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Lead-induced Hormesis in *Corchorus olitorius*: Growth, Antioxidants and Micronutrients Accumulation

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Abstract

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Corchorus olitorius, a leafy vegetable with characteristically high content of minerals and antioxidants, is known for its ability to grow in Pb-contaminated soils with minimum influence on its morphological parameters. Its growth can be stimulated by low Pb concentrations, although Pb is not a nutritional element and is known for its toxicity to plants. This phenomenon is referred to as hormesis, the stimulatory impact of low doses of some toxins on the growth and metabolism of living organisms. Therefore, plants subjected to Pb contamination may have better crop yield despite of their potential negative influences when entering the food chain. In this study, Pb was added to the cultivated soil in concentrations that are within permissible levels for cultivated soils. Growth parameters significantly increased under Pb treatment. Hormesis in plants has been previously reported to be related mainly to growth parameters, pigments and antioxidants, all are tested in this work, in addition to the contents, accumulation and translocation factors of Pb as well as the essential micronutrients Fe, Zn, Cu and Ni. Antioxidants significantly decreased and the accumulation of some essential micronutrients as Fe and Zn is disturbed. The tested parameters were measured in two different plant life stages, early and late vegetative stages. The hormetic effect was more significant in older plants. Hormesis could be used as an agricultural practice for increasing crop productivity. However, the characteristic nutritional and medicinal value of this plant is negatively affected.

Keywords: Antioxidants; *Corchorus olitorius*; Hormesis; lead; micronutrients

Introduction

Corchorus olitorius (also known as jute or Jew's mallow) is a green leafy vegetable known for high contents of proteins, lipids and minerals particularly iron and zinc. It is also a good source of vitamins (A, C, and E) and characterized by high phenols content and antioxidant activity. Its leaves are widely consumed as food in many countries in Africa and Asia and are used for medicinal purposes (Isuosuo et al., 2019). Contamination of agricultural soils with lead (Pb) is considered a major problem for both agricultural production and human health since because Pb is a heavy metal that is toxic and with unknown physiological functions for either plants or humans. Sources of lead soil contamination include smelting, combustion of leaded gasoline and application of lead-contaminated sewage sludge as fertilizer (Fattahi et al., 2019). The maximum Pb concentration that is allowable in agricultural soils is 300 mg kg^{-1} (Xiao et al., 2018).

Hormesis or hormetic effect is a bi-phasic concentration response phenomenon, characterized by low dose stimulatory effect and high dose inhibitory effect of toxic substances, including heavy metals. The hormetic effect is described by an inverted U-shaped curve. Hormesis protects some plants exposed to toxic substances at low doses against stress and increases productivity (Ndlovu et al., 2020). It is a universal phenomenon that is independent of the organism in which it occurs or the type of stress. The concept of hormesis is supported by numerous studies that included a wide range

of organisms (from microorganisms through plants to mammals). Growth stimulation might be due to an adaptive compensatory process following an initial disruption in homeostasis (Calabrese & Baldwin, 2003). Thus, metal ions can act as elicitors of defense mechanism which can increase plant growth (Muszynska & Labudda, 2019). The most frequently examined physiological parameters by scientists interested in hormesis in plants are growth, oxidative stress and photosynthetic activity. Many scientists have connected the stimulation of plant growth during hormesis with a low level of oxidative stress. Their results emphasize the involvement of reactive oxygen species (ROS) in the hormetic effect (Malkowski et al., 2020). The growth stimulation resulted by hormetic effect can be applied to agricultural systems to reduce environmental change impacts, enhance resistance to microbes and pests, and increase productivity at much lower cost. However, such practices need to be very cautious about the risks that could be associated with the incorrect use of hormesis-based solutions (Agathokleous & Edward, 2019)

Most previous researches studying Pb effect on jute plants involved toxicity of high Pb concentrations through on-site polluted soil or wastewater (Uddin et al., 2016; Ahmed & Slima, 2018). Jute was recorded to grow in soils contaminated with high levels of Pb with minimum influence on their growth rates and morphological characters (Liu et al., 2018; Ndlovu et al., 2019). However, plants grown in Pb-contaminated soils might have disturbed metabolism or high Pb concentrations inside their tissues which can result in severe health problems for consumers as accumulated in their bodies over time.

The aim of the present study was to evaluate the influence of slightly increased soil Pb concentrations within the allowable limits on *C. olitorius* for better understanding the Pb hormetic effect on this plant. The study also aimed to confirm the safety of using *C. olitorius* grown under such Pb concentrations for human consumption and to evaluate the potential Pb influence on its growth, metabolites, antioxidant system and accumulation of essential micronutrients.

