

## HEAVY METALS PHYTOREMEDIATION POTENTIAL OF NAPIER GRASS (PENNISETUM PURPUREUM) CULTIVATED ON TANNERY SLUDGE

Md. Ariful Islam Juel<sup>\*1</sup>, Thuhin Kumar Dey<sup>2</sup>, Md. Ibrahim Sardar Akash<sup>3</sup> and Kushol Kumar Das<sup>4</sup>

<sup>1</sup> Faculty of Leather Engineering Department, Khulna University of Engineering & Technology, Bangladesh, e-mail: [juelbclt@gmail.com](mailto:juelbclt@gmail.com)

<sup>2</sup> Faculty of Leather Engineering Department, Khulna University of Engineering & Technology, Bangladesh, e-mail: [tuhinndey@gmail.com](mailto:tuhinndey@gmail.com)

<sup>3</sup> Student of Leather Engineering Department, Khulna University of Engineering & Technology, Bangladesh, e-mail: [ibrahimakash17@gmail.com](mailto:ibrahimakash17@gmail.com)

<sup>4</sup> Student of Leather Engineering Department, Khulna University of Engineering & Technology, Bangladesh, e-mail: [kusholkdas163@gmail.com](mailto:kusholkdas163@gmail.com)

### ABSTRACT

Phytoremediation is a cost effective and eco-friendly method for cleanup of contaminated soil. This study focused on the assessment of phytoremediation potential of Napier grass (*Pennisetum purpureum*) and Indian mustard (*Brassica juncea*) yielding in tannery sludge. Initial characterization of tannery sludge showed a high concentration of chromium, lead, copper and zinc which were  $6845.5 \pm 50.2$  mg/kg,  $73 \pm 2.5$  mg/kg,  $93 \pm 1.5$  mg/kg, and  $29 \pm 2.5$  mg/kg, respectively. Both seeds of these plants were sown on tannery sludge kept in baskets and harvested after 8 and 12 weeks of plantation. The study indicated that both Napier grass and Indian mustard accumulated heavy metals in the order of  $Cr > Zn > Cu > Pb$  at different parts of these plants. Indian mustard accumulated highest concentration of Cr, Cu, and Pb whereas Napier grass showed highest Zn uptake and good Cr accumulating capacity. The uptake rate of Cr increased in Napier grass with the increase of plant age. Moreover, Napier grass is one of the rapid yielding tropical grasses and can retain for a longer period than Indian mustard. For that attribute, it may accumulate more heavy metals than Indian mustard within its life span. Both of these plants can be used for remediating heavy metals from contaminated tannery sludge.

**Keywords:** Napier grass, Indian mustard, phytoremediation, heavy metals, tannery sludge

### 1. INTRODUCTION

Tannery sludge contains elevated concentration of heavy metals like Cr, As, Pb, Ni, Cu, Zn and Cd due to the use of basic chromium salt, different syntans, dyes, pigments, retanning agents during the leather production (Juel, Mizan, & Ahmed, 2017). These kinds of heavy metals are non-biodegradable and accumulated in soils, waters and contaminate the food chain at later (Ali, Khan, & Sajad, 2013). In recent years, although there are some methods available for reuse or recycling of tannery sludge, most of the tannery sludge is disposed of as landfilling without any treatment. But this can alter the physicochemical properties and fertility of the soil. When crops are grown on such kind of contaminated soil, the heavy metal can be accumulated in its vegetative parts with incorporation into the food chain and leading to bio-amplification (Pergent & Martini, 1999). These metals are toxic and can form irreversible effects even at low

