed symptions of sickness, suffering from chronic arsenic poisoning and skin cancer. These areas have a history of extensive mining of bedrock and alluvial material. The wastes which are typically rich in arsenopyrite (FeAsS) and related alteration products contaminated widely by the surrounding environment. The concentration of arsenic in the soil ranged between 0 – 3,931 mgAs/kg soil (average is 222.8 mgAs/kg). (Pollution Control Department, 1998). Remediation of contaminated soil by soil washing or the cut off wall method are high investment. Phytoremediation, which is a form of ecological engineering, has emerged as an alternative that has proven to be effective and relatively inexpensive (Raskin *et al.* 1997). Therefore, it was strongly considered as an alternative in this case. Phytoremediation is the use of vegetation for *in situ* treatment of contaminants such as heavy metals and pesticides in soils and water. The ideal characteristics of plant species used to remove toxic contaminants from soil should be as follows: high biomass, short life span, tolerable and able to accumulate high concentrations of contaminants (Raskin *et al.* 1997). The degree of arsenic uptake by plants varies widely from species to species and from parts of the plant. Several studies have shown that higher levels of arsenic accumulate in the roots compared to the leaf or stems (O'Neill, 1993; Tlustos *et al.*1998,) Moreover, the uptake of arsenic and other heavy metals would be difficult in neutral pH soil, high clay levels and soils with the presence of organic matter content (Tlustos *et al.* 1997)

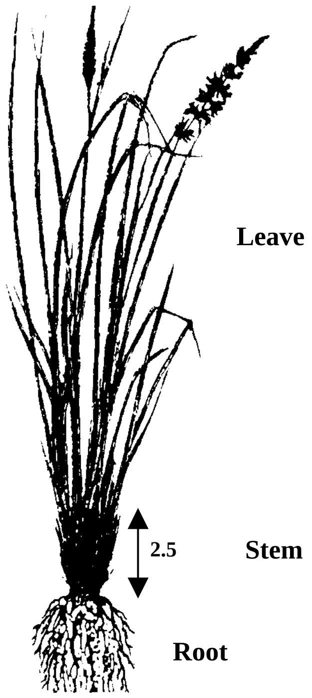


Figure 1. Vetiver grass

Vetiver is a perennial grass with strong ecological adaptability, large biomass and is easy to manage and grow in different soil conditions. It has great potential for various applications including hillside soil and water conservation, sustainable agriculture, fixing sandy riverbank and pollution control. Vetiver is an extremely hardy grass species with many characteristics that makes it ideal for environmental protection, i.e. its roots reach 3-4 m. in the first year. The application of vetiver grass was first developed by the World Bank for soil and water conservation in India in the 1980s. In Thailand, 2 types of vetiver grass have been founded, *V. zizanioides* and *V. nemoralis*. Both *V. zizanioides,* Surat Thani ecotype and for *V. nemoralis*, Prachuabkirikhan ecotype are easily found (Fig 1). They have the ability to grow well in various climates and in the different geographical areas present in Thailand. Besides, *V. zizanioides* can tolerate and grow in high metal contaminated soil (Truong, 2000). The total dry weight of *V. zizanioides* grown in 250 mgAs/Kg soil significantly decreased with the arsenic accumulating more in the roots than in the leaves (Truong, 1996; Truong and Baker, 1998). Because of their favorable characteristics, two types of vetiver grass (*V. zizanioides* (Surat Thani ecotype) and *V. nemoralis* (Prachuabkiri-khan ecotype) were selected for this study. The objectives of this research was to study the growth of both vetivers mentioned above in different soil arsenic concentrations, to study the accumulation of arsenic in various parts of the vetiver and to compare the efficiency of arsenic removal rate of the chosen vetiver.

2 METHODOLOGY

This study was conducted as a laboratory experiment. The two chosen vetivers' were grown in pots containing soil treated with sodium arsenate ($Na_2HAsO_4.7H_2O$) with no leaching. The chosen plants were grown in 6 different soil concentrations from 0-150 mgAs/Kg for 90 days. The plants were harvested and analyzed every 15 days for arsenic accumulation in the roots, stems and leaves.

