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Phytotoxicity of Lead and Chromium on Germination, Seedling Establishment and Metal Uptake by Kenaf and Mesta

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ABSTRACT: Heavy metal contaminated soil raises major global environmental and agricultural concern. Recently soil pollution through lead (Pb) and chromium (Cr) becoming serious problem and remediation or utilization of those contaminated soil with potential crops is of the outmost importance. The objectives of present study were to examine the effects of Pb and Cr on three different kenaf and mesta varieties for seed germination, seedling establishment and amount of Pb and Cr uptake by tested varieties in laboratory condition. Three varieties were used for the study namely, HC-95 (kenaf), CPL-72126 (mesta) and Samu-93 (mesta) and the treatments were combination of Pb and Cr chemical at (0,0), (60,60), (80,80), (100,100) and (120,120) mg/L. Increased level of lead and chromium gradually reduced the germination percentage and primary growth parameters compared to control. The shoot and root lengths were affected only little, whereas, the biomass showed a considerable reduction with the increase of Pb and Cr toxicity. Stress tolerance indices showed a gradual and negative response by the plant with the increase of metal concentrations. However, in all the levels of Pb and Cr treatment, the seedlings were capable to tolerate the toxicity and seedlings were established. Bioaccumulation of Cr was higher than that of Pb in all varieties and in all treatments. The interaction of Pb and Cr reduced the toxic effect of both metals to the plants. The findings are helpful for selecting fiber crop varieties for cultivation in contaminated soils or phytoremediation of Pb and Cr from the contaminated soils.

Keywords: Lead, chromium, phytoremediation, kenaf, mesta.

INTRODUCTION

Soil contamination by heavy metals due to increased human activities including mining, industry activities, transportation, and agriculture raises major global environmental and human health concern (Marichali *et. al.*, 2014). Due to their toxicity and non-biodegradability metal ions pose a threat to plants by accumulating in edible parts which eventually could enter into the food-chain posing threat to human health (Clemens, 2006). Lead (Pb) and chromium (Cr) are regarded as the toxic environmental pollutants, as they display the most profound mobility in the soil environment (Lou *et al.*, 2013). In Bangladesh, lead contamination is increasing day by day due to indiscriminate

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use and throwing of used batteries of automobile in the agricultural land and deposition of contaminants to the soil or aquatic environment from vehicular emission (Hossain *et al.,* 2014). Cromium is widely used in industries like steel, leather and textile. Tannery and textile discharges cause a huge pollution of Cr in our soils and water. It is well documented that excess Cd or Pb inhibits plant growth, and directly or indirectly interferes with their physiological processes through disrupting their metabolism (Yourtchi and Bayat, 2013; He *et al.*, 2015). Lead also affects seed germination of *Lens culinaris* L. (Cokkizgin and Cokkizgin, 2010). Excess level of Cr was found to be harmful for plant life, reducing the protein contents, inhibiting the enzyme activity, and causing chlorosis and necrosis (Puyang, 2015). Chromium led to an inhibition of seed germination, nutrient imbalance, antioxidant enzymes, significant growth inhibition and chlorophyll content and proteins (Ali *et al.*, 2015; Zeid, 2001; Panda and Choudhury, 2005; Panda *et al.*, 2001, Shanker, 2003; Han 2004, and Shanker *et al.*, 2004), root growth and biomass, chlorosis, photosynthetic impairing and finally, plant death (Scoccianti *et al.*, 2006).

Seed is a stage in the plant life cycle that is well protected against various stresses and soon after imbibition and subsequent vegetative developmental processes, they become stress-sensitive in general (Li *et al.*, 2005). Therefore, germination of seed is thought to carefully monitor such external parameters as light, temperature and nutrient in order to maintain the protective state until external conditions become favorable for following developmental processes (Karssen, 1982; Pritchard *et al.*, 1993; Bungard *et al*., 1997). Seed germination is the first physiological process to encounter abiotic stress, the ability of a seed to germinate in a medium containing metal ions would be indicative of its level of tolerance to this metal (Mrozek Jr and Funiccilli, 1982). Inhibition of germination may result from the interference of metal ions with crucial enzymes. That is why we preferred the germination and early growth stage of the tested varieties to reveal the capability of the plants to tolerate Pb and Cr contamination.

Due to increase population, urbanization and rapid industrialization our cultivable land is reduced day by day. So, inventing cultivation technology that are capable in both successful crop production and also remove or decrease pollutants from soil is prime need to us. Jute is the most important fiber crop of Bangladesh. Besides these, it is a non-edible crop. If it is possible to reduce or remove lead and chromium pollutants from soil by growing jute crops, it will be helpful for increasing farmer's income by utilizing fallow land. Kenaf and mesta (a crop similar to jute known as mesta jute) are also fiber and commercial crops like jute. As a non-edible crop, there is no danger of bioaccumulation of the lead and chromium in to the food chain. There would be less possibility to return these pollutants into the soil within short time that uptake by a fiber crop. Considering above facts, the research was conducted with two varieties of kenaf and one variety of mesta with the objectives to examine the phytotoxic effect of Pb and Cr on kenaf and mesta and their tolerance to Pb and Cr and uptake ability of those metals by the plants from the growth media at germination stage.

