



Removal of arsenic and manganese from the tailing storage facility of a gold mine using *Vetiveria zizanioides*, *Bambusa bambos* and *Pennisetum purpureum*

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Abstract

Phytoremediation is a promising technology to remediate heavy metal contaminated soil. The main objective of this study was to investigate the potential of using *Vetiveria zizanioides*, *Bambusa bambos* and *Pennisetum purpureum* to remove arsenic (As) and manganese (Mn) from the metal (loid) tailings pond of a gold mine. The aerial or aboveground parts (shoots and leaves) and underground parts (roots) of these plants, as well as the metal (loid) tailings soil, were analyzed for the As and Mn contents using the USEPA technique. The samples were collected every 30 d over a 180 d cultivation period. This study has shown that the relative growth rate (RGR) of all three plants' dry weight decreased with increasing cultivation time to 180 d. The results exhibited that *P. purpureum* had the highest growth rate and accumulated the highest As and Mn levels in the aerial parts at 15.5 and 943.3 mg/plant, respectively. On the other hand, the accumulation of As and Mn in the underground parts were 1.8 and 93.7 mg/plant, respectively. Moreover, *P. purpureum* is very tolerant to various soil and weather conditions; and it provides abundant biomass. Hence, rather than using it as phytoremediation only, *P. purpureum* could be utilized as biomass feedstock for producing heat and electricity.

Keywords: Phytoremediation, phytotolerant, phytotoxicity, gold mine, metalloid

Introduction

Heavy metal contamination occurs naturally, but especially from anthropogenic activities, such as mining (Niyomthai and Wattanawan, 2014). In mining activities, the soil surface has to be exposed to extract the relevant precious mineral, but these activities can result in heavy metal leaching into the environment. Thailand has one area of open gold mining (Parinyachet and Leepowpanth, 2015), and arsenic (As) and manganese (Mn) are found in association with the gold. With poor mining practice, the leaching of these metal (loid) can adversely affect the environmental quality as well as the physical and mental health of nearby communities (Jaruvakul, 2007).

Heavy metal phytoremediation uses a green plant to extract the heavy metal contaminants from the environment (surface water or topsoil) and accumulate it in the plant tissue (Akkajit, 2015). This is a cost effective method that generates insignificant negative effects on the environment. To maximize the phytoremediation efficiency, the selected green plant species should have a high growth rate and high biomass content, and this biological treatment can be more efficient than the more costly chemical and physical

treatment (Tananonchai and Sampanpanish, 2014). Moreover, the green plants should be non-edible in order to prevent the heavy metal contamination from entering into the food chain (Ghosh and Singh, 2005; Sampanpanish, 2015).

In this study, the three non-edible monocotyledon plants of *Vetiveria zizanioides* (L.), *Bambusa bambos* (L.) and *Pennisetum purpureum* (cv. Mott.) were evaluated for their potential to remove As and Mn from the tailing storage soil of an open gold mine. The objectives were to study the growth rate and toxicity symptoms of the plants, to investigate the As and Mn uptake efficiency of the three selected monocot species when cultivated in an As and Mn contaminated area and to compare the accumulation of As and Mn in the three selected plant species.

Materials and Methods

Site preparation

The selected study area was the Tailing Storage Facility (TSF) or metal (loid) reservoir in the gold mine area at GPS location X=676128, Y=1801325. Soil in the metal (loid) reservoir was excavated, each as a 30 x 30 cm

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square with a depth of 30 cm, for planting one plant per plot. At the bottom of each plot, 1 kg of polymer absorber was placed as a base layer and then 30 g of chemical fertilizer (15-15-15 NPK formula) which was equivalent to 500 kg of elements/1,600 square meter was added to the soil since there was no nutrient in the soil in the selected study area. Afterward, plant species with the same size and weight were planted in each experimental plot. The control plots were the experimental plots without fertilizer addition. About 1 L of water was applied into each plot during 9.00-12.00 am everyday throughout the 180 d cultivation period. In addition, the fertilizer was added again in the second month of the cultivation to each plot.

