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Metalliferous conditions induce regulation in antioxidant activities, polyphenolics and nutritional quality of *Moringa oleifera* L.

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ABSTRACT

Moringa oleifera L. was grown under cadmium and lead stress conditions and the variations in its mineral content, polyphenolics, and antioxidant activities were studied and how these heavy metals affect plant growth and development. In this study, the metal translocation factor was found <1 which indicates more metal accumulation in moringa roots than stem. A significant increase in enzymatic and non-enzymatic antioxidant activities was observed in leaves, stem, and roots under metal stress which shows moringa can withstand under metalliferous conditions by regulating its antioxidant system. Various parts of moringa plants exhibited good nutritional quality; even significant variation was recorded in nutritional attributes. A significant variation was also noted in the expression of polyphenolics in moringa stem, roots, and leaves which are indicators of plant defense system under abiotic stress conditions. The results of the present study clearly manifest that the nutritional quality and concentration of polyphenolics in moringa plants are least affected by cadmium and lead uptake. These findings suggested the cultivation of moringa plants on cadmium and lead affected soils which cannot only remediate soil metalliferous conditions but can also provide nutritious fodder for livestock. For better understanding of the involved mechanisms, there is need to study the genes which are associated with moringa tolerance under metalliferous conditions.

KEYWORDS

Ascorbate; cadmium; catalase; flavonoids; hydroxycinnamic acids; lead; peroxidase; superoxide dismutase

Introduction

Metallophytes naturally grow on metalliferous soils while their adaptation and changes in physiological and biochemical properties need confirmatory tests (Verbruggen *et al.* 2009). It looks very interesting that the hyperaccumulating plants do not belong to only one genus or family, which shows that a few species have acquired hyperaccumulating character through an evolutionary process but the reasons for this evolutionary growth are still unknown (Baker and Brooks 1989). Till the time, nearly 450 angiosperms have been studied for their hyperaccumulating potential which is only <0.2% of the known species. The increase in metal-contaminated lands is insisting the plant and soil scientists to discover new plant species which are better to withstand under metalliferous conditions. The other objective in selecting metallophytes might be their nutritional or medicinal quality which can be included in our daily cuisines or can be used as animal fodder. Considering these points, the scientists are continuously working on identifying metallophyte

species, this number is increasing (Sun *et al.* 2006; Karimi *et al.* 2009).

Among heavy metals, cadmium (Cd) and lead (Pb) have gained more consideration because of their extensive and high toxicity to plant functions (Öncel *et al.* 2000). High levels of Cd contamination in soils cause oxidative damage to plants, resulting in the generation of reactive oxygen species (ROS) by displacing Fe from proteins and inhibiting the electron transport chain in the chloroplast and mitochondria in plants (Scandalios 2005). Moreover, Cd toxicity also affects photosynthetic system inhibiting photosynthetic electron transport and the activity of photosystem (McCarthy *et al.* 2001; Romero-Puertas *et al.* 2007). Pb is another toxic metal in agricultural soils which is not biodegradable and can even last for 150–5000 years in the environment (Saxena *et al.* 1999). So, once Pb enters in the soil environment, it is nearly impossible to remove it from the soil (Traunfeld and Clement 2001). It has a growth-inhibiting impact on plants affecting physiological and metabolic functions associated with photosynthesis and oxidative stress (Fahr *et al.* 2013).

It is nearly impossible in the natural environment that soil is contaminated with single metal and it is uniformly distributed. The combined impacts of heavy metals are recent research topics for plant and soil scientists and they are working on these aspects. The combined impacts of Cd and Pb have been previously reported on *Hordeum vulgare* and *Cucumis sativus* (Luo and Rimmer 1995; An *et al.* 2004). Till recent times, very few species have been identified as Cd hyperaccumulators like *Thlaspi caerulescens* and *Arabidopsis halleri*, and *Moringa oleifera* is not an exception (McGrath *et al.* 2001).

Yadav and Jyoti (2017) reported that *M. oleifera* roots retain Cd at high levels which shows its potential for phytoextraction of Cd by rhizofiltration. The researchers reported the effect of Cd on the growth of moringa plants and Cd translocation while the impact of Pb and the combined impact of these two heavy metals need to be studied. This is also an important question that how the uptake of these heavy metals affects polyphenolics of edible moringa parts. Moringa leaves, and roots are important dietary sources for humans and livestock. It is very necessary to investigate moringa cultivation on metalliferous soils or if moringa crop is irrigated with metal-contaminated water, how it affects plant growth, physiological attributes, and nutritional quality and in which plant part Cd and Pb are stored and how do these translocate from roots to other plant parts. To address these questions, the present research study was designed. Moringa was selected in this research study for its fast growth (as can be used for phytoremediation), prolific biomass production, and nutritional quality with a strong antioxidant system (Nouman *et al.* 2013; Nouman, Basra, Siddiqui, *et al.* 2014; Basra *et al.* 2015).

As the farmers are extensively cultivating moringa as fodder for their livestock, there is a dire need to assess moringa potential to be cultivated on metal contaminated soils or where sewage wastewater is applied to fodder crops due to less availability of canal water. A few reports are available on the growth potential of moringa under Cd contaminated soils (Soliman and Sugiyama 2016; Yadav and Jyoti 2017) but the changes in growth behavior, physiology, antioxidant activities and especially the polyphenolics have not been well defined. So, there is a dire need to investigate the physiological and biochemical changes in different edible parts of *M. oleifera* when grown under Cd and Pb metalliferous conditions. In this study, moringa seedlings were exposed to the individual and combined stress of Cd and/or Pb for studying their toxic impact on moringa seedlings' growth, translocation of Cd and Pb, changes in mineral contents, regulation of antioxidant activities and polyphenolics' distribution and concentration in its roots, stem, and leaves to determine the ability of moringa plants to withstand under Cd and Pb contaminated soils.

Materials and methods

The present investigation was carried out through a series of experiments under controlled conditions to investigate the phytoremediation potential of *M. oleifera* under Cd and Pb

affected soil with the use of cadmium sulfate (CdSO_4) and lead nitrate (PbNO_3), respectively in the glass house of the Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University, Multan, Pakistan.

Seed collection and experimental design

The seeds of *M. oleifera* were collected from a single tree located in Forestry Research Area of Bahauddin Zakariya University, Multan, Pakistan. The seeds were firstly surface sterilized with 0.1% H_2O_2 for 10 min and then hydroprimed for 12 h following the findings of Nouman, Basra, Yasmeen, *et al.* (2014) to improve seed germination and plant vigor. The hydroprimed seeds were sown in sand culture for germination and 1 week after the germination, the seedlings were transferred to hydroponic culture. In hydroponics, half-strength Hoagland solution was applied which was prepared following the protocol of Hoagland and Arnon (1950).

The experiment was designed in completely randomized design (CRD) with six replications of each treatment at $35 \pm 2^\circ\text{C}$ and 16 h daylight and 8 h night conditions. In each replication, eight plants were retained. Of these, two plants from each container were used for determination of photosynthetic pigments, two plants were used for determining ascorbate contents and antioxidant assays, and two plants were used for determining polyphenolics, while the remaining two plants were used for plant vigor evaluation, and mineral analysis. Roots, stem, and leaves were studied separately for the above-mentioned variables.

Three Cd levels (1, 3, and 5 mM) and three Pb levels (5, 10, and 20 mM) with one control (no Cd or Pb application) were induced separately by using CdSO_4 and PbNO_3 . Cd and Pb treatments were applied in two different experiments while in the third experiment, these treatments were applied in combination (nine combinations of Cd and Pb with one control). The treatments were applied 2 weeks after the transfer of moringa seedlings to hydroponics. The nutrient solution and mentioned treatments were changed three times on 10 days interval and the pH was maintained at 6.9 using 1 N H_2SO_4 and/or NaOH and electrical conductivity was maintained at 6 dS m^{-1} by using NaCl (Nouman, Basra, Yasmeen, *et al.* 2014; Soliman and Sugiyama 2016). Continuous aeration was applied throughout the experiment using micro aeration pumps.

Plant vigor evaluation

After 4 weeks of treatment, the plants were harvested and their shoot and root length, and fresh and dry weight of shoot and root were recorded with the help of measuring rod and weighing balance. Before harvesting, the number of leaves (leaf score) and the number of branches were noted. The fresh samples of root, stem, and leaves were collected and placed in separate polythene bags after uprooting the plants. After noting down the fresh weight of root and stem, these were air-dried for 24 h and then placed in an oven at $70 \pm 2^\circ\text{C}$ for 72 h till constant weight achieved. After

completing the drying procedure, the dry weight of the roots and shoots was noted. After oven drying roots, stem and leaves were ground separately in fine powder to pass 2 mm sieve and stored the samples separately in polythene bags with respective name tags and treatment levels for mineral analysis.

Spectrophotometric assay of chlorophyll contents

Before harvesting, 0.5 g leaf sample from selected moringa plants of each treatment was collected and chlorophyll *a* and *b* contents were determined by following the protocol devised by Nagata and Yamashita (1992). Briefly, 0.5 g sample of moringa leaves was ground in 5 ml of 80% acetone and filtered through Whatman's filter papers No. 1. The filtered extract was poured in cuvette and absorbance was noted at 663, 645, 505, and 453 nm by using UV-spectrophotometer (UV-4000, O.R.I. Germany).

Total flavonoid contents (TFC) and total phenolic contents (TFC)

TFC was determined by using the protocol described by Hussain *et al.* (2012) and modified by Gull *et al.* (2018). TPC in moringa parts was quantified by using the method described by Singleton and Rossi (1965) revised by Waterhouse (2002).

Dry ashing of plant material for macro and micronutrients

For mineral contents quantification, the dry ashing method was followed according to the protocol described by Campbell and Plank (1998). Sodium and potassium were separately measured in moringa roots, stem and leaves with the help of flame photometer (Jenway PEP-7) and Cu, Zn, Cd, Pb, Fe, and Mn were measured using atomic absorption spectrophotometer (Model: Z-8200, Hitachi, Japan).

Translocation of Cd and Pb from root to stem was calculated by using the formula given here (Rezvani and Zaefarian 2011).

$$\text{Translocation Factor (TF)} = \frac{\text{Concentration of metal in shoot}}{\text{Concentration of metal in root}}$$

Ascorbate estimation and antioxidants assay

Ascorbic acid contents in moringa roots, stem, and leaves were quantified by following the procedure devised by Intrigliolo *et al.* (1999).

For analyzing activities of enzymatic antioxidants, in other words, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), fresh moringa leaves, stem, and roots were plucked at dawn time and stored at -70°C to avoid heat and light effect on enzymatic antioxidants. Superoxide dismutase (SOD) activity was noted at 560 nm by using UV spectrophotometer (UV-4000, O.R.I. Germany)

according to the procedure described by Giannopolitis and Ries (1977) while peroxidase (POD) and catalase (CAT) activities were determined by following the procedure of Chance and Maehly (1955) at 240 and 470 nm wavelength, respectively.