MATERIALS AND METHODS

Three fiber crops varieties *viz.* HC-95 (kenaf), CPL-72126 (kenaf) and Samu-93 (mesta) from two fiber crops namely kenaf (*Hibiscus cannabinus* L.) and mesta, also known as roselle (*Hibiscus sabdariffa* L.), were used for the study. Four treatments *viz*. (0,0 as control), (60,60), (100,100), (120,120) mg/L of Pb and Cr combinations were used. These doses were selected based on our previous experiment (Sultana *et al*., 2019) with the same three varieties where single treatment of Pb and Cr were used up to 120 mg/L and plant growth was found a little affected till the highest level of metal stress. Therefore, the combined doses were also selected up to 120 mg/L. The source of chemical was lead nitrate [$Pb(NO_3)_2$] for Pb and chromium oxide (CrO_3) for Cr. Desired amount of lead and chromium were calculated for all the treatments from their respective chemicals. A volume of 10 mL solution from each treatment was added in each petridish over the cotton sheet before seed placement.

Seeds of different kenaf and mesta varieties were collected from Regional Jute Research Institute, Khepupara, Patuakhali, Bangladesh. A total of 25 seeds were placed in each petridish and 5-10 mL water was added in each petridish as and when necessary. The germination of the seeds was checked after 5 days of seed placing and the percent germination was calculated accordingly. After one week of germination, the root and shoot length and the fresh weight were measured. The seedlings were then placed in an oven at 80℃ for 48hr and the dry weights were taken. Twenty seedlings were selected for each parameter and the average results were calculated from the collected plants. The experiment was carried out in Completely Randomized Design (CRD) with three replications.

For chemical analysis, 0.5 g of oven dry sample was taken into a 100 mL conical flask. A volume of 5 mL HNO_3 was added into the flask and stand overnight. The flask was placed on a hot plate at low heat until the brown fumes of NO_2 ceased and cooled with content. Then, 2.5 mL 70% $HClO_4$ was added and raised the temperature 120^{o}C. The digestion was stopped when dense white fumes evolved and 3-5 mL contents were remaining in the flask. Expected volume (50 mL) was made by several rinsed after cooling the digest. Then the samples were analyzed by Atomic absorption spectrophotometry (AA-7000, Shimadzo) in the central laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The detection limit of both metals is 0.1 ppb using graphite furnace. The relative standard deviation (RSD) in the analysis was set at 2 % prior to analysis. To get consistency of measurement standard was run at every10 samples intervals and the recovery was kept within 95–105 %.

Stress tolerance index was calculated to reveal the yield and stress tolerance potential of varieties or genotypes. Stress tolerance indices for different growth parameters were calculated using following formulae (Wikins, 1957)

RLSTI = (Root length of stressed plant/Root length of control plant) × 100

SLSTI = (Shoot length of stressed plant/Shoot length of control plant) × 100

FWSTI = (Fresh weight of stressed plant/Fresh weight of control plant) × 100

DWSTI = (Dry weight of stressed plant/Dry weight of control plant) × 100

All the data were analyzed using Microsoft® Excel software. The results were expressed as a mean of three replicates with± standard error (SE). Student’s t-test and analysis of variance were used to evaluate the statistical significance test.

RESULTS AND DISCUSSION

Lead and chromium toxicity in a combined Pb, Cr treatment at different increasing levels, negatively and gradually affected the seed germination of kenaf and mesta varieties. Among three varieties, the maximum germination percentage (100 %) was observed in CPL-72126 (kenaf) variety in control treatment and the lowest germination percentage (58.33%) was found at Samu-93 (Mesta) at combination of lead (120 mg/L) and chromium (120 mg/L) (Figure 1). All the kenaf and mesta varieties were affected in germination in different levels of Pb and Cr toxicity. The combined effect of Pb and Cr reduced the germination percentage to 60% at the highest level of Pb and Cr (120 mg/L) in the variety Samu-93 (mesta) compared to that of control, indicated that 40% of the seeds were failed to germinate at the highest level of both Pb and Cr combined treatment. The two kenaf varieties were found to have more tolerance to the combined metal toxicity where the seed germination percentages were 91.66 and 70 at the highest combined metal dose. Aydinalp and Marinova (2009) examined the effects of the concentrations of Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} on seed germination of alfalfa (cultivar Malone) grown in solid media (agar). They presented result in which significant reduction in seed germination as metal concentrations increased in the growing media. They showed that 10 mg/L of Cd^{+2} and $Cr^{+6,}$ and the 20 mg/L of Cu^{+2} and Ni^{+2} significantly reduced seed germination and the concentration of 40 mg/L $Cd^{+2,}$ Cr^{+6}, Cu^{+2}, and Ni^{+2} inhibited significantly seed germination by 44.0%, 54.0%, 39.0%, and 24.0%, respectively. Samanta (2009) reported that, with the increasing concentrations of Pb, germination of jute seeds was reduced. Pandey (2008) reported that the negative stress of the non-essential Cr is more vigorous than other heavy metals like copper in germination stage. This similar trend was also observed in our experiment. However, in our experiment the reduction of germination up to 60% was found only at Samu-93 at the level of 120 mg/L, revealed the better tolerance of all the tested variety to Pb and Cr toxicity.