Materials and Methods

Cultivation Conditions

The experiment was carried out at the Botanical Garden of the Faculty of Sciences, Minia University in the middle of August 2020 for 45 days. Seeds of *Corchorus olitorius* were obtained from the Agricultural Research Unit, Minia, Egypt and were cultivated in pots filled with 3.5 kg of clay soil obtained from the Botanical Garden of the Faculty of Sciences, Minia University. Three pots were cultivated for each treatment as replicates. Ten seeds were sown per pot, irrigated by tap water till full germination and afterwards thinned to 3 per pot. Fifteen-days old seedlings were treated with lead (Pb) added as $Pb(CH_3COO)_2$ in three different concentrations (30, 60 and 120 mg Pb kg^{-1} soil), referred to as T1, T2 and T3, respectively. Pots were kept in the open field and irrigated with tap water until the end of the experimental period. No fertilizers were added. Samples were collected at 15 days after treatment, the plants were 30 days old. This stage was referred to as the first stage or 30 days after sowing (DAS) stage. The samples were collected again at the maturity stage at the end othe experimentnt, 30 days after treatment, the plants were 45 days old (second stage or 45 DAS stage).

Growth Parameters

In both tested stages (30 and 45 DAS) stem lengths (cm) and leaves numbers were estimated. The disk method (Watson & Watson, 1953) was used to measure average leaf (expressed as cm^2/plant leaf). Fresh weights of harvested plant stems and leaves were determined. Roots, stems and leaves were then washed thoroughly with deionized water and oven dried at 65ºC for 48 h until a constant weight was reached and dry weights were determined. Water contents in stems and leaves were calculated as a percentage of fresh weight (Alhassan et al., 2015) according to the equation

$$\text{Water content (W.C.), \%} = [(\text{fresh weight-dry weight})/\text{fresh weight}]*100$$

Dried roots, stems and leaves were ground into fine powder, passed through a 2 mm sieve and kept in paper bags for analysis. Samples of fresh plant leaves were kept in deep freezer for protocols that required fresh tissues.

Soil Analysis

A sample of the cultivated soil was air-dried, ground and passed through a 2 mm sieve and kept for analysis. Five g of soil was weighed and soaked in 50 ml of double distilled water and put on a shaker for 24 hours for extraction. The soil extract was then filtered through Whatman no. 1 filter papers, acidified by drops of nitric acid and completed to 50 ml with double distilled water and used for determining concentrations of Cu, Zn, Ni, Fe and Pb using an atomic absorption spectrophotometer (PerkinElmer AAnalyst 400).

Determination of Photosynthetic Pigments

The photosynthetic pigments chlorophyll a, chlorophyll b and carotenoids were extracted from 0.1 g fresh leaf samples suspended in 5 ml of 95% ethyl alcohol in a test tube at 60˚C until they turn into colorless. The extracts were then made up to 10 ml with 95% ethyl alcohol and the absorbance

was measured at 470 nm, 647 nm and 664 nm. Chlorophyll a, chlorophyll b and carotenoids were calculated using the equations cited by Lichtenthaler (1987).

Chlorophyll a (μg mL^{-1}) = 12.25*A664 – 2.79*A647

Chlorophyll b (μg mL^{-1}) = 21.50*A647 – 5.1*A664

Carotenoids (μg mL^{-1}) = (1000*A470 – 1.8*chla – 85.02*chlb)/ 198

DPPH Assay

Dried powdered plant leaves were used for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Tusevski et al., 2014). A sample of 0.05 g dried leaves was extracted with 5 ml of 50% methanol overnight. The samples were centrifuged at 14000 rpm for 15 min and the supernatants were used for DPPH assay.

For measuring free radical scavenging activity (McCune & Johns, 2002) 1 mL of 0.1 mM solution of DPPH in methanol was mixed with 1 mL of methanol extract of each sample. The mix was incubated in the dark for 30 min and absorbance at 517 nm was measured. The DPPH solution without sample was used as control and the 50% methanol was used as a blank. A freshly prepared DPPH solution exhibits a deep purple color with a maximum absorption at 517 nm. The purple color disappears when an antioxidant is present in the medium, antioxidant molecules quench DPPH free radicals and convert them to a colorless product (2,2-diphenyl-1-picrylhydrazine), resulting in a decrease in absorbance. The percentage scavenging activity was calculated using the following equation:

$$\% SA = [(A0 - A1)/ A0] \times 100,$$

where % SA= percentage scavenging activity, A0 = absorbance of control, A1 = absorbance of sample. The higher % SA indicates higher antioxidant activity.

Determination of Ascorbic Acid Content

A sample of 0.1 g of fresh plant leaves was homogenized in 2 mL of 0.75 M metaphosphoric acid. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was collected and 200 μL of 3% meta-phosphoric acid and 200 μL of Folin reagent (1:5) were added to 400 μL of the supernatant and the total volume completed with distilled water to 2 mL. The content was mixed for 10 min, and the absorbance read at 760 nm. A standard curve constructed with known concentrations of ascorbic acid (50-1000 μg mL^{-1}) was used used to calculate the ascorbic acid concentration (Jagota & Dani, 1982).

