

## MERCURY ACCUMULATION IN GOLD MINE TAILING BY SWEET SORGHUM INOCULATED WITH CHROMIUM UPTAKE ENHANCING RHIZOBACTERIA

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### ABSTRACT

Small scale gold mining provides an important source of income for miners in rural communities where economic alternatives are extremely limited. However, it releases mercury which damages the environment and poses risks to those in the nearby community. Phytoremediation is considered as a simple and cost-effective method for the cleanup of heavy metal from contaminated soil. Phytoremediation is a technology that uses plants to degrade, extract, contain, or immobilize contaminants from soil and water. In particular, phytoextraction is the uptake of contaminants by plant roots and translocation within the plants to shoots or leaves. Contaminants are then removed by harvesting the plants. The objective of this research is to examine the ability of sweet sorghum and its inoculation using chromium uptake enhancing rhizobacteria for phytoremediation of gold mine tailing. Two varieties of sweet sorghum were used in this experiment, i.e. FS501, and KCS105. The seed of the sweet sorghum was sown in mercury containing tailing or a mixture of it with uncontaminated soil. Plant biomass and its mercury content were determined 35 days after sowing. Only FS501 was able to grow on tailing containing 26.94ppm of mercury. Inoculation of the sweet sorghum with the rhizobacteria does not affect its biomass but increase its mercury uptake significantly. Mixing the tailing material with uncontaminated soil causes an increase in biomass but reduce the mercury content in plant significantly. As a result, the amounts of removable mercury decrease significantly. Inoculation with the rhizobacteria has no effect on mixed material.

**Keywords:** Sweet sorghum, mercury, uptake enhancing, rhizobacteria

### INTRODUCTION

Small scale gold mining provides an important source of income for miners in rural communities where economic alternatives are extremely limited. It uses simple practices with little economic investment. Mercury is often used in small scale gold mining to separate the metal from the ore and is generally handled by people with little or no awareness of its risks toward health and environment. Mine tailing arising from this process contains mercury which may be released to the environment and damages it. With gold prices rising, the number of miners using mercury may increase in the coming years. Mercury release to the environment should be reduced by simple and cost-effective means (Limbong *et al.*, 2002).

The reclamation of tailing pile is important for every mine operation. The most appropriate technologies which can be suggested for this purpose is vegetation. Plants have a natural propensity to take up metals. Plant-based environmental remediation technology has been widely pursued in recent years as an *in situ*, cost-effective potential strategy for the cleanup of trace metals from contaminated sites (Salt *et al.*, 1995). Several technological subsets have been proposed for the technology. Phytoextraction is the use of plants to remove metal contaminants from polluted soil. In this approach, plants capable of accumulating high levels of metals are grown in contaminated soil. Metal-enriched above ground biomass of the plants is then harvested and a fraction of soil metal contaminants is removed (Lasat, 2002). Phytoextraction efficiency is determined by two

key factors: metal accumulating capacity and biomass production of the plants. Plants with both high accumulation and translocation factors have the potential to be used in phytoextraction (Yoon *et al.*, 2006). Metal hyperaccumulator plants have been demonstrated to be potentially useful in soil cleanup, as they can take up significant amounts of metal from contaminated soils (Baker *et al.*, 1994), but their low annual biomass production tends to limit their phytoextraction ability. The other possible alternative is the use of nonaccumulator plants (McGrath and Zhao, 2003), either high biomass plants or fast-growing trees that can be easily cultivated using established agronomic practices (Ghosh and Singh, 2005; Meers *et al.*, 2005; Solhi *et al.*, 2005). Extensive work has been done on the heavy metal uptake capacity of high biomass crop plants, such as Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), and maize (*Zea mays*) (Szabo and Fodor, 2006; Cui *et al.*, 2004; Turgut *et al.*, 2004). Sweet sorghum (*Sorghum bicolor* var. *saccharatum*) has also been demonstrated to be able to accumulate copper (Szekely *et al.*, 2011). Clearly, if affecting factors can be optimized, phytoextraction by high biomass crop plants could be accelerated.

