EVALUATION OF PHYTOREMEDIATION POTENTIALS OF *Phytolacca dodecandra*, *Adhatoda schimperiana* AND *Solanum incanum* FOR SELECTED HEAVY METALS IN FIELD SETTING LOCATED IN CENTRAL ETHIOPIA.

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I declare that the above thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

I further declare that I submitted the thesis to originality checking software and that it falls within the accepted requirements for originality.

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Abstract

Pollution of soil by trace metals has become one of the biggest global environmental challenges resulting from anthropogenic activities, therefore, restoration of metal contaminated sites needs due attention. The use of phytoremediation technologies as nature-based solution to pollution, could support successful implementation of green economic development strategies; with economically affordable and environmentally friendly benefits. The present investigation employed an exploratory study on the phytoremediation potentials of three selected native plants; ***Phytolacca dodecandra*** (L'Herit), ***Adhatoda schimperiana*** (Hochst) **and *Solanum incanum*** L, dominating areas close to heavy metal contamination sources; in metropolitan centers of Addis Ababa. In this work, concentration of six heavy metals of interest chromium (Cr), lead (Pb), cadmium (Cd), nickel (Ni) copper (Cu) and zinc (Zn) were examined in soil and in different tissues (leaves, stems and roots) of selected plants (both seedlings and mature plants), in dry and rainy seasons using atomic absorption spectrophotometer. Efficiency of phytoremediation is discussed based on calculated values of Bio-concentration Factor (BCF), Translocation Factors (TF) and Bioaccumulation Coefficient (BAC). *Phytolacca dodecandra* showed BCF, TF and BAC > 1 for Zn, Pb, Ni, Cu and Cd *Adhatoda schimperiana* gave BCF, TF and BAC > 1 for Zn, Cu, Ni and Cr; likewise, BCF, BAC and TF values of > 1 were noted in *Solanum incanum* for Zn, Cu, Pb and Ni. Based on these scenarios, the three plants could be utilized for phytoextraction of contaminated soil. Conversely, BCF and BAC for Cr levels in tissues of *Phytolacca dodecandra* were all < 1, which indicates unsuitability for phytoremediation of Cr in contaminated soils. Besides, *Adhatoda schimperiana* retained Pb and Cd in their roots showing root BCF > 1, while BAC and TF < 1, which highlights its suitability for phytostabilization. Moreover, BCF, TF and BAC values of < 1 noted for Cr and Cd in *Solanum incanum* reveal that *Solanum incanum* may not be a good candidate for remediation of Cr and Cd contaminated environments. In conclusion, results from this study revealed that the selected plants can accumulate substantial amounts of the above trace metals in their tissues and can serve as prospective phytoremediators of most of these metals. Phytoextraction and phytostabilization were the main mechanisms of remediation in this study.

Key words: Contaminated soil; Heavy metals; Plant uptake; Translocation factor; Bioconcentration factor; Phytoremediation, Phytoextraction; Phytostabilization

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List of abbreviations and acronyms

AAS	Atomic absorption spectrophotometer
BAC	Bioaccumulation Coefficient
BCF	Bio-concentration Factor
Cd	Cadmium
Cr	Chromium
Cu	Copper
CEC	Cation exchange capacity
dS /m	Deci Siemens per meter
EC	Electrical conductivity
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
HCL	Hydrochloric acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
$K_2Cr_2O_7$	Potassium dichromate
Mg/kg	Milligram per kilogram
Ml	Milliliter
Ni	Nickel
OM	Organic matter
Pb	Lead
TF	Translocation Factors
WHO	World Health Organization
Zn	Zinc

CHAPTER ONE

1. Introduction

1.1. Background of the study

Environmental pollution is of a serious ecological concern, and several inorganic and organic contaminants have been noted as sources of environmental contamination (Ali *et al*., 2018; Gul *et al*., 2019; Saxena *et al*., 2019). Heavy metals are one of the leading environmental pollutants and critical threats to human health in many countries (Ashraf, *et al*., 2019). Heavy metal refers to trace elements with an atomic number above 20 and density of larger than 5 g/cm^3 (Singh *et al*., 2019). There are 53 trace elements documented as heavy metals (Padmavathiamma and Li, 2007; Sarma, 2011; Prieto *et al*., 2018).

Pollution of soils by heavy metals or excessive accumulation of trace metals in agricultural soils and edible crops have become a potential threat to the environment and human health since the start of the industrial revolution (Xiong *et al*., 2016; Chaoua *et al*., 2019; Woodford, 2019; Elshamy *et al*., 2019). Heavy metal contaminants can have both anthropogenic and natural sources, however, the anthropogenic activities are major sources of metal contaminants (EPA, 2000; Kabata-Pendias, 2011; Saif and Khan, 2017; Singh *et al*., 2019). Contamination of soil by toxic metals can occur from a variety of sources the main ones are the use of fossil fuel (Muradoglu *et al*., 2015), industrial practices (Belouchrani *et al*., 2016; Singh *et al*., 2019), mine tailings (Chen *et al*., 2016; Gul *et al*., 2019), agricultural pesticides (Belouchrani *et al*., 2016), automobile exhaust or transportation (Luo *et al*., 2018) and illegal dumping of wastes (Khan *et al*., 2016; Gul *et al*., 2019).

Common soil contaminant trace metals with tremendous health risk to organisms include Cd, As, Ni, Pb, Cr, Zn, Cu, and Hg (Sharma *et al*., 2014; Singh *et al*., 2019). Trace metals especially; Cr, Pb, Cd, Hg, Ni, Cu, and Zn are considered as potentially toxic heavy metals with negative environmental impacts (Antoniadis *et al*., 2017; Cabral-Pinto *et al*., 2019; Hasan *et al*., 2019). Similarly, Nagajyoti *et al*. (2010) reported urban areas with intensive anthropogenic activities have been contaminated by environmental pollutants including: Cr, Pb, Cd, Ni, Co, Zn, Fe and others. Some of these heavy metals, including Mn, Ni, Cu and Zn are essential elements having biological

importance. However elevated concentrations of these heavy metals could be harmful to organisms (Shi *et al*., 2016). Conversely, metals including Cr, Cd, Hg, As and Pb are non-essential metals and have no apparent biological importance (Swapna *et al*., 1987).

Despite the presence of low contaminated areas (pristine areas) in some parts of the world, anthropogenic environmental pollution influences are well spread throughout the world. Even though the contribution of developed countries is very high and they are the most polluting countries in the world, the negative impact of pollution is worse in developing countries mainly because of limited financial resources and technical expertise that hamper the progress to comply with universal environmental guidelines and remediation measures (Azam, 2016).

Deterioration of natural environments caused by unsustainable use of resources and contamination of environmental medium is a serious problem in the developing world, like Africa. Africa is a continent with rapid population growth accompanied by urbanization and industrialization which places huge demand on resources and impacts the quality of environment (Fayige *et al*., 2018; Odoh *et al*., 2019). Central Ethiopian highlands are under increasing pressure due to expansion of industries and population growth (Minase *et al*., 2016). Even though Addis Ababa is a rapidly growing metropolitan in Africa, solid waste management systems, sewerage treatment facilities and regulatory compliances have not progressed well in proportion to its development (Colombani *et al*., 2018; Aschale *et al*., 2019). Pollution of local environments in Addis Ababa is connected to uncontrolled solid waste and sewage disposal from industrial and domestic sources, urbanization and unsustainable use of resources, vehicle washing effluents and toxic chemicals used for agriculture (Regassa *et al*., 2011; Woldetsadik *et al*., 2018; Eriksson and Sigvant, 2019).

There are several conventional treatment technologies (physical and chemical approaches) to deal with metal contaminated environments (Antoniadis *et al*., 2017; Yang *et al*., 2018), but most of these are expensive, more technical and environmentally destructive (Luo *et al*., 2018). Phytoremediation is identified as a non-destructive and promising technology for cleaning toxic metals from polluted water or soil (Prieto *et al*., 2018; Eid *et al*., 2019). This plant-based clean-up method is an alternative and emerging technology, which is both economically and environmentally sound (Gong *et al*., 2018; Ashraf, *et al*., 2019). This technology is an efficient and feasible technique for clean-up of sites contaminated by toxic metals (Fu *et al*., 2019). Phytoremediation technologies can be classified based on the mechanisms involved. These

include: phytoextraction, phytodegradation, phytostabilization, phytovolatilization and rhizofiltration (Pantola and Alam, 2014; Eid *et al*., 2019).

Plants that can absorb metals, mainly in the aboveground parts, are usually referred to as hyperaccumulators. These plants can collect and build up exceedingly higher level of metals than nearby plants. Hyperaccumulators can take up and retain the following metals with a minimum concentrations of: 10,000 mg/kg of Mn or Zn, 1,000 mg/kg of Cu, Ni, Cr, Co, Se or Pb, 100 mg/kg of Cd or As (Turgut *et al*., 2004; Saxena *et al*., 2019).

Numerous studies have been conducted on phytoremediation of metal contaminants, and over 500 plant species have been identified as metal hyperaccumulators (Luo *et al*., 2016; Singh *et al*., 2019). Phytoremediation methods depend on potential absorption and translocation of contaminants into the aerial parts of plants (Rehman *et al*., 2017; Gul *et al*., 2019). Metal mobility and availability for plant uptake can be influenced by cation exchange capacity, organic matter, soil pH, soil texture, soil microorganisms, root type and other factors (Eid *et al*., 2019; Gul *et al*., 2019). A potential phytoremediation candidate plant has to be tolerant to harsh environmental conditions, has unpalatable nature and must grow in polluted soil (Evangelou *et al*., 2015; Prabakaran, *et al*., 2019). Hence, sound evaluation of plant capacity to remediate a polluted site and a practical approach for implementations are essential prerequisite of any phytoremediation plan.

Three locally available native plants namely: *"endod"* (*Phytolacca dodecandra*) "sensel" (*Adhatoda schimperiana*) and Thorne apple (*Solanum incanum*) were chosen for this study. The selection of these plants was based on fulfillment of basic characteristics of plants for phytoremediation. Most importantly, tolerance and survival in contaminated sites, large biomass, perennial nature and availability at a local level are used as a preliminary selection criteria.

It is evident from literature that there are no previous reports using these plants for phytoremediation of heavy metals contaminated soils. Currently, phytoremediation research is dominantly green-house and laboratory-based; however, these conditions do not reflect accurately the accumulation capability in terrestrial application (Paz-Alberto and Sigua, 2013; Rostami and Azhdarpoor, 2019). Real field evaluation of phytoremediation properties of plants is critical since several environmental factors which cannot be simulated in controlled environment could have

different impact in natural environments (Luo *et al*., 2018). Findings in natural field conditions could have significant differences from those in controlled environments since field is an actual world where numerous factors in the environment concurrently affect removal efficiency of plants (Ji *et al*., 2011; Singh and Malaviya, 2019). Several factors can play a role that could influence phytoremediation in the field; these include temperature, soil pH, nutrients, moisture, microorganisms, contaminant distribution pattern, low metal bioavailability and soil type (Saxena *et al*., 2019). Consequently, this study evaluated the heavy metal phytoremediation potentials of the 'Endod' *Phytolacca dodecandra*, 'Sadom Apple' *Solanum incanum* and 'Sensel'*Adhatoda schimperiana* plants under field conditions.

1.2. Statement of problem

Heavy metal contaminants causing ecological health problems are receiving global attention (Gajic *et al*., 2018, Fu *et al*., 2019), and the condition is even worse in developing countries, where there are no stringent environmental regulations. These pollutants should be managed in an environmentally safe and economically sustainable way.

Application of nature's self-purification strategies, such as phytoremediation can be alternatives to conventional remedial technologies including chemical leaching, solidification, excavation, filtration, soil washing, vitrification, thermal treatment which are expensive, labor-intensive, and known to cause undesirable effects on the ecosystem and soil characteristics (Suthar *et al*., 2014; Yi and Sung, 2015; Luo *et al*., 2018). Phytoremediation is the bioremediation method using either terrestrial or aquatic plants to clean-up polluted sites. This technology is natural solar-driven clean-up method, economical and ecologically pleasant with high public acceptance (Sidhu *et al*., 2017; Hrynkiewicz *et al*., 2018). It uses selected plants and associated microorganisms to remediate contaminated air, water or soil (Hrynkiewicz *et al*., 2018).

Several plants are known as metal hyper accumulators; however, many of these species are leafy vegetables and edible crops. Edible plants are not preferred for phytoremediation due to the toxic effect of certain metals and short life span of most edible crops (Evangelou *et al*., 2015; Saxena *et al*., 2019). Previous studies identified few non-edible tree species, shrubs and energy crops as suitable plants for clean-up of metal contaminated sites (Evangelou *et al*., 2015; Tauqeer *et al*., 2019). The utilization of these plants for remediation purpose is advantageous due to their

perennial nature, high biomass, public acceptance, adaptation to polluted sites, erosion control and aesthetic values (Cristaldi *et al*., 2017; Ting *et al*., 2018). Thus, there is a need to look for a wider variety of wild plants for phytoremediation of contaminated sites.

Phytoremediation of contaminants from polluted sites could be affected by plant variety, soil properties and climate of the region. Plants must be appropriate for soil and climatic conditions of the area and introduction of exotic plant species for phytoremediation purposes could also cause disruption of the local flora, take over local plants and might have impact on soil and biodiversity (EPA, 2000; Jeschke *et al*., 2014; Potgieter *et al*., 2019). Therefore, there is a need to concentrate on locally available native flora for remediation of metal polluted soils (Lajayer *et al*., 2017; Eid *et al*., 2019).

Studies on phytoremediation properties of plants are prerequisite and also have a positive impact on the success of phytoremediation project plan. Even though there are numerous research reports on phytoremediation potentials of plants, phytoremediation studies on mixed metal contaminants and the use of plants to remediate multiple contaminants are very limited (Tauqeer *et al*., 2019). And, most of the available studies draw attention to plant families: *Brassicaceae, Caryophyliaceae, Euphorbiaceae, Cunouniaceae, Cyperaceae, Asteraceae, Flacourtiaceae, Fabaceae, Lamiaceae, Poaceae and Violaceae* (Anjum *et al*., 2014, Amin *et al*., 2018). A review article published by Luo *et al*. (2016); also noted most of the studied plants were families of *Asteraceace*, *Brassicaceae*, *Caryophyllaceae*, *Fabaceae*, *Poaceae* and *Violaceae*. Further, based on review of studies conducted so far, it can be concluded that little attention has been given to: *Phytolaccaceae, Acanthaceae* and *Solanaceae* plant families; except for few edible members of *Solanaceae.*

Phytoremediation studies on mixed metal contaminants could be essential since mixed metal contamination is common in most sites (Singh *et al*., 2019). Besides, based on local personal observation, plant species selected for this study: "Endod" (*Phytolacca dodecandra*), "Sensel" (*Adhatoda schimperiana*) and "Embuaye" (*Solanum incanum*) seem to grow vigorously and adapt well in extremely contaminated industrial areas in Ethiopia. On the contrary, too much concentration of pollutants especially heavy metals, could inhibit plant growth and can even result in death of plants and limit application of phytoremediation (Fu *et al*., 2019). However, plants used in phytoremediation projects are often adapted to contaminated sites (Keller *et al*., 2006) and sites

with multiple contaminants could be good candidates for phytoremediation plan. Consequently, in view of the fact that adaptation to a metal contaminated area could be due to capacity to accumulate or exclude pollutant (EPA, 2000; Mehes-Smith *et al*., 2013; Kalubi *et al*., 2016; Fu *et al*., 2019); there is also a need to investigate this fact and the mechanisms of adaptation.

The selection of these plants for this study was based on several criteria. Some of these conditions are: ability to survive, tolerate, grow and reproduce in a highly contaminated sites; Non-invasive and locally available; non edible to human and unpalatable to most herbivores; adaptation to a wide range of climatic and soil conditions; large biomass production ability and regrowth after harvesting the aboveground portion. Similarly, contaminants of interest (Cd, Pb, Cr, Ni, Zn and Cu) were selected for this study based on the reports on their potential toxicity, tremendous health risk on organisms, negative environmental impacts and abundance in urban areas.

The emphasis of the present study was therefore to assess phytoremediation potentials of *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum* by determination of elemental concentrations in plants collected from multiple metal contaminated industrial sites. In addition, this research is the first to report phytoremediation potential investigations of these plants in a field setting.

1.3. Aim and Objectives

- The overall objective of this research was to assess the use and efficiency of naturally growing plants, *A. schimperiana*, *P. dodecandra,* and *S. incanum L*, in phyto-remediating multiple metals (Pb, Cd, Cu, Ni, Zn and Cr) from contaminated soils under natural field conditions.

Specific study objectives are:

- To evaluate the correlations between soil physicochemical characteristics and metal absorption by the selected plants.
- To determine and compare the heavy metal accumulation potentials of seedlings and mature counterparts of the selected plants.
- To examine the effect of seasonal variations on elemental absorption and accumulation properties of the selected plants.
- To determine the mechanisms used by the plants for remediation.

- To determine the suitability of the selected plants for field restoration of metal contaminated soil.

1.4. Research questions

In evaluating each aspect of selection, the following questions were addressed:

Are there any differences between the studied seedlings and mature plants in heavy metal accumulation?

What mechanisms are involved in the phytoremediation process?

Does seasonal variation affect absorption and translocation of metals by the selected plants?

Is there correlation between soil physicochemical characteristics and metal uptake by the selected plants?

How suitable are the selected plants for field restoration of metal contaminated soil?

1.5. Significance of the study

Contaminated land, a global problem, is becoming an important research topic in many countries since it has a considerable impact on environment and human health (Sarwar *et al*., 2017; Li *et al*., 2019). Soil pollution is an insidious risk since it is difficult to detect and observe soil erosion and other soil degradation processes and it is making a hidden impact that is based on contaminant characteristics, properties of soil and speed of entry. Anthropogenic activities and poor environmental management standards are principal soil pollution causes.

Toxic metals can contaminate soil and water resources which can directly affect biological diversity and human health. Excessive buildup of metals in the environment could result in degradation of natural environments and ecological imbalance which will in turn affect yield and quality of crops (Mayor *et al*., 2013). Similarly, accumulation of toxic metals in food crops and biomagnification of metals in animals along food chains could also affect health.

Environmental clean-up is crucial for mitigating pollution risk on the human health and the natural ecosystem. However, physical treatment methods and chemical treatments of heavy metal contaminated sites are very expensive, labor intensive and results in secondary pollution. Net

environmental benefits of remediation technologies should be considered before commencing the remedial measures to maximize net environmental benefits. Phytoremediation technology could be highly suitable and can have enormous benefits for low income countries (Waoo *et al*., 2014). Plant-based remediation of polluted sites is an environmentally friendly alternative to other treatment technologies (Nanda and Abraham, 2013; Thijs *et al*., 2017; Li *et al*., 2019). Besides the remediation purpose, phytoremediation also contributes to the reduction of greenhouse gases via fixation of atmospheric CO_2, reduction of soil erosion, energy production and aesthetic values (Novo *et al*., 2018).

Phytoremediation using native plants is often better because of their adaptation, survival and growth under environmental stress compared to non-native ones (Chandra and Kumar, 2017; Guarino *et al*., 2019) and it will also reduce environmental risks associated with this technology. However, planning for a phytoremediation project without having initial information about the nature of the plants to be used and relying on non-native plants might not be economically viable and environmentally sustainable. Successful remediation depends on adaptation or tolerance of plants to multiple metal contaminants and the capacity to uptake and translocate metals (Singh *et al*., 2019). Hence, in-depth research and selection of metal accumulator plants, along with metal-resistant plants, and those best adaptable to contaminated environment and a deeper understanding of their heavy metal absorption, translocation and accumulation is vital to make the whole project a success (Reeves *et al*., 2018; Singh *et al*., 2019).

This field experimental study on selected plants growing in metal contaminated soil, examined the remediation potentials of these plants to clean up contaminants of interest (Pb, Ni, Cu, Zn, Cd, and Cr) in actual environmental conditions. Findings of the study addresses the application of *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum* for phytoremediation heavy metal contaminated soils. This could contribute to the existing broader scientific knowledge on the use of indigenous, locally adapted and commonly available plant species for the management of polluted soils in urban environments. Results could provide important insight on the potential benefits of these plants and the research output could add value to environmental management efforts, especially on remediation of heavy metal contaminated sites. Further, results from this study can also be used as an input for further research and provide information on the

selected plants so that there could be informed consideration of them for the implementation of phytoremediation projects.

1.6. Scope of Study

The research is delimited to assess the potential use of selected native plants; *Adhatoda schimperiana, Phytolacca dodecandra* and *Solanum incanum* for phytoremediation of selected heavy metals: Pb, Ni, Cu, Zn, Cd, and Cr from contaminated industrial sites. Predefined heavy metal concentration ratios between the plants and the contaminated soil were used for evaluation of the degree and mechanism of phytoremediation process.

In the current study, no controlled pot experiment was conducted, but an investigation on metal absorption and translocation by selected plant species growing naturally on the industrially contaminated soil was done. Exclusion of metals accumulated in seeds and flowers of the studied plants can also be mentioned as a limitation of this study, which is basically because the study also comprises samples of young plant seedlings that did not flower. Plant age estimation was done using growth ring examination, stem girth comparison and other morphological characterization. Although this study compared the phytoremediation of seedlings and 'mature plants' the exact age of mature plants remains unestablished. Similarly, the study did not include comparative study on the plant growth rate. On the other hand, this study did not assess the impacts of microbial activity on the phytoremediation activities of plants.

The other limitation of this study is that there was no 'before' and 'after' assessment to evaluate metal contaminant's reduction after phytoremediation, as no predetermined or artificially contaminated soil was employed. However, the assessment of phytoremediation potential was conducted by quantification of metal accumulation efficiency by calculating the Bioconcentration factors and Translocation factors.

1.7. Ethical considerations

1.7.1. Site and societal value considerations

Sampling sites involved in the sampling campaign were of low-intensity public uses and sites that belong to the municipality, company or industries. No samples were collected without consent with the concerned bodies. Necessary precautions were taken to reduce conflict with stakeholder

communities. Study ethical clearance was obtained from UNISA and the ethics details and other consent letters are placed in the Appendix part.

1.7.2. Regulatory concerns

All regulatory issues were taken into account and agreement was made and ethical clearance and support was obtained from all concerned bodies; government (Addis Ababa City Municipality and Environmental Protection Authority), private companies including industries around the study area.

1.7.3. Ecological risk considerations

The study took into account all possible ecological risks, all study plants are native to the country and no risk of invasive species expected. Study plant samples were carefully discarded, in best available hazardous waste disposal facility based on the consent of EPA, to avoid cross contamination to any environmental medium. In addition, plant root samples were safely collected without extensive excavation and the excavated soil was filled back, to avoid the risk associated with erosion.

1.7.4. Health risk considerations

Appropriate protective measures that can avoid hazard exposure routes such as inhalation, contact and ingestion were considered while doing the experiments and during disposal of analyzed samples. All possible risk reduction procedures and materials including masks, safety gloves, and lab apron were used. All field and laboratory equipment were cautiously cleaned to prevent contamination.

CHAPTER TWO

2. Literature review

2.1. Environmental contamination

Environmental pollution attributable to anthropogenic and natural sources is increasing rapidly with human population, industrialization, urban development and consumption of natural resources (Panayotou, 2016; Irfan and Shaw, 2017; Liang and Yang, 2019). Discharges of several chemical contaminants by industries into different environmental media (soil, water, air) disturb ecosystem balance. Dealing with environmental contaminants that endanger the normal functioning of the environment becomes a paradox for scientists and politicians (Basak and Dey, 2016). In general, the rate of contaminant accumulation surpasses the ecological capacity of the planet to remove it. Thus, reversing the potential impacts of these contaminants on the natural environment needs collaboration with nature (Prieto *et al*., 2018).

Several toxic chemicals and materials are available in the environment and people get exposed to these substances in many ways. Exposure to these contaminants can be through air, water and polluted soil pathways (Moore, 2019). Consumption of vegetables and seafood exposed to contaminated water or soil are potential sources of exposure. Industrial activities are most significant sources of environmental pollution especially in developing countries (Mingkhwan and Worakhunpiset, 2018). Ecosystem degradation, elevated levels of hazardous contaminants in the environment, and potential impacts of these contaminants were aggravated due to industrialization (Nayak *et al.*, 2018; Moore, 2019).

Heavy metals are the major naturally occurring toxic materials and heavy metal pollution is of great ecological and health concern (Nayak *et al*., 2018; Almalki *et al*., 2019). Resistance to degradation, bioaccumulation and biomagnification are main factors that exacerbate the likely effects of metals on health of human being, biodiversity and the ecosystem.

2.2. Soil pollution

Soil is an exceedingly complex and vital constituent of the environment. Pollution or contamination of soil is extensive and a severe global environmental problem (Mizutani *et al*., 2016). Good soil lacks pollutants and contains key minerals and other components for better plant

growth. However, due to the continuous unsustainable exploitation of natural resources and the negligent disposal of wastes, it is difficult to find good soil. Soil is final receptor of several environmental contaminants that are produced by human activities (Cristaldi *et al*., 2017). Land disposal of solid wastes and toxic contaminants is a common practice for many of us. However, the impact on soil can be long term, degrading the quality of productive land and leaving it wasted. The pollution of soil is an important environmental problem seriously impacting the normal functioning of the ecosystem today (Huang *et al*., 2018; Rostami and Azhdarpoor, 2019). With increasing human population and industrial development, contamination of life supporting environmental media is inevitable. Rapid urbanization accompanied by increasing agricultural and industrial practices has severely degraded soil quality worldwide (Adrees *et al*., 2015; Li *et al*., 2019). Soil pollution can adversely impact soil quality, productivity and other natural ecological parameters connected to soil. Contamination of soil can directly impact food security through crop yield reduction due to toxicity or making the produced one unsafe for consumption (Eugenio *et al*., 2018).

Contamination of land with heavy metals has been given considerable attention to date, due to the increased health effects, persistence in the environment and deterioration of soil quality (Kidd *et al*., 2007; Kankia and Abdulhamid, 2014; Ashfaque *et al*., 2016; Azeez *et al*., 2019). Anthropogenic activities such as wastewater irrigation, poor solid waste disposal practices, mining, excessive use of agrochemicals, accidental leakage of chemicals and oil, atmospheric pollution can also increase soil contaminants, especially concentration of trace metals in soil (Jarosz-Krzeminska and Adamiec, 2017; Eugenio *et al*., 2018).

Effect of soil contamination depends on the nature of the pollutant, dosage of pollution and agro-ecological regions because of the complex linkage between the climate of the region, soil type, plant or crop types and management practices involved (Saha *et al*., 2017). Ecological states of the biosphere are closely interconnected, pollution therefore will have an impact on the quality of the other. Therefore, soil contaminated with chemical substances becomes a pollution source for other environmental components including air, water and plants (Kalandadze and Matchavariani, 2019). Appropriate remedial measures for contaminated soil need detailed study on pollutant type and dose, pollution source and assessment of possible impacts.

2.3. Soil remediation

Soil remediation is the method of cleansing and revitalizing contaminated soil, to reduce environmental impacts and effects on health. The main target of soil remediation is restoration to its natural state. But, complete removal of contaminant or purifying to the level suitable for all-purpose does not seem economically feasible and technically achievable (Azam, 2016). Thus, most remediation approaches are predetermined to deal with contaminated soil and make the soil suitable for a specific future use or achieving 'fitness for use' status (US EPA, 2009).

The removal of toxic contaminants from polluted soil is usually expensive, labor intensive and time consuming. Currently, there are several technologies dealing with heavy metal contaminated soil recovery; however, some are still in the experimental stage (Prieto *et al*., 2018). Contaminated soil can be remediated by several mechanisms; mechanical, thermal or biological processes. Conventional remediation techniques, including chemical extraction methods, physical excavation, and thermal decontamination methods use diesel fuel motorized machineries which, could have harmful environmental impacts through their emissions of contaminants such as greenhouse gases (Sner and Anderson, 2011; Yang *et al*., 2018).

Ex-situ and *in-situ* remediation methods are two main strategies for remediation of contaminated sites. Remediation at the original site is termed as *in-situ* remediation and *ex-situ* clean-up method includes excavation and transportation of contaminated soil into a new location for treatment (Leguizamo *et al*., 2017). *Ex-situ* remediation of polluted soil carried out by chemical and physical methods carry a large price tag (Lajayer *et al*., 2017). However, *in-situ* remediation methods are economical and have limited environmental impacts (Song *et al*., 2017; Li *et al*., 2019). *In-situ* vitrification, soil washing, incineration, soil flushing, landfilling, stabilization and solidification are common conventional remediation methods (Rahman *et al*., 2016).

In general, decontamination of soil can be done using chemical, physical or biological techniques (Khalid *et al*., 2017; Liu *et al*., 2018). Chemical and physical remediation methods are disadvantaged by limitations such as an irreversible change in soil properties, high cost, labor-intensive, disruption of biological diversity and native flora, secondary pollution problem and the need for technological advancement (Rahman *et al*., 2016). Adequate remediation of contaminated sites or environmental media needs an integrated approach, cooperation and

utilization of biotechnological methods together with other traditional remediation and natural resource conservation methods (Mani *et al*., 2015; Singh and Singh, 2016).

2.3.1. Physical remediation

Physical remediation involves physical techniques such as thermal treatment, isolation, containment method, and soil replacement methods for reversal of polluted soil (Yao *et al*., 2012). Thermal treatment is common for volatile contaminants such as Hg; it involves remediation by surface heating (Li *et al.*, 2019). Conductive heating methods, electrical heating, and steam heating are common methods in thermal treatment techniques (Song *et al*., 2017). Contaminated soil can be isolated or contained in a physical barrier wall or impermeable materials for the reduction of further migration and distribution of contaminants such as heavy metals into groundwater.

Blending contaminated soil with a large amount of uncontaminated soil involves soil replacement methods and this method also includes landfilling and surface capping techniques. Replacement techniques are suitable for heavily polluted soil with a small area because of the high cost associated.

2.3.2. Chemical remediation

Chemical remediation method is a system of removal of contaminants or decontamination of environmental medium using chemical reagents or reactions (Song *et al*., 2017). Remediation techniques involved in this method include stabilization, solidification, soil flushing, soil washing, vitrification and electro-kinetics (Liu *et al*., 2018). However, stabilization technique is temporary *in-situ* remediation technique that is inexpensive and easy to apply for high contamination. Solidification is a regularly practiced fast, efficient technique involving chemical reaction for contaminant immobilization and physical enclosing of pollutants in a solid medium, like asphalt and cement (Li *et al.*, 2019). Solidification is an expensive process, both *in-situ* or *ex-situ* conditions are possible, while stabilization technique is common in an *in-situ* condition. However, in both cases, contaminants remain in the soil and the land loses its original function. Soil flushing is another *in-situ* remediation technique applicable to moderate to highly contaminated conditions; it is an economical contaminant removal process with a limited disturbance on soil, however, the potential to pollute groundwater remains as a disadvantage of this technique (Liu *et al*., 2018). Similarly, soil washing is fast remediation method using water or other suitable solution for

decontamination of soil (U.S EPA, 2006; Li *et al*., 2019). It is a regularly practiced technique involving solvent and mechanical method for remediation of polluted soil, and the method involves extreme soil disturbance and common in an *ex-situ* condition (Song *et al*., 2017; Ashraf *et al*., 2019).

Vitrification technique is another chemical remediation technique regularly practiced both in an *ex-situ* and *in-situ* condition and this technique involves mixing of contaminated soil with glass forming mixtures under heat of thermal energy (1400–2000°C) (Yao *et al*., 2012; Li *et al*., 2019). The final product after vitrification process gives amorphous homogeneous glass that contains contaminants like heavy metals immobilized in a glass matrix via chemical bonding encapsulation (Navarro, 2012).

Electrokinetics is also newly developed *in-situ* contaminant removal technique applicable for fine texture soil with moderate to high contamination condition. This process uses direct electric current for effective heavy metals removal from contaminant matrix which involves process mechanisms like electrolysis, electrophoresis, electroosmosis and electromigration (Liu *et al*., 2018).

2.3.3. Biological remediation

Biological remediation methods are an environmentally sustainable techniques for remediation of polluted soils (Agnello *et al*., 2016). Considering negative impacts on the environment and the high cost associated with physical and chemical treatments; biological treatments are better alternatives for clean-up of contaminated sites (Prieto *et al*., 2018). Reclamation of contaminated sites using biological methods can be done via bioremediation or phytoremediation, or combination of both (Ashraf *et al*., 2019). Removal, immobilization and decontamination of pollutants via biological remediation techniques could be done by plants and associated microorganisms (Ayangbenro and Babalola, 2017). Microorganisms cannot breakdown toxic metals but they can minimize the mobility and bioavailability by changing the chemical and physical characteristics of the polluted environment (Ashraf *et al*., 2019).

2.4. Phytoremediation Methods

"The term phytoremediation comes from the Greek word "phyto" meaning plant, and Latin 'remedium' meaning restoring balance" and it can be defined as remediation of contaminated environment using an inherent characteristic of plants (Rahman *et al*., 2016). It is an *in-situ* technique of utilizing plants to uptake, assimilate or stabilize different contaminants from water, soils or sediments (Leguizamo *et al*., 2017; Rostami and Azhdarpoor, 2019). Phytoremediation is an emerging, cost effective, environmentally sound, *in-situ* clean-up and remediation approach for contaminated soil (Razzaq, 2017; Yang *et al*., 2019). It has been reported to be tenfold cheaper than chemical and physical methods of environmental remediation methods and other engineering-based soil removal and replacement techniques (Marques *et al*., 2009).

The process can occur by means of plant metabolic processes alone or in association with microorganisms which play an important role in phytoremediation (Nwoko, 2010; Wang *et al*., 2017). An effective phytoremediation process relies on site characteristics, plant type and the capacity of plants to accumulate, assimilate and degrade the pollutants (Kvesitadze *et al*., 2006; Tauqeer *et al*., 2019). Natural phytoremediation approach uses the natural accumulating properties of plants and concomitant microorganisms, while an induced phytoremediation method; involves different mechanisms for enhancing availability of contaminants and uptake properties of plants (Rahman *et al*., 2016). Induced phytoremediation approach involves chelators, chemicals, genetically modified plants or other enhancing methods applied on the target plant or the environmental compartment under treatment or investigation.

Plants can use several strategies to decontaminate toxic heavy metals, however the common strategy is metal uptake. Excessive absorption and concentration of toxic metals can deleteriously damage plant tissue (Khan and Faisal, 2018). Plants can also use several methods to avoid the toxic effect of over-accumulation. The restriction of metal movement into plant root with the help of mycorrhizal fungi is one way of avoiding the toxic effect (Marques *et al*., 2009). Plants can also be metal "accumulators" or "excluders"; plants that can store metals and remain vigorous are metal accumulators, while plants that can control the uptake of toxic metals can be categorized as metal excluders (Tangahu *et al*., 2011; Khan and Faisal, 2018). Various phytoremediation mechanisms are used by plants to accomplish detoxification of contaminated sites these include;

phytoextraction, phytodegradation, phytostabilization, phytovolatilization and rhizofiltration (Pantola and Alam 2014; Gupta *et al*., 2019; Ashraf *et al*., 2019).

2.4.1. Phytoextraction

Phytoextraction can also be termed as phytosequestration, phytoaccumulation or phytoabsorption (Mahar *et al*., 2016). Pollutant accumulation (mostly metals) in plant biomass is a common process in phytoextraction. Phytoextraction process can be applied to polluted soil and water, and this kind of extraction can be realized by accumulation or hyperaccumulation (Wang *et al*., 2017). In this method, transfer of contaminants to the aerial part of plants is vital since harvest and safe disposal of contaminants is desired (Bosiacki *et al*., 2014; Razzaq, 2017; Prabakaran *et al*., 2019). The phytoextraction method has been tried more on toxic metal extraction than organic contaminants. This process remediates metal contaminated soils without disturbing soil properties, and it is also termed as biomining or phytomining (Singh and Bhargava, 2017). Translocation of contaminants from root to the aboveground portion is vital for effective phytoextraction since removal or harvest of below ground portion is difficult (Rahman *et al*., 2016).

Phytoextraction method can clean-up metal polluted sites and can be applied for phytomining (plant assisted mining) of precious metals including Pt (Platinum), Au (Gold), Pd (Palladium) and Ag (Silver) (Rahman *et al*., 2016). Application of phytomining process along with mining rehabilitation project could compensate for the cost of remediation; and revenue generated from energy recovery (bioenergy generation) and extraction of precious metals can boost the economic feasibility of the process (Robinson *et al*., 2015). Profitable phytomining could depend on metal stored in soil and the aboveground plant tissue, existing market value of the target metal and eco-environmental benefit obtained from bioenergy generation and possible benefits from carbon credit sale (Mahar *et al*., 2016; Chaney and Baklanov, 2017; Saxena *et al*., 2019). Phytomining can also generate revenue from combustible biomass of plants by applying it in the agricultural field or Agro-mining site (Mahar *et al*., 2016).

2.4.2. Phytodegradation

Phytodegradation is the process of transformation of pollutants using plants either through internal metabolic processes or externally via release of compounds and plant enzymes (Verma and Gupta, 2013; Muthusaravanan *et al*., 2018). It is a common remediation process for biodegradable organic

pollutants, rather than heavy metals (Fasani *et al.*, 2018). Compounds produced by plants will convert contaminants to nontoxic/less toxic ones (Verma and Gupta, 2013). Phytodegradation can occur in the absence of microorganisms and can serve as a promising remediation method if the environment lacks microorganisms due to elevated pollutant level and plant enzymes play roles in degradation process (Pandey and Bajpai, 2019). Phytodegradation efficiency can be affected by factors including pollutants concentration in soil, uptake efficiency of pollutants, soil moisture (Muthusaravanan *et al*., 2018).

2.4.3. Phytostabilization

It is the process of immobilizing/reducing contaminant mobility of using chemicals produced by plants or it is the plant-based inactivation of contaminants (Singh, 2012; Oosten and Maggio, 2015; Ramanjaneyulu *et al*., 2017). It is a relatively easier phytoremediation technique to implement (Chen *et al*., 2015). Plant species that can store metal contaminants in their roots can be considered suitable for phytostabilization (Giovanni *et al*., 2019). Contaminant mobility/solubility can be changed by the action of plants, and metal contaminants will be adsorbed and form a precipitate or accumulate in below ground parts of phytostabilizing plants (Liu *et al*., 2018; Zeng *et al*., 2019). Phytostabilization also reduces contaminant availability for transfer into food chain, reduce heavy metal leaching and can serve as promising long-term solution for contaminated sites (Wang *et al*., 2017).

Phytostabilization of metal polluted site can be enhanced through combining biological activities and soil amendment methods (Giovanni *et al*., 2019). The process needs no disposal of hazardous contaminants and it is suitable to natural remediation of polluted sites to attain site-specific remediation objectives (Gupta *et al*., 2019; Pandey and Bajpai, 2019). Most important processes involved in phytostabilization technology includes removal and storage of heavy metals/other contaminants in the below ground part; immobilization of contaminants via alteration of soil characteristics (organic matter, pH); soil cover and reduction of physical contact with human and other animals; erosion control through mechanical stabilization of soil and regulation of contaminant movement by controlling leaching (Bolan *et al*., 2011; Chen *et al*., 2015).

Sites established for phytostabilization will always remain polluted, vegetated and unsuitable for other uses, especially, for the production of edible crops. Migration of contaminants and transfer

to the food chain could happen due to high rainfall and flooding and other extreme weather events (Bolan *et al*., 2011). However, biomass production from phytostabilized sites can generate income and add other ecological benefits.

2.4.4. Phytovolatilization

Contaminants can be volatilized to the atmosphere through the opening of stomata after being taken up by the plant root (Pantola and Alam, 2014). In this process contaminants (inorganic pollutants like metal ions or volatile organics) could be volatilized or evaporated in a modified form or in a normal form (Vanek *et al*., 2010). Phytovolatilization can be utilized for remediation of either inorganic or organic contaminants for instance vinyl chloride, Hg, Se and As (Pantola and Alam, 2014; Gomes *et al*., 2016; Giovanni *et al*., 2019).

Phytovolatilization of soil contaminated by toxic heavy metals could not be sustainable and reliable because of the difficulty to control the fate of volatilized materials. Even though the phytovolatilization process seems to be easy; loss of control over the volatilized element is the main drawback of the process which would result in potential health impact on human and the secondary deposition of contaminants that would re-contaminate soil and water bodies (Chen *et al*., 2015).

2.4.5. Rhizofiltration

Rhizofiltration is also termed as phytofiltration. It is a contaminant removal method which involves either absorption or adsorption of contaminants into plant roots (Rezania *et al*., 2016; Gupta *et al*., 2019). There is similarity between rhizofiltration and phytoextraction, however it is applicable for remediation of groundwater rather than soil. The process also concentrates or precipitates contaminants like heavy metals and hence reduces the migration of contaminants (Yang *et al*., 2005, Pandey and Bajpai, 2019). Metal extraction via rhizofiltration can be done using either aquatic or terrestrial plants, but long rooted terrestrial plants are commonly used (Ashraf *et al*., 2019).

2.5. Phytoremediation in practice

Phytoremediation has been accepted as a sustainable technique for remediation of contaminated sites (Ashraf *et al*., 2019). This green technology uses both terrestrial and aquatic plants, and associated microorganisms to deal with several contaminants especially heavy metals. Phytoremediation process could be applied using different methods (Ali *et al*., 2013): natural (traditional method for using natural accumulation capacity of plants) and induced (modern way of enhancing the efficiency of the process by technological manipulation of different conditions).

Several researchers have screened a wide range of plants and examined their phytoremediation potentials (Pandey and Bajpai, 2019). However, this technology is struggling to shift from controlled experiment systems to field level applications (Saxena *et al*., 2019; Agnihotri and Shekhar, 2019). Field scale applications of phytoremediation were observed in few countries including United States, Germany, Canada, Belgium, Italy, and others (Greenberg *et al*., 2014; Pandey and Bajpai, 2019). In addition, the review conducted by Odoh *et al*. (2019) reported active utilization of phytoremediation in some areas of UK, India and USA, and substantial achievement in Germany, France, Peru, and China.

In general, practical applications on the field or commercial application did not progress well, mainly due to sustainability issues, time consuming nature, influence of climatic conditions, lower biomass of hyperaccumulator plants, limited bioavailability of metals and difficulty of complete decontamination using a single approach, especially the traditional ways (Dotaniya *et al*., 2018; Pandey and Bajpai, 2019). Therefore, the use of combined phytoremediation approaches and modern ways of plant and site parameter manipulation were observed to enhance remediation potential by improving plant biomass (rapid growth) and accelerating contaminant removal.

The other main challenge to put into action the concept of phytoremediation method for large field remediation is, scarcity of enough documentation on the phytoremediation potentials of nonedible plants. Most of phytoremediation studies worked so far focused on food crops, however edible crops should not be used for remediation (Pandey and Bajpai, 2019). Therefore, successful practical application of phytoremediation in the coming years needs careful selection of non-edible, perennial, fast growing, easily harvestable and native plant species with sufficient biomass and decontamination potential.

For commercialization and practical application, phytoremediation has to be further tested at field level and other factors contributing to underutilization of practical applications of techniques could be tackled by using potential efficiency enhancement methods. Efficiency of phytoremediation, especially heavy metal phytoextraction can be induced by using modern techniques such as chelating, pH manipulation, using electric current in soil, using microbes, organic amendment, using genetically engineered plants, improved agronomic practices, applying plant growth promoters, etc.(Gerhardt *et al*., 2017; Pandey and Bajpai, 2019). Genetically engineered and transgenic plants resulted in a great achievement and advancement in phytoremediation; however, there are critical safety concerns especially a risk of horizontal gene transfer is a major concern (Agnihotri and Shekhar, 2019). Another recently advocated novel and efficient remediation technique for clean-up of heavy metal polluted site is washing-coupled phytoremediation, which uses soil washing reagents for enhancing metal accumulation in plants (Xiao *et al*., 2019).

2.6. Factors affecting the phytoremediation of heavy metals

Performance of phytoremediation, especially for heavy metal remediation, could be affected by metal availability in soil, tolerance of plants, translocation of metals, environmental factors, plant-microbial interaction, soil properties and others (Hasan *et al*., 2019; Giovanni *et al*., 2019). This emerging technology and research on it is mostly restricted to pot and laboratory level with few field studies (Ashraf *et al*., 2019; Kumar and Thakur, 2019). However, there could be entirely different situations in the natural field conditions. In field soil, factors such as OM and extent of metal mobility, uneven distribution of contaminants, soil pH, temperature, seasonal variability, moisture content, available nutrient and microbes could affect phytoremediation efficiency (Ashraf *et al*., 2019; Rostami and Azhdarpoor, 2019).

