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EFFECT OF Cr AND Pb ON GROWTH OF *MORINGA OLEIFERA* CHARACTERIZATION OF *MORINGA OLEIFERA* SEEDLINGS AS INFLUENCED BY Cr AND Pb ADDED WATER

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Abstract

Moringa *oleifera* has several applications in the food, feed, and supplement industries. Urban water contamination poses a risk to our food supply. Heavy metals (HM) such as Cd, Ni, Al, Cr, Pb etc. are toxic substances that have a negative impact on plant growth and are often found in urban wastewater. Seedlings of two Moringa strains: "Faisalabad white" (M1); "Original PKM1" (M2) grown (in the Department of Agronomy at the University of Agriculture in Faisalabad (UAF), Pakistan) in earthen pots (for six months) were monitored to assess the impact of Cr and Pb when treated with two different dosages of each metal [Cr, 0.2 mg L-1 (T_1) and 0.4 mg L-1(T_2); Pb, 10 mg L-1 (T_3) and 20 mg L-1 (T_4)]. Results revealed that metal (Cr and/or Pb) added water resulted in decreased the survival rate, growth and fresh and dry biomass in M1 and M2 seedlings under greenhouse conditions. However, higher concentration of HMs significantly enhanced metal contents in root, stem and leaves portion of both cultivars. It was noticed that Pb was more toxic as all the studied attributes had reduced values as compared to Cr. The decreasing pattern in terms of growth for both moringa types was M1 > M2 and for the metal treatments $T_0 > T_1 > T_2 > T_3 > T_4$ during study period while, for the Cr contents was $T_2 > T_1 > T_0$ and for Pb contents in various plant parts was $T_4 > T_3 > T_0$. Data revealed that different levels of Cr and Pb strongly affected growth, biomass, and metals absorption characteristics of moringa. Furthermore, the ability of the used moringa types to take up Cr and Pb varied accordingly and low levels Cr and Pb contents were noted in Moringa M1 (local). This suggests to use this plant in advanced moringa breeding programmers for better moringa production.

Keywords: Environmental Concern, Phytoaccumulation, HM Accumulation, Moringa Strain, Tolerance Potential, Translocation Factor.

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1. INTRODUCTION

Woody plants play a significant role in an agro forestry systems designated for timber, fuel, food and forage production (Nouman et al., 2006; Hashim et al., 2023). They can grow easily in a sustainable manner as they need no intensive care as compared to common fodder/forage crops. Fast growing forage trees with high foliage production are rich in digestible protein (15-29%) and other essential minerals giving nutritious forage for farm animals (Nouman et al., 2006).

Moringa oleifera, a fast-growing drought-tolerant tree of the tropical belts including Pakistan below 600 m altitudes (Makkar and Becker, 1997; Fahey, 2005). It does well in light frost giving maximum growth (tree height up to 6-7 m with 0.2-0.4 m stem diameter in a single year) at moderate temperature (19-29 ^{o}C) and 225-1500 mm of rainfall (Jahn, 1988; Odee, 1998; Foild et al., 2001). Its leaf extract promotes seed germination and plant growth when used for seed priming (Nouman et al., 2012) cleaning turbid water (Suarez et al., 2003) and for phytomedic chemicals (Anwar et al., 2006). Moringa leaves are good animal feed (Gidamis et al., 2003; Sánchez et al., 2006) while, seeds are used for purification of drinking water and to treat the water bearing pathogen (Berger et al., 1984; Gassenschmidt et al., 1995). It is a good nutritional source for undernourished and/or malnourished countries (Fahey, 2005) like Pakistan as its 100 g dry leave contains S (137 mg), Fe (7 mg), AsA (200 mg), vitamin A (6800 mg), choline (vitamin B) (423 mg), thiamine (2.6 mg), riboflavin (20.5 mg), nicotinic acid (vitamin B_3) (0.8 mg), protein(6.7 mg), K (259 mg), (Bau et al., 1994; Sarwar and Peace, 1994; Fuglie, 2000; Aregheore, 2002; Mathur, 2006). Crude protein (CP) contents in dry moringa leaves are 271.0 g kg^{-1} to 332.5 g kg^{-1} (Soliva et al., 2005; Ferreira et al., 2008; Mendieta-Araica et al., 2011)**.**

The upsurge in urbanization and industrialization in last century associated with exponential population with declined economic growth have generated various environmental concerns such as pollution, population pressure, poor draining, and improper infrastructure, thus impacting the human health (Song et al., 2020). Elements having density 5 times heavier than water with inorganic nature are termed as heavy metals (HM). Nowadays, these HM are widespread in each and every part of the biosphere especially in developing countries causing various types of pollution (Adil et al., 2023). Residues of industrial, mining and vehicular and agrochemical are the pools of various HMs occurring in our environment and harm in each and every aspect of life (Grimm et al., 2008). Sludge material (toxic pollutants) when directly applied as a source of nutrient to agricultural soil in cropland allows the build-up of HM making un-hygenic ecosystem (Mosquera et al., 2000). Metal accumulation by adding waste water and its soil profile reactions depends on prevailing conditions of environment (Song et al., 2020).

These metals have a deadly effect on animals and plants due to aggregation of soils (Sardar et al., 2013; Briffa et al., 2020). Because of the significant exposure to HM contamination is very harmful for life. Based on above facts, this study was conducted to test two different *Moringa* strain ("Faisalabad white" (M1); "Original PKM1" (M2) for their

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performance against HM stress and to determine the amount of HM absorbed and their translocation to various parts of the *Moringa* species.