The design test method involved a closed drainage system to prevent leakage of arsenic from the system. The arsenic was directly added to the pot and totally spread in the soil. During the experiment, the plants were showered with water, which was not allowed leach out of the pot. Before running the experiment, the soil was analyzed for arsenic contamination.

2.1 Soil and Plants Preparation

Topsoil, al silt loam texture from Pathumtani Province was used in the study, (it was collected 40 km north of Bangkok). It was mixed to homogeneity then analyzed for physical and chemical properties such as pH level, conductivity, organic matter, N, P, K and total arsenic. Then 4 kg of arsenic non-detectable soil was put into 30 cm deep pots and unleached prior to planting.

In this experiment, *V. zizanioides* and *V.* were cleaned and cut into 35 cm. long pieces. Then, they were planted and nursed for one month. During this nursing period, they were randomly sampled to analyze for arsenic contamination but the results showed that arsenic was not detected. (Table 1)

Table 1. Characteristic of Soil used

Characteristic	Value	Analyzed Method
Moisture - air dry - oven dry	 20.21% 22.25%	Gravimetric Method
pH	6.44	pH meter
Soil type sand : silt : clay	Silt loam 16.83 : 58.17 : 25.01	Hydrometer Method
Ion exchange capacity	13.7518 me/100 g	Ammonium acetate Method
Organic matter	0.9433%	Walkey-Black Method
Nitrogen	0.05%	Kjeldahl Method
Potassium	369.3 ppm	Flame photometer
Phosphorus	**575 ppm**	**Perchloric acid ($HClO_4$) Digestion**

Arsenic	**Non detectable**	**Nitric acid and Sulfuric acid Digestion Atomic Absorption Spectrophotometer**

After the nursing period, every experimental pot with the exception the control pots were spiked with sodium arsenate ($Na_2HAsO_4.7H_2O$) solution to obtain 5 different concentrations 50, 75, 100, 125, and 150mgAs/kg respectively. All pots were placed outdoor accordingly to complete randomized design with approximately 0.75 m intervals.

2.2 Growth Observation

After treatment with arsenic, three of the plants in each treatment were harvested every 15 days up to 90 days. The growth parameters observed were based on height, diameter of clump and number of plants per clump. The wet and dry weight was also recorded for each plant. The growth data was analyzed and compared by using ANOVA at 95% confidence level.

2.3 Arsenic Accumulation in Vetiver

After recorded growth parameters, each plant was cleaned, cut and separated into roots and leaves. To get stable dry weight, every part was put into an oven at 60 ^{o}C for 3 days. Both wet and dry weights were recorded. All dried parts were ground and mixed thoroughly and then digested with acid by the method of US EPA-3030, 1982. Sequentially, the sample solutions were analyzed for arsenic content by atomic absorption spectrophotometer (Varian: model Specter AA-10 Plus, VGA-76). Arsenic accumulation in each part of the plant was calculated and defined as milligram arsenic per kg of dry weight.

2.4 The Efficiency of Arsenic Removal

Total arsenic accumulation in the plant was determined and compared to the total amount of arsenic of that treatment as % arsenic removal. Then the collected data was analyzed for arsenic removal efficiency in two-ways. By first using the ANOVA and then, comparing the differences by DMRT (Duncan Multiple Range Test).

The efficiency of arsenic removal was calculated as per the equation below:

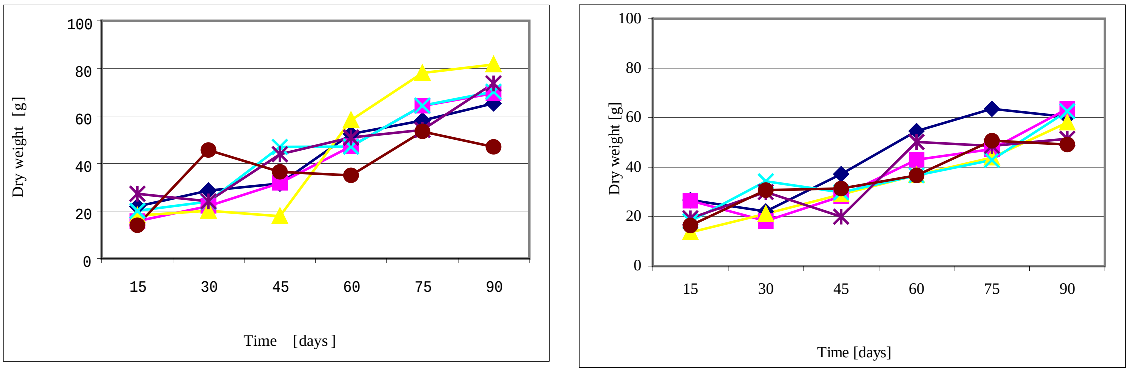
$$\text{Efficiency of arsenic removal (\%)} = \frac{\text{[As in leave + As in root] (mg)}}{\text{Total As in pot (mg.)}} \times 100$$

