Accumulation and distribution pattern of lead and cadmium and their effect on vegetative and reproductive traits of *Corchorus olitorius*

By

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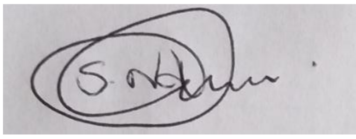
Dedications

This work is dedicated to my supervisors, Dr. NR Ntuli, Prof. VSR Rajasekhar Pullabhotla and the late Prof. AM Zobolo. I also dedicate it to my late father Mr. MN Ndlovu and my brothers who supported and encouraged me to follow my dreams; and lastly, to my son Nkosenhle Ndlovu.

Declaration

I, Sibongokuhle Ndlovu, certify that the material reported in this thesis represents my original work, except where acknowledged. I further declare that these results have not otherwise been submitted in any form for any degree or diploma to any university.



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Sibongokuhle Ndlovu

I certify that the above statement is correct.

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Dr. NR Ntuli

List of publications

Ndlovu S, Rajasekhar Pullabhotla VSR, Ntuli NR, 2019. Agro-morphological changes caused by the accumulation of lead in *Corchorus olitorius*, a leafy vegetable with phytoremediation properties. *Journal of Applied Botany and Food Quality*. 92, 371–377.

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Abstract

Lead and cadmium heavy metal toxicity are some of the major concerns on agriculture because they can enter the food chain through the consumption of contaminated vegetables and cause serious health issues to humans. *Corchorus olitorius* L. is a wild leafy vegetable that can be harvested from areas such as roadsides and mining areas, which are prone to contamination by these heavy metals. However, limited studies focus on determining the effect of toxic Pb and Cd accumulation on morphological features of wild edible plants. *C. olitorius* seeds were sown in potted soil treated with 0, 5, 10, 15, 20 and 25 mg kg^{-1} $Cd(NO_3)_2$ and 0, 150, 300, 600, 900, and 1000 mg kg^{-1} $Pb(NO_3)_2$. The experiment was arranged in a randomized complete block design and each concentration had five replications. Differences in germination percentage, seedling mortality, vegetative and reproductive traits were recorded among treatments. Harvested immature and mature plants were separated into leaves, stems, and roots; dried and grounded into powder; and analyzed for heavy metal accumulation using Atomic Absorption Spectroscopy (AAS) and Scanning

Electron Microscopy (SEM) in combination with Energy Dispersive X-ray (EDX) techniques. Concentrations up to the maximum of 600 mg kg^{-1} Pb and 10 mg kg^{-1} Cd soil treatments resulted in toxic accumulation of these heavy metals in different plant parts, but either promoted or did not affect germination percentage, seedling growth, as well as vegetative and reproductive traits, when compared with the control. Pb was relatively restricted to and highly accumulated in the roots, whereas Cd was easily translocated and was concentrated in the aerial parts. In three and two informative principal components of morphological and accumulation analyses, respectively, almost all investigated traits were associated with principal component one which had the highest variability. The biplots and dendrograms in both analyses mainly grouped Pb and Cd treatments into separate clusters. Results of metal accumulation analysis using both AAS and SEM coupled with EDX techniques were complementary to each other. *C. olitorius* is a potential plant for the use in the phytoremediation of Pb and Cd contaminated soils but is toxic for harvest and consumption from such areas.

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Abbreviations

ASS:	Atomic Absorption Spectroscopy
C. olitorius:	*Corchorus olitorius*
Cd:	Cadmium
EDX:	Energy dispersive X-ray
HMs:	Heavy Metals
Pb:	Lead
NPK:	Nitrogen: Phosphorus: Potassium
DAS:	Days after sowing
mg kg^{-1}:	milligrams per Kilogram
mg L^{-1}:	milligrams per litre
PCA:	Principal Components Analysis
PC1:	Principal Component 1
PC2:	Principal Component 2
SEM:	Scanning electron microscope

Chapter 1
Introduction

1. Introduction

Corchorus olitorius L. commonly known as jute mallow is a leafy vegetable, which belongs to the Tiliaceae family (Nwangburuka *et al*., 2012). Its origin is not yet known, but both tropical and subtropical Africa have been suggested because of the high genetic diversity of this plant in these areas (Adedosu *et al*., 2015). *C. olitorius* is used as an important food source in the Middle East, Africa and Asia (Adebo *et al*., 2015). Its leaves are rich in iron, protein, calcium, thiamine, riboflavin niacin, folate and dietary fiber (Adediran *et al*., 2015). *C. olitorius* leaves are also used as herbal remedy to treat malaria or typhoid fever (Adebo *et al*., 2015). This plant is the world's second-best source of fiber, used to make clothing, bags, ropes, and packaging (Adebo *et al*., 2015).

Soil heavy metal contamination has become a major concern in agricultural improvement, especially in crop production around the globe (El-khawaga, 2014; Alves *et al*., 2016; Dutta *et al*., 2018). Therefore, there has been a growing interest to study heavy metal remediation by plants, termed phytoremediation (Abubacker and Sathya. 2017). Heavy metals are normally present in the soil. However, the use of fertilizers in agriculture, mining, dumping of sewage waste (Akintola *et al*., 2019; Hosman *et al*., 2017) and emissions from car exhaust near the roadsides increase their levels in the soil and lead to soil contamination and toxicity (Kabir, 2010: Aslam *et al*., 2015). Common heavy metals include arsenic, cadmium, copper, cobalt, lead and zinc (Nas and Ali, 2018). Heavy metals can enter the food chain (Hosman *et al*., 2017) through plants' absorption, including traditional leafy vegetables (Akubugwo *et al*., 2012) and then eaten by humans and animals (Chang *et al*., 2014; Aslam *et al*., 2015). The absorption is through the roots and leaves (Huang *et al*., 2012) and then translocate to other parts of the plant (Chang *et al*., 2014; Pourrut *et al*., 2014).

Lead (Pb) and cadmium (Cd) are some one of the most common environmental pollutants and potential hazards (Aslam *et al*., 2015; Andjelkovic *et al*., 2019). High accumulation of these metals in plants has a certain morphological impact on plant growth (Ambike *et al*., 2016). High Pb levels inhibit seed germination and retard seedling growth (Abraham *et al*., 2013). Lead decreases root, shoot length, dry mass of roots, shoots, and causes leaf chlorosis (Naz *et al*., 2015). High levels of Pb in the soil also reduces the stem diameter and the number of leaves per plant (Khan *et al*., 2013), reduces the leaf size and leaf chlorophyll content (Yilmaz, 2009; Brandt, 2012). Increasing Pb concentration in the soil also reduces the moisture content in most leafy vegetables (Naz *et al*., 2015). However, some flowering plants such as *Raphanus sativus* maintain their seed and floral traits at high levels of Pb in the soil (Hladun *et al*., 2015).

Plants that are grown in soil with high levels of Cd usually have reduced seed germination and plant growth, chlorosis, and browning of root tips leading to plant death (Laghlimi *et al*., 2015; Ambika *et al*., 2016). An increase in Cd levels in the soil also results in the reduction of the number of leaves (Khan *et al.*, 2013), stem diameter, total leaf area, and chlorophyll content (Rehman *et al*., 2011; Ibrahim, 2017). Plants' moisture content also decreases with an increase of Cd in the soil (Naz *et al*., 2015; Nazarian *et al*., 2016). High levels of Cd also reduce the number of flowers and fruits per plant (Rehman *et al*., 2011).

Lead and cadmium are the two heavy metals that are highly accumulated by leafy vegetables (Chang *et al*., 2014) and some staple crops (Marquez *et al*., 2018). Some of these leafy vegetables with high heavy metal accumulation potential, known as bioaccumulators (Juson *et al*., 2016) sometimes have much greener and broader leaves, which promote an appealing morphological characteristic for vegetable harvesting (Dinis and Fiuza, 2011). When people harvest bio-accumulators from such areas they end up being intoxicated by high levels of heavy metals (Marquez *et al*., 2018). *C*. *olitorius* is also one of the leafy vegetables that grow naturally in the wild near road sides (Senyaulo *et al*., 2011) and in cultivated fields (Mgius *et al*., 2014). It is consumed because of its richness in certain nutrients such as abundant β-carotene and other carotenoids, vitamins B1, B2, C and E, and minerals (Adediran *et al*., 2015).

Assessment of heavy metal toxicity on selected leafy vegetables has been conducted in other African countries such as Nigeria (Nkafamiya *et al*., 2010); Ivory Coast (Otitoju *et al*., 2012) and Kenya (Nwajei *et al*., 2014). However, no such studies have been conducted in South Africa. A study by Sanyaulu *et al*. (2011) focused on *C. olitorius* leaves and the spatial distribution of heavy metals according to differences in distance from roadsides. Another study by Hassan *et al*. (2016) focused on Cd accumulation by *C. olitorius* in hydroponics. *C. olitorius* is one of the most preferred leafy vegetables in northern KwaZulu-Natal and is mostly collected in areas, which are prone to high heavy metal content.

1.1 Problem statement

People from rural communities consume leafy vegetables from areas with heavy metal deposition, especially near mining sites or on roadsides. These vegetables may have a healthy physical appearance but can be contaminated with certain heavy metals, thus toxic for consumption. Consumption of vegetables with high concentrations of heavy metals can lead to damage of the central nervous system; alter blood composition and can damage body organs such as lungs, kidneys and liver. However, no assessments of heavy metal toxicity in leafy vegetables such as *C. olitorius* have been conducted in South Africa and there is a need to assess the uptake and accumulation of heavy metals by this vegetable.

1.2 Aim

This study aimed to determine the accumulation and distribution pattern of lead (Pb) and cadmium (Cd) heavy metals, as well as their effect on vegetative and reproductive traits of *C. olitorius* leafy vegetable.

1.3 Objectives

The objectives of this study were to:

- Establish the variation of vegetative and reproductive traits of *C. olitorius* plants grown under different Pb and Cd concentrations.
- Determine the accumulation of Pb and Cd by roots, stems and leaves of *C. olitorius* at different stages of growth using the atomic absorption spectroscopy technique.
- Evaluate the distribution patterns of Pb and Cd in *C. olitorius* roots, stems and leaves using the scanning electron microscopy technique coupled with energy dispersive X-ray.

1.4 Research questions

- How do high levels of Pb and Cd in the soil affect the vegetative and reproductive traits of *C. olitorius*?
- Which *C. olitorius* plant parts accumulate elevated quantities of Pb and Cd and at what stages of growth?
- What are the distribution patterns of both Pb and Cd in roots, stems, and leaves of *C. olitorius* at different stages of growth?

1.5 Research hypotheses

H_0: Toxic Pb and Cd levels in the soil does not affect vegetative and reproductive traits of *C*. *olitorius* leafy vegetable.

H_1: An increase in Pb and Cd soil levels retards the growth of *C*. *olitorius* vegetative and reproductive traits.

H_0: Both Pb and Cd are accumulated in similar quantities throughout the *C*. *olitorius* plant, at both immature and mature stages of growth.

H_1: Pb is accumulated in high quantities in roots than aerial parts, whereas Cd is heavily accumulated in aerial parts than in roots, at both stages of growth.

H_0: The distribution pattern of both Pb and Cd is similar in roots, stems, and leaves, throughout the growth cycle of *C*. *olitorius* plant.

H_1: Maximum Pb distribution is recorded in roots than stems and leaves; whereas high Cd distribution is present in leaves than roots and stems of *C. olitorius* leafy vegetable particularly at an immature stage of growth.

1.6 Structure of the dissertation

Chapter 1 is a general introduction of the study and included the problem statement, aims, objectives, research questions and hypotheses

Chapter 2 reviews literature on taxonomy, distribution, uses, and nutritional value of *C. olitorius*; sources and chemical properties of heavy metals and their toxicity to humans and animals; as well as the effect of Pb and Cd heavy metals on vegetative and reproductive traits of several plants. Chapters three, four, and five are each composed of an introduction, materials and methods, results, discussion, and conclusion.

Chapter 3 focuses on the effect of Pb and Cd on the vegetative and reproductive traits of *C. olitorius*.

Chapter 4 investigates the variation in the accumulation of Pb and Cd to different parts of *Corchorus olitorius* at different stages of growth using Atomic Absorption Spectroscopy.

The distribution patterns of both Pb and Cd were further analyzed in roots stems and leaves at immature and mature stages, using the Scanning Electron Microscopy coupled with Energy Dispersive X-ray, in **Chapter 5**.

Chapter 6 presents conclusions and recommendations on the variation in accumulated and distributed Pb and Cd on vegetative and reproductive traits of *C. olitorius* vegetable species, at different stages of growth.

Chapter 2

Literature Review

2. Literature review

This review provides the information on leafy vegetables in general and it also discusses the origin, distribution, uses and nutritional value of *Corchorus olitorius*. Descriptions of heavy metals; their sources and toxicity towards plant growth and reproduction as well as human consumption are also discussed in this chapter. This review then focuses precisely on the uptake of lead (Pb) and cadmium (Cd) by leafy vegetables and their effect on their growth and yield. It also explains differences in the accumulation and distribution patterns of Pb and Cd by roots, stems, and leaves of plants.

2.1 Leafy vegetables

Traditional leafy vegetables are herbaceous plants whose leaves, tender stems, flowers, and sometimes immature fruits are consumed as vegetables (Mabala, 2018). These plants are also called wild vegetables and African leafy vegetables (Maseko *et al*., 2018; Senyolo *et al*., 2018). There are more than 100 different vegetable plants in South Africa (Maseko *et al*., 2018) and they are referred to as *imifino* in isiZulu and IsiXhosa, *Morogo* in Sesuthu and *Muhuro* in Tshivenda (Njeme *et al*., 2014; Senyolo *et al*., 2018). These vegetables grow in the wild and do not require formal cultivation. They are inexpensive, easily accessible and easy to cook (Essack *et al*., 2017).

Traditional leafy vegetables are an important source of nutrition (Kamble and Jadhav, 2013) especially vitamin A and C, iron, calcium, folic acid, and dietary fiber. In recent years, the consumption of leafy vegetables has increased in both rural and urban areas (Gupta *et al*., 2013). In African countries, both indigenous and introduced leafy vegetables can be collected as weeds from the wild, near mining industries, waste sites and roadsides (Essack *et al*., 2017). These plants also occur in cultivated soils, gardens, seasonally wet areas and cattle pens where manure accumulates (Maseko *et al*., 2018).

The consumption of leafy vegetable in South Africa varies depending on factors such as poverty status, degree of urbanization, distance to fresh produce markets and time of the year (Njeme *et al*., 2014). They are mostly consumed by resourcepoor households who lack finances to either purchase or produce their modern vegetables (Gupta *et al*., 2013; Kamble and Jadhav, 2013). These plants hold a vital role in well-balanced diets of these people (Kamble and Jadhav, 2013; Maseko *et al*., 2018). Rural communities generally use leafy vegetables to survive poverty (Kamble and Jadhav, 2013; Essack *et al*., 2017). This is because they are cheap yet giving an adequate amount of nutrients, almost similar to commercial vegetables (Kamble and Jadhav, 2013).

2.2 Taxonomy and distribution of *Corchorus olitorius*

Corchorus olitorius L. commonly known as Jute Mallow belongs to the family Tiliaceae. It is an erect annual herbaceous plant, which grows between 20 cm and 1.5 m in height (Mguis *et al*., 2014). *C. olitorius* is a popular leafy vegetable in both dry or semi-arid regions and humid areas of Africa (Musa and Ogbadoyi, 2012). It usually occurs as a weed in cultivated fields and prefers sandy loam soils rich in organic matter (Mguis *et al*., 2014).

Figure 2.1: An image of a mature *C. olitorius* at flowering stage

Corchorus olitorius is one of the common tropical leafy vegetables found in Asia, Africa and some parts of America (Adebo *et al*., 2015). In Africa, it is a leading vegetable in countries like Benin, Cote d'Ivoire, Nigeria, Cameroon, Kenya, Sudan, Uganda and Zimbabwe (Adediran *et al*., 2015).

2.3 Uses and nutritional value of *C. olitorius*

Corchorus olitorius is consumed as a leafy vegetable (Adebo *et al*., 2015), where its young leaves are harvested along with young stems (Adideran *et al*., 2015). *C. olitorius* is a preferred healthy leafy vegetable because of its high content in betacarotene and other carotenoids; vitamins B1, B2, C and E; as well as minerals such as calcium and iron (Oyedeji and Bolarinwa, 2013; Mguis *et al*., 2014). It also contains different proportions of dietary fiber and proteins needed for human health (Ilori *et al*., 2015)

This plant is demulcent, diuretic, lactagogue, purgative and tonic (Oyedeji and Bolarinwa, 2013). Its leaves are often used in folk medicines for ascites, pain, piles, tumours, cystitis, dysuria, fever and gonorrhoea. The cold infusion is also used to restore appetite and strength (Adediran *et al*., 2015). Stem fiber of *C. olitorius* is the world's most vital bagging and wrapping textile. *C. olitorius* is ranked second from cotton in its fiber uses such as to make clothing, ropes, bags, and packaging, to name a few (Adebo *et al*., 2015).

2.4 Heavy metals and their chemical properties

Heavy metals are metallic elements of densities higher than 5 g kg^{-1} (Brandt, 2012; Akintola *et al*., 2019). They exhibit metallic properties such as ductility, malleability, conductivity, cation stability and ligand specificity (Chibuike and Obiora, 2014). Some heavy metals are essential for human metabolism and plant growth, for example, Fe, Mn, Zn, Cu, Co, Mo, Ni and Cr (Khan *et al*., 2015; Akintola *et al*., 2019). Others are non-essential to both plants and humans and toxic at very low concentrations, those heavy metals include mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) (Dinis and Fiuza, 2011; Laghlimi *et al*., 2015).

Lead is one of the toxic elements. It is a group 4 period 8 element with an atomic mass of 207.2 and the atomic number of 82. It has a density of 11.4 g cm^{-3}, a melting and boiling point of 327.4 and 1725 ˚C respectively. It is mostly preferred for its softness, malleability, ductility, poor conductivity and resistance to corrosion (Nas and Ali, 2018). Lead is naturally occurring and is silvery-white in colour (Pinho and Ladeiro, 2012). It is usually found in combination with other elements such as sulphur in a form of PbS or $PbSO_4$, or oxygen in $PbCO_3$. The estimated amount of Pb in the earth's crust is 10 to 30 mg kg^{-1} of soil (Wuana and Okieimen, 2011).

Cadmium is a transitional element located at the second row of metals on the periodic table. It has an atomic number and mass of 48 and 112.4 g in the periodic table. Its density is 8.65 g cm^{-3} with a melting and boiling point of 320.0 and 765 ˚C, respectively. It is one of the big three poisonous heavy metals which include mercury (Hg) and chromium (Cr) and has no essential use in plants and humans (Tran and Popova, 2012). It usually occurs as a divalent compound in the form of Cd^{+2} ions and is not found in a pure form in nature. Cadmium in the air is quickly oxidized into cadmium oxide. It reacts with carbon dioxide, water vapour, sulphur dioxide, sulphur trioxide, hydroxide, sulphite or chloride (Wuana and Okieimen, 2011).

2.5 Sources of heavy metals

Heavy metals occur naturally in the soil. Geological and anthropogenic processes increase the amounts of these elements in the soil (Chibuike and Obiora, 2014; Akintola *et al*., 2019). The elevated heavy metals in the soil lead to toxicity, which is harmful to humans, plants, and animals (Messou *et al*., 2013). Activities leading to soil contamination include mining and smelting of metals; burning of fossil fuels; use of fertilizers and pesticides in agriculture; production of batteries and other metal products in industries; sewage sludge; and municipal waste disposal (Chibuike and Obiora, 2014; Laghlimi *et al*., 2015).

Lead is readily available in the earth's crust from the natural process of chemical weathering in the soil (Khan *et al*., 2014). The external sources of Pb are fumes from automobiles near the roads; chimneys from Pb-using factories; battery manufacturing industries; mining and smelting (Kaur *et al*., 2015a); Pb ores; metal plating and fishing operations; use of fertilizers in agriculture; additives in pigments and gasoline (Codling, 2014; Aziz, 2015). Sewage sludge deposited in garden soils is also a source of lead and other heavy metals (Aziz, 2015; Kaur *et al*., 2015a).

Cadmium naturally enters the environment through weathering of rocks and by anthropogenic impacts as such heating systems; generation of power; industrial metal processing and car fuel fumes (Demirezen *et al*., 2012; Chetan and Ami, 2015). It also results from the burning of Cd-containing metals and the use of phosphate fertilizers. The other Cd sources include the manufacture of several different products such as batteries, chipsets, pigments, television receivers, and semi-conductors (Ibrahim *et al*., 2017). Cadmium also comes from natural emissions and bioaccumulation, which occur in certain plants, mammals and filter feeder organisms through decomposition (Fasahat, 2015).

2.6 Heavy metal uptake by plants

Certain plant species are more resistant to heavy metal toxicity in the soil (Akintola *et al*., 2019). *Arabidopsis halleri* and *Arabidopsis arenosa* are the example of previously reported heavy metals resistant plants (Singh *et al*., 2016). They can absorb and accumulate large amounts in their internal tissues (Al-Saadi *et al*., 2013; Nazarian *et al*., 2016). The factors affecting heavy metal uptake or the availability in the soil are soil pH (Chang *et al*., 2014), organic matter content, cation exchange capacity, soil texture and soil microorganisms (Al-Saadi *et al.,* 2013; Khan *et al*, 2015). Heavy metal uptake also varies with plant species, varieties within a species and level of soil contamination (Aldoobie and Beltagi, 2013; Nwajei *et al*., 2014). The time of harvest and the stage of maturity of the plant are also another factors affecting the uptake of heavy metals in leafy vegetables (Naser *et al*., 2011; Revelli *et al*., 2014).

The uptake of heavy metals by plants is either from soil to plant, water or air to plant (Huang *et al*., 2012). However, the soil-to-plant pathway is the major source of contamination. Some plants accumulate more heavy metals in the roots than in the stems and leaves (Revelli *et al*., 2014). For example, in *Populas nigra* (Barbes et al., 2014) and *Trigonella foenum-graecum* (Kaur *et al*., 2015), most of the accumulated heavy metals were found in the roots. This is due to the plants' low ability to translocate heavy metals from roots to other parts of the plant (Anoliefo *et al*., 2008). Accumulation and distribution of Pb in *Amaranthus dubius* was in the order of roots > stem > leaves in a descending manner (Mellem *et al*., 2012). In most plants, the accumulation of Pb is usually higher than Cd because of its higher potential to translocate from roots to other plant parts (Oluwatosin *et al*., 2010).

Lead is readily available to plants from the soil and it uptake is through roots (Baunthiyal *et al*., 2015). The uptake mechanism is through adsorption onto roots and binding to carboxyl groups of mucilage uranic acid (Pinho and Ladeiro, 2012) or directly to the polysaccharides of the rhizoderm cell surface (Pourrut *et al*., 2014). Once on the root surface, Pb enters the roots passively and follows the water translocation streams (Pinho and Ladeiro, 2012). There is no uniformity in Pb concentration in the roots as a concentration gradient is usually observed from the root apex to the entire root. High amounts of Pb are found in the root apices, where the cells are still young and have thin cell walls. In the entire plant, the concentration of Pb decreases from roots to above plant parts (Pourrut *et al*., 2014).

Roots can take up a huge amount of Pb while at the same time restricting its movement to the above-ground parts (Pinho and Ladeiro, 2012: Baunthiyal *et al*., 2015). The restriction in translocation is because Pb precipitates as phosphate, which is mostly found in the roots (Pinho and Ladeiro, 2012). The uptake of Pb depends on the plant species and its availability in the soil. Some plants such as *Zea maize* can acquire Pb from the air via the leaves (Codling, 2014). In the soil, Pb uptake occurs through the cation exchange between plants and soil (Codling, 2014). The uptake of Pb by plants also depends on its concentration in the soil, which increases with an increase in its concentration (Pinho and Ladeiro, 2012; Ambika *et al*., 2016). The minimum and maximum acceptable levels of Pb in agricultural soil range from 50-300 mg kg^{-1} (Mohammed and Folorunsho, 2015) (Table 2.1).