Determination of Total Anthocyanins Content

A weight of 0.1 g of fresh, frozen leaves was ground in liquid nitrogen and extracted with 2 mL of 1% HCl acidified methanol (1% (v:v) HCl in methanol) for 2 h at room temperature (20-25°C). Samples were then centrifuged at 12000 rpm for 15 min and the absorbance of supernatant was read at 535 nm. Acidified methanol was used as a blank (Strack & Wray, 1989). The dilution factor and the cyanidin 3-galactoside coefficient of extinction (98.2) were taken into account in the anthocyanins concentration calculations (Lees & Francis, 1972) according to the equation:

Total anthocyanins (mg cyaniding g^{-1} fresh plant material) = = (Absorbance x dilution factor)/ 98.2

Determination of Free and Wall-bound Phenolic Compounds

Free and wall bound phenolics were determined (Kofalvi & Nassuth, 1995) by extracting 0.25 g of fresh leaves in 5 mL of 50% methanol (1:1 v/v) for 90 min at 80°C. The extract was centrifuged for 15 min at 14000 rpm. The supernatant was collected and used for free phenolics determination and the pellet was used for bound phenolic extraction. The pellet was mixed to release bound phenolics with 2 mL of 0.5 N NaOH for 24 h at room temperature (20-25°C), neutralized with 0.5 mL of 2 N HCl and centrifuged at 14000 rpm for 15 min. The supernatant was collected and used for the estimation of bound phenolics.

Folin Ciocaltaeu method was used for free and bound phenolics determination. One hundred μL of each sample was diluted to 1 mL with deionized water and mixed with 0.5 mL of 1 N Folin Ciocalteau reagent (1:1 diluted commercial 2N reagent). After 5 minutes, 2.5 mL of 20% Na_2CO_3 was added to each sample. Samples were incubated at room temperature (20-25°C) for 20 min and absorbance measured at 735 nm against a blank. A standard curve was constructed using known concentrations of gallic acid (100-1000 μg mL^{-1}) in 50% methanol. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg g^{-1} fresh plant material.

Determination of Total Flavonoids Content

Total flavonoids were estimated in methanol extracts of fresh plant leaves. Five hundred μL of 2% $AlCl_3$ were added to 0.5 mL of samples extracted in methanol. After incubation of 1 h at room temperature (20-25°C), the absorbance was measured at 420 nm. A standard curve was prepared using quercetin of known concentrations (20-200 μg mL^{-1}) prepared in 50% methanol. Total flavonoids content was expressed as quercetin equivalents (QE) in mg g^{-1} dried plant material (Ordonez et al., 2006).

Estimation of Heavy Metals Concentrations

The concentrations of the heavy metals Cu, Zn, Ni, Fe and Pb were estimated in roots, stems and leaves of *C. olitorius*

plants subjected to control, T2 and T3 treatments. One gram of each of the selected samples was weighed and dissolved in 5 ml of 60% hydrochloric acid and 10 ml of 70% nitric acid and digested at 50 °C until white fumes evolved and the solution changed to a brownish color. The heat was further intensifieda few minutes few minutes to expel most of the HCl. Distilled water (50 ml) was added, ha few minutesew minutes, allowed to cool and filtered through a Whatman's No. 1 filter paper (Ndlovu et al., 2019). The concentrations of heavy metals in soil and plant tissues were determined using atomic absorption spectrometer AAnalyst 400, PerkinElmer.

The leaves translocation factor (TF) was determined as a ratio of metal in plant leaves to that in plant root, calculated as follows (Gupta et al., 2008):

$$\text{Translocation factor (TF)} = \text{metal (leaves) in } \mu g\ g^{-1}/\text{metal (root) in } \mu g\ g^{-1}$$

Metals accumulation in stems and leaves was calculated as follows (Shakir et al., 2020):

Tissue metal accumulation (µg) = tissue dry weight (g)* metal content (µg g^{-1})

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS (ver. 21.0) and represented as means of three replicates ± SE. Least significant difference (L.S.D) was used to compare means of control and treated plants.

Results

Table 1 represents fresh and dry weights as well as water contents of *C. olitorius* stems and leaves under different treatments at 30 DAS and 45 DAS stages, respectively. Stems and leaves recorded fresh and dry weights higher than their controls in response to Pb treatments, particularly T2, in both tested growth stages. This increased growth was more pronounced in older (45 DAS) than in younger plants (30 DAS). In 30 DAS plants at T2 treatment (Table 1), fresh weights of stems and leaves recorded 173% and 158% in relation to control, respectively, and dry weights recorded 180% and 152% of control, respectively. Stems and leaves of T2-treated plants at 45 DAS stage (Table 2) recorded fresh and dry weights more than double their control values (214% and 200% for fresh weights of stems and leaves, respectively, and 211% and 196% for dry weights of stems and leaves, respectively, in relation to their controls). Only water content did not record significant differences in Pb treated plants when compared to control. All moisture content values were in the range of 70% of fresh weight, either in stems or leaves.