Successful phytoremediation technique starts from selection of appropriate plants for individual metal contaminants (Yadav *et al*., 2018). Plant characteristics and species can affect heavy metal absorption from the contaminated soil. Using plants with better accumulation potential or application of hyperaccumulators can give significant clean-up of toxic metals (Yadav *et al*., 2018). However, the same plant species growing under the same concentration of contaminants, in the same site can accumulate different levels of the same heavy metals (Lone *et al*., 2008).

Soil pH is a principal factor governing plant available metal concentrations, and solubility of metals tends to rise in acidic pH and decline in alkaline pH conditions (Kumar and Thakur, 2019). Soil pH has a significant impact on heavy metal bioavailability for plant uptake and lower pH increases metal absorption and concentration in plants tissues (Bader *et al*., 2019). Lower pH of the soil can break dissolution–precipitation equilibrium between metals and allow the leaching of heavy metals into solutions (Sheoran *et al*., 2016).

Soil texture can considerably influence heavy metal availability; fine textured soils including clay soil can adsorb higher amounts of metals and have lower bioavailability. However, higher metal ion availability is a characteristic of coarse textured soils including sand (Sheoran *et al*., 2016). The higher clay content also increases the Cation Exchange Capacity (CEC) and lowers metal availability. The higher soil CEC, the higher heavy metal immobilization and the lower availability of metals (Yadav *et al*., 2018). Lower soil pH and the replacement of metal cation with H^+ will result in leaching metals. Soil moisture content, temperature, and several other minor factors can also affect heavy metal phytoremediation potentials of plants.

Plant interaction with microorganisms can enhance plant potential for uptake of heavy metal pollutants (Mandal *et al*., 2017; Yadav *et al*., 2018). Organic compounds produced by plants can be potential foods for microorganisms in soil, increase microbial count and diversity that will accelerate remediation process (Dotaniya and Meena, 2017). Rhizospheric bacteria can make metals available to the roots of plants by changing mobility of metallic ions, by producing chelators or using tolerance properties of microorganisms (Nayak *et al*., 2018). Metal tolerant bacteria can also enhance plant growth through production of plant-growth-promoting compounds, enhancing nutrient uptake, increasing metal tolerance, reduce toxicity and facilitate the phytoremediation process (Nayak *et al*., 2018; Dotaniya *et al*., 2018).

Plant associated factors including root density and depth, plant biomass, transpiration rates and others can also play significant role in phytoremediation process (Sheoran *et al*., 2016). Sufficient root depth that can reach soil solution and dense root system that can have large area of contact with contaminant will increase removal efficiency. Plants with higher above ground biomass can have better phytoextraction efficiency. Similarly, metal translocation through uptake and transpiration of soil solution are important factors. In general, to achieve effective decontamination or phytoremediation, plants should have increased growth rate, have high resistance to toxicity and

able to grow in highly contaminated sites, be native to a particular environment, disease and pest resistant, should be inedible or less attractive to herbivores and insects so as to reduce transfer through food chain and it shall remediate multiple contaminants (Rezania *et al*., 2016; Khan and Faisal, 2018).

2.7. Limitations of phytoremediation

Challenges in the phytoremediation technologies includes accumulation capacity of plants, limited bioavailability, impact of invasive plants, transfer into food chain, restriction by lower biomass of plants, soil toxicity level, suitability for low-polluted territories and root depth (Burges *et al.*, 2018; Hasan *et al*., 2019). These limitations need to be considered and possible improvement and management technologies need to be included before the implementation of large scale and commercial phytoremediation projects (Kumar and Thakur, 2019).

Time is the most important limitation of phytoremediation, and various companies and industries lost interest to implement it due to its time-taking nature (Mahar *et al*., 2016; Saxena *et al*., 2019). Time required for growth of plants and slow removal of contaminants, which may take 2-3 or several years, made phytoremediation time intensive (Agnihotri and Shekhar, 2019). The time-consuming nature of the technology might involve additional cost for treatment and safety especially for field level application of phytoextraction (Mahar *et al*., 2016). Plant growth can be restricted by adverse climatic conditions such as drought, flood, pests and etc. (Agnihotri and Shekhar, 2019). However, we could think time as such is not a problem with phytoremediation, especially considering the lasting environmental solution phytoremediation could bring to ecosystems and possibilities to enhance the remediation process by integrating with other management practices.

In addition, phytoremediation might be suitable for sites where human interference is limited and it may not be the remediation method of choice for sites that pose an acute risk to humans and receiving ecosystems (Tangahu *et al*., 2011). Especially, the main challenge of using hyperaccumulator plants for clean-up of metal contaminated sites includes slow growth, lower biomass yield and finding the hyperaccumulator plant itself (Sumiahadi and Acar, 2018). This makes the process not feasible for sites that need quick remediation. In addition, the use of artificial chelators or mobilizing agents can also be expensive and could have negative environmental

impacts including change in physicochemical properties of soil, leaching to the groundwater and toxicity to plants and soil microorganisms (Saxena *et al*., 2019).

Likewise, phytotoxicity issue is another limitation of phytoremediation. This technology is rarely applicable in highly contaminated sites because a toxic effect in the environmental matrices could hinder plant germination and growth. However, many stakeholders take no notice of these limitations being impressed by the economic and environmental aspects of the technology (Petruzzelli *et al*., 2018). The gap between the real field-scale remediation and results obtained from controlled laboratory or greenhouse studies is another drawback of the technology, particularly, field scale application of heavy metal phytoextraction has encountered many challenges, basically due to the need for understanding detailed interaction between soil-plant-contaminant interactions and underlying molecular mechanisms (Petruzzelli *et al*., 2018). In addition, metals in soil under the natural field condition could be unavailable or insoluble for plant uptake principally because of trace metal adsorption to the corresponding soil particles and precipitation (Li *et al*., 2017).

2.8. Advantages of phytoremediation

Expensive environmental clean-up methods using engineering techniques and heavy machinery that involve the installation of artificial barriers, massive excavation and coverage with clean soil and others can be replaced by cheap plant-based solutions. The phytoremediation approach is a quite innovative method for the restoration of polluted sites. Environmental remediation using this method has several benefits over other conventional physical and chemical treatment technologies. Remediation through phytoremediation has several advantages including low cost, little disturbance on the landscape, simple to use, added aesthetic value, applicability to a wide range of contaminants, have implications on human health (Kumar and Thakur, 2019); Gupta *et al*., 2019).

Apart from the remediation of polluted sites, the phytoremediation method produces green cover and has numerous environmental and socioeconomic importance (Pandey and Bajpai, 2019). Phytoremediation approach of environmental remediation has numerous indirect benefits, including aesthetic value, sequestration of carbon, conservation of biological diversity, economic benefits and additional income through utilization of contaminated land after remediation, reduces

hazardous waste disposal cost, benefits from biomass production and metal recovery (Tangahu *et al*., 2011; Pandey and Bajpai, 2019).

Phytoremediation technology can be applied for decontamination of wide-ranging environmental pollutants (Tangahu *et al*., 2011). Phytoremediation is very much economical when applied in large contaminated sites or large volumes of contaminated water, and it offers cost effective alternative solution for remediation of hazardous wastes. Lately, phytoremediation has been receiving attention as a most ecological and promising green technology for remediation of polluted soil (Tangahu *et al*., 2011; Laghlimi *et al*., 2015).

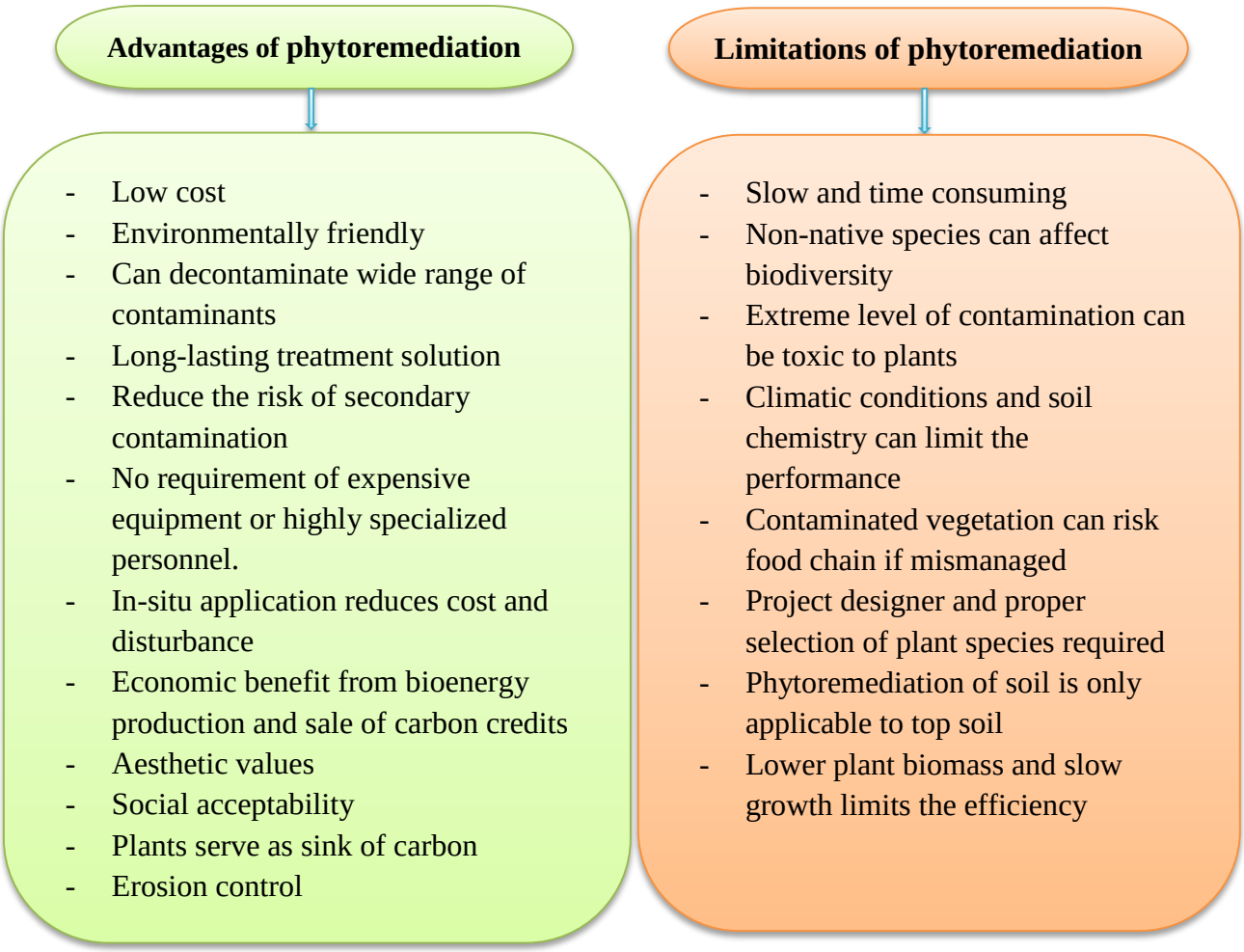


Figure 1. Advantages and disadvantages of phytoremediation solutions for contaminated sites.

2.9. Bio-concentration factors and translocation factors

Quantification of phytoextraction efficiency or potential of plants to uptake and translocate metals can be computed by calculating Bioconcentration Factor (BCF) and Translocation Factor (TF). The BCF signifies the potential of selected plants in accumulating trace metals (environmental contaminants) into its tissues (Ladislas *et al*., 2012). Values of TF displays the potential of plants in translocating pollutants into their above ground parts (Antoniadis *et al*., 2017). Screening plants for phytoremediation and selection of hyperaccumulator plants entirely relies on the values of TF and BCF (Antoniadis *et al*., 2017; Saxena *et al*., 2019). Identification of suitable plants for remediation of contaminated medium relies on the computed values of BCF and TF (Pandey, 2012; Sidhu *et al*., 2017). Heavy metal hyperaccumulator plants can also be selected by computing values of BCF and TF. Similarly, TF and BCF > 1 indicates the suitability of plant species for phytoextraction of metals (Yoon *et al*., 2006). In addition, in the selection of potential plants for phytoextraction, BCF is effective than shoot metal concentration and it is an appropriate technique for quantitative estimation and comparison of bioavailability of metal to plants (Naseem *et al*., 2009; Sakakibara *et al*., 2011).

2.10. Heavy metal pollution and phytoremediation

Metals are non-biodegradable or persistent elements accumulating in the environment for a long run (Oosten and Maggio, 2015); identified as major carcinogens (Wekpe *et al*., 2019), and these metals are extremely contributing to environmental contamination throughout the world (Razzaq, 2017). Heavy metals can cause detrimental toxicological and environmental effects even in a little amount in the environment. Excessive trace metal accumulation in soil could transfer through the food chain; bio-accumulate and reduce microbial density in soil (Khan *et al*., 2010; Alloway, 2013).

Both non-essential and essential metals are naturally available in soil; however, an increase in their concentration due to anthropogenic activities made them the most represented soil contaminants and major abiotic stresses that cause environmental pollution (Kabata-Pendias, 2001; Shehata *et al*., 2019). The essential ones include Ni, Fe, Zn, Cu, Mn, and non-essential metals include Pb, Cr,

Cd, As and Hg (Dabonne *et al*., 2010; Lu *et al*., 2015). Several industries, manufacturing companies, and agricultural processes commonly use heavy metals including Pb, Cr, Cd, Ni, Cu, Zn, Hg and As and dispose their wastes into the receiving environment (Tchounwou *et al*, 2014; Cristaldi *et al*., 2017). Among these metals Hg, Pb, Cd, Cr, Zn, Cu, As and Co are reported as most toxic ones and commonly known as potentially toxic heavy metals (Ghosh, 2010; Alloway, 2013; Eugenio *et al*., 2018).

Phytoremediation technique can be extensively applied to several environmental restorations, however selection of suitable plant/s is very essential for phytoremediation process (Cristaldi *et al*., 2017; Wang *et al*., 2017). The most important criteria, in addition to their metal removal properties, could include tolerance to extreme soil conditions including very high or very low pH; excessive metal concentration (Wu *et al*., 2013). Further, adaptability to the desired local environment; dense rooting systems and high biomass production (Marques *et al*., 2009; Oosten and Maggio, 2015) and fast-growing properties (Doty *et al*., 2007) needs to be considered. Plants that have large biomass and translocate moderate amount of trace metals in the aerial parts are suggested as suitable for field scale phytoremediation (Fiorentino *et al*., 2013).

"Viola calaminaria and *Thlaspi caerulescens* were the first plant species known to accumulate metals" as reported by Hartman, 1975 (Pantola and Alam, 2014). Previous investigators identified several plants that can accumulate heavy metals and today over 101 families have been found to contain hyperaccumulator plant species (Sarma, 2011; Ahmadpour *et al*., 2012), *Brassicaceae* plant family contain several hyperaccumulators of heavy metals species like Pb, Cd, Cu, Zn, Ni, and Se (Bouquet *et al*., 2017; Yahaghi *et al*., 2018).

Chromium is a potentially toxic metal largely used in chemical industry, metal manufacturing, textile, pesticide manufacturing, dyeing, tanneries, industrial coolants, mining and others, has an ecological risk and carcinogenic effect on living organisms (Zhitkovich, 2011; Vimercati *et al*., 2017; Xia *et al*., **Error! Reference source not found.**). Long exposure to Cr can cause cancer on human beings, it can also bring about other health impacts such as hair fall, skin irritation, eye irritation and impact on nervous system (Dotaniya *et al*., 2018). This toxic metal can be accumulated by plant species such as *Leersia hexandra* (Zhang *et al*., 2007), *Typha angustinfolia*

(Dong *et al*., 2007), *Cynodon dactylon* (Sampanpanish *et al*., 2006, *Helianthus annuus* (Farid *et al*., 2017), *Eichhornia crassipes* (Sarkar *et al*., 2017), and others.

Lead is a toxic heavy metal with no identified importance or essential cell activities in plants (Azeez *et al*., 2019). Anthropogenic sources such as industrial and atmospheric deposition, lead acid batteries, heavy-traffic load, coal-based power plants, paints industrial effluent; mineral extraction and others could increase concentration of Pb in soil (Dotaniya *et al*., 2018). Exposure to lead can cause mental retardation, nervous system disorder, cardiovascular disease, gastrointestinal cancer, kidney disease and other health impacts on human (Dotaniya *et al*., 2018). Lead polluted soil can also be remediated by using different plant species such as, *Brassica junicca,* which is a good accumulator of Pb (Clemente *et al*., 2005; Yahaghi *et al*., 2018). Other researchers also investigated other potential accumulators of Pb. For instance, Zea maize L. and *Pisum sativum* L. that absorbed large concentrations of Pb in their aerial parts (Tariq and Ashraf, 2016). Several plants such as *Piptatherum miliaceum, Thlaspi praecox, Hemidesmus indicus, Thlaspi rotundifolium* were identified as good remediators of Pb contaminated soil (Sekhar *et al*., 2005; Oh *et al*., 2013). Similarly, *Linum usitatissimum L.* was regarded as hyperaccumulator of Pb (Hosman *et al*., 2017).

Industrial activities like mining, smelting, electroplating, plumbing, brass manufacture, agriculture fertilizers, sewage disposal and other anthropogenic activities are most important sources of zinc pollution (Furini, 2012; Dotaniya *et al*., 2018). Even though it is an essential element at high concentrations it is toxic (Agnello *et al*., 2014). Potential effect on human health includes damage to the nerve system, skin irritation, vomiting and weakness (Dotaniya *et al*., 2018). Plant species such as: (*Arabidopsis halleri*, *Thlaspi praecox*, *Thlaspi goesingense* and *Thlaspi caerulescens*) can remediate Zn contaminated soils (Pantola and Alam, 2014).

Other researchers also reported *Helianthus annus* and Z*ea mays* (Spirochova *et al*., 2003) and *Juncus effuses* (Favas *et al*., 2016) as good accumulators of Zn among others. Zn was found to be accumulated by *Salix viminalis* (Hammer *et al*., 2003) and *Sonchus asper* and *Corydalis pterygopetata* (Yanqun *et al*., 2005). Dhiman *et al*. (2016) noted Zn phytoextraction using *Brassica napus* is possible. *Linum usitatissimum L* was reported as hyperaccumulator of Zn (Hosman *et al*., 2017). *Sedum alfredii* was reported as hyperaccumulator or Zn (Cui *et al*., 2018). Plants including *Arabidopsis halleri, Thlaspi goesingense, Thymus praecox* and *Sedum alfredii* were also reported

as Zn hyperaccumulators (Tian *et al*., 2017; Liu *et al*., 2018). Recently, Guarino *et al*. (2019) reported that *Populus alba* and *Eucalyptus camaldulensis* are good candidates for Zn phytoextraction.

Main sources of Cd in the environment are mining, paint industries, battery industries electronic waste, welding, electroplating, agricultural pesticides, fertilizers, and others (Dotaniya *et al*., 2018). Cd is an abundant, toxic and most serious metal pollutant in agricultural sites, causing oxidative stress in a plant, subsequently affecting plant germination, growth, fruiting and nutrients translocation (Shang *et al*., 2018; Azeez *et al*., 2019). Cadmium transfer via food chain and health impacts are also of significant concern (Huang *et al*., 2017; Rizwan *et al*., 2018). Effects on human health includes softening of the bones, enzymatic disorder, kidney damage, carcinogenic effect, lung cancer, renal dysfunction, and Ca imbalance (Dotaniya *et al*., 2018).

Experiments showed different plants can accumulate Cd. (Spirochova *et al*., 2003) reported *Helianthus annuus* and Z*ea mays* can store a considerable amount of Cd in their biomass. Alaboudi *et al*. (2018) also recommended *Helianthus annuus* for remediation of Cd contaminated soil. *Thlaspi caerulescens* was also identified to remediate soil polluted with Cd (Wu *et al*., 2004). A field investigation and laboratory dose-gradient experiments by Liu *et al*. (2019) reported *Lantana camara* L plants Cd-hyperaccumulating plants suitable for remediation of Cd polluted sites. Rasheed *et al*. (2019) reported *Conocarpus lancifolius* as potential candidate for Cd phytoextraction. *Sedum alfredii* was reported as a hyperaccumulator of Cd (Tian *et al*., 2017; Cui *et al*., 2018). Field investigation combined with dose dependent laboratory experiment conducted by Liu *et al*. (2019) also reported *Lantana camara* L as a hyperaccumulator of Cd. Phytoextraction of Cd using *Ricinus communis* was also reported by (Yang *et al*., 2017).

Nickel, in its toxic level, can have potential health impacts for instance: a disorder of the nervous system, lungs and impact on cardiovascular tissues (Axtell *et al*., 2003). Successful phytoremediation of Ni contaminated soil could be attained using plants such as *Bidens pilosa*, *Conyza Canadensis*, *Crotalaria micans*, *Leucaena leucocephala*, and *Pueraria lobata* (Ho *et al*., 2013). Similarly, Ni Hyperaccumulator plant species (*Stackhousiatryonii*, *Helianthus anus*, *Thlaspi goesingens*) were reported by Bhatia *et al*. (2005), Turgut *et al*. (2004) and Kramer *et al*., (2000). A plant known as *Sebertia acuminate* was reported as a Ni hyperaccumulator tree (Jaffré *et al*., 2013). Successful phytoextraction of Ni form contaminated soil using *Brassica juncea* was

reported (Kathal *et al*., 2016). *Alyssum bertolonii* was reported as an efficient accumulator of Ni (Bini *et al*., 2017). *Noccaea caerulescens* was reported as multi-metal hyperaccumulator including Ni (Milner and Kochian, 2008; Deng *et al*., 2019).

Copper is an essential plant micronutrient with an extensive source in the environment and excessive concentration can cause great harm to plants (Lin *et al*., 2019). Major anthropogenic sources of Cu include Cu mining, smelting chemical industry, pesticide, sulphuric acid plant, metal piping and others (Dotaniya *et al*., 2018). Long exposure to Cu can significantly affect human brain, kidney and liver, and it also results in chronic anemia, stomach irritation, and lethargy among others (Dotaniya *et al*., 2018).

High concentration of Cu in the leaves of *Avicennia marina* plant was reported by (Lotfinasabasl *et al*., 2012). *Brachiaria decumbens* was reported as efficient phytoextractor of Cu (Andreazza *et al*., 2013). Similarly, plant species *Brassica juncea L* was also described as best accumulator of Cu (Pantola *et al*., 2013; Purakayastha *et al*., 2008). Accumulation and tolerance of Cu was observed in *Eichhornia crassipes* (Sarkar *et al*., 2017).

Studying the properties of plants under their natural environment will be important as the above-listed factors can affect the potentials of plants to accumulate heavy metals. Even though most phytoremediation studies available in the literature were conducted under controlled environment or green house conditions, few field-based experiments assessing plant phytoremediation potentials are also available.

The first field trial on plant metal extraction potential was conducted at Woburn, UK in 1991 and natural hyperaccumulator plants grown in sewage treated field spots were investigated (McGrath *et al*., 1993). The authors reported *Thlaspi caerulescens* was found accumulating 2000-8000 mg Zn per kg of shoot dry weight when growing in soil containing 150-450 mg of Zn per kg of soil. Jose *et al*. (2011) conducted a survey on phytoremediation potentials of plants grown in mining sites. Plant and soil samples in this study were obtained from mining area, and results from the study revealed that hyperaccumulation was found in *Thlaspi caerulescens* for Pb, Cd, and Zn and in *Armeria vulneraria* for Zn and Pb. Another research conducted by Usman *et al*. (2019) on naturally growing shrub plant *Tetraena qataranse* reported the plant as a suitable candidate for phytostabilization of toxic metals such as Cr, Cu, Ni and Pb phytoextraction of Cd.

Lotfinasabasl *et al*. (2012), studied heavy metal phytoremediation potentials of *Avicennia marin*, grown in mangrove forest, revealed that both seedling and mature plants of *Avicenniamarin* can take up elements (Cr, Cu, Co, Cd, Ni and Fe) and accumulate in their tissues. This study finally proved the suitability of *Avicennia marina* for clean-up of metal polluted soil. Similar research was also conducted on plant *Rhizophora mucronata* by Pahalawattaarachchi *et al*. (2009) in Alibaug mangrove forest, and the plant accumulated considerable amounts of Cr, Cu, Cd, Ni and Fe in their tissues.

Yahya *et al*. (2014) examined accumulation of metals in five native plant species (*Dipterygium glaucum, Indigofera spiniflora*, *Salsola kali*, *Suaed aaegyptiaca,* and *Zygophyllum album*) grown in an industrial area. Samples of soil and plants were obtained from contaminated sites and analysed for their heavy metal content. BCF, BAC and TF were calculated to understand phytoremediation potentials of the plants. Accordingly, the highest of both TF and BAC was shown for Zn in *Dipterygium glaucum,* and they concluded this species as an excellent candidate for decontamination of Zn-polluted soils. The highest value of BCF recorded for *Indigofera spiniflora* was also noted as a good candidate to immobilize Zn. The study concluded that the potential of the studied plants to remediate the Zn contaminated soils, in the order of *Zygophyllum album* being the most suitable plant followed by *Dipterygium glaucum*, *Salsola kali*, *Suaedaa egyptiaca* and *Indigofera spiniflora*.

The most important hyperaccumulator *Thlaspi caerulescens* can accumulate a large concentration of metals especially Zn and Pb without showing signs of toxicity (Dinh *et al*., 2018). Brassicaceae plant families represent a large number of metal accumulating plants principally, *Alyssum, Arabidopsis*, *Bornmuellera*, *Thlaspi* plant genera of Brassicaceae can remove multiple metals (Dar *et al*., 2015; Gupta *et al*., 2019). Naturally growing *Portulaca oleracea* L. plant samples were collected from industrial areas by Elshamy *et al*. (2019) to study their suitability for phytoremediation, and results showed efficient decontamination of multiple metal contaminated soil using *Portulaca oleracea.* Similarly, large amounts of more than single metal could be accumulated by plants such as *Malva parviflora*, *Amaranthus viridus*, *Echinochloa colonum*, and *Chenopodium murale* (Elshamy *et al*., 2019).

Another field survey conducted by Favas *et al*. (2016) revealed that *Holcus lanatus* can accumulate As, Cu, Zn and Pb; *Pteridium aquilinum* can remove As, Pb and Zn, and *Rumex induratus* and

Cistus salvifolius were reported as good accumulators of Cu from polluted sites. Field study conducted by Kumari *et al*. (2016) for screening plant species for remediation of metals also reported metal accumulators and hyper accumulator plants. The hyperaccumulator terrestrial plant species reported are *Cannabis sativa* for Ni, Cd and Cr; *Parthenium hysterophorus* hyperaccumulation of Pb; and *Amepelopteris prolifera* for Ni.

It was evident that most of the studies were done in a controlled environment, green-house and laboratory, while field trials on phytoremediation potentials of plants to remediate contaminated sites are very limited and there have been gaps in this regard. Therefore, field scale studies and documentation of findings will be key preconditions that need to be carried out for successful implementation of phytoremediation projects. Consequently, this study examined heavy metal remediation potential of three selected plants: *Adhatoda schimperiana*, *Phytolacca dodecandra* and *Solanum incanum* from contaminated metropolitan areas of Ethiopia.

2.11. Phytoremediation studies in Ethiopia

Industrial wastes have been increasingly discharged into water and soil, and causing environmental pollution in Ethiopia. Release of toxic heavy metals from industries, manufacturing companies, domestic sources and agricultural sources is becoming serious problem to the environment and biological systems.

There are several previous studies on determination of heavy metal concentrations, sources of contamination, effects of heavy metals on environment and potential health impacts in Ethiopia. Similarly, studies on heavy metals uptake and bioaccumulation in edible crops and vegetables are abundant. However, researches on phytoremediation potentials of non-edible plants targeting cleanup of heavy metal contamination in soil are very few in literatures, in Ethiopia.

An overview of few selected literatures relevant to the theme of present study are presented as follows: An investigation on phytoremediation potential of commonly grown tree species (*Millittia ferruginea*, *Ricinus communis* and *Eucalyptus camaldulensis*) was conducted by Mehari *et al*. (2010). The total Cr uptake and the calculated Cr Accumulation factor reported for *Ricinus communis* was highest and followed by *Millittia ferruginea* and *Eucalyptus camaldulensis*. The study finally recommended the three plant species for remediation of Cr contaminated sites.

A study by Itanna and Coulman, (2003) on Phyto-extraction of Cu, Fe, Mn, and Zn from contaminated site using threes grass species, reported concentrations in grass plant tissue indicate generally that grass species, rhodesgrass (*Chloris gayana*) and setaria *Setaria Sphacelata* could extract all the selected metals more efficiently than oat (*Avena sativa*).

Phytoremediation of chromium from tannery wastewater using swamp smartweed (*Polygonum coccineum*), para grass (*Brachiara mutica*) and papyrus (*Cyprus papyrus*) was reported by (Kassaye *et al*., 2017). Phytoremediation potentials of *Lemna minor* and *Azolla filiculoides* was investigated under field condition by (Amare *et al*., 2018) in semi-arid regions of Ethiopia. Then *Lemna minor* was reported as phytoaccumulator for Fe, Mn, Zn and Co. Similarly, higher accumulation of Fe, Mn, Zn and Cu was noted for *Azolla filiculoides.*

Four local plant species (*Pennisetum purpureum*, *Typha domingensis*, *Cyprus latifolius*, and *Echinochloa pyramidalis*) were studied for their potentials to remove Cr from contaminated tannery wastewater under constructed wetland system (Alemu *et al*., 2020). The BCF values of > 1 were noted for all these plants, but TF < 1 reveals larger accumulation in roots (non-harvestable portion) and inadequacy for phytoextraction of Cr (III) was reported. However, even though TF of < 1 was noted for *Pennisetum purpureum*, it has been recommended for Cr treatment based on its potential to store Cr in the shoot part, rapid growth and produce biomass (Alemu *et al*., 2020).

2.12. Metal tolerance in plants

Heavy metal toxicity can disturb the redox status, cause oxidative stress and can affect plant physiological, biochemical processes, growth and yield. Heavy metals effects on plants might be due to absorption of plant nutrients, interaction with plant functional protein groups, the formation of reactive oxygen species (Dotaniya *et al*., 2018). Tolerance to toxic effects of metals is a principal reason for implementation of phytoremediation technique for restoration of contaminated soils (Thakur and Singh, 2016). Plants have self-mechanism to survive in contaminated sites and a variety of biomolecules that govern metal uptake and accumulation process (Mehes-Smith *et al*., 2013).

During reduction of metal toxicity, physical barriers in which trichomes, plant cell wall, and microbial association are used as principal defense mechanisms (Harada *et al*., 2010). Plants can avoid metal toxicity by accumulating in their vacuoles and synthesis of biomolecules that can

detoxify toxic metals (Fahr *et al*., 2013; Dotaniya *et al*., 2018). Plant proteins can play key roles for metal tolerance. Metal uptake and transport via plant xylem can be accomplished by proteins such as heavy metal transporting ATPases, copper transporters (COPTs), cation diffusion facilitator (CDF), Zn–Fepermease (ZIP), and Multidrug And Toxin Efflux (MATE) (Dotaniya *et al*., 2018). The protein P1B ATPases type protein in hyperaccumulator plants regulates homeostasis and metal tolerance (Ali *et al*., 2013). Uptake and transport of heavy metals through the cell membrane and decontamination can be done by heavy-metal ATPase (HMA) (Saxena *et al*., 2019). Likewise, Chen *et al*. (2015) reported metal binding proteins, metallothiones (MTs), and phytochelatins (PCs) can detoxify toxic metals.

Metal accumulation or exclusion strategies can be used by plants to grow in contaminated medium and tolerate the toxic effects (Li *et al*., 2017). Similarly, Thakur and Singh (2016), noted plants have several means of metal tolerance and detoxification. Plants can limit excessive accumulation of heavy metals in the cytoplasm using two defense strategies: avoidance and tolerance. The avoidance mechanism is the plant's capability to hinder metal uptake, while tolerance implies continued existence under excess metal condition (Thakur and Singh, 2016).

CHAPTER THREE

3. Research design and methodology

Quantitative research design was applied in this study and the method employed was exploratory research method. Experimental values obtained from laboratory measurement, using reliable devices, are interpreted using mathematical and statistical procedures. The sampling sites were purposively selected and samples were collected purposively. Plant samples from non-polluted sites (control) and polluted sites (Industrial area) were examined along with their corresponding soil. Sampling plan and location was based on availability of target plant and site characteristics.

3.1. Site description

Samples of plants and soil for this research were obtained from metal contaminated sites located in Akaki River Basin industrial area and contaminated sites of Addis Ababa, Ethiopia. Akaki River Basin is an industrial belt area, in central Ethiopia, majority of effluents originating from different industrial units: steel melting furnaces, re-rolling mills, food and beverages, paints, wineries, rubber and plastic products, soap, textile and other metalliferous industries around Addis Ababa are channeled to natural drains within the industrial estates ultimately to Akaki River Basin (Aschale *et al*., 2019).

Therefore, the area is highly polluted with multiple contaminants as it is also evident from previous researches (Alemayehu, 2006; Woldetsadik *et al*, 2017; Aschale *et al*., 2019). These sites also receive contaminants from domestic, agricultural sources, schools, hospitals, small manufacturing business and others.

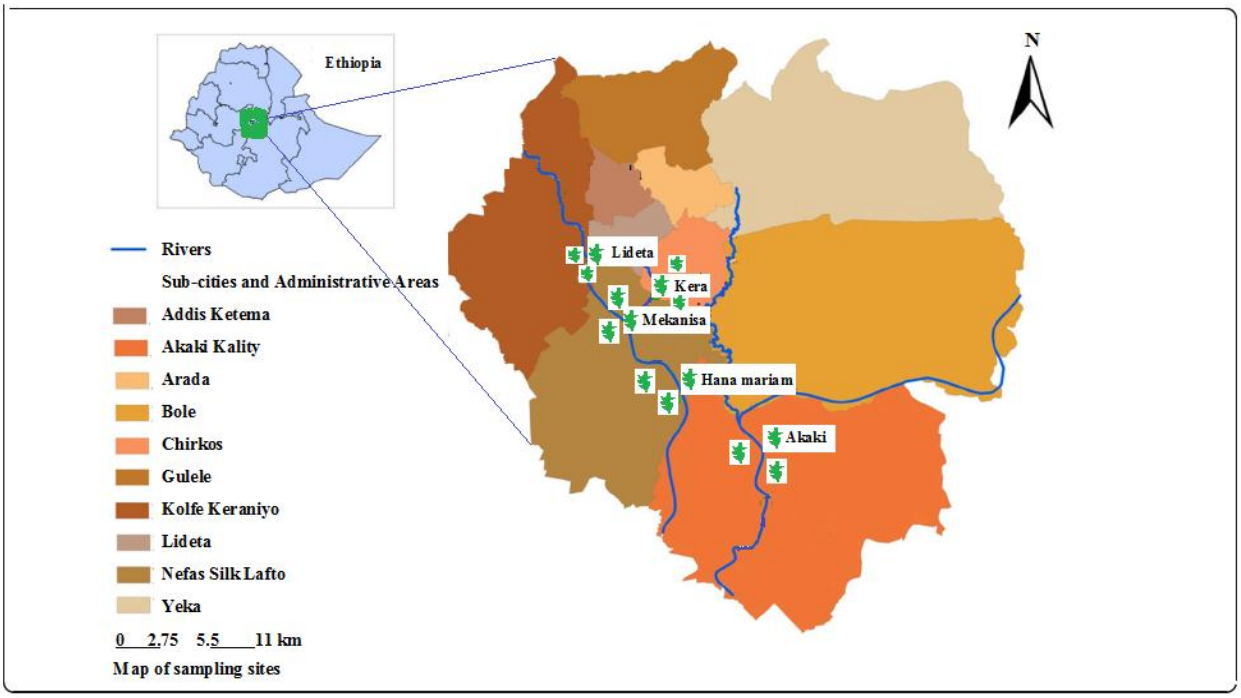


Figure 2. Map of the study area and sampling sites.

3.2. Plants used in this study

Three different plants *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum* were chosen in this study as they are locally available plants dominating contaminated industrial areas, dump sites and road sides of many cities in Ethiopia. These plants were chosen after studying basic properties of plants for phytoremediation such as tolerance to high concentrations of metals, adaptability to specific environment/sites, non-edible, large biomass, fast growing and extended root system, as in (Sheoran *et al*., 2016; Pandey and Bajpai, 2019).

3.2.1. *Phytolacca dodecandra*

Phytolacca dodecandra is a woody plant commonly found in South Africa; Sub-Saharan Africa, Madagascar; South America, and Asia (Hanelt *et al*., 2001; Esser *et al*., 2003). It is a plant from family of Phytolaccaceae, commonly known as 'endod' or 'gopo berry' (Hanelt *et al*., 2001; Zelalem *et al*., 2016). *Phytolacca dodecandra* is relatively common in Ethiopia, it is well known for its local benefit as soap. An Ethiopian scientist Aklilu Lema discovered that it as lethal to snails and effective for control of schistosomiasis (Esser *et al*., 2003).

Figure 3. ***Phytolacca dodecandra*** **plant pictures**

3.2.2. *Adhatoda schimperiana*

Adhatoda schimperiana belongs to the plant family of Acanthaceae, commonly known as 'sensel', 'simiza' or 'dhumuga', it is fast growing plant (Zelalem *et al*., 2016). This plant is very common

in towns as well as in cities growing on waste places or serve as fence. It has several medical importance, especially used in the treatment of pelegra or "kuruba" local name (Getahun, 1976).

Figure 4. ***Adhatoda schimperiana*** **plant pictures.**

3.2.3. *Solanum incanum*

Solanum incanum is a shrubby herb from plant family Solanaceae, it can grow up to 4 ft high and it is locally available and abundant throughout Ethiopia. It is called ‘apple of Sodam’ or ‘thorn apple’ (Eng.) and have local names ‘Imbuay’ or ‘hidi’ (Sambo *et al*., 2016). The fruits of *solanum*

incanum are poisonous; however, it has several medicinal properties, especially for treatment of gonorrhea and fruits mixed with cattle urine can be used for tanning leather.

Figure 5. *Solanum incanum* plant pictures.

Several East African communities use fruits of *Solanum incanum* as a remedy for toothache, stomachache, fever, snakebite and earache (Kokwaro, 1993). Treatment of tumors, warts and sore-throat using *Solanum incanum* plant extracts was also reported (Dold and Corps, 2000; Schemelzer and Gurib-Fakim, 2008).

3.3. Sampling procedures and design

Preceding the main survey, a pilot investigation was carried out to determine possible sampling points. Five sampling sites (SS1- SS5) were established from Akaki River Basin (industrial belt in central Ethiopia), distances between sampling points varied based on the availability of plants and industries. These sampling sites are Lideta (SS-1), Mekanisa (SS-2), Kera (SS-3), Hana Mariam (SS-4) and Akaki sites (SS-5) representing contaminated sites. These sites are situated along the river basin SS-1 the upper catchment/upstream (located in urban center) and SS-5, Lower catchment/downstream (the periphery). Sampling points with in the sample site were selected based on the availability of desired plants and proximity to contaminant sources like factories. Preliminary investigation and survey of sample points was done before the actual sampling. Accordingly, sites along the river bank, exposed to the polluted river water through urban irrigation or receiving direct effluent from factories, were selected as contaminated site. In addition, sites under intense anthropogenic pressure, receiving leachate from open dump sites, wastewater from car wash and garages were targeted.

On the other hand, control sampling site (CSS/ SS_6) was identified in non-industrial area, with limited human interference (rural area of the town of Debrezeit) to serve as control and the same trend was applied for collection of control soil samples; adapting methods followed by (Jose *et al*., 2011; Lotfinasabasl *et al*., 2012).

The GPS coordinates of the different sampling points with in the different sites are as follows: Lideta (9°00'42.9"N 38°44'27.5"E, 9°00'28.5"N 38°44'23.2"E and 9°00'31.4"N 38°44'11.9"E); Mekanisa (8°58'31.9"N 38°44'02.5"E, 8°58'29.3"N 38°44'01.8"E and 8°58'27.3"N 38°43'58.2"E); Kera (8°58'59.4"N 38°45'09.6"E, 8°58'57.7"N 38°45'12.7"E and 8°59'19.5"N 38°44'58.9"E); Hana (8°55'49.3"N38°45'24.3"E, 8°55'53.0"N 38°45'26.2"E and 8°55'32.8"N 38°45'06.1"E) and Kality (8°54'43.1"N 38°44'56.4"E, 8°54'41.4"N 38°44'49.3"E and 8°54'20.1"N 38°44'47.1"E).

Within each sampling site SS1-SS6, there were three sampling plots (Plant 1, Plant 2 and Plant 3) and within each sampling plot, for instance; *Phytolacca dodecandra* sample plot (Plant 1), there were eight sampling points, 2 + 2 for mature (having flower) and 2 + 2 for seedlings (younger plants with no flower), representing dry and wet season samples. Eight (4 mature and 4 seedlings) individual study plants from each species were carefully dug and removed for analysis, following

the work of Nazir *et al.* (2011) and Lotfinasabasl *et al.* (2012) as a reference. In this case, In addition to the presence and absence of flowers, seedlings were selected and differentiated from the mature plants, based on plant age estimation done using growth ring examination, stem girth comparison and other morphological characterization. Dwarf plants with thick stem that seems seedling were identified using morphological parameters (external structure of plants) and excluded from sampling. A total of 144 plant samples were taken and separated into leaves, stems and roots; and, covered with carefully labeled plastic bags, stored in ice boxes and taken to Laboratory of the Geological Survey of Ethiopia and Addis Ababa City Environmental Protection Authority for analysis of contaminants of interest: Zn, Ni, Cu, Cr, Cd and Pb.

The soil in which plants grew, representing surface and rhizosphere soil 0 to 40 cm depth range (0 - 15 cm, 15 - 30 cm and 30 – 40 cm, combined as one sample) was taken immediately after plant sampling. Five surface samples of soils adjacent to sample plants (that is directly influenced by root secretions), one from the midpoint and others from four vertexes, were taken and a composite sample was made by mixing (Ashraf, 2011; Yahya and Hajar, 2014).

A total of 36 composite soil samples (1.5 kg each) were taken during the dry season, T^0 ranging from 21-25^{0}C (November-December, 2017) and 36 soil samples were taken during wet season, T^0 ranging from 18-21^{0}C (June-July, 2018). During the sampling process, the topsoil, which is composed of litter, wet spots, was cleared out to have clear and fully decomposed soil sample. Composite soil samples (1.5kg) from each point were thoroughly mixed, carefully labeled and bagged using polyethylene bags. Analysis of soil and plant samples run from November 2017-February, 2018 for dry season samples and June 2018- August, 2018 for wet season samples.

Figure 6, shows photographs taken during sampling campaign and the points 6.a, 6.b, 6.c and 6.d represents different sampling sites. Accordingly, 6.a (Sampling of *Phytolacca dodecandra,* Mekanisa site); 6.b (Sampling of *Adhatoda schimperiana,* Kera site); 6.c (Sampling of *Adhatoda schimperiana,* Hana mariam site) and 6.d (Sampling of *solanum incanum*, Kality site). The GPS coordinates of the sampling points 6.a, 6.b, 6.c and 6.d were 8°58'31.9"N 38°44'02.5"E; 8°58'57.7"N 38°45'12.7"E; 8°55'53.0"N38°45'26.2"E; 8°54'20.1"N 38°44'47.1"E, respectively.

6. a

6. c

6. b

6. d

Figure 6. Photographs of sample collection.

3.4. Analytical procedures

3.4.1. Characterization of soil and plant samples

Samples of plants and soil were examined for their metal (Pb, Cr, Cd, Zn, Cu and Ni) levels and physicochemical properties of soil samples: texture, pH, conductivity, organic matter, Cation Exchange Capacity (CEC), and moisture content were also analysed.

Table 1. Analytical method for determination of soil physicochemical parameters.

Parameters	Methods	References
pH	1:2.5 Jackson method	Jackson, 1973
Moisture content (%)	Gravimetric method	Jackson, 1973; Joel and Amajuoyi, (2009)
Conductivity (dSm-1)	1:2 soil/water slurry	Jackson, 1973
Organic matter (%)	Wet combustion with $K_2Cr_2O_7$	Nelson and Sommers, 1996
CEC (mequ/100g)	Sodium saturation Method	Rowell, 1994
Texture	Hydrometer method	Bouyoucos, 1962
Heavy metal concentration (mg/kg)	Atomic Absorption Spectrophotometer	EPA, 1996

Soil pH measurement was performed using the water suspension method, soil to water ratio (1:2.5) described by Jackson, (1973). First soil samples were mixed well, powdered, put through a sieve (2 mm). Then 20 g sample of soil was added into a beaker and mixed with 50 ml deionized water. The mixture was systematically blended by stirring with disposable plastic stirrer for about 10 min. Then pH meter was adjusted using standard methods, and finally the reading of pH value was taken by immersing the electrode of the pH meter (ELMETRON, CPI-501, Poland) into the solution.

