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Agro-morphological changes caused by the accumulation of lead in *Corchorus olitorius*, a leafy vegetable with phytoremediation properties

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Summary

Lead (Pb) can enter the food chain through the consumption of contaminated plants and can cause serious health issues. However, research on how Pb accumulation affects morphology of leafy vegetables in South Africa is minimal. This study tested the effect of lead accumulation on vegetative and reproductive traits of *Corchorus olitorius*. Plants were grown under varying Pb concentrations, and studied for their variation in vegetative and reproductive traits as well as Pb accumulation in leaves, stems and roots. Plants grown within allowable soil concentrations of 150 mg kg^{-1} Pb accumulated toxic (≥ 10 mg kg^{-1}) Pb in all plant parts without causing any morphological defect, except for a decrease in chlorophyll content. Minor reductions in growth and yield were evident only at 900-1000 mg kg^{-1} concentration. Pb accumulation increased as its concentration increased in the soil, with a higher accumulation in roots in comparison to aerial parts. In conclusion, *C. olitorius* can grow and reproduce under toxic Pb levels (≥ 300 mg kg^{-1}) and accumulate toxic amounts of Pb (≥ 10 mg kg^{-1}) without visible morphological defects. Therefore, it is suitable for phytoremediation but unsafe for consumption when it is collected from sites prone to Pb contamination.

Keywords: *Corchorus olitorius*, lead heavy metal, plant accumulation, vegetative and yield traits

Introduction

Contamination of agricultural soils with lead (Pb) is a major problem for agricultural production and human health (FATTAHI et al., 2019). Lead, a heavy metal that is not essential for plant growth, can be accumulated in excess by plants and become toxic to them (SHEORAN et al., 2016). Lead soil contamination results from sources such as smelting, combustion of leaded gasoline and application of lead-contaminated sewage sludge as fertilizer (POURRUT et al., 2011). The maximum allowable Pb concentration in agricultural soils is 300 mg kg^{-1} (XIAO et al., 2018). The lowest detected value is 0.3 mg kg^{-1} (AHMAD et al., 2019) and the maximum permissible limit is 10 mg kg^{-1} (WHO, 1998 cited in JEZLER et al., 2015) Pb within edible plant parts.

Phytoremediation is a process of growing plants in contaminated soils to either remove heavy metals (phytoextraction and phytovolatilization) or stabilize them into a harmless status (phytostabilization or phytoimmobilization) (KHALID et al., 2017; Liu et al., 2018). Hyperaccumulators are plants that can accumulate metals and metalloids at concentrations 100 times greater than that of normal plants growing in the same environment (SHEORAN et al., 2016). Certain plants within the Asteraceae and Brassicaceae families can accumulate > 1 000 mg kg^{-1} of Pb (SHEORAN et al., 2016). Some wild leafy vegetables are good accumulators of Pb, too. *Amaranthus viridis* is recommended for Pb phytoextraction, whereas *Solanum nigrum* is recommended for phytostabilization of Pb-contaminated soils (MALIK et al., 2010).

Corchorus olitorius L., a leafy vegetable commonly known as Jute mallow, is an annual erect herb that grows in fields, home gardens (TOVIHOUDJI et al., 2015) and on roadsides (SANYAOLU et al., 2011). Its leaves are in abundance of iron, folate, protein, fibre, calcium, riboflavin, carotene, vitamin C and phenols, and have high zinc bioavailability and appreciable amounts of other proximate components and minerals (ISUOSUO et al., 2019). Cooked leaves and tender shoots that are eaten along with food staples are recommended for pregnant and nursing mothers because of their high iron content (SANYAOLU et al., 2011). Leaves of *C. olitorius* are used in folk medicines for different ailments and they possess antioxidant, hepatoprotective and antidiabetic properties (SALIU et al., 2019). This species has the ability to accumulate 0.31 mg kg^{-1} of Pb in its leaves when it grows at ≤ 10 m away from the major roads, which is slightly above the lowest detected value (0.3 mg kg^{-1}) for consumption purposes (SANYAOLU et al., 2011). Consumption of lead contaminated food including vegetables in particular, cause health problems such as brain and kidney diseases (FATTAHI et al., 2019).

Lead uptake is primarily through the roots, but some plants such as *Brassica napus* also acquire it through the leaves (RUBIO et al., 2019). Roots can accumulate large amount of Pb but restrict its movement towards the shoots (POURRUT et al., 2011). The uptake of Pb from the soil by plants increases with an increase of its concentration in the soil – availability being the highest in loam and sandy soils – at higher soil moisture levels – and low pH (SHEORAN et al., 2016). Vegetable and medicinal plants such as *Ocimum basilicum* show similar plant height, internode length, leaf length and width as the control when exposed to 400 mg kg^{-1} Pb (FATTAHI et al., 2019); which is a major concern because this concentration is above the maximum allowable soil concentration (300 mg kg^{-1}) (XIAO et al., 2018).