concentration (Kara, 2005). Moreover, Heavy metals can cause oxidative stress by the generation of free radicals which can disrupt cell's inherent antioxidant defenses and can cause cell damage (Das, Das, & Dhundasi, 2008; Mudipalli, 2008). It's imperative to recover metals from the soil in order to mitigate the contamination of ecosystem as well as environment. But it's not a facile task with respect to cost and technical complexity (Barceló & Poschenrieder, 2003). Different kinds of physical, chemical and biological techniques are used for this purpose. The traditional processes are soil incineration, excavation, soil washing, soil flushing, solidification, and stabilization of electro-kinetic systems (Sheoran, Sheoran, & Poonia, 2011). But these physical and chemical methods have some basic limitations like high cost, intensive labor, irreversible changes in soil properties and disturbance of native soil microflora (Ali, Khan, & Sajad, 2013). As a result it's inevitable to find out cost effective, efficient and environment friendly remediation methods for the decontamination of heavy metal-polluted soils. In that scenario, Phytoremediation is a cost effective and eco-friendly technique which can be a good alternative to treat or stabilize hazardous wastes. Plants are supplied with the ability to corrupt the toxins in their vegetative parts and by this way it's possible to expel, exchange and stabilize heavy metals from the contaminated soil (Salt, Smith, & Raskin, 1998). This process of heavy metal extraction, utilizes a particular group of plants known as hyper-accumulators. The hyper-accumulator plant species have 100 times higher capability to accumulate metals than those typically found in common plants (Choudhury, Islam, Ahmed, & Nayar, 2015). In this study, Indian mustard (*Brassica Juncea*) and Napier grass (*Pennisetum Purpureum*) were employed to investigate their phytoremediation potential cultivated on tannery sludge. Both plant species are widely available in Bangladesh. Indian mustard is a well-known hyper-accumulator plant for its abnormal heavy metals extraction capacity form the soil (Choudhury, Islam, Ahmed, & Nayar, 2015). In recent, Napier grass (*Pennisetum Purpureum*) has drawn attention in phytoremediation due to its long, deep root system and resistance to an extensive variety of unfavorable climatic and edaphic conditions. Very few researcher investigated Napier grass in phytoremediation of heavy metals cultivated in contaminated sites. Ishii, Hamano, Kang, Idota, & Nishiwaki, 2015 investigated Cadmium phytoremediation capacity of Napier grass cultivated in Japan. Moreover, both Indian mustard and Napier grass were not studied to investigate their performance cultivated in high concentration of chromium contaminated site like tannery sludge.

## **2. METHODOLOGY**

### **2.1 Collection and preparation samples**

Tannery sludge was collected from sludge dumping site of Apex Tannery Ltd, unit-2, Gazipur, Bangladesh. After collection, the sludge was dried to drive out extra moisture and the sample was prepared for seedling. Seeds of Indian mustard & Napier grass were collected from local market. Then the seed samples were sown in two different pots containing sludge sample. The seeds were grown through proper care and the plant samples were harvested after 8<sup>th</sup> and 12<sup>th</sup> weeks of plantation. The samples were then washed with distilled water and then sorted into three parts: roots, shoots, and leaves. After grinding and homogenization the samples stored in refrigerated condition (4°C) in sealed container until the next analysis.



Figure 1: Growth of Napier grass after 8<sup>th</sup> and 12<sup>th</sup> weeks



Figure 2: Growth of Indian mustard after 8<sup>th</sup> and 12<sup>th</sup> weeks

## 2.2 Heavy metal analysis

### 2.2.1 Digestion for plant analysis

0.5 g of pulverized sample was taken in a 200 ml beaker and 5 mL of concentrated Nitric acid ( $\text{HNO}_3$ ) was added. The beaker was covered with watch glass and allowed to stand for overnight. After that, the covered beaker was placed on hot plate and heated at  $125^\circ\text{C}$  for one hour and then cooled. The digestion was continued at same temperature with the presence of 1-2 mL of 30%  $\text{H}_2\text{O}_2$  until the digest was clear. When the digested sample was clear, temperatures was reduced to  $80^\circ\text{C}$  and continue heating until dryness. Then diluted nitric acid and deionized water were added as a ratio of 1:2 to dissolve digest residue and bring sample to final volume of 50 ml. Finally, the sample was filtered through a  $0.45\ \mu\text{m}$  filter paper and preserved for the analysis of Cr, Cu, Zn, and Pb by Atomic Absorption Spectrophotometer (AAS) (Shimadzu AA 6800).

