Tithonia diversifolia (HEMSL.) A. GRAY AND *Chromolaena odorata* (L.) KING AND ROBINSON AS POTENTIAL PHYTOREMEDIATORS

BY

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CERTIFICATION

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DEDICATION

This research is dedicated to the Omniscient God, I am that I am, who made the completion of this work possible; my loving, caring, and ever ready mother, who with her diligent struggle made me what I am today; my amiable and adorable wife; and my children.

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ABSTRACT

Heavy Metal (HM) pollution of soil is of global concern in view of the great risk HMs pose to human and animal health through the food chain. Remediation by plants has become an attractive means of HM removal because of its eco-friendliness. However, most plants often used are crops and vegetable needed as foods. *Tithonia diversifolia* (Td) and *Chromolaena odorata* (Cho) are fast growing ubiquitous weeds whose phytoremediation capabilities are yet to be fully understood. Therefore, the aim of this study was to determine the physiology and HM phytoremediation capabilities of Td and Cho.

Three dumpsites (Irese, New stadium area and Onyearugbulem) and a control site (Ijare), all in Ondo State were purposively selected for this study. Three soil samples on which Td and Cho grew were collected from each dumpsite. Thirty-six Uncontaminated Soil Samples (USS) were collected from Ijare. A screen house experiment was conducted using soils from dumpsites and control. Ten grammes (g) of $CdCl_2$, $ZnCl_2$, $FeCl_2$, $CuCl_2$ and Pb $(NO_3)_2$ were introduced to 6.5 kg of six USS (3 for Td and 3 for Cho) to simulate contaminated soils. Six USS were used as control. Soil analysis was done before planting and after harvest. Viability test (Emergent %) was carried out on both plants. Growth parameters (shoot-length, root-length and stem-girth), Relative growth rate (RGR) and plant biomass were also determined. Chlorophyll content (photosynthetic rate), HMs concentration in roots and shoots were analysed using atomic absorption spectrophotometry. Data obtained were subjected to descriptive analysis and ANOVA with Duncan Multiple Range Test at $\alpha_{0.05}$.

The HMs concentrations (mg/kg) in dumpsite soils were: Cd, 0.01-0.08; Zn,0.48-1.92; Fe,0.99-353.87; Cu,0.10-3.04 and Pb,0.20-1.40, while in simulated-polluted-soil, the HMs were; Cd, 942.88; Zn,630.40; Fe,919.45; Cu,612.60; Pb,962.33. Mean HMs concentrations in soil planted with Td after harvest were: Cd, 0.01-0.18±0.008; Zn,0.24-1.92±0.04; Fe,4.97-12.05±0.263; Cu,0.10-3.04±0.038; Pb,0.30-1.00±0.079 , while in Cho were:Cd, Not-detectable-0.20±0.009; Zn, 0.12-1.91±0.014; Fe, 4.99-9.10±0.37; Cu,0.07-3.04±0.014 and Pb,0.20-0.60±0.07. The HMs concentration in simulated-polluted soils indicated a reduction in HM. The seed emergent for Td was 93.33% and Cho, 86.77%. This indicated that both passed the viability test. Shoot-length (185cm), root-length (45.40cm) and stem-diameter (6.90cm) values for Td were more than that of Cho (90.00, 26.11 and 2.61 cm). The RGR of Td ranging from 0.06 to 0.16 showed more tolerance to pollution load than Cho (0.01 to 0.09). Fresh weight (mg) of shoots (5.60-110.61) and roots (1.02-47.98) for Td produced more biomass than shoot (2.41-53.11) and roots (0.39-7.18) of Cho. Likewise, the dry weight (mg) of Td shoots (0.89-20.66) and roots (0.22-16.58) was higher than the corresponding shoot (0.10-4.57) and root (0.05-0.36) of Cho. Generally, total chlorophyll content (mg/) was higher in Td (3.20-29.59) than Cho (0.92-17.39). Uptake of HMs concentration in shoots and roots of Td were more than in Cho. Data analysed showed that Td values were significantly higher than Cho.

The phytoremediation abilities possessed by *Tithonia diversifolia* and *Chromolaena odorata* enabled photosynthetic activities and heavy metal uptake. The plants reduced heavy metals in the polluted soil and can be used for phytoremediation.

Keywords: Heavy metals pollution; Plant biomass; Plant viability test; Soil remediation

Word count: 499

CHAPTER ONE

INTRODUCTION

1.1 Heavy Metals

Heavy metals refer to metals and intermediate elements of viscocity above 5 g cm^{-3}, (Adriano, 2001) that are often connected to contamination and toxins. Adriano (2001) asserts that these trace metals are required in small amount in plants or animals. A good example of these heavy metals is Zinc (Zn), which has parts of collections of chemical substances such as, proteinases and peptidases in animals and plants. Zinc also participates in the catabolism and anabolism of food as well as configuration of ribonucleic acid (RNA) and ribosomes in plants (Mengel and Kirkby, 1982).

Copper (Cu), another trace element, contributes to many functioning operations in living organisms including prevention of pathogen's attack (Kabata-Pendias and Pendias, 2001). Trace metals are harmful on cells at large amount (Baker and Walker, 1989). Cadmium (Cd), a minor element, does not partake in any life operations It poses threat to living organisms when proliferated in their tissues or system (Peng *et al.* 2006; Suzuki *et al.* 2001). Cadmium, considered a chemical substance with inhibiting roles disturbs deoxyribonucleic acid (DNA)-intermediate transposition in minute organisms, is involved in mutual and commensal interaction between microorganisms and plants, and aids assist susceptibility of plants to incursion of mycology (Kabata-Pendias and Pendias, 2001).

The increase in the demand for agricultural products and fall in oil price have led to diversification of economy and production of varieties of agricultural crops. AlAlthough fertilizer, herbicides and pesticides enhance quality and high biomass of these crops, overuse of these agro-chemicals causes danger in the ecosystem (Sahibin *et al.* 2002). Introduction of phosphate fertilizers to the soil is responsible for absorption of cadmium, copper, zinc and arsenic in any soil (Zarcinas *et al.* 2004).

Owing to urbanization and industrialization, pollution level is high in the ecosystem (Ahmadpour *et al.* 2012). Heavy metal pollution is a serious security challenge to the soil and water body, particularly man's wellness (Macek *et al.* 2000; Meagher, 2000; Eapen and D'Souza, 2005; Yoon *et al.* 2006). The yearly extensive introduction of heavy metal in metric tons got to 21000 for cadmium, 888000 for copper, 1249000 for zinc and 7390000 for lead (Singh *et al.* 2003).

Environmental contamination via agro-chemicals is a global menace. There is, therefore, the need for new technologies for clean-up of polluted sites. In decontaminating polluted sites, harmonization of physical, chemical and biological strategies is needed (Wirtz *et al.* 2000; Kummling *et al.* 2001; Perrin-Ganier *et al.* 2001; Matsunaga and Yashuhara, 2003).

Welch (1995) avert that heavy metal can be introduced into the ecosystem via man's role in the environment. This can be through smelting of iron, indiscriminate disposal of waste, and rigorous agriculture practice, among others. This high concentration of these heavy metals is hazardous to the environment (Page *et al.* 1982). Remediation of contaminated environment using plants that are capable of absorbing these pollution loads in their roots and shoots has a promising future (Gurbisu and Alkorta, 2003). Since many roots are found below soil level, they are involved in pollution uptake by inducing it with chemicals (Dunbabin and Bowmer, 1992; Wright and Otte, 1999). Some researchers who worked on some plant species, like *Sterculiar acuminata* and *Typha caerulescens* (Cunningham and Ow, 1996), *Arabidopsis thaliana* (Delhaize, 1996), as well as *Thlaspi latifolia*, and *Phragmites austra*lis (Ye *et al*., 2001) documented that they can store heavy metals in their systems. Ye *et al*. (1997a, b) also confirmed successful use in *Thlaspi latifolia* and *P. australis* for clean-up lead and zinc contaminated sites.

1.2 Phytoremediation

Phytoremediation is coined from two Latin words, *phyto* which depicts plant, and *remedium*, meaning revitalise (Cunningham *et al.,* 1997). This word specifically points to different forms of phyto-based technologies using indigenous or altered plants genetically for restoring polluted soil (Flathman and Lanza, 1998). Although making use of plants tolerant to pollution loads in order to clean up heavy metals and other chemical substances started 1983, the ideology actually started 300 years ago with waste-water effluents (Blaylock, 2008).

Phytoremediation has attracted global endorsement because of its cost effectiveness documented in research on dangerous refuse site (Susarla *et al.* 2002; Jadia and Fulekar, 2009; Zhang *et al.* 2010). It is considered a publicly appealing (green) remediation technology owing to its eco-friendliness, using leafage and microbes and soil remediation strategy to clean up the heavy metal (Cunningham and Ow, 1996; Vyslouzilova *et al.*, 2003).

1.3 Merits of green technology

The following are advantages of phytoremediation:

1) It minimizes decomposition of soil and the immediate surroundings reducing the spread of contamination through the air and water-borne wastes.
2) When properly used, the vegetation becomes eco-friendly and aesthetically pleasing to the public, which has social and psychological benefits (Raskin and Ensley, 2000; Ghosh and Singh, 2005; Lewis, 2006).
3) It is cost effective and very cheap (Vidali, 2001; Prasad and Freitas, 2003).
4) It is easy to execute, as it does not require skills, costly machines or special technology.
5) There is no need of digging away top soil and moving of contaminated soil.
6) Extension of pollutants into air and water is also checked, which prevents washing away of top soil and erosion (Pivetz, 2001; Ghosh and Singh, 2005).

1.4 Demerits of green technology

The demerits of green technology are highlighted below:

1) It is restricted to the rooting depth of remediating plants.
2) Clean-up of contaminated site using plants may take years (USEPA, 2000a; Vidali, 2001; Rajakaruna *et al.* 2006).
3) Using fast-growing and allelopathic plants can be detrimental to biodiversity of exotic species.
4) Feeding on contaminated plants by animal is dangerous (Pivetz, 2001).
5) Climatic condition not favourable to the plant can serve as an obstacle to growth and biomass production (USEPA, 2000b).

Industrial activities are a primary source of heavy metal contamination (Deo *et al.,* 2011). Some of dangerous trace metals have found their ways into the ecosystem, thus posing threat to life (Rahman and Zaim, 2015).

According to the World Health Organization (WHO), the commonest heavy metal pollutants are usually cadmium, chromium, cobalt, copper, lead, Mercury, Nickel and Zinc. As a result of the impact on environment, clean-up is expedient (Okoronkwo and Olasehnde, 2007).

1.5 Response of plant to heavy metals

Plants have mechanisms for surviving on polluted sites (Raskin *et al.,* 1994) owing to certain characteristics they exhibit. The major ones are discussed below.

1.6 Metal excluders

This strategy inhibits entry of pollution load to aerial parts of a particular plant or immobilizing pollution in the soil for large amounts of heavy metals by confining them to the root region through alteration of osmosis (Cunningham, 1995). E.g. *Commelina communis,*

1.7 Metal indicator species

These are plants that operationally amass pollution load in their roots, stems and leaves. They accommodate some amounts of pollution load by destabilizing pollution-enclosed space strategy. This is done by reserving elements in non-delicate parts (Li *et al.,* 2017).

1.8 Metal accumulator plant species

This species accumulates large amounts of trace elements in their shoots. Hyperaccumulators are plants that uptake large amounts of pollution load which may be in roots, stems or leaves (Raskin *et al.*, 1994; Bakers *et al.*, 1994; Cunningham and Ow, 1996). These plants are got from areas thickly dominated by these elements (Gleba *et al.*, 1999). Close to 400 plants in this category has been documented. The *Brassicaceae* family has high amount of hyperaccumulator species with diverse elements. These include 87 species from11 genera (Bakers and Brooks, 1989). Examples are *Thlaspi caerulescence, T. rotundifolium, T. ochoroleucum*.

1.9 Mechanism of phytoextraction

For metals to be phytoextracted, they must be mobilized in media in a diluted form in order for plants to absorb them. The presence of elements is raised through different routes. Plants can attain this by oozing out phytosidophores into the rhizosphere to combine and dilute elements that are compacted by soil (Kinnersely, 1993). Both acidification of the rhizosphere and flowing out of carboxylates are regarded could raise uptake of elements. After movement, an element has to be

trapped by root cells. Elements are first tied by the cell wall, as charge exchanger of comparatively small affinity and low selectivity.

Transport system is introduced and there is intracellular high-affinity binding, which drives uptake across the plasma membrane. Accumulation of element charge assumedly moves into action through secondary transporters, such as channel proteins and/or hydrogen ion coupled carrier proteins. The membrane capability, which is negative on the inside of the plasma membrane, and might go beyond –200 mV in root epidermal cells, provides a strong driving force for the accumulation of positive charge ion via 2° mobiliser (Hirsch, 1998).

After being taken up by plants, many elements are too undiluted to be mobilised freely in the root and phloem. So they often form carbonate, sulphate or phosphate precipitates stagnating them in apoplastic (extracellular) and symplastic (intracellular) partitions (Raskin *et al.*, 1997). Provided the element charge are moved as non-cationic element combination, apoplastic movement is further restricted by increased positively charged exchange capacity of cell walls (Raskin *et al.,* 1997).

The apoplast continuum of the root epidermis and cortex are readily permeable for solutes. Apoplastic pathway is not checkmated owing to the fact that solute can enter and move without passing a membrane. The cell walls of the endodermal cell layer act as an obstacle for apoplastic diffusion into the root and phloem.

In general, dissolved substances must be absorbed inside the root symplasm before they can enter the root (Tester and Leigh, 2001). Subsequent to element accumulation into the root symplasm, three processes dictate the transport into the root of element: sequestration of metals inside root cells, symplastic transport into the stele, and release into the root (Bubb and Lester, 1991; Gaymard, 1998).

Heavy metals strongly compete for the same transmembrane carriers used by major elements (Crowley *et al.,* 1991; Karley *et al.,* 2000).

1.10 Types of Phytoextraction

1.11 Natural phytoextraction

Thlaspi caerulescens is one of the most popular hyperaccumulator species often called *Alpine pennycress* (Kochain, 1996). It is capable of gulping 26,000 mg/kg of Zinc; and up to 22% of soil exchangeable cadmium out of polluted sites (Brown *et*

al., 1995; Gerard *et al.,* 2000). This plant is capable of translocating Pb from the root to stems and leaves.

1.12 Induced phytoextraction

Heavy metals propel manufacturing of oligopeptide ligands called plant-chelatin (PCs) and metallothioneins (MTs) in cells of plants (Cobbett, 2000). These poly nucleiotide chains hold and form stable complex with heavy metals and therefore neutralize the poisonous charges on elements (Grill *et al.,* 1987).

Chelators are extracted from plants that fully participate in cleaning-up of heavy metals and neutralizing their poisons. Compound forming complexes like ethylenediaminetetraacetic acid (EDTA) are introduced to soil impregnated with heavy metals, which enhances the quantity of bioavailable Pb in media and increased uptake in plants (Huang *et al.,* 1997).

1.13 Genetic engineering to improve phytoremediation

Introduction of new hybrid tall plants has greater potential phytoremediation techniques to aid clean-up of polluted sites. Moreover, biomass is often orchestrated by multiple genes, and increasing single gene insertion will be a problem. This hybrid of plants for heavy metal potential take-up is an alternative proposed by most scientists (Brown *et al.,* 1995; Cunningham and Ow, 1996; Zhu *et al*., 1999; Chaney *et al.,* 2000). But the breakthrough may be restricted owing to internal structure constraints (Ow, 1996).

1.14 Limitations of phytoextraction

Phytoremediation and other related techniques will be much viable only if soil polluted within 0.9 m topmost soil and groundwater within 3.048 m of the surface (Raskin *et al.,* 1994; Cunningham *et al.*, 1997). Phytoextraction can have prospect where there is little or moderate pollution over a large portion of land and to areas with high volume of ground water with little strata of pollution being removed to low (strict) standards (Salt *et al.,* 1995). Provision for degraded soil restoration contributes to pollution when ammonium sulphate ($(NH_4)_2SO_4$) is proposed for use to acidify the soil (Chaney *et al.,* 2000). Natural binding agents that suppress chemical activities by forming compounds having ring structures that often contain an element charge held by coordinate bonds of plants or phylogeny of microbes seem to have prospects than synthetic chemical chelators (Rauser, 1999). A strategy based on chemical chelators needs to be applied to enhance remediation using plants, since chemical chelators

contribute to poisoning of the plant, those plants might enhance taking up of pollution load thereby reduce size of plant resulting to restricted benefit.

1.15 Utilization of phytoremediation by-products

Phytoremediation requires harvesting of plants in polluted sites until the amount of pollution loads reduces to an acceptable level. The capability of plant to reduce pollution in the soil is determined by regulatory bodies. Pollution remediation can be valid through knowing the amount of pollution load in plant in conjunction with high plant production in relation to decrease in soil pollution loads (Gosh and Singh, 2005). Part of the challenges for large practice of plant-based remediation is disposal of polluted plants. This is because they would have gulped up high amount of pollution, which makes them a threat to the populace (Gosh and Singh, 2005).

Hetland *et al.* (2001) claimed that compost can appreciably decrease harvested plant biomass. Weight loss of polluted plant products via stiffing is a plus, because this will reduce expenses for movement to hazardous dumpsite. This squashing proposed by Blaylock and Huang (2000) was to rebrand resiowing to rich element. Today, people are getting sensitised on how to turn waste to money which will alleviate indiscriminate disposal of heavy metals. This will boost the economy.

1.16 Future of Phytoremediation

Phytoremediation is the best approach to clean-up polluted areas. But the factor to make it work and survive is its result and how the residues are being turned for economic gains. This technique would have gone far. It is being limited by weighing it side by side with other technologies that are not eco-friendly in restoring polluted locations on a commercial basis. Most remediation processes using plant take place in the screenhouse. The outcomes are promising, but screenhouse cannot be likened to the natural environment where they are liberated from restricted climate or environmental conditions. In addition, most heavy metals are compacted in undiluted state, making them unavailable, which is a serious challenge (Kochian, 1996). Although phytoremediation is still developing, some vital parameters are supposed to be put into consideration: studying the physiology of hyperaccumulator plants, their mechanism of absorption and how they are being disposed of.

1.17 Justification

The increase in the concentrations of heavy metals in the environment has attracted global attention. Conventional and physicochemical way of cleaning-up

polluted soil is yet to yield eco-friendly results. Therefore, the clean-up of heavy metal-contaminated soil is very important for maintenance of environmental health and ecosystem restoration using a natural extraction mechanism. This need for urgent action brought about the idea of using two plant species (*Tithonia diversifolia* and *Chromolaena odorata*) to clean-up the havoc wreaked by heavy metals. These two flowering plants were chosen typically on their speed sprouting and high biomass production capabilities.

1.18 Plant species

1.19 *Chromolaena odorata*

Chromolaena odorata and *Tithonia diversifolia* are of the Asteraceae family, which is widely spread family of plants. Asteraceae family is known to be well defined, fast-growing, ubiquitous and very successful. Many of the species in the Asteraceae are either shrubs or herbaceous; trees are hardly found. Although the family is large, they are of low economic importance, with relatively few crop plants. Many members of this family are ornamental plants. Introduction of *C. odorata* was by accident via Indies of the West (Toelken, 1983). Bennett and Rao (1968) asserts that rapid spread of *C. odorata* at Burma, Assam and Bengal in India was supposedly through attachment of their seeds to the clothing of soldiers going back home.

Chromolaena odorata is restricted to temperate and cool tropical zones (Woodward and Williams, 1987). Favourable climatic conditions have impact on the spread but yearly low temperature might restrict the spread of the plant (Woodward, 1986). The spread of *C. odorata* has geographically being restricted to around 250˚N and S latitudes and around 1000 m in height close to the equatorial plane. Moreover, the spread of *C. odorata* is limited to regions of 200 mm/h rainfall yearly and heat range from 20 to 37˚C (Woodward, 1986). In addition to precipitation and temperature, within a micro climatic zone, intensity of light also influences the distribution of *C. odorata*.

Tithonia diversifolia and *Chromolaena odorata* are not shade-loving plant species which make it difficult for it to invade indigenous forest vegetation but it becomes a serious weed in pastures and vacant lands. Their ability to spread fast makes them dominate newly areas they are being introduced.

In the southern Nigeria, *C. odorata* produces flowers between November and December and spreading of its seeds is in February, while *T. diversifolia* flowers between October and November and the seed dispersal takes place January. *Tithonia diversifolia* and *Chromolaena odorata* become dried up during the dry season. As a result of this, they become dangerous during fire incidents. When fires goes through the bush of *T. diversifolia* and *C. odorata*, the stems burn leaving the underground root of *C. odorata* alive (Alvanrenga *et al*., 2009). The remaining underground roots of *C. odorata* regenerate whenever the environmental conditions are favourable, especially at the beginning of the wet season. Whenever some plant species have been destroyed by inferno, *T. diversifolia* and *C. odorata* emerge and invade such area, in the following season thereby inhibiting the growth of other species of plant because they possess allelopathic properties (Alvanrenga *et al*., 2009).

1.20 *Tithonia diversifolia*

Tithonia diversifolia (Hemsl). A. Gray belongs to the Asteraceae family (formerly *Compositae*). *Tithonia diversifolia*, simply recognised as the Mexican sunflower, found its way to Africa as an ornamental plant (Akobundu and Agyakwa, 1987) but has become weed of field crops and roadsides (Akobundu and Agyakwa, 1987). Opinions differ as regards the introduction and subsequent establishment of *T. diversifolia* in Nigeria. The most authentic opinion suggests *T. diversifolia* penetrated into West Africa, especially Nigeria, via Oyo State, Ogbomoso to be precise, in company of maize seeds imported from Israel (Akobundu and Agyakwa, 1987; Lordbanjou, 1991; Chukwuka, 2003) by the then Oyo State Phased Agricultural Development Project (OSPADP) in the late 1970s (Chukwuka, 2003). The plant has since then spread to various parts of the southern states of Nigeria, especially in the last forty-four years, where the conditions favouring its development exist. The plant has established itself as a serious weed of arable crops, abandoned plantations, lawns and roadsides (Chukwuka *et al.,* 2007).

Tithonia diversifolia is an environmental weed that invades open lands, often influencing the thriving of indigenous vegetation (Chukwuka *et al.,* 2007). This invasive plant exists either as annual, herbs or shrubs. An increased segment of this colonial terrestrial flora are of open and temperate region while some survive at

different phases of low grade of light as found under density of shrubbery or rainforest regions (Akobundu and Agyakwa, 1987).

However, *T. diversifolia* is known to survive in gradient of environmental measure. This adaptable feature to different phases of medium, gradient humidity, sunlight and heat regions gives them room to colonise a wide range of population and community types. They are capable of colonizing and occupying wide range of environment owing to their numerous viable seeds that last long in storage room.

The activities of man in interaction with the ecosystem enhance the distribution and escalation of *T. diversifolia* (Chukwuka, 2003). Environmental factors are put in place to elevate distribution and escalation of *T. diversifolia* that replaces and contends with indigenous plants. This evading plant was not introduced by accident, although accidental introductions happened through importation feed, as pollutant with seed in heavy materials (such as rocks or water) and soil (Chukwuka, 2007). Movement within different ecological zones should be responsible for the escalation and wide distribution, with the assistant of wind, animals and water.

Recently, *T. diversifolia* and *C. odorata* have found themselves rooted in West Africa, especially in Nigeria. These two plants species are aggressively invading the vegetation without considering the fertility of the soil. The population of *T. diversifolia* and *C. odorata* has increased geometrically. They are never seen in the midst of other indigenous species. If they are found there, they use their fast-growing, abundant seed production and allelopathic substance emission to colonise and displace the species met on ground.

1.21 Economic importance of *C. odorata* and *T. diversifolia*

Chromolaena odorata has been considered a menace in crop plantation, such as *Elaesis guiniensis*, *Mangifera indica*, *Tectonia grandis* and *Hevea brasiliensis,* and a weed of vegetation, roadsides and uncultivated land. In some areas, there are few abandoned segments owing to non-productivity of such soil occupied by *C. odorata* and *T. diversifolia*. It is poisonous to livestock. It is well known that *C. odorata*, apart from hindering thriving of other plant species, have insects and mites that are dangerous to different crops (Bennett and Rao, 1968; Joy *et al*., 1979; Ramani and Haq, 1983; Muniappan and Viraktamath, 1986). In Cambodia, *C. odorata* serves as nourishment for black pepper, *Oryza sativa* and *Manihot esculentus* production

(Garry, 1963; Litzenberger *et al*., 1961) but kills aquatic organisms. In Nigeria, *C. odorata* is used for medicinal and ornamental purposes. The young leaves are crushed; the liquid extracted out is used in treating wounds and also in curing of malaria when used in bathing with caustic soda.