One approach is to add chemical agents to the soil that increase metal uptake by high biomass producing plants. However, *in situ* application of such chelators may pose a potential risk of groundwater pollution. Several lines of evidence suggest that soil microorganisms possess mechanisms capable of altering environmental mobility of metal contaminants with subsequent effects on the potential for root uptake and accumulation in plants (Lasat, 2002). Prijambada *et al.* (1999) have reported that inoculation of *Brassica juncea* with fluorescent pseudomonads stimulate heavy metal accumulation in plant. Rosariastuti *et al.* (2012) reported that chromium uptake by maize could be enhanced by inoculating it with their rhizobacterial isolate. This study was launched to examine the capability of sweet sorghum in accumulating mercury and the effect of its inoculation with chromium uptake enhancing rhizobacteria.

## MATERIALS AND METHODS

A rhizobacterial isolate which was proved to be able to enhance chromium uptake by maize, and two varieties of sweet sorghum, i.e. FS501 and KCS105, were used in this study. Tailing from small scale gold mine at Wonogiri Regency, Central Java was used for growth medium.

### Seed preparation

The seeds of two varieties of sweet sorghum were germinated at room temperature for 1 week in petri dishes in which a piece of filter paper that has been soaked in water was placed to keep the humidity inside the dishes. Three plants of similar size and appearance were chosen to be transplanted on the growth media.

### Rhizobacteria preparation

Chromium uptake enhancing rhizobacterial isolate was grown in liquid LB medium for 24 hours until its cell concentration reached  $10^8$ /ml.

### Growing media

Sweet sorghum was grown on mercury containing tailing material, and mixtures of tailing material with uncontaminated agricultural soil (1:1 and 1:3).

### Mercury accumulation in sweet sorghum grown on tailing material

Before planting, tailing material and mixtures of tailing materials with uncontaminated agricultural soil were amended with nitrogen in the form of urea (0.451 g/pot), phosphorus in the form of rock phosphate (0.56 g/pot) and potassium in the form of potassium chloride (0.44 g/pot). Three sorghum seedlings were transplanted in pots containing 3.3 kg of amended tailing material. Rhizobacterial inoculation was done in the same time with the transplantation of sorghum seedlings by pouring 240 mL of LB broth containing the cells. Each treatment was done in three replications. Two weeks after transplantation, thinning was

carried down to one plant for each pot. Plants were watered using 240 mL sterile distilled water/ potdaily. Plants were harvested 35 days after transplantation. Dry weight of the plants and their mercury content were determined after harvesting.

#### Determination of mercury content in plant

Plant materials were air dried for 24 hours, cut into small pieces and ground. Powdered plant tissues were dissolved in 5ml of dilute acid mixture (60% HClO<sub>4</sub> : 65% HNO<sub>3</sub>; 1:1 v/v) in around bottom flask. Mercury content in plant and soil were analyzed using a FIMS 100 CVAAS (cold vapor atomic absorption spectrometry) from Perkin Elmer.

## RESULTS AND DISCUSSION

Tailing material from small scale gold mine at Wonogiri Regency was found to contain mercury at 26.94 ppm. The use of heavy metal resistant microbes-capable of reducing metal and supporting the growth of plants was supposed to reduce mercury release to the environment by the use of phytoextraction. Mercury toxicity in plants was marked by impaired growth of plants. It affects plants dry weights. From two varieties of sweet sorghum which were examined, only FS501 was able to grow on the tailing material, while KCS105 plant died two weeks after transplantation.

The effect of mixing tailing materials with uncontaminated agricultural soil and rhizobacterial inoculation were shown in Table 1. It showed that the dry weight of plants grown

in mercury containing tailing material was the lowest compared to the ones that were grown in mercury containing tailing material mixed with uncontaminated agricultural soil at ratios 1:1 as well as 1:3. Mixing of mercury containing tailing material with uncontaminated agricultural soil causes increase of dry weight up to nearly 10 times.