The EC of sample soil solution was determined using conductivity meter and analytical procedure stated by Jackson, (1973) was applied. 10 g sample of soil was placed in 250 ml beaker and mixed with 10 ml of distilled water. Then the mixture was then stirred with disposable plastic stirrer for 30 minutes and allowed to settle. Finally, conductivity was measured using an electrical conductivity meter (SCHOTT handy-lab LF11, Germany) after standard calibration and temperature adjustment (25 ^{o}C).

Soil MC% was estimated by using oven drying gravimetric method (Jackson, 1973). For this, first dry clean crucible was prepared and 10 g sample of soil was added to it, then the weight of crucible containing moist soil was determined (W_1) and it was oven dried at (105 °C) for 24 hours and the weight was recorded for the second time (W_2).

Using the formula indicated by (Joel and Amajuoyi, 2009; Tellen and Yerima, 2018), moisture content was calculated using equation 1 and presented in percentage as follows:

$$\text{Moisture content (\%)} = \frac{(\text{Wet weight of soil} - \text{Dry weight of soil})}{\text{Dry weight of soil}} \text{X } 100 \qquad \text{Equation 1}$$

Soil OM% content was calculated from the value of organic carbon (OC%) in soil by using a mathematical equation presented in (Nelson and Sommers, 1996) Equation 2. Majority of soil contain 2-6% organic matter and 58% of the organic fraction is OC, which is 1.2-3.5% of the soil composition (Manns *et al*., 2016). OC% was determine using wet digestion technique Walkley and Black (1934), and 5 g of air-dry soil was crushed and put through a sieve (0.5 mm). Then fine powder of 1g soil was transferred to 500 ml wide-mouth Erlenmeyer flask (Here: 2 other blank flasks were prepared without soil and run the same way) and then 1N $K_2Cr_2O_7$ (10 ml) and H_2SO_4 (20 ml conc.) were added into the flask. The flask was properly swirled until the soil and the reagents are mixed. After that distilled water (200 ml), H_3PO_4 (10 ml), and then diphenylamine indicator (1 ml) was added. In successive steps solutions in the blank flasks and sample flask were titrated with Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ (0.5 N) and the titration process was stopped when the color changed sharply to green. Finally, the OC percentage was calculated using the formula (Van Reewijk, 2002; Addis and Abebaw, 2015; Tellen and Yerima, 2018).

$$\text{Organic C\%} = \text{N} \times \frac{(\text{V1} - \text{V2})}{\text{S}} \times 0.39 \times \text{MCF} \qquad \text{Equation 2}$$

Where,

N = Normality of ferrous ammonium sulfate

V1 = Volume of ferrous ammonium sulfate used in titration of blank

V2 = Volume of ferrous ammonium sulfate used in the titration of soil sample

S = Weight of soil sample (g)

$0.39 = 3 \times 10^3 \times 100\% \times 1.3$ (3 = equivalent weight of carbon)

MCF = Moisture correction factor

Lastly, the estimation of organic matter was done using the following formula

Organic matter (%) = Organic carbon (%) x 1.724

Soil CEC value was determined using the standard procedure of Rowell. (1994), modified by Herk (2012) Equation 3. First, previously crushed sample of soil (5 g) was placed in a centrifuge tube of 50 ml and 1 M sodium acetate solution (30 ml) was added. After 5 min shaking and 10 min centrifuge the liquid was decanted and 1 M sodium acetate solution (30 ml) was added, and the sample was re-suspended 3 times. Then solution ethanol (30 ml) was poured and re-suspended for 3 cycles. After that, NH_4OAc (30 ml) was added and then re-suspended and another 3 cycles was started. Then, the liquid was filtered using the filter paper (Whatman No 42), and poured into a flask (100 ml). Finally, the solution in the flask was made 100 ml with NH_4OAc pH 7 solution. In the end, the flame photometer was adjusted by a standard Na solution. CEC of the soil was then computed using the mathematical formula (Herk, 2012).

$$\text{CEC, cmol(+) kg} - 1 \text{ soil} = \frac{10 * \text{Na concentration in meq L} - 1}{\text{Mass of sample (g)}} \qquad \text{Equation 3}$$

Particle size of soil was obtained using hydrometer (Bouyoucos, 1962). Accordingly, 50 g of sample soil (< 2 mm) was added into a beaker (400 ml) and socked with 50ml calgon solution overnight. The solution was then poured to a 1000ml cylinder. Then it was covered with rubber and the suspension was mixed (10 times) by inverting the cylinder. After that, 2 drops of amyl alcohol were poured into soil suspension and finally hydrometer was inserted. The first hydrometer reading (H_1) and the temperature (T_1) was recorded after 40 seconds. The suspension was covered with the tight rubber and mixed by inverting the cylinder (10 times) for the second time, and left

for 3 hours. The second reading of the hydrometer (H_2) and the temperature (T_2) were recorded after 3 hours. Lastly, percentage of the textural class was obtained using procedures used by (Olayinka *et al*., 2017).

$$\text{Sand (\%)} = 100 - [H_1 + 0.2\,(T_1 - 68) - 2.0]^2$$

$$\text{Clay (\%)} = [H_1 + 0.2\,(T_1 - 68) - 2.0]^2$$

$$\text{Silt (\%)} = 100 - (\%\ \text{sand} + \%\ \text{clay})$$

3.4.2. Soil heavy metal analysis

For analytical experiment soil samples were crushed to a size of 2mm following methods used by Spirochova *et al*. (2003). EPA method 3050 B (1996) was applied to determine total metal concentration. About 1g sample of soil was added to a solution of 1:1 HNO_3 10 ml and the solution was then heated at 95^0C for 15 minutes on hotplate. Then the samples were digested with repeated addition of concentrated HNO_3 (5 ml). After that, samples were digested with 30% H_2O_2 and H_2O_2 (1 ml) was repeatedly added. Lastly, HCl (10 ml) was applied for 15 minutes to digest samples. A 100 ml flask was then used to collect digested samples, and dilution was made using 100 ml distilled water and analysis was done using Atomic Absorption Spectrometry (AAS), model, Varian Spectr AA 20Plus.

3.4.3. Plant sample analysis

Plant samples were washed carefully using distilled water as in (Spirochova *et al*., 2003). Leaf stem, and root samples were added in different crucible and heated in furnace up to 450^0C for 1.10 hour and then plant sample was then heated and for 4 hours (Mathew, 2005). The final ash residue was then mixed with 25% HNO_3 (5 ml) (Soylak *et al*., 2004). The solution was filtered using Whatman No. 42 and then poured into flask (25 ml) and the flask was filled up to 25 ml with distilled water. Finally, AAS was used to measure metal concentrations.

3.5. Evaluation of phytoremediation efficiency

The following factors were calculated for the evaluation of phytoremediation potentials of sample plants: Calculation of BCF, TF and BAC. BCF is an index for calculation of metal accumulation in plants (Ghosh and Singh, 2005). Consequently, bioaccumulation of metals in the plant tissues or the uptake and accumulation in plant parts was estimated by computing BCF applying the formula (Liu *et al*., 2009) given in Equation (4).

$$BCF = \frac{C\ harvested\ tissue}{C\ soil} \qquad \text{Equation 4}$$

Where,

C harvested tissue = represents metal concentration in different plant tissues

C soil = is a concentration of metal in soil

Further, BCF can also be presented in percentage by applying following the formula used in (Wilson and Pyatt, 2007) for calculation of metal concentration percentages in leaves, stem and root of the particular plant (Equation 5).

$$BCF\% = \frac{C\ Plant\ tissue}{C\ soil} \times 100\% \qquad \text{Equation 5}$$

Metal movement from root to aerial part (stem and leaves) was determined by calculating TF. TF was calculated by using the formula of translocation ratio applied in (Padmavathiamma and Li, 2007) (Equation 6).

$$TF = \frac{C\ Shoot}{C\ Root} \qquad \text{Equation 6}$$

Biological Accumulation Coefficient (BAC) or otherwise called Bioaccumulation Coefficient was calculated using the formula presented in (Moffat, 1995) (Equation 7).

$$BAC = \frac{Metal\ in\ plant\ shoot}{Metal\ in\ soil} \qquad \text{Equation 7}$$

Some authors consider BCF as a relationship between concentration of metal in root and metal in soil (Ghosh and Singh, 2005; Mahdavian *et al*., 2017). Others define it as metal concentration in plant shoot in relation to that of metal concentration in soil (Lu *et al*., 2015; Saravanan *et al*., 2019). Other authors define BCF as a ratio of metal in plant and metal in soil (Kulkarni *et al*., 2014). However, other authors (Hesami *et al*., 2018) applied another term called Extraction Factor (EF) for calculation of shoot metal concentration (Equation 8).

$$EF = BCF * TF \text{ or } EF = \frac{C\ shoot}{C\ soil}, \text{ while BCF in this case is BCF of root}$$

$$EF = \frac{C\ root}{C\ soil} * \frac{C\ shoot}{C\ root} \qquad \textbf{Equation 8}$$

However, even though different authors used a different approach to calculate this EF, BCF_{shoot} and BAC intended to explain the same thing (metal build up in shoot in relation to soil).

Several authors recommended the use of BCF and TF for the identification of suitable plants for phytoremediation purpose (Pandey *et al*., 2014; Sidhu *et al*., 2017). Plants showing BCF root > 1 and a TF < 1 could be used for phytostabilization and plants could be selected for phytoextraction if their TF and BCF values are > 1 (Yoon *et al*., 2006). However, in cases where; BCF is less than one, but with very large TF (> 1) another approach could be used since larger TF could compensate lower BCF. Explicitly plants having a larger metal concentrations in their shoot could be identified by calculating EF and EF > 1 could be considered as an indicator of plants with phytoextraction potential (Hesami *et al*., 2018).

Therefore, in this research, combination of most of these are used, for instance: the method of Yoon *et al*. (2006) was used for selection of plants with phytostabilization properties; BCF of shoot as in (Lu *et al*., 2015; Saravanan *et al*., 2019) and EF values as in Hesami *et al*. 2018, were also used to evaluate phytoextraction potentials and since one has to consider plants that can accumulate trace metals in their shoot (aboveground harvestable portion) to the level higher than the soil metal concentration to give shoot BCF >1 and TF >1.

3.6. Statistical analysis

Relationships between data (metal accumulation and translocation potentials of selected plant species and their seedlings, soil metal concentration and others) were presented statistically and statistical interpretations such as ANOVA and post-hoc (LSD) were applied for significant differences. Differences between soil metal concentrations of dry and wet season samples; seedlings and mature plants, were evaluated by performing T-test (Mean difference test). Similarly, correlation coefficient (r) was computed to evaluate association between concentrations of metals in plant tissues and soils. Significance values were presented as $p < 0.05$ and $p < 0.01$levels. For all analysis and for comparison of mean differences, SPSS version 22 and Microsoft Office Excel were used.

CHAPTER FOUR

4. Results and Discussions

4.1. Characteristics of sample site

Characteristics of sample sites (Lideta, Mekanisa, Kera, Hana Mariam, Kality) chosen on the basis of the possible presence of wide-ranging environmental pollution and presence of desires plants was described in the following sections. The study area, Addis Ababa City is a metropolitan city located in the center of Ethiopia, East Africa. The City is situated at the latitude of 9.005401 and the longitude is 38.763611 and the GPS coordinates are 9° 0' 19.4436" N and 38° 45' 48.9996" E. The city covers an area of about 540 Km^2 and the elevation lies between 2,200 to 2,500 m above sea level. The United Nations population projections estimated the population of Addis Ababa in 2020 is 4,794,000, a 4.4% increase from 2019. However, the estimation made by (CSA, 2019) was 4,592,000 with an annual growth rate of 4.4 percent. Furthermore, the average low-temperature of 12°C and an average high -temperature of 20°C.

The control samples were collected from a non-industrial site at the periphery of Bishoftu/ Debrezeit town located at south east of Addis Ababa, and the GPS coordinates of 8° 44' 4.74" N and 39° 0' 30.726" E. The average altitude of the town is about 1877.8 m above sea level. The average annual minimum and average annual maximum temperatures are 8°C and 22°C, respectively.

4.2. Characteristics of sampled soils

Soil characteristics and physicochemical properties can considerably affect the uptake characteristics of different elements and trace metals by plant roots. Removal of metal contaminants by plants depends on several factors; these include the plant species, biomass, root depth, age, growing season; soil pH, organic matter, temperature, moisture content, aeration; availability of competing ions and form and magnitude of trace metals (Vangronsveld *et al*., 2009; Pandey and Bajpai, 2019; Saxena *et al*., 2019).

Table 2. Characteristics of selected soil parameters within the plant growth areas.

Parameters		Soil samples						*P* (0.05)
		Lideta	**Mekanisa**	**Kera**	**Hana Mariam**	**Kaliti**	**Control**	
DRY SEASON								
pH		6.34 ± 0.25	5.74 ± 0.94	5.09 ± 0.4	6.5 ± 0.5	7.22 ± 0.41	6.91 ± 0.15	**0.002***
		6.07- 6.6	5.19 -6.83	5.06 – 5.14	6.06 – 7.1	6.75 – 7.47	6.75 – 7.03	
EC		296.9 ± 8.96	415.9 ± 28.	418.7 ± 16.6	385.4 ± 9.1	318.2 ±7.3	123.12 ± 7.9	**0.000***
		290-307	394- 447.6	401 - 434	376- 394	312.4 -326.4	117.3 – 132	
CEC		43.73 ± 3.2	45.6 ± 8.9	47.6 ± 7.14	50.2 ± 7.52	49.2 ± 2.8	39.3 ± 2.3	**0.305**
		41.8 – 47.4	36. 4 -54.2	42.7 – 55.8	43 - 58	47.2 – 52.4	37.34 – 42.6	
OM%		4.11 ± 0.8	3.7 ± 1.2	4.24 ± 0.3	4.8 ± 1.35	5.2 ± 0.22	4.3 ± 0.26	**0.343**
		3.2 – 4.62	2.59 – 4.93	3.94 – 4.5	3.51 – 6.2	4.91 – 5.4	4.03 – 4.52	
MC%		18.51 ± 1.4	20.5 ±2.20	18.81 ± 1.7	20.9 ± 2.14	21.8 ± 1.5	21.12 ± 0.6	**0.248**
		17.03 – 19.7	18.02 – 22.3	17.1 – 20.4	16.7 - 22.4	20.85 – 23.6	20.43 – 21.5	
Texture	Sand	16 ± 4	18.3 ± 2.83	19.97 ± 3.8	19.82 ± 2.2	21.2 ± 1.4	17.8 ± 0.62	**0.304**
		12 – 20	15.2 - 20.7	16.6 – 24	18.4 – 22.3	19.8 – 22.6	17.1 – 18.2	
	Silt	35.4 ± 2.44	34.1 ± 5.9	34.5 ± 5.8	29.1 ± 3.84	30.9 ± 2.97	39.3 ± 2.4	**0.117**
		33.2 – 38	28.3 – 40	28.5 – 40	26.7 – 33.5	28.54 – 34.2	37 – 41.8	
	Clay	48.61 ± 1.62	47.6 ± 4.4	45.53 ± 2.1	51.1 ± 3.44	48 ± 2.21	43 ± 1.9	**0. 054**
		46.8 – 50	44.8 – 52.6	43.4 – 47.5	47.7 – 54.6	46 – 50.4	41 – 44.8	
WET SEASON								
PH		7.02 ± 0.2	6.38 ± 0.9	5.04 ± 0.1	7.4 ± 0.4	7.6 ± 1.31	6.6 ± 0.41	**0.009***
		6.8 – 7.2	5.37 – 7. 1	4.95 -5.12	7.1 – 7.82	6.14 – 8.71	6.14 – 6.95	
EC		283.74 ± 5.74	352.9 ± 12.	407.4 ± 5.4	362 ± 4.8	308.2 ± 31.6	125.9 ± 10.3	**0.000***
		279 - 290	343 - 366.4	402.5 - 413	357.5 - 367	289 - 344.7	115 - 135.8	
CEC		41.9 ± 3.64	46.92 ± 2.21	46.1 ± 6.9	47.44 ± 6.2	47.95 ± 3.52	40.3 ± 1.2	**0.244**
		39.2 - 46	44.95 – 49.3	38.8 - 52.5	41.4 – 53.8	44.4 – 51.4	39 – 41.4	
OM%		3.7 ± 1.04	3.4 ± 0.71	3.81 ± 0.41	3.9 ± 0.62	4.9 ± 0.6	3.98 ± 0.2	**0.178**
		2.48 – 4.4	2.98 – 4.2	3.35 – 4.12	3.17- 4.33	4.22 – 5.32	3.81 – 4.19	
MC%		31.1 ± 3.5	30.14 ± 2.8	32.32 ± 2.5	28.9 ± 1.4	34.3 ± 1.3	30.4 ± 1.4	**0.145**
		28 – 34.93	27.9 – 33.34	29.6 – 34.4	27.53 – 30.3	32.83 – 35.2	28.84 – 31.7	
Texture	Sand	20.4 ± 3.82	23.13 ± 2.9	19.9 ± 3.2	20.53 ± 1.9	20.41 ± 2.3	22.8 ± 1.9	**0.589**
		17.5 – 24.7	19.8 - 25	16.6 - 23	18.5 – 22.2	18.43 – 22.9	20.7 -24.3	
	Silt	35.96 ± 1.2	33.7 ± 5.01	33.9 ± 3.5	33.3 ± 6.24	31.53 ± 1.12	38.1 ± 2	**0.389**
		34.7 - 37	29.1 - 39	31.1 – 37.8	29.4 – 40.5	30.4 – 32.6	36.1 – 40.03	
	Clay	43.63 ± 3.96	43.2 ± 2.4	46.23 ± 5.4	46.2 ± 4.6	48.1 ± 2.9	39.1 ± 1.6	**0.123**
		39.1 – 46.3	41.2 – 45.9	42.2 – 52.3	41 - 49	45.5 – 51.2	37.5 – 40.6	

*Mean values significantly different, $p < 0.05$; all parameter levels reported as Mean ± SD, N=3. Units used for EC and CEC are (mS/m) and (meq/100g), respectively.

Composite soil samples surrounding plant samples were collected for analysis. Accordingly, corresponding soil physicochemical properties and concentrations of metals were measured to evaluate correlation between soil parameters and plant uptake and transfer potential. The following vital soil parameters were examined in the present study: soil pH, cation exchange capacity (CEC), organic matter (OM), texture, electrical conductivity (EC), and moisture content. Values of soil parameters determined in this study are presented in Table 2 above.

4.2.1. Soil pH

Soil pH is an important soil parameter used for measuring acidity or alkalinity of soil solution which shows the activities of H^+ and OH^- ions (Motsara, and Roy, 2008). Chemical process occurring in soil, possible toxicity, plant nutrient deficiency can be estimated via soil pH (Hazelton and Murphy, 2016). Soil pH also impacts heavy metal solubility, speciation and availability for uptake (Sheoran *et al*., 2016). Alkaline pH, higher percentage of OM and larger fraction of clay substances lowers metal movement and availability for uptake (Mkumbo *et al*., 2012). Conversely, a lower pH enhances the cation mobility, absorption, and transfer of trace metals within the plant tissue.

The data presented in Table 2, clearly indicated that, the pH values of soil samples collected from different sampling sites ranged from acidic to slightly alkaline, and pH ranged between 4.95 to 8.7 in rainy season and 5.06 to 7.47 for dry season. ANOVA also revealed there is significant seasonal variation $p < 0.05$ in pH of soil. Mean soil pH in samples at Lideta site was 6.34 ± 0.25 for dry season and 7.02 ± 0.22 for rainy season. Soil taken from samples at Mekanisa site had pH 5.74 ± 0.94 during dry season and 6.38 ± 0.9 in wet season. Samples of soil collected from Kera site showed relatively low pH values of 5.04 ± 0.1 and 5.09 ± 0.04 in rainy and dry seasons, respectively. Soil pH of samples at Hana Mariam site varied between 7.08 to 7.82 during dry season and 6.06 and 7.06 during rainy season. In addition, soil pH values obtained from samples at Akaki site were all slightly alkaline both in dry season and wet seasons, the mean values of 7.22 ± 0.41 and 7.6 ± 1.31 are presented in Table 2.

Mean pH values of soil samples collected from contaminated sites, except for samples collected from Kality site, during dry season are lower than mean pH value of control samples (6.91 ± 0.15). However, during wet season soil samples collected from contaminates sites, with the exception of

Kera and Mekanisa site, gave a higher mean pH values than Control sample collected from the vicinity of Bishoftu/Debrezeit Town.

The mean pH values of soil samples obtained in this research are comparable to results reported by other recent studies for instance: Woldetsadik *et al*. (2017), reported mean pH values ranging from 5.99 to 7.16 and Mean pH value reported by (Mengesha *et al*., 2017) was 6.97 or it ranges between 6.5 and 7.4.

A lower probability value of $p = 0.000 < 0.05$ obtained from ANOVA also revealed that there is significant variation in pH values among samples from different sites. Moreover, LSD ($p = 0.000 < 0.05$) was observed between Kera site and Akaki site which are sites with the least and the highest mean pH values, respectively. A wide range of variation between pH values of different sample sites of present could be attributed to the levels of anthropogenic interferences. An increase in pH values of soil samples during wet season was probably attributable to broken floor tiles, block and leaching from other construction and demolition wastes containing calcium carbonate ($CaCO_3$), which could serve as buffer and dilute and raise pH values (Oluyemi *et al*., 2008).

Control soils sampled from Bishoftu area showed pH recordings ranging from 6.14 to 6.95 and 6.75 to 7.03 during rainy and dry seasons, respectively. Reduction in pH values during rainy season in control sample sites might be due to lower pH of rain water or leaching of cations due to precipitation (Brady and Weil, 2002) or production of organic acid due to cultivation of land in the upper catchments which in turn adds H^+ ion to the soil. Mean pH value (6.75) noted in the present study was slightly lower than neutral pH; which is nearly comparable to pH values ranging between 6.38 and 8.08 (average 7.00) reported by (Minase *et al*., 2016).

4.2.2. Soil texture

Soil texture refers to the particle size of soil or it represents fineness and roughness of soil particles. It elucidates relative proportions of different particle size clay, silt and sand (ISSS, 2002). Soil texture is an essential factor that affects metal mobility, availability and soil to plant transfer of trace metals. Clay particles can significantly impact trace metal availability (Beyer and Cromartie, 1987; Sheoran *et al*., 2016).

Soil samples investigated in this research are composed of mixtures of sand, silt and clay. Results showed, clay and silt are dominant soil textures in the studied soil. Clay fraction is a predominant texture in almost all studied sites including the control samples site chosen as unpolluted. Particle size distribution in soils of the study site shows the largest portion of clay followed by silt and sand.

The highest mean clay fraction (51.1 ± 3.4%) was recorded at Hana Mariam site during dry season, while the minimum recording of (45.5 ± 2.1) for clay was noted at Kera site. Slightly lower mean values of clay fraction were noted in rainy season. The lower and higher mean values of 48.1 ± 2.9 and 43.2 ± 2.4% were obtained for Akaki site and Mekanisa site, respectively (Table 2). Comparison with the mean values of the control soil showed significant variation $p = 0.015 < 0.05$ and based on LSD value, highly significant difference ($p = 0.001 < 0.05$) was recorded for Hana Mariam site and the control site.

Similarly, sample soils also had significant amount of silt, minimum mean fraction (29.1%) and maximum (35.4%) were recorded for Hana Mariam site and Lideta site, respectively during dry season (Table 2). During wet season lowest mean portion of silt 31.5 ± 1.12% was recorded at Akaki site and maximum portion amounting to 35.96 ± 1.2% was noted for Lideta site. Control soil collected from non-contaminated site showed largest mean silt fraction. Mean values of 38.1 ± 1.98 and % 39.3 ± 2.4% were noted for wet and dry season samples, correspondingly. Probability value of $p = 0.011 < 0.05$ indicated control soil samples had significantly higher mean values than those from contaminated sites.

Data in Table 2, also showed, a sand fraction was the lowest in its proportion in all sample sites, the minimum percentage of 16 ± 4% was recorded for Lideta site and the maximum mean value 21.2 ± 1.4 was noted for Kality site in dry season. The average value of sand fraction noted during wet season was slightly higher than the value in dry season, however the difference remains statistically insignificant $p = 0.722 > 0.05$. During rainy season minimum (19.9 ± 3.2%) and maximum (23.1 ± 2.9%) values were recorded at Kera site and Mekanisa site, respectively.

4.2.3. Moisture content (MC)

The quantity of water that exists in the soil mass was represented as moisture content. Soil MC could vary depending on level of precipitation. During dry season, high temperature enhances

evapotranspiration that will in turn reduces soil MC. Knowing the MC of soil is vital for evaluating water uptake in plants, water holding capacity, water movement, infiltration, leaching of chemicals and other physical processes (Foth, 1990). Soils under different land uses vary in their MC and soil texture especially silt and clay fractions are strongly correlated with the MC (Mishra and Defera, 2018; Medeiros *et al*., 2018).

Seasonal variation and difference in MC of different sampling sites in this study is summarized in Table 2. Mean values of MC vary between 27.5 to 35.2%, and 16.7 to 23.6% during wet and dry seasons, respectively. The lowest (16.7%) mean soil moisture was noted in the soil of Hana Mariam site and the highest (35.2%) was recorded at Akaki site. However, ANOVA, both in dry season ($p = 0.248 > 0.05$) and wet season ($p = 0.145 > 0.05$) revealed variation in moisture content of different sites was not significant.

Further, even though the moisture difference during the wet and dry season is obvious, the MC of soil samples were also positively affected by the organic matter content in soil, which could be explained by the Pearson Correlation Coefficients recorded in dry season r (0.603), $p = 0.008$ and wet season r (0.769), $p = 0.000$ whereas $p < 0.01$. Likewise, clay fraction has a positive effect on the soil moisture content, which is, significant positive effect in wet season r (0.534), $p < 0.05$ and insignificant positive effect r (0.186), $p > 0.05$ during dry season. This positive relationship could explain that clay-rich soil could retain more water. Likisa and Gejea (2017) reported a similar finding concluding elevated moisture content due to higher clay fraction.

4.2.4. Organic Matter (OM)

Soil OM represents the portion of soil containing living things and dead (debris) of plants and decomposition products from animals. OM in soil includes debris of plants and animals that contains protein, carbohydrate and other organic species (Foth and Ellis, 1997). OM composition of soil can influence soil properties including soil structure, nutrient contributions, infiltration rate and biological activity. Soil with high OM content could have acidic pH due to decomposition of OM that can produce inorganic acids, carbonic acids and carboxylic acids (Brady and Weil, 2002). Higher OM in soil increases CEC, MC, and improves soil stability and aeration.

The percentage values of organic matter were found to vary with sample sites. Maximum of 4.8% ± 1.35 was recorded in samples at Kera site and the minimum percentage of 3.4% ± 0.71was

recorded at Mekanisa site. Soils having higher clay content are likely to retain more OM than soils with low clay content. Compositions of OM noted in control site 3.75% ± 1.34 for dry season and 3.44% ± 0.82 for wet season which is near compare to values reported by (Woldetsadik *et al*., 2017) in wastewater irrigated sites of Addis Ababa. Mean OM percentage recorded in dry season was greater than recordings taken during wet season. However, statistical analysis (ANOVA) revealed seasonal differences are insignificant, $p = 0.087 > 0.05$. Similarly, the variation in percentage values of organic matters in soil samples of different sites were not statistically significant, $p > 0.05$ both for dry and wet season samples (Table 2).

4.2.5. Electrical Conductivity (EC)

EC explains the capacity to carry electric current. It is usually given in units of deciSiemens per meter (dS/m) or millisiemens per meter (mS/m). The conductivity property depends on availability of ions and temperature (Maria, 1997). Electric conductivity also expresses soluble salts in the soil solution, which impacts plant metal.

Relatively, lowest mean conductivity value (296.9 ± 8.96 mS/m) was observed at Lideta site soils and highest mean value of electrical conductivity (418.7 ± 16.6 mS/m) was observed at Kera site soils during dry season. Similarly, minimum and maximum mean EC values recorded in wet season are 283.74 ± 5.74 mS/m and 407.4 ± 5.41 mS/m, displayed in (Table 2). The electric conductivity of the present soil samples had positive relationship with the clay fraction r (0.476), $p = 0.003$ and soil CEC r (0.470), $p = 0.004$ which indicates that, an increase in clay content and CEC could increase the EC. This is in agreement with Mosseler and Major. (2017) who indicated clay soil has greater EC than sandy due to its higher cation exchange capacity. In addition, Corwin and Lesch. (2005) and Medeiros *et al*. (2018) also reported positive correlation among clay content and the soil EC.

ANOVA revealed the mean difference of EC between different sampling sites is highly significant $p = 0.000$ at 0.05 level. Conversely, comparison of mean differences in EC of soil samples collected in dry and wet season shows $t(34) = 0.591$, $p = 0.558$. Explicitly, mean EC of dry season sample (M = 326.35 mS/m, SD = 105.57) showed insignificant difference from the mean EC of wet season soil samples (M = 306.71 mS/m, SD = 93.39). Control soil samples procured from Debrezeit/Bishoftu area, showed the lowest mean values of EC ranging from 115 to 135 mS/m

and 117.3 to 132 mS/m for wet and dry season, respectively (Table 2). The lower values of EC in uncontaminated site could be explained as due to limited concentration of ions.

4.2.6. Cation exchange capacity (CEC)

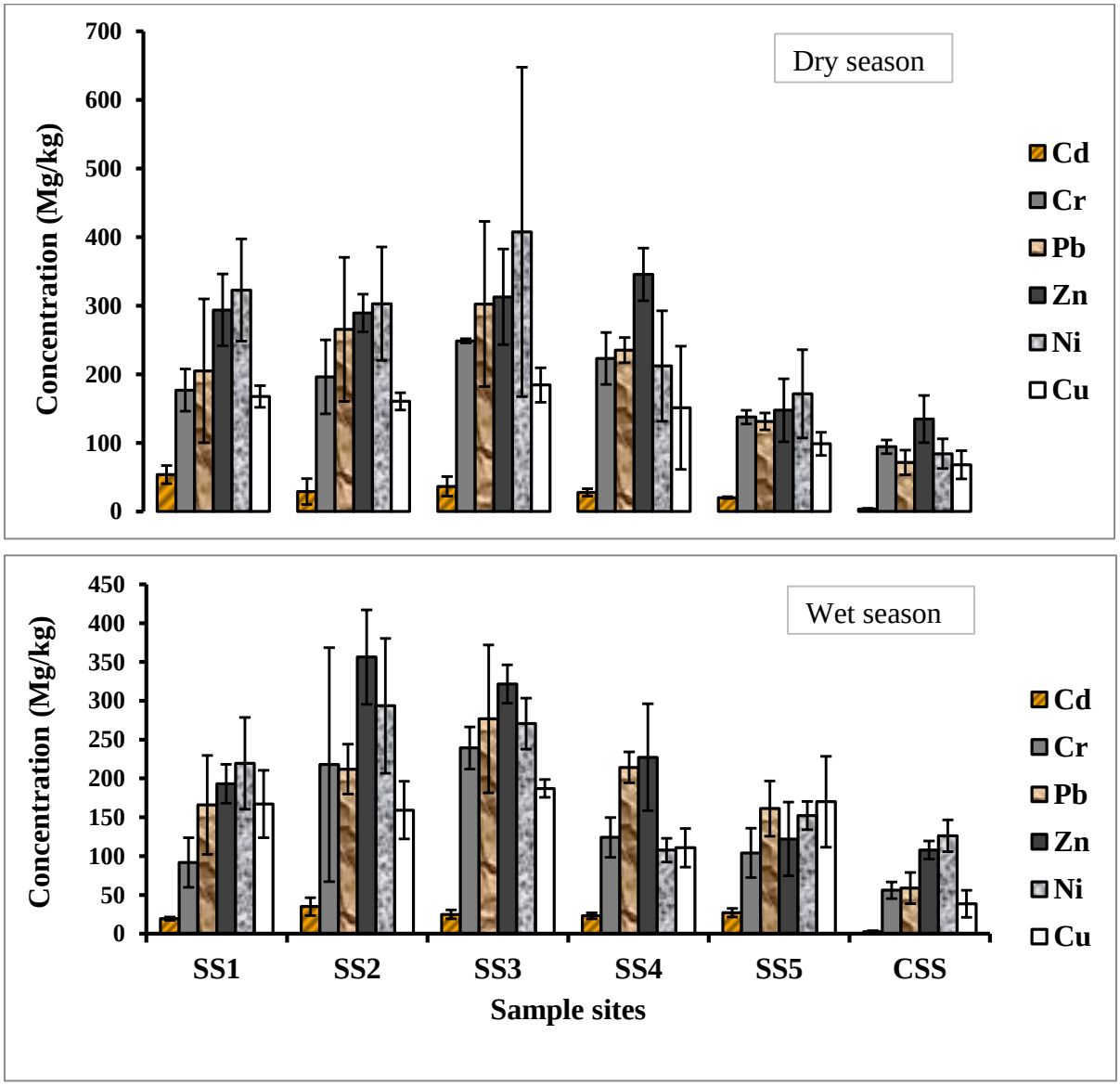
CEC explains soil capacity to hold and exchange cations (Brady and Weil, 2002) or keep cations in available form for plant uptake (Ilaco, 1985). Mean values of CEC in soil from Lideta, Mekanisa, Kera, Hana Mariam and Kality sites were 42.8 ± 3.2, 46.24 ± 5.9, 46.8 ± 6.3, 48.8 ± 6.35 and 48.6 ± 2.92 meq/100g soil, respectively. CEC value is highest in soils of Hana Mariam (48.82 ± 6.35 meq/100g) and the minimum was noted in soils of Lideta site, 42.8 ± 3.23 meq/100g. The higher CEC exceeding 25 meq/100g indicates the higher clay fraction in soil samples, which enhances heavy metal adsorption (Bulluck *et al*., 2002). The correlation coefficient r (0.505) and *p* value of 0.002 which is <0.01 revealed there is significant relationship between the OM percentage composition and the CEC of soil samples. Soil samples with higher organic matter tend to have higher CEC and those with lower OM fraction had lower CEC. This is in concurrence with McAlister *et al*. (1998) who reported CEC have strong association with OM content. Olaniran *et al*. (2013) also reported soil OM has strong influence on CEC.

Similarly, mean values of CEC were significantly impacted by the clay content in the soil samples correlation coefficient (r) 0.570, $p = 0.000 < 0.05$ reveal that the higher the clay content the higher is the CEC, which is in line with Bulluck, *et al*. (2002). However, a negative and significant relationship r (-0.642), $p = 0.000$ was noted between CEC and silt content while, the sand fraction had insignificant ($p = 0.461 > 0.05$) effect on the CEC of soils.

Mengesha *et al*. (2017), reported soil samples taken from Akaki River catchment located in central Ethiopia have moderate to high CEC in the range of 31.44 to 51.12 Meq/100mg. Similarly, control soil collected from Bishoftu has mean CEC value of 47.95 Meq/100 gm was comparable with a moderate to high CEC values varying between 45 - 58 Meq/100 gm reported by (Minase *et al*., 2016).

Heavy metal (Pb, Cr, Cd, Zn, Cu and Ni) concentration in soil samples obtained from five different polluted industrial sites and control sites are presented in Figure 7 below. ANOVA, $p < 0.05$ showed significant difference in concentration of trace metals among different sampling sites. Soil

samples collected from all contaminated sites gave significantly higher mean values of analysed heavy metals than samples collected from control sites, except for Ni which showed insignificant difference $p = 0.054 > 0.05$. Heavy metal concentration in control soil samples might be associated with phosphate fertilizers which contain toxic heavy metals (Bitew and Alemayehu, 2017).



SS: Sample site, CSS control sample site; SS1 (Lideta site), SS2 (Mekanisa site), SS3 (Kera site), SS4 (Hana Mariam site), SS5 (Kality site)
Bar charts represent mean of 3 samples and vertical error bars represent standard deviation (±SD)

Figure 7. Heavy metal concentrations in the soil surrounding plant samples.

High concentration of heavy metals in soils can negatively impact absorption of essential nutrients (Khan *et al.*, 2016). Heavy metal bioaccumulation in edible plants has detrimental effect on human health (Mortensen *et al.*, 2018). Therefore, edible crops grown in metal contaminated sites are not

safe for consumption. Heavy metals toxicity can also limit plant growth, crop yield and change native microbial populations (Asati *et al*., 2016; Xia *et al*., **Error! Reference source not found.**). Based on their level of toxicity, heavy metals are classified as relatively less poisonous, moderately poisonous and extremely poisonous (Mukesh *et al*., 2008). Metals studied in this research, Pb, Cd, and Zn were classified as extremely poisonous and Cr, Ni and Cu are classified as moderately poisonous. And maximum permissible limits for metals (Cr, Pb, Cd, Cu, Zn, and Ni) concentration in soil presented by European Union, 2002 and joint WHO/FAO, (2007), were 300, 3, 300, 150, 140 and 50, respectively in mg/kg.

Cd detected in all sampling sites from contaminated regions of Addis Ababa, are far greater than maximum permissible limit (3 mg/kg) while, the highest mean value (53.95 ± 13.27 mg/kg) was noted at Lideta site and minimum recording 2.6 ± 1.3 mg/kg was noted for control site. The total mean metal values in soil are in order of Zn > Ni > Pb > Cu > Cr > Cd during wet season and Ni > Zn > Pb > Cr > Cu > Cd during dry season. Mean values of metal concentrations of in contaminated sites of Addis Ababa ranged between: 108.7-385.6, 61.2-390, 14.79-69.2, 70.4 - 419.7, 82.2 - 253.8 and 93.2- 664.8 in mg/kg's for Pb, Cr, Cd, Zn, Cu and, Ni respectively (Figure 7). The mean values recorded for studied metals particularly, Cd (29.74 ± 12.8 mg/kg), Pb (217 ± 80.12 mg/kg), Cr (175.99 ± 72.7 mg/kg) and Ni (246 ± 118.5 mg/kg) are higher than values noted by previous studies in the same city (Alemayehu, 2006; Aschale *et al*, 2017, Mengesha, *et al*., 2017; Melaku, 2018). For instance; Mengesha, *et al*. (2017) reported lower mean values of 139.9 ± 9.57, 40.52 ± 12.97, 13.91 ± 10.96 and 0.31 ± 0.04 for Ni, Cr, Pb, and Cd, correspondingly.

However, the mean concentration of Cu (155.73 ± 44.15 mg/kg) obtained from the present investigation was higher than mean value of Cu (103.16 ±7.22) reported by Mengesha *et al*. (2017). Conversely, mean value of Zn (261.01 ± 90.01mg/kg) recorded in soil samples of this study was considerably lower than mean concentration of Zn (5856.74 ± 642.61) noted by Mengesha *et al*. (2017). Elevated levels of metals, especially Pb and Cu in high-traffic density urban centers indicate traffic related sources (Chen *et al*., 2016).

Mean concentrations of 61.6 ± 7.32 mg/kg, 43.3 ± 4.41mg/kg, 29.6 ± 3.59 mg/kg, 145 ± 26.4 mg/kg, 48.7 ± 4.75 mg/kg and, 2.27 ± 0.31 mg/kg for Cr, Cu, Pb, Zn, Ni and Cd, respectively on soils of Mekanisa site as also noted by Woldetsadik *et al*. (2017). However, concentration of trace

metals in soils of Mekanisa site obtained in the present investigation are as follows: Cd (29.08 ± 18.82 mg/kg), Cr (196.23 ± 54.04 mg/kg), Pb (265.67 ± 104.98 mg/kg), Zn (289.37 ± 27.64 mg/kg), Ni (303.02 ± 82.76 mg/kg), Cu (160.69 ± 12.53 mg/kg) during dry season, and mean values of Cr (217.83 ± 150.7 mg/kg), Cd (34.99 ±11.62), Pb (212.04 ± 32.01 mg/kg), Zn (356.35 ± 60.76 mg/kg), Cu (159.23 ± 37.18 mg/kg) and Ni (293.55 ± 86.92 mg/kg) during wet season (Figure 7). Woldetsadik *et al*. (2017), also examined mean metal concentrations in soils of Kera, Hana and Akaki areas of Addis Ababa. The respective values of trace elements (Cd, Cr, Pb, Zn, Ni and Cu) reported were 2.95 ± 0.42, 76.3 ± 6.74, 81.1 ± 10.9, 160 ± 8.35 and 49.9 ± 6.2 mg/kg; 1.37 ± 0.21, 56.3 ± 2.52, 33.1 ± 1.88, 130 ± 16.6, 39.9 ± 4.85 and 38.3 ± 4.92; 1.19 ± 0.27, 69.1 ± 8.51, 35.9 ± 5.22, 154 ± 28, 46.6 ± 3.27 and 27.9 ± 1.6 mg/kg at Kera, Hana and Kality sites correspondingly.

Control soils collected from the non-industrial site gave considerably lower trace elements than all the sample soils collected from around industrial sites of Addis Ababa. Mean levels of metals in control soil during dry season were as follows: Cr (94.3 ± 10.1 mg/kg), Cd (3.74 ± 1.03 mg/kg), Pb (71.64 ± 18.12 mg/kg), Zn (134.7 ± 34.4 mg/kg), Ni (84.3 ± 21.7 mg/kg) and Cu (68.14 ± 21.7 mg/kg). The soil Cr, Cd, Pb, Zn, Ni and Cu concentrations recorded for control soil samples collected in wet season were 56 ± 10.6, 2.6 ± 1.28, 58.7 ± 20.1, 107.9 ± 11.65, 126.13 ± 20.6 and mg/kg, respectively (Figure 7).

Mean concentrations of heavy metals higher than Maximum Permissible Limit (MPL, European Union, 2002 and joint WHO/FAO. (2007), were recorded at Lideta site for Cd, Cr, Ni and Cu during dry season and only Cd was higher than MPL during the wet season. Mekanisa site had average values of > MPL for Cr, Cu, Cd, and Ni during dry season, while wet season soil samples had mean values > MPL for all studied metals except Pb. Soil heavy metals at Kera sites are all higher than MPL, except for Pb which is slightly lower than MPL. Cadmium was the only metal with concentration higher than MPL at Hana Mariam site, while all studied elements, except Pb, were higher than MPL in dry season. Among studied elements, Cu Cd, and Ni during wet season and only Cd and Ni during dry season were higher than MPL in soils of Akaki site. Control soils collected from the non-industrial site perceived to be uncontaminated, showed mean concentrations of studied heavy metals lower than MPL, except for a slightly higher mean value recorded for Ni during wet season and Ni and Cd during dry season (Table 3).

Table 3. Total heavy metal concentrations in the soil samples surrounding the plant samples.