### 2.2.2 Digestion for sludge analysis

At first, 1 g of pulverized sample (dry weight) was taken to a beaker. 10 mL nitric acid was mixed with the slurry and covered with a watch glass. The samples were then heating at  $95 \pm 5^\circ\text{C}$  and refluxed for 15 minutes without boiling. Next, the sample was allowed to cool. Then 5mL concentrated  $\text{HNO}_3$  was added and refluxed for 30 minutes.  $\text{HNO}_3$  was added repeatedly until no brown fumes were emitted that indicates the complete reaction with  $\text{HNO}_3$ . Sample was

then allowed to evaporate approximately 5 mL solution without boiling. After cooling the sample, 2 mL of water and 3 mL of 30% H<sub>2</sub>O<sub>2</sub> were added and warmed to initiate the peroxide reaction. The addition of 30% H<sub>2</sub>O<sub>2</sub> was continued with warming until the effervescence was minimal or until the general sample appearance was unchanged. After the completion of peroxide reaction, the sample volume was reduced to approximately 5 mL. Finally, the sample was cooled, diluted to 15 mL with water and filtered through a 0.45 µm filter paper. Analysis of Cr, Cu, Zn, and Pb was carried out by Atomic Absorption Spectrophotometer (AAS) (Shimadzu AA 6800).

### 3. RESULTS AND DISCUSSION

#### 3.1 Heavy metal contents in tannery sludge:

Heavy metal concentrations of sludge sample were shown in the Table 1. The concentration of metals was 6881.55 mg/kg, 73.515 mg/kg, 93.6 mg/kg and 29.73 mg/kg for the Cr, Cu, Zn, and Pb, respectively. Metal concentration order from higher to lower in the sludge is Cr>Zn>Cu>Pb. Relatively higher metal contents were found in other papers for tannery sludge of Bangladesh (Juel, Mizan, & Ahmed, 2017; Juel, Chowdhury, Mizan, & Alam, 2016). This variation may be due to the variation in the tanning process. These metal concentrations of sludge were compared with several international regulatory limits for utilization.

Table 1: Heavy metals concentration in tannery sludge and their permissible limits for utilization

Parameter	Heavy metal concentration of tannery sludge (mg/kg dry wt.)			
	Cr	Cu	Zn	Pb
Sludge sample	6845.5±50.2	73±2.5	93±1.5	29±2.5
Permissible limit in Bangladesh <sup>a</sup>	900	800	2500	900
USEPA limit <sup>b</sup>	3000	4300	7500	840

<sup>a</sup> Bangladesh standards and guidelines for sludge management (2015)

<sup>b</sup> USEPA, Land Application of Sewage Sludge. Web link: <http://www3.epa.gov/npdes/pubs/sludge.pdf>

The present study showed that the average concentrations of Copper, Zinc and Lead in tannery sludge were lower than permissible limit where the concentration of chromium exceeds both Bangladesh and USEPA sludge application limit. Cr concentration was about 8 fold higher than Bangladesh limit and about 3 fold higher than USEPA limit.

#### 3.2 Accumulation of Heavy Metals in Napier and Indian mustard:

Figure 3 showed the accumulation of Cr, Cu, Zn, and Pb per unit dry mass of root, shoot, and leaf of Napier grass. The figure also indicates the heavy metal uptake data of 8 and 12 weeks of harvesting. It revealed that all metal extraction except lead by different parts of Napier grass increased from 8 weeks to 12 weeks though the accumulation pattern was not same for these metals. After 8 weeks of harvesting, the concentration of chromium in root portion was 452.1 mg/kg which increased to 1623.1 mg/kg after 12 weeks of plantation, i.e., about a 4 fold higher uptake rate accounted during the last 4 weeks of its growth (Table 2). But, the uptake rate of copper in root was comparatively lower in last 4 weeks than that of chromium. In case of zinc and lead, accumulation rate in all portion of the plant was better within early 8 weeks of growth compared to 12 weeks. This may be happened due to high bioavailable nature of zinc (Juel, Chowdhury, Mizan, & Alam, 2016; Rahman et al., 2012)

