PHYTOEXTRACTION OF CHROMIUM AND IRON FROM CONTAMINATED SOIL USING *PSORALEA PINNATA*

By

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DECLARATION

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I, Oluchuku Richie Ochonogor, declare that the work, **PHYTOEXTRACTION OF CHROMIUM AND IRON FROM CONTAMINATED SOIL USING *PSORALEA PINNATA*** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

_____ _____

SIGNATURE DATE

Mr. O. R. OCHONOGOR

DEDICATION

This master's dissertation is dedicated to my entire family of Ochonogor and the generations to come.

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ABSTRACT

The overall efficiency of plants to remediate soils contaminated by metals depends on their growth ability especially on soils with low-fertility. For twelve weeks, the ability of *Psoralea pinnata* to grow well and remove chromium and iron from artificially contaminated soil was tested. The concentrations of chromium and iron in two soils obtained from different sources namely, University of South Africa premises (US) and commercial potting soil (PS) were 80 ppm, 130ppm, 180ppm, 230ppm, 280ppm, 330ppm, 380pp, 430ppm and 480ppm. *Psoralea pinnata* was transplanted into the contaminated soils and the experiments were watered daily to maintain 70% moisture at field capacity in a greenhouse. Shoot height and root length of *Psoralea pinnata* before and after planting were measured. Other parameters that were measured were number of leaves, wet shoot and dry weights, and wet root and dry weights. The growth of *Psoralea pinnata*, after 12 weeks of experimentation was noticeably affected by the concentrations of chromium and iron in the soil. The percentage increases in shoot height of *Psoralea pinnata* in the PS Soil (C-PS, 48cm from initial shoot height of 12.6cm) treatments were generally higher than the increases in the US Soil (C-US, 45.2cm from initial shoot height of 12.8cm) treatments.

Psoralea pinnata in the (US) treatments accumulated Fe (50.02 ppm) from the soil more than Cr (32.38ppm). In the (PS) treatments, *Psoralea pinnata* also accumulated more Fe (60.57 ppm) than Cr (38.34 ppm). In the experiments containing both Fe and Cr, the US treatments with 40 ppm each of Cr and Fe, chromium was initially mostly accumulated by *Psoralea pinnata* (68%). At higher concentrations (320 ppm) of the combined metals (Cr and Fe) treatment, more Fe (55%) was accumulated in *Psoralea pinnata*. This study however showed that *Psoralea pinnata* may not be an efficient phytoextraction plant for hyperaccumulation.

KEYWORDS: Chromium, Co-contamination, Iron, Metal accumulation factor, Phytoextraction, Phytoremediation, *Psoralea pinnata*.

LIST OF ABBREVIATIONS

$BaCl_2$	Barium chloride
CAS	Chemical Abstract Service
CEC	Cation Exchange Capacity
C-US	Soil control sample A
C-PS	Soil control sample B
Cr	Chromium
EPA	Environmental Protection Agency
Fe	Iron
$Fe(NO_3)_3$	Ferric Nitrate
FLAA	Flame Atomic Absorption
HCl	Hydrochloric acid
HNO_3	Nitric acid
ICP	Inductively coupled plasma spectroscopy
$KCrO_4$	Potassium Chromate
MAF	Metal Accumulation Factor
$MgSO_4$	Magnessium sulphate

MLPP	Mature leaves per plant
NIRS	Near-infrared reflectance spectroscopy
OM	Organic matter
PPM	Parts per million
PSB	Potting soil contaminated with both iron and chromium metals
PSC	Potting soil contaminated with chromium
PSI	Potting soil contaminated with iron
PS	Potting soil
SVOCs	Semi volatile organic compounds
USI	UNISA soil contaminated with iron
USC	UNISA soil contaminated with chromium
USB	UNISA soil with both iron and chromium metals
US	UNISA soil