Lead toxicity can induce different morphological, physiological and biochemical effects on plants at different stages of plant growth and contamination (POURRUT et al., 2011). Increase in Pb levels in the soil retards seed germination, seedling growth and fresh weight of roots and shoots (YAHAGHI et al., 2019). It also impairs mitosis, root and shoot growth, transpiration, chlorophyll production, lamellar organization in the chloroplast and results in leaf chlorosis (POURRUT et al., 2011). Increased Pb concentration in the soil also reduces the moisture content of roots, stems and leaves (YILMAZ et al., 2009).

Improved and/or similar morphological features of some edible plants such as *Ocimum basilicum* grown under toxic Pb concentrations (≥ 300 mg kg^{-1}) and had accumulated toxic concentrations (> 0.3 mg kg^{-1}), when compared with untreated plants (FATTAHI et al., 2019), have high potential of intoxicating consumers. When people from rural areas accidentally harvest such Pb contaminated vegetables for food and medicinal purposes, they can be intoxicated by high concentrations or gradual Pb accumulation in their system. As *C. olitorius* is one of the leafy vegetables that grow in areas prone to Pb contamination, it is essential to study its Pb accumulation potential. Depending on the type of species, plants that have accumulated toxic Pb amounts can either have better, retarded or similar

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growth and yield than plants without Pb. Therefore, the objective of this study was to determine variation in vegetative and reproductive traits of *C. olitorius* accumulating different toxic Pb concentrations.

Materials and methods

Seed sourcing, study area and experimental design

Seeds of *Corchorus olitorius* L. were sourced from the Agricultural Research Council in Roodeplaat, Pretoria. Subsequent experiments were conducted at the University of Zululand (28.85416° S, 31.84565° E), Department of Botany, in a rain-free environment. A black, humus-rich soil was collected from the university's farm and had its properties analysed using a method described by Manson and Roberts (2000) (Tab. 1). Twenty-litre plastic pots were filled with soil mixed with lead in the form of lead acetate [$Pb(NO_3)_2$], applied at 0, 150, 300, 600, 900 and 1000 mg kg^{-1} soil as modified from Khodaverdiloo et al. (2011). These values were chosen to include the maximum allowable (≤ 300 mg kg^{-1}) and toxic (> 300 mg kg^{-1}) Pb levels in agricultural soils (Xiao et al., 2018). The experiment was laid out in a randomized complete block design with five replications. Ten seeds of *C. olitorius* were germinated in each pot and later thinned into one plant per pot, followed by application of 2:3:2 (27) NPK fertilizer at a rate of 1 g kg^{-1} of soil. Plants were regularly irrigated with deionised water (50 ml per pot), which was then collected from the base of the pots and reused to irrigate, in order to avoid nutrient or heavy metal loss.

Measurement of agronomic traits

Germination percentage was recorded at seven days after sowing (DAS), prior to thinning. All vegetative traits were measured at 44 DAS, whereas the number of branches and all reproductive traits were measured at 85 DAS, in quintuplicate. Plant height (cm) was measured from the soil level to the tip of the stem using a ruler. The numbers of leaves and branches were counted manually. Vernier callipers were used to measure stem width (mm) at 10 cm from the soil level. Leaf area (length × width) (cm^2) was recorded on the fourth leaf from the apex using a ruler. The leaf chlorophyll content of the fifth oldest leaf (from the apex of the main stem) was captured with a chlorophyll content meter (CCM-200). Five different spots were randomly measured in the leaf and provided the average leaf chlorophyll content.

Plants were uprooted, washed with distilled water and blot-dried with a paper towel, and had their root length (cm) measured from the root tip to the base of the stem using a ruler. The fresh mass (g) of roots, stems and leaves of uprooted plants were measured separately. Separated plant parts were oven dried at 60 °C until constant dry mass. The dry mass (g) and moisture content of roots, stems and leaves were also determined separately. The number of pods per plant was counted manually, and pod length (cm) and width (cm) determined with Vernier callipers. Seed traits were determined from five pods per plant per treatment. The number of seeds per pod was counted manually; total seed mass (g) per pod and 100-seed mass (g) in each pod were also recorded.

Tab. 1: Properties of the soil used in the research

Soil property	Value (mg kg^{-1} for elements)
P	10
K	170
Mg	1432
Na	525
Zn	22
Cu	3
Mn	6
Fe	395
pH	4.5
Clay content	23.0%
Organic matter	4.3%

Lead content of plant parts

Plants were analysed for lead accumulation in roots, stems and leaves at both seedling (44 DAS) and termination or maturity (85 DAS) stages. Three pots were selected for harvesting and each pot was used as a replicate for each plant part. Plants were carefully uprooted, thoroughly washed with distilled water and blot-dried with a paper towel. Plants were then separated into leaves, stems and roots. Each plant part (in three separate replicates) was cut into pieces and further dried in an oven at 80 °C until they reached a constant weight. Dried samples were ground into powder and packaged in air-tight plastic containers and stored in a fridge (–4 °C) for further analysis.

