RESEARCH ARTICLE

LEAD TOLERANT DYNAMICS OF KENAF (*HIBISCUS CANNABINUS* L.) AND MESTA (*HIBISCUS SABDARIFFA* L.) AT GERMINATION STAGE

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ABSTRACT

Efforts were given to evaluate the lead (Pb) tolerant dynamics of kenaf (*Hibiscus cannabinus* L.) and mesta (*Hibiscus sabdariffa* L.) varieties at germination stage against high levels of Pb toxicity. Six levels of Pb viz. 0, 200, 400, 600, 800, 1000 mg Pb L⁻¹ were applied to the germination medium of kenaf (var. HC-95, HC-3, HC-2) and mesta varieties (var. Samu-93 and VM-1). Results showed significant reduction of germination, root and shoot growth, fresh and dry biomass production due to elevated levels of Pb. With respect to germination, kenaf HC-95 and HC-2 were more dynamic to tolerate Pb than kenaf HC-3, mesta Samu-93 and VM-1. Seed germination of mesta Samu-93 and VM-1 were affected at elevated concentrations of Pb and degenerated just after sprouting. At higher levels of Pb, the kenaf and mesta seedlings showed some deformities like twisting, curling, discoloring and growth stunting. Pb contents were increased in seedlings with the increasing of Pb treatment. Highest Pb was accumulated by kenaf HC-2 (19492±239 mg kg⁻¹) at highest treatment. Maximum amount of Pb (4.45±0.40 mg petridish⁻¹) was uptaken by kenaf HC-95 followed by kenaf HC-3. It is concluded that the degree of Pb tolerant dynamics was kenaf HC-95>kenaf HC-3>kenaf HC-2>mesta Samu-93>mesta VM-1. The research findings will be helpful for selecting kenaf and mesta varieties for grown in Pb contaminated soil and phytoremediation of Pb contaminated site.

KEYWORDS

Contamination, Kenaf, lead, mesta, tolerant

1. INTRODUCTION

Soil pollution is an imminent danger for Bangladesh and the minimization of this pollution will be a great challenge. Pollutants like Pb, Cd, Cr, Hg, As, Zn, Cu, Mn and Fe are mainly released in soil from different anthropogenic activities. Human activities released 333, 20, 14, 4 and 2 times more Pb, Cd, Cu, As and Cr, respectively in the environment than natural release (Khopkar, 2005). The response of plant metabolism to heavy metals has become a subject of great interest in recent years because of their high toxicity to plants. Some metals are regarded as essential nutrients, excess concentrations of all metals lead to various toxic effects such as oxidative stress and inhibition of enzymatic activities (Islam et al., 2017, 2019; Dietz and Samuel, 1999). Unlike organic pollutants, heavy metals are not biodegradable hence display the immediate cause of bioaccumulation and its harmful subsequences on food chain (Hamidian et al., 2014). Metals are the only hazardous constituents that cannot be destroyed or altered by chemical or thermal methods. They must either be treated or converted into the most insoluble form to prevent their entry into the environment (Lakshmanan et al., 1993).

The major portion of Pb in soil comes from industrial application followed by painting; Pb based pesticides application, burning of fossil fuel and disposal of municipal sewage sludges, Pb batteries etc. (Saifullah et al., 2009; Waranusantigul et al., 2011; Zheng et al., 2011). Bangladesh soils contained Pb range from 11–213 mg kg⁻¹ during wet season and 59.83–

222.21 mg kg⁻¹ during dry season in industrially polluted areas; average 22.80 mg kg⁻¹ in calcareous soils and 24.10 mg kg⁻¹ in non-calcareous soils (Jahiruddin et al., 2000; Mondol et al., 2011). Lead contamination is increasing day by day due to indiscriminate use and throwing of used batteries of automobile in the agricultural land.