Heavy metal concentration (mg kg^{-1})								
Season	Sites		**Cd**	**Cr**	**Pb**	**Zn**	**Ni**	**Cu**
	1	Mean	53.95 ± 13.3	177 ± 30.6	205.1±104.7	293.8 ± 52.4	322.8±74.43	167.84±15.9
		Range	45.05-69.2	145-206	108.7-316.5	260.2-354.1	270.41-408	151.4-183
	2	Mean	29.1±18.8	196.23±54.0	265.7±104.98	289.37±27.6	303.02±82.8	160.7±12.5
		Range	14.79-50.4	141-249	182.1-383.5	266.3-320	249.23-398	149.64-174
	3	Mean	36.6±14.3	248.7±3.03	302.5±120.4	312.97± 69.9	407.43 ± 240	184.5±25.1
		Range	21.3-49.6	246.9-252	164.1-382.6	264.21-393	189.5-664.8	158-208
	4	Mean	27.8±5.54	223±37.9	235.4±18.5	345.62±38.4	212.2±80.7	151.27±89.7
		Range	22.7-33.7	193.8-265.9	220.74-256	319.2-389.7	159.95-305	87.42-253.8
	5	Mean	20.3 ±1.4	137.67±10	131.2±12.4	147.67±45.9	171.6±64.3	98.76±16.9
		Range	19-21.7	130-149	118-142.6	104-195.5	116-242	82.23-116
	Control	Mean	3.74±1.03	94.33±10.1	71.64±18	134.7±34	84.28±21.7	68.14± 20.7
		Range	2.96-4.91	85-105	51.7-87.1	110-174	66.51-108.4	49.91-90.6
ANOVA	**F-Value**		**6.541**	**10.446**	**3.599**	**11.017**	**3.014**	**3.735**
	***p* (*Sig*)**		**0.004**	**0.000**	**0.032**	**0.000**	**0.054**	**0.029**
Wet	**1**	Mean	19.5±1.99	91.9±31.97	165.9±63.8	193.17±25	219.50±59.1	167.13±43.6
		Range	17.22-21.03	61.2-125	112-236.4	168.2 - 218	174.2-286.3	118-201
	2	Mean	34.99±11.6	217.83±150.	212 ±32.01	356.4±60.8	293.6±86.9	159.2±37.2
		Range	27.97-48.4	110.21-390	175.4-234.6	298.6-419.7	225.3-391.4	125.74-199
	3	Mean	24.9±5.64	239.33±27.1	276.8±95.3	321.7±24.7	270.64±32.8	187.1±11.4
		Range	18.8-29.93	217-269.5	208.5-385.6	299.7-348.4	247.2-308	178.4-200
	4	Mean	23.13±3.9	124.10±25.6	214.3± 20	227.3±68.8	107.63±15.3	110.7±24.8
		Range	18.9-26.5	94.7-141.6	196.3-235.9	159.7-297.3	93.2-123.7	86.5-136
	5	Mean	27.14±5.5	104.08±31.8	161.19±35.5	122.1±47.6	152.1±18.2	170±58.64
		Range	22.4-33.12	80.86-140.3	126-197.06	70.4-164	134-170.3	103-212
	Control	Mean	2.56±1.3	56.02±10.6	58.67±20.1	107.9±11.7	126.13±20.6	38.57±17.5
		Range	1.34-3.9	48.05-68	46.22-81.84	98.7-121	102.4-138	28.5-58.7
ANOVA	**F- Value**		**9.761**	**3.680**	**5.949**	**15.471**	**8.309**	**7.122**
	***p* (*Sig*)**		**0.001**	**0.030**	**0.005**	**0.000**	**0.001**	**0.003**
	MPL (Mg Kg^{1})		**3**	**150**	**300**	**300**	**50**	**140**

NB: N= 3 for all samples and all samples value is representation of a composite of samples taken from 5 points
$P < 0.05$ represents significant difference based on results of ANOVA
MPL: Maximum permissible Limit, European Union, 2002 and joint WHO/FAO, 2007

The higher values of trace elements in most of studied sample spots could be associated with motor vehicle emission in urban center (Wekpe *et al*., 2019), industrial effluent (Belouchrani *et al*., 2016) or poor waste management systems (Khan *et al*., 2016). Heavy metals in control soils could be attributed to use of agricultural pesticides, fungicides and artificial fertilizers in a nearby agriculture fields or from geological processes (Prieto *et al*., 2018). Seasonal variation in level of metals in soil samples were estimated by computing independent sample t-test. Cadmium concentrations were higher in dry season, however t-test t (20.32) = 1.677, p = 0.109 revealed there were no statistically different variations between their mean values of dry season (M = 33.54 mg/kg, SD = 15.81) and wet season (M = 25.93 mg/kg, SD = 7.72).

Seasonal differences in Cr mean concentrations of dry season soil samples (M = 195.5 mg/kg, SD = 48.27) and wet season samples (M = 155.4 mg/kg, SD = 87.9) was also statistically insignificant t (28) = 1.588, p =0.124. However, the maximum mean chromium 248.7 ± 3.03 mg/kg was noted in soils at Kera site followed by Hana Mariam (223 ± 37.9 mg/kg) and Mekanisa area (196.2 ± 54.04 mg/kg) in dry season (Table 3). Similarly, mean values recorded in wet season are maximum at Kera site 239.3 ± 27.1 mg/kg followed by Mekanisa (217.8 ± 150.7 mg/kg) and Hana Mariam (124 ± 25.6 mg/kg). These sites had elevated levels of Cr that is larger than recommended maximum limit (100 mg/kg) because they are urban centers where anthropogenic interference is intense.

Lead showed insignificant mean differences t (28) = 0.743, p = 0.464. However, the mean value of dry season soil sample 227.96 mg/kg ± 94.38 was slightly higher than wet season samples (206 mg/kg ± 64.3). The mean values of Zn available in dry season (M = 277.9 mg/kg, SD = 81.7) and wet season (M = 244.14 mg/kg, SD = 97.47) are insignificantly different t (28) =1.027, p = 0.313. The higher content of lead in all sites could be due to traffic related emission of trace metals in metropolitan areas (Feng *et al*., 2011).

Mean difference test (t-test) of soil Ni and Cu also showed insignificant seasonal variation. Ni concentration of 283.4 mg/kg ± 137.9 noted in dry seasons was higher than values recorded during rainy season 208.7 mg/kg ± 84.12. However, t-test gave a value of t (28) =1.792, p = 0.84 which is statistically not significant. Mean value of Cu recorded in rainy season (158.84 mg/kg ± 42.13) was higher than values recorded in dry season (152.6 mg/kg ± 47.35). It was noted Cu in soil samples had insignificant seasonal variation t (28) = 0.38, p = 0.706.

Even though seasonal variation showed statistically insignificant mean differences, the higher concentration in dry season soil samples could be due to the dumping of solid wastes and limited wash in dry season which permit deposition of heavy metals. The larger mean values of Zn and Cu recorded in soils of Kera site, Mekanisa site and Hana Mariam area could be associated to an input of wastewater from different anthropogenic activities and garages. Which is in concurrence with findings of Tekere *et al*. (2016), who reported car wash effluent characterized by Cu and Zn pollutants.

Table 4. Correlations matrix of the six studied heavy metals in soil samples.

Correlation							
		Cd	Cr	Pb	Zn	Ni	Cu
Cd	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	36					
Cr	Pearson Correlation	.619**	1				
	Sig. (2-tailed)	.000					
	N	36	36				
Pb	Pearson Correlation	.582**	.653**	1			
	Sig. (2-tailed)	.000	.000				
	N	36	36	36			
Zn	Pearson Correlation	.648**	.782**	.636**	1		
	Sig. (2-tailed)	.000	.000	.000			
	N	36	36	36	36		
Ni	Pearson Correlation	.508**	.479**	.640**	.567**	1	
	Sig. (2-tailed)	.002	.003	.000	.000		
	N	36	36	36	36	36	
Cu	Pearson Correlation	.471**	.343*	.610**	.464**	.622**	1
	Sig. (2-tailed)	.004	.040	.000	.004	.000	
	N	36	36	36	36	36	36

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Regardless of other factors, the relationships between levels of different metals in soil were investigated by computing the correlation matrix. Statistically significant values ($p < 0.05$) or highly significant values ($p < 0.01$), and the correlation between occurrence of each trace metals were as follows: strong positive correlations were observed between Cd and Cr (r =.619, n = 36, $p < 0.01$), Pb and Cd (r =.582, n = 36, $p < 0.01$), Zn and Cd (r = .648, n 36, $p < 0.01$), Cd and Ni (r = .508, n 36, $p < 0.01$), Pb and Cr (r = .653), Cr and Zn (.782), Pb and Zn (.636), Ni and Pb (r = .640), Ni and Zn (r = .567), Cu and Pb (r = .610), Cu and Ni (r = 622) and moderate positive correlations were recorded between Ni and Cr (r = .479 n 36, $p < 0.01$), Cu and Cd (r = .471 n 36, $p < 0.01$), Cu and Cr (r = .343 n 36, $p < 0.01$) and Cu and Zn (r =.464 n 36, $p < 0.01$) presented

in Table 4. These positive relationships explain that potential sources of pollution are similar and sites that had higher concentration of one metal tend to have higher concentration of the other metal (Shen *et al*., 2016).

Further, based on R^2 values, presence of Cd could indicate 38.32%, 42%, 34%, 26% and 22.18% availability of Cr, Zn, Pb, Ni and Cu in soil samples, respectively. Similarly, presence of Cr in soil samples could explain availability of Pb (42.64%), Zn (61.15%), Ni (22.94) and Cu (11.77%). In addition, Pb concentration in soil could indicate availability of Zn, Ni, and Cu by 40.45%, 40.96% and 37.2%, respectively. Finally, Ni availability could explain presence of Zn by 32.15% and Cu could explain 38.69% possible presence of Ni in the same soil (Table 4).

4.3. Metal accumulation and phytoremediation potentials of plants

All of the selected plants; *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum* species can grow in both dry and wet seasons, under varied ecological conditions. These plants also share characteristics of fast growth, perennial, large biomass production and re-growth after pruning. Plants can take up and accumulate both essential and non-essential metals, however the uptake properties could be affected by plant type, the availability of metals in sufficiently mobile form in the growth medium, metal type, other physicochemical parameters and plant type and age environmental parameters (Gomes *et al*., 2016; Usman *et al*., 2019). Most importantly, plant growth is the most essential parameter to evaluate phytoremediation efficiency of plants to clean-up polluted sites (Beauchamp *et al*., 2018; Rasheed *et al*., 2019). In other terms, plant adaptability or vitality in metal stress is a supplementary indicator to recognize plants for phytoremediation or phytoextraction (Li *et al*., 2017). Accordingly, all plants selected in this study were found dominating contaminated sites.

4.3.1. *Phytolacca dodecandra* plant characteristics and phytoremediation properties

Phytolacca dodecandra is a plant from the family Phytolaccaceae (Adams *et al*., 1989) and it is native to Madagascar and sub-saharan Africa (Schemelzer and Gurib-Fakim, 2008). This plant is commonly known in Ethiopia with its local name “endod” or named as African soapberry in Ethiopia (Esser *et al*., 2003; Matebie *et al*., 2019). *Phytolacca dodecandra* is also named as ‘Gopa berry’ in some parts of east Africa. It also has several traditional medical and historical

backgrounds in East Africa, especially Ethiopia for its detergent properties and molluscicidal potencies or snail control purposes.

The most important characteristics of this plant that received the researcher's attention is that: *Phytolacca dodecandra* has high adaptability and can grow comfortably producing a high above ground biomass in highly contaminated soil; it is a perennial rapid growing plant with deep root system, unpalatable by livestock and it can re-grow after cutting. This plant was observed to grow and adapt to heavily polluted soil and concentration of heavy metals without showing phytotoxic symptoms such as leaf stunting and tip withering (Chanu and Gupta, 2016; Luo *et al*., 2018).

4.3.1.1.Metal accumulation and distribution pattern of *Phytolacca dodecandra*

Phytolacca dodecandra (mature and seedlings) plants gathered from five different contaminated sites were examined and content of Cr, Pb, Cd, Ni, Cu, and Zn in different parts-root, stem and leaves. *Phytolacca dodecandra* plants can deposit metals in the vegetative tissues. *Phytolacca dodecandra* accumulated larger portions of heavy metals in its roots and substantial portions were distributed in aboveground biomass.

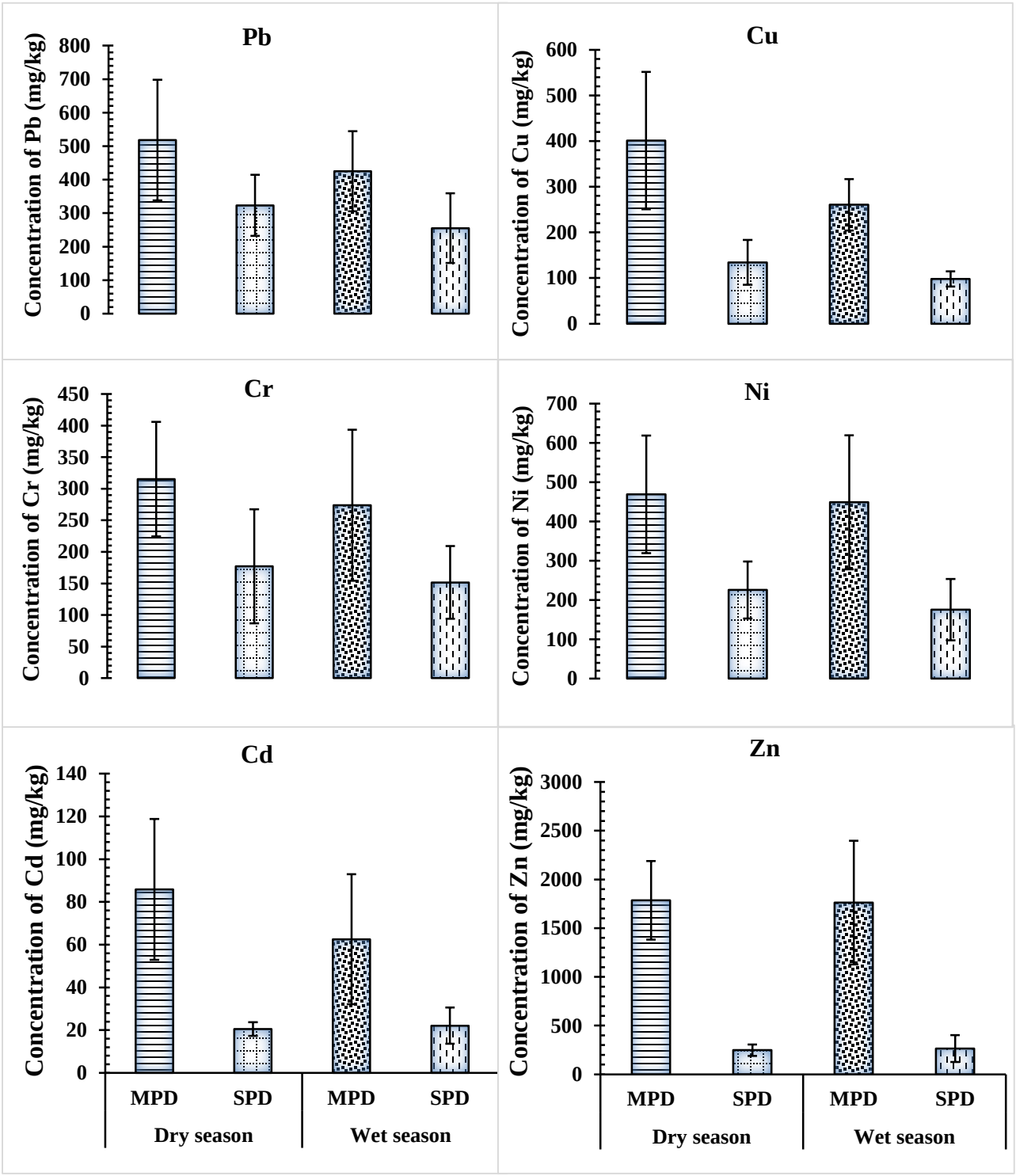
Based on initial investigations, *Phytolacca dodecandra* is capable of taking up and transporting several heavy metals within their above ground tissues and root zone. As it can be seen in Table 5, both seedlings and mature plants of *Phytolacca dodecandra* accumulated analysed metals.

Table 5. Distribution of heavy metals (mg/kg) in different tissues of *Phytolacca dodecandra*.

Metal	Phytolacca /Plant parts	Dry season (mg/kg)		Wet season (mg/kg)	
		Mature plants	Seedlings	Mature plants	Seedlings
Cd	**Root**	24.7 ± 8.62	8.35 ± 2.96	20.32 ± 10.8	6.9 ± 2.24
	Stem	23.3 ± 12.23	5.2 ± 1.92	17.1 ± 7.8	7.9 ± 3.8
	Leaf	37.9 ± 16.1	6.97 ± 1.8	25.12 ± 12.2	7.3 ± 4.1
	Total concentration plant	**85.87 ± 32.95**	**20.53 ± 3.17**	**62.5 ± 30.42**	**22.1 ± 8.5**
Cr	**Root**	139.5 ± 69.4	73.5 ± 34.5	120.78 ± 67.58	65.11 ± 30.2
	Stem	90.7 ± 13.3	55.7 ± 31.5	83.34 ± 24.64	43.51 ± 18.4
	Leaf	85.1 ± 16.1	47.98 ± 27	69.69 ± 34.14	43.03 ± 10.7
	Total concentration plant	**315.3 ± 90.63**	**177.2 ± 90.4**	**273.81 ± 119.5**	**151.65 ± 57.5**
Pb	**Root**	160.9 ± 54.4	92.64 ± 26.5	157 ± 56.1	85.07 ± 23.7
	Stem	132.6 ± 53.6	96.94 ± 20.7	102.5 ±10.8	66.65 ± 19.2
	Leaf	224.6 ± 89.5	133.8 ± 59.4	165.7 ± 57.2	103.4 ± 68.7
	Total concentration plant	**518.11 ± 179.7**	**323.37 ± 90.9**	**425.19 ± 119.5**	**255.14 ± 104**
Zn	**Root**	517.64 ± 113.9	69.34 ± 7.25	577.24 ± 212.6	78.03 ± 38.7
	Stem	528.59 ± 126.7	64.63 ± 26.7	486.9 ± 167.1	65.97 ± 47.7
	Leaf	739.38 ± 171.4	113.69 ± 39.96	697.74 ± 266.8	120.5 ± 68.4
	Total concentration plant	**1785.6 ± 404**	**247.7 ± 59.2**	**1761.8±634.9**	**264.5±137.7**
Ni	**Root**	156.4 ± 60.8	79.25 ± 35	130.7 ± 46.7	50.3 ± 23.6
	Stem	107.1 ± 37.72	64.5 ± 19.53	136.6 ± 47.8	65.54 ± 28.8
	Leaf	205.5 ± 81.6	81.68 ± 35.4	181.4 ± 106	59.6 ± 32.2
	Total concentration plant	**468.9 ± 149.6**	**225.42 ± 72.6**	**448.6 ± 170.5**	**175.4 ±77.9**
Cu	**Root**	151.8 ± 64.5	37.5 ± 12.5	102 ± 32.2	32.1 ± 13.1
	Stem	142.4 ± 60.9	46.1 ± 18.7	85.74 ± 17.04	39.4 ± 7.31
	Leaf	106.9 ± 43.6	50.72 ± 22.5	72.75 ± 16.6	26.55 ± 6.7
	Total concentration plant	**401 ± 150**	**134.3 ± 49.4**	**260.5 ± 56.2**	**98.02 ± 16.6**

NB: Values are grand means of 5 samples with 2 trials for each analysis ± the standard deviation (SD).

However, results observed for metal uptake and translocation patterns in different tissues indicated that there is substantial difference in elemental content and abundance in different tissues. The mean content of Zn (1785.61 ± 404.05 mg/kg) was the largest among analysed trace metals and Cd concentration (62.51 ± 30.42 mg/kg) was the lowest (Table 5).



NB: MPD represents mature *Phytolacca dodecandra* SPD represents seedlings of *Phytolacca dodecandra*

Figure 8. Metal concentration in *Phytolacca dodecandra* plants

Table 6. Mean BCF and TF values of trace metals in different tissues of *Phytolacca dodecandra* in dry season.

Elements	Maturity (Dry- Season)		Biological concentration factor (BCF) *Phytolacca dodecandra*				Translocation factor (TF) *Phytolacca dodecandra*		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	TF aerial
Cd	**mature**	**M ± SD**	0.67±0.30	0.55±0.12	0.93±0.25	**1.48***±0.35	0.98±0.47	**1.53***±0.50	2.50***±0.95**
		Range	0.35-**1.1***	0.43-0.72	0.63-**1.28***	**1.06*-2.00***	0.63-**1.79***	**1.16*-2.32***	1.81*-4.11*
	seedlings	**M ± SD**	0.23±0.07	0.15±0.09	0.20±0.08	0.35±0.16	0.72±0.40	0.92±0.38	1.64***±0.72**
		Range	0.1-0.29	0.07-0.31	0.11-0.29	0.18-0.57	0.12-1.15	0.45-**1.29***	**0.76**-4.11*
Cr	**mature**	**M ± SD**	0.63 ± 0.26	0.42±0.10	0.40±0.15	0.83±0.25	0.72±0.18	0.68±0.214	1.40***±0.4**
		Range	0.37-**1.06***	0.28-0.57	0.24-0.65	0.52-**1.22***	0.41-0.85	0.38-0.97	**0.79**-1.82*
	seedlings	**M ± SD**	0.34±0.17	0.25± 0.14	0.23±0.16	0.48±0.29	0.75±0.13	0.64±0.14	1.38***±0.13**
		Range	0.11-0.52	0.09-0.42	0.06-0.45	0.15-0.81	0.55-0.88	0.52-0.86	1.07*-1.57*
Pb	**mature**	**M ± SD**	0.61±0.21	0.50±0.19	0.79±0.17	**1.30***±0.33	0.86±0.34	**1.202***±0.63	2.06*±**0.96**
		Range	0.34-0.84	0.22-0.68	0.65-**1.08***	0.87-**1.74***	0.46-**1.39***	0.38-**2.10***	**0.84**-3.49*
	seedlings	**M ± SD**	0.35±0.14	0.39±0.17	0.47±0.09	0.86±0.22	**1.14***±0.45	**1.45***±0.48	1.95*±**1.10**
		Range	0.24-0.59	0.23-0.65	0.38-0.59	0.63-**1.17***	0.71-**1.81***	0.89-**1.69***	**0.35**-2.25*
Zn	**mature**	**M ± SD**	**1.79***±0.3	**1.85***±0.38	**2.56***±0.41	**4.40***±0.77	**1.02***±0.09	**1.43***±0.04	2.45*±**0.06**
		Range	**1.49*2.12***	**1.3*-2.21***	**2.13*-2.98***	**3.5*-5.18***	0.87-**1.08***	**1.39*-1.48***	2.35*-2.51*
	seedlings	**M ± SD**	0.25±0.08	0.24±0.15	0.40±0.15	0.64±0.26	0.92±0.31	**1.46*** ± 0.44	2.35* ± **0.37**
		Range	0.19-0.39	0.11-0.48	0.21-0.57	0.35-**1.05***	0.5-**1.24***	**1.14*-2.21***	1.91*-2.71*
Ni	**mature**	**M ± SD**	0.65±0.22	0.45±0.12	0.82±0.34	**1.27***±0.37	0.69±0.07	**1.42***±0.633	2.09*±**0.68**
		Range	0.37-0.98	0.28-0.59	0.49-1.26	0.9-1.79	0.6-0.77	0.5-1.89	**1.1-2.62**
	seedlings	**M ± SD**	0.37±0.28	0.31±0.18	0.36±0.23	0.67±0.39	0.89±0.34	**1.07***±0.45	1.76*±**0.82**
		Range	0.16-0.86	0.09-0.50	0.14-0.68	0.30-**1.18***	0.56-**1.28***	0.72-**1.65***	**0.54**-2.65*
Cu		**M ± SD**	**1.18***±0.54	**1.05***±0.24	0.83±0.39	**1.88***±0.61	**1.01***±0.39	0.73±0.14	1.74***±0.49**
		Range	0.59-**1.95***	0.85-**1.36***	0.45-**1.47***	**1.36*-2.83***	0.58-**1.55***	0.49-0.84	1.07*-2.32
	seedlings	**M ± SD**	0.31±0.13	0.36±0.14	0.39±0.17	0.75±0.25	**1.24***±0.29	**1.34***±0.34	2.58***±0.44**
		Range	0.12-0.43	0.17-0.54	0.18-0.61	0.35-0.95	0.72-**1.43***	0.76-**1.64***	2.11*-3.01*

***NB: M* ± SD** stands for mean ± standard deviations, **N=5** **Bold** and asterisks **(*)** denote values are **> 1**

Table 7. Mean BCF and TF values of trace metals in different tissues of *Phytolacca dodecandra* in wet season.

Element	Maturity (Wet- Season)		Biological concentration factor (BCF) *Phytolacca dodecandra*				Translocation factor (TF) *Phytolacca dodecandra*		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	**TF aerial**
Cd	**mature**	**M ± SD**	0.91±0.66	0.76±0.50	**1.09***±0.71	**1.85***±1.21	0.91±0.24	**1.29***±0.25	**2.20***±0.47
		Range	0.04-**1.97***	0.35-**1.60***	0.11-**2.29***	0.93-**3.89***	0.71-**1.33***	**1.07*-1.64***	**1.78***-2.97
	seedlings	**M ± SD**	0.32±0.17	0.37±0.23	0.32±0.24	0.89±0.45	**1.10***±0.35	**1.06***±0.47	**2.16***±0.48
		Range	0.10 -0.5	0.08-0.59	0.16-0.74	0.25-1.32	0.74-**1.62***	0.69-**1.62***	**1.45*-2.65***
Cr	**mature**	**M ± SD**	0.77± 0.27	0.60± 0.30	0.44± 0.19	**1.054***± 0.44	0.76 ± 0.17	0.594± 0.22	**1.35*** ± 0.28
		Range	0.45-**1.14***	0.3 - 0.98	0.25 - 0.65	0.56 - **1.63***	0.52 - 0.92	0.36 - 0.87	0.94 - **1.71***
	seedlings	**M ± SD**	0.41±0.14	0.30± 0.15	0.31 ± 0.15	0.61± 0.274	0.70± 0.23	0.73± 0.20	**1.44** ± 0.34
		Range	0.26- 0.57	0.16 - 0.51	0.13 - 0.45	0.31 - 0.96	0.56 - 0.06	0.51 - 0.95	**1.19*- 2.01***
Pb	**mature**	**M ± SD**	0.79±0.22	0.48±0.12	0.83±0.17	**1.31***±0.23	0.64±0.183	**1.07***±0.17	**1.71***±0.28
		Range	0.55-**1.12***	0.42-0.73	0.63-**1.03***	**1.1*-1.53***	0.48-0.96	0.86-**1.24***	1.34*-2.01*
	seedlings	**M ± SD**	0.44±0.12	0.34±0.11	0.51±0.27	0.85±0.33	0.78±0.05	**1.15***±0.58	**1.93***±0.58
		Range	0.25-0.57	0.18-0.46	0.17-0.9	0.35-**1.24***	0.72-0.85	0.67-**2.11***	**1.63*-2.9***
Zn	**mature**	**M ± SD**	**2.10*±** 0.83	**1.81** ± 0.73	**2.57*± 1.18***	**4.36*** ± 1.91	**0.87** ± 0.12	**1.21***± 0.08	**2.08** ± 0.16
		Range	**1.48*-3.48***	**1.08*-2.98***	**1.85*-4.63***	**2.96*-7.61***	0.67-0.99	**1.12*-1.33***	1.84-2.24
	seedlings	**M ± SD**	0.30±0.14	0.24±0.14	0.41±0.164	0.65±0.28	0.78±0.31	**1.81***±1.44	**2.60±1.41**
		Range	0.08-0.44	0.05-0.39	0.31-0.70	0.4-**1.09***	0.46-**1.25***	0.74-**4.33***	**1.20*-4.91***
Ni	**mature**	**M ± SD**	0.83±0.66	0.81±0.57	0.98±0.51	**1.79***±1.03	**1.07***±0.25	**1.44***±0.74	**2.50***±0.95
		Range	0.28-**1.94***	0.35-**1.80***	0.42-**1.60***	0.77-**3.4***	0.73-**1.34***	0.82-**2.66***	**1.65*-4.00***
	seedlings	**M ± SD**	0.29±0.15	0.39±0.24	0.31±0.12	0.7±0.32	**1.32***±0.28	**1.28***±0.56	**2.60***±0.43
		Range	0.06-0.47	0.07-0.67	0.14-0.42	0.21-**1.08***	**1.05*-1.75***	0.74-**2.17***	**2.30*-3.34***
Cu	**mature**	**M ± SD**	0.68±0.23	0.59±0.24	0.49±0.13	**1.08***±0.36	0.88±0.19	0.76±0.26	**1.44***±0.31
		Range	0.32-0.87	0.31-0.92	0.35-0.65	0.68-**1.57***	0.67-**1.15***	0.52-**1.18***	**1.16*-1.96***
	seedlings	**M ± SD**	0.21±0.09	0.27±0.11	0.18±0.08	0.43±0.21	**1.37***±0.44	0.92±0.34	**2.29***±0.77
		Range	0.1-0.30	0.17-0.44	0.102-0.26	0.18-0.7	0.61-**1.73***	0.37-**1.25***	0.98-**2.98***

***NB: M* ± SD** stands for mean ± standard deviations **Bold** and asterisks **(*)** denote values are **> 1**

a. Cd concentration in different parts of *Phytolacca dodecandra*

Table 4 shows mean uptake values of Cd in root and aerial parts of *Phytolacca dodecandra* during dry and rainy seasons, respectively. Uptake for root ranged from 10.6 - 33.02 mg/kg for dry and 7 - 37.00 mg/kg for wet seasons. There is also large difference in absorption of heavy metals among plants from different sites. Mature *Phytolacca dodecandra* plants were found to uptake and transfer large amounts of Cd in their aerial parts as compared to seedlings and this could be due to Cd dilution within the plant throughout its growth (Ismael *et al*., 2019). The maximum uptake of 123.1 mg/kg Cd was noted in mature plant samples taken from samples at Lideta site during dry season and the minimum Cd of 2.34 mg/kg was noted from samples taken from the control sample site. The largest uptake of 33.18 mg/kg seedling was recorded in samples at Kera site and wet season and the minimum of 1.43 mg/kg was recorded for control site samples during wet season. Liu *et al.* (2010) reported *Phytolacca americana* can grow in heavily contaminated soils with Cd level of more than 1083mg/kg at Datianwan site. Liu *et al.* (2010) also reported *Phytolacca americana* can accumulate 637 mg/kg and 402 mg/kg of Cd in their aerial parts and leaves respectively.

The grand mean results of cadmium 24.71 ± 8.6 mg/kg, 23.3 ± 12.2 mg/kg and 37.9 ± 16.1 mg/kg were recorded for the uptake in root, stem and leaf of mature plants *Phytolacca dodecandra* respectively; during dry season (Table 5). In addition, means of Cadmium uptake by seedlings of *Phytolacca dodecandra* was also computed as 8.35 ± 2.96 for root, 5.2 ± 1.92 for stem, and 6.98 ± 1.75 mg/kg for leaves, which is in order of root > leaves > stem. Mean and standard deviations of Cadmium uptake in root, stem and leaf of mature *Phytolacca dodecandra* recorded in wet season samples were 20.32 ± 10.8, 17.07 ± 7.8 and 25.12 ± 12.2 mg/kg, respectively. Values recorded for the mean uptake in root, stem and leaf of seedlings are also in order of 6.9 ± 2.24, 7.9 ± 3.8 and 7.3 ± 4.1 mg/kg (Table 5).

The results were further interpreted using BCF and TF, mean BCF of Cd in root, stem and leaves of mature *Phytolacca dodecandra* plant in dry season are in order of leaf (0.932 ± 0.25) > root (0.67 ± 0.30) > stem (0.552 ± 0.12) and that of seedlings showed, in roots (0.23 ± 0.074) > leaf (0.196 ± 0.08) > stem (0.15 ± 0.094). Biological concentration factors of Cd computed for the harvestable portion or BAC (shoot = 1.484 ± 0.35) and aerial translocation factors (2.50 ± 0.95) were found to be > 1 showing suitability of *Phytolacca dodecandra* for phytoextraction of Cd,

while values computed for seedlings shoot BCF (0.35 ± 0.16) and aerial TF (1.644 ± 0.72) showed BCF < 1 and TF > 1 (Table 6). Similarly, rainy season samples were also subjected to computation of BCF and TFs and results showed BCF of Cd in leaf (1.09 ± 0.71) > root (0.91 ± 0.66) > stem (0.764 ± 0.50). Whereas, seedlings showed BCF of Cadmium in stem > leaf > root or 0.366 ± 0.21 > 0.324 ± 0.24 > 0.318 ± 0.171, respectively which are all less than one, however the aerial TF was found to be 2.20 ± 0.47 which is >1 (Table 7). The mean leaf BCF of mature plants was 0.93 ± 0.25 in dry season and 1.09 ± 0.71 in wet season, and the mean values are ranging from 0.63-1.28 for dry and 0.51- 2.29 for wet. During wet season, 40% of plants samples had BCF leaf > 1, which is slightly greater than the 29% noted by Liu *et al*. (2010) in leaf of *Phytolacca americana.*

In general, Cd uptake and accumulation properties tend to increase with the maturity of the plants, to be precise; the mature plants accumulate more heavy metals in their tissues than seedlings. Mean difference test (t-test) to assess the difference between mature and seedlings of *Phytolacca dodecandra* plants t (12.19) = 3.72, p = 0.003 revealed a significant difference in mean Cd accumulated in mature (M = 62.39 mg/kg ± 40.18) and seedlings (18.07 mg/kg ± 9.35).

In addition, Cd accumulation in the aerial parts of *Phytolacca dodecandra* seedlings tends to be greater during the rainy season. This can be further elucidated by BCF of shoot ranging from 0.18-0.57 and aerial TF ranging 0.76-4.11 for dry season seedling samples and the mean shoot BCF ranging from 0.25-1.32 and mean TF values ranging from 1.45-2.65 for seedling samples collected in rainy season. However, seasonal variation in Cd uptake and accumulation patterns has no considerable differences. Values computed for means of shoot BCF or BAC (1.484 ± 0.35) and aerial TF (2.504 ± 0.953) of dry season samples and shoot BCF 1.854 ± 1.21 and aerial TF 2.20 ± 0.47 for the wet season can be observed. In addition, ANOVA of p = 0.087 > 0.05 revealed that the seasonal variation has insignificant effect on Cd uptake patterns.

Samples of mature plants and seedlings of *Phytolacca dodecandra* from the same soil showed wide variation in metal concentration. The correlation between Cd accumulation in mature *Phytolacca dodecandra* and Cd concentration in soil was statistically significant. Which could be explained by significant value of p = 0.003 at level of 0.01, while that of seedling showed value of p = 0.103 which is not statistically significant. The impact of pH on the Cd accumulation pattern of *Phytolacca dodecandra* was indirect both in dry and rainy season, an increase in pH of soil results in a decrease in Cd uptake which is concurring with Feng *et al*. (2011). However,

correlation coefficient of r (-0.501), p = 0.311 and r (-0.544), p = 0.264 for mature and seedlings of *Phytolacca dodecandra* indicates intermediate negative relationship which is not significant. An increase in EC may have also positively impacted Cd uptake in seedlings of *Phytolacca dodecandra* both in dry season r (0.863), p = 0.027 and wet season r (0.94), p = 0.006. However, positive and insignificant relationship was noted between EC of soil and uptake of Cd by *Phytolacca dodecandra* plant $p > 0.05$ plants.

Other analysed physicochemical parameters (CEC, soil texture, MC and OM) showed insignificant relationship ($p > 0.05$) with the uptake and accumulation of Cd by *Phytolacca dodecandra*. In summary, the value of TF, shoot BCF or BAC > 1 for mature plants all together point toward the conclusion that *Phytolacca dodecandra* can be a promising plant for decontamination of Cd polluted sites. According to Hesami *et al*. (2018), trace metal concentration of plants having lower root BCF and higher TF can be estimated by Enrichment Factor (EF) which is a product of TF and roots BCF, consequently, the values computed for Cd in *Phytolacca dodecandra* gave EF of > 1; therefore, *Phytolacca dodecandra* could be potential plant for remediation of Cd (Ghavri *et al*., 2013).

b. Cr concentration in different parts of *Phytolacca dodecandra*

Findings from this study revealed that *Phytolacca dodecandra* can take up and distribute Cr in its different parts. The maximum grand mean of 315.3 ± 90.63 mg/kg Cr uptake was recorded in dry season for mature plants out of which the largest mean of 139.45 ± 69.4 mg/kg was recorded in root followed by means of 90.7 ±13.3 mg/kg and 85.12 ±16.1 for stem and leaf, respectively. Similarly, seedlings showed maximum total mean of 177.2 ± 90.4 in the entire plant while mean values of 73.5 ± 34.5 mg/kg, 55.72 ± 31.49 mg/kg and 47.98 ± 27.02 mg/kg Cr were recorded in roots, stems and leaves, respectively.

Phytolacca dodecandra plant samples collected during rainy season showed slightly lower uptake and accumulation characteristics for Cr, which is not statistically significant. Cumulative mean of 273.8 ± 119.5 mg/kg and 151.7 ± 57.5 mg/kg Cr were recorded for mature and seedling plants, respectively. Distribution of Cr in different parts of *Phytolacca dodecandra* can be observed from mean recordings of 120.8 ± 67.6 mg/kg, 83.34 ± 24.64 and 69.7 ± 34.14 for root, stem and leaves of mature plants, respectively. Likewise, mean values of Cr recorded for root, stem and leaves of

Phytolacca dodecandra seedlings in wet season are in order of root (65.1 ± 30.2 mg/kg) > stem (43.51 ± 18.4 mg/kg) > leaf (43.03 ± 10.7 mg/kg).

The mean values recorded for both mature and seedlings of *Phytolacca dodecandra* plants both during dry and wet seasons revealed that the plant has tendency to retain most of the Cr absorbed in their roots. BCF values recorded were 0.63 ± 0.26, 0.422 ± 0.103 and 0.404 ± 0.15 for root, stem and leaf, respectively in dry season and mean BCF values computed for wet season samples were also 0.77 ± 0.27, 0.604 ± 0.30 and 0.442 ± 0.193 for root, stem and leaf, respectively.

However, even though, mean aerial TF values of 1.40 ± 0.38 for mature plants and 1.38 ± 0.13 for seedlings collected during dry season and the TF values 1.35 ± 0.28 and 1.44 ± 0.34 computed for the Cr content in mature and seedlings of *Phytolacca dodecandra* in rainy season are all > 1, values of BCF < 1 computed for root, stem and leaves of the plant, limits reliability of *Phytolacca dodecandra* for remediation of Cr contaminated sites. However, based on BCF of shoot or BAC (1.054 ± 0.44) recorded in mature plants during wet season the possibility to harvest (Marques *et al*., 2009) and dispose the aboveground parts and allowing re-growth can be considered for remediation purpose.

However, chromium levels in leaves remained quite low in all seasons and at all levels of chromium in soil. And *Phytolacca dodecandra* plants predominantly retained substantial amounts of Cr in their roots. Cr concentration in different tissues of *Phytolacca dodecandra* was: root > stem > leaf. The content of Cr in *Phytolacca dodecandra* increases with metal content in soil. Concentration soil Cr level on the uptake of Cr in *Phytolacca dodecandra* can be elucidated by the correlation coefficient r (0.834), $p = 0.001 < 0.01$ and value r (0.64), $p = 0.023 < 0.05$ for mature plants and seedlings of *Phytolacca dodecandra*, respectively. This is in concurrence with Jadia and Fulekar. (2009) who mentioned that an increase in metal concentration of growing medium results in a linear response in concentration of the metal in plants.

Uptake of Cr was negatively impacted by soil pH; plants samples obtained from high pH soil absorbed lower Cr than those from acidic soil. Positive impacts of soil physicochemical parameters including CEC, EC, OM%, MC% and texture on Cr uptake were statistically insignificant $p > 0.05$ for dry and wet seasons. In addition, statistical analysis for mean comparison $t(21) = 2.552$, $p = 0.019$ revealed that mature plants and seedlings of *Phytolacca dodecandra* had significant

differences. While majority of accumulated Cr, both in mature and seedlings of this plant were sequestered in the root.

c. Pb concentration in different parts of *Phytolacca dodecandra*

Lead concentration in *Phytolacca dodecandra* plants varied among sampling sites $p < 0.05$, and the maximum Pb concentration was noted at Lideta site (795.13 mg/kg) followed by Kera site (543.07 mg/kg) and Mekanisa site (512.14 mg/kg). Likewise, *Phytolacca dodecandra* plant samples collected during wet season gave the maximum uptake at Mekanisa site 551.51 mg/kg followed by Kera site 548.57 mg/kg and Lideta site (390 mg/kg). Although accumulation of Pb in some soils were higher there were lower uptake levels by plants; this could be due to limited availability of Pb attributable to its strong association with clay particles, organic matter, precipitation as carbonate and other environment factors (Shen *et al*., 2002).

However, *Phytolacca dodecandra* can take up and remove Pb from contaminated soil. Plant samples showed slightly higher potential of Pb uptake during dry season than that of rainy season samples for both seedlings and mature plants. The highest accumulation in dry season was in the leaves with metal concentration of 224.61 ± 89.46 mg/kg followed by root 160.88 ± 54.43 mg/kg and stem 132.62 ± 53.62 mg/kg for mature plants. The same previous trend was observed for mature *Phytolacca dodecandra* collected during wet season, with the highest Pb accumulation 165.68 ± 57.15 mg/kg recorded for leaves followed by roots 157.02 ± 56.06 mg/kg and stem 102.49 ± 10.80 mg/kg.

Similarly, seedlings gave maximum transfer in their leaves 133.79 ± 59.39 mg/kg followed by stem 96.94 ± 20.69 mg/kg and root 92.64 ± 26.52 mg/kg during dry season. While, during rainy season highest accumulation of Pb (103.42 ± 68.66 mg/kg) was noted in seedlings leaves, followed by root 85.07 ± 23.72 mg/kg and stem 66.65 ± 19.23 mg/kg. In the present finding, Pb absorbed by roots of *Phytolacca dodecandra* was supposedly transported and accumulated both in stems and leaves which is not concurring with Gupta *et al*. (2013) who noted most absorbed Pb remains in root.

In addition, results from ANOVA $p = 0.172$ and 0.237 for mature plants and seedlings, respectively indicated seasonal variation had insignificant effect on absorption and concentration

pattern of Pb in *Phytolacca dodecandra*. However, the mean difference test t (22) = 2.50, $p = 0.020$ show the mean concentration of Pb uptake by mature plants significantly exceeds the value recorded in the seedlings, which could be explained as metal uptake is a continuous process.

It is also evident from BCF and TF values that *Phytolacca dodecandra* can extract and accumulate Pb in its different parts. BCF of Pb in tissues of *Phytolacca dodecandra* in dry season is in order of leaf (0.794 ± 0.17) > root (0.612 ± 0.21) > stem (0.504 ± 0.19) and that of wet season is in order of leaf (0.825 ± 0.173) > root (0.79 ± 0.22) > stem (0.48 ± 0.12). The values BCF are slightly higher during the rainy season the BCF of Pb in shoot of *Phytolacca dodecandra* ranged from 0.87-1.74 and the mean BCF of shoot or BAC value computed was 1.30 ± 0.33 in dry season, while values recorded in rainy season ranged from 1.1-1.53 and the mean value computed was 1.31 ± 0.23 (Table 7).

Further, relationship between Pb available in soil versus Pb contained in mature plants and seedlings of *Phytolacca dodecandra* was presented using Pearson correlation coefficient. The correlation coefficients and probability values r (0.808), $p = 0.001$ and r (0.819), $p = 0.001$ were recorded for mature plants and seedlings of *Phytolacca dodecandra*, respectively. Therefore, it has been recognized that, Pb concentration in soil positively significantly ($p < 0.01$) affected plant metal uptake pattern. This relatively high positive correlation (R^2 =0.6529) highlights Pb concentration in soil which could explain 65.29% amount in mature *Phytolacca dodecandra*, irrespective of other factors.

The effect of different soil physicochemical parameters was evaluated using Pearson correlation. For instance, pH had an indirect relationship with the Pb in *Phytolacca dodecandra* both in dry r (-0.463), $p = 0.36$ and wet season r (-0.536), $p = 0.273$ which indicates a lower pH will enhance Pb uptake but the impact was insignificant for both. However, the impact of pH was significant on seedlings especially for dry season samples (r = - 0.89, $p = 0.018$). This is in agreement with findings of Nanda and Abraham. (2013). Further, EC, CEC and clay fraction had insignificant positive effect on uptake of Pb ($p > 0.05$).

In general, the BCF and TF larger than 1 indicated that *Phytolacca dodecandra* is capable of taking up Pb and accumulating Pb in above ground parts, especially in the harvestable portions of the plant. Aerial TF of 2.06 ± 0.96 for mature plants and 1.95 ± 1.094 for seedlings dry season and the

mean values 1.71 ± 0.28 for mature plants and 1.93 ± 0.58 for seedlings in wet season, presented in Table 6 and Table 7, indicates the suitability of this plant to remove Pb from polluted sites via phytoextraction mechanism.

d. Zn concentration in different parts of *Phytolacca dodecandra.*

Zinc is an essential element that plants require in low concentrations (Jadia and Fulekar, 2008). It is an available element for plant uptake found abundantly in soil; relatively mobile and most plants absorb this metal using their special zinc transporters (Jadia and Fulekar, 2008; Kumar and Thakur, 2019). Excessive level of Zn could result in retarded plant growth, senescence and leaf chlorosis (Golubev, 2011).