One gram of each milled sample was dissolved in 5 ml of 60% hydrochloric acid and 10 ml of 70% nitric acid, and then digested at a moderate temperature of 50 °C until white fumes evolved, and the solution changed to a brownish colour. The heat was further intensified for few minutes to expel most of the HCl. 50ml distilled water were added, heated for few minutes and allowed to cool. The solution was filtered through a Whatman's No. 1 paper into a transparent plastic container, and was allowed to settle for a few minutes for the aspiration of the lead accordingly. The digested sample was analysed for lead concentration using an atomic absorption spectrophotometer.

Statistical analysis

Data were subject to analysis of variance (one-way ANOVA) in GenStat 12.1 version. Means were separated using Tukey's Multiple Range Test in GenStat at a 5% level of significance. Correlation matrix analysis also determined the relationship between vegetative and reproductive traits.

Results

Vegetative traits

Some vegetative traits of plants treated with lead differed significantly ($p < 0.05$) from each other and/or the untreated plants except for root length; number of branches, and leaf fresh mass (Tab. 2). Lead application at 300 mg kg^{-1} was associated with the maximum germination rate (100%) of *C. olitorius* seeds, whereas the minimum germination rate (60%) corresponded with 1000 mg kg^{-1}. A significant reduction in seed germination was recorded for plants exposed to 900-1000 mg kg^{-1} Pb compared with the control. Plants exposed to 150-600 mg kg^{-1} Pb were taller than untreated plants, whereas the shortest plants were exposed to 1000 mg kg^{-1}. The plants treated with 1000 mg kg^{-1} Pb had the thinnest stems compared with the control, but their stem girth was similar to all other treated plants.

Exposure of plants to 150 mg kg^{-1} Pb promoted the formation of numerous leaves, whereas 1000 mg kg^{-1} Pb drastically reduced the number of leaves per plant compared with the control. Leaves gradually became smaller as the concentration of Pb increased above 300 mg kg^{-1}. Plants treated with Pb had a significantly lower chlorophyll content than untreated plants. Only stems and leaves of plants exposed to 150 mg kg^{-1} Pb had increased fresh and dry mass, respectively. This treatment also caused an increase in stem moisture content, but a reduction in leaf moisture content. However, a reduction in leaf moisture content was only significant when compared with untreated plants and 1000 mg kg^{-1} Pb.

Tab. 2: Effect of lead on the vegetative traits of *C. olitorius*.

Conc.	GP	RL	PH	SG	NB	NL	LA	LCC	LFM	SFM	RFM	LDM	SDM	RDM	LMC	SMC	RMC
0.00	90.0^{ab}	24.7^{a}	38.00^{c}	5.09^{a}	8.0^{a}	25.8^{bc}	54.13^{a}	54.07^{a}	7.27^{ab}	5.55^{a}	1.34^{abc}	1.08^{b}	0.70^{b}	0.51^{a}	84.90^{b}	61.70^{b}	87.20^{b}
150	86.7^{ab}	26.3^{a}	43.60^{b}	4.86^{ab}	8.8^{a}	34.4^{a}	49.90^{ab}	44.54^{b}	8.59^{a}	8.77^{a}	1.93^{a}	2.64^{a}	1.29^{a}	0.16^{b}	69.33^{d}	91.70^{a}	89.30^{ab}
300	100^{a}	26.0^{a}	44.20^{b}	4.68^{ab}	8.0^{a}	30.4^{ab}	47.11^{b}	44.04^{b}	4.79^{c}	9.16^{a}	1.44^{ab}	0.89^{b}	0.83^{b}	0.46^{a}	81.50^{bc}	65.20^{b}	91.07^{ab}
600	70.0^{ab}	23.7^{a}	49.80^{a}	4.79^{ab}	7.8^{a}	24.0^{c}	46.73^{b}	37.77^{c}	6.04^{bc}	6.41^{a}	1.27^{abc}	1.28^{b}	0.83^{b}	0.44^{a}	78.83^{c}	65.90^{b}	87.13^{b}
900	73.3^{ab}	26.3^{a}	36.00^{c}	4.44^{ab}	7.4^{a}	20.8^{c}	36.22^{c}	43.53^{b}	6.02^{bc}	5.15^{a}	0.99^{bc}	1.14^{b}	0.65^{b}	0.32^{ab}	81.10^{bc}	67.90^{b}	87.07^{b}
1000	60.0^{b}	16.0^{a}	29.80^{d}	4.05^{b}	7.2^{a}	14.2^{d}	34.76^{c}	34.52^{c}	6.30^{bc}	6.11^{a}	0.57^{c}	0.72^{b}	0.41^{c}	0.21^{b}	91.33^{a}	71.20^{b}	93.27^{a}
GM	80.0	23.8	40.23	4.65	7.87	24.93	44.84	43.08	6.50	6.86	1.26	1.29	0.79	0.35	81.17	70.60	89.17
CV%	16.3	20.3	6.3	9.2	14.7	12.1	6.6	4.5	8.9	38.1	23.9	18.5	9.9	18.8	2.2	8.8	2.0
LSD	23.72	8.81	3.35	0.57	1.53	3.98	3.91	2.56	1.06	4.75	0.55	0.44	0.14	0.12	3.23	11.33	3.19
P-value	0.033	0.159	<.001	0.02	0.36	<.001	<.001	<.001	<.001	0.35	0.005	<.001	<.001	<.001	<.001	0.002	0.006