Growth rate and phytotoxicity

The relative growth rate (RGR) of the plants was observed by measurement of the plant dry weight (DW) mass every 30 d (Hoffmann and Porter, 2002) and was derived using Eq. (1). In addition, phytotoxicity was monitored by visual observation of the monocot leaves as previously reported (Brown *et al.*, 1991) and then recorded. The percentage phytotoxicity was calculated from Eq. (2).

$$\text{RGR} = \frac{[\ln(W_2) - \ln(W_1)]}{(t_2 - t_1)} \quad (1)$$

Where: W_1 and W_2 are the dry weight (g) of plants at the beginning and harvesting time, respectively, and t_1 and t_2 are the time (d) at the beginning and harvesting time, respectively.

$$\text{Phytotoxicity (\%)} = \frac{(A_0 \times B_0) + (A_1 \times B_1) + (A_2 \times B_2) + (A_3 \times B_3) + (A_4 \times B_4) + (A_5 \times B_5) \times 100}{(A_r \times B_r)} \quad (2)$$

Where: A is the number of leaves with toxic symptoms at a different level of toxicity, ranked from non-toxic (A0) to severe (A5), B is the phytotoxicity score from 0 to 5 (B0–B5), A_r is total number of leaves and B_r is the highest phytotoxicity score (5).

As and Mn in the plants and soil samples

Both the plant and soil samples were collected every 30 d over the 180 d cultivation period. Plant samples were first cleaned in tap water and then in deionized water. They were then cut into two parts of aerial and underground parts and then oven dried to constant weight (dry weight) at 105 °C for 24–48 h. Soil samples were likewise oven dried. Subsequently, each dry sample was ground and meshed to pass through a 10 mesh-sieve, before the As and Mn levels were analyzed using the USEPA 3052 method (US.EPA, 1996) by Atomic

Absorption Spectrophotometry (AAs). The results showed that original soils had As and Mn levels at 50 and 1,670 mg/kg, respectively. Whereas, all plants had none detectable metal (loid) of As and Mn.

Statistical analysis

Variation in the As and Mn accumulation levels was assayed by analysis of variance (ANOVA) and the significance of any differences between means was tested using Duncan's new multiple range test (DMRT), accepting significance at the 95% ($p < 0.05$) level. All statistical analyses were performed using the Statistical Package for the Social Science (SPSS) software.

Results and Discussion

Growth rate

All three selected monocot species (*V. zizanioides*, *B. bambos* and *P. purpureum*) showed increase in dry weight of plants biomass with time during the 180 d cultivation period, having the highest plant biomass at 180 d (Figure 1). However, the growth of the three monocot species were significantly different between each other ($p < 0.05$), with by far the highest growth (as DW of plant biomass) being observed for *P. purpureum*, which attained 3,343 g (208.6 g and 3,134.1 g for underground and aerial parts, respectively), followed by 1,083 g for *V. zizanioides* (223.3 g and 859.3 g for underground and aerial parts, respectively) and 811 g for *B. bambos* (179.2 g and 631.7 g for underground and aerial parts, respectively).

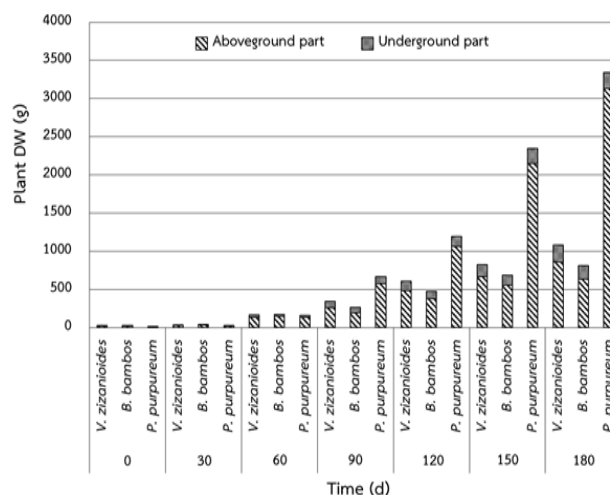


Figure 1: Growth (as DW mass) of the selected plants over 180 d cultivation. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT).



