ASSESSMENT OF PHYTOREMEDIATION POTENTIAL OF INDIGENOUS FLORA AROUND AJAOKUTA STEEL COMPANY LIMITED, AJAOKUTA, KOGI STATE, NIGERIA.

BY

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16/27/MEHS002

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DECLARATION

I, hereby declare that the dissertation titled "Assessment of Phytoremediation Potential of Indigenous Flora around Ajaokuta Steel Company Limited, Ajaokuta, Kogi State, Nigeria" is a product of my own work under the supervision of Dr. Henry O. SAWYERR and has not been submitted for the award of a degree, diploma or any other qualification in any institution. All information and excerpts from the work of others have been acknowledged by means of references.

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CERTIFICATION

This is to certify that this dissertation has been read, approved and accepted as meeting the requirement for the award of Master of Science Degree in Environmental Health Science, School of Allied Health and Environmental Science, Kwara State University, Malete.

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DEDICATION

This research work is dedicated to my family, to also Dr (Mrs) Gladys Ihunda and all lovers of Environmental Health.

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I wish to appreciate Almighty God the most merciful and the most benevolent who made it possible for me to have made it this far. I pray for more of His grace.

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ABSTRACT

In recent years, with the development of the global industrialization, the content of heavy metals in the soil caused by industrial activities have gradually increased, resulting in environmental deterioration. There is a need to provide viable option that is economical, environmental friendly and sustainable for clean-up of environmental contamination such as phytoremediation. Series of studies conducted in the past on heavy metals content in soil around Ajaokuta Steel Company Limited (ASCL) without considering the remediation technologies of such contents. This study primarily assessed the phytoremediation potential of indigenous flora around Ajaokuta Steel, Company Limited, Ajaokuta, Kogi State, Nigeria. To achieve this, top and sub soil samples along with control were collected around the vicinity of ASCL for heavy metals analysis. Four different indigenous plants (Imperata cylindrical (Spear grass or cotton wool grass), Sida acuta (Wire weed), Helianthus annuus (Sunflower) and Chromoleana odarata (Siam weed) grown within the vicinity of the ASCL were randomly collected for heavy metals analysis. The samples were digested and analysed using Atomic Absorption Spectrophotometer (AAS) (PerkinElmer® Analyst 100 model). The data obtained were analysed using SPSS 20 for descriptive and inferential statistics. The metal transfer factors were also determined. The results obtained revealed that the potential of remediating Lead by C. odarata was high compared to other plant species which uptake 10.33mg/kg, 20.11mg/kg, and 25.32mg/kg in the leaves, stem and root respectively. The bioaccumulation level of Lead in C. odarata and H. annuss were recorded to be 0.91mg/kg and 0.71mg/kg respectively which indicated highest bioaccumulation factor. Unlike the bioaccumulation factors, the translocation factors were high in all the heavy metals investigated. The study revealed that C. odarata, I. cylindrica and S. acuta are good accumulators of heavy metals and they should therefore be encouraged to be cultivated. The plant species may be regarded as hyper-accumulators, which is characterized by their ability to accumulate high quantities of metals in their tissues.
Keywords: *Hyper-accumulators; Heavy metals; Phytoremediation; Indigenous Flora*

CHAPTER ONE

1.1 Background of the Study

Environmental contamination by heavy metals is a serious and worldwide problem that accompany with the rapid industrialization and urbanization in many countries (Alhassan *et al.,* 2012). This contamination by heavy metals can equally result in environmental degradation. Environmental degradation is a result of socio-economical, technological and institutional activities. It is the deterioration of the environment through depletion of resources, the destruction of ecosystems and the extinction of wildlife.

Environmental degradation has been defined as any change or disturbance to the environment perceived to be deleterious or undesirable. Degradation occurs when Earth's natural resources are depleted. These resources which are affected include: water, air and soil. The degradation also impacts our wildlife, plants, animals and micro-organisms. The global problem concerning contamination of the environment as an aftermath of anthropogenic activities is on the increase which has resulted in environmental build up of waste products of which heavy metals are of particular concern (Yashim *et al*, 2015).

Many clean-up technologies exist for the treatment of contaminated soils, but only few are applicable to heavy metal contaminated soils (Wedepohl, 2008). The use of plants and associated microorganisms to contain, inactivate, remove or degrade harmful environmental contaminants and to revitalize contaminated sites is gaining more attention (Vangronveld *et al.*, 2009).

Soils are not just essential parts of ecosytem as they give supplements to living life forms, yet additionally fill in as stores for pernicious substance species which cause negative impacts on amphibian framework and human wellbeing (Ng *et al.,* 2016). Heavy metals are harmful to the biota and the earth. The Ecosystem around Ajaokuta Steel Company has been progressively contaminated with different sorts of poisonous toxins and one of the contaminations is heavy metal which is found in different natural media including the dirt (Massa *et al.,* 2010).

Soil is commonly regarded as one of the significant natural resources that provide numerous essential elements and interrelating functions which include as a store for biodiversity, as a natural habitat for living organisms, food and biomass production as well as a relatively stable reservoir for the whole ecosystem. It is a limited resource that can easily deteriorate by both anthropogenic and natural changes. Soil pollution is the form of which pollutant materials are present at concentrations above naturally occurring levels and are likely to cause a direct and/or long term danger to humans and the environment (DOE, 2009). Urban soil contamination has greatly affected many countries, including the United States, Germany, United Kingdom, China and India (Belluck *et al*., 2006; Meuser, 2010) meanwhile heavy metal soil contamination itself has gained a serious attention at the global perspective (Van de Velde, *et al*., 2005).

Heavy metal contamination refers to the excessive deposition of toxic heavy metals in the soil caused by human activities. This includes some significant metals of biological toxicity, such as mercury, cadmium, lead, chromium, arsenic, zinc, copper, nickel, and vanadium. The rapid increase in population in Ajaokuta Local Government and expanding level of industrialization and urbanization has prompted natural contamination (Filazi *et al,* 2003). Industries have to a great extent been in charge of releasing of effluents containing metals, for example, Zinc (Zn), Cadmium (Cd), Mercury (Hg), Lead (Pb) and Chromium (Cr) into our environment (Inuwa, 2004).

The uptake of toxic heavy metals from contaminated soils by food and foliage plants comprises a prominent path for such elements to enter the food chain and finally be ingested by human. Ingestion and eventually accumulation of toxic heavy metals pose a threat to human health and should be minimized (Dieckmanni, *et al*, 2001). Heavy metals can be very toxic even in low concentrations and are not easily degraded or destroyed. They are generally harmful to humans and other living organisms as it easily bio-accumulate and cause food chain contamination. Nevertheless, heavy metals often exist in small amounts in soils and plants as some of the trace metals play an essential role in promoting biological growth (Dimitiru, 2014).

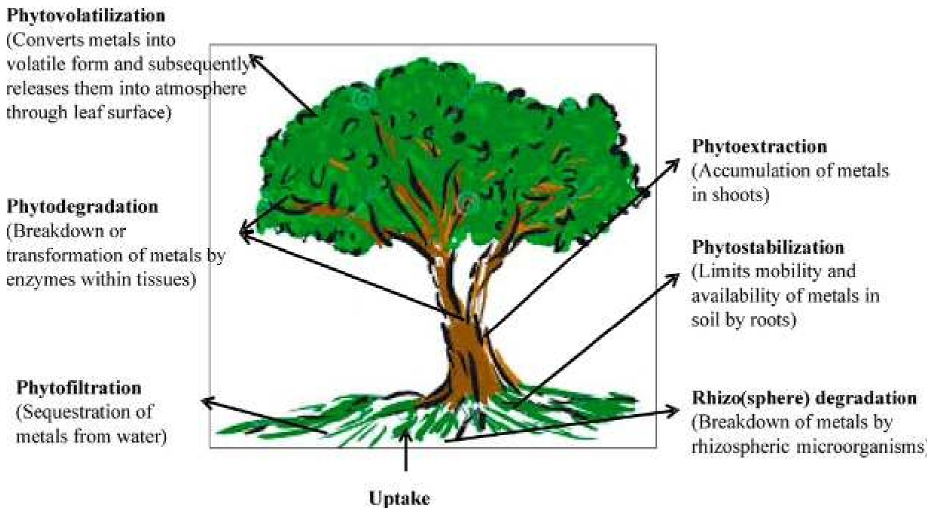
In general, heavy metal can be categorized into essential and non-essential. Essential 5x such as nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) are required by living organisms in trace amounts to support their metabolic functions while non-essential heavy metals such as chromium (Cr), arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) are not needed for the growth of living organisms (Kabata-Pendias, 2011; Cuypers *et al*., 2013).

Increasing mining activities in many countries are contributing to heavy metal pollution of water, soil and air (Massa *et al.,* 2010). This indiscriminate pollution is likely to have long-term environmental and public health implications unless immediate action is taken (Meuser, 2010).

Plants have developed the ability to absorb relatively less abundant micronutrients such as Cu, Zn, Ni and Mn from the soil. These essential micronutrients are also highly reactive and potentially toxic to plants; hence the uptake, transport and accumulation are thereby highly coordinated and regulated by necessity in plants.

Therefore, plants have evolved ability mechanisms like detoxification of metals in the roots and tolerance to heavy metal stress which may involve primarily the avoidance of build-up of toxic concentrations at sensitive sites. Escaping from heavy metal toxicity by reducing or excluding the uptake of heavy metals may be another strategy for plants to resist metal toxicity.

Some plants are also tolerant to specific metal and are able to accumulate such metals in substantial quantities in their biomass due to their effective uptakes and translocations and are regarded as accummulators (Nan, *et al.,* 2002). Such plants with effective transfer and translocation concentrate metals in above ground parts from low to great soil concentrations and are regarded as accumulators (Turgut *et al*., 2004).



Phytoremediation can be defined as the process of utilizing plants to absorb, accumulate, detoxify and/or render harmless, contaminants in the growth substrate (soil, water and air) through physical, chemical or biological processes (Cunningham and Berti, 2009). In the last decade, phytoremediation has gained a great deal of interest in both public and private sectors as a cost-efficient alternative to the conventional remediation technologies. Effective phytoremediation depends upon correct selection of local plant species, preferably with hyper-accumulator characteristics, that can grow on land of marginal quality in terms of fertility, age, texture and structure of polluted soil and mixture of metals present (Mwegoha, 2008, Lin *et al.,* 2009;). Introduction of exotic species carries a high risk of disturbing the natural ecosystem if the foreign species proves to be invasive (Mwegoha, 2008).

Phytoremediation of soil has pulled in much consideration as of late because of its numerous points of interest, for example, keeping up the natural movement and physical structure of soils, being conceivably modest and outwardly inconspicuous, and giving the likelihood of bio-recovery of metals (Strange *et al.,* 2011).

Phytoremediation is an alternative or complimentary technology that can be used along with or, in some cases in place of mechanical conventional clean-up technologies that often require high capital inputs and are labour and energy intensive. Phytoremediation is an 'in situ' remediation technology that utilizes the inherent abilities of living plants. It is also an ecologically friendly, solar-energy driven clean-up technology, based on the concept of using nature to cleanse nature (Delia, 2014).

Bioremediation is characterized as the utilization of plants and microorganisms to remove of adsorb the natural or inorganic contaminants. Regarding hydrocarbon removal, microorganisms exhibit in the plant rhizosphere (root zone) have been appeared to be powerful at remediating the contaminants; this is called rhizoremediation. Plant roots increment microbial exercises (counting hydrocarbon corrupting exercises) through the arrival of root exudates, for example, supplements, oxygen, and hydrocarbon analogs, which may add to the increasing speed of the debasement of petrogenic hydrocarbons. Be that as it may, the poisonous quality related with numerous hydrocarbon items towards the plants combined with unwanted soil conditions, for example, saltiness may restrain the adequacy of phytoremediation (rhizoremediation). For this situation, necro-phytoremediation can be utilized as an elective technique; this is characterized as the utilization of dead plant biomass (e.g. straw) and its related microflora to debase the contaminant (Eric and Andrew, 2017).

Phytoremediation is a plant based bioremediation propels which uses the assembled use of green plants and their related little scale biota for the in-situ treatment of corrupted soil and ground water (Sadowsky, 2010). The method is common genial and endeavors the unique and specific take-up limits of plant root systems, together with the translocation, bioaccumulation, and contaminant accumulating/corruption limits of the entire plant body. Phytoremediation is fiscally keen and gorgeously fulfilling as the plants can be successfully watched and metals devoured by the plants may be expelled from assembled plant biomass and after that reused.

Heavy metals contamination of soils, water and plants has become a serious global issue, and as such both government and regulatory agencies including the Federal Ministry of Environment have to ensure the implementation of environmental quality guidelines "to protect, sustain, and enhance the quality" of the environment and to assist with the assessment and remediation of contaminated sites. Water and soil contaminated by heavy metals can pose health risks to both humans and animals (Rascio, 2011). The mutagenic potential of heavy metals that are toxic can cause DNA damage and have carcinogenic effects on animals and humans (Baudouin *et al.*, 2002).

Present solutions for removal of heavy metals from industrial polluted sites include excavation or entombment, chemical and thermal treatments, which are not cost effective (Fillips, 2010). A form of in situ biological remediation may have the least impact and be the most cost effective option to treat heavy metal contaminated areas (Fillips, 2010). Phytoremediation uses plants to clean up contamination from soil, sediments and water to improve environmental quality. Presently phytoremediation technologies are effective, affordable and environmentally friendly in situ technological solutions to extract metals from contaminated soil (Tangahu *et al.,* 2011).

1.2 Statement of Problem

The mitigation of the potential environmental impacts of iron and steel production operations require information and understanding of the environmental status of the location of the industry, vicinities near and further away. Current practice for remediating substantial metal-polluted soils depends intensely on 'burrow and-dump' or epitome, neither of which tends to the issue of cleaning of the dirt (Khan, *et al.,* 2013). As human activities began to undergo industrialization, the amount of waste thrown into the environment has increased tremendously (Inuwa, 2004). Heavy metals such as arsenic (As), chromium (Cr), mercury

(Hg), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are hazardous and the metal toxicity can be severely hazardous if the concentration of heavy metal exceeds its threshold level (DOE, 2009; Ng *et al.*, 2016).

Among all heavy metals; Cadmium (Cd), Lead (Pb), Zinc (Zn) and Copper (Cu) are the most commonly found metals in contaminated sites (Wang *et al.*, 2009). Immobilisation or extraction by chemicals is expensive, requires a technically complex process and is often appropriate only for small areas where rapid, complete decontamination is required (Mwegoha, 2008). This process generally has adverse effects on biological activity, soil structure and fertility. The necessities of these techniques make them unreasonably expensive for poor nations (Mwegoha, 2008).

When heavy metals are present in high concentrations in the environment, it may enter the food chain from soils and result in becoming a health hazard. Heavy metals are the most common of all metabolic poisons. The mechanism of metals toxicity is different from other metabolic poisons. Metal toxicity can affect enzymes, the cellular proteins that react with and inhibit *sulfhydryl* (SH) *enzyme* systems, such as those involved in the production of cellular energy. Heavy metals toxicity tends to bioaccumulate in plants and animals, bioconcentrate in the food chain, or attack specific organs of the body (Rascio, and Izzo, 2011).

Heavy metal pollution not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community. Therefore, heavy metals are considered as one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd, Zn, Cr, and Pb. The adverse effects of heavy metals on soil biological and biochemical properties are well documented. The soil properties i.e. organic matter, clay contents and pH have major influences on the extent of the effects of metals on biological and biochemical properties (Singh and Kalamdhad Ajay, 2011). Heavy metals in urban soils may go into the body directly through ingestion, skin contact and so on. However, heavy metals in agricultural soils are absorbed and accumulated by crops. Ingesting heavy metals through the soil–crop system is a major way of damaging human health (Aeliona *et al*., 2008).

1.3 Justification of the Study

In recent years, with the development of the global industrialisation, both the type and content of heavy metals in the soil caused by human activities have gradually increased, resulting in the deterioration of the environment (Chao *et al.,* 2014). Heavy metals are highly hazardous to the environment and organisms. As heavy metals get into the ecosystem they can be enriched through the food chain. Once the soil accumulates heavy metal and is contaminated, it is difficult to get it remediated. In the past, soil contamination was not considered as important as air and water pollution, because soil contamination was not often and was more difficult to be controlled and taken care of than air and water pollution. However, in recent years soil contamination in developed countries has become an environmental concern (Chao *et al.,* 2014).

Heavy metal contamination is an increasing worldwide environmental concern. Contamination of soil-water-plant system with heavy metals is a form of chemical environmental load, which has health, economic and ecological importance (Alloway, 2005). Numerous studies have been conducted on remediation of heavy metal contaminated soil by employing thermal, chemical, physical and biological treatments, and significant progress has been made. These conventional methods are usually very expensive (Salt *et al*, 2005). While there have been many studies conducted on heavy metals content in soil in the past, many of them have looked specifically at the contents of such heavy metals around Ajaokuta Steel Company without considering the remediation technologies of such contents.

The study seeks to provide viable option for clean-up of environmental contamination because phytoremediation is more cost-effective with fewer side effects than physical and chemical techniques (Nasser *et al.*, 2013).

This study is justified with the rationale of assessing the ability of native plants in the Ajaokuta Steel Company Limited environment to phytoremediate the existing heavy metals.

1.4 Aim of the Study

To identify plant species with potential for phytoremediation of heavy metals in soils around Ajaokuta Steel Company Limited, Kogi State, Nigeria.

1.5 Objectives of the Study

1) To determine the concentration of heavy metals in the indigenous plant samples

2) To estimate ability of plants to reduce concentrations of heavy metals in soils around Ajaokuta Steel Company Limited

3) To determine which plant species presently growing around Ajaokuta Steel Company may contribute to phytoremediation.

4) To compare the ability of these plants to accumulate metals against the soil concentration of the respective metal at the plant sampling point.

1.6 Scope of the Study

This study sought to assess phytoremediation potential of indigenous flora around the industrial area of Ajaokuta Steel Company Limited, Kogi State, Nigeria. It covers the assessment of the native plants' ability to bioremediate heavy metals around the industrial area.

CHAPTER TWO

2.0 Literature Review

2.1 Conceptual Framework

Every living organism has the ability to withstand a specific quantity of essential and non-essential metals present in the environment, and utilize them for their growth processes, but at higher level these metals can be toxic (Bradshaw, 2010). In contrast to essential metals, which serve as metabolic precursors for the plants, the elements classified as non-essential elements do not have any known physiological function in the plant. The most commonly occurring non-essential elements in the environment can be grouped into one major category, termed as heavy metals.

There are two routes of entrance of metals into the environment: natural processes or human activities. Natural contamination originates from either excessive withering of minera1 and metal ions from rocks or from displacement of certain contaminants from the ground water or subsurface layers of the soi1. Humans have been introducing trace metals into the environment since they first gained knowledge of their many useful properties. Despite the benefits that society has had after this discovery, the harsh consequences of metal pollution were encountered at the turn of the 19th century, when the Industrial Revolution resulted in a greater demand of various metals (Nriagu, 2009).

Bioremediation is the use of plants and/or their associated microorganisms for environmental cleanup. Phytoremediation can be defined as the process of utilizing plants to absorb, accumulate, detoxify and for render harmless, contaminants in the growth substrate (soil, water and air) through physical, chemical or biological processes. This technology makes the use of the naturally occurring processes by which plants and their associated rhizospheric microflora degrade and sequester organic and inorganic pollutants (Inuwa, 2004).

Though, bioremediation as a technology has been used for hundreds of years to treat human wastes, reduce soil erosion and protect water quality, research focusing specially on the phytoremediation of contaminated soils has only grown significantly in the last 25 years (Robinson *et al,* 2003).

United Nations Environment Programme defines Phytoremediation as "the efficient use of plants to remove, detoxify or immobilize environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants". Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. Phytoremediation involves growing plants in a contaminated matrix, for a required growth period, to remove contaminants from the matrix, or facilitate immobilization (binding/containment) or degradation (detoxification) of the pollutants. The plants can be subsequently harvested, processed and disposed.

UNEP (2016) further stated that plants have evolved a great diversity of genetic adaptations to handle the accumulated pollutants that occur in the environment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is a passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. Phytoremediation can be used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates. It can also be used for river basin management through the hydraulic control of contaminants.

The concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were first proposed for use in the treatment of wastewater (Hartrnan, l975). At the end of the 19' century, *Thiaspi caerulescens* and *Viola calaminana* were the first plant species documented to accumulate high levels of metals in leaves (Baumann, 1885). Byers (1935) found that plants of the genus *Astragalus* were capable of accumulating up to 0.6 %

seleniurn in dry shoot biomass. Plants able to accumulate up to 1% Ni in shoots were reported by Minguzzi and Vergnano (1948) and recently, Rascio, (1977) reported high Zn accumulation in shoots of *Thlaspi caerulescens***.**

The idea of using plants to extract metals from contaminated soi1 was reintroduced and developed by Utsunamyia (1980) and Chaney (1983), and the first field trial on Zn and Cd phytoextraction. The use of plants for remediating heavy metal contaminated soils has multifold advantages such as:

❖ Growing plants is relatively inexpensive

❖ Large scale application, as plants can be sown or planted in large areas large

❖ Plants provide an aesthetic value to the landscape of contaminated sites

❖ Plants concentrate the contaminants within their tissues, thereby reducing the amount of hazardous waste

❖ Concentrated hazardous waste would require smaller reclamation facilities for extracting the heavy metals

❖ Increased aeration of the soi1 which in turn enables microbial degradation of organic contaminants and microbe-assisted uptake of metal contaminants

❖ Reduced top soi1 erosion due to plant stand

❖ Enhancement of rhizospheric micro-fauna and flora for maintaining a healthy ecosystem (Saxena *et al.,* 2010)

Disadvantages of phytoremediation such as:

❖ Root system it is required that the contaminants be in contact with the root zone of the plants. Either the plants must be able to extend the roots to the contaminants, or the contaminated media must be moved to within range of the plants.

❖ Growth rate and seasonality will prolong the phytoremediation time of site as compared with other more traditional cleanup technologies.

❖ Contaminant concentration only sites with low medium level contamination within the root zone are the best candidates for phytoremediation processes.

❖ Performance monitoring demonstrates whether phytoremediation has successfully cleaned up contaminated soi1 or groundwater to a predetermined standard. This requires continuous measurements of changes in the mass balance of contaminant concentrations in plants, soil, and for ground water, or by measuring the plant stress response.

❖ Risk monitoring is necessary to assure that accumulation of contaminants in plants does not cause unacceptable ecological risks regulatory acceptance is currently being developed (DOE, 1999; US EPA; 2000)

2.2 Mechanism of Phytoremediation

Nature has blessed us with a wide variety of plants which may exhibit diverse mechanisms for the removal of environmental toxicants depending upon their inherent abilities. Although the basic concept of utilizing plants to remediate contaminated sites remains the same.

Contaminants uptake by plants and its mechanisms have been being explored by several researchers (Alhassan *et al*., 2012). It could be used to optimize the factors to improve the performance of plant uptake. According to Sinha *et al.,* (2013), the plants act both as "accumulators" and "excluders". Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass. Plants have evolved highly specific and very efficient mechanisms to obtain essential micronutrients from the environment, even when present at low concentration levels.

Plant roots, aided by plant-produced chelating agents and plant-induced pH changes and redox reactions, are able to solubilize and take up micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation.

Schmidt (2003) established that there are several ways in which plants are used to clean up, or remediate, the contaminated sites. To remove pollutants from soil, sediment and/or water, plants break down, or degrade, organic pollutants or contain and stabilize metal contaminants by acting as filters or traps.

Delia (2014) stated that the uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The root system provides an enormous surface area that absorbs and accumulates the water and nutrients essential for growth, as well as other non-essential contaminants. Researchers find out that the use of trees (rather than smaller plants) is effective in treating deeper contamination because tree roots penetrate more deeply into the ground (Delia, 2014). In addition, deep-lying contaminated ground water can be treated by pumping the water out of the ground and using plants to treat the contamination.

Lors, *et al.,* (2012) noted that plant roots also cause changes at the soil-root interface as they release inorganic and organic compounds (root exudates) in the rhizosphere. These root exudates affect the number and activity of the microorganisms, the aggregation and stability of the soil particles around the root, and the availability of the contaminants. Root exudates, by themselves can increase (mobilize) or decrease (immobilize) directly or indirectly the availability of the contaminants in the root zone (rhizosphere) of the plant through changes in soil characteristics, release of organic substances, changes in chemical composition, and/or increase in plant-assisted microbial activity.

Aleer, *et al.,* (2011) explained that phytoremediation is a cost-effective plant-based approach of remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. It refers to the natural ability of certain plants called hyperaccumulators to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air.

Erkelens, *et al.,* (2012) described the different types of phytoremediation and their mechanisms of action

2.2.1 Phytosequestration/phytostabilization

Phytostabilization is defined as immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants, and at the same time it can also be described as the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching, and soil dispersion (EPA, 2000). Phytostabilization, involves use of plants especially roots and for plant exudates to stabilize, demobilize and bind the contaminants in the soil matrix thereby reducing their bioavailability. This approach is suitable for both organic and metal contaminated soils (Vangronsveld and Cunningham, 2008).