Analysis of phenolic compounds by HPLC-PDA-ESI-MSⁿ and HPLC-PDA

All LC-MS grade solvents were obtained from J.T. Baker (Phillipsburg, NJ). Formic acid was purchased from Panreac (Barcelona, Spain). The standards (–)-epigallocatechin, quercetin-3-*O*-glucoside, 5-*O*-caffeoylquinic acid were from Sigma Aldrich (Steinheim, Germany). Ultrapure water was produced using a Millipore water purification system. Samples of the selected parts including roots, stem, and leaves of Cd and Pb affected moringa samples were prepared for the analysis of phenolic compounds using HPLC-PDA-ESI-MSⁿ and HPLC-PAD following the protocol as described by our research group (Nouman *et al.* 2016; Gull *et al.* 2019).

Statistical analysis

The statistical difference of individual and combined effects of Cd and Pb was tested by analysis of variance (ANOVA). The analysis was carried out using JMP (version 4; SAS Institute, Cary, NC). The experiment design followed one way completely randomized layout incorporating three replications. A multifactorial analysis of variance (ANOVA) and multiple range tests (Tukey's test) were carried out to achieve statistical differences. The level of significance was set at $p < 0.05$. The correlation coefficient was analyzed among variables to understand their associations.

Results and discussion

Contamination of soil and water with heavy metals is considered as one of the serious problems facing the environment. Heavy metals spread into the environment through agricultural practices, wastewater, sewage sludge, metallurgy, and manufacturing. Some heavy metals have a long soil retention time such as Cd, Pb, Cr, and As (Kumar *et al.* 1995). These metals may transfer and accumulate into human bodies throughout the food chain, causing serious threats not only to humans but also to animals and ecosystems (McIntyre 2003). This study was conducted to estimate the individual and combined phytotoxic impact of Cd and Pb on moringa plants. Table 1 represented the effects of Cd and Pb on growth parameters, Cd and Pb translocation factor, and chlorophyll contents. It has been reported previously that Cd has a negative effect on plant shoot and root growth and development even at low concentrations (Farinati *et al.* 2010), while in the present investigation, although the ANOVA showed that the individual and combined addition of Cd and Pb significantly affected the growth of moringa plants, while these effects were not negative especially in term of shoot yield (fresh and dry weight).

Table 1. Mean and standard error values of *Moringa oleifera* growth parameters, Cd and Pb translocation factor and photosynthetic pigments under different concentration of cadmium and lead.

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Number of branches	Leaf score	Root score	Cd translocation	Pb translocation	Chlorophyll a (µg g ⁻¹)	Chlorophyll b (µg g ⁻¹)	Beta carotene (µg g ⁻¹)
Control	19.76±1.42 b	2.85±0.15 bc	0.84±0.05 b	12.27±0.81 b	132.22±15.4 b	8.53±0.38 b	3.24±0.12 c	12.27±0.81 b	32.22±15.4 b	19.10±1.18 b	0.54±0.011 b	0.61±0.039 b	17.43±0.79 b	4.67±0.32 b	3.50±0.23 b
1 mM Cd	34.42±1.34 a	4.18±0.29 a	1.21±0.08 a	17.45±1.24 a	182.69±8.44 a	12.43±1.09 a	4.79±0.10 a	17.45±1.24 a	182.69±8.44 a	23.01±1.19 a	0.39±0.005 c	0.61±0.011 b	22.07±0.99 a	9.47±0.38 a	4.97±0.36 ab
3 mM Cd	32.69±2.43 a	3.30±0.24 b	0.93±0.08 b	10.56±0.43 b	142.30±12.6 b	11.58±0.89 a	3.85±0.13 b	10.56±0.43 b	142.30±12.6 b	25.11±0.91 a	0.47±0.005 bc	0.85±0.026 a	24.57±1.39 a	10.40±0.89 a	6.03±0.52 a
5 mM Cd	23.44±1.32 b	2.52±0.22 c	0.89±0.04 b	9.64±1.51 b	53.73±6.85 c	11.49±0.76 a	3.98±0.28 b	9.64±1.51 b	53.73±6.85 c	16.43±1.29 b	0.63±0.055 a	0.93±0.006 a	21.83±1.955 ab	6.30±0.56 b	4.73±0.81 ab
Critical value for comparison	5.53	0.77	0.21	3.52	37.01	2.67	0.57	3.52	37.01	3.76	0.09	0.079	4.42	1.89	1.83
Control	19.76±1.42 c	2.85±0.15 b	0.84±0.05 b	12.27±0.81 a	132.22±15.4 a	8.53±0.38 b	3.24±0.12 b	12.27±0.81 a	132.22±15.4 a	19.10±1.18 a	0.54±0.011 b	0.61±0.039 c	17.43±0.79	4.67±0.32 c	3.50±0.23
5 mM Pb	25.45±1.69 b	2.99±0.14 b	0.92±0.11 ab	10.98±0.77	84.65±7.87 b	9.83±0.83 b	3.43±0.24 b	10.98±0.77	84.65±7.87 b	21.49±1.96 a	0.58±0.060	0.87±0.068 b	21.73±2.24	6.10±1.08 bc	6.33±0.88
10 mM Pb	31.54±1.35 a	3.72±0.14 a	1.13±0.07 a	11.45±1.34	120.33±6.30 a	15.93±0.66 a	4.61±0.35 a	11.45±1.34	120.33±6.30 a	17.92±0.50 b	0.47±0.003	0.96±0.003 ab	17.00±2.41	10.07±0.88 a	6.47±1.36
20 mM Pb	24.51±0.79 b	3.25±0.14 ab	1.00±0.04 ab	11.15±0.92	82.67±6.62 b	9.75±1.35 b	4.50±0.25 a	11.15±0.92	82.67±6.62 b	14.22±1.26 b	0.53±0.050	0.96±0.003 a	17.40±1.77	8.90±1.61 ab	4.47±1.18
Critical value for comparison	4.41	0.47	0.23	3.21	31.93	2.87	0.82	3.21	31.93	4.33	0.13	0.20	6.22	3.50	3.29
Control	19.76±1.42 e	2.85±0.15 cd	0.84±0.05 c	12.27±0.81 c-e	132.22±15.4 a	8.53±0.38 cd	3.24±0.12 c-e	12.27±0.81 a	132.22±15.4 a	19.10±1.18 a	0.54±0.011 cd	0.61±0.039 c-e	17.43±0.79	4.67±0.32 b-c	3.50±0.23
1 mM Cd × 5 mM Pb	23.39±1.69 de	2.33±0.13 d	0.91±0.05 bc	8.96±1.55 bc	83.08±7.17 cd	9.34±0.55 b-d	4.97±0.40 a	8.96±1.55 bc	83.08±7.17 cd	13.77±0.97 bc	0.90±0.027 a	0.79±0.133 a-c	21.63±0.43	5.60±1.19 a-c	5.00±0.35
1 mM Cd × 10 mM Pb	30.37±1.57 b	3.26±0.15 bc	1.17±0.06 ab	7.31±0.63 cd	106.33±7.63 bc	17.87±1.89 a	4.01±0.13 bc	7.31±0.63 cd	106.33±7.63 bc	17.64±1.72 ab	0.83±0.070 ab	0.45±0.027 e	15.30±3.75	8.77±2.22 a	3.93±0.89
1 mM Cd × 20 mM Pb	28.87±1.16 bc	3.17±0.11 bc	0.94±0.18 bc	10.88±1.38 ab	115.87±10.3 ab	12.51±1.46 b	4.44±0.51 ab	10.88±1.38 ab	115.87±10.3 ab	12.15±1.02 c	0.81±0.062 ab	0.93±0.021 de	20.80±0.68	2.90±0.27 bc	7.10±1.64
3 mM Cd × 5 mM Pb	21.17±1.48 de	4.34±0.36 a	1.47±0.18 a	5.29±0.23 d	44.11±4.55 ef	9.51±0.86 b-d	3.53±0.19 cd	5.29±0.23 d	44.11±4.55 ef	13.78±1.05 bc	0.93±0.138 a	0.53±0.011 a	17.07±0.98	6.30±1.91 a-c	3.37±1.37
3 mM Cd × 10 mM Pb	22.32±1.49 de	4.84±0.20 a	1.16±0.06 a-c	8.40±1.02 bc	32.59±2.29 f	11.50±0.47 bc	3.86±0.19 bc	8.40±1.02 bc	32.59±2.29 f	15.67±1.66 a-c	0.69±0.037 bc	0.86±0.115 ab	15.23±2.01	6.83±1.02 a-c	3.07±1.23
3 mM Cd × 20 mM Pb	24.56±1.21 cd	3.53±0.18 b	0.99±0.08 bc	5.36±0.53 d	34.19±2.40 f	9.86±0.63 b-d	3.32±0.27 c-e	5.36±0.53 d	34.19±2.40 f	17.84±1.79 ab	0.53±0.036 cd	0.84±0.084 ab	16.23±2.74	8.27±2.71 a	4.73±0.74
5 mM Cd × 5 mM Pb	30.86±1.74 b	2.50±0.22 d	0.86±0.06 bc	8.29±0.54 bc	94.85±6.61 bc	6.89±1.02 d	2.70±0.28 e	8.29±0.54 bc	94.85±6.61 bc	12.94±1.26 c	0.61±0.011 cd	0.68±0.012 b-d	17.43±2.37	7.77±2.97 ab	4.07±1.13
5 mM Cd × 10 mM Pb	39.30±1.56 a	3.17±0.21 bc	0.88±0.13 bc	9.65±1.03 a-c	113.11±6.7 ab	11.82±1.54 b	2.89±0.24 de	9.65±1.03 a-c	113.11±6.7 ab	17.79±1.12 ab	0.64±0.022 cd	0.75±0.065 a-d	20.23±0.32	2.77±0.86 c	5.13±0.44
5 mM Cd × 20 mM Pb	24.83±1.22 cd	3.33±0.19 bc	0.95±0.12 bc	10.09±1.12 a-c	60.81±8.85 de	11.73±0.83 b	4.53±0.20 ab	10.09±1.12 a-c	60.81±8.85 de	14.24±2.11 bc	0.46±0.016 d	0.90±0.096 a	16.17±2.06	5.00±0.62 a-c	5.53±1.32
Critical value for comparison	4.32	0.60	0.32	2.85	24.44	3.18	0.80	2.85	24.44	4.24	0.17	0.22	6.77	4.97	3.07

Means not showing same letters in a column differ significantly.

Cd and Cd × Pb treatments significantly affected the number of branches while Pb treatments had no significant impact on the number of branches. Leaf and root scores were significantly affected by all Cd and Pb treatments (Table 1). Regarding root length, a 45% increase was recorded when moringa plants were subjected to 3 mM Cd stress while 63% increase was noted under 10 mM Pb stress. Under combined stress of Cd and Pb, 54% increase was found when 1 mM Cd × 10 mM Pb was subjected to moringa plants (Table 1). The increase in root length under stress conditions might be attributed to less osmotic pressure as it supports root elongation and expansion in search of water and essential nutrients (Hsiao and Xu 2000). Such behavior of moringa roots' elongation and expansion has been previously reported and discussed by Nouman *et al.* (2012), Nouman, Basra, Yasmeen, *et al.* (2014) and Azam *et al.* (2020) under saline and drought stress conditions. It is well-established fact that reduction in shoot length and leaf score, and increase in root length and its biomass is a plants' survival strategy to improve water uptake through roots (Houle *et al.* 2001). A similar concept can be considered for metallophytes as these plants also try to withstand under metalliferous conditions sometimes avoiding the uptake of heavy metals.

