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Efficiency of Arsenic Removal From Soil by *Vetiveria zizanioides* (Linn.) Nash and *Vetiveria nemoralis* (Balansa) A. Camus

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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 used for arsenic removal experiments. Both plants were grown one month, then put in experimental pots, the soil of which contained sodium arsenate ($Na_2HAsO_4.7H_2O$) at different concentrations (control, 50, 75, 100, 125 and 150 mg As/kg soil). Three experiments were conducted for each concentration. Plants were harvested, observed for their growth, and analyzed for the arsenic accumulation in roots, stems and leaves after every 15 days up to 90 days. It was observed that all of the plants grew well in every concentration of arsenic, with 100% survival. *V. zizanioides* (Linn.) Nash had a higher number of plants per clump and diameters of clumps than *V. nemoralis* (Balansa) A. Camus. In contrast, the height and dry weight of *V. nemoralis* (Balansa) A. Camus were higher than those of *V. zizanioides* (Linn.) Nash. Accumulation of arsenic in the root of both species was higher than in the leaf. The amount of arsenic accumulation in *V. zizanioides* (Linn.) Nash was more than in *V. nemoralis* (Balansa) A. Camus. In addition, the arsenic removal efficiency of both species increased with increasing exposure time. The highest efficiency of *V. zizanioides* (Linn.) Nash was 0.05% after 90 days at an As concentration 75 mg As/kg soil dry weight, and the highest efficiency of *V. nemoralis* (Balansa) A. Camus was 0.04%, after 90 days at an As concentration of 125 mg As/kg soil dry weight.

KEYWORDS: Vetiver grass, Arsenic removal.

INTRODUCTION

In Thailand, human health problem caused by the exposure to arsenic was first recognized in 1987. People living in 12 villages at Ron Phibun District and their vicinity in Nakhon Sri Thammarat Province, southern Thailand showed sign of sickness symptoms suffering from chronic arsenic poisoning and skin cancer. These areas have a history of extensively mining of bedrock and alluvial. The wastes which are typically rich in arsenopyrite (FeAsS) and related alteration products contaminated the surrounding environment widely. The concentration of arsenic in soil ranged between 0–3,931 mgAs/kg soil (average is 222.8 mg As/kg).[1]

Practically, remediation of contaminated soil by soil washing or cut off wall methods require a high investment. Phytoremediation, which is a form of ecological engineering has emerged as an alternative that has proven to be effective and relatively inexpensive.[2] Therefore, it should be strongly consider 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 soil and water. The ideal characteristics of plant species to be used to remove toxic contaminants from soil should be as follow: high biomass, short life span and able to tolerate and accumulate high concentration of contaminants.[2] The degree of arsenic uptake by plants varies widely from species to species and from parts to parts. Several studies have shown that higher levels of arsenic accumulate in the root compared to the leaf or stem.[3,4] Moreover, the uptake of arsenic and other heavy metals for remediation in neutral pH soil, high clay level and soil where organic matter content is present would be difficult.[5]

Vetiver is a kind of perennial grass with strong ecological adaptability and large biomass and is easy to manage and grow at different soil conditions. It has great potential for various applications including hillside soil and water conservation, sustainable agriculture, fixing sandy river banks and pollution control. Vetiver is an extremely hardy grass species with many characteristics that makes it ideal for environmental protection. Its root reaches 3-4 m in the first year. The application of vetiver grass (*Vetiveria zizanioides*) 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* (Linn.) Nash and *V. nemoralis* (Balansa) A. Camus. For *V. zizanioides* (Linn.) Nash, the Surat Thani ecotype is most easily found. And for *V. nemoralis* (Balansa) A. Camus, the Prachuabkirikhan ecotype is also most easily found (Fig 1). They have the ability to grow well in various climates and in different geographical areas of Thailand. Besides, *V. zizanioides* can tolerate and grow in soil with high metal contamination.[6] It was found that the total dry weight of *V. zizanioides* grown in 250 mg As/kg soil was significantly decreased and arsenic accumulated more in the root than in the leaf.[7,8] Because of these characteristics, two types of vetiver grass (*V. zizanioides* (Linn.) Nash (Surat Thani ecotype) and *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) were selected as the study target. The objectives of this research were to study the growth ability of the vetiver grasses mentioned above in different concentrations of arsenic contaminated soil, to study the accumulation of arsenic in various parts of the vetiver and to compare the efficiency of arsenic removal of the chosen vetivers.