Many different processes fall under this category which can involve absorption by roots, adsorption to the surface of roots or the production of biochemical by the plant that are released into the soil or groundwater in the immediate vicinity of the roots, and can sequester, precipitate, or otherwise immobilize nearby contaminants.

Phytostabilization occurs when plant roots bind and stabilize contaminants from the soil reducing their bioavailability. Phytostabilization, the third option, is the use of vegetation to contain soil contaminants *in situ,* through modification of the:

- ❖ Chemical;
- ❖ Biological;
- ❖ Physical conditions in the soil.

Contaminant transport in soil, sediments, or sludge can be reduced through absorption and accumulation by roots; adsorption to roots; precipitation, complexation, or metal valence reduction in soil within the root zone.

Phytostabilization reduces the mobility of the contaminant and prevents further movement of the contaminant into groundwater or the air and reduces the bioavailability for entry into the food chain. Phytostabilization aims to reduce the mobility/bioavailability of heavy metals in the soil and the re-vegetation of the site, often in combination with adding adsorbents and other chemicals to the soil. The traditional means by which metal toxicity is reduced at these sites is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals.

Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater; and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Schnoor, 2005). This technique is actually a modified version of the in-place inactivation method in which the function of plants is secondary to the role of soil amendments. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

2.2.2 Rhizodegradation

This takes place in the soil or ground water immediately surrounding the plant roots. Exudates from plants stimulate rhizosphere bacteria to enhance biodegradation of soil contaminants. Enhanced rhizosphere biodegradation takes place in the soil immediately surrounding plant roots. Natural substances released by plant roots supply nutrients to microorganisms, which enhances their biological activities. Plant roots also loosen the soil and then die, leaving paths for transport of water and aeration.

This process tends to pull water to the surface zone and dry the lower saturated zones. The most commonly used flora in phytoremediation projects are poplar trees, primarily because the trees are fast growing and can survive in a broad range of climates. In addition, poplar trees can draw large amounts of water (relative to other plant species) as it passes through soil or directly from an aquifer. This may draw greater amounts of dissolved pollutants from contaminated media and reduce the amount of water that may pass through soil or an aquifer, thereby reducing the amount of contaminant flushed though or out of the soil or aquifer. Rhizofiltration is the adsorption onto plant roots or absorption into plant roots of contaminants that are in solution surrounding the root zone (rhizosphere). Rhizofiltration is used to decontaminate groundwater. Plants are grown in greenhouses in water instead of soil. Contaminated water from the site is used to acclimate the plants to the environment. The plants are then planted on the site of contaminated ground water where the roots take up the water and contaminants (Schnoor, 2005).

Rhizofiltration utilizes plant roots to take up and sequester metal contaminants and or excess nutrients from aqueous growth substrates (waste water streams, nutrient-recycling systems). This approach is suitable for remediating most metals such as Lead (Pb), Cadmium (Cd), Nickel (Ni), Copper (Cu), Chromium (Cr), vanadium (V), excess nutrients, and radio nuclides such as Uranium (U), Caecium (Cs), Strontium (Sr) contaminated water. Once the roots are saturated with the contaminant, the plants are harvested including the roots. Examples of plant species used *are Helianthus* sp., *Brassica* sp., (Dushenkov *et* al., 2005) *Populus* sp., *Lemna* sp., and *Thlaspi* sp., (Salt *et al.,* 2005).

Dushenkov and Kapulnik, (2000) described the characteristics of the ideal plant for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable plants to produce significant amounts of root biomass or root surface area. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth. However, these plants have limited potential for rhizofiltration, because they are not efficient at metal removal, a result of their small, slow-growing roots (Dushenkov *et al.,* 2005).

2.2.3 Phytofiltration/Phytohydraulics

Use of deep-rooted plants (usually trees) to contain, sequester or degrade ground water contaminants that come into contact with their roots. In one example of this, poplar trees were used to contain a ground water plume of methyl-tert-butyl-ether (MTBE). Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration), is similar in concept to phytoextraction, but is used to absorb or adsorb pollutants, mainly metals, from groundwater and aqueous-waste streams rather than the remediation of polluted soils (Hukabee *et al.,* 2011).

Rhizosphere is the soil area immediately surrounding the plant root surface, typically up to a few millimeters from the root surface. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly in situ but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place, the water supply is substituted for a polluted water supply to acclimatize the plant. After the plants become acclimatized, they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely. Repeated treatments of the site can reduce pollution to suitable levels as was exemplified in Chernobyl where sunflowers were grown in radioactively contaminated pools.

2.2.4 Phyto-extraction/Phyto-accumulation.

Phyto-extraction is the process of growing plants in metal contaminated soil. Plant roots then translocate the metals into aboveground portions of the plant. After plants have grown for some time, they are harvested and incinerated or composted to recycle the metals. Phyto-extraction is the uptake/absorption and translocation of contaminants by plant roots into the above ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash (U.S.EPA, 2005).

It involves specific plant species which can absorb and accumulate metal contaminants and for excess nutrients in harvestable root and shoot tissue, from the growth substrate (soil). This approach is suitable to remove most metals such as nickel, zinc, copper, lead, chromium and cadmium and excess nutrient from contaminated soils. Examples of plants species used are plants belonging to Brassicaceae family such as *Thlaspi* sp., (Baker *et al.,* 1994) *Brassica sp.,* (Kumar *et al.,* 2005) and *Alyssum sp.* (Kramer *et al.,* 2006).

Several crop growth cycles may be needed to decrease contaminant levels to allowable limits. If the plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of the ash is much smaller than the volume of contaminated soil if dug out and removed for treatment.

Plants take up or hyper-accumulate contaminants through their roots and store them in the tissues of the stem or leaves. The contaminants are not necessarily degraded but are removed from the environment when the plants are harvested. This is particularly useful for removing metals from soil and, in some cases, the metals can be recovered for reuse, by incinerating the plants, in a process called phytomining (Fulekar, 2016).

Hyper-accumulators contain more than 1,000 milligrams per kilogram of cobalt, copper, chromium, lead, or nickel; or 10,000 milligrams per kilogram (1 %) of manganese or zinc in dry matter. One or more of these plant types are planted at a particular site based on the kinds of metals present and site conditions.

Phyto-extraction occurs when plants accumulate metals in their harvestable tissues. Through this phyto-extraction, the contaminant is up taken by roots with subsequent accumulation in the above ground portion of a plant, generally to be followed by harvest and ultimate disposal of the plant biomass. It is a contaminant removal process (Dimitriu, 2014).

Phyto-extraction applies to:

- Metals (e.g. Ag, *Cd*, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn);
- Metalloids (e.g. As, Se);
- Radionuclides (e.g. 90Sr, 137Cs, 234U, 238U);
- Non-metals (e.g. B).

The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix (Kumar *et al.,* 2005). In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution.

Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar *et al.,* 2005) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years (Kumar *et al.,* 2005).

This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar *et al.,*2005). Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites. The selection of a site that is conducive to this remediation technology is of primary importance.

Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue.

Assessing the Efficiency of Phytoextraction

Depending on heavy metal concentration in the contaminated soil and the target values sought for in the remediated soil, phytoextraction may involve repeated cropping of the plant until the metal concentration drops to acceptable levels. The ability of the plant to account for the decrease in soil metal concentrations as a function of metal uptake and biomass production plays an important role in achieving regulatory acceptance. Theoretically, metal removal can be accounted for by determining metal concentration in the plant, multiplied by the reduction in soil metal concentrations. Phytoextraction aims to remove theheavy metal using specific plants, often in combination with specific soil additives.

2.2.5 Phytovolatilization

Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant. Phytovolatilization occurs as growing trees and other plants take up water along with the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Plants take up volatile compounds through their roots, and transpire the same compounds, or their metabolites, through the leaves, thereby releasing them into the atmosphere.

The uptake of contaminants by plants and their subsequent release into the atmosphere in volatile form. Phytovolatilization, uses the plants ability to absorb and subsequently volatilize the contaminant into the atmosphere (Vangronsveld and Cunningham, 2008). This approach is suitable for remediating metals such as Hg and Se from contaminated soils. Recently, Rugh *et al.* (2000) has genetically engineered plants (*Arabidopsis thaliana L., Nicotiana tabacum and Linodendron tulipifera*) with bacterial genes that convert organic and ionic mercury compounds to the volatile and less toxic forms.

2.2.6 Phytodegradation/Phytostimulation

Contaminants are taken up into the plant tissues where they are metabolized, or biotransformed. Where the transformation takes place depends on the type of plant, and can occur in roots, stem or leaves. Phyto-degradation is the metabolism of contaminants within plant tissues. Plants produce enzymes, such as dehalogenase and oxygenase that help catalyze degradation. It includes the enzymatic breakdown of organic pollutants such as TCE (Trichloroethane), herbicides etc., both internally and with the help of secreted enzymes (Rugh *et al.* 2000).

Phytodegradation/phytostimulation, utilizes the rhizospheric associations between plants and soi1 microorganisms to degrade complex organic-metal contaminant mixtures. This approach is suitable for remediating TNT, PAH, petroleum hydrocarbons from contaminated soils (Schnoor *et al.,* 2005). Examples of plant species used are: *Medicago sp*. and several grasses (Gordon *et al.,* 2007). Investigations are proceeding to determine if both aromatic and chlorinated aliphatic compounds are amenable to phyto-degradation.

Understanding these basic mechanisms involved in the removal of contaminants can lead us to design strategies to achieve more efficient removal of environmental pollutants.

2.3 Physiology of Metal Tolerance Mechanism

Tolerance mechanisms are of primary importance for accumulator plant species, as these plants accumulate large amounts of metal ions and sequester them appropriately without incurring cellular tissue damage. Important tolerance mechanism identified to date, include, production of intracellular binding facilitate toxic metal ions sequestration, alteration of metal compartmentalization patterns to avoid damage and/or disturbances to basic cellular processes, modulation of cellular metabolism to alleviate damage due to accumulation of toxic metals, and modification of membrane structure (Cobbert and Goldsbrough, 2000).

a. Production of intracellular binding compounds

Plants are stationary in a fluctuating environment, and therefore undergo a constant modulation of internal levels of metal ions. In general, plants synthesize and accumulate a variety of metal chelating compounds upon exposure too exceedingly high levels of available metals. Natural chelating compounds viz. amino acids and their derivatives, citric acid, malic acid and/or phytochelatins are produced by the plants to alleviate metal-induced toxicity symptoms (Prasad, 2009).

The family of proteins called metallothioneins (MT) also serves to bind metal ions by wordinating metal ions to closely spaced cysteine thiol groups. The role of metallothioneins and phytochelatins in imparting metal tolerance in plants is well established, has been reviewed in depth (Cobbert and Goldsbrough, 2000). In brief, phytochelatins are a unique family of thiol-wntaining metal binding polypeptides. These polypeptides are synthesized in the plant in response to treatment with metals, and their biosynthesis is connected with glutathione metabolism rather than to ribosomal protein synthesis.

Among the metals, Cd is the strongest inducer of phytochelatins while higher concentration of Zn also elicits a similar induction response. Phytochelatins appear to be the primary metal binding polypeptide in plants, and the enzyme catalyzing their biosynthesis, phytochelatin synthase is constitutively expressed in plants. Phytochelatins may be also involved in trace-metal homeostasis, which can detoxm excess metals. Although the reasons for the apparent dichotomy in metal sequestration by animals (metallothioneins) and plants (phytochelatins) remains poorly understood, recent evidence of metalothionein - like proteins in plants suggests that phytochelatins may not be the exclusive heavy metal binding polypeptides in plants (Steffens, 2000).

The role of organic acids, like citric acid or malic acid, as metal buffering substances has been suggested by a number of researchers (Prasad; 2009; Rauser, 2009). The mechanisms by which this may occur remain unclear as the accumulation of these organic acids upon metal exposure may merely reflect disturbances in metabolism.

b. Alteration of metal compartmentalization patterns.

Although metal tolerance may be prevalent at the cellular, tissue, organ level in plants, the metal compartmentalization processes taking place at the level of tissues and organs that contribute to survival of the intact plant. Significant differences have been reported between tolerant and non-tolerant plants of the same species, in the distribution of metals ions in their tissues. Organic complexes mediate transport of heavy metal ions from root to shoot via xylem**,** at least partially. It is therefore possible that low metal concentration in the shoots of any species could be a direct result of a reduced metal transport, instead of decreased translocation rate.

At the subcellular level, the central vacuole often contains high concentration of heavy metals: Zn and Ni, Cu and Pb or Cd (Brooks *et al.,* 2001). Altered compartmentalization patterns at the subcellular level, due to changes in tonoplast transferase system, modulation likely play an important role indifferential tolerance to heavy metals in plants.

c. Alteration of cellular metabolism

Plants have the ability to mode their metabolic processes in order to survive and grow in adverse conditions including the presence of phytotoxic metals (Cobbert and Goldsbrough, 2000). It has been hypothesized that changes in metabolism, other than the production of chelators or metal compartmentation could provide the plants with an adsorptive advantage (Prasad, 2009).

Hyperaccumulator plant species on the other hand may survive in a metal rich environment by avoiding damage to metal sessile metabolic processes by activation of alternative pathways or through over-production of cytosolutes, which detoxify the metal ions. Alternatively, enhanced metabolism and increased levels of metabolic energy for metal sequestration could represent potential mechanism involved in the survival of hyperaccumulator plants. An increased production of metal sensitive enzyme could also help counteract the metal inactivation of these critical enzymes, thereby maintaining biosynthetic processes and normal growth and (Verkleij and Schat, 2010).

d. Alteration of membrane structure

The final potential mechanism for metal ion-tolerance is the alteration of membrane structure. Evidence for metal ion induced changes in membrane structure originates from the studies quant wing ion leakage from roots or cells exposed to a variety of metal ions. Several ions including, Ni, CO, and Zn enhance K efflux from Zea may root segments. While Cd enhances solute leakage from leaf discs of *Phaseolus vulgans* (Cumming and Taylor, 2010). Copper induced potassium loss has also been observed in *Agrosfis capillans*, *Silene vulgaris* (De Vos and Schatt. 2009) and *Mimulus guttafus* (Strange and McNair, 2011). These reports provide circumstantial evidence for heavy metal induced alterations in plasma membrane structure.

2.4 Physiology of Metal up-take by Hyper-accumulators

2.4.1 Mechanism of absorption

The unusually high level of metal found in hyper-accumulating plants can be attributed to the physiological, biochemical and genetic processes inherent to these species. For the purpose of this review, we shall limit the discussion to the metal accumulation process, which has been recognized as an extreme physiological response in metal tolerant plants. The uptake of metals by plant roots involves several processes like: a) root interception of metal ions, b) entry of the metal ion into the roots through mass flow and diffusion, and c) translocation of the metal ions from the root to the shoot (Marschner, 2005).

The root uptake process is achieved by mobilizing metal bound to soi1 particles in the soi1 solution. The mobilization is reached in several ways (Salt *et al.,* 2005): **(a)** metal chelating molecules (phytosiderophores) secreted in to the rhizosphere, **(b)** specific plasma membrane bound metal reductases and **(c)** proton extrusion from roots.

The transport across of metal ions the plasma membrane has also gained attention recently. It is likely that the entry of metalions inside the plants, either through the symplast (intercellular) or the apoplast (extracellular), depends on the type of metal and the plant species under investigation. For example, cadmium is thought to enter the tissues by both **ML** indicates the illumination of the continuous modulated light, SP indicates abrief saturating pulse (~3s). +AL indicates the illumination of the continuous of the actinic light. - AL indicates the when the actinic light is switched off, and +FR is the illumination of the far red light (Schreiber *et al.,* 2006).

2.4.2 Mechanism(s) of Translocation

The mechanisms of metal translocation seem to be very similar to those for essential nutrients such as Fe or Ca, in as much as the transport from the root to the shoot takes place principally through the xylem. According to Hardiman *et al.,* (2004), Cd enters the stele through leakage and its translocation rate to the shoot is dependent on the root concentration. It is, therefore, reasonable to expect that a large part of metals would be retained in the stem during translocation through the xylem fluid. Prezemeck and Haase (2001) suggested phytochelatinmediated metal binding in the xylem Sap as a possible mechanism for metal translocation. However, Salt *et al.,* (2005) found that the translocation of Cd through the xylem Sap was not dependent of the production of phytochelatins in roots.

Several types of compounds have been proposed to be involved in metal absorption and translocation in hyperaccumulator plant species (Salt and Kramer, 2006). Among these, low molecular weight chelators such as citrate (Lee *et al.,* 2008) and free histidine in *Alyssumlesbiacum* (Kramer *et al.,* 2006) have been reported to play an important role in metal tolerance by forming nickelhistidine complexes. These complexes can reduce the solution concentration of the free metal ion preventing metal toxicity at cellular and sub-cellular level. Malate and malonate were reported to be the predominant nickel ligands complexes isolated from *Alyssumberfolonii* (Brooks *et al.,* 2001).

Similarly, high malate concentrations were also reported in the leaves of zinc accumulator *Thfaspicaerulescens* (Lee *et al.,* 2008). The presence of Pb-EDTA complexes in the xylem exudates of *Brassicajuncea*suggests a potential involvement of chelators in metal transport within the plants (Vassil et al., 2008)- It is likely that, phytochelatins are involved in trace-metal homeostasis, while organic acids, like citric acid or malic acid, merely act as metal buffering substances. The mechanisms by which this may occur still remain unclear, as the accumulation of chelators such as organic acids upon metal exposure, might in part reflect disturbances in metabolic status of the plant. Apart from these factors, heavy metal ion translocation within the plant is also influenced by other factors such as transpiration rate, root uptake, radial transport and xylem loading (Salt *et al.,* 2005).

According to Nan *et al.,* (2002), the uptake and transfer of heavy metals from soil to plants is a process of significant importance and determining of transfer factor (TF) and translocation index (Ti) have been considered as a key parameter to assess the availability of elements in soil and hyper-accumulation capacity of the plants. This is to say, a detailed study on the metal transfer from soils to plants and translocation to aerial parts could possibly shed more light on the metal accumulation potentials of plants species and their phytoremediation potentials. D'Souza *et al.,* (2013) stated that all plants with phytoremedial ability are not alike; some maybe metal-specific while others may perform well for metals in combination. The phytoremedial ability of plants is reported to be influenced by the mobility and availability of heavy metals in soil and plants, and thus the transfer factor (TF) and translocation index (Ti) can be used to assess the accumulation potential and hence the phytoremedial potential of plants.

2.5 Benefits and Application of Phytoremediation Technology.

The many benefits of phytoremediation include: helping industrial sites to comply with water quality guidelines using relatively inexpensive methodology, approximately 60-80 % less expensive, compared to the operational costs of traditional mining water treatment facilities such as high density sludge treatment and being aesthetically pleasing. Further internationally phytoremediation has been found effective at reducing contaminants, less disruptive and more environmentally friendly than current physical and chemical processes (Prasad as cited in Tangahu *et al.,* 2011).

Salido *et al.,* (2007) explained that phytoremediation techniques may also be more publicly acceptable, aesthetically pleasing, and less disruptive than the current techniques of physical and chemical process. Advantages of this technology are its effectiveness in contaminant reduction, low-cost, being applicable for wide range of contaminants, and in overall it is an environmental friendly method.

According to Salido *et al.,* (2007) phytoremediation benefits include but not limited to the following:

- ❖ Aesthetically pleasing
- ❖ Less disruptive than current techniques
- ❖ The effectiveness in contaminant reduction
- ❖ Low cost
- ❖ Applicable for wide range of contaminants
- ❖ Environmentally friendly method

The major advantages of the heavy metal adsorption technology by biomass are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials. Phytoremediation as possibly the cleanest and cheapest technology can be employed in the remediation of selected hazardous sites. Phytoremediation encompasses a number of different methods that can lead to contaminant degradation.

Phytoremediation is a low-cost option and inexpensive approach for remediating environmental media, particularly suited to large sites that have relatively low levels of contamination. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites

(Mwegoha, 2008). Phytoremediation potentially offers unique, low cost solutions to many currently problems of soil contamination. It is inexpensive (6–80% or even less costly) than conventional physicochemical methods, since it does not require expensive equipment or highly specialized personnel.

It is cost-effective for large volumes of water having low concentrations of contaminants and for large areas having low to moderately contaminated surface soils. It is applicable to a wide range of toxic metals and radionuclides and also useful for treating a broad range of environmental contaminants, including organic and inorganic contaminants. Phytoremediation is regarded as a new approach for the cleanup of contaminated soils, water, and ambient air. Phytoremediation research can also contribute to the improvement of poor soils such as those with high aluminum or salt levels. It is applicable to a range of toxic metals and radionuclides, minimal environmental disturbance, elimination of secondary air or water-borne wastes, and public acceptance.

Phytoextraction is considered as an environmentally friendly method to remove metals from contaminated soils in situ. This method can be used in much larger-scale clean-up operations and has been applied for other heavy metals. It is an aesthetically pleasing, solar-energy-driven cleanup technology and there is minimal environmental disruption and in situ treatment preserves topsoil. In Situ applications decrease the amount of soil disturbance compared to conventional methods. It can be performed with minimal environmental disturbance with topsoil left in a usable condition and may be reclaimed for agricultural use. The organic pollutants may be degraded to Carbon monoxide and Oxide of Hydrogen, removing environmental toxicity.

Phytoremediation can be an alternative to the much harsher remediation technologies of incineration, thermal vaporization, solvent washing, or other soil washing techniques, which essentially destroy the biological component of the soil and can drastically alter its chemical and physical characteristics as well as creating a relatively nonviable solid waste.

Many soil remediation technologies have been used over the last few decades, and phytoremediation has emerged to be one of the most cost effective and eco-friendly solution for soil metal contamination (Glass, 2000). In phytoremediation, plants are utilized to remove various hazardous substances present in the environment including organic compounds, inorganic ions, heavy metals and radioactive materials. As a consequence, the phytoremediation approach has gained much attention and numerous plants species have been tested for phytoremediation properties, including vegetable crops, ornamental flowers, trees, weeds and grasses.

Phytoremediation actually benefits the soil, leaving an improved, functional soil ecosystem at costs estimated at approximately one-tenth of those currently adopted technologies. It is the most ecological cleanup technology for contaminated soils and is also known as a green technology.

Another advantage of phytoremediation as asserted by Rakhshaee, *et al.,* (2009) is the generation of a recyclable metal-rich plant residue. Phytoremediation could be a viable option to decontaminate heavy-metal-polluted soils, particularly when the biomass produced during the phytoremediation process could be economically valorized in the form of bioenergy. The use of metal- accumulating bioenergy crops might be suitable for this purpose. If soils, contaminated with heavy metals, are phytoremediated with oil crops, biodiesel production from the resulting plant oil could be a viable option to generate bioenergy. In large-scale applications, the potential energy stored can be utilized to generate thermal energy. The success of the phytoextraction technique depends upon the identification of suitable plant species that can hyperaccumulate heavy metals and produce large amounts of biomass using established crop production and management practices.

Phytoremediation has been applied to or proposed for cleanup of many types of hazardous wastes, including toxic metals and man-made organic compounds.

Metals:

❖ Certain plants can take up large amounts of some toxic heavy metals from the soil. For example, some trees adapted to growth on serpentine soils in the South Pacific, which are naturally high in nickel, take up the metal and concentrate it in their tissues, so much that the sap of the trees is a bright blue.

❖ This phenomenon has inspired scientists and engineers to propose to develop plants that can "hyperaccumulate" heavy metals in their above-ground tissues so that, by harvesting them, the metals can be economically removed in an ecologically friendly manner.

Organics:

❖ Soluble Compounds

- The most important and widespread of groundwater pollutants are the chlorinated hydrocarbons, such as trichloroethylene. Plants can take up these carcinogens and break them down to harmless products such as chloride and carbon dioxide. Pioneering work in the UW phytoremediation labs has demonstrated biochemical pathways for plant transformations of trichloethylene and carbon tetrachloride.
- Another class of soluble compounds are some of the chemicals found in munitions, especially triazines such as RDX. We are also engineering plants to degrade these dangerous pollutants.

❖ Insoluble compounds (hydrophobic)

▪ Less soluble organic pollutants include polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls, as well as the munitions compound, TNT. Plants have a limited capability to take up these pollutants, but bacteria associated with their roots play a role in the degradation of many of these chemicals.

2.6 Significance of Phytoremediation Technologies in Environmental Clean-up

Plants can bioaccumulate xenobiotics in their above-ground parts, which are then harvested for removal. Plants may contribute to remediation in several ways, by reducing the leaching of contaminants, aerating soil, phytodegradation/transformation, phytovolatilization, evapotranspiration, and rhizoremediation (Galadima *et al.,* 2010).

The popularity of phytoremediation is based in part on the relatively low cost of phytoremediation, combined with the limited funds available for environmental cleanup as the costs associated with environmental remediation are staggering.

Currently, $6–8 billion per year is spent for environmental cleanup in the United States, and $25–50 billion per year worldwide (Eric and Andrew, 2017). Because biological processes are ultimately solar-driven, phytoremediation is on an average tenfold cheaper than engineering-based remediation methods such as soil excavation, soil washing or burning, or pump-and-treat systems (Glass, 2000). Since phytoremediation is usually carried out in situ, it is cost-effective and may reduce the exposure of the polluted substrate to humans, wildlife, and the environment.