Means followed by different superscript(s) within a column vary significantly ($P < 0.05$). Conc. (concentration (mg kg^{-1})); GP (germination percentage (%)); RL (root length (cm)); PH (plant height (cm)); SG (stem girth (mm)); NB (number of branches); LA (leaf area (cm^2)); NL (number of leaves); LCC (leaf chlorophyll content (mg cm^{-2})); LFM (leaf fresh mass (g)); SFM (stem fresh mass (g)); RFM (root fresh mass (g)); LDM (leaf dry mass (g)); SDM (stem dry mass (g)); RDM (root dry mass (g)); LMC (leaf moisture content (%)); SMC (stem moisture content (%)); RMC (root moisture content (%)).

Reproductive traits

Reproductive traits of Pb-treated plants differed significantly among each other and from the untreated plants, except for the number of pods per plant (Tab. 3). A reduction in pod length was recorded in plants treated with 900-1000 mg kg^{-1} Pb, when compared with the control. Only pods from plants treated with 1000 mg kg^{-1} were lighter than those of untreated plants. Although the number of seeds per pod of the untreated plants were insignificantly different from all treated plants, 150 mg kg^{-1} produced plants with more numerous seeds per pod than plants treated with 900–1000 mg kg^{-1}. Total seed mass of plants grown under 150 mg kg^{-1} was higher than seeds of untreated plants and those treated with ≥ 600 mg kg^{-1}. Also, treatment with 150 mg kg^{-1} resulted in plants with a heavier 100-seed weight than untreated plants and those exposed to 600 and 100 mg kg^{-1}.

Lead accumulation and correlation matrix

Lead accumulation differed significantly within each plant part and among different plant parts at seedling (44 days after sowing) and termination or maturity (85 days after sowing) stages (Tab. 4). Among the treated plants, mature roots of plants exposed to 900 mg kg^{-1} had accumulated the most Pb (634.0 mg kg^{-1}); whereas mature leaves treated with 1000 mg kg^{-1} had the least (22.8 mg kg^{-1}). Accumulation of a range from 22.8-78.8 mg kg^{-1} in immature and mature stems and leaves of plants exposed to different Pb concentration in the soil did not differ significantly with the untreated plants without Pb.

At seedling stage, application of 1000 mg kg^{-1} resulted in plants with the highest Pb accumulation (448.8 mg kg^{-1}) in roots; but the lowest (67.0 mg kg^{-1}) in stems, when compared with all other treatments. At both stages of growth, an increase in Pb soil content resulted in its high accumulation in the roots. However, in immature stems and leaves, the increase in accumulation was evident up to the maximum of 900 mg kg^{-1} Pb and then declined drastically at 1000 mg kg^{-1}. In mature stems, the increase was only evident in plants exposed to 1000 mg kg^{-1}; whereas leaves were not significantly different from each other. Also, in each Pb concentration in the soil, the accumulation was the highest in roots and then decreased relatively similarly in stems and leaves, at both stages of growth.

Almost all traits had a significant positive correlation with at least 50% of the traits investigated, except for root dry mass and leaf moisture content (Tab. 5). Most vegetative traits correlated significantly with one another except for leaf chlorophyll content, root dry mass, and moisture content of leaves, stems and roots. Further, pod and seed traits were significantly correlated with one another and to most of the vegetative traits, except root fresh mass; leaf and root dry mass, as well as leaf, stem and root moisture content.

Tab. 3: Effect of lead on the reproduction traits of *C. olitorius*.

Conc.	NP	PL	PM	SPP	TSM	100-SM
0	7.00^{a}	6.35^{ab}	5.50^{a}	114.4^{ab}	0.12^{b}	0.11^{bc}
150	5.50^{a}	4.97^{c}	5.40^{a}	139.6^{a}	0.25^{a}	0.15^{a}
300	6.75^{a}	6.48^{ab}	5.38^{a}	121.4^{ab}	0.17^{ab}	0.17^{a}
600	6.25^{a}	6.62^{a}	5.37^{a}	113.0^{ab}	0.13^{b}	0.14^{ab}
900	2.25^{a}	4.77^{c}	4.58^{ab}	98.4^{b}	0.15^{b}	0.15^{a}
1000	6.50^{a}	5.42^{bc}	4.11^{b}	86.6^{b}	0.10^{b}	0.09^{c}
GM	5.71	5.77	5.06	112.2	0.15	0.13
CV%	40.5	8.4	9.1	16.0	31.3	14.8
LSD	3.48	0.73	0.69	23.74	0.06	0.03
P-value	0.092	<.001	0.003	0.003	0.001	<.001

Means followed by different superscript(s) within a column vary significantly ($P < 0.05$). NP (number of pods); PL (pod length (cm)); PM (pod mass (g)); SPP (number of seeds per pod); TSM (total seeds mass (g)); 100-SM (100 seed mass (g)).