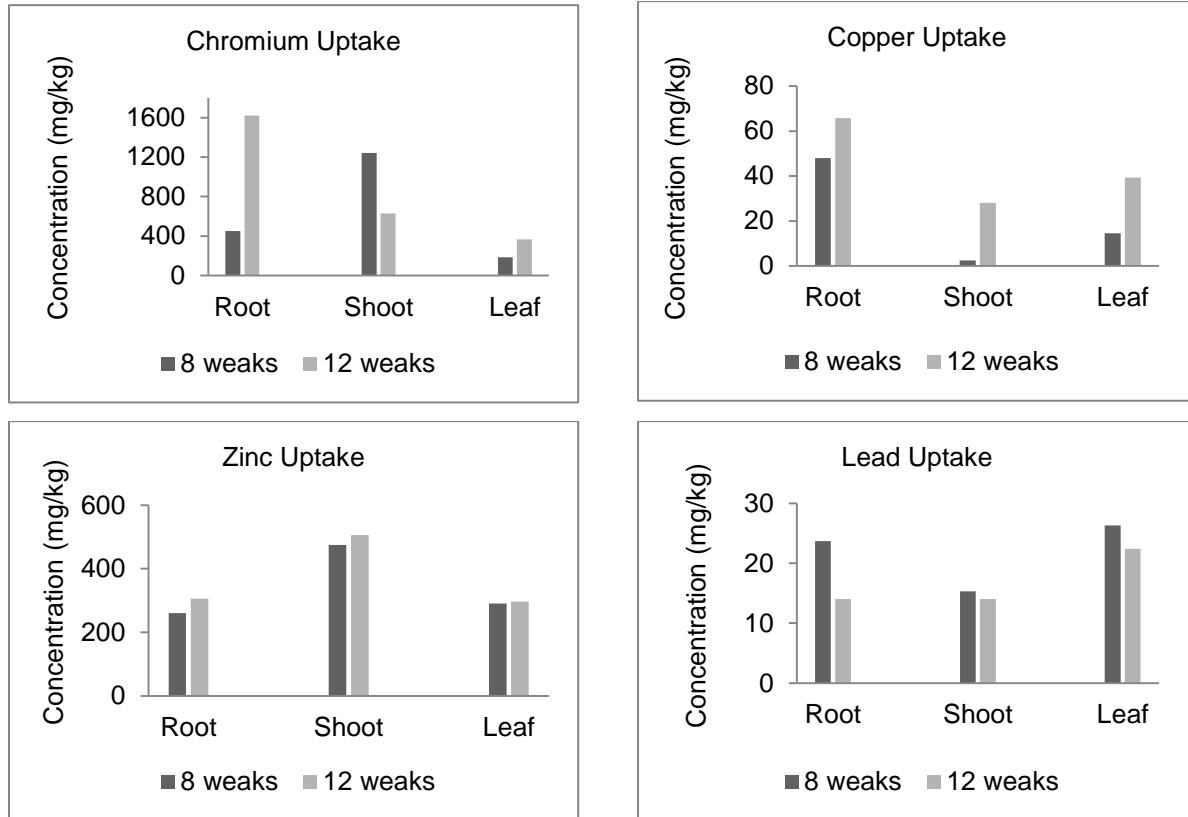


Figure 3: Uptake of different metals after 8 and 12 weeks in various parts of Napier grass

Table 2: Accumulation of heavy metals (mg/kg) in different parts of the Napier grass and Indian mustard after 8 weeks and 12 weeks of plantation.