Moreover, phytoremediation also enjoys popularity among the general public since it's a green clean technology (Dimitriu, 2014). When mechanisms like phytoextraction are adopted by the plant ultimately the plant material needs to be harvested. The resulting biomass can be utilized for heat and bioenergy production under specialized facilities that makes the process to be a potentially profitable technology (Chao *et al.,* 2014).

Phytoremediation can also help in controlling run off, soil erosion, lower air, water emissions and secondary waste production thus making it an attractive technology. Phytoremediation can thus offer a modest way to decrease the effects of global change by lowering energy usage, CO2 emission and waste emission during waste water treatment (Sridhar *et al,* 2011).

Bioremediation using microorganisms is often performed on extracted soil, in the controlled environment of a bioreactor. It can also be used for in situ remediation, but it has often been found that the cleaning bacteria can compete with local microbes, and that keeping them at high concentrations requires the addition of a lot of nutrients.

In comparison, phytoremediation is easier to manage because it is an autotrophic system of large biomass that requires little nutrient input.

2.7 Limitations of Phytoremediation Technology

According to European and Mediterranean Plant Protection Organization (EPPO), 2007, one of the limitations of phytoremediation is that this technology is not feasible if growing conditions are phytotoxic. They further stated that even though certain plants have adapted to growing in soil with high metal concentrations, plants can show short term or long term damage in phytotoxic environments where heavy metal concentrations are too high. Another limitation is that phytoremediation is a slow remediation technology that is dependent on the length of the growing season. Other limitations are that phytoremediation poses a risk to herbivores if they consume plants that have bioconcentrated heavy metals and bioremediation only works in shallow soils.

Phytoremediation is a time consuming process, potentially taking several growing seasons to remediate an area, further it is limited by the number of plants in the area and plant growth rates, root depth, soil chemistry, level of site contamination, contaminant concentrations, impacts of contaminated vegetation and climatic condition. The age of the plants can also be a limitation as younger plants have a greater ability to absorb ions, however, larger plants may compensate by their larger size (Tangahu *et al.,* 2011).

Inuwa *et al.,* (2007) asserted that despite the best attempts at waste avoidance, reduction, reuse, and recovery, landfill and disposal of metal still constitute a principal focus by environmental scientist. It has been observed that the larger the urban area, the lower the quality of the environment. So solid waste disposal and management have reached a critical stage in major towns and cities of Nigeria.

W. H. O. (2000) stated that environmental restoration of metal-polluted soils using a plant-based technology has attracted increasing interest in the last two decades. Phytoremediation has been developed as a cost effective and environmentally friendly remediation method of contaminated soils. It is an economically attractive approach to decontaminate soils polluted by heavy metals. Because of its relatively low costs, phytoremediation poses a viable approach to cleaning up soils.

In a study by Smical *et al.,* (2008) shows that the use of plants to extract and translocate metals to their harvestable parts (phytoextraction) is aimed at reducing the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. Some plant species have developed tolerance towards metals and others (hyper-accumulators) are characterised by their ability to accumulate high quantities of metals in their tissues. Hyper-accumulators are plants that achieve a plant-to-soil metal-concentration ratio (bioaccumulation factor) and shoot-to-root metal-concentration ratio (transfer factor) greater than one. The accumulation of these metals may vary from plant to plant and soil to soil. The metal availability to plants depends on total concentration of metals in the soil and the forms in which they occur, pH, organic carbon, cation-exchange capacity, stage of growth of plants, and microorganisms around the root zone (Dimitriu, 2014). If these factors are constant, the uptake of a metal by different plant species may be compared.

The work of Zakka *et al.,* (2014) identified that *Solanum melongena* showed bioaccumulation factor (BF) and transfer factor (TF) greater than 1 for Cd, Pb, and Mn; *Rumex acetosa* showed BF and TF greater than 1 for Mn and Zn, and TF was greater than 1 for Cu and Fe; *Lycopersicon esculentum* had only the TF for Fe, Pb, Mn, and Zn greater than 1. They asserted that the results of the experiment imply that *Solanum melongena* and *Rumex acetosa* plants can be effectively used for phytoremediation of Cd, Pb, Mn, and Zn from a dumpsite in Zaria, Kaduna State, Nigeria.

2.8 Phytoremediators

The term "hyper-accumulator' has been first used by Brooks, (1977) to describe plants that can survive (tolerate) or even thrive in soils contaminated with toxic metals. By definition metal hyper-accumulators are herbs, shrubs or even trees with the ability to concentrate heavy metals in their biomass to levels greater than 100 times the normal without exhibiting phytotoxicity symptoms (Lasat, 2000). Baker *et al.,* (2004) suggested that for a plant to be termed as a must accumulate > 0.1% (by dry weight) of Co, Cu. Cr, Pb or Ni and > 1% (by dry weight) of Mn or Zn in its natural habitat. To date, approximately 400 plant species from at least 45 plant families have been reported to hyper-accumulate metals (Salt *et al.,* 2005). Most hyper-accumulators accumulate Ni, about 30 absorb either CO, Cu, and/or Zn**,** even fewer species accumulate Mn and Cd, and there are no known natural Pb-hyperaccumulators (Reeves and Baker, 2000). The accumulation of metals could represent a defense mechanism against insect and herbivores (Boyd and Martens, 2004).

An ideal plant species for phytoremediation should have either one of the following characteristic combinations (US EPA**,** 2000): **a)** a low biomass plant with a very high metal accumulation capacity, or **b)** a high biomass plant with enhanced metal uptake potential. In addition to these characteristics, versatility of the candidate plant to tolerate and at the same time accumulate multiple metal contaminants and/or metal-organic mixtures would be an asset for any phytoremediation system (Chaney *et al.,* 2000). Despite increasing reports, most species of metal-accumulator plants are metal-specific, have a small biomass, slow growth rate, and require careful management of agricultural techniques under field conditions (Gleba *et al.,* 2009). It is also important to acknowledge the possible environmental impacts of introducing a new plant species that could represent an environmental risk (US EPA 2000). In addition, most metal accumulator plants are collected from natural habitats and the use and performance of these plants in other regions could be limited by the lack of optimized agronomic practices in the new regions. In this context, the identification domesticated metal accumulator plant species, coupled with a better understanding of the physiological mechanisms underlying metal accumulation, would facilitate development of effective remediation systems.

Preliminary surveys of domesticated horticultural plants species as candidates for phytoremediation resulted in identification of plants of the genus *chromolaena odarata, Imperata cylindrical, Sida acuta* and *Helianthus annuus.*

Additionally, Murch *et al.*, (2007) found that *Pelargonium*sp. plants when treated with a growth regulator (Thidiazuron) exhibited increased uptake of nutrients and heavy metals (such as iron). In view of these, plants belonging to the *Pelargonium* sp. were selected as candidate plants for assessing their efficiency in phytoremediation of metal contaminated soils.

2.8.1 *Imperata cylindrica*

Imperata cylindrica (commonly known as cogon grass, spear grass, kunai grass, blady grass, alang-alang, cotton wool grass) is a perennial rhizomatous grass and it belongs to the family Poaceae. Red cultivars of the species grown as ornamental plants are known as Japanese bloodgrass.

Imperata cylindrica is an extremely aggressive invader with the capability of invading a range of sites. It forms dense, usually circular infestations that exclude all other vegetation. It is a perennial rhizomatous grass native to east and southeast Asia, India, Micronesia, Melanesia, Australia, and eastern and southern Africa. It grows from 0.6 to 3 m (2 to 10 feet) tall. The leaves are about 2 cm wide near the base of the plant and narrow to a sharp point at the top;

the margins are finely toothed and are embedded with sharp silica crystals. The main vein is a lighter colour than the rest of the leaf and tends to be nearer to one side of the leaf. The upper surface is hairy near the base of the plant while the underside is usually hairless. Roots are up to 1.2 meters deep, but 0.4m is typical in sandy soil.

Wunderlin (2008), analyse that *Imperata cylindrica* is planted extensively for ground cover and soil stabilization near beach areas and other areas subject to erosion. Other uses include paper-making, thatching and weaving into mats and bags. It is used in traditional Chinese medicine.

A number of cultivars have been selected for garden use as ornamental plants, including the red-leaved 'Red Baron', also known as Japanese blood grass. Young inflorescences and shoots may be eaten cooked, and the roots contain starch and sugars and are therefore easy to chew. It is used as an ingredient in the skincare brand Kiehl's Ultra Facial Cream for its high concentrations of potassium which provides a hydrating effect. Their website also states that indigenous native Australians may have used the plant as a substitute for salt due to its high saline content.

Ali *et al.*, (2013; Sinha *et al.*, (2013) stated that tropical grasses are fast growing plants with good tolerance for growth under a wide range of soil, rainfall and temperature conditions. Due to its good adaptation to environmental stress, high biomass production and fast growth rate; grasses are often used to be the preferable choice for phytoremediation compared to shrubs and trees. Hence, Ng et al., (2016) carefully selected three tropical grasses, viz: Vetiver (*V. zizanoides*), Imperata (*I. cylindrical*) and Pennisetum (*P. purpureum*) in their study.

2.8.2 *Helianthus annuus*

Helianthus annuus, the common sunflower, is a large annual forb of the genus Helianthus grown as a crop for its edible oil and edible fruits. This sunflower species is also used as bird food, as livestock forage (as a meal or a silage plant), in some industrial applications, and as an ornamental in domestic gardens. The plant was first domesticated in the Americas. Wild *Helianthus annuus* is a widely branched annual plant with many flower heads. The domestic sunflower, however, often possesses only a single large inflorescence (flower head) atop an unbranched stem. The name sunflower may derive from the flower's head's shape, which resembles the sun, or from the impression that the blooming plant appears to slowly turn its flower towards the sun as the latter moves across the sky on a daily basis.

The plant has an erect rough-hairy stem, reaching typical heights of 3 metres (9.8 ft). The tallest sunflower on record achieved 9.17 metres (30.1 ft). Sunflower leaves are broad, coarsely toothed, rough and mostly alternate. What is often called the "flower" of the sunflower is actually a "flower head" or pseudanthium of numerous small individual five-petaled flowers ("florets"). The outer flowers, which resemble petals, are called ray flowers. Each "petal" consists of a ligule composed of fused petals of an asymmetrical ray flower. They are sexually sterile and may be yellow, red, orange, or other colors. The flowers in the center of the head are called disk flowers. These mature into fruit (sunflower "seeds"). The disk flowers are arranged spirally. Generally, each floret is oriented toward the next by approximately the golden angle, 137.5°, producing a pattern of interconnecting spirals, where the number of left spirals and the number of right spirals are successive Fibonacci numbers. Typically, there are 34 spirals in one direction and 55 in the other; however, in a very large sunflower head there could be 89 in one direction and 144 in the other.

Sunflower oil, extracted from the seeds, is used for cooking, as carrier oil and to produce margarine and biodiesel, as it is cheaper than olive oil. A range of sunflower varieties exist with differing fatty acid compositions; some 'high oleic' types contain a higher level of monounsaturated fats in their oil than even olive oil.

The cake remaining after the seeds have been processed for oil is used as a livestock feed. The hulls resulting from the dehulling of the seeds before oil extraction can also be fed to domestic animals. Heuzé *et al.,* (2018) some recently developed cultivars have drooping heads. These cultivars are less attractive to gardeners growing the flowers as ornamental plants, but appeal to farmers, because they reduce bird damage and losses from some plant diseases. Sunflowers also produce latex, and are the subject of experiments to improve their suitability as an alternative crop for producing non allergenic rubber.

Traditionally, several Native American groups planted sunflowers on the north edges of their gardens as a "fourth sister" to the better known three sisters combination of corn, beans, and squash. Annual species are often planted for their allelopathic properties. (Adler & Tina 2006)

However, for commercial farmers growing commodity crops, the sunflower, like any other unwanted plant, is often considered a weed. Especially in the Midwestern US, wild (perennial) species are often found in corn and soybean fields and can have a negative impact on yields (Singh and Kalamdhad, 2011).

Sunflowers can be used in phytoremediation to extract toxic ingredients from soil, such as lead, arsenic and uranium, and used in rhizofiltration to neutralize radionuclides and other toxic ingredients and harmful bacteria from water. They were used to remove caesium-137 and strontium-90 from a nearby pond after the Chernobyl disaster, and a similar campaign was mounted in response to the Fukushima Daiichi nuclear disaster.

The sunflower (*Helianthus annuus L.*) is an annual plant in the family *Asteraceae* has thus been identified as one of the target species that has great potential as a phytoextractor due to the fact that it produces large amounts of biomass, capable of hyper accumulating heavy metals in its harvestable parts (Stems, leaves and roots) and it grows quickly (Encheva *et al.,* 2014).

Other species of sunflowers:

Encheva *et al.,* (2014) stated that there are many species in the sunflower genus Helianthus and many species in other genera that may be called sunflowers. The Maximillian sunflower (*Helianthus maximiliani*) is one of 38 species of perennial sunflower native to North America. The Land Institute and other breeding programs are currently exploring the potential for these as a perennial seed crop. The sunchoke (Jerusalem artichoke or *Helianthus tuberosus*) is related to the sunflower, another example of perennial sunflower. The Mexican sunflower is Tithonia rotundifolia. It is only very distantly related to North American sunflowers. False sunflower refers to plants of the genus Heliopsis.

Sunflower hybrids

In today's market, most of the sunflower seeds provided or grown by farmers are hybrids. Hybrids or hybridized sunflowers are produced by crossbreeding different types and species of sunflower, for example crossbreeding cultivated sunflowers with wild species of sunflowers. By doing so, new genetic recombinations are obtained ultimately leading to the production of new hybrid species. These hybrid species generally have a higher fitness and carry properties or characteristics that farmers look for, such as resistance to pathogens.

Hybrid, *Helianthus annuus* dwarf does not contain the hormone gibberellin and does not display heliotropic behavior. Plants treated with an external application of the hormone display a temporary restoration of elongation growth patterns. This growth pattern diminished by 35% 7–14 days after final treatment.

Hybrid Male Sterile and Male Fertile flowers that display heterogeneity have a low crossover of honeybee visitation. Sensory cues such as pollen odor, diameter of seed head, and height may influence pollinator visitation of pollinators that display constancy behavior patterns.

Threats and diseases

Martin, *et al.,* (2016) lamented that one of the major threats that sunflowers face today is Fusarium, a filamentous fungi that is found largely in soil and plants. It is a pathogen that over the years has caused an increasing amount of damage and loss of sunflower crops, some as extensive as 80 percent of damaged crops.

Downy mildew is another disease to which sunflowers are susceptible. Its susceptibility to downy mildew is particular high due to the sunflower's way of growth and development. Sunflower seeds are generally planted only an inch deep in the ground. When such shallow planting is done in moist and soaked earth or soil, it increases the chances of diseases such as downy mildew.

Another major threat to sunflower crops is broomrape, a parasite that attacks the root of the sunflower and causes extensive damage to sunflower crops, as high as 100 percent.

The work of Saurabh *et al.,* (2017) revealed that accumulation of metal contaminants in soil as a result of various industrial and anthropogenic activities has reduced soil fertility significantly. Phytoextraction of metal contaminants can improve soil fertility and provide inexpensive feedstock for biorefineries. They further investigated the hyperaccumulation capacity of sunflower (*Helianthus annuus*) biomass by cultivating these plants in various concentrations of metal contaminants. Sunflowers were grown in soils contaminated with various levels of heavy metals (10–2,000 mg/kg dry soil). The degree of metal uptake by different parts of the biomass and the residual concentration in the soil were estimated through inductively coupled plasma mass spectrometry. An almost 2.5-fold hyperaccumulation of Zn was observed in the leaf and flower biomass compared with the concentration in the soil.

Lin *et al.,* (2009) explained that apart from industrial applications of dry sunflower biomass, growing sunflowers (SF) have shown the potential to absorb various metal contaminants such as Ni, Cu, As, Pb, and Cd. Most of the heavy metals are non-biodegradable and toxic to numerous organisms, including humans; therefore, they must be removed from ecosystems. Because growing SF can accumulate high concentrations of metal contaminants, they are considered "hyperaccumulators" of heavy metals.

The work of Lin *et al.,* (2009) equally revealed that Sunflowers are highly adsorbent and have been used to bioaccumulate heavy and toxic metals. In the case of Ni, As, and Cd at low concentrations (10–100 mg/kg dry soil), Cd (161 mg/kg dry wt) accumulated to its highest concentration in sunflower leaves and flowers, whereas in the stems only 78.4 mg/kg dry wt was accumulated. Similarly, for Ni and As, flowers and leaves showed higher phytoremediation capacity than stems. In the case of Zn, as the initial concentration of the metal ion in the soil increased, its absorption by the plant also increased. Nearly 2.5-fold more Zn was observed in flowers and leaves compared with the concentration in the soil (i.e., 300 mg-Zn/kg dry soil). The concentration absorbed by the stems alone was nearly 1.5-fold more than the supplied Zn. When Cu was supplied in moderate concentrations (300 and 450 mg/kg dry soil) to the growing *H. annuus*, the least accumulation was observed in all plant parts. Only 75.3 mg of Cu was present in the leaves and flowers, and only 10.3 mg was present in the stems of the Sunflowers. With regard to the accumulation of the highest concentrations of Pb (2000 mg/kg dry soil), the stems showed the highest capacity, storing 252 mg of Pb compared to the 149 mg accumulated by leaves and flowers. Even at a moderate concentration of Pb (1000 mg/kg dry soil), the stems showed the highest accumulation capacity (136 mg). However, when a lower concentration (250 mg/kg dry soil) of Pb was supplied, both stems (29.1 mg) and leaves and flowers (26.3 mg) accumulated the metal at similar concentrations. The heavy metal concentrations in sunflower tissues growing in control soil were analyzed and used as a control.

2.8.3 *Sida acuta*

Sida acuta, the common wireweed, is a species of flowering plant in the mallow family, Malvaceae. It is believed to have originated in Central America, but today has a pantropical distribution and is considered a weed in some areas.

In northern Australia, *Sida acuta* is considered an invasive species, and the beetle Calligrapha pantherina has been introduced as a biological control agent in an attempt to control the plant.

Description

Plant - Undershrub, with mucilagenous juice, aerial, erect, cylindrical, branched, solid, green.

Leaves - Alternate, simple, lanceolate to linear, rarely ovate to oblong, obtuse at the base, acute at the apex, coarsely and remotely serrate; petiole much shorter than the blade; stipulate, stipules free-lateral, unequally paired at the node, reticulate venation.

Inflorescence - Cymose

Flower- Small, axillary, 2-3 in a cluster; pedicels jointed at the middle, epicalyx absent, complete, bisexual, regular, actinomorphic, hypogynus, pentamerous, yellow.

Calyx- Sepals 5, gamosepalous, campanulate, slightly accrescent, persistent, valvate.

Corolla - Petals 5, polypetalous but slightly connate below and jointed with the staminal column, twisted.

Androecium - Stamens many, monadelphous, arranged on the staminal column; staminal column is shorter than the petals, divided above into numerous filaments, anthers monothecus, reniform, basifixed, filament short, extrorse.

Gynoecium- Carples 5, syncarpous, ovary superior, penta or multilocular with axile placentation, one ovule in each locule; style 1, passing through the staminal tube; stigma globular, correspond to the number of carples.

Fruit - A schizocarpic mericarp, seed 1 in each mericarp.

2.8.4 *Chromolaena odorata*

Chromolaena odorata is a tropical and subtropical species of flowering shrub in the sunflower family. It is native to the Americas, from Florida and Texas in the United States south through Mexico and the Caribbean to South America. It has been introduced to tropical Asia, West Africa, and parts of Australia. Common names include Siam weed, Christmas bush, devil weed, camphor grass, common floss flower, communist green and triffid (Padey and Madhuri, 2009).

Description

Chromolaena odorata is a rapidly growing perennial herb. It is a multi-stemmed shrub to 2.5m (100 inches) tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas it becomes etiolated and behaves as a creeper, growing on other vegetation. It can then become up to 10m (33 feet) tall. The plant is hairy and glandular and the leaves give off a pungent, aromatic odour when crushed. The leaves are opposite, triangular to elliptical with serrated edges. Leaves are 4–10 cm long by 1–5cm wide (up to 4 x 2 inches). Leaf petioles are 1–4cm long. The white to pale pink tubular flowers are in panicles of 10 to 35 flowers that form at the ends of branches. The seeds are achenes and are somewhat hairy. They are mostly spread by the wind, but can also cling to fur, clothes and machinery, enabling long distance dispersal. Seed production is about 80000 to 90000 per plant. Seeds need light to germinate. The plant can regenerate from the roots. In favorable conditions the plant can grow more than 3cm per day.

Chromolaena odorata contains carcinogenic pyrolizidine alkaloids. It is toxic to cattle. It can also cause allergic reactions. Recent research has shown the plant is larvicidal against all major mosquito vectors (Gunasekera, 2009).

Ali *et al.,* (2013) carried out an analysis on the Siam weed, *Chromolaena odorata* Family Asteraceae, and it was found to be a new Lead (Pb) hyperaccumulator by means of field surveys on Pb soil and hydroponic studies. Plants from field collection accumulated 1377 and 4236 mg kg^{-1} Pb in their shoots and roots, respectively, and could tolerate soil Pb concentrations up to 100 000 mg kg^{-1} with a translocation factor of 7.62. They further investigated that the percentage uptakes of Pb, Cd, and Zn by *C. odorata* increased with increasing metal concentrations.

2.9 Selection of Plant Systems for Remediation of Heavy Metals

For the removal of heavy metals from the environment, the selection of an appropriate plant with certain desirable characteristics is one of the most important preliminary steps in phytoremediation research. Though several plants have shown the ability to remediate contaminated soils; non edible plants are generally selected to be applied onto heavy metal contaminated sites. Most of the studies on phytoremediation of heavy metals demonstrate their removal through either degradation of the heavy metals, extraction of the heavy metals or the adsorption and/or accumulation of the heavy metals (Bharti, 2012).

Compounds accumulated in the plant roots could be further translocated to shoots and leaves. To prevent the accumulated heavy metals or their metabolites from entering the food chain, the use of non edible plants is always preferred. Different types of grasses, ferns, weeds or agricultural wastes have been suggested and tested for the removal of heavy metals. *Phragmites* species have shown immense potential to remediate waste waters from industries such as textile industry. *Phragmites australis,* a reed which is a component of the wetland community has been extensively studied for remediation of textile effluents and mainly with respect to the removal of the dye, Acid Orange 7 (Encheva *et al.,* 2014).

Many native populations of *Phragmites autralis* are benign in that they pose little or no threat to other species. Among 10 different species of macrophytes screened, *Phragmites karka* was found to have broad amplitude of pH tolerance and was found to be growing well in alkaline, neutral and acidic textile wastewaters resulting in considerable shoot density and biomass to achieve maximum translocation of water and assimilation of nutrients. This makes the plants highly suitable for the treatment of industrial waste waters that may be contaminated with different types of acidic as well as basic heavy metals. Moreover, the good growth of underground organs in these species thereby provides maximum surface area to assimilate pollutants (Dimitiru, 2014).

Thus, the plant should be fast growing and should have a deep rooted system that enables it to reach the pollutants easily. Larger biomass and surface area of the plant system can facilitate more efficient removal of the heavy metals. Thus, a plant that has the ability to remove heavy metals from the environment and also have a good biomass can prove to be potent for phytoremediation. Not all plants will be able to demonstrate similar responses or have similar removal rate for all heavy metals. Hence, extensive screening of different plant species can help us to understand the selective abilities of a particular plant to remove a heavy metal or a group of heavy metals. Plants that degrade the heavy metals into non toxic products are preferable for phytoremediation.

Hence, the selection of the plant will depend upon the genetic makeup of the plant that manifests in terms of varied enzyme activities in the plant and differential absorptive capacities resulting into variable patterns for the removal of heavy metals. Moreover, all the plants selected should be in the same stage of growth and should have almost equivalent dry weights and should have almost similar root and shoot lengths that can help to achieve reproducibility of results. Further, the plants selected should preferably be from the same area since factors such as age of the plant, soil conditions, nutrient status, light availability etc. are factors that can affect the removal of the heavy metal. In addition, the use of flowering plants for the removal of heavy metal would offer aesthetically appealing systems and will serve dual purposes of bioremediation and will also allow the flowers to be used for decorative purposes, while a few other plants could also be used for bio-energy production, thus serving economic benefits (Jenne *et al.,* 2011).

The plants selected for studies concerned with this dissertation have also shown some characteristics that have been desirable for phytoremediation studies.

2.9.1 Use of Whole Plants

In vitro plantlets can offer excellent tools for phytoremediation studies. In whole plants, the metabolic fate of a compound could depend on its translocation from roots to leaves if the enzymes required for further conversion are located in the aerial parts of the plant. In contrast, metabolism in plant suspension or hairy root cultures is not subject to this restriction. Although differences in the xenobiotic metabolism between whole plants and cell cultures are mostly quantitative than qualitative, the significant discrepancies do occur. Toxicity and tolerance mechanisms may also show differences in whole plants and hairy roots or suspension cultures. Plantlets of *Brassica juncea* have been used for studying copper tolerance (Singh *et al,* 2011).