2. MATERIALS AND METHODS

A pot experiment was conducted at the research area of Department of Agronomy, University of Agriculture Faisalabad University of Agriculture Faisalabad, Pakistan (31o26 N, 73o06E; 184.4 m) respectively from July 2018 to April 2019. Seedlings of two Moringa strains: “Faisalabad white” (M1); “Original PKM1” (M2) in earthen pots (for six months).

One-month old healthy seedlings of both types Moringa (M1 and M2) were grown and transplanted in the earthen pots each filled with 7 kg sandy loam soil per pot comprising sand, silt clay @ 43%, 50% and 7%. Induction of heavy metal Cr and Pb (HM) to the seedlings was done after complete establishment of seedlings and were tested when treated with two different levels of Cr and Pb each i.e. T1= Cr (0.2 mg L-1); T2 = Cr (0.4 mg L-1); T3= Pb (10 mg L-1); T4 = Pb (20 mg L-1) followed by comparison with T0 (Control). Levels of HMs were fixed keeping in view the maximum permissible limit of the metal in irrigation water (Westcot and Ayers, 1985) i.e. two times and 4 times of the limit.

Seedlings of each pot were watered with metal added as per designated treatment every other day in almost equal quantities to keep the soil at the field capacity during the experiment. The trial was performed for six months. After 6 months of experiment, three plants from each replication were selected randomly for observing growth and metal analysis. The mean value of each attribute was calculated from fifteen plants.

Plant Survival percent (PSP) (%) was assessed by recording dead and alive plants after every 15 days and the percentage of survived plants was calculated using the formula: “PSP (%) = (Number of survived seed/Number of seeds sown) × 100”.

Growth Parameters like plant height (cm), plant collar diameter (cm), plant collar diameter (cm), shoot length (cm) and soot length (cm) were measured at the end of experiment and were averaged for each treatment. Similarly, fresh and dry shoot and roots of all seedlings were weighed (in gram) for all the prescribed treatments.

Concentration of lead and chromium in moringa leaves, stem and roots were determined according to the method of Estefan et al. (2013). Ground samples (1 g) and di-acid mixture (10 mL) (nitric acid and perchloric acid in 2:1 ratio) was added in digestion tubes. The digestion tubes were heated at 150 °C up to 1 hand then at 235 °C on block digester until fumes disappeared and solution became colorless.

Tubes were cooled, few drops of distilled water were added. Volume of solution was made upto100 mL with distilled water and filtered. A blank was also included in batch for digestion. The filtrate was used to determine Pb and Cr concentration by using (AAS) atomic absorption spectrophotometer.

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Statistical Analysis

Collected data from all the trials was analyzed using Fisher's analysis of variance technique. Treatment means were compared using Tukey HSD ("honestly significant difference" or "honest significant difference") test at 5% level of probability and determined levels of significance (HSD values) for means.

3. RESULTS

Results revealed that metal treatments used in this study positively decreased the survival rate, growth and fresh and dry biomass of moringa cultivars under greenhouse conditions (Table 1). However, higher concentration of Pb and Cr significantly enhanced the Pb and Cr contents in root, stem and leaves portion of moringa types (Table 2). Lead cause toxic effects in reduction of all the studied attributes as compared to the chromium metal. Chromium application @ 0.2 and 0.4 mg L-1 significantly reduced the survival rate (4.39 and15.56%), plant height (14.61 and 22.70%), collar diameter (9.75 and 26.82%), shoot length (10.64 and 16.99%), root length (17.53 and 35.54%), shoot fresh weight (5.61 and 15.16%), shoot dry weight (10.83 and 24.09%), root fresh weight (13.50 and 25.27%), root dry weight (12.01 and 31.25%) respectively. However, application of Pb at 10 and 20 mg L-1 significantly reduced the survival rate percentage (27.52 and 36.35%), plant height (30.30 and 41.47%), collar diameter (34.14 and 48.78%), shoot length (32.74 and 39.60%), root length (50.23 and 57.81%), shoot fresh weight (39.88 and 53.58%), shoot dry weight (44.60 and 69.85%), root fresh weight (52.55 and 58.72%), root dry weight (53.12 and 72.59%) respectively. Better response in terms of growth was observed in local moringa; Faisalabad White (M1) as compared to Indian PKM 1 (M2). The decreasing pattern in terms of growth for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period while for the Cr contents was T2 > T1 > T0 and for Pb contents in various plant parts was T4 > T3 > T0 (Table 1).

Plant Survival Percent (PSP) (%)

Results revealed that metal treatments used in this study positively decreased the survival rate of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa types (M1 and M2) and metal treatments (T) exhibited statistically significant result, the interactive effect between the (M1 and M2) × T showed significant results for the survival rate. For moringa strains maximum survival rate (76.53%) was observed in M1 (Faisalabad White) where Faisalabad White was sown, while minimum survival rate was noticed in M2 (Indian Variety: Original PKM 1) (74.19%).

Regarding the metal treatments maximum survival rate was observed in T0 (90.87%) where no metal was applied followed by in T1 (86.48%) where Cr metal (0.2 mg L-1) was applied during study period. While, the minimum survival rate (57.83%) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. While, discussing about the interactive effect of (M1 and M2) × T maximum survival rate (91.30%) was observed for the moringa variety Faisalabad White when sown under the controlled conditions,

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while minimum survival rate (57.57%) was noticed in those pots where M2 was sown in those pots having (20 mg L-1) of Pb (Figure 1). Better response in terms of survival rate was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of survival rate for moringa types was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Plant Height (cm)