Detection of photosynthetic pigments is considered as a low cost, highly sensitive, reliable, and suitable technique to assess a plant's adaptability and its physiological status under stress conditions because chlorophyll contents absorb light energy and convert it into carbohydrates which determine the biomass production of any plant. If photosynthetic pigments show increasing trend under metalliferous conditions, it manifests the plant's adaptability in that environment (Baker and Oxborough 2004; Murchie and Lawson 2013). In the present study, mostly, Cd and Pb translocation factors increased under stress, while chlorophyll *a*, *b*, and β -carotene content showed significant differences under Cd treatments while Pb treatments and combined addition of Cd and Pb did not show clear responses (Table 1). The impact of Cd has been previously reported in the literature which shows inconsistency regarding the expression of photosynthetic efficiency in wheat plants (Vassilev, Lidon, Matos, *et al.* 2004; Vassilev, Lidon, Scotti, *et al.* 2004). Cd translocation factor, which represents the transfer of metal from root to stem, maintained values <1 under all treatments. Also, Pb translocation factor maintained values <1 under all treatments except at the highest level of Pb (20 mM Pb) treatment only (1.12). These results reflected the tolerance ability of moringa plants to Cd and Pb stress, in addition to the accumulation of Cd and Pb in roots rather than in stem. This is consistent with previous findings that indicated the tolerance ability of moringa to Cd stress (Zhu *et al.* 1999; Dong *et al.* 2008; Fowotade and Abdallah 2012; Soliman and Sugiyama 2016; Yadav and Jyoti 2017). Comparatively less storage of Cd and Pb in aerial parts of moringa plants might be due to several physical barriers like possible Cd/Pb translocation depending on binding to the extracellular matrix, Cd immobilization, Cd accumulation in plasma membranes and Cd sequestration across the cell wall

Table 2. Mean and standard error values of antioxidant activities in leaves of *Moringa oleifera* under different concentration of cadmium and lead.

Treatments	CAT (unit mg ⁻¹ Protein)	POD (unit mg ⁻¹ Protein)	SOD (unit mg ⁻¹ Protein)	Ascorbate (μg mg ⁻¹)	TFC (GAE)	TPC (CE)
Control	22.53 ± 1.27 c	244.90 ± 7.32 d	231.90 ± 8.74 c	82.53 ± 9.94 b	144.03 ± 34.70	292.73 ± 12.06 b
1 mM Cd	29.37 ± 1.45 b	501.10 ± 12.03 b	334.40 ± 7.61 b	95.23 ± 3.98 b	164.87 ± 33.50	325.67 ± 7.06 ab
3 mM Cd	61.60 ± 2.02 a	346.50 ± 8.24 c	454.80 ± 9.33 a	119.30 ± 4.08 a	213.17 ± 21.45	347.87 ± 20.39 ab
5 mM Cd	32.70 ± 2.33 b	537.80 ± 11.02 a	329.60 ± 10.51 b	103.00 ± 7.68 ab	228.60 ± 23.02	376.33 ± 42.36 a
Critical value for comparison	5.93	22.59	22.59	22.50	93.90	79.96
Control	22.53 ± 1.27 c	244.90 ± 7.32 d	231.90 ± 8.74 d	82.53 ± 9.94	144.03 ± 34.70	292.73 ± 12.06
5 mM Pb	51.70 ± 1.76 b	511.80 ± 14.69 c	518.40 ± 16.93 b	103.37 ± 12.97	195.67 ± 17.61	267.33 ± 12.86
10 mM Pb	49.50 ± 2.02 b	819.90 ± 23.71 b	540.70 ± 14.25 a	122.43 ± 10.77	214.67 ± 8.65	317.97 ± 18.17
20 mM Pb	114.90 ± 2.89 a	861.30 ± 21.09 a	486.70 ± 14.01 c	92.50 ± 2.91	202.00 ± 8.62	351.33 ± 21.26
Critical value for comparison	6.75	22.59	22.59	32.27	66.50	53.91
Control	22.53 ± 1.27 f	244.90 ± 7.32 i	231.90 ± 8.74 f	82.53 ± 9.94 cd	144.03 ± 34.70	292.73 ± 12.06
1 mM Cd × 5 mM Pb	75.07 ± 3.21 c	538.30 ± 11.34 d	247.10 ± 6.98 ef	93.00 ± 6.11 bc	183.47 ± 18.79	255.17 ± 53.79
1 mM Cd × 10 mM Pb	26.57 ± 4.84 ef	503.90 ± 10.98 e	395.20 ± 8.64 c	99.97 ± 8.17 a-c	223.73 ± 21.82	309.83 ± 51.55
1 mM Cd × 20 mM Pb	32.57 ± 1.82 e	749.90 ± 19.72 b	396.70 ± 7.22 c	108.63 ± 5.18 ab	224.33 ± 24.85	323.27 ± 60.03
3 mM Cd × 5 mM Pb	29.57 ± 1.27 ef	328.40 ± 8.20 g	257.90 ± 10.24 e	115.80 ± 3.22 a	217.67 ± 18.19	361.33 ± 24.50
3 mM Cd × 10 mM Pb	103.63 ± 2.03 a	544.20 ± 14.58 d	505.00 ± 18.68 b	99.70 ± 8.75 a-c	227.67 ± 26.97	365.53 ± 35.46
3 mM Cd × 20 mM Pb	34.03 ± 2.64 e	784.60 ± 21.37 a	556.10 ± 16.49 a	105.60 ± 8.03 ab	215.23 ± 31.77	380.17 ± 62.82
5 mM Cd × 5 mM Pb	108.27 ± 3.76 a	651.10 ± 23.04 c	563.10 ± 17.11 a	82.83 ± 3.09 cd	207.80 ± 38.42	317.13 ± 2.05
5 mM Cd × 10 mM Pb	94.57 ± 2.60 b	476.50 ± 16.68 f	403.90 ± 13.27 c	70.23 ± 11.61 d	204.53 ± 17.68	343.60 ± 23.04
5 mM Cd × 20 mM Pb	46.03 ± 3.07 d	267.70 ± 9.82 h	365.30 ± 10.08 d	81.20 ± 3.14 cd	238.47 ± 32.51	349.03 ± 35.08
Critical value for comparison	8.43	17.14	20.44	21.58	81.13	121.16

Means not showing same letters in a column differ significantly.

or Casparian strips (Arias *et al.* 2010; Jiang and Liu 2010). Furthermore, Cd concentration in plant tissues often increases with increasing Cd concentration in the soil. The distribution of Cd in plant tissues differs from plant to plant (Zhao *et al.* 2014). On the other hand, Pb is difficult to be absorbed by plants because its reaction with soil components and forming complexes or chelate and so form insoluble compounds, such as PbCO₃, PbSO₄, and Pb(OH)₂. Many authors reported that Pb is low in mobility, and preferably accumulates in roots rather than the stem of plants (Abbas *et al.* 2019). Zhou *et al.* (2008) reported that 79–88% of Pb was mainly distributed in the cell walls and soluble parts of the ribosomes in the roots of *Potentilla griffithii* var. *velutina*. Marmiroli *et al.* (2005) identified that Pb would form a complex with oxygen at the cell walls in the roots of the European walnut. Pb, which is a “soft” cation, shows a strong affinity for the organic ligands and tends to form inner-sphere complexes (Zaccone *et al.* 2007). This is also an important reason why it is difficult for Pb to transfer from roots to shoots. As moringa leaves are edible for both livestock and human beings, the less translocation and storage of these heavy metals in moringa leaves make it a healthier food which will not exert any harmful effect on kidney and liver even when cultivated under metalliferous conditions.

Tolerance to heavy metal stress might be correlated with an effective antioxidant system (Verma and Dubey 2003; Yadav and Jyoti 2017). Exposure to heavy metals provoked responses of antioxidant systems, but the direction of response is dependent on the plant species, tissue analyzed, the metal used for treatment, and also the intensity of the metal stress (Shainberg *et al.* 2000). In this study, in addition to growth parameters, antioxidant activities of; catalase (CAT), peroxidase (POD), superoxide dismutase (SOD); ascorbate contents, total flavonoid contents (TFC), and total phenolic contents (TPC) as well as mineral contents; sodium (Na), potassium (K), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn) were measured in

leaves, stem, and roots. CAT, POD, and SOD activities were significantly affected by Cd and Pb in all parts, while TFC did not show significant responses in all plant parts (Table 2). On the other hand, ascorbate content was affected by Cd, but not affected by Pb stress. TPC content showed different trends in each part of moringa plants under individual and combined stress of Cd and Pb. In leaves, Cd stress significantly affected TPC contents. TPC content in moringa stem were significantly affected by Cd and Pb treatments while roots' TPC content were significantly affected by combined treatment of Cd and Pb (Table 2). Na, K, Cu, Cd, and Pb content showed significant changes to Cd and Pb stress in all plant parts, while Zn and Mn mostly did not show significant responses. Fe showed significant responses to Cd and Pb in stem and roots, but not in leaves. The data showed that Cd and Pb reflected significant increases in antioxidant activities; CAT, POD and SOD and ascorbate content, TFC, and TPC in leaves, stem, and roots (Tables 2–4, respectively). The lowest values of antioxidant activities mostly were shown under control. These increments in antioxidants were associated with increments of Cd and Pb contents in all plant parts under stress. Abiotic stress cause degeneration of proteins which can be minimized by improving the antioxidant activities. The results of the present investigation argue that the antioxidant activities were increased under Cd and Pb stress which may impart resistance in moringa plants to survive better under metalliferous conditions. The higher antioxidant activities in moringa plants under abiotic stress conditions have been previously reported by Nouman, Basra, Yasmeen, *et al.* (2014) and Azam *et al.* (2020). Moreover, Cu content significantly increased under Cd and Pb stress in leaves and stem, but significantly decreased in roots (Tables 5–7, respectively) while K content mostly decreased under Cd and Pb treatments. Other minerals did not show a clear trend of response to Cd and Pb treatments. The present investigation manifested that moringa plants can be easily grown as a fodder crop under Pb and Cd contaminated soils and these

Table 3. Mean and standard error values of antioxidants in stem of *Moringa oleifera* under different concentration of cadmium and lead.