1.22 Aim and objectives

The aim of this study is to clean up heavy metals from the polluted soil and to know if these plants can be classified as hyperaccumulators. The objectives are to;

a) determine the germination indices of the two wild plant species grown in metal-contaminated pots;

b) determine their tolerance to pollution loads and the effect of the heavy metals on the growth of the species of plants used;

c) determine the effect of the heavy metals on the photosynthetic rate of the two plant species,

d) determine the extents to which the plant species (*T. diversifolia* and *C. odorata*) can help in removing the toxic contaminants (heavy metals) in the soil, so as to make the soil free or reduce the presence of heavy metals in the soil and environment to an acceptable standard.

CHAPTER TWO

LITERATURE REVIEW

The foundation of our agricultural resources, food security, global economy and environmental quality is basically soil (Oh *et al.,* 2013). The contamination of our soil with heavy metals and manure contamination through industrialization and urbanization cause problem to the ecosystem (Li *et al.,* 2009). Therefore, good technologies are needed to address polluted soil.

There have been some established soil remedy approaches, such as soil removal, complete burning, curing, evaporation extraction, thermal desorption, and disposal as waste. Many of these have not solved the problem owing to the fact that they usually make secondary hair pollution. Ground water contamination affects plant productivity. Moreover, it is extremely high cost that limits their application extensively especially in developing countries (Oh *et al.,* 2013).

Chromolaena odorata (Siam weed) known as fast-growing shrub of Neotropical region, has also been introduced to other parts of the world, Africa inclusive. The implication of *C. odorata* in its medicinal attributes (Taiwo *et al.,* 2001; Anyasi, 2011) is owing to the fact that it helps in addressing soil contamination (Tessier and Campbell, 1988, Tanhan *et al.,* 2007, 2011). The physical and chemical attributes of a soil polluted with heavy metals also determines the level of pollution (Gerber *et al*., 1991; Chlopecka *et al.,* 1996; Singh *et al.,* 2009).

Cadmium has been considered a major interest as soil and environmental contaminant owing to its toxic quality at small pollution load. Its poisonous nature is 2-20 % times higher than other HMs (Banavides *et al.,* 2005). Some factors contribute to Cd pollution. These include indiscriminate emission of cadmium, minning and smelting of iron, disposal of used batteries, metals-polluted refuse and sewage disposal, and use of chemicals to control pests and phosphate fertilizers.

Bioavailability of heavy metals in soil affects plant uptake (accumulated in foliage safe to eat, fruits and seeds) and subsequent man uptake (anabolism of milk in and fatty tissues), which affect agricultural activities, building up and breaking of food in plants, and the well-being of man and society (Sao *et al.,* 2006; ATSDR, 2008; Singh *et al.,* 2009). Cadmium has been reported by ATSDR (2011) to be 7th among 20 most dangerous elements, as indicated by Comprehensive Environmental Response Compensation and Liability Act (CERCLA) priority list.

Cadmium can be immediately absorbed by man through different means, one of which is taking products that wear Cd-coat in a confinement. It can be through smoking at about 1-3 mg per day (Zeneli *et al.,* 2009). The known "itai-itai", meaning bone disease of the Japanese, happens as an outcome of constant vulnerability to cadmium exposure (Bernard, 2008). When the presence of Cd concentrations is between 3 and 8 mg/kg, it becomes very harmful in most plants, thereby causing growth retardation in plants, chlorosis and stunting (Banavides *et. al.,* 2005).

Major heavy metals are elements required by plants and animals for growth, development and proper functioning. These include Mn, Fe, Ni, Cu and Zn (Gohre and Paszkowski, 2006). Some heavy metals are non-essential to plants and animals. These include Cd, Pb, Hg and As (Mertz, 1981; Suzuki and Sano, 2001; Bidar *et al.,* 2006; Peng *et al.,* 2009) but in small portion.

Lead has the tendency of accumulating more in roots than in shoots of plants during movement owing to some barriers preventing mobilization. Other elements, like cadmium, move freely in plants (Garbisu and Alkorta, 2001). The heavy presence of minor elements in the soil helps the uptake by plants, which has adverse effects on the development and sometimes interfere with their metabolism, resulting in death of such plant species (Schmidt, 2003; Schaller and Diez, 1991).

Light Harvesting Complex (LHC) II and photosystem I and II used in building of carbohydrates can be destroyed by Cd. Totality of chlorophyll contents. Non-light chemical extinguishing increases in some plant species, especially in *Brassica napus*. It is assumed that cadmium also alters the mobility of K^+, Ca^{2+} and abscisic acid in safe cells, in the name of preventing stomata exposure (Shaw, 1995). Contamination by heavy metals is responsible for transformation in functional steps at the cellular and molecular levels as a result of natural chemical substance stoppage or fencing of

functional groups of catabolism and anabolism special molecules (Mohebbi *et al.* 2012).

Brassicia sp. is a phytoextraction plant, useful in treating cadmium-polluted soil as a result of high crop production when subjected to increased pollution load of cadmium. This is because of its capacity to move big volume of cadmium into aerial parts of plant (Rajeev *et al*., 2014). In applying crop rotation procedures, *Brassica juncea* may be of assistance in controlling and reducing heavy deposits of harmful substances in the soil (Ginneken *et al.,* 2007). For a plant to be cadmium hyperaccumulator, it should be able to fuse at least 10 mg/kg of dry matter/weight in shoot (Ginneken *et al.,* 2007).

In some plants, quantities of cadmium are basically smaller than 2.89 mg/kg, but may be up to 20 kg/mg or higher in Cd-sufficient soil. A plant keeping 100 mg/kg of cadmium in the system might be considered as special in Cd-sufficient soil (Yang *et al.,* 2004).

Pot experiment to determine accumulation of cadmium with *Brassica* sp. at certain concentration was conducted by Rajeev *et al*. (2014). It was discovered that there was more accumulation in the roots than shoots. According to Zheng *et al.* (2010), cadmium ions normally accumulate in roots and few quantities are moved to stems and leaves, because roots of plants stand as blockage to upward movement of pollutants. This same outcomes were noticed in *Amaranthus tricolor*, *Brassica chinensis* and *Lupinus albus* (Zoronza *et al.,* 2002; Liu *et al.,* 2007; Watanabe *et al.,* 2009).

Heavy metals are contained in soil generally. There are procedures in removing heavy metals from contaminated soil. Phytoextraction of heavy metals has become a popular technology for removing heavy metals from polluted soil. This is because of low cost of execution and eco-friendliness (Vassilev *et al.,* 2002; Alizadeh *et al.,* 2012).

Phytoextraction procedure is widely preferred to procedures like verification, electrokinetics, filling of land's top soil and digging procedure was reported by Gosh *et al.* (2005). The approach aid plants' capacity to function as a solar pump driver, removal and filtering systems to uptake contaminants through their root to shoot. For this procedure to be more helpful, the plant used must be able to move heavy metals trapped in their roots to shoots of plants. According to Chinmayee *et al.* (2012), few

publications have documented proof that frequent uptake and movement to shoots by plants will assist removal of heavy metals.

Chromolaena odorata, an invasive plant, grows in several soil types because of its acceptance to a large soil pH range (Woodward and Willians, 1987). It also prefers well-absorbed soil because it is terminated by water logging and saline soil. *Chromolena odorata* is susceptible to pathogens like fungi, which cause yellowing of leaves, stem blackening and dying when waterlogged (Woodward and Willians, 1987).

Moreover, *C. odorata* enjoys fertile soil, but when it finds itself in an infertile soil, the development will be slow. The plant thrives in an uninterrupted region with proper light and temperature ranging from 20-37^{o}C. The vital thriving scheme of this plant is the total non-structural carbohydrates, which is the pathway of carbon stored in the root and is applied for the survival specifically in times of disturbance and winter.

The amount of total non-structural carbohydrates dictates a plant's capacity to develop and recreate. It helps plants to survive in times of contention with immediate plants, giving room for fast intrusion, response to stress, as it applies what is available to plants when carbohydrate is broken down (Bennett and Rao, 1968).

Mexican sunflower is broadly dispersed in rainforest regions, like Asia and Africa. It used for different purposes, for example as animal hunt, green fertilizer, insecticide, an ornament shrub, and a honey plant (Rios, 1999). The presence of phosphorus to crops to stimulate the species results in its prescription for high mass production techniques (George *et al.,* 2002 a,b). In whatever manner, it has similar features with stubborn and aggressive weeds, and it is abundant in Nigeria (Ayeni *et al.,* 1997). There are different ecological zones which species are adapted to, namely: temperate, tropic, savanna, waste lands, road sides and polluted and unpolluted sites.

Researches on biochemical stimuli to pollution loads are important. In agroforestry, for animal production, this species is recommended in the tropics. It is also a weed that has the ability to take over vacant lands or polluted sites. The beneficial effects of this plant on the sprouting phase of selected plants have been reported (Ademiluyi and Omotoso, 2008). *Tithonia diversifolia* also has a negative impact on the sprouting of others (Otusanya *et al.,* 2007).

Using plant to clean up polluted sites, also known as phytoremediation, has been deployed in the screenhouse with cost-effective plans for cleaning contaminant from affected locations (Salt *et al.* 1995). Different factors affect the deployment of commercializing phytoremediation. These factors include identification of hyperaccumulator plants, high crop production development method and management of crops (Blaylock *et al*., 1997)

Cleaning of toxic elements from soil is achieved via two main strategies. The first strategy is the phytoextraction approach using element hyperaccumulator species (Baker *et al*., 1994). It has been proven that metal hyperaccumulator plants are effectually used in cleaning-up contaminated soil because they have capability to absorb large amounts of heavy elements from polluted soil. Phytoextraction ability is restricted because of low yield. For example, *Thlaspi caerulescens* is known as zinc/cadmium hyperaccumulator that can gather and accommodate 10,000 mg/kg of Zn and 100 mg/kg of cadmium in stems and leaves (dry matters). Sign of toxicity was not shown (Tanhan *et al.,* 2011). There are about 400 species of hyperaccumulator plants (Baker *et al.,* 2000). The productivity of high biomass indigenous plants that can absorb more pollution loads outside screenhouse has only being investigated by few researchers. Practically, the zinc/cadmium hyperaccumulator *Thlaspi caerulescens* (Zhao *et al.,* 2003*)* and the cadmium hyperaccumulator *Viola baoshanensis* (Zhuang *et al*., 2005) might be suitable in phytoremediation of trace elements in polluted sites. Another substitute is utilizing plants that are not pollution-tolerant, use of non-accumulator plants, either high biomass plants or fast-growing trees that can be cultivated without stress (Ghosh and Singh, 2005; Meers *et al*., 2005; Solhi *et al*., 2005). Studies have been carried out on heavy metals absorption capability of high agronomic plants, such as *Brassica juncea*, *Helianthus annuus*, and *Zea mays* (Cui *et al.,* 2004; Turgut *et al*., 2004).

Apart from shrubs and herbs, trees like *Salix spp* and *Populus spp* have high biomass, and have been investigated. High capability in forwarding ability to build phytoremediation was discovered (Liphadzii *et al*., 2003; Vervaekea *et al*., 2003). Plants with little potential to gather pollution load can be compensated by a sufficient high number of plant biomass, which will result in collection of high-concentration heavy metals removed from polluted sites (Liphadzii *et al*., 2003).

Many have shown that introduction of chelator, like dissolved EDTA, N-(2-hydroxyethyl)-ethylene-di-amine-triacetic acid (HEDTA), can stimulate phytoremediation (Nowack *et al.,* 2006). Groundwater pollution may be caused by introduction of chelating agent by the screenhouse sometimes. Processes in which plants biomass are produced via agronomic administrative exhibit vital influence in large remediation techniques using plants (Liphadzii *et al*., 2003).

Transport factor shows that metal are transported via root to stem and leaf in plants. Efficient upward movement is favourable to metal clean-up using plants in the following areas: (i) reduction of amount of pollution load and reduction in poison capability to vascular bundle; and (ii) uptake by root and shoot is a system of intolerance to increase pollution load (Liphadzii *et al*., 2003).

2.1 Phytoextraction

This is described as intake of pollution in soil using plants and transfer of these metals to shoots where they compile (Sheoran *et al.,* 2016). To eliminate contaminating agents from a growing soil, the roots and shoots are reaped. Salt *et al.,* (1995) note that the expenses involved in removing pollutants by plants are ten times cheaper than a typical soil pollution removal approach. This phytoextraction technique can aid recycling of these heavy metals, producing them in large quantity, especially in mineral industries (Sheoran *et al.,* 2016).

Nascimento and Xing (2006) observe that, in the days to come, phytoextraction may be considered as economical automation. Jiang *et al.* (2004) examined the maturation enforcement and capability for copper phytoextraction for *Elshotzia splendens.* The convertible form of cadmium was incompletely taken out by plant absorption, which followed the absorption of nutrients in the company of crops. Cadmium phytoextraction was carried out on *Zea mays*, the quantum of substitutive type of cadmium lowered with soil treated with phytoextraction. Similar observation of reduction in cadmium state with *Zea mays* has been reported (Mojiri, 2011)

2.2 Phytostabilization

Phytostabilization can be defined as stable mollification. It is basically utilized in upturning degraded sites, debris and slime. It uses plant roots to curb pollution load movement and bioavailability on earth. The plant reduces the flow of hazardous solution. It hinders straight connection with contaminated soil by posing as a bridge and interposing in soil abrasion, which emanates from transmission of destructive

heavy elements to separate locations. Photostabilization can also be considered as one of the convenient procedures in cleaning sites polluted with cadmium, copper, arsenic, zinc and chromium. Alvarenga *et al.* (2009) examined the impact of organic cleaning, sludge slime, hard throwaway and decayed mixture of plants on photostabilization of polluted sites. This technique aids removal of dangerous substances to conserve the soil and water.

2.3 Rhizofiltration

Rhizofiltration is mainly utilized for the purpose of remediating withdrawn groundwater, water body and sludge with little toxic concentration. This is typically usage of plants to clean different surroundings of water bodies. It could also be utilized for heavy metals that are mainly stored and kept in the ambient of the xylem and phloem. The capability to extract Pb out of water was examined using sunflower possessing the highest capability (Sheoran *et al.,* 2016).

2.4 Phytovolatilization

As noted by Banuelos (2000), some plants have the capability to change Selenium (Se) into dimethyldiselenide in increased Se soil. Unlike different correction methods, one has no control over their movement to other areas once the contaminant has been removed via volatilization. There have been recently published reports of similar cases of volatilization-based soil correction (Tangahu *et al.,* 2011). Contamination of different environments has been caused by the movement of heavy metals by man through fertilizer application, toxic effluent discharge and processing for different applications, and mining from ores. Heavy metals like these are hazardous to the environment and man's health by the contamination of the food chain. These pollutants are accumulated by living organisms. Phytovolatilization usage of plants, which collect contaminants, like Selenium and Mercury in contaminated soil and transmit them to the atmosphere via leaf (Karami and Shamsuddin, 2010). This approach is also usage of plants to absorb pollution loads in soil, changing them to vapourised state and later to the atmosphere (United States Protection Agency, 2000).

Trace elements are required in small quantities in living organisms for their biological optimization, such as Fe, B, Mn, Zn, Cu, Mo, Ni (Mertz, 1981; Sano and Suzuki, 2001; Bidar *et al.* 2006; Peng *et al.* 2009). High concentrations of heavy elements disrupt proper biochemistry and physiology of biosystems. The major

dangerous heavy elements are Hg, Pb, Cu, Se, Cr, As, Zn and Cd (Wright, 2007; Gosh, 2010). Among these, Pb and Cd are most hazardous heavy metals for man (Sekara *et al.* 2005).

The essential mineral element required in large quantities for the development of plants and animals include nitrogen, phosphorous, potassium, calcium, Magnesium, sulphur (Lenntech, 2011). Increased amount of pollution load in plants, leading to decrease dry matter production, is caused by increase in Ni in the soil (Chhotu *et al.,* 2009 and Giordani *et al.,* 2008). Root and shoot growth in seed germination was discovered to be significantly influenced by increased in this element (Chhotu *et al.,* 2008). Higher green plants have Nickel as an important minor nutrient (Brown *et al.,* 1987). Nickel may be poisonous to plants at upward strata (Bingham *et al.,* 1986). Physiologico-chemical steps, like reducing leaf pigment (Piccini and Malavolta, 1992) and leaf photosynthetic and transpiration activities (Jones and Hutchinson, 1988) and crippling permeability by membrane in connection, aid extracellular peroxidase activities (Pandolfini *et al.,* 1992) owing to excess nickel. Nutrients from the root to the shoot possess reduced translocation (Yang *et al.,* 1996).

The most prominent element in soil is copper. Irritating to the nose, mouth, and eye can be caused by long-term exposure to this element. Cancer of the liver and kidney caused by high intake of copper can lead to death. In the environment copper cannot be broken down. Because of this, it can accumulate in animals and plants. Some plants have slight opportunity to survive on Cu-rich soil as a result of copper interference of the soil. This is because it adversely affects the performance of Annelids, microbes, and organic matter degradation (Lenntech, 2011).

In the modern age, cadmium is regarded as serious contaminant (Singh *et al.* 2009). Pollution loads of Cd over the average figure have been discovered mutagenic, carcinogenic and teratogenic in animal species of huge population (Degraeve, 1981). An endocrine disruptor was reported for Cd (Awofolu, 2005).

Some complications in man, like chronic neurological disorders specifically foetuses and young ones, has been discovered to be caused by Pb. This implies that attitude and behaviour change with slow intellectual development (Awofolu, 2005). Toxicity in young ones owing to Pb causes failure of short-term events, decreased intelligence, coordination problems and mastering disabilities (Padmavathiamma and Li, 2007).

Shu *et al*. (2000) reported that *Commelina communis* on Cu mine spoils at Huangshi, in their shoots had increased consumption of copper beyond total dry weight of 1%. Hyperaccumulation of Zn and Cd, namely, *Cardaminopsis halleri*, was reported by Dahmani-Muller *et al*. (2000); their amounts in leaves were greater than 20000 and 100 mg/kg, respectively. It was recommended by Madejon *et al*. (2003) that firmness protocol soil polluted by heavy element can be effective by use of *Helianthus annus*. This crop thrives well, which aids soil firmness. Animals rarely eat its stubborn leaves and stems. There is little amount of poisonous metals in their seeds (actively eaten by birds); they showed low risk for the food web. Annually, heavy metals increase in concentration in the environment (Govindasamy, 2011). Therefore decontamination of the soil of heavy metals is essential for environmental health maintenance and restoration of the ecosystem. Chemical and physical strategies are commonly seen as costly, stressful and risky (Padmavathiamma and Li, 2007; Wu *et al.,* 2010). Turan and Esringu (2007), Singh *et al.* (2009), Saier and Trevors (2010) and Revathi *et al.* (2011) claim that, in evaluation, plant-used remediation is a simple, cheaper, eco-friendly approach.

Heavy metals are metalloids. Elements with densities greater than 3 cm and 5 g are often connected with toxicity and contamination. Low concentrations of these metals are needed by microbes (Adriano, 2001). For example, Zn is made up of different chemical substances (dehydrogenases, peptidases, and proteinases) but it is also involved in a complete set of chemical reactions that occur in the living cells of phosphate, auxins, proteins, ribosome formation and RNA in plants (Mengel and Kirkby, 1982).

Copper contributes to many functional procedures in plants (cell wall metabolism, seed production, photosynthesis, respiration, nitrogen and carbohydrate distribution) and disease resistance (Kabata-Pendias and Pendias, 2001). According to Baker and Walker (1989) metals exhibit toxic effect on cells at a high concentration. Cadmium might be dangerous after being used up by living organisms (Suzuki *et al.,* 2001; Peng *et al.,* 2009). Kabata-Pendias and Pendias (2001) reported that these chemical substances are known to interfere with their processes, to prevent deoxygenated ribonucliec acid operation change in microbes, to disturb mutualism or commensalism between plants and microorganisms, symbiosis between microbes and plants, and up to enlarge plants susceptibility to fungal attack.

Heavy metals are not degradable, unlike organic compounds, and the needs stagnation and removal. Kuzovkina *et al.* (2004) state that, engineers and scientists have commenced deploying less expensive techniques, such as employment of microorganism or plants, for removal of contaminants.

According to Boonyapookana *et al.* (2005), Su and Wong (2004) view phytoremediation as a thriving technique that can be recommended for clean-up of polluted soil. It is cheap and eco-friendly, and permits repeated usage. It is applied best at sites with deep contamination of organic nutrient pollutants that are amenable to these five applications mentioned by Schnoor (1997), Yang *et al.* (2005) and Ciura *et al.* (2005): phytotransformation, phytostabilization, phytoextraction, rhizofilteration, and rhizosphere bioremediation.

Lasat (2002) and Tang *et al.* (2003) define phytoextraction as the usage of green plants to remove organic pollutants, trace elements domiciled in contaminated soil to be absorbed in roots and shoots that are harvested. Deo *et al.* (2012) aver that phytoremediation can not only remove metals like Ag, Co, Cr, and Cu but also radionuclides, such as Sr, Cs, and Pu, and certain organic compounds.

The ability to tolerate increased amount of pollution load with poisonous influence has been shown by plants. Heavy metals in a low dosage are important micronutrients for green plants though, in high dose, it is likely to come up with building and breaking down of food disorder, and for most plants, development inhabitation. Peralta *et al.* (2000) observe that some species of plants could actually accommodate more concentration of heavy compound or other elements and are common to metallic ferrous soil.

According to Schmidt (2003), Tang *et al.* (2003) and Pilon-Smits (2005), plants such as Indian mustard or sunflower, like *Helianthus annus* showed an increased accommodation of heavy elements. Therefore, they are employed in the study of plant-used remediation. The materials of plants can be used for non-food reasons. In other words, refining of the residue from burnt remnants is also a *C. odorata*ice (Bennett *et al.,* 2003). The capability of *H. annus* was examined for removal of heavy elements. This was because of its fast-growing nature (Ximenez-Embun *et al*., 2001).

Clean up of heavy elements effectively from contaminated areas is dependent on the soil conditions. According to Arthur *et al.* (2005), soil continuous exposure to

insecticides, pesticides and fungicides showed increased amount of heavy elements that can be extracted. This resulted high concentration of heavy metals, chemical and fertilizers always pollute air, land and water, and besides annual price increase. When the fertilizers are added, then hinder the uptake of some essential contaminants, like Pb. Malik *et al.* (2000) asserts the combination of metal with other compounds to form chelate held by bond action of plants look promising than artificial chemical chelating agents because they have excessive poison to plants, aid their storage in plants, and reduce yield of plants.

Phytoremediation has become popular, with government agencies and industries. The technique was widely known for its cost-effectiveness. This plant-based technique is an important farming practice used in the location where the soil are contaminated with HMs. Some chemical reactions are important to living organisms, like decaying organic matter (*Maharashtra Nature Park Bulletin*, 2003). Suthar *et al.* (2005) reported that the vermin-compost have high nutrient value, improves richness of soil and maintains the health of the soil. The use of compost and vermin-compost in polluted soil makes soil fertility better and helps in phytoremediation (Zheljazkov and Warman, 2004). Rahman and Zaim *et al.* (2015) asserts that it improves the growth of plant and enhances its productivity. The use of vermin-compost provides natural environments for phytoremediation.

Plant-based remediation can also aid intake of heavy elements from soil through the vascular system of plants and their movement to the upward part of the plant. Heavy metals affect the ecosystem owing to anthropogenic activities like mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and melting operations (Welch, 1995).

Some plants have been successfully used in plant-based remediation. Many factors affect metal accumulation by plants. The growth stages of plants influence use-up, absorption by separation mechanism through the vascular system to the heavy elements (Guilizzoni, 1991). Three essential uses of plants in environmental studies have been indentified by Gareeb (2007): indicators of pollution, excluders and accumulators.

Excluders are plants limiting stage of heavy metals' mobilisation in plants. They contain little amount of pollution loads. Baker *et al*. (2000) claims that plant excluders are employed in restoring sites polluted by heavy elements.

Heavy elements can be taken-up by almost all higher plants. Some plants are capable of taking in large quantities of metals. Baker and Brook (1989) note that some groups of plants are hyperaccumulators for some heavy elements.

The plant types used for remediation are those that absorb, store and, under certain conditions, detoxify contaminants. Hyperaccumulators are plant species that can accumulate and tolerate shoot concentrations of heavy metals. It is important to know plant species which can both accumulate and tolerate contaminants at a given site. Various factors, like soil nutrients, pH, and plant type affect the interaction between plants and microbes and, hence, influence heavy metal intake by plants.