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CHAPTER ONE

Introduction

1.1 Overview/Description of Problem.

Global industrialisation, including human, social and agricultural activities is a serious source of soil pollution (Suciu et al., 2008). The period from the industrial revolution has witnessed a dramatic increase in the level of toxic metal pollution in the environment (Nriagu, 1996).). The emission of metals into the environment now threatens health human beings (Yagdi, et al. 2000). Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity to the biota (Alkorta, et. al., 2004). These heavy metals are extremely persistent in the environment; they are non-biodegradable by microbial activity or through chemical oxidation (Beiergrohslein, 1998) and are non-thermo-degradable (i.e. cannot be degraded by heat) and thus their accumulation readily reaches to toxic levels (Bohn, et. al., 1985). Metals are natural constituents of the earth's crust which plants absorb and pass into the food chain; however, an excessive concentration of these heavy metals is introduced into the environment by human activities. These human activities include smelting, electroplating, and mining. According to Kuhndt (2008), there is a belief that during the extraction process up to smelting, a ton of copper generates about 100 to 350 tons of residues, a situation almost always not handled correctly. South Africa is Africa's most important country in terms of the variety and quantity of minerals produced. South Africa, being the world's largest producer of ferrochrome, holds about 70% of the world's total chrome reserves, mostly located in the Bushveld Igneous Complex (BIC) ores, and produces 75% of the world's ferrochrome with about 6,000 abandoned mines in areas most of which have been damaged by mine residues (Cramer, et. al., 2004). The contamination of soil with heavy metals in each of the sites is dependent on length of time of

operation of mining or smelting. At these abandoned mines, rain and runoff water increases the chance of extended metal contamination beyond the boundaries of primary contaminated sites. The biological half-lives of these metals are long with the potential of accumulating in the human body when ingested through plants in the food chain. These produce unwanted side effects (Jarup, 2003; Sathawara, 2004; Ata, 2009). Until now, traditional methods have been used for heavy metal remediation. These traditional methods include soil flushing, solidification/stabilisation, vitrification, thermal desorption and encapsulation (Bio-Wise, 2003). Reports of other methods include burying contaminated soil and dilution of contaminated soil using clean soil. These have contributed to long term risks associated with contaminants leaching into groundwater and surrounding soil (Beiergroshlein, 1998). In countries where these methods are practiced, it can be said that site remediation is not a priority and are considered time consuming, too expensive and in some cases result in additional risks to remediation workers.

Studies have been conducted aimed at developing an effective technology that is efficient, economic and feasible to remediate soils contaminated by heavy metals. Due to the expensive nature of these conventional remediation methods of heavy metals which include physical, thermal and chemical treatments (Danh, 2009), phytoremediation technologies are continuously researched. The level of heavy and toxic metals (Pb, Cr, Hg etc.) can be reduced in contaminated sites or media using a number of aquatic and terrestrial plants. The metals are taken up by the root system and transported to the stems and leaves without showing a toxicity syndrome and this has been supported by many studies (Cardwell, et al 2002; Chatterjee and Chatterjee, 2000). The use of plants to remediate contaminants is a developing technology and an approach of phytoremediation which is called phytoextraction applies to metals (e.g. Ag, Cr, Fe, Cu, Hg, Mn, Mo Ni, Pb, Zn), metalloids (e.g. As, Se), radionuclides (e.g. 90Sr, 137Cs, 234U, 238U) and non-