The uptake and distribution of Cd inside the plant depends on factors such as soil type, pH, the presence of competing ions and the plant species. Among these factors, organic matter and soil pH are the two most effective factors (Fasahat, 2015). The level of Cd uptake also depends on the amount of metal present in the soil. There is a linear negative relationship between soil pH and Cd accumulation. An increase in soil pH leads to a decrease in Cd uptake by plants (Chen et al, 2011; Chang et al., 2014). An increase soil pH increases the availability of many cations like Fe^{2+}, Mn^{2+}, Zn^{2+}, and Ca^{2+} which increase the competation of Cd availability ti plants (Chang *et al*., 2014).

It is assumed that Cd uptake is through active transport but most evidence supports the passive uptake (Pinho and Ladeiro, 2012). This assumption suggests that the uptake of Cd after root absorption follows the water transport mechanism through the xylem (Chang *et al*., 2014). The absorption of Cd by plants takes place in competition with other metal elements especially Zinc (Zn) (Kassir *et al*., 2012). Much of the accumulated Cd is retained in the roots and small portions are translocated to the aerial portions of the plant and the seeds (Tran and Popova, 2012). The minimum and maximum allowable Cd levels in agricultural soils range from 1–3 mg kg^{-1} (Mohammed and Folorunsho, 2015) (Table 2.1).

Table 2.1 Toxic and acceptable levels of heavy metals in agricultural soil in general (mg kg^{-1}) (Ali et al., 2019)

Heavy metals	Toxic values in the soil (mg kg^{-1})	Toxic values in Leafy vegetables (mg kg^{-1})

Pb	50-300	0.3
Cd	1-3	0.2

2.7 Effects of heavy metals on plant growth

Plants react differently to heavy metal toxicity in the soil. Some plants accumulate certain heavy metals at a high concentration without physiological effects, while others show effects at the same and low concentrations (Al-Saadi *et al*., 2013; Usu and Okereke, 2015). High levels of heavy metals in the soil are known to prevent plant growth, uptake of plant nutrients, as well as physiological and metabolic processes. This leads to leaf chlorosis; damage in root tips; minimized nutrient and water uptake and non-functional enzymes. High heavy metals also promote increased antioxidant enzymatic processes in plants (Kamran *et al*., 2013). Heavy metals toxicity also leads to a decrease in seed germination, an increase in seedling mortality and a decrease in reproductive capabilities (Al-Saadi *et al*., 2013).

Most plants grow well in lead containing soil; hence, its toxicity in humans from plants consumption is a major threat (Chen *et al*., 2011). However, high levels of Pb interfere with physiological and biochemical processes in plants (Naz *et al*., 2015). It retards photosynthesis and respiration (Bhardwaj *et al*., 2009) and inhibits plant growth and seed germination. High Pb also decreases seedling biomass and moisture content (Naz *et al*., 2015) as well as root and shoot lengths (Khan *et al*., 2013). Increased Pb levels in the soil do not affect flower formation and seed production in most plants (Hladun *et al*., 2015).

High Cd levels lead to leaf chlorosis; growth inhibition; reduced water uptake; and deficiency of some nutrients (Naz *et al*., 2015; Ambika *et al*., 2016). It interferes with physiological and biochemical processes such as photosynthesis and nutrient uptake and deposition (Chen *et al*., 2011; Brandt, 2012; Naz *et al*., 2015). Cadmium also interferes with water uptake in plants resulting in reduced moisture content

(Nazarian *et al*., 2016; Naz *et al*., 2015). Increased Cd levels in the soil also retard flower formation and inhibit seed production (Rehman *et al*., 2011).

Growth inhibition is due to Cd effects on the nucleus and interaction with plant hormones in the leaves, which adversely affects photosynthesis (Hirve and Bafna, 2013). Cadmium at high concentrations in the soil does not only inhibit growth but also can result in plant death (Khan *et al*., 2013). The permissible limit of Cd on Agricultural soil is 1−3 m g kg^{-1} of soil (Table 2.1) (Ali *et al*., 2019).

2.8 Heavy metal toxicity in humans and animals

Food plant contamination with heavy metals and their impact on plant nutrition is a major health issue (Juson *et al*., 2016). The common route of the entrance of heavy metals to humans and animals is through inhalation, ingestion and dermal contacts (Baunthiyal *et al., 2015*; Fasahat, 2015; Andjelkovic *et al*., 2019). Heavy metal toxicity in humans leads to diarrhoea, nausea, lung disease, anaemia, kidney disorders, stomach problems, skin diseases, neurological disorders and cancer (Dinis and Fiuza, 2011; Adesuyi *et al*., 2015). Non-essential heavy metals can also affect the reproductive capacity of living organisms (Khan *et al*., 2015).

Lead toxicity in humans, which is a 0.025 mg kg^{-1} per body weight, leads to growth retardation; decreased intelligence and loss of hearing in children (Zhou *et al*., 2016). It also results in brain damage with disorders such as mental retardation and behavioural problems (Amirmoradi *et al*., 2012; Kamran *et al*, 2012). Dullness, headache, poor attention span, and irritability are also signs of Pb toxicity in humans (Amirmoradi *et al*., 2012). Lead interferes with the reproductive system (Kamran *et al*., 2012; Andjelkovic *et al*., 2019), causes acute and chronic nephropathies and affects bone metabolism (Carocci *et al*., 2016).

Cadmium contamination in humans, value above 0.007 mg kg^{-1} per body weight, leads to acute toxicity in the liver and lungs; induces nephrotoxicity and steotoxicity, and impairs functions of the immune system (Zhou *et al*., 2016). Cadmium toxicity disorders include cardiovascular, nervous, kidney and bone disorders (Kamran *et al*., 2012; Andjelkovic *et al*., 2019). It induces human tubular renal dysfunctioning (Wuana and Okieimen, 2011), severe bone damage and carcinogenic effects (Fasahat, 2015).

2.9 Scanning electron microscope and energy dispersive X-ray

Scanning electron microscope (SEM) coupled with energy dispersive X-ray is another advanced method used by scientists to determine the distribution of heavy metals in plants (Babaoğlu aydaş *et al*., 2013). SEM is a technique that measures the binding of heavy metals to biomolecules. It allows researchers to study the morphological changes in the internal structure of the plants after metal binding (Baruah *et al*., 2012). Changes in the vascular cells of the roots stem and leaves of *Eichhornia crassipes* plants were observed with the EDX and SEM technique explosed to different concentrations of Pb (Baruah *et al*, 2012). The presence of Cd also resulted in internal structure changes in *Solanum lycopersicum* viewed with the EDx and SEM method (Godinho *et al*., 2018). The SEM machine operates at a 15 kV and 500x magnification power for clear image observation. The powder samples from plants are mounted on the conductive carbon tape with an electron beam from an SEM gun, which scans across the surface (Jamarie *et al*., 2014).

2.10 Conclusion

Heavy metal toxicity is now a serious problem in agriculture especially plant production. Some plants accumulate high content of heavy metals in their tissues with no visible effects on morphological features. *Corchorus olitorius* is also one of the traditional leafy vegetables growing near roadsides and mining areas, which are prone to heavy metal contamination. Such plants are a pathway for the entry of heavy metals to the circle of the food chain. Once in the body of humans and animals at high concentrations, heavy metals result in serious health issues when they interact with the body system. Therefore, to address this issue, a study was conducted with *Corchorus olitorius* to warn the people, especially those from rural poor communities who largely rely on leafy vegetable harvesting for food. The following chapter addresses the effect of Pb and Cd on the vegetative and reproductive growth of *Corchorus olitorius*.

Chapter 3 Effect of lead and cadmium on vegetative and reproductive traits of *Corchorus olitorius*

Part of this chapter is published as follows:

Ndlovu S, Rajasekhar Pullabhotla VSR, Ntuli NR, 2019. Agro-morphological changes caused by the accumulation of lead in *Corchorus olitorius*, a leafy vegetable with phytoremediation properties. *Journal of Applied Botany and Food Quality*. 92, 371–377.

3.1 Introduction

Corchorus olitorius is a leafy vegetable commonly known as jute mallow (Musa and Ogbadoyi, 2012). It is an annual erect herb that grows between 60 and 150 cm in height (Nwangburuka *et al*., 2012; Tovihoudji *et al*., 2015). Its leaves are largely consumed along with staple food in many African countries (Senyaolu *et al*., 2011; Ilori *et al*., 2015). *C. olitorius* is mostly preferred for its high carotenoids, vitamin B_1, B_2, C and E and minerals (Adebo *et al*., 2015). *Corchorus olitorius* grows in the fields, home gardens and on roadsides, and can accumulate toxic and anti-nutrient substances (Sanyaulo *et al*., 2011). Other uses of *C. olitorius* include folk medicines for ascites, pain block, piles, tumours, cystitis, dysuria, fever and gonorrhoea (Makinde *et al*., 2009). Its cold infusion restores appetite and strength (Adideran *et al*., 2015).

Heavy metal toxicity has certain morphological effects on certain plants (bio-indicators) at different levels of plant growth and heavy metal contamination (Bhardwaj *et al*., 2009; Juson *et al*., 2016). High lead (Pb) levels inhibit seed germination and retard seedling growth (Abraham *et al*., 2013). An increase in Pb concentration in the soil results in a decline in seed germination of *Spartiana alterflora* and *Pinus helipensis* (Gill, 2014). It also reduces root and shoot growth, leaf area and results in leaf chlorosis in *Sesanum indicum* (Gill, 2014). Elevated Pb levels in the soil reduce soil productivity and inhibit some important plant processes, such as photosynthesis, mitosis and water absorption. These Pb effects result in dark leaves, wilting of older leaves, stunted foliage and brown short roots (Aziz, 2015). Increased Pb concentration in the soil also reduces root, stem and leaf moisture content (Yilmaz *et al*., 2009; Naz *et al.*, 2015).

Cadmium (Cd) accumulated by plants can adversely affect the morphology and functioning of the plants (De Maria *et al*., 2013). Plants that are grown in soil with high levels of Cd usually have decreased seed germination and plant growth, chlorosis and browning of root tips leading to plant death (Laghlimi *et al*., 2015; Ambika *et al*., 2016). Germination of *Leucaena leucocephala* is decreased by an increase in Cd concentration in the soil (Shafiq *et al.,* 2008). In most plants, cadmium also reduces the number of leaves and leaf area (Ambika *et al*., 2016) as well as moisture content of the whole plant (Nazarian *et al*., 2016).

Heavy metal toxicity has become a major problem in environmental pollution (Alves *et al*., 2016; Hosman *et al*., 2017). These metals are also deposited into agricultural soil from the mining processes, cars near roadsides and fertilizers in crop production (Kabir, 2012; Juson *et al*., 2016). Most of these heavy metals have growth promotion tendencies in certain traditional leafy vegetables such as *Brassica campestris* Sigh *et al*., 2010) and *spinacial oleracea* (Nuz *et al*., 2017), known as bio-accumulators (Juson *et al*., 2016), which promote appealing morphological features (Dinis and Fiuza, 2011). People from rural areas accidentally harvest such vegetables as their source of food and end up eating heavy metal contaminated plants (Marquez *et al*., 2018). This raises a need to study the growth and yield of leafy vegetables that grow in soils contaminated with heavy metals. Therefore, the objective of this study was to determine variation in vegetative and reproductive traits of *C. olitorius* grown under different lead and cadmium concentrations.

3.2 Materials and Methods

3.2.1 Study Area and seeds sourcing

This research was conducted at the University of Zululand (S 28.85416° E31.84565°), Department of Botany under a rain-free environment. *C. olitorius* seeds were provided by Agricultural Research Council, Lindley Road R76, Bethlehem, Roodeplaat, Pretoria.

3.2.2 Soil sampling, sample preparation and fertility examination

Sand-humus rich soil mixture, manures and soil-manure mixture were collected at 30–60 cm soil depth, air dried for seven days and then sieved through a two millimetre sieve net to eliminate unnecessary access stones and debrides. Thereafter, they were sent to CEDARA (Department of Agriculture and Rural Development, KwaZulu-Natal section) for physio-chemical analysis. To examine soil nutrients and other components present such as; soil pH, carbon and N ratio, organic matter, moisture, total N, phosphorus and exchangeable cations (Ca, Cu, Mg, Mn, Na, K and Zn) as well as soil components (clay, slit and sand) proportions. The results are presented in Table 3.1.

Table 3.1 Properties of the soil used in the research

Soil property	Value
pH	4.5
P	10.1
K	169.9
Mg	1432.3
Na	525.4
Zn	22.4
Cu	2.7
Mn	6. 0
Fe	395
Clay content	23
Organic matter	4.3

All the mineral values are in milligrams per kilogram (mg kg^{-1}) and the clay content and organic matter in percentage (%).

3.2.3 Treatments and experimental design

A black humus-rich soil was collected from the University of Zululand farm. The effect of an increase in Pb and Cd concentrations on vegetative and reproductive traits of *Corchorus olitorius* plants was tested separately. Lead acetate [Pb $(NO_3)_2$] was applied at the rates of 150, 300, 600, 900 and 1000 mg kg^{-1} of soil. Cadmium acetate [Cd $(NO_3)_2$ $4H_2O$] was applied at 5, 10, 15, 20 and 25 mg kg^{-1} of soil rates as modified from Khodaverdiloo *et al*. (2011). The 0 mg kg^{-1} Pb and 0 mg kg^{-1} Cd was shared by both the Pb and Cd experiments as the control treatments. These values were chosen to include the minimum and maximum (50 and 300 mg kg^{-1}) and (1 and 3 mg kg^{-1}) toxic levels of Pb and Cd respectively, in agricultural soils (Ali *et al*., 2019). This study was conducted in 20 L plastic pots under a rain-free environment. The experiment was laid in a randomized complete block design with five replications.

3.2.4 Plant management

Ten seeds of *Corchorus olitorius* were germinated in pots with treated soil. At a four to five leaves growth stage, the seedlings were thinned into one plant of equal growth and vigour per pot. Dead plants because of seedling mortality were replaced by transplanting from pots with more than one plant. After thinning and transplanting, a 2:3:2 (27) NPK fertilizer was applied to all pots at 1 g kg^{-1} of soil. Plants were irrigated daily using deionized water (50 ml per pot) and the irrigation water was collected from the base of the pots and reused to irrigate, to avoid nutrient or heavy metal loss.

3.2.5 Data collection

Seed germination percentage was recorded at seven days after sowing before the plants thinned into one plant per pot. Seedling mortality was also recorded before transplanting. As this experiment was replicated three times, 15 pots for each treatment were used for seed germination and seedling mortality recording. Means were calculated per five pots and used as replicates for both germination and mortality.

Agronomic traits were measured at 44 days after sowing using five plants (5 pots of the same treatment) as replicates. Plant height (cm) was measured from the soil level to the tip of the stem using a ruler. The number of leaves and branches was counted manually. Tesa Vernier Callipers (Microntesa products, 6 Derrick Road, Spartan, Kempton Park, South Africa) were used to measure stem girth (mm) at 10 cm from the soil level. Leaf area (width × length) (cm^2) was measured on the fourth leaf from the apex using a ruler. The leaf chlorophyll content was measured on the fifth oldest leaf (from the apex of the main stem) in each plant using a CCM-200 chlorophyll content meter (Opti-Sciences, ADC BioScientificLtd, Hoddesdon, UK). Five different spots were randomly selected in each leaf for measurements and the average chlorophyll content was recorded.

Three plants per treatment were harvested by uprooting the whole plant at 44 days after sowing to determine fresh and dry mass. Plants had their fresh and dry mass (g) of roots, stems and leaves measured separately. Root length (cm) was measured from the root tip to the base of the stem using a ruler. The number of pods per plant was counted manually. Pods length and pods width (cm) was measured at 90 days after sowing. Seed traits were determined from five pods per plant per treatment and used as replicates. The number of seeds per pod was counted manually. Total seed mass (g) per pod and 100-seed mass (g) per 100 seeds in each pod was recorded.

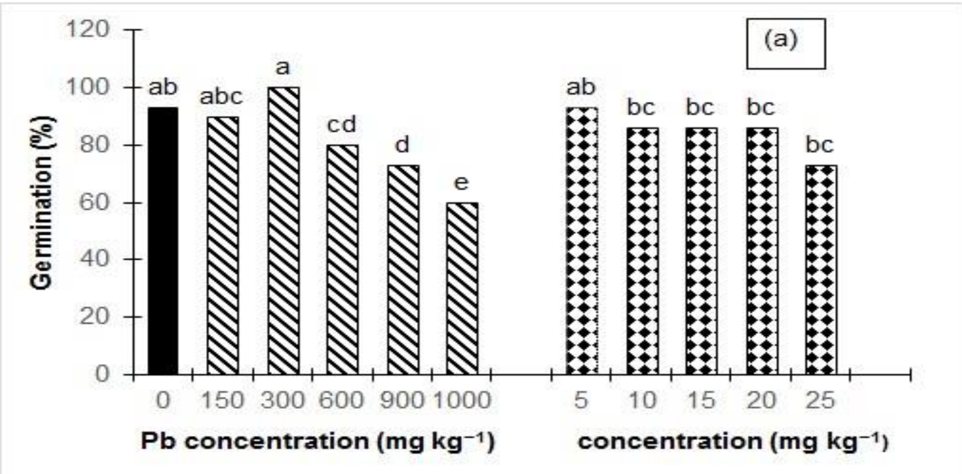
3.2.6 Data analysis

Data were analyzed using analysis of variance (one-way ANOVA) in Genstat 12.1 version. Treatment means were separated using Tukey's Multiple Range Test in Genstat at a 5% level of significance. Correlation, principal component analysis, biplot, and dendrogram analysis was also done using XLSTAT2018.6.54215 (Addinsoft, Paris, France) to access the relationship between heavy metals concentration in the soil and vegetative and reproductive growth of the plant.

3.2 Results

3.3.1 Seed germination and seedling mortality

Lead application at 300 mg kg^{-1} had maximum *C. olitorius* seeds germination (100%), while the minimum germination (60%) was at 1000 mg kg^{-1} Pb concentration (Figure 3.1a). A significant ($p < 0.05$) reduction in seed germination was recorded from 600 to 1000 mg kg^{-1} in Pb-treated plants compared with the control. In Cd-treated plants, germination percentage was the highest (93%) at 5 mg kg^{-1} and the lowest (73%) at 25 mg kg^{-1}. Significant germination inhibition was recorded only at 25 mg kg^{-1} in Cdtreated plants when compared with the control.



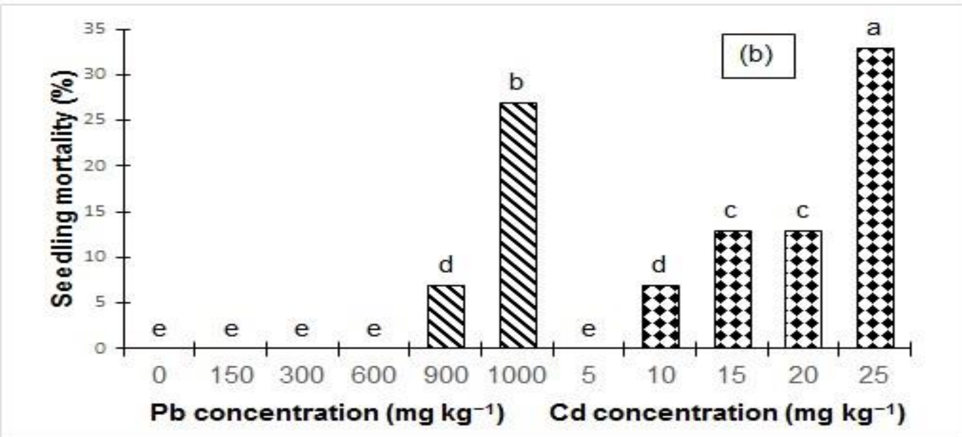


Figure 3.1: Effect of Pb and Cd on germination percentage (a) and seedling mortality (b) of *C. olitorius*

Bars with same letter do not vary significant at p ≥ 5.

Lead application from 150 to 600 mg kg^{-1} and Cd concentration of 5 mg kg^{-1} did not result in seedling mortality; same as the control (Figure 3.1b). The maximum seedling mortality (33%) was attained at 25 mg kg^{-1} Cd and the minimum (7%) was recorded at Cd 10 Cd and 900 mg kg^{-1} Pb levels. Seedling mortality in Pb-treated plants significantly increased from 900 to 1000 mg kg^{-1} of Pb in the soil. In Cd-treated soil, the highest seedling mortality (33%) was recorded at 25 mg kg^{-1} and the lowest (7%) in 10 mg kg^{-1} of Cd in the soil. The seedling mortality also increased with an increase in Cd concentration in the soil.

3.3.2 Root length, plant height, stem girth, and stem branching

Cadmium application of 5 mg kg^{-1} resulted in plants with the longest roots (32 cm), while 25 mg kg^{-1} Cd resulted in the shortest roots (13 cm) (Table 3.2). In Pb-treated plants, the longest roots (25.51 cm) were recorded at 600 mg kg^{-1} and the shortest (15.67 cm) were obtained at 1000 mg kg^{-1} Pb applications. The root length was significantly decreased by the application of Cd only at 20 mg kg^{-1}. However, root length under all Pb concentrations did not differ significantly from the control. The highest root growth in both treatments was found at a concentration above the heavy metals toxic levels in the soil, 5 mg kg^{-1} Cd and 600 mg kg^{-1} Pb. Therefore, this means toxic Pb and Cd concentrations do not hinder the growth of *C. olitorius* roots.

The tallest plants (49.80 cm) were recorded at 600 mg kg^{-1} Pb application and the shortest (13.00 cm) were attained at 25 mg kg^{-1} Cd levels in the soil (Table 3.2). Lead application from 150 to 600 mg kg^{-1} significantly promoted plant height. In Pb-treated soil, the tallest plants (49.80 cm) were recorded at 600 mg kg^{-1} and the shortest (29.80 cm) were obtained at 1000 mg kg^{-1}. In Cd-treated soils, the tallest plants (24.60 cm) were recorded at 5 mg kg^{-1} and the shortest (13.00 cm) at 25 mg kg^{-1}. Cadmium application at all concentrations caused a significant decline in plant height compared with the control.

Table 3.2 Effects of Pb and Cd on growth parameters of *C. olitorius* at 44 days after sowing.

Tt	Conc.	RL	PH	SG	NOB	NOL	LA	LCC
0	0.00	25.00 ab	38.00 c	5.09 a	8.0 ab	25.8 bc	54.13 a	54.07 a
Pb	150	25.00 ab	43.60 b	4.86 ab	8.8 a	34.4 a	49.90 ab	44.54 b
	300	25.33 ab	44.20 b	4.68 ab	8.0 ab	30.4 ab	47.11 b	44.04 b
	600	25.51 ab	49.80 a	4.79 ab	7.8 ab	24.0 c	46.73 b	37.77 c
	900	24.00 ab	36.00 c	4.44 abc	7.4 abc	20.8 c	36.22 c	43.53 b
	1000	15.67 bc	29.80 d	4.05 bcd	7.2 abc	14.2 de	34.76 c	34.52 c
Cd	5	32.00 a	24.60 d	4.41 abc	8.6 a	15.4 d	30.75 c	20.38 de
	10	25.00 ab	16.00 e	3.65 cd	5.0 bcd	12.4 de	15.16 de	23.47 d
	15	20.67 bc	15.87 e	3.63 cd	4.4 cde	11.6 de	14.66 de	16.97 e
	20	13.00 c	13.80 e	3.16 de	3.0 de	11.6 de	17.27 d	10.40 f
	25	–	13.00 e	2.23 e	1.6 e	9.6 e	10.70 e	20.64 de
Stats	GM	21.33	30.75	4.19	6.35	19.57	33.61	33.34
	CV %	15.7	7.9	10.2	23.5	12.5	8.5	5.2
	LSD	5.67	3.09	0.55	1.96	3.12	3.66	2.21
	P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Means followed by the different letter (s) within a column vary significantly at 5% level of significance. Tt = treatments, Conc = concentrations (mg kg^{-1}), RL = root length (cm), PH = plant height (cm), SG = stem girth (mm), NOB = number of branches, LA = leaf area (cm^2), NOL = number of leaves, LCC = leaf chlorophyll content. GM = grand mean, CV% = co-efficient of variation percentage, LSD = least significant difference, p = probability value. Em dash (–) means that there was no harvest because of seedling mortality.