Chaney (1983) examined the development of plants used to clean-up pollutants from contaminated soil. The absorption of heavy metals from the soil and water differ from plant to plant. Brooks and Lee (1977) label some plants as hyperaccumulators based on the quantity of their absorption. Most of the absorption activities take place in rhizosphere. Brown *et al.* (1994) note that pH is a controlling factor for the bioavailability of heavy elements in soil. Robinson *et al.* (1998) found opposite correlation between heavy metal absorption and alkalinity and acidity of soil. The current study was designed to determine the potential of *Canna indica* absorbing heavy elements in various parts of plants.

The deposition of heavy elements in the soil started thousands of years ago. This is why, unlike pollutants, metals are not degraded biologically but are transformed from non-reduction stage to another form of complexes (Gisbert *et al*., 2006). Accordingly, environmental concerns have led to setting up of strict guidelines to avert the increasing amount of heavy elements in soil. Consequently, various techniques are required to decrease the level of harmful elements in polluted soil, particularly those applied in crop production. The conventional methods employed in restoration of metal-polluted soil were based on civil or chemical engineering. These include vitrification, excavation followed by land filing, chemical treatment, and electrokinetics (Salt *et al*., 1995a; Glass, 1999; Kumpiene *et al*., 2008; Aboulroos *et al.,* 2006). Clean-up of soil polluted by heavy elements compared to old method, is expensive (Salt *et al.,* 1995a).

Hyperaccumulating plants have the tendency to remove high quantities of elements in soil, and could translocate pollution loads from root to shoot. They could also assemble and permit high pollution loads (Gosh *et al*., 2005). Despite the

introduction of chelators to clean up heavy elements, movement to other parts of plant is enhanced with chelators. Soil plants have mostly reduced the capacity to accommodate increasing pollution loads thereby surviving within little time after pollutants have been accumulated (Nriago, 1979; Adriano, 2001; Kratz and Schnug, 2006; Nascimento and Xing, 2006). Another significant difference between plant-based remediation and chelator-facilitated techniques is that plant-based techniques are known to sprout slowly, associated with decreased crop production, but the facilitated techniques apply fast, sprouting with increased biomass production (Nascimento and Xing, 2006). Plants capable of moving pollution loads from root to shoot could increase the concentration of heavy elements at the surface.

2.5 Mechanism of metal hyperaccumulation

Accommodation of pollution load in plant takes series of mechanism involving movement within the root region to other parts of the plant; translocation to shoot of plants (Clement *et al.,* 2002). For adequate metal hyperaccumulation, plants must possess the ability to move elements in soil to solution state. They must also have capacity to easily and quickly absorb pollutants in the vascular system; and increase the translocation process from root to shoot. Besides, they must have the ability of extracellular storage or intracellular sequestration in the leaf cell. These areas have been looked at by Salt *et al*. (1995), Chaney *et al.* (1997) and Clemens *et al*. (2002) and some others.

Rosa *et al*. (2004) showed *Sinosa kali* absorbs and moves cadmium simply to stem and leaf sections as a vibrant cadmium hyperaccumulator. Stingu *et al*. (2011) showed that *Zea mays* is a potential cadmium hyperaccumulators.

2.6 Metal phytoavailability

Phytoremediation of heavy metals in soil depends on quantity of procurator, the activity, ionic ratios of the elements in solution in soil, and the quality movement in soil to solution stage and then to root of the plant. In soil, metals exist in five different pools:

1. Fraction one, solubility, that is elements present or available in the solution of soil;
2. Fraction two, transferable that is elements adsorbed on charges transfer location and on inorganic growing soil compositions;
3. Fraction three, organic that is metals bonds with the organic matter;

4. Fraction four, insoluble that is elements precipitated mainly as oxides, carbonates and hydroxides;
5. Fraction five, residual that is metals incorporated in the silicate minerals.

According to Salt *et al*. (1995a) and Aboulroos *et al*. (2006), anthropogenic contaminations affect the metal contents in fractions 1-4, while fraction 5 reflects the background of pollution load of these elements. The metal concentration in plants corresponds with soil metal concentration in the soluble fraction as the most essential indicator of the metal phytoavailability.

Phytoavailability of heavy metals in the soil is primarily for achieving in plant-based remediation. A vital portion of metals in the soil lives as the tight part which requires preparation in soil solution in making it accessible to uptake. However, this can be accomplished artificially through soil amendments. Plant-based absorbing more than the required amount of pollution load in the tissue of the plants could deal with the constraint decreasing the bonding of soil elements with plasmalemma tight metal reductases. This is through the vascular bundle solution coming out of natural ligands, like phytosiderophores. Reduced genetic heavy natural acids results in chelation, acidifying the rhizosphere via the operation of proton pump and plant solution of low molecular weight of organic acids (Salt *et al.*, 1995); Nascimento and Xing, 2006; Quartacci *et al.,* (2009).

Roots of pea plants germinating under iron or copper deficiency can reduce them, thus increasing their uptake (Welch *et al.,* 1993). Hypothetically, root solution should involve major activities in organizing heavy metals, furthering their assimilation of non artificial hyperaccumulators. Furthermore, the procedure of root transpiration and organization of permeability in hyperaccumulating plants is comparatively poorly for most of the environmental issues related to heavy metals (Rahman and Zaim *et al*., 2015). Nickel hyperaccumulation by *Thlapsi geosingense* has already been applied to the ligand produced for the conclusion of Ni accepting minerals in the rhizosphere (Wenzel *et al.,* 2002).

The low molecular weight of organic acids (LMWOAs) are essential in organizing soil metals owing to their function of soil acidification and forming complexes with heavy metals. The metal complexity is a key in metal mobilization and uptake for plants. The use of root showing LMWOSAs enjoys better acceptance from the public, as they are degradable by microorganisms. According to Renella *et*

al. (2004), the accuracy of natural acids without artificial chemicals in organizing metals in the rhizosphere soil is governed by their environmental-friendly nature, the aspect which is poorly understand.

2.7 ROOT UPTAKE

Tandy *et al*. (2006) and Lu *et al*. (2009) argue that elements enter roots through either active or passive pathways. This is contrary to the route of passing solute from one plant cell to another outside the plasmalema, which is root conduit (apoplastic pathway) where cation and anion or elements forming complexes enter the vascular system via intra cellular spaces. Passing of solute from one plant cell to another via plasmodesmata (symplastic transport), minor element ion strive movement across memebrane carrier employed by heavy metals. For example, nickel and cadmium strive for the transmembrane carrier employed by copper and zinc (Clarkson and Luttge, 1989). Even metal chelates, like iron phytosiderophore, can be moved by symplastic pathway via particular messengers (Crowley *et al.*, 1991). Not only hyperaccumulator plant species but also populations within a species may significantly differ in metal uptake.

2.8 Transportation

There are different non hyperaccumulator plants, in which metals are put together within root cells, which later become unnecessary for root loading. Hyperaccumulators are effective transport metals from stem. For instance, variations in contrasting ecotypes is responsible for *Sedium alfredi* hyperaccumulation of Zn unlike in Zn transport crossways tonoplasts in the stem cells (Yang *et al*., 2006). Similarly, in Cd hyperaccumulating ecotypes of *Sedium alfredi* Cd assimilate and root fill is an active procedure as compared to plants that cannot absorb or accumulate more pollution load in their systems (Lu *et al*., 2009). For mobilisation to shoot, metals must be stacked into the root. Metals first have to move from one side of casparian band on endodermis to another. This is a liquid resistant that hinders movement of solutions within root conduit into the stele (Marshner, 1995). Hence, to overcome this barrier and to get to the root conduit, elements have to cross by passing solution via plasmodesmata. This is factor determining the level of element transfer from root to shoot. Endodermis, is not an ideal obstacle against apoplastic transport of metals from cortex to the usually cylindrical central vascular portion of the axis of vascular plants (Marshner, 1995). Apart from possessing apoplastic or symplastic

movement, the innermost tissue of the cortex in most roots and stems could have hole that allows solutions to pass out through at least two locations around the root axis, at the root apex (Huang *et al*., 1989). Endodermis is ruptured momentarily by oblique root emerging from the pericycle of the stele via root conduit (Marshner, 1995).

Interference of the innermost tissues can be influenced by chemical-controlling herbs, which collaborate with metal complexes. These can amplify element collection (Ensley *et al*., 1999). Studies on chelant-induced phytoextraction, which examined the root uptake of metal complexes, including their elemental system, principally followed passage cell within the root conduit pathway (Collins *et al*., 2002). The root pack is an unyielding regulated action moderated by the skin transport proteins (Clemens *et al*., 2002). Before, it was accumulated into the root region. The extended separation of movement metal ions can be restricted because of increase in positively charged ion interchange capacity of the root cell wall (Przemeck and Haase, 1991). A pH based on equilibrium exists between LMWOAs and elemental complexes while moving solution from soil, via plants to the atmosphere (Clemens *et al*., 2002). These organic ligands play a significant role in metal uptake by hyper- accumulators. Applied chelants play an important role as components of hyperaccumulators is well formed (Lu et al*.,* 2009).

2.9 Translocation of heavy metal to leaves

Translocation is the movement of water molecules from the root of a plant to the aerial parts. Heavy metals are moved to the leaves where photosynthesis takes place (Vacchina *et al*., 2003). When water and nutrients are located where they are not needed, translocation helps in directing the movement to the aerial parts where they are needed for production of food. Hyperaccumulator plants are capable of translocating metals from the root to other parts of the plant (Peer *et al*., 2005).

The above refer to the mechanism of the antioxidants positive regulations enzymes which discuss the toxic heavy metals of tolerance (Freeman *et al*., 2004). The cell types where metals are placed vary with the element, including the plant species (Cosio *et al*., 2005). In *Thlaspi caerulescens*, Zn accumulation was several folds higher in vacuoles of the outer layer and sub epidermal cells than of the mesophyll cell (Kulli *et al.,* 1999). In *Arabidopsis halleri*, zinc and cadmium were sequestered according to scale of preference in storage of the mesophyll then the epidermal cell (kupper *et al*., 2000). Leaf tri*C. odorata*mes could be the vital

sequestered parts for cadmium in *Brassica juncea* (Salt *et al.,* 1995b). Proteins involved in the contrasting dispensation of metals between leaf cells have not been identified, but different approaches have been suggested (Clemens *et al.*, 2000).

2.10 Natural phytoextraction

With the exception of Ni phytoextraction, plant-based is mainly hindered by little crop production. To such plants might need tens of years to reduce heavy metals in soil to the normal level for the environment according to standardize amount prescribed by European Regulatory Standard (ERS) and World Health Organization (WHO) (McGrawth *et al*., 2001). For instance, most efficient zinc hyperaccumulators, *Thlaspi caerulescens*, would need non-cropping in reducing Zn-concentrated polluted soil ranging from 440 to 300 mg/kg (Mcgrath *et al*., 2000). Cleaning a soil polluted with nickel and zinc in 13-14 years of repeated harvesting of *T. caerulescens* is needed (Baker *et al*., 1994).

Brown *et al*. (1994) note that it took almost 28 years of repeated planting to clean up a soil loaded with 2100 mg/kg of Zn. The continuous planting in a soil polluted with cadmium and zinc for almost 14 months using *T. caerulescence* separated almost 22 % of Cd and 4 % of Zn (McGrawth *et al*., 2001)

Toxic effect by a compound on plant growth influenced by intake of compounds that are not part of the objective might be a means of restricting plant-based remediation (Lombi *et al*., 2001). For example, 3 crops of *T. caerulescens*, separated 43% of Cd and 7% of Zn from an industrial polluted soil (19 mg/kg, cadmium; 2920 mg/kg, zinc and 78 mg/kg, copper). Introduction of copper pollution load influenced the uptake capability of cadmium and zinc in an agricultural soil contaminated with sludge (Lombi *et al*., 2001).

The most successful and prominent use of plant-based remediation documented for nickel, resulting in the planting of vegetation that selectively concentrated nickeliferous soil containing Ni content has been reported by Baker *et al.* (1994). As noted by Anderson *et al.* (1999), these have not been exploited for phytoextraction of this rare but extremely toxic metal. *Poecilotheria vittata*, sometimes called Pederson's ornament, was reported to remediate an arsenic-contaminated site in 10 years or less (Salido *et al.,* 2003). The involvement of growing plant on low grade nickel-contaminated soil has not been exploited for this rare but extremely toxic metal (Nicks and Chambers, 1995, 1998).

2.11 Induced phytoextraction

This is the introduction of chelating compound to an already contaminated site to make the pollutant (heavy metals) available for uptake of plants that produces increased crop yield through the vascular system to other parts of the plants resulting in identification of hyperaccumulator species (Salt *et al.* 1995a). Some plants have been noted for action when it comes to moving pollutants from the root conduit to the shoot, especially sunflower and *Brassica* sp (Vandenhove and Hees, 2004). Soil with pH ranging from 7-14 rendered the heavy metals immobile and not available for plant's absorption, especially lead and chromium (Gray *et al*., 1999). However, element movement and plant-based remediation are considered aided by natural and artificial element ammendment (Schmidt, 2003). Since inorganic and natural mobilisers move metals by different mechanisms, these are discussed separately.

2.12 Inorganic amendments

Inorganic agents mobilize metals mainly by removal (Brümmer *et al.,* 1986), which is effected either by lowering the soil pH (Gray *et al.,* 1999; Schremmer *et al.,* 1999), or by alkaline salt addition (Smolders *et al.,* 1998). Soil acidity or alkalinity determines the dilution state of heavy metals, available quantity and mechanism for use-up and every content of heavy metals in the growing soil (Brümmer *et al.,* 1986; Hornburg and Brümmer, 1993; Gray *et al.,* 1999). The proportion of soluble cadmium, zinc, lead, and copper increases at soil pH below 6.5, 5.3, 4.5 and 3.5, respectively (Hornburg and Brümmer, 1993).

After introduction of metals into soil solvent and removal by plant use-up or leaching, further acidification will dissolve many soil elements (Brümmer *et al.* 1986). The ability to go depends on the buffering capacity of soil; in aerated soil, the process follows the Ist-order moving energy for many elements (Aringhieri and Pardini, 1985). Since soil physicochemical characteristics strongly influence the soil's buffering capacity, the quantity of protons required to reach successful pH target may vary for different soil (Wang *et al.,* 2006). Moreover, the extreme acidification of soil (pH < 4) is detrimental for plant growth (Marschner, 1995). Therefore, to achieve efficient phytoextraction, pH optimal level should be worked out for different soil-plant systems. Decreasing the soil pH through application of mineral acids (Gray *et al.,* 1999) or elemental sulphur (Wang *et al.,* 2006) is an effective strategy to remove elemental pollutants out of the soil to the solution phase, thus increasing uptake by

plants. Physiological acidification of rhizosphere by application of ammonium fertilizers has also been reported as a low-cost strategy to increase the heavy metal mobilization (Schremmer *et al.,* 1999). However, it is relatively less effective in enhancing phytoextraction as compared to the acidification of bulk soil. To avoid nitrification of the applied ammonium, nitrification inhibitors may also be needed. The commercial nitrification inhibitors are hardly effective at high soil temperatures (Ali *et al.,* 2008). Some metals in soil are also bound or adsorbed on oxides of iron, manganese, and aluminum; dissolution of these oxides at low pH will simultaneously release these metals into the soil solution. Citric acid addition to a U-contaminated soil not only mobilized Uranium but also released Fe and Al (Salt *et al.,* 1995b; Huang *et al.,* 1998). In some cases, natural hyperaccumulation may also be limited by the metal mobility in soil that needs to be enhanced through soil amendments. For example, after optimizing the soil pH through elemental S amendments, *T. caerulescens* extracted 36% of Cd out of soil contaminated with 25 mg/kg of cadmium (Wang *et al.,* 2006).

Salt amendments, like NaCl or KCl, can increase phytoextraction by two mechanisms: swap of elements from take-up and hold areas in soil by positively charged and building of balance chelators with negatively charge chloride (Schmidt, 2003). Introduction of sodium chloride into the soil dilution state enhanced cadmium load and accumulation in the leaves of Swiss chard *Beta vulgaris, Cicla* sp. (Bingham *et al.,* 1983). In other studies, adsorption of cadmium in *B. vulgaris* was attributed to formation of chloro-complexes of cadmium instead of increased cadmium concentration in soil solution (Smolder *et al.,* 1998). The use of KCl at 3 g/kg enhanced Cd accumulation in corn similar to that achieved by 0.6 g/kg of EDTA, suggesting KCl as a preferred amendment over EDTA owing to the lower cost and relatively shorter persistence of the Cd-Cl complex in soil (Maxted *et al.,* 2001). Usage of 10 $mmolkg^{-1}$ of NH_4Cl to a Zn-enriched soil, though increased the metal solubility by 1.5 folds, caused only a slight increase in the Zn accumulation by *Salix aurita* (Keller *et al.,* 1999). Schmidt (2003) attributed low ability of NH_4Cl in Zn phytoextraction to the small cation exchange effect (owing to low NH_4Cl application rate) and the much lower extent of complexing Zn with Cl as compared to Cd. Since

application of NaCl damages the growing soil structure more than other salts, Schmidt (2003) suggested the need of exploring other Cl^- salts for enhancing phytoextraction.

2.13 Organic complexing agents

The naturally made agents remove elements out of different parts of soil resulting in solutions (Schmidt, 2003). An examples of chelating agents is synthetic amino polycarboxylic acids (APCAs), for example, ethylenediaminetetra acetic acid (EDTA), hydroxyl-ethyl-ethylene-di-amine-tri-acetic acid (HEDTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA) and diethylenetriaminepentaacetic acid (DTPAcid). Other examples are naturalaminopolycarboxylates, such as ethylenediamine-di-succinate (EDDS) and nitriloacetic acid (NTA); and low molecular weight organic acids (LMWOAs), for instance, citric acid, oxalic acid, gallic acid and acetic acid (Collins *et al.,* 2002).

Ethylene-di-amine-tetra-acetic acid is a commonly investigated organic amendment in phytoextraction, and it has been successfully used to increase removal of Pb and other heavy metals using plant-based technology (Blaylock *et al.,* 1997; Shen *et al.,* 2002; Wu *et al.,* 2003; Santos *et al.,* 2006). Relatively few studies demonstrated the heavy element fractions targeted by chelating agents.

There are also risks connected to application of combining different substances of APCAs under field conditions, as the quantity of pollutant moved by synthetic chelants usually overshoots the load used-up by plants (Römkens *et al.,* 2002). Not only the synthetic APCAs, like EDTA, are stubborn to obey owing process, their mineral groups of apartment are also, to a great degree, firm and capable of continuing in soil holes solution for many months (Lombi *et al.,* 2001). Washing away of the topmost soil risk that was associated with the application of combining different substances forming mineral solution was decreased owing to the few attempts made on it. For example, Salt *et al.* (1998) reported that, at the time of maximum plant biomass banding agent was used. According to Li *et al.* (2005), the risk of metal leaching can decrease through the articulation of little-free EDTA small pieces covering with silicate. Examples of biodegradable APCAs are EDDs, NTA, methylglycinediacetate (MGDA) and Hydroxyiminodisuccinic acid (HIDS).

As reported by Greman *et al*. (2003), Tamura *et al.* (2005), Freitas and Nascimento (2009), and Rehman *et al.* (2009), biodegradables have been estimated as environmentally safe alternatives to EDTA. Luo *et al.* (2005) noted that EDTA was

more functional than EDDS for Pb and Cd. Malic acid, oxalic acid and citric acid are natural combining agents. They have been assessed for mobilizing heavy metals, such as cadmium, zinc, uranium, chromium and nickel in soil. The metal uptake increased just as the translocation to shoot. According to Jean *et al.* (2008), in mobilizing Cr, citric acid was more productive. Serpentine mine tailings was a study on EDTA and DTPA, organized more Cr and Ni than LMWOAs tested citric and oxalic acids, but decreased the biomass of *B. juncea* owing to metal phytotoxicity (Hsiao *et al.,* 2007). Citric acid was more productive in mobilizing U from the soil and enlarged its uptake by *Brassica* spp (Huang *et al.,* 1998) by more than 1000 folds and it was tested with the various synthetic APCAs and LMWOAs. Estimating different biodegradable adjustments for assembling U, Duquene *et al*. (2008), described citric acid as the most productive adjustment that brought about up to 479 folds increase in soluble U, to be induced by oxalic acid, EDDS and NTA.

Different heavy metals also differ by the effectiveness of diverse natural combining agents in plant-based remediation. As asserted by Wu *et al.* (2003), potency of different chelants for organizing HMs followed the normal process, which is EDTA>citric acid=oxalic acid=malic acid; for Zn, EDTA>malic acid>citric acid >oxalic acid (Wu *et al*., 2003). Elkhatib *et al.* (2001) confirmed that cadmium accumulating in sunflower was elevated in citric acid, followed by malic acid, CTAB and EDTA. Blaylock (1997) reported that accumulation of Pb by *B. juncea*, expanded in the order EDTA>>CDTA>>DTPA>>EGTA>> Citric acid (Schmidt, 2003).

The mortification rates of metal chelant complex rely on their firm constant, the activities of microbes and concentration of free ionic metals. Henneken *et al.* (1998) aver that Fe^2-EDTA, which is an example of heavy metal types of binding agent, reduced drastically when compared to chelates of other metals, such as Na, Ca, or Mg. Wenger *et al.* (1998), reported that APCA, as a biodegradable, at a much slower rate NTA, deteriorated when it is mixed with Zn in contrast to Na_3NTA (Tabatabai and Bremner, 1975). Meers *et al.* (2005) and Evangelou *et al.* (2008) assert biodegradability of organic composite agents plays a vital role in deciding their ability and their welfare in photoextraction and this has been well documented for APCAs and organic acids (Meers *et al.,* 2005).

Apparently, *H. annus* did not show stress from heavy metals with expanding EDTA application rate and this is owing to the high amount of metals mobilized.

Metal photoextraction by citric acid was also not sufficient due to its immediate degradation (Lesage *et al*., 2005). Evangelou *et al.* (2008) examined the fast biodegradation of LMWOAs for photoextraction. The same microorganisms were broken down and the successive application was not effective.

Apart from biodegradation, higher concentrations of LMWOAs may require the high buffering ability of calcareous soil. Lesage *et al*. (2005) argue that citric acid can be important in assembling heavy metals only when its demand rate passed the buffering capability of the soil. Therefore, for phytoremediation of polluted calcareous soil, proper acidification with mineral acids or elemental S may be predicted as a useful strategy to reduce the amount of citric acid needed. Huang *et al.* (1998) and Shahandeh and Hossner (2002) note that the acidification have been illustrated in studies with phytoremediation of U-contaminated soil for which citric acid has been reported to be a highly achievable amendment. Besides, soil acidification alone (combining mineral acid or elemental sulphur) did not enlarge the U uptake by plants; since U is taken up by plants as free uranylcation ,which is the foremost U species at pH<5.5. Ebbs *et al.* (1998) claim that it has powerful tendency to attach to soil solids and organic matter; thus it is inaccessible for plant uptake (Sheppard *et al.,* 1989). Ebbs *et al.* (1998b) observe that citric acid which, apart from reducing the pH of the soil, liberating uranyl ion, forms binuclear complex together with uranyl cations and decreasing the U solubility over 200 folds and U photoaccumulation over 1000 folds (Huang *et al.,* 1998).

The level of U solubilisation is dependent on the amount of the citric acid and the inceptive pH of the soil. For example, soil with a pH of 7.7, double the citric acid application rate from 10 to 20 mmole kg^{-1}, produced 5-fold enlargement in stem and root with large amount *by B*. *juncea* (Huang *et al.,* 1998). Similarly, at pH of 4-5, proportionately low appeal rate, that is 2 mmole kg^{-1} and citric acid, was very functional in solubilising U than at pH 6-8 (Ebbs *et al.,* 1998b).

2.14 Phytotoxicity of chelants

The chelants phytotoxicity will seldom reduce the phytoextraction potential, that depends on chelating agents. Biomass reduction by synthetic APCAs has been frequently reported. For instance, EDTA and EDTA-heavy metals combining substances are poisonous growing soil (Grčman *et al.,* 2001) in connection to plants promoting drastic decrease in sprouting of many plants (Chen and Cutright, 2001;

Nascimento *et al.,* 2006; Jean *et al.,* 2008). Biomass reduction in different plant species has also been reported with other synthetic APCAs, like EGTA (5-10 mmol/kg), DTPA (5-10 mmol/kg), EDDHA (1.39 mmol/kg, HEDTA (1.45 mg/kg), EDTA (2.32 mg/kg) and CDTA (5-10 mg/kg) (Blaylock *et al.,* 1997; Huang *et al.,* 1997; Sun *et al.,* 2009).

Degradable APCAs, like EDDS and NTA, are also sometimes toxic. For instance, EDDS, applied at 5-10 mmol/kg, caused severe growth reduction in *Zea mays* (Luo *et al.,* 2005), whereas its application in 4 splits each of 10 mmol/kg caused biomass reduction in *Brassica rapa* (Grčman *et al.,* 2003).