metals (e.g. B) (Salt 1995; Cornish, et al 1997; Banuelos and Ajwa 1999). Phytoextraction employs plants to transport high quantities of metals from soil and to accumulate them in the harvestable parts of roots and above ground shoots (Chaney 1983; Chaney 1997), and has emerged as a cost effective, environment friendly clean up alternative (Itana and Coulman, 2003). The phytoextraction or hyperaccumulation of metals in various plant species have been extensively investigated and substantial progress has been made. The potential of duckweed was investigated by Zayed, (1998) for the removal of Cd, Cr, and Cu from nutrient-added solutions and the results indicated that duckweed is a good accumulator for Cd and Cu, but his research was unable to uncover a potential plant for abstracting Cr from the soil. Brooks and Robinson (1998) investigated the uptake of Cr from soil by the use of some plants including Indian mustard (*Brassica juncea,* L). He indicates that there is no evidence of Cr hyperaccumulation by any vascular plants. Robbinson, et al (2010) investigated the potential of the South African high-biomass Cd hyperaccumulator *Berkheya Coddii,* L to phytoextract Co from artificial metalliferous media. Though Co was readily taken up by *Coddii,* cobalt phytotoxicity above a total Co concentration in plant growth was observed. To date, the majority of phytoextraction work has focused on Cd, Pb and Zn (Suciu, 2008). However, Fe contamination is a problem in many soils especially where efforts are made to extract iron from iron ore, and the conversion of the raw iron from the furnace into various kinds of steel.

The hyperaccumulators that have been extensively studied by scientific community include *Thlaspi spp., Arabidopsis spp., sedum alfredii spp.,* all genera belong to the family *Brassicaceae* and *alyssum* (Prasad and Freitas, 2003*). Psoralea pinnata* (L) belongs to the family *Fabaceae* thriving well in both wetland and upland habitats. So far, the use of *Psoralea pinnata* as a possible plant for phytoextraction has not been investigated. Furthermore, the leaves of this plant

are very green (Abou-Shanab, et al 2003), which might help to study occurrence of physiological changes in the growing process of this plant (i.e. as an indicator).

1.2 Typical Ferrochrome Smelting Operation:

South Africa as the world's largest producer of ferrochrome has many companies involved in production. Ferrochrome is the alloy formed when the natural chromite mineral is reduced in the presence of fluxes (which reduce the mixture melting point) with carbonaceous reductants such as coal, anthracite, coke or char. Its main components are: Cr-Fe-C-Si in decreasing order of concentration. In most cases, ferrochrome has been described as an alloy of chromium and iron (Daavittila, et al 2004). Ferrochrome with chrome content between 50-55 % is known as charge chrome. Ferrochrome is one of the major ingredients for the manufacture of stainless steels. Ferroalloy production processes are very traditional and no revolutionary new technologies have been launched in the markets (Daavittila, et al 2004). The components of ferrochrome have been identified as heavy metals and heavy metals have been defined as chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C. Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. (Lide, 1992).

1.3 Aim of the study

The aim of this study is to test the use of *Psoralea Pinnata*, in a phytoextraction experiment.

1.4 Objectives of study

The objectives of this study were to:

- To determine the ability of *Psoralea pinnata* to grow in a soil contaminated with Cr and Fe.

- To determine optimum pH for growth of *Psoralea Pinnata*
- To determine whether *Psoralea pinnata* can extract Cr and Fe from contaminated soil.
- To determine the amount of Cr and Fe present in the *Psoralea pinnata* tissues.
- To determine whether *Psoralea pinnata* can bioaccumulate heavy metals of Cr and Fe.

CHAPTER TWO

Review of related literature

2.1 Chemistry of iron and chromium.

Heavy metal is a term applied to a large group of elements or any metallic chemical element that has a relatively high density, specific gravity or atomic weight and is toxic or poisonous at low concentrations (having both industrial and biological importance) (Lubomir, et al 2007). Soil is both a source of heavy metals and also a receptacle of heavy metal contamination. The factors controlling the total and bio-available concentrations of heavy metals in the soil are of high importance with regard to both toxic threats to humans and agricultural productivity.