2.9.2 Use of Hairy Roots

Hairy roots are produced by genetic transformation using *Agrobacterium rhizogenes*; shooty teratomas are generated using particular strains of *Agrobacterium tumefaciens*. Hairy roots grow relatively quickly and do not require exogenous hormones in the medium. They offer high genetic and biochemical stability. Extensive root proliferation by *Agrobacterium rhizogenes*, generally considered an undesirable characteristic, may find good utility for phytoremediation as roots will portray larger penetrating ability to retrieve thecontaminant from deeper soils, and also the enzymatic degradation (Encheva *et al.,* 2014).

As an organized tissue, roots are closer in structure and function to the organs of whole plants, thereby offering a greater degree of authenticity with regard to their biological behaviour and properties. *Chromolaena odarata* hairy roots have been studied for the degradation of the heavy metals (Oladipo *et al.,* 2012).

2.9.3 Use of cell suspension cultures

Plant cell cultures provide excellent tools for investigating the metabolic fate of heavy metals. Because callus and suspended plant cells lack many of the barriers used by whole plants to regulate penetration of chemicals from the environment, such as leaf wax, bark, cuticles, epidermis, and endodermis, and do not depend on translocation processes for tissue-specific metabolic activity, greater and more uniform uptake of external components is generally expected in plant cell cultures.

Thus, there is a general expectation that uptake of contaminants will be greater in plant cell cultures can whole plants because of the uniform exposure of plant cells to the contaminant. Plant cell cultures are relatively homogenous and standardization procedures can be easier which helps improve the reproducibility of results as compared to whole plants (Doran, 2009).

Rhubarb cell suspension culture has been used for its dye accumulative properties. Plant cell cultures can thus enable us to work with systems which are easier to work with than whole plants. Because of the reduced amount of starch and chlorophyll in cultured plant cells, isolation of the products formed after metabolism of the dye molecules could be easier and require fewer purification steps. Thus, plant cell cultures form ideal systems for dye degradation studies with respect to understanding the biochemical basis for the removal of heavy metals.

Plant tissue cultures have been employed for the removal of a variety of pollutants from the environment. But, to the best of our knowledge there are no reports where *in vitro* plantlets have been used for heavy metal degradation studies.

Moreover, there are hardly any reports where plant cell suspension cultures and hairy root cultures have been used for heavy metal degradation studies.

2.10 Plant Species for Phytoremediation, an ideal prototype

The potential for any plant species to remediate successfully heavy metal contaminated sites depends on al1 of the following prerequisite factors: **a)** the amount of metals that can be accumulated by the candidate plant, **b)** the growth rate of the plant in question, and **c)** the planting density (Saxena *et al.,* 2009).

The growth rate of a plant in a chemically contaminated soi1 is important from the perspective of biomass. The rate of metal removal from the soils can be calculated if information on the above mentioned parameters is available. An ideal plant species for phytoremediation should have either one of the following characteristic combinations (US EPA, 2000): a) a low biomass plant with a very high metal accumulation capacity, or b) a high biomass plant with enhanced metal uptake potential. In addition to these characteristics, versatility of the candidate plant to tolerate and at the same time accumulate multiple metal contaminants and/or metal-organic mixtures would be an asset for any phytoremediation system.

2.11 Metal Hyper-accumulators for Terrestrial Ecosystems

The first report of a hyper-accumulator plant goes as far back as 1885 when Baurnann analysed for zinc specimens of Viola *calaminana* and *Thlaspicalaminare* growing over the calamine deposits of Aachen, Germany. In 1948, Vergagno Garnbi discovered the unusual hyperaccumulation of nickel by the Tuscan serpentine plant *AIyssumbertolloni.* Hyperaccumulators are taxonomically well represented throughout the plant kingdom (Baker *et al.,* 2004).

For example, *Seberfiaacuminata* (a small tree) exudes Sap that contains up to 25% Ni by dry weight (Baker *et al.,* 2004). Another example is *Thlaspicaerulescens*a member of the Brassicaceae family that can accumulate up to 4% zinc in its tissue without any visible signs of damage (Brown *et al.,* 1994). Despite a large distribution throughout the plant kingdom, most of the commonly known hyperaccumulators belong to the Brassicaceae family (Kumar *et al.,* 2005). They probably inherited this characteristic from wild members of the family, which are known to thrive in metal rich environments and accumulate metals in their roots and shoots (Baker *et al.,* 2004).

Environment Canada's Environmental Technology Advancement Directorate has recently released the PHMOREM data base, an interactive electronic database of more than 700 plants, lichens, algae, fungi and bryophytes that have demonstrated an ability to tolerate, accumulate or hyperaccumulate a range of 19 different metals. Species that show considerable potential to date include sunflowers, ragweed, cabbage, lndian mustard, geranium and jack pine. Accompanying this database are 35 different search fields containing additional geographical, regulatory and eco-physiological data on each species. This allows the owners and managers of contaminated sites to choose the species that suit their site conditions, and take the steps necessary to secure regulatory approval for their use (An additional database (PhytopetO - available in CD format)- has been developed in order to describe plants species with a demonstrated ability for tolerance to petroleum hydrocarbons and the capacity to reduce contaminant levels in terrestrial or wetland environments (Farrell *et al.,* 2000). These plants grow in the Canadian prairies and boreal plains, but may have useful applications in other environments as well. The rigorous of the seasonal changes of Canada's climate have forced Canadian species to be highly adaptive.

These plants may possess genetic material that supports the development of agricultural crops that can withstand greater temperature ranges. The database contains also botanical surveys of a number of "historkal" (weathered) hydrocarbon contaminated (adjacent and non-contaminated) sites in Alberta and Saskatchewan.

2.12 Factors Influencing Heavy Metal Availability and Uptake by Plants

The composition of the soi1 at a contaminated site can be extremely diverse and the heavy metals present can exist as components of several different fractions (Salt *et al.,* 2005): (1) in soi1 solution, as free metal ions and soluble metal complexes, (2) adsorbed to inorganic soil constituents at ion exchange sites, (3) bound to soi1 organic matter, (4) precipitated such as oxides, hydroxides, carbonates, and (5) embedded in structure of the silicate minerals.

Soi1 sequential extractions are employed to isolate and quantify metals associated with different fractions. For phytoextraction to occur, contaminants must be associated with the first two fractions listed above (Lasat, 2000). Some metals, such as Zn and Cd, occur primarily in exchangeable, readily bioavailable form while Pb, occur as soil precipitate, which a less bioavailable form (Lasat, 2000). Plants grown in metal-enriched substrate take up metal ions at varying degrees. This uptake is largely influenced by the bioavailability of the metals which is, in turn, determined by both external (soil associated) and internal (plant-associated) factors.

2.12.1 Soil Associated Factors

The success of any phytoremediation scheme relies on the availability of metals in the soil, which in turn is controlled by: a) chemical (pH, Eh, CEC, metal speciation), b) physical (size, texture, clay content, % organic matter) and c) biological (bacteria, fungi) processes and their interactions (Ernst et al. 2002).

2.12.1.1 PH

The chemical forms of heavy metals in soil are affected by modifications to the soil pH. An increase in pH (basic range) results in higher adsorption of Cd, Zn, Cu to soi1 particles and reduces the uptake of Cd, Zn, Pb by plants (Kuo *et al.,* 2005). On other hand, acidification increases the metal absorption by plants through a reduction of metal adsorption to soi1 particles (Brown *et al.,* 2004). For example, the metal cation concentration was estimated at 9080 pgll cations in an acidic soil, compared to 17 pgll cations at neutral pH. Soil pH affects not only metal bioavailabilty, but also the process of metal uptake by roots. This effect could be metal specific. For example, in *Thlaspi caerulescens,* Zn uptake in roots showed a small pH dependence, while in the case of Mn and Cd the uptake was more dependent on soi1 acidity (Brown *et al.,* 2004).

2.12.1.2 Redox potential (Eh)

The redox potential of the soil is a measure of the tendency of the soi1 solution to accept or donate electrons. As the redox potential decreases, heavy metal ions are converted from insoluble to soluble forms, thus increasing - bioavailability. It is likely that a lower pH and Eh of the soil would enhance the mobility of most metals Cation Exchange Capacity **(CEC).**

The cation exchange capacity of the soi1 is a measure of the ability of the soi1 to retain metal ions. The cation exchange capacity increases with increasing clay content in the soi1 while the availability of the metal ions decreases. Thus, the higher the cation exchange capacity (CEC) of the soil, the greater the sorption and immobilization of the metals.

In acidic soils, metal desorption from soi1 binding sites into solution is stimulated due to H+competition for binding sites. Modulating the CEC would, therefore, result in increased or decreased availability of metals to plants-

2.12.1.3 Soil type

The bioavailability of heavy metals in the soi1 also depends on the texture of the soil. A gradient of metal ion availability exists in varying soi1 types with the availability being lowest in clay soils followed by clay loam and finally loam and sand. Similarly, heavy metal concentrations in soi1 are also dependent on the soi1 order; Gleysols and Luvisols have the highest concentration, followed by Brunisols and Podzols. However, this observation can also be related to soil texture because Gleysols and Luvisols have a higher clay content, compared to Brunisols and Podzols (Webber and Singh, 2005). Normally, a higher level of heavy metal can be retained in fine-textured soils such as clay and clay loam, compared to coarse textured soils such as sand. This is in part due to the low bioavailability of these metal ions, or reduced leaching as metals are bound to the soi1 matrix in fine-textured soils (Webber and Singh, 2005). The complexation of heavy metals with organic matter, humic acid in particular, has been well documented (Friedland, 2000). A high organic matter content enhances the retention of the metals, drastically reducing the metal availability.

2.12.1.4 Chelates

An essential component of the bioavailability process is the exudation of metal chelating compounds by plant roots (ex. phytosiderophores). These chelators are synthesized by plants and can mobilize heavy metals such as copper, lead and cadmium by formation of stable complexes (Mench *et al.,*2005). Chelators are usually low molecular weight compounds such as sugars, organic acids, amino acids and phenolics that can change the metal speciation and thus, metal bioavailability.

Apart from the chelating agents produced by plants, addition of synthetic chelating agents to contaminated soils was shown to increase substantially the metal solubility in the soi1 (Salt *et al.,* 2005, Cunningham and Berti, 2009). It is likely that in contaminated soils, chelator application enhances the formation of metal-chelate complexes reducing the sorption of metals to the soi1 particles (Huang *et al.,* 2007). To date, numerous studies have focussed on evaluating the effect of adding synthetic chelates such as ethylene diaminetetracetic acid (EDTA), ethyleneglycoltetracetic acid (EGTA) and citrate on the uptake of metals by plants (Salt *et al.,* 2005). For example, the addition of EDTA to a Pb contaminated soi1 increased the shoot Pb concentration of corn (Zea *mays)* and pea (Pisum sativum) from 500 mglkg to more than 10,000 mg/kg (Huang *et al.* 1997) with a concomitant, more than 1000-fold, increase in available metal content of the soi1 solution (Cunningham and Berti, 2009). Increased absorption of heavy metal from soi1 into the roots of *Brassica juncea* has also been attributed to chelators. Chelatar-assisted phytoextraction approach is applicable to several metals of interest (Zn, Cd, Ni, Se, As, Cr, U) (for an in depth review, see Salt *et al.,* 2005).

However, the adverse effects of applying EDTA or other chelators such as the leaching of **Pb EDTA** to subsurface water has been encountered in an in situ phytoextraction field test (Minnesota) and in and ex situ phytoextraction, were the leachate collected had 160 -1 80 mg PbIL (Chaney, Phflonet Network, 2001).

2.12.2 Plant Associated Factors

The genetic make-up of the plant greatly influences its metal uptake potential (Chen *et al.,* 2007). Irrespective of which approach of phytoremediation is used, two major plant-associated factors need to be satisfied in order to devise a successful remediation strategy (Saxena *et al.,* 2009). These characteristics, viz., metal tolerance and metal hyperaccumulation potential, clearly define phytoremediation candidate plants. The metal accumulation potential of different plant species varies markedly and is highly correlated to their genotype. Huang *et al.* (2007) have found that Pb accumulation varies significantly in different species grown in similar environments. It is therefore crucial that a selection scheme be employed to select the best hyperaccumulator genotypes even within a specific species to ensure the success of phytoremediation procedure.

Rhizospheric processes

The conditions that exist in root-soi1 interface (rhizosphere) differ in many aspects from those of the bulk soi1 and are, in part, responsible for the manifestation of certain plants adaptations. Conditions in the rhizosphere have a significant influence on the mineral nutrient uptake and water relations (Marschner, 2005). The extent to which plant roots can modify these conditions is extremely important for the survival of plants under adverse conditions (Marrschner, 2005). The pH of the rhizosphere can, in some instances, differ from the bulk soi1 pH by up to two units. Any modifications to the rhizospheric pH values would, in turn, have an impact on the cation/anion uptake ratio, the release of carbonated and the excretion of organic acids. Acidification of the rhizosphere through exudation of protons from the roots has also been shown to enhance uptake and accumulation of metals (Marschner, 2005).

Rhizospheric microorganisms also play a significant role in metal availability. For instance, several strains of *Pseudomonas* and *Bacillus* were able to increase the amount of Cd accurnulated by 2-week-old *Brassica juncea* seedlings grown in hydroponics (Salt *et al.,* 2005). Finally, the association between plant root system and mycorrhizai colonies has been suggested to influence metal uptake in plants. The plant-mycorrhizal associations in phosphorus deficient plants led to enhanced uptake of phosphates as well as copper and zinc. A similar enhancement in cadmium and lead uptake was observed when plants were infected with vesicular arbuscular mychorrizae. A large number of ectomychomzal (ECM) fungi have also been found to be effective in increasing heavy metal availability to their host plant (Marschner, 2005). For example, birch seedlings inoculated with the ECM fungi Lactarius *nifus* or *Sclerofinia navidium* had increased Ni levels under high Ni exposure. The increased tolerance to heavy metals in this scenario was a result of sequestration of heavy metals in the fungal structure either at the hexametrical mycelium or in the sheath, which resulted in lower concentration of metal ions in the soil solution around the host roots.

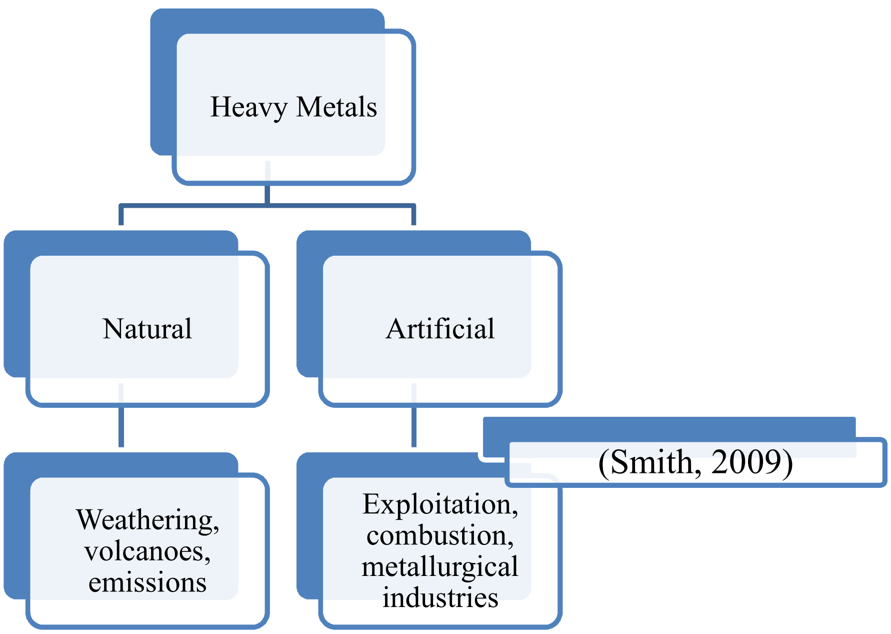
2.12.3 Agronomic practices

Chaney *et al.* (2009) has discussed the importance of employing effective agronomic practices. These authors investigated the effect of soi1 acidification on Zn and Cd phytoextraction and proposed the use of (NH&S04 as a soi1 additive to provide nutrients (N and S) needed for high yield, and to acidify the soil for greater metal bioavailability. These authors found that after phytoremediation the soi1 can be limed to elevate the pH near a neutral value, so that normal farm uses or ecosystem development could resume. However, premature liming may increase soi1 capacity for metal binding and restrict the potential for phytoextraction. The addition of organic fertilizers could generate the same effects. Phosphorus is a major nutrient, and plants respond favourably to the application of P fertilizer by increasing biomass production. The addition of P fertilizer, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloro-pyromorphite (Chaney *et al.,* 2000).

2.13.1 Heavy Metals

Accordng to U.S.EPA, (1998), Heavy metals are elements having an atomic weight between 63.546 and 200.590 which in their standard state, have a specific gravity (density) of more than about 5 g/cm3 in their elemental form. Although many of the elements listed under 'heavy metals' have specific gravities greater than five, major exception to this rule remain. In hindsight, metals which have the specific gravities of more than five should preferably have been referred to as 'toxic elements', for they are all included in the United States Environmental Protection Agency's (USEPA's) (Alhassan *et al.,* 2012). Metal being elements cannot be degraded nor =created. They are an example of ultimate persistence (Samsiah, 2007). Examples of heavy metals are chromium (Cr), cadmium (Cd), lead (Pb), mercury (Hg) and manganese (Mn).

2.13.2 Sources of Heavy Metals in Contaminated Soils



Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000 mg kg−1) and rarely toxic (Zhao and Kaluarachchi, 2002).

The major sources of metal pollution arise mainly from combustion of fossil fuels (coal, oil, natural gas), metal manufacturing plants and foundries, mines urban/agricultural runoff and sewage effluent use of agricultural chemicals such as pesticides, herbicides and fertilizers (Seward and Richardson, 2000).

The metal species commonly found in the soi1 as a result of aforementioned human activities include Copper (Cu), Lead (Pb), Zinc (Zn), Nickel (Ni), Cobalt (Co), Mercury (Hg), Cadmium (Cd) and Arsenic (As) (US EPA 2000, Environment Canada 2000). In order to alleviate the problems caused by these pollutants several criteria have to be taken into consideration such as: characteristics of the contaminant land, form and concentration of the contaminant as well as the end use of the remediated land (Saxena *et al.,* 2009**).**

In general, the conventional technologies colloquially termed as 'pump-and treat' and 'dig-and-dump' techniques are limited in their applicability to small area and have their own inherent limitations (Vangronsveld and Cunningham, 2008).

At sites where the contaminants are slightly higher than the industrial criterion (governmental regulations), the use of conventional technologies is not economically viable due to the cost involved. So far, irrespective of the technology being selected, the cost estimates for utilizing conventional remediation techniques have remained high (Lasat, 2000, Glass, 2009). The overall remediation budget includes design, construction, operation and maintenance costs of the process associated with each technology in addition to mobilization, demobilization and **pre-** and **post-** treatment costs which are determined on a site-to-site basis. Also, in the case of most ex situ treatment technologies, excavation and transport costs need to be factored in, to arrive at the final cost for remediating a contaminated site. Market survey indicates that the world remediation market ranged between is expected to grow to approximately US$ 50 billion by the year 2002 (Glass, 2009).

Smith (2009) identifies natural sources of Heavy Metals (HM) such as volcanoes emissions transport of continental dusts, and weathering of metal-enriched rocks due to long exposure to air, greatly adds higher amounts of Heavy Metals to soils. In addition, Heavy Metal can also contaminate the soil through other human activities, such as: (i) exploitation of mines and smelters (ii) application of metal-based pesticides and metal-enriched sewage sludge in agriculture (iii) combustion of fossil fuel, metallurgical industries, and electronics, and (iv) military training and weapons, etc.

Khan, *et al.,* 2010 further categorized anthropogenic activities into five groups: (i) metalliferous mining and smelting (e.g., As, Cd, Pb and Hg), (ii) industry (e.g., As, Cd, Cr, Co, Cu, Hg, Ni and Zn), (iii) atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg and U), (iv) agriculture (e.g., As, Cd, Cu, Pb, Se, U and Zn), and (v) waste disposal (e.g., As, Cd, Cr, Cu, Pb, Hg and Zn). Agricultural practices such as excessive application of phosphatic fertilizers for optimum crop production, extensive and injudicious usage of toxic pesticides, and use of sewage sludge can result in soil pollution.

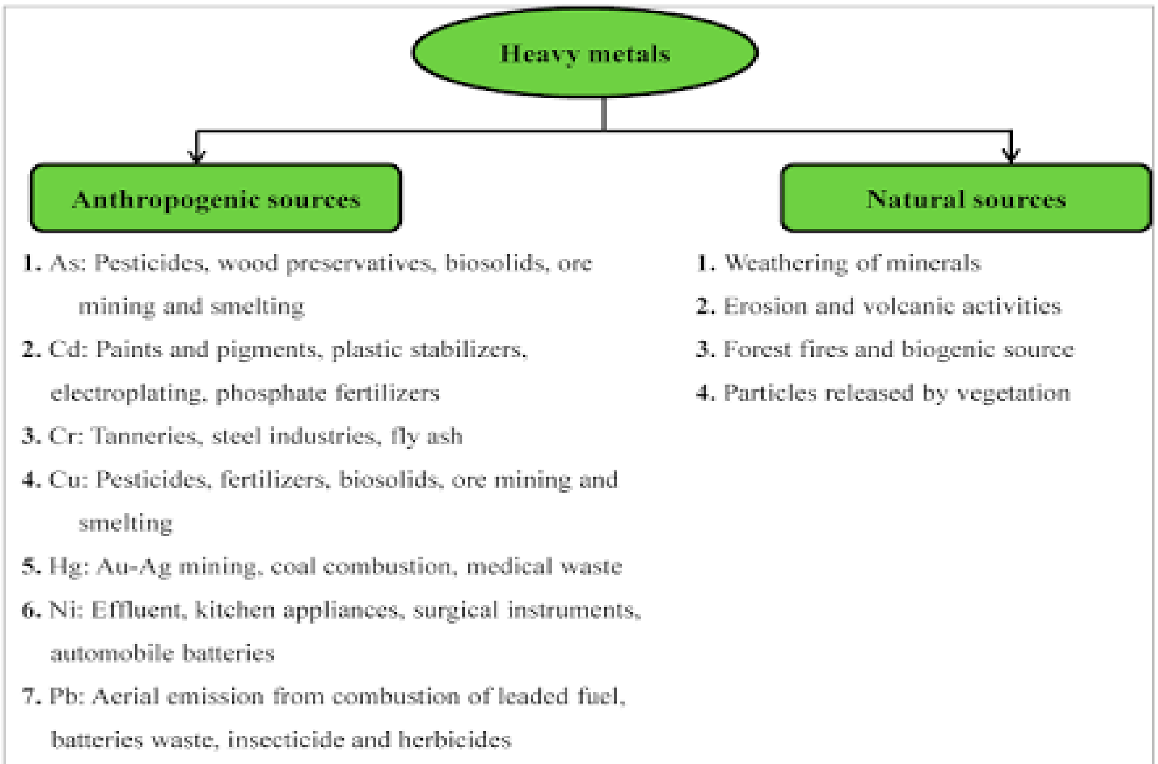
Singh, *et al.,* (2003) recorded that the annual estimate of heavy metals release from all sources in worldwide is around (in metric tons) 22,000 of Cd, 939,000 of Cu, 783,000 of Pb, and 1,350,000 of Zn. The USGS (2009) Minerals Yearbook, gave the worldwide estimation of total production of ferrochromium and chromites to be 7,000,000 and 19,300,000 metric tons, respectively. Moreover, Pb, Cd, Zn and Ni are notable metals that originate from heavy traffic on roads and may also cause soil pollution. Previous studies have reported that high concentrations of metals like Fe, Zn, Pb, and Cr can also be found in road dust, which may originate from the electric arc and furnace dust.

In a study by Rascio, and Izzo, (2011) shows that high concentrations of Fe, Zn, Cu, Cr and Ni can also be produced by serious wear and tear of tires and brake linings. Other sources that may result in Heavy Metal pollution includes: fly ash discharged from coal dependent power plants, PolyVinyl Chloride (PVC) products, colour pigments, several alloys, and chargeable Ni-Cd batteries. Another major source of soil contamination is the unprocessed industrial wastewater. Currently, the environmentalists around the world are paying more attention to the contamination of agronomic soils with heavy metals and their consequent adverse effect on the agro-ecosystems. This is because many chemical, physical, and biological activities are going on inside the soil constantly, which makes it an active and dynamic system. Moreover, the fertility of soil (nutrient pool) is mainly determined by the massive interactions between living and non-living components of soil. In addition, maintaining the soil in good quality is very important in any sustainable agriculture system. However, the nutrient status of soil is changed according to the following factors: time, prevailing conditions of climate and plant cover, and microbial composition of soil. When some stressors such as Heavy Metal, temperature, extreme pH, or chemical pollutions are imposed on a natural environment, soil biota can be negatively affected and subsequently the whole ecological processes mediated by them are disturbed. The assessment of soils for metal levels has therefore become important in order to assess its impact on different forms of water (groundwater and surface water), microbial communities, plant genotypes, and animals and human health.

Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils of rural and urban environments may accumulate one or more of the heavy metals above defined background values high enough to cause risks to human health, plants, animals, ecosystems, or other media.