Treatments	CAT (unit mg ⁻¹ Protein)	POD (unit mg ⁻¹ Protein)	SOD (unit mg ⁻¹ Protein)	Ascorbate (µg mg ⁻¹)	TFC (GAE)	TPC (CE)
Control	29.20 ± 1.74 c	321.4 ± 9.63 c	305.2 ± 7.92 d	69.77 ± 8.96 b	176.6 ± 50.8	236.3 ± 23.5 b
1 mM Cd	40.27 ± 2.33 b	720.3 ± 11.93 b	486.3 ± 12.08 b	70.57 ± 7.16 b	200.0 ± 20.5	319.9 ± 6.0 a
3 mM Cd	81.43 ± 2.02 a	320.8 ± 8.29 c	614.9 ± 19.22 a	102.9 ± 3.69 a	216.1 ± 12.3	366.6 ± 27.0 a
5 mM Cd	25.33 ± 1.20 c	1064.9 ± 30.04 a	373.2 ± 8.91 c	112.12 ± 7.87 a	225.8 ± 26.2	361.4 ± 12.9 a
Critical value for comparison	6.10	72.14	32.18	23.46	101.09	62.78
Control	29.20 ± 1.74 d	321.4 ± 9.63 d	305.2 ± 7.92 c	69.77 ± 8.96 b	176.6 ± 50.8	236.3 ± 23.5 b
5 mM Pb	39.27 ± 1.75 c	407.8 ± 15.87 c	412.9 ± 13.81 b	74.53 ± 5.02 b	233.1 ± 15.8	362.3 ± 30.6 a
10 mM Pb	45.77 ± 1.75 b	537.0 ± 12.05 b	473.0 ± 12.37 a	107.5 ± 8.14 a	255.9 ± 26.0	382.7 ± 16.2 a
20 mM Pb	98.87 ± 1.49 a	825.1 ± 28.05 a	433.2 ± 14.22 b	99.30 ± 6.96 a	241.1 ± 15.4	416.5 ± 11.8 a
Critical value for comparison	5.50	81.73	20.09	24.19	99.86	30.74
Control	29.20 ± 1.74 e	321.4 ± 9.63 i	305.2 ± 7.92 f	69.77 ± 8.96 b-d	176.6 ± 50.8 b-d	236.3 ± 23.5
1 mM Cd × 5 mM Pb	75.37 ± 2.03 b	556.9 ± 16.68 f	262.2 ± 6.86 g	100.0 ± 6.97 a	108.2 ± 32.9 d	327.0 ± 8.0
1 mM Cd × 10 mM Pb	28.47 ± 1.44 e	348.3 ± 8.91 h	488.8 ± 13.26 a	85.43 ± 7.35 a-c	155.4 ± 18.0 b-d	394.2 ± 17.5
1 mM Cd × 20 mM Pb	37.10 ± 2.31 d	843.4 ± 26.37 e	410.3 ± 12.39 c	93.23 ± 4.68 a	135.2 ± 23.4 cd	281.2 ± 21.7
3 mM Cd × 5 mM Pb	33.33 ± 1.45 de	405.5 ± 14.20 g	321.5 ± 10.28 f	99.67 ± 2.89 a	232.8 ± 48.5 a-c	364.2 ± 9.9
3 mM Cd × 10 mM Pb	87.73 ± 2.33 a	1314.8 ± 37.43 c	434.0 ± 15.62 b	89.10 ± 4.97 ab	222.3 ± 60.0 a-c	341.4 ± 20.5
3 mM Cd × 20 mM Pb	20.63 ± 0.88 f	1036.3 ± 27.15 d	358.5 ± 12.17 e	81.70 ± 8.66 a-c	280.9 ± 23.5 a	401.1 ± 40.0
5 mM Cd × 5 mM Pb	71.97 ± 2.89 b	1066.6 ± 41.28 d	383.3 ± 13.86 d	98.93 ± 6.23 a	242.3 ± 25.3 ab	214.4 ± 121.0
5 mM Cd × 10 mM Pb	91.03 ± 2.31 a	1838.7 ± 55.14 a	399.9 ± 16.22	58.67 ± 10.47 d	205.2 ± 9.1 a-d	293.0 ± 33.9
5 mM Cd × 20 mM Pb	51.37 ± 2.03 c	1753.1 ± 49.26 b	400.6 ± 15.23 cd	68.53 ± 2.86 cd	215.5 ± 19.7 a-c	314.2 ± 176.9
Critical value for comparison	5.94	50.04	28.12	20.21	102.87	209.77

Means not showing same letters in a column differ significantly.

Table 4. Mean and standard error values of antioxidants in roots of *Moringa oleifera* under different concentration of cadmium and lead.

Treatments	CAT (unit mg ⁻¹ Protein)	POD (unit mg ⁻¹ Protein)	SOD (unit mg ⁻¹ Protein)	Ascorbate (µg mg ⁻¹)	TFC (GAE)	TPC (CE)
Control	74.6 ± 2.39 d	527.2 ± 15.81 d	175.7 ± 6.93 d	50.7 ± 1.32 b	195.7 ± 60.4	327.1 ± 4.8
1 mM Cd	92.8 ± 3.82 c	788.4 ± 26.79 c	258.0 ± 9.26 c	54.7 ± 5.50 b	209.4 ± 13.6	374.4 ± 23.6
3 mM Cd	164.7 ± 5.36 b	956.5 ± 32.68 b	415.7 ± 12.39 a	92.5 ± 5.94 a	233.5 ± 52.4	395.8 ± 34.1
5 mM Cd	182.8 ± 4.34 a	1457.8 ± 40.79 a	365.8 ± 10.28 b	85.5 ± 2.77 a	262.4 ± 15.3	344.7 ± 15.8
Critical value for comparison	13.45	102.29	40.18	14.11	134.67	72.81
Control	74.6 ± 2.39 c	527.2 ± 15.81 d	175.7 ± 6.93 c	50.7 ± 1.32 b	195.7 ± 60.4	327.1 ± 4.8
5 mM Pb	170.9 ± 4.04 b	1217.6 ± 42.59 c	500.8 ± 15.68 b	64.0 ± 3.82 ab	218.0 ± 12.5	322.6 ± 13.8
10 mM Pb	165.8 ± 3.19 b	1479.2 ± 43.71 b	516.5 ± 14.27 b	73.6 ± 4.45 a	239.0 ± 31.6	372.3 ± 7.1
20 mM Pb	324.7 ± 9.90 a	1844.1 ± 53.28 a	599.8 ± 17.11 a	81.7 ± 10.77 a	226.0 ± 9.7	377.6 ± 4.7
Critical value for comparison	18.61	130.24	32.42	20.11	114.11	27.51
Control	74.6 ± 2.39 f	527.2 ± 15.81 i	175.7 ± 6.93 g	50.7 ± 1.32 d	195.7 ± 60.4	327.1 ± 4.8 ab
1 mM Cd × 5 mM Pb	247.0 ± 7.23 a	1585.8 ± 41.93 b	300.4 ± 9.84 e	79.2 ± 1.78 ab	213.1 ± 17.8	267.8 ± 18.0 c
1 mM Cd × 10 mM Pb	125.5 ± 5.48 e	1065.7 ± 36.26 f	358.8 ± 11.20 d	73.4 ± 5.59 a-c	200.0 ± 17.2	262.8 ± 18.0 c
1 mM Cd × 20 mM Pb	121.0 ± 6.66 e	1168.0 ± 34.27 e	340.2 ± 10.27 d	78.2 ± 3.55 a-c	205.2 ± 18.0	298.1 ± 16.8 bc
3 mM Cd × 5 mM Pb	90.4 ± 2.60 f	660.4 ± 21.06 h	201.6 ± 8.92 f	83.0 ± 2.20 ab	222.0 ± 26.9	355.7 ± 21.7 a
3 mM Cd × 10 mM Pb	215.9 ± 8.08 b	1229.3 ± 36.81 d	484.2 ± 12.41 c	79.7 ± 3.93 ab	240.4 ± 22.8	371.4 ± 8.9 a
3 mM Cd × 20 mM Pb	194.5 ± 2.92 c	1777.0 ± 49.37 a	610.8 ± 17.82 a	88.2 ± 12.14 a	243.9 ± 25.9	305.2 ± 9.8 bc
5 mM Cd × 5 mM Pb	249.9 ± 5.20 a	1510.9 ± 45.30 c	584.8 ± 21.02 b	82.5 ± 4.71 ab	214.5 ± 2.5	364.1 ± 12.0 a
5 mM Cd × 10 mM Pb	166.3 ± 4.91 d	924.8 ± 29.57 g	341.5 ± 12.04 d	62.1 ± 2.47 cd	191.3 ± 9.6	278.2 ± 17.8 c
5 mM Cd × 20 mM Pb	111.2 ± 6.35 e	654.5 ± 18.97 h	288.6 ± 8.21 e	69.3 ± 7.67 bc	179.3 ± 21.5	268.7 ± 14.9 c
Critical value for comparison	16.28	20.41	25.13	16.25	78.41	44.54

Means not showing same letters in a column differ significantly.

metals exert least impact on nutritional quality of moringa leaves. It will help the farmers to bring their contaminated lands under moringa cultivation which can be a good alternative nutritious fodder for their livestock.

In the present investigation, shoot fresh weight negatively correlated with stem Cd content, but did not correlate significantly with Pb content (Figure 1, $r = -0.50^*$ and 0.08^{ns} , respectively), and positively correlated with Zn content in leaves and roots ($r = 0.69^{**}$ and 0.53^* , respectively). Also, shoot dry weight correlated positively with leaf Zn content (0.52^*). On the other hand, stem Cd content negatively correlated with leaf Zn content ($r = -0.56^*$). Meanwhile stem Pb content positively correlated with leaf Cu and Mn content ($r = 0.67^{**}$ and 0.54^* , respectively). On the other hand, leaf POD, SOD, TFC, and TPC positively correlated with leaf Pb content but did not show a significant correlation

with Cd content (Figure 2). Cd is a nonessential element and exerts hazardous effects on plant growth, while Zn is an essential element for plant growth but its excess amount exerts toxic effects on the plant (Chaves *et al.* 2011). These results reflected the important role of Zn to maintain plant growth by reducing Cd absorption by plants. This is consistent with Faquin (2005) who found competition between Zn and Cd in the plant absorption when the concentration of the former is lesser than that of Cd. On the other hand, Cu and Mn may play an important role in maintaining plant growth under Pb stress. In addition, the antioxidant may play an important role to scavenge heavy metal stress, especially Pb stress.

The correlation between Cd translocation and Pb translocation was not significant. Leaf Cu and Mn contents negatively correlated with Cd translocation and positively

Table 5. Mean and standard error values of mineral content in leaves of *Moringa oleifera* under different concentration of cadmium and lead.