Based on the present investigation, *Phytolacca dodecandra* was able to accumulate Zn and can bring down the pollution load in soil. Zn uptake by the whole plant tends to increase with the maturity of plants, and similarly uptake of Zn by *Phytolacca dodecandra* increases with the concentration of Zn in the soil. Roots of the plant were observed to have characteristics that can remove Zn from soil. The total mean Zn uptake by mature *Phytolacca dodecandra* collected during dry season was 1785.61± 404.05 mg/kg while that of seedlings is 247.66 ± 59.15mg/kg. In the same way, wet season samples showed mean concentrations recordings of 1761.84 ± 634.91 mg/kg and 264.49 ± 137.74 mg/kg for mature plants and seedlings, respectively (Table 5). ANOVA results suggested that there is insignificant (p > 0.05) difference in Zn accumulation patterns between samples collected during dry and in rainy season.

Accumulation patterns of Zn in different parts of *Phytolacca dodecandra* are in order of leaf (739.4 ± 171.4 mg/kg) > stem (528.6 ± 126.7 mg/kg) > root (517.6 ± 113.9 mg/kg). During rainy season Zn was taken up in the following order: leaf 697.7 ± 266.8 mg/kg > root 577.2 ± 212.6 mg/kg > stem 486.9 ± 167.1 mg/kg for mature plants and leaf 120.5 ± 68.4 mg/kg > root 78 ± 38.7 mg/kg > stem 65.97 ± 47.7 mg/kg for seedlings (Table 5). This is in concurrence with Drew *et al*. (1987), who reported Zn accumulation mostly occurs in actively growing tissues like young leaves and shoot.

The mean values of total Zn accumulated in *Phytolacca dodecandra* showed mature plants can accumulate six-fold more Zn from the soil. Significant correlation was observed from a correlation coefficient value of r (0.767), p = 0.004. However, Pearson correlation r (0.405), p = 0.191 > 0.001

showed Zn concentration in soil had insignificant effect on uptake of Zn in seedlings of *Phytolacca dodecandra*.

Higher mean values recorded for BCF (4.40 ± 0.773) and aerial TF (2.45 ± 0.062) computed for mature plants in dry season and the mean values of BCF 4.36 ± 1.91 and aerial TF of 2.08 ± 0.16 for wet season samples (Table 6 and 7) showed *Phytolacca dodecandra* plants can be utilized phytoremediation of Zn through phytoextraction mechanism. The highest shoot BCF (7.61) was noted at Lideta site in rainy season and maximum shoot BCF noted in dry season was noted for Akaki site.

BCF of Zn in different tissues of *Phytolacca dodecandra* ranged between 2.13 - 2.98, 1.3 - 2.21, 1.49 - 2.12 for leaves, stems and roots, respectively. Whereas, the mean values of Zn BCF were in order of leaf 2.56 ± 0.41 > stem 1.85 ± 0.38 > root 1.794 ± 0.3 (Table 6). Similarly, wet season samples had BCF ranging 1.85 - 4.63, 1.08 - 2.98 and 1.48 - 3.48 for leaves, stems and roots, respectively. The mean values achieved in each compartment were in order of leaf (2.57 ± 1.18) > root (2.10 ± 0.83) > stem (1.81 ± 0.73) as noted in Table 7. The BCF values noted for seedlings of *Phytolacca dodecandra* were all < 1 both in dry and wet season. However, mean values of TF recorded were > 1; this could be explained as the uptake and transfer of Zn into the vegetative tissues and young leaves of seedlings were higher in the initial growing season.

e. Ni concentration in different parts of *Phytolacca dodecandra.*

Nickel is one of biologically important metals, and it is vital component required in limited amount for growth and metabolic activities of plants (Panda and Choudhury, 2005; Kumar *et al*., 2019). Elevated concentrations of Ni in soil did not affect *Phytolacca dodecandra;* the plant remains green and healthy and removal level increased with metal concentration in soil. Seedlings of this plant are also able to uptake nickel at a lower concentration.

Seasonal variation showed insignificant effect on Ni uptake capabilities of *Phytolacca dodecandra* $p > 0.05$. Leaves of mature plants and seedlings of *Phytolacca dodecandra* showed higher accumulation of Ni both during dry and wet seasons. Mature plants removed mean concentration of 448.64 ± 170.48 mg/kg during rainy season and 468.99 ± 149.6 mg/kg in dry season. The mean values of Ni concentrated in seedlings of *Phytolacca dodecandra* were 175.38 ± 77.86 mg/kg during wet season and 225.42 ± 72.59 mg/kg during dry seasons (Figure 8). The lowest uptake of

98.7 mg/kg was recorded at non-contaminated control sample site and while highest uptake of 683.15 mg/kg Ni was recorded at Mekanisa sample site during dry season. Similarly, during wet season, lower Ni concentration 184.6 mg/kg was noted for control plants and highest mean recording of 689 mg/kg was noted for plants at Kera sample site.

The distribution level of Ni in *Phytolacca dodecandra* tissues was in the following order: leaves (205.5 ± 81.6) > root (156.4 ± 60.8) > stem (107.1 ± 37.7) for dry season samples and leaves (181.4 ± 106) > stem (136.6 ± 47.8) > root (130.7 ± 46.7) for rainy season (Table 5). And concentration of Ni in seedlings of *Phytolacca dodecandra* showed same trend. The concentration of Ni in soil had positive but insignificant ($r = 0.32$, $p = 0.31 > 0.05$) impact on the uptake and accumulation properties of seedlings but the concentration of Ni in mature plants significantly increased with soil concentration ($r = 0.644$, $p = 0.024$). The independent sample t- test for comparison of the differences between means of Ni in mature plants ($M = 405.95$, $SD = 185.41$) and seedlings ($M = 178.75$, $SD = 85.18$) plants of *Phytolacca dodecandra* revealed, $t(15.45) = 0.01$, which indicates significance of the difference.

BCF of Ni in different tissues of *Phytolacca dodecandra* showed higher mean values during rainy season. BCF noted during dry season showed higher removal rate in leaf of *Phytolacca dodecandra* and the BCF values noted for root, stem and leaves ranged between 0.37-0.98, 0.28-0.59 and 0.9-1.79, respectively (Table 6). The shoot BCF or the BAC was found to be more than 1, the mean value recorded was 1.27 ± 0.37. Maximum (1.79) shoot BCF was recorded in site 3 and the minimum (0.9) was noted in site 1. Comparison using the TF also showed the higher TF of Ni was contributed by leaves and mean value computed was 1.42 ± 0.63 and aerial TF of 2.094 ± 0.68 was noted.

Similarly, in wet season BCF values ranging between 0.28-1.94, 0.35-1.80 and 0.42-1.60 were found in root, stem and leaves of *Phytolacca dodecandra* (Table 7). The maximum (3.4) and minimum (0.77) values of BAC were recorded at Hana Mariam site and Lideta site, respectively. The mean value of 1.794 ± 1.03 was noted for BAC of Ni in *Phytolacca dodecandra* in wet season. In addition, aerial TF were found to be more >1 in all samples with minimum and maximum TF values of 1.65 and 4, respectively.

In general, based on shoot metal concentration EF and TF values > 1 *Phytolacca dodecandra* can be utilized for phytoremediation of Ni, while the phytoextraction mechanism seems to be the most appropriate technique. However, even though TF values of > 1 were recorded for seedlings of *Phytolacca dodecandra* the EF or shoot metal concentration of < 1 reveals, seedlings shall grow to mature plants for better removal.

f. Cu concentration in different parts of *Phytolacca dodecandra*

Copper is a trace element required in a low concentration and hence mentioned as micronutrient and it serves as structural component in regulatory proteins (Jadia and Fulekar, 2008; Furini, 2012). However, excessive amount of Cu results in phytotoxicity problem on plants (Adrees *et al*., 2015; Lin *et al*., 2019).

Findings from this research have indicated the following Cu concentration ranges: mature plants in dry season (106.9 -600.5 mg/kg), mature plants in wet season 91.1-350 mg/kg, seedlings in dry season (49.3-197 mg/kg), and seedlings in rainy season (34.8-109.1 mg/kg). It appears that, the largest share of Cu removed by *Phytolacca dodecandra* remains in the root tissue. Therefore, distribution of Cu in plant tissue was in the order of root > stem > leaf for mature plants in both season and seedlings leaf > stem > root in dry season and stem > root > leaf in wet season. In addition, it was found that seasonal variation had considerable effect on the Cu uptake patterns of *Phytolacca dodecandra.* Total mean of 401.1 ± 150.2 mg/kg and 134.3 ± 49.4 mg/kg recorded for mature and seedling plants of the dry season and values of 260.5 ± 56.2 mg/kg and 98.02 ± 16.6 mg/kg recorded for mature and seedling plants in wet season (Table 5).

Plants from control site also absorbed considerable amount of Cu in their tissues; which could be due to an increase in availability and in-plant mobility of Cu attributed to fertilizer application in nearby sites (Yang *et al*., 2005). Cu accumulated in mature plants of *phytolacca dodecandra* was significantly higher than those recorded for seedlings. The value of T test indicated t (13.12) = 4.201, p = 0.001, M ± SD = 292.15 mg/kg ± 148.30 of Cu in mature plants is significantly higher than M ± SD = 103.82 mg/kg ± 46.13 of Cu in seedlings reveals that there is continuous uptake of Cu in *Phytolacca dodecandra* plants (Ebere *et al*., 2016).

The level of Cu in corresponding soil had insignificant effect on the metal uptake in *Phytolacca dodecandra.* Correlation coefficient between Cu concentration of soil and Cu uptake in plant r

(0.53), $p = 0.077$ for mature plants and seedlings r (0.423), $p = 0.171$ revealed there is insignificant positive relationship. This was not in concurrence with Cui *et al*. (2004), who noted metal accumulation in plant is a function of metal level in corresponding medium.

Values of BCF supported the higher concentration in shoot, which is attributable to the larger biomass of the shoot than root. The uptake and concentration of Cu in the aboveground parts of *Phytolacca dodecandra* explained by computation of TF and EF or shoot BCF of more than 1 shows remediation of Cu contaminated soil can be approached through *Phytolacca dodecandra* uptake measures or phytoextraction. However, the BCF values recorded in root (1.18 ± 0.54) > Stem (1.05 ± 0.24) > leaf (0.83 ± 0.39) and root (0.68 ± 0.23) > stem (0.59 ± 0.24) > leaf (0.494 ± 0.13) were noted for mature plants in dry and rainy season sample, respectively. Further, the BCF and TF s computed for seedlings of *Phytolacca dodecandra* revealed BCF in roots, stems and leaves are all less than 1. However, larger TF values were recorded and the mean aerial TF's of 2.29 ± 0.77 and 2.58 ± 0.44 were recorded for seedlings of *phytolacca dodecandra* during wet and dry season respectively (Table 6 and Table 7).

4.3.1.2. Summary of metal accumulation status in *Phytolacca dodecandra*

Present investigation revealed *Phytolacca dodecandra* can grow and regenerate under stress exerted by multi-metal contamination. The plant can absorb and transport significant levels of heavy metals into its aboveground tissues. Plants collected from a metal-contaminated industrial environment accumulated more metals in their tissues than plants from a common environment used for reference or control.

The mean levels of metals in *Phytolacca dodecandra* in dry season were in the following order; Zn (1785.6 ± 404.1) > Pb (518.1 ± 179.7) > Ni (468.99 ± 149.6) > Cu (401.1±150.2) > Cr (315.3 ± 90.6) > Cd (85.9 ± 32.95) in mg/kg. The trace metal accumulation in *Phytolacca dodecandra* during wet season showed the following order; Zn (1761.8 ± 634.9) > Ni (448.6 ± 170.5) > Pb (425.2 ± 119.5) > Cr (273.8 ± 119.5) > Cu (260.5 ± 56.2) > Cd (62.5 ± 30.4) in mg/kg (Figure 8). Higher concentrations of metals (lead, cadmium, zinc and nickel) were found in aboveground tissues or predominantly in the leaf of *Phytolacca dodecandra* collected from polluted sites. On the contrary, chromium and copper were abundant in below ground tissues (roots) of *Phytolacca dodecandra.* Zn also attained its largest concentration in *Phytolacca dodecandra* plants both in dry

and rainy season; this could be associated to the relatively higher mobility of Zn in soils and its abundance in soil.

The present finding reveals; *Phytolacca dodecandra* could be used for phytoremediation of analyzed trace metals. The potential of *Phytolacca dodecandra* to clean-up metal contaminated sites is promising, based on values of BCF >1 and TF > 1. However, mechanisms involved for remediation of metals vary based on the type of trace metal under investigation. Among the studied trace metals *Phytolacca dodecandra* can remove Cd, Pb, Zn, Ni and Cu via phytoextraction mechanism, however; Cr was not efficiently accumulated by *Phytolacca dodecandra*. Further, pot experiments with artificial contamination (increasingly adding contaminant of interest) could give a better information on the maximum metal concentration levels that the plant could tolerate.

4.3.2. *Adhatoda schimperiana* plant characteristics and phytoremediation properties

Adhatoda schimperiana (local name: simiza/sensel) is characterized by producing biomass faster than most other plants growing around it. It is a shrub, up to 5 m height, stems and branches can grow up to 1.5 cm thick (Janses, 1981). Households commonly use this plant as a fence or green barrier and for medical purpose; and the plant can be propagated by seed or cuttings (Janses, 1981). *Adhatoda schimperiana* has been widely known in Ethiopia for its rapid growth, medicinal values; especially for malaria treatment (Abdela *et al*., 2019; Bobasa *et al*., 2018), erosion control and fencing.

The plant could grow in a variety of climatic conditions, and it can grow and appear green the entire year. This plant can also grow in multi-metal contaminated soils. Massive and deep root systems support quick growth and its ability to consume large quantities of water enhance metal removal.

4.3.2.1. Metal accumulation and distribution pattern of *Adhatoda schimperiana*

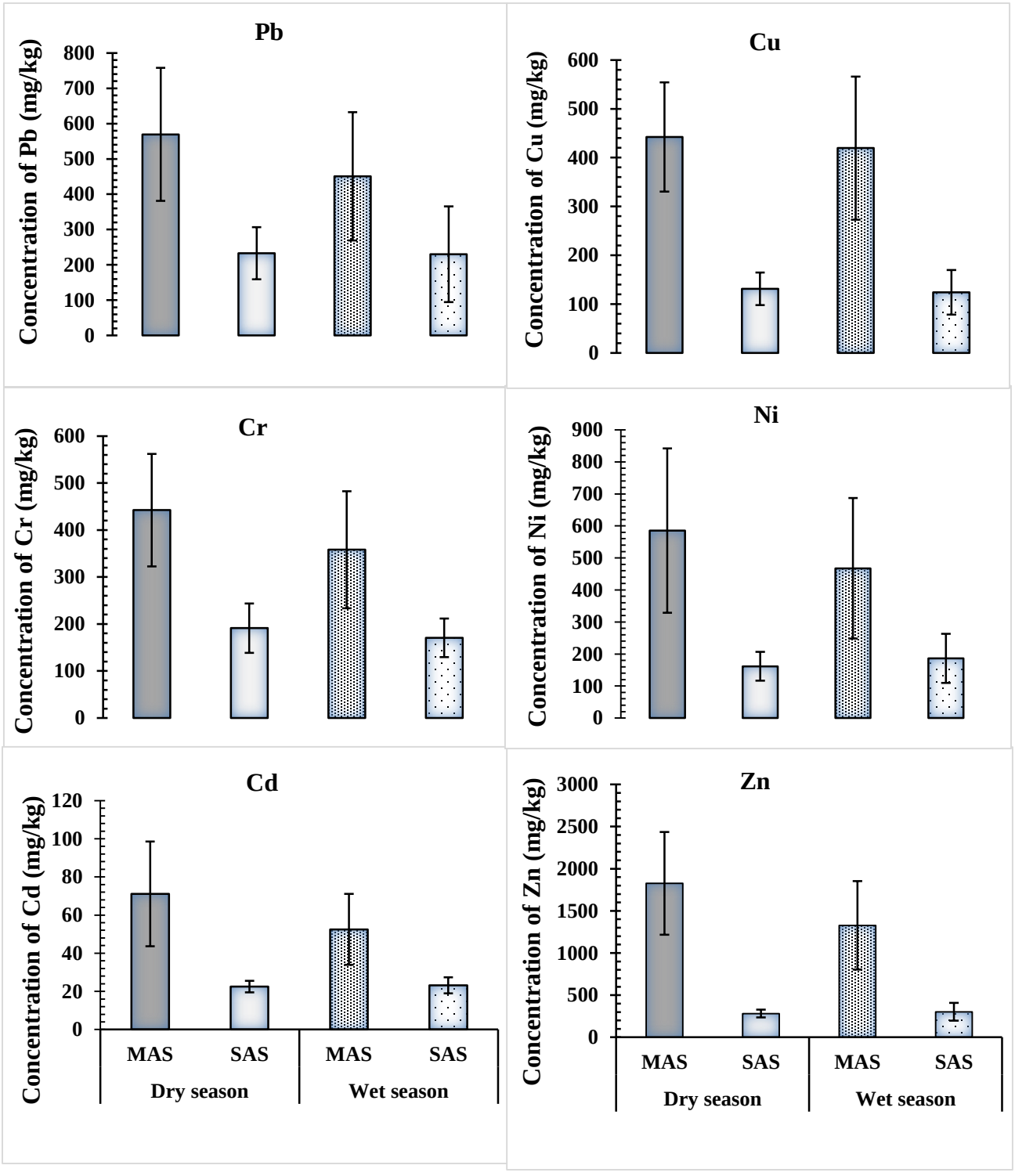
Data from this study and statistical analysis revealed that *Adhatoda schimperiana* has reasonable potential as an alternative for accumulation of Pb, Cr, Cd, Ni, Zn, and Cu. Every part of plant (root and above ground parts) has a significant contribution for removal of metal contaminants. An interaction between ecological factors and metal uptake potential and the impact of different soil physicochemical properties on metal removal were also determined.

The concentrations of Ni and Zn were larger than all the studied trace metals. The highest total metal uptake of 1825.8 ± 609.3 mg/kg was noted for Zn, while minimum recording of 81.14 ± 24.06 mg/kg was for Cd in dry season (Table 8). However, even though the mean value of Cd absorbed by *Adhatoda schimperiana* is lower, the higher biomass could increase the total Cd in the plant (Babaeinejad *et al*., 2015).

Table 8. Distribution of heavy metals (mg/kg) in different tissues of *Adhatoda schimperiana.*

Metal	*Adhatoda* /Plant parts	Dry season (mg/kg)		Wet season (mg/kg)	
		Mature plants	Seedlings	Mature plants	Seedlings
Cd	Root	47.4 ± 16.3	15.54 ± 1.99	36.4 ± 13.3	15.81 ± 3.4
	Stem	15.5 ± 8.9	4.33 ± 1.65	9.94 ± 2.4	5.23 ± 1.33
	Leaf	8.24 ± 3.52	2.66 ± 1.33	6.38 ± 4.16	2.30 ± 0.54
	Total concentration plant	**71.1 ± 27.3**	**22.5 ± 2.99**	**52.5 ± 18.6**	**23.2 ± 4.2**
Cr	Root	195.4 ± 50	75.24 ± 26.4	170.2 ± 66.6	68.1 ± 26.4
	Stem	135.9 ± 48.4	63.3 ± 21.7	98.6 ± 31.35	61.50 ± 9.99
	Leaf	111 ± 41.4	52.7 ± 16.8	89.4 ± 41.3	41.13 ± 11.2
	Total concentration plant	**442.4 ± 119.8**	**191.2 ± 52.4**	**358.1 ± 124.3**	**170.7 ± 41.03**
Pb	Root	411.1 ± 131.3	175.25 ± 53.4	322.7 ± 134.2	174.5 ± 123.3
	Stem	101.9 ± 54.4	37.25 ± 15	90.8 ± 37.13	34.4 ± 9.6
	Leaf	56.53 ± 27.2	20.63 ± 7.34	51.3 ± 31.53	29.3 ± 9.5
	Total concentration plant	**569.5 ± 188.6**	**233.1 ± 73.9**	**464.8 ± 159.7**	**238.1 ± 125.5**
Zn	Root	539.5 ± 173.4	65.524 ± 7.6	412.6 ± 114.9	94.6 ± 42.8
	Stem	565. ± 218	74.56 ± 12	414.2 ± 161.3	72.6 ± 14.5
	Leaf	720.6 ± 247	141.95 ± 33.2	500.6 ± 262.8	135.7 ± 61.4
	Total concentration plant	**1825.8 ± 609.3**	**282.03 ± 47.1**	**1327.4 ± 524.5**	**302.9 ± 105.9**
Ni	Root	200.1 ± 133.7	63.03 ± 25.3	146.7 ± 39.2	64.67 ± 15.1
	Stem	132.1 ± 48.04	47.54 ± 24.24	125.1 ± 55.2	54.55 ± 28.1
	Leaf	253.3 ± 109.2	50.98 ± 22.2	195.7 ± 126.8	67.03 ± 37.06
	Total concentration plant	**585.5 ± 256.7**	**161.6 ± 44.9**	**467.6 ± 219.6**	**186.3 ± 76.5**
Cu	Root	202.2 ± 77.6	36.97 ± 16.9	172.4 ± 81.8	45.48 ± 25.7
	Stem	141.5 ± 37.2	54.94 ± 17.1	154.6 ± 82.1	47.3 ± 14.1
	Leaf	98.5 ± 11.96	39.3 ± 8.1	92.6 ± 6.9	31.2 ± 13.2
	Total concentration plant	**442.2 ± 111.8**	**131.2 ± 33.4**	**419.6 ± 146.5**	**124.02 ± 45.7**

NB: values are means of 5 samples and ± standard deviation



NB: MAS represents mature *Adhatoda schimperiana* **SAS represents seedlings of *Adhatoda schimperiana***

Figure 9. Metal concentration in *Adhatoda schimperiana* plants.

Adhatoda schimperiana plants have also demonstrated the capability to absorb considerable amount of toxic metals during wet season. Metal concentrations in mature plants during wet season was in order of: Zn > Ni > Pb > Cu > Cr > Cd with higher and lower mean recordings of 1327.4 ± 524.5 and 52.5 ± 18.6 mg/kg for Zn and Cd, respectively. Conversely, it was also examined that, uptake and concentrations of metals in seedlings of *Adhatoda schimperiana* showed higher values in wet season, except for Cr and Cu.

Table 9. Mean BCF and TF values of trace metals in different tissues of *Adhatoda schimperiana* in dry season.

Element	Maturity (Dry)		**Biological concentration factor BCF** *Adhatoda schimperiana*				**Translocation factor (TF)** *Adhatoda schimperiana*		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	**TF aerial**
Cd	**mature**	**M ± SD**	**1.76*±**0.51	0.57±0.32	0.31±0.07	0.88±0.36	0.31±0.12	0.174±0.04	0.48±0.12
		Range	**1.13*-2.27***	0.19-**1.08***	0.22-0.39	0.47-**1.45***	0.15-**1.45***	0.13-0.21	0.36-0.64
	seedlings	**M ± SD**	0.67±0.32	0.20±0.13	0.12±0.08	0.32±0.20	0.29±0.12	0.18±0.13	0.46±0.21
		Range	0.26-**1.12***	0.08-0.35	0.05-0.23	0.13-0.55	0.16-0.44	0.09-0.32	0.26-0.76
Cr	**mature**	**M ± SD**	**1.08*±**0.23	0.77±0.30	0.62±0.19	**1.38*±**0.46	0.70±0.19	0.59±0.21	**1.29*±**0.37
		Range	0.88-**1.48***	0.49-**1.21***	0.38-0.85	0.87-**1.91***	0.48-0.92	0.38-0.84	0.86-**1.76***
	seedlings	**M ± SD**	0.41±0.08	0.37±0.15	0.29±0.06	0.66±0.19	0.92±0.38	0.53±0.25	**1.45*±**0.26
		Range	0.31-0.49	0.16-0.5	0.21-0.35	0.43-0.81	0.41-**1.43***	0.10-0.73	**1.08*-1.68***
Pb	**mature**	**M ± SD**	**2.3***±**1.31***	0.62±0.59	0.30±0.13	0.91±0.71	0.25±0.09	0.14±0.05	0.39±0.12
		Range	**1.37*-4.49***	0.29-**1.67***	0.17-0.50	0.46-**2.17***	0.12-0.37	0.07-0.20	0.19-0.48
	seedlings	**M ± SD**	0.93±0.25	0.20±0.07	0.12±0.05	0.31±0.12	0.21±0.04	0.12±0.03	0.33±0.06
		Range	0.65-1.16	0.1-0.28	0.05-0.16	0.15-0.44	0.15-0.25	0.08-0.144	0.23-0.39
Zn	**mature**	**M ± SD**	**2.14*±**0.36	**2.15*±**0.24	**2.96*±**0.45	**5.11*±**0.62	**1.04*±**0.25	**1.35*±**0.3	**2.38*±**0.54
		Range	**1.77*-2.51***	**1.96*-2.55***	**2.29*-3.42***	**4.29*-5.83***	0.85-**1.44***	**1.06*-1.85***	**1.91*-3.29***
	seedlings	**M ± SD**	**0.3±**0.18	**0.32±**0.14	**0.58±**0.15	**0.91±**0.28	**1.14*±**0.15	**2.18*±**0.51	**3.32*±**0.65
		Range	0.2-0.62	0.22-0.57	0.44-0.82	0.66-**1.39***	0.91-**1.32***	**1.32*-2.68***	**2.23*-3.88***
Ni	**mature**	**M ± SD**	0.61±0.15	0.47±0.24	0.85±0.33	**1.33*±**0.50	0.80±0.42	**1.41*±**0.59	**2.21*±**0.93
		Range	0.44-0.78	0.27-0.83	0.43-**1.14***	0.78-**1.92***	0.41-**1.37***	0.88-**2.3***	**1.29*-3.67***
	seedlings	**M ± SD**	0.22±0.17	0.20±0.184	0.18±0.10	0.38±0.26	0.80±0.30	**1.12*±1.22***	**1.93*±**1.40*
		Range	0.02-0.46	0.07-0.52	0.08-0.3	0.16-0.82	0.52-**1.14***	0.38-**3.29**	1.11*-**4.37***
Cu	**mature**	**M ± SD**	**1.35*** ±0.60	0.94 ± 0.23	0.67 ± 0.18	**1.42*** ± 0.67	0.57 ± 0.35	0.54±0.172	**1.11*±**0.45
		Range	0.85-2.37	0.61-1.18	0.51-0.97	0.4-2.11	0.09-1.07	0.37-0.8	0.69-**1.87***
	seedlings	**M ± SD**	0.25±0.12	0.37±0.13	0.27±0.08	0.63±0.13	**1.60*±**0.55	**1.23*±**0.6	**2.82*±**0.86
		Range	0.11-0.42	0.23-0.54	0.18-0.38	0.44-0.81	**1.15*-2.3***	0.63-**1.98***	**1.9*-3.91***

NB: M* ± SD** stands for mean ± standard deviations **Bold** and ** denote values are > **1**

Table 10. Mean BCF and TF values of trace metals in different tissues of *Adhatoda schimperiana* in dry season.

Element	Maturity (Wet)		Biological concentration factor BCF *Adhatoda schimperiana*				Translocation factor (TF) *Adhatoda schimperiana*		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	**TF aerial**
Cd	**mature**	**M ± SD**	**1.40*±**0.40	0.40±0.10	0.25±0.13	0.65±0.20	0.29±0.09	0.17±0.07	0.46±0.12
		Range	0.89-**1.80***	0.23-0.5	0.11-0.44	0.34-0.81	0.2-0.44	0.08-0.24	0.38-0.66
	seedlings	**M ± SD**	0.59±0.10	0.2±0.06	0.11±0.03	0.32±0.08	0.37±0.07	0.16±0.07	0.56±0.11
		Range	0.46-0.72	0.13-0.27	0.06-0.15	0.22-0.42	0.28-0.45	0.08-0.24	0.42-0.69
Cr	**mature**	**M ± SD**	**1.27***±0.45	0.75±0.18	0.69±0.36	**1.44***±0.49	0.66±0.36	0.55±0.19	**1.21***±0.50
		Range	0.75-1.93	0.48-0.96	0.32-1.22	0.80-2.06	0.43-1.28	0.29-0.78	0.72-2.06
	seedlings	**M ± SD**	0.51±0.14	0.49±0.17	0.32±0.10	0.81±0.25	**1.01***±0.42	0.634±0.13	**1.65***±0.53
		Range	0.34-0.73	0.33-0.74	0.24-0.49	0.57-**1.09***	0.66-**1.57***	0.5-0.76	**1.16*-2.31***
Pb	**mature**	**M ± SD**	**1.98*±**0.61	0.49±0.23	0.21±0.09	0.69±0.31	0.24±0.06	0.10±0.04	0.34±0.10
		Range	**1.34*-2.95***	0.28-0.08	0.12-0.33	0.4-**1.07***	0.15-0.31	0.06-0.16	0.21-0.47
	seedlings	**M ± SD**	0.99±0.4	0.19±0.12	0.14±0.06	0.33±0.16	0.21±0.12	0.15±0.04	0.36±0.16
		Range	0.56-**1.60***	0.12-0.40	0.08-0.21	0.2-0.61	0.08-0.39	0.12-0.21	0.2-0.60
Zn	**mature**	**M ± SD**	**2.23***±0.74	**2.24*±**0.83	**2.40***±0.58	**4.65*±1.25***	**1**.00±0.20	**1.15***±0.36	**2.14***±0.50
		Range	**1.37*-3.32***	0.97-**3.27***	**1.47*-2.97***	**2.44*-5.53***	0.71-**1.22***	0.68-**1.65***	**1.66*-2.87***
	seedlings	**M ± SD**	0.54±0.32	0.42±0.22	0.68±0.16	**1.10***±0.35	0.86±0.30	**1.46*±**0.46	**2.32*±0.69**
		Range	0.24-**1.06***	0.24-0.78	0.51-0.92	0.78-**1.70***	0.42-**1.13***	0.87-**2.11***	**1.60*-3.24***
Ni	**mature**	**M ± SD**	0.79±0.28	0.63±0.17	0.914±0.33	**1.54*±**0.47	0.82±0.18	**1.224***±0.54	**2.05***±0.70
		Range	0.52-**1.26***	0.48-0.88	0.35-**1.17***	0.84-**2.03***	0.7-**1.08***	0.5-**1.77***	**1.20*-2.81***
	seedlings	**M ± SD**	0.37±0.20	0.27±0.13	0.32±0.08	0.59±0.19	0.81±0.25	**1.00*±**0.39	**1.81*±**0.59
		Range	0.2-0.72	0.18-0.46	0.2-0.40	0.38-0.84	0.6-1.20	0.53-**1.43***	**1.17*-2.45***
Cu	**mature**	**M ± SD**	**1.17***±0.60	**1.13***±0.84	**0.66±0.31**	**1.79*±1.06***	**1.00*±**0.40	**0.69±**0.41	**1.68**±0.75
		Range	0.37-**2.03***	0.52-**2.54***	0.41-**1.12***	0.97-**3.39***	0.55-**1.41***	0.36-**1.21***	0.91-**2.62***
	seedlings	**M ± SD**	0.31±0.19	0.35±0.20	0.22±0.13	0.57±0.33	**1.29***±0.77	0.83±0.54	**2.12*±**1.30
		Range	0.11-0.55	0.14-0.60	0.08-0.34	0.22-0.94	0.62-**2.52***	0.37-**1.75***	0.99-**4.27***

NB: M* ± SD** stands for mean ± standard deviations **Bold** and ** denote values are **> 1**

a. Cd concentration in different parts of *Adhatoda schimperiana.*

Soil naturally contains much lower concentration of cadmium; however it is relatively mobile in soil solution (Jadia and Fulekar, 2009; Kumar and Thakur, 2019). It is observed that *Adhatoda schimperiana* plants can absorb and store Cd in their roots. The uptake of Cd recorded during dry season is higher than values noted during rainy season. The total Cd uptake recorded for the whole plant, during dry season was 71.1 ± 27.3 mg/kg, while that of wet season was 52.5 ± 18.6 mg/kg, and this could be due to the transpiration. The distribution of Cd in different tissues was as follows: root > stem > leaf for both seedlings and mature plants. Likewise, studies reported most plants tend to store larger fraction of absorbed Cd in their roots (Boominathan and Doran, 2003; Jadia and Fulekar, 2009).

Cd concentration in *Adhatoda schimperiana* varied among sampling sites and seasons. In dry season, the highest Cd concentration value was noted in plant samples obtained from Hana Mariam site (95.8 mg/kg in average) whereas, minimum value of (38.54 mg/kg in average) was measured at Akaki site. Similarly, the maximum (77.72 mg/kg) and minimum (31 mg/kg) average values of Cd found in mature plants of *Adhatoda schimperiana* in wet season were computed for Kera site and Akaki site, respectively. However, plant samples from uncontaminated (control) sites, gave the lowest Cd concentration of 1.54 mg/kg in rainy seasons and 2.01 mg/kg in dry season.

The highest uptake of Cd (Table 8) was observed in the roots rather than in the above ground tissues. Therefore, this uptake pattern will make a difficult situation to make use of *Adhatoda schimperiana* for phytoremediation on a practical scale, because below ground plant parts are not as easily harvestable as shoots. However, even though the plant fails to translocate the Cd, it can be used for phytostabilization since we need to take advantage of the multiple metal removal properties of *Adhatoda schimperiana*. However, considering the advantages of much higher concentrations of Cd in some cases; substantially higher biomass of this plant and the possibility of re-growing after harvesting the aboveground tissues can be kept for phytostabilization.

Movement of Cd from soil to roots of *Adhatoda schimperiana* and throughout the plant and translocation to the upper part were also computed using BCF and TFs. Data in Table.9 and 10 indicate that Cd retained in the below-ground parts of *Adhatoda schimperiana* was much higher

than those accumulated in the above-ground parts. That is they avoid toxicity via exclusion mechanism and therefore it is suitable for phytostabilization of cadmium. BCF of root in mature plant ranged from 1.13 - 2.27, and the mean value was 1.76 ± 0.51 in dry season. The value of BCF in wet season was in the range of 0.89 -1.80 and the mean value computed was 1.40 ± 0.40. However, TF values recorded were all < 1 both for seedlings and mature plants indicating poor transfer of Cd to the aerial part and unsuitability of the plant for phytoextraction.

Regardless of the accumulating tissue, the total uptake of Cd in *Adhatoda schimperiana* increases with the level of Cd in soil. Correlation coefficient r (0.815), $p = 0.001$ calculated for mature plants and the value of r (0.632), $p = 0.027$ computed for seedlings showed a positive relationship, indicating metal uptake and accumulation in plants increases with metal concentration in corresponding soil, which is, similar both for seedlings and mature plants. Among soil properties examined, soil pH, organic matter, moisture content and clay content had negative but insignificant impact on the Cd uptake pattern, however CEC and EC impacted Cd uptake positively $p > 0.05$. The test for equality of means indicated that mean values recorded for mature plants (M = 55.99, SD = 34.12) was significantly different from that of seedlings (M =19.24, SD = 9.04). That is, $t(12.54) = 3.61$, $p = 0.003 < 0.05$.

b. Cr concentration in different parts of *Adhatoda schimperiana.*

The trend of chromium uptake was: root > stem > leaf for mature plants and seedlings collected during dry season. The highest accumulation of chromium (585.2 mg/kg) was detected in plant samples collected at Hana Mariam site during dry season and a minimum (297.6 mg/kg) was recorded for samples from Akaki site (Figure 9). Mean values of Cr concentration 442.4 ± 119.8 mg/kg and 191.2 ± 52.4 mg/kg were recorded for mature plants and seedlings of *Adhatoda schimperiana* samples collected during dry season (Table 8).

During wet season mature *Adhatoda schimperiana* plants from soil containing (217 mg/kg) of Cr showed highest recording of chromium uptake (476.3 mg/kg) which is noted at Kera site. Mean values of 358.1 ± 124.3 mg/kg was noted for Cr in mature plants of *Adhatoda schimperiana* and seedlings of *Adhatoda schimperiana* accumulated remarkable concentration of 170.7 ± 41.03 mg/kg chromium that was distributed in all tissues. The larger portion of Cr concentration was

noted in the root and the lower values were recorded for leaves. The trend of chromium accumulation by *Adhatoda schimperiana* in wet season was: root > stem > leaf (Table 8).

Data also illustrates that, Cr uptake and distribution in different sampling sites differ significantly ($p < 0.05$). Cr concentration in *Adhatoda schimperiana* samples collected from different sites were in order of: Hana Mariam > Kera > Mekanisa > Lideta > Akaki for dry season samples and Kera > Mekanisa > Hana Mariam > Lideta site > Akaki for wet season samples. However, variation in concentration levels of Cr could be related to plant condition or Cr levels in soil. The lower plant uptake could depend the dominant Cr species trivalent Cr (III) and hexavalent Cr (VI). The presence of higher level of hexavalent Cr (highly soluble, mobile and the most available form of Cr), in soil solution could significantly increase the Cr levels in plats. In addition soil properties, competition of other cations and other reasons could play a role in changing the trends of uptake.

Quantification and remediation efficiency of chromium using *Adhatoda schimperiana* was also further explained by TF and BCF. According to results in Table 9 and 10, BCF for different parts of *Adhatoda schimperiana* could be arranged in the sequence: root (1.08 ± 0.23) > stem (0.77 ± 0.30) > leaves (0.62 ± 0.19) for dry season. Similarly, BCF of Cr in different tissues of *Adhatoda schimperiana* during wet season was also in the order of root (1.27 ± 0.45) > stem (0.75 ± 0.183) > leave (0.692 ± 0.36). Bioconcentration values recorded for seedlings are all measured to be less than 1, however TF of Cr from root to above-ground portion was higher in seedlings of *Adhatoda schimperiana* as compared to that of mature ones. This illustrates that uptake and translocation of Cr in *Adhatoda schimperiana* decreases with plant age. Mean TF values of Cr in mature plants are < 1 in dry season. This demonstrates the preference of *Adhatoda schimperiana* in storing Cr in the below ground part. This finding supports Yu *et al*. (2010) where it was reported that Cr mainly is retained in roots parts of plants.

Statistical analysis also revealed, chromium accumulation in *Adhatoda schimperian*a correlates positively ($p < 0.01$) with soil metal concentration. Correlation coefficient of r (0.791), $p = 0.002$ was computed for the mature plants and value of r (0.789), $p = 0.002$ for Cr uptake in seedlings. Further independent sample t test for comparison mean concentrations t (14.33) = 3.89, $p = 0.002$ revealed a significant difference in uptake Cr properties of mature plants and seedlings of *Adhatoda schimperiana*.

Generally, Cr concentration in aerial parts computed by BCF of shoot or the BAC gave value > 1 and aerial TF > 1. The higher TF > 1 in both seedlings and mature plants of *Adhatoda schimperiana* reveals the plant had efficient potential to translocate metals to aerial parts (Mkumbo *et al*., 2012). This shows shoots of *Adhatoda schimperiana* plant can concentrate Cr to the level higher than Cr available in soil. Hence, Cr phytoextraction can be approached by using *Adhatoda schimperiana*. Moreover, it was observed from this investigation that, the plant could grow in soil samples containing Cr levels higher than maximum allowable level of Cr in soil (150 mg/kg).

c. Pb concentration in different parts of *Adhatoda schimperiana.*

Lead was a trace metal reported to be immobile with lower bioavailability due to the formation of precipitation with low solubility (Dede *et al*., 2012; Usman *et al*., 2019). However, mobility of metal elements like Pb in soil solution can be impacted by soil OM content, pH or clay fraction (Amin *et al*., 2018). *Adhatoda schimperiana* plants removed and retained the largest fraction of Pb (72.96%) in their roots followed by stem (18.7%) and roots (8.98%). Similarly, percentage values of Pb accumulation marked for seedlings 75.9% for root, 14.36% for stem and 9.74% for leaves were found to be comparable with the values computed for mature plants. The highest Pb concentration in root could be due to immobilization of Pb in soil or precipitation, and this also supports reports of other researches that Pb does not easily move into the aboveground portion of plants (Yu *et al*., 2010; Usman *et al*., 2019). Study using transmission electron microscopy reported Pb mainly remains in below ground part of plants particularly in cell wall roots (Wenger *et al*., 2003).

Accumulation in different tissues of the plant can be ordered as (root > stem > leaf) for mature plants and (root > stem > leaf) for seedlings. Mean of total uptake and absorption recorded for the whole plant 569.5 ± 188.6 mg/kg in dry season and 464.8 ± 159.7 mg/kg in wet season suggest better removal characteristics in dry season. Conversely, even though the total uptake recorded in seedlings are far less than that of mature plants, mean uptake recordings in wet season (238.1 ± 125.5 mg/kg) exceeds that of dry season 233.1 ± 73.9 mg/kg (Table 8).

Lead content in *Adhatoda schimperiana* gradually increases with the levels of Pb in soil. The highest absorption of 746.9 mg/kg Pb was recorded in site 3 soil containing 382.6 mg/kg lead. The

lower Pb contents of 131 and 146 mg/kg were measured at control soil containing 46.2 and 51.7 mg/kg lead in dry and rainy season, in that order. This can also be further explained by the Pearson correlation, which showed strong positive correlation (r = 0.711, p = 0.010 < 0.05) for mature plants and (r = 0.755, p = 0.022) for seedlings of *Adhatoda schimperiana*.

BCF and TF values of Pb in *Adhatoda schimperiana* are computed and presented in Table 8 and Table 9. BCF values (> 1) of Pb was observed only in roots of mature *Adhatoda schimperiana* and value computed for stem and leaf tissue were all < 1 indicating lead tolerance. All values of BCF computed for seedlings were < 1 and similarly, significantly lower mean TF values (TF < 1) of Pb in stems and leaves of both seedlings and mature plants.

Thus, larger BCF in roots and lower TF values, highlights that *Adhatoda schimperiana* has limited tendency of transferring Pb to its upper tissues. *Adhatoda schimperiana* can possibly be utilized for phytostabilization (immobilization) of lead polluted soil (Mendez and Maier, 2007). However, lower TF of Pb indicates *Adhatoda schimperiana* plants had poor potential to transfer Pb from belowground part to the aboveground tissue, which could be caused by Pb toxicity (Yoon *et al*., 2006).

Statistical significance in the difference between the mature and the seedling of *Adhatoda schimperiana* was also computed by using independent sample test. Thus, t-test for equality of means indicated there was significant difference between mature and seedlings of *Adhatoda schimperiana* in Pb uptake. That is, t (17.30) = 4.04, p = 0.001 at the 95% confidence level.

d. Zn concentration in different parts of *Adhatoda schimperiana*

Zinc concentration in *Adhatoda schimperiana* was higher than the entire trace metals investigated in this study. The higher Zn concentration in mature plants of *Adhatoda schimperiana* indicated that metal removal was elevated with the plant age. Irrespective of Zn level in soil, the mean uptake and accumulation level of Zn (1825.8 ± 609.3 mg/kg) in mature *Adhatoda schimperiana* was higher during dry season than mean value in rainy season (1327.4 ± 524.5 mg/kg) and this could be due to the lower pH values during dry season. However, mean Zn accumulated in sample seedlings collected during wet season (302.9 ± 105.9 mg/kg) was greater than values (282.03 ± 47.1 mg/kg) in dry season (Table 8).

Zn concentration in *Adhatoda schimperiana* plants increased with the level of Zn in corresponding soil; which is concurring with trend observed in *Linum usitatissimum* studied by Hosman *et al.* (2017). In addition, seedlings of *Adhatoda schimperiana* can remove considerable level of Zn in contaminated sites, while mature plants can accumulate an average of 6.93 times more Zn than available in soil in dry season. Similarly, in wet season 1.03 and 5.63 times more Zn was deposited in seedlings and mature plants of *Adhatoda schimperiana*, respectively.

Subsequently, the pattern of Zn concentration in different tissues decreased in the order of leaves > stem > roots. Explicitly, the highest accumulation Zn was examined in leaves while roots had the lowest recordings. Therefore, based on the very common indices used to evaluate metal accumulating capacity BCF and TF values (> 1) computed in this research, *Adhatoda schimperiana* could be considered as hyperaccumulator of Zn (Wu *et al*., 2011).

Zinc uptake and distribution in plant samples collected from different sites are in order of Hana site (2360.2 mg/kg) > Mekanisa site (2140.7 mg/kg) > Kera site (1943.5 mg/kg) > Lideta site (1898.6 mg/kg) > Akaki site (786 mg/kg) for dry season. In addition, Zn levels of different sites during wet season were in the following order: Kera site (2091.2 mg/kg) > Lideta site (1392 mg/kg) > Mekanisa site (1332.9 mg/kg) > Hana Mariam site (1198 mg/kg) > Akaki site (623 mg/kg). The variation in levels of Zn in *Adhatoda schimperiana* plant tissue could be also dependent on the microbial activities. The reduction in activity of microorganism's results in reduction the release of zinc from organic materials and an increase in microbial activity can also increase the release of Zn from organic materials (Mousavi, 2011).