Depending on the properties of individual metals, heavy metals dissolve in a soil solution. Metals are present in soil in any of five different fractions. The various fractions are 1) dissolved in soil solution, 2) attached to exchange sites on inorganic soil constituents, 3) adsorbed to inorganic soil constituent, 4) attached to insoluble organic matter, and 5) precipitates of pure or mixed solids (Radwan and Salama, 2006). Iron is the most abundant transition metal on earth and is essential for growth and functioning of plants. It is important for the respiration and photosynthesis processes implying that it is involved in many enzymatic systems like chlorophyll synthesis. However, iron and other metals including chromium have been identified as heavy metals that pollute the environment, especially when in high concentrations typically found in contaminated environments of ferrochrome smelting sites. The oxides of iron which are commonly referred to as hydrous oxide play an important role in the behaviour of metals in soil (George, 2008). Precipitation of iron is usually in the form of gelatinous ferrihydrite ($Fe_2O_3.9H_2O$) initially and it gradually dehydrates to more stable forms such as goethite (FeOOH) (Krauskopf, 1967). Goethite

is the most common oxide of Fe found in the soil and hematite (alpha-Fe_2O_3) is mainly found in tropical soils. Lepidocrocite (gamma-FeOOH) is characteristic of the fluctuating redox conditions in gleyed soils.

Chromium occurs naturally in rocks, animals, plants, soil, and in volcanic dust and gases. In the periodic table, chromium is a first-row d-block transition metal of group VIB. It has an atomic number 24, atomic mass 52, density 7.19gcm^{-3}, melting point 1875^{o} C, and a boiling point 2665^{o}C. Chromium can be found in the environment in several different forms. The most common forms are chromium (0), trivalent [or chromium (III)], and hexavalent [or chromium (VI)]. Chromium (III) is naturally found in the environment and as an essential nutrient, it is required by the human body to promote the action of insulin in body tissues so sugar, protein, and fat can be used by the body. The two valence states trivalent Cr (III), the most stable and hexavalent Cr (VI) are the most important. Chromium (0) and Chromium (VI) are not found in these forms in the environment and are released by industrial processes of human activity. No known taste or odour is associated with chromium compounds (Kotas and Stasicka, 2000). The chromium (0) form is a steel-gray solid with a high melting point, used for making steel and other alloys. Chromium compounds, mostly in chromium (III) and chromium (VI) forms, which are produced by the chemical industry, are used for chrome plating, the manufacture of dyes and pigments, leather tanning, and wood preservation from where a lot of the concentration goes unchecked into the environment (Pandey and Sharma, 2003). Chromium (VI) is the dominant form of Cr commonly found at contaminated sites. Chromium (VI) is the dominant form in shallow aquifers where aerobic conditions exist. Chromium (VI) can be reduced to Cr (III) by soil organic matter (Smith, et al 1995). Major Cr (VI) species are chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). Chromate and dichromate also absorb on soil surfaces, especially iron and aluminium

oxides. Chromium (III) is the most dominant one at pH less than 4.0. Cr^{3+} forms solution complexes with OH^-, Cl^-, F^-, CN^-, SO_4^{2-} and insoluble organic ligands. Chromium (VI) is the more toxic form of chromium and is also more mobile (Zou, et al 2006). Chromium mobility depends on absorption characteristics of the soil, including clay content, iron oxide content, and the amount of organic matter present. Small amounts are used in drilling mud, rust and corrosion inhibitors, textiles, and toner for copying machines. The Chemical Abstracts Service (CAS) Registry numbers and the solubility of a few important hexavalent chromium compounds are given in Table 1. Hexavalent chromium, in solution, exists as hydrochromate ($HCrO_4^-$), chromate (CrO_4^{2-}), and dichromate ($Cr_2O_7^{2-}$) ionic species. The proportion of each of these ions in solution is dependent on pH. In neutral and basic pH, the chromate form predominates. As the pH is lowered (6.0 to 6.2), the hydrochromate concentration increases. At very low pH, the dichromate species predominate (U.S. EPA, 1989).

Table 1. CAS numbers and aqueous solubilities of selected hexavalent chromium compounds.