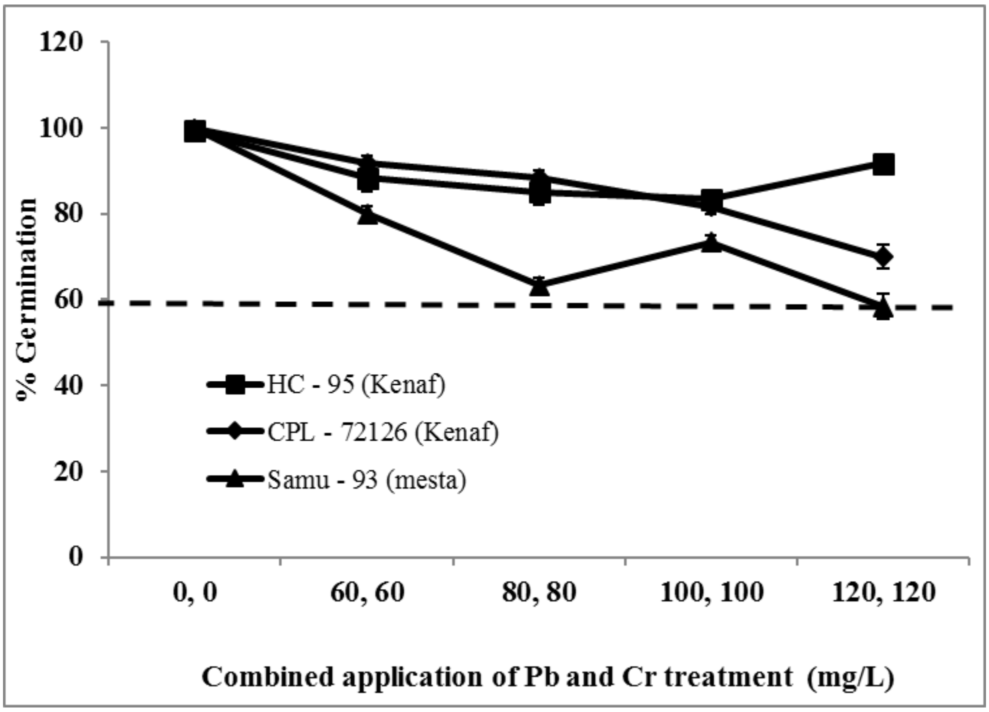

Fig. 1. Combined effect of Pb and Cr on germination of kenaf and mesta seeds (Data represents the mean of three replicates; each with 25 seeds; bar represents ± standard errors)

Increasing level of combined lead and chromium toxicity gradually reduced the shoot growth of all of the three kenaf and mesta varieties (Fig. 2A). However, the reduction was not very sharp rather very little with the increase of Pb and Cr application level and the inhibitory effects were not similar in all concentrations.

Maximum shoot length was observed in HC-95 (kenaf) variety at control treatment and minimum shoot length was found in Samu-93 at the highest treatment of Pb and Cr (120 mg/L). In all the three varieties, after control the shoot lengths were more or less similar in the next two levels of combined metal applications with a gradual decrease at 100 and 120 mg/L metal applications. In the case of HC-95 (kenaf), growth of plants exposed to 60 and 80 mg/L Pb and Cr combined treatment, were statistically similar to the control plants. Under higher exposures (100 and 120 mg/L), growth inhibition was consistently higher with the increase in metal concentration. The decreasing trend of shoot growth of the seedlings against combined effect of lead and chromium toxicity was also observed by Samanta (2009) which was similar to our research findings. The reason behind the decreasing trend might be due to the toxic effect of lead and chromium on the meristematic growth of the seedlings at germination stage.