3 RESULTS AND DISCUSSIONS

3.1 Growth Observation

During the experimental period, both ecotypes of vetiver grass survived under all conditions of soil arsenic concentration and grew well in the control group. From statistical analysis, the growth of plants in the terms of height and diameter of clump and the number of plants per clump were not significantly different between the arsenic treated group and the control, at 95% confidence level. This finding has confirmed that vetiver grasses are highly tolerable and thus could grow in high arsenic contaminated soil (Truong, 2000). Moreover, the number and diameter of clumps of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) was higher than *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype). On the contrary, the height and dry weight of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) was higher than the other ecotype as shown in Figure 2.

Figure 2. Dry weight of vertiver grasses during the experimental period



V. nemorali (Prachuabkirikhan ecotype) *V. zizanioides* (Surat Thani ecotype)

0 mg As/kg 50 mg As/kg 75 mg As/kg 100 mg As/kg 125 mg As/kg 150 mg As/kg

3.2 Arsenic Accumulation in Vetiver

Arsenic accumulation was found in all parts of the grasses with different levels of concentration. The arsenic accumulation in both ecotypes of vetiver grass was higher in the roots than the leaves. For the 150 mgAs/kg soil pot, the arsenic accumulations in leaves of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) and *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) were 0.463 and 0.494 mgAs/kg (in leaves dry matter), respectively whereas in root, they were 6.197 and 11.775 mgAs/kg (in roots dry matter), respectively. The results are quite similar to the study performed by Truong and Baker, 1998. The maximum arsenic accumulations in roots of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) and *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) were 9.795 and 11.775 mgAs/kg (in root dry matter). However, arsenic accumulation in roots of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) was more than *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) as shown in Table 1 and Table 2. In addition, the level of arsenic accumulation tended to decrease with growth over time because increases in arsenic accumulation increased at a lower rate than the plant dry weight. Roontanakiat and Chairoj (2000) also reported decreasing arsenic accumulation with time.

Table 1. Arsenic accumulation in various part of *Vetiveria nemoralis* (mgAs/kg/plant).

Part of *V. nemoralis*	$Na_2HAsO_4.7H_2O$ concentration (mg As/kg soil)	Time (days)					
		15	30	45	60	75	90
As in leaves	50	$^{a}0.4306^{a}$	$^{b}0.2597^{a}$	$^{c}0.2578^{a}$	$^{d}0.2726^{a}$	$^{e}0.1310^{a}$	$^{f}0.1472^{a}$
	75	$^{a}0.4448^{b}$	$^{b}0.4343^{b}$	$^{c}0.3343^{b}$	$^{d}0.3367^{b}$	$^{e}0.2532^{b}$	$^{f}0.1371^{b}$
	100	$^{a}0.4811^{c}$	$^{b}0.5884^{c}$	$^{c}0.3568^{c}$	$^{d}0.4641^{c}$	$^{e}0.3274^{c}$	$^{f}0.2887^{c}$
	125	$^{a}0.5160^{d}$	$^{b}0.4969^{d}$	$^{c}0.4082^{d}$	$^{d}0.5786^{d}$	$^{e}0.3552^{d}$	$^{f}0.2759^{d}$
	150	$^{a}0.5782^{e}$	$^{b}0.5925^{e}$	$^{c}0.5097^{e}$	$^{d}0.6450^{e}$	$^{e}0.3544^{e}$	$^{f}0.4940^{e}$
As in root	50	$^{a}5.2377^{a}$	$^{b}4.7181^{a}$	$^{c}1.4961^{a}$	$^{d}4.6323^{a}$	$^{e}1.6473^{a}$	$^{f}1.9704^{a}$
	75	$^{a}4.8945^{b}$	$^{b}5.5359^{b}$	$^{c}2.9090^{b}$	$^{d}2.3585^{b}$	$^{e}2.9390^{b}$	$^{f}2.2774^{b}$
	100	$^{a}5.6138^{c}$	$^{b}8.4321^{c}$	$^{c}2.7610^{c}$	$^{d}4.5315^{c}$	$^{e}4.0765^{c}$	$^{f}5.3828^{c}$
	125	$^{a}5.1276^{d}$	$^{b}9.4097^{d}$	$^{c}5.5909^{d}$	$^{d}5.6653^{d}$	$^{e}4.3703^{d}$	$^{f}6.5655^{d}$
	150	$^{a}8.4115^{e}$	$^{b}5.5959^{e}$	$^{c}5.6695^{e}$	$^{d}9.0023^{e}$	$^{e}5.9108^{e}$	$^{f}11.7750^{e}$