The thickest stems (5.09 mm) were found in the control while the thinnest stems (2.23 mm) were recorded at 25 mg kg^{-1} Cd (Table 3.2). A significant reduction in stem girth was only recorded at 1000 mg kg^{-1} in Pb-treated plants. The lowest Pb concentration (150 mg kg^{-1}) resulted in the thickest stems (4.86 mm), whereas the highest Pb concentration (1000 mg kg^{-1}) gave the thinnest stem (4.05) among Pb-treated plants. Among Cd treatments, plants exposed to 5 mg kg^{-1} Cd had the widest stems (4.41 mm), whereas those treated with 25 mg kg^{-1} Cd had the thinnest (2.23 mm). All concentrations above 5 mg kg^{-1} significantly reduced the stem girth of *C. olitorius* plants. This reduction in stem girth increased with an increase in Cd soil concentrations.

The maximum number of branches (8.8) occurred in plants exposed to 150 mg kg^{-1} Pb and the minimum (1.6) was recorded in those treated with 25 mg kg^{-1} Cd (Table 3.2). Lead application into the soil resulted in an insignificant reduction in plant branching when compared with the control treatment. The most numerous branches (8.8) were recorded in 150 mg kg^{-1} Pb-treated plants and the fewest (7.2) were attained in plants grown under 1000 mg kg^{-1} Pb. In Cd-treated plants, maximum branches (8.6) were recorded at 5 mg kg^{-1} treatment, whereas the least branches (1.6) were attained at 25 mg kg^{-1} treatment. A significant decline in the number of branches was recorded at 15 mg kg^{-1} Cd, which then increased with an increase of Cd concentration in the soil.

3.3.3 Number of leaves, leaf area and leaf chlorophyll content

Lead application of 150 mg kg^{-1} resulted in the highest number of leaves (34.4) and the lowest leaves number (9.6) was recorded at 25 mg kg^{-1} cadmium (Table 3.2). A significant reduction in the number of leaves was only observed at 1000 mg kg^{-1} in Pb-treated plants, compared with the control. The highest number of leaves in Pbtreated plants was recorded at 150 mg kg^{-1}, whereas the lowest was attained at 1000 mg kg^{-1}. In Cd-treated plants, the most numerous leaves (15.4) were recorded at 5 mg kg^{-1} but the fewest (9.6) were obtained at 25 mg kg^{-1} of soil. Cadmium application showed a severe decline in the number of leaves, compared with the control. This decline increased with an increase in Cd levels in the soil.

The broadest leaves (54.13 cm^2) were obtained from in control plants, whereas the narrowest (10.70 cm^2) were recorded at 25 mg kg^{-1} Cd (Table 3.2). Application of both Pb and Cd in the soil significantly retarded leaf growth. A Pb application of 150 mg kg^{-1} resulted in the highest leaf area (49.90 cm^2) and further application reduced leaf area with the minimum (34.76 cm^2) obtained at 1000 mg kg^{-1}. This significant reduction was first observed at 300 mg kg^{-1} in Pb-treated plants and increased with an increase in Pb in the soil. Cadmium application of 5 mg kg^{-1} resulted in the highest leaf area (30.75 cm^2) with the lowest (10.70 cm^2) obtained at 25 mg kg^{-1}. This significant reduction of leaf area by Cd also increased with an increase in Cd in the soil.

The highest value of the chlorophyll content (54.07) was attained in the control while the lowest (10.40) was obtained at 20 mg kg^{-1} Cd (Table 3.2). The application of Pb and Cd significantly decreased leaf chlorophyll content compared with the control. Among Pb treatments, the highest chlorophyll content (44.54) was recorded at 150 mg kg^{-1} and the lowest (34.52) was obtained at 1000 mg kg^{-1}. Application of 10 mg kg^{-1} resulted in the highest chlorophyll content (23.47), but 20 mg kg^{-1} had the lowest (10.40) in Cd-treated plants. The reduction in the chlorophyll content was quite significant, but it did not follow any trend as per the increase in heavy metal concentrations.

3.3.4 Roots, stem and leaves fresh and dry mass

Lead concentration of 150 mg kg^{-1} resulted in the highest leaves fresh mass (8.19 g), whereas, leaves with the lowest fresh mass (2.09 g) were obtained at 20 mg kg^{-1} Cd (Table 3.3). The presence of Pb in the soil had an insignificant reduction in leaf fresh mass compared with the control. The heaviest fresh leaves (8.19 g) were obtained at 150 mg kg^{-1}, while the lightest (6.05 g) were recorded at 1000 mg kg^{-1} Pb application. Under Cd treatments, leaves with the greatest mass (7.25 g) were recorded at 10 mg kg^{-1} and the ones with the least mass (2.09 g) were found at 20 mg kg^{-1}. A significant decline in leaf fresh mass was observed from 15 mg kg^{-1} upwards, in Cd-treated plants

Table 3.3 Effect of lead and cadmium on plant biomass and moisture content of *C. olitorius*

Tt	Conc.	LFM	SFM	RFM	LDM	SDM	RDM	LMC	SMC	RMC
Control	0.00	7.47 ab	5.5 bc	1.39 abc	1.11 bc	0.70 a	0.51 a	84.90 a	61.73 c	87.20 a
Pb	150	8.19 a	12.10 a	1.94 a	2.62 a	1.29 a	0.17 a	69.33 bc	91.73 a	89.30 a
	300	7.28 b	9.25 b	1.36 a	0.89 bc	0.89 a	0.47 a	81.50 ab	65.20 c	91.07 a
	600	6.26 bc	6.42 bc	1.35 abc	1.28 bc	0.89 a	0.47 a	78.83 abc	65.93 c	87.13 a
	900	6.36 bc	6.59 bc	1.18 bc	1.14 bc	0.65 a	0.32 a	81.10 ab	67.87 bc	87.07 a
	1000	6.05 bc	5.41 bc	0.72 c	0.68 c	0.77 a	0.22 a	91.33 a	71.17 bc	93.27 a
Cd	5	7.23 ab	6.86 bc	1.52 ab	1.51 b	1.07 a	0.48 a	78.07 abc	71.53 bc	84.64 a
	10	7.25 ab	4.76 bc	1.58 ab	1.18 bc	0.61 a	0.24 a	86.07 a	84.40 ab	90.93 a
	15	5.12 c	4.36 bc	0.89 bc	1.00 bc	0.61 a	0.25 a	83.20 ab	70.87 bc	86.10 a
	20	2.09 d	2.12 c	0.85 bc	0.44 bc	0.22 a	0.09 a	65.20 c	76.20 abc	72.40 a
	25	–	–	–	–	–	–	–	–	–
Stats	GM	6.20	5.95	1.25	1.19	0.77	1.39	80.40	86.9	71.7
	CV%	9.4	33.1	21.6	20.7	44.9	5.7	6.3	10.5	8.1
	LCD	0.99	3.35	0.46	0.42	0.59	5.09	8.86	15.57	9.91
	P-value	0.18	0.18	<.001	<.001	0.18	0.04	<.001	0.43	<.001

Means followed by the different letter (s) within a column vary significantly at 5% level of significance. 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 (%). GM = grand mean (cm), CV% = co-efficient of variation percentage (%), LSD = least significant difference, p = probability value. Em dash (–) means that there was no harvest because of high seedling mortality.

The heaviest fresh stems (12.10 g) were obtained at 150 mg kg^{-1} Pb while the lightest (2.12 g) were recorded at 20 mg kg^{-1} Cd (Table 3.3). A 150 mg kg^{-1} Pb resulted in significantly heavier fresh stems than the control. Stem fresh mass was highest at 150 mg kg^{-1} and lowest at 1000 mg kg^{-1} among Pb treatments. The maximum mass of fresh stem under Cd treatments was 6.86 g recorded at 5 mg kg^{-1}, whereas the minimum was 2.12 g recorded at 20 mg kg^{-1}. The significant effect of the Cd application was evident at 20 mg kg^{-1} when compared with the control.

The highest root fresh mass (1.92 g) was obtained at Pb 150 mg kg^{-1}, while the lowest (0.72 g) was attained at 1000 mg kg^{-1} (Table 3.3). None of the two heavy metals had a significant effect on root fresh mass compared with the control. In the presence of Cd in the soil, the heaviest fresh roots (1.58 g) were at 10 mg kg^{-1} whereas the lightest (0.85 g) were at 20 mg kg^{-1}.

The highest leaf dry mass (2.62 mg) was recorded at 150 mg kg^{-1} Pb, while the lowest (0.68 g) was obtained at 1000 mg kg^{-1} Pb compared with the control andother treatments (Table 3.3). There was a significant mass increase obtained at 150 mg kg^{-1} Pb. Results from Pb concentrations above 150 mg kg^{-1} did not vary significantly from the control. For the Cd-treated plants, leaf dry mass declined from 1.51 g (maximum) attained at 5 mg kg^{-1} to 0.44 g (minimum) recorded at 20 mg kg^{-1} comapared to the control. Cadmium application resulted in insignificant variations in leaf dry mass.

The highest stem dry mass (1.29 g) was recorded at 150 mg kg^{-1} Pb and the lowest (0.22 g) was obtained at 20 mg kg^{-1} Cd levels (Table 3.3). Also, the highest root dry mass (0.51 g) was obtained in the control plants and the lowest (0.09 g) was recorded at 20 mg kg^{-1} Cd. Application of both Pb and Cd in the soil caused insignificant differences in dry mass of both stems and roots when compared with the control and among treatments.

The highest leaf moisture content (91.33 %) was obtained at 1000 mg kg^{-1} Pb while the lowest (65.20 %) was found at 20 mg kg^{-1} Cd (Table 3.3). A significant reduction in leaf moisture content was only recorded at 150 mg kg^{-1} in Pb-treated plants.

Application of 1000 mg kg^{-1} resulted in the highest leaf moisture content (91.33%) and

150 mg kg^{-1} gave the lowest (69.33%). There was no linear relationship between Pb content in the soil and leaf moisture content. Cadmium application of 10 mg kg^{-1} gave the highest leaf moisture (86.07%), whereas 20 mg kg^{-1} resulted in the lowest moisture content (65.20%). A significant reduction in leaf moisture content was observed at 20 mg kg^{-1} Cd-treated plants.

Lead application of 150 mg kg^{-1} resulted in the highest stem moisture content (91.73%) but the lowest (61.75%) was recorded in the control plants (Table 3.3). A significant increase in stem moisture content was observed at 150 mg kg^{-1} in Pbtreated plants. Lead levels above 150 mg kg^{-1} did not vary significantly from the control. The maximum stem moisture content of 91.73% was recorded at 150 mg kg^{-1} and the minimum moisture content of 61.73% was found in the control plants, in plants grown under Pb treatments. For cadmium, 10 mg kg^{-1} obtained the highest stem moisture of 84.40% while the control resulted in the lowest moisture of 61.73%. Only cadmium treatment of 10 mg kg^{-1} resulted in a significant increase in stem moisture content, but concentrations above and below 10 mg kg^{-1} did not differ significantly from the control.

Lead concentration of 1000 mg kg^{-1} resulted in the highest root moisture content (93.29%), while 20 mg kg^{-1} of Cd obtained the lowest root moisture content (72.40%) (Table 3.3). However, the application of both Pb and Cd had an insignificant effect on root moisture content. Plants grown under 25 mg kg^{-1} were burned by the Cd, hence nothing was harvested for analysis at this concentration.

3.3.5 Reproductive traits

All plants treated with Pb produced pods at varying levels but only 5 mg kg^{-1} Cdtreated plants produced pods (Table 3.4). The number of pods; pod length and width; the number of seeds per pod; as well as total and 100-seed masses of plants treated with 5 mg kg^{-1} Cd had insignificant differences with the control. Applications 150 and 300 mg kg^{-1} Pb; 5 mg kg^{-1} Cd, as well as the control plants, resulted in the highest number of pods (7), while the lowest number of pods (3) was recorded at 1000 mg kg^{-1} Pb in the soil (Table 3.4). However, the application of Pb had an insignificant effect on the number of pods per plant when compared with the control.

The longest pods (6.90 cm) were recorded at Pb 150 mg kg^{-1} while the shortest (4. 58 cm) were attained at 1000 mg kg^{-1} Pb (Table 3.4). A significant reduction in pod length started only at 900 mg kg^{-1} Pb application. The control contained the heaviest pods (5.46 g), while the lightest pods (4.18 g) were obtained at 1000 mg kg^{-1} Pb. In the Pbtreated plants, the biggest pod mass (5.44 g) was observed at 150 mg kg^{-1} while the lowest (4.18 g) was recorded at 1000 mg kg^{-1} Pb levels. The only significant reduction in pod mass was found at 1000 mg kg^{-1}. Lead concentrations less than 1000 mg kg^{-1} did not differ significantly from the control.

Table 3.4: Effect of lead and cadmium on the reproduction traits of *C. olitorius*

Tt	Conc.	PN	PL	PM	NS/ pod	TSM	100-SM
Control	0	7 a	6.36 ab	5.46 a	114.4 abc	0.12 b	0.11 b
Pb	150	7 a	6.90 a	5.42 a	139.9 a	0.25 a	0.18 a
	300	7 a	6.08 a	5.44 a	121.4 ab	0.17 ab	0.14 ab
	600	6 a	5.50 bc	5.26 a	113.0 abc	0.13 b	0.12 b
	900	6 a	4.96 c	4.81 ab	98.4 bc	0.15 b	0.15 ab
	1000	3 a	4.58 c	4.18 b	86.6 c	0.11 b	0.12 b
Cd	5	7 a	6.20 ab	5.22 ab	113.8 abc	0.17 ab	0.15 ab
	10	–	–	–	–	–	–
	15	–	–	–	–	–	–
	20	–	–	–	–	–	–
	25	–	–	–	–	–	–
Stats	GM	5.97	5.89	5.13	112.7	0.14	0.15
	CV%	36.5	7.0	9.2	14.8	19.6	30.4
	LSD	0.61	3.16	0.69	21.24	0.03	0.06
	P-value	0.088	0.001	0.003	0.002	0.001	0.001

Means followed by different letter (s) within a column vary significantly at 5% level of significance. PN = number of pods, PM = Pod mass per 5 pods, PL = Pod length (cm), NS/pod = number seeds per pod, TSM = total seeds mass (g), 100-SM = seeds mass per 100 seeds (g). GM = grand mean, CV% = co- efficient of variation percentage, LSD = least significant difference, p = probability value. Em dash (–) means that pods were not produced in these Cd concentrations.

The number of seeds per pod was highest (139.9) at 150 mg kg^{-1} Pb and the lowest (86.6) at 1000 mg kg^{-1} Pb (Table 3.4). A significant decline in the number of seeds was observed only at 1000 mg kg^{-1} Pb, but Pb levels below this concentration resulted in an insignificant decline. Lead application of 150 mg kg^{-1} resulted in the largest total seed mass (0.25 g), whereas the lowest (0.11 g) was recorded at 1000 mg kg^{-1} Pb. A significant seed mass increase was obtained at 150 mg kg^{-1} in Pb-treated plants. Concentrations above 150 mg kg^{-1} did not vary significantly from the control.

The highest 100-seed mass was recorded at 150 mg kg^{-1} Pb, whereas the lowest (0.12 g) was at 600 and 1000 mg kg^{-1} Pb in the soil. A significant increase in 100-seed mass was only obtained at 150 mg kg^{-1} Pb, but concentrations above this one caused insignificant differences compared with the control. The reproductive traits in Cd treated plants were only observed at 5 mg kg^{-1} and gave similar properties as the control plants.

3.3.6 Correlation matrix between vegetative and reproductive traits of *C. olitorius*

All traits were positively correlated to one another. Root length exhibited a significant correlation with most of the traits except for plant height, leaf area, number of leaves and leaf chlorophyll content (Table 3.5). The plant height was also significantly correlated to most traits except for all moisture content, root fresh mass, and leaf dry mass. The stem girth was significantly correlated to almost all traits apart from root dry mass. The number of branches also showed a significant relationship with almost all traits expect with root dry mass and stem moisture. The root dry mass was only significantly linked with root length (0.64) and plant height (0.81).

3.3.7 Principal component analysis

The principal component analysis grouped traits into three informative components, which accounted for the total variability of 90.56% (Table 3.6). The first principal component, which accounted for 69.56% of the total variability, was positively defined by almost all the traits except the root dry mass and stem moisture content.

Table 3.5 Correlation matrix among investigated traits

Variables	RL	PH	SG	NOB	NOL	LA	LCC	LFM	SFM	RFM	LDM	SDM	RDM	LMC	SMC	RMC	PN	PL	PW	NS/Pod	TSM
PH	0.53																				
SG	**0.84**	**0.86**																			
NOB	**0.83**	**0.82**	**0.95**																		
NOL	0.52	**0.9**	**0.81**	**0.76**																	
LA	0.56	**0.94**	**0.91**	**0.87**	**0.9**																
LCC	0.40	**0.85**	**0.78**	**0.73**	**0.83**	**0.9**															
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	**0.8**	**0.77**	**0.68**	**0.6**	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**						
PN	**0.67**	**0.88**	**0.88**	**0.90**	**0.79**	**0.9**	**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**	**0.8**	**0.64**	**0.69**	0.56	0.55	**0.72**	0.45	0.42	0.27	0.48	**0.97**	**0.99**			
NS/POD	**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**	**0.80**	**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**

Variables: RL = Root length, PH = Plant height, SG = Stem girth, NOS =Number of shoots, LA = Leaf area, NOL = Number of leaves, LCC = leaf chlorophyll content, LFM = Leaf fresh mass, SFM = Stem fresh mass, RFM = Root fresh mass, LDM = Leaf dry mass, SDM = Stem dry mass, RDM = Root dry mass, TMC = Total moisture content, PN = Pod number, PL = Pod length, PW = Pod Width, NS/pod = Number of seeds per pod, TSM = total seed mass. Values ≥ 0.6 in **bold** are significant

The second and the third principal components contributed 14.63% and 6.37% variability, which were associated with stem moisture content and root dry mass, respectively.

Table 3.6 Principal components analysis of vegetative and reproductive traits.

Variables	PC1	PC2	PC3
RL	**0.84**	0.45	0.16
PH	**0.87**	-0.35	-0.15
SG	**0.98**	0.04	0.07
NOS	**0.98**	0.00	0.07
NOL	**0.85**	-0.24	-0.34
LA	**0.90**	-0.31	0.04
LCC	**0.77**	-0.38	-0.01
LFM	**0.84**	0.43	0.03
SFM	**0.83**	0.13	-0.46
RFM	**0.82**	0.45	-0.20
LDM	**0.75**	0.35	-0.49
SDM	**0.89**	0.22	-0.27
RDM	0.54	0.16	**0.60**
LMC	**0.65**	0.58	0.39
SMC	0.58	**0.76**	-0.06
RMC	**0.73**	0.58	0.22
PN	**0.91**	-0.36	0.08
PL	**0.90**	-0.38	0.20
PW	**0.92**	-0.35	0.07
ND/POD	**0.89**	-0.40	0.20
TSM	**0.87**	-0.37	0.07
100-SM	**0.89**	-0.31	0.07
Eigenvalue	15.30	3.22	1.40
Variability (%)	69.56	14.63	6.37
Cumulative %	69.56	84.19	90.56

Variables: RL = Root length, PH = Plant height, SG = Stem girth, NOB =Number of branches, LA = Leaf area, NOL = Number of leaves, LCC = leaf chlorophyll content, LFM = Leaf fresh mass, SFM = Stem fresh mass, RFM = Root fresh mass, LDM = Leaf dry mass, SDM = Stem dry mass, RDM = Root dry mass, TMC = Total moisture content, PN = Pod number, PL = Pod length, PW = Pod width, NS/pod = Number of seeds per pod, TSM = total seed mass

The principal components biplots indicated the link between the studied traits with Pb and Cd treatments (Table 3.2). The biplot also grouped the Pb and Cd treatments into four informative clusters. The first cluster contained the Cd treatments from 5 to 20 mg kg^{-1}, whereas the second cluster combined the controls, 150, 300, 600 and 900 mg kg^{-1} Pb treatments. The highest concentrations of both Cd and Pb (25 and 1000 mg kg^{-1}) in cluster IV and III, respectively, both had a severe reduction in plant growth such that Cd even resulted in death of seedling at a seedling stage.

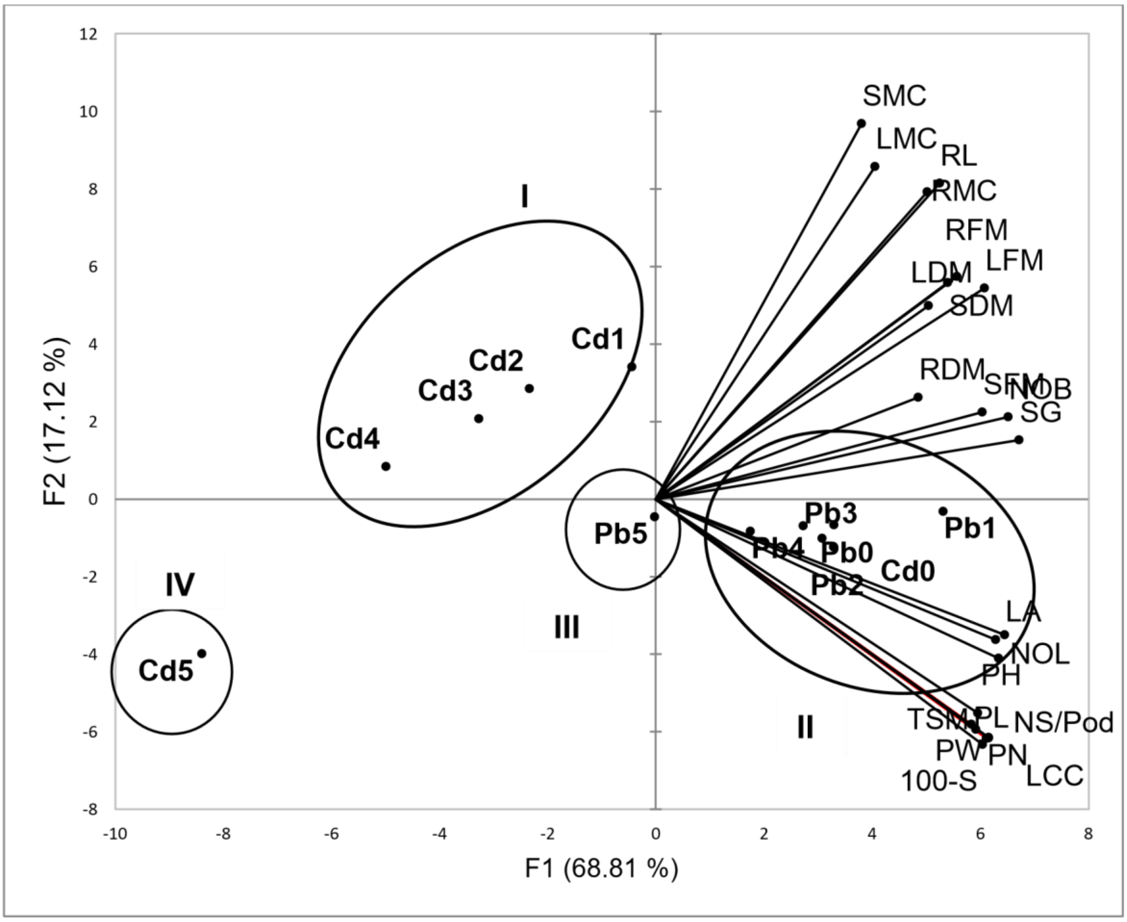


Figure 3.2 Biplot of traits as well as Pb and Cd treatments.