Phytotoxicity

Within the 180 d cultivation period, the phytotoxicity level of *V. zizanioides*, *B. bambos* and *P. purpureum* was 53%, 56% and 56%, respectively. The observed symptoms in *V. zizanioides* were the presence of yellow dots at the rim of leaves along with leaf curl, while in *B. bambos* and *P. purpureum* both plants showed some yellow dots at the rim and along all the length of the leaves. This may be due to the photosynthesis resistance effect, when As and Mn were absorbed and subsequently accumulated in plant tissues (Pangta and Sampanpanish, 2009). This result is in accordance with that reported previously by Sampanpanish et al. (2008), who found that *Chromolaena odorata* and *V. zizanioides* (L.) cultivated in heavy metal contaminated soil showed phytotoxicity symptoms, such as white, curled and burnt leaves, due to heavy metal accumulation in the plant tissues.

Correlation between RGR and phytotoxicity

The RGR of all the three monocots evaluated in this study decreased with an increasing cultivation time over the 180 d period (Figure 2). This could be due to the resistance process and growth inhibition of plants in order to survive and adjust themselves to tolerate the As and Mn contaminants at a certain level. In addition, the highest RGR of all three monocot species were significantly different to each other ($p < 0.05$), but reached maximum at 60 d of cultivation and decreased steadily with cultivation time thereafter, with the RGR of *P. purpureum* at 180 d cultivation being lower than the initial RGR at 30 d.

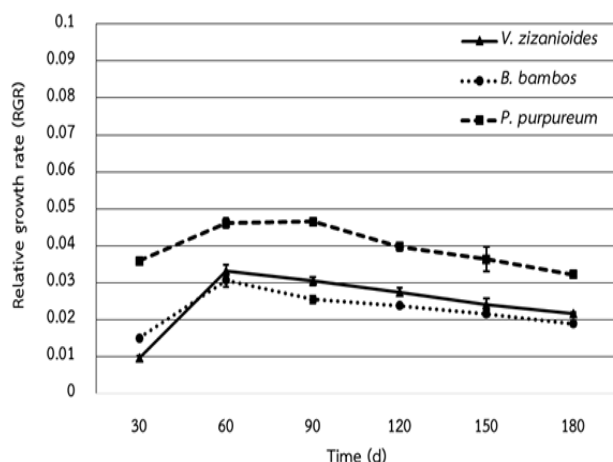


Figure 2: The RGR of the three selected monocots. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT).

Total As accumulation in plants

For the total As accumulation in the plants, the highest As accumulation in the aerial part was 4.9 mg/kg DW in *P. purpureum* after 180 d cultivation, followed by *B. bambos* and *V. zizanioides* at 4.7 and 4.6 mg/kg DW, respectively. For As accumulation in the underground parts, in contrast the highest As accumulation was found in *V. zizanioides* at 11.5 mg/kg DW, followed by *B. bambos* and *P. purpureum* at 10.6 and 8.6 mg/kg DW, respectively.

In addition, the total As accumulation in plant material was higher in the aerial parts than in the underground parts of all three monocots after 180 d of cultivation. The highest level of As accumulation (15.5 mg/plant) was found in the aerial parts of *P. purpureum*, followed by *V. zizanioides* and *B. bambos* at 4.0 and 3.0 mg/plant, respectively. For As accumulation in the underground parts, the highest level was in *V. zizanioides* (2.6 mg/plant), followed by *B. bambos* and *P. purpureum* at 1.9 and 1.8 mg/plant, respectively. Overall, *P. purpureum* had the highest total As accumulation in the whole plant, followed by *V. zizanioides* and *B. bambos*, respectively. That, the aerial parts of the plants played an important role in the As uptake and accumulation may be due to the markedly higher biomass of the aerial parts than the underground parts, ranging from 3.5-fold for *B. bambos* to 15-fold for *P. purpureum*. The total As accumulation per plant is shown in Figure 3.