Table 2 represents the results of plant height, number of leaves and average leaf area of control and treated jute plants at 30 and 45 DAS. Most of these growth parameters were also stimulated in response to Pb treatment, more significantly in the 45 DAS stage. Data of T2-treated older plants were the highest, recording plant height of 168% compared to control, number of leaves of 172% of control and average leaf area of 207% of control.

Table 3 shows the results of pigments contents in jute leaves under different Pb treatments at 30 and 45 DAS. Chlorophyll a and carotenoid contents in younger leaves were not significantly affected by Pb treatment while chlorophyll b contents increased, particularly at T1 and T2 where chlorophyll b contents recorded 165% and 140% compared to control, respectively. On the other hand, older jute leaves recorded decreased pigments contents in response to Pb treatment,

Table 1. Fresh weights (g), dry weights (g) and water content (% of fresh weight) for stems and leaves of jute plants in the first (30 DAS) and second (45 DAS) stages of growth under control, T1, T2 and T3 treatments. Results are represented as means of three replicates ± SE, n=3

Treatment	Stem f.w.	% of control	Leaves f.w.	% of control	Stem d.w.	% of control	Leaves d.w.	% of control	Stem water content	Leaf water content
First stage (30 DAS)										
Control	3.5±0.10	100	5.44±0.12	100	0.8±0.06	100	1.64±0.12	100	77.14	69.85
T1	4.72*±0.12	134.86	7.28*±0.18	133.82	1.28*±0.10	160	1.9±0.14	115.84	72.88	73.9
T2	6.04*±0.16	172.57	8.60*±0.25	158.09	1.44*±0.16	180	2.5*±0.18	152.44	76.16	70.93
T3	4.64*±0.14	132.57	6.04*±0.24	111.03	1.36*±0.11	170	1.96*±0.14	119.51	70.69	67.55
Second stage (45 DAS)										
Control	3.68±0.16	100	5.8±0.18	100	0.9±0.06	100	1.68±0.14	100	75.54	71.03
T1	5.76*±0.17	156.52	7.76*±0.24	133.79	1.38*±0.08	153.33	2.04*±0.18	121.43	76.04	73.71
T2	7.88*±0.28	214.13	11.6*±0.38	200	1.9*±0.08	211.11	3.3*±0.22	196.43	75.89	71.55
T3	5.72*±0.14	155.44	6.64±0.27	114.48	1.56*±0.12	173.33	2.1*±0.12	125	72.73	68.37

Value with (*) are statistically different from their control at $p < 0.05$.

particularly at the highest tested Pb concentration. Contents of chlorophyll a, chlorophyll b and carotenoids of T3-treated leaves at 45 DAS stage were 80%, 73% and 73% of their controls, respectively. A general reduction in the absolute values of different pigments was observed in older plants when compared to younger plants in both control and treated leaves.

Table 4 represents the results of total antioxidant activity as estimated by DPPH assay, the values of free and wall-bound total phenolic compounds (TPC), total flavonoids content (TFC), total anthocyanins content (TAC) and ascorbic acid content (AAC) in the leaves of *C. olitorius* plants in both young and old stages of growth. The total antioxidant activity decreases as a result of Pb treatment in both stages. Control values of percentage scavenging activity are the highest, particularly in older plants.

The lowest antioxidant activity was recorded in T2-treated plant leaves (59% of control in young plants and 51% of

Table 2. Plant height (cm), number of leaves and average leaf area (cm^2) of jute plants in both first and second stages of growth under control, T1, T2 and T3 treatments. Results are represented as means of three replicates ± SE, n=3

Treatment	First stage						Second stage					
	Plant height	% of control	No. of leaves	% of control	Average leaf area	% of control	Plant height	% of control	No. of leaves	% of control	Average leaf area	% of control
Control	16.13±0.82	100	24±1	100	8.45±0.28	100	20.83±1.12	100	25±1.5	100	8.87±0.32	100
T1	18.66*±0.89	115.69	25±1.33	104.17	11.43*±0.35	135.27	29.5*±1.5	141.62	29*±1.75	116	16.45*±0.74	185.46
T2	22*±1.05	136.39	26.5±1	110.42	9.73*±0.24	115.15	35*±2.4	168.03	43*±2	172	18.34*±0.89	206.76
T3	19.66*±0.96	121.89	27.7±1.9	115.42	7.08*±0.19	83.79	24*±1.25	115.22	29.33*±1.67	117.32	12.96*±0.46	146.11

Value with (*) are statistically different from their control at $p < 0.05$.

Table 3. Contents of chlorophyll a, chlorophyll b and carotenoids (mg g^{-1} fresh weight) of jute plants in both first and second stages of growth under control, T1, T2 and T3 treatments. The results are represented as means of three replicates ± SE, n=3.