Tab. 4: Accumulation of Pb in roots, stems and leaves of *C. olitorius*.

Harvest stage (days after sowing)	Pb in soil (mg kg^{-1})	Pb in plant parts (mg kg^{-1})		
		Roots	Stem	Leaves
44	0	0.0^{l}	0.0^{l}	0.0^{l}
	150	185.5^{ef}	$78.8^{g–l}$	$110.5^{f–k}$
	300	276.8^{de}	$78.2^{g–l}$	$136.2^{f–i}$
	600	340.8^{cd}	159.2^{fgh}	179.5^{efg}
	900	409.5^{bc}	202.5^{ef}	212.0^{ef}
	1000	448.8^{b}	$67.0^{h–l}$	$68.6^{h–l}$
85	0	0.0^{l}	0.0^{l}	0.0^{l}
	150	$129.2^{f–j}$	$33.2^{i–l}$	$34.5^{i–l}$
	300	212.5^{ef}	$37.5^{i–l}$	$39.2^{i–l}$
	600	483.8^{b}	$33.2^{i–l}$	$42.0^{i–l}$
	900	634.0^{a}	$41.0^{i–l}$	26.2^{jkl}
	1000	596.8^{a}	$116.0^{f–k}$	22.8^{kl}

Means followed by different superscript(s) within a column and a row vary significantly ($P < 0.05$)

Tab. 5: Correlation matrix among vegetative and reproductive traits.

Traits	RL	PH	SG	NB	NL	LA	LCC	LFM	SFM	RFM	LDM	SDM	RDM	LMC	SMC	RMC	NP	PL	PW	SPP	TSM
PH	0.53																				
SG	**0.84**	**0.86**																			
NB	**0.83**	**0.82**	**0.95**																		
NL	0.52	**0.90**	**0.81**	**0.76**																	
LA	0.56	**0.94**	**0.91**	**0.87**	**0.90**																
LCC	0.40	**0.85**	**0.78**	**0.73**	**0.83**	**0.90**															
LFM	**0.89**	0.57	**0.85**	**0.86**	0.56	**0.63**	0.57														
SFM	**0.90**	**0.71**	**0.75**	**0.79**	**0.86**	**0.71**	0.59	**0.72**													
RFM	**0.90**	0.56	**0.80**	**0.77**	**0.68**	**0.60**	0.45	**0.88**	**0.80**												
LDM	**0.77**	0.54	**0.71**	**0.73**	**0.68**	0.56	0.43	**0.81**	**0.90**	**0.88**											
SDM	**0.86**	**0.68**	**0.83**	**0.86**	**0.75**	**0.68**	0.49	**0.82**	**0.89**	**0.90**	**0.92**										
RDM	**0.64**	**0.81**	0.54	0.56	0.29	0.37	0.39	0.55	0.23	0.47	0.24	0.43									
LMC	**0.79**	0.39	**0.71**	**0.66**	0.30	0.44	0.33	**0.78**	0.42	**0.65**	0.44	0.53	0.54								
SMC	**0.73**	0.28	**0.60**	0.55	0.35	0.34	0.15	**0.75**	**0.61**	**0.80**	**0.68**	**0.63**	0.31	**0.84**							
RMC	**0.83**	0.48	**0.77**	**0.71**	0.43	0.51	0.37	**0.83**	**0.58**	**0.76**	0.58	**0.65**	0.51	**0.98**	**0.91**						
NP	**0.67**	**0.88**	**0.88**	**0.90**	**0.79**	**0.90**	**0.77**	**0.61**	**0.64**	0.59	0.54	**0.74**	0.48	0.38	0.23	0.44					
PL	**0.62**	**0.87**	**0.86**	**0.91**	**0.75**	**0.91**	**0.78**	**0.60**	**0.61**	0.52	0.45	**0.67**	0.50	0.43	0.23	0.47	**1.00**				
PW	**0.62**	**0.89**	**0.88**	**0.93**	**0.79**	**0.93**	**0.80**	**0.64**	**0.69**	0.56	0.55	**0.72**	0.45	0.42	0.27	0.48	**0.97**	**0.99**			
SPP	**0.61**	**0.88**	**0.87**	**0.90**	**0.77**	**0.93**	**0.83**	0.59	**0.59**	0.51	0.44	**0.64**	0.52	0.43	0.21	0.47	**1.00**	**0.99**	**0.98**		
TSM	**0.61**	**0.87**	**0.8**	**0.86**	**0.79**	**0.84**	**0.69**	0.51	**0.67**	0.53	0.46	**0.72**	0.45	0.37	0.23	0.44	**0.93**	**0.95**	**0.93**	**0.93**	
100-SM	**0.66**	**0.81**	**0.81**	**0.90**	**0.74**	**0.81**	**0.65**	0.58	**0.69**	0.58	0.55	**0.76**	0.45	0.37	0.27	0.45	**1.00**	**0.96**	**0.95**	**0.93**	**0.96**

Values ≥ 0.6 in **bold** are significant. Traits are described in Tab. 2 and 3.