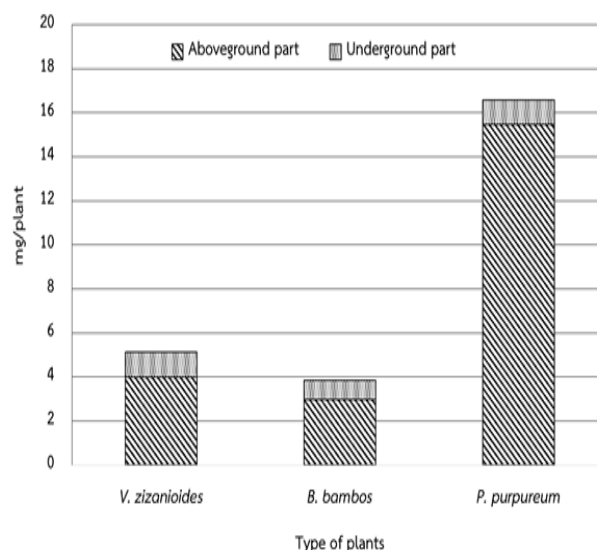


Figure 3: Total As accumulation level in each plant after 180 d cultivation. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT).



Total Mn accumulation in the plants

The level of Mn accumulation in the plants after 180 d cultivation was higher in the underground parts of both *V. zizanioides* and *P. purpureum* than in the aerial parts. However, there was no significant difference in the Mn accumulation level between the underground and aerial parts of *B. bambos*. The highest Mn accumulation level (301.0 mg/kg DW) was found in the aerial part of *P. purpureum*, followed by *B. bambos* and *V. zizanioides* at 290.2 and 182.9 mg/kg DW, respectively. Moreover, the underground parts of *P. purpureum* clearly had the highest Mn accumulation level (450.5 mg/kg DW), followed by *V. zizanioides* and *B. bambos* at 309.9 and 278.5 mg/kg DW, respectively.

Considering the total Mn accumulation in different parts of the plants after 180 d cultivation, the aerial parts of the three monocots accumulated more Mn than the underground parts, with the highest level in *P. purpureum* followed by *B. bambos* and *V. zizanioides* at 943.3, 183.5 and 157.2 mg/plant, respectively. Within the underground parts, *P. purpureum* accumulated the highest Mn level at 93.8 mg/plant, followed by *V. zizanioides* and *B. bambos* at 69.0 and 50.2 mg/plant, respectively. The total Mn accumulation per plant is shown in Figure 4.

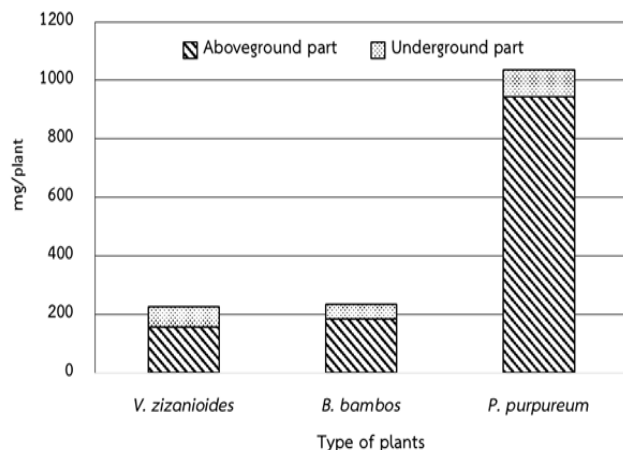


Figure 4: Total Mn accumulation level per plant after 180 d cultivation. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT).

Overall, of the three plant species studied, *P. purpureum* had the highest capacity for Mn accumulation. This may reflect its fibrous root system which has a complex structure of permanent tissue that can uptake and subsequently transport soluble Mn^{2+} from the soil solution through the xylem and partially through the phloem to

various parts of the plant. Xylem is recognised as an important part of the plant that has a major function in transportation of both water and other nutrients in ion form or as a chelate (Tananonchai and Sampanpanish, 2014). For example, the transportation of manganese citrate or malate from the root to various other parts of the plant by conduction (Tananonchai and Sampanpanish, 2014; Nadeem *et al.*, 2016.). The transportation of nutrients through the phloem is different for different parts of the plant, which might be due to greater ease of Mn transport in the underground parts than in the aerial parts (Mazzolini *et al.*, 1985). Therefore, Mn accumulation per kg DW plant tissue in the underground parts tended to be higher than in the aerial parts, but overall the total Mn accumulation per plant was higher in the aerial parts due to the greater above ground mass.