Treatment	Na (mg kg ⁻¹)	K (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Control	184.80 ± 12.1 a	7615.7 ± 200 a	33.34 ± 2.95 b	29.17 ± 1.73 c	0.25 ± 0.01 ab	1.63 ± 0.16 b	270.2 ± 42.71 b	53.40 ± 3.73
1 mM Cd	137.00 ± 13.1 b	6392.3 ± 251 b	36.83 ± 2.60 b	59.67 ± 6.60 a	0.28 ± 0.02 ab	2.19 ± 0.29 b	398.4 ± 48.48 a	59.97 ± 7.48
3 mM Cd	90.83 ± 7.1 c	6593.4 ± 225 b	68.30 ± 4.60 a	47.50 ± 6.19 ab	0.32 ± 0.03 a	3.95 ± 0.33 a	513.0 ± 29.19 a	54.50 ± 3.19
5 mM Cd	190.27 ± 5.4 a	7155.2 ± 348 ab	42.63 ± 5.09 b	38.03 ± 2.72 bc	0.22 ± 0.02 b	4.33 ± 0.50 a	421.5 ± 7.60 a	64.07 ± 4.03
Critical value for comparison	32.49	853.80	12.90	15.66	0.07	1.13	116.27	16.00
Control	184.80 ± 12.1 ab	7615.7 ± 200 a	33.34 ± 2.95 b	29.17 ± 1.73	0.25 ± 0.01 b	1.63 ± 0.16 d	270.2 ± 42.71	53.40 ± 3.73
5 mM Pb	188.00 ± 9.3 a	6901.8 ± 349 ab	93.21 ± 3.93 a	44.60 ± 2.86	0.25 ± 0.01 b	4.14 ± 0.27 c	483.2 ± 69.04	59.73 ± 3.02
10 mM Pb	146.03 ± 7.1 c	6569.1 ± 332 ab	81.41 ± 3.39 a	51.57 ± 4.06	0.42 ± 0.03 a	5.87 ± 0.36 b	530.6 ± 102.5	64.40 ± 7.55
20 mM Pb	156.57 ± 7.7 bc	6237.4 ± 391 b	92.69 ± 5.42 a	49.80 ± 14.93	0.40 ± 0.03 a	8.65 ± 0.35 a	376.7 ± 50.72	70.83 ± 4.85
Critical value for comparison	30.17	1062.8	13.15	25.81	0.08	0.97	228.72	16.59
Control	184.80 ± 12.1 cd	7615.7 ± 200 b	33.34 ± 2.95 bc	29.17 ± 1.73	0.25 ± 0.01 de	1.63 ± 0.16 e	270.2 ± 42.71 c	53.40 ± 3.73
1 mM Cd × 5 mM Pb	244.83 ± 18.5 b	5805.7 ± 235 c	38.17 ± 4.45 bc	51.07 ± 6.30	0.20 ± 0.02 e	3.15 ± 0.47 d	345.2 ± 69.04 bc	55.07 ± 4.86
1 mM Cd × 10 mM Pb	148.33 ± 8.1 e	7524.8 ± 338 b	36.54 ± 2.22 bc	44.87 ± 8.67	0.23 ± 0.02 de	3.76 ± 0.25 d	353.0 ± 34.24 bc	48.23 ± 5.37
1 mM Cd × 20 mM Pb	204.60 ± 10.6 c	7529.8 ± 316 b	45.08 ± 5.04 b bc	36.27 ± 7.16	0.28 ± 0.02 c-e	5.95 ± 0.39 a-c	495.1 ± 51.84 a	54.13 ± 1.75
3 mM Cd × 5 mM Pb	97.37 ± 5.7 f	4233.2 ± 267 d	31.50 ± 2.83 bc	50.13 ± 12.43	0.27 ± 0.03 de	4.86 ± 0.44 c	396.5 ± 48.20 a-c	41.57 ± 4.13
3 mM Cd × 10 mM Pb	157.00 ± 9.1 de	6054.3 ± 273 c	37.92 ± 6.39 bc	63.93 ± 8.67	0.30 ± 0.02 cd	6.13 ± 0.29 ab	360.9 ± 59.86 a-c	49.47 ± 4.59
3 mM Cd × 20 mM Pb	144.60 ± 12.2 e	6946.0 ± 287 b	84.61 ± 8.77 a	55.73 ± 6.51	0.30 ± 0.04 cd	6.65 ± 0.42 ab	291.9 ± 37.64 c	40.57 ± 9.36
5 mM Cd × 5 mM Pb	93.13 ± 4.8 f	7031.9 ± 292 b	28.81 ± 2.35 c	42.20 ± 6.99	0.36 ± 0.04 bc	4.87 ± 0.34 c	406.3 ± 41.21 a-c	61.33 ± 5.23
5 mM Cd × 10 mM Pb	291.47 ± 16.7 a	8642.7 ± 423 a	36.09 ± 6.21 bc	49.80 ± 5.46	0.41 ± 0.05 b	5.55 ± 0.42 bc	477.3 ± 37.79 ab	49.80 ± 5.46
5 mM Cd × 20 mM Pb	272.40 ± 12.7 ab	7470.5 ± 256 b	72.66 ± 4.60 a	46.83 ± 6.63	0.60 ± 0.04 a	6.75 ± 0.42 a	368.8 ± 36.86 a-c	67.27 ± 7.01
Critical value for comparison	34.78	868.46	14.74	22.15	0.09	1.10	139.18	16.20

Means not showing same letters in a column differ significantly.

Table 6. Mean and standard error values of mineral content in stem of *Moringa oleifera* under different concentration of cadmium and lead.

Treatments	Na (mg kg ⁻¹)	K (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Control	3527.4 ± 291 a	12888.8 ± 257 a	31.73 ± 3.00 c	47.60 ± 10.1	0.50 ± 0.01 b	3.92 ± 0.26 b	333.1 ± 25.7 ab	26.93 ± 4.27 b
1 mM Cd	2572.2 ± 213 b	13089.9 ± 455 a	72.27 ± 10.87 a	63.23 ± 6.84	0.39 ± 0.02 c	5.16 ± 0.29 b	381.4 ± 27.6 a	39.70 ± 4.86 ab
3 mM Cd	2487.2 ± 180 b	12704.3 ± 526 a	56.93 ± 5.50 ab	50.30 ± 4.76	0.53 ± 0.01 b	9.93 ± 0.57 a	284.0 ± 11.9 b	54.47 ± 5.67 a
5 mM Cd	1418.1 ± 155 c	6333.1 ± 236 b	49.50 ± 4.91 bc	38.23 ± 2.50	0.77 ± 0.03 a	9.83 ± 0.36 a	288.3 ± 21.9 b	36.70 ± 2.95 b
Critical value for comparison	704.44	1268.2	21.97	21.73	0.07	1.30	73.62	14.82
Control	3527.4 ± 291 bc	12888.8 ± 257 bc	31.73 ± 3.00 b	47.60 ± 10.1	0.50 ± 0.01 a	3.92 ± 0.26 d	333.1 ± 25.7 b	26.93 ± 4.27
5 mM Pb	2728.4 ± 176 c	12500.5 ± 539 c	81.23 ± 16.54 a	64.67 ± 7.61	0.47 ± 0.03 a	8.36 ± 0.40 c	357.6 ± 22.5 b	48.17 ± 6.88
10 mM Pb	3752.8 ± 342 ab	15110.3 ± 408 a	108.67 ± 13.97 a	52.33 ± 12.91	0.37 ± 0.02 b	11.47 ± 0.64 b	444.9 ± 32.9 a	52.90 ± 10.22
20 mM Pb	4424.4 ± 233 a	13942.0 ± 354 ab	95.49 ± 12.26 a	80.33 ± 6.08	0.49 ± 0.02 a	14.08 ± 1.02 a	387.9 ± 24.6 ab	37.53 ± 5.08
Critical value for comparison	873.56	1312.3	40.87	31.09	0.07	1.78	87.01	22.82
Control	3527.4 ± 291 b	12888.8 ± 257 ab	31.73 ± 3.00 e	47.60 ± 10.1	0.50 ± 0.01 d-f	3.92 ± 0.26 e	333.1 ± 25.7 a	26.93 ± 4.27 c
1 mM Cd × 5 mM Pb	4630.7 ± 227 a	11047.3 ± 558 c	78.60 ± 7.94 a	53.47 ± 9.84	0.51 ± 0.02 de	4.93 ± 0.42 de	346.0 ± 35.0 a	34.40 ± 6.89 bc
1 mM Cd × 10 mM Pb	4544.1 ± 136 a	10966.9 ± 434 c	56.83 ± 8.91 bc	68.60 ± 16.15	0.66 ± 0.03 bc	4.27 ± 0.39 de	283.6 ± 14.5 ab	35.20 ± 3.41 bc
1 mM Cd × 20 mM Pb	2681.8 ± 182 cd	11957.4 ± 311 bc	71.90 ± 8.95 ab	87.60 ± 6.32	0.83 ± 0.03 a	7.66 ± 0.40 c	323.1 ± 43.5 a	49.33 ± 5.17 a
3 mM Cd × 5 mM Pb	4274.4 ± 243 a	11294.4 ± 455 c	45.77 ± 2.95 c-e	75.87 ± 13.09	0.39 ± 0.03 f	5.82 ± 0.53 d	201.4 ± 13.0 cd	39.50 ± 4.83 a-c
3 mM Cd × 10 mM Pb	2118.9 ± 151 d	10889.9 ± 575 c	47.77 ± 3.73 c-e	51.23 ± 8.99	0.46 ± 0.04 ef	9.68 ± 0.93 b	244.2 ± 20.0 bc	35.97 ± 5.99 a-c
3 mM Cd × 20 mM Pb	4300.8 ± 188 a	10977.6 ± 354 c	36.93 ± 3.87 de	46.20 ± 7.28	0.39 ± 0.05 f	9.26 ± 0.76 bc	162.6 ± 17.0 d	29.10 ± 3.76 c
5 mM Cd × 5 mM Pb	3291.4 ± 202 bc	13934.7 ± 473 a	41.13 ± 3.24 c-e	60.47 ± 11.2	0.59 ± 0.03 cd	8.12 ± 0.51 bc	214.3 ± 22.7 b-d	40.50 ± 4.12 a-c
5 mM Cd × 10 mM Pb	4383.2 ± 201 a	11144.6 ± 436 c	39.63 ± 3.89 de	64.37 ± 10.75	0.72 ± 0.05 b	9.45 ± 0.78 b	174.5 ± 17.2 cd	47.59 ± 3.77 ab
5 mM Cd × 20 mM Pb	2200.9 ± 228 d	12934.3 ± 526 ab	50.43 ± 2.47 cd	49.83 ± 8.70	0.59 ± 0.04 cd	12.91 ± 0.90 a	221.9 ± 10.9 b-d	36.77 ± 3.67 a-c
Critical value for comparison	617.50	1322.7	16.18	31.23	0.11	1.67	70.87	13.90

Means not showing same letters in a column differ significantly.

correlated with Pb translocation (Figure 3). Stem TFC negatively correlated with Cd translocation ($r = -0.54^*$). Also antioxidants; CAT, POD, SOD in roots positively correlated with Pb translocation ($r = 0.56^*$, 0.56^* , and 0.55^* , respectively). These results suggested that Cu and Mn as well as antioxidants may play an important role not only in tolerance mechanism but also in regulation of metal transfer from roots to stem. It was further observed that under Pb stress, lesser uptake of Cd was observed in moringa stem and leaves. Similar observations have been previously reported for *Cucumis sativus* and *Lemna polyrrhiza* (An et al. 2004; John et al. 2008).