The study highlights that *Adhatoda schimperiana* plants can take up and accumulate Zn in both heavily contaminated sites and from soils with relatively low Zn contamination levels. A rise in level of Zn in soil has considerable influence ($p < 0.05$) on metal retained in biomass of plants. The coefficients correlation r (0.865), $p = 0.000$ recorded for mature plants and values of r (0.503), $p = 0.096$ recorded for seedlings, shows 86.5% of Zn in mature plants and 50.3% of Zn in seedlings could be due to the levels of Zn in the corresponding soil. Further, t-test for equality of means, $t(11.41) = 5.292$, $p = 0.000$ reveals the difference between Zn uptake in mature plants and seedlings of *Adhatoda schimperiana.*

Translocation factors of Zn in A*dhatoda schimperiana* plants collected from all sites were > 1, and the higher TF values of Zn could be because of its metabolic roles and activities in plants or enzymes such as proteinases and dehydrogenases. Jepkoech *et al*. (2013) also concluded higher mobility and transfer potential of Zn from soil to root and root to aerial part.

e. Ni concentration in different parts of *Adhatoda schimperiana.*

Higher absorption of Ni in *Adhatoda schimperiana* was observed in plants growing on at Kera site (995.3 mg/kg) followed by Lideta site (602.3 mg/kg) and Mekanisa site (564.1 mg/kg) during dry season. Similarly, higher Ni content was noted at Mekanisa site (751 mg/kg) followed by Kera site (636.6 mg/kg) and Lideta site (410 mg/kg). Whereas total concentration of Ni in seedlings collected during dry season gave the highest recording of 218.5 mg/kg at Hana Mariam followed by 196.45 recorded in Kera and 151.7 mg/kg at Akaki. In addition, maximum total content of Ni content in seedlings of *Adhatoda schimperiana* samples collected during wet season was found at Kera site (272.64 mg/kg) followed by Mekanisa site (263.8 mg/kg) and Hana Mariam site (145.3 mg/kg) (Figure 9). Soil containing toxic level of Ni (>100 mg/kg) could result in chlorosis of plants (Kabata-Pendias, 2001; Shaw *et al*., 2004), however, *Adhatoda schimperiana* plants were found grown vigorously in soils containing Ni concentrations ranging from 93 to 664.8 mg/kg.

The Ni concentrations of *Adhatoda schimperiana* plant from contaminated sites gave metal accumulation in the roots which ranged between 116-434.2 mg/kg, and in the stem ranged between 79.82 - 178.05 mg/kg, and in the leaf varied between 103 - 383 mg/kg during dry season. Similarly, the lower and higher levels of Ni in wet season are: 106 to 203 in root, 74 to 189 in stem and 53 to 359 mg/kg in leaves. The leaves of *Adhatoda schimperiana* are capable of accumulating the highest amount of Ni with a mean of 253.3 ± 109.2 mg/kg during dry season, while 195.73 ± 126.76 mg/kg for rainy season. The smallest Ni accumulation was recorded in stems with the mean values of 132.1 ± 48.04 and 125.1 ± 55.23 mg/kg for dry season and rainy season, respectively (Table 8).

Adhatoda schimperiana was observed to uptake Ni starting from the seedling stage and it was revealed that it can increase its uptake if its exposure to Ni contaminated soil was prolonged. Explicitly, statistical analysis for the relationship between Ni level in soil and Ni in plant biomass showed, correlation coefficients and probability values of r (0.91), $p = 0.000$ and r (0.47), $p = 0.13$

for mature and seedling plants of *Adhatoda schimperiana*. Which indicates, the positive impact is highly significant in mature plants. The mature and seedlings of *Adhatoda schimperiana* from similar soil had significantly different mean uptake values, which could be explained by independent sample t test, t (12.39) = 4.09, $p = 0.001 < 0.05$.

Tables 9 and 10 revealed the potential of plant for taking up Ni from contaminated soil as also determined by computing BCF. Data in the tables also illustrate that, *Adhatoda schimperiana* gave root BCF < 1 in most of the examined plants except for plants grown at Hana Mariam site which gave root BCF of 1.26 and we could explain this as it could be because of the level of Ni in the corresponding soil (171.45 mg/kg) or other ecological parameters. However, shoot BCF ranged from 0.78 - 1.92 for mature plants in dry season and 0.84 - 2.03 for plants in wet season which testifies that uptake properties are better in wet season. And values computed for shoot BCF in seedlings ranged from 0.16 - 0.82 and 0.38 - 0.84 for dry and wet seasons, respectively. The TF computed to evaluate transfer potential of absorbed Ni from root to the upper tissues and values computed for mature plants ranged from 1.29 - 3.67 and 1.2 - 2.81 for dry and wet season, showing better transfer potential in dry season. Further aerial TF values recorded for seedlings 1.11- 4.37 and 1.17 - 2.45 computed for dry season and rainy seasons, respectively.

Finally, based on the higher biomass the values of TF and BAC or BCF of shoot > 1 *Adhatoda schimperiana* could have good potential for phytoextraction of Ni contaminated sites (Kandziora-Ciupa *et al*., 2017). Even though BCF values of root were lower than 1; plants with TF > 1 and shoot BCF > 1 could be considered suitable for phytoextraction of contaminated sites (Yoon *et al*., 2006; Malik *et al*., 2010).

f. Cu concentration in different parts of *Adhatoda schimperiana*

Generally, the Cu absorption in different tissues of *Adhatoda schimperiana* was in the order of; roots > stems > leaves both in dry and rainy season. A portion of Cu accumulated in *Adhatoda schimperiana* was transferred to the above ground biomass (stem and leaf). Mean values recorded for Cu absorbed by mature *Adhatoda schimperiana* plant in dry and wet season are 442.2 ± 111.8 mg/kg and 419.6 ± 146.5 mg/kg, respectively. Similarly mean uptake values recorded in seedlings are 131.2 ± 33.4 and 124.02 ± 45.7 mg/kg for dry season and rainy season, respectively (Figure 9).

Cu uptake in *Adhatoda schimperiana* had positive correlation with Cu concentration in soil. Strong correlation with r (0.591), $p = 0.043 < 0.05$ was observed in mature plants and insignificant correlation r (0.471), $p = 0.123 > 0.05$ was noted for seedlings. In addition, values of t (12.499) = 5.11, $p = 0.000$ reveal significant difference between mean values of Cu uptake in mature plants and seedlings of *Adhatoda schimperiana.*

Mean values of BCF and TF recorded for roots and shoots of mature plants were > 1 in both seasons of the year. Plants collected during wet season showed better absorptive and translocation ability than those collected during dry season. The maximum mean values of shoot BCF (1.79 ± 1.06) recorded for wet season samples are bigger than mean values of shoot BCF (1.42 ± 0.67) noted for dry season (Table 9 and Table 10). However, root BCF recorded for dry season samples (1.35 ± 0.60) were higher than that of wet season samples 1.17 ± 0.60. In addition, aerial TF recorded both in wet (1.684 ± 0.75) and dry seasons (1.11 ± 0.45) are > 1, indicating *Adhatoda schimperiana* is suitable for phytoextraction of Cu contaminated sites. Further the higher mean values of TF in seedlings of *Adhatoda schimperiana*, shows immediate transfer of absorbed Cu to the aerial parts during growth season. This could be due to the importance of Cu as an essential nutrient.

4.3.2.2. Summary of metal accumulation status in *Adhatoda schimperiana*

The results indicated *Adhatoda schimperiana* plants stored substantial concentrations of metals in their tissues and the plant exhibited better remediation capacity for Zn than other studied trace metals. The trends in variation of metal accumulation (mg/kg) in *Adhatoda schimperiana* in wet season were: Zn (1825.8 ± 609.3) > Ni (585.5 ± 256.7) > Pb (569.5 ± 188.6) > Cu (442.4 ± 119.8) > Cr (442 ± 111.8) > Cd (71 ± 27.3); while during dry season observed trends were: Zn (1327.4 ± 524.5) > Ni (467.6 ± 219.6) > Pb (464.8 ± 159.7) > Cr (419.6 ± 146.5) > Cu (358 ± 124.3) > Cd (52.5 ± 18.6). And an irregular trend in some cases could be associated to the impact of soil parameters or planting density. However, the lower Cd concentration in the plant could be because of higher level of Zn in the tissues of the plant (Eid *et al*., 2019).

Heavy metals were consistently higher in plant materials as concentration in soil increases, and a significant relationship was observed from correlation analysis ($p < 0.05$). The present work demonstrated, elemental contents in roots, stems and leaves of *Adhatoda schimperiana* varied

significantly ($p < 0.05$). Metal content in root systems was higher for Cd, Cr, Pb and Cu. Conversely, it was noticed that Zn and Ni were abundant in leaves.

Using BCF and TF factors as an indicator of phytoremediation efficiency, *Adhatoda schimperiana* plants are suitable plants for phytoremediation of multiple trace metals. And based on these estimators *Adhatoda schimperiana* plants can clean Cr, Zn, Ni and Cu through phytoextraction mechanism and Cd and Pb could be immobilized through phytostabilization.

4.3.3. *Solanum incanum* plant characteristics and phytoremediation properties

Solanum incanum plants are known to grow vigorously in a great variety of geographic and climatic conditions, including highly contaminated areas and needs little care and marginal cost if planted. It is spiny and familiar plant found anywhere in roadsides and it also has extensive root system and large biomass.

Solanum incanum is a perennial bushy herb or shrub and also known with its common names (bitter garden egg or thorn apple) in most places, belongs to the family *Solanaceae* and genus *Solanum*. This plant can grow up to 1.8 meters of height; has alternate leaves and flowers in the leaf axils (Sambo *et al*., 2016). The fruits of which are yellow with short lifespan due to high moisture content. It is a common medicinal plant with multiple traditional applications in Ethiopia.

4.3.3.1. Metal accumulation and distribution pattern of *Solanum incanum*

Uptake and translocation status of metals, Cr, Pb, Zn, Ni, Cu and Cd, by *Solanum incanum* and their concentration in roots, stems and leaves were examined. Consequently, present findings revealed *Solanum incanum* plants can absorb considerable levels of heavy metals from polluted soils. It was found that Cr and Zn attained their highest uptake concentrations (418.06 and 1983.16 mg/kg) at Kera site, while Pb and Ni (630.21 and 605 mg/kg) at Hana Mariam site. Moreover, the highest mean levels of Cu (697) and Cd (9.94) mg/kg, were recorded at Lideta (Table 11).

Table 11. Distribution of heavy metals (mg/kg) in different tissues of *Solanum incanum*.

Metal	*Solanum* I /Plant parts	Dry season (mg/kg)		Wet season (mg/kg)	
		Mature plants	Seedlings	Mature plants	Seedlings
Cd	Root	4.30 ± 1.38	2.09 ± 0.68	3.07 ± 1.43	1.66 ± 0.85
	Stem	2.15 ± 0.57	1.23 ± 0.6	1.39 ± 0.47	0.82 ± 0.27
	Leaf	1.34 ± 0.54	0.67 ± 0.31	0.92 ± 0.69	0.42 ± 0.28
	Total concentration plant	**7.79 ± 2.29**	**3.99 ± 1.55**	**5.38 ± 2.06**	**2.9 ± 1.22**
Cr	Root	129.7 ± 51.6	71.1 ± 15.2	99.8 ± 26.14	62.35 ± 17.6
	Stem	97.12 ± 28.04	56.6 ± 14.5	68.74 ± 26.9	42.5 ± 13.7
	Leaf	75.6 ± 19.9	49.7 ± 14.3	48.1 ± 26.4	42.9 ± 19.7
	Total concentration plant	**302.5 ± 92.95**	**177.51 ± 31.5**	**216.6 ± 73.4**	**147.7 ± 43.5**
Pb	Root	201.9 ± 22.1	90.56 ± 22.5	181.7 ± 52.6	73.3 ± 28
	Stem	149 ± 33.44	61.99 ± 11.7	136.6 ± 48.6	60.7 ± 23.8
	Leaf	156.2 ± 40.7	69.73 ± 4.5	125.8 ± 41.6	52.6 ± 22.6
	Total concentration plant	**507.05 ± 94.5**	**222.3 ±177.2**	**444.1 ± 108.8**	**186.6 ± 73**
Zn	Root	568.41 ± 145.8	106.8 ± 21.2	494.2 ± 121.1	115.4 ± 62.6
	Stem	661 ± 236.98	163.5 ± 56.9	535.8 ± 153.2	137.8 ± 64.8
	Leaf	324.5 ± 23.57	85 ± 30.13	332 ± 71.4	99.7 ± 52.04
	Total concentration plant	**1553.9 ± 380.4**	**355.3 ± 91.3**	**1362 ± 260.7**	**352.9 ± 175.3**
Ni	Root	199.3 ± 82	69.68 ± 33.7	147.02 ± 82.2	59.4 ± 26.95
	Stem	145.98 ± 44.3	58.95 ± 30.4	124.7 ± 43.04	38 ± 14.5
	Leaf	179.8 ± 67.9	65.67 ± 65.7	115.6 ± 17.04	35.1 ± 3.36
	Total concentration plant	**525.1 ± 148.52**	**194.3 ± 77.99**	**387.3 ± 119.5**	**132.5 ± 40.8**
Cu	Root	112 ± 16.9	43.97 ± 12.3	125.9 ± 49.6	32.8 ± 9.9
	Stem	223.6 ± 58.1	88.04 ± 11.5	212.4 ± 47	57.1 ± 25.4
	Leaf	74.4 ± 17.6	30.45 ± 8.2	66.9 ± 27.3	21.5 ± 5.99
	Total concentration plant	**410.1 ± 87.54**	**162.5 ± 27.9**	**405.2 ± 95.3**	**111.4±37.4**

NB: values are means of 5 samples and ± standard deviation

In addition, the lowest absorptions for all metals were attained at control sampling sites examined for reference levels of trace elements in non-anthropogenic sites as in Navas and Machin, (2002). Mean values of trace metals accumulated in the mature *Solanum incanum* were in order of: Zn (1553.9 ± 380.4) > Ni (525.1 ± 148.5) > Pb (507.05 ± 94.5) > Cu (410.05 ± 87.54) > Cr (302.5 ± 92.95) > Cd (7.79 ± 2.29 mg/kg). Likewise, wet season samples of mature *Solanum incanum* absorbed mean values of Zn (1362 ± 260.7) > Pb (444.1 ± 108.8) > Cu (405.2 ± 95.3) > Ni (387.31 ± 119.5) > Cr (216.6 ± 73.4) > Cd (5.4 ± 2.06 mg/kg) as noted in (Table 11).

Table 12. Mean BCF and TF values of trace metals in different tissues of *Solanum incanum* in dry season.

Element	Maturity (S. incanum) Dry		Biological concentration factor BCF				Translocation factor (TF)		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	**TF aerial**
Cd	**mature**	**M ± SD**	0.15±0.05	0.08±0.02	0.05±0.02	0.123±0.05	0.52±0.07	0.31±0.07	0.83±0.06
		Range	0.05-0.15	0.02-0.08	0.02-0.05	0.05-0.12	0.074-0.52	0.07-0.31	0.06-0.83
	seedlings	**M ± SD**	0.07±0.02	0.04±0.02	0.02±0.01	0.06±0.03	0.56±0.16	0.31±0.07	0.87±0.22
		Range	0.02-0.15	0.02-0.08	0.01-0.05	0.03-0.12	0.16-0.56	0.07-0.31	0.22-0.87
Cr	**mature**	**M ± SD**	0.70±0.17	0.53±0.07	0.42±0.09	0.94±0.15	0.78±0.12	0.622±0.15	**1.40***±0.26
		Range	0.46-0.89	0.43-0.61	0.32-0.52	0.76-**1.13***	0.65-0.93	0.38-0.78	**1.03*-1.66***
	seedlings	**M ± SD**	0.39±0.04	0.31±0.04	0.29±0.14	0.72±0.24	0.80±0.12	0.74±0.33	**1.54***±0.40
		Range	0.35-0.46	0.28-0.36	0.17-0.51	0.46-**1.03***	0.61-0.92	0.49-**1.29***	**1.20*-2.15***
Pb	**mature**	**M ± SD**	**1.12***±0.33	0.82±0.27	0.86±0.32	**1.67***±0.59	0.73±0.10	0.76±0.12	**1.49***±0.22
		Range	0.89-**1.70***	0.6-**1.28***	0.64-**1.42***	**1.26*-2.70***	0.57-0.82	0.63-0.89	**1.20*-1.69***
	seedlings	**M ± SD**	0.48±0.06	0.35±0.12	0.39±0.14	0.74±0.24	0.71±0.15	0.81±0.23	**1.52*±0.35***
		Range	0.38-0.54	0.21-0.49	0.25-0.6	0.5-**1.09***	0.56-0.93	0.52-**1.15***	**1.14*-2.08***
Zn	**mature**	**M ± SD**	**2.16***±0.57	**2.40***±0.50	**1.29***±0.48	**3.69***±0.68	0.94±0.40	0.60±0.16	**1.54***±0.47
		Range	**1.43*-2.79***	**1.76*-3.17***	0.84-**2.10***	**2.84*-4.43***	0.29-**1.23***	0.42-0.76	0.75-**1.99***
	seedlings	**M ± SD**	0.41±0.12	0.59±0.14	0.33±0.12	0.92±0.22	**1.53*±**0.53	0.78±0.16	**2.31*±**0.55
		Range	0.28-0.55	0.41-0.76	0.18-0.46	0.59-**1.17***	0.94-**2.39***	0.64-**1.03***	**1.61*-3.12***
Ni	**mature**	**M ± SD**	0.76±0.15	0.61±0.23	0.75±0.31	**1.37***±0.54	0.82±0.32	**1.02***±0.45	**1.84***±0.76
		Range	0.64-**1.02***	0.37-0.89	0.4-**1.09***	0.77-**1.94***	0.45-**1.15***	0.50-**1.52***	0.95-**2.65***
	seedlings	**M ± SD**	0.29±0.13	0.24±0.10	0.26±0.08	0.49±0.13	0.85±0.07	**1.31*±1.32***	**1.96*±1.54***
		Range	0.09-0.45	0.08-0.34	0.14-0.34	0.36-0.68	0.76-0.91	0.50-**3.65***	0.52-**4.56***
Cu	**mature**	**M ± SD**	0.71±0.15	**1.37*±**0.18	0.47±0.11	**1.83*±**0.28	**1.98*±**0.35	0.67±0.124	**2.65*±**0.44
		Range	0.53-0.94	**1.14*-1.63***	0.31-0.62	**1.45*-2.25***	**1.45*-2.27***	0.53-0.86	**1.98*-3.13***
	seedlings	**M ± SD**	0.28±0.10	0.58±0.21	0.19±0.05	0.77±0.234	**2.08*±**0.39	0.70±0.16	**2.78*±**0.42
		Range	0.17-0.41	0.30-0.87	0.13-0.25	0.43-**1.04***	**1.60*-2.50***	0.50-0.91	**2.21*-3.28***

NB: M ± SD stands for mean ± standard deviations
Bold and * denote values are > 1

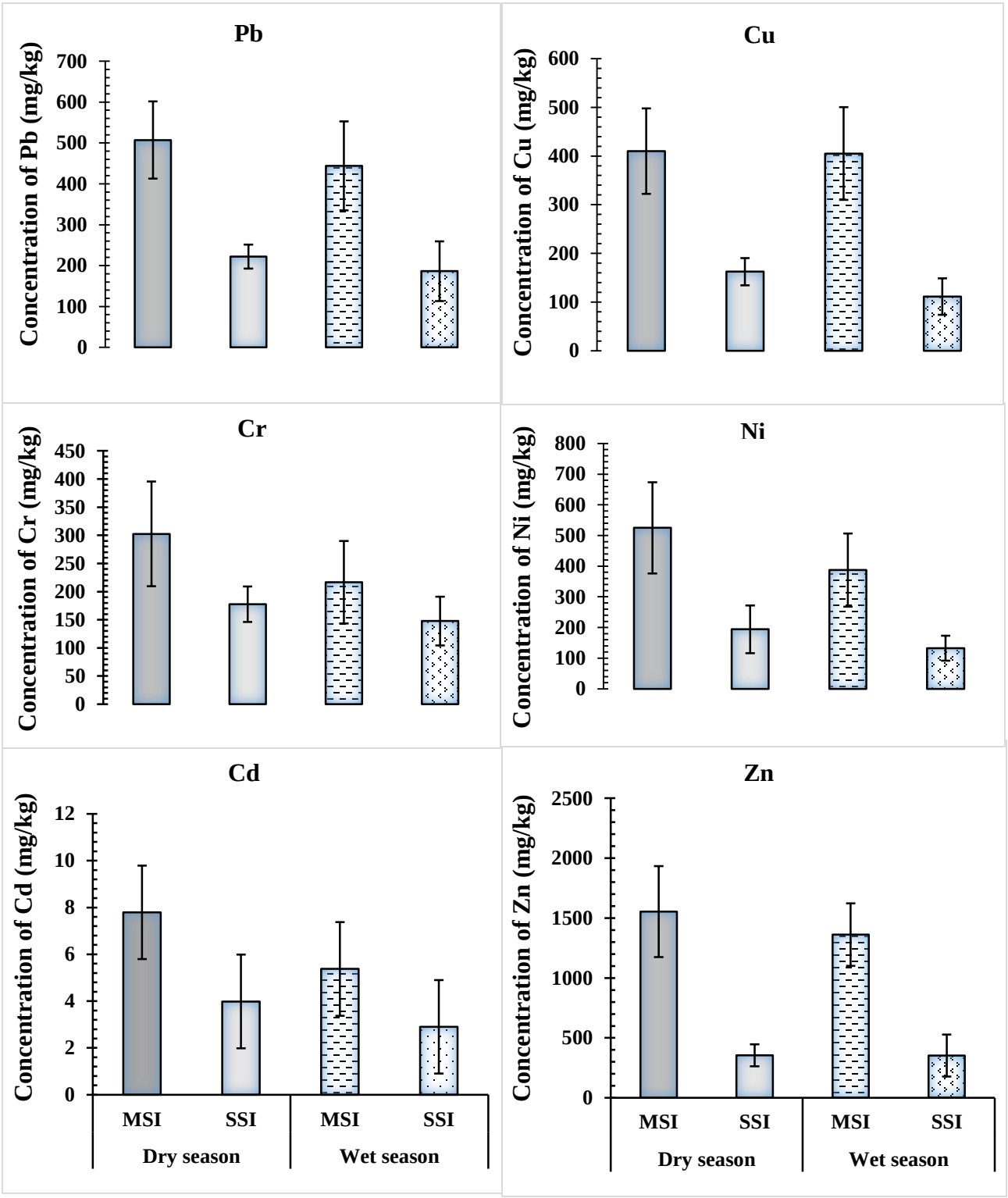
Most of the metals absorbed from soil, are uniformly distributed between shoot and root systems of *Solanum incanum* plants. However, it was noted that *Solanum incanum* has higher tendency to

use above ground tissues for metal accumulation instead of roots, except for Cd and Cr. The highest concentrations of Cd and Cr were recorded in roots of *Solanum incanum*. Further, concentrations and relative distributions of analysed heavy metals were calculated and displayed by BCF and TF values in the following sections, see (Table 12 and Table 13).

Table 13. Mean BCF and TF values of trace metals in different tissues of *Solanum incanum* in wet season.

Element	Maturity (S. Incanum-Wet)		Biological concentration factor BCF *Solanum incanum*				Translocation factor (TF) *Solanum incanum*		
			BCF root	**BCF stem**	**BCF leaf**	**BCF shoot**	**TF stem**	**TF leaf**	**TF aerial**
Cd	**mature**	**M ± SD**	0.12±0.04	0.06±0.02	0.04±0.03	0.11±0.03	0.54±0.28	0.29±0.17	0.83±0.392
		Range	0.04-0.15	0.02-0.08	0.03-0.05	0.03-0.12	0.28-0.56	0.17-0.31	0.39-0.87
	seedlings	**M ± SD**	0.06±0.03	0.03±0.01	0.02±0.01	0.05±0.02	0.55±0.23	0.28±0.212	0.81±0.41
		Range	0.03-0.15	0.01-0.08	0.01-0.05	0.02-0.12	0.23-0.56	0.212-0.31	0.41-0.87
Cr	**mature**	**M ± SD**	0.75±0.21	0.50±0.132	0.334±0.08	0.832±0.15	0.69±0.22	0.464±0.15	**1.16*±**0.31
		Range	0.6-**1.08***	0.35-0.71	0.2-0.39	0.69-1.07	0.31-0.85	0.31-0.62	0.62-**1.36***
	seedlings	**M ± SD**	0.47±0.14	0.33±0.14	0.35±0.26	0.68±0.39	0.67±0.10	0.70±0.28	1.37±0.36
		Range	0.36-0.71	0.23-0.57	0.16-0.80	0.4-**1.37***	0.55-0.80	0.42-**1.11***	**1.03*-1.91***
Pb	**mature**	**M ± SD**	0.80±0.26	0.58±0.09	0.57±0.20	**1.15*±**0.25	0.77±0.23	0.74±0.32	**1.52***±0.49
		Range	0.54-**1.20***	0.46-0.69	0.26-0.81	0.8-**1.50***	0.5-**1.11***	0.41-**1.16***	0.94-**2.27***
	seedlings	**M ± SD**	0.31±0.03	0.26±0.04	0.22±0.04	0.47±0.06	0.83±0.08	0.71±0.10	1.54*±0.04
		Range	0.27-0.35	0.21-0.29	0.17-0.26	0.39-0.55	0.74-0.96	0.57-0.84	1.48*-1.58*
Zn	**mature**	**M ± SD**	**2.23*±**0.52	**2.56*±**1.12	**1.51*±**0.41	**4.07*±**1.45	**1.13*±**0.35	0.68±0.05	**1.81*±**0.36
		Range	**1.72*-3.03***	**1.23*-3.77***	**1.03*-2.08***	**2.64*-5.77***	0.6-**1.55***	0.6-0.72	**1.29*-2.27***
	seedlings	**M ± SD**	0.50±0.16	0.59±0.23	0.43±0.20	**1.02*±**0.41	**1.17*±**0.28	0.82±0.21	**1.99*±**0.43
		Range	0.25-0.64	0.22-0.76	0.14-0.65	0.36-**1.38***	0.9-**1.55***	0.58-**1.09***	**1.48*-2.56***
Ni	**mature**	**M ± SD**	0.68±0.22	0.60±0.12	0.63±0.33	**1.23*±**0.43	0.92±0.21	0.952±0.40	**1.88*±**0.56
		Range	0.4-0.93	0.51-0.80	0.34-**1.15***	0.92-**1.95***	0.68-**1.25***	0.37-**1.34***	**1.05*-2.50***
	seedlings	**M ± SD**	0.30±0.15	0.19±0.09	0.18±0.06	0.37±0.13	0.68±0.13	0.812±0.70	**1.49*±**0.82
		Range	0.10-0.51	0.09-0.33	0.12-0.26	0.28-0.59	0.56-0.89	0.42-2.05	0.99-2.94
Cu	**mature**	**M ± SD**	0.79±0.16	**1.36*±**0.14	0.43±0.16	**1.79*±**0.29	**1.78*±**0.40	0.584±0.25	**2.36*±**0.65
		Range	0.59-**1.04***	**1.19*-1.56***	0.20-0.624	**1.39*-2.18***	**1.14*-2.16***	0.19-0.803	**1.33*-2.96***
	seedlings	**M ± SD**	0.22±0.07	0.42±0.12	0.14±0.05	0.56±0.15	**1.98*±**0.44	0.68±0.19	**2.66*±**0.54
		Range	0.15-0.32	0.22-0.50	0.11-0.22	0.33-0.713	**1.47*-2.47***	0.43-0.943	**1.99*-3.19***

NB: M* ± SD** stands for mean ± standard deviation **Bold** and ** denote values are **> 1**



NB: MSI represents Mature *Solanum incanum* SSI represents Seedlings of *Solanum incanum*

Figure 10. Metal concentration in *Solanum incanum* plants.

a. Cd concentration in different parts of *Solanum incanum*

Mean concentration (mg/kg) of Cd in whole *Solanum incanum* plant was 7.79 ± 2.29 and 5.38 ± 2.06 in dry season and rainy seasons, respectively. Similarly, in seedlings of *Solanum incanum* also mean uptake levels of Cd (3.99 ± 1.55 and 2.90 ± 1.22, mg/kg) were recorded in dry and rainy seasons, respectively (Figure 10). Different seasons of the year had insignificant effect ($p > 0.05$) on Cd uptake levels of *Solanum incanum*. The elemental distribution in different tissues of *Solanum incanum* appears in the decreasing order from root > stem > leaf, for dry season and rainy seasons. This shows roots obtained highest Cd concentrations among the plant organs. Conversely, Cd accumulation trend reported, by Dwivedi *et al*. (2014), for *Solanum nigrum* was in order of; leaf > stem > root. Similarly, Peng *et al*. (2009) also reported *Solanum nigrum* can accumulate (262 mg/kg) Cd in the leaves.

Based on the TF and BCF values of < 1, noted for Cd, *Solanum incanum* can be considered as an excluder of cadmium (Hosman *et al*., 2017). However, plants of the same family *Solanum nigrum* have been reported to have Cd hyperaccumulation ability (Jiang *et al*., 2016). Likewise, Wei *et al*. (2006) described *Solanum nigrum* had greater potential to uptake bioavailable Cd. Further, Bao *et al*. (2011) reported *Solanum lycopersicum* as a non-hyperaccumulator and *Solanum nigrum* as hyperaccumulator of Cd. Another study by Yashim *et al*. (2014) noted BCF and TF > 1 and reported *Solanum melongena* as an effective phytoremediator of Cd.

Independent sample t test t (16.70) = 2.90, $p = 0.010$ revealed Cadmium concentration in tissues of mature *Solanum incanum* was significantly higher than from that of seedlings. Cd uptake in mature *Solanum incanum* was positively correlated with Cd levels in the soil r (0.831), $p = 0.001$ and that of seedling was also correlated significantly r (0.782), $p = 0.003$, while significant value was $p = 0.01$. In addition, Cd uptake was also dependent on soil physicochemical parameters; there was strong correlation ($p < 0.05$) between Cd uptake by *Solanum incanum* and clay fraction contained in corresponding soil; CEC and EC also positively but insignificantly affected the uptake of Cd. According to Liu *et al*. (2009), positive correlation was noted between the organic matter fraction and the Cd uptake by plant, however an insignificant relationship ($p > 0.05$) was noted for OM in soil and Cd uptake in *Solanum incanum* plant. Conversely, soil pH had negative impact on the uptake level of Cd in *Solanum incanum* and significant impact ($p < 0.05$) was noted for seedlings, while impact of pH on mature plants was not statistically significant. This was also

shown in similar studies by Nanda and Abraham (2013).The present investigation also revealed, the values of BCF (root, stem and leaf), TF and shoot BAC are all less than 1; hence *Solanum incanum*, can absorb limited amount of Cd but it does not accumulate it. Therefore, this plant can be placed under the listing of Cd excluders.

b. Cr concentration in different parts of *Solanum incanum*

As observed in Table 10, the chromium content of the *Solanum incanum* plant samples varied over a wide range at 159.6 - 418.1 in dry season and 98.3 to 341.02, mg/kg in wet season. The concentration of chromium in *Solanum incanum* plants varied among sampling sites ($p < 0.05$). Correlation coefficient r (0.944) $p = 0.000 < 0.01$ revealed a significant positive correlation between levels of Cr in soil and total Cr content in *Solanum incanum*. Seedlings of *Solanum incanum* also showed mean concentration which is positively and significantly r = (0.736) $p = 0.006 < 0.01$ affected by concentration in soil. ANOVA, $p < 0.05$ also revealed there is significant variation in Cr concentration among plants from different sampling stations. However, the variation could be due to the level of Cr in different soils or metal availability differences; that could be impacted by ecological factors or soils properties (Verbruggen *et al*., 2009; Yuan *et al*., 2016).

Total mean levels of chromium absorbed in mature plants of *Solanum incanum* displayed significantly higher value than seedlings. Independent sample t test gave t (17.624) = 2.89, $p = 0.010$ which shows mean values of mature (M = 237.78 ± 97.63) and seedling (M = 143.72 ± 56.49) to be significantly different. However, the higher concentration of Cr in seedlings could be explained by the better absorptive properties in the first growing seasons and substantial decline with maturity. The decline in metal concentration might be because of metal dilution in the large biomass of plant or saturation (Eid *et al*., 2012; Chang *et al*., 2014).

Distribution of Cr levels in plant tissues of *Solanum incanum* was found to be in order of root > stem > leaf both for dry and wet season samples. And mean BCF values of Cr for different parts of *Solanum incanum* were 0.70 ± 0.17 (root) 0.53 ± 0.07 (stem) 0.42 ± 0.093 (leaves) for dry season samples and BCF of wet season samples were 0.754 ± 0.21 (root) 0.50 ± 0.132 (stem) and 0.334 ± 0.08 (leaves). From Table 11, mean BCF values in root stem and leaves were < 1; similarly, BCF values recorded for shoots were < 1 and TF values recorded for shoot (aerial part) > 1; which

implies the plant has no Cr accumulation potential (Liu *et al*., 2009). Further, the relative levels of Cr in shoots is less than soil Cr content and based on TF of < 1; therefore *Solanum incanum* cannot be utilized for clean-up of Cr contaminated sites (Haque *et al*., 2008).

c. Pb concentration in different parts of *Solanum incanum*

Lead is a relatively immobile non-essential heavy metal and considered as a very toxic environmental pollutant (Kabata-Pendias, 2007; Amin *et al*., 2018). As shown in Table 11, the concentrations of Pb in *Solanum incanum* roots varies between 173.2 and 234 mg/kg, the stem segment between 99.2 to 187.1 mg /kg, and Pb levels in the leaf section varied between 109 to 209 mg/kg in dry season. Similarly, Pb concentration in different tissues of *Solanum incanum* collected during wet season were in ranges of 106 - 244 mg/kg, 90 - 209.13 mg/kg and 80-19 mg/kg for root, stem and leaves, correspondingly. The pattern of accumulation of Pb are as follows: root (201.9 ± 22.1 mg/kg) > leaves (156.2 ± 40.7 mg/kg) > Stem (149 ± 33.4 mg/kg) for dry season and the mean values in wet season are in order of root (181.7 ± 52.6 mg/kg) > stem (136.6 ± 48.6 mg/kg) > leaves (125.8 ± 41.6 mg/kg). The same trends were observed for Pb in seedlings of *Solanum incanum*, both in wet and dry season.

The mean values of Pb in *Solanum incanum* had linear relationship with the soil Pb concentration. The correlation coefficients r = .757, $p = 0.004 < 0.01$ and $R^2 = 0.573$ shows Pb in soil explains 57.30% of Pb in mature plants of *Solanum incanum*. Similarly, r = 0.831, $p = 0.001 < 0.01$ and $R^2 = 0.6906$ shows 69.06% of Pb in seedlings of *Solanum incanum* could be estimated by Pb soil.

The total concentration of Pb in mature plants of *Solanum incanum* in dry season 507.05 ± 94.52 mg/kg was larger than mean values of Pb recorded in wet season (444.1 ± 108.8 mg/kg). The total uptake of Pb in seedlings of *Solanum incanum* in dry season (222.3 ± 177.2 mg/kg) was also larger than mean values of Pb (186.6 ± 73 mg/kg) absorbed in wet season; this could be due to evapotranspiration during dry season.

Based on the concentration of Pb obtained, BCF and TF were calculated; Mean BCF values of Pb for various tissues were 1.12 ± 0.33 (root), 0.814 ± 0.27 (stem), 0.86 ± 0.32 (leaf). These reveals that Pb bioavailability was high (> 1) in roots, while that of stem and leaves were <1 during dry season. Likewise, BCF obtained for different tissues in wet season samples were 0.80 ± 0.26 (root), 0.58 ± 0.09 (stem) and 0.57 ± 0.20 (leaves) which are all < 1 (Table 13).

On average, BAC or EF values of > 1 and TF > 1 reveals that *Solanum incanum* has the potential of translocation of Pb to the easily harvestable aerial parts or its suitability for phytoextraction of Pb (Ghavri *et al*., 2013; Gul *et al*., 2019). Similarly, Malik *et al*. (2010), studied metal concentration in *Solanum nigrum* and reported the plant can be used for phytostabilization of Pb contaminated sites. *Solanum melongena* was also reported as good phytoremediator of Pb contaminated sites (Yashim *et al*., 2014).

d. Zn concentration in different parts of *Solanum incanum*.

Zinc is an essential element having physiological importance in plants and it is required for chlorophyll biosynthesis and has key role in carbonic enzyme present in photosynthetic tissues of plants (Mousavi, 2011). However, excessive amount of Zn causes toxicity to plants; resulting in reduction in development of leaves and root length. Zn uptake by plants and its mobility in soil could be affected by factors including total Zn in soil, organic matter content, soil type, soil pH, and others (Mousavi *et al*., 2013).

Solanum incanum can also take up and accumulate Zn in its tissues. Uptake and concentration patterns of Zn in plant tissues was in the order of: stem > root > leaves in all sites are similar. The highest Zn accumulation in *Solanum incanum* plant samples during dry season 1983.16 mg/kg was observed in acidic soil (pH = 5.07) of sample at Kera site and wet season samples also gave the higher Zn recording 1711.17 mg/kg in soils of Kera site pH (5.04). Conversely, the lowest mean Zn uptakes of 1037 mg/kg during dry season and 1049 mg/kg for rainy season were recorded in Akaki sample site with a calcareous pH of 7.45 and 7.82, respectively. These results of, higher Zn accumulation in acidic soil and lower values of Zn accumulated in calcareous soils, are in agreement with Abedin *et al.* (2012) and Mousavi *et al*. (2013). The lowest total Zn uptake values of 364.5 and 500 mg/kg were recorded in rainy and dry seasons of control sites, respectively.

Similarly, seedlings of *Solanum incanum* attained maximum uptake of 639.06 mg/kg in rainy season and maximum uptake value recorded during dry season was 445.53 mg/kg. These relatively higher values for seedlings were recorded at Mekanisa site and Kera site samples, respectively. Zn accumulation has been observed in all parts of both mature plant and seedlings of *Solanum incanum*.

Zinc concentration in *Solanum incanum* showed a linear relationship with zinc concentration in soil. A strong positive relationship (r = .778, $p = 0.003 < 0.01$) was observed between Zn uptakes in mature plants and soil Zn concentration. Total Zn absorbed by seedlings of *Solanum incanum* was also significantly impacted by the amounts of Zn in soil (r = .642, $p = .024$, at the 0.05 level).

Mature *Solanum incanum* showed a better Zn uptake and accumulation properties than seedling plants. Mean value of Zn (M = 1287.02 ± 495.9 mg/kg) accumulated in mature *Solanum incanum* was significantly higher than mean value M = 315.8 ± 149.7 mg/kg obtained in seedlings. This is computed and displayed by independent sample t test t (12.99) = 6.495, $p = 0.000$. Zinc content in the aboveground part (shoot) of *Solanum incanum* was higher than Zn levels contained in roots of *Solanum incanum.* In concurrence with findings of this study, previous researches reported the likelihood of Zn accumulation in green parts of plants (Liu *et al*., 2011).

BCF and TF values > 1 for root, stem and leaves of *Solanum incanum* indicated Zn can be accumulated in tissues of this plant. The observed mean values of BCF in tissues of *Solanum incanum* were in order of stem (2.40 ± 0.50) > root (2.16 ± 0.57) > 1.29 ± 0.48 for dry season samples and likewise, BCF for rainy season samples was in order of stem (2.56 ±1.12) > root (2.23 ± 0.52) > leaves (1.512 ± 0.41) (Table 12 and Table 13).

Zinc accumulation properties of *Solanum incanum* plants were found to be better during wet season. The mean values of BCF of Zn in shoots of *Solanum incanum* were 3.69 ± 0.68 in dry season and 4.072 ± 1.454 in wet season, showing shoots of mature *Solanum incanum* can absorb and accumulate Zn to the level higher than concentration available in soil. Mean values of BCF and TF of Zn in plants from control soil were also found to be > 1. This could be due to the metabolic importance of Zn as an essential nutrient for production of protein and development (Mousavi *et al*., 2013).

e. Ni concentration in different parts of *Solanum incanum.*

The mean value of Ni accumulated in mature *Solanum incanum* plants collected from contaminated sites of Addis Ababa during dry season was 525.06 ± 148.52 mg/kg and ranging from 314.6 to 712 mg/kg. Nickel concentration in mature *Solanum incanum* plant samples collected during wet season was within the range of 280 to 589.9 mg/kg and the mean value was 387.3 ± 119.5 mg/kg. The levels of Ni in *Solanum incanum* tissues were in order of root (199.3 ±

82.02 mg/kg) > leaves (179.8 ± 67.9 mg/kg) > stem (145.98 ± 44.3 mg/kg) in dry season. Whereas, trend noted during wet season was root (147.02 ± 82.2 mg/kg) > stem (124.7 ± 43.04 mg/kg) > leaves (115.6 ± 17.04 mg/kg) (Table 11).

Ni content in mature plants of *Solanum incanum* recorded higher values than seedling plants. This can be observed from values of independent sample t test t (14.061) = 4.801, p = 0.000; showing, mean values of total Ni moped up in mature *Solanum incanum* (M = 408.9 ± 53.2 mg/kg) exceeds the value taken up by seedlings (M =153.50 ± 64.98) significantly. This is in agreement with Sharma *et al*. (2006) who stated elemental content depends on growth stages.

The concentration of Ni in *Solanum incanum* plants was proportional to Ni content in corresponding soil, correlation coefficients of r (0.801), p = 0.002 for mature plants r (0.779), p = 0.003 noted for seedlings shows a strong positive effect of soil metal concentration on plant uptake characteristics. This result concurs with findings of other researchers, and metal uptake in plants depends on metal available in soil (Jung, 2008). However, soil physicochemical parameters including pH, OM% and MC% affected the uptake level negatively and the EC, CEC and clay affected the Ni uptake positively. Moreover, significant impacts were noted for EC r (0.871), p = 0.024) and clay fraction r (0.830), p = .041 for mature plants collected during wet season; similarly, significant impacts observed in dry season were observed for seedlings; pH r (-0.884), p = 0.019 and CEC r (0.883), p = 0.020).

The BCF investigated for root, stem and leaves of both mature and seedlings of *Solanum incanum* plant samples collected during both seasons of the year were low (< 1). Values of TF > 1 also indicates that *Solanum incanum* plants can effectively transfer Ni from root to the aboveground parts (Ghosh and Singh, 2005; Syam *et al*., 2016). Translocation factors in leaves varied from 0.50 to 1.52 in dry season and 0.37 to 1.34, while in stem TF ranges form 0.45-1.15 and 0.68-1.25 for dry and wet season samples, respectively (Table 12). Based on mean values of shoot BCF (1.37 ± 0.54) and aerial TF (1.834 ± 0.76) values obtained in mature plants during dry season and shoot BCF (1.23 ± 0.43) and aerial TF (1.88 ± 0.56) recorded in wet season we can conclude *Solanum incanum* potentials for phytoremediation of Ni contaminated soil (Ghosh and Singh, 2005).

f. Cu concentration in different parts of *Solanum incanum.*

In dry season samples, total Cu absorption recorded in roots of *Solanum incanum* ranged from 44 to 135 mg/kg and accumulation levels in stems and leaves varied between 132 to 288 mg/kg and from 32.3 to 97.06 mg/kg, respectively. The minimum and the maximum levels of Cu recorded for root, stem and leaves of *Solanum incanum* collected in wet season are as follows: 20.5 to 64.08 mg/kg, 60.8 to 102.45 mg/kg and 13.4 to 39 mg/kg, respectively (Table 11).

Solanum incanum plants store most of Cu mopped up from the soil in their stem parts. Of the total Cu absorbed, 54.54% was accumulated in stem followed by 27.31% in root and 18.15% in leaves of mature plants during dry season. Portions of 52.42%, 31.08% and 16.50% were distributed in stems, roots and leaves of mature *Solanum incanum* plant samples collected during rainy season. Additionally, mature *Solanum incanum* exhibited the highest average Cu removal (502.08 mg/kg) in site 4 during dry season, and followed by 467.06 mg/kg at Kera site and 417 mg/kg at Lideta site. In rainy season, the maximum uptake (490 mg/kg) was recorded in Lideta site followed by Kera site (464 mg/kg) and Mekanisa site (455.3 mg/kg).