Findings of current study revealed that metal treatments used in this study positively decreased the plant height of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa types (M1 and M2) and metal treatments (T) showed statistically significant results, while the interactive effect between the (M1 and M2) × T also showed significant results for the plant height. For moringa strains maximum height of plant (111.3 cm) was noticed in M1 (Faisalabad White), while minimum plant height was noticed in M2 (Indian: Original PKM 1) (106.9 cm). Regarding the metal treatments, maximum plant height was observed in T0 (139.6 cm) where no metal was applied followed by in T1 (119.2 cm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum plant height (81.7 cm) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. While discussing about the interactive effect of (M1 and M2) × T maximum value (140.2 cm) was observed for the moringa strain Faisalabad White when sown under the control conditions, while minimum value (78.2 cm) was noticed in those pots where PKM 1 was sown in those pots having (20 mg L-1) of Pb (Figure 2). Better response in terms of plant height was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of plant height for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Collar Diameter (cm)

Current study revealed that metal treatments used in this study positively decreased the collar diameter of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 and M2)) and metal treatments (T) showed statistically significant results, while the interactive effect between the (M1 and M2) × T showed non-significant results for the collar diameter. For moringa strains maximum collar diameter (3.2 cm) was observed in M1 where Faisalabad White was sown, while minimum collar diameter was noticed in M2 (3.0 cm). Regarding the metal treatments maximum collar diameter was observed in T0 (4.1 cm) where no metal was applied followed by in T1 (3.7 cm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum collar diameter (2.1 cm) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. Better response in terms of collar diameter was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of collar diameter for moringa types was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

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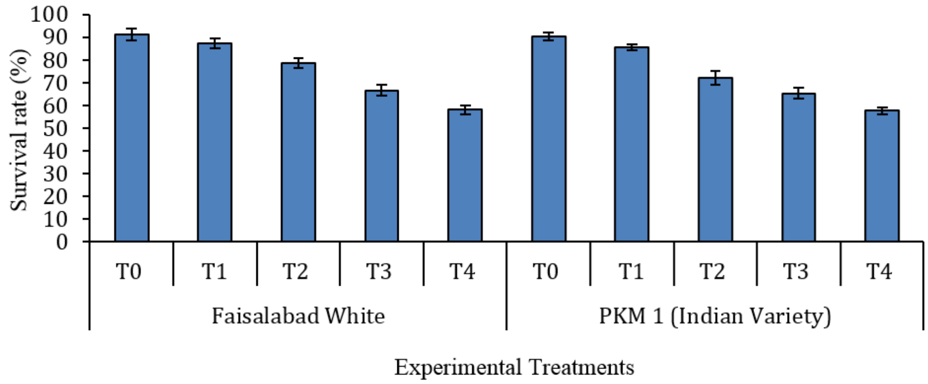

Figure 1: Interactive Effect of Different Levels of Cr and Pb on the Survival Rate (%) of Various Moringa Strains under Greenhouse Conditions

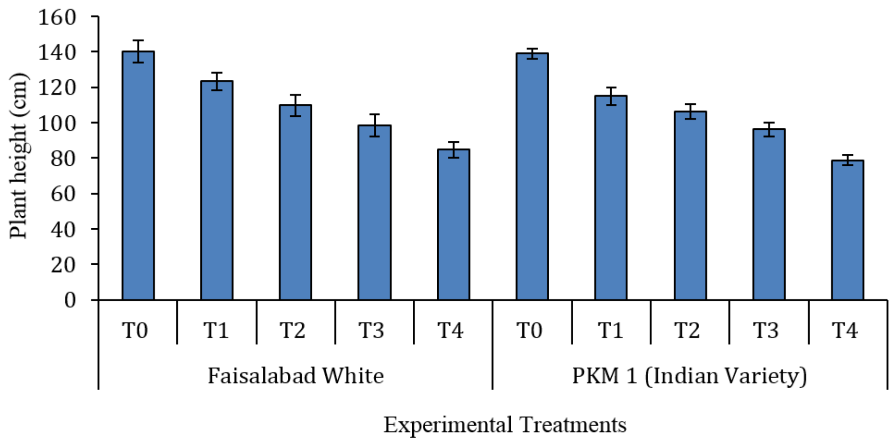

Figure 2: Interactive effect of different levels of Cr and Pb on the plant height (cm) of various Moringa strains under greenhouse conditions

Root Length (cm)

Results of current investigation revealed that metal treatments used in this study positively decreased the root length of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed significant results for the root length. For moringa strains highest length of root (15.7 cm) was observed in M1 where Faisalabad White was sown, while lowest root length was noticed in M2 (12.9 cm). Regarding the metal treatments maximum root length was observed in T0 (21.1 cm) where no metal was applied followed by in T1 (17.4 cm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum root length (8.9 cm) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. While discussing about the interactive effect of moringa strains and metals treatments maximum value (22.1 cm) was observed for the moringa variety Faisalabad White when sown under the control, while lowest value (8.0 cm) was noticed

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in those pots where PKM 1 was sown in those pots having (20 mg L-1) of Pb (Figure 3). Better response in terms of root length was noticed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of root length for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Shoot Length (cm)

Current investigation revealed that metal treatments used in this study positively decreased the shoot length of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed significant results for the shoot length. For moringa strains highest length of shoot (113.2 cm) was noticed in M1 where Faisalabad White was sown, while minimum length of shoot was noticed in M2 (108.8 cm).

Regarding the metal treatments maximum length of shoot length was noticed in T0 (137.1 cm) where no metal was applied followed by in T1 (122.5 cm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum shoot length (82.8 cm) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. While discussing about the interactive effect of V × T maximum shoot length (138.9 cm) was observed for the moringa variety Faisalabad White when sown under the control conditions, while minimum shoot length (77.2 cm) was noticed in those pots where PKM 1 was sown in those pots having (20 mg L-1) of Pb (Figure 4). Better response in terms of length of shoot was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of shoot length for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Root Fresh Weight (g)

Data revealed that metal treatments used in this study positively decreased the root fresh weight of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) showed statistically significant results, while the non-significant results were noticed for the interactive effect between the (M1 & M2) × T for the root fresh weight. For moringa strains highest value (45.51 g) was noticed in M1 where Faisalabad White was sown, while lowest root fresh weight was noticed in M2 (42.25 g).