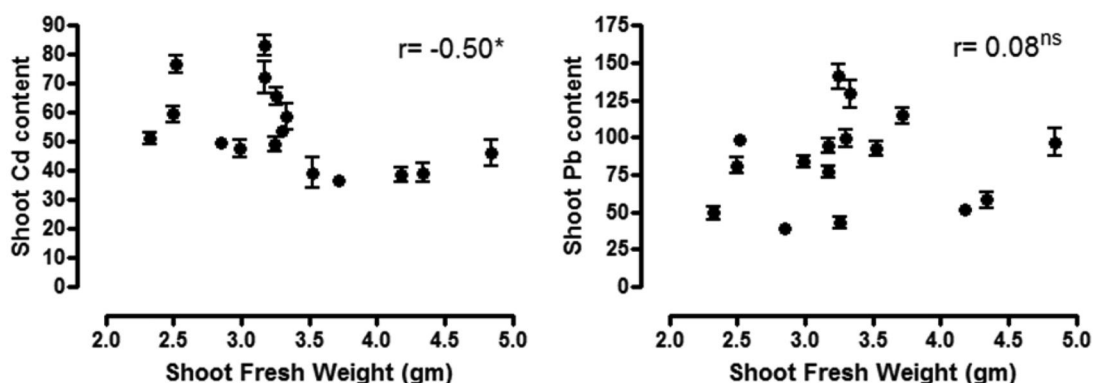
The defense system of plants can be detected by various mechanisms like transmembrane recognition and production of secondary metabolites to mitigate abiotic stress conditions (Jones and Dangl 2006; Dawid and Hille 2018). Secondary metabolites like phenolics, flavonoids, flavons, and flavonols are produced by plants naturally for protecting against

abiotic stress conditions. In the present investigation, polyphenolic profiling of moringa leaves, stem, and roots were analyzed to study the changes in the expression of secondary metabolites under metalliferous conditions (Tables 8–10). The data showed that Cd and Pb treatments significantly affected the composition of polyphenolics in different parts of the moringa plants (Supplemental data/tables). The LC/MS analysis of polyphenolic profile showed 15 compounds that were mainly related to flavonoids (10 compounds) and hydroxycinnamic acids (5 compounds) (Figure 4). The first compound at m/z 625 corresponds to quercetin-3,7-diglucoside which was further identified with m/z 505, 463, and 301. The second compounds was identified at $R_{t_{min}}$ 15.46 at m/z 431 corresponds to apigenin-6-C-glucoside (isovitexin) with fragments m/z 341 and 311, third compound at m/z 463 was detected at $R_{t_{min}}$ (17.0) corresponds to quercetin-3-glucoside with fragments m/z 505 and 301, fourth compound at m/z 577 corresponds to apigenin-7-C-glucoside

Table 7. Mean and standard error values of mineral content in roots of *Moringa oleifera* under different concentration of cadmium and lead.

Treatments	Na (mg kg ⁻¹)	K (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Control	6207 ± 177 a	11160 ± 139 a	79.30 ± 5.73 a	33.33 ± 2.69	0.93 ± 0.04 b	6.39 ± 0.20 c	279.0 ± 60.7	52.92 ± 4.48 c
1 mM Cd	3613 ± 118 d	7438 ± 261 c	70.50 ± 4.28 ab	39.40 ± 3.10	0.98 ± 0.05 b	8.39 ± 0.33 b	232.6 ± 23.9	61.11 ± 5.55 bc
3 mM Cd	4572 ± 238 c	7717 ± 183 c	24.27 ± 3.03 c	43.53 ± 2.05	1.13 ± 0.05 a	10.68 ± 0.40 a	293.2 ± 43.6	84.18 ± 6.81 a
5 mM Cd	5397 ± 174 b	9735 ± 239 b	57.30 ± 4.35 b	36.90 ± 4.50	1.21 ± 0.05 a	11.55 ± 0.39 a	174.3 ± 44.7	77.51 ± 6.62 ab
Critical value for comparison	592.61	687.63	14.51	10.48	0.15	1.03	147.31	19.37
Control	6207 ± 177 a	11160 ± 139 a	79.30 ± 5.73 a	33.33 ± 2.69 c	0.93 ± 0.04 a	6.39 ± 0.20 c	279.0 ± 60.7 b	52.92 ± 4.48
5 mM Pb	3606 ± 112 b	7553 ± 133 c	34.50 ± 3.00 c	36.10 ± 1.22 bc	0.83 ± 0.04 ab	9.65 ± 0.29 b	241.1 ± 43.9 b	89.70 ± 14.36
10 mM Pb	5860 ± 170 a	9746 ± 224 b	71.80 ± 3.30 ab	50.57 ± 4.79 a	0.78 ± 0.04 b	11.90 ± 0.53 a	456.1 ± 21.3 a	78.89 ± 5.24
20 mM Pb	3825 ± 111 b	6832 ± 166 d	58.63 ± 4.82 b	45.03 ± 3.49 ab	0.93 ± 0.04 a	12.61 ± 0.33 a	508.1 ± 38.5 a	57.41 ± 7.37
Critical value for comparison	475.80	552.31	14.21	10.80	0.13	1.17	141.72	28.62
Control	6207 ± 177 a	11160 ± 139 b	79.30 ± 5.73 a	33.33 ± 2.69	0.93 ± 0.04 c	6.39 ± 0.20 e	279.0 ± 60.7 cd	52.92 ± 4.48 cd
1 mM Cd × 5 mM Pb	4013 ± 46 f	7852 ± 162 e	42.03 ± 4.36 d	52.27 ± 3.00	0.57 ± 0.03 e	6.42 ± 0.52 e	548.3 ± 26.0 a	53.72 ± 7.24 cd
1 mM Cd × 10 mM Pb	5547 ± 128 b-e	9360 ± 231 c	48.13 ± 3.62 cd	60.53 ± 5.27	0.79 ± 0.03 d	9.49 ± 0.30 d	495.5 ± 48.9 ab	79.85 ± 4.38 a
1 mM Cd × 20 mM Pb	5926 ± 108 ab	13040 ± 247 a	59.73 ± 5.73 bc	44.93 ± 10.05	1.03 ± 0.04 bc	8.27 ± 0.33 d	342.8 ± 38.8 b-d	67.46 ± 5.76 a-c
3 mM Cd × 5 mM Pb	3916 ± 124 f	9387 ± 246 c	82.23 ± 5.51 a	38.13 ± 4.10	0.43 ± 0.03 f	10.89 ± 0.56 c	435.9 ± 50.6 a-c	59.79 ± 6.86 b-d
3 mM Cd × 10 mM Pb	5268 ± 120 c-e	8656 ± 137 d	68.13 ± 5.68 ab	90.57 ± 43.98	0.67 ± 0.03 de	11.40 ± 0.44 bc	336.7 ± 103.7 b-d	75.13 ± 4.83 ab
3 mM Cd × 20 mM Pb	5184 ± 88 de	6871 ± 80 f	26.67 ± 5.37 e	46.93 ± 19.30	0.74 ± 0.05 d	11.16 ± 0.59 c	220.4 ± 50.3 d	63.92 ± 4.29 a-d
5 mM Cd × 5 mM Pb	5896 ± 582 a-c	6431 ± 120 f	48.97 ± 2.98 cd	29.70 ± 4.16	0.97 ± 0.06 c	11.95 ± 0.56 bc	363.0 ± 11.3 b-d	48.77 ± 6.99 d
5 mM Cd × 10 mM Pb	5762 ± 115 a-d	8131 ± 203 de	61.40 ± 4.12 bc	37.43 ± 1.67	1.12 ± 0.05 b	12.73 ± 0.47 b	360.1 ± 28.3 b-d	62.54 ± 4.14 b-d
5 mM Cd × 20 mM Pb	5025 ± 161 e	10765 ± 280 b	57.07 ± 6.73 b-d	30.73 ± 4.66	1.27 ± 0.05 a	14.46 ± 0.52 a	307.1 ± 72.1 cd	73.75 ± 4.51 ab
Critical value for comparison	644.06	574.79	15.01	46.74	0.19	1.38	162.17	16.16

Means not showing same letters in a column differ significantly.

**Figure 1.** The correlation coefficient between shoot fresh weight and stem Cd and Pb content.

(isorhoifolin) with fragments m/z 431, 341 and 311, fifth compound was detected at $R_{t_{min}}$ (19.58) at m/z 609 corresponds to kaempferol-3,7-diglucoside with fragments at m/z 447 and 285, sixth compound detected at m/z 577 was identified as apigenin-7-O- rutinoside with fragments at m/z 431, 341, 311, 283, 269, 225, and 156, seventh compound identified at $R_{t_{min}}$ 22.49 was detected at m/z 463 was identified as quercetin-3-rhamanoside with fragments at m/z 301, 271, 179, and 926, eighth compound was identified as quercetin-3-sophoroside at m/z 625 with fragments at m/z 463, 301, 271, and 151, ninth compound detected at $R_{t_{min}}$ 26.08 at m/z 505 was identified as kaempferol-3-glucoside with fragments at m/z 301 while the last (10th) flavonoid was identified as kaempferol-7-glucoside at $R_{t_{min}}$ 27.66 with fragments at m/z 285, 255, and 173 (Table 8–10).

In the case of hydroxycinnamic acids, 5 compounds were identified as dicaffeoylquinic acid, 5-caffeoylquinic acid, 3-caffeoylquinic acid, 3-*p*-coumaroylquinic acid and feruloylquinic acid at m/z 515, 353, 353, 337, and 367, respectively. The first hydroxycinnamic acid was identified with fragments at m/z 353, 191, 179, 173, and 135, the second

compound was with fragments at m/z 179, 173, and 135, the third compound with fragments at same m/z as of the second compound, the fourth compound was detected with fragments at m/z 191 and 179 while fifth hydroxycinnamic acid was identified with fragments at m/z 191, 173, 179, and 163 (Tables 8–10).