Traits: RL = Root length, PH = Plant height, SG = Stem girth, NOB =Number of branches, LA = Leaf area, NOL = Number of leaves, LCC = leaf chlorophyll content, LFM = Leaf fresh mass, SFM = Stem fresh mass, RFM = Root fresh mass, LDM = Leaf dry mass, SDM = Stem dry mass, RDM = Root dry mass, TMC = Total moisture content, PN = Pod number, PL = Pod length, PW = Pod width, NS/pod = Number of seeds per pod, TSM = total seed mass. Treatments: Pb = Lead, Cd = Cadmium, Pb1 = 150 mg kg^{-1}, Pb2 = 300 mg kg^{-1}, Pb3 = 600 mg kg^{-1}, Pb4 = 900 mg kg^{-1}, Pb5 = 1000 mg kg^{-1}, Cd1 = 5 mg kg^{-1}, Cd2 = 10 mg kg^{-1}, Cd3 = 15 mg kg^{-1}, Cd4 = 20 mg kg^{-1} and Cd5 = 25 mg kg^{-1}. mg kg^{-1} = milligram per kilogram

The dendrogram clustered treatments into two groups based on the 22 investigated traits (Figure 3.3). The first cluster contained control, all Pb treatments as well as 5 mg kg^{-1} Cd. These treatments were closely linked by the stem girth and number of branches per plant, which was no affected by the increase of either Pb and Cd in the soil application. The second cluster that was divided into two sub-clusters associated 10–25 mg kg^{-1} Cd treatments, where 25 mg kg^{-1} was a singleton. All these treatments uniformly reduced all the morphological traits, such that 25 mg kg Cd application even resulted in death of seedlings.

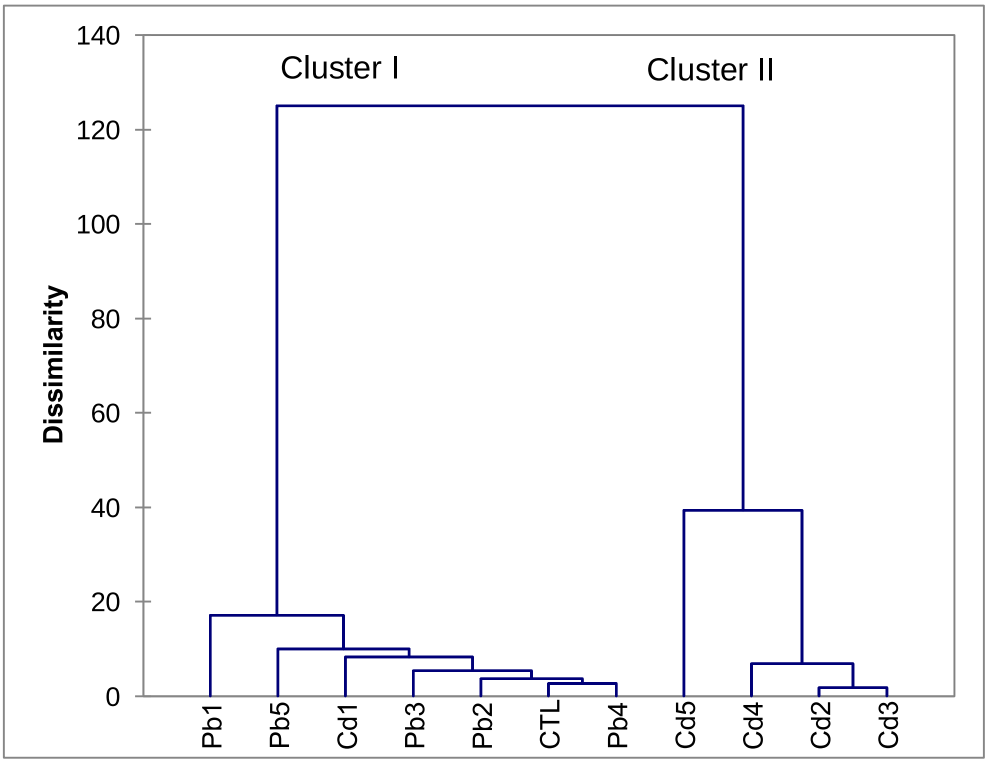


Figure 3.3: A Hierarchical cluster analysis dendrogram displaying the relationship among Pb and Cd treatments.

Pb = Lead, Cd = Cadmium, Pb1 = 150 mg kg^{-1}, Pb2 = 300 mg kg^{-1}, Pb3 = 600 mg kg^{-1}, Pb4 = 900 mg kg^{-1}, Pb5 = 1000 mg kg^{-1}, Cd1 = 5 mg kg^{-1}, Cd2 = 10 mg kg^{-1}, Cd3 = 15 mg kg^{-1}, Cd4 = 20 mg kg^{-1} and Cd5 = 25 mg kg^{-1}. Mg kg^{-1} = milligrams per kilogram

3.4 Discussion

3.4.1 Seed germination and seedling mortality

An increase in Pb concentration from 600 to 1000 mg kg^{-1} in the soil resulted in a decline in seed germination of *C. olitorius* (figure 3.1a). Similarly, an increase of Pb concentration in the soil causes a decline in the germination of *Arachis hypogeae* (Abraham *et al*., 2013) and *Pisum sativum* (Deswal and Laura, 2018) seeds. High levels of Pb in the soil are known to inhibit the germination of seeds (Kabir *et al*., 2010). When Pb is in excess in the soil, it interferes with hydrolytic enzymes that break down the cotyledons to initiate the germination process (Kabir *et al*., 2010: Ambika *et al*., 2016). However, treatments 150 and 300 mg kg^{-1} were not significantly different from the control plants.

The increased levels of Cd in the soil to 25 mg kg^{-1} triggered a significant decline in seed germination in a treatment dependent manner when compared with the control (Figure 3.1b). High concentrations of Cd also cause a decline in seed germination in *Suaeda salsa* (Liu *et al.*, 2012) and *Arachis hypogeae* (Abraham *et al*., 2013). This may be because Cd affects the plasma membrane and reduces water uptake, and therefore affects the seed germination process (Ambika *et al*., 2016). Although 5 mg kg^{-1} Cd application is above the world permissible limits in agricultural soils (3 mg kg^{-1}), it caused an insignificant effect on the germination of seeds compared with the control. Therefore, *C. olitorius* seeds can be successfully germinated at toxic levels of Cd in the soil.

Seedling mortality began only at 900 mg kg^{-1} and increased ith an increase of Pb in the soil (Figure 3.1a). Similarly, high Pb concentrations in the growth medium also cause an increase in seedling mortality in *Arachis hypogeae* (Abraham *et al*., 2013) and *Pisum sativus* (Deswal and Laura, 2018). The presence of Pb in the soil inhibits root growth owing to a decrease in water and nutrients uptake, resulting in mortality in most plants (Ali *et al*., 2018). However, *Corchorus olitorius* seedlings survived mortality at concentrations higher than the maximum permissible limits of Pb in the soil. In most plants, Pb at low levels does not have a negative effect of plant metabolism, which results in normal seedling growth (Abbasi *et al*., 2017).

Seedling mortality began at the lowest Cd level (5 mg kg^{-1}) and increased with an increase of its application in the soil (Figure 3.1b). An increase in Cd contents in the soil also results in dying of *Pisum sativum* seedlings (Deswal and Laura, 2018) and retards seedling growth in *Ricinus communis* (Dutta *et al*., 2018). Death of plants exposed to high Cd levels is caused by its uptake which follows a similar path as some of the essential elements such as Zn, competes with them, and then results in the deficiency of such elements (Ali *et al*., 2018). Certain plants survive heavy metals toxicity by the use of antioxidance compouds with protects thecells from being damaged by the high levels of heavy metals takan by the plant (Singh *et al*., 2015).

3.4.2 Root length, plant height, stem girth, and number of branches

The presence of Pb in the soil at any concentration resulted in an insignificant impact on root growth (Table 3.2). Contrary to *C. olitorius*, Pb application of 150 and 300 mg kg^{-1} in *Solanum melongena* (Yilmaz *et al*., 2009) and *Spinacia oleracea* (Naz *et al*., 2015) results in a severe decline in root length. Again, increasing the Pb application also results in a decline in the length of *Oryza sativa* roots (Alfaraas *et al*., 2016: Marquez *et al*., 2018). In the current study *C. olitorius* roots grew well under toxic Pb concentrations in the soil (above 300 mg kg⁻1) without any morphological changes. In most plants, Pb inhibits root growth and even results in death of roots (Gill, 2014). This is because roots are more suscebtible to Pb toxicity when compared to the aerial parts of the plants, hence they are ones very close to heavv metals contacts (Al-Akeel *et al*., 2016).

The effect of Cd in root growth varied with different treatments (Table 3.2). The lowest concentration of Cd (5 mg kg^{-1}) promoted root growth while 20 mg kg^{-1} Cd application resulted in a severe decline in root growth when compared with the control. High Cd application also results in root growth inhibition in *Anethum graveolens* (Aghaz *et al*., 2013), *Spinacia oleracea* (Khan *et al.,* 2013), *Ocimum basilicum* and *Ocimum basilicum var. purpurescens* (Gharebaghi *et al*., 2017). Similar trends were obserevd in *Miscanthus sinensis, Miscanthus floridulus, Iscaanthus sacchariflorus* (Gou *et al*.,

2016) and *Corchorus olitorius* (Hassan *et al*., 2016). Cadmium inhibits iron (Fe) reduction in the root growth and therefore disturbs the metabolic activities in the roots, which leads to reduced plant growth (Naz *et al*., 2015: Ambika et al., 2016).

The treatments of plants with Pb from 150 to 600 mg kg^{-1} promoted plant growth and resulted in longer plants, whereas treatments from 900 to 1000 reduced the plant height in a concentration-dependent manner (Table 3.2). However, Pb treatments similar to 150 – 600 mg kg^{-1} reduces the height of *Mentha piperita* main stems (Amirmoradi *et al*., 2012). High Pb levels result in a decrease in plant height in *Ocimum tenuifolium* (Dwivedi *et al*., 2012), *Spinacia oleracea* (Khan *et al*., 2013) and *Cedrela odorata* (Akintola *et al*., 2019). Most of the accumulated Pb is stored in the roots, therefore blocking the passage of nutrients from the roots to the aerial parts of the plants, which results in stunted growth (Gill, 2014).

Cadmium application at all concentrations resulted in a significant decline in plant height and the effect increased with an increase of its application in the soil (Table 3.2). An increase in Cd concentration in the soil also causes a decrease in the height of *Phaselous vulgaris*, *Thespesia populnea*, (Kabir *et al*., 2010), *Spinacia oleracea* (Naz *et al*., 2015), *Hordeum vulgare* (Januškaitienė and Klepeckas, 2015), *Miscanthus sinensis, Miscanthus floridulus, Miscanthus sacchariflorus* (Ghou *et al*., 2016) and *Corchorus olitorius* (Hassan *et al*., 2016) plants. An Increase in concentrations of Cd in the soil interferes with cell formation in the roots which results in reduced plant growth (Naz *et al*., 2015). Heavy metals toxicity interferes with cell division, which reduces the uptake of water and nutrients by roots, resulting in reduced plant growth (Sigh *et al*., 2015).

Lead application in the soil had an insignificant impact on the stem girth of *C. olitorius* compared with the control (Table 3.2). However, the presence of Pb causes a decrease in shoots diameter in *Spinacia oleracea* (Khan *et al*., 2013) and *Ligustrum vulgare* seedling (Zhou *et al*., 2016) and *Cedrela odorata* (Akintola *et al*., 2019). This reaction of *C. olitorius* to Pb toxicity may be explained by the fact that different plants react differently to Pb toxicity in the soil (Wang *et al*., 2015).

Cadmium application from 10 to 20 mg kg^{-1} resulted in a drastic reduction in stem girth when compared with the control (Table 3.2). Increasing levels of Cd in the soil results in a decline in the diameter in *Spinacia oleracea* (Khan *et al*., 2013) and *Gynura procumbens* (Ibrahim *et al*., 2017) shoots. High Cd content in plant tissues induces oxidative stress that affects photosynthesis in plants, and thus decreases the production of carbohydrates, which are responsible for plant growth (Mizushima *et al*., 2019). The decline in stem diameter at 5 mg kg^{-1} (which is also above the World Cd safe limits) did not differ significantly from the control plants. The stem of *C. olitorius* is not susceptible to Cd toxicity.

The application of Pb in the soil had an insignificant effect on the number of branches even at high concentrations (Table 3.2). On contrary, an increase in Pb levels results in a decrease in the number of surviving *Mentha piperita* (Amirmoradi *et al*., 2012), *Salix matsudana* and *Salix babylonica* (Wang *et al*., 2016) shoots per stem. On the other hand, the number of branches was significantly reduced by Cd application from 10 to 20 mg kg^{-1} when compared with the control. The reduction increased with an increase in Cd concentration in the soil. This can be attributed to Cd inactivations of enzymesresponsible for cell divisions during roots growth and direct exposure of cellular enxymes to toxic environment which might cause injuries in cells (Azhar e*t al*., 2019).

3.4.3 Leaf number, leaf area and leaf chlorophyll content

Lead concentrations of 150 and 300 mg kg^{-1} increased the number of leaves, whereas the application of 1000 mg kg^{-1} decreased the number of leaves when compared with the control (Table 3.2). *Cedrela odorata* plants when grown in 260 mg kg^{-1} Pb, which is within the range between 150 and 300 mg kg^{-1}, results in a reduction in leaf number (Antikola et al., 2019). In other findings, an increase in concentrations of Pb in the soil reduces the leaf number in *Thespesia populnea* (Kabir *et al*., 2010), *Spinacia oleraceae* (Khan *et al*., 2013) and *Ocimum basilicum* and *Ocimum basilicum* var. *purpurescens* (Gharebaghi *et al*., 2017). The leaf number at Pb concentrations above the allowable limit (300 mg kg^{-1}) was not significantly different from the control. Therefore, toxic levels of Pb can result in healthy, appealing plants that are tempting for vegetable harvest because some plants have the potential to exclude tocix metals from the soil (Sigh et al., 2010).

There was a severe decline in leaf number with an increase from 10–25 mg kg^{-1} Cd content in the soil (Table 3.2). High Cd levels in the soil also result in a decline in the number of *Mentha piperita* (Amirmoradi *et al*., 2012) and *Spinacia oleraceae* (Khan *et al*., 2013) leaves. However, there are insignificant changes in the number of leaves in *Raphanus sativus* with an increase in Cd concentration in the soil (Hladun *et al*., 2015). This decline in leaf number may be caused by leaf senescence and leaf chlorosis caused by high Cd contents in the soil. Cadmium effect on root tips and root death results in the failure of plants to translocate water and nutrients to the above ground plant parts and then results in leaf abscission and chlorosis (Kamran *et al*., 2013: Gill, 2014). Cadmium toxicity stress also affects the colour of the leaves and results in leaf yellowing (Tran and Popova, 2012).

A reduction in leaf area because of an increase in Pb concentration recorded in *C. olitorius* was similar to the scenario that also occurs in *Mentha piperita* (Amirmoradi *et al*., 2012) and *Taraxacum officinale* (Bini *et al*., 2012). A possible reason or process for the reduction in leaf area is Pb interference with the cell membranes and reduces the uptake of water and nutrients, which also results in reduced photosynthesis rate, and then finally affects the leaf size (Ambika et al., 2016).

Cadmium application in the soil resulted in a drastic decline in the leaf area compared with the control (Table 3.2). The presence of Cd in high quantities in the soil also causes a reduction in *Helianthus annuus* (Chaves *et al*., 2011), *Ocimum basilicum* (Nazarian *et al*., 2016), *Gynura procumbens* (Ibrahim *et al*., 2017) and *Ricinus communis* (Dutta *et al*., 2018) leaf area. Cadmium toxicity in the soil results in plants with poor leaf size and structure. This may be attributed to the fact that high Cd content in the soil inhibits Fe uptake and seriously reduces the rate of photosynthesis (Ambika *et al*., 2016). The negative relationship of Cd to phosynthesi is because of the Cd reduction of chlorophyll content which also part of the process (Ahzar *et al*., 2019).

A decline in leaf chlorophyll content with an increase in Pb concentration recorded in this study is similar to the reduction found in *Triticum aestivum* and *Spinacia oleracea* (Lamhamdi *et al*., 2013) leaf chlorophyll content. However, there was no linear relationship between the leaf chlorophyll content and the level of Pb in the soil. Therefore, leaf chlorophyll content can be used as an indicator of Pb toxicity stress in *C. olitorius*. This reduction was probably a result of toxic levels of Pb that altered the relative proportion of chlorophyll a and chlorophyll b and thus reduced total chlorophyll production and the rate of photosynthesis (Khan *et al*., 2013).

The application of cadmium severely reduced the leaf chlorophyll content even at low concentrations (Table 3.2). Similarly, high Cd concentrations in the soil also result in a decline in leaf chlorophyll content in *Brassica nigra* (Chen *et al*., 2011) *Oryza sativa* (Fasahat, 2015), *Miscanthus sinensis, Miscanthus floridulus, Miscanthus. sacchariflorus* (Gou *et al*., 2016) and *Gynura procumbens* (Ibrahim *et al*., 2017). Again, there was no linear relationship between the level of Cd in the soil and the leaf chlorophyll content. *Corchorus olitorius* had the same reaction on the toxic and allowable Cd levels when it comes to the leaf chlorophyll content. The decline in chlorophyll content may be attributed to that Cd reacts with biosynthetic enzymes and blocks chlorophyll synthesis (Mohammed *et al*., 2017).

3.4.4 Leaf, stem and root fresh mass

An increase in the levels of Pb in the soil resulted in an insignificant decline in the leaf fresh mass when compared with the control (Table 3.3). Contrary to these results, high levels of Pb in the soil results in a decrease in the fresh mass of *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015) and *Spinacia oleracea* (Naz *et al*., 2015) leaves. In this study, the fresh mass of *C. olitorius* leaves grown at concentrations above the maximum lead allowable limits in the soil (300 mg kg^{-1}) was almost similar to that of control plants. Therefore, toxic levels of Pb do not affect the leaf fresh mass and can be a threat for human consumption.

The application of 15–20 mg kg^{-1} Cd in the soil resulted in a significant decrease in leaf fresh mass, compared with the control (Table 3.3). The high concentration of Cd in the growth medium causes a decline in the fresh mass of *Medicago sativ*a (Aslam *et al*., 2015) and *Ocimum basilicum* (Nazarian *et al*., 2016) leaves. Leaf fresh mass of plants exposed to the lower two Cd treatments (5 and 10 mg kg^{-1}), which are also above the Cd permissible limits in the soil (3 mg kg^{-1}), did not differ significantly from the control. Therefore, *C. olitorius* leaf fresh mass cannot be used as an indicator of Cd toxicity in the soil. The shoot fresh mass of *Helianthus annuus* is also not afftected by an increase of Cd in the soil up to toxic levels (Chaves *et al*., 2011).

The application of 150 mg kg^{-1}, resulted in a significant increase in stem fresh mass, whereas concentration above that resulted in an insignificant influence on the stem fresh mass (Table 3.3). On contrary, an increase in Pb concentration resulted in a decline in the fresh mass of *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015) and *Spinacia oleracea* (Naz *et al*., 2015) stem. These include Pb values above 300 mg kg^{-1}, which are above the world safe limits of Pb concentration in the soil. For cadmium, a significant decline in stem fresh mass was recorded at 20 mg kg^{-1} while values below that did not differ significantly from the control. A constant decline in stem fresh mass with an increase in Cd content in the soil also occurs in *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015) and *Ocimum basilicum (*Nazarian *et al*., 2016). The stem fresh mass was also not a good indicator of Cd toxicity because there was an insignificant reduction at Cd concentrations above the safe limits.

Root fresh mass of plants exposed to Pb concentrations ranging from 150 to 900 mg kg^{-1} had insignificant differences when compared with the control (Table 3.3). On contrary, high Pb concentrations resulted in a reduction in the fresh mass of *Spinacia oleracea* (Khan *et al*., 2013), *Triticum aestivum* (Lamhamdi *et aI*., 2013) and *Ceratophyllum demersum* (Afaj *et al*., 2017) roots. The only significant reduction in fresh mass of *C. olitorius* roots was recorded at 1000 mg kg^{-1} Pb treatment. This decrease in dry weight can be caused by the shortage of some nutrients elements, like N, P and K caused by the inhibitin of their uptake under Pb toxicity (Abassi et al., 2017).

The application of Cd at all concentrations (below and above toxic levels) showed an insignificant effect on *C. olitorius* root fresh mass (Table 3.3). However, elevated levels of Cd in the soil causes a drastic reduction in the fresh mass of *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015) and *Spinacia oleracea* (Naz *et al*., 2015) roots. This reduction occurs because when Cd is present in the plant it interferes with metabolism and affects plant growth (Ambika *et al*., 2016). As from the current results, the root fresh mass cannot be a factor used to determine Cd toxicity in the soil.

3.4.5 Leaf, stem and root dry mass

The application of Pb from 150 to 900 mg kg^{-1} resulted in an insignificant effect on the leaf dry mass (Table 3.3). Similarly, a high application of Pb also had an insignificant effect in the leaf dry mass of *Medicago sativa* leaves (Aslam *et al*., 2015). A clear reduction in leaf dry mass recorded at 1000 mg kg^{-1} Pb treatment in the current study was similar to a reduction recorded in *Mentha pepirita* (Amirmoradi *et al*., 2012) and *Spinacia oleracea* (Naz *et al*., 2015) shoot dry mass. These differences possibly happen because plants react differently towards Pb accumulation in their tissues, with some plants accumulating high Pb content but do not affect their biological activities (Abbasi *et al*., 2017).

The application of Cd in the soil led to an insignificant impact on leaf dry mass when compared with the control (Table 3.3). Similarly, the shoot dry mass of *Helianthus annuus* is not negatively affected by an increase in levels of Cd in the soil (Chaves *et al*., 2011). All the Cd treatments were above 3 mg kg^{-1} and they did not expose toxicity in terms of leaf mass. However, *Medicago sativa* (Aslam *et al*., 2015), *Ocimum basilicum* (Nazarian *et al*., 2016), *Miscanthus sinensis, Miscanthus floridulus and Miscanthus sacchariflorus* (Gou *et al*., 2016) that are grown under toxic Cd concentrations have a decline in their shoot dry mass. This is because High Cd concentration triggers the inactivation of enzymes responsible for sell division in the roots and retard plant growth (Ahzar *et al*., 2019).

Both Pb and Cd treatments also did not affect the dry mass of *C. olitorius* stems (Table 3.3). This differed from a constant decline in *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015) stem dry mass when plants were grown in soil with increasing Pb and Cd concentrations. In most plants, a decrease in dry mass is caused by the inhibition of nutrient uptake in the presence of high Pb, resulting in nutrients deficiency in plants (Abbasi *et al*., 2017). Again, the lack of water in the plant is also caused by the interaction of Cd with the plasma membrane that results in reduced water uptake (Ambika *et al*., 2016).