Parameter	Heavy metals in the sample (mg/kg)			
	Chromium (Cr)	Copper (Cu)	Zinc (Zn)	Lead (Pb)
Napier grass (8 weeks)	Root	48	260	23.7
	Shoot	2.4	306.1	15.3
	Leaf	14.5	290.5	26.3
Napier grass (12 weeks)	Root	65.8	475	14
	Shoot	28	505.9	14
	Leaf	39.3	296.5	22.4
Indian mustard (12 weeks)	Root	284.6	235.6	34.8
	Shoot	69.3	222.3	47.3
	Leaf	58.1	403	80.3

Figure 4 indicates the difference of Cr, Cu, Zn, and Pb extraction capacity of Napier grass and Indian mustard after 12 weeks of plantation. It indicated that the magnitude and distribution of these metals were different. The total metal concentration order from higher to lower in both plants is Cr>Zn>Cu>Pb, which varied from one part to another. This order of metal uptake by plants was similar with the order of metal concentration found in sludge. Generally, the process of metal uptake and accumulation by plants depend on the available metal concentration in soil, solubility sequences and plant species growing on these soils (Gupta & Sinha, 2007). The transport of metals from roots to shoot, and leaf includes long distance affected by many factors. The accumulation of Cr was better in root of Napier plant whereas leaf of Indian mustard showed highest Cr accumulation. Cr uptake in different parts (root, shoot, leaf and fruit) varies plants to plants. (Gupta & Sinha, 2006) reported that the concentration of metals accumulated in root is higher than shoot of *Sesamum indicum* whereas same author found vice versa for other plants (Gupta & Sinha, 2007). Both plants showed similar pattern in case of Cu, and Pb accumulation, i.e., highest concentration accounted at lower part of plants. Zn was almost evenly distributed at lower and upper parts of both plants. (Choudhury, Islam, Ahmed & Nayar, 2015) also found similar type of pattern in case of Zn distribution in different parts of Indian mustard and Marigold grown at Buriganga river sediment.