In present research work, flavonols profile was found in line with the previous research studies conducted to analyze polyphenolics in different parts of moringa plants (Amaglo *et al.* 2010; Nouman *et al.* 2016) while this is the first time that the variation in these compounds was studied based on independent and mixed toxic effects of Cd and Pb. Nouman *et al.* (2016) reported 17 compounds in different cultivars of *Moringa oleifera* while in the present study, 15 compounds were noted with varying concentrations (Tables 8–10, Supplemental Data). The differences might be attributed to growing conditions and metal toxicity. Again galloylated or other flavonoids were not detected in contrast to the information provided by Manguro and Lemmen (2007). The presence of relatively high concentrations of acetyl derivatives have been previously described in plant materials, and,

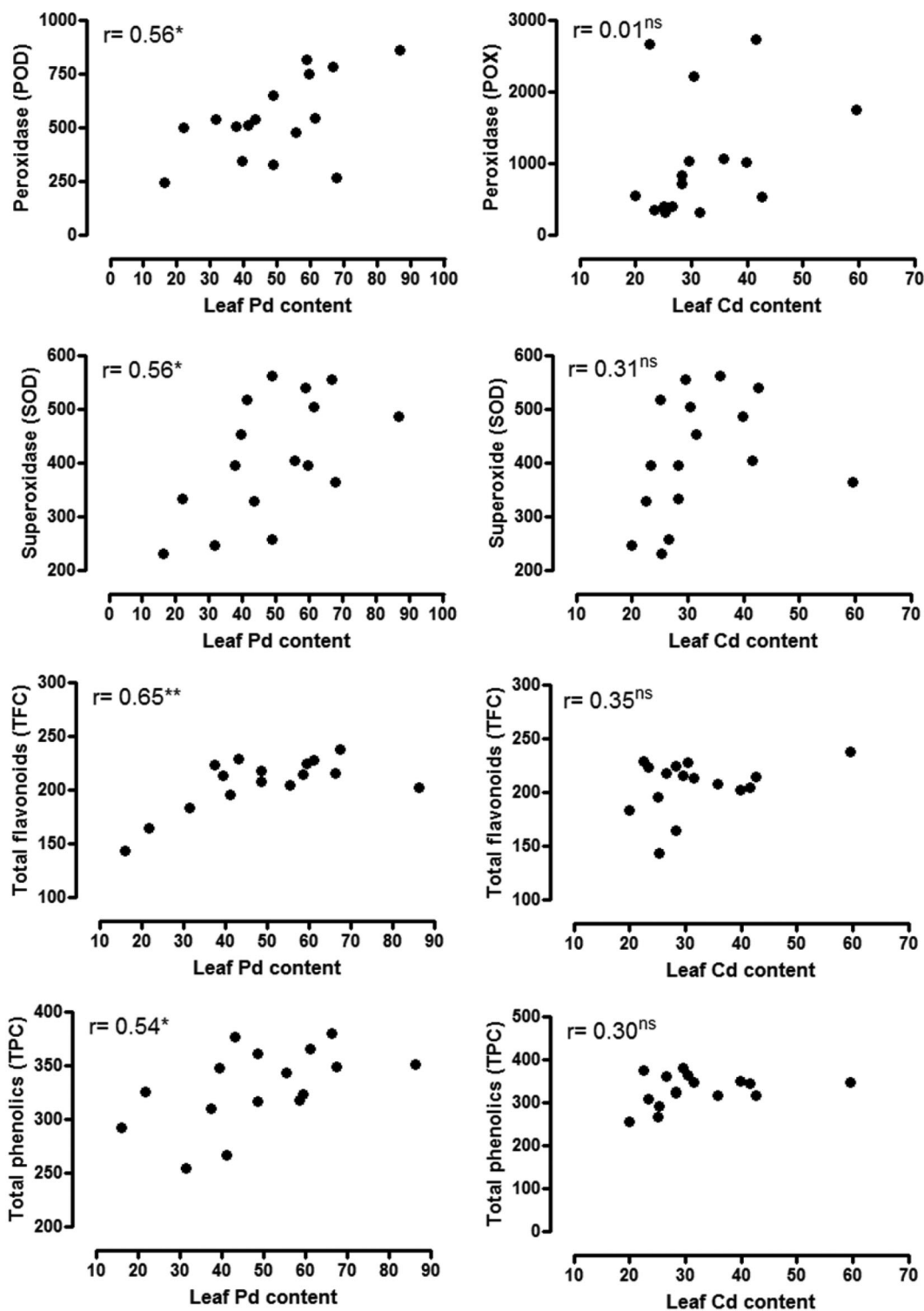


Figure 2. The correlation coefficient of leaf Pb and Cd content with peroxidase (POD), superoxidase (SOD), total flavonoid content (TFC), and total phenolic content (TPC).

although this not constitute a frequent flavonoid derivative, its presence could be due to the decomposition of malonylglucosides during material processing, according to the instability described for these compounds (Švehlíková *et al.* 2004).

Nouman *et al.* (2016) reported caffeoylquinic acids (5- and 3-isomers) as the main hydroxycinnamic acids while in

present investigation 3-caffeoylquinic acid and feruloylquinic acid ranked higher in all three parts of moringa plants (Tables 8–10). These findings are also consistent with Bennett *et al.* (2003) and Karthivashan *et al.* (2013). Similar findings have already been reported by Nouman *et al.* (2016) and Gull *et al.* (2019). In their research work, the researchers reported variation in flavonoids and

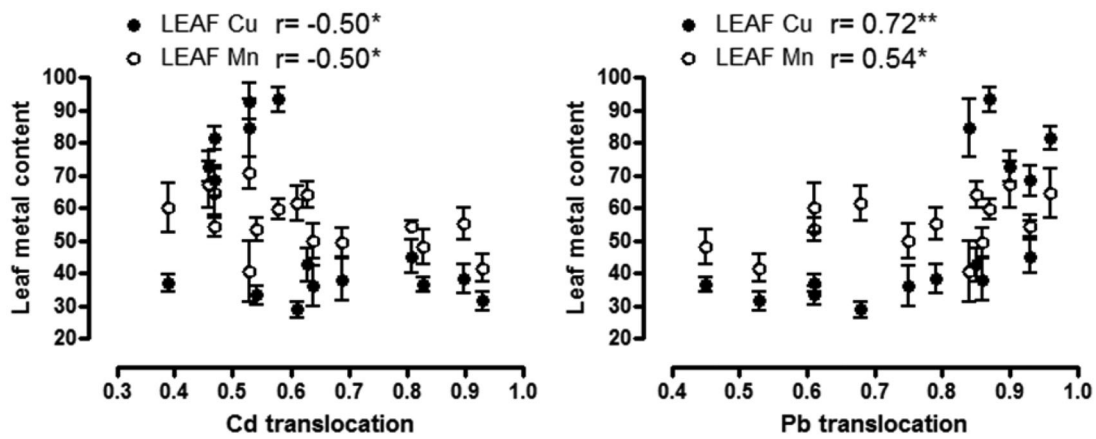


Figure 3. The correlation coefficient of Cd and Pb translocation with leaf Cu and Mn content.

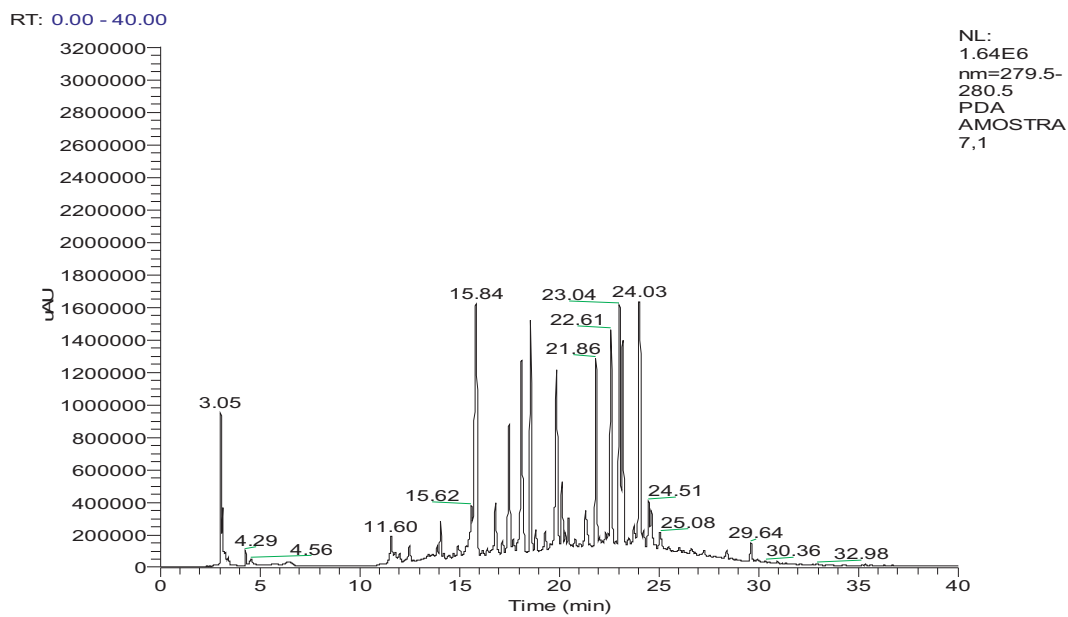


Figure 4. A representative chromatogram showing the separation of selected polyphenolics.

hydrocinnamic acids in *Moringa oleifera*. Naikoo *et al.* (2019) reported that plants accumulate bioactive compounds like phenolic acids in their tissues as a commutable response to abiotic stress like salinity, drought, high or low temperature, nutrients deficiency, and metal toxicity. Under such conditions, polyphenolics play an important role in regulating the physiological mechanisms of plants. Moreover, the production of secondary metabolites or polyphenolics also changed under stress as these compounds are responsible for regulating the antioxidant system of the plants. Berini *et al.* (2018) discussed in detail the production of secondary metabolites in plants under abiotic stress. They reported that the plants respond differently to expressing secondary metabolites in their different parts at different stages. The researchers further reported that such environmental stresses may affect biomass production and biosynthesis of polyphenolics. In another study, the researchers reported that the biosynthesis of polyphenolics is an adaptive response of

plants under environmental stress conditions (Edreva *et al.* 2008). In present investigation, the production of polyphenolics in different moringa parts might be attributed to higher uptake of Cd and Pb by moringa plants which resultantly improved moringa growth response.

Conclusions

Based on the present investigation, it can be concluded that moringa has the ability to withstand under Cd and Pb contaminated soils with the least impact on its nutritional quality and polyphenolics. The translocation factors of Cd and Pb increased under stress but maintained values less than 1 indicating accumulation of Cd and Pb in roots rather than in shoots and leaves. Antioxidant activities such as CAT, POD, SOD, and ascorbate content were significantly increased in all plant parts under Cd and Pb stress while TFC and TPC content behaved differently. The results of

Table 8. *Moringa oleifera* leaves polyphenolic ($\mu\text{g g}^{-1}$ dry weight) profiling under different concentration of cadmium and lead.