Root dry mass of Pb- and Cd-treated plants did not differ from that of the control plants (Table 3.3). The same observations are reported in Pb-treated *Oryza sativa* (Marquez *et al*., 2018). Contrary to these findings, high Pb concentrations in the soil results in a decrease in dry mass of *Spinacia oleracea* (Khan *et al*., 2013) *Triticum aestivum* (Lamhamdi *et al*. 2013) and *Ceratophyllum demersum* (Afaj *et al*., 2017) roots. Again, elevated levels of Cd in the soil results in a reduced root dry mass in *Mentha pepirita* (Amirmoradi *et al*., 2012), *Medicago sativa* (Aslam *et al*., 2015), *Spinacia oleracea* (Naz *et al*., 2015), *Corchorus olitorius* (Hassan *et al*., 2016) and *Oryza sativa* (Marquez *et al*., 2018). This might also be associated with the relationship of Cd with water uptake by the plants (Ambika *et al*., 2016).

3.4.6 Leaf, stem and root moisture content

Lead treatments did not affect the moisture content of *C. olitorius* leaves, except for a significant reduction at 150 mg kg^{-1}, when compared with the control (Table 3.3). On the contrary, high Pb concentrations in the soil decrease leaf moisture content in *Spinacia oleracea* (Naz *et al*., 2015). However, as in *C. olitorius*, the moisture content in *Eichhornia crassipes* leaves is not affected by Pb application (Malar *et al*., 2014). The presence of Pb in the plant results in in cell membranes blockage which results in reduced water uptake (Ambika *et al*., 2016).

A decrease in the moisture content of *C. olitorius* leaves because of an increase in Cd concentrations in the soil was similar to a reduction that occurred in the moisture content *Spinacia oleracea* (Naz *et al*., 2015) and *Ocimum basilicum* (Nazarian *et al*.,

2016) leaves. High Cd concentrations in the soil interfere with cell formation in the roots which results in reduced water uptake (Naz *et al*., 2015).

The lowest concentration of Pb (150 mg kg^{-1}) promoted stem moisture content, whereas concentrations above it caused insignificant differences when compared with the control (Table 3.3). Similarly, high Pb levels do not affect stem moisture content in *Eichhornia crassipes* (Malar *et al*., 2014), but in contrast, they decrease it in *Spinacia oleracea* (Naz *et al*., 2015). Almost all the Cd concentrations used in this study did not affect the moisture content of *C. olitorius* stems, in the same way as the water content of *Solanum lycopersicum* plant remains unaffected by the application of Cd in the soil (Godinho *et al*., 2018). However, in many plants, Cd affects the permeability of the plasma membrane and results in reduced water uptake by the cells (Ambika *et al*., 2016).

Both Pb and Cd treatments did not affect the root moisture content of *C. olitorius* roots (Table 3.3). Similarly, the root moisture content of *Eichhornia crassipes* (Malar *et al*., 2014) and *Solanum lycopersicum* (Godinho *et al*., 2018) is not affected by the application of Pd and Cd, respectively. However, an increase in Pb application in the soil results in a severe decrease in moisture content in *Spinacia oleracea* (Naz *et al*., 2015) roots. Lead adversely affects plant biomass by reducing the accumulation and translocation of other essential nutrients in plants and by blocking their entry or binding to the nutrient carriers, and thus make them unavailable for other elements (Pinho and Ladeiro, 2012). Also, Cd a non-nutrient element that uses the same absorption pathways as other essential elements, and competes with the absorption of water and other nutrients and results in both nutrients and water deficiencies (Ambika *et al*., 2016).

3.4.6 Fruit traits

An increase in Pb levels in the soil did not affect the reproductive traits of *C. olitorius* (Table 3.4). However, the presence of Pb in the soil results in a decrease in seeds mass in *Zea mays* (Ghani, 2010). Again, Pb concentrations above the world safe limits of 300 mg kg^{-1} did not have a severe impact on the seed production potential of the plant. Therefore, the pods of *C. olitorius* may appear healthy even when plants are grown in Pb-contaminated soil.

C. olitorius plants exposed to almost all Cd treatments failed to bear reproductive traits. Similarly, an increase in Cd concentration in the soil also results in a decline in seeds mass of *Zea mays* (Ghani, 2010); number of pods per plant and seed mass per 100 seeds in *Pisum sativum* (Alyemeni *et al*., 2014) and reproductive traits in *Solanum lycopersicum* (Rehman *et al*., 2011). Reproductive traits that were produced by *C. olitorius* plants treated with 5 mg kg^{-1} Cd did not differ significantly from the control plants. Similarly, the presence of Cd in the soil did not affect reproductive traits of *Raphanus sativus* (Hladun *et al*., 2015). The reason for the absence of reproductive traits in Cd treated plants is due to the effect of Cd in metabolic activities in the plant, leading reduced vegetative and reproductive growth (Naz *et al*., 2015).

3.4.7 Correlation among the studied traits

The correlation matrix showed that all the studied traits were positively correlated to one another (Table 3.5). These correlation results mean that traits are similarly affected by Pb and Cd. A positive significant correlation existed between the root length and most traits, except for the plant height, the number of leaves, leaf area, and leaf chlorophyll content. Another strong positive correlation between the increase of Cd levels in the soil and its effects in plant morphological traits was reported in *Lycopersicon esculentum* (Rehman *et al*., 2011) and *Aeluropus littoralis* (Rezvani and Zaefarian, 2011). However, a significant negative correlation between the effect of Pb and Cd on plant growth and biomass was observed in *Mentha arvensis* (Nigam *et al*., 2019).

3.4.8 Principal component and cluster analyses

The principal component analysis grouped traits into two informative components, which accounted for the total variability of 90.56% (Table 3.6). The first principal component, which accounted for 69.56% of the total variability, was positively defined by almost all the traits except the root dry mass and stem moisture content. The first principal was also responsible for 65% variation on the effect of Pb and Cd in the growth of *Mentha arvensis* (Nigam *et al*., 2019). However, in the effect of Pb and Cd in *Zea mays*, the first principal component was responsible for 48% total variation (Ali *et al*., 2017).

Biplot and dendrogram clustered the controls with 150–900 mg kg^{-1} Pb treatments together (Figure 3.2 and 3.3, respectively). These treatments together with the Cd treatments did not affect the stem girth and the number of branches. The second cluster separated all the other Cd treatments from the Pb treatments possibly because of its severe reduction of plant growth, such that 25 mg kg^{-1} Cd application even resulted in death of seedlings. However, high degrees of similarities were observed in the cluster analysis of the effect of both Pb and Cd in the growth and photosynthetic properties of *Mentha arvensis* (Nigam *et al*., 2019). The principal component and its biplots help the researchers to select the best treatments and genotypes that are intended to be improved (Ali *et al*., 2017).

3.5 Conclusion

It can be concluded from this study that the effect of Pb and Cd in germination, vegetative and reproductive features of *C. olitorius* depends on the concentration of each heavy metal in the soil. Concentrations from 150–300 mg kg^{-1} Pb and 5 mg kg^{-1} Cd promoted seed germination and hindered seedling mortality. However, concentrations above these treatments resulted in a drastic decline in germination and an increase in mortality. Certain Pb and Cd treatments above their world safe limits in the soil also promoted vegetative and reproductive growth of *C. olitorius*, resulting in taller plants with broader leaves. This causes *C. olitorius* plants that grow in Pb- and Cd-intoxicated soils to be morphologically appealing for vegetable harvesting and consumption, which is in line with the problem statement. The association of almost all traits with the first principal component, render these traits as essential for determining the effect of these heavy metals in *C. olitorius* plants. The separate grouping of Pb and Cd in cluster analysis shows that these heavy metals have different effects on *C. olitorius* species. The next chapter, therefore, focuses on determining quantities of Pb and Cd that are accumulated in roots, stems, and leaves of *C. olitorius*, using the atomic absorption spectroscopy.

Chapter 4 Uptake and accumulation of lead and cadmium in roots, stems and leaves of *Corchorus olitorius*

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4.1 Introduction

Lead (Pb) and cadmium (Cd) are not essential for plant growth but they can cause adverse effects on plant morphology even at low concentrations (Chibuike and Obiora, 2014). These heavy metals are normally present in the soil (Al-Keel, 2016), and their contamination in agricultural soils has become a major problem in the agricultural industry (Alves *et al*., 2016; Dutta *et al*., 2018). Lead and cadmium are emitted from industries associated with smelting and mining, battery manufacturers, pigments and ceramic products (Andjelkovic *et* al., 2019). Sewage sludge disposal and vehicle exhausts are additional sources of Pb contamination in agricultural soil (Abbasi *et al*., 2017). Lead and cadmium can be highly accumulated in leafy vegetables and the magnitude of their accumulation depends on the nature of the vegetables (Aldoobie and Beltagi, 2013; Ali *et al*., 2017). *Amaranthus spinosus* is a good bio-accumulator of Pb and Cd and is successfully used in phytoremediation of these heavy metals (Anoliefo *et al*., 2008). Consumption of such contaminated vegetables can lead to health issues in humans and animals feeding on them (Chen *et al*., 2011).

Lead uptake is through the roots (Baunthiyal *et al*., 2015), but some plants such as *Zea maize* acquire it from the air through leaves by the process of gaseous exchange in the atmosphere (Codling, 2014). Roots can take up a huge amount of Pb while restricting its movement towards the aerial plant parts (Baunthiyal *et al*., 2015). The uptake of Pb from the soil by plants increases with an increase in its concentration in the soil (Ambika *et al*., 2016). Most plants grow well in Pb contaminated soil, which is why it is a major concern in traditional vegetable consumption (Chen *et al*., 2011). The highest limit of Pb in agricultural soils is 300 mg kg^{-1} of soil (Khodaverdiloo *et al*., 2011). Cadmium is easily taken up by plants through the root system and translocated to other plant parts (Ibrahim *et al*., 2017). Its uptake follows the same paths as for essential elements, thus creating competition in translocation, which results in the decline of essential elements in plants (Kao, 2014). The amount of Cd in the soil is one of the factors affecting the uptake of Cd by plants (Chen, 2011).

Some heavy metals including Cd and Pb have growth promotion tendencies in leafy vegetables, resulting in undamaged, dark-green and big leaves (Ali *et al*., 2017). Some people consider such plants as good quality leafy vegetables. Therefore, people from rural communities harvest such vegetables as their source of food and end up eating plants contaminated with heavy metals. Consumption of such contaminated vegetables can lead to health issues in humans and animals feeding on them. Since *Corchorus olitorius* is also one of the leafy vegetables that grow in areas prone to Pb and Cd contaminations, therefore there is a need to study the heavy metal accumulation potential for this plant. Thus, the objective of the study was to determine the accumulation of Pb and Cd in roots, stem, and leaves of *C. olitorius* at different stages of plant growth.

4.2 Materials and methods

The description of the study area and seeds sourcing, treatments and experimental design and plant management are reported in Chapter 3.

4.2.1 Lead and cadmium content of plant parts

The contents of Pb and Cd were analyzed in roots, stems, and leaves of *C. olitorius* plants harvested at 44 and 85 days after sowing, using a method adapted from

Adayusi *et al*. (2015). The plants in each pot, at each stage of growth, were separated into leaves, stem, and roots. Three pots were selected for harvesting and each pot was used as a biological replicate. Each plant part (replicated three times) was cut into pieces and air-dried and then further dried in an oven at 80˚C, and milled with an electric blender into powder for consistency. The milled plant part sample was mixed properly by rumbling and sieved through a mesh filter.

A gram of each milled homogenized sample was weighed with a digital weighing balance into a conical flask. A 5 ml of 60% hydrochloric acid (HCl) and 10 ml of 70% nitric acid were added into the weighed samples. The sample mixture was digested with moderate heat at 50˚C on a hot plate until white fumes evolved, and the solution changed to brownish. Then the heat was intensified further for few minutes to expel off most of the HCl.

Fifty milliliters of distilled water were added to the solution and heated for a few minutes; allowed to cool before it was filtered through Whitman's No 1 paper into a dispensed transparent plastic container. The plastic container was cleaned with detergent, treated successively with HCl, and rinsed with deionized water. The filtered sample was settled for a few minutes for the proper aspiration of the elements. The digested sample was analyzed for heavy metal concentration using atomic absorption spectroscopy (AAS). To find Pb or Cd concentration, all the AAS reading were multiplied by 1000 to convert the values from grams to kilograms:

[Pb] in mg kg^{-1} = ASS reading × 1000

The translocation factor (TF) was calculated according to Rangnekar *et al*. (2013) and Al-Farraj *et al*. (2010), which is the ratio of the total Cd or Pb content in the plant part to its total content in the roots.

$$TF = \frac{\text{Pb leaves}}{\text{Pbroots}} \text{ and } \frac{[\text{Pbstem}]}{[\text{Pbroots}]} \text{ or } TF = \frac{\text{Cdstem}}{\text{Cdroots}} \text{ and } \frac{[\text{Cdleaves}]}{[\text{Cdroots}]}$$

4.2.2 Statistical analysis

Data were analyzed using analysis of variance (one-way ANOVA) in GenStat 12.1 version. Means were separated using Turkey's Multiple Range Test at a 5% level of significance. Correlation matrix, principal component analysis, biplot, and dendrogram were also determined using XLSTAT2018.6.54215 (Addinsoft, Paris) to access the relationship between the concentrations of heavy metals in the soil and their uptake and accumulation in the plant parts.

4.3 Results

4.3.1 Cadmium accumulation at seedling stage

Cadmium was not detected from the control plant parts. The amount of Cd accumulated in the roots first increased with an increase in its concentration in the soil and later decreased as levels get much higher (Table 4.1). The highest amount (920.5 mg kg^{-1}) of Cd was accumulated in the roots exposed to 10 mg kg^{-1}, while the lowest (125.5 mg kg^{-1}) was also attained in the roots from 20 mg kg^{-1} Cd treatment. In stems, Cd accumulation increased as its concentration increased in the soil. It ranged from 456.8 mg kg^{-1} at 5 mg kg^{-1} Cd treatment to 791.3 mg kg^{-1} at 15 mg kg^{-1} Cd treatment. A similar trend was also observed in the leaves. The highest (642.3 mg kg^{-1}) and lowest accumulations (360.0 mg kg^{-1}) were recorded at 20 and 5 mg kg^{-1} Cd treatments, respectively in the leaves. Plants treated with 25 mg kg^{-1} Cd were not harvested for analysis at the seedling stage because of insufficient seedlings as most suffered from high seedling mortality.

Accumulation and translocation of Cd decreased gradually from roots, stems to leaves at 5, 10 and 15 mg kg^{-1} Cd treatments (Table 4.1). Under all these Cd concentrations, the highest accumulation was in roots and the lowest in leaves. However, the differences were not significant between roots and stems at 5 and 15 mg kg^{-1} Cd soil concentrations. For the 20 mg kg^{-1} Cd, the accumulation followed the order of stems, leaves then roots, from highet to smallests.

4.3.2 Accumulation of Cd at termination stage

The highest Cd accumulation (1 340.8 mg kg^{-1}) in roots was recorded at 15 mg kg^{-1} Cd soil treatment, while the lowest accumulation (394.5 mg kg^{-1}) was obtained at 5 mg kg^{-1} Cd treatment, at second harvest (Table 4.1). Cadmium accumulation again increased with an increase in concentration in the soil until 15 mg kg^{-1} and then declined in concentrations above that. In stems, Cd accumulation ranged from 479.3 mg kg^{-1} at 5 mg kg^{-1} Cd treatment to 1 169.0 mg kg^{-1} at 25 mg kg^{-1} Cd treatment. Cadmium accumulation in stems continually increased with an increase in its soil levels. In leaves, the maximum amount (912.3 mg kg^{-1}) of accumulated Cd was obtained in plants treated with 20 mg kg^{-1} Cd and the minimum (420.8 mg kg^{-1}) was attained in those exposed to 5 mg kg^{-1} Cd. This was a similar trend as in the roots.

Table 4.1 Accumulation of Cd in roots, stem and leaves of *C. olitorius*

Harvest stage	Cd conc. In the soil (mg kg^{-1})	Cd concentration in plant parts (mg kg^{-1})		
		Roots	Stem	Leaves
44 DAS	0	0.0 i	0.0 i	0.0 i
	5	510.8 de	456.8 ef	360.0 g
	10	920.5 a	668.0 c	395.8 fg
	15	793.3 b	791.3 b	546.3 d
	20	124.5 h	787.0 b	642.3 c
	25	–	–	–
85 DAS	0	0.0 h	0.0 h	0.0 h
	5	394.5 g	479.3 g	420.8 g
	10	1051.8 bc	975.5 c	636.3 f
	15	1340.8 a	794.3 de	649.0 f
	20	684.0 ef	1033.3 bc	912.3 d
	25	624.5 f	1169.0 b	762.0 ef

Means followed by different letter(s) within a column vary significantly at 5% level of significance.

Accumulation of Cd within a plant, in its particular soil concentration, differed significantly among plant parts, except for plants exposed to 5 mg kg^{-1} Cd (Table 4.1). In plants exposed to 10 and 15 mg kg^{-1} Cd treatments, the accumulation decreased gradually from roots to stems and then leaves. However, in both 20 and 25 mg kg^{-1} Cd treatments, plants accumulated the highest Cd in the stems than roots and leaves.

4.3.3 Lead accumulation at the seedling stage

In roots at 44 days after sowing, the highest Pb accumulation (448.8 mg kg^{-1}) was recorded at 1000 mg kg^{-1} treatment, while the lowest accumulation (185.5 mg kg^{-1}) was obtained at 150 mg kg^{-1} treatment (Table 4.2). The accumulation of Pb in the roots increased with an increase in its concentration in the soil. In the stem, Pb accumulation ranged from 67.0 mg kg^{-1} at 1000 mg kg^{-1} to 202.5 mg kg^{-1} at 900 mg kg^{-1}. For the stem, accumulation increased until 900 mg kg^{-1} and then declined at 1000 mg kg^{-1} Pb treatment when compared with the control. The highest accumulation (212.0 mg kg^{-1}) in the leaves was obtained at 900 mg kg^{-1} treatment, whereas the lowest (68.8 mg kg^{-1}) was attained at 1000 mg kg^{-1} treatment. Similar to the stems, the accumulation increased until 900 mg kg^{-1} and then declined at 1000 mg kg^{-1} treatment.

Table 4.2 Accumulation of Pb in roots, stem and leaves of *C. olitorius*

Harvest stage	Pb conc. In the soil (mg kg^{-1})	Pb concentration in plant parts (mg kg^{-1})		
		Roots	Stem	Leaves
44 DAS	0	0.0 i	0.0 i	0.0 i
	150	185.5 de	78.8 h	110.5 gh
	300	276.8 c	78.3 h	136.3 fg
	600	340.8 b	159.3 ef	179.5 def
	900	409.5 a	202.5 de	212.0 d
	1000	448.8 a	67.0 h	68.8 h
85 DAS	0	0.0 e	0.0 e	0.0 e
	150	129.3 cd	33.3 de	33.8 de
	300	212.5 c	37.5 de	38.8 de
	600	481.8 b	38.3 de	42.0 de

	900	634.0 a	41.0 de	26.3 de
	1000	596.8 ab	128.8 cde	22.8 de

Means followed by different letter(s) within a column vary significantly at 5% level of significance.

Seedlings treated with different Pb concentrations, all had the highest amount of Pb accumulated in the roots than aerial plant parts. Lead accumulated in stems and leaves was relatively the same within each soil concentration, except for 300 mg kg^{-1} Pb treatment, where leaves accumulated more Pb than stems.

4.3.4 Lead accumulation at the termination stage

At the second harvest (85 DAS), Pb accumulation increased within the roots as its concentration in the soil increased, but such differences were not significant in both stems and leaves. Roots treated with 900 mg kg^{-1} Pb accumulated the highest amount (634.0 mg kg^{-1}), while those exposed to 150 mg kg^{-1} Pb accumulated the lowest (129.3 mg kg^{-1}). In stems, the accumulated Pb amount ranged from 33.3 mg kg^{-1} at 150 mg kg^{-1} treatment to 128.8 mg kg^{-1} at 1000 mg kg^{-1} Pb treatment. In leaves, the accumulation ranged from 22.8 mg kg^{-1} at 1000 mg kg^{-1} treatment to 42.0 mg kg^{-1} at 600 mg kg^{-1} Pb treatment. Within each Pb treatment, roots accumulated the highest Pb than stems and leaves, and both did not differ significantly from each other.

4.3.5 Translocation of Pb and Cd within *C. olitorius* plants

Translocation of Pb from roots to stems and from roots to leaves is generally lower than that of Cd, both at the seedling and termination stages, except for root to leaf translocation at seedling stage (Table 4.3). At the seedling stage, the highest root to stem Pb translocation factor (0.49) was obtained at 900 mg kg^{-1}, while the lowest (0.15) was attained at 1000 mg kg^{-1} treatment (Table 4.3). The movement of Pb from roots to the stem showed a significant increase only in plants treated with 600 and 900 mg kg^{-1} when compared with the control. However, Pb translocation from roots to leaves did not differ among the treatments, with a range from 0.60–0.15 at 150 and 1 000 mg kg^{-1} treatment, respectively. Similarly, at the termination stage, differences in root to stem and root to leaf Pb translocation were not significant among the treatments.

In Cd-treated plants, root to stem and root to leaf translocation factor increased with an increase in Cd content in the soil, at both stages of growth (Table 4.3). At the seedling stage, the highest (6.30 and 5.74) and the lowest (0.73 and 0.69) translocation factors were obtained in 20 mg kg^{-1} as well as 10 and 5 mg kg^{-1} Cd treatments, for root to stem and root to leaf, respectively. At the termination stage, 25 mg kg^{-1} Cd treatment resulted in the highest translocation factors (1.88 and 1.24), but 5 mg kg^{-1} Cd treatment resulted in the lowest translocation factors (0.79 and 0.59), for both root to stem and root to leaf translocation.

Table 4.3 The translocation factors for Pb and Cd in *C. olitorius*

		Seedling stage (44 DAS)		Termination stage (85 DAS)	
Tt	Conc.	R to S	R to L	R to S	R to L
Control	0	0.00 e	0.00 d	0.00 d	0.00 e
Pb	150	0.43 cde	0.60 b	0.02 d	0.02 e
	300	0.29 def	0.49 bc	0.03 d	0.03 e
	600	0.47 cd	0.53 bc	0.06 d	0.08 e
	900	0.49 cd	0.52 bc	0.06 d	0.05 e
	1000	0.15 ef	0.15 bc	0.02 d	0.03 e
Cd	5	0.82 b	0.51 bc	0.79 c	0.59 d
	10	0.73 bc	0.69 b	0.93 c	0.69 d
	15	1.00 b	0.71 b	1.16 b	0.89 c
	20	6.30 a	5.74 a	1.22 b	1.09 b
	25	-	-	1.88 a	1.24 a
Stats	GM	0.97	0.90	0.48	0.38
	LCD	0.19	0.33	0.10	0.06
	CV%	15.2	25.2	14.5	11.8
	P-value	<.001	<.001	<.001	<.001

Means followed by different letter(s) within a column vary significantly at 5% level of significance Legend: Tt = Treatments, Cinc = concentrations, R to S = Roots to stem, R to L = roots to leaves, 44 DAS = 44 days after sowing and 85 DAS = 90 days after sowing.

4.3.6 Correlation

Almost all the accumulation and translocation traits were positively correlated with one another, except for translocation from roots to stems both at 44 and 85 days after sowing (Table 4.4). Accumulation of Pb and Cd recorded in the roots at first harvest was significantly correlated with accumulation in the stem (0.61) and leaves (0.62) at first harvest. A positive linear relationship between the accumulation in the roots with the amount found in the stem and leaves was only observed at first harvest. The amount of Pb and Cd found in the stem at first harvest was significantly correlated with almost all the traits except for accumulation in the roots at both harvest stages. Translocation from roots to leaves at first harvest was significantly correlated with the accumulation in the leaves at first and second harvest (0.65 and 0.60), accumulation in the stem at second harvest (0.72) and translocation from roots to stem at second harvest (1.00).