The higher amount of Pb in soil exhibit a wide range of adverse effects on plants, like prevention of root proliferation, shoot growth stunting, reducing of leaves number and diminish biomass production (Gupta et al., 2013). Similarly, plant species also have diverse adaptability and surviving capacity to grow in this toxic metal contaminated medium. These characteristics are used to remediate toxic metal contaminated site and the process of using plants for decontamination is the phytoremediation which is a suitable, sustainable and eco-friendly technique (Islam et al., 2013a; Nizam, 2014). The plants, which accumulate metals in their aerial tissues, are called hyper accumulator (Yanqun et al., 2004; Ali et al., 2013). *Xanthium italicum*, *Cynodon dactylon*, *Pistia stratiotes*, *Eichhornia crassipes*, *Echinochloa crusgalli* L., *Micranthemum umbrosum*, *Ipomoea aquatica*, *Alternanthera philoxeroides* and *Monochoria hastata* L. and *Jassieua repens* are identified as metal hyper accumulator in the naturally contaminated soil and water (Islam et al., 2013b, 2017, 2019; Zaman et al., 2005).

Some of the researchers use seedlings of different plant species for searching toxic metals accumulation. Others are analyzing plants, growing

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in contaminated site to evaluate their metal accumulation capacity (Islam et al., 2013a, 2013b; Sarwat et al., 2013; Zabir, 2014). Pb at lower concentrations promoted seedling growth, but at higher concentrations reduced growth and chlorophyll content (Maiti et al., 2002). Lead also reported to affect seed germination of *Lens culinaris* L. (Cokkizgin and Cokkizgin, 2010). A group researchers conducted an experiment using different kenaf and mesta varieties at 0-120 mg L⁻¹ Pb and Cr treated medium in germination stage and found that these varieties can tolerate Pb but failed in Cr at highest level of treatment (120 mg L⁻¹) (Sultana et al., 2019).

It is concluded that Pb pollution in the environment is one of the major concern in developing countries which is increasing day by day. Crops accumulated toxic Pb in their body when grown in Pb contaminated soil and contaminate the food chain and associate risks are arise in human health. In most cases commercial crop varieties cannot be grown in the contaminated sites due to heavy metals toxicity reduces the plants growth and yields and farmers became looser. So it is urgent to find out suitable crop species with high tolerance to heavy metals and their maximum tolerance capacity to these toxic metals grown in contaminated soil. Therefore, the present study was carried out to know the Pb tolerant dynamics of kenaf and mesta at germination stage in media containing different high levels of Pb (200-1000 mg L⁻¹) and its effect on germination, seedling growth, biomass production and uptake of Pb by seedlings.

Here germination stage is considered using commercial fibrous crop (kenaf and mesta) varieties because seed germination is the first step for further growth and development of plants in contaminated soil. Plant species usually have more tolerance in toxic metal contaminated soils, compared to direct toxic metals in solution because a lot of diversified factors including microbes are active in soil medium that are commonly absent in artificial medium (Nizam et al., 2013). On the other hand, these crops are non-edible hence there is no danger of bioaccumulation of Pb in food chain. In addition, farmers will be benefitted (increasing income) by cultivating these varieties in Pb contaminated soils and also reduces or remove the Pb pollution.

2. MATERIALS AND METHODS

2.1 Seeds collection and processing

The experiment was carried out in the postgraduate research laboratory of Agricultural Chemistry, Patuakhali Science and Technology University, Dumki, Patuakhali. Kenaf (*Hibiscus cannabinus* L. Var. HC-95 and HC-3) and mesta (*Hibiscus sabdariffa* L. Var. Samu-93 and Vegetable Mesta-1) were used in this study. Uniform textured seeds of jute, kenaf and mesta were separated from the collected seeds. Seeds of different kenaf and mesta varieties were collected from Regional Jute Research Institute, Khepupara, Patuakhali, Bangladesh. To protect fungal attack the seeds were surface sterilized by dipping 95% ethanol for 3 minutes and washed with deionized water. A thin layer of cotton was placed at the bottom of the clean dry petridishes. Then 20 seeds of each variety were placed in each dish.

2.2 Treatments of the experiment

After placing seeds, 10 mL of deionized water was added to control and 10 mL solution of each of different levels of Pb like 0, 200, 400, 600, 800, 1000 mg Pb L⁻¹ were applied from analytical grade lead nitrate [Pb(NO₃)₂] was added in each dish. Then the dishes were covered with lids and kept in the incubator at 25°C temperature. After two days the lids were removed and the germinating medium were further moistened with 10 mL of deionized water for control dishes and 10 mL solution of each of different levels of Pb. At 4th days 5 mL of deionized water for control dishes and 5 mL solution of each of different levels of Pb also added. Completely Randomized Designs (CRD) with three replications was followed for applying all the treatments.