Residual level of As and Mn in the cultivated soil

The As and Mn levels in the soil decreased over the 180 d cultivation period (Figure 5). This result conformed to the increasing As and Mn accumulation level in the plants over the same period. Of the three plants, *P. purpureum* had the highest capacity to deplete the soil of residual As and Mn, in accordance with its highest As and Mn accumulation compared to *V. zizanioides* and *B. bambos*.

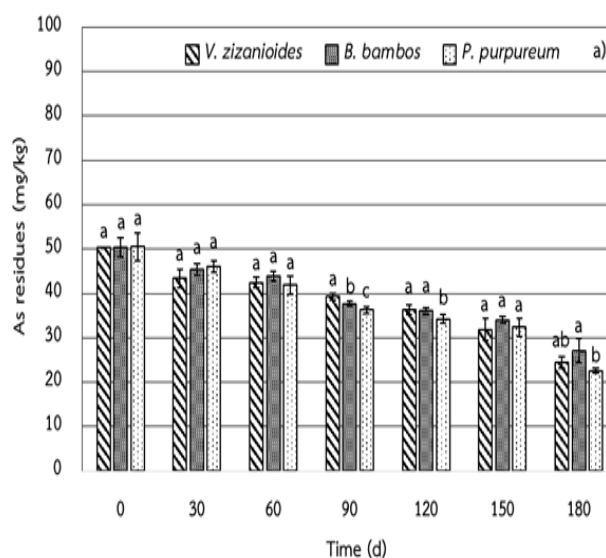


Figure 5: Residual (a) As and (b) Mn concentration in the soil over the 180 d cultivation period with the indicated plant species. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT).

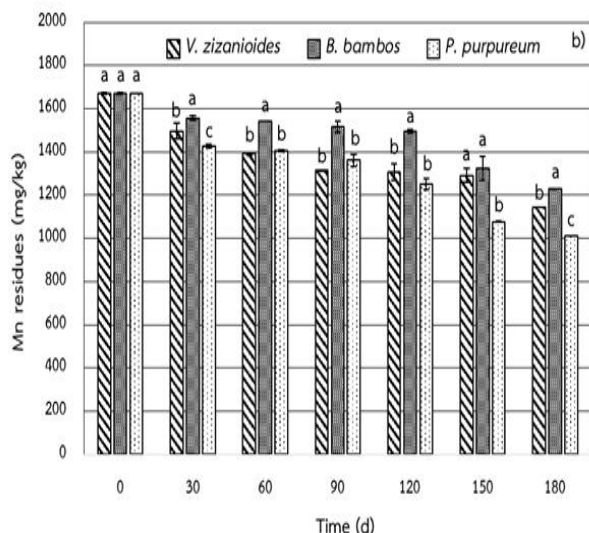


Figure 5: Residual (a) As and (b) Mn concentration in the soil over the 180 d cultivation period with the indicated plant species. Data are shown as mean $\pm$ SD, derived from three replicates. Means with a different letter are significantly different ($p < 0.05$; DMRT). (Cont.)

Conclusion

All plants in this study should be cultivated until it can no longer deplete the contaminated metals or until the contaminated metals are below the standards and safety. A higher proportion of metal (loid) was accumulated in the aerial parts rather than in the underground parts of each plant since the shoot parts had a by far higher biomass at 3.5- to 15-fold by DW. Of the three studied plants, *P. purpureum* showed the highest accumulation level of As and Mn as well as the highest metal (loid) removal efficiency from the tailing storage soil, which was reduced continuously over the 180 d cultivation period. This was likely to be due to it having the fastest growth rate (nearly double than those of the two other species).

In the case of As and Mn toxicity to *P. purpureum*, yellow leaves and some leaf curl was observed, but this did not have an effect on the growth rate during the 180 d cultivation period. Moreover, the biomass increased (as DW) continuously across the 180 d cultivation period, and so *P. purpureum* appears to be a suitable plant for As and Mn removal in this contaminated site. However, it should be noted that the limitation of using *P. purpureum* in phytoremediation is its short fibrous roots, and so it would only have this high potential for metal (loid) removal when applied in a shallow tailing storage pond. In addition to its

high growth rate, biomass and metal (loid) accumulation, *P. purpureum* can be utilized as a feedstock for producing electricity (Buranasin, 2015).

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