Table 4.4: Correlation matrix of Pb and Cd accumulation and translocation to different plant parts of *Corchorus olitorius*

Variables	RFH	SFH	LFH	RSH	SSH	LSH	RTSFH	RTLFH	RTSSH
SFH	**0.61**								
LFH	**0.62**	**0.81**							
RSH	0.27	**0.71**	0.51						
SSH	0.50	**0.76**	**0.96**	0.53					
LSH	0.18	**0.82**	**0.70**	**0.84**	**0.74**				
RTSFH	-0.12	0.30	**0.65**	0.41	**0.72**	**0.60**			
RTLFH	0.01	**0.68**	0.39	**0.80**	0.39	**0.86**	0.32		
RTSSH	-0.12	0.32	**0.65**	0.44	**0.73**	**0.63**	**1.00**	0.34	
RTLSH	0.01	**0.78**	0.58	**0.85**	**0.63**	**0.98**	0.49	0.92	0.83

Variables: RFH = accumulation in roots at first harvest, SFH = stem at first harvest, LFH = leaves at first harvest, RSH = roots second harvest, SSH = stem at second harvest, LSH = leaves at second harvest, RTSFH = translocation from roots to stem at first harvest, RTLFH = translocation from roots leaves at first harvest, RTSSH = translocation from roots to stem at second harvest, RTLSH = translocation from roots to leaves at second harvest.

4.3.7 Principal component and cluster analyses

The principal component analysis grouped the accumulation and translocation of Pb and Cd into two informative components, which accounted for 81.94% variation (Table 4.5). The first component which was responsible for 65.5% of the total variation, had a significant positive correlation with almost all traits except for accumulation in the roots at first harvest. The second principal component which accounted for 16.4 of the total variability was positively defined by translocation from roots to stem at both first and second harvest and negatively defined by the accumulation at first harvest.

Table 4.5 Principal component analysis of the accumulation and translocation of Pb and Cd in *C. olitorius*

Variables	PC1	PC2
RFH	0,43	**-0,70**
SFH	**0,88**	-0,41
LFH	**0,88**	-0,07
RSH	**0,84**	-0,18
SSH	**0,88**	0,06
LSH	**0,96**	0,01
RTSFH	**0,70**	**0,67**
RTLFH	**0,79**	-0,14
RTSSH	**0,71**	**0,67**
RTLSH	**0,90**	-0,03
Eigenvalue	6,55	1,64
Variability (%)	65,52	16,41
Cumulative %	65,52	81,94

Variables: RFH = accumulation in roots at first harvest, SFH = stem at first harvest, LFH = leaves at first harvest, RSH = roots second harvest, SSH = stem at second harvest, LSH = leaves at second harvest, RTSFH = translocation from roots to stem at first harvest, RTLFH = translocation from roots leaves at first harvest, RTSSH = translocation from roots to stem at second harvest, RTLSH = translocation from roots to leaves at second harvest.

In a biplot, all accumulation and translocation traits (variables) were positively associated with the first principal component (Figure 4.1). The biplot also grouped Pb and Cd treatments into three clusters. Cluster I contained control and all Pb treatments. Cluster II had 5, 10, 15 and 25 mg kg^{-1} Cd treatments (Cd 1, 2, 3 and 5), whereas 20 mg kg^{-1} Cd (Cd 4) formed a singleton in Cluster III.

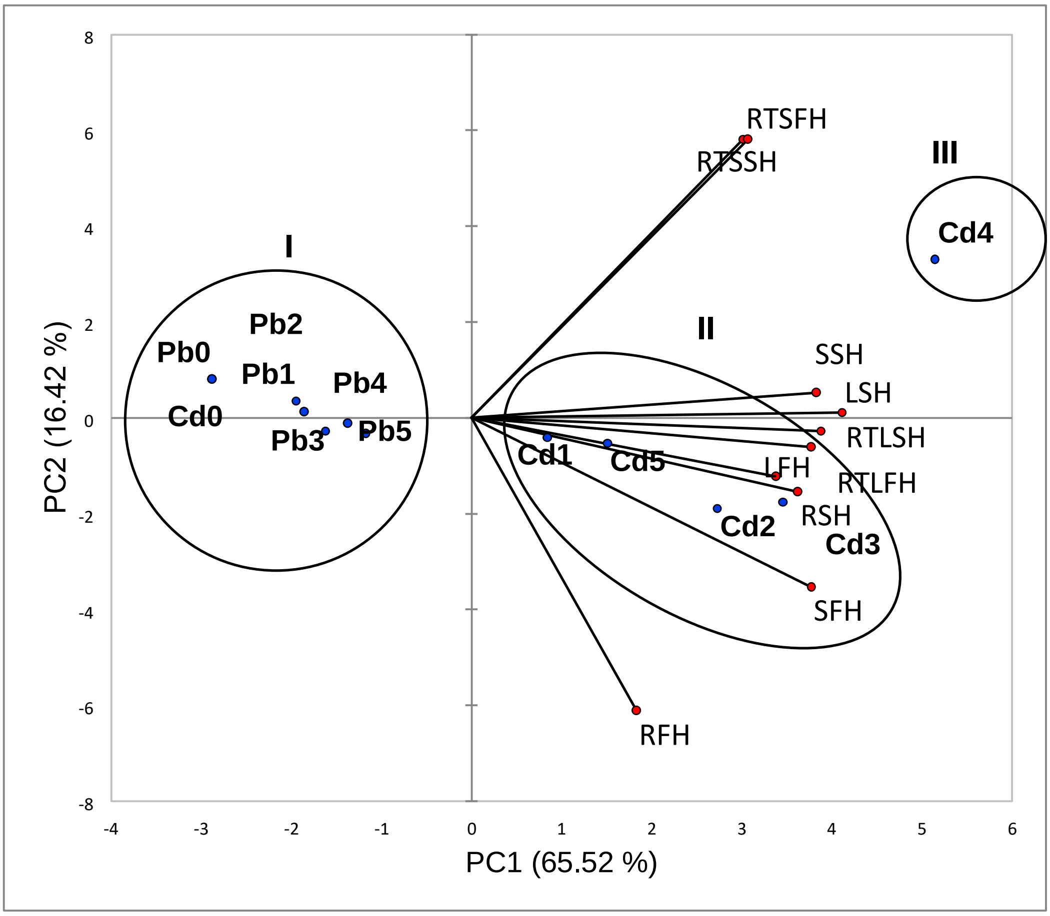

Figure 4.1 Biplot for the accumulation and translocation of Pb and Cd in *C. olitorius*.

Traits: RFH = accumulation in roots at first harvest, SFH = stem at first harvest, LFH = leaves at first harvest, RSH = roots second harvest, SSH = stem at second harvest, LSH = leaves at second harvest, RTSFH = translocation from roots to stem at first harvest, RTLFH = translocation from roots leaves at first harvest, RTSSH = translocation from roots to stem at second harvest, RTLSH = translocation from roots to leaves at second harvest. Treatments: Cd0 and Pb0 = control, Cd1 = 5 mg kg^{-1}, Cd2 = 10 mg kg^{-1}, Cd3 = 15 mg kg^{-1}, Cd4 = 20 mg kg^{-1}, Cd5 = 25 mg kg^{-1}, Pb1 = 150 mg kg^{-1}, Pb2 = 300 mg kg^{-1}, Pb3 = 600 mg kg^{-1}, Pb4 = 900 mg kg^{-1}, Pb5 = 1000 mg kg^{-1}

The dendrogram clustered treatments into three groups based on the accumulation and translocation (traits) (Figure 4.2). The first cluster was divided into two subclusters, where Sub-Cluster Ia contained the control and 150 and 300 mg kg^{-1} Pb, whereas Sub-Cluster Ib had 600–1000 mg kg^{-1} Pb treatments joined with 5 mg kg^{-1} Cd. The high degree of similarities in these treatments was based on the low metal detected in the control and 150 to 300 5 mg kg^{-1} Pb, while in the second cluster accumulated metals were kept in the roots. The second cluster grouped 10 and 15 mg kg^{-1} Cd, whereas the third cluster contained 20 and 25 mg kg^{-1} Cd.

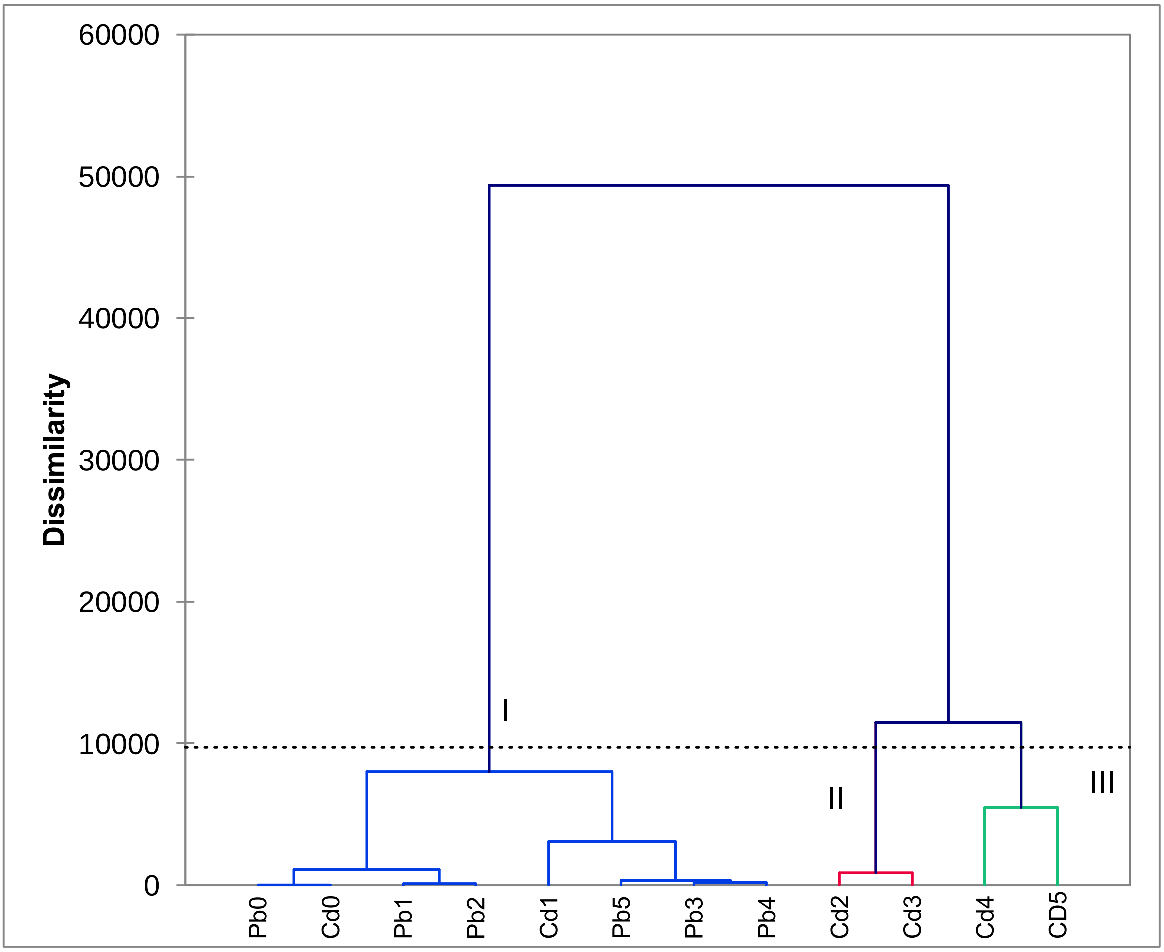

Figure 4.2 A dendrogram clustering the accumulation and translocation of Pb and Cd in roots, stem and leaves of *C. olitorius*.

Treatments: Cd0 and Pb0 = control, Cd1 = 5 mg kg^{-1}, Cd2 = 10 mg kg^{-1}, Cd3 = 15 mg kg^{-1}, Cd4 = 20 mg kg^{-1}, Cd5 = 25 mg kg^{-1}, Pb1 = 150 mg kg^{-1}, Pb2 = 300 mg kg^{-1}, Pb3 = 600 mg kg^{-1}, Pb4 = 900 mg kg^{-1}, Pb5 = 1000 mg kg^{-1}

4.4 Discussion

4.4.1 Accumulation of Cd at the seedling stage

The accumulation of Cd in the roots, stem, and leaves increased with an increase in its application in the soil (Table 4.1). An increase in levels of Cd in the soil also leads to an increase in Cd in the roots, stem, and leaves of *Corchorus olitorius* (Sanyaolu *et al*., 2011; Hassan *et al*., 2016). The accumulation of Cd also increases with an increase in its concentration in the soil in other plants, like *Brassica napus* (Angelova *et al*., 2010), *Eruca sativa* (Cannata *et al*., 2013), *Populus nigra* (Barbeş *et al*., 2014), *Miscanthus. sinensis, Miscanthus floridulus, Miscanthus. sacchariflorus* (Gou *et al*., 2016) and *Solanum melongena* (Youssef and El-Gawad, 2018). The accumulation of Cd at first harvest was above the world safe limits of Cd contents in leafy vegetables (< 0.2 mg kg^{-1}) in all plant parts, which indicates that *C. olitorius* is potential for Cd phytoremediation purposes. Similar results were observed in *Brassica oleracea var. capitata* (Hashim *et al*., 2017).

The amount of Cd accumulated in *C. olitorius* was in a descending order of roots > stems > leaves (Table 4.1). A reduction in Cd accumulation in an order of roots > shoots > fruits also occurs in *Pisum sativum* (Singh *et al*., 2012), *Myriophyllum spicatum* (Yabanli *et al*., 2014) and *Solanum Melongena* (Youssef and El-Gawad, 2018). However, the accumulation of Cd in *Brassica napus* (Angelova *et al*., 2010), *Spinacia oleracea*, *Amaranthus cruentus*, *Trigonellafoenum-graecum (*Rangnekar *et al*., 2013) and *Miscanthus spicatum* (Yabanli *et al*., 2014) is in a descending manner of roots > leaves > stems. However, Cd is one of the heavy metals known to have high mobility from the soil to the plant particularly from the roots to the leaves, resulting in its abundance in the leaves in many plants (Ali *et al*., 2018).

4.4.2 Accumulation of Cd at the termination stage

In the roots and leaves at 85 DAS, Cd uptake increased with an increase of application in the soil until 15 mg kg^{-1}, whereas in the stem the accumulation increased with an increase in Cd application throughout the treatments (Table 4.1). An increase in the levels of Cd in the soil also increased the accumulation in *Solanum lycopersicum*, *Brassica oleracea var. capitata* (Bvenura and Afolayan, 2012), *Solanum lycopersicum* (Huang *et al*., 2012), and *Brassica oleracea var. capitata* (Hashim *et al*., 2017). The amount of Cd accumulated at this stage and all treatments was much higher than the safe limits in vegetables. The amount of Cd that accumulates in *Brassica oleracea var. capitata* (Hashim *et al*., 2017) and *Solanum melongena* (Youssef and El-Gawad, 2018) is also double or three times more than the permissible limits in leafy vegetables which makes these plants dangerous for human consumption, especially when grown in heavy metal prone areas.

Within the plant parts, the uptake and accumulation of Cd declined from roots to the leaves in an order of roots > stem > leaves in a descending manner. *Populus nigra* (Barbeş *et al*., 2014) and *Brassica oleracea var. capitata* (Hashim *et al*., 2017) also show a decrease in Cd accumulation from roots to the leaves. A uniform accumulation and distribution of Cd in roots, stem, and leaves occur in *Bougainvillea spectabilis* and *Leucaena lecocephala* (Juson *et al*., 2016). This reduction in Cd transport to the aerial parts of most plants including the current plant attributed to the production of phytochelatins and their precursors that bind to cd and prevent it from upwaed movements (Yadzi *et al*., 2019).

4.4.3 Accumulation of Pb at the seedling stage

At first harvest, the accumulation of Pb increased with an increase in its application in the soil (Table 4.2). An increase in Pb soil levels also results in an increase in its uptake in *Brassica napus* (Angelova *et al*., 2010), *Taraxacum officinale* (Bini *et al*., 2012), *Brassica oleracea* var. *capitata* (Hashim *et al*., 2017) and *Solanum melongena* (Youssef and El-Gawad, 2018). The accumulation of Pb in *C. olitorius* roots, stem and leaves separately in values much above the world safe limits in leafy vegetables is a good indication of this plant as possessing some phytoremediation properties. Similarly, Pb accumulation is 15 times more than the permissible limits in *Solanum lycopersicum* (Huang *et al.,* 2012) and *Brassica oleracea* var. *capitata* (Hashim *et al*., 2017). High Pb accumulation in the plant is usually related to the level of its concentration in the growth environments and accumulation gets higher with an increase in Pb contents in the environment (Hashim *et al*., 2017).

Distribution of Pb to different plant organs decreased from the roots towards the aerial plant parts in a roots > leaves > stems descending manner. Similarly, the accumulation of Pb at a seedling stage in *Brassica napus* (Angelova *et al*., 2010) *Brassica oleracea* var. *capitata* (Singh *et al*., 2012), *Spinacia oleracea*, *Amaranthus cruentus* and

Trigonellafoenum-graecum (Rangnekar *et al*., 2013) and *Myriophyllum spicatum* (Yabanli *et al*., 2014) follows the same descending order of roots > leaves > stems. However, the accumulation of Pb in *Taraxacum officinale* (Bini *et al*., 2012), *Myriophyllum spicatum* (Yabanli *et al*., 2014), *Eruca sativa* (Cannata *et al*., 2013), *Brassica oleracea* var. *capitata* (Hashim *et al*., 2017) and *Solanum melongena* (Youssef and El-Gawad, 2018) was in an order of roots > stems > leaves. Binding of Pb into the roots and restricting its movement to the aerial parts of the plants is one of the mechanisms that most plants use to respond to the toxic effects of Pb in the growth medium (Al-Keel, 2016).

4.4.5 Accumulation of Pb at the termination stage

At 85 days after sowing, Pb taken up by the roots increase with an increase in its concentration in the soil compared with the control (Table 4.2). Similarly, an increase in Pb application in the growth medium also increases the accumulation in *Brassica oleracea* var. *capitata* (Corley and Mutiti, 2017) and *Brassica oleracea* var. *capitata* (Hashim *et al*., 2017). There was no specific trend as observed in the accumulation of Pb in the stem and leaves at this stage of harvest. The order of Pb accumulation at 85 days after sowing was from roots to the stems than leaves in a descending manner. The accumulation of Pb in *Populus nigra* (Barbeş *et al*., 2014) and *Trigonella foenumgraecum* (Kaur *et al*., 2015[b]), is also in the order of roots > stem > leaves, in a descending manner. This can be attributed to Pb's low mobility in the soil which is caused by the roots restriction of Pb movement to the above plant parts, except for too much high levels (Ali et al., 2018).

4.4.6 Lead and Cd translocation at seedling stage

At 44 days after sowing, the translocation factor of Pb in *Corchorus olitorius* from roots to aerial parts of *C. olitorius* was very low since most of Pb was found in the roots (Table 4.3). Lead application in the soil also results in a decline in the translocation factor in *Linum usitatissimum* (Hosman *et al*., 2017). Contrary to *C. olitorius*, an increase in the translocation factor because of an increase in Pb concentration in the soil has been reported in *Brassica oleracea* (Hashim *et al*., 2017). In other plants, Pb accumulation is uniformly distributed from roots to stem and leaves in *Spinacia oleracea, Trigonella foenum-graecum, Amaranthus cruentus* (Rangnekar *et al*., 2013), *Brassica oleracea* (Hashim *et al*., 2017) and two varieties of *Vaselious valgaris* (Saadaoui *et al*., 2017). For most plants, a high amount of the accumulated lead is kept in the roots and a very small fraction is transported to aerial parts (Pourrut *et al*., 2014). This is due to the fact that Cd is a highly mobile element, easily taken up by the roots and then trasnorted to the leaves and evenly distributed to the above plant parts (Youssef and El-Gawad, 2018).

In the Cd-treated plants at 44 days after sowing, transportation increased with an increase of Cd in the soil (Table 4.3). The same increase in the Cd translocation factor as results on increased Cd levels in the soil occurs in *Brassica oleracea* (Hashim *et al*., 2017). However, the opposite in Cd translocation occurs in *Spinacia oleracea, Trigonellafoenum-graecum, Amaranthus cruentus* (Rangnekar *et al*., 2013), *Brassica oleracea* (Hashim *et al*., 2017) and *Vaselious valgaris* (Saadaoui *et al*., 2017). Cadmium uptake goes through the Zn carriers or channels which result in it being easily transported from roots to the above plant parts (Rangnekar *et al*., 2013).

4.4.7 Lead and Cd translocation at second harvest

Most of the Pb accumulated at second harvest was kept in the roots with little or none being taken to the above plant parts (Table 4.3). The same result occurs in *Linum usitatissimum* (Hosman *et al*., 2017). The translocation of Pb at this stage of growth had no linear relationship with the amount of Pb present in the soil. However, the translocation of Pb from roots to the aerial parts of *Solanum melongena* (Youssef and El-Gawad, 2018) and *Vaselious valgaris* (Saadaoui *et a*l., 2017) is high resulting in more Pb recorded in the leaves. High contents of Cd in the roots results in the formation of phytochelatines which combine with Cd in an Cd-PC complex to aid its transport to the shoots (Cannata *et al*., 2013).

Again, at 85 days after sowing, Cd translocation from roots to the aerial parts of *C. olitorius* also increased with an increase of its concentration in the soil in all plant parts (Table 4.3). The Cd translocation factor also increases with an increase in its concentration in the soil in *Brassica oleracea* var. *capitata* (Hashim *et a*l., 2017), *Vaselious valgaris* (Saadaoui *et a*l., 2017) and *Solanum melongena* (Youssef and ElGawad, 2018). However, increasing concentration of Cd results in an insignificant decline in the translocation factor in *Linum usitatissimum* (Hosman *et al*., 2017). Within the plant parts, the Cd translocation factor followed the order from roots to stems > roots > leaves in a decreasing manner. Similar results in Cd translocation is found in *Spinacia oleracea, Trigonella foenum-graecum, Amaranthus cruentus* (Rangnekar *et al*., 2013) and *Brassica oleracea* (Hashim *et al*., 2017). This is because roots are the first plant parts to contact and accumulated the element and only pass it through to the aerials parts one the concentration reach e certain level required by the roots (Saadaoui *et al*., 2017).

4.4.8 Correlation of Pb and Cd accumulation and translocation

The correlation matrix revealed a positive significant correlation ($p > 0.6$) between Pb and Cd accumulation in the roots and the values recorded in the stem and leaves at first harvest (Table 4.4). The amount of Pb and Cd recorded in the stem was significantly correlated with almost all the traits, except for translocation from roots to stem at both first and second harvest. A positive correlation between the accumulation of Pb and the soil application was also observed in *Solanum melongena* (Yilmaz *et al*., 2009) and *Taraxacum officinale* (Bini *et al*., 2012). Lead uptake was also reported to be positively correlated to Cd accumulation in vegetable species planted in heavy metal contaminated soil (Zhou *et al*., 2016). This is due to the fact heavy metal uptake has a positive relationship with the amount of heavy metal found in the soil (Nwajei *et al*., 2014). An increase in heavy metal concentration in the soil also results in an increase in their uptake by plants (Pinho and Ladeiro, 2012).