2.3 Data collection

Five days after completing germination and establishment of seedlings, data were recorded (Islam and Rahman, 2008). Data included percent germination, shoot and root length (cm) fresh and dry weight (g) and Pb contents of seedlings. Germination (%), shoot length (cm), root length (cm) and fresh weights (g) were recorded in the day of seedlings harvesting and the dry weights (g) were recorded after air drying followed by oven dried at 65°C for 48 hrs (Islam et al., 2015). Root and shoot lengths were calculated from the average of 5 seedlings. On the other hand, fresh and dry weights of all the seedlings per petri dish were measured.

2.4 Chemical analysis

Exactly 0.2 g ground plant sample was taken in a 150 mL digestion tube and then 10 mL of 65 %HNO₃ (MERCK, Germany) was added to it and heated at 130°C for 1hr in a digestion chamber (Islam et al., 2019). After that the sample was cooled and 1 mL of 30%H₂O₂ (Scharlau, Spain) was added to it and again heated at 130°C until a colorless aliquot (approx. 1 mL) remained at the bottom of the tube. The tube was then allowed to cool down and the content was diluted with deionized water and made up to 100 mL volume (Islam et al., 2015). All the samples were filtered through Whatman No. 42 filter paper. Pb content in plant extracts were determined following the method described using atomic absorption spectrophotometer (Shimadzu AA 7000) (Sparks, 1996; Singh et al., 1999).

2.5 Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984). Results were expressed as the means ± standard deviation (SD) of three replicates. Significance degree was calculated using a *t*-test and curve fitting was done using the computer package Microsoft Excel program (Microsoft Office 2007 Professional).

3. RESULTS AND DISCUSSION

3.1 Effects of Pb treatments on seed germination of kenaf and mesta varieties

The data of Table 1 reflected that the percent germination of almost all the varieties was decreased with the increasing of Pb concentration. But the variations of germination with control were very minimum. The highest (98.33±2.89%) germination was found in kenaf (HC-95 & HC-2) at control and the lowest (20%) was in mesta VM-1 at 1000 mg Pb L⁻¹ treatment. Maximum rate of germination was also observed in kenaf HC-2 at 200 mg Pb L⁻¹ followed by kenaf HC-95 (96.67 ± 2.89%) at the same level of Pb. All the tested varieties showed comparatively better performance up to 1000 mg Pb L⁻¹. None of the variety was found completely without germination up to 1000 mg Pb L⁻¹ (Table 1). Besides these, some other varieties perform better on different concentrations. Kenaf HC-95 variety showed 93.33 ± 11.55% germination at 400 and 600 mgL⁻¹ Pb, 91.67 ± 5.77% and 83.33 ± 2.89% germination were also observed at 800 and 1000 mgL⁻¹ Pb, respectively in the same variety. Only mesta VM-1 variety was comparatively inferior in germination and similar trends was also found (Nizam et al., 2014).

Table 1: Effects of increasing levels of Pb on the germination of different varieties of kenaf and mesta

Concentrations of Pb (mg L ⁻¹)	Germination (%)				
	HC-95 (Kenaf)	HC-3 (Kenaf)	HC-2 (Kenaf)	Samu-93 (Mesta)	VM-1 (Mesta)
00	98.33 ± 2.89	88.33 ± 7.64	98.33 ± 2.89	90.00 ± 13.23	43.33 ± 7.64
200	96.67 ± 2.89	88.33 ± 2.89	98.33 ± 2.89	80.00 ± 17.32	33.33 ± 2.89
400	93.33 ± 11.55	88.33 ± 12.58	96.67 ± 2.89	76.67 ± 14.43	30.00 ± 5.00
600	93.33 ± 7.64	86.67 ± 7.64	85.00 ± 5.00	76.67 ± 10.41	28.33 ± 12.58
800	91.67 ± 5.77	83.33 ± 7.64	80.00 ± 5.00	75.00 ± 8.66	26.67 ± 2.89
1000	83.33 ± 2.89	83.33 ± 7.64	76.67 ± 2.89	73.33 ± 8.55	20.00 ± 13.23

Note: * Means significant at 5%, ** means significant at 1% and ***means significant at 0.1% level of probability. ± Values are standard deviation with mean value.