Treatmet	First stage						Second stage					
	Chl. a	% of control	Chl. b	% of control	Carotenoids	% of control	Chl. a	% of control	Chl. b	% of control	Carotenoids	% of control
Control	2.49±0.18	100	0.80±0.04	100	1.28±0.09	100	1.37±0.11	100	0.33±0.02	100	0.70±0.05	100
T1	2.6±0.21	104.42	1.32*±0.10	165	1.25±0.11	97.66	1.36±0.09	99.27	0.34±0.02	103.03	0.63±0.06	90
T2	2.64±0.25	106.02	1.12*±0.09	140	1.25±0.10	97.66	1.48±0.08	108.03	0.28±0.01	84.85	0.61±0.05	87.14
T3	2.61±0.17	104.82	0.91*±0.09	113.75	1.24±0.08	96.88	1.1*±0.12	80.29	0.24*±0.01	72.73	0.51*±0.04	72.86

Value with (*) are statistically different from their control at $p < 0.05$.

Table 4. Effect of control, T1, T2 and T3 treatments on the antioxidants activity of jute leaves in their first stage of growth (DPPH percentage scavenging activity, free and wall bound total phenolic compounds (TPC) in mg GAE g^{-1} fresh weight, total flavonoids content (TFC) in mg QE g-1 dry weight, total anthocyanins contents (TAC) in mg cyanidin g^{-1} fresh weight and ascorbic acid content (AAC) in mg g^{-1} fresh weight. Values are represented as means of three replicates ± SE, n = 3

Treatment	DPPH %SA	% of control	Free TPC	% of control	Bound TPC	% of control	TFC	% of control	TAC	% of control	AAC	% of control
First stage (30 DAS)												
Control	47.89±3.25	100	4.35±0.28	100	8.51±0.46	100	20.85±0.98	100	171.35±4.82	100	7.65±0.0.38	100
T1	38.50*±2.48	80.39	4.62±0.26	106.21	6.91*±0.38	81.2	22.59±1.14	109.77	202.69*±5.18	118.29	5.79*±0.0.19	75.69
T2	28.4±1.59	59.3	5.39*±0.31	123.91	6.53*±0.42	79.73	20.99±0.87	100.67	187.12±4.82	109.2	5.27*±0.27	68.89
T3	36.15* ±2.12	75.45	6.16*±0.34	141.61	6.14*±0.30	72.15	29.01*±1.74	139.14	123.50*±3.88	72.08	5.05*±0.22	66.01
Second stage (45 DAS)												
Control	55.4±3.45	100	10.84±0.68	100	12.53±0.75	100	29.17±1.68	100	283.64±14.78	100	10.45±0.52	100
T1	42.72*±3.28	77.11	14.32±0.38	132.1	14.66*±0.52	117	22.28*±1.28	76.38	443.83*±22.15	156.48	9.76±0.39	93.4
T2	28.17*±1.65	50.85	15.12*±0.95	139.48	18.96*±1.04	151.32	22.38*±1.43	76.72	325.87*±18.87	114.89	11.63±0.40	111.29
T3	48.36*±2.74	87.29	9.29*±0.32	85.7	12.82±0.78	102.31	18.73*±0.95	64.21	316.42±15.62	111.56	10.09±0.27	96.56

Value with (*) are statistically different from their control at $p < 0.05$.

control in older plants). A general disturbance was recorded in the contents of different antioxidants under Pb treatment, particularly in young plants. Free phenolics significantly increased in response to increasing soil Pb concentrations at the expense of bound phenolics whose concentrations decreased with Pb treatment. T3-treated leaves at this stage recorded free phenolic of 142% of control while bound phenolics recorded 72% of control. Total flavonoids increased significantly at the highest tested Pb concentration (139% of control) while the ascorbic acid content dropped sharply with increasing soil Pb levels and recorded 66% of control at the highest Pb concentration. In older plants, most of the measured antioxidants were either increased or maintained around control values in response to Pb treatment, with the exception of total flavonoids content that decreased significantly as the Pb concentration increased in soil recording 64% of control at T3 treatment. In this stage (45 DAS) the highest values were 139% of control for free phenolics, 151% of control for binding phenolics and 111% of control for ascorbic acid content under T2 treatment. Anthocyanins content recorded their highest value at T1 treatment (156% of control).

Table 5 represents roots, stems and leaves contents of the heavy metals Cu, Zn, Ni, Fe and Pb in µg g^{-1} tissue dry weight in both tested stages of plant life as well as in cultivated soil. The absorbed Zn sharply reduced as a result of Pb treatment in both tested growth stages in the three plant organs.