4.4.9 Principal component and cluster analysis

The PCA analysis results showed that the first three principal components accounted for 95.64% of the total variability, with PC1 contributing 65.52% and PC2 accounting for 81.94% variation (Table 4.5). Similarly, in *Mentha arvensis* the first principal component was responsible for 65% variation in Pb and Cd accumulation and translocation study (Nigam *et al*., 2019). Almost all the traits except for accumulation in the roots at first harvest significantly contributed to the variation. The principal component biplot indicated the link between accumulation and translocation within the Pb and Cd treatments. The Cd treatments (5, 10, 15 and 25 mg kg^{-1}) were grouped in the first, while 20 mg kg^{-1} was in the second quadrant separately from other Cd treatments, the control, and all the Pb treatment

The separate clustering of Pb and Cd treatments in both the biplot and dendrogram can be attributed to their major differences in their accumulation and translocation within the *C. olitorius* plant (Figure 4.1 and 4.2, respectively). Lead is mostly accumulated and restricted in roots with low translocation to the stems and leaves, whereas Cd is easily translocated from the roots to the aerial parts. Again, clustering of the lowest Cd concentration (5 mg kg–1) with higher Pb concentration (600-1000 mg kg–1) in Sub-Cluster Ib of the dendrogram probably shows the related effective accumulation and translocation of Cd in low concentrations but of Pb at high concentrations, within *C. olitorius* plants. The Cd treatments were also closely related in the dendrogram in the study of Cd accumulation in *Iris sibirica, Acorus calamus, Typha orientalis Presl and Cyperus alternifolius* (Wang *et al*., 2016).

4.5 Conclusion

The accumulated amounts of Pb and Cd were all above the safe limits leafy vegetables and their accumulation increased with the application in the soil. However, accumulation of Pb from 150 to 600 mg kg^{-1} and Cd 5 mg kg^{-1} had a minor effect on the vegetative and reproductive traits of *C. olitorius* species. This species is advantageous for phytoremediation use in Pb- and Cd-contaminated soils with the previously mentioned values. However, *C. olitorius* plants that grow under Pb and Cd contaminated soils can look healthy and very appealing for harvesting, but then intoxicate people upon consumption. Results on Pb and Cd accumulation within different *C. olitorius* plant parts were obtained using the atomic absorption spectroscopy (AAS). However, further studies on the accumulation and distribution of Pb and Cd in this plant will be conducted in the following chapter using the scanning electron microscope (SEM) and energy dispersive X-ray (EDX) techniques.

Chapter 5

Study of cadmium and lead distribution in roots, stem and leaves of *C. olitorius* using scanning electron microscope and energy dispersive X-ray techniques

5.1 Introduction

Lead and Cd are the major environmental contaminants that are released into the environment and cause serious heavy metal toxicity concerns (Rezvani and Zaefarian, 2011; Cikili *et al*., 2015). Lead can be easily taken up by plants from the soil and accumulated to different plant parts (Li *et al*., 2016). However, a large amount of Pb taken up by plants is restricted to the roots because of its low mobility in plants (AlAkeel, 2016). On the other hand, Cd has high mobility in plants; and thus, most of the accumulated Cd can be found in the edible parts of the plant. This makes Cd more accessible to animals feeding on leaves and tender stems (Cikili *et al*., 2015; Ismael *et al*., 2019). The distribution of these metals to the roots, stem or leaves can depend on various factors such as growth stage of the plant; concentration of competing nutrients; growth condition and the amount of Cd present in the soil (Ismael *et al*., 2019).

The accumulation and distribution of heavy metals in plant tissues are commonly detected by inductively coupled plasma-mass spectrometry system (ICP-MS), atomic absorption spectroscopy (AAS), and other procedures designated for specific elements (Cikili *et al*., 2015). These tools are the best for analyzing both specific and multiple compositions of elements in plants. Scanning electron microscope (SEM) coupled with energy dispersive X-ray (EDX) is another advanced method used by scientists to determine the distribution of heavy metals in plants (Babaoğlu aydaş *et al*., 2013). This is a well-known technique that investigates metals bindings to biomolecules and can provide adequate inputs to determine the distribution of various elements in plant tissues (Baruah *et al*., 2012). The SEM results show morphological changes in the intercellular structure of the plant in the form of images. The EDX gives the quantity of the target metal in weight percentage that is normally expressed in tables and figures (Nikolic *et al*., 2019).

However, no study has been conducted to localize the distribution of Pb and Cd in roots, stem, and leaves of *C. olitorius* using SEM coupled with EDX in South Africa. Therefore, the objective of this study was to determine the accumulation and distribution of Pb and Cd in *C. olitorius* roots, stem and leaves tissues using scanning electron microscopy and energy dispersive X-ray.

5.2 Materials and methods

The description of the study area and seeds sourcing, treatments and experimental design and plant management are reported in Chapter 3.

The actual amount of Pb and Cd in $Pb(NO_3)_2$ and $Cd(NO_3)_2$ are presented in Table 5.1 after being calculated as follows:
A percentage of Pb and Cd in lead nitrate and cadmium nitrate was calculated as follow:

$$\text{Pb\%} = \frac{\text{Pb molar mass (g/mol)}}{\text{Pb(NO3)2 molar mass (g/mol)}} \text{ X } 100$$

$$= \frac{207.2\ \text{(g/mol)}}{331.21\ \text{(g/mol)}} \text{ X } 100$$

$$= 0.626 \text{ X } 100$$

$$= \underline{62.26\%}$$

$$\text{Cd\%} = \frac{\text{Cd molar mass (g/mol)}}{\text{Cd(NO3)2 molar mass (g/mol)}} \text{ X } 100$$

$$= \frac{112.41\ \text{(g/mol)}}{236.42\ \text{(g/mol)}} \text{ X } 100$$

$$= 0.475 \times 100$$

$$= \underline{47.5\%}$$

An actual value of Pb and Cd in these salts was calculated by multiplying each Cd and Pb treatments with the ratio of Pb molar mass over $Pb(NO_3)_2$ molar mass and Cd molar mass and $Cd(NO_3)_2$ molar mass.

Table 5.1 Applied Pb and Cd nitrates and the actual amount of Pb and Cd in the salts.

$Pb(NO_3)_2$	Actual Pb	$Cd(NO_3)_2$	Actual Cd
150	93.75	5	2.374
300	187.5	10	4.75
600	375.0	15	7.13
900	562.5	20	9.5
1000	625.0	25	11.88

Values are all in mg kg^{-1}.

5.2.1 Heavy metal analysis

Plants were harvested at 44 and 85 days after sowing (DAS). Three pots were selected for harvesting and each pot was used as a biological replicate. Plants in each pot, at each stage of harvest, were separated into leaves, stem, and roots. Each plant part was cut into pieces and air-dried and then further dried in an oven at 80 ^{o}C. The dried plant materials were then milled with an electric blender into powder for consistency.

For heavy metal analysis using the scanning electron microscope (SEM) coupled with energy dispersive X-rays (EDX), a small amount of the dry plant material was deposited on an aluminium stub lined with carbon tape. This was sputter-coated in a Quorum Q150 RES (UK) gold coater. The powder samples were analyzed in a Zeiss Ultra Plus FE-SEM coupled to an Oxford (UK) EDX system. The acceleration voltage was set at 20 kV. The obtained EDX results were then multiplied by 1000 to convert the weight percentage (wt%) values to milligrams per kilogram.

5.2.2 Statistical analysis

Data were subjected to analysis of variance (one-way ANOVA) in GenStat 12.1 version. Treatment means were separated using Turkey's Multiple Range Test in GenStat at a 5% level of significance.

5.3 Results

5.3.1 Cadmium distribution in leaves, stem and roots of *C. olitorius*

Cadmium was not detected in control plants, both at first and second harvests (Table 5.2; Appendixes 1, 2 and 3). At first harvest, leaves of plants treated with 5 mg kg^{-1} had accumulated the lowest Cd concentration (30 mg kg^{-1}), whereas roots exposed to 20 mg kg^{-1} had the highest concentration (350 mg kg^{-1}). The lowest and highest Cd distribution in roots (100 and 350 mg kg^{-1}), stems (140 and 181 mg kg^{-1}) and leaves (30 and 130 mg kg^{-1}), were recorded at 5,10 and 20 mg kg^{-1}; 10 and 20 mg kg^{-1}; as well as 5 and 15 mg kg^{-1} soil treatments, respectively. An increase in Cd soil concentration increased Cd detected in roots and leaves, but the trend was unstable in stems. Distribution of Cd among the plant parts within each treatment showed that stems of plants treated with 5 and 10 mg kg^{-1} Cd had the highest distribution when compared with other plant parts, in descending order of stems > roots > leaves. Also, roots exposed to 15 and 20 mg kg^{-1} Cd had the highest Cd distribution, followed by stems and then leaves. Plants from the highest concentration of Cd (25 mg kg^{-1}) were not included in the data for the first harvest because of the high seedling mortality rate and were spared for the second harvest.

At second harvest, the lowest Cd distribution (80 mg kg^{-1}) was recorded in roots of plants treated with 5 mg kg^{-1}, whereas the leaves of plants treated with 20 mg kg^{-1} accumulated the highest Cd content (760 mg kg) (Table 5.2; Appendixes 1, 2 and 3).

The lowest and highest Cd distribution in the roots (80 and 270 mg kg^{-1}), stems (340 and 560 mg kg^{-1}) and leaves (350 and 760 mg kg^{-1}), were recorded in plants treated with 5 and 15 mg kg^{-1}, 5 and 25 mg kg^{-1} as well as 5 and 20 mg kg^{-1} Cd, respectively. An increase in Cd application in the soil resulted in an increase in Cd distribution in stems and leaves. In the roots, the distribution increased from 5 to 15 mg kg^{-1} and the began to decline from 20 to 25 mg kg^{-1}. Distribution of Cd among the plant parts within each treatment revealed that the leaves of almost all the Cd treatments except for 5 and 10 mg kg^{-1} had the highest Cd distribution when compared with other plant parts, in a descending distribution order of leaves > stem > roots. In plants treated with 5 and 10 mg kg^{-1}, Cd was evenly distributed between the stems and leaves and then the lowest was recorded in the roots.

Table 5.2 The distribution of cadmium on leaves, stem and roots of *C. olitorius* using SEM and EDX techniques.

Harvest stage	Cd concentration (mg kg^{-1})	Cd concentration (mg kg^{-1})		
		Roots	Stem	Leaves
First	0	0 j	0 j	0 j
	5	100 f	190 bc	30 i
	10	100 f	140 e	70 g
	15	200 b	160 d	130 e
	20	350 a	181 c	51 h
	25	-	-	-
Second	0	0 j	0 j	0 j
	5	80 i	340 f	350 ef
	10	253 g	360 ef	360 ef
	15	270 g	370 e	510 d
	20	260 g	370 e	760 a
	25	130 h	560 c	600 b

Means followed by different letter(s) within columns and rows vary significantly at 5% level of significance. The data for first and second harvests were analysed separately.

The internal surface of the plant tissue from treated plants was spongy-like and slightly porous when compared with the control plants (Figure 5.1; Appendixes 1, 2 and 3). This indicated the distribution of Cd to different plant parts of *C. olitorius* at each treatment.

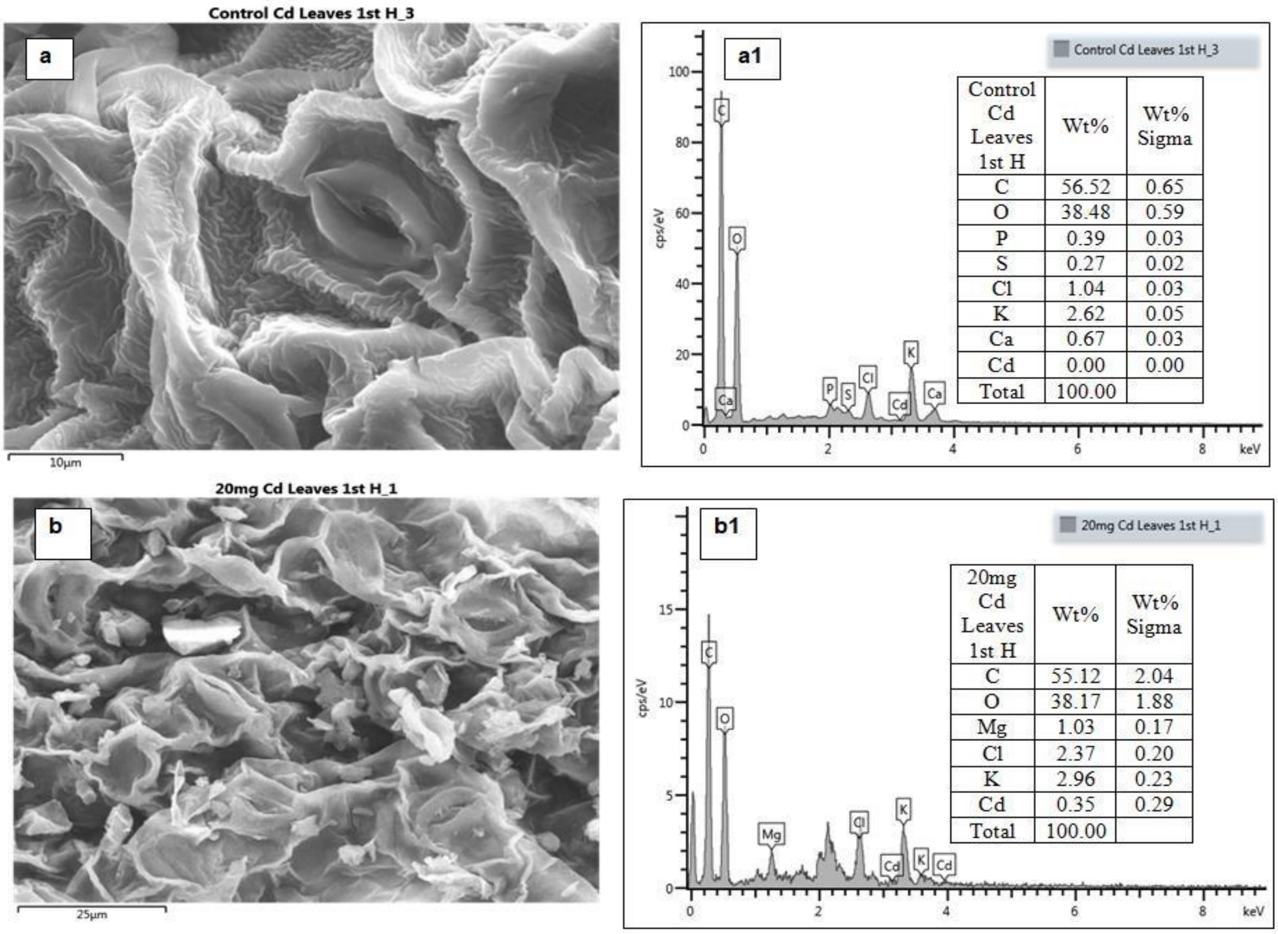

Figure 5.1 SEM images and corresponding EDX spectra tables of leaves from the control (a, a_1) and 20 mg kg^{-1} (b, b_1) Cd-treated plants, respectively

5.3.2 Lead distribution in leaves, stem and roots of *C. olitorius*

Lead was not detected from the control plants at both harvest stages (Table 5.3; Appendixes 1, 2 and 3). At first harvest, the distribution of Pb ranged from 110 mg kg^{-1} in the leaves of the plants exposed to 150 mg kg^{-1} Pb treatment to 970 mg kg^{-1} in the stems of those that received 1000 mg kg^{-1} treatment. The lowest and highest Pb distribution in the roots (240 and 900 mg kg^{-1}), stems (120 and 970 mg kg^{-1}) and leaves (110 and 800 mg kg^{-1}), were attained from plants treated with 150 and 1000 mg kg^{-1} for roots and stem and at 150 and 900 mg kg^{-1} in the leaves. An increase in Pb treatment in the soil increased its distribution within all plant parts. The distribution of Pb to different plant parts within each treatment revealed that the roots of the plants treated with 150 and 300 mg kg^{-1} Pb had the highest Pb when compared with other plant parts. The leaves of the plants exposed to 600 had the highest Pb distribution, whereas the lowest was detected in the stems. The leaves of the plants treated with 900 mg kg^{-1} accumulated the highest Pb, while the lowest was detected in the roots. For those plants exposed to 1000 mg kg^{-1}, the highest Pb was detected in the stem while the lowest was recorded in the roots. The order of distribution of Pb varied with different Pb treatments at this stage.

Table 5.3: Distribution of Pb on leaves, stem and roots of *C. olitorius* using SEM and EDX techniques

Harvest stage	Pb concentration, mg kg^{-1}	Pb concentration in plant part (mg kg^{-1})		
		Roots	Stem	Leaves
First	0	0 l	0 l	0 l
	150	240 j	120 k	110 k
	300	300 gh	290 h	120 k
	600	310 g	270 i	340 f
	900	480 e	690 d	800 c
	1000	900 b	970 a	270 i
Second	0	0i	0 i	0 i
	150	220 fg	160 h	250 ef
	300	320 d	170 h	163 h
	600	690 b	280 e	210 h
	900	660 b	240 fg	343 d
	1000	1970 a	233 fg	540 c

Means followed by different letter(s) within columns and rows vary significantly at 5% level of significance. The data from the first and second harvests were analysed separately.

Control plants did not have any alteration in their internal structures for roots, stems and leaves, at both first and second harvest (Figure 5.2; Appendixes 1, 2 and 3). Lead distribution resulted in a loss of smoothness in the structure of all tissues from the treated plants, with destructed stomata in the leaves when compared with the control plants. An increase in Pb accumulation resulted in the severe loss of tissue smoothness in each plant part. The EDX spectrum also indicated the presence of Pb in different plant parts in weight percentage.

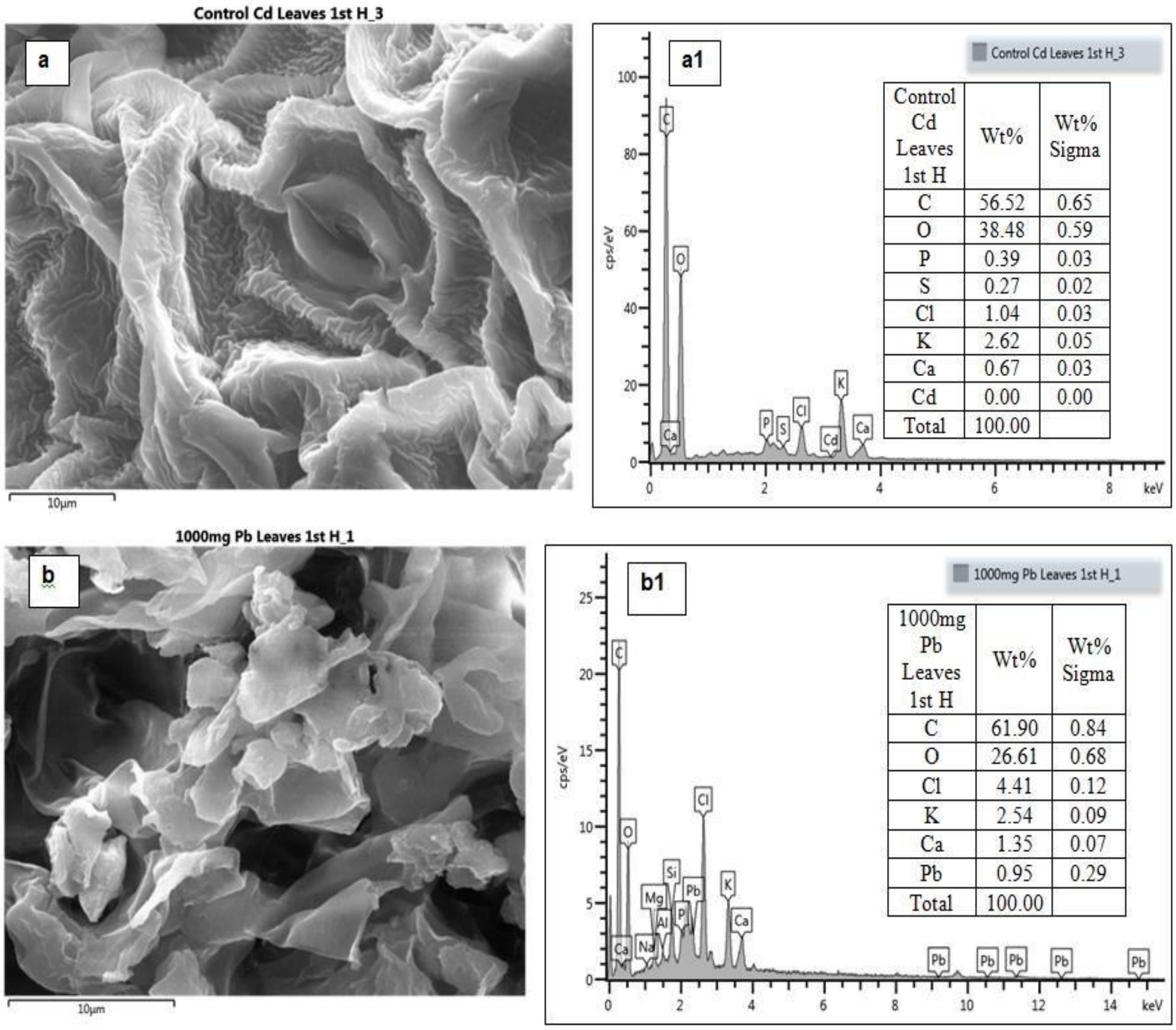

Figure 5.2 SEM images and corresponding EDX spectra tables of leaves from the control (a, a$_1$) and 1000 mg kg^{-1} (b, b$_1$) Pb-treated plants, respectively

At the second harvest, the highest Pb (1970 mg kg^{-1}) was recorded in roots of the plants exposed to 1000 mg kg^{-1} Pb treatment, whereas the stems of plants treated with 150 mg kg^{-1} had the lowest Pb (160 mg kg^{-1}) detection. The lowest and the highest Pb in the roots (220 and 1970 mg kg^{-1}), stems (160 and 240 mg kg^{-1}) and leaves (163 and 540 mg kg^{-1}) were recorded in plants exposed to 150 and 1000 mg kg^{-1}, 150 and 600 mg kg^{-1} and 300 and 1000 mg kg^{-1}, respectively. In all the plant parts (roots, stems, and leaves), an increase in the Pb concentration in the soil increased Pb distribution. Lead distribution to different plant parts as within each treatment showed that the leaves and the roots of the plants treated with 150 mg kg^{-1} had the highest Pb detection while the lowest was attained in the stems. From 300 to 1000 mg kg^{-1}, the highest Pb was recorded in the roots while the lowest was detected in the leaves at 300 and 600 mg kg^{-1} Pb treatments and in the stems at 900 and 1000 mg kg^{-1}. Lead mobility within the plant was highest at the lowest application in the soil, but concentrations above 150 mg kg^{-1} restricted the movement of Pb to aerial parts of the plant, hence more Pb was detected in the roots.

The SEM images and EDX spectra also did not detect Pb in the control plants, thus, there were no changes in the cell structure in all plant parts (Figure 5.2; Appendixes 1, 2 and 3). The presence of Pb was also indicated by the changes in the subcellular structure of the leaves, stem, and roots in Pb-treated plants. The alteration of the tissue structure became more severe with the increase of the Pb accumulation in the plant tissue. The EDX spectrum also shows the accumulation of Pb by the *C. olitorius* leaves at the highest Pb treatment compared with the control plants in weight percentage.

5.4 Discussion

5.4.1 Cadmium distribution in the roots, stem and leaves of *C. olitorius*

In this study, the quantity of Cd detected in the *C. olitorius* roots harvested at 44 days after sowing also increased with an increase of its application in the soil from 5 to 20 mg kg^{-1} Cd treatments (Table 5.2). In the stem and leaves, the accumulation increased with an increase in soil Cd from plants treated with 10 to 20 and 5 to 15 mg kg^{-1}, respectively. The same increase in Cd accumulation occurs in *Solanum tuberosum* (Xu *et al*., 2013) and *Linum usitatissimum* (Hasman *et al*., 2017) grown in 10, 20, 40 and 1, 5, 25 mg kg^{-1} Cd that are harvested at 22 and 10 weeks after planting, respectively. Cadmium accumulation in plants usually increases with an increase in its levels in the soil (Hassan *et al*., 2016).