Compound	CAS No.	Water solubility
Ammonium chromate $(NH_4)_2CrO_4$	7788-98-9	40.5 g/100 mL at 30 ^OC
Calcium chromate $CaCrO_4$	13765-19-0	2.23 g/100 mL at 20 ^OC
Chromic acid CrO_3	1333-82-0	61.7 g/100 mL at 0 ^OC
Potassium chromate K_2CrO_4	7789-50-6	62.9 g/100 mL at 20 ^OC
Potassium dichromate $K_2Cr_2O_7$	7789-50-9	4.9 g/100 mL at 0 ^OC
Sodium chromate Na_2CrO_4	7775-11-3	87.3 g/100 mL at 30 ^OC
Sodium dichromate dihydrate	7789-12-0	230 g/100 mL at 0 ^OC

Sources: Weast, 1980; Hartford, 1979.

2.2 Role of Chromium in Plants

Chromium is considered not essential for plant growth and development. Studies have, however shown that at low concentrations like 1μM, Cr promote plant growth (LvWeili, et al 2004). Cr (VI) compounds are comparatively more toxic than Cr (III) due to their better solubility in water (Kader, 2007). Rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Shahida and Indu, 2007) also play a role in making Cr (VI) more toxic. At small concentrations of about 0.5 to 5.0 mg l^{-1} in nutrient solution and 5 to 100 mg kg^{-1} of available Cr in soil, chromium is said to be toxic to plants (Hossner, et al 1998). The species found to accumulate Cr are largely exotic (that is non-indigenous plants) and studies into Cr hyperaccumulating mechanisms are scarce. Only few Cr hyperaccumulator species have currently been identified, an example being *Brassica juncea* (L). In this study, Cr (VI) was added to soil as potassium dichromate solution at various concentrations.

2.3 Role of Iron in Plants

Iron is a micronutrient essential for all higher plants. Unlike plant requirements for carbon, oxygen, hydrogen, nitrogen, potassium, phosphorus and sulfur, only a small amount of iron is required to satisfy plant needs. Fe^{2+} is the specie said to be taken up by plants but has low mobility in plants. The symptoms of iron deficiency in plants are leaves of plants becoming nearly white while youngest leaves become pale yellow, but like animals and people, too much iron can have a toxic effect on the plant, weakening and eventually killing it.

2.4 Environmental occurrences

Because chromium occurs in ores, environmental levels are increased due to mining, smelting and industrial uses. The interest in chromium (Cr) speciation originates from widespread use of this metal in various industries such as metallurgy (steel, ferro- and nonferrous alloys), refractories

(chrome and chrome-magnetite), and chemical (pigments, electroplating, and tanning). Due to these industry processes, large quantities of Cr compounds are discharged as liquid, solid, and gaseous wastes into the environment and can ultimately have significant adverse biological and ecological effects (Kotas and Stasicka, 2000).

2.5 Sources of Heavy metals (Chromium and Iron).

The Toxics Release Inventory in 1997 estimated the chromium release was 706,204 pounds to the air, coming from 3,391 large processing facilities which accounted for about 2.2% of total environmental releases. Cr (III) and Cr (VI) are released to the environment through stationary point sources (that is facilities that are identified individually by name and location) resulting from human activities. The estimates of atmospheric chromium emissions in 1976 and 1980 in the Los Angeles, CA and Houston, TX areas indicated that emissions from stationary fuel combustion are about 46-47% of the total, and emissions from the metal industry range from 26 to 45% of the total (ATSDR, 2000).

2.6 Health and environmental effects of Heavy metals (Toxicity)

Cr (III) is said to be nutritionally beneficial as an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids (e.g., impaired glucose tolerance, elevated fasting insulin, elevated cholesterol and triglycerides, and hypoglycemic symptoms) (Anderson and Walsh, 1997) when ingested in small amounts. However, Cr (III) in large amounts may also cause health problems, e.g. lung cancer (Costa, 1997; Zhitkovich, et al 1996). At high concentrations, Cr (VI) is also toxic to humans. Hexavalent Cr (Cr^{6+}) is a potent, extremely toxic carcinogen and may cause death to animals and humans if ingested in large doses (Beaumont, et al 2008). According to the guidelines for drinking water issued by World Health Organization (WHO), the maximum permissible limit for hexavalent

chromium (chromate) and total chromium is 0.05 and 2mgL^{-1}, respectively (Oliva and Espinosa, 2007).