Regarding the metal treatments maximum value was depicted in T0 (62.70 g) where no metal was applied followed by in T1 (54.23 g) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum root fresh weight (25.88 g) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied.

Better response in terms of root fresh weight was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of root fresh weight for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

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Shoot Fresh Weight (g)

Findings of current investigation revealed that metal treatments used in this study positively decreased the shoot fresh weight of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed statistically significant results for the shoot fresh weight. For moringa strains maximum value (79.14 g) was observed in M1 where Faisalabad White was sown, while minimum value was noticed in M2 (73.48 g). Regarding the metal treatments maximum value was observed in T0 (98.91 g) where no metal was applied followed by in T1 (93.36 g) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum value (45.91 g) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. While discussing about the interactive effect of V × T highest value (100.47 g) was noticed for the moringa variety Faisalabad White when sown under the control conditions, while minimum shoot fresh weight (43.07 g) was noticed in those pots where PKM 1 was sown in those pots having (20 mg L-1) of Pb (Figure 5). Better response in terms of shoot fresh weight was observed in Faisalabad White in comparison with PKM 1. The decreasing pattern in terms of shoot fresh weight for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Root Dry Weight (g)

Data revealed that metal treatments used in this study positively decreased the root dry weight of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) showed statistically significant results, while the interactive effect between the (M1 & M2) × T depicted non-significant results for the root dry weight. For moringa strains maximum value (2.95 g) was observed in M1 where Faisalabad White was sown, while minimum value was noticed in M2 (2.67 g). Regarding the metal treatments maximum value was observed in T0 (4.16 g) where no metal was applied followed by in T1 (3.66 g) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum value (1.14 g) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. Better response in terms of root dry weight was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of root dry weight for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

Shoot Dry Weight (g)

Results revealed that metal treatments used in this study positively decreased the shoot dry weight of moringa strains under greenhouse conditions as shown in (Table 1). Both factors moringa strains (M1 & M2) and metal treatments (T) showed statistically significant results, while the interactive effect between the (M1 & M2) × T depicted non-significant results for the shoot dry weight. For moringa strains highest (13.86 g) shoot dry weight was noticed in M1 where Faisalabad White was sown, while lowest dry weight

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of shoot was depicted in M2 (12.80 g). Regarding the metal treatments highest dry weight of shoot was noticed in T0 (19.01 g) where no metal was applied followed by in T1 (16.95 g) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum shoot dry weight (5.73 g) of moringa strains were noticed in those pots where Pb (20 mg L-1) was applied. Better response in terms of shoot dry weight was observed in Faisalabad White as compared to PKM 1. The decreasing pattern in terms of shoot dry weight for moringa strains was M1 > M2 and for the metal treatments T0 > T1 > T2 > T3 > T4 during study period (Table 1).

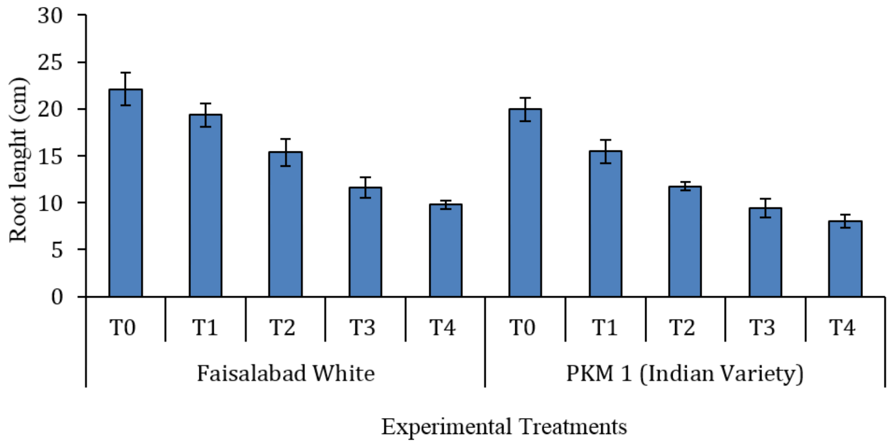

Figure 3: Interactive effect of different levels of Cr and Pb on the root length (cm) of various Moringa strains under greenhouse conditions

Table 1: Growth response of various Moringa strains to different levels of Cr and Pb stress under greenhouse conditions

		Parameters								
Treatments		Survival rate (%)	Plant Height (cm)	Collar diameter (cm)	Root length (cm)	Shoot Length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Moringa Strain (M1 & M2)	M1*	76.53 A	111.3 A	3.2 A	15.7 A	113.2 A	79.14 A	45.51 A	2.95 A	13.86 A
	M2**	74.19 B	106.9 B	3.0 B	12.9 B	108.8 B	73.48 B	42.25 B	2.67 B	12.80 B
		1.95	2.48	0.17	0.52	0.96	2.24	2.22	0.22	0.64
HMs (T)	T0	90.87 A	139.6 A	4.1 A	21.1 A	137.1 A	98.91 A	62.70 A	4.16 A	19.01 A
	T1	86.48 B	119.2 B	3.7 B	17.4 B	122.5 B	93.36 B	54.23 B	3.66 B	16.95 B
	T2	75.31 C	107.9 C	3.0 C	13.6 C	113.8 C	83.91 C	46.85 C	2.86 C	14.43 C
	T3	65.86 D	97.3 D	2.7 D	10.5 D	99.2 D	59.46 D	29.75 D	1.95 D	10.53 D
	T4	57.83 E	81.7 E	2.1 E	8.9 E	82.8 E	45.91 E	25.88 E	1.14 E	5.73 E
		3.08	3,88	0.24	0.83	1.42	2.44	2.91	0.36	0.98
Level of Significance	M1 & M2	**	**	**	**	**	**	**	**	**
	T	**	**	**	**	**	**	**	**	**
	(M1& M2) ×T	**	**	NS	**	**	**	NS	NS	NS
Any two means followed by same letter are not significantly different, n = 3. NS = non-significant; ** = significant at p ≤ 0.01										