The distribution of Cd to different parts within each treatment varied with an increase in Cd application in the soil. Cadmium was highest in the stem followed by roots then leaves in the plants exposed to 5 and 10 mg kg^{-1} Cd. In many plants, Cd content is usually lower in the roots compared with the shoots (Mizushima *et al*., 2019). However, in *C. olitorius*, seedlings exposed to 15 and 20 mg kg^{-1} Cd treatments had Cd distribution in the order of roots > stems > leaves. Similarly, the Cd application of 15 mg kg^{-1} in *Monochoria hastate* results in the same trend (Baruah *et al*., 2017). Again, *Avicennia schaueriana* plants exposed to 16, 32 and 64 mg kg^{-1} Cd treatments had the highest Cd distribution in their roots (Mizushima *et al*., 2019). In many plants, Cd is restricted in the roots because it competes with the essential nutrients which are divalent cation, such as Zinc (Tran and Popova, 2012). Plants treated with Cd had alterations in the cellular structure of their roots, stems, and leaves when compared with the control, which indicated the presence of Cd in these plants (Figure 5.1). The presence of heavy metals in plant tissues also causes the destruction in cell structure in *Pteris vittata* (Sridhar *et al*., 2011) and *Galerina vittiformis* (Damodaran *et al*., 2013).

At 85 days after planting, the distribution of Cd to different plant parts was in the order of leaves > stems > roots in a descending manner at all treatment levels (Table 5.2). The accumulation of Cd is in the order of leaves > roots > stems in *Solanum tuberosum* when treated with 1, 5 and 25 mg kg^{-1} and harvested at 10 weeks after planting (Xu *et al*., 2013). This can be attributed to Cd being one of the mobile elements in plants and also easily dissolves in water (Fasahat, 2015). However, Cd is only restricted in the roots in *Mentha arvensis* grown in soils when treated with 32 and 320 mg kg^{-1} Cd, harvested at 90 days after planting (Nigam *et al*., 2019).

The presence of Cd even at low levels resulted in damage in the intercellular structure of roots, stem, and leaves of *C. olitorius,* compared with the control plants. Distribution of Cd results in changes in leaf cell structure in *Solanum lycopersicum* (Godinho *et al*., 2018). The amount of Cd distributed in all plant parts at all treatments at both first and second harvest was above the Cd safe limits in leafy vegetables (>0.2 mg kg^{-1}). Cadmium contents recorded in *Zea mays*, *Cucurbita pepo* and *Manihot esculenta* grown in farms with inorganic fertilization also exceed the words safe Cd limits in vegetables (Emurotu and Onianwa, 2017).

5.4.2 Lead distribution in roots, stem and leaves of *C. olitorius*

The amount of Pb detected in *C. olitorius* roots of the treated plants increased with an increase of Pb application in the soil at 44 and 85 days after sowing (Table 5.3). In *Trigonella foenum-graecum* and *Lemna polyrrhiza* plants the accumulation of Pb in the roots and leaves also increases with an increase of its application in the growth medium (Abubacker and Sathya. 2017). The amount of Pb detected in the leaves increased with an increase in its concentration from 150 to 900 mg kg^{-1} at 44 DAS and from 300 to 1000 mg kg^{-1} Pb application at 85 DAS. Similar results also occur in *Trigonella foenum-graecum* grown in soils treated with 100 and 800 mg kg^{-1} Pb (Kaur *et al*., 2015b). Lead is known to be easily absorbed by the plants and transported to the above plant parts (Wang *et al*., 2015) and the uptake increases with an increase in concentration in the soil (Ambika *et al*., 2016; Li *et al*., 2016).

Lead distribution trend in plant parts within each treatment was in the descending order of roots, stem and leaves in plants exposed to 150 and 300 mg kg^{-1} and harvested at 44 days after sowing. The same trend occurs in *Raphanus sativus* (Wang *et al*., 2015) and *Conyza canadensis* (Li *et al*., 2016) grown at 200 mg kg^{-1} Pb treatment for 14 and 8 days, respectively. The restriction in translocation is because Pb precipitates as phosphate, which is mostly found in the roots (Pinho and Ladeiro, 2012). These results are however in disagreements with the Pb accumulated in *Cymodocea serrulata* which is in the order of leaves > stem > roots when exposed to 5 to 15 mg kg^{-1} Pb treatments (Rosalina *et al*., 2019). Soil Pb concentration of 900 mg kg^{-1} resulted in most Pb recorded in the stems and the lowest in the leaves. Similarly, more Pb is found in the stem in *Solanum lycopersicum* when grown in the soil containing 18 mg kg^{-1} Pb (Salem *et al*., 2016). The presence of Pb in plant tissues also destroyed the internal structure of the roots, stem, and leaves in *C. olitorius* (Figure 5.2). Roots and stems of the treated plants had rough and porous texture compared with the control plants. A clear difference in the surface of the control compound to metal-bonded biomass sample is also observed in *Galerina vittiformis* (Damodaran *et al*., 2013)

The order of distribution of Pb in plants exposed to 150 mg kg^{-1} was from leaves > roots > stem and roots > stems > leaves in those treated with 300 and 600 mg kg^{-1} in descending order. Most of the Pb is detected in the roots *Conyza canadensis* treated with 200 µM, when compared with other plant parts (Li *et al*., 2016). Lead distribution within plant parts at 85 DAS was highest in the roots followed by leaves and then stems, in plants treated with 900 to 1000 mg kg^{-1}. Contrary to *C. olitorius*, the distribution of Pb in *Brassica juncea-arawali* grown at 800 mg kg^{-1} Pb and harvested at the termination stage is in the order of stem > roots > leaves (Kaur *et al*., 2015b).

The amounts of Pb detected in the edibles parts of *C. olitorius* were above the Pb safe limits in leafy vegetables (0.3 mg kg^{-1}) at both first and second harvest, which make the plants grown under these conditions unsafe for human consumption. Lead contents recorded in *Zea mays*, *Cucurbita pepo*, and *Manihot esculenta* also exceed the world's safe limits in vegetables (Emurotu and Onianwa, 2017). Changes in cell structure were observable from roots to leaves and became severe as Pb application increased in the soil. A portion of Pb in weight percentage was also observed on the EDX spectra and confirms the accumulation of Pb by C. *olitorius*. The presence of Pb also results in an irregular and rougher cell surface of the roots and leaves in *Lemna polyrrhiza* (Abubacker and Sathya. 2017) and *Shewanella oneidensis* (Xu *et al*., 2018).

5.5 Conclusion

In conclusion, the actual amounts of Pb and Cd accumulated by *C. olitorius* increased with an increase in concentrations of both metals in the soil, at 44 and 85 days after sowing. The distribution values attained with the scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) were related to the ones obtained with the atomic absorption spectroscopy in the previous chapter. The Cd and Pb values were also above the safe limits of these metals in leafy vegetables (< 0.2 Cd and < 0.3 Pb, respectively). The SEM images and EDX spectra also indicated the presence of both heavy metals in the roots, stems, and leaves of the treated plants when compared with the control plants. The SEM images showed the alteration of internal structures even at the lowest Pb and Cd treatments, whereas vegetative and reproductive traits were not affected by these concentrations. The internal cells had irregular shaped and sized pores because of the binding of the non-biochemical important heavy metals in the absorption pores. This indicates that internal structures are suitable for use to investigate the presence of heavy metals in plants than external morphological plant features.

Chapter 6 Conclusions and recommendations

6.1 Conclusions and recommendations

The effect of both lead (Pb) and cadmium (Cd) on vegetative and reproductive features of *C. olitorius* were insignificant at their lower concentrations compared with the control plants, although they were above safe limits of 300 mg kg^{-1} Pb and 3 mg kg^{-1} Cd agriculture soils, respectively. The toxic effects were recorded from 900 to 1000 mg kg^{-1} in Pb and 10 to 25 mg kg^{-1} in Cd-treated plants. The Pb and Cd concentrations were above their world safe limits on agriculture soil, but below 600 mg kg^{-1} Pb and 10 mg kg^{-1} Cd, promoted growth, resulting in broad green appealing *C. olitorius* leaves. These results prove that *C. olitorius* grows well in Pb and Cd intoxicated soil with minor extended signs of toxicity but shows healthy appealing vegetative features. Therefore, communities should be advised not to harvest this leafy vegetable from places of Pb and Cd contaminations, especially near roadsides, battery production factories, mining industries, and heavily-fertilized agriculture soils or gardens.

The assessment of Pb and Cd accumulation in *C. olitorius* using atomic absorption spectroscopy (ASS) indicated that an increase in the application of both Pb and Cd in the soil resulted in an increase in their accumulation in the plant both at immature and mature stages of growth. The order of distribution to different plant parts within each treatment varied with different concentrations. Cadmium was translocated easily within the plant and was mostly accumulated in the leaves than other plant parts. However, Pb was restricted in the roots and less translocated to aerial parts. Therefore, *C. olitorius* is one of the wild leafy vegetables that can accumulate Pb and Cd contents above their safe limits in leafy vegetables with no visible effects in the vegetative and reproductive traits. These findings recommend the use of *C. olitorius* as a phytoremediation agent for Pb and Cd contaminated soils.

The study of the distribution of Pb and Cd to the roots, stems, and leaves of *C. olitorius* using scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX) revealed that the distribution of both heavy metals to different plant parts increased with an increase of their application in the soil. The order of distribution to roots, stems and leaves varied with different treatments, where most of the accumulated Cd was distributed to the aerial plant parts whereas Pb was generally restricted to the roots especially at its highest application in the soil. Again, according to the SEM coupled with EDX, *C. olitorius* accumulates a high amount of Pb and Cd, much above their maximum toxic levels in leafy vegetables in its plant tissues, grown under intoxicated soil. This did not affect the vegetative features of this plant but altered their cell morphology even at low concentrations, as per SEM images. These results also confirm the phytoremediation potential of *C. olitorius* for Pb and Cd intoxicated soils.

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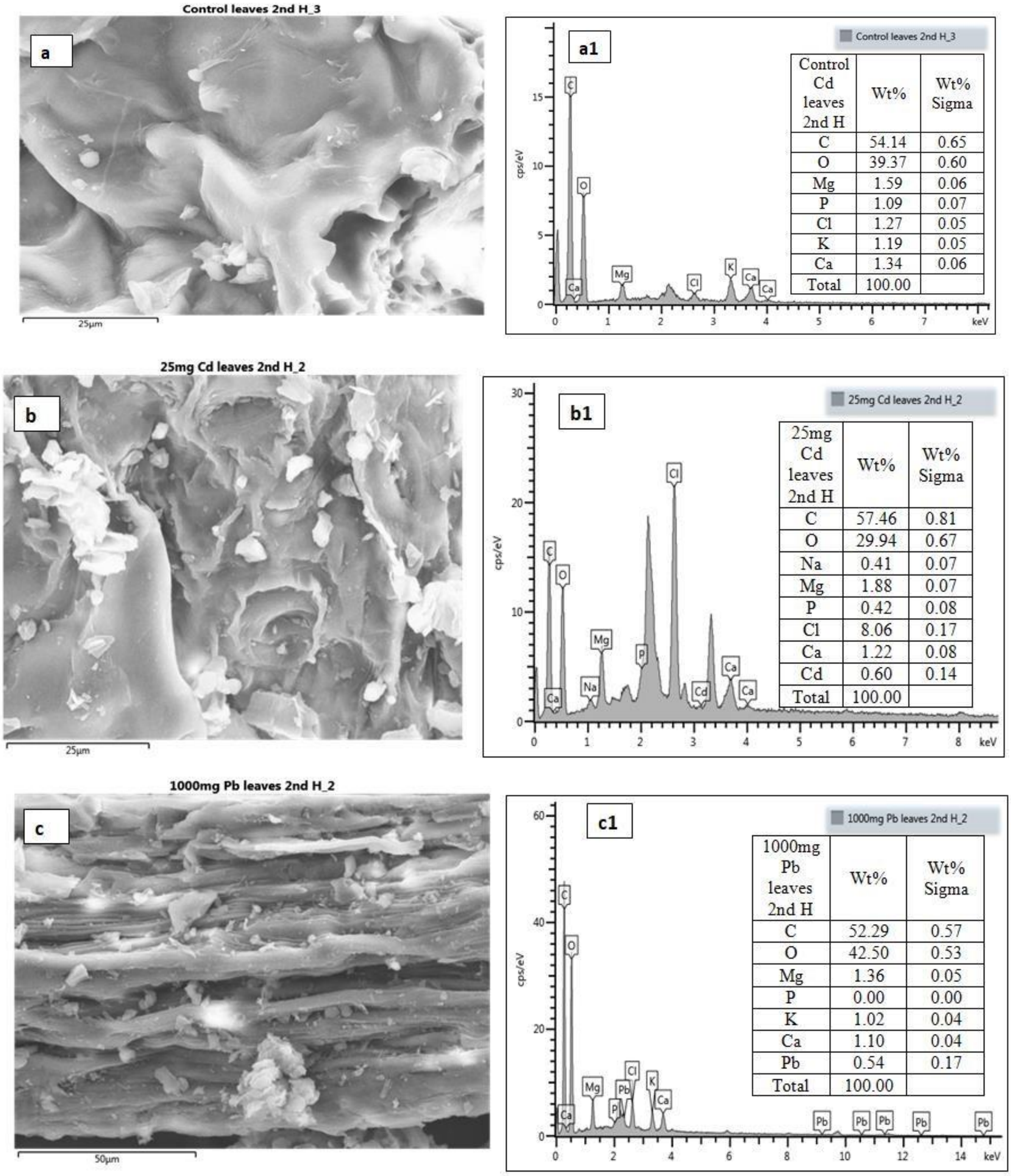
List of Appendices

Appendix 1: SEM images and EDX spectrum combined corresponding tables of the leaves from the control plants (a and a1) compared with the highest treatments of Cd (b and b1) and Pb (c and c1).

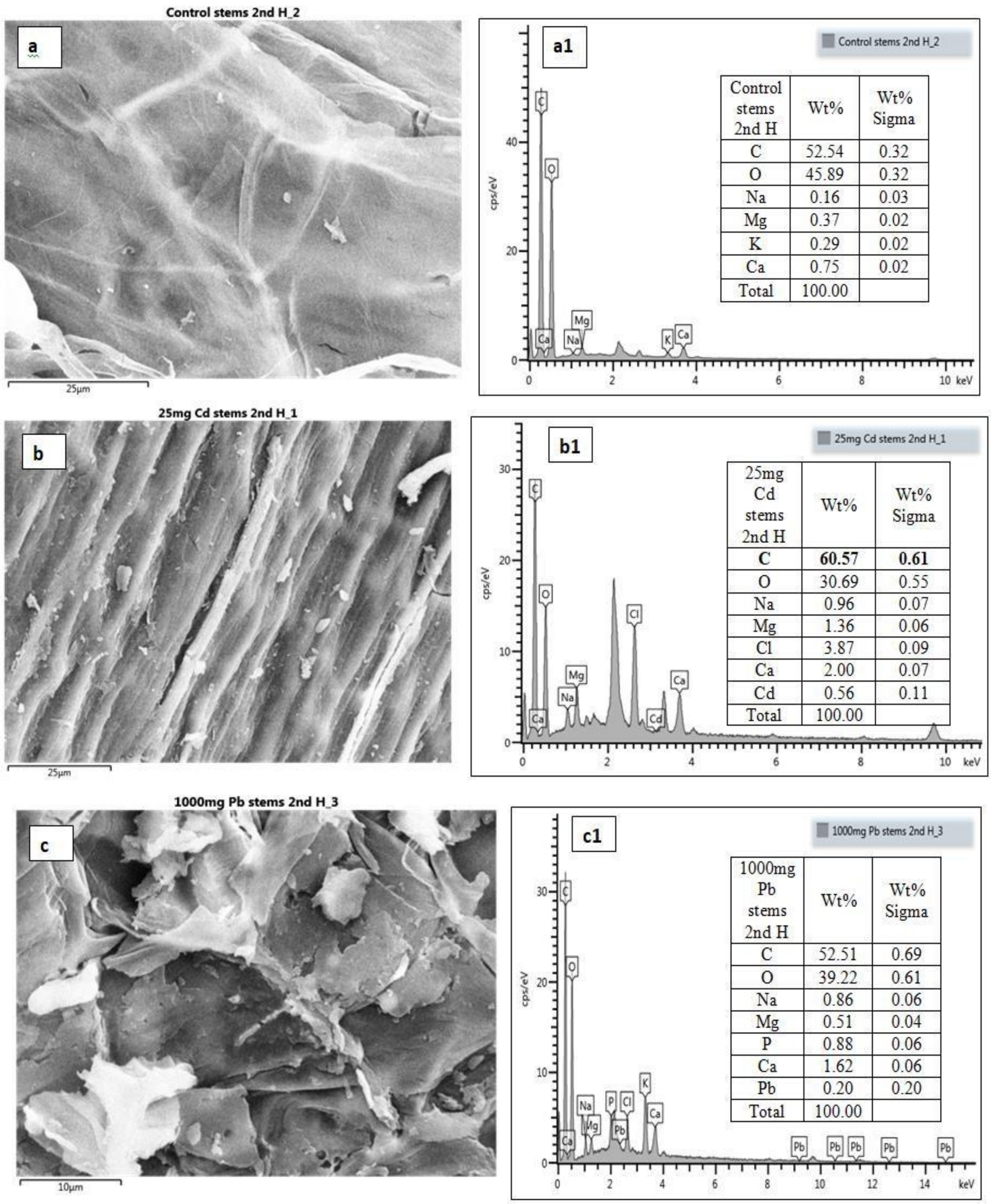


Control Cd leaves 2nd H	Wt%	Wt% Sigma
C	54.14	0.65
O	39.37	0.60
Mg	1.59	0.06
P	1.09	0.07
Cl	1.27	0.05
K	1.19	0.05
Ca	1.34	0.06
Total	100.00	

25mg Cd leaves 2nd H	Wt%	Wt% Sigma
C	57.46	0.81
O	29.94	0.67
Na	0.41	0.07
Mg	1.88	0.07
P	0.42	0.08
Cl	8.06	0.17
Ca	1.22	0.08
Cd	0.60	0.14
Total	100.00	

1000mg Pb leaves 2nd H	Wt%	Wt% Sigma
C	52.29	0.57
O	42.50	0.53
Mg	1.36	0.05
P	0.00	0.00
K	1.02	0.04
Ca	1.10	0.04
Pb	0.54	0.17
Total	100.00	

Appendix 2: SEM images and EDX spectrum combined corresponding tables of the stem from the control plants (a and a1) compared with the highest treatments of Cd (b and b1) and Pb (c and c1).

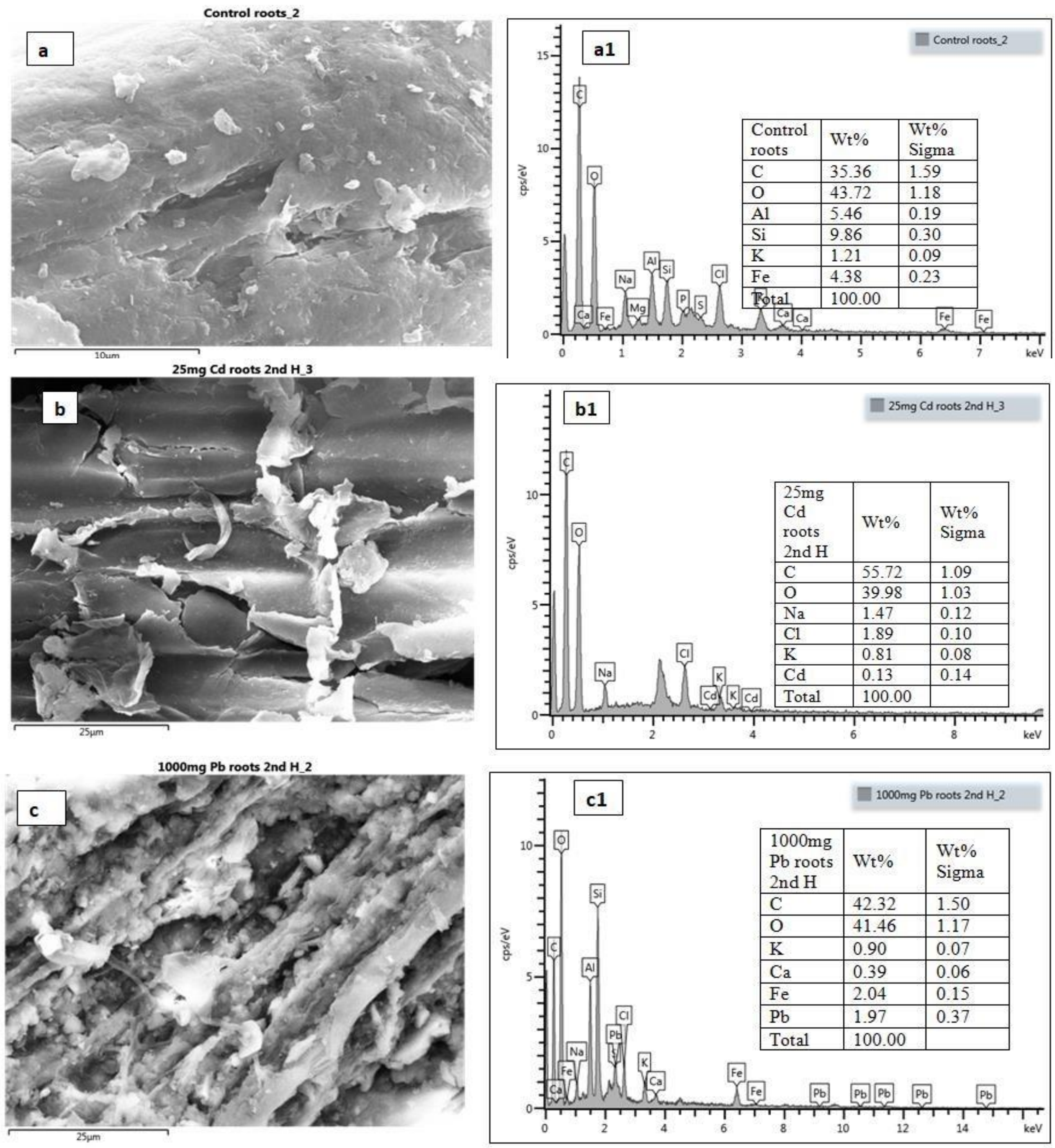


Control stems 2nd H	Wt%	Wt% Sigma
C	52.54	0.32
O	45.89	0.32
Na	0.16	0.03
Mg	0.37	0.02
K	0.29	0.02
Ca	0.75	0.02
Total	100.00	

25mg Cd stems 2nd H	Wt%	Wt% Sigma
C	**60.57**	**0.61**
O	30.69	0.55
Na	0.96	0.07
Mg	1.36	0.06
Cl	3.87	0.09
Ca	2.00	0.07
Cd	0.56	0.11
Total	100.00	

1000mg Pb stems 2nd H	Wt%	Wt% Sigma
C	52.51	0.69
O	39.22	0.61
Na	0.86	0.06
Mg	0.51	0.04
P	0.88	0.06
Ca	1.62	0.06
Pb	0.20	0.20
Total	100.00	

Appendix 3: SEM images and EDX spectrum combined corresponding tables of the roots from the control plants (a and a1) compared with the highest treatments of Cd (b and b1) and Pb (c and c1).



Control roots	Wt%	Wt% Sigma
C	35.36	1.59
O	43.72	1.18
Al	5.46	0.19
Si	9.86	0.30
K	1.21	0.09
Fe	4.38	0.23
Total	100.00	

25mg Cd roots 2nd H	Wt%	Wt% Sigma
C	55.72	1.09
O	39.98	1.03
Na	1.47	0.12
Cl	1.89	0.10
K	0.81	0.08
Cd	0.13	0.14
Total	100.00	

1000mg Pb roots 2nd H	Wt%	Wt% Sigma
C	42.32	1.50
O	41.46	1.17
K	0.90	0.07
Ca	0.39	0.06
Fe	2.04	0.15
Pb	1.97	0.37
Total	100.00	