As shown in the Figure 2B, root growth was affcetd more than that of shoot growth at the same level of combined toxicity of Pb and Cr. In all the varieties, root lenghts were lower than the coresponding shoot lenght. The root growth was highest in CPL-72126 (Kenaf) in all the treatmnets except the highest level where HC-95 (kenaf) yielded the longest roots. At the level of 60 mg/L Pb and Cr level, all the three varieties yielded root lengths which were very close to their respective root lengths in control. Maximum root length was observed in CPL-72126 (Kenaf) variety at control treatment. The color of the roots in higher combined treated media became browner in color and root growth was stunted. Shadot (2009) reported similar type of decreasing trend of root lengths with increasing combined lead and chromium concentrations in different jute seeds. The stunted root might be the tolerance mechanism of the plants against metal stress.

Observation from the experiment revealed that increased level of Pb and Cr combined treatment decreased the biomass production of the kenaf and mesta varieties (Fig. 3). In case of fresh weight, the kenaf varieties, CPL-72126 and Samu-95 produced maximum biomass yield at control experiment. With the increase of Pb and Cr level, the biomasses gradually reduced. In the case of dry weight HC-95 (kenaf) produced the highest dry biomass at control treatment. In each treatment level, the CPL-72126 produced dry biomasses very close to HC-95 in the respective treatment. The Samu-93 (mesta) produced the lowest fresh and dry biomass in all the treatments of combined Pb and Cr toxicity. Rashid (2010) conducted an experiment having five jute varieties such as *Shonpat*, HC-95 (Kenaf), CVE-3, OM-1 and O-92 in which he mentioned that fresh and dry weights of jute seedlings was significantly affected by increased level of combined toxicity of As, Cd, Pb, and Cr. This result was at par with our findings.

In one of our different experiment, Pb and Cr were applied separately to all the three kenaf and mesta varieties tested here. It was found that at the level of 120 mg/L Cr , seedling failed to established and the shoots and roots were diminished (Sultana *et al.*, 2017). When, Pb and Cr applied together at the same level (120, 120 mg/L Pb and Cr), the seedlings successfully established and shoot and root were not diminished. Although, the root growth was stunted and root color were brownish and produced more lateral secondary roots, the plants could well tolerate the level of combined Pb and Cr than the individual Cr level. The result suggests that the interaction of Pb and Cr might reduce the toxicity of both metals, especially the Cr toxicity to the plants.

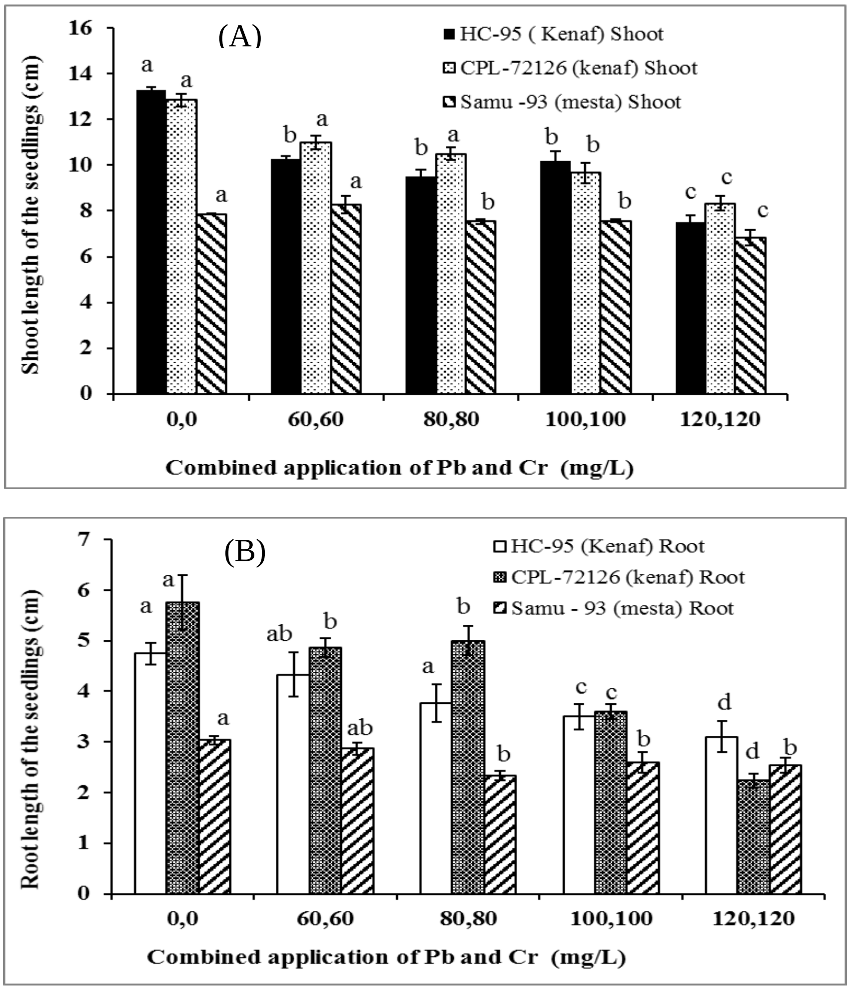

Fig. 2. Effect of Pb and Cr toxicity on (A) shoot and (B) root growth of kenaf and mesta seedlings (Data represents the mean of three replicates; each with 25 seedlings; bar represents ± standard errors. Shoot and root length were compared among the treatments in each varieties; column with same letters are not significantly differ according to Tuky's combined test at p<0.01).