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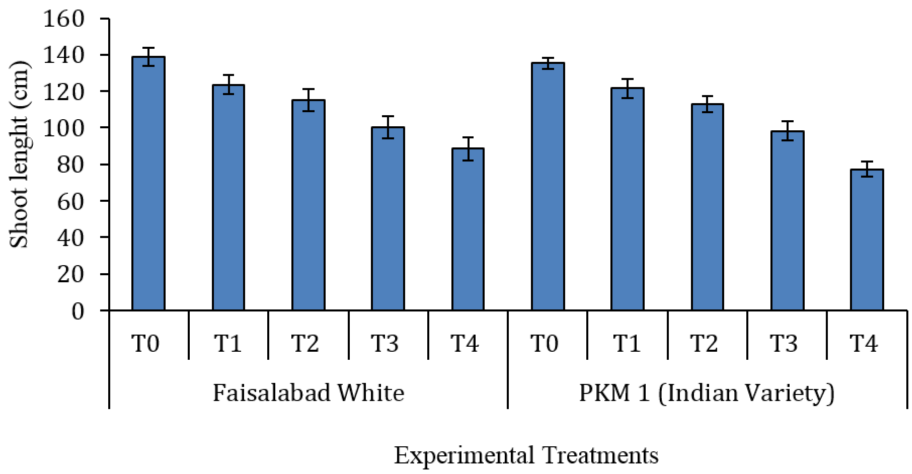

Figure 4: Interactive effect of different levels of Cr and Pb on the shoot length (cm) of various Moringa strains under greenhouse conditions

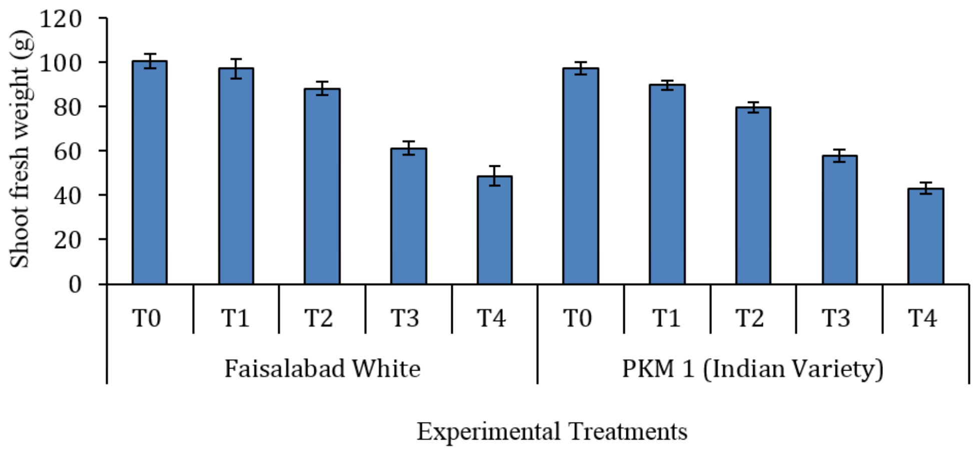

Figure 5: Interactive effect of different levels of Cr and Pb on the shoot fresh weight (g) of various Moringa strains under greenhouse conditions

Root Pb Contents (ppm)

Data revealed that metal treatments used in this study positively enhanced the root lead contents of moringa strains under greenhouse conditions as shown in (Table 2). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed significant results for the root lead contents. For moringa strains maximum root lead contents (0.48 ppm) was observed in M2 where PKM 1 was sown, while minimum root lead contents were noticed in M1 (0.44 ppm). Regarding the metal treatments maximum root lead contents were observed in T4 (1.11 ppm) where Pb at 20 mg L-1was applied followed by in T3 (0.89 ppm) where Pb metal (10 mg L-1) was applied during study period.

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Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

While the minimum root lead contents (0.06 ppm) of moringa strains were noticed in those pots where non-metal was applied. While discussing about the interactive effect of (M1 & M2) × T maximum root lead contents (1.15 ppm) were observed for the moringa variety PKM 1 when sown under the T4 treatment, while minimum root lead contents (0.06 ppm) was noticed in those pots where PKM 1 was sown in those pots having control conditions (Figure 6). The decreasing pattern in terms of root lead contents for moringa strains was M2 > M1 and for the metal treatments T4 > T3 > T2 > T1 > T0 during study period (Table 2).

Root Cr Contents (ppm)

As shown in Table 2, metal treatments in this study positively enhanced the root Cr contents of moringa strains. Both factors moringa types (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also had significant root Cr contents. For moringa strains maximum root chromium contents (0.49 ppm) was observed in M2 where PKM 1 was sown, while minimum root Cr contents were noticed in M1 (0.46 ppm). Regarding the metal treatments maximum root Cr contents were observed in T2 (1.14 ppm) where Cr at 0.4 mg L-1 was applied followed by in T1 (0.99 ppm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum root Cr contents (0.06 ppm) of moringa strains were noticed in those pots where non-metal was applied. While discussing about the interactive effect of V × T maximum root Cr contents (1.17 ppm) were observed for the moringa variety PKM 1 when sown under the T2 treatment, while minimum root Cr contents (0.06 ppm) were noticed in those pots where PKM 1 was sown in those pots having control conditions (Figure 7). The decreasing pattern in terms of root Cr contents for moringa strains was M2 > M1 and for the metal treatments T2 > T1 > T3 > T4 > T0 during study period (Table 2).