Khan, *et al,* (2008) states that heavy metals essentially become contaminants in the soil environments because (i) their rates of generation via man-made cycles are more rapid relative to natural ones, (ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the concentrations of the metals in discarded products are relatively high compared to those in the receiving environment, and (iv) the chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable.

It is projected that the anthropogenic emission into the atmosphere, for several heavy metals, is one-to-three orders of magnitude higher than natural fluxes. Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence bioavailable than pedogenic, or lithogenic ones. Metal-bearing solids at contaminated sites can originate from a wide variety of anthropogenic sources in the form of metal mine tailings, disposal of high metal wastes in improperly protected landfills, leaded gasoline and lead-based paints, land application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition.



Mahar *et al.,* (2016)

2.13.3 Effects of Heavy Metal on the Environment

U.S.EPA., (1998) stated that human activity effects the natural geological and biological redistribution of heavy metals through pollution of the air, water and soil and also by altering the chemical form of heavy metals released in the environmental.

The work of Rascio, and Izzo, (2011) revealed that Mines, foundries, smelters and coal-burning power plants, as well as combustion by-products and vehicles emissions are the primary anthropogenic sources of heavy metals.

Singh and Kalamdhad Ajay, (2011) stated that, soil contamination by heavy metals is of most important apprehension throughout the industrialized world. Heavy metal pollution not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community. Therefore, heavy metals are considered as one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd, Zn, Cr, and Pb. The adverse effects of heavy metals on soil biological and biochemical properties are well documented. The soil properties i.e. organic matter, clay contents and pH have major influences on the extent of the effects of metals on biological and biochemical properties.

Sinha *et al.,* (2013), states that heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes. Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms. Conversely, long-term heavy metal effects can increase bacterial community tolerance as well as the tolerance of fungi such as arbuscular mycorrhizal (AM) fungi, which can play an important role in the restoration of contaminated ecosystems.

In the work of Wang, *et al.,* (2009), the order of inhibition of urease activity generally decreased according to the sequence Cr > Cd > Zn > Mn > Pb. Diversity and activity of soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals and the control of plant pests and plant growth communities are important indices of soil quality. It is important to investigate the functioning of soil microorganisms in ecosystems exposed to long-term contamination by heavy metals. Chromium is commonly present in soils as Cr (III) and Cr (VI), which are characterized by distinct chemical properties and toxicities. Cr (VI) is a strong oxidizing agent and is highly toxic, whereas Cr (III) is a micronutrient and a non-hazardous species 10 to 100 times less toxic than Cr (VI). Cr (VI) has been reported to cause shifts in the composition of soil microbial populations, and known to cause detrimental effects on microbial cell metabolism at high concentrations. Ashraf and Ali also reported that the heavy metals exert toxic effects on soil microorganism hence results in the change of the diversity, population size and overall activity of the soil microbial communities and observed that the heavy metal (Cr, Zn and Cd) pollution influenced the metabolism of soil microbes in all cases. In general, an increase of metal concentration adversely affects soil microbial properties e.g. respiration rate, enzyme activity, which appears to be very useful indicators of soil pollutions. In case of soil contaminated with lead (Pb) slight change was observed in the soil microbial profile.

2.13.4 Impact of Heavy Metal on Soil Microorganisms and Enzymatic Activity

Microbial activity and enzymatic activity of soil can reflect the quality of the soil, Chao, *et al.* (2014) stated that microbial biomass of soil is an important indicator for determining the extent of soil contamination. Microbial activity is inhibited significantly in heavy metal contaminated soil. The microbial biomass in soil contaminated by Cu, Zn, Pb and other heavy metals are inhibited severely. The soil's microbial biomass near the mine was significantly lower than that far away from the mine. And the effects of different concentrations of heavy metals and different heavy metals on soil microbial biomass were different.

Chander *et al*. (2005) studied the effect of different concentrations of heavy metals on soil microbial biomass, and found out that it is only if the concentration of heavy metals in the soil is three times above the environmental standard, established by the European Union, that it could inhibit microbial biomass. Fliepbach *et al*. (2004) also discovered that low concentrations of heavy metals could stimulate microbial growth and increase microbial biomass; while high concentrations could decrease soil microbial biomass significantly. The enzymes in the soil play an important role in the process of organic matter decomposition and nutrient cycling. Studies have showed that the activities of enzymes in the soil are related to the heavy metal contamination. Chander *et al*. (2005) found that the activities of almost all enzymes in the soil are significantly reduced by 10 to 50 times with increase in the concentration of heavy metals.

2.13.5 Impact of Heavy Metals on the Plants

Low concentration of soil heavy metals, regardless of whether it is necessary or unnecessary to plants, will not affect the growth of plants in a certain range. But if the concentration is too high, the content of heavy metals bioaccumulated by the plant exceeds its tolerance threshold, and thus the plant physiological composition is altered and it may even lead to death of the plant. In Florida, it was found that if the copper content in soil was more than 50 mg/kg, it would affect citrus seedlings; if soil copper content reached 200 mg/kg, wheat would wither (Zhao *et al.,* (2006). Research has indicated that the growth of cabbage and bean seedling under Cd concentration of 30 μmol/L was inhibited: the root length decreased, and the plant height and leaf area dropped (Qin *et al.,* 2008). Cd may interfere with crop photosynthesis and protein synthesis, and may cause membrane damage etc (Chao *et al.,* 2014).

2.13.6 Impact of Heavy Metals on Humans

In recent years, studies have shown that the characteristics of heavy metal contamination in urban soils and agricultural soils are different (Babula *et al*., 2008). Heavy metals are natural constituents of the environment, but indiscriminate use for human purposes has led to pollution of the natural environment (Malik, & Khan, 2016). This results in excess release of heavy metals such as cadmium, mercury, arsenic, copper, lead, nickel, zinc etc. into the atmosphere and natural resources like the soil and aquatic environments. Natural phenomena such as weathering and volcanic eruption have also been reported to significantly contribute to heavy metal pollution Bharti (2012). Prolonged exposure and higher accumulation of such heavy metals can have deleterious health effects on humans, animals and plants (Wedepohl, 2008). Most environmental contamination and human exposure result from anthropogenic activities such as mining, smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds (Yashim *et al.,* 2015).

Cadmium may damage the metabolism of calcium, which will cause calcium deficiency and result in cartilage disease and bone fractures, etc. Agency for Toxic Substances Management Committee has listed Cd as the sixth most toxic substance that damages human health. Pb mainly enters human body through the digestive tract and respiratory tract, and then goes into the blood circulation in the form of soluble salts, protein complexes or ions, etc. It is reported that 95% of the insoluble lead phosphate accumulates in bones. Pb is strongly pro-organizational, it affects and damages many of the body organs and systems, such as kidney, liver, reproductive system, nervous system, urinary system, immune system and the basic physiological processes of cells and gene expression. Cu, Zn and Ni are essential trace metals in the human body, but if the body takes excessive Cu, Zn and Ni from the outside environment, they will damage human health. Ni and Cu are tumour promoting factors, whose carcinogenesis effect has attracted global concerns. Workers who are in close contact with the nickel powder are more likely to suffer from respiratory cancer, and the content of Ni in the environment is positively correlated with nasopharyngeal carcinoma (Chen, 2011).

Numerous studies indicated that the major sources of heavy metal contamination in urban soils include emissions from transport (exhaust, tire wear debris particles, particles formed by weathering street, etc.), industrial wastes (from power plants, coal combustion, metallurgical industry, automobile repair plants, chemical plants and so on), house garbage, building and weathered particles of sidewalk and precipitation in the atmosphere, among others. However, the major sources of heavy metal contamination in agricultural soils include the impact from the city, smelting minerals, waste treatment (such as landfill, etc.), sewage sludge, automobile exhaust, fertilizers, pesticides and so on (Montagne *et al*., 2007).

The range and mean concentrations (mg/kg) of some heavy metals in the sediments collected from River Niger via Ajaokuta Steel Company, North Central, Nigeria between 2004 and 2005 were Zn 36.64 – 96.23 (70.70 ± 10.68); Mn 4.97 to 21.77 (13.24 ± 2.04); Pb 8.84 to 17.52 (12.35 ± 1.14); Ni 2.65 to 18.61 (9.67 ± 2.91); Cu 0.89 to 8.21 (3.58 ± 1.32); and Cr 0.48 to 13.08 (3.38 ± 0.76) and Cd 0.07 to 0.62 (0.27 ± 0.07) (Olatunde and Oladele, 2003).Thermal neutrons activation analysis technique (TNAA) indicated that some farmland soil in Lokoja contained Mg, K, Ca in large concentration; Sodium (Na),Manganese (Mn) and Vanadium (V) were present in minor concentration in all the samples; Al and Titanium (Ti) were present in minor concentration while Dysprosium (Dy) was present in relatively low concentration (Oladipo *et al.,*2012).

2.13.7 Heavy Metals under Study in this Research

2.13.7.1 Zinc

The major uses of zinc metal are in galvanizing iron and steel against corrosion and in making brass and alloys for die-casting. Zinc itself forms an impervious coating of its oxide on the surface when exposed to the atmosphere, and hence the metal is more resistant to ordinary atmosphere than iron and corrodes at a much lower rate. Zinc is an essential trace element in the human body, where it is found in high concentration in the red blood cells serving as an essential part of the enzyme carbonic anhydrase, which promotes many reactions related to carbon (IV) oxide metabolism. The zinc present in the pancreas may aid in the storage of insulin. Zinc is a component of some enzymes that digest protein in the gastrointestinal tract. Zinc deficiency in nut-bearing and fruit trees causes such diseases as pecan rosette, little leaf and mottle leaf (Noltze *et al*., 2003).

The toxicity of zinc is low, in drinking water zinc can be detected by taste only when it reaches a concentration of 15 mg/kg; water containing 40 mg/kg zinc has a definite metallic taste. Vomiting is induced when the zinc content exceeds 800 mg/kg. Cases of fatal poisoning have resulted through the ingestion of zinc chloride or sulphide, but these are rare. Both zinc and zinc salts are well tolerated by the human skin. Excessive inhalation of zinc compounds can cause such toxic manifestations as fever, excessive salivation and a cough that may cause vomiting; but the effects are not permanent (Prasad, 2009).

2.13.7.2 Lead (Pb)

Lead (Pb) is a major anthropogenic pollutant that has been released to the environment since the industrial revolution and accumulated in different terrestrial and aquatic ecosystems (Verma & Dubey, 2003). It is an extremely toxic metal whose effects on human health have been widely described. For example it occur through multiple pathways, through inhalation of air, water, soil or dust, as it is emitted in excessive lead (Pb) exposure can cause mental retardation and behavioural disorder and its exposure can the environment from vehicles and automobiles. It can also enter the food chain via plants. In plants, its accumulation has been reported in stem, leaves, roots and seeds that increase with increase in exogenous lead levels (Singh *et al.,* 2008). It detrimentally influences plant growth particularly by hampering enzymatic activities.

Lead and its compounds are toxic and are retained by the body, accumulating over a long period of time, a phenomenon known as cumulative poisoning until a lethal quantity is reached. The toxicity of lead compounds increases as their solubility increases. In children and adults the accumulation of lead may result in progressive renal disease. Symptoms of lead poisoning include abdominal pain and diarrhea followed by constipation, nausea, vomiting, dizziness, headache and general weakness. Elimination of contact with a lead source is normally sufficient to effect a cure (Singh *et al.,* 2011).

WHO (2001) Lead (Pb), with atomic number 82, atomic weight 207.19, and a specific gravity of 11.34, is a bluish or silvery-grey metal with a melting point of 327.5°C and a boiling point at atmospheric pressure of 1740°C. It has four naturally occurring isotopes with atomic weights 208, 206, 207 and 204 (in decreasing order of abundance). Despite the fact that lead has four electrons on its valence shell, its typical oxidation state is $^{+}2$ rather than $^{+}4$, since only two of the four electrons ionize easily. Apart from nitrate, chlorate, and chloride, most of the inorganic salts of $lead^{2+}$ have poor solubility in water. Lead (Pb) exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals. Soil and plants can be contaminated by lead from car exhaust, dust, and gases from various industrial sources.

Pb^{2+} was found to be acute toxic to human beings when present in high amounts. Since Pb^{2+} is not biodegradable, once soil has become contaminated, it remains a long-term source of Pb^{2+} exposure. Metal pollution has a harmful effect on biological systems and does not undergo biodegradation (Vaclavikova, *et al.,* 2008).

In humans the main sources of lead are usually lead - based paint and drinking water carried through lead pipes; lead-based paints are especially harmful to children who chew on painted toys and furnishings and eat paint peelings from walls. Industries in which workers encounter lead-containing solids, dusts or fumes include the petroleum industry, mining, smelting, printing, cutlery, storage-battery manufacture, plumbing, gas fitting, paint and pigment manufacture and manufacture of ceramics, glass and ammunition. Other possible sources of lead poisoning include the agricultural use of insecticides containing lead compounds; the spraying of fruits and vegetables that may affect the workers and eventually, the consumers. In the mid-20th century, constant exposure to exhaust fumes of motor vehicles powered by fuel containing tetraethyl lead became a significant cause of lead poisoning, especially in children (Sadowsky, 2010).

Soil can be contaminated with Pb from several other sources such as industrial sites, from leaded fuels, old lead plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile.

Contamination is long-term. Without remedial action, high soil lead levels will never return to normal.

In the environment, lead is known to be toxic to plants, animals, and microorganisms. Effects are generally limited to especially contaminated areas. Pb contamination in the environment exists as an insoluble form, and the toxic metals pose serious human health problem, namely, brain damage and retardation.

Symptoms of lead poisoning vary, they may develop gradually or appear suddenly after chronic exposure. The poisoning affects the entire body especially the nervous system, the gastrointestinal tract and the blood forming tissues. The victim usually becomes pallid, moody irritable and may complain of a metallic taste. Digestion is deranged, the appetite fails and there may be severe abdominal pain, with spasms of the abdominal muscles and constipation. A black line (lead line) may appear at the base of the gums. There is often anaemia, in later stages, headache, dizziness, confusion and visual disturbances may be noted. Peripheral nerve involvement results in a paralysis (lead palsy) that generally first affects the fingers, hands and wrists (wrist drop). The most serious effects are seen in children under the age of six, in whose brain and nervous system development is still occurring. In these children, even a small amount of lead can result in permanent damage and loss of function of the affected area of the brain. Complications may occur, such as learning disabilities, slowed growth, blindness, deafness, and, in extreme cases, convulsions and coma ending in death. Brain injury may also occur in adults after massive exposure (Rascio and Izzo, 2011).

2.13.7.3 Copper

The work of Hukabee *et al.,* (2011) reviewed that the major portion of copper produced in the world is used by electrical industries; most of the remainder is combined with other metals to form alloy. Important series of alloys in which copper is the chief constituents are brass (copper and zinc) and bronze (copper and tin). Copper resists the action of the atmosphere and seawater on exposure for long periods to air, however, these results in the formation of a thin green protective coating (patina) that is a mixture of hydroxocarbonate, hydroxosulphate and small amounts of other compounds. Copper is a moderately noble metal, being unaffected by non-oxidizing or non-complexing dilute acids in the absence of air; it will however, dissolve readily in HNO_3 and in H_3SO_4 in the presence of oxygen (Frits *et al.,* 2000).

Copper is among heavy metals that are essential to life but could be toxic at elevated levels. It is toxic at low concentration in water and is known to cause brain damage in mammals. Elevated levels of this metal has however, been found to be toxic (Hukabee *et al.,* 2011). Toxicity of Copper in plants as a result of high level in sewage treated agricultural soil has been reported. Contribution of copper to environmental burden could be by atmospheric deposition from metal industries, dumpsites and power plants that burn fuels (Baryla *et al.,* 2011).

2.13.7.4 Cadmium

Cadmium occurs in a few minerals and in small quantities in other ores, especially zinc ores, from which it is produced as a by - product. Some lead ores also contain small quantities of cadmium and if it is present in sufficient quantity it is recovered by a cycle of operations similar to that used by zinc smelters. The concentrations of cadmium in agricultural soils depend upon the amounts present in the parent rocks from which the soil is formed, the amounts added in the form of fertilizers and soil amendments, the amounts deposited onto soils from the atmosphere and the amounts removed by harvested crops and by leaching.

On the average, sedimentary rocks contain greater concentrations of Cd than either igneous or metamorphic rocks and therefore, recent soils derived from sedimentary rock should contain greater concentrations of Cd than those derived from igneous or metamorphic rocks. Among the commercial fertilizers, phosphorus fertilizers contain somewhat elevated levels of Cd.

Results show that the long-term use of phosphorus fertilizers will slightly increase the concentration of Cd in surface soils (Wedelpohl, 2008).

In acute cadmium poisoning by ingestion, irritation of the gastrointestinal tract is the major toxicity, causing nausea, vomiting, diarrhea and abdominal cramps. With chronic exposure by inhalation, however, kidneys and lungs are the target organs. Cadmium compounds do damage many organs. They cause skin lesions, decrease heart contractility, damage blood vessel and injuries of the nervous system, kidney and liver. Cadmium compounds also produce skin and lung tumours in humans (Jenne *et al.,* 2011).

2.13.8 Analysis of Heavy Metal Contamination

Chao, *et al.,* (2014) showed that South Korea's agricultural soils are almost not polluted by heavy metals, and the Cd contamination in Spain, the United States and Xuzhou is more serious. Heavy metal contamination in the agricultural soils of Wuxi and Chengdu is relatively serious than other cities. Hg contamination in Guangzhou and Chengdu are relatively serious. Worldwide speaking, the contents of Cu and Hg in most agricultural soils reach light contamination, which is different from the Pb and Cd in cities' soil.

In Nigeria today several ways were identified through which specific heavy metal can be transmitted to living species. Continuous use of leaded gasoline has contributed greatly to the number of cases of childhood lead poisoning. Leaded gasoline in Nigeria contain lead in the concentration range of 0.65 to 0.74 g/L, the Clean Air Initiative is proposed to reduce the 24concentration to 0.15 g/L and finally to zero level. However, numerous studies revealed that, the initiative is just on paper (Gordon *et al.,* 2017) due to government negligence. The consequences have been severe environmental problems. Upon the combustion of the leaded petrol in the engine, the organic lead is oxidized to lead oxide (equation 2.3).

i.e. $2Pb(C_2H_5)4 + 27O_2$ $2PbO + 16CO_2 + 20H_2O$…………………………2.3 The lead oxide formed reacts with the halogen carriers to form lead halides like $PbCl_2$,$PbBr_2$, or PbClBr, which escapes in to the air through vehicles exhaust pipes. About 80% of lead in petrol was noticed to escape. Lead pollution from automobile emissions in Nigeria has been extensively studied and documented in various Nigerian and international publications. Nriagu *et al.* (2007) investigated blood lead levels in 87 children aged 1-6 years from Kaduna state. An average of 10.6 μg/dl was found, with some children having up to 30 μg/dl. The values exceed the maximum allowed limit of 10 μg/dl recommended by Centre for Disease Control (CDC) and correlated linearly with the distance of house from highly trafficking roads, as well as, whether a family owns a car or not. At the beginning of the 21^{st}century, the Federal Environmental Protection Agency (FEPA) of Nigeria examined the lead concentrations in soils from roads, markets and motor parks of Lagos, Aba, Abuja, Ibadan, Kaduna and Port Harcourt. The results revealed elevated and health threatening concentrations. It could be seen that, the highly trafficking cities of Lagos, Ibadan and Kaduna recorded the highest lead levels. Sridhar *et al.,* (2011) reported high degree of contamination in different samples from Ibadan and Lagos. Drinking waters exhibited up to 2.16 mg/L, foods contained 18.5 mg/kg and soil samples from residential and mechanic areas showed 81.91-4060.7 and 140.0- 5454.6 mg/kg respectively. The highest concentrations in all cases correlated linearly with level of traffic and gasoline usage.

Human beings, animals and soil are the ultimate recipients of the lead particulate. It sometimes exists in soil as lead (II) sulphate (PbSO4). Concentrations of about 100 to 1000 ppm have been recorded depending on the nature of the activities, carried out in a particular area (Galadima *et al.,* 2010; Garba *et al.,* 2010). Other anthropogenic sources include mining and metallurgic industries, manufacture of batteries, sheet, ammunition, pipe, cable sheeting, solder, saint and trash incineration. The principal route of exposure for people in the general population is food and lead in contaminated drinking water, working and hand to mouth activities of young children living in polluted environments and the lead dust brought home by industrial workers on their clothes and shoes. Paints containing lead are the most common high dose sources of lead exposure for school and pre-school children. Most of them contain up to 50% of lead in the form of Lead Sulphide (PbS). Children can get seriously lead poisoned when renovations, modeling and construction activities take place in a house or class that contains lead paints. Inhalation or swallowing of debris of the paints during regular playing causes the accumulation of the metal in the children body.

Cadmium is a by-product in the production of zinc and lead and the pyrometallurgy production of Zinc (Zn) is the most important anthropogenic source to the environment. Other major sources are fossil fuel combustion and waste incineration (Su and Wong, 2003). Cadmium is used in a wide spectrum of applications including alloys, pigments, metal coatings, batteries and in the electronic industry. It is also a contaminant in chemical fertilizer, manure and sewage sludge (Ademoroti, 1996a; Ademoroti, 1996b; Ladigbolu and Balogun, 2011).

The EU Directive 94/62/EC (amended by 2004/12/EC) on Packaging Waste sets out a maximum limit of 100 mg/kg for all Pb, Cd, Cr (VI), Cd and Hg in packaging materials. This limitation must be controlled for polymeric packaging materials and to calculate a possible heavy metal enrichment provoked by recycling processes of organic polymers. World Health Organisation sets a tolerance limit of 0.05, 0.05, 0.01, 0.05, 0.05, 1.0, 5.0 and 0.3 ppm for Mn, Pb, Cd, Cr, Ni, Cu, Zn and Fe in drinking water respectively. The Federal Environmental Protection Agency of Nigeria (FEPA) approved a maximum tolerance limit of 1.0, 1.0, 1.0, 5.0, 28 20 and 1.0 ppm for Pb, Cr, Cu, Mn, Fe and Zn for industrial effluents respectively (Galadima and Garba, 2011).

2.13.9 The Implication of Soils and Plants Polluted by Heavy Metals

The fate of various metals in the natural environment is of great concern particularly near mine sites, dumps, tailing piles and impoundments including urban areas and industrial centres. Soil, sediment, water and organic materials in these areas may contain higher than average abundances of heavy metals. In order to estimate the effects and the potential risks associated with elevated elemental concentrations in soils, resulting from mineral deposits and anthropogenic activities, the fraction of the total elemental abundances in water, sediment and soil to the bioavailable amounts in soil need to be identified.

Bioavailability is the proportion of total metals that are available for incorporation into the biota. The total metal concentrations do not necessarily correspond with metal bioavailability. The extent of bioavailability is largely controlled by elemental speciation, which determines the solubility of heavy metals in soils. A number of soil testing methods and partial or sequential chemical extraction techniques and methods are used to determine elemental behavior (Joel *et al.,* 2001).

Bioavailability of metals released from deposits is very complex and depends on many interrelated chemical, biological and environmental processes. These processes may vary overtime and among micro - organisms, plants and animals. Field and laboratory studies of some particular sites using soil, plants and selective chemical extraction methods may enhance understanding of this concept which is of environmental concern (Joel *et al.,* 2001).

Okoronkwo *et al.* (2006) carried out a study on the risk and health implications of metals pollution of soils of urban Umuahia, capital of Abia State in Southern Nigeria presently used for cultivation of crops for crop production. The results of the analyses showed that there was heavy metal contamination of the soils of the flooded area. They concluded that there is higher risk of exposure to heavy metal as a result of the plant uptake of these toxic elements, this makes the use of polluted soils for agricultural purposes to be environmentally risky. Of all the five toxic elements studied, Okoronkwo *et al.* (2006) stated, the highest mean concentration (mgkg-1) of 133.74 ± 10.60 for Pb followed by Cr (22.27 ± 3.03), Ni (8.14 ± 0.33) and As (5.97 ± 0.32) in the soils of urban Umuahia, capital of Abia State in Southern

Nigeria presently used for cultivation of crops, while the least mean concentration of 1.64 ± 0.11 was recorded for Cd. The toxic elements were examined for dependency upon some soil factors through the use of correlation analysis. Sand, organic matter and effective cation exchange capacity (CEC) correlated significantly and positively with Cr and Pb, indicating that these factors largely control the concentration of these elements in soils.

Chowdhury *et al*. (2010) carried out the assessment of the phytoavailable Cd, Pb and Zn using various extraction procedures. The phytoavailability of cadmium, lead and Zinc in the soils from Bangladesh was assessed. The uptake by *Ipomea aquatica and Oryza sativa* was measured and a range of extractants tested on the soils and plant tissue samples. The results of the study suggested that agricultural soils of Bangladesh in the vicinity of different industrial areas were at risk of heavy metal contamination/pollution. There was a strong correlation between the extractable metal fractions and the metal content in plants, which indicated the phytoavailability of the heavy metals. The assessment of the phytoavailability of the elements considered in this study was found to be dependent on the method of extraction, the crop and metal species, as well as the soil type. The present study indicates that a mild extractant such as 1M HCl can be used to assess the phytoavailability of the heavy metals Cd, Pb and Zn.