Plant growth stage has significant effects on tolerance against synthetic APCAs and this varies with plant species. For example, moderate rates of EDTA increased Cd phytoextraction by *Solanum nigrum* when applied near flowering stage (Sun *et al.,* 2009). The other degradable APCA, NTA showed no toxicity symptoms when applied in the range of 1–20 mmol kg^{-1} (Quartacci *et al.,* 2005), whereas in another study it caused moderate to severe growth reduction with application rate of 2.7–26.6 $mmolkg^{-1}$ (Kulli *et al.,* 1999). Also, LMWOA, citric acid applied in the range of 3-20 $mmolkg,^{-1}$ was not phytotoxic (Huang *et al.,* 1998; Shahandeh and Hossner, 2002; Wu *et al.,* 2003; Quartacci *et al.,* 2005). At a higher application rate (25 mmol kg^{-1}) though citric reduced the dry matter yield of mustard and rye grass, it enhanced U phytoextraction by both species (Vandenhove and Hees, 2004).

2.15 Challenges and opportunities

Plants that are really effective for the removal of contaminants are supposed to be expanded, increase crop production with vascular bundle deeply rooted, and simple when it comes to harvesting. They should also tolerate large amount of pollution loads in their absorbing tissues in parts of gathered plants (Alkorta *et al.,* 2004). As of now, no plant has fulfilled the requirements mentioned above. Many plant-based techniques capable of accumulating large amount of pollution loads are restricted to small elements. The prospect of this technology is that it is still at initial stage (Alpana *et al.,* 2007). There is great possibility for biodiversity to discover more effective natural hyperaccumulators (Alloway, 1995).

Besides, there is the need to explore various agronomic strategies to intensify the biomass prospective of natural hyperaccumulators. Producing high crop yield of

capable plant-based remediators, non-artificial pollutant absorbers, in large quantity by optimizing NPK fertilization seems to be a viable option (Adriano, 2011). Although chelant-induced phytoextraction by high biomass plants has always been considered as a promising substitute to the natural phytoextraction, it has not yet been widely assessed under field conditions (Chaney *et al.,* 1997).

There are several limitations that challenge the commercialization of chelant-assisted phytoextraction. For example, to solve the plant-poison and leaching challenge connected to application of chelants, spilt-application of chelants lowers the desired improvement effects, whereas the sub-irrigation drainage systems to deal with the leaching loss is not cost effective (Salt *et al.,* 1995). Besides, the cost of chelating agents considered to operate phytoextraction at the field level has not been duly addressed (Salido *et al.,* 2003). For example, in most studies carried out with citric acid, the latter was productive at 20 m-mol kg, which is equivalent to > 8 tons ha. Therefore, the cost-effectiveness of chelant-assisted phytoextraction needs reconsideration (Lombi *et al.,* 2001). Although external use of LMWOAs, like citric acid, was successful in moving heavy metals in growing soil and enlarging the plant use-up, the potential of citric acid restricting plants has not been achieved for metal phytoextraction (Vandenhove and Hees, 2004). Members of the family Proteaceae G*lupinus* species are well known for denying high amounts of LMWAOs that play a part in mobilizing Phosphorous in P-deficient soil. Under P-deficient conditions, the proteoid roots of *Labium albus* predominantly restrict citric acid, which may be as high as 23% of the acquired carbon (Wang *et al.,* 2006).

Therefore, it is necessary to explore citric acid-eliminating plant species with characteristics recommendable for phytoextraction (Maxted *et al.,* 2001). Co-culturing citric acid-eliminating plants with known metal hyperaccumulators may also be utilized for expanding the metal phytoavailability and uptake by hyperaccumulators, thus reducing the outermost input of citric acid (Smolder *et al.,* 1998).

Useful parts of bacteria also need to be utilized in raising phytoextraction of massive metal-polluted soil. Soil inoculation with cadmium-repellant strains *Pseudomonas* sp. and *Bacillus* sp. enlarged Cd and Pb phytoextraction by hyperaccumulating tomato plant (Mertz, 1981). *Pseudomonas* sp. is known to manufacture rhamnolipid, which is a metal-confiscating agent and has a powerful

affinity for Cd. Rhamnolipid may exist in the soil long enough to enhance metal phytoextraction, but it is not resistant, like EDTA, to raise concerns regarding metal leaching (Pawlowska et al., 1996; Quartacci et., 2009).

Phytoextraction could also be fused with profit making operations, like forestry and bioenergy production (Welch et al., 1993). For instance, hydroponically grown castor beans known to gather Cd and Pb, needs to be tracked in metal-contaminated soil (Welch et al., 1993). Phytoextraction of Cd by a high-biomass tree CARAMBOLA presents a realistic option in Clean up cadmium-polluted growing soil (Romheld, 1991). In a soil contaminated with Cd, the tree would extract half of total cadmium content in a soil for 13 years (Wenzel, 2002). To produce biofuels, valorization of the bioenergy crops, like poplar, willow trees, castor, *Jatropha* and *Brassica* spp., can be carried out by diverse ATP retrieval methods, such as incineration, gasification, anaerobic digestion and pure plant oil output (Clemens et al., 2002).

An unspecified number of these compounds are trace elements essential for growth of plants, like zinc, copper, manganese, cobalt and nickel (Marschner and Romheld, 1995). The rest trace elements do not have specific roles to play in plant growth, like cadmium, lead and mercury (Clemens et al., 2002). The dispersion rate of these heavy metals from polluted region via particles, erosion, flood, sludge and indiscriminate disposal of refuse, contributes to degradation of the soil and the ecosystem in general (Govindasamy et al., 2011).

Several studies have established the fact that these compounds cannot be reduced far below global standards but need to be removed or rendered stationary manually (Kroopnick, 1994). Besides, as a matter of necessity, it is important to develop herbaceous plants that can have a firm grip on the vegetation with functional removal processes and are capable of increased biomass of root, stem and leaf (Zak and Parkinson, 1982*;* Leyval *et al.,* 1997) in order to stop strong and incessant erosion either by wind or water. Relevant plants in this regard lack symbiotic association of the mycelium of a fungus with the root of a seed plant and are often portrayed with underdeveloped urbanized vascular bundles and stem and leaf, especially for availability of heavy metals (Pawlowska *et al.,* 1996).

Plant-based remediation technique is a most successful strategy in cleaning-up contaminant, simply out of the soil and separating without compromising the soil

profile and soil fertility (United States Protection Agency Reports, 2000; Ghosh and Singh, 2005). Seed plants are best fit agents for restoring soil and water owing to the fact that they have genetic composition that can never be shared, with respect to biochemistry and functional description. Plant-based remediation is a new approach called biotechnology (Rajeev et al., 2014).

Plants have different approaches for increase in size on polluted soil (Raskin *et al.,* 1994). Some of them focus on elements when they are above the ground level to a state beyond that of the soil. Heavy metals, like manganese, copper, zinc, nickel and iron, are major mineral elements needed by plants. Ideal quantities of these heavy metals have vital role in plant growth and biomass. Usually, heavy metals could cause oxidative stress, elicit enzymatic and non-enzymatic anti-oxidative reaction stimuli, and cause fat and oil peroxidation in plant. Also, ideal quantities of Cu play role of co-factors in body building and chemical substances that aid configuration. In spite of the fact that it is an important component of food builder and respiratory electron chains (cytochrome oxidase), large presence of Cu in an environment where plants are being grown is capable of causing alteration in the passage of substances via membrane, chromatin structure, protein synthesis, activities of natural-occurring chemical substance procedures via poisonous influence. Garg and Kataria (2013) discovered alterations in catabolism and anabolism liveliness and degree of green pigment measurement, in justifying the capability of the cultivated plant to restore the polluted soil without wasting resources.

Gathering of *C. odorata*ice elements differs among plants. Removal of pollutants by plant is dependent on plant species, its natural grip, and soil value (Norman, 1974). There are parameters that control the state of any solution in a growing soil: the alkalinity and acidity of the soil, amount of positively charged ions, exchange of element ability, natural carbon content and oxidation state of the system (Willey and Osiru, 1972).

Zu *et al*. (2014) and Yoon *et al.* (2006) found that plant accumulating higher pollution load in lead, copper, manganese and cadmium beyond normal limits (4 mg/kg, Pb and Cu) which are 5 and 10 mg/kg. Although manganese and copper are very important to the growth of plants, they are required in small quantities. However, higher amount of these in plant tissues could result in detrimental effects. If the amount of pollution load of copper is greater than 40 mg/kg in the tissue of any

species of plant, it can cause poisoning in plant and fauna grazing them; sheep is a good example (Zu *et al*., 2014).

Mohebbi *et al.* (2012) reported that the amount of copper in tissues of corn, alfalfa and sunflower in monoculture or intercropping were >14 and 21 mg/kg, lower when compared with the research of Cui *et al.* (2007), but higher than the result of Malik *et al.* (2010). That of lead went beyond 5 mg/kg and ranged from 12.64 to 23.78 which could definitely have detrimental effect on the building food for plants (Mohebbi *et al.,* 2012).

McGinty, (1996) established a high bioconcentration factors (BCF) data for Cu and Mn, that showed how the pollution loads restricted movement to the vascular region and sidelined the stem and leaf during absorption by plants. Increased amount of heavy metals in root and little amount in the shoot showed the suitability of plants stabilization of heavy metals in the soil. The data generated by McGinty (1996) from accumulated elements in the tissues of corn or sunflower with or without date palm and alfalfa showed that BCF was greater than 1 and TF was less than 1. This implies that BCF>1 and TF<1 is possible for this plant to be an important agent for 1-4 heavy metal stabilization in a growing soil. Mohebbi *et al.* (2012) also established that BCF of Cu was higher than that of Mn with all values more than 1. He also stated that Cd had BCF higher than 1 as well.

Rajeev *et al*. (2014) further documented that raising different compounds along with gradient amount of cadmium in a growing soil will enhance high uptake in the tissues of plants. Immediately elements with charge are taken up, they are stored in the root region, which could be transported to the shoot through active transport (Ximenez *et al*., 2002).

Larger amount of cadmium concentration absorbed by *Brassics juncea* were more pronounced in root when compared with shoot (Bonnet et al., 2010). Zheng et al. (2010) aver that, under normal conditions, higher amounts of Cd^{2+} are trapped in the root and only little amount are allowed to escape to the parts above soil level. It is obvious that the vascular region hinders the movement of heavy metals, which might be an ability or mechanism /strategy in accommodating heavy metals in the root (Bonnet *et al*., 2010). Some plants have been exhibiting series of high accumulation of cadmium in their tissue systems in the order of root>stem>leaf, such as *Brassica*

juncea, B. chinensis, L. albus, Amaranthus tricolor when contaminated with 0.09 M (Liu *et al*., 2007; Watanabe *et al*., 2009).

The translocation factor is a mechanism possessed by the root of a plant to mobilise heavy metals from root to stem and leaf. When a plant is capable of moving substances from the root to shoot, it is a merit to plant-based remediation strategy. This is capable of decreasing heavy metals quantity thereby reducing poisonous capacity to the vascular region. Besides, mobilisation of heavy metals from root to shoot is a strategy for minimizing large quantities of pollution loads in the root region (Wani *et al*., 2011; Gonzalez *et al*., 2012).

Peer *et al.,* (2005) assert that the plant to be used for remediation must possess potential capability to clean up a polluted site and ability to accommodate heavy metals in their tissues. Prasad *et al*. (2004) note that some environmental factors, like light and temperature and length of time exposed to these environmental factors, enhances uptake of heavy metals. Failure of some plants to absorb poisonous heavy metals, like cadmium, lead and chromium, may be as a result of poisonous effect on plants. The soil has different mineral interrelationship and surrounding conditions, like control of water, increased temperature and availability of competing charges, which render the element usable for plants (Chaney *et al*., 1997; Prasad *et al*., 2004). Some plants exhibit defensive mechanisms, to defend themselves against taking up and storing increased amount of heavy metals. Some plants have developed adaptive mechanisms to accommodate selected heavy metals in their vascular tissues and parts above the soil level (Ebbs et al., 1998a).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study area

This research work was conducted in ScreenHouse of Bethel College of Education, Ijare, Ondo State in the south western part of Nigeria. It can be located within latitude 7˚ 21' 49.442" N and longitude 5˚ 10' 25.784" E: this means that the state lies entirely in the tropics having average temperature of 34˚C, rainfall of 2002.41 mm and relative humidity of 53-65%. It is classified as wetland of flat terrain in whose hydrological cycle is generally affected by its location.

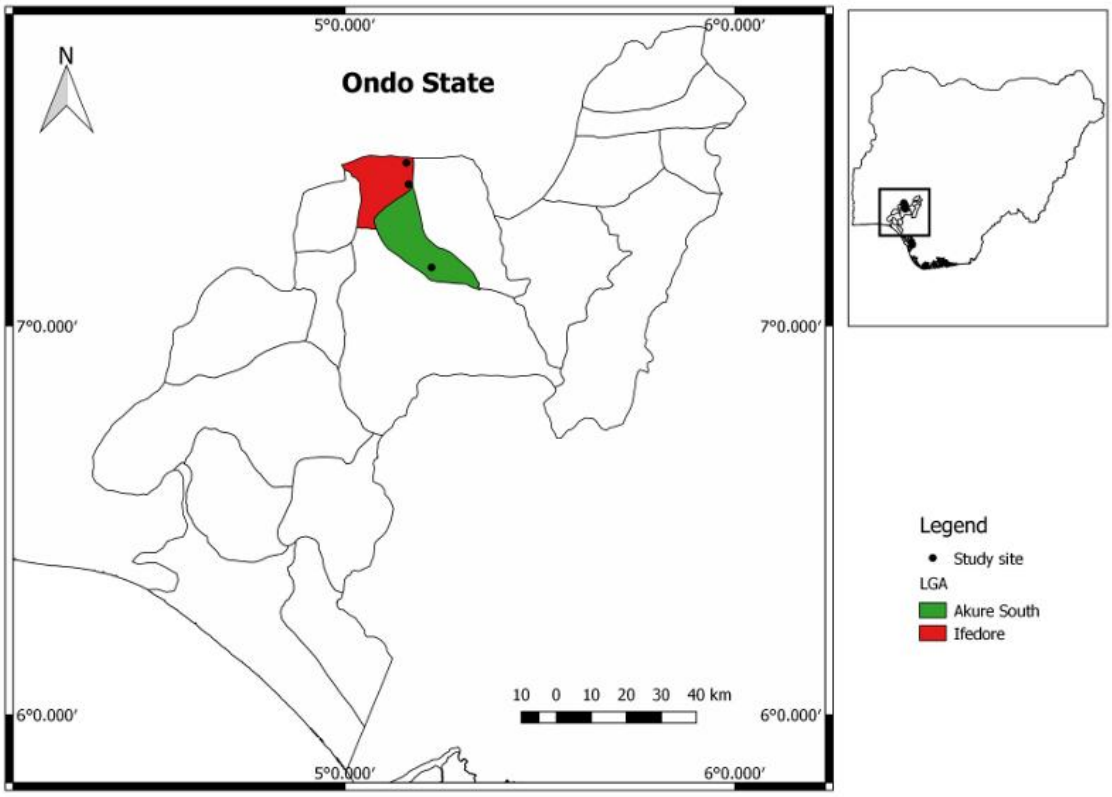


Figure 3.1: Soil sample location.

3.2 Experimental design

The study adopted complete randomized design (CRD).

3.2 Experimental plant

Tithonia diversifolia (*T. diversifolia*) and *Chromolaena odorata* (*C. odorata*) seeds obtained from natural population were broadcast in both contaminated and uncontaminated soil to test their capabilities for clean-up contaminated soil.

3.3 Experimental soil

Soil samples were collected from three selected dumpsites: Irese (ID), Onyearugbulem (OD) and New Stadium (NSD), and one uncontaminated site, Ijare (IJ). Irese and Ijare are in Ifedore Local Government Area (LGA) while OD and NSD are from Akure-South LGA.

3.4 Soil treatments

Soil samples obtained were all taken into the ScreenHouse. The soil samples obtained were air-dried for 48 hours and sieved with 2mm wire mesh. Soil samples of 6.5 kg were fell into the experimental pots, that is, six soil samples each from ID (3 each for *T. diversifolia* and *C. odorata*); NSD (3 each for *T. diversifolia* and *C. odorata*); six soil samples from OD (3 each for *T. diversifolia* and *C. odorata*) totaling 18].From the uncontaminated soil samples (USS), obtained from Ijare, 42 USS were collected, in which 10 g of $CdCl_2$ (Cd), $ZnCl_2$ (Zn), $FeCl_2$ (Fe), $CuCl_2$ (Cu) and Pb $(NO_3)_2$ (Pb) each were introduced to 6.5 kg of (3 for *T. diversifolia* and 3 for *C. odorata*) totaling 30 polluted soil samples. Another six USS (3 each for *T. diversifolia* and *C. odorata*) were contaminated with composite, that is collection of 2 g each from all the heavy metals (Cd+Zn+Fe+Cu+Pb). Further six USS (3 each for *T. diversifolia* and *C. odorata*) were used without self-contamination (no contaminant). The simulated heavy metals for Cd was calculated using: [Mass of heavy metal (Cd)*atomic number of the heavy metal]/[atomic number of compound ($CdCl_2$) * mass of soil] in mg/kg. This was repeated for other heavy metals.

3.5 Seeding

Fifteen seeds of *Tithonia diversifolia* and *Chromolaena odorata* each were broadcast on the experimental bags, which were later thinned to one after 2 weeks of planting. The plants were watered daily.

3.6 Estimation of emergence indices

After emergence, emergent counts were taken 7, 11 and 14 Days After Planting (DAP) and values obtained were used to estimate germination rate indices [Percentage Emergence (%E), Emergence Index (EI), and Emergence Rate Index (ERI)] according to Fakorede and Ayoola (1980) and Fakorede and Ojo (1981)

The plants E% was done at 14^{th} DAP when all seeds ought to have germinated. That is, %E = 100 (No. of seedlings emerged 14 DAP)/Total No. of seeds planted.

Emergent Index is the speed of emergence, that is, EI = $\sum$(Nx)(DAP)/seedlings emerged 14 DAP where Nx is the number seedlings emerged on a day X (7, 11 and 14).

Emergent Rate Index is the rate at which the total plant emerges at 14 DAP, that is, ERI = EI/%E. The seedlings of these two plant species were thinned down to one at 12 DAP which were used to determine the relative growth rate and allowed to grow under monitoring for 97days. The plants were harvested and analysed for heavy metals accumulation.

3.6 Measurement of growth parameters

The growth parameter measured for the two plant species were: Relative Growth Rate, which was calculated after thinning down *T. diversifolia* and *C. odorata* from fifteen to one at different days (7 days interval) The seedlings of this two plant species were thinned down to one at 14 DAP, which were subjected to relative growth rate using this formular :$(\ln w_2 - \ln w_1)/(T_2 - T_1)$ where W_1= first weeding weight, W_2 = second weeding weight, T_1= first weeding day, and T_2= second weeding day.

Plant biomass was taken, that is, fresh and dry weight of roots and shoots at maturation were measured using digital weighing balance.

Plant height, shoot length, and root length were measured using metre rule and vernier caliper for stem diameter.

3.7 Determination of chlorophyll contents

The extraction procedure followed modification in the trend of Booker and Fiscus (2005). Leaf samples of 0.2 g were soaked inside test-tube containing 30 ml of absolute ethanol at 5˚C in the laboratory until the leaf turned blanch. The leaf extract was used to measure absorbance value at wavelength of 649 and 665 nm. The concentration values were used in calculating chlorophyll content; Chlorophyll content = C (mg/L)*total content of extract solution (ml)* dilution factor/ fresh weight of leaf (g) (Oyerinde *et al.* 2009).

$$\text{Chlorophyll a(Chla)} = 13.36*A_{649} - 5.19*A_{665}$$

$$\text{Chlorophyll b (Chlb)} = 27.43*A_{649} - 8.12*A_{665}$$

$$\text{Total chlorophyll (Chlt)} = \text{Chla} + \text{Chlb}$$

3.8 Pre-soil analysis

3.81 Determination of pH

A pH electrode was washed with distilled water and it was placed in sample. It was allowed to stay few minute to stabilize for reading to take place. This procedure was replicated in triplicate.

3.82 Determination of Nitrogen

The crude protein content was determined using micro Kjeldahl method as described in AOAC (1996). Soil sample weighed 0.2077 g was put inside a long-necked Kjeldahl flask. 1 tablet of Kjeldahl catalyst was added to the sample in the flask with 25 cm^3 of conc. H_2SO_4. The flask was swirled, gently clamped in an inclined position and heated electricity in a fume cupboard. The heating continue until a clear solution was obtained. The clear solution was cooled, poured into a 100 cm^3 volumetric flask and made up to mark with distilled water 10 ml of the resulting mixture was measured into the distillation set through the funnel. 5 cm^3 of boric acid

was pipetted into a 100 cm^3 conical flask and placed at the receiving end of the distillatory. The conical flask was placed such that the delivery tube dipped completely into the boric acid inside the flask. 40% NaOH was used to liberate ammonia out of the digest under alkaline condition during the distillation. 2 drops of methyl orange were always added to the round bottom flask containing the digested sample before 40% NaOH was added.

As soon as the contents became alkaline, the red colour changed to yellow showing NaOH to be in excess. Steam was then generated into the distillation set using a steam chest. The liberated ammonia was trapped in the boric acid solution and about 50 cm^3 of the solution collected into a conical flask. The solution in the flask was titrated against 0.1M HC1 until the first permanent colour change was observed.

A blank sample was carried out through the sample procedure and the titre value for the blank was used to correct the titre value for samples.

$$\% N = \frac{\text{Molarity of HC1 X (Sample titre – Blank titre) X 0.014 X 6.25 X 100}}{\text{Weight of sample used.}}$$

% N was converted to the percentage crude protein by multiplying by 6.25.

3.83 Determination of phosphate

Pipette of 50 ml solution of digestate was put into a 100 ml graduated flask and make volume up to 60 ml with distilled water. Then added 25 ml Ammonium molybdate vanadate reagent and shake and dilute to 100ml with distilled water. The colour was measured at 470 ml in a 1 cm cell until a colour changed from red to blue is obtained at least 15 minutes after adding reagent. A blank was prepared using 25 ml reagent plus 75 ml of distilled water.

3.9 Soil and plant analyses for heavy metals after harvest

The contaminated and uncontaminated soil samples were analysed before simulation and planting and after harvest. At maturity, the treatments were cropped. The plant bodies were thoroughly bathed with clean solution and thereafter used distilled water to put off dusts and later divided into shoot and root. The treatments

segments were later subjected to drying using oven at 85°C for 48 hours and subjected to digestion using the method of Awofolu (2005).

3.91 Digestion of plant samples

The plant samples (1g) were air dried to a constant weight after which they were ground into powder. Plant samples were first pre-digested in concentrated HNO_3 followed by digestion in a 3:2 diacid mixture of HNO_3 and $HClO_4$. Deionized water was added followed by filtration with Whatmann No 1 filter paper. The digestate was then diluted appropriately and analyzed for mineral uptake using AAS Buck scientific VGP 210 model (Deo *et al.,* 2011).

3.92 Digestion of soil

The soil samples were first dried in the drying oven at the temperature of 50ºC for three days (or air drying to constant weight) and ground to pass through a 2mm soil sieve to get a homogenized particle size. The soil samples were thereafter weighed (1g of soil) and placed in the 250 mL glass beaker. 24mL of aqua regia is added to the soil followed by mixing. The mixture of soil and aqua regia is placed over a hot plate and digested at 110^0C for 3 hrs. After evapouration to near dryness, the mixture is diluted with 20 ml of 2% (v/v) nitric acid and then filtered through Whatman no. 42 filter paper into a 100-mL volumetric flask. The mixture in the 100mL volumetric flask is further diluted with deionized water to the 100 mL mark (Rahman and Zaim, 2015).

3.10 Calculation of bioconcentration factor (BF) and translocation factor (TF)

Bioconcentration Factor (BF) was described by Liu *et al*. (2009) and Tanhan *et al*. (2007). This was modified as; the ratio of heavy metal accumulation in root to heavy metal retaining in soil after harvest. Also, heavy metal accumulation in shoot to that of soil after harvest, that is,

BF = Heavy metal accumulation in the root plant/ Heavy metal in the soil after harvest……1

BF^1 = Heavy metal accumulation in the shoot/ Heavy metal in the soil after harvest ……..2

Translocation factor (TF) describes ability of plants to transport heavy metals from root to shoot (Mattina *et al*. 2003). Translocation factor was calculated according to Liu *et al*. (2009):

TF = accumulation of heavy metal in shoots/ accumulation of heavy metal in roots, that is, TF = (BF^1/BF).