Stem Pb Contents (ppm)

Current study revealed that metal treatments used in this study positively enhanced the stem Pb contents of moringa strains under greenhouse conditions as shown in (Table 2). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T showed non-significant results for the stem lead contents. For moringa strains maximum stem lead contents (0.32 ppm) was observed in M2 where PKM 1 was sown, while minimum stem Pb contents were noticed in M1 (0.30 ppm).

Regarding the metal treatments maximum stem Pb contents were observed in T4 (0.69 ppm) where Pb at 20 mg L-1 was applied followed by in T3 (0.63 ppm) where Pb metal (10 mg L-1) was applied during study period. While the minimum stem Pb contents (0.06 ppm) of moringa strains were noticed in those pots where nonmetal was applied. The decreasing pattern in terms of stem Pb contents for moringa strains was M2 > M1 and for the metal treatments T4 > T3 > T1 > T2 > T0 during study period (Table 2).

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

Stem Cr Contents (ppm)

Data revealed that metal treatments used in this study positively enhanced the stem Cr contents of moringa strains under greenhouse conditions as shown in (Table 2). Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed significant results for the stem Cr contents. For moringa strains maximum stem Cr contents (0.38 ppm) was observed in M2 where PKM 1 was sown, while minimum stem Cr contents were noticed in M1 (0.34 ppm).

Regarding the metal treatments maximum stem Cr contents were observed in T2 (0.88 ppm) where Cr at 0.4 mg L-1was applied followed by in T1 (0.71 ppm) where Cr metal (0.2 mg L-1) was applied during study period. While the minimum stem Cr contents (0.06 ppm) of moringa strains were noticed in those pots where non-metal was applied. While discussing about the interactive effect of (M1 & M2) × T maximum stem Cr contents (0.92 ppm) were observed for the moringa variety PKM 1 when sown under the T2 treatment, while minimum stem Cr contents (0.06 ppm) were noticed in those pots where PKM 1 was sown in those pots having control conditions (Figure 8). The decreasing pattern in terms of stem chromium contents for moringa strains was M2 > M1 and for the metal treatments T2 > T1 > T3 > T4 > T0 during study period (Table 2).

Leaf Pb Contents (ppm)

As clear from the Table 2, metal treatments used in this study positively enhanced the leaf lead contents of moringa strains under greenhouse conditions. Metal treatments (T) exhibited statistically significant results, while the moringa strains (M1 & M2) and interactive effect between the (M1 & M2) × T showed non-significant results for the leaf lead contents. Regarding the metal treatments maximum leaf lead contents were observed in T4 (0.27 ppm) where Pb at 20 mg L-1 was applied followed by in T3 (0.20 ppm) where Pb metal (10 mg L-1) was applied during study period.

While the minimum leaves lead contents (0.06 ppm) of moringa strains were noticed in those pots where non-metal was applied which are statistically at par with T1 and T2. The decreasing pattern in terms of leaves lead contents for moringa strains was M2 > M1 and for the metal treatments T4 > T3 > T1 > T2 > T0 during study period (Table 2).

Leaf Cr Contents (ppm)

As clear from the Table 2, metal treatments used in this study positively enhanced the leaf Cr contents of moringa strains under greenhouse conditions. Both factors moringa strains (M1 & M2) and metal treatments (T) exhibited statistically significant results, while the interactive effect between the (M1 & M2) × T also showed significant results for the leaf Cr contents. For moringa strains maximum leaves Cr contents (0.21 ppm) were observed in M2 where PKM 1 was sown, while minimum leaf Cr contents were noticed in M1 (0.18 ppm). Regarding the metal treatments maximum leaf Cr contents were observed in T2 (0.45 ppm) where Cr at 0.4 mg L-1 was applied While the minimum leaf Cr contents (0.06 ppm) of moringa strains were noticed in those pots where non-metal was applied.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

The decreasing trend in terms of leaf Cr contents for moringa strains was M2 > M1 and for the metal treatments T2 > T1 > T3 > T4 > T0 during study period (Table 2).

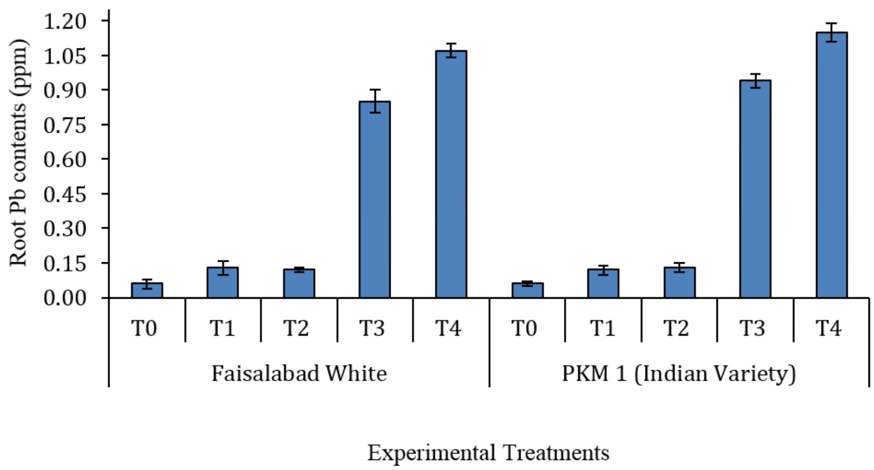

Figure 6: Interactive effect of different levels of Cr and Pb on the root Pb contents (ppm) of various Moringa strains under greenhouse conditions