Discussion

Phytoremediation and toxic consumption potential of *C. olitorius* exposed to lead contaminated soils

Corchorus olitorius species accumulated toxic lead content (> 10 mg kg^{-1}) even when grown at concentrations below the maximum allowable soil levels (300 mg kg^{-1}), but had very low morphological defects even at the exposure to high Pb concentrations in the soil. However, its herein observed maximum Pb accumulation of 634 mg kg^{-1} does not qualify it as hyperaccumulator (SHEORAN et al., 2016). This plant is good for phytoextraction purposes, because it can grow well in Pb contaminated soil, remove it and accumulate it within its plant parts (LIU et al., 2018). It can also be recommended for phytostabilization purpose because it accumulates high levels of Pb in its roots and transport limited amount to the aerial parts (KHALID et al., 2017). The low phytotoxicity was probably caused by the retention of more Pb in roots and less translocation to the stems and leaves (shoots) (SHI et al., 2019). Although this retention was evident in the study, but the concentration that ranges from 22.8-212.0 mg kg^{-1} accumulated in its stems and leaves, is very high for the maximum recommended 10 mg kg^{-1} Pb within the consumed plant part (JEZLER et al., 2015). Statistical analysis showed insignificant differences of accumulated concentrations from 0-100 mg kg^{-1} Pb among the *C. olitorius* plant parts; but this can be disputed and consider the maximum allowable concentration of 10 mg kg^{-1} Pb for consumption purposes.

A 150 mg kg^{-1} Pb concentration in the soil, which is half the maximum limit of 300 mg kg^{-1} (XIAO et al., 2018), either promoted growth and yield in *C. olitorius* or induced traits that were similar to the untreated plants. These included plants with numerous leaves that were relatively large, with higher fresh and dry masses — increased shoot dry mass and moisture content — as well as heavier total and 100-seeds weight. These features can make the plant to be selected for vegetable harvest by rural communities when they had accumulated toxic amounts of 78.8 and 33.2 mg kg^{-1} in stems as well as 110.5 and 34.5 mg kg^{-1} in leaves at immature and mature stages, respectively. *C. olitorius* accumulates Pb at low soil concentrations, but Pb becomes intensified within the plant leading to toxic amounts for consumption purposes. There was also a significant decline in Pb concentration of stems (202.5–41.0 mg kg^{-1}) and leaves (212.0–26.2 mg kg^{-1}) from immature to mature stages of *C. olitorius* plants exposed to 900 mg kg^{-1} Pb soil concentration, respectively. This might result from an increased level of phytostabilization in this plant (KHALID et al., 2017; LIU et al., 2018), and enhanced passive mechanisms as even small amount of Pb penetrated root cell membranes, interacted with cellular components and increased thickness of the cell walls (POURRUT et al., 2011), as plants were forming nodule-like swellings in their roots in concentrations from 900–1000 mg kg^{-1} at maturity.

Effects of consumption of plants such as vegetables that are contaminated with Pb differs among individuals and their different stages of growth (DINIS and FIÚZA, 2011). As *C. olitorius* is recommended for pregnant and nursing mothers (SANYAOLU et al., 2011), its consumption with trace amounts of Pb might lead to severe health issues of both the mother and the foetus. Pregnant mothers, foetus and breastfeeding individuals are more susceptible to renal failure, cardiovascular diseases as well as neurological and mental disorders as a result of Pb toxicity (DINIS and FIÚZA, 2011; SARWAR et al., 2017). Therefore, precautions are necessary for the collection sites of these vegetables by pregnant women.

High accumulation of Pb in *C. olitorius* roots and relatively less translocation to the aerial parts is similar to the records in edible plants such as *Amaranthus viridis*, *Malvastrum coromandelianum and Chenopodium album* (MALIK et al., 2010), as well as *Mentha arvensis* (JEZLER et al., 2015). However, in *Ocimim bacilicum* Pb concentration was higher in leaves than roots when plants were exposed to 100 and 200 mg kg^{-1} Pb (FATTAHI et al., 2019). C. *olitorius* is a potential species for phytoremediation in Pb contaminated areas with advantages such as high germination rate, growth and yield rates on Pb intoxicated soils. It also quickly accumulates lead at very juvenile stages without any morphological defects (phytotoxicity), which is a requirement for a good species for phytoremediation purposes (SARWAR et al., 2017, LIU et al., 2018).