3.11 Statistical analysis

Values generated were analysed descriptively, with the statistical software, SPSS (version 16.0). A separation of means was done using Duncan Multiple Range Test at $\alpha_{0.5}$ and correlation coefficient using MATLAB.

CHAPTER FOUR

RESULTS

4.1. Concentration of heavy metals in soil samples from dumpsites and after simulation with heavy metal

The heavy metals were in small quantities except for Fe (Fig. 4.1). The heavy metals from dumpsites were higher than the uncontaminated site (Table 4.1). After introducing more heavy metals into the soil from the uncontaminated site, the heavy metals became registered largely compared to soil obtained from uncontaminated site (IJ) and dumpsites (ID, NSD and OD) (Table 4.1) .

The uncontaminated soil had increased amount of Iron (Fe), 353.87 mg/kg before simulation (Table 4.1). In soil samples from dumpsites (ID, NSD and OD), Fe was not heavily present, unlike the soil sample from USS. After polluting the USS, the heavy metal concentrations (mg/kg) present in polluted soil samples (PSS) were in the order; Cd, 942.88; Zn, 630.40; Fe, 919.45; Cu, 612.60 and Pb, 962.33, unlike initial concentration in USS (Table 4.1).

Table 4.1: Soil properties of soil samples before planting

Parameters	IJ	ID	NSD	OD	PSS
pH	6.779	6.227	5.668	5.190	-
% O.C	0.046	5.209	0.020	0.017	-
% T.N	0.148	6.935	0.079	0.062	-
Gkg^{-1} P	0.031	0.052	0.0140	0.011	-
% Sand	63.738	82.391	93.212	95.813	-
% Silt	14.083	1.676	2.691	1.892	-
% Clay	21.982	14.971	4.041	2.033	-
$CmolKg^{-1}$ Ca	2.092	0.011	0.008	0.006	-
$CmolKg^{-1}$ Mg	1.000	1.110	0.004	0.002	-
$CmolKg^{-1}$ K	0.381	0.251	0.001	0.001	-
$CmolKg^{-1}$ Na	0.210	0.560	0.006	0.004	-
MG/KG Cd	0.011	0.011	0.072	0.081	942.88
MG/KG Zn	0.541	0.093	0.483	1.924	630.40
MG/KG Fe	353.865	0.999	10.057	8.437	919.45
MG/KG Cu	0.100	1.110	2.421	3.044	612.60
MG/KG Pb	0.441	0.204	0.401	1.401	962.33

IJ= Ijare, ID= Irese Dumpsite, NSD= New Stadium Dumpsite, OD= Onyearugbulem Dumpsite and PSS= Polluted Soil Sample

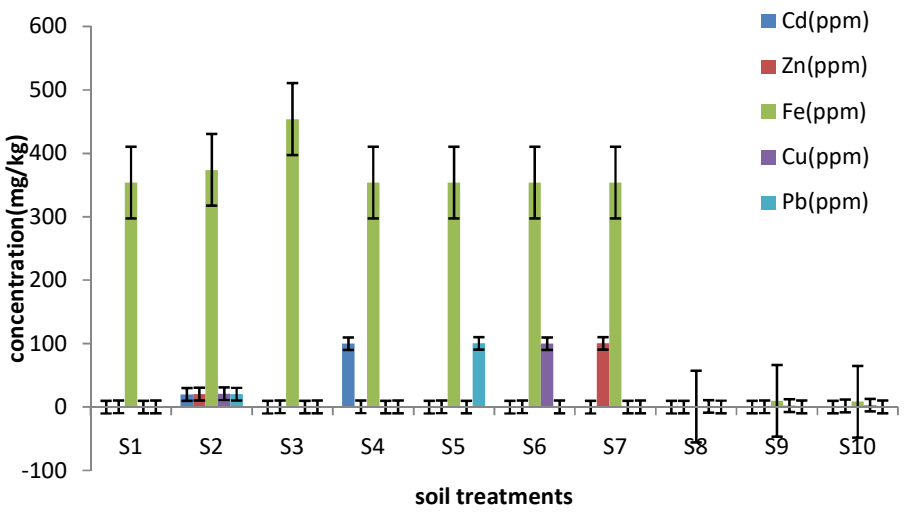


Figure 4.1: Soil properties before treatment.

S_1 (Control: no pollution), S_2 (soil polluted with 2 g each of all heavy metal), S_3 (soil polluted with Iron-Fe), S_4 (soil polluted with Cadmium-Cd), S_5 (soil polluted with Lead-Pb), S_6 (soil polluted with Copper-Cu), S_7 (soil polluted with Zinc-Zn),and dumpsite soil; S_8 (soil collected from Irese dumpsite-ID), S_9 (soil collected from New stadium dumpsite-NSD), S_{10} (soil collected from Onyearubulem dumpsite-OD)

Note: Bars means standard error of the mean

4.2 Emergence indices and relative growth rate of *Tithonia diversifolia* and *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil.

Monitoring of growing of *T. diversifolia* and *C. odorata* started the 6th day after planting in the ScreenHouse experiments. The emergent indices were taken into consideration. Emergent Index (EI), known as the speed of emergence per day, had the higher emergence for *T. diversifolia* grown on USS (29.99) and Fe-polluted (29.85) soil without significant difference (Table 4.2), but showed significant differences when compared with other polluted and dumpsite soil samples at p= 0.05. In *C. odorata*, highest EI was recorded in *C. odorata* grown on Cd-polluted (28.51) and Pb-polluted (27.09) soil when compared with polluted and dumpsite soil samples with significant differences at p= 0.05 (Table 4.3). Emergence Rate Index (ERI) is the rate at which the total plant emerges which was 14 DAP.

Tithonia diversifolia completed its emergent 11 DAP in T1,=T2,=T3,= =T4,= T5,= T6,= T7 and T9 growing soil. *Chromolaena odorata* completed its emergent at 14 DAP. The rate at which they emerged differs according to heavy metal concentration. The two plant species grown on USS, PSS and dumpsites soil samples exhibited significant differences in ERI of *T. diversifolia* (Table 4.2) and *C. odorata* (Table 4.3)

Percentage emergent (%E) is the total number of plants that emerged 14 DAP. *Tithonia diversifolia* had different percentage emergent in the sequence T1>T3>T8>T5>T2andT9>T6>T7>T4. *Tithonia diversifolia* grown on USS soil (T1) had the highest emergent percentage (92.93%) while the least was recorded for *T. diversifolia* grown on Cd-polluted soil with 12.83%. Soil samples obtained from Onyearubulem dumpsite (T10) had no emergence for *T. diversifolia* (Table 4.2).

In *C. odorata*, E% were in the sequence C1>C6>C8>C3>C9>C2>C5>C7>C4 at 14 DAP. Chromolaena odorata had highest E% on USS (C1) with 86.77% while the least was recorded for *C. odorata* grown on Cd-polluted soil with 13.28%. Soil samples collected from Onyearubulem dumpsite (C10) had no emergence for *C. odorata* (Table 4.2).

For mean relative growth rate, *T. diversifolia* had the highest on USS (T1) with 0.16 followed by *T. diversifolia* grown on NSD (T9) with 0.14 while *T. diversifolia* grown on Cd-polluted soil (T4) had the least mean relative growth rate with 0.06 (Table 4.2).

Chromolaena odorata likewise had highest mean relative growth rate grown on USS (C1) with 0.09 followed by *C. odorata* grown on soil sample polluted with all heavy metal composite (C2) with 0.08 while *C. odorata* grown on Cd-polluted soil (T4) had the least mean relative growth rate with 0.01 (Table 4.3). Generally, *T. diversifolia* had higher growth rate than *C. odorata* (Fig 4.2)

Table 4.2: Emergence Indices of *T. diversifolia* from soil polluted with different heavy metals

TREATMENTS	Emergent Index (EI)	Emergent percentage (E%)	Emergent Rate Index (ERI)	Mean Relative Growth Rate (mRGR)
T1	29.989^{cd}	92.933^{f}	0.317^{a}	0.163^{g}
T2	27.842^{bc}	67.012^{bcd}	0.415^{c}	0.120^{c}
T3	29.852^{d}	87.329^{ef}	0.337^{ab}	0.100^{b}
T4	28.501^{bcd}	12.833^{a}	2.138^{f}	0.063^{a}
T5	25.554^{a}	72.733^{cde}	0.345^{b}	0.130^{de}
T6	28.592^{bcd}	60.002^{bc}	0.479^{d}	0.125^{d}
T7	28.592^{bcd}	52.933^{b}	0.538^{e}	0.110^{bc}
T8	27.202^{ab}	79.986^{def}	0.337^{ab}	0.133^{e}
T9	27.101^{ab}	67.027^{bcd}	0.415^{c}	0.143^{f}
T10	-	-	-	

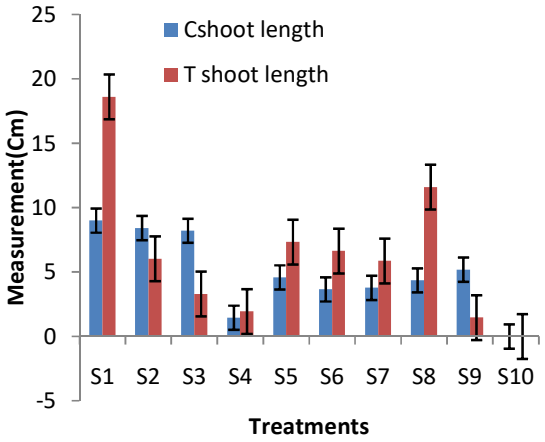
Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyearubulem dumpsite-OD)

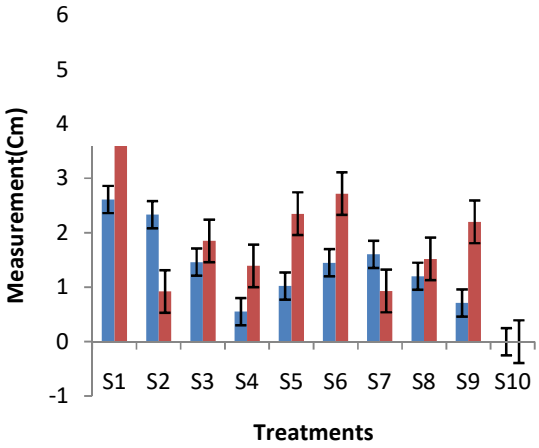
Means with same letters are not significantly different along column at P=0.05

Table 4.3: Emergence Indices for *C. odorata* from soil polluted with different heavy metals

TREATMENTS	Emergent Index (EI)	Emergent percentage (E%)	Emergent Rate Index (ERI)	Mean Relative Growth Rate (mRGR)
C1	25.708^{bcd}	86.767^{e}	0.302^{a}	0.090^{e}
C2	24.394^{abc}	60.102^{c}	0.411^{c}	$0.081d^{e}$
C3	23.112^{a}	73.293^{d}	0.311^{a}	0.064^{d}
C4	28.506^{e}	13.282^{a}	2.137^{f}	0.010^{a}
C5	27.089^{de}	60.101^{c}	0.446^{d}	0.030^{bc}
C6	26.214^{cd}	73.333^{d}	0.358^{b}	0.021^{b}
C7	23.864^{ab}	46.667^{b}	0.507^{e}	0.020^{b}
C8	26.819^{de}	73.328^{d}	0.369^{b}	0.030^{bc}
C9	23.402^{a}	66.665^{cd}	0.346^{b}	0.029^{c}
C10	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyearubulem dumpsite-OD).
Note that Means with same letters are not significantly different along column at P=0.05





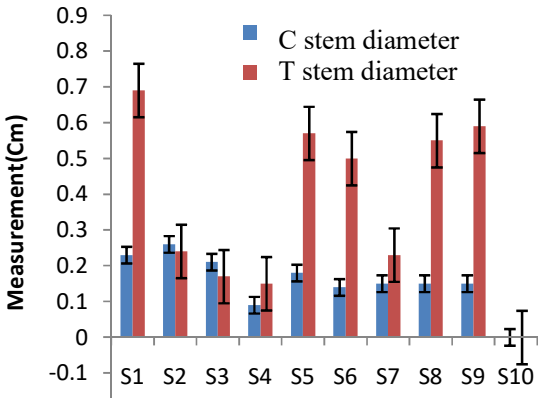


Figure 4.2: Comparison of growth parameters for *T. diversifolia and C. odorata*

Note: C shoot length=shoot length of *C. odorata*, T shoot length=shoot length of *T. diversifolia*
C root length= root length of *C. odorata*, T root length=root length of *T. diversifolia*
C stem diameter=stem diameter of *C. odorata*, T stem girth=stem diameter of *T. diversifolia,* S_1 (Control: no pollution), S_2 (soil polluted with 2g each of all heavy metal), S_3 (soil polluted with Iron-Fe), S_4 (soil polluted with Cadmium-Cd), S_5 (soil polluted with Lead-Pb), S_6 (soil polluted with Copper-Cu), S_7 (soil polluted with Zinc-Zn),and dumpsite soils; S_8 (soil collected from Irese dump site-ID), S_9 (soil collected from New stadium dump site-NSD), S_{10} (soil collected from Onyarubulem dump site-OD). Bars means standard error of the mean.

4.3 Growth parameters and biomass production of *Tithonia diversifolia* and *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil.

Analysis in growth gives information about the activities of plant depending the soil where the plant germinates from. Regarding growth, *T. diversifolia* and *C. odorata*, the *T. diversifolia* grown on USS (T1), had the highest shoot length (185.50 cm), follwed by *T. diversifolia* grown on NSD (T9), 147.00 cm. The soil treated with cadmium (T4) had the least shoot length, 20.00 cm. *Tithonia diversifolia* grown on USS was significantly higher than *T. diversifolia* grown on other soil samples (Table 4.4). *C. odorata* grown on USS (C1) had the highest shoot length with 90.00 cm followed by *C. odorata* grown on soil treated with composite of all heavy metals (C2) with 84.20 cm. The *C. odorata* grown on Cd-polluted (C4) had the least shoot length with 14.00 cm. *Chromolaena odorata* grown on USS was significantly higher than *T. diversifolia* grown on other soil samples (Table 4.5).

The root length of *T. diversifolia* grown on USS (T1) had the highest mean with 45.00 cm followed by *T. diversifolia* grown on Cu-polluted soil (T6) with 27.20 cm. *T. diversifolia* grown on soil sample treated with composite of all heavy metals had the least root length with 9.20 cm. The root length of *T. diversifolia* grown on USS was significantly higher than *T. diversifolia* grown on other soil samples (Table 4.4). *Chromolaena odorata* grown on USS (C1) had the highest root length with 26.10 cm followed by *C. odorata* grown on soil treated with heavy metals composite (C2) with 23.30 cm. *Chromolaena odorata* grown on Cd-polluted soil (C4) had the least root length with 5.50 cm. *Chromolaena odorata* grown on USS was significantly higher than *T. diversifolia* grown on other soil samples (Table 4.5).

Considering the stem diameter, *T. diversifolia* had the highest in the USS (T1) with 6.90 cm followed by *T. diversifolia* grown on NSD (T9) with 5.90 cm but they did not show much significant difference (Table 4.4). *Tithonia diversifolia* grown on Cd-polluted soil (T4) had the least stem diameter with 1.50 cm which showed significant difference when compared *T. diversifolia* grown on USS (Table 4.4).

Chromolaena odorata grown on soil treated with heavy metal composite (C2) had the highest stem diameter with 2.60 cm followed by *C. odorata* grown on USS (C1) with 2.30 cm with no significant difference. The least stem diameter was found *C. odorata* grown on Cd-polluted soil (C4) with 0.9 cm with significant differences when compared to *C. odorata* grown on other soil samples (Table 4.5). The mass (mg) of shoots and roots of *T. diversifoilia* and *C. odorata* were in trend of shoots and roots length. The *T. diversifolia* grown on USS had the highest shoot and root weight (110.58 g and 48.41 g respectively), followed by *T. diversifolia* grown on NSD with 52.29 g and 20.64 g, respectively. The least was recorded *T. diversifolia* grown on Cd-polluted soil with 5.58 g and 1.02 g, respectively. In *C. odorata*, the above biomass and below biomass weight had the highest USS with 52.9 g and 7.18 g followed by *C. odorata* grown on heavy metal composite soil with 45.63 g and 5.94 g. The least was recorded in Cd-polluted soil with 2.41 g and 0.39 g, respectively (Table 4.5). The metal extraction rate (MER) was in the sequence Zn > Cu > Fe > Cd > Pb in *T. diversifolia* and Zn ≥ Fe > Cu > Pb > Cd in *C. odorata* (Figures 4.3 and 4.4).

Table 4.4: Growth and amount of living matter in *T. diversifolia* grown in dumpsites and heavy metal polluted soil.

TREATMENT	SHOOT LENGTH (cm)	ROOT LENGTH (cm)	SHOOT DIAMETER (cm)	SHOOT WEIGHT (gFW)	ROOT WEIGHT (gFW)	SHOOT WEIGHT (gDW)	ROOT WEIGHT (gDW)
T1	185.500^{g}	45.400^{f}	6.900^{c}	110.611^{g}	47.981^{g}	20.661^{g}	16.576^{e}
T2	60.310^{c}	9.200^{a}	2.400^{a}	19.095^{c}	6.793^{c}	3.795^{c}	1.213^{bc}
T3	33.000^{b}	18.500^{c}	1.700^{a}	8.817^{ab}	3.059^{b}	1.318^{b}	0.589^{b}
T4	19.420^{a}	13.900^{b}	1.500^{a}	5.602^{a}	1.017^{a}	0.892^{a}	0.217^{a}
T5	73.300^{d}	23.500^{d}	5.700^{b}	35.967^{d}	9.118^{de}	8.697^{e}	1.613^{c}
T6	66.310^{cd}	27.200^{e}	5.000^{b}	29.210^{cd}	6.957^{c}	5.198^{d}	1.502^{c}
T7	58.600^{c}	9.300^{a}	2.300^{a}	19.110^{c}	7.189^{d}	3.611^{c}	0.701^{b}
T8	116.000^{e}	15.200^{bc}	5.500^{b}	45.793^{e}	12.989^{e}	10.078^{ef}	3.038^{d}
T9	147.000^{f}	22.000^{d}	5.900^{b}	52.309^{f}	20.586^{f}	11.511^{f}	3.248^{d}
T10	-	-	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD).

FW=Fresh weight; DW= Dry weight
Means with same letters are not significantly different along column at P=0.05

Table 4.5: Growth and amount of living matter in *C. odorata* grown in dumpsites and heavy metal polluted soil.

TREATMENT	SHOOT LENGTH (cm)	ROOT LENGTH (cm)	SHOOT DIAMETER (cm)	SHOOT WEIGHT (gFW)	ROOT WEIGHT (gFW)	SHOOT WEIGHT (gDW)	ROOT WEIGHT (gDW)
C1	90.000^{g}	26.110^{e}	2.310^{de}	53.112^{f}	7.178^{e}	4.568	0.356^{e}
C2	84.200f	23.300^{d}	2.610^{e}	45.5863^{e}	5.978^{d}	0.927^{c}	0.278^{d}
C3	82.100^{f}	14.600^{c}	2.100^{cd}	43.209 ^{e}	3.521^{c}	0.865 ^{c}	0.256 ^{d}
C4	14.600^{a}	5.500^{a}	0.900^{a}	2.405^{a}	0.389^{a}	0.100^{a}	0.045^{a}
C5	45.900^{d}	10.200^{b}	1.800^{bc}	20.428^{cd}	2.509^{b}	0.589^{bc}	0.220^{c}
C6	36.600^{b}	14.500^{c}	1.400^{b}	9.356^{b}	2.627^{bc}	0.167^{a}	0.079^{ab}
C7	37.800^{b}	16.000^{c}	1.500^{b}	11.300^{b}	2.401^{b}	0.378^{b}	0.090 ^{ab}
C8	43.600^{c}	12.000^{b}	1.500^{b}	18.100^{c}	2.610^{b}	0.510 ^{b}	0.121^{b}
C9	51.900cf^{e}	7.100^{a}	1.050^{b}	25.632^{d}	3.938^{c}	0.619^{bc}	0.220^{c}
C10	-	-	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyearubulem dumpsite-OD)
FW= Fresh weight; DW= Dry weight
Means of the same letter means no significant difference at P=0.05

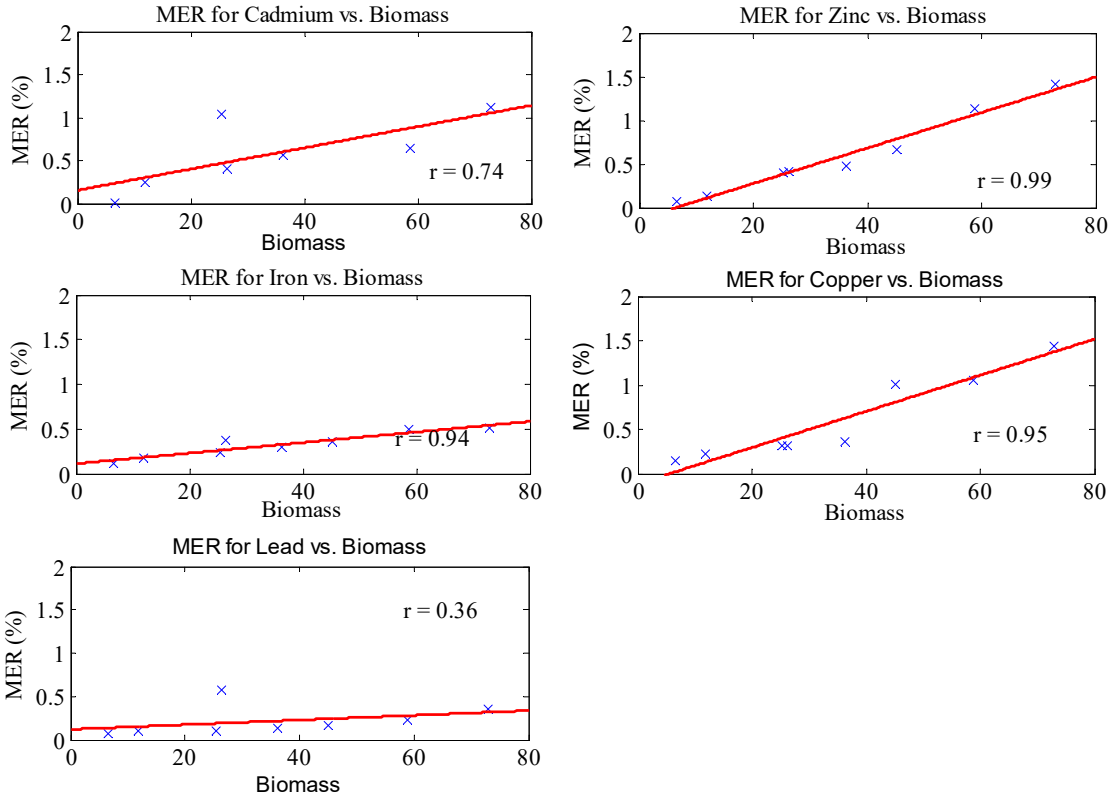


Figure 4.3: The correlation between the Metal Extraction Rate (MER) and Plant Biomass for *Tithonia diversifolia*

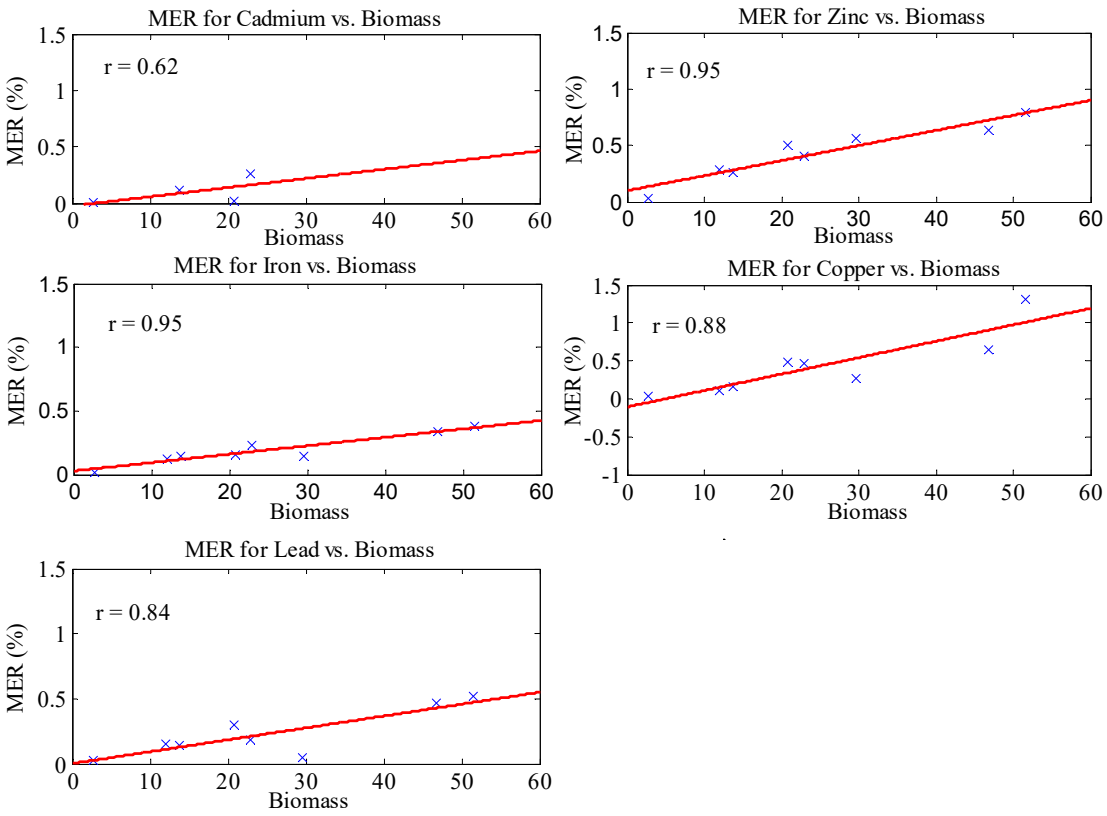


Figure 4.4: The correlation between the Metal Extraction Rate ((MER) and Plant Biomass for *Chromolaena odorata*

4.4: Chlorophyll contents in the plants grown on dumpsite and heavy metal polluted soil.