In addition, total uptake and accumulations of Cu in mature plants had a higher mean value than seedlings (410.1 ± 87.54 mg/kg and 162.5 ± 27.9 mg/kg) during dry season than that of values in rainy season (405.2 ± 95.3 mg/kg and 111.4 ± 37.4), respectively (Table 11). However, plants from a non-contaminated site (control) showed total Cu uptakes of 208.3 and 69.6 mg/kg in dry and rainy seasons, correspondingly. Concentration of Cu in the soil had a direct impact on the amount of Cu stored in *Solanum incanum* which could be shown by the correlation coefficient r = .936, $p = 0.000 < 0.001$.

Further, a negative relationship was noted between metal in plant and soil pH; however a significant negative impact r (-0.884), $p = 0.019$ was only noted in seedlings of *Solanum incanum* collected during dry season. Conversely, Cu plant uptake by plants showed positive correlation with clay content, CEC and EC of soil. However, soil EC and Clay content gave positive and significant correlation ($p < 0.05$) with metal concentration in *solanum incanum* plant samples collected during wet season. The correlation coefficients and significance level displayed were; r (0.871), $p = 0.024$ for EC and r (0.830), $p = 0.041$ for clay fractions.

Accumulation of Cu in *Solanum incanum* showed an increasing trend as plant growth advanced, and this concurs with Swapna *et al*. (1987) who noted a linear rise in Cu concentration in plant tissues during growth. However, total Cu accumulated in mature plants had a mean value (362.8 ± 133.8 mg/kg) of significantly higher than seedlings (128.3 ± 42.9 mg/kg). An independent sample t test displayed t (13.24) = 5.78 p = 0.000 which is highly significant.

Tables 12 and Table 13 showed, Mean BCF of Cu accumulated in tissues of *Solanum incanum* followed the order of: stem (1.37 ± 0.18) > root (0.71 ± 0.15) > leaves (0.47 ± 0.11) for mature plants collected during dry season; and BCF of mature plants collected in wet season exhibited a trend of: stem (1.36 ± 0.14) > root (0.79 ± 0.164) > leaf (0.433 ± 0.16). Similarly, considerable amount of Cu in root was translocated to the aerial parts (stem and leaf) and TF recorded for stems and leaves gave values > 1. This could be due to its biological importance as an essential metal (Swapna *et al*., 1987).

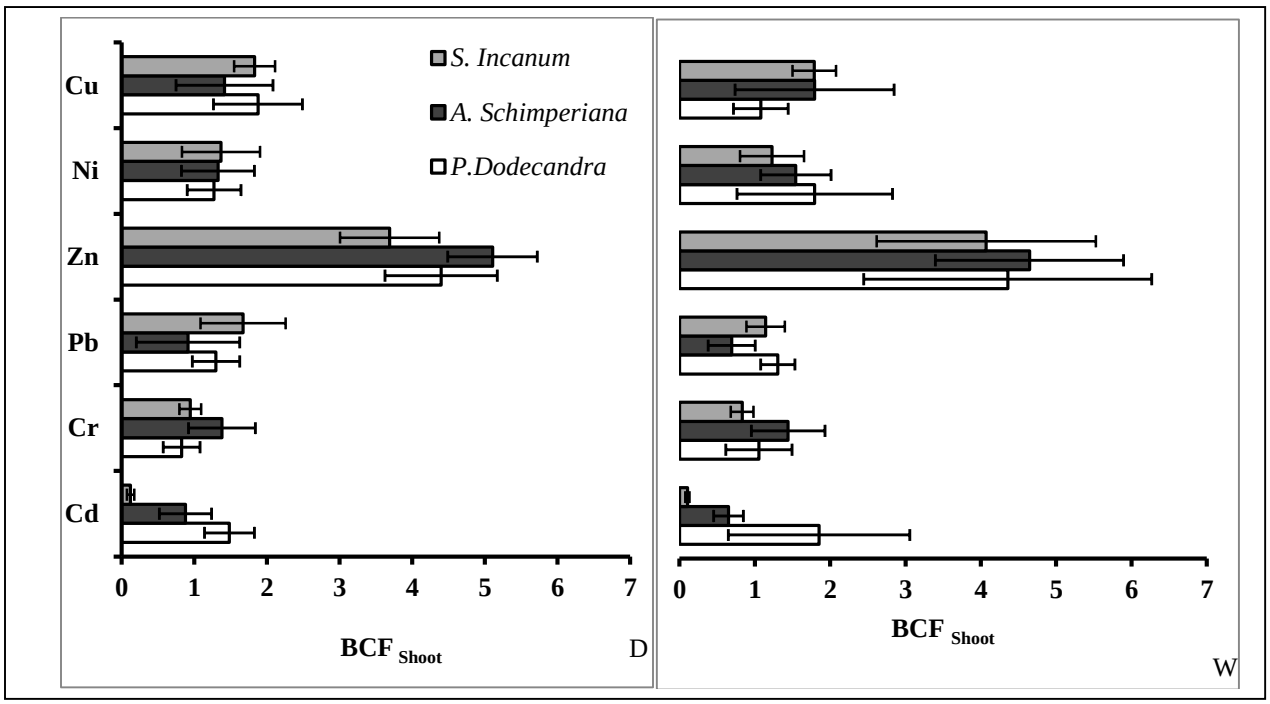
Seedlings of *Solanum incanum* had a higher aerial translocation factor (>1), especially due to higher absorption in their stem; however total shoot BCF were < 1. Hence, founded on the present findings, for mature plants of *Solanum incanum* (having both TF and shoot BCF > 1) which indicates the suitability of *solanum incanum* for phytoextraction or phytoremediation of Cu contaminated sites.

4.3.3.2. Summary of metal accumulation status in *Solanum incanum*

Potential of *Solanum incanum*, to uptake and accumulate heavy metals (mg/kg's) appears in order of Zn (1553.9 ± 380.4) > Ni (525.1 ± 148.5) > Pb (507.05 ± 94.5) > Cu (410.05 ± 87.54) > Cr (302.5 ± 92.95) > Cd (7.79 ± 2.29) during dry season. Similarly, during wet season Zn had the highest concentration in plants (1362.03 ± 260.7) followed by Pb (444.06 ± 108.79), Cu (405.2 ± 95.3), Ni (387.31 ± 119.53), Cr (216.6 ± 73.41) and Cd (5.38 ± 2.06). Results from the present investigation suggest that *Solanum incanum* has good potential for the phytoextraction of Pb, Ni, Zn, and Cu based on BCF and TF > 1; however, the TF and BCF obtained (<1) for Cd and Cr reveals that this plant could uptake a certain amount of these metals but not accumulate these trace metals from polluted soil; therefore *Solanum incanum* does not seem appropriate for the phytoremediation of Cd and Cr polluted sites.

4.4. Comparative discussion

Phytoremediation of contaminated sites can be attained via mechanisms including phytoextraction, phytodegradation, rhizofiltration, phytostabilization, phytotransformation (Saha *et al*., 2017; Eid *et al*., 2019). However, the most common and vital phytoremediation mechanisms for heavy metal contaminated sites are phytostabilization and phytoextraction (Anjum *et al*., 2014; Sidhu *et al*., 2017). Successful eco-friendly heavy metal phytoextraction process needs careful identification of plant species which can effectively uptake and translocate to the aerial parts (Chandrasekhar and Ray, 2019; Galal *et al*., 2018).



NB: D represent dry season samples; W represent wet season samples. Error bars represent standard deviations.

Figure 11. Bioconcentration factors of heavy metals in shoots of *Solanum incanum*, *Adhatoda Schimperiana* and *Phytolacca dodecandra*.

Studied plants can absorb and concentrate high amount of selected metal contaminants (Pb, Cr, Cd, Zn, Cu, and Ni) into their aerial parts, and phytoextraction and phytostabilization mechanisms were dominantly noted for all sampled plants. However, the possibility of phytodegradation or phytovolatilization mechanism, in this case, needs studies in controlled and dose-dependent investigations. Heavy metal stored in different parts of investigated plants were directly correlated to metal concentration in soil, and similar observation was reported by Elshamy *et al*. (2019). Even

though these plants can absorb selected heavy metals starting from the early stages of their development, only mature plants were considered here for comparison due to the continuous metal removal properties. Among analysed heavy metals Cd was the lowest in concentration both in soil and in different tissues of studied plants, and this concurs with Galal *et al*. (2018); Eid *et al*. (2019) who noted Cd is usually very low. The lower accumulation of Cd in plants could be due to higher accumulation of Zn because Cd is a chemical analogue to Zn and various plants fail to differentiate among these ions (Yashim *et al*., 2014; Eid *et al*., 2019). However, other factors including the biological importance of metals, metal concentrations at the sampling sites, detainment in the root part or other mechanisms at play could affect accumulation of metal in plant.

TF and BCF are the two main factors applied for evaluation of the efficacy of metal extraction by plants (Sidhu *et al*., 2017; Eid *et al*., 2019). Phytoremediation efficiency and metal mobility inside the tissues of studied plants was compared by calculating TF and BCF values. The highest shoot BCF was recorded for Zn in all studied plants both in dry and wet season and the lowest values were noted for Cd. Zinc was the most abundant metal obtained in different tissues of studied plants. Shoot BCF and aerial TF of Zn in *Adhatoda schimperiana* and *Phytolacca dodecandra* showed higher values during dry season, while *Solanum incanum* showed an opposite trend.

The mean values of shoot BCF (5.11 ± 0.62) and aerial TF (2.38 ± 0.54) recorded for Zn in *Adhatoda schimperiana* during dry season was higher than values of shoot BCF (4.65 ± 1.25) and aerial TF (2.14 ± 0.50) during wet season. Similarly, *Phytolacca dodecandra* showed a higher mean values of shoot BCF (4.40 ± 0.77) and aerial TF (2.45 ± 0.06) during dry season than values of shoot BCF (4.36 ± 1.91) and aerial TF (2.08 ± 0.16) recorded during wet season. Conversely, mean values of shoot BCF (3.69 ± 0.68) and TF (1.54 ± 0.47) recorded for Zn in *Solanum incanum* during dry season was lower than values of shoot BCF (4.07 ± 1.45) and TF (1.81 ± 0.36) noted during wet season.

Heavy metal BCF in aboveground parts of selected plants were calculated and presented in Figure 12. BCF of various heavy metals in *Phytolacca dodecandra* shoot during dry season were recorded in the following order: Zn (4.398 ± 0.77) > Cu (1.878 ± 0.61) > Cd (1.48 ± 0.35) > Pb (1.30 ± 0.33) > Ni (1.27 ± 0.37) > Cr (0.83 ± 0.25), while the trend of shoot BCF during wet season were Zn (4.36 ± 1.91) > Cd (1.85 ± 1.20) > Ni (1.79 ± 1.03) > Pb (1.31 ± 0.23) > Cu (1.08 ± 0.36) > Cr (1.05 ± 0.44). The Mean BCF values of Pb, Cr, Cd, Ni, Zn, and Cu in shoots of *Phytolacca*

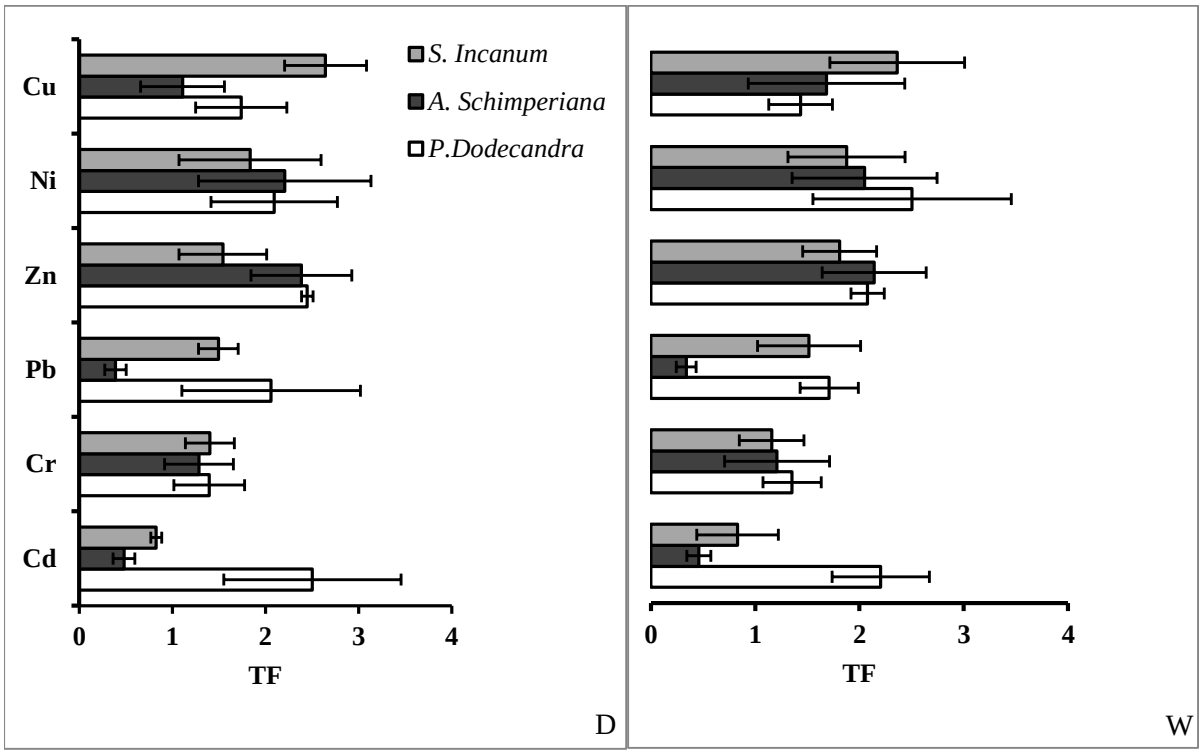
dodecandra were > 1, which reveals the potential of *Phytolacca dodecandra* for phytoremediation (Pandey, 2012; Sidhu *et al*., 2017).

In addition, higher aerial TF values of Cd (2.50 ± 0.95) > Zn (2.45 ± 0.06) > Ni (2.09 ± 0.68) > Pb (2.06 ± 0.96) > Cu (1.74 ± 0.49) > Cr (1.40 ± 0.38) were observed in dry season samples, and TF values of metals in the aerial parts of *Phytolacca dodecandra* were Ni (2.50 ± 0.95) > Cd (2.20 ± 0.47) > Zn (2.08 ± 0.16) > Pb (1.71 ± 0.28) > Cu (1.44 ± 0.31) > Cr (1.35 ± 0.28). The data in Figure 12 indicates all aerial TF values recorded were > 1 which means *Phytolacca dodecandra* can effectively transfer heavy metals into the above-ground parts and can be used for phytoextraction of metals. This agreed with Bazan and Galizia, (2018) who stated plants with adaptation to different environmental conditions and having BCF and TF > 1 can be good phytoextractors.

Adhatoda schimperiana was also observed to take up and accumulate multiple heavy metal contaminants. BCF values and TF of studied heavy metals in shoots of *Adhatoda schimperiana* were all > 1 during both seasons of the year except for Cd and Pb. Shoots of *Adhatoda schimperiana* showed mean BCF values in order of Zn (5.11 ± 0.62) > Cu (1.42 ± 0.67) > Cr (1.38 ± 0.46) > Ni (1.33 ± 0.50) > Pb (0.91 ± 0.71) > Cd (0.88 ± 0.36) in dry season, and Zn (4.65 ± 1.25) > Cu (1.79 ± 1.06) Ni (1.54 ± 0.47) > Cr (1.44 ± 0.49) > Pb (0.69 ± 0.31) > Cd (0.65 ± 0.20) in wet season samples. According to the mean shoot BCF's, heavy metal accumulation by *Solanum incanum* was in increasing order as Zn > Cu > Pb > Ni > Cr > Cd during dry season. The mean BCF value was 3.69 ± 0.68, 1.83 ± 0.28, 1.67 ± 0.59, 1.37 ± 0.54, 0.94 ± 0.15, respectively (Figure. 11). Similarly, calculated BCF's for *Solanum incanum* collected during wet season were 4.07 ± 1.45, 1.79 ± 0.29, 1.23 ± 0.43, 1.14 ± 0.25, 0.83 ± 0.15, 0.11 ± 0.03 for Zn, Cu, Ni, Pb, Cr, and Cd, respectively. Mean BCF values recorded for shoots of *Solanum incanum* plants were > 1 for Cu, Zn, Ni, Pb while Cd and Cr gave values < 1.

In addition to the biomass yield, tolerance to multiple contaminants and bioaccumulation factors, translocation factors or metal mobility to the aerial parts of plants are very crucial to evaluate the effectiveness of phytoremediation (Ali *et al*., 2013; Sidhu *et al*., 2017). Accordingly, based on the mean values calculated for aerial TF of trace metals studied plants can be ordered as follows: *Phytolacca dodecandra* > *Solanum incanum* > *Adhatoda schimperiana* for Cd, Cr and Pb during both seasons. However, seasonal variation was noted in the translocation characteristics of Zn, Ni

and Cu within plants. The highest mean TF of Zn was observed in *Phytolacca dodecandra* followed by *Adhatoda schimperiana* and *Solanum incanum* in dry season, while during wet season *Adhatoda schimperiana* showed a higher TF followed by *Phytolacca dodecandra* and *Solanum incanum.* The higher TF of Zn, Cu and Ni in aerial biomass of plants is coherent since these metals are essential to the plants (Guarino *et al*., 2019).



D-represent dry season samples, W-represent wet season samples. Error bars represent standard deviations.

Figure 12. Translocation Factors of heavy metals in shoots of *Solanum incanum*, *Adhatoda Schimperiana* and *Phytolacca dodecandra.*

Aerial TF of Ni calculated was higher for *Adhatoda schimperiana* during dry season followed by *Phytolacca dodecandra* and *Solanum incanum*, whereas *Phytolacca dodecandra* showed a better mean value during wet season. *Solanum incanum* was found to be superior in translocation of Cu followed by *Phytolacca dodecandra* and *Adhatoda schimperiana.* Mean values of aerial TF were all > 1, except for Pb in *Adhatoda schimperiana* and Cd in *Solanum incanum* and *Adhatoda schimperiana.* The ability of plant's to accumulate metal contaminants from polluted soil, or phytoremediation potential to clean-up heavy metals can be better explained by the calculated values of BCF and TF than simple total metal available in plant. Thus, in this study, the mean

values of BCF >1 and TF >1 noted for the native plants indicated, majority of studied heavy metals can be removed from contaminated soil using these plants. The total mean values of BCF and TF, regardless of the contributions of seasonal variation and soil physicochemical properties, selected plants are suitable for remediation of heavy metal contaminated sites.

CHAPTER FIVE

5. Conclusions and recommendations

5.1. Conclusions

The current investigation indicated, soil physicochemical parameters organic matter (OM), moisture content (MC), clay content, cation exchange capacity (CEC); showed no significant variation ($p > 0.05$) among different sampling sites; while pH and EC varied significantly ($p < 0.05$). Thus, these had limited differential influence on the phytoremediation studies of different sites. Investigation of trace metals in experimental soil revealed trace metals Cr, Cd, Pb, Ni and Cu had mean values significantly higher than recommended maximum limit, but Zn showed mean value of less than recommended maximum limits. The current level of toxic metals in the soil of studied sites could have significant effect on human health and receiving ecosystem. Therefore, there is a need for urgent remediation and restoration of this soil.

Three different plant species, viz. *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum,* were evaluated for their heavy metal phytoremediation potentials. These plants grow in multi-metal contaminated sites without showing stress related morphological symptoms. The outcomes of this research have shown Cr, Pb, Cd, Cu, Zn and Ni were distributed in tissues of all selected plants (*Phytolacca dodecandra, Adhatoda schimperiana and Solanum incanum*). Mature plants of all selected plants showed significantly higher ($p < 0.05$) metal removal capacity, as shown by the BCF and TF's, than younger plants. A gradual rise in metal uptake by plants was observed with an increase in metal concentration of soil. Correlation analysis ($p < 0.05$) revealed; the levels of individual metal in the corresponding soil positively and significantly correlated to levels of metals contained in plants. Thus, it can be concluded that selected plants are appropriate for phytoremediation of sites contaminated by the studied heavy metals.

Each metal absorbed by *Phytolacca dodecandra* had peculiar characteristics of accumulation; for instance, Cd and Cr were abundant in the roots of *Phytolacca dodecandra*; while Pb, Zn and Ni were higher in leaves; whereas Cu showed higher values in roots during dry season and higher values in stem during wet season. The order of total metal concentrations in *Phytolacca dodecandra* is: Zn > Ni > Pb > Cr > Cu > Cd for wet season and Zn > Pb > Ni > Cu > Cr > Cd for dry season samples. In addition, mean values of studied metals accumulated in plants collected

during dry season were higher than those collected during wet season. Further, based on the TF, BCF, and BAC values it can be suggested that *Phytolacca dodecandra* is a potential accumulator of Zn, Cu, Ni, Pb, and Cd; while Cr was not sufficiently accumulated in the shoot.

Adhatoda schimperiana has good potential to accumulate Ni and Zn, in its upper tissues; leaves contain the largest share of metals taken up by the entire plant. Distribution of Cd, Cr and Pb in *Adhatoda schimperiana* highlighted higher concentration in roots. However, *Adhatoda schimperiana* can be effectively utilized for phytoremediation of metal contaminated sites. Mechanism involved for removal of Cd and Pb was phytostabilization; while Cr, Zn and Ni, was phytoextraction as based on values of (BCF > 1, TF > 1 and BAC > 1). The result from this study indicates that *Solanum incanum* is good in taking up and distributing trace metals in their tissues. Among all investigated metals, the plant *Solanum incanum* accumulated higher concentration of Zn. The BCF of Cd in *Solanum incanum* plants was < 1, TF was also < 1, indicating these plants retain larger portion of metals in their roots than other parts; similarly, Cr was more in roots and the BCF of shoot < 1 and TF > 1, revealing that the plant may not be suitable for phytoremediation of Cr contaminated sites. Pb was also higher in root, but BCF and TF > 1 shows its potential for phytoextraction. Ni, Cu and Zn had the highest accumulation in the stems of *Solanum incanum,* showing the suitability for phytoextraction of Zn, Cu and Ni contaminated sites.

Finally, multiple metal uptake properties, adaptability of candidate native plant species to multiple metal contamination and prevailing environmental conditions could make these plants suitable for phytoremediation of metalliferous sites. Besides, using plants as suitable green filters, such as *Solanum incanum, Adhatoda schimperiana* and *Phytolacca dodecandra* for remediation of contaminated sites, has a great potential contribution towards the successful implementation of zero carbon economy. The contribution of these plants for reduction of soil erosion, energy recovery, and other environmental services could be an additional advantage. Hence, the finding of this study is very useful and worthwhile for researchers and government policy makers to propose low cost and nature-based solution for environmental pollution. It can pave the way for further study and implementation of the phytoremediation project using these plants to clean heavy metal contaminants from soil and mitigate harmful environmental impacts of heavy metals released through anthropogenic activities. Finally, it can be concluded that potential adoptions of phytoremediation in the Ethiopian as well as the study area setting is very promising. Summary of the most important findings from this study are presented in Schematic diagram (Figure. 13).

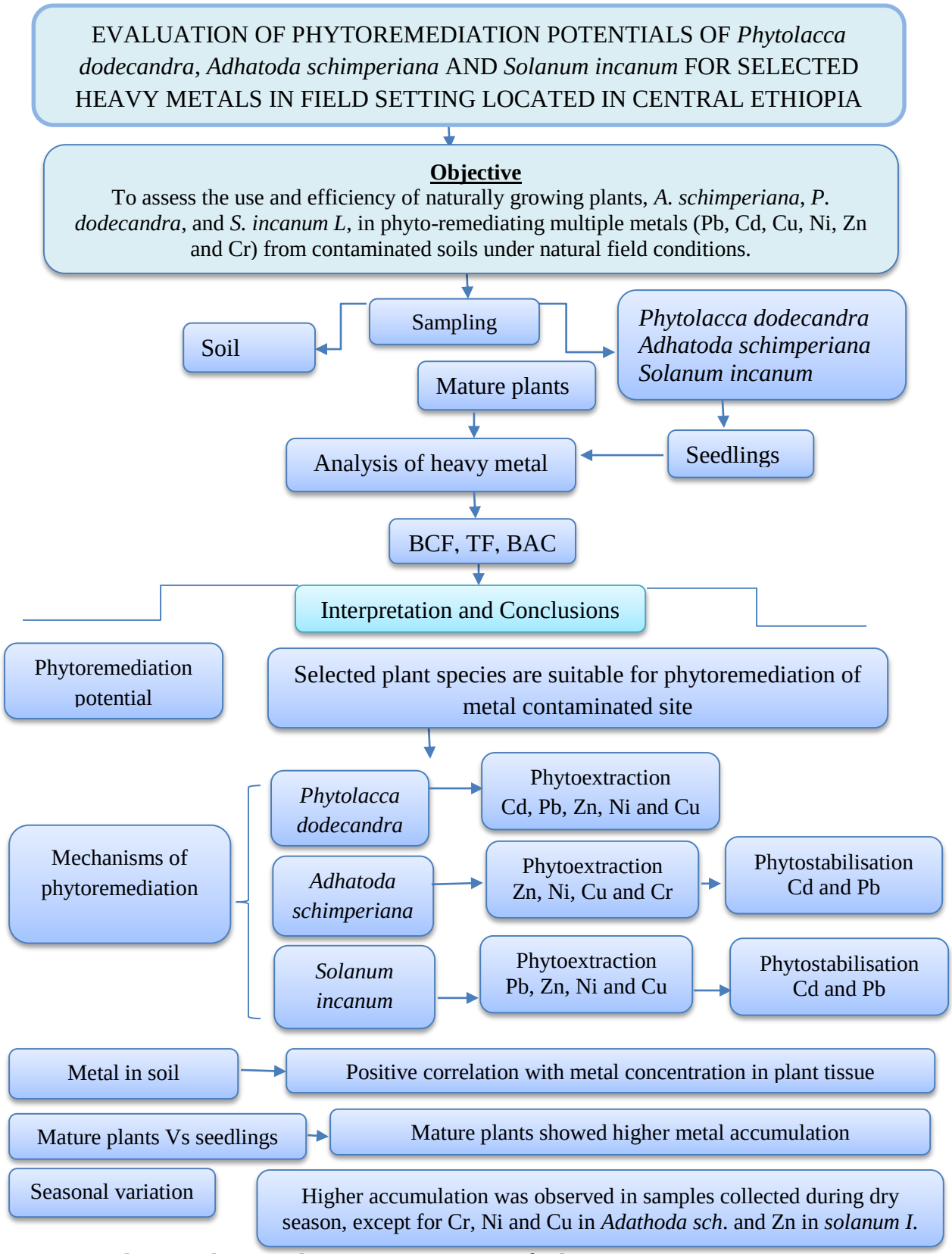


Figure 13. Schematic diagram showing most important findings

5.2. Recommendations

Screening of suitable plants, especially native ones, for phytoremediation and implementation of projects along with other development works can have numerous environmental relevant advantages. Since different plants native to contaminated sites have different unique growth and phytoremediation potentials, robust plants that will aid the remediation process at high efficiencies and economically promising way shall be discovered.

Using this primary investigation as an open window of opportunity; one can further investigate the applicability of these plants to other contaminants.

Even though the removal efficiencies were high in the early growth stages; allowing the plant grow to maturity could give a better result; or using the continuous removal properties and advantages of larger biomass could meet the ultimate goal of complete heavy metal removal. There is need to establish the best life stage at which the most remediation is realized and use such as the target in actual field applications.

The use of harvesting and re-growth methods and multiple planting for better removal or successful extraction of target contaminant and using mixed planting in case of multiple metal contaminated sites should be evaluated and applied.

Further studies in controlled environments can investigate the age dependent variation and trends of metal removal based on time of exposure and dose of contaminant.

Research on these plants will be desirable to clarify the biochemistry of metal accumulation, translocation in the plant shoots and tolerance mechanisms.

Finally, further investigations on plant physiologies, the use of advanced agronomic practice, management systems that enhance the metal availability, accumulation and translocation may play important role for an efficient and better remediation of heavy metals.

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7. APPENDICES

7.1. Appendix 1. Summaries of selected statistical datas

T-test for mean differences of metal accumulation in mature and seedlings of *phytolacca dodecandra*

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cd_ Phyto _T-test	mature	12	62.3867	40.18075	11.59918
	seedlings	12	18.0675	9.35153	2.69955

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_ phyto	Equal variances assumed	20.085	.000	3.721	22	.001	44.31917	11.9092	19.62103	69.0173
	Equal variances not assumed			3.721	12.188	.003	44.31917	11.90918	18.41565	70.2227

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cr_ Phyto _T-test	mature	12	262.6850	118.94936	34.33772
	seedlings	11	155.7973	74.64250	22.50556

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cr_ phyto	Equal variances assumed	1.611	.218	2.552	21	.019	106.88773	41.87666	19.80045	193.97500
	Equal variances not assumed			2.603	18.687	.018	106.88773	41.05581	20.85948	192.91597

Group Statistics

	maturity	N	Mean	Std. Deviation	Std. Error Mean
Pb_ phyto _ T_ test	mature	12	417.3400	188.06968	54.29104
	seedlings	12	258.1275	115.60013	33.37088

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Pb_ Phyto	Equal variances assumed	2.119	.160	2.498	22	.020	159.21250	63.72702	27.05075	291.37425
	Equal variances not assumed			2.498	18.274	.022	159.21250	63.72702	25.47058	292.95442

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Ni_ Phyto_ T-test	Mature	12	405.9533	185.40697	53.52238
	seedlings	12	178.7525	85.18303	24.59022

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ni_ Phyto	Equal variances assumed	7.349	.013	3.857	22	.001	227.20083	58.90097	105.04770	349.35397
	Equal variances not assumed			3.857	15.446	.001	227.20083	58.90097	101.97121	352.43046

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Zn_ phyto_ T-test	mature	12	1550.7492	691.19411	199.53055
	seedlings	12	242.7725	96.64428	27.89880

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zn_ Phyto	Equal variances assumed	17.631	.000	6.492	22	.000	1307.97667	201.47155	890.15025	1725.80308
	Equal variances not assumed			6.492	11.430	.000	1307.97667	201.47155	866.56738	1749.38595

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cu_ Phyt_ T-test	Mature plant	12	292.15417	148.297588	42.809826
	seedlings	12	103.81833	46.128995	13.316294

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cu_ Phyto	Equal variances assumed	10.877	.003	4.201	22	.000	188.335833	44.833078	95.357720	281.313947
	Equal variances not assumed			4.201	13.109	.001	188.335833	44.833078	91.561583	285.110084

T-test for mean differences of metal accumulation in mature and seedlings of *Adhatoda schimperiana*

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cd_ Adha _T-test	mature plants	12	55.9900	34.11938	9.84942
	seedlings	12	19.2367	9.03856	2.60921

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_ Adha	Equal variances assumed	18.044	.000	3.607	22	.002	36.75333	10.18916	15.62231	57.88436
	Equal variances not assumed			3.607	12.536	.003	36.75333	10.18916	14.65794	58.84872

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cr_ Adha _ T-test	Mature plants	12	352.1842	158.41288	45.72986
	Seedlings	12	160.9358	62.35892	18.00147

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cr_ Adha	Equal variances assumed	12.865	.002	3.891	22	.001	191.24833	49.14543	89.32695	293.16971
	Equal variances not assumed			3.891	14.329	.002	191.24833	49.14543	86.06858	296.42809

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Adha _ Pb_ T- test	Mature plants	12	470.2817	196.61645	56.75828
	Seedlings	12	207.0983	110.31502	31.84520

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Adha _Pb	Equal variances assumed	4.337	.049	4.044	22	.001	263.18333	65.08164	128.21228	398.15439
	Equal variances not assumed			4.044	17.301	.001	263.18333	65.08164	126.05489	400.31177

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Zn_ Adha _T-test	Mature plants	12	1359.4775	741.14341	213.94967
	seedlings	12	216.8775	100.67629	29.06274

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zn_ Adha	Equal variances assumed	24.091	.000	5.292	22	.000	1142.60000	215.91458	694.82057	1590.37943
	Equal variances not assumed			5.292	11.406	.000	1142.60000	215.91458	669.43000	1615.77000

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Ni_ Adha _T -test	Mature plants	12	467.3925	252.48776	72.88694
	seedlings	12	160.1875	63.58001	18.35397

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ni_ Adha	Equal variances assumed	13.336	.001	4.087	22	.000	307.20500	75.16232	151.32790	463.08210
	Equal variances not assumed			4.087	12.389	.001	307.20500	75.16232	144.00949	470.40051

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cu_ Adha_ T-test	mature plants	12	375.8858	170.53047	49.22791
	seedlings	12	115.4917	44.62191	12.88123

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cu_ Adha	Equal variances assumed	13.271	.001	5.117	22	.000	260.39417	50.88529	154.86453	365.92380
	Equal variances not assumed			5.117	12.499	.000	260.39417	50.88529	150.01383	370.77450

T-test for mean differences of metal accumulation in mature and seedlings of *Solanum Incanum*

Group Statistics

	maturity	N	Mean	Std. Deviation	Std. Error Mean
Cd_ Sola _T-test	mature	12	5.8258	2.81486	.81258
	seedling	12	3.1625	1.48693	.42924

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_ Sola	Equal variances assumed	3.979	.059	2.898	22	.008	2.66333	.91899	.75747	4.56919
	Equal variances not assumed			2.898	16.695	.010	2.66333	.91899	.72174	4.60492

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Cr_ Sola	mature	12	237.7833	97.62596	28.18219
	seedlings	12	143.7208	56.49417	16.30846

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cr_ Sola	Equal variances assumed	6.695	.017	2.889	22	.009	94.06250	32.56074	26.53567	161.58933
	Equal variances not assumed			2.889	17.624	.010	94.06250	32.56074	25.55026	162.57474

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Pb_ Sola_ T-test	mature	12	415.4567	168.40244	48.61360
	seedlings	12	183.1333	71.16581	20.54380

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Pb_ Sola	Equal variances assumed	5.946	.023	4.402	22	.000	232.32333	52.77622	122.87215	341.77451
	Equal variances not assumed			4.402	14.807	.001	232.32333	52.77622	119.70595	344.94072

Group Statistics

	Maturity	N	Mean	Std. Deviation	Std. Error Mean
Zn_ Sola_ T- test	Mature	12	1287.0158	495.90480	143.15539
	seedling	12	315.8192	149.66368	43.20418

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zn_ sola	Equal variances assumed	9.532	.005	6.495	22	.000	971.19667	149.53283	661.08457	1281.30877
	Equal variances not assumed			6.495	12.987	.000	971.19667	149.53283	648.11861	1294.27472

Group Statistics

	maturity	N	Mean	Std. Deviation	Std. Error Mean
Ni_ Sola_ T-test	mature	12	408.9017	172.46483	49.78631
	seedlings	12	153.4992	64.98080	18.75834

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ni_ Sola	Equal variances assumed	11.703	.002	4.801	22	.000	255.40250	53.20293	145.06637	365.73863
	Equal variances not assumed			4.801	14.061	.000	255.40250	53.20293	141.34032	369.46468

Group Statistics

	maturity	N	Mean	Std. Deviation	Std. Error Mean
Cu _sola_ T-test	mature	12	362.8275	133.80116	38.62507
	seedlings	12	128.3367	42.91534	12.38859

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cu_ Sola	Equal variances assumed	11.373	.003	5.781	22	.000	234.49083	40.56320	150.36791	318.61376
	Equal variances not assumed			5.781	13.240	.000	234.49083	40.56320	147.02026	321.96141

Correlation coefficients for relationships between metal concentrations in soils and metal accumulation in mature and seedlings of *phytolacca dodecandra*

Correlations

		soil	Cd_ Phytolacca _mature
soil	Pearson Correlation	1	.778**
	Sig. (2-tailed)		.003
	N	12	12
Cd_ Phytolacca _mature	Pearson Correlation	.778**	1
	Sig. (2-tailed)	.003	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		soil	Cd_ Phytolacca _seedlings
soil	Pearson Correlation	1	.493
	Sig. (2-tailed)		.103
	N	12	12
Cd_ phytolacca_ seedlings	Pearson Correlation	.493	1
	Sig. (2-tailed)	.103	
	N	12	12

Correlations

		Soil	Cr_ phytolacca _mature
Soil	Pearson Correlation	1	.834**
	Sig. (2-tailed)		.001
	N	12	12
Cr_ phytolacca _mature	Pearson Correlation	.834**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil	Cr_ phytolacca seedlings
Soil	Pearson Correlation	1	.647*
	Sig. (2-tailed)		.023
	N	12	12
Cr_ phytolacca seedlings	Pearson Correlation	.647*	1
	Sig. (2-tailed)	.023	
	N	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Soil	Pb_ Phytolacca _mature
Soil	Pearson Correlation	1	.808**
	Sig. (2-tailed)		.001
	N	12	12
Pb_ phytolacca _mature	Pearson Correlation	.808**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil	Pb_ Phytolacca _seedlings
Soil	Pearson Correlation	1	.819**
	Sig. (2-tailed)		.001
	N	12	12
Pb_ Phytolacca _seedlings	Pearson Correlation	.819**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil	Zn_ mature_ phytolacca
Soil	Pearson Correlation	1	.767**
	Sig. (2-tailed)		.004
	N	12	12
Zn_ mature_ phytolacca	Pearson Correlation	.767**	1
	Sig. (2-tailed)	.004	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil	Zn _phytolacca _seedlings
Soil	Pearson Correlation	1	.405
	Sig. (2-tailed)		.191
	N	12	12
Zn _phytolacca _seedlings	Pearson Correlation	.405	1
	Sig. (2-tailed)	.191	
	N	12	12

Correlations

		Soil	Ni_ phytolacca_ mature
Soil	Pearson Correlation	1	.644*
	Sig. (2-tailed)		.024
	N	12	12
Ni_ phytolacca _ mature	Pearson Correlation	.644*	1
	Sig. (2-tailed)	.024	
	N	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Soil	Ni_ phytolacca _ seedlings
Soil	Pearson Correlation	1	.320
	Sig. (2-tailed)		.310
	N	12	12
Ni_ phytolacca _ seedlings	Pearson Correlation	.320	1
	Sig. (2-tailed)	.310	
	N	12	12

Correlations

		Soil	Cu_ phytolacca_ mature
Soil	Pearson Correlation	1	.530
	Sig. (2-tailed)		.077
	N	12	12
Cu_ phytolacca _mature	Pearson Correlation	.530	1
	Sig. (2-tailed)	.077	
	N	12	12

Correlations

		Soil	Cu_ Phytolacca _seedlings
Soil	Pearson Correlation	1	.423
	Sig. (2-tailed)		.171
	N	12	12
Cu_ Phytolacca _seedlings	Pearson Correlation	.423	1
	Sig. (2-tailed)	.171	
	N	12	12

Correlation coefficients for relationships between metal concentrations in soils and metal accumulation in mature and seedlings of *Adhatoda schimperiana*

Correlations

		Cd_ soil	Cd_ mature_ Adhatoda
Cd_ soil	Pearson Correlation	1	.815**
	Sig. (2-tailed)		.001
	N	12	12
Cd_ mature_ Adhatoda	Pearson Correlation	.815**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Cd_ soil	Cd _seedling _ Adhatoda
Cd_ soil	Pearson Correlation	1	.632*
	Sig. (2-tailed)		.027
	N	12	12
Cd _seedling _ Adhatoda	Pearson Correlation	.632*	1
	Sig. (2-tailed)	.027	
	N	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Cr	Cr _Adhatoda_ Mature
Cr	Pearson Correlation	1	.791**
	Sig. (2-tailed)		.002
	N	12	12
Cr _ Adhatoda _Mature	Pearson Correlation	.791**	1
	Sig. (2-tailed)	.002	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Cr	Cr _ Adhatoda _ Seedling
Cr	Pearson Correlation	1	.789**
	Sig. (2-tailed)		.002
	N	12	12
Cr _ Adhatoda _ Seedling	Pearson Correlation	.789**	1
	Sig. (2-tailed)	.002	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Pb_ soil	Pb_ Adhatoda _mature
Pb_ soil	Pearson Correlation	1	.711**
	Sig. (2-tailed)		.010
	N	12	12
Pb_ Adhatoda _mature	Pearson Correlation	.711**	1
	Sig. (2-tailed)	.010	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Pb_ Soil	Pb_ Adhatoda seedling
Pb_ Soil	Pearson Correlation	1	.755**
	Sig. (2-tailed)		.005
	N	12	12
Pb_ Adhatoda seedling	Pearson Correlation	.755**	1
	Sig. (2-tailed)	.005	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil_Zn	Zn_ Adhatoda _mature
Soil_ Zn	Pearson Correlation	1	.865**
	Sig. (2-tailed)		.000
	N	12	12
Zn_ Adhatoda _mature	Pearson Correlation	.865**	1
	Sig. (2-tailed)	.000	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil_ Zn	Zn_ Adhatoda_ seedling
Soil_ Zn	Pearson Correlation	1	.503
	Sig. (2-tailed)		.096
	N	12	12
Zn_ Adhatoda _ seedling	Pearson Correlation	.503	1
	Sig. (2-tailed)	.096	
	N	12	12

Correlations

		Ni_ soil	Ni_ mature _Adhatoda
Ni_ soil	Pearson Correlation	1	.906**
	Sig. (2-tailed)		.000
	N	12	12
Ni_ mature _Adhatoda	Pearson Correlation	.906**	1
	Sig. (2-tailed)	.000	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Ni_ soil	Ni_ Adhatoda _seedling
Ni _soil	Pearson Correlation	1	.465
	Sig. (2-tailed)		.128
	N	12	12
Ni_ Adhatoda _seedling	Pearson Correlation	.465	1
	Sig. (2-tailed)	.128	
	N	12	12

Correlations

		Soil_ Cu	Cu_ Adhatoda_ mature
Soil_ Cu	Pearson Correlation	1	.591*
	Sig. (2-tailed)		.043
	N	12	12
Cu_ Adhatoda_ mature	Pearson Correlation	.591*	1
	Sig. (2-tailed)	.043	
	N	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Soil_ Cu	Cu_ Adhatoda_ seedling
Soil_ Cu	Pearson Correlation	1	.471
	Sig. (2-tailed)		.123
	N	12	12
Cu_ Adhatoda_ seedling	Pearson Correlation	.471	1
	Sig. (2-tailed)	.123	
	N	12	12

Correlation coefficients for relationships between metals concentrations in soils and metal accumulation in mature and seedlings of *Adhatoda schimperiana*

Correlations

		Cd in soil	Cd _ solanum_ mature
Cd in soil	Pearson Correlation	1	.831**
	Sig. (2-tailed)		.001
	N	12	12
Cd _ solanum_ mature	Pearson Correlation	.831**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Cd in soil	Cd _seedlings_ solanum
Cd in soil	Pearson Correlation	1	.782**
	Sig. (2-tailed)		.003
	N	12	12
Cd _seedlings_ solanum	Pearson Correlation	.782**	1
	Sig. (2-tailed)	.003	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil_ Cr	Cr_ solanum _mature
Soil_ Cr	Pearson Correlation	1	.944**
	Sig. (2-tailed)		.000
	N	12	12
Cr_ solanum _mature	Pearson Correlation	.944**	1
	Sig. (2-tailed)	.000	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil_ Cr	Cr_ solanum _seedlings
Soil_ Cr	Pearson Correlation	1	.736**
	Sig. (2-tailed)		.006
	N	12	12
Cr_ solanum _seedlings	Pearson Correlation	.736**	1
	Sig. (2-tailed)	.006	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Pb_ soil	Pb_ solanum _ mature
Pb_ soil	Pearson Correlation	1	.757**
	Sig. (2-tailed)		.004
	N	12	12
Pb_ solanum _ mature	Pearson Correlation	.757**	1
	Sig. (2-tailed)	.004	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Pb_ soil	Pb_ solanum _seedling
Pb_ soil	Pearson Correlation	1	.831**
	Sig. (2-tailed)		.001
	N	12	12
Pb_ solanum _seedling	Pearson Correlation	.831**	1
	Sig. (2-tailed)	.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Zn_ soil	Zn _ solanum_ mature
Zn_ soil	Pearson Correlation	1	.778**
	Sig. (2-tailed)		.003
	N	12	12
Zn _ solanum_ mature	Pearson Correlation	.778**	1
	Sig. (2-tailed)	.003	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Zn_ soil	Zn _ seedlings_ solanum
Zn_ soil	Pearson Correlation	1	.642*
	Sig. (2-tailed)		.024
	N	12	12
Zn _ seedlings_ solanum	Pearson Correlation	.642*	1
	Sig. (2-tailed)	.024	
	N	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations

		Soil_ Ni	Ni_ solanum_ mature
Soil_ Ni	Pearson Correlation	1	.801**
	Sig. (2-tailed)		.002
	N	12	12
Ni_ solanum_ mature	Pearson Correlation	.801**	1
	Sig. (2-tailed)	.002	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Soil_ Ni	Ni_ solanum_ seedlings
Soil_ Ni	Pearson Correlation	1	.779**
	Sig. (2-tailed)		.003
	N	12	12
Ni_ solanum_ seedlings	Pearson Correlation	.779**	1
	Sig. (2-tailed)	.003	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Cu_ soil	Cu_ solanum _ mature
Cu_ soil	Pearson Correlation	1	.936**
	Sig. (2-tailed)		.000
	N	12	12
Cu_ solanum _ mature	Pearson Correlation	.936**	1
	Sig. (2-tailed)	.000	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Cu_ soil	Cu_ solanum _ seedling
Cu_ soil	Pearson Correlation	1	.568
	Sig. (2-tailed)		.054
	N	12	12
Cu_ solanum _ seedling	Pearson Correlation	.568	1
	Sig. (2-tailed)	.054	
	N	12	12

Correlation coefficient between soil physicochemical parameters and metal accumulation in *Phytolacca dodecandra* (Dry season)

		Cd_ in mature (Phyto /Dry)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd in mature (Phyto /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd in seedling	Pearson Correlation	.913*	1						
	Sig. (2-tailed)	.011							
	N	6	6						
pH	Pearson Correlation	-.501	-.544	1					
	Sig. (2-tailed)	.311	.264						
	N	6	6	6					
EC	Pearson Correlation	.720	.863*	-.520	1				
	Sig. (2-tailed)	.107	.027	.291					
	N	6	6	6	6				
CEC	Pearson Correlation	.553	.578	.020	.743	1			
	Sig. (2-tailed)	.255	.229	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.096	.083	.098	.142	.448	1		
	Sig. (2-tailed)	.856	.876	.853	.789	.373			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.453	.633	-.321	.636	.683	.814*	1	
	Sig. (2-tailed)	.366	.178	.535	.174	.134	.049		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.368	-.293	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.473	.574	.508	.497	.419	.311	.612	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Cr_ in mature (Phyto /Dry)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ in mature (Phyto /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cr_ in seedlings	Pearson Correlation	.819*	1						
	Sig. (2-tailed)	.046							
	N	6	6						
pH	Pearson Correlation	-.805	-.502	1					
	Sig. (2-tailed)	.053	.310						
	N	6	6	6					
EC	Pearson Correlation	.803	.660	-.520	1	.743			
	Sig. (2-tailed)	.055	.153	.291		.090			
	N	6	6	6	6	6			
CEC	Pearson Correlation	.203	.161	.020	.743	1			
	Sig. (2-tailed)	.699	.761	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.244	-.239	.098	.142	.448	1		
	Sig. (2-tailed)	.642	.648	.853	.789	.373		.049	.311
	N	6	6	6	6	6	6	6	6
Clay	Pearson Correlation	.287	.072	-.321	.636	.683	.814*	1	.265
	Sig. (2-tailed)	.581	.893	.535	.174	.134	.049		.612
	N	6	6	6	6	6	6	6	6
MC	Pearson Correlation	-.167	-.241	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.752	.646	.508	.497	.419	.311	.612	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Pb_ in mature (Phyt /Dry)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ in mature (Phyt /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ in seedlings	Pearson Correlation	.660	1						
	Sig. (2-tailed)	.154							
	N	6	6						
pH	Pearson Correlation	-.463	-.887*	1					
	Sig. (2-tailed)	.355	.018						
	N	6	6	6					
EC	Pearson Correlation	.524	.819*	-.520	1				
	Sig. (2-tailed)	.286	.046	.291					
	N	6	6	6	6				
CEC	Pearson Correlation	.331	.327	.020	.743	1			
	Sig. (2-tailed)	.521	.527	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.116	-.148	.098	.142	.448	1		
	Sig. (2-tailed)	.827	.780	.853	.789	.373			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.384	.418	-.321	.636	.683	.814*	1	
	Sig. (2-tailed)	.453	.410	.535	.174	.134	.049		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.248	-.081	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.636	.879	.508	.497	.419	.311	.612	
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Zn_ in mature (Phyto /Dry)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Phyto /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ in seedlings	Pearson Correlation	.381	1						
	Sig. (2-tailed)	.456							
	N	6	6						
pH	Pearson Correlation	-.093	-.302	1					
	Sig. (2-tailed)	.860	.561						
	N	6	6	6					
EC	Pearson Correlation	.827*	.356	-.520	1				
	Sig. (2-tailed)	.042	.489	.291					
	N	6	6	6	6				
CEC	Pearson Correlation	.962**	.249	.020	.743	1			
	Sig. (2-tailed)	.002	.634	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	.326	.571	.098	.142	.448	1		
	Sig. (2-tailed)	.529	.237	.853	.789	.373			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.683	.774	-.321	.636	.683	.814*	1	
	Sig. (2-tailed)	.134	.071	.535	.174	.134	.049		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.487	.307	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.327	.555	.508	.497	.419	.311	.612	
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Ni_ in mature (Phyto /Dry)	Ni_ in seedlings	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Phyto /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Ni_ in seedlings	Pearson Correlation	.476	1						
	Sig. (2-tailed)	.340							
	N	6	6						
pH	Pearson Correlation	-.744	-.148	1					
	Sig. (2-tailed)	.090	.779						
	N	6	6	6					
EC	Pearson Correlation	.761	.791	-.520	1				
	Sig. (2-tailed)	.079	.061	.291					
	N	6	6	6	6				
CEC	Pearson Correlation	.549	.751	.020	.743	1			
	Sig. (2-tailed)	.259	.085	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	.294	-.230	.098	.142	.448	1		
	Sig. (2-tailed)	.572	.661	.853	.789	.373			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.761	.228	-.321	.636	.683	.814*	1	
	Sig. (2-tailed)	.079	.664	.535	.174	.134	.049		
	N	6	6	6	6	6	6		
MC	Pearson Correlation	.119	-.793	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.823	.060	.508	.497	.419	.311	.612	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Cu_ in mature (Phyto /Dry)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Phyto /Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6	6						
Cu_ in seedlings	Pearson Correlation	.827*	1						
	Sig. (2-tailed)	.042							
	N	6	6						
pH	Pearson Correlation	-.542	-.325	1					
	Sig. (2-tailed)	.267	.529						
	N	6	6	6					
EC	Pearson Correlation	.733	.486	-.520	1				
	Sig. (2-tailed)	.097	.328	.291					
	N	6	6	6	6				
CEC	Pearson Correlation	.356	.126	.020	.743	1			
	Sig. (2-tailed)	.489	.813	.970	.090				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.493	-.415	.098	.142	.448	1		
	Sig. (2-tailed)	.321	.413	.853	.789	.373			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.097	.082	-.321	.636	.683	.814*	1	
	Sig. (2-tailed)	.855	.877	.535	.174	.134	.049		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.581	-.506	-.341	-.350	-.411	.502	.265	1
	Sig. (2-tailed)	.226	.306	.508	.497	.419	.311	.612	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

Correlation coefficient between soil physicochemical parameters and metal accumulation in phytolacca *dodecandra*

(Wet season)

		Cd_ in mature (Phyt /Wet)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd_ in mature (Phyt /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd_ in seedlings	Pearson Correlation	.784	1						
	Sig. (2-tailed)	.065							
	N	6	6						
pH	Pearson Correlation	-.572	-.104	1					
	Sig. (2-tailed)	.236	.845						
	N	6	6	6					
EC	Pearson Correlation	.802	.939**	-.100	1				
	Sig. (2-tailed)	.055	.006	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.489	.678	.260	.687	1			
	Sig. (2-tailed)	.325	.138	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.069	-.116	.465	.173	.192	1		
	Sig. (2-tailed)	.896	.827	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.122	.440	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.817	.382	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.167	-.237	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.752	.651	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cr_ in mature (Phyt /Wet)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ in mature (Phyt /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cr_ in seedlings	Pearson Correlation	.976**	1						
	Sig. (2-tailed)	.001							
	N	6	6						
pH	Pearson Correlation	-.490	-.401	1					
	Sig. (2-tailed)	.324	.430						
	N	6	6	6					
EC	Pearson Correlation	.665	.759	-.100	1				
	Sig. (2-tailed)	.149	.080	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.523	.663	.260	.687	1			
	Sig. (2-tailed)	.287	.151	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	.091	.021	.465	.173	.192	1		
	Sig. (2-tailed)	.863	.969	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.343	.420	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.506	.407	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.031	-.189	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.954	.720	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Pb_ in mature (Phyto /Wet)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ in mature (Phyto /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ in seedlings	Pearson Correlation	.786	1						
	Sig. (2-tailed)	.064							
	N	6	6						
pH	Pearson Correlation	-.536	-.373	1					
	Sig. (2-tailed)	.273	.467						
	N	6	6	6					
EC	Pearson Correlation	.812*	.726	-.100	1				
	Sig. (2-tailed)	.050	.103	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.452	.662	.260	.687	1			
	Sig. (2-tailed)	.369	.152	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	.211	.034	.465	.173	.192	1		
	Sig. (2-tailed)	.688	.950	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.285	.437	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.584	.387	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.325	-.217	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.530	.679	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

		Zn_ in mature (Phyt /Wet)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Phyt /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ seedlings	Pearson Correlation	.375	1						
	Sig. (2-tailed)	.463							
	N	6	6						
pH	Pearson Correlation	-.336	-.507	1					
	Sig. (2-tailed)	.515	.305						
	N	6	6	6					
EC	Pearson Correlation	.553	.503	-.100	1				
	Sig. (2-tailed)	.255	.309	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.459	.399	.260	.687	1			
	Sig. (2-tailed)	.360	.433	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	.428	-.606	.465	.173	.192	1		
	Sig. (2-tailed)	.397	.203	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.269	-.207	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.607	.694	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.428	-.263	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.397	.615	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

		Ni_ in mature (Phyto /Wet)	Ni_ in seedlings	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Phyto /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Ni_ in seedlings	Pearson Correlation	.858*	1						
	Sig. (2-tailed)	.029							
	N	6	6						
pH	Pearson Correlation	-.274	-.586	1					
	Sig. (2-tailed)	.599	.222						
	N	6	6	6					
EC	Pearson Correlation	.814*	.787	-.100	1				
	Sig. (2-tailed)	.049	.063	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.699	.359	.260	.687	1			
	Sig. (2-tailed)	.122	.485	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.293	-.401	.465	.173	.192	1		
	Sig. (2-tailed)	.574	.431	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.240	.010	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.647	.985	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.306	-.067	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.555	.900	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cu_ in mature (Phyt /Wet)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Phyt /Wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cu_ in seedlings	Pearson Correlation	.460	1						
	Sig. (2-tailed)	.359							
	N	6	6						
pH	Pearson Correlation	.098	-.025	1					
	Sig. (2-tailed)	.853	.963						
	N	6	6	6					
EC	Pearson Correlation	.568	.974**	-.100	1				
	Sig. (2-tailed)	.239	.001	.850					
	N	6	6	6	6				
CEC	Pearson Correlation	.575	.708	.260	.687	1			
	Sig. (2-tailed)	.233	.116	.618	.132				
	N	6	6	6	6	6			
OM	Pearson Correlation	.741	.153	.465	.173	.192	1		
	Sig. (2-tailed)	.092	.772	.353	.744	.716			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.621	.666	.622	.598	.750	.670	1	
	Sig. (2-tailed)	.189	.149	.187	.210	.086	.146		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.358	-.174	-.338	-.040	-.534	.463	-.230	1
	Sig. (2-tailed)	.487	.742	.513	.940	.275	.356	.661	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Correlation coefficient between soil physicochemical parameters and metal accumulation in *Adhatoda schimperiana* (Dry season)

		Cd_ in mature (Ada/Dry)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd_ in seedlings	Pearson Correlation	.741	1						
	Sig. (2-tailed)	.092							
	N	6	6						
pH	Pearson Correlation	-.418	-.575	1					
	Sig. (2-tailed)	.410	.233						
	N	6	6	6					
EC	Pearson Correlation	.708	.974**	-.677	1				
	Sig. (2-tailed)	.116	.001	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.122	-.066	.742	-.181	1			
	Sig. (2-tailed)	.818	.901	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.200	-.354	.809	-.516	.853*	1		
	Sig. (2-tailed)	.704	.491	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.468	.096	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.349	.856	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.579	-.635	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.228	.175	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cr_ in mature (Ada/Dry)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cr_ in seedlings	Pearson Correlation	.963**	1						
	Sig. (2-tailed)	.002							
	N	6	6						
pH	Pearson Correlation	-.676	-.478	1					
	Sig. (2-tailed)	.140	.338						
	N	6	6	6					
EC	Pearson Correlation	.927**	.848*	-.677	1				
	Sig. (2-tailed)	.008	.033	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.306	-.116	.742	-.181	1			
	Sig. (2-tailed)	.555	.827	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.623	-.448	.809	-.516	.853*	1		
	Sig. (2-tailed)	.187	.373	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.204	.386	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.698	.450	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.649	-.474	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.163	.342	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Pb_ in mature (Ada/Dry)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ in seedlings	Pearson Correlation	.675	1						
	Sig. (2-tailed)	.141							
	N	6	6						
pH	Pearson Correlation	-.675	-.760	1					
	Sig. (2-tailed)	.142	.080						
	N	6	6	6					
EC	Pearson Correlation	.720	.891*	-.677	1				
	Sig. (2-tailed)	.107	.017	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.408	-.177	.742	-.181	1			
	Sig. (2-tailed)	.422	.737	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.373	-.403	.809	-.516	.853*	1		
	Sig. (2-tailed)	.467	.428	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.159	-.364	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.763	.478	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.838*	-.792	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.037	.060	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Zn_ in mature (Ada/Dry)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ in seedlings	Pearson Correlation	.955**	1						
	Sig. (2-tailed)	.003							
	N	6	6						
pH	Pearson Correlation	-.706	-.690	1					
	Sig. (2-tailed)	.117	.129						
	N	6	6	6					
EC	Pearson Correlation	.829*	.809	-.677	1				
	Sig. (2-tailed)	.041	.051	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.528	-.417	.742	-.181	1			
	Sig. (2-tailed)	.282	.411	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.679	-.577	.809	-.516	.853*	1		
	Sig. (2-tailed)	.138	.231	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.299	.269	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.565	.607	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.729	-.732	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.100	.098	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Ni_ in mature (Ada/Dry)	Ni_ in seedling	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Ni_ in seedling	Pearson Correlation	.512	1						
	Sig. (2-tailed)	.299							
	N	6	6						
pH	Pearson Correlation	-.796	-.255	1					
	Sig. (2-tailed)	.058	.625						
	N	6	6	6					
EC	Pearson Correlation	.720	.698	-.677	1				
	Sig. (2-tailed)	.106	.123	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.334	.230	.742	-.181	1			
	Sig. (2-tailed)	.518	.661	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.334	-.082	.809	-.516	.853*	1		
	Sig. (2-tailed)	.517	.877	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	-.271	.345	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.604	.503	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.928**	-.311	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.007	.549	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cu_ in mature (Ada/Dry)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Ada/Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cu_ in seedlings	Pearson Correlation	.285	1						
	Sig. (2-tailed)	.584							
	N	6	6						
pH	Pearson Correlation	-.740	.012	1					
	Sig. (2-tailed)	.093	.983						
	N	6	6	6					
EC	Pearson Correlation	.911*	.332	-.677	1				
	Sig. (2-tailed)	.012	.521	.140					
	N	6	6	6	6				
CEC	Pearson Correlation	-.303	.100	.742	-.181	1			
	Sig. (2-tailed)	.559	.850	.091	.731				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.489	.200	.809	-.516	.853*	1		
	Sig. (2-tailed)	.325	.704	.051	.295	.031			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.095	.321	.452	.032	.232	.191	1	
	Sig. (2-tailed)	.859	.536	.368	.952	.658	.718		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.810	-.207	.948**	-.676	.649	.624	.362	1
	Sig. (2-tailed)	.051	.694	.004	.140	.163	.186	.481	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

Correlation coefficient between soil physicochemical parameters and metal accumulation in *Adhatoda schimperiana* (Wet season)

		Cd_ in mature (Ada wet)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd_ in seedlings	Pearson Correlation	.764	1						
	Sig. (2-tailed)	.077							
	N	6	6						
pH	Pearson Correlation	-.384	.060	1					
	Sig. (2-tailed)	.452	.910						
	N	6	6	6					
EC	Pearson Correlation	.976**	.875*	-.222	1				
	Sig. (2-tailed)	.001	.023	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	.042	.299	.496	.190	1			
	Sig. (2-tailed)	.937	.565	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.517	.141	.595	-.354	.152	1		
	Sig. (2-tailed)	.293	.789	.212	.491	.773			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.157	.756	.439	.347	.372	.744	1	
	Sig. (2-tailed)	.766	.082	.384	.501	.468	.090		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.489	.141	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.325	.790	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cr_ in mature (Ada wet)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cr_ seedlings	Pearson Correlation	.871*	1						
	Sig. (2-tailed)	.024							
	N	6	6						
pH	Pearson Correlation	-.396	-.443	1					
	Sig. (2-tailed)	.438	.379						
	N	6	6	6					
EC	Pearson Correlation	.920**	.927**	-.222	1				
	Sig. (2-tailed)	.009	.008	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	.145	.232	.496	.190	1			
	Sig. (2-tailed)	.785	.659	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.683	-.362	.595	-.354	.152	1		
	Sig. (2-tailed)	.135	.481	.212	.491	.773			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	-.034	.320	.439	.347	.372	.744	1	
	Sig. (2-tailed)	.949	.537	.384	.501	.468	.090		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.631	-.274	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.179	.599	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Pb_ in mature (Ada wet)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ in seedlings	Pearson Correlation	.953**	1						
	Sig. (2-tailed)	.003							
	N	6	6						
pH	Pearson Correlation	-.547	-.707	1					
	Sig. (2-tailed)	.261	.116						
	N	6	6	6					
EC	Pearson Correlation	.891*	.813*	-.222	1				
	Sig. (2-tailed)	.017	.049	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	-.240	-.241	.496	.190	1			
	Sig. (2-tailed)	.647	.646	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.421	-.454	.595	-.354	.152	1		
	Sig. (2-tailed)	.406	.366	.212	.491	.773			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.182	.093	.439	.347	.372	.744	1	
	Sig. (2-tailed)	.730	.861	.384	.501	.468	.090		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.415	-.363	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.413	.479	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Zn_ in mature (Ada wet)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ seedlings	Pearson Correlation	.948**	1						
	Sig. (2-tailed)	.004							
	N	6	6						
pH	Pearson Correlation	-.600	-.594	1					
	Sig. (2-tailed)	.208	.214						
	N	6	6	6					
EC	Pearson Correlation	.868*	.896*	-.222	1				
	Sig. (2-tailed)	.025	.016	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	-.175	.067	.496	.190	1			
	Sig. (2-tailed)	.740	.900	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.477	-.493	.595	-.354	.152	1		
	Sig. (2-tailed)	.338	.321	.212	.491	.773			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.139	.160	.439	.347	.372	.744	1	
	Sig. (2-tailed)	.792	.763	.384	.501	.468	.090		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.474	-.409	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.342	.421	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Ni_ in mature (Ada wet)	Ni_ in seedlings	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Ni_ in seedlings	Pearson Correlation	.964**	1						
	Sig. (2-tailed)	.002							
	N	6	6						
pH	Pearson Correlation	-.594	-.714	1					
	Sig. (2-tailed)	.214	.111						
	N	6	6	6					
EC	Pearson Correlation	.687	.712	-.222	1				
	Sig. (2-tailed)	.131	.113	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	.263	.138	.496	.190	1			
	Sig. (2-tailed)	.615	.795	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.605	-.668	.595	-.354	.152	1		
	Sig. (2-tailed)	.203	.147	.212	.491	.773			
	N	6	6	6	6	6	6		
Sand	Pearson Correlation	-.011	.184	-.664	-.347	-.402	-.276		
	Sig. (2-tailed)	.984	.727	.150	.500	.430	.597		
	N	6	6	6	6	6	6		
MC	Pearson Correlation	-.513	-.531	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.298	.279	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cu_ in mature (Ada wet)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Ada wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cu_ seedlings	Pearson Correlation	.582	1						
	Sig. (2-tailed)	.226							
	N	6	6						
pH	Pearson Correlation	-.254	-.441	1					
	Sig. (2-tailed)	.627	.381						
	N	6	6	6					
EC	Pearson Correlation	.601	.795	-.222	1				
	Sig. (2-tailed)	.207	.059	.673					
	N	6	6	6	6				
CEC	Pearson Correlation	-.073	-.368	.496	.190	1			
	Sig. (2-tailed)	.891	.473	.317	.718				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.081	-.169	.595	-.354	.152	1		
	Sig. (2-tailed)	.878	.749	.212	.491	.773			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.398	.332	.439	.347	.372	.744	1	
	Sig. (2-tailed)	.434	.520	.384	.501	.468	.090		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.211	-.171	.469	-.335	.237	.954**	.712	1
	Sig. (2-tailed)	.688	.746	.348	.516	.652	.003	.112	
	N	6	6	6	6	6	6	6	6
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Correlation coefficient between soil physicochemical parameters and metal accumulation in *Solanum incanum* (Dry season)

		Cd_ in mature (Sola Dry)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd_ in seedlings	Pearson Correlation	.888*	1						
	Sig. (2-tailed)	.018							
	N	6	6						
pH	Pearson Correlation	-.729	-.926**	1					
	Sig. (2-tailed)	.100	.008						
	N	6	6	6					
EC	Pearson Correlation	.593	.655	-.513	1				
	Sig. (2-tailed)	.215	.158	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.536	.616	-.666	.806	1			
	Sig. (2-tailed)	.273	.193	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	.095	-.084	-.069	.165	.473	1		
	Sig. (2-tailed)	.859	.874	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.744	.546	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.090	.262	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.524	-.551	.365	.025	.234	.739	.106	1
	Sig. (2-tailed)	.286	.257	.477	.962	.656	.094	.842	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Cr_ in mature (Sola Dry)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cr_ in seedlings	Pearson Correlation	.714	1						
	Sig. (2-tailed)	.111							
	N	6	6						
pH	Pearson Correlation	-.908*	-.444	1					
	Sig. (2-tailed)	.012	.378						
	N	6	6	6					
EC	Pearson Correlation	.781	.962**	-.513	1				
	Sig. (2-tailed)	.067	.002	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.701	.783	-.666	.806	1			
	Sig. (2-tailed)	.121	.065	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.038	.019	-.069	.165	.473	1		
	Sig. (2-tailed)	.943	.972	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.543	.430	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.266	.395	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.387	-.056	.365	.025	.234	.739	.106	1
	Sig. (2-tailed)	.449	.916	.477	.962	.656	.094	.842	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Pb_ in mature (Sola Dry)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ in seedlings	Pearson Correlation	.853*	1						
	Sig. (2-tailed)	.031							
	N	6	6						
pH	Pearson Correlation	-.061	-.550	1					
	Sig. (2-tailed)	.908	.258						
	N	6	6	6					
EC	Pearson Correlation	.750	.816*	-.513	1				
	Sig. (2-tailed)	.086	.048	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.484	.697	-.666	.806	1			
	Sig. (2-tailed)	.331	.124	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	.408	.369	-.069	.165	.473	1		
	Sig. (2-tailed)	.421	.472	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.776	.898*	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.070	.015	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.160	-.127	.365	.025	.234	.739	.106	1
	Sig. (2-tailed)	.763	.811	.477	.962	.656	.094	.842	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Zn_ in mature (Sola Dry)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ in seedlings	Pearson Correlation	.800	1						
	Sig. (2-tailed)	.056							
	N	6	6						
pH	Pearson Correlation	-.717	-.462	1					
	Sig. (2-tailed)	.109	.356						
	N	6	6	6					
EC	Pearson Correlation	.750	.772	-.513	1				
	Sig. (2-tailed)	.086	.072	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.609	.337	-.666	.806	1			
	Sig. (2-tailed)	.199	.514	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.220	-.238	-.069	.165	.473	1		
	Sig. (2-tailed)	.676	.650	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.450	.527	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.370	.283	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.586	-.480	.365	.025	.234	.739	.751	1
	Sig. (2-tailed)	.221	.335	.477	.962	.656	.094	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

		Ni_ in mature (Sola Dry)	Ni_ in seedlings	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Ni_ in seedlings	Pearson Correlation	.412	1						
	Sig. (2-tailed)	.417							
	N	6	6						
pH	Pearson Correlation	-.564	-.884*	1					
	Sig. (2-tailed)	.243	.019						
	N	6	6	6					
EC	Pearson Correlation	.573	.691	-.513	1				
	Sig. (2-tailed)	.235	.128	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.334	.883*	-.666	.806	1			
	Sig. (2-tailed)	.517	.020	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.086	.438	-.069	.165	.473	1		
	Sig. (2-tailed)	.872	.385	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.675	.647	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.141	.165	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.627	.080	.365	.025	.234	.739	.106	1
	Sig. (2-tailed)	.183	.880	.477	.962	.656	.094	.842	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

		Cu_ in mature (Sola/ Dry)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Sola Dry)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cu_ in seedlings	Pearson Correlation	.763	1						
	Sig. (2-tailed)	.078							
	N	6	6						
pH	Pearson Correlation	-.800	-.727	1					
	Sig. (2-tailed)	.056	.102						
	N	6	6	6					
EC	Pearson Correlation	.758	.913*	-.513	1				
	Sig. (2-tailed)	.081	.011	.298					
	N	6	6	6	6				
CEC	Pearson Correlation	.640	.852*	-.666	.806	1			
	Sig. (2-tailed)	.171	.031	.148	.053				
	N	6	6	6	6	6			
OM	Pearson Correlation	.139	-.014	-.069	.165	.473	1		
	Sig. (2-tailed)	.792	.980	.897	.755	.343			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.791	.419	-.470	.597	.580	.657	1	
	Sig. (2-tailed)	.061	.408	.347	.211	.228	.156		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.388	-.180	.365	.025	.234	.739	.106	1
	Sig. (2-tailed)	.448	.732	.477	.962	.656	.094	.842	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									

Correlation coefficients between soil physicochemical parameters and metal accumulation in *Solanum incanum* (Wet season)

		Cd_ mature (Sola wet)	Cd_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cd_ mature (Sola wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cd_ seedlings	Pearson Correlation	.848*	1						
	Sig. (2-tailed)	.033							
	N	6	6						
pH	Pearson Correlation	-.308	-.738	1					
	Sig. (2-tailed)	.553	.094						
	N	6	6	6					
EC	Pearson Correlation	.760	.725	-.416	1				
	Sig. (2-tailed)	.080	.103	.412					
	N	6	6	6	6				
CEC	Pearson Correlation	.917*	.866*	-.376	.586	1			
	Sig. (2-tailed)	.010	.026	.463	.222				
	N	6	6	6	6	6			
OM	Pearson Correlation	.543	.346	.085	-.125	.673	1		
	Sig. (2-tailed)	.266	.501	.873	.813	.143			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.898*	.945**	-.651	.659	.890*	.516	1	
	Sig. (2-tailed)	.015	.004	.161	.155	.017	.294		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.750	.709	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.086	.115	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Cr_ in mature (Sola wet)	Cr_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cr_ mature (Sola wet)	Pearson Correlation	1	.729	-.773	.829*	.731	.026	.805	.587
	Sig. (2-tailed)		.100	.072	.041	.099	.961	.053	.221
	N	6	6	6	6	6	6	6	6
Cr_ in seedlings	Pearson Correlation	.729	1	-.388	.911*	.682	.172	.810	.427
	Sig. (2-tailed)	.100		.448	.012	.136	.745	.051	.399
	N	6	6	6	6	6	6	6	6
pH	Pearson Correlation	-.773	-.388	1	-.416	-.376	.085	-.651	-.274
	Sig. (2-tailed)	.072	.448		.412	.463	.873	.161	.599
	N	6	6	6	6	6	6	6	6
EC	Pearson Correlation	.829*	.911*	-.416	1	.586	-.125	.659	.360
	Sig. (2-tailed)	.041	.012	.412		.222	.813	.155	.483
	N	6	6	6	6	6	6	6	6
CEC	Pearson Correlation	.731	.682	-.376	.586	1	.673	.890*	.944**
	Sig. (2-tailed)	.099	.136	.463	.222		.143	.017	.005
	N	6	6	6	6	6	6	6	6
OM	Pearson Correlation	.026	.172	.085	-.125	.673	1	.516	.744
	Sig. (2-tailed)	.961	.745	.873	.813	.143		.294	.090
	N	6	6	6	6	6	6	6	6
Sand	Pearson Correlation	-.800	-.536	.873*	-.454	-.729	-.369	-.895*	-.667
	Sig. (2-tailed)	.056	.273	.023	.366	.100	.471	.016	.148
	N	6	6	6	6	6	6	6	6
MC	Pearson Correlation	.587	.427	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.221	.399	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Pb_ in mature (Sola wet)	Pb_ in seedlings	pH	EC	CEC	OM	Clay	MC
Pb_ mature (Sola wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Pb_ seedlings	Pearson Correlation	.836*	1						
	Sig. (2-tailed)	.038							
	N	6	6						
pH	Pearson Correlation	-.408	-.769	1					
	Sig. (2-tailed)	.422	.074						
	N	6	6	6					
EC	Pearson Correlation	.951**	.829*	-.416	1				
	Sig. (2-tailed)	.004	.041	.412					
	N	6	6	6	6				
CEC	Pearson Correlation	.600	.754	-.376	.586	1			
	Sig. (2-tailed)	.208	.084	.463	.222				
	N	6	6	6	6	6			
OM	Pearson Correlation	.007	.114	.085	-.125	.673	1		
	Sig. (2-tailed)	.989	.830	.873	.813	.143			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.739	.882*	-.651	.659	.890*	.516	1	
	Sig. (2-tailed)	.093	.020	.161	.155	.017	.294		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.379	.618	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.459	.191	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Zn_ in mature (Sola wet)	Zn_ in seedlings	pH	EC	CEC	OM	Clay	MC
Zn_ in mature (Sola wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Zn_ in seedlings	Pearson Correlation	.769	1						
	Sig. (2-tailed)	.074							
	N	6	6						
pH	Pearson Correlation	-.461	-.770	1					
	Sig. (2-tailed)	.358	.073						
	N	6	6	6					
EC	Pearson Correlation	.893*	.720	-.416	1				
	Sig. (2-tailed)	.016	.106	.412					
	N	6	6	6	6				
CEC	Pearson Correlation	.383	.591	-.376	.586	1			
	Sig. (2-tailed)	.453	.217	.463	.222				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.392	.014	.085	-.125	.673	1		
	Sig. (2-tailed)	.442	.979	.873	.813	.143			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.440	.779	-.651	.659	.890*	.516	1	
	Sig. (2-tailed)	.382	.068	.161	.155	.017	.294		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	.252	.504	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.629	.308	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Ni_ in mature (Sola wet)	Ni_ in seedlings	pH	EC	CEC	OM	Clay	MC
Ni_ in mature (Sola wet)	Pearson Correlation	1	.871*	-.731	.871*	.757	.053	.830*	.603
	Sig. (2-tailed)		.024	.099	.024	.081	.920	.041	.205
	N	6	6	6	6	6	6	6	6
Ni_ in seedlings	Pearson Correlation	.871*	1	-.710	.708	.758	.253	.865*	.547
	Sig. (2-tailed)	.024		.114	.116	.081	.629	.026	.261
	N	6	6	6	6	6	6	6	6
pH	Pearson Correlation	-.731	-.710	1	-.416	-.376	.085	-.651	-.274
	Sig. (2-tailed)	.099	.114		.412	.463	.873	.161	.599
	N	6	6	6	6	6	6	6	6
EC	Pearson Correlation	.871*	.708	-.416	1	.586	-.125	.659	.360
	Sig. (2-tailed)	.024	.116	.412		.222	.813	.155	.483
	N	6	6	6	6	6	6	6	6
CEC	Pearson Correlation	.757	.758	-.376	.586	1	.673	.890*	.944**
	Sig. (2-tailed)	.081	.081	.463	.222		.143	.017	.005
	N	6	6	6	6	6	6	6	6
OM	Pearson Correlation	.053	.253	.085	-.125	.673	1	.516	.744
	Sig. (2-tailed)	.920	.629	.873	.813	.143		.294	.090
	N	6	6	6	6	6	6	6	6
Clay	Pearson Correlation	.830*	.865*	-.651	.659	.890*	.516	1	.751
	Sig. (2-tailed)	.041	.026	.161	.155	.017	.294		.085
	N	6	6	6	6	6	6	6	6
MC	Pearson Correlation	.603	.547	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.205	.261	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

		Cu_ in mature (Sola wet)	Cu_ in seedlings	pH	EC	CEC	OM	Clay	MC
Cu_ in mature (Sola wet)	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	6							
Cu_ in seedlings	Pearson Correlation	.811	1						
	Sig. (2-tailed)	.050							
	N	6	6						
pH	Pearson Correlation	-.313	-.481	1					
	Sig. (2-tailed)	.545	.334						
	N	6	6	6					
EC	Pearson Correlation	.840*	.863*	-.416	1				
	Sig. (2-tailed)	.036	.027	.412					
	N	6	6	6	6				
CEC	Pearson Correlation	.152	.207	-.376	.586	1			
	Sig. (2-tailed)	.773	.694	.463	.222				
	N	6	6	6	6	6			
OM	Pearson Correlation	-.590	-.455	.085	-.125	.673	1		
	Sig. (2-tailed)	.218	.365	.873	.813	.143			
	N	6	6	6	6	6	6		
Clay	Pearson Correlation	.223	.426	-.651	.659	.890*	.516	1	
	Sig. (2-tailed)	.672	.399	.161	.155	.017	.294		
	N	6	6	6	6	6	6	6	
MC	Pearson Correlation	-.001	-.087	-.274	.360	.944**	.744	.751	1
	Sig. (2-tailed)	.998	.870	.599	.483	.005	.090	.085	
	N	6	6	6	6	6	6	6	6
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

7.2. Appendix 2. CAES Research ethical clearance

UNISA university of south africa

CAES RESEARCH ETHICS REVIEW COMMITTEE

Date: 05/03/2015

Ref #: **2015/CAES/031**
Name of applicant: **Mr AS Debela**
Student #: **55761372**

Dear Mr Debela,

Decision: Ethics Approval

Proposal: Evaluation of phytoremediation potentials of plants: *Phytolacca dodecandra, Adhatoda schimperiana* and *Solanum incanum*

Supervisor: Dr Mekibib David Dawit

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Research Ethics Review Committee for the above mentioned research. Final approval is granted for the duration of the project.

Please consider points 4 and 5 below for further action.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 05 March 2015.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable*

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
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Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

7.3.Appendix 3. UNISA study fees finance statement

099859

UNISA university of south africa

DEBELA A S MR
P O BOX 667
WACHEMO UNIVERSITY
ETHIOPIA

e-mail: FINAN@UNISA.AC.ZA
Fax: (012) 429-4150
Reference: 55761372
Date: 2019-09-30
Qualification: 98009
Academic Year: 2019

Statement of Account
UNISA STUDY FEES

Date	Ref. No.	Allocation	Details	Debit	Credit	Balance
2019-02-26	7095/009	5400/1000	TFENS01-STUDY FEES	15500.00		15500.00
2019-06-22	2840/683	5400/1015	TUITION FEE ADJUSTMENT		95.00	15405.00
2019-09-21	1923/025	5400/7464	LICENSEE REGISTERED FOR: TFENS01		15405.00	0.00

Payable on or before	Immediately	2019/03/31	2019/05/15	2019/08/15	2019/11/15	2020/03/15	Balance	0.00

Please return this detachable slip with your payment

NB: Cash deposits only at any Standard Bank branch in S.A.
UNISA STUDY FEES

Reference: 55761372 **Name:** DEBELA A S MR
Bank: STANDARD BANK **Account:** 096R **Ref:** 55761372 5400374721
Cell nr: +251911060663 **E-mail:** 55761372@mylife.unisa.ac.za

Please consult the "My Registration" brochure for the other methods of payment

Payable on or before	Immediately	2019/03/31	2019/05/15	2019/08/15	2019/11/15	2020/03/15	Balance	0.00

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tswane
PO Box 392 UNISA 0003 South Africa
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national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.

4) *It is not clear whether any of the samples will be taken from privately owned land. Should this be the case, the researcher must first obtain written permission from the owner to access his land and take the samples. This permission must be submitted to the Committee as it is obtained.*
5) *The researcher did not indicate whether the three targeted plant species are protected or not. This must be clarified in a memorandum to the Committee. No data collection may take place until the clarification has been submitted.*

Note:
The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

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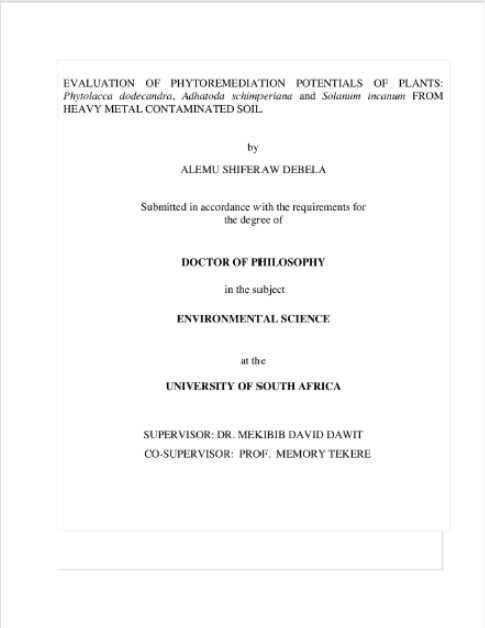


EVALUATION OF PHYTOREMEDIATION POTENTIALS OF PLANTS: *Phytolacca dodecandra*, *Adhatoda schimperiana* and *Solanum incanum* FROM HEAVY METAL CONTAMINATED SOIL

by

ALEMU SHIFERAW DEBELA

Submitted in accordance with the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the subject

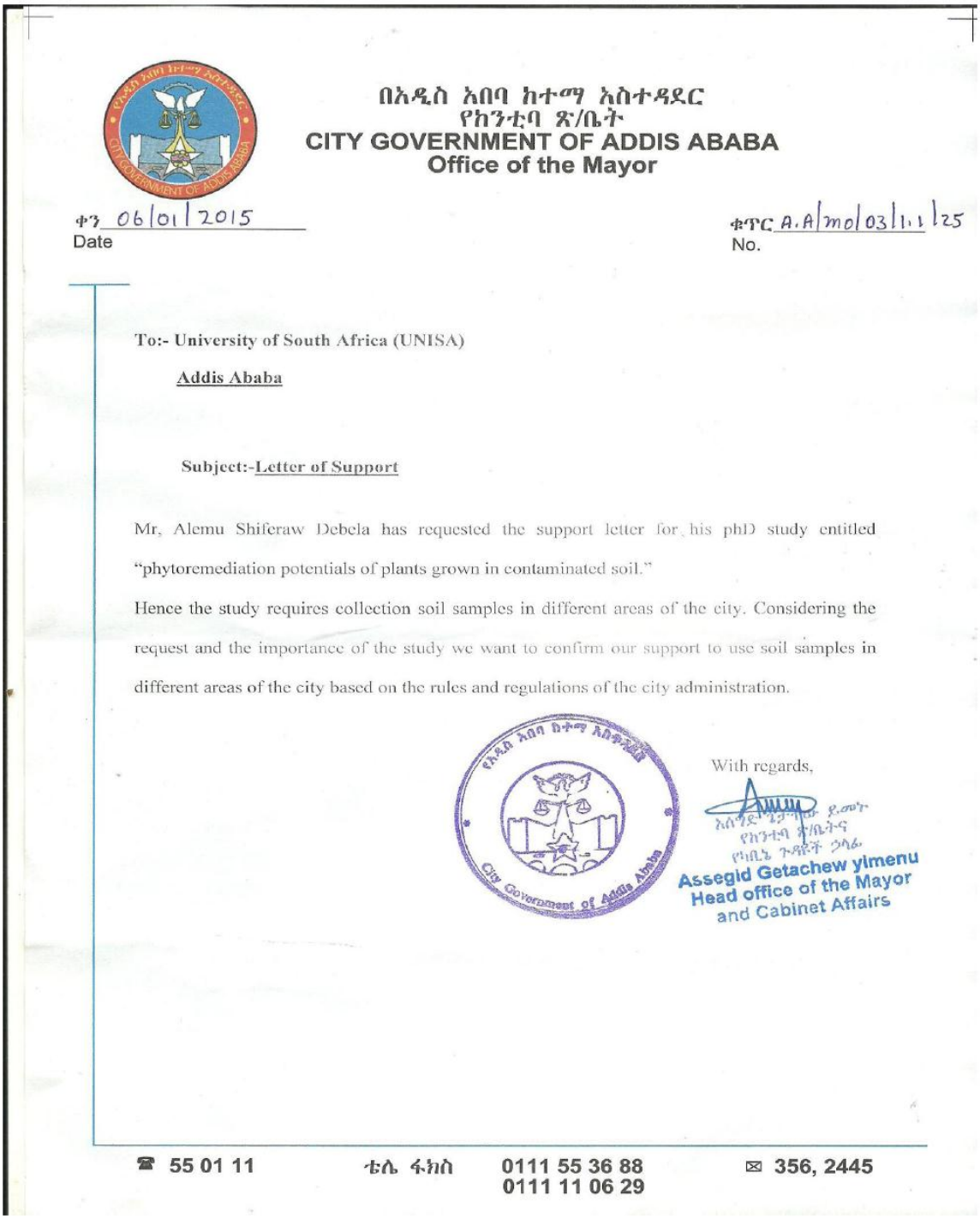
ENVIRONMENTAL SCIENCE

at the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: DR. MEKIBIB DAVID DAWIT
CO-SUPERVISOR: PROF. MEMORY TEKERE

7.4. Appendix 4. Support letters from local authorities



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የከንቲባ ጽ/ቤት
CITY GOVERNMENT OF ADDIS ABABA
Office of the Mayor

ቀን 06/01/2015
Date

ቁጥር A.A/mo/03/1.1/25
No.

To:- University of South Africa (UNISA)

Addis Ababa

Subject:-Letter of Support

Mr, Alemu Shiferaw Debela has requested the support letter for his phD study entitled "phytoremediation potentials of plants grown in contaminated soil."

Hence the study requires collection soil samples in different areas of the city. Considering the request and the importance of the study we want to confirm our support to use soil samples in different areas of the city based on the rules and regulations of the city administration.

With regards,

አሰግድ ጌታቸው ይመኑ
የከንቲባ ጽ/ቤትና
የካቢኔ ጉዳዮች ኃላፊ
Assegid Getachew yimenu
Head office of the Mayor
and Cabinet Affairs

☎ 55 01 11 ቴሌ ፋክስ 0111 55 36 88 0111 11 06 29 ✉ 356, 2445

በአዲስ አበባ ከተማ አስተዳደር
Addis Ababa City Government

የአካባቢ ጥበቃ ባለሥልጣን
Environmental Protection Authority

ቁጥር Ref. No. 80/18-01/315

ቀን Date 08/12/2014

To : University of South Africa (UNISA)

Subject: - letter of support

Mr Alemu Shiferaw who is now involving in PhD program in your university requested AAEPA to write a letter regarding environmental principle and ethics on conducting a research proposal entitled 'EVALUATION OF PHYTOREMEDIATION POTENTIALS OF PLANTS: *Phytolacca dodecandra, Adhatoda schimperiana and Solanum incanum* GROWN IN HEAVY METAL CONTAMINATED SOIL' by taking sample of some species of plants and soil around Akaki River.

Accordingly, the research proposal which Mr Alemu wants to conduct is in line with environmental principles and ethics hence, we want to appreciate and support the work since it will be an input for our future work too.

In addition, we do also have all the necessary laboratory material and technicians that can support the work as our laboratory rules and regulations.

With regards

Adugna Mekonnen Beyene
Deputy Manager and Head for
Environmental Pollution &
Monitoring & Control Core Process

Government of Addis Ababa Environmental Protection Authority

011-6-46 38 43
011-6-46 38 44
011-6-46 38 45

መለስ ዜናዊ ለሕዝብ የተፈጠረ ለሕዝብ የኖረ
ለሕዝብ የተሰዋ ታላቅ መሪ!!
ሌጋሲህ ይቀጥላል ራዕይህም በትውልዶች ቅብብሎሽ ይሳካል::

Fax 251-11-6-46 38 41 P.O.Box 8968
Email:- aaepa04@gmail.com
Website:- www.aaepa.gov.et