CHAPTER THREE

METHODOLOGY

3.1.1 Study Area

3.1.2 Location and Population:

Kogi State is found in the North Central region of Nigeria, it occupies 29,833 square kilometre, it lies on 7030`N and 6042`E. It shares common borders with the Federal Capital Territory (Nigeria) to the north, Nasarawa State to the North East, Benue State to the east, Enugu State to the South East, Anambra State to the South, Edo State to the South West, Ondo and Ekiti States to the West, Kwara State to the North West and Niger State to the North. It is popularly called the Confluence State because the confluence of River Niger and Benue is at its capital, Lokoja, which is the first administrative capital of modern-day Nigeria.

Ajaokuta Steel Company is located in Ajaokuta Local Government Area of Kogi State, along the bank of lower river Niger region in North Central Nigeria, with Latitude 7^0 33` 44.24``N and Longitude $6^0$ 39` 17.89``E and altitude range 74-187 metres of the river Niger alluvium at Ajaokuta, North Central Nigeria with population of 122,321 at the 2006 census. As of 2008 Ajaokuta town had an estimated population of 160,039 (NPC, 2010)

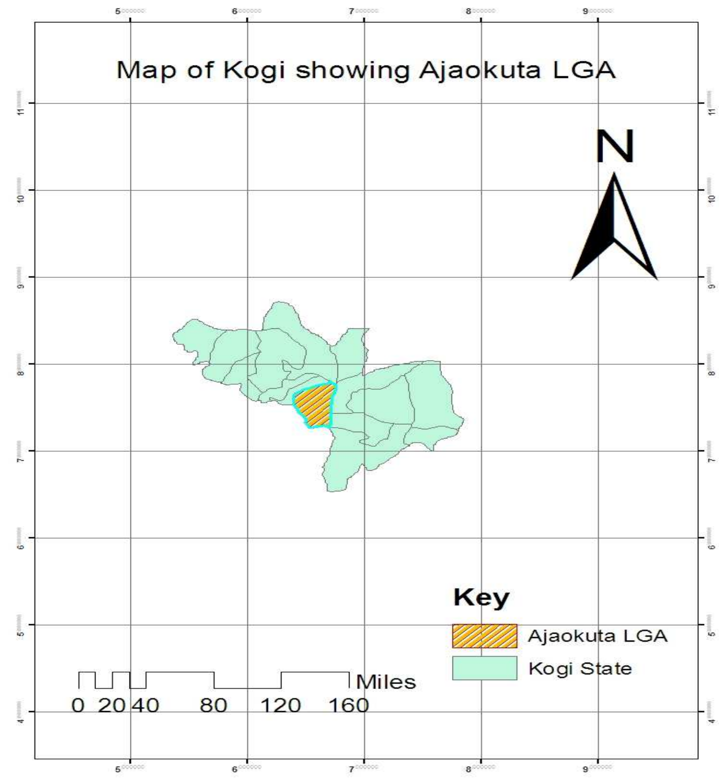


Fig 3.1: Map of Kogi State showing Ajaokuta LGA

Source: Field survey 2018

Table 3.1 Ajaokuta climate table/historical weather data

Months	Avg. Temperature (°C)	Avg. Temperature (°F)	Precipitation/Rainfall (mm)
January	26.6	79.9	6
February	28.5	83.3	11
March	29.7	85.5	41
April	29.4	84.9	96
May	28.1	82.6	161
June	26.7	80.1	173
July	26.1	79.0	173
August	25.9	78.6	165
September	26.1	79.0	231
October	26.7	80.1	141
November	27.3	81.1	18
December	26.4	79.5	4

The difference in precipitation is 227 mm between the lowest and the highest precipitation during the year. Throughout the year, temperatures vary by 3.8 °C.

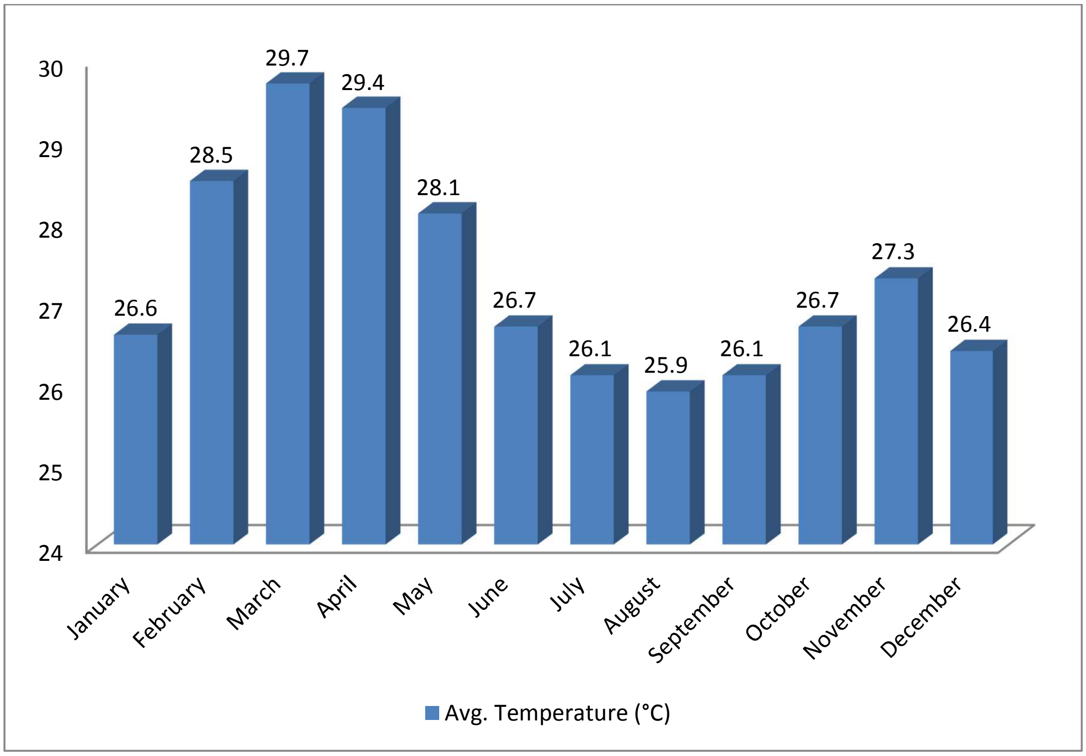


Fig 3.3: Average Temperature (^{O}C) graph of Ajaokuta

Source: Field Survey 2018

At an average temperature of 29.7 °C, March is the hottest month of the year. August is the coldest month, with temperatures averaging 25.9 °C.

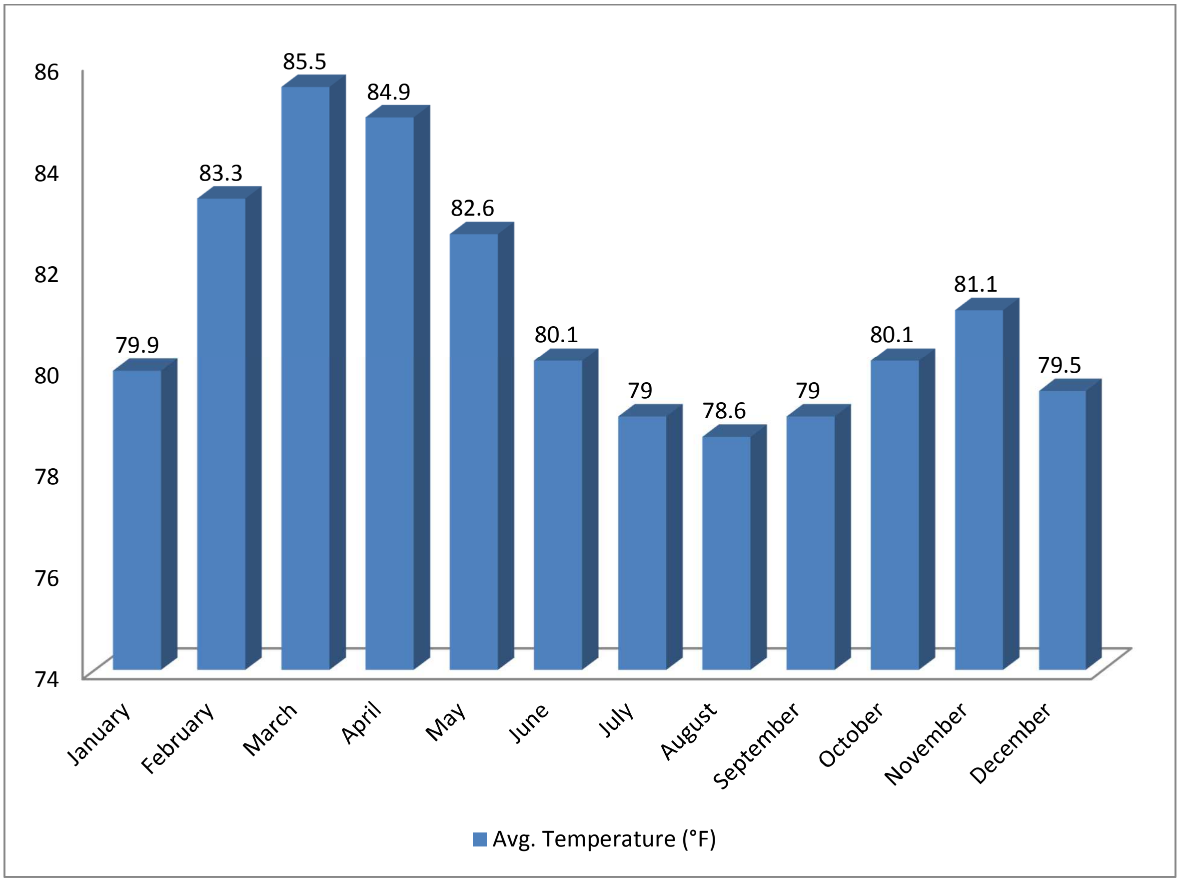


Fig 3.4: Average Temperature (^{O}F) graph of Ajaokuta

Source: Field Survey 2018

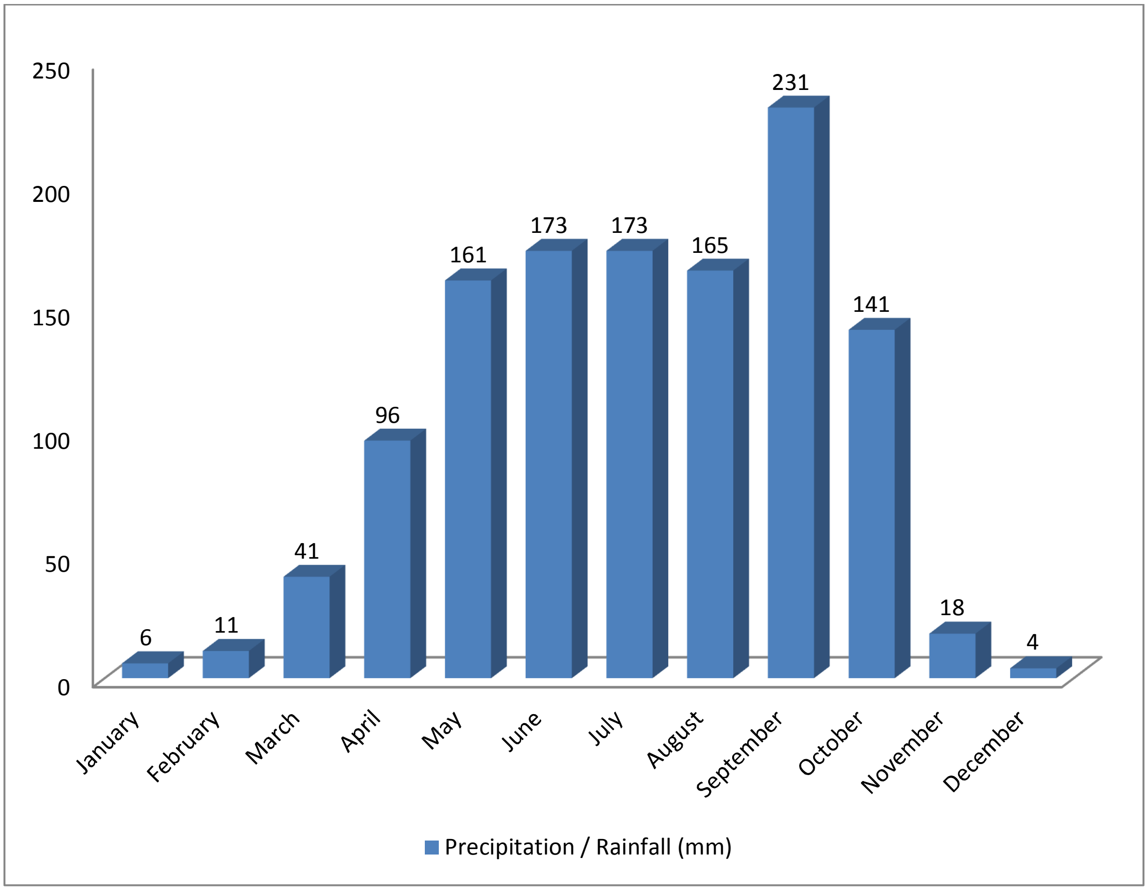


Fig 3.5: Precipitation (mm)

Source: Field Survey 2018

3.1.3 VEGETATION

Located around the guinea savannah, Ajaokuta is dominated with savannah species of plants, scattered and scanty trees on the soil surface. With the presence of mountain and hills around and especially with the rocky nature of the metropolis forest vegetation is not visible. Because of the presence of water/river, there is a presence of water plants in the metropolis especially places along the River Niger.

3.1.4 Historical Background of Ajaokuta Steel Company Limited (ASCL)

The Federal Government of Nigeria established the Nigerian Steel Development Authority (NSDA) in 1971 through Decree No. 19 in order to advance the development of the Nigerian Steel Industry. NSDA carried out detailed market studies and investigations on local availability of raw materials. The Preliminary Project Report (PPR) of 1974, the Detailed Project Report (DPR) of 1977 and the Global Contract (1979) for construction of steel plant at Ajaokuta were all commissioned and executed during the NSDA period.

The NSDA was thereafter dissolved through decree No. 60 on the 18th of September 1979. This decree also created Ajaokuta Steel Company Limited, ASCL being the successor of NSDA. The Ajaokuta integrated steel complex was conceived and steadily developed with the vision of erecting a Metallurgical Process Plant cum Engineering Complex with other auxiliaries and facilities. The complex is meant to be used to generate important upstream and downstream industrial and economic activities that are critical to the diversification of our economy into an industrial one. Ajaokuta Steel Plant is therefore aptly tagged as the Bedrock of Nigerians industrialization.

Ajaokuta Steel Company Limited (ASCL) popularly known as Ajaokuta Steel Mill is a steel mill in Nigeria, located in Ajaokuta, Kogi State, Nigeria. Built on a 24,000 hectares (59,000 acres) site starting in 1979, it is the largest steel mill in Nigeria, and the coke oven and byproducts plant are larger than all the refineries in Nigeria combined. However, it has never produced any steel, and it is non-operational as of December 2017.

The project was embarked upon as a strategic industry, a job creator and a foreign exchange saver and earner. It was envisaged that the project would generate a myriad of socio-economic benefits and increase the productive capacity of the nation through its linkages to other industrial sectors. It would provide materials for infrastructural development, technology acquisition, human capacity building, income distribution, regional development and employment generation. While the project would directly employ about 10,000 staff at the first phase of commissioning, the upstream and downstream industries that will evolve all over the nation will engage not less than 500,000 employees.

The plant by 1994 was reckoned to be at 98% completion in terms of equipment erected. Some completed units of the Plant operated at different times but had to short down due to non-availability of fund. The project was undertaken by the Soviet Union under a cooperation agreement with Nigeria. In 1967, Soviet experts recommended prospecting for iron ore in Nigeria, as the known deposits were of poor quality for steelmaking. In 1973, iron ore of the required quality was discovered in Itakpe, Ajabanoko, and Oshokoshoko. The Ajaokuta Steel Company Limited was incorporated in 1979, and the steel mill reached 98% completion in 1994. To supply the Ajaokuta Steel Mill with raw materials and connect it with the world market, a contract was awarded in 1987 for the construction of Nigeria's first standard gauge railway, from the iron mines at Itakpe to the steel mill at Ajaokuta and continuing to the Atlantic Ocean at Warri.

3.2 Plants Sample Collection

Some of the indigenous plants within Ajaokuta metropolis in Kogi State, Nigeria were sampled. Random sampling was done within the four cardinal points of ASCL viz; North, South, East and West. Vegetations study was carried out based on the predominant presence of the existing native flora. Four different indigenous plants were sampled which include *Imperata cylindrical* (Spear grass or cotton wool grass), *Sida acuta* (Wire weed), *Helianthus annuus* (Sunflower) and *Chromoleana odarata* (Siam weed). Plant species was randomly collected, properly labeled, and packed in polyethylene bags. The plant species were identified and classified taxonomically with the help of taxonomist.

3.3 Sample Preparation

In the laboratory, the roots, stems and leaves of the studied plants were separated in each case and the components were cut into pieces. The plant samples were rinsed with tap water to remove firmly attached soil particles from the leaves, stems and roots. The samples were then rinsed with distilled or deionised water. Plant samples were air-dried for two weeks at room temperature by spreading them on nylon fabric, followed by oven-drying at 70 °C for 48 hours so as to deactivate the enzymatic activities. Thereafter, each part of the samples was mechanically grounded into fine powdery with the aid of an agate mortar and was further dried at 65ºC in an oven to obtain a constant weight. The samples were appropriately stored in treated plastic bottles for Atomic Absorption Spectrophotometer (AAS) analysis.

3.4 Plant Analysis

One gram (1.0g) of the dry plant sample was placed in a test tube, treated with 5ml of a 5:1 mixture of aqua regia (HNO_3-HCl) and perchloric acid ($HClO_4$) and then digested in a hot air oven at 95°C for approximately two hours until complete digestion as described by Massa *et al*., (2010). After digestion, the samples were left to cool and then transferred to 100-ml volumetric flasks. The test tubes used for digestion were rinsed with distilled water and added to the volumetric flasks to make the volume up to 100 ml. The solution was then filtered using Whatman® Ashless qualitative filter paper (Grade No. 41), followed by Atomic Absorption Spectrophotometer (AAS) (PerkinElmer® AAnalyst 100 model) for the analysis of Pb, Zn Cd and Cu.

Plant ability to take up heavy metals from the soil was evaluated by determining the ratio of element concentration in plants to element concentration in soil, i.e. the plants with a high Bioaccumulation Factors value (BAF>1, i.e. metal concentration ratio of plant roots to soil) are suitable for phytoextraction. Plants with low Translocation Factor, TF (i.e. metal concentration ratio of plant shoots to roots) (TF<1) have the potential for phytostabilisation as described by Cheraghi *et al.,* (2011).

3.5 Soil Sampling Collection and Analysis

Top and sub soil samples, 0-15cm of A-horizon were randomly collected northward, eastward, westward and southwards by scooping surface soils in the study area using a stainless steel hand trowel. The soil samples were stored in a nitric acid pre-treated and dried polypropylene bags and well labeled.

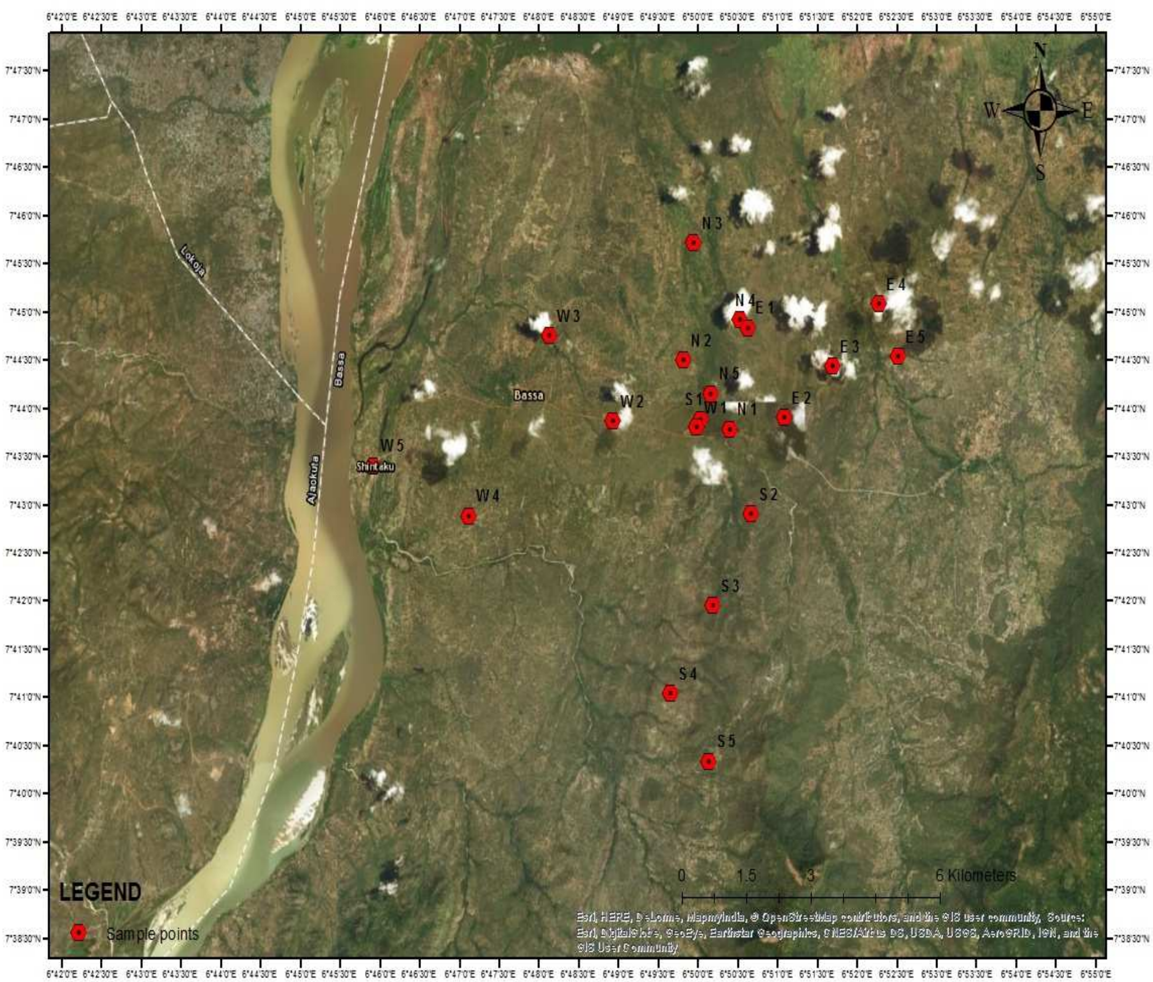


Fig 3.6: Geo-reference coordinates of plants and soils sampling points around Ajaokuta steel company

Source: Field survey (2018)

The soil samples were manually sorted to eliminate pebbles and coarse materials and air-dried under ambient conditions in the laboratory for 72 hours. The dried soil samples were pulverized and sieved to obtain < 2 mm by screening through a nylon sieve of 2 mm mesh size. About 5g each of soil samples were digested in 50mL 2M analar grade HNO_3 in a water bath for 2 hours. The resulting sample digests were filtered into 100 cm^3 volumetric flasks and made up to 100 cm^3mark with distilled water. The digested sample solutions were quantified for the heavy metals Cd, Cu, Zn and Pb, using Atomic Absorption Spectrometry (AAS).

In addition to metal concentration, the pH, percentage of organic matter and percentages of sand, silt, and clay of the soil were determined using Orion 920A pH meter; Walkley & Black method and Hydrometer method respectively (White, 2006).

3.6 Determination of soil pH

Hydrogen ion (H^+) concentration was determined using the method of McLean (1982). Briefly, 1:1 mixture of soil and distilled water and top soil samples were prepared (10g of soil mixed with 10mL of distilled water). The resulting solution was mixed on a mechanical shaker for 0.5hours and then allowed to stand for another 0.5hours and the pH determined using a pre-calibrated pH meter.

3.7 Statistical Analysis

The samples were assayed and analyzed in triplicates and data generated from FAAS were reported as Mean±Standard Error. One way analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) were used to determine significant difference within and between groups, considering a level of significance of less than 5% and from the generated data, PI, BAF and TF were calculated.

$$\text{Pollution Index (PI)} = \frac{\text{Concentration of heavy metal in the soil}}{\text{Concentration EU, 2002 Standard}}$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1.1 Results:

Table 4.1 showed the permissible limits of heavy metals in soil and plants as recommended by European Union (EU) and the World Health Organization (WHO). The Table 4.2 presented the results of mean concentrations of heavy metals in soil around ASCL and the PI, BAF and TF calculated from the generated data. European Economic Community maximum acceptable concentration guideline threshold for arable agricultural soils was used as basis for establishing the environmental status and implications of the analyzed soil samples.