Generally, the entry routes of chromium into the human body are inhalation of airborne particulates, ingestion of food and water, and dermal absorption. Occupational exposure generally occurs through inhalation and dermal contact, whereas the general population is exposed most often by ingestion through chromium content in soil, food, and water (Park and Bena 2004).

2.7 Remediation Techniques for Heavy Metal-Contaminated Soil

Metals are natural soil constituents but increased human interference results in increased concentration of heavy metals exceeding compliance with environmental regulations. An overall objective of any soil treatment/remediation approach is to create a final medium that is protective of human health and brings the concentration of contaminants below or to the level of compliance (Martin and Ruby, 2004). However, regulatory authorities will normally accept remediation approaches that aim at reducing metal bioavailability if reduced bioavailability is equated with reduced risk, and if the bioavailability reductions are demonstrated to be long-term (Martin and Ruby, 2004). The physical and chemical forms of the heavy metals affect the selection of an appropriate remediation approach in the case of heavy metal contaminated soils. Information about the physical characteristics of the site and the type and level of contamination at the site is needed for an accurate assessment of site contamination and treatment (Jadia and Fulella 2009).

Several technologies have been identified recently for the remediation of metal contaminated soil. Gupta et al. (2001) classified remediation technologies for contaminated soils into three categories of hazard-lessening measures: (i) gentle *in situ* (in place) remediation, (ii) *in situ* harsh soil restrictive measures, and (iii) *in situ* or *ex situ* harsh soil destructive measures. The objective

of the latter two harsh alleviating measures is to avert hazards either to man, plant, or animal while the main goal of gentle *in situ* remediation is to restore the functionality of soil (soil fertility), for safe soil usage. Most recently, a variety of approaches have been suggested for remediating contaminated soils. Stegmann, et al (2001) opines that there are four alternatives for treatment of heavy-metal contaminated soils. They include: natural attenuation and restriction of land utilisation, encapsulation of the contaminated site, excavation (physical removal of the contaminated material) and then land filling and stabilisation of the metals in the soil on site. Stegmann, et al (2001) noted that in practice, actual remediation involved the use of mechanical, thermal or biological processes.

Another classification places remediation approaches for heavy metal-contaminated soils in five categories of remediation; isolation, immobilisation, toxicity reduction, physical separation, and extraction (GWRTAC 1997). In practice, it may be more convenient to employ a hybrid of two or more of these approaches for more cost effectiveness. The main factors that may determine the applicability and selection of any of the available remediation technologies are: (i) cost, (ii) long-term effectiveness/ permanence, (iii) commercial availability, (iv) general acceptance, (v) applicability to high metal concentrations, (vi) applicability to mixed wastes (heavy metals and organics), (vii) toxicity reduction, (viii) mobility reduction, and (ix) volume reduction. In 2007, USEPA broadly classified remediation technologies for contaminated soils into (i) source control and (ii) containment remedies. Source control involves *in situ* and *ex situ* treatment technologies for the exact sources of contamination. In situ (is the treatment of contaminated soil in its original place, immobilised, and unexcavated, remaining at the site or in the subsurface. *In situ* treatment technologies treat or remove the complex forming processes of the contaminant (Hashimoto, 2009). *In situ* processes are preferred due to the lower labour and energy requirements (since

treatment will be on site), but implementation of *in situ* treatment will depend on immediate site conditions.

Different methods to remediate metal-contaminated soils have been developed. They include physical, chemical, electrical, thermal and biological methods. Physical, chemical and electrical mechanisms are grouped into one, called physico-chemical. Often times, due to the presence of varying types of contaminants in a media like soil, it is important to apply more than one remediation method to reduce the concentrations of pollutants to an acceptable or recommended level.