Leaf chlorophyll content expressed significant differences among treatments in *T. diversifolia* and *C. odorata* (Table 4.6). For *T. diversifolia*, the highest Total Chlorophyll Content (TCC) was recorded in *T. diversifolia* grown on USS with 29.59 mg/L followed by *T. diversifolia* grown on Fe-polluted soil with 17.58 mg/L with significant difference at p=0.05. The least TCC was recorded for *T. diversifolia* grown on NSD soil with 3. 20 mg/L. There were significant differences among treatments in the TCC in *T. diversifolia* (Table 4.6).

For *C. odorata*, the highest Total Chlorophyll Content (TCC) was recorded in *C. odorata* grown on USS with 17.39 mg/L followed by *C. odorata* grown on Fe-polluted soil with 13.57 mg/L with significant difference at p=0.05. The least TCC was recorded for *C. odorata* grown on Zn-polluted soil with 0.92 mg/L. There were significant differences among treatments in the TCC in *C. odorata* (Table 4.6).
Tithonia diversifolia was higher in TCC value when compared to *C. Odorata* (Fig. 4.5)

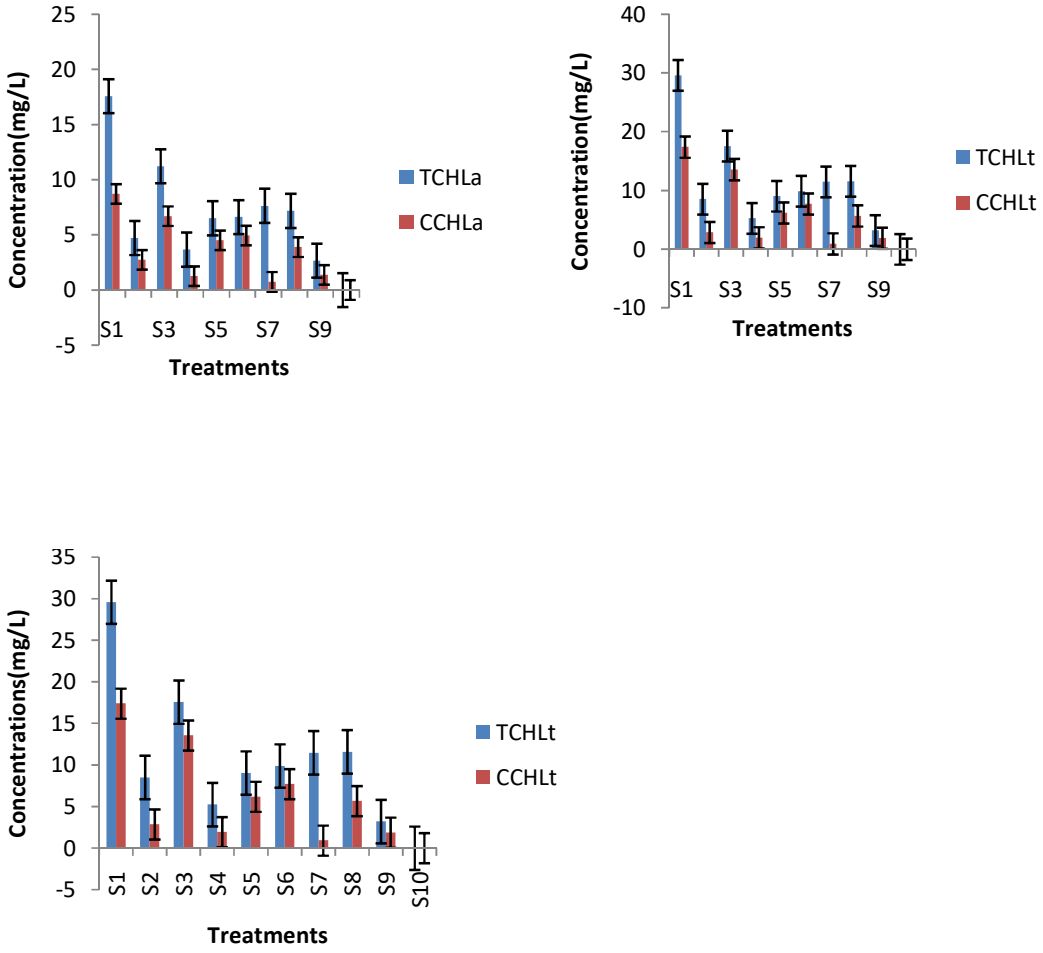


Figure 4.5: Chlorophyll Content in *T. diversifolia* and *C. odorata* grown in dumpsite and heavy metal polluted soil.

Note: TCHLa= *T. diversifolia* chlorophyll*a*, TCHL*b*=*T. diversifolia* chlorophyll*b*, TCHL*t*= *T. diversifolia* chlorophyll total, CCHL*a*=*C. odorata* chlorophyll*a*, CCHL*b*=*C. odorata* chlorophyll*b*, CCHL*t*= *C. odorata* chlorophyll total

S_1 (Control: no pollution), S_2 (soil polluted with 2g each of all heavy metal), S_3 (soil polluted with Iron-Fe), S_4 (soil polluted with Cadmium-Cd), S_5 (soil polluted with Lead-Pb), S_6 (soil polluted with Copper-Cu), S_7 (soil polluted with Zinc-Zn),and dumpsite soil; S_8 (soil collected from Irese dumpsite-ID), S_9 (soil collected from New stadium dumpsite-NSD), S_{10} (soil collected from Onyearubulem dumpsite-OD).

Bars means standard error of the mean

.**Table 4.6: Chlorophyll contents (Mg/L) in leaves of *T. diversifolia* and *C. odorata* grown in dumpsite and heavy metal polluted soil.**

TREATMENTS	T. CHLa	C. CHLa	T. CHLb (Mg/L)	C. CHLb	T. CHLt	C. CHLt
1	17.58^{g}	8.72^{g}	8.72^{g}	4.43^{g}	29.59^{g}	17.39^{f}
2	4.72^{c}	2.75^{c}	2.43^{b}	1.13^{c}	8.52^{c}	2.86^{b}
3	11.23^{f}	6.70^{f}	5.59^{f}	3.63^{f}	17.58^{f}	13.57^{e}
4	3.67^{b}	1.26^{b}	1.35^{a}	0.79^{bc}	5.25^{b}	1.95^{ab}
5	6.51^{d}	4.51^{e}	2.53^{b}	2.08^{d}	9.04^{cd}	6.19^{c}
6	6.61^{d}	4.95^{e}	3.12^{c}	2.78^{e}	9.89^{d}	7.70^{d}
7	7.64^{e}	0.75^{a}	3.66^{d}	0.22^{a}	11.48^{e}	0.92^{a}
8	7.18^{de}	3.89^{d}	4.37^{e}	2.29^{de}	11.59^{e}	5.68^{c}
9	2.67^{a}	1.37^{b}	1.33^{a}	0.49^{ab}	3.20^{a}	1.88^{ab}
10	-	-	-	-	-	-

Note: TCHLa= *T. diversifolia* chlorophyll*a*, TCHL*b*=*T. diversifolia* chlorophyll*b*, TCHL*t*= *T. diversifolia* chlorophyll total, CCHL*a*=*C. odorata* chlorophyll*a*, CCHL*b*=*C. odorata* chlorophyll*b*, CCHL*t*= *C. odorata* chlorophyll total.
S_1 (Control: no pollution), S_2 (soil polluted with 2g each of all heavy metal), S_3 (soil polluted with Iron-Fe), S_4 (soil polluted with Cadmium-Cd), S_5 (soil polluted with Lead-Pb), S_6 (soil polluted with Copper-Cu), S_7 (soil polluted with Zinc-Zn),and dumpsite soil; S_8 (soil collected from Irese dumpsite-ID), S_9 (soil collected from New stadium dumpsite-NSD), S_{10} (soil collected from Onyarubulem dumpsite-OD)
Means with same letter are not significantly different along the column at P=0.05

4.5 Pollution load in shoot of *T. diversifolia* after harvest from dumpsite and heavy metal polluted soil.

The presence of cadmium was not detected in the leaf of *T. diversifolia* grown on USS and PSS except in *T. diversifolia* grown on heavy metal composite with 0.02 mg/kg (Table 4.7). Likewise, copper was not detectable in PSS (Table 4.7). It was on Zn and Fe that were much detected in the leaf of *T. diversifolia* with significant differences among the treatments except soil samples from Oyearugbulem (OD) as a result of no germination (Table 4.7).

In the stem of *T. diversifolia*, Cd, Zn, Fe, Cu and Pb had highest in *T. diversifolia* grown on USS with 0.05, 0.64, 3.06, 0.51 and 0.42 mg/kg, respectively (Table 4.8). Considering above soil level biomass (shoot), *T. diversifolia*, Cd, Zn, Fe, Cu and Pb had highest in *T. diversifolia* grown on USS with 0.05, 0.84, 3.98, 0.53 and 0.46 mg/kg, respectively (Table 4.9). The least heavy metal was recorded in the sequence of Cd<Cu<Pb<Zn<Fe with ND, 0.018, 0.019, 0.047 and 0.98 mg/kg, respectively in *T. diversifolia* grown on Cd-polluted and Pb-polluted soil (Table 4.9). There were significant differences among treatments for heavy metals along column at P= 0.05 (Table 4.9). Heavy metals were accumulated in the tissue of *T. diversifolia* in the sequence of Fe > Zn > Cu > Pb > Cd (Figure 4.6).

Table 4.7: Pollution load in leaf of *Tithonia diversifolia* grown on dumpsite and heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
T1	ND	0.21 ± 0.04^{d}	0.92 ± 0.26^{e}	0.02 ± 0.038^{b}	0.04 ± 0.01^{c}
T2	0.02±0.02	ND	0.02 ± 0.23^{a}	ND	ND
T3	ND	0.03 ± 0.02^{bc}	1.02 ± 0.30^{f}	ND	0.002 ± 0.08^{a}
T4	ND	ND	ND	ND	ND
T5	ND	0.02±0.03b	0.49 ± 0.30^{d}	ND	ND
T6	ND	0.02 ± 0.04^{b}	0.21 ± 0.26^{c}	ND	0.01 ± 0.07^{b}
T7	ND	0.038 ± 0.01^{bc}	0.03 ± 0.21^{a}	ND	ND
T8	ND	0.04 ± 0.04^{f}	0.10 ± 0.30^{b}	0.05 ± 0.034^{a}	ND
T9	ND	0.01 ± 0.02^{a}	ND	0.02 ± 0.038^{b}	ND
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD). ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

Table 4.8: Pollution load in stem of *Tithonia diversifolia* grown on dumpsite and heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
T1	0.047±0.002^{b}	0.636±0.020^{g}	3.06±0.010^{g}	0.51±0.013^{f}	0.42±0.122^{c}
T2	0.064±0.003^{a}	0.305±0.019^{d}	1.42±0.061^{c}	0.107±0.013^{c}	0.048±0.119^{ab}
T3	0.001±0.001^{a}	0.105±0.024^{b}	2.40±0.100^{f}	0.176±0.013^{d}	0.084±0.119^{b}
T4	ND	0.047±0.021^{a}	0.978±0.062^{a}	0.018±0.013^{a}	0.076±0.119^{ab}
T5	ND	0.125±0.017^{b}	1.00±0.059^{c}	0.065±0.013^{b}	0.019±0.117^{a}
T6	0.009±0.003^{a}	0.200±0.02^{c}	1.588±0.060^{d}	0.058±0.013^{b}	0.019±0.121^{a}
T7	ND	0.213±0.02^{c}	1.22±0.057^{b}	0.058±0.013^{b}	0.316±0.123^{bc}
T8	0.009±0.003^{a}	0.54±0.012^{f}	2.09±0.120^{e}	0.31±0.013^{e}	0.096±0.115^{ab}
T9	ND	0.380±0.019^{e}	1.009±0.070^{a}	0.14±0.013^{d}	0.027±0.120^{ab}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

Table 4.9: Pollution load in shoot of *Tithonia diversifolia* grown on dumpsite and simulated heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
T1	0.047±0.003^{b}	0.836±0.019^{g}	3.976±0.062^{g}	0.527±0.013^{f}	0.459±0.119^{c}
T2	0.066±0.003^{a}	0.305±0.019^{d}	1.436±0.062^{c}	0.107±0.013^{c}	0.048±0.119^{ab}
T3	0.009±0.003^{a}	0.108±0.019^{b}	3.419±0.062^{f}	0.176±0.013^{d}	0.084±0.119^{b}
T4	ND	0.047±0.019^{a}	0.978±0.062^{a}	0.018±0.013^{a}	0.076±0.119^{ab}
T5	ND	0.135±0.019^{b}	1.486±0.062^{c}	0.065±0.013^{b}	0.019±0.119^{a}
T6	0.009±0.003^{a}	0.238±0.019^{c}	1.247±0.062^{b}	0.058±0.013^{b}	0.019±0.119^{a}
T7	ND	0.223±0.019^{c}	1.768±0.062^{d}	0.058±0.013^{b}	0.316±0.119^{bc}
T8	0.009±0.003^{a}	0.578±0.019^{f}	2.186±0.062^{e}	0.316±0.013^{e}	0.096±0.119^{ab}
T9	ND	0.390±0.019^{e}	1.009±0.062^{a}	0.157±0.013^{d}	0.027±0.119^{ab}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

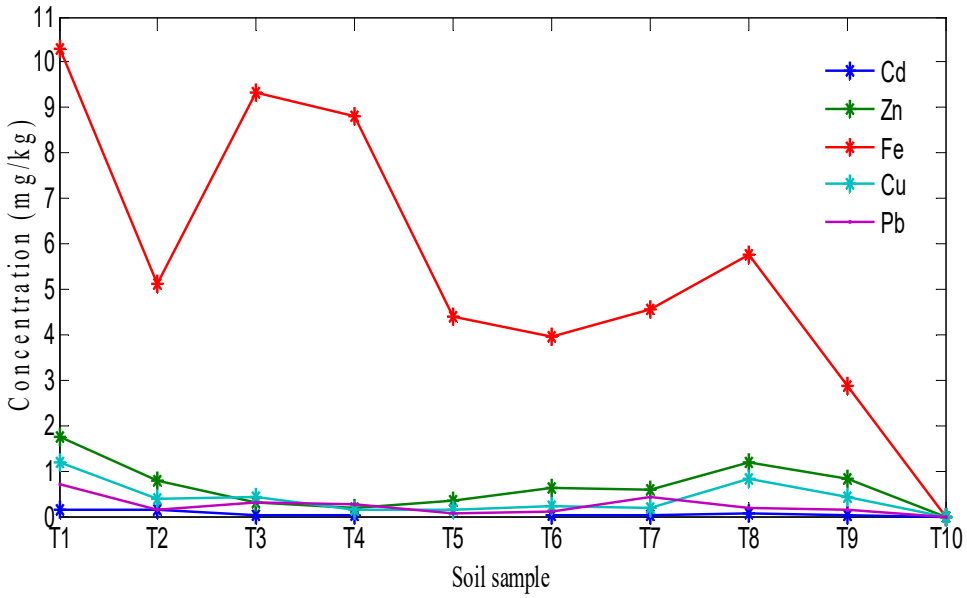


Figure 4.6: Heavy metals accumulation in the tissue of *Tithonia diversifolia*

4.6 Pollution load in shoot of *C. odorata* after harvest from dumpsite and heavy metal polluted soil.

In the leaf of *C. odorata*, Cd was not detected in all the treatment (Table 4.10). Zinc was not detected only in the leaf of *C. odorata* grown on heavy metal composite (C2) and NSD soil. Copper was not detected in *C. odorata* grown on USS (C1), heavy metal composite (C2), Fe-polluted (C3), Zn-polluted (C7) and NSD (C9) soil. Lead concentration not detected among treatments except Pb-polluted (C5), Cu-polluted (C6) and ID (C8) soil (Table 4.10).

Cadmium concentration not detected in the stem of *C. odorata* except for *C. odorata* grown on Pb-polluted soil while there was presence of heavy metal concentration in stem of Zn, Fe, Cu and Pb (Table 4.11).

Zinc had highest concentration in shoot for *C. odorata* grown on USS with 0.81 mg/kg and least concentration was recorded for *C. odorata* grown on heavy metal composite with 0.04 mg/kg. Iron had highest concentration in shoot for *C. odorata* grown on Pb-polluted soil with 2.13 mg/kg and least concentration was recorded for *C. odorata* grown Fe-polluted soil with 0.5 mg/kg. Copper had highest concentration in shoot for *C. odorata* grown on ID soil with 0.2 mg/kg and least concentration for *C. odorata* grown on NSD soil with 0.01 mg/kg. Lead had highest concentration in shoot for *C. odorata* grown on ID soil with 0.18 mg/kg and least concentration for *C. odorata* grown on NSD soil with 0.01 mg/kg (Table 4.12). There were significant differences among treatments along columns at P= 0.05 (Tables 4.10, 4.11 and 4.12). Heavy metals were accumulated in the tissue of *C. odorata* in the sequence of Fe > Zn > Cu > Pb > Cd (Figure 4.7).

Table 4.10: Pollution load in leaf of *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
C1	ND	0.06±0.012^{e}	0.36±0.031^{c}	ND	ND
C2	ND	ND	0.22±0.031^{c}	ND	ND
C3	ND	0.02±0.012^{bc}	0.01±0.031^{a}	ND	ND
C4	ND	0.015±0.012^{b}	0.12±0.031^{d}	0.02±0.014^{a}	ND
C5	ND	0.025±0.012^{bc}	0.13±0.031^{d}	0.02±0.014^{a}	0.03±0.01^{d}
C6	ND	0.04±0.012^{cd}	0.04±0.031^{b}	0.02±0.014^{a}	0.02±0.01^{b}
C7	ND	0.007±0.012^{a}	0.03±0.031^{b}	ND	ND
C8	ND	0.03±0.012^{c}	0.12±0.031^{d}	0.02±0.014^{a}	0.05±0.01^{e}
C9	ND	ND	0.15±0.031^{d}	ND	ND
C10	-	-	-	-	-

C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Values with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

Table 4.11: Pollution load in stem of *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
C1	ND	0.75 ± 0.012^{f}	0.63 ± 0.031^{d}	0.069 ± 0.014^{bc}	0.065 ± 0.01^{cd}
C2	ND	0.036 ± 0.012^{a}	0.69 ± 0.031^{c}	0.088 ± 0.014^{cd}	0.028 ± 0.01^{ab}
C3	ND	0.063 ± 0.012^{b}	0.51 ± 0.031^{a}	0.026 ± 0.014^{a}	0.028 ± 0.01^{ab}
C4	ND	0.055 ± 0.012^{b}	0.86 ± 0.031^{d}	0.09 ± 0.014^{d}	0.071 ± 0.01^{c}
C5	0.009	0.065 ± 0.012^{bc}	2.00 ± 0.031^{e}	0.14 ± 0.014^{e}	0.077 ± 0.01^{d}
C6	ND	0.30 ± 0.012^{e}	1.02 ± 0.031^{d}	0.14 ± 0.014^{e}	0.08 ± 0.01^{d}
C7	ND	0.103 ± 0.012^{c}	0.98 ± 0.031^{d}	0.056 ± 0.014^{b}	0.036 ± 0.01^{b}
C8	ND	0.125 ± 0.012^{d}	0.86 ± 0.031^{d}	0.18 ± 0.014^{f}	0.13 ± 0.01^{e}
C9	ND	0.076 ± 0.012^{b}	0.62 ± 0.031^{b}	0.009 ± 0.014^{a}	0.009 ± 0.01^{a}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

Table 4.12: Pollution load in shoot of *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
C1	ND	0.807±0.012^{f}	0.987±0.031^{d}	0.069±0.014^{bc}	0.065±0.01^{cd}
C2	ND	0.036±0.012^{a}	0.905±0.031^{c}	0.088±0.014^{cd}	0.028±0.01^{ab}
C3	ND	0.079±0.012^{b}	0.518±0.031^{a}	0.026±0.014^{a}	0.028±0.01^{ab}
C4	ND	0.065±0.012^{b}	0.976±0.031^{d}	0.107±0.014^{d}	0.071±0.01^{c}
C5	0.009	0.088±0.012^{bc}	2.129±0.031^{e}	0.157±0.014^{e}	0.099±0.01^{d}
C6	ND	0.335±0.012^{e}	1.046±0.031^{d}	0.157±0.014^{e}	0.099±0.01^{d}
C7	ND	0.109±0.012^{c}	1.018±0.031^{d}	0.056±0.014^{b}	0.036±0.01^{b}
C8	ND	0.147±0.012^{d}	0.976±0.031^{d}	0.198±0.014^{f}	0.176±0.01^{e}
C9	ND	0.076±0.012^{b}	0.766±0.031^{b}	0.009±0.014^{a}	0.009±0.01^{a}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

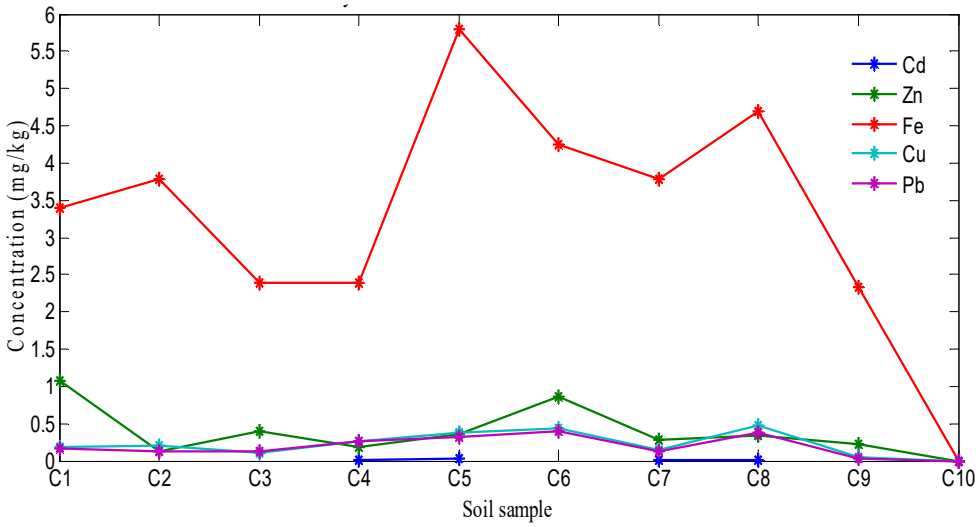


Figure 4.7: Heavy metals accumulation in the tissue of *Chromolaena odorata*

4.7 Pollution load in root of *T. diversifolia* after harvest from dumpsite and heavy metal polluted soil.

The roots of *T. diversifolia* in the USS (T1) had highest concentration of cadmium with 0.11 mg/kg, followed by *T. diversifolia* root grown on ID (T8) with 0.04 mg/kg. The least *T. diversifolia* concentration in root for cadmium was recorded on *T. diversifolia* grown on Cd-polluted soil (T4), Cu-polluted (T6), Zn-polluted (T7) and NSD (T9) with 0.01 mg/kg (Table 4.13). Cadmium was not detected in *T. diversifolia* root grown on Pb-polluted soil (Table 4.13). The highest concentration for zinc in the root of *T. diversifolia* was recorded on *T. diversifolia* grown on USS (T1) with 0.91 mg/kg, followed by *T. diversifolia* grown on ID (T8) with 0.61 mg/kg. The least concentration for zinc in roots of *T. diversifolia* was recorded for *T. diversifolia* grown on Cd-polluted soil (T4) with 0.12 mg/kg (Table 4.13).