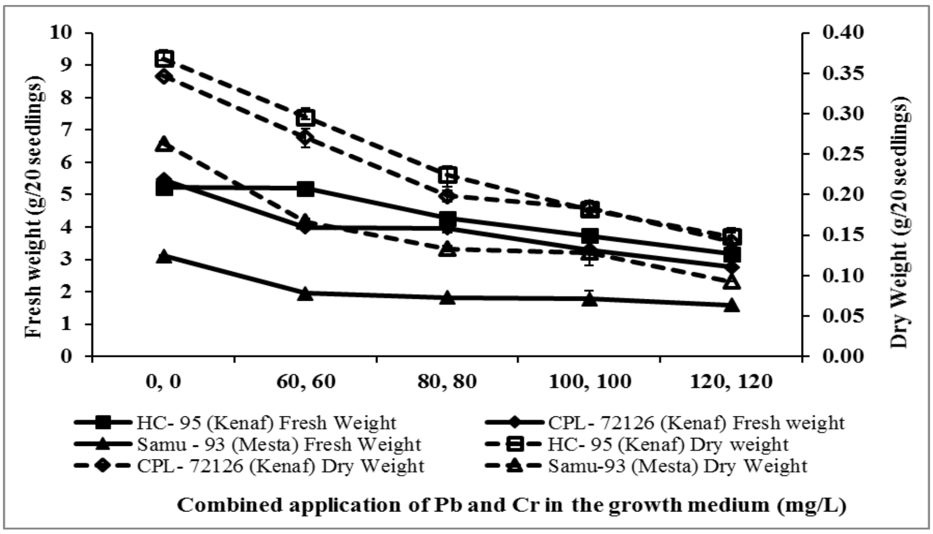

Fig. 3. Biomass of kenaf and mesta seedlings as affected by Pb and Cr treatment (Data represents the mean of three replicates; bar represents ± standard error)

Increased levels of Pb and Cr negatively affected the stress tolerance ability of the seedlings in all the three varieties. Unlike to the root and shoot length where CPL-12726 (kenaf) produced the highest value, Samu-93 (mesta) was found to be best tolerant up to 120 mg/L Pb and Cr treatment in terms of shoot and root length stress tolerance indices. As shown in the table 1, in Samu-3(mesta) at the highest level of Pb and Cr (120,120 mg/L), the stress tolerance index was lowered only by 14% and 17% for SLSTI (Shoot Length Stress Tolerance Index) and RLSTI (Root Length Stress Tolerance Index), respectively, compared to control. At the first treatment (60,60 mg/L Pb and Cr), SLSTI and RLSTI were 105.13 % and 94.48 %, indicated that seedlings were enough capable to tolerate that level of Pb and Cr in terms of shoot and root length. The shoots of two kenaf varieties HC-95 tolerate he metal stress from 77.40% to 56.58 % and CPL-72126 tolerate 85.80% - 65.05% metal stress. Root length stress tolerance index was found lowest in case of CPL-72126 kenaf variety (39.71%).

Both fresh and dry weight stress tolerance indices were found highest in the case of HC-95 (kenaf) followed by CPL-72126 (kenaf) and Samu-93 (mesta) at the first metal treatment (60,60 Pb and Cr). The trend was similar to the rest treatments. Fresh weight stress tolerance indices (FWSTI) were 99.67%, 72.45%, 62.58% in case of HC-95 (kenaf), CPL-72126 (kenaf) and Samu-93 (mesta), respectively at the initial dose of Pb and Cr.

Table 1. Stress tolerance index (%) of the seedlings at increased levels of Pb and Cr