3.2 Effects of Pb treatments on shoot length of kenaf and mesta seedlings

Almost all the varieties of kenaf and mesta showed their gradual reduction in shoot growth but the shoots were healthy and normal. In case of kenaf (HC-95), shoot length was statistically identical from 200 and 800 mg Pb L⁻¹, whereas it was identical at 200 and 400 mg Pb L⁻¹ for kenaf HC-2, and also 800 and 1000 mg Pb L⁻¹. The kenaf and mesta varieties continued their shoot growth up to 1000 mg Pb L⁻¹ but the root lengths were lower at higher concentration (Table 2). Highest shoot lengths were recorded at control treatment for all the varieties. But with the increasing of Pb concentrations, the variations of shoot growth decreasing trends were minimum. At 200 mgL⁻¹ of Pb application, maximum shoot length

(8.57±0.16cm) was measured from kenaf HC-95 followed by 7.68±0.07cm shoot length from the same variety (Table 2). At the highest levels of Pb treatment, the shoots were somewhat curled. Shoot growth reduction might have occurred due to the toxic effect of Pb that retards the cell division of the meristematic zone of shoot (Rashid, 2010).

Table 2: Effects of increasing levels of Pb on the shoot length of seedlings of different varieties of kenaf and mesta.

Concentrations of Pb (mgL ⁻¹)	Shoot length (cm)				
	HC-95 (Kenaf)	HC-3 (Kenaf)	HC-2 (Kenaf)	Samu-93 (Mesta)	VM-1 (Mesta)
00	9.30 ± 0.23	8.75 ± 0.19	8.48 ± 0.07	8.13 ± 0.15	7.73 ± 0.21
200	**8.57 ± 0.16	**7.60 ± 0.32	***7.36 ± 0.07	***7.19 ± 0.18	***6.53 ± 0.14
400	***7.68 ± 0.07	6.85 ± 0.08	***7.02 ± 0.11	5.80 ± 0.20	5.07 ± 0.15
600	***7.39 ± 0.06	5.30 ± 0.06	5.55 ± 0.16	5.08 ± 0.30	4.75 ± 0.25
800	5.95 ± 0.11	4.43 ± 0.42	***4.90 ± 0.11	3.28 ± 0.39	3.48 ± 0.08
1000	4.55 ± 0.09	***3.72 ± 0.10	***3.59 ± 0.18	***2.70 ± 0.13	2.45 ± 0.05

Note: ** means significant at 1 % and ***means significant at 0.1 % level of probability. ± values are standard deviation with mean value.

3.3 Effects of Pb treatments on root growth of kenaf and mesta seedlings

Gradual reduction of root growth was found for almost all the varieties of kenaf but drastic root growth reduction was measured in case of mesta Samu-93 and mesta VM-1. Roots were not normal at higher concentrations of Pb. In case of kenaf HC-3, almost identical root length was found at 200 and 400 mg Pb L⁻¹. All the kenaf and mesta varieties were able to continue their root growth up to 1000 mg Pb L⁻¹ though the root lengths were lower at higher concentration (Table 3). At higher levels of Pb application the tap root system of the seedlings of all the varieties were destroyed and, in that case, adventitious root system were developed by the seedlings to survive. Besides this with higher concentrations of Pb in germinating media the color of the roots of some varieties were turned into brown. This phenomenon was confirmed because he found that radicals (primary root) were more adversely affected by heavy metals than shoot growth (Yemeni, 2001).

Table 3: Effects of increasing levels of Pb on the root length of seedlings of different varieties of kenaf and mesta.