Materials and Methods

This study was conducted in a laboratory experiment. The two chosen types of vetiver were grown in pots containing soil treated by addition of sodium arsenate ($Na_2HAsO_4.7H_2O$) with no leaching. The chosen plants were grown in 6 different concentrations of 0-150 mg As/kg soil for 90 days.

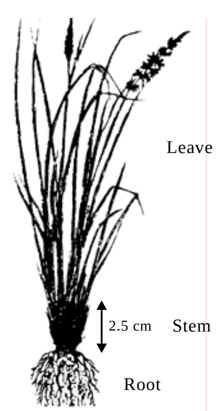


Fig 1. Vetiver grass.

Plants were harvested and analyzed every 15 days for arsenic accumulation in roots, stems and leaves.

The test method was designed as a closed system in order to control the leakage of arsenic from the system. The arsenic was directly added into the pot and thoroughly mixed in the soil. There was no leakage of water from the plant pots. During the experiment, the showering water was not allowed to come out of the pot. Before running the experiment, the soil was analyzed for arsenic contamination.

Soil and Plants Preparation

Topsoil (silt loam) 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 for planting.

In this experiment, *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) and *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) 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 no arsenic was detected (Table 1).

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

Growth Observation

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

Arsenic Accumulation in Vetivers

After having recorded the 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 °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 with an atomic absorption spectrophotom-

Table 1. Characteristic of Soil studied.

Characteristic	Value	Analyzed Method
Moisture		
- air dry	20.21%	Gravimetric Method
- oven dry	22.25%	
pH	6.44	pH meter
Soil type	Silt loam	
sand : silt : clay	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

eter (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.

The Efficiency of Arsenic Removal

Total arsenic accumulation in plant was determined and compared with the total amount of arsenic of that experimental pot as % arsenic removal. Then data collected were analyzed for their 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 using the equation below:

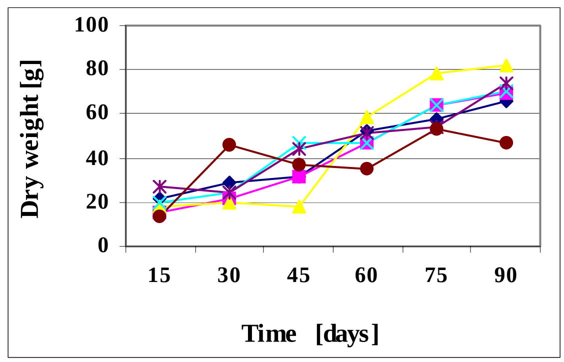
Efficiency of arsenic removal (%)

$$= \frac{\text{[As in leaves + As in root] (mg)}}{\text{Total As in pot (mg)}} \times 100$$

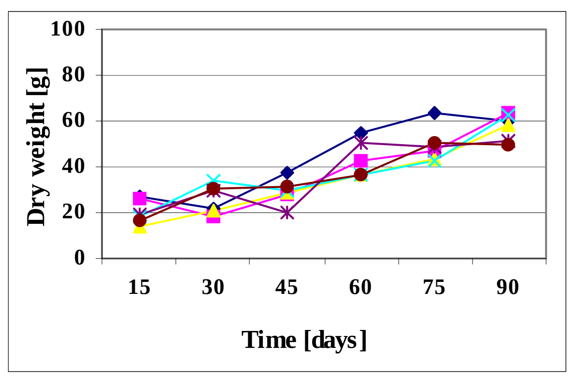
Results and Discussion

Growth Observation

During the experimental period, both ecotypes of vetiver grasses survived under all conditions of arsenic concentration in soil and grew as well as the control group. From statistical analysis, it was found that the growth of plants, in terms of the height and diameter of clumps, and the number of plants per clump, were not significantly different between the arsenic treated group and control at 95% confidence level. This finding confirmed that vetiver grasses are highly tolerant, and thus could grow in highly arsenic- contaminated soil.[6] The number of clumps and diameter of clump of *V. zizanioides* (Linn.) Nash (Surat



V. nemoralis (Balansa) A. Camus
(Prachuabkirikhan ecotype)



V. zizanioides (Linn.) Nash
(Surat Thani ecotype)

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

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

Table 2. Arsenic accumulation in various parts of *Vetiveria nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype)

Unit: mg As/kg

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	$^{a}8.4115^{e}$	$^{b}5.5959^{e}$	$^{c}5.6695^{e}$	$^{d}9.0023^{e}$	$^{e}5.9108^{e}$	$^{f}11.7750^{e}$

Note: - The same letters on the same corner means that there is no significant difference at 95% confidence level.
- The letters on the right corner show differences in concentrations.
- The letters on the left corner show differences in periods of time.