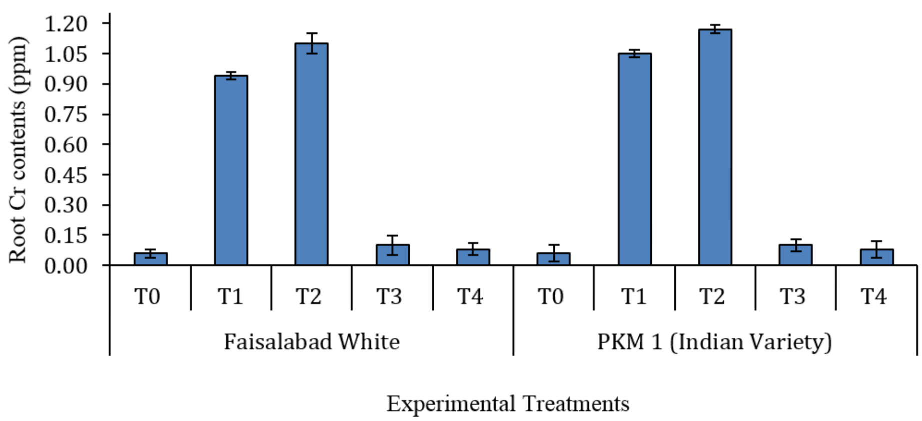

Figure 7: Interactive effect of different levels of Cr and Pb on the root Cr contents (ppm) of various Moringa strains under greenhouse conditions

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

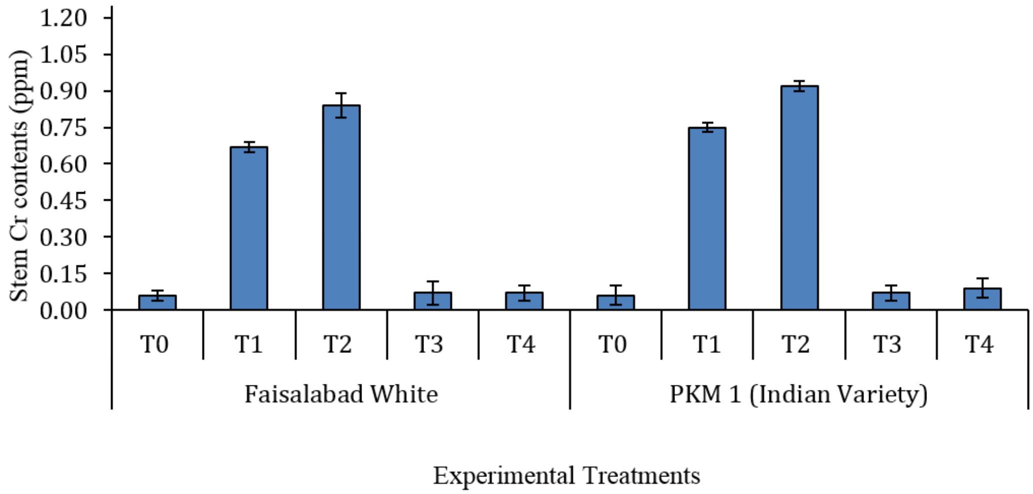

Figure 8: Interactive effect of different levels of Cr and Pb on the stem Cr contents (ppm) of various Moringa strains under greenhouse conditions

Table 2: Metal accumulation in various parts of Moringa seedlings under Pb and Cr stress

Treatments		Pb			Cr		
		Root	Stem	Leaves	Root	Stem	Leaves
Moringa Strains (M1 & M2)	M1*	0.44 B	0.30 B	0.12	0.46 B	0.34 B	0.18 B
	M2**	0.48 A	0.32 A	0.13	0.49 A	0.38 A	0.21 A
		0.24	0.012	0.012	0.024	0.024	0.012
HMs (T)	T0	0.06 D	0.06 C	0.06 C	0.06 D	0.06 C	0.06 C
	T1	0.12 C	0.09 C	0.06 C	0.99 B	0.71 B	0.36 B
	T2	0.12 C	0.08 C	0.06 C	1.14 A	0.88 A	0.45 A
	T3	0.89 B	0.63 B	0.20 B	0.10 CD	0.07 C	0.07 C
	T4	1.11 A	0.69 A	0.27 A	0.09 D	0.08 C	0.06 C
		0.038	0.038	0.024	0.04	0.036	0.024
Level of Significance	(M1 & M2)	**	**	NS	**	**	**
	T	**	**	**	**	**	**
	(M1 & M2) × T	**	NS	0.12	**	**	NS
Any two means followed by same letter are not significantly different, n = 3. NS = non-significant; ** = significant at $p \leq 0.01$							

4. DISCUSSION

The findings of the present investigation showed that Pb and Cr at various levels caused a momentous reduction of germination and seedling growth of moringa cultivars. However, by increasing the levels of metal stress a momentous increase in the concentration of the metal was also observed in various plant parts of moringa strains. Similarly, the growth and biomass reduction of plants under Cr and Pb were observed in previous studies (Guo et al., 2007; Shamsi et al., 2007; Liu et al., 2008; Zeng et al., 2010). The reduction in plant biomass was found more pronounced in PKM 1 as compared to

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

Faisalabad White under both Pb and Cr. It might be considered that Pb and Cr have the same means in moringa plants.