Concentrations of Pb (mgL ⁻¹)	Root length (cm)				
	HC-95 (Kenaf)	HC-3 (Kenaf)	HC-2 (Kenaf)	Samu-93 (Mesta)	VM-1 (Mesta)
00	5.24 ± 0.93	7.83 ± 0.08	5.17 ± 0.15	4.33 ± 0.15	4.10 ± 0.13
200	**1.68 ± 0.21	***2.06 ± 0.12	1.96 ± 0.04	2.08 ± 0.20	1.85 ± 0.18
400	**1.49 ± 0.08	***1.23 ± 0.08	1.17 ± 0.03	2.25 ± 0.23	1.05 ± 0.05
600	**0.98 ± 0.02	***0.86 ± 0.08	***0.78 ± 0.08	***1.14 ± 0.13	***0.97 ± 0.08
800	**0.86 ± 0.05	***0.60 ± 0.04	0.43 ± 0.03	***0.65 ± 0.14	***0.45 ± 0.05
1000	**0.57 ± 0.04	***0.40 ± 0.05	0.27 ± 0.03	***0.27 ± 0.08	***0.31 ± 0.06

Note: ** means significant at 1 % and ***means significant at 0.1 % level of probability. ± Values are standard deviation with mean value.

3.4 Effects of Pb treatments on fresh biomass of kenaf and mesta seedlings

Fresh weights were gradually decreased for all the studied varieties of kenaf and mesta. For kenaf (HC-95, HC-3 & HC-2) fresh weights were identical from 200 to 800 mg Pb L⁻¹ applications. The fresh biomass weight of seedlings of mesta Samu-93 and VM-1 were identical at 200 and 400 mg Pb L⁻¹. Statistically similar fresh biomass also measured in kenaf HC-95 and kenaf HC-2 from 200 to 800 mg Pb L⁻¹ applications. All the varieties were able to continue their fresh biomass production up to 1000 mg Pb L⁻¹ but the amount of fresh biomass was lower at higher concentration (Table 4). For all the varieties highest fresh weights were measured without application of Pb. At the application of highest concentration of Pb

(1000mg L⁻¹), highest fresh biomass from kenaf HC-95 (2.48 ± 0.28 g petridish⁻¹) and lowest biomass from mesta VM-1 (2.48 ± 0.28 g petridish⁻¹) were obtained. Similar had been the observation, they observed visible decrease in plant (*Cicer arietinum* L.) biomass with increase in the concentration of heavy metals like Pb and Cr in the soil (Dasgupta et al., 2011).

Table 4: Effects of increasing levels of Pb on the fresh weight of seedlings of different varieties of kenaf and mesta

Concentrations of Pb (mgL ⁻¹)	Fresh weight (g petridish ⁻¹)				
	HC-95 (Kenaf)	HC-3 (Kenaf)	HC-2 (Kenaf)	Samu-93 (Mesta)	VM-1 (Mesta)
00	5.31 ± 0.36	3.61 ± 0.34	4.22 ± 0.26	2.04±0.60	1.04 ± 0.19
200	**4.18 ± 0.29	*2.92 ± 0.14	***3.56 ± 0.06	*1.60±0.26	*0.66 ± 0.04
400	***3.21 ± 0.17	**2.68 ± 0.32	***2.97 ± 0.15	**1.59±0.28	*0.63 ± 0.02
600	***3.02 ± 0.07	**2.12 ± 0.07	***2.36 ± 0.19	*1.46±0.55	*0.52 ± 0.29
800	***2.95 ± 0.13	***1.31 ± 0.27	***1.96 ± 0.10	*1.32±0.21	**0.41 ± 0.04
1000	2.48 ± 0.28	***1.17 ± 0.26	***1.64 ± 0.10	*1.25±0.54	**0.33 ± 0.20

Note: * Means significant at 5 %, ** means significant at 1 % and ***means significant at 0.1 % level of probability. ± Values are standard deviation with mean value.

3.5 Effects of Pb treatments on dry biomass of kenaf and mesta seedlings

The dry weights of seedlings were significantly reduced in (Mesta) Samu-93 VM-1 with the increasing concentrations of Pb. No statistical variations were found in dry biomass production for kenaf (HC-95, HC-3, & HC-2). All the varieties were able to continue their dry biomass production up to 1000 mg Pb L⁻¹ and the differences in dry weight from treatment to treatment are very minimum. For all the varieties highest dry biomass was measured from control when no Pb was applied. At highest level of Pb application, comparatively higher amount (0.27± 0.01g) of dry biomass was obtained from kenaf HC-95 and lowest amount (0.04 ± 0.03g) of biomass from mesta VM-1. The present findings completely harmonized with the findings of who revealed that the dry biomass of jute, kenaf and mesta seedlings were decreased with the increased levels of Pb (Akter, 2011; Nizam et al., 2014).