Agro-morphological traits

Insignificant changes in the germination rate of *C. olitorius* seeds sown in Pb treated soils compared with the control indicate that it can germinate successfully even in Pb concentrations far above the maximum allowable limit of 300 mg kg^{-1} in agricultural soils (Xiao et al., 2018). However, seeds of *Ocimum basilicum* had a significant decline in their germination percentage with an increase in Pb concentration from 0–80 mg L^{-1} (Fattahi et al., 2019). This also reveals that Pb toxicity varies between plant species, as plants with phytoremediation properties tolerate more Pb than sensitive ones (Pourrut et al., 2011). When Pb is in excess in the soil for a particular plant, it interferes with hydrolytic enzymes, including proteases and amylases that breaks down the cotyledons to initiate the germination process (Pourrut et al., 2011).

The insignificant differences between control and Pb exposed *C. olitorius* plants in terms of root length, number of branches, stem and root fresh mass and number of pods indicate that this species possesses some phytoremediation properties. The same holds for insignificances with the control for stem girth, number of leaves, dry mass of leaves, stems and roots, root moisture content, pod length and mass, number of seeds per pod and total seed mass when plants were exposed to toxic soil Pb levels (≥ 300 mg kg^{-1}) (Xiao et al., 2018).

Although Pb is a non-nutrient heavy metal (Sheoran et al., 2016), exposure of *C. olitorius* to mild soil concentration of 150 mg kg^{-1} resulted in taller plants, numerous leaves, heavier dried leaves and stems, higher stem moisture content, numerous seeds per pod as well as higher total seed and 100-seed mass than the control. A similar trend was recorded in *Ocimum basilicum* where Pb treatments (100–400 mg kg^{-1}) resulted in taller flowering stems as well as leaf collars and stem that are wider, than the control (Fattahi et al., 2019). This might result from prolific cell division and intensive reproduction as plants were exposed to heavy metal stress, which is the similar case as in *Eclipta prostrata* (Chandrasekhar and Ray, 2019).

High dry mass and low moisture content in leaves of plants exposed to 150 mg kg^{-1}, could have resulted from the loading of photosynthetic products in them, as they are the site of photosynthesis. However, the retain of both high dry weight and moisture content of stems was related to the taller plants at this Pb concentration. Therefore, more secondary tissues contributed to dry mass and moisture content relates to the stem as the passage for the transpiration and assimilation streams which both works with adequate moisture content. Roots at this concentration had a drastic reduction in their dry mass because they function primarily for water absorption, although their moisture content was basically the same as that of the control. However, the increase in root moisture content in plants exposed to 1000 mg kg^{-1} is evident of high Pb accumulation (448.8 mg kg^{-1}) at this concentration, which will facilitate more water uptake from the soil (Zhang et al., 2019).

Insignificant effect of Pb application on the length of *C. olitorius* roots was contrary to a decline in root length of *Solanum melongena* (Yilmaz et al., 2009) and *Spinacia oleracea* (Alia et al., 2015) caused by 150 and 300 mg kg^{-1} Pb levels, respectively. Resistance of *C. olitorius* is an indication of its phytoremediation potential and therefore reacts differently to Pb toxicity in the soil. The Pb inhibition on root growth depends on the lead and ionic composition and the pH of the medium (Pourrut et al., 2011). Interaction of Pb with the chemicals found in the soil results in reduced cell growth and formation, which results in root and shoot growth inhibition (Alia et al., 2015). Increase in height of plants exposed to 150–600 mg kg^{-1}, but a decrease only at 1000 mg kg^{-1} Pb concentration probably means that *C. olitorius* can resist and undergo proper stem cell division and elongation at toxic Pb levels (> 300 mg kg^{-1}). On contrary, high Pb levels results in a decrease in plant height in *Spinacia oleracea* (Lamhamdi et al., 2013).

Stem girth and number of branches of *C. olitorius* were not affected by Pb application, which is indicative of species resistance towards toxic Pb concentrations. Similarly, an insignificant decline in stem diameter at increasing Pb levels in the soil was also recorded in *Ligustrum lucidum* seedling (ZHOU et al., 2018). However, diameter of *Spinacia oleracea* shoots decreased as a result of an increase in Pb in the growth medium (Lamhamdi et al., 2013). Also, increasing Pb resulted in a decrease in number surviving shoots in *Salix* species (Wang et al., 2014).

The lowest Pb concentration (150 mg kg^{-1}) increased the number of *C. olitorius* leaves, with a decline only at the highest (1000 mg kg^{-1}) concentration in the soil. Increase in Pb soil concentration (100–1 600 mg kg^{-1}) did not affect the number of *Eclipta prostrata* and *Scoparia dulcis* leaves but reduced that of *Phyllanthus niruri* leaves at the maximum dose of 1 600 mg kg^{-1} (Chandrasekhar and Ray, 2019). Differences in response towards Pb contamination in the current and previous studies shows that Pb effect is species dependant. The decline in leaf number at highest Pb concentrations results from leaf senescence, chlorosis, and later abscission, because of the disturbed plant metabolic activities (Chandrasekhar and Ray, 2019; Shi et al., 2019).