This reduction was more pronounced in the second stage of growth, particularly in roots and stems. Only T2-treated leaves of older plants maintained Zn tissue content that was not significantly different from control (90%). Nickel tissue contents did not show significant differences from control regardless the tested plant organ, Pb treatment or growth stage. Iron tissue contents increased in response to Pb treatment in roots and stems of young plants, but not in leaves. T2-treated young plants recorded Fe contents of 187%, 276% and 66% in roots, stems and leaves, respectively. In the 45 DAS stage Fe contents decreased in roots and stems in response to Pb treatment while maintainingthe valuesthe controls around

Table 5. Properties of cultivated soil and the effect of control, T1, T2 and T3 treatments on the contents of Cu, Zn, Ni, Fe and Pb (µg g^{-1} tissue dry weight) in roots, stems and leaves of jute plants in the first and second stages of growth. Values are represented as means of three replicates ± SE, n=3

Soil properties		pH	Organic matter (%)	Elements (µg g^{-1} soil)							
				Cu	Zn	Ni	Fe	Pb	K	Ca	Mg
		7.9±0.3	9.2± 0.47	1.57±0.11	6.92± 0.59	6.62±0.52	6.03± 0.48	2.56±0.19	450± 6.89	320±4.5	170± 3.82
Organ	Treatment	First stage (30 DAS)									
		Cu	%	Zn	%	Ni	%	Fe	%	Pb	%
Roots	Control	7.65±0.88	100	9.75±0.74	100	24±0.1.65	100	378±28.78	100	1.35±0.15	100
	T2	7.2±0.61	94	4.2±0.16*	43	26.55±1.15	111	705.1±58.4*	187	2.55±0.31*	189
	T3	8.25±0.44	108	6.45±0.46*	66	24.75±2.65	103	405±29.25	107	1.8±0.16*	133
Stems	Control	4.95±0.45	100	18.15±1.34	100	12.3±0.91	100	41.25±3.31	100	2.85±0.31	100
	T2	8.1±0.75*	164	14.1±1.05*	78	13.35±1.18	109	114±9.75*	276	3.6±0.29*	126
	T3	4.5±0.3	91	12.45±0.95*	69	13.95±1.2	113	44.25±4.5	107	2.55±0.16	90
Leaves	Control	10.65±0.9	100	10.8±0.76	100	14.1±1.33	100	127.5±9.7	100	2.4±0.14	100
	T2	5.55±0.3*	52	3.9±0.30*	36	11.85±1.03*	84	84±7.22*	66	1.65±0.13*	69
	T3	7.2±0.45*	68	5.55±0.32*	51	13.35±0.89	95	122.25±12.3	96	4.8±0.3*	200
Second stage (45 DAS)											
Roots	Control	7.8±0.61	100	13.35±1.23	100	23.7±1.81	100	897±47.5	100	3±0.14	100
	T2	7.95±0.59	102	4±0.25*	30	23.7±2.54	100	579±43.48*	65	2.55±0.17	85
	T3	8.1±0.74	104	4.54±0.44*	34	22.95±2.1	97	477±39.5*	53	5.55±0.44*	185
Stems	Control	7.21±0.6	100	21.75±1.76	100	22.8±2.9	100	138±12.75	100	4.95±0.28	100
	T2	5.42±0..57*	75	2.1±0.14*	10	19.05±1.65	84	81±6.6*	59	5.4±0.58	109
	T3	4.51±0.33*	63	2.1±0.16*	10	19.5±1.34	86	51±5.85*	37	4.35±0.42	88
Leaves	Control	6.75±0.72	100	10.8±0.74	100	13.05±0.89	100	161.25±11.1	100	3.6±0.28	100
	T2	7.21±0.63	107	9.75±0.88	90	10.5±0.87*	81	171.75±13.05	107	2.85±0.33*	79
	T3	4.79±0.44*	71	4.65±0.45*	43	10.95±1.21	84	165±13.95	102	3.3±0.43	92

Value with (*) are statistically different from their control at $p<0.05$.

control in leaves. Increased soil Pb concentrations increased Pb accumulation in the first growth stage, particularly in roots. Root Pb contents in comparison with control recorded 189% and 133% under T2 and T3 treatments, respectively. Pb contents in leaves in this first stage decreased to 69% of control under T2 treatment, whiledoubling doubled to 200% of control under T3 treatment. In the second stage, T2 treatment also resulted in decreased tissue accumulation of Pb in leaves (79% of control) as well as in roots (85% of control). With the exception of T3-treated old roots that recorded 185% of control Pb content, other values of Pb tissue contents in the second stage recorded values compared to their controls. It is noteworthy that young leaves under T2 treatment recorded the least tissue contents of all the five estimated heavy metals in comparison with their controls (52%, 36%, 84%, 66% and 69% for Cu, Zn, Ni, Fe and Pb, respectively). This trend was not recorded in older plants under the same treatment.