Table 1 also showed that inoculation of sweet sorghum with chromium uptake enhancing rhizobacteria did not affect plant dry weight significantly. This result did not conform with the report of Prijambada *et al.* (1999) who found that inoculation of *Brassica juncea* with fluorescent pseudomonads stimulate plant growth.

Mercury content in sweet sorghum grown in tailing materials as well as in mixtures of tailing materials with uncontaminated agricultural soil and rhizobacterial inoculation were shown in Table 2. It showed that no differences was observed in mercury content except for plant grown in tailing material inoculated with chromium uptake enhancing rhizobacteria. Inoculation of plant with chromium uptake enhancing rhizobacteria could increase mercury content in plant up to nearly 6 times. Increasing mercury content without reducing growth in plant grown in tailing material inoculated with chromium uptake enhancing rhizobacteria indicates that the rhizobacteria could reduce mercury toxicity in the tailing material.

To determine plant capability to remove mercury from the tailing material, plant weight was multiplied with its mercury content. Table 3 showed the results of multiplication. Mercury

**Table 1.** Dry weight of plant grown in tailing and its mixture with uncontaminated agricultural soil with and without inoculation of rhizobacteria.

Treatment	Plantdry weight (g)		
	Mercury Containing Tailing	Tailing: Uncontaminated Agricultural Soil (1:1)	Tailing: Uncontaminated Agricultural Soil (1:3)
Inoculated	0.25 <sup>b</sup>	2.33 <sup>a</sup>	1.93 <sup>a</sup>
Uninoculated	0.37 <sup>b</sup>	1.44 <sup>ab</sup>	1.84 <sup>a</sup>

**Table 2.** Mercury content of plant grown in tailing and its mixture with uncontaminated agricultural soil with and without inoculation of rhizobacteria.

Treatment	Hg Content (ppm)		
	Mercury Containing Tailing	Tailing: Uncontaminated Agricultural Soil (1:1)	Tailing: Uncontaminated Agricultural Soil (1:3)
Inoculated	34,49 <sup>a</sup>	3,79 <sup>b</sup>	1,07 <sup>b</sup>
Uninoculated	6,26 <sup>b</sup>	2,77 <sup>b</sup>	0,35 <sup>b</sup>

**Table 3.** Mercury uptake of plant grown in tailing and its mixture with uncontaminated agricultural soil with and without inoculation of rhizobacteria.

Treatment	Hg (µg)		
	Mercury Containing Tailing	Tailing: Uncontaminated Agricultural Soil (1:1)	Tailing: Uncontaminated Agricultural Soil (1:3)
Inoculated	8,57 <sup>a</sup>	7,54 <sup>ab</sup>	2,19 <sup>bc</sup>
Uninoculated	2,30 <sup>bc</sup>	3,54 <sup>abc</sup>	0,66 <sup>c</sup>

uptake of sweet sorghum grown in tailing material inoculated with chromium uptake enhancing rhizobacteria was found to be the highest with no significant differences compared to only the one grown in tailing material mixed (1:1) with uncontaminated agricultural soil and inoculated with chromium uptake enhancing rhizobacteria. These results showed that chromium uptake enhancing rhizobacteria could also enhance mercury uptake. This result also did not conform with the report of Prijambada *et al.* (1999) who found that inoculation of *Brassica juncea* with fluorescent pseudomonads did not affect heavy metal uptake ability of the plant growth. However, the result conformed to the result of Rosariastuti *et al.* (2012) who reported that the same isolate enhance chromium uptake by maize. The effect of rhizobacterial inoculation on the growth of plant grown on heavy metal containing growth media was supposedly to be dependent on the microbe involved.

From the current studies, it can be concluded that sweet sorghum var. FS501 was able to grown on growth medium containing high mercury content and their ability to uptake mercury was affected by inoculation of chromium uptake enhancing rhizobacteria.

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