A gradual reduction in *C. olitorius* leaf area in concentrations ≥ 300 mg kg^{-1} indicated lead toxicity in the soil, which is known to reduce the leaf area in plants, such as *Taraxacum officinale* (Bini et al., 2012). The decline in leaf chlorophyll content in the current study is similar to the reduction recorded in *Triticum aestivum* and *Spinacia oleracea* (Lamhamdi et al., 2013) when Pb concentration was increase in the soil. This was probably a result of toxic levels of Pb that altered relative proportion of chlorophyll a and chlorophyll b and thus reduced total chlorophyll production and the rate of photosynthesis (Khan et al., 2013; Hou et al., 2018).

Insignificant difference in the fresh and dry weight of *C. olitorius* leaves, stems and roots treated with Pb than the control, with few exceptions, might imply the potential of these plants to grow relatively well under Pb contaminated soils. Different responses towards Pb contamination were recorded in different plants, where fresh and dry weights of shoots and roots of *Eclipta prostrata* were increased by an increase in Pb concentrations (100–1 600 mg kg^{-1}); were not affected in *Scoparia dulcis*; but they were drastically decreased in both *Phyllanthus niruri* (Chandrasekhar and Ray, 2019) and *Helianthus annus* (with Pb increase from 300–900 mg kg^{-1}) (Saleem et al., 2018). The increase in *C. olitorius* leaf and stem dry mass at 150 mg kg–1 might result from enhanced growth under Pb application as in *Eclipta prostrata* (Chandrasekhar and Ray, 2019) but reduction in stem and root dry mass at 1000 mg kg–1 was caused by growth retardation at high Pb concentrations as in *Spinacia oleracea* and *Triticum aestivum* (Lamhamdi et al., 2013), *Ceratophyllum demersum* (Afaj et al., 2017), *Helianthus annus* (Saleem et al., 2018) and *Phyllanthus niruri* (Chandrasekhar and Ray, 2019).

Moisture content of *C. olitorius* leaves, stems and roots was hardly affected by Pb application, except for its decrease in leaves (150 and 600 mg kg^{-1} Pb) and increase in stems (150 mg kg^{-1}) as well as leaves and roots (1 000 mg kg^{-1}). Similarly, the relative water content of *Eichhornia crassipes* seedlings increased at low Pb concentrations and started to decrease at higher concentrations, when compared with the control (Malar et al., 2014). However, increase of Pb in the soil resulted in a decline in moisture content in *Spinacia oleracea* (Alia et al., 2015).

Increasing concentrations of Pb in the soil interfere with cell formation in the roots which results in reduced plant growth (Alia et al., 2015). Lead adversely affects plant biomass by reducing the accumulation and translocation of other essential nutrients and by blocking their entry or binding to the nutrient carriers making them unavailable for other elements (Pinho and Ladeiro, 2012).

An insignificant effect of Pb in most *C. olitorius* reproductive traits, with few exceptions, probably means that plants that reach the reproductive stages (maturity) are less affected by the amount of Pb in the soil. Increase in total and 100–seed mass at lower Pb concentrations (150 and 300 mg kg^{-1}) might be a response toward stress, where plants maximizes their resources for reproduction than vegetative growth (Cho et al., 2017). However, in *Zea mays* the presence of Pb in the soil results in a decrease in seeds mass (Ghani, 2010).

Correlation matrix

Vegetative and reproductive traits correlated with each other in terms of how they are affected by Pb content in the soil. Moisture content of leaves, stems and roots had a strong positive correlation with one another and they were all less affected by Pb content. Although leaf chlorophyll content was drastically reduced by the increase in Pb concentration, it correlated positively with less-affected reproductive traits as well as stem and leaf traits. Significant positive correlations among vegetative and reproductive traits are essential to indicate similar effect of Pb on all traits. Insignificant correlation of reproductive traits with some vegetative traits such as root fresh and dry mass; leaf dry mass as well as moisture content of roots, stems and leaves probably means that these plants do not entirely depend on these traits for reproduction. The growth of *Ligustrum lucidum* seedlings under Pb stress had a significant negative correlation with dry mass of roots, stems and leaves (Zhou et al., 2018). Several studies mainly focus on correlation matrix among different heavy metals (Bini et al., 2012) and their accumulation within plants (Hashim et al., 2017; Huang et al., 2017; Fattahi et al., 2019; Shi et al., 2019), but less among plant traits that are affected by heavy metals.

Conclusions

Corchorus olitorius accumulates toxic amounts of Pb when growing in Pb-contaminated soils, but continues to grow successfully. This shows its potential for phytoremediation. However, its consumption can be fatal to humans when it is collected from Pb-contaminated areas. Pb is less translocated from roots to aerial plant parts, but continuous shoot consumption could lead to gradual Pb accumulation in humans over time resulting in its toxicity. Therefore, collection and consumption of this vegetable species from areas that are prone to Pb contamination is not recommended. Future research will focus on variation on growth and yield of Pb-treated *C. olitorius* plants, grown in different soil types under different climatic conditions. This will also include the measurement of pH and ion conductivity of the collected run-off water in order to source information on the mobility of Pb ions, such as availability of Pb to the roots.

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