2.7.1 Physical/Chemical Treatments

Physical/chemical treatment employs the physical and chemical properties of the contaminants or of the contaminated medium to destroy or chemically convert, separate, or contain the contamination (U.S. EPA 2006). In the physical processes the phase transfer of pollutants is induced. In the chemical processes the chemical structure of the pollutants is changed by chemical means (i.e. reactions) to produce less toxic or better separable constituent compounds from the solid matrix. Equipment is readily available and is generally not engineering or energy-intensive. Examples of these techniques include: (1) Soil washing and (2) Isolation and Containment.

2.7.1.1 Soil washing

Soil washing is a method for the remediation of metal-contaminated soils. It is a volume reduction/waste minimisation treatment method done on excavated soil. It can also be used on on-site soil. During soil washing, there is physical separation based on the particles of the soil, especially those that hold the majority of the contamination of the soil. There is also the removal of contaminants by aqueous chemicals, and contaminants are recovered from the solution on a

solid substrate. The soil washing technique employs the principles of the difference in grain size, setting velocity, specific gravity, surface chemical behaviour and magnetic properties of soil particles (Peters, 1999). It is a process that removes contaminants by dissolving or suspending them in the wash solution (Stegmann, 2001). Soil washing in general is a technique that targets semi-volatile organic compounds (SVOCs), fuels, and heavy metals. The technology offers the ability for recovery of metals and can clean a wide range of organic and inorganic contaminants from coarse-grained soils. The limitations of the soil washing technique include inefficiency on complex waste mixtures (e.g., metals with organics) which makes formulating the washing fluid difficult. Another limitation may be possible high humic content in soil which may require pretreatment. Also additional treatment steps may be required to address hazardous levels of washing solvent remaining in the treated residuals. It may be difficult to remove organics adsorbed onto clay-size particles.

2.7.1.2 Isolation and containment

This is sometimes called solidification/stabilisation (S/S). Solidification involves the addition of binding agents to contaminated mediums. These binders prevent the movement of contaminants or reduce the permeability of the waste to a minimum. Cement-based binders and stabilisers are a few of the most used materials for the implementation of the S/S method (EPA, 1989). According to Mulligan (2001), capping is another technology to prevent water infiltration into the soil, but it is site specific (directed to particular sites depending on site properties).

2.7.2. Thermal Treatments

Thermal treatment of heavy metal contaminated soils has been described as a promising way for the decontamination of residues by incineration. Thermal treatments offer fast cleanup times but

is a method seen as very expensive because of energy and equipment costs and is both capital and operation and maintenance (O & M) intensive (Stegmann, 2001). A thermal process employs heat in the increase of contaminant volatility, in burning, in the decomposition, and in the destruction or melting of contaminants. Cleaning soil with thermal methods may take only a few months to several years. The time it takes depends on three major factors that vary from site to site: type and amounts of chemicals present; size and depth of the polluted area; type of soil and conditions present.

2.7.3. Electrokinetics

Electrokinetics employs direct electrical current to remove organic, inorganic and heavy metal particles from the soil. Electrokinetic processes make use of the low intensity electric current between a cathode and an anode plugged in the contaminated soil of interest causing the passage of ions and of small charged particles between the electrodes. A buffer solution ensures that the pH at the electrodes is maintained. Metals which can be bound to soils as oxides, hydroxides and carbonates are removed by this method. Mulligan (2001) opines that one major advantages of this method is that it can be used as a very effective way for low permeable soils.

2.7.4. Biological Treatments

The use of biological processes for the removal of heavy metals in contaminated sites is generally termed bioremediation. A biological treatment employs the use of living organisms to remove contamination, especially of heavy metals and organic compounds from soil. Some basic principles of bioremediation are bioaccumulation, biosorption and biocrystalisation. The use of plants in bioremediation is termed *phytoremediation* (Evangelou, 1998). The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the root zone is called *rhizodegradation*. This process uses microorganisms to consume and digest organic

substances for nutrition and energy. Natural substances released by the plant roots, such as sugars, alcohols, and acids, contain organic carbon that provides food for soil microorganisms and establish a dense root mass that takes up large quantities of water. This process is suitable for organic substance contaminants in soil medium (Prasad and Freitas, 2003). Biological processes are low cost processes and contaminants can be destroyed or made harmless either by transformation or by degradation into a stable form.