The influence of Pb treatment of translocation factors to leaves anthe total accumulationon of metals in stems and leaves of the five estimated heavy metals is represented in Table 6. In young plants, Pb treatment resulted in decreased translocation factors of all tested heavy metals, except Pb under T3 treatment. In particular, TF of Fe and Pb decreased sharply under T2 treatment at this stage (0.12 compared to 0.34 of the controls for Fe and 0.65 compared to 1.78 of control for Pb). On the contrary, the second growth stage recorded higher translocation factors under Pb treatment in comparison to control in most cases except Pb TF which decreased in T2 and T3 plants. Pb treatment resulted in a high increase in TF of Zn in 45 DAS stage in comparison to control (0.81, 2.44 and 1.03 under control, T2 and T3 treatments, respectively). Stem total accumulation of all tested metals increased under different Pb treatments in both stages, except stem total accumulation of Zn under T2 and T3 treatments in the second stage as well as stem accumulation of Fe under T3 treatment on the same stage. In leaves total accumulation of Cu and Zn decreased due to T2 and T3 Pb treatments in the first stage and also due to T3 treatment in the second stage. Otherwise, all values of leaves total accumulation were higher than their corresponding controls.

Discussion

The results of this work recorded increased growth parameters of *Corchorus olitorius* stems and leaves in response to increased Pb soil concentrations. The tested Pb concentrations are within the permissible levels for cultivated soils (<300 mg kg^{-1}) (Xiao et al., 2018). However, since Pb is not a nutritional element and does not have any known physiological function in plant metabolism, it is not expected to stimulate plant growth even if added in low concentrations. This growth stimulation is due to the hormetic effect of Pb as a toxic heavy metal on *C. olitorius*. Although many researchers have studied the effects of heavy metal toxicity on plants, only few works were conducted to study the stimulatory effect of sub-toxic levels of heavy metals, the phenomenon of hormesis. However, hormesis has been gaining more and more interest (Reuscher et al., 2016). It was reported that Pb had a stimulatory effect on growth of *C. olitorius* (Ndlovu et al., 2019), corn (Malkowski et al*.,* 2020), *Arabis paniculata* (Milner et al., 2013), *Anthyllis vulneraria* (Piwowarczyk et al., 2018) and *Pisum sativum* (Wo´zniak et al., 2017).

According to Ndlovu et al. (2019) studying the effect of Pb soil concentrations of 150-1000 mg kg^{-1} soil on *C. olitorius*, this plant had the ability to grow under toxic Pb soil levels without showing visible morphological signs of toxicity. Some treatments even resulted in stimulating growth, yet

Table 6. Leaves translocation factors (TF), stems total accumulation and leaves total accumulation (µg/organ) of Cu, Zn, Ni, Fe and Pb under control, T2 and T3 treatments of jute plants in their first and second stages of growth. Values are represented as means of three replicates ± SE, n=3

	Treatment	30 DAS					45 DAS				
		Cu	Zn	Ni	Fe	Pb	Cu	Zn	Ni	Fe	Pb
TF	Control	1.39±0.11	1.11±0.14	0.59±0.05	0.34±0.02	1.78±0.12	0.87±0.06	0.81±0.07	0.55±0.07	0.18±0.02	1.2±0.01
	T2	0.77±0.08*	0.93±0.09	0.45±0.07*	0.12±0.01*	0.65±0.04*	0.91±0.1	2.44±0.18*	0.44±0.03*	0.30±0.04*	1.12±0.09
	T3	0.87±0.07*	0.86±0.09*	0.54±0.08	0.30±0.02	2.67±0.18*	0.59±0.05*	1.03±0.07*	0.48±0.06	0.35±0.04*	0.60±0.04*
Stems total accumulation (µg)	Control	3.9±0.44	14.56±1.21	9.91±1.18	33±2.86	2.25±0.14	6.45±0.58	30.15±2.1	20.52±0.91	124.2±9.73	4.51±0.29
	T2	1.71±0.1.05*	20.25±2.23*	19.18±1.64*	164.1±14.25*	5.27±0.58*	10.21±0.78*	61.04±2.55*	36.15±2.86*	153.9±14.1*	10.21±1.2*
	T3	6.15±0.73*	16.95±1.23*	19.05±1.48*	60.14±6.29*	3.46±0.29*	7.07±0.33	47.41±4.34*	30.46±3.31*	79.51±7.34*	6.76±0.73*
Leaves total accumulation (µg)	Control	17.4±0.1.35	17.71±1.78	23.11±1.82	209.11±14.69	3.9±0.45	11.41±1.35	272.25±19.5	21.91±1.36	270.89±18.5	6±0.47
	T2	13.95±1.38*	9.74±0.77*	29.69±2.39*	210±15.26	4.21±0.32	23.69±2.55*	483.6±40.5*	34.65±4.66*	566.85±31.5*	9.45±0.89*
	T3	14.1±0.16*	10.96±0.89*	26.09±2.12	239.55±17.24	10.05±0.74*	10.05±1.19	146.3±15.9*	22.96±3.14	346.5±22.81*	6.9±0.88

Value with (*) are statistically different from their control at $p < 0.05$.