Treatments of Pb and Cr	Variety	SLSTI	RLSTI	FWSTI	DWSTI
0,0	HC-95 (Kenaf)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.0
60,60		77.40 ± 1.66	90.90 ± 5.56	99.67 ± 1.79	80.43 ± 0.46
80,80		71.65 ± 2.95	79.21 ± 5.43	81.83 ± 3.31	60.96 ± 3.86
100,100		76.68 ± 4.06	74.05 ± 6.16	71.64 ± 4.23	`49.39 ± 2.73
120,120		56.58 ± 2.78	66.02 ± 8.51	60.84 ± 1.75	40.30 ± 2.89
0,0	CPL-72126 (Kenaf)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.0
60,60		85.80 ± 3.85	86.48 ± 10.45	73.10 ± 1.46	77.90 ± 3.46
80,80		81.81 ± 2.53	88.46 ± 10.42	72.45 ± 2.31	57.21 ± 2.01
100,100		75.21 ± 2.17	63.98 ± 7.8	60.30 ± 1.24	53.09 ± 2.60
120,120		65.05 ± 4.01	39.71 ± 5.24	50.56 ± 1.80	40.86 ± 3.30
0,0	Samu-93 (Mesta)	100.00 ± 0.00	100 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
60,60		105.13 ± 5.18	94.48 ± 2.24	62.58 ± 1.18	63.28 ± 1.01
80,80		95.76 ± 0.86	77.14 ± 4.56	58.29 ± 0.58	50.64 ± 1.33
100,100		96.20 ± 1.26	85.81 ± 7.10	57.25 ± 7.64	48.68 ± 5.77
120,120		86.85 ± 2.95	83.38 ± 2.36	51.01 ± 0.74	35.19 ± 0.31

SLSTI = Shoot length stress tolerance index, RLSTI= Root length stress tolerance index, FWSTI= Fresh weight stress tolerance index and DWSTI= Dry weight stress tolerance index

Dry weight stress tolerance index was found to be affected as lowest as 40 % in case of HC-95 (kenaf) and CPL-72126 (kenaf). Samu-93 variety was found to be less tolerant in case of dry weight stress tolerance index. At highest level of Pb and Cr the DWSTI was found 35.19 % in Samu-93 (mesta). The effects of Cr and Pb treatments on the stress tolerance of plants have been reported over the last few years (Murtaza *et al.*, 2018, Akinchi and Akinchi, 2010). It is suggested that mechanism of stress tolerance helps plant to maintain growth even in the presence of potentially toxic metal concentrations (Clemens, 2006; Diwan *et al.*, 2010) where the root and shoot growth used as an important parameter of heavy metal tolerance At the cellular level, oxidative stress has been reported as a common mechanism in stress situations (Akinchi and Akinchi, 2010). Among our experimental varieties based on RLSTI and SLSTI mesta or roselle variety was more tolerant. However, the two kenaf varieties, HC-95 and CPL-72126 might tolerated the stress by producing more lateral roots resulting in more fresh and dry weight.

Bioaccumulation of lead and chromium by kenaf and mesta varieties were evaluated at germination stage and the result showed that all the three varieties tested in the experiment, took up Pb and Cr at an increasing rate with the increase of the metals treatment (Fig.4). Maximum lead uptake was found in HC-95 (Kenaf) 100 mg/L Pb and Cr combined treatment and it is similar to that in 100 mg/LPb and Cr combined treatment. All the three varieties accumulated Pb from the Pb and Cr treated media, gradually increased the Pb accumulation and then decreased. At the maximum level of Pb and Cr, the Pb uptake by the all three varieties was reduced a little compared the previous level. The minimum Pb accumulation was found in Samu-93 (mesta) at 60 mg/L Pb and Cr combined treatment. The accumulation pattern of Cr was also the similar to Pb, i.e, the accumulation of Cr first increased with the increase of Pb and Cr combined treatment and then decreased a little at the highest level of Pb and Cr. However, the CPL-72126 (kenaf) was exception, where, the accumulation of Cr increased with the increase of Pb and Cr treatment till the highest treatment level. The highest Cr accumulation (0.741 mg/g of dry weight) was found at100 mg/L and 120 mg/L Pb and Cr combined treatment in HC-95 (Kenaf) and in CPL-72126 (kenaf) respectively. The lowest Cr uptake was recorded in Samu-93 (mesta) variety at 80 mg/L Pb and Cr combined treatment (0.025 mg/g of dry wt).