3.6 Effect of applied Pb on the Pb content in seedlings of different kenaf and mesta varieties

At germination stage the concentrations of Pb in seedlings of kenaf and mesta were increased by the application of increasing Pb levels (200 – 1000 mg Pb L⁻¹) in the germinating medium. Intra and inter variatals variations of Pb concentrations were detected in seedlings with increasing Pb application. At the highest level of Pb (1000 mg Pb L⁻¹), Pb concentrations in the seedlings were 16012 ± 10, 19492 ± 239, 12043 ± 41, 15418 ± 609 and 17902 ± 401mgkg⁻¹ in kenaf HC-95, kenaf HC-3, kenaf HC-2, mesta Samu-93 and mesta VM-1 respectively. On the other hand for all the varieties the lowest (0.00 mg kg⁻¹) Pb concentrations were detected at control. Similar wide concentration range (4 – 500 mg L⁻¹) of Pb removal with Indian mustard had also been described (Raskin and Ensley, 2000).

It is assuming that higher concentration of Pb in the germinating medium had slight adverse effect on the growth and development of seedlings that influence the biomass production, but it promotes the Pb absorption. A group researcher stated that plants which are able to accumulate more than 1000 ppm Pb considered as hyper-accumulator, according to their comments all the varieties of the present study are hyper-accumulator of at germinating stage, because the concentration of Pb in all the varieties were > 1000 mgkg⁻¹ (Wei et al., 2004). Discussed similar nature of Pb accumulation; they revealed that *Brassica juncea* plants accumulated higher amounts of Pb and Zn than *Brassica carinata* (Rio et al., 2004).

3.7 Effect of applied Pb on Pb uptake by seedlings of kenaf and mesta varieties

At germination stage, the uptake of Pb by seedlings of kenaf and mesta plants were increased significantly with the increasing levels (5.00 – 25.00 mg Pb pot⁻¹) of Pb application in the germinating medium (Figure 3). The

uptake patterns of Pb varied on the amount of applied Pb and plants variety. For all the varieties, maximum uptakes were calculated against the highest amount of applied Pb. At the highest level (25.00 mg Pb pot⁻¹) of Pb in the germinating medium, maximum uptakes of Pb in the seedlings were 4.45 ± 0.40 , 3.70 ± 0.72 , 2.81 ± 0.48 , 2.62 ± 0.38 and 1.07 ± 0.18 mg pot⁻¹ in kenaf HC-95, kenaf HC-3, kenaf HC-2, mesta Samu-93 and mesta VM-1, respectively (Figure 3). On the other hand the lowest (0.00 mg pot⁻¹) Pb uptake was detected at control for all the varieties.

Figure 1: Effects of applied Pb on the dry weight of seedlings of different varieties of kenaf and mesta

Figure 2: Effect of Pb on the Pb contents of seedlings of different varieties of kenaf and mesta

Figure 3: Effect of total amount of applied Pb on the uptake of Pb by the different varieties of kenaf and mesta

4. CONCLUSIONS

Germination of seeds in contaminated media is very important to grow them as phytoremediator in contaminated environment to reduce

pollutants. The results of the research showed that the increasing concentration of applied Pb decreased the percent germination, root and shoot growth of seedlings and fresh and dry biomass for almost all the varieties of kenaf and mesta. Since the varieties of kenaf and mesta were able to germinate up to 1000 mg PbL⁻¹ and none was found to complete failure of germination, so it would also be able to germinate and grow in high Pb contaminated soil (1000 mg PbL⁻¹). Therefore, these fibrous kenaf and mesta varieties could be used for growing in Pb contaminated soil where other crop plants cannot be able to grow and farmers become benefitted. On the other hand, Pb bioaccumulation within seedlings of kenaf and mesta plants were increased significantly with the increasing levels of Pb application indicating the amount of Pb in soil will be reduced with time by cultivating these species and soil become reclaimed.

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