Considering Fe, root of *T. diversifolia* grown on Cd-polluted soil (T4) had the highest concentration with 7.84 mg/kg, followed by root of *T. diversifolia* grown on USS (T1) with 6.31 mg/kg (Table 4.13). The least was recorded at *T. diversifolia* grown on NSD soil (T9) with 1.88 mg/kg (Table 4.13). Copper concentration had highest in *T. diversifolia* root grown on USS (T1) with 0.66 mg/kg followed by *T. diversifolia* root grown on ID soil (T8) with 0.49 mg/kg (Table 4.13). Soil samples treated with lead (T5) had the least concentration of Fe with 0.09 mg/kg. Likewise for Pb, the highest concentration was recorded for *T. diversifolia* root grown on USS (T1), Fe-polluted soil and Cd-polluted soil with 0.199 mg/kg, 0.199 mg/kg and 0.198 mg/kg, respectively without significant differences (Table 4.13). The least Pb concentration was recorded for *T. diversifolia* root grown on Pb-polluted soil (T5) with 0.05 mg/kg. There were significant differences among treatments along column at P= 0.05 (Table 4.13).

Table 4.13: Pollution load in roots of *Tithonia diversifolia* grown on dumpsite and heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
T1	0.106±0.005^{c}	0.905±0.018^{g}	6.307±0.053^{g}	0.657±0.042^{e}	0.199±0.011^{c}
T2	0.027±0.005^{b}	0.476±0.018^{e}	3.689±0.053^{e}	0.265±0.042^{c}	0.080±0.011^{b}
T3	0.027±0.005^{b}	0.189±0.018^{b}	5.906±0.053^{f}	0.247±0.042^{bc}	0.199±0.011^{c}
T4	0.008±0.005^{a}	0.116±0.018^{a}	7.836±0.053^{h}	0.118±0.042^{a}	0.198±0.011^{c}
T5	ND	0.218±0.018^{b}	2.919±0.053^{c}	0.089±0.042^{a}	0.048±0.011^{a}
T6	0.007±0.005^{a}	0.368±0.018^{c}	2.689±0.053^{b}	0.167±0.042^{ab}	0.087±0.011^{b}
T7	0.007±0.005^{a}	0.355±0.018^{c}	2.768±0.053^{b}	0.115±0.042^{a}	0.089±0.011^{b}
T8	0.038±0.005^{b}	0.609±0.018^{f}	3.566±0.053^{d}	0.487±0.042^{d}	0.085±0.011^{b}
T9	0.009±0.005^{a}	0.425±0.018^{d}	1.877±0.053^{a}	0.248±0.042^{bc}	0.088±0.011^{b}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.8 Pollution load in root of *C. odorata* after harvest from dumpsite and heavy metal polluted soil.

The root of *C. odorata* had the highest concentration of cadmium in *C. odorata* grown on Pb-polluted soil (C5) with 0.02 mg/kg, followed by *C. odorata* root grown on ID soil (C8), Zn-polluted soil (C7) and Cd-polluted soil (C4) 0.01 mg/kg each (Table 4.14). Cadmium was not detected in remaining soil samples. For Zn, highest concentration was found in *C. odorata* root grown on Cu-polluted soil (C6) with 0.52 mg/kg, followed by *C. odorata* root grown on Fe-polluted soil (C3) with 0.31 mg/kg. The least Zn concentration was recorded for *C. odorata* root grown on heavy metal composite soil (C2) with 0.09 mg/kg (Table 4.14).

Furthermore, the highest Fe concentration was recorded for *C. odorata* root grown on ID soil (C8) with 3.72 mg/kg, followed by *C. odorata* root grown on Pb-polluted soil (C5) with 3.66 mg/kg, which was not significantly from each other (Table 4.14). The least Fe concentration was recorded for *C. odorata* root grown on Cd-polluted (C4) with 1.42 mg/kg (Table 4.14). Copper concentration had highest in *C. odorata* root grown on Cu-polluted soil (C6) with 0.28 mg/kg followed by *C. odorata* root grown on ID soil (C8) with 0.27 mg/kg with no significant difference (Table 4.14). The least copper concentration was recorded for *C. odorata* root grown on NSD soil (C9) with 0.03 mg/kg. There were significant differences among treatments along column at p= 0.05 (Table 4.14)

Similarly, Pb concentration in the root of *C. Odorata* had the highest in *C. odorata* root grown on Cu-polluted soil (C6) with 0.30 mg/kg. The least was recorded *C. odorata* root grown on NSD soil (C9) with 0.01 mg/kg. There were significant differences among treatments along column at p= 0.05 (Table 4.14)

Table 4.14: Pollution load in roots of *Chromolaena odorata* grown on dumpsite and heavy metal polluted soil.

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
C1	ND	0.257 ± 0.033^{c}	2.376 ± 0.075^{c}	0.089 ± 0.03^{bc}	0.089 ± 0.01^{b}
C2	ND	0.089 ± 0.033^{a}	2.865 ± 0.075^{d}	0.107 ± 0.03^{bc}	0.087 ± 0.01^{b}
C3	ND	0.306 ± 0.033^{c}	1.867 ± 0.075^{b}	0.067 ± 0.03^{ab}	0.087 ± 0.01^{b}
C4	0.008 ± 0.003^{a}	0.109 ± 0.033^{ab}	1.419 ± 0.075^{a}	0.145 ± 0.03^{c}	0.189 ± 0.01^{c}
C5	0.017 ± 0.02^{b}	0.257 ± 0.033^{c}	3.656 ± 0.075^{f}	0.207 ± 0.03^{d}	0.211 ± 0.01^{c}
C6	ND	0.515 ± 0.033^{d}	3.196 ± 0.075^{e}	0.278 ± 0.03^{e}	0.298 ± 0.01^{d}
C7	0.008 ± 0.003^{a}	0.167 ± 0.033^{b}	2.767 ± 0.075^{d}	0.087 ± 0.03^{bc}	0.087 ± 0.01^{b}
C8	0.008 ± 0.003^{a}	0.178 ± 0.033^{b}	3.718 ± 0.075^{f}	0.265 ± 0.03^{e}	0.189 ± 0.01^{c}
C9	ND	0.138 ± 0.033^{ab}	1.557 ± 0.075^{a}	0.028 ± 0.03^{a}	0.009 ± 0.01^{a}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.9: Pollution load in soil after the harvest of *Tithonia diversifolia*

After the contaminants had been absorbed by harvested *T. diversifolia*, the soil samples were subjected for post-analysis to know the pollution load remaining in the soil. The highest concentration of cadmium was recorded for USS (T1) with 0.18 mg/kg. It was low in other soil treatments within the range of 0.01-0.03 mg/kg. There were little significant differences (Table 4.15). For zinc, the concentration was very high in OD soil (T10) with 1.89 mg/kg. The least was recorded in Cd-polluted soil (T4) with 0.24 mg/kg after harvest (Table 4.15).

Considering Fe, the concentration was the highest in USS (T1) with 12.05 mg/kg. The least concentration was recorded in Zn-polluted soil (T7) with 4.97 mg/kg (Table 4.15). Copper concentration had the highest in OD soil (T10) at 2.98 mg/kg while the least concentration was recorded in Cd-polluted soil (T4) with 0.10 mg/kg after harvest (Table 4.15). For Pb, the highest concentration was recorded in USS (T1) with 1.00 mg/kg. The least concentration was recorded in Zn-polluted soil (T7) and Pb-polluted soil (T5) with 0.30 mg/kg each (Table 4.15). There were significant differences among treatments for all heavy metals along the column at p= 0.05 (Table 4.15).

Table 4.15: Pollution load in soil after the harvest of *Tithonia diversifolia*

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
T1	0.01±0.008^{a}	1.09±0.04^{g}	12.05±0.263^{f}	0.98±0.038^{f}	1.00±0.079^{e}
T2	0.05±0.008^{c}	0.79±0.04^{e}	8.44±0.263^{d}	0.48±0.038^{d}	0.60±0.079^{c}
T3	0.03±0.008^{b}	0.42±0.04^{b}	10.06±0.263^{e}	0.36±0.038^{c}	0.50±0.079^{bc}
T4	0.18±0.008^{e}	0.24±0.04^{a}	7.56±0.263^{c}	0.10±0.038^{a}	0.40±0.079^{ab}
T5	0.01±0.008^{a}	0.38±0.04^{b}	8.41±0.263^{d}	0.11±0.038^{a}	0.30±0.079^{a}
T6	0.02±0.008^{ab}	0.70±0.04^{de}	7.30±0.263^{c}	0.36±0.038^{c}	0.50±0.079^{bc}
T7	0.01±0.008^{a}	0.56±0.04^{c}	4.97±0.263^{a}	0.23±0.038^{b}	0.30±0.079^{a}
T8	0.07±0.008^{d}	0.94±0.04^{f}	10.50±0.263^{e}	0.69±0.038^{e}	0.80±0.079^{d}
T9	0.01±0.008^{a}	0.65±0.04^{cd}	6.32±0.263^{b}	0.32±0.038^{c}	0.40±0.079^{ab}
T10	0.074±0.008^{d}	1.89±0.04^{h}	7.78±0.263^{d}	2.98±0.038^{g}	0.36±0.079^{ab}

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.10: Pollution load in soil after the harvest of *C. odorata*

Cadmium concentration was very high in Cd-polluted soil (C4) and ID soil (C8) when comparing with other treatments with 0.20 mg/kg each. The remaining soil treatments had low cadmium concentration recorded except in USS (C1) where cadmium concentration was not detected (Table 4.16). The highest Zn concentration was recorded in OD soil (C10) with 1.92 mg/kg while the least was recorded in Heavy metal composite (C2) at 0.12 mg/kg (Table 4.16).

The highest Fe concentration was recorded in ID soil (C8) with 9.10 mg/kg mg/kg. The least Fe concentration was recorded for Fe-polluted soil (C3) with 4.99 mg/kg (Table 4.16). Likewise copper concentration had the highest in OD soil (C10) at 2.94 mg/kg. The least Cu concentration was observed in NSD soil (C9) with 0.07 mg/kg (Table 4.16).

Considering Pb, highest concentration was observed in Pb-polluted soil (C5) with 0.60 mg/kg. The least Pb concentration was recorded in heavy metals composite soil (C2), Zn-polluted soil (C7), Fe-polluted soil (C3) and ID soil (C8) with 0.20 mg/kg each (Table 4.16). There were significant differences among treatments for all heavy metals along the column at p= 0.05 (Table 4.16).

Table 4.16: Pollution load in soil after the harvest of *Chromolaena odorata*

Treatment	Cadmium (Mg/kg)	Zinc (Mg/kg)	Iron (Mg/kg)	Copper (Mg/kg)	Lead (Mg/kg)
C1	ND	0.41±0.014^{f}	7.44±0.37^{ef}	0.14±0.014^{b}	0.40±0.07^{b}
C2	0.01±0.009^{a}	0.12±0.014^{a}	7.84±0.37^{fg}	0.12±0.014^{b}	0.20±0.07^{a}
C3	0.01±0.009^{a}	0.44±0.014^{g}	4.99±0.37^{a}	0.11±0.014^{b}	0.20±0.07^{a}
C4	0.20±0.009^{d}	0.25±0.014^{d}	6.06±0.37^{b}	0.32±0.014^{e}	0.40±0.07^{b}
C5	0.04±0.009^{b}	0.31±0.014^{e}	8.78±0.37^{h}	0.28±0.014^{d}	0.60±0.07^{c}
C6	0.01±0.009^{a}	0.54±0.014^{h}	6.33±0.37^{bc}	0.84±0.014^{f}	0.50±0.07^{bc}
C7	0.02±0.009^{a}	0.23±0.014^{cd}	5.49±0.37^{ab}	0.20±0.014^{c}	0.20±0.07^{a}
C8	0.20±0.009^{d}	0.21±0.014^{c}	9.10±0.37^{h}	0.31±0.014^{e}	0.40±0.07^{b}
C9	0.01±0.009^{a}	0.18±0.014^{b}	6.89±0.37^{de}	0.07±0.014^{a}	0.20±0.07^{a}
C10	0.071±0.009^{c}	1.91±0.014^{i}	7.46±0.37^{gh}	2.94±0.014^{g}	0.37±0.07^{b}

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD), ND (Not Detected).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.11 Bioconcentration Factor (BCF) of pollution load in the root of *T. diversifolia* from soil

Tithonia diversifolia grown on Zn-polluted soil (T7), Fe-polluted soil (T3) and NSD soil (T9) had their BCF ≤ 1 for cadmium ranging from 0.50 to 1.00 (Table 4.17). *Tithonia diversifolia* grown on other soil treatments had their BCF > 1 (Table 4.17). Zinc had BCF < 1 in *T. diversifolia* grown on soil treatments ranged from 0.45 to 0.83 (Table 4.17). The BCF for Fe ranged from 0.30 to 0.59 with significant differences among treatments along the column at p= 0.05 (Table 4.17). Copper had BCF < 1, ranged from 0.47 to 0.82 having significant difference among treatments along the column at p= 0.05 (Table 4.17). Lead had BCF < 1 ranged from 0.13 to 0.75 with significant difference among treatments along the column at p= 0.05 (Table 4.17).

Table 4.17: Bioconcentration Factor (BCF) from soil to root in *T. diversifolia* grown in dumpsite and heavy metal polluted soil.

TREATMENT	Cd	Zn	Fe	Cu	Pb
T1	0.61 ± 0.155^{a}	0.83 ± 0.087^{c}	0.53 ± 0.053^{cd}	0.67 ± 0.053^{b}	0.2 ± 0.089^{b}
T2	0.6 ± 0.155^{a}	0.61 ± 0.087^{ab}	0.44 ± 0.053^{bc}	0.56 ± 0.053^{a}	0.17 ± 0.089^{ab}
T3	1.00 ± 0.155^{b}	0.45 ± 0.087^{a}	0.59 ± 0.053^{d}	0.69 ± 0.053^{b}	0.4 ± 0.089^{bc}
T4	0.50 ± 0.155^{a}	0.5 ± 0.087^{ab}	0.32 ± 0.053^{ab}	0.7 ± 0.053^{bc}	0.75 ± 0.089^{c}
T5	-	0.58 ± 0.087^{ab}	0.35 ± 0.053^{b}	0.82 ± 0.053^{d}	0.17 ± 0.089^{ab}
T6	0.50 ± 0.155^{a}	0.53 ± 0.087^{ab}	0.37 ± 0.053^{b}	0.47 ± 0.053^{a}	0.2 ± 0.089^{b}
T7	1.00 ± 0.155^{b}	0.64 ± 0.087^{abc}	0.56 ± 0.053^{d}	0.52 ± 0.053^{a}	0.33 ± 0.089^{bc}
T8	0.57 ± 0.155^{a}	0.65 ± 0.087^{abc}	0.34 ± 0.053^{b}	0.71 ± 0.053^{bcd}	0.13 ± 0.089^{a}
T9	1.00 ± 0.155^{b}	0.66 ± 0.087^{bc}	0.3 ± 0.053^{a}	0.81 ± 0.053^{cd}	0.25 ± 0.089^{b}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.12 Bioconcentration Factor (BCF) of pollution load in the root of *C. odorata* from soil

The bioconcentration factor (BCF) of root pollution load in C. odorata had their BCF < 1 for all soil treatments (Table 4.18). For zinc, the BCF < 1 ranged from 0.44 to 0.96 in all soil treatments with significant differences among treatments along the column at p= 0.05 (Table 4.18). Iron had BCF < 1, which ranged from 0.23 to 0.51 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.18).

For Cd, BCF < 1 was only observed for *C. odorata* grown on Pb-polluted soil, 0.5 (Table 4.18). Furthermore, Cu had BCF < 1, which ranged from 0.33 to 0.92 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.18). Also, Pb had BCF < 1, which ranged from 0.25 to 0.60 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.18).

Table 4.18: Bioconcentration Factor (BF) from soil to root in *C. odorata* grown in dumpsite and heavy metal polluted soil.

TREATMENT	Cd	Zn	Fe	Cu	Pb
C1	-	0.63 ± 0.046^{b}	0.32 ± 0.046^{b}	0.71 ± 0.031^{d}	0.25 ± 0.168^{a}
C2	-	0.75 ± 0.046^{cd}	0.37 ± 0.046^{b}	0.92 ± 0.031^{e}	0.5 ± 0.168^{b}
C3	-	0.7 ± 0.046^{bc}	0.37 ± 0.046^{b}	0.64 ± 0.031^{c}	0.5 ± 0.168^{b}
C4	-	0.44 ± 0.046^{a}	0.23 ± 0.046^{a}	0.47 ± 0.031^{b}	0.5 ± 0.168^{b}
C5	0.50±0.077	0.84 ± 0.046^{de}	0.42 ± 0.046^{c}	0.75 ± 0.031^{d}	0.35 ± 0.168^{ab}
C6	-	0.96 ± 0.046^{f}	0.51 ± 0.046^{c}	0.33 ± 0.031^{a}	0.6 ± 0.168^{b}
C7	-	0.74 ± 0.046^{cd}	0.5 ± 0.046^{c}	0.45 ± 0.031^{b}	0.45 ± 0.168^{b}
C8	-	0.86 ± 0.046^{e}	0.41 ± 0.046^{c}	0.87 ± 0.031^{e}	0.5 ± 0.168^{b}
C9	-	0.78 ± 0.046^{ce}	0.23 ± 0.046^{a}	0.43 ± 0.031^{b}	0.5 ± 0.168^{b}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD).

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.13 Bioconcentration Factor (BCF^1) of pollution load in the shoot of *T. diversifolia* from soil

Tithonia diversifolia in all soil treatments had their $BCF^1 < 1$. Cadmium had its $BCF^1 < 1$ ranging from 0.14 to 0.50 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.19). Likewise Zn had its $BCF^1 < 1$, which ranged from 0.21 to 0.77 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.19).

Iron had its $BCF^1 < 1$ ranged from 0.13 to 0.36 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.19). Furthermore, Cu had its $BCF^1 < 1$, which ranged from 0.20 to 0.64 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.19). Similarly, Pb had its $BCF^1 < 1$ ranged from 0.04 to 0.5 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.19).

Table 4.19: Bioconcentration Factor (BCF[1]) from soil to shoot of *Tithonia diversifolia* grown on dumpsite and heavy metal polluted soil.

TREATMENT	Cd	Zn	Fe	Cu	Pb
T1	0.28±0.064^{b}	0.77±0.073^{d}	0.33±0.03^{c}	0.54±0.09^{b}	0.50±0.064^{c}
T2	0.20±0.064^{ab}	0.39±0.073^{b}	0.17±0.03^{ab}	0.23±0.09^{a}	0.08±0.064^{a}
T3	0.33±0.064^{b}	0.26±0.073^{ab}	0.34±0.03^{c}	0.50±0.09^{b}	0.20±0.064^{ab}
T4	-	0.21±0.073^{a}	0.13±0.03^{a}	0.20±0.09^{a}	0.20±0.064^{ab}
T5	-	0.37±0.073^{ab}	0.18±0.03^{ab}	0.64±0.09^{b}	0.07±0.064^{a}
T6	0.50±0.064^{c}	0.34±0.073^{ab}	0.17±0.03^{ab}	0.22±0.09^{a}	0.04±0.064^{a}
T7	-	0.39±0.073^{b}	0.36±0.03^{c}	0.26±0.09^{a}	0.27±0.064^{b}
T8	0.14±0.064^{a}	0.62±0.073^{c}	0.21±0.03^{b}	0.46±0.09^{b}	0.13±0.064^{bc}
T9	-	0.60±0.073^{c}	0.16±0.03^{ab}	0.52±0.09^{b}	0.08±0.064^{a}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean.

4.14 Bioconcentration Factor (BCF^1) of pollution load in the shoot of *C. odorata* from soil

Chromolaena odorata had its $BCF^1 \leq 1$ and $BCF^1 \geq 1$ in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.20). Cadmium had its $BCF^1 < 1$ with 0.25 for *C. odorata* grown on Pb-polluted soil (Table 4.20). Zinc had its $BCF^1 \geq 1$ ranged from 0.18 to 1.98 in all soil treatments with significant differences among treatments along the column at p= 0.05 (Table 4.20).

Furthermore, Fe had its $BCF^1 < 1$, which ranged from 0.10 to 0.24 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.20). Similarly, Cu had its $BCF^1 < 1$ ranged from 0.14 to 0.75 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.20). Also, Pb had its $BCF^1 < 1$, which ranged from 0.05 to 0.25 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.20).

Table 4.20: Bioconcentration Factor (BCF[1]) from soil to shoot of *Chromolaena odorata* grown in dumpsite and heavy metal polluted soil

TREATMENT	Cd	Zn	Fe	Cu	Pb
C1	-	1.98 ± 0.039^{e}	0.13 ± 0.015^{ab}	0.57 ± 0.074^{c}	0.2 ± 0.032^{bc}
C2	-	0.25 ± 0.039^{ab}	0.12 ± 0.015^{a}	0.75 ± 0.074^{d}	0.15 ± 0.032^{b}
C3	-	0.18 ± 0.039^{a}	0.1 ± 0.015^{a}	0.27 ± 0.074^{ab}	0.15 ± 0.032^{b}
C4	-	0.28 ± 0.039^{b}	0.16 ± 0.015^{bc}	0.34 ± 0.074^{b}	0.18 ± 0.032^{bc}
C5	0.25±0.026	0.29 ± 0.039^{b}	0.24 ± 0.015^{d}	0.57 ± 0.074^{c}	0.17 ± 0.032^{b}
C6	-	0.63 ± 0.039^{d}	0.17 ± 0.015^{c}	0.19 ± 0.074^{ab}	0.2 ± 0.032^{bc}
C7	-	0.48 ± 0.039^{c}	0.19 ± 0.015^{c}	0.3 ± 0.074^{ab}	0.2 ± 0.032^{bc}
C8	-	0.71 ± 0.039^{d}	0.11 ± 0.015^{a}	0.65 ± 0.074^{cd}	0.25 ± 0.032^{c}
C9	-	0.44 ± 0.039^{c}	0.11 ± 0.015^{a}	0.14 ± 0.074^{a}	0.05 ± 0.032^{a}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.15: Translocation Factor (TF) of heavy metal from root to shoot of *T. diversifolia*

Mobilisation of heavy metals from vascular region to the aerial parts of plants is regarded as translocation factor. *T. diversifolia* had their TF ≤ 1 and TF ≥ 2 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21). Cadmium had its TF ≤ 1, which ranged from 0.25 to 1.00 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21). Zinc had its TF < 1, which ranged from 0.42 to 0.95 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21).

Furthermore Fe had its TF < 1, which ranged from 0.39 to 0.64 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21). Also, Cu had its TF < 1, which ranged from 0.29 to 0.81 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21). However, Pb had its TF ≥ 2.5, which ranged from 0.2 to 2.5 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.21).

Table 4.21: Translocation Factor (TF) from root to shoot of *Tithonia diversifolia* harvested from dumpsite and heavy metal polluted soil.

TREATMENT	Cd	Zn	Fe	Cu	Pb
T1	0.46±0.038^{b}	0.93±0.038^{c}	0.63±0.052^{de}	0.81±0.145^{d}	2.5±0.149^{d}
T2	0.33±0.038^{a}	0.64±0.038^{b}	0.39±0.052^{a}	0.41±0.145^{b}	0.47±0.149^{b}
T3	0.33±0.038^{a}	0.58±0.038^{b}	0.58±0.052^{de}	0.72±0.145^{cd}	0.50±0.149^{b}
T4	-	0.42±0.038^{a}	0.41±0.052^{ab}	0.29±0.145^{a}	0.27±0.149^{a}
T5	-	0.64±0.038^{b}	0.51±0.052^{bcd}	0.78±0.145^{c}	0.41±0.149^{b}
T6	1.00±0.038^{c}	0.64±0.038^{b}	0.46±0.052^{abc}	0.47±0.145^{bc}	0.20±0.149^{a}
T7	-	0.61±0.038^{b}	0.64±0.052^{e}	0.50±0.145^{c}	0.82±0.149^{c}
T8	0.25±0.038^{a}	0.95±0.038^{c}	0.62±0.052^{de}	0.65±0.145^{c}	1.04±0.149^{c}
T9	-	0.91±0.038^{c}	0.53±0.052^{cde}	0.64±0.145^{c}	0.32±0.149^{a}
T10	-	-	-	-	-

Note: T_1 (Control: no pollution), T_2 (soil polluted with 2g each of all heavy metal), T_3 (soil polluted with Iron-Fe), T_4 (soil polluted with Cadmium-Cd), T_5 (soil polluted with Lead-Pb), T_6 (soil polluted with Copper-Cu), T_7 (soil polluted with Zinc-Zn),and dumpsite soil; T_8 (soil collected from Irese dumpsite-ID), T_9 (soil collected from New stadium dumpsite-NSD), T_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean

4.16 Heavy metal translocation factor (TF) from root to shoot of *C. odorata*

Translocation factor (TF) of heavy metals from root to shoot. *C. odorata* had its TF ≤ 1 and TF ≥ 3 in all soil treatments with significant differificant difference among treatments along the column at p= 0.05 (Table 4.22).