Table 2 Arsenic accumulation in various part of *Vetiveria zizanioides* (mgAs/kg/plant).

Part of *V. zizanioides*	$Na_2HAsO_4.7H_2O$ concentration (mg As/kg soil)	Time (days)					
		15	30	45	60	75	90
As in leaves	50	$^{a}0.5384^{a}$	$^{b}0.4321^{a}$	$^{b}0.3076^{a}$	$^{c}0.2379^{a}$	$^{d}0.2722^{a}$	$^{e}0.2311^{a}$
	75	$^{a}0.6139^{b}$	$^{b}0.3638^{b}$	$^{b}0.3535^{b}$	$^{c}0.3585^{b}$	$^{d}0.3115^{b}$	$^{e}0.3706^{b}$
	100	$^{a}0.7561^{c}$	$^{b}0.4509^{c}$	$^{b}0.4496^{c}$	$^{c}0.3003^{c}$	$^{d}0.3512^{c}$	$^{e}0.4553^{c}$
	125	$^{a}0.8546^{d}$	$^{b}0.4020^{d}$	$^{b}0.5289^{d}$	$^{c}0.5043^{d}$	$^{d}0.3481^{d}$	$^{e}0.3699^{d}$
	150	$^{a}1.2002^{e}$	$^{b}0.4672^{e}$	$^{b}0.5139^{e}$	$^{c}0.5712^{e}$	$^{d}0.3831^{e}$	$^{e}0.4628^{e}$
As in root	50	$^{a}1.8460^{a}$	$^{b}5.0495^{a}$	$^{a}1.5972^{a}$	$^{c}3.2676^{a}$	$^{d}3.0161^{a}$	$^{e}2.2818^{a}$
	75	$^{a}5.0952^{b}$	$^{b}5.7300^{b}$	$^{a}4.0749^{b}$	$^{c}6.4072^{b}$	$^{d}5.1377^{b}$	$^{e}5.0202^{b}$
	100	$^{a}5.6390^{c}$	$^{b}6.0212^{c}$	$^{a}5.2110^{c}$	$^{c}6.7332^{c}$	$^{d}5.9817^{c}$	$^{e}5.5798^{c}$
	125	$^{a}7.7237^{d}$	$^{b}8.6163^{d}$	$^{a}9.7955^{d}$	$^{c}7.0086^{d}$	$^{d}8.1342^{d}$	$^{e}6.9393^{d}$
	150	$^{a}7.3066^{e}$	$^{b}8.0711^{e}$	$^{a}6.9412^{e}$	$^{c}8.4242^{e}$	$^{d}8.2939^{e}$	$^{e}6.1969^{e}$

Note : - The same alphabets on the same corner mean there is no significant difference at 95% confidence level.

- The alphabets on the right corner are the difference of concentrations.
- The alphabets on the left corner are the difference of period of times.

3.3 Efficiency of Arsenic Removal

The arsenic removal efficiency of vetiver grass increased as arsenic concentrations in soil was increased. The arsenic removal efficiency of *V. nemoralis* showed the highest efficiency was very low at 0.0398% at the 90th day in the 125 mgAs/kg soil group.

For *V. zizanioides* (Linn.) Nash (Surat Thani ecotype), the arsenic removal efficiency of 0.488% was very low at maximum efficiency of 75 mgAs/kg soil at the 90th day (Table 3).