Table 4.1 Permissible Limits of Heavy Metals in Soil and Plants

Elements	**Threshold concentration in soil (mg/kg) (EU, 2002)**	**Background concentration in soil (mg/kg) (CEPA, 1995)**	**Permissible value in plants (mg/kg) (WHO, 1996)**
Cu	140	22.6	10
Zn	300	74.2	20
Pb	300	26	2
Cd	3	0.097	0.2

Table 4.2: Mean concentrations of heavy metals in soil around ASCL and their pollution indices (Average pH = 6.5±0.29)

Metal (mg/kg)	Topsoil	PI	Subsoil	PI	EU, 2002
Cd	0.13±0.16	0.04	0.05±0.02	0.02	3
Cu	40.80±5.84	0.29	29.01±1.22	0.21	140
Pb	36.30±3.06	0.12	25.20±2.01	0.08	300
Zn	60.80±11.94	0.20	55.41±5.01	0.18	300

Table 4.3: Properties of soil from ASCL

S/No	Properties	(n=3)
1	Sand (%)	61.28±5.2
2	Silt (%)	7.12±0.8
3	Clay (%)	31.60±2.6
4	Organic matter (%)	1.34±0.5

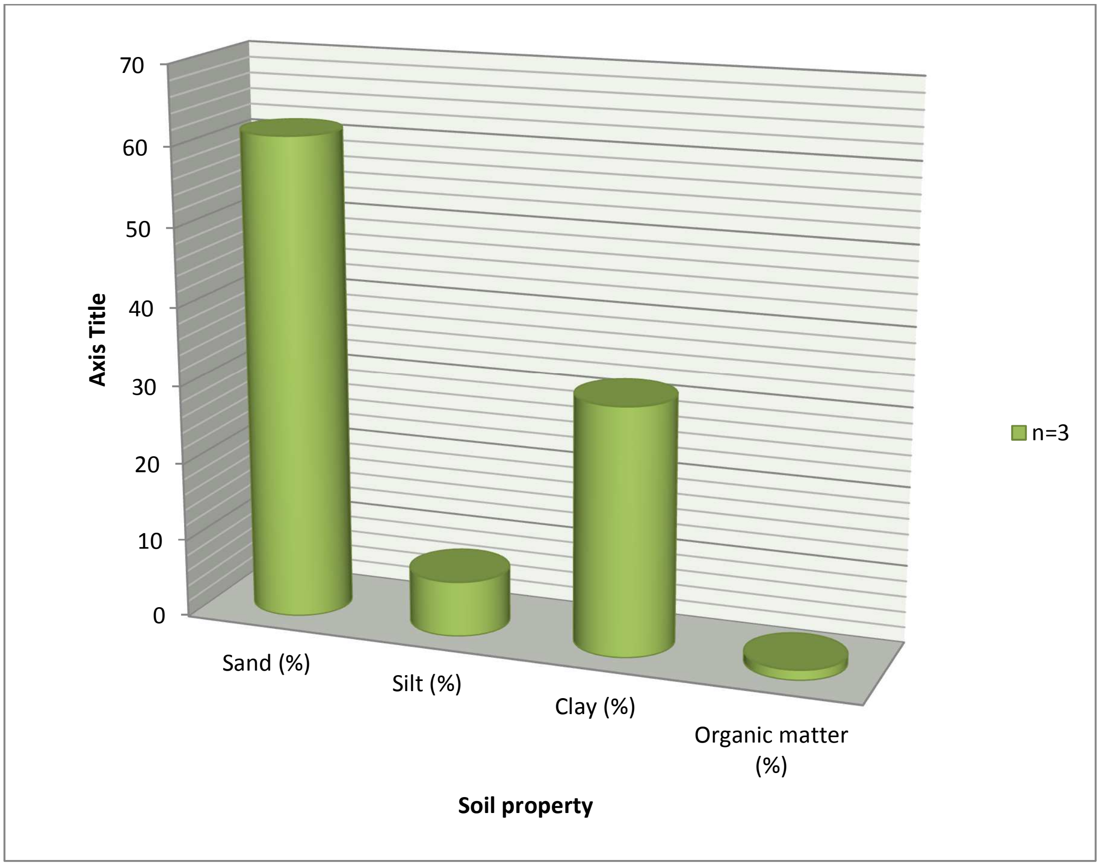


Fig 4.1: Properties of soil from ASCL

Table 4.4: Mean concentrations of selected heavy metal in leaves of different plants around Ajaokuta Steel Company Limited

Plants (mg/kg)	Cd	Cu	Pb	Zn
IC	0.03±0.01	7.10±2.01	5.32±9.12	9.33±2.23
SA	0.02±0.001	6.33±3.22	3.33±0.55	8.12±3.32
HA	0.001±0.01	3.11±1.21	8.23±2.32	4.11±1.22
CO	0.03±0.02	8.45±3.24	10.33±2.55	10.4±4.23

IC: = *Imperata cylindrica*, **SA:** = *Sida acuta,* **HA:** = *Helianthus annuus*, **CO:** = *Chromolaena odarata*

Table 4.5: Mean concentrations of selected heavy metals in stems of different plants around Ajaokuta Steel Company Limited

Plants (mg/kg)	Cd	Cu	Pb	Zn
IC	0.04±0.01	8.23±3.32	7.44±2.43	13.33±6.42
SA	0.03±0.01	9.21±6.51	5.0±1.42	11.34±4.55
HA	0.002±0.02	5.22±2.12	15.40±6.42	6.21±2.54
CO	0.04±0.01	10.00±4.23	20.11±9.66	14.00±6.23

Table 4.6: Mean concentrations of selected heavy metals in roots of different plants around Ajaokuta Steel Company Limited

Plants (mg/kg)	Cd	Cu	Pb	Zn
IC	0.05±0.02	12.33±3.21	10.12±3.43	18.45±9.43
SA	0.05±0.01	12.22±2.32	8.21±2.22	15.03±7.24
HA	0.003±0.001	8.12±4.33	20.34±6.43	9.23±2.45
CO	0.06±0.03	12.55±5.32	25.32±9.32	22.33±9.34

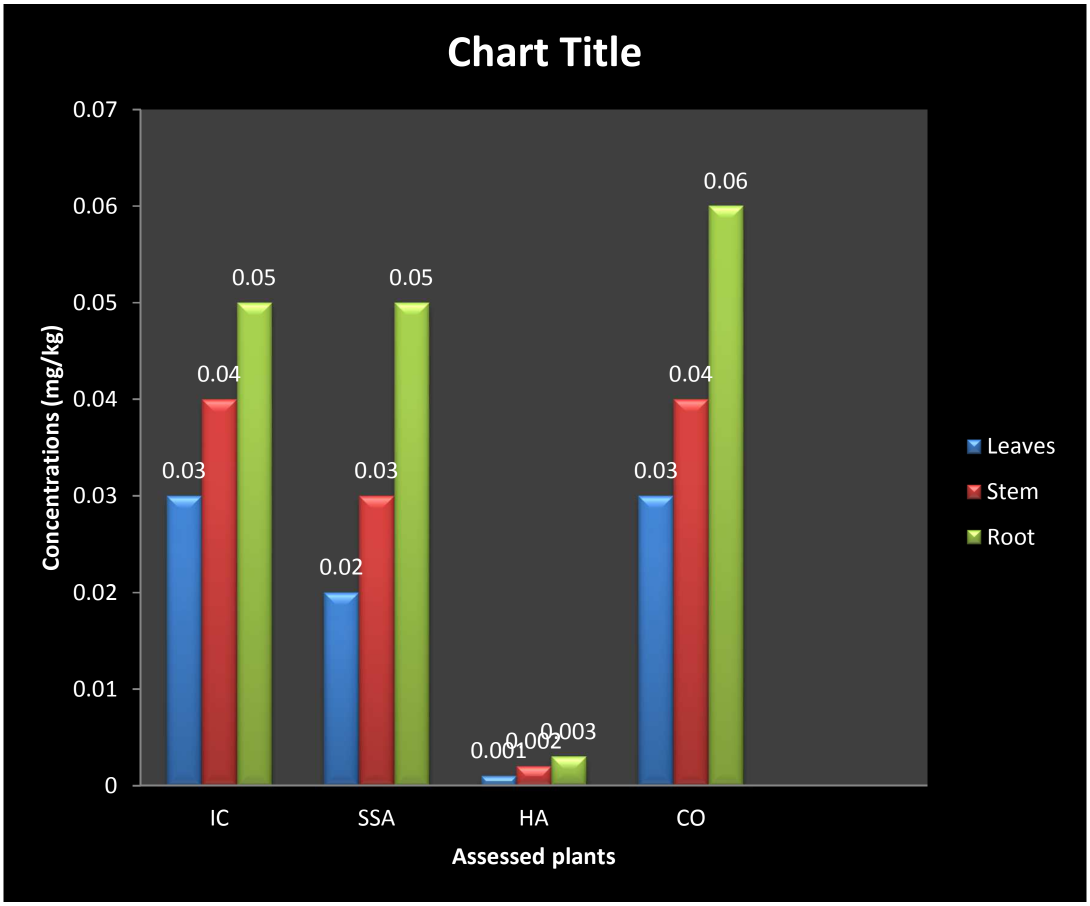


Fig 4.2: Level of Cadmium (Cd) in plants.

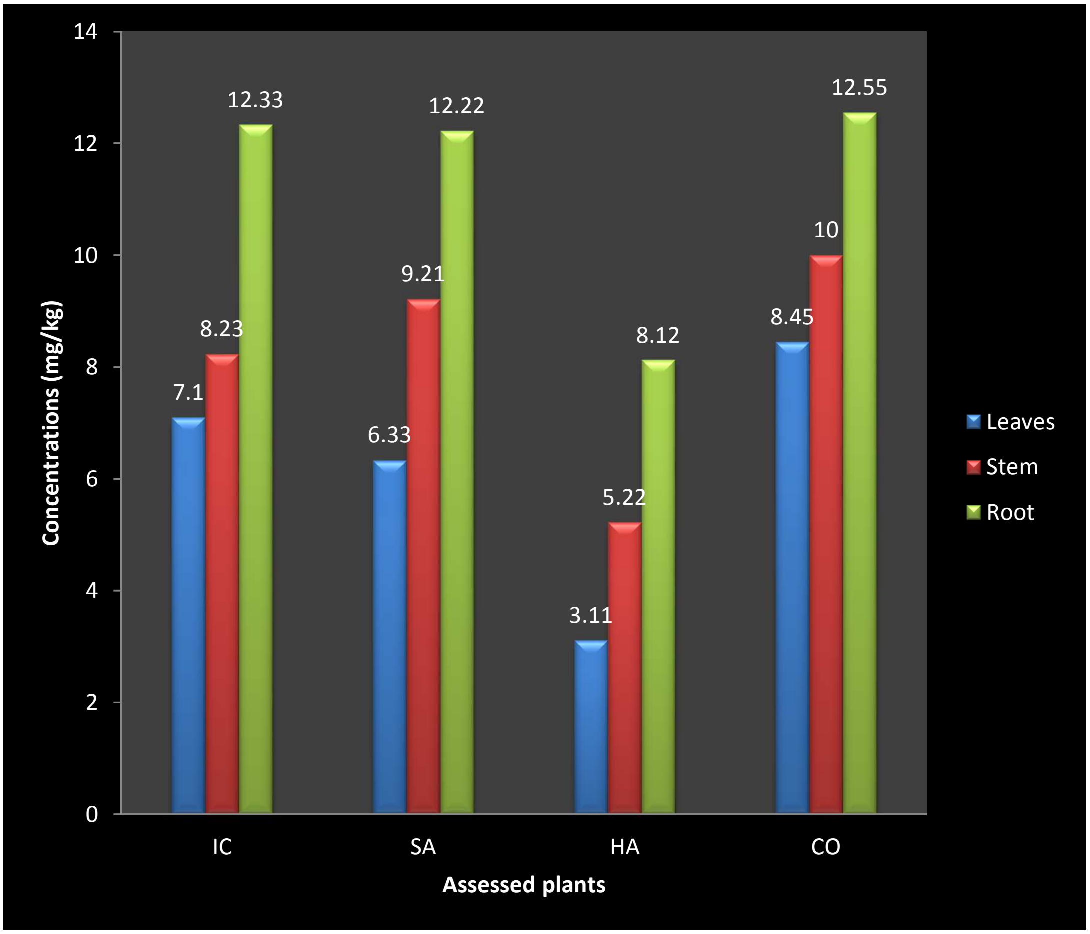


Fig 4.3: Level of Copper (Cu) in plants.

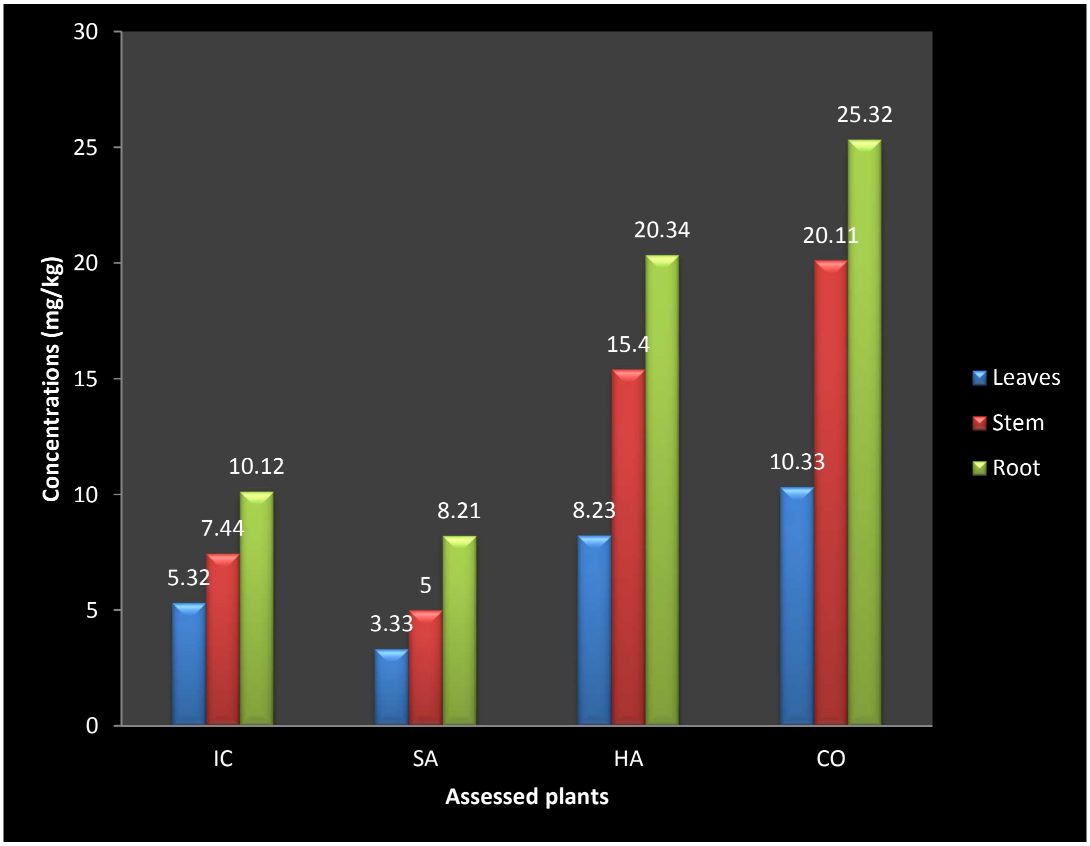


Fig 4.4: Level of Lead (Pb) in plants.

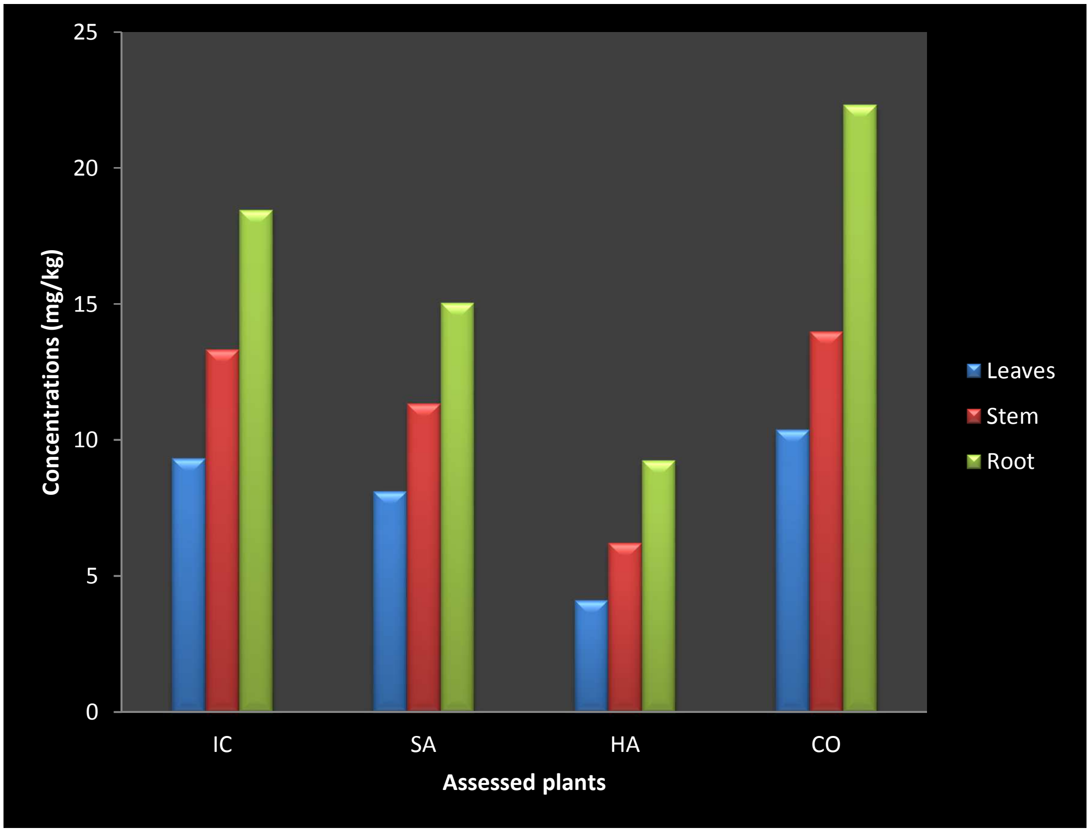


Fig 4.5: Level of Zinc (Zn) in plants.