Table 3. Arsenic accumulation in various part of *Vetiveria zizanioides* (Linn.) Nash (Surat Thani ecotype)

Unit: mg As/kg

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 meaning of superscript symbols is as described in Table 2.

Thani ecotype) were higher than *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype). In contrast, the height and dry weight of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) was higher than the other ecotype, as shown in Fig 2.

Arsenic Accumulation in Vetivers

Arsenic accumulation was found in all parts of the grasses at different concentrations. The arsenic accumulation in both ecotypes of vetiver grasses was more in the roots than in its leaves. For the 150 mg

Table 4. Efficiency of arsenic removal both Vetiver grass types

Unit: %

Vetivergrass	$Na_2HAsO_4.7H_2O$ concentration (mg As/kg soil)	Time (days)					
		15	30	45	60	75	90
Prachuab-kirikhan ecotype	50	$^a0.0139^a$	$^a0.0209^a$	$^c0.0116^a$	$^d0.0384^a$	$^d0.0249^a$	$^e0.0288^a$
	75	$^a0.0124^a$	$^a0.0150^b$	$^c0.0081^b$	$^d0.0198^b$	$^d0.0332^b$	$^e0.0275^b$
	100	$^a0.0123^c$	$^a0.0159^c$	$^c0.0157^c$	$^d0.0238^c$	$^d0.0271^c$	$^e0.0390^c$
	125	$^a0.0118^d$	$^a0.0123^d$	$^c0.0195^d$	$^d0.0251^d$	$^d0.0201^d$	$^e0.0398^d$
	150	$^a0.0109^e$	$^a0.0147^e$	$^c0.0150^e$	$^d0.0200^e$	$^d0.0228^e$	$^e0.0357^e$
Surat Thani ecotype	50	$^a0.0157^a$	$^b0.0194^a$	$^c0.0138^a$	$^d0.0233^a$	$^d0.0365^a$	$^f0.0344^a$
	75	$^a0.0154^b$	$^b0.0193^b$	$^c0.0201^b$	$^d0.0312^b$	$^d0.0391^b$	$^f0.0488^b$
	100	$^a0.0122^a$	$^b0.0264^a$	$^c0.0170^a$	$^d0.0180^a$	$^d0.0281^a$	$^f0.0421^a$
	125	$^a0.0132^c$	$^b0.0228^c$	$^c0.0148^c$	$^d0.0217^c$	$^d0.0326^c$	$^f0.0285^c$
	150	$^a0.0100^d$	$^b0.0192^d$	$^c0.0167^d$	$^d0.0177^d$	$^d0.0322^d$	$^f0.0228^d$

Note: - The meaning of superscript symbols is as described in Table 2.

As/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 mg As/kg (in leaves dry matter), respectively, whereas in root, they were 6.197 and 11.775 mg As/kg (in root dry matter), respectively. The results are quite similar to the study performed by Truong and Baker, 1998.[8] 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 mg As/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 2 and Table 3. In addition, the level of arsenic accumulation tended to decrease with time of growth because the increase in arsenic accumulation was less than that of the dry weight of plant. Roontanakiat and Chairoj, 2000[9] also reported decreasing in accumulation with time.

Efficiency of Arsenic Removal

The arsenic removal efficiency of the vetiver grasses increased as the arsenic concentration in the soil increased.

The arsenic removal efficiency of *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype) was very low. The highest efficiency was 0.0398% at the 90^{th} day in the 125 mg As/kg soil group.

For *V. zizanioides* (Linn.) Nash (Surat Thani ecotype), the arsenic removal efficiency was also quite low. The maximum efficiency, found in the 75 mg As/kg soil at 90^{th} day was 0.0488% (Table 4).

In addition, both types of vetiver grass showed significant differences between treatments and harvest times at the 95% confidence level and the percent efficiency 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 the Surat Thani ecotype was slightly higher than that of the Prachuabkirikhan ecotype. One possible reason may be that Surat Thani ecotype has more clump and root hairs, and thus poses more surfaces area than Prachuabkirikhan ecotype, leading to more arsenic absorption.

Both ecotypes of vetiver grasses could grow well in arsenic contaminated soil, up to 150 mg As/kg soil. Our results indicated that the number of clumps and diameter of clumps of *V. zizanioides* (Linn.) Nash (Surat Thani ecotype) were higher than those 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 *V. nemoralis* (Balansa) A. Camus (Prachuabkirikhan ecotype). It is recommended that vetiver grasses could be used to remove arsenic from contaminated soil. However, it is proposed that further research should be done on ways to increase the arsenic uptake rate of vetiver.

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