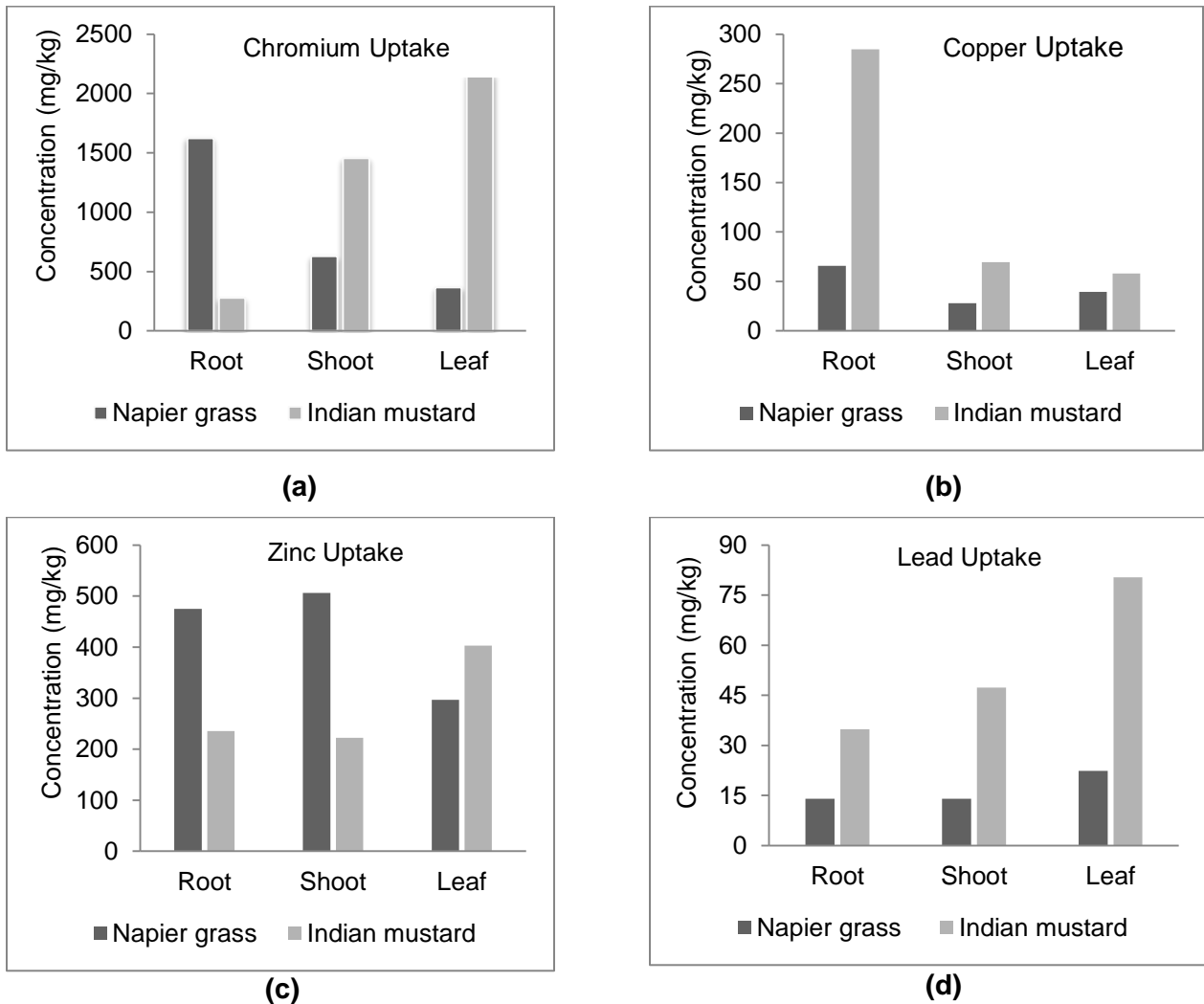


Figure 4: Comparison of heavy metals accumulation in different parts of Napier grass and Indian mustard after 12 weeks of plantation grown on tannery sludge.

The maximum concentration of chromium, copper and lead were accounted in Indian mustard whereas Napier grass showed highest Zn extraction ability. Moreover, Napier grass is one of the highest yielding tropical grass in Bangladesh and it can sustain for a longer periods than Indian mustards. Harvesting of that plant can continue at an interval of 6-8 weeks for 3-5 years<sup>1</sup>. For its longer life period than Indian mustard it may has more extraction capability of heavy metals.

#### 4. CONCLUSION

The results of the present research works showed that both Napier grass (*Pennisetum purpureum*) and Indian mustard (*Brassica juncea*) plants were grown well on tannery sludge and accumulated high concentration of heavy metals in different parts of the plant grown on tannery sludge. Indian mustard showed good performance in Cr, Cu, and Pb accumulation whereas Napier grass was good at Cr, and Zn uptake. Chromium accumulated rapidly in Napier grass during last 4 weeks of growth. Although the Cr accumulation rate of Napier grass was comparatively lower than Indian mustard for first 12 weeks of plantation, the total life span of Napier grass was higher than Indian mustard and the metal accumulation rate of Napier grass was in increasing trend with time. Hence, it indicates the higher possibility of heavy metals accumulation than Indian mustard. During the study period, Indian mustard and Napier grass were harvested in the basket for a short period of time and the distribution of the roots of the plants were random within the sludge. Hence no attempt was made to measure the post-harvesting changes of heavy metals in soil. To have an idea about the average reduction of heavy metal concentration extensive sampling should have been required, which was not done in the present study. Assessment of heavy metal concentration in sludge sample and multiple plants sample could have given some information about the average lowering of heavy metal concentration in the sludge after application of phytoremediation.

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<sup>1</sup> [www.kalro.org/CultUtiliseNgrass](http://www.kalro.org/CultUtiliseNgrass)

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