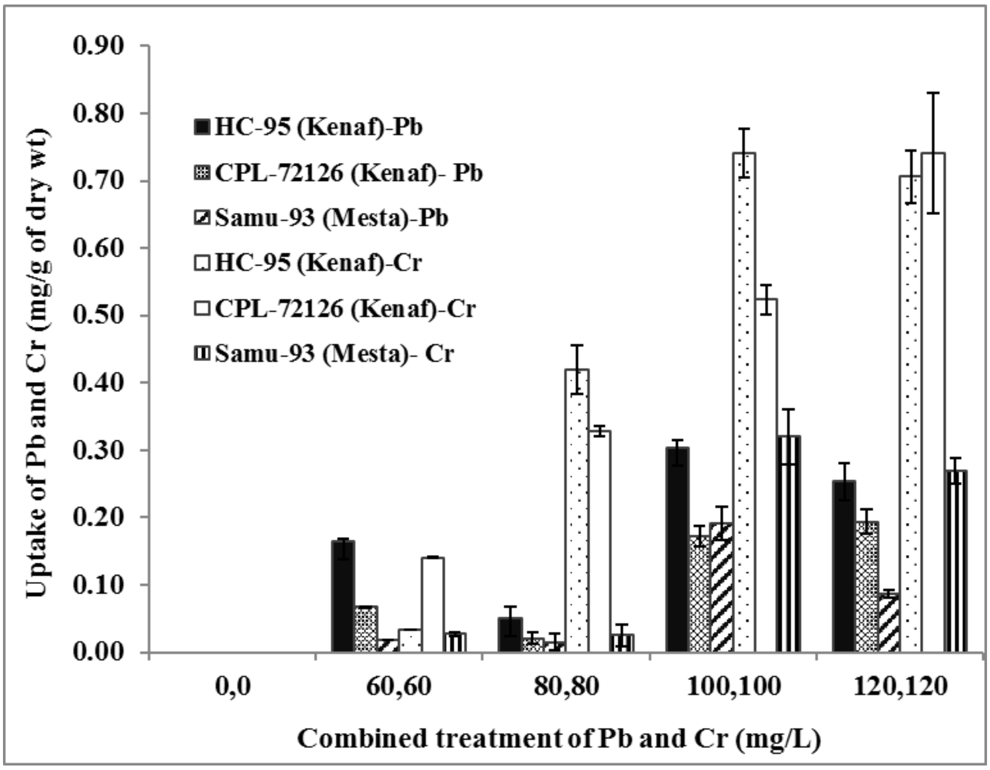

Fig. 4. Uptake of Pb and Cr by kenaf and mesta seedlings at increasing levels of Pb and Cr treatment (Data represents the mean of three replicates; bar represents ± standard errors)