Table 3 Efficiency of arsenic removal for both Vetiver grass types (%).

Vetiver grass	$Na_2HAsO_4.7H_2O$ concentration (mg As/kg soil)	Time (days)					
		15	30	45	60	75	90
Prachuab-kirikhan ecotype	50	$^{a}0.0139^{a}$	$^{a}0.0209^{a}$	$^{c}0.0116^{a}$	$^{d}0.0384^{a}$	$^{d}0.0249^{a}$	$^{e}0.0288^{a}$
	75	$^{a}0.0124^{a}$	$^{a}0.0150^{b}$	$^{c}0.0081^{b}$	$^{d}0.0198^{b}$	$^{d}0.0332^{b}$	$^{e}0.0275^{b}$
	100	$^{a}0.0123^{c}$	$^{a}0.0159^{c}$	$^{c}0.0157^{c}$	$^{d}0.0238^{c}$	$^{d}0.0271^{c}$	$^{e}0.0390^{c}$
	125	$^{a}0.0118^{d}$	$^{a}0.0123^{d}$	$^{c}0.0195^{d}$	$^{d}0.0251^{d}$	$^{d}0.0201^{d}$	$^{e}0.0398^{d}$
	150	$^{a}0.0109^{e}$	$^{a}0.0147^{e}$	$^{c}0.0150^{e}$	$^{d}0.0200^{e}$	$^{d}0.0228^{e}$	$^{e}0.0357^{e}$
Surat Thani ecotype	50	$^{a}0.0157^{a}$	$^{b}0.0194^{a}$	$^{c}0.0138^{a}$	$^{d}0.0233^{a}$	$^{d}0.0365^{a}$	$^{f}0.0344^{a}$
	75	$^{a}0.0154^{b}$	$^{b}0.0193^{b}$	$^{c}0.0201^{b}$	$^{d}0.0312^{b}$	$^{d}0.0391^{b}$	$^{f}0.0488^{b}$
	100	$^{a}0.0122^{a}$	$^{b}0.0264^{a}$	$^{c}0.0170^{a}$	$^{d}0.0180^{a}$	$^{d}0.0281^{a}$	$^{f}0.0421^{a}$
	125	$^{a}0.0132^{c}$	$^{b}0.0228^{c}$	$^{c}0.0148^{c}$	$^{d}0.0217^{c}$	$^{d}0.0326^{c}$	$^{f}0.0285^{c}$
	150	$^{a}0.0100^{d}$	$^{b}0.0192^{d}$	$^{c}0.0167^{d}$	$^{d}0.0177^{d}$	$^{d}0.0322^{d}$	$^{f}0.0228^{d}$

Note : - The same alphabets on the same corner mean there is no significant difference at 95% confidence level.
- The alphabets on the right corner is the different of concentrations.
- The alphabets on the left corner is the different of period of times.

In addition, the results of both types of vetiver grass showed that there were significant differences between treatments and harvest times at 95% confidence level. The efficiency percentage of arsenic removal increased with harvest times.

While comparing the efficiency of arsenic removal of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) and *V. zizanioides* (Linn.) Nash (Surat Thani ecotype), it was found that the average efficiency of *V. zizanioides* ecotype was slightly higher than those of *V. nemoralis* ecotype. The suggested reason may be that *V. zizanioides* ecotype has more clump and root hairs, thus possess more surface area than *V. nemoralis* ecotype leading to more arsenic absorption.

4 CONCLUSIONS

Vetiver grasses could grow well in arsenic contaminated soil, up to 150mgAs/kg soil. Both ecotypes of vetiver grass also posses the ability to grow well in every soil arsenic concentration. Our results indicated that the number of clumps and diameter of clumps of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) was higher than that of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype). Nevertheless, the height and dry weight of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) were higher. The arsenic accumulation in roots was higher than that in leaves for both ecotypes of vetiver grasses. Moreover, the efficiency of arsenic removal increased with time of growth. However, *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) had better arsenic removal efficiency than that of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype). It is recommended that vetiver grass could be used to remove arsenic contaminated soil. However, it is proposed that further research be done to increase the up-take rate of vetiver by chemical processes.

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