plants accumulated toxic concentrations of Pb inside their edible tissues. Plants with stimulating growth due to subjection to higher Pb concentrations are more likely to be chosen for harvest by rural communities for their better fresh and dry weights, number of leaves and other morphological parameters. Therefore, studying metabolites of these plants is crucial to evaluate the Pb influence on their nutritional value. This work studies the influence of lower Pb concentrations (up to 120 mg kg^{-1} soil) on *C. olitorius*. All of the three tested Pb concentrations stimulated growth parameters of stems and leaves of jute plants in the two tested stages, particularly T2-treated older plants. Different parameters in this work were measured in two stages of plant life, 30 and 45 days after sowing (DAS) in order to follow up plant response to increasing soil Pb concentrations at different stages. Moisture content of *C. olitorius* leaves and stems from this work was hardly affected by Pb application, which is in accordance with the results obtained by (Ndlovu et al., 2019). They also recorded declineschlorophyllrophylls contents in response to Pb treatment. In the present study, Pb treatment did not have significant effects on pigment contents in young plants, chlorophyll b contents were even stimulated in this stage. At the second stage, the negative impact of Pb on photosynthetic pigments was recorded only at the highest Pb concentration. This might be related to the long term subjection to the elevated soil Pb levels, which also a general reduction in the absolute values of pigments in older plants compared to young plants. Pb is known for its interference with photosynthesis (Khan et al., 2013).

The total antioxidant activity in leaves as estimated by the DPPH assay was inversely proportional to fresh and dry weights. The least growth and higher total antioxidant activity were recorded in control leaves while the highest fresh and dry weights accompanied with the least total antioxidant activity were recorded in T2 treated leaves in both stages (60% and 50% of control plants antioxidant activity in young and old plants, respectively). In the 30 DAS stage, ascorbic acid contents decreased sharply in parallel to decreased total antioxidant activity, which might refer to the essential role of ascorbic acid in the antioxidant system of jute plants, particularly in the young stage of growth. Jute is well known for its high contents of ascorbic acid (Ahmed & Slima, 2018). Most experiments studying Pb effect on plants have reported an increase in the reactive oxygen species (ROS) level in response to Pb treatment that was accompanied by an inhibition of growth (Sidhu et al., 2016; El-Banna et al., 2018). However, in experiments where growth stimulation due to heavy metal treatment was recorded, there was no significant change in the level of oxidative stress (Lin et al., 2007; Jia et al., 2013).

Previous work (Ndlovu et al., 2019) recorded a significant decline in Pb concentration of stems and leaves from immature to mature stages of *C. olitorius* plants exposed to 900 mg kg^{-1} Pb soil concentration and attributed this to increased Pb phytostabilization in roots of *C. olitorius* rather than aerial parts as suggested by other researchers (Shi et al., 2019). This is in accordance with the data obtained from the present study. In T3-treated plants, Pb accumulated in roots recorded 133% of control in young plants compared to 185% of control in old plants. On the other hand, Leaves contents of Pb recorded 200% of control in young plants compared to 92% of control in older plants. Therefore, Pb translocation factors recorded 2.67 in T3-treated young plants against 0.6 in T3-treated old plants. High accumulation of Pb in roots of *C. olitorius* and relatively less translocation to aerial plant parts is similar to the records in edible plants, including *Chenopodium album, Malvastrum coromandelianum* and *Amaranthus viridis* (Malik et al., 2010), as well as *Mentha arvensis* (Jezler et al., 2015). Pb is characterized by low translocation from roots to shoots (Malkowski et al. 2019) therefore it causes a disturbance in the proper functioning of roots, which has a negative influence on plants mineral nutrition (Ismail et al., 2013). T2 treatment, the treatment that resulted in the highest growth and most prominent hormetic effect in this work, recorded the least leaves tissue accumulation of Pb, as well as the other estimated heavy metals, in comparison with control young plants. This might refer to an exclusion mechanism adapted by jute plants against Pb toxicity. The same trend was not recorded in the second stage of growth, which suggests different defense mechanisms in different growth stages. Other heavy metals were estimated to study potential interactions between these elements and Pb treatment. Dramatic reduction in Zn tissue contents was notable in the different organs in both stages in response to Pb treatment. Despite the decreased plant contents of Zn under Pb treatment, T2-treated jute plants (treatment that caused the best hormetic effect) directed most of its absorbed Zn contents towards leaves, where they are most needed. The translocation factor of Zn was 2.44 in T2-treated 45 DAS leaves compared to 0.81 for control plants. On the contrary, no antagonism was recorded between increased Pb tissue contents and Fe in this experiment. Fe contents decreased in the second growth stage. However, most of the absorbed Fe was directed towards leaves where it was required for its critical role in photosynthesis. T2-treated plants which showed the best hormetic response to Pb were characteristically successful in the internal distribution of essential elements (particularly Zn and Fe) towards the leaves, where they were majorly required.

Conclusion

The results of this work concluded that cultivating *C. olitorius* in soils with Pb concentrations within the permissible levels had a hormetic effect and stimulated plant growth. However, it might affect the nutritional value of this plant as a part of human diet and as a medicinal plant by influencing its characteristic contents of antioxidants as well as absorption and accumulation of essential micronutrients.

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