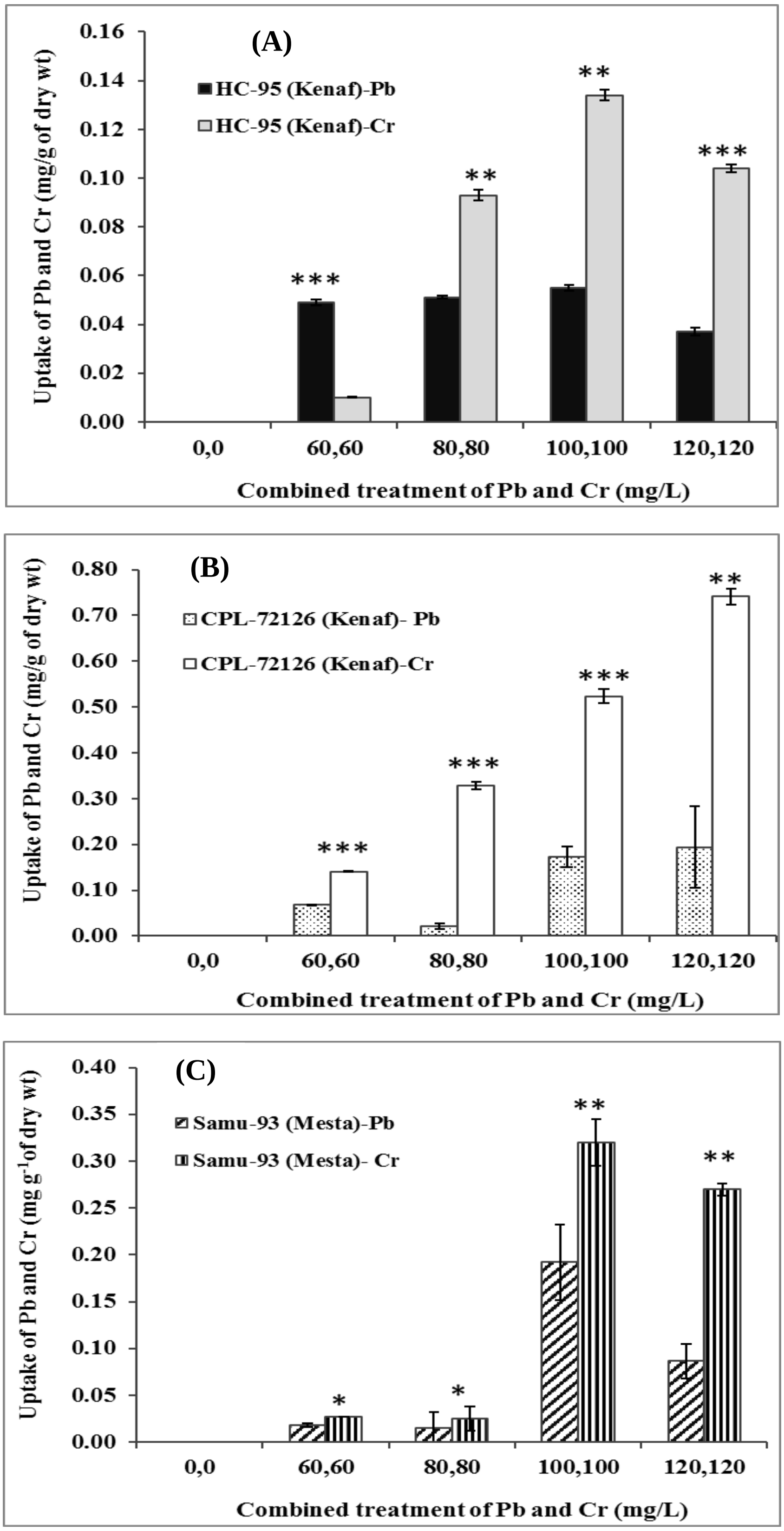

Fig. 5. Interaction effect of Pb and Cr treatment on these metal accumulations in the (A) HC-95 (kenaf), (B) CPL-72126 (kenaf) and (C) Samu-93 (mesta) seedlings (Bars indicate the ± standard error of the means (n= 3). * Significantly different according to paired T-test (* at *P*< 0.05, ** at *p* <0.01, * at *p* <0.001) between Pb and Cr accumulation. (T-test table included in Supplementary table)**

The bioaccumulation of Cr was much higher than Pb in all treatments and in all varieties. It is very interesting to note that, in the single application of Pb and Cr separately, from 0 to 120 mg/L, the bioaccumulation of Pb was higher than Cr by the three same kenaf and mesta varieties in all the treatments (Sultana *et al*., 2019). As shown in the Figure 5, in the case of combined application in all varieties and in all treatments, the Cr accumulation was significantly higher than that of Pb accumulation. The only exception is in the case of HC-95 (kenaf) at 60,60 mg/L Pb and Cr combined treatment, where Pb accumulation was higher than Cr accumulation. Student-t test revealed a significant variation among Pb and Cr accumulation in all three varieties. It is also found that, in combined treatment of Pb and Cr, accumulation of lead was lower than in single treatment of Pb (Sultana *et al.*, 2019), whereas, the accumulation of Cr was not found to be affected very much by the interaction of the chemicals. These results indicated that, interaction of the lead and chromium in the growth media reduced the metal uptake by the seedlings, but in same treatments increase the chromium uptake compared to their single application as explained by Sultana *et al.*, 2019. The reason could be the interaction effect of Pb and Cr in the growth media might reduce the Pb toxicity, while Cr become more active in the growth media and taken up more by the seedlings than the bioaccumulation of Pb.

CONCLUSION

Phytotoxic effects of Pb and Cr on two kenaf and one mesta varieties, namely, HC-95 (kenaf), CPL-72126 (Kenaf), Samu-93 (mesta) were evaluated against different levels of Pb and Cr in a combined treatment at seedlings stage. Increased levels of lead and chromium negatively affected the germination. The other early growth parameters of the test varieties such as root length, shoot length, fresh weight and dry weight were affected slowly and gradually with the increase of treatments of those chemical. The germination percentage was found similar in HC-95 and CPL-72126 kenaf, except at the highest treatment level. The highest root and shoot lengths were observed in CPL-72126 (Kenaf) variety. Both fresh and dry weights were found higher in HC-95 (kenaf) which was very close to CPL-72126 (kenaf) in all the levels of lead and chromium combined treatments. All the tested varieties resulted a gradual negatively responded to the increase of combined metal stress. Root and Shoot length stress tolerance was found higher in Samu-93 (mesta) in all Pb and Cr levels, whereas, fresh and dry weight stress tolerance were found higher in two kenaf varieties compared to mesta.

The accumulation of Pb and Cr by the three varieties was also determined. The results revealed that, the bioaccumulation of Cr was much higher than Pb in all treatments and in all varieties. Maximum Pb uptake was found in HC-95 (Kenaf) 100 mg/L Pb and Cr combined treatment. The highest Cr accumulation was also found in HC-95 (Kenaf) at 120 mg/L Pb and Cr combined treatment. The interaction effect of Pb and Cr might reduce the toxic effects of the metals, especially the toxicity of Pb on the seedlings.

It can be concluded that, all the three varieties are efficient to tolerate lead and chromium in the growth media contained the heavy metals up to120 mg/L at germination and early growth stage. Among the three varieties HC-95 (kenaf) and CPL-72126 (kenaf) varieties showed the better performance in respect to germination and growth and HC-95 (Kenaf) showed best performance in terms of bioaccumulation of the metals. Based on the findings, it can be suggested that HC-95 and CPL-72126 kenaf varieties can be selected to cultivate as a cash crop in the soils contaminated with Pb and Cr or for phytoremediation of Pb and Cr contaminated soil.

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The present research did not receive any financial support.

CONFLICT OF INTEREST
The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING
No life science threat was practiced in this research.

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