Treatment	Control		1 mM Cd		5 mM Cd		10 mM Cd		20 mM Cd		1 mM Cd × 3 mM Pb		5 mM Cd × 3 mM Pb		10 mM Cd × 3 mM Pb		5 mM Cd × 5 mM Pb		10 mM Cd × 5 mM Pb		5 mM Cd × 20 mM Pb		10 mM Cd × 20 mM Pb		5 mM Cd × 50 mM Pb		F-value		
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	
Flavonoids																													
Quercetin-3,7-diglucoside	1.7	3.5	5.5	3.4	3.5	4.2	4.9	13.3	15.1	8.6	6.9	12.1	Traces	5.8	4.4	3.7	45.7***												
Apigenin-6-C-glucoside(isovitexin)	17.1	20.2	13.6	10.9	11.8	19.6	Traces	16.9	12.9	Traces	9.0	28.1	19.8	8.7	6.9	20.9	18.3***												
Quercetin-3-glucoside	16.0	19.6	21.6	14.2	28.4	24.6	20.5	12.6	20.1	19.0	16.5	Traces	12.2	13.2	2.7	7.7	13.4***												
Apigenin-7-C-glucoside (isorhoifolin)	4.0	7.3	6.4	5.5	4.7	9.9	6.6	5.0	6.3	Traces	Traces	5.5	Traces	10.2	12.1	4.0	20.2***												
Kaempferol-3,7-diglucoside	18.1	16.2	10.0	11.4	16.6	9.5	Traces	25.6	21.5	Traces	Traces	Traces	Traces	Traces	7.2	6.0	28.5***												
Apigenin-7-O- rutinoside	16.0	18.2	27.3	26.3	30.6	10.1	Traces	11.9	9.6	Traces	12.7	17.3	11.8	4.1	Traces	Traces	30.3***												
Quercetin-3-rhamanoside	2.3	3.4	7.3	6.2	Traces	13.9	18.2	5.5	14.1	10.4	Traces	4.7	8.4	3.5	Traces	Traces	34.9***												
Quercetin-3-sophoroside	28.7	34.7	33.9	26.0	30.5	26.5	32.0	56.8	41.6	32.6	45.6	23.3	20.4	4.8	4.1	Traces	45.7***												
Kaempferol-3-glucoside	15.1	43.2	53.5	37.3	17.2	23.1	31.1	16.9	21.1	17.8	12.6	14.7	Traces	8.4	8.7	4.6	82.7***												
Kaempferol-7-glucoside	23.4	30.0	38.4	30.7	23.8	35.1	25.1	35.2	44.2	31.9	25.5	32.2	17.8	22.5	32.2	19.4	9.2												
Hydroxycinnamic acids																													
Dicaffeoylquinic acid	10.5	12.8	13.8	17.1	13.4	18.0	15.9	14.8	15.9	17.3	15.6	16.4	14.0	10.9	12.7	12.9	5.4***												
5-Caffeoylquinic acid	2.5	3.6	5.9	4.7	6.1	7.9	5.1	5.5	5.6	6.7	5.7	6.4	5.8	Traces	Traces	Traces	30.2***												
3-Caffeoylquinic acid	63.8	91.3	81.8	42.9	55.2	88.4	91.9	77.0	53.4	Traces	46.8	64.4	51.5	Traces	Traces	Traces	24.0***												
3-p-coumaroylquinic acid	3.0	5.2	8.9	Traces	14.7	18.3	8.9	9.6	10.6	9.5	18.9	9.1	4.2	4.1	2.3	Traces	57.8***												
Feruloylquinic acid	24.6	34.1	47.6	20.6	55.1	31.1	44.0	36.8	31.1	29.8	28.9	35.2	12.5	23.5	11.3	3.7	13.4***												

Table 9. *Moringa oleifera* stem polyphenolic ($\mu\text{g g}^{-1}$ dry weight) profiling under different concentration of cadmium and lead.

Treatment	Control		1 mM Cd		5 mM Cd		10 mM Cd		20 mM Cd		1 mM Cd × 3 mM Pb		5 mM Cd × 3 mM Pb		10 mM Cd × 3 mM Pb		5 mM Cd × 5 mM Pb		10 mM Cd × 5 mM Pb		5 mM Cd × 20 mM Pb		10 mM Cd × 20 mM Pb		5 mM Cd × 50 mM Pb		F-value		
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	
Flavonoids																													
Quercetin-3,7-diglucoside	0.9	2.3	3.1	1.7	1.5	1.7	3.2	7.9	12.4	6.8	3.6	7.7	4.8	1.0	4.9	Traces	32.7***												
Apigenin-6-C-glucoside(isovitexin)	14.5	18.9	12.7	15.0	12.1	15.9	7.4	11.7	9.8	7.6	9.4	18.8	14.6	4.2	6.2	4.6	18.6***												
Quercetin-3-glucoside	14.7	17.3	21.0	19.7	24.7	20.9	16.8	8.9	12.6	9.4	16.6	12.9	7.1	9.2	4.4	Traces	18.1***												
Apigenin-7-C-glucoside (isorhoifolin)	5.7	9.7	12.1	10.2	10.5	6.2	8.2	4.5	2.3	Traces	4.8	3.3	Traces	8.9	10.1	Traces	16.3***												
Kaempferol-3,7-diglucoside	14.5	16.3	11.8	10.2	12.9	9.4	5.7	18.6	20.5	17.9	12.6	14.5	Traces	7.2	6.0	2.0	29.4***												
Apigenin-7-O- rutinoside	16.0	18.2	27.3	26.3	30.6	10.1	Traces	15.1	18.6	18.9	27.9	13.4	Traces	16.8	Traces	Traces	37.7***												
Quercetin-3-rhamanoside	2.2	2.5	6.2	4.1	3.1	5.9	2.0	3.4	7.2	8.3	2.2	4.7	2.2	5.2	3.4	Traces	12.7***												
Quercetin-3-sophoroside	20.5	25.1	31.6	23.9	19.1	23.2	30.2	42.0	38.9	29.6	35.7	20.8	15.3	10.4	5.8	Traces	20.3***												
Kaempferol-3-glucoside	13.2	34.7	51.2	29.4	13.5	18.7	27.7	17.8	18.2	13.8	11.5	12.1	8.9	8.4	8.7	2.5	28.7***												
Kaempferol-7-glucoside	19.9	27.9	36.1	28.6	20.1	31.6	23.4	32.6	41.5	25.2	21.6	29.7	12.6	Traces	Traces	Traces	48.1***												
Hydroxycinnamic acid																													
Dicaffeoylquinic acid	7.9	11.4	9.6	5.5	9.8	13.3	9.2	10.4	18.7	20.0	11.7	16.6	9.3	6.6	9.1	7.1	10.7***												
5-Caffeoylquinic acid	1.6	2.6	1.6	2.6	2.9	4.3	1.5	2.2	3.1	6.5	2.1	4.9	Traces	Traces	Traces	Traces	15.6***												
3-Caffeoylquinic acid	55.5	74.4	80.0	37.8	42.9	79.3	88.3	32.1	56.2	36.2	37.7	47.3	34.9	22.8	Traces	Traces	15.1***												
3-p-coumaroylquinic acid	1.6	3.8	6.0	6.3	10.1	5.6	6.9	5.5	8.8	6.2	15.0	8.3	9.4	5.7	4.7	Traces	22.5***												
Feruloylquinic acid	19.9	28.4	43.3	18.9	27.7	35.0	40.4	36.8	27.2	18.6	25.4	33.0	20.2	18.6	7.5	8.3	10.8***												

Table 10. *Moringa oleifera* roots polyphenolic ($\mu\text{g g}^{-1}$ dry weight) profiling under different concentration of cadmium and lead.

Treatment	Control		1 mM Cd		5 mM Cd		10 mM Cd		20 mM Cd		5 mM Pb		10 mM Pb		20 mM Pb		5 mM Cd × 5 mM Pb		10 mM Cd × 5 mM Pb		5 mM Cd × 20 mM Pb		F value
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	
Flavonoids																							
Quercetin-3,7-diglucoside	8.6		13.0	16.2	11.9	9.9	12.7	8.2	13.9	17.2	13.4	8.6	12.0	19.9	10.5	7.6	Traces	3.1	7.6	Traces	Traces	20.8***	
Apigenin-6-C-glucoside(isovitexin)	16.1		20.0	25.0	15.9	16.9	24.6	18.3	18.0	15.8	8.1	10.8	Traces	17.9	10.5	7.6	Traces	10.5	7.6	Traces	Traces	10.2***	
Quercetin-3-glucoside	16.8		20.6	22.8	19.6	19.0	29.2	22.7	15.4	23.4	20.4	17.5	17.9	14.4	7.1	Traces	14.4	7.1	Traces	Traces	Traces	20.2***	
Apigenin-7-C-glucoside (isorhoifolin)	2.7		3.2	3.6	Traces	3.4	11.0	9.2	9.7	7.4	7.5	2.7	9.6	Traces	6.8	Traces	Traces	6.8	Traces	Traces	Traces	23.0***	
Kaempferol-3,7-diglucoside	12.2		16.7	14.9	13.1	20.1	12.4	Traces	28.0	16.2	7.9	6.2	Traces	25.6	18.6	13.4	Traces	Traces	Traces	Traces	Traces	24.1***	
Apigenin-7-O- rutinoside	16.3		21.3	28.6	31.7	25.2	11.3	19.1	17.5	16.9	25.6	28.5	22.1	12.3	18.6	15.4	Traces	18.6	13.4	15.4	17.7***		
Quercetin-3-rhamnoside	3.2		4.3	6.1	3.9	6.4	15.0	4.2	6.3	15.2	Traces	3.2	4.7	Traces	4.6	Traces	4.6	Traces	Traces	Traces	38.8***		
Quercetin-3-sophoroside	31.2		36.7	41.4	27.6	33.6	23.7	28.2	62.8	43.1	34.5	49.9	23.2	21.2	9.9	5.9	Traces	9.9	5.9	Traces	Traces	24.9***	
Kaempferol-3-glucoside	15.9		50.6	60.2	40.8	18.6	26.7	34.4	17.7	22.5	22.4	14.0	19.2	17.9	7.9	Traces	7.9	Traces	Traces	Traces	Traces	67.6***	
Kaempferol-7-glucoside	25.9		31.8	40.4	35.3	26.8	37.4	28.8	37.7	45.7	33.8	27.0	33.9	19.7	23.6	33.4	19.7	23.6	33.4	17.5	17.5	10.7***	
Hydroxycinnamic acids																							
Dicaffeoylquinic acid	11.3		13.7	15.0	18.7	14.6	20.1	18.0	16.1	20.0	22.0	19.4	21.9	24.5	17.7	13.5	24.5	17.7	13.5	13.5	13.5	8.8***	
5-Caffeoylquinic acid	3.3		4.6	7.1	6.2	7.2	9.0	6.5	6.7	6.7	6.7	5.7	6.4	7.1	4.7	4.8	7.1	4.7	4.8	4.8	4.8	6.8***	
3-Caffeoylquinic acid	69.6		94.3	83.4	62.1	54.6	77.7	89.9	66.0	41.1	59.9	46.9	68.2	51.6	35.8	67.4	51.6	35.8	67.4	67.4	67.4	7.3***	
3-p-coumaroylquinic acid	4.1		6.3	9.0	6.9	15.2	17.2	10.8	11.1	8.1	9.9	19.1	10.7	Traces	5.3	4.1	10.7	5.3	4.1	4.1	4.1	21.9***	
Feruloylquinic acid	23.2		43.6	58.4	16.3	56.3	32.8	29.1	35.7	32.0	26.2	32.8	35.7	15.2	40.1	12.7	15.2	40.1	12.7	12.7	12.7	23.2***	

the present investigation are very helpful in selecting moringa as a phytoremedial plant to grow under Cd and Pb contaminated soils or when irrigated with sewage wastewater. This study does not end here, as there is further need to identify the molecular basis or changes being taken place in cells and to identify the responsible genes which are associated with moringa tolerance under metalliferous conditions.

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