Statistically significant differences in the survival rate of moringa genotypes to Cr and Pb treatments have been found (Table 1). Similar observations were also noticed in previous studies, momentous changes in survival rate were reported among field crops exposed to Mo, Cr and Pb (Rout and Das, 2002; Mahmood et al., 2007). Similar behavior of various tree species (Morus Alba, Vachellia nilotica, Acacia ampliceps, and Azadirachta indica) was noticed by (Hussain et al., 2021) with irrigation of Pb contaminated water. Mahmood et al. (2007) also described the survival rate of seedlings of crops belonging to the cereals family were inversely associated to HM treatments. This might be due to maximum germination inhibition of seeds followed by a reduction in the length, fresh and dry biomass of roots and shoots due to HM toxicity (Mishra and Choudhuri, 1998). By avoiding or tolerating HM stress or both, tolerance to HM by plants can be accomplished (Sharma and Dubey, 2005). Reduced metal uptake was related to avoidance that can be obtained by cell wall components deposition or by secretion of chelates (Meharg, 2005). Plant's ability to detoxify metals entered in cells depends on tolerance to heavy metals (Ghani et al., 2010). In the present study, similar responses in Faisalabad white as compared to PKM 1 were reported.

In fresh and dry weights of roots and shoots significant variations were noticed for both moringa cultivars (Table 1) in response to applied Pb and Cr at various levels. The shoot biomass was higher than root biomass in moringa genotypes at applied Pb and Cr stress. By the application of Pb and Cr the roots of the moringa genotypes depicted a reduction in fresh and dry weights compared to their respective controls. Same results by various researchers for willows (Zhivotovsky et al., 2011) rice strains (Verma and Dubey, 2003), cereals (Mahmood et al., 2007), castor bean (Romeiro et al., 2006) and maize (Gupta et al., 2009) grown under monitored conditions. The root growth rate was decreased in response to the absorption of heavy metals, and branching patterns were modified. Root growth inhibition of can be observed due to the reduction of calcium contents in the tips of roots that results in the reduction of cell elongation and division processes (Eun et al., 2000). So, the results of the present investigation revealed similar findings for the Faisalabad white as compared to PKM 1 (Niaz et al., 2010).

The present results showed that roots of Faisalabad white had less fresh and dry weights in all Pb and Cr levels followed by PKM 1 (Table 1). The fresh and dry biomass of root and shoots of moringa genotypes decreased with mounting Pb and Cr concentrations in the pot cultures. The increasing levels of Pb from 10 to 20 mg L-1 also reduced the root and shoot biomass of moringa genotypes tested in the present experiment. This is due to the reason that exposure to various levels of HM changes the biomass of their roots and shoots as compared with their control plants. Niaz et al. (2010) observed similar results in rice crops and reported that Pb and Cr inhibit rice shoot growth because of their harmful effects on plants (Sharma and Dubey, 2005). Verma and Dubey (2003) found that the growth of shoot and root over control was reduced with enhancing HM levels. Mahmood

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Vol: 67 Issue 03 | 2024
DOI: 10.5281/zenodo.10897303

et al. (2007) stated that by enhancing the levels of Pb from 0 to 10 μM, the shoot length of the wheat seedlings decreased by 15%. In another similar study presence of Pb at 10 μM in the medium, significantly reduced the growth, and fresh and dry biomass of cereals crops (Mishra and Choudhuri, 1998).

In the present study, significant variations in shoot and root and leaf Pb, Cr concentration (Table 2) of moringa plants have been observed. Already published material revealed that Pb and other HM accumulation, transport and tolerance varied greatly among field crops (Yang et al., 2000; Liu et al., 2003, 2007; Verma and Dubey, 2003; Niaz et al., 2010). Similar findings were reported by Gupta et al. (2009) and Saifullah et al. (2010) who recorded low HM translocation from root to shoot as we observed in our study (Table 2). The fact is that the binding of HM to exchangeable sites on extracellular precipitation and root cell wall (Sharma and Dubey, 2005).

In the present study, among moringa genotypes faced with different Pb and Cr levels, PKM 1 had the highest accumulation of lead and chromium in root, stem and leaf, while Faisalabad white had the lowest accumulation of both metals in root, stem and leaf because of its mechanism for genotypic tolerance. This might be due to organic acids higher root secretions for crop plants (Liu et al., 2003, 2007; Zhivotovsky et al., 2011). Translocation from roots to shoots of HM took place by loading in the sap of the xylem and translocation to the areal parts of the plants through the transpiration pull (Sharma and Dubey, 2005; Zhivotovsky et al., 2011).

5. CONCLUSIONS

In the present pot culture study, the growth and biomass production were significantly influenced under different Pb and Cr added water and moringa strains showed differences in accumulation, translocation, and tolerance to lead and chromium when applied at various rates. The Faisalabad White was observed tolerant to chromium and lead because of improved growth, lower metals toxicities, the maximum percentage of survival rate, and lower concentration of chromium and lead in the various parts of moringa species. Thus, moringa strains with low translocation and accumulation of metals, for now with less relative toxicity of heavy metals and high metals tolerance can be measured as an effectual loom for resolving the issues and threats related to heavy metals. Therefore, based on our results we can conclude that moringa strains can be candidate species for tree plantations in areas irrigated with municipal or industrial wastewater.

6. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Author Contributions

R.R., M.A.T. and M.F.N. conceived the idea and designed the experiment. R.R. and M.A.T. performed experiment and data analysis. R.R. and M.A.T. prepared the original draft of this work. M.A.K., S.M.A.B. and M.F.N. contributed to the revision and proofreading of this manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data sheets are available and can be requested from the corresponding author on reasonable request.

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Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
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