However, Fe had its TF < 1, which ranged from 0.27 to 0.57 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.22). Also, Cu had its TF < 1, which ranged from 0.33 to 0.82 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.22). Similarly, Pb had its TF < 1, which ranged from 0.1 to 0.80 in all soil treatments with significant difference among treatments along the column at p= 0.05 (Table 4.22).

Table 4.22: Translocation Factor (TF) from root to shoot in *C. odorata* harvested from dumpsite and heavy metal polluted soil

TREATMENT	Cd	Zn	Fe	Cu	Pb
C1	-	3.14 ± 0.027^{f}	0.41 ± 0.058^{bc}	0.80 ± 0.059^{cd}	0.8 ± 0.040^{e}
C2	-	0.33 ± 0.027^{c}	0.32 ± 0.058^{ab}	0.82 ± 0.059^{d}	0.30 ± 0.040^{b}
C3	-	0.26 ± 0.027^{a}	0.27 ± 0.058^{a}	0.42 ± 0.059^{ab}	0.30 ± 0.040^{b}
C4	-	0.64 ± 0.027^{d}	0.70 ± 0.058^{e}	0.72 ± 0.059^{cd}	0.36 ± 0.040^{bc}
C5	0.5±0.0026	0.35 ± 0.027^{b}	0.57 ± 0.058^{d}	0.75 ± 0.059^{cd}	0.49 ± 0.040^{d}
C6	-	0.66 ± 0.027^{d}	0.33 ± 0.058^{ab}	0.58 ± 0.059^{b}	0.33 ± 0.040^{b}
C7	-	0.65 ± 0.027^{d}	0.38 ± 0.058^{abc}	0.67 ± 0.059^{c}	0.44 ± 0.040^{cd}
C8	-	0.83 ± 0.027^{e}	0.27 ± 0.058^{a}	0.75 ± 0.059^{cd}	0.50 ± 0.040^{d}
C9	-	0.56 ± 0.027^{c}	0.48 ± 0.058^{cd}	0.33 ± 0.059^{a}	0.10 ± 0.040^{a}
C10	-	-	-	-	-

Note: C_1 (Control: no pollution), C_2 (soil polluted with 2g each of all heavy metal), C_3 (soil polluted with Iron-Fe), C_4 (soil polluted with Cadmium-Cd), C_5 (soil polluted with Lead-Pb), C_6 (soil polluted with Copper-Cu), C_7 (soil polluted with Zinc-Zn),and dumpsite soil; C_8 (soil collected from Irese dumpsite-ID), C_9 (soil collected from New stadium dumpsite-NSD), C_{10} (soil collected from Onyarubulem dumpsite-OD)

Means with same letters are not significantly different along column at P=0.05
± = Standard error of the mean.

CHAPTER FIVE

DISCUSSION

The negative influence of man on the ecosystem led to contamination of the soil purely by heavy metal pollutants. In most developing nations, like Nigeria, research revealed that there is little knowledge of soil contamination. Availability of pollution load in the soil could influence the nature of edibles, water, microbial roles and growth of organisms negatively (Antoaneta *et al.* 2009). The abandonment of polluted sites for some time and later cultivating for crop production makes plants absorb heavy metals. Their non-biodegradability could have dangerous effect on agricultural products. These pollution loads pose threat to biotic component, inhabiting the soil, because there is heavy presence of the elements (Antoaneta *et al.,* 2009).

In Akure (Ondo State), owing to man's activities on the ecosystem such as smelting, weldering, indiscriminate toxic effluent discharge and dumping sites for refuse and accident cars (body parts, grease, battery electrodes and electrolytes and used engine oil), pollution loads are easily noticed. The commonest heavymetal pollution that is easily noticed in the soil includes cadmium, lead, copper, zinc and iron. This reflects in the soil got from NSD and OD, with 0.07 and 2.42, and 0.08 and 3.04 all in mg/kg for cadmium and copper, respectively, when compared to the soil got from the control site before simulation, at 0.01 mg/kg, as shown in Table 4.1. The reason for this significant difference could be as a result of the presence of body parts of accident cars, grease, battery electrodes and electrolytes and used engine oil in the areas.

Soil has significant role in determining the biomass of agricultural products. This research documented the influence of pollution loads on sprouting of *Tithonia diversifolia* and *Chromolaena odorata* (Tables 4.2, 4.3).

5.1 Emergence indices

The outcome of this study showed that seed emergence was on the high side when considering uncontaminated soil that could be adduced to low pollution load and Fe availability. The seeds of *T. diversifolia* and *C. odorata* planted on the soil samples obtained from Onyearugbulem Dumpsite did not germinate at all, which could be traced to used engine oil availability and blood of butchered cows on the soil that prevented percolation or penetrance of water into the soil and therefore affects seed imbibitions. This promotes reduction in seed emergence. Cadmium is one of the poisonous heavy metals that affect water absorption and movement. This could be accountable for the low yield recorded for soil sample polluted with Cd load (Barcelo *et al.,* 1990). Moreover, there is possibility of low yield if the hormones in the seeds prevents sprouting (Simiri *et al.,* 2009) leading to reduction in Adenosine tri phosphate (ATP) production. Sprouting of *T. diversifolia* and *C. odorata* was late in all the contaminated soil samples, which may be owing to pollution loads or inabilities of the seed of plant species to imbibe water.

Reduction of pollution loads on the polluted sites commenced with germination where absorption of liquid substances was in high amount. Uptake of pollution load ions into the roots bioaccumulated and later got transferred to other segments of plants through transpiration pull (Ximenez-Embun *et al.,* 2001). Similar occurrence was noticed by Claire *et al*. (1991), with use of nickel and other heavy metals in agricultural crops. The pollution loads (Cd and Ni) in larger amount can prevent growth of sunflower (Khan and Moheman, 2006). This may also be the case for *T. diversifolia* and Co in an uncontaminated soil obtained from OD in connection with slow absorption of water, mitotic division prevention and imbibition, reducing metabolism. Stoppage of any of these steps will disrupt growth progress (Shaddad *et al.,* 1989). Bazzaz *et al*. (1974) reported disturbance in opening of the subsidiary cells performance is peculiar to cadmium and many remaining minor metals.

5.2 Root growth

The length of root and shoot in early sprouting is critical for plant production. Gelmond (1978) reported that it determines the crop stand, density and yield of resultant crop. In this study, the contaminated soil reduced the length of the root when compared with the control treatment soil of *T. diversifolia* and *C. odorata* with root length of 45.40 cm and 26.1 cm, respectively (Table 4.4). This is in agreement with the report of Udita *et al*. (2013) who had decrease in the root length of *Brassica napus* owing to pollution load. The root growth of *T. diversifolia* was tolerance to Cu-contaminated soil, Pb-contaminated soil and Fe-contaminated soil while the root growth of *C. odorata* was promoted by Zn-contaminated soil, Fe-contaminated soil and Pb-contaminated soil.

Gabbrielli *et al.* (1990) note that the adaptive feature, shape occassioned by heavy metals in plants were to reduce formative, elongative and maturation phases of the root, depriving them of mineral nutrients and leading to low yield. This was in agreement with this study owing to the fact that *T. diversifolia* and *C. odorata* grown on PSS had decrease in root length while *T. diversifolia* and *C. odorata* grown on USS were not affected.

In this study, the root hairs of *T. diversifolia* and *C. odorata* grown on PSS got decreased, which may be as a result of the presence of heavy metal. This was in agreement with the submission of Xiong (1998), who state that heavy metal accumulation in roots exhibit defects like decrease in number of root hairs and cell division. Although Cd decreased arrangement of 2º roots and root hairs, Zinc contamination improved the root growth.

There were significant influences of heavy metal on the root of *T. diversifolia* and *C. odorata* because Pb reduced their emergence. Emergence of roots in Pb-contaminated soil decreased compared with that of the control soil treatment. Part of the reason why roots are more exposed to risk or heavy metal threat is as a result of direct contact with them and they are responsible in the uptake of water and mineral nutrients from the soil and which bioaccumulate in them. This is why root is considered as a yardstick for pollution load tolerance (Xiong, 1998).

Generally, all the soil treatments, except the USS, experienced reduction in their roots, most especially soil polluted with cadmium in *T. diversifolia* and *C. odorata*. The root growth reductions was also reported by Punz and Sieghardt (1993)

and Nandkumar *et al.* (1995), who claim that heavy metals seriously prevented root increament.

Chaignon and Hinsinger (2003) and Boonyapookana *et al*. (2005) reported inhibition of root growth first before extending to other parts of the plants by Cu. Previous studies showed that root elongation of *Thlapsi caerulescens* was not hindered by pollution load of Cd, compared with unpolluted soil (Boominathan and Doran, 2003). The length and extension of roots are very important in phytoextraction. In this study, though there were depressed root elongations in both *T. diversifolia* and *C. odorata* in PSS, *T. diversifolia* developed much root hair, which enhanced the accumulation or tolerance to pollution loads.

Shaddad *et al.* (1989) documented also compressed elongation of root in *Zea mays* owing to availability of Cd. The decrease in root length of *T. diversifolia* and *C. odorata* in the contaminated soil treatment might be as a result of reduced water intake, potency of zinc, cadmium and copper charges also reduce metabolism. Atlassipak *et al.* (2009) also documented the influence of brackish water on sprouting of plant, which causes injurious connection of metabolic assimilation.

5.3 Shoot growth

Stunted growth and physical impairment in sunflower's shoot was observed by Singh (2006). This was similarly experienced in this study for *T. diversifolia* and *C. odorata* grown on PSS. Heavy metals, such as cadmium, copper, iron and zinc, did not favour shoot growth of *T. diversifolia* and C. odorata. Shoot length of *T. diversifolia* and *C. odorata* got reduced, ranged from 185.5 cm to 19.40 cm and 90 cm to 14.60 cm by soil polluted with heavy metals. When the amount of pollution load was analysed, appreciable decrease in shoot length was observed: *T. diversifolia* and *C. odorata* (19.40 cm and 14.60 cm) from the Cd-polluted soil, compared to the control (185.50 cm and 90 cm, respectively). *T. diversifolia* and C. odorata grown on Cu-polluted soil showed more decrease on stem length, unlike *T. diversifolia* and *C. odorata* grown on uncontaminated soil samples. *Tithonia diversifolia* and *Chromolaena odorata* appeared to be healthy, without any negative symptom, which suggests that a micronutrient action effect on the plants was efficient. There were significant negative influences of Pb-polluted soil on shoots of *T. diversifolia* and Co, unlike the ones from the uncontaminated soil samples.

With respect to transfer of mineral nutrient to plants, Pb was not part of the major elements. Its bioconcentration in shoots and roots often occured with increament in Pb concentration in the superficial tissues (Singh, 1998; Zhu, 2007). Lead penetration in the tissues of plants negatively affected growth of plant, leading to decreased leaf area thereby hindering enzymes' exercise (Nandkumar *et al.,* 1995; Reddy, 2005; Zhu, 2007; Islam *et al.,* 2008). The Pb-polluted soil experienced stunted growth. This agreed with Nandkumar *et al.* (1995), who reported that Pb treated plant species will exhibit reduced sprouting and decreased leaf area. The reduction of shoot length in the Pb-contaminated soil was also reported by Mohebbi *et al.* (2012). Ardakani *et al.* (2009) found decrease in the growth of *Hordeum volgare* grown on soil polluted with Pb.

Concentrations of Cu in soil reduced significantly plant size. Stem and leaf of the plants raised in concentration of Cu reduced. Wenger *et al*. (2003) reported intolerance of Cu in plant tissue when pollution load is > 20 mg/kg. Christodoulakis and Margaris (1996) documented adverse effects of Nickel (Ni), resulting in reduced and stunted growth of stem and leaf showing chlorosis. But in this study, there were no symptoms of chlorosis in Ni polluted soil. Chatterjee and Chatterjee (2000) assert that *Brassica oleracea* is affected because of decreased solution capability and iron pollution load influenced by Cu. Effect of increased copper pollution load in wheat cultivar, *Vergina* sp., causes decreased size of stem and leaf (Athar andAhmad, 2002). The outcome of this research revealed that the growth of *T. diversifolia* and *C. odorata* was affected by the contaminated soil treatments. Heavy metals added to the soil samples greatly influenced the growth of the plant tissues examined. The biomass of the studied plant species also got reduced. It was documented that pollution load of 100 mg/kg Cd decreased plant tissues of rice by 31.98% and 20.67% (Muramoto *et al*., 1990) and decreased products of biomass (Di-Toppi and Gabrielli, 1999; Zadeh, 2008; John, 2009), posing threat via food chain.

5.4 Chlorophyll contents (mg/L)

Chlorophyll is very vital for photosynthesis that serves as light energy receptor from sunlight. Its significant role is to absorb light and transfer it to other parts of the photosystem. Green plants make use of light energy to tap mineral element from the soil into their tissues based on mineral requirements (Rajeev *et al*., 2014). Different pigments are available in green plants; chlorophyll, carotenoid, xanthophyll and many

others. But of all the pigments, chlorophyll is readily available and significant in plants.

Most often, pollution loads decrease biomass by affecting production of food (Udita *et al.,* 2013). The result of chlorophyll content in this study showed influences of these heavy metals on chlorophyll compositions. Chlorophyll *a* of *T. diversifolia* and *C. odorata* was reduced up to 2.67 mg/L and 1.39 mg/L grown on NSD soil and 3.67 mg/L and 1.26 mg/L grown on Cd-polluted soil. Other heavy metals used for this study had influence on chlorophyll composition, except Fe, because the plants make use of Fe for their photosynthetic activities, when compared with the control soil treatment. Change in plant biochemistry owing to pollution loads led to decrease of in *T. diversifolia* and *C. odorata*. Heavy metals such as Pb, Cu, and Cd had negative strong influence on *T. diversifolia* and *C. odorata*, which led to reduction in pigment content of *T. diversifolia* and *C. odorata*. This was in agreement with Abdul (2012) who assert that among all tested chlorophyll fluorescence; plants grown on heavy metal polluted soil were most frequently reduced.

Likewise, Chlorophyll *b* experienced reduction under the PSS. Decrease in green pigments can be traced to barrier caused by pollution loads in *T. diversifolia* and *C. odorata*. Jaleel *et al.* (2008) assert that decrease is also connected to deformation of pigments and their unstable state. Reduction in plant pigment amount was established as a result of plants with salt stress (Mickelbart and Arpaia, 2002; Musyimi *et al.* 2007). The results in Ardakani *et al*. (1997) did not agree with the outcomes of this study owing to the fact that salinity did not have significant impact on the green pigment. But the finding of this study agreed with the work of Fikriye and Omer (2005) that, in the presence of pollution loads, there is decrease of chlorophyll content in different plants.

5.5 Heavy metal accumulation in plant tissues

The result of trace metal concentration in plant tissues of *T. diversifolia* and *C. odorata* differed significantly among soil treatments at p=0.05, indicating their capability difference in metal uptake. Metal concentration varies, depending on particular plants (Alloway *et al.,* 1990). *Chromolaena odorata* accumulated Cd (0.01 mg/kg and 0.02 mg/kg), in its shoots and roots, respectively. These results were higher than the ones in other soil treatments. Most plant species were not detected of

cadmium in their shoots and roots. But for *T. diversifolia*, Cd accumulation in the shoots and roots were 0.05 mg/kg and 0.11 mg/kg, respectively, which were higher than *T. diversifolia* for the other soil treatments.

The quantity of pollution loads in shoot was lower than that of the root of *T. diversifolia* and *C. odorata*, respectively. Increased levels of heavy metals in soil used for this study increase their loads in the tissues of *T. diversifolia* and *C. odorata*. This was in line with the works of Zadeh *et al.* (2008) and John *et al.,* (2009), who established that introduction of Cd increased the amount of such metals in plants. Several heavy metals were accumulated in the root. Similar outcomes were established in the work of Chaturvedi (2004) and Zadeh *et al.* (2008). In all the soil treatments and the two plants used, heavy metal accumulation was higher in the root than the aerial parts. These findings are in tandem with Boominathan and Doran (2003), who claim that many researchers have experimented on observed cadmium which is stored more in the root than the stem and leaf. Nouri *et al.* (2009) also argue that *Chenopodium botrys* accumulated copper in the root more than the shoot.

Further studies revealed that the solution that flows out slowly from the root of plants affects dilution absorption (Klassen *et al.,* 2000) through their influence on microoganism, rhizosphere physical inheritance, and root-growth change (Yang *et al.,* 2005). The solution that flows out slowly from the root of plants symbolises their ability in forming keratin, which helps in movement of mineral elements and heavy metals (Jauert *et al.,* 2002). For the components of trace metals in shoots and roots, proportion of heavy metals was higher in *T. diversifolia*, except Pb, that was higher in the root of *C. odorata*. The present study disagreed with the work of Freeman (2004) that *T. diversifolia* has higher amount of Pb in the stem and leaf than in the root but agreed with work of Wierzbicka (1999), who note that, usually, the root has heavy pollution load of Pb when compared with the shoot. This was also supported by McGrath *et al.* (2001), who claim that heavy metal concentrations is more in the root than stem and leaf. When looked at from the toxicological perspective, *T. diversifolia* and *C. odorata* may be a good candidate for polluted sites clean up. This is because a heavy metal will not have its entry into the food chain through herbivorous animals. Although Cu is one of the requirements for growth in plants, pollution accumulated in the shoot exceeding 20 mg/kg of of copper can cause toxicity (Borkert *et al*., 1998).

This study showed that iron was more accumulated by *Chromolaena* and *Tithonia* than any other heavy metal. This was corroborated by Kamal *et al.* (2004), Thien (2005), Noor (2006) and Bonnet *et al*. (2010). This might be owing to its (Fe) contribution in the development of chlorophyll. Heavy metals are contaminants that have been known to introduce dangerous threat to man's well-being owing to the fact that they are poisonous (Boran and Altınok, 2010). Chromium and cadmium are also known to be strongly poisonous even at little amount (Shukla *et al.,* 2007). The interactions of cadmium with other heavy metals can alter the amount of nutrient and their arrangement in some plants (Scebba *et al.,* 2006).

5.6 Uptake and accumulation of heavy metals

The result on heavy metals concentration showed that in the roots and shoots of *T. diversifolia* and *C. odorata* exhibited significant differences with regard to heavy metal pollution. Any step that hinders usual absorption and building and breaking of nitrogen and magnesium in plants will consistently influence pigment build-up and always influence biomass production. This implies that phytoextraction allows the fundamental minerals needed for pigment build-up. Green pigment influences the rate at which light is receptive.

Pollution load in the environment has recently called for global attention. These elements bioconcentrate in food chain, which poses a risk to man when consumed. This threat to life is as a result of indiscriminate discharge of refuse and effluent, and human practices injurious to the environment (Alloway, 1994). Recent research has revealed that some plants can actually invade and populate a land with different levels of heavy metal concentration in soil. Anyakora *et al.* (2013) state that different bodies like World Health Organisation (WHO), United States Environmental Protection Agency (USEPA) and European Regulatory Standards (ERS), have attached various highest contaminant boundaries for heavy metals. The highest threshold recommended by ERS for soil are as follows: cadmium, 3 mg/kg; copper, 30 mg/kg and lead, 150 mg/kg. The amount of pollution load in plants differs from species to species (Alloway *et al.,* 1990; Istvan and Benton, 1997).

5.7 Bioconcentration Factor (BCF) and Translocation Factor (TF)

Identifying plants for phytoextraction in a polluted site, "tolerant" and "hyperaccumulator" must be put into consideration. An accommodating plant is any species that sprouts on contaminated site, that is poisonous to the remaining species (MacNair *et al.,* 1999; Assuncao *et al.,* 2001; Bert *et al.,* 2003). The plant species used in this research were tolerant to the measured heavy metals.

Another requirement for categorizing hyperaccumulator plant species is that the amount of pollution load in them must be 10-500 folds higher than the species sprouting on the unpolluted site (Yanqun *et al.,* 2005). The results of this study showed a TF $\geq$ 1 for zinc in *C. odorata* and cadmium and lead in *T. diversifolia*, which implies that zinc, cadmium and lead could actively be transported from the root to other aerial parts. The findings of this study were in line with Mohebbi *et al.* (2012). They also found TF to be more than one (TF>1) in *Aeluropus littoralis*. The important role of pollution load hyperaccumulator is active upward movement by translocation factor, resulting in values > 1 (Zho *et al.,* 2006). *Chromolaena odorata* could be regarded as a hyperaccumulator of zinc (0.26-3.14) as shown in Table 4.16, based on the fact that the TF was greater than one (TF=1.98). Likewise *T. diversifolia* could be considered an accumulator of cadmium (0.25-1.00) and lead (0.20-2.50).

Any plant that must be considered a hyperaccumulator of a contaminated site, its bioconcentration factor should be > 1 (Mohebbi *et al.,* 2012). In this study, the bioconcentration factor (BCF) of Zn in *C. odorata* was 0.28-1.98 and for *T. diversifolia*, the bioconcentration factor of Cd was 0.50-1.00 (Table 4.13), which implies high accomplishment of *T. diversifolia* and Co in phytoextraction of cadmium and zinc, respectively. The above outcomes were in accordance with the result of Mohebbi *et al.* (2012). The highest BCF was recorded for Zn (1.98) in *C. odorata* and Cd (1.00) for *T. diversifolia*, which were higher than the results documented by Shu *et al.* (2002). The value found in *Physalis distichum* was 0.2.

CHAPTER SIX

Conclusion and recommendations

This phytoremediation method of clean-up pollution loads on polluted areas with heavy metals should be intensified on. Application of plants to remove soil with pollution loads should be seen as a prospect technique to clean up and hold polluted soil.

Tithonia diversifolia and *Chromolaena odorata*, studied in this research, sprouted well with high biomass product planted on the contaminated soil samples. This is advantageous in revamping and restoring contaminated sites at little cost in an eco-friendly manner. Analysis of heavy metal accumulation in the *T. diversifolia* and *C. odorata* roots and shoots was done to know the BCF and TF of the two plant species for hyperaccumulation potential which turned to be a favourable plant species in cleaning-up heavy metal polluted sites.

Tithonia diversifolia and *Chromolaena odorata* were tolerant to pollution loads. They showed the trait of potential of phytoremediation of heavy metals polluted sites because they were able to uptake heavy metal without showing negative symptoms despite the availability of contaminant. This suggests that they possess sequestration mechanism or possess higher willingness to accept pollution than easily upset species when in contact with pollution. The ability to exclude those pollution loads in their tissues may be the reason for their success.

It is known that only species of plants that have their BCFs and TFs > 1 are capable of clean-up polluted sites. The present investigation has shown that the *T. diversifolia* and Co accumulated appreciable amounts of heavy metals and were tolerant to it, with their BCFs and TFs as one or greater than one. *Tithonia diversifolia* was more efficient in taking up cadmium, iron and lead while *C. odorata* was more

effective in taking up zinc. However, *C. odorata* and *T. diversifolia* were effective in taking up copper. The two plant species (*T. diversifolia* and *C. odorata*) were regarded as plant species with prospect in cleaning the polluted soil used in this work.

Based on the findings of this study, *T. diversifolia* and *C. odorata* accumulated pollution loads more in the root than the stem and the leaf. They could also be categorised as hyperaccumulator species because their BF and TF values were > 1. However, they may be excluders to other heavy metals. *T. diversifolia* will be a suitable candidate for removing heavy metals, especially, Fe, Cu, Pb and Cd; while *C. odorata* will be a suitable candidate for removing heavy metals, especially, Zn and Cu. Generally, *T. diversifolia* and *C. odorata* both reduced heavy metals in the contaminated soil samples. However, *C. odorata* better reduced heavy metals in the selected dumpsites and the polluted soil better than *T. diversifolia*.

Uprooting should be the best method of harvest in order not to leave the stumps underground since the root accumulates more heavy metals than the shoot. However, *T. diversifolia* and *C. odorata* possess the capability of translocating the heavy metals.

6.1 Contribution to Knowledge

Tithonia diversifolia and *Chromolaena odorata* were found suitable for cleaning-up contaminated sites and are potential hyperaccumulator of heavy metals used in this study, hence, the two plant species are good candidates for phytoremediation.

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