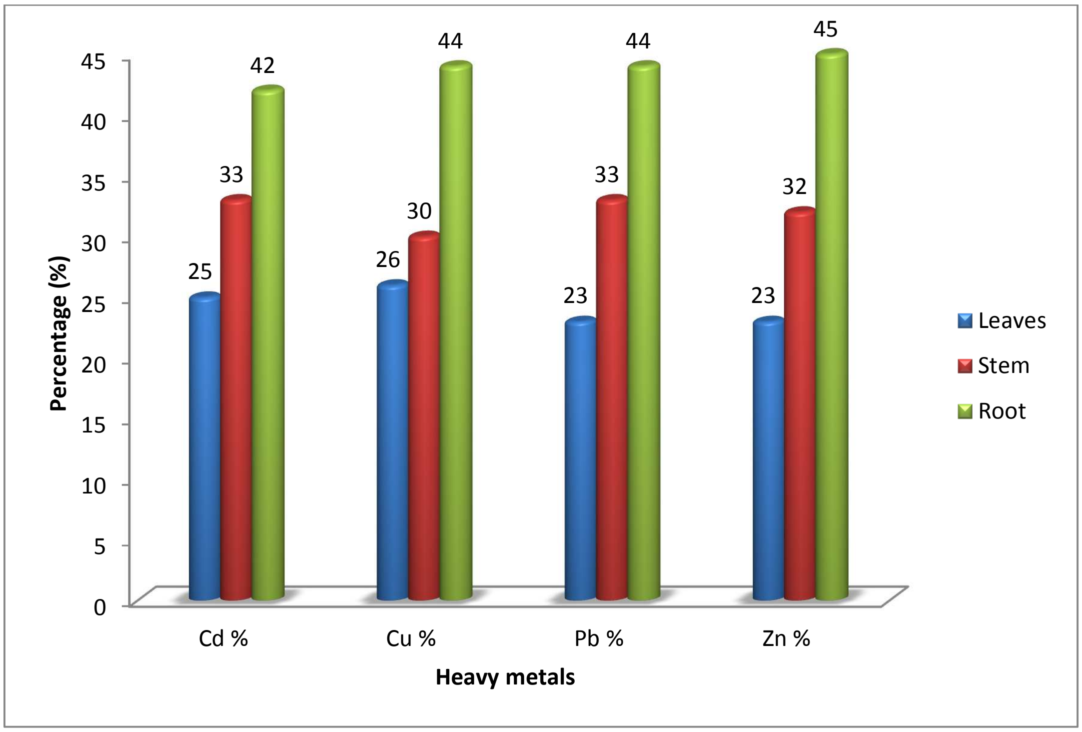


Fig 4.6: Percentage of heavy metals in *Imperata cylindrica*,

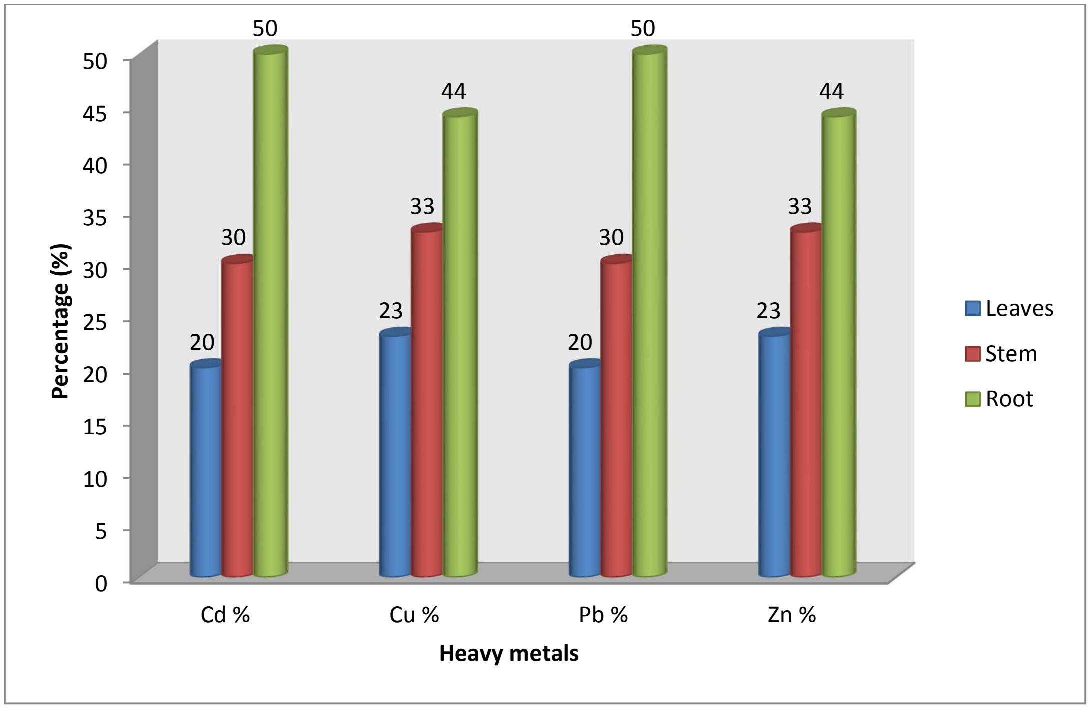


Fig 4.7: Percentage of heavy metals in *Sida acuta*

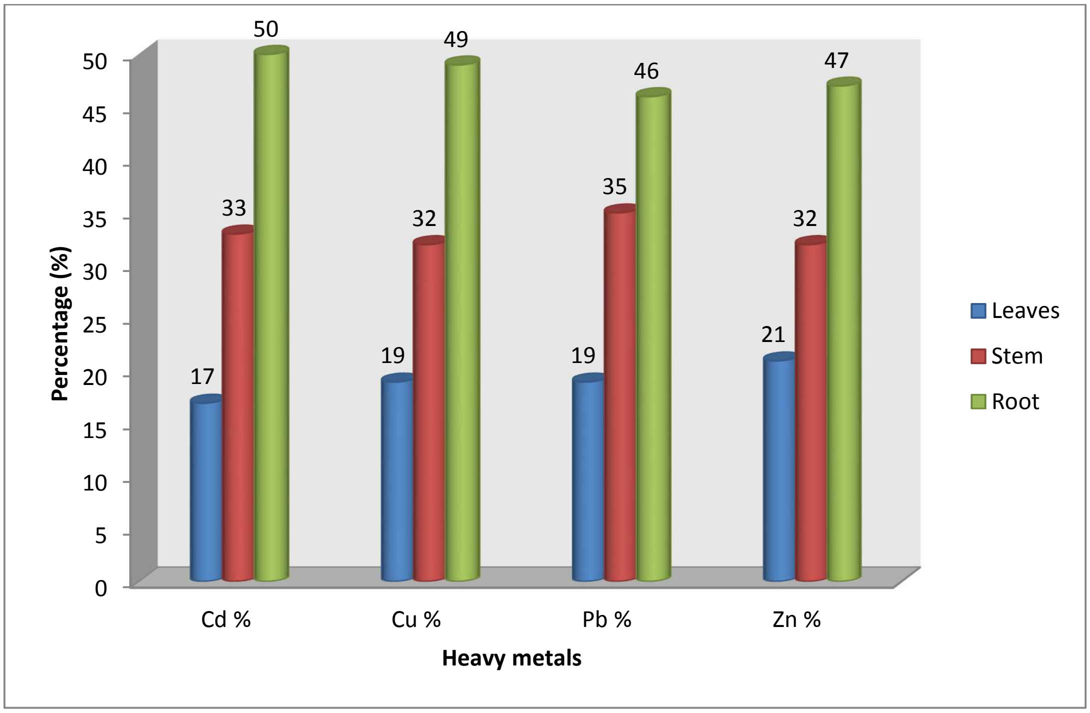


Fig 4.8: Percentage of heavy metals in *Helianthus annuus*

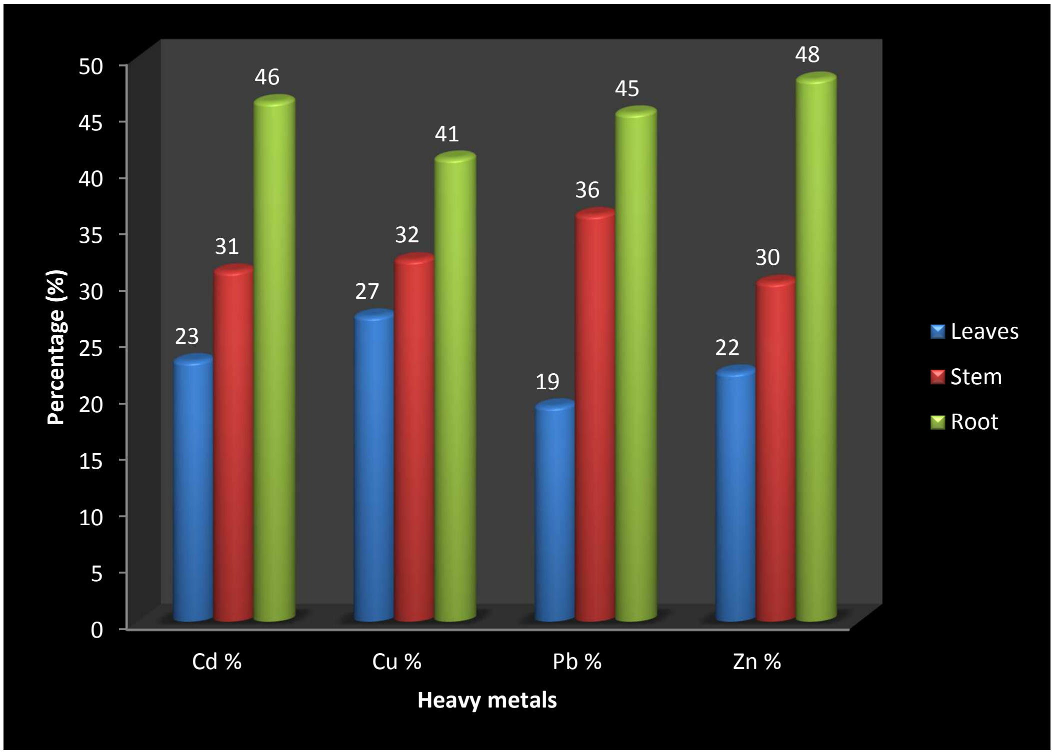


Fig 4.9: Percentage of heavy metals in *Chromolaena odarata*

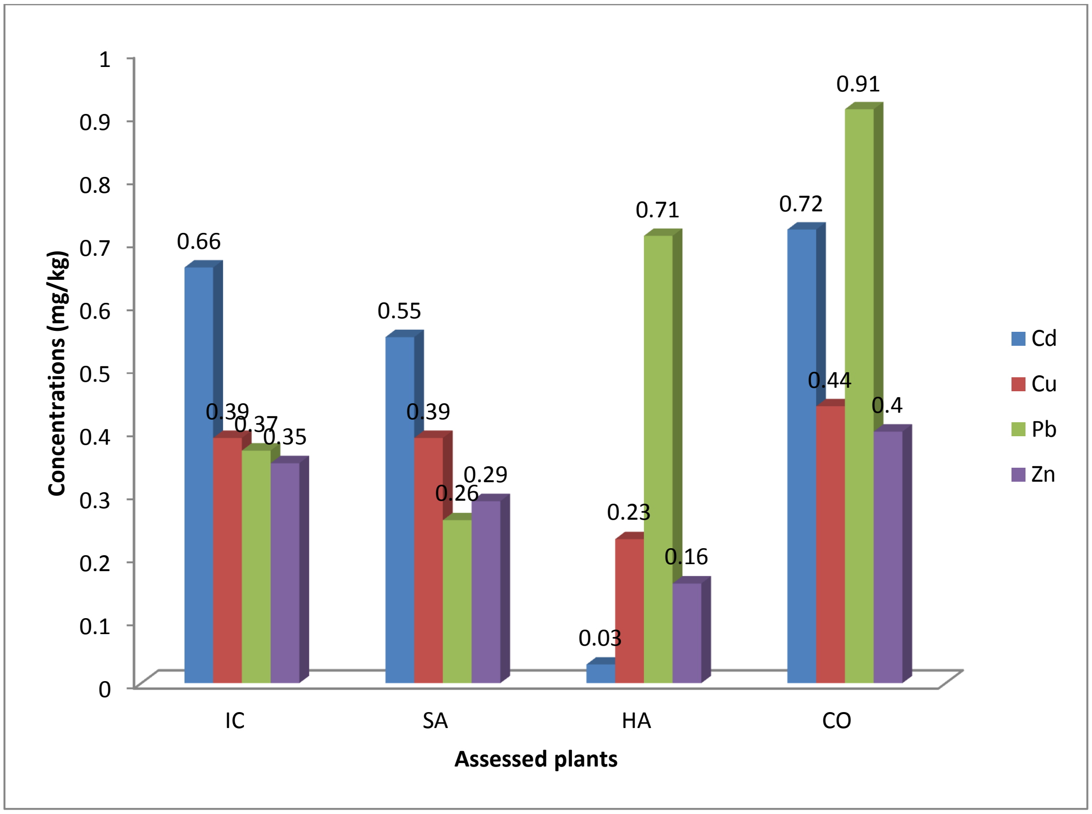


Fig 4.10: Bioaccumulation factors (BAF = C_{plant}/C_{soil})

C_{plant} = Concentration of heavy metal in plant

C_{soil} = Concentration of heavy metal in soil

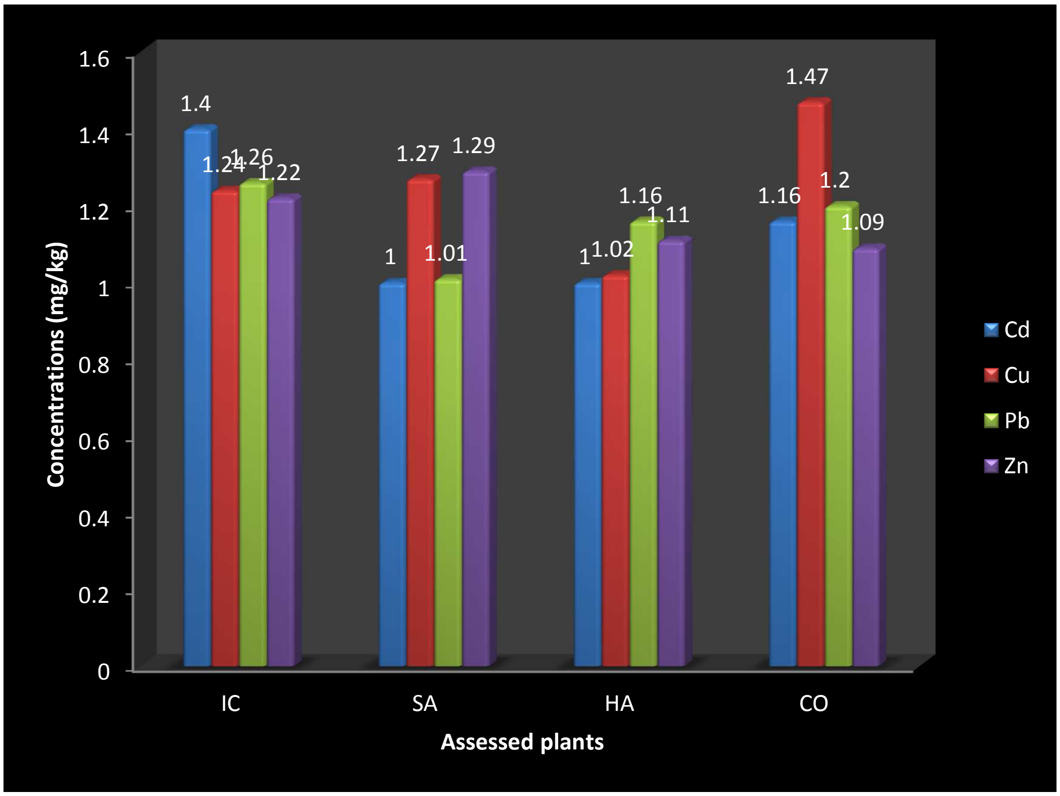


Fig 4.11: Translocation factors (TF = C_{shoot}/C_{root})

C_{shoot} = Concentration of heavy metal in shoot

C_{root} = Concentration of heavy metal in root

4.2 Discussion:

From the Table 4.2 it showed that all the heavy metals under study from the top soil around ASCL do not exceed the limits with their pollution index (PI). These findings agreed with with the work of Olatunde and Osibanjo, (2014) that Cadmium, Lead, Zinc and Copper are heterogeneously distributed in soils around ASCL. Zinc was the most prevalent (39.15- 76.15 mg/kg) among the selected heavy metals while Cadmium had the least concentration of (0.05- 0.23 mg/kg).

Tables 4.4, 4.5 and 4.6 showed the mean concentrations of heavy metals (mg/kg) in leaves, stems and roots respectively of different plants around ASCL. These showed that the levels of heavy metals in the plants do not follow a particular trend. However, in most cases heavy metal concentrations were found to be higher in the root than in the stems and leaves reason been that, the roots have the first contact with the soil where the heavy metals are stored before translocation. The concentrations of metals at the roots of the plants were higher than that of the shoots. The findings agreed with the work of Fulekar (2016) who their study showed higher accumulation of all metals (Cd, Pb and Zn) in the roots than in the shoots. The results based on the statistical analysis showed an increasing trend as the concentration of heavy metals increased from 5, 10, 20 and 50 ppm. The accumulation of metal was found more in roots followed by the shoots of sunflower. The concentration of Cd in sunflower was found 29,550 µg gm and 7,657 µg gm in roots and shoots, respectively at the higher exposure concentration i.e. 50 ppm

Fig 4.2 showed the level of Cadmium in the four different plants around ASCL. It is obvious that *Helianthus annuus* has no potential of remediating cadmium as the levels both in leaves, stem and root show 0.001mg/kg, 0.002mg/kg and 0.003mg/kg respectively. This indicates that *Helianthus annuus* is a poor phyto-extractor of Cadmium. Unlike the *Chromolaena odarata* and *Imperata cylindrica* which uptake the highest Cadmium both on their leaves, stems and roots as 0.03mg/kg, 0.04mg/kg, 0.06mg/kg and 0.03mg/kg, 0.04mg/kg and 0.05mg/kg respectively. In this case, *Chromolaena odarata* and *Imperata cylidrica* are hyper-accumulators of Cadmium.

The uptake of Copper by the indigenous plants around ASCL was relatively high as shown in Fig 4.3. The levels of Cu uptake in leaves, stems and roots were high in both *Imperata cylindrica* (7.10mg/kg, 8.23mg/kg, 12.33mg/kg), *Sida acuta* (6.33mg/kg 9.21mg/kg, 12.22mg/kg), and *Chromoleana odarata* (8.45mg/kg, 10.00mg/kg, 12.55mg/kg) respectively. This affirmed the Ali *et al.*, (2013) and; Sinha *et al.*, (2013) who stated that the tropical grasses are fast growing plants with good tolerance for growth under a wide range of soil, rainfall and temperature conditions. Due to its good adaptation to environmental stress, high biomass production and fast growth rate; grasses are often used to be the preferable choice for phytoremediation compared to shrubs and trees (Ng *et al.,* 2016).

The potential of remediating Lead by *Chromoleana odarata* is high compares to other plants as shown in Fig 4.4. It recorded (10.33mg/kg, 20.11mg/kg, and 25.32mg/kg) in the leaves, stem and root respectively. This is in agreement with the work of King and Robinson, (2006) who carried out an analysis on the *Chromolaena odorata*(Siam weed) Family Asteraceae, and it was found to be a new Lead (Pb) hyperaccumulator by means of field surveys on Pb soil and hydroponic studies. According to their study, plants from field collection accumulated 1377 and 4236 mg kg−1 Pb in their shoots and roots, respectively, and could tolerate soil Pb concentrations up to 100 000 mg kg−1 with a translocation factor of 7.62. They further investigated that the percentage uptakes of Pb, Cd, and Zn by *C. odorata* increased with increasing metal concentrations.

In the same way, *Helianthus annuus* recorded high uptake of Lead which are 8.23mg/kg, 15.40mg/kg and 20.34mg/kg as against the leaves, stem and root respectively. Encheva *et al.,* (2014) supported this as seen from the literature reviewed that "the sunflower (*Helianthus* *annuus L.*) is an annual plant in the family *Asteraceae* has thus been identified as one of the target species that has great potential as a phytoextractor due to the fact that it produces large amounts of biomass, capable of hyper accumulating heavy metals in its harvestable parts (Stems, leaves and roots)and it grows quickly".

From the study, it was discovered that almost all the selected plant species have the ability to remediate the Zinc except the *Heliantus annus* which low values (4.11mg/kg, 6.21mg/kg and 9.23mg/kg) in their phytoremediating parts (Fig 4.5).

In general, the phytoremediation potential of the selected plants was high in the roots. This affirmed the fact that the roots accumulate the heavy metals from the soil as the first contact before transferring it to the stems and the leaves respectively. The percentages in the transferring of heavy metals from the roots through the stems to the leaves were virtually not different in both plants. Delia (2014) affirmed that uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The percentages of heavy metals content in the roots vary averagely at 3.5%. While that of the stems is at 3.2% and the leaves vary at 4.25% in all the plants under study.

The root system provides an enormous surface area that absorbs and accumulates the water and nutrients essential for growth, as well as other non-essential contaminants. Hence, the use of trees (rather than smaller plants) in treating deeper contamination because tree roots penetrate more deeply into the ground and more effective.

Low bioaccumulation factors (BAF<1) were generally observed for all the investigated heavy metals except for Pb. The bioaccumulation level of Pb in *Chromolaena odarata* and *Helianthus annus* are 1.0mg/kg and 0.71mg/kg respectively. Cadmium (Cd) also had a little bit high bioaccumulation factors in *Chromoleana odarata* and *Imperata cylindrica* which were 0.72mg/kg and 0.66mg/kg respectively. This therefore means that Pb has the highest

BAF. Bioaccumulation factor is known to decrease with increasing metal concentrations in the soil (Alam *et al.,* 2003).

Unlike the bioaccumulation factors, the translocation factors were high in all the heavy metals investigated. Smical *et al.,* (2008) showed that the use of plants to extract and translocate metals to their harvestable parts (phytoextraction) is aimed at reducing the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. Some plant species have developed tolerance towards metals and others (hyperaccumulators) are characterised by their ability to accumulate high quantities of metals in their tissues. However, high Translocation Factors (TF>1) were observed in all the plants. A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots being, characterized by the TF>1 (Zhao *et al.,* 2006).

The levels of heavy metals in soil environment around Ajaokuta Steel Company Limited, Kogi State, Nigeria were low and within the European Economic Community maximum acceptable concentration limits for agricultural soils. Also, the levels were the WHO permissible limits of heavy metals in plants. Therefore, the soil environment are not negatively impacted by heavy metals, hence could be regarded as pristine. Be that as it may, Dieckmanni, *et al*, (2001), stated that the uptake of toxic heavy metals from contaminated soils by food and foliage plants comprises a prominent path for such elements to enter the food chain and finally be ingested by human. Ingestion and eventually accumulation of toxic heavy metals such as lead, cadmium, chromium etc. pose a threat to human health and should therefore be minimized. Heavy metal can be very toxic even in low concentration and are not easily degraded or destroyed. It is generally harmful to humans and other living organisms as heavy metals can easily bio-accumulate and cause food chain contamination.

From the study, *Chromolaena odarata* and *Imperata cylindrica* are very good for remediating cadmium as they recorded the highest uptake. In remediating Pb, *Chromolaena odarata* is of paramount importance as it recorded the highest. *Chromolaena odarata*, *Imperata cylindrica* and *Sida acuta* showed high potential for Cu phytostabilisation. All the plants under study are hyper accumulators of Zinc except for *Heliantus annus.*

Apart from industrial applications of dry sunflower biomass, growing sunflowers have shown the potential to absorb various metal contaminants (Lin *et al*., 2009). Sunflower accumulates high concentrations of metal contaminants, they are considered "hyperaccumulators" of heavy metals.

Most of the heavy metals are non-biodegradable and toxic to numerous organisms, including humans; therefore, they must be removed from ecosystems.

The concentrations of heavy metals in the indigenous plants under study were determined. The results showed that except for *Helianthus annuus* which had low phytoremediating potential in Cd, the other three plants are hyper-accumulators of the heavy metals examined (Cd, Pb, Zn and Cu). This goes in-line with the research carried out by D'Souza et al., (2013) which stated that all plants with phyto-remedial potentials are not alike; some maybe metal-specific while others may perform well for metals in combination. But the phytoremedial potential of plants is reported to be influenced by the mobility and availability of heavy metals in soil and plants, and thus the transfer factor (TF) (ratio of bioavailable metal content in root to that in soil) and translocation index (Ti) (ratio of metal content in leaves to that in root) can be used to assess the accumulation potential and hence the phytoremedial potential of plants.

The plants had the ability of reducing concentrations of heavy metals in soil around Ajaokuta Steel Company as agreed in one of the literatures reviewed by by Smical *et al.,* (2008) which shows that the use of plants to extract and translocate metals to their harvestable parts (phytoextraction) is aimed at reducing the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. Some plant species have developed tolerance towards metals and others (hyperaccumulators) are characterised by their ability to accumulate high quantities of metals in their tissues. Hyperaccumulators are plants that achieve a plant-to-soil metal-concentration ratio (bioaccumulation factor) and shoot-to-root metal-concentration ratio (transfer factor) greater than one. The accumulation of these metals may vary from plant to plant and soil to soil. The metal availability to plants depends on total concentration of metals in the soil and the forms in which they occur, pH, organic carbon, cation-exchange capacity, stage of growth of plants, and microorganisms around the root zone. If these factors are constant, the uptake of a metal by different plant species may be compared. From the study, it has been determined that all the plants species under study contribute to phytoremediation.

The abilities of these plants were compared in phyto-accumulation of heavy metals against the soil concentration of the respective metal at the plant sampling points. It indicated that, *Chromolaena odarata, Sida acuta* and *Imperata cylindrical* posses such ability to accumulate all the heavy metals. But *Helianthus annuus* has phyto-accumulation potential only in accumulating Lead (Pb).

Some plants are also tolerant to specific metals and are able to accumulate such metals in substantial quantities in their biomass due to their effective uptakes and translocations. Such plants with effective transfer and translocation concentrate metals in aboveground parts from low to great soil concentrations and are regarded as accumulators. According to Nan *et al.,* (2002), the uptake and transfer of heavy metals from soil to plants is a process of significant importance and determining of transfer factor (TF) and translocation index (Ti) have been considered as a key parameter to assess the availability of elements in soil and hyper-accumulation capacity of the plants. So a detailed study on the metal transfer from soils to plants and translocation to aerial parts could possibly shed more light on the metal accumulation potentials of plants species and their phytoremediation potentials.

Plants take up or hyperaccumulate contaminants through their roots and store them in the tissues of the stem or leaves. The contaminants are not necessarily degraded but are removed from the environment when the plants are harvested. This is particularly useful for removing metals from soil and, in some cases; the metals can be recovered for reuse, by incinerating the plants, in a process called phytomining (Fulekar, 2016).

CHAPTER FIVE

5.1 Conclusion

Heavy metals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavy-metals-contaminated environment. It has some advantages compared with other commonly used conventional technologies. Several factors must be considered in order to accomplish a high performance of remediation result. The most important factor is a suitable plant species which can be used to uptake the contaminant. Even the phytoremediation technique seems to be one of the best alternatives, it also has some limitations. Prolong research needs to be conducted to minimize this limitation in order to apply this technique effectively.

From the study, *Chromolaena odarata*, *Imperata cylindrica* and *Sida acuta* are good accumulators of heavy metals and they should therefore be encouraged to be cultivated. The plant species have developed tolerance towards metals and they are hyperaccumulators. This is characterised by their ability to accumulate high quantities of metals in their tissues.

Since heavy metals are hazardous to human health and the metal toxicity can be severely hazardous if the concentration of heavy metal exceeds its threshold level, the process of remediation aid in reducing if not eliminating the effects of such heavy metals. Phytoremediation has greatly reduced the content of these metals in the soil through bioaccumulation and onward translocation in the plants. Heavy metals in urban soils may go into the body directly through ingestion, skin contact and so on. The use of plants to remediate the heavy metals in the urban soils will reduce the ingestion by man which may cause health problem to man. However, heavy metals in agricultural soils are absorbed and accumulated by crops. Ingesting heavy metals through the soil–crop system is a major way of damaging human health.

Heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes. Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms (Sinha *et al.,* 2013). Conversely, long-term heavy metal effects can increase bacterial community tolerance as well as the tolerance of fungi such as arbuscular mycorrhizal (AM) fungi, which can play an important role in the restoration of contaminated ecosystems.

5.2 Recommendation

1. Analysis of heavy metals should be carried out regularly to ascertain the level of pollution of heavy metals in the soils around Ajaokuta Steel Company. The acquired data may be of importance in making informed choices on the rational managements of steel production industries environment, as well as in the development environmental regulations concerning metallurgical operations/industries.

2. The study of other indeginous plants' potential in phytoremediation should also be carried out.

3. The researcher equally recommends that the government should establish Environmental Health Laboratory at the ASCL to conduct analysis on regular basis of the potential ability of plants around the ASCL and other possible ways of remediating them.

4. Heavy metals contamination of soils, water and plants has become a serious global issue, and as such both government and regulatory agencies including the Federal Ministry of Environment have to ensure the implementation of environmental quality guidelines "to protect, sustain, and enhance the quality" of the environment and to assist with the assessment and remediation of contaminated sites.

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Appendix 1

Table 4.7: Heavy metal contents in both plants and soil around ASCL (mg/kg).

(mg/kg)	**Cd**	**Cu**	**Pb**	**Zn**
IC	0.12	27.66	22.88	41.11
SA	0.1	27.76	16.54	34.49
HA	0.006	16.45	43.97	19.55
CO	0.13	31	55.76	46.73
Soil	0.13	40.80	36.30	60.80

Appendix 2

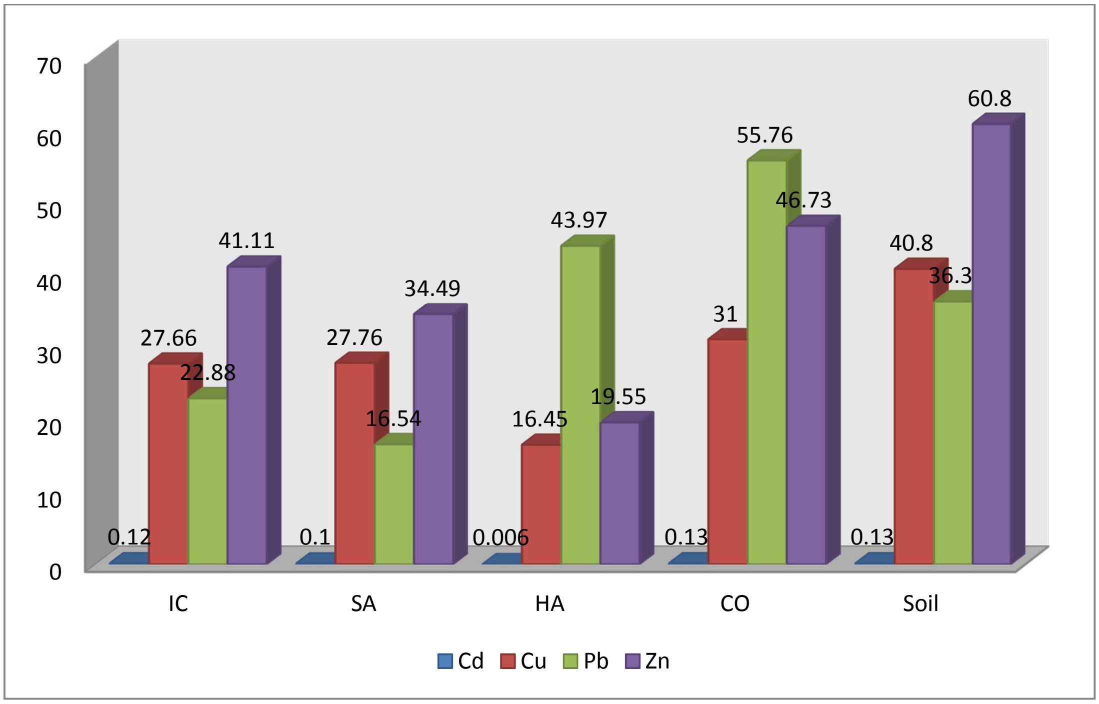

Fig 4.12: Graph of heavy metal contents in both plants and soil around ASCL.