2.7.4.1 Phytoremediation

Phytoremediation ("phyto" which means plant, and the Latin suffix "remedium" meaning to make clean or reclaim) refers to the various categories of green technologies that make use of either naturally occurring or genetically engineered plants to remediate polluted air, soil, and water (Cunningham et al. 1997; Flathman and Lanza, 1998). With remediation of heavy metal contamination still a difficult task and selection of remediation technique still a complex process, there is the challenge of finding a method that is accessible (i.e. perceivable, operable, understandable and robust), available (the technique can be adapted for use) and economically achievable or feasible. McIntyre (2003) itemizes some factors that play an important role in the selection of a suitable procedure for remediating metal contaminated soils. They include size of remediation area, location and history of site. Others include accessibility and availability of technical expertise, financial resources and extent of contamination.

As an emerging technology, phytoremediation can be successfully applied in the remediation of metal contaminated sites (Dong Jianxin, 2007). According to Wang Zi (2011), the bioavailabilty of metals to plants is determined by factors such as soil metal concentration, soil processes and properties, physical processes such as root intrusion, water, and ion fluxes and their relationship to the kinetics of metal solubility in soils. Worthy of note are factors such as biological

parameters, including kinetics of membrane transport, ion interactions, and metabolic fate of absorbed ions. Furthermore, bioavailability of metals to plants is also influenced by the plants' ability to adapt metabolically to dynamic metal stresses in the environment. Plant species and age of vegetation also affect metal uptake (McIntyre, 2003).

As a means of reducing the cost of other methods of remediation, especially where it is impractical, phytoremediation is applied to waste sites. It has also been advanced as a method applicable to low-level contaminated sites especially where only polishing treatment is required with time. As a final cap and closure to sites, phytoremediation can be used in conjunction with other technologies (e.g. isolation and containment).

2.7.4.2 Types of Phytoremediation

Phytoremediation includes six types of cleanup categories. The first is phytoextraction, which refers to the use of plants to remove contaminants from soils, sediments or water into harvestable plant biomass. Secondly, rhizofiltration refers to the approach of using cultivated plant roots in the removal of contaminants in water through absorption, concentration, and precipitation of pollutants (Sengupta et al., 2008). This has been described to be a hydroponical process (cultivation of plants in a nutrient solution rather than in soil). The third category is phytovolatilisation which refers to plants' uptake and transpiration of contaminants, organic compounds being the primary target forms of contamination. Fourth, is phytostabilisation which has been described as the reduction of the mobility of heavy metals in soil by plants. A very good example is that plants' presence can reduce wind erosion, or the plants' roots preventing water erosion and the immobilisation of the pollutants by absorption or accumulation. This provides a zone around the roots where the contaminants can precipitate and stabilise (Blaylock, 1995; Miller, 1996). More so, phytotransformation refers to the breakdown of organic contaminants

sequestered by the metabolic processes of plant or the effect of compounds produced by the plant (EPA, 1998). Finally, phytostimulation make up the sixth category. It is the breakdown of organic contaminants in the soil through enhanced microbial activity in the plant root zone or rhizosphere. The table below shows the advantages and disadvantages of pytoremediation.

Table 2: Advantages and disadvantages of phytoremediation.

Advantages	Disadvantages
Cost reduced over traditional/ conventional methods.	Long remediation time required
Low volume of secondary waste	Effective depth limited by plant roots
Improved aesthetics	Phytotoxicity limitations
Habitat creation – biodiversity	Fate of contaminants often unclear
Green technology	Climate dependent/variable
More publicity accepted	Seasonal effectiveness
Provide erosion control	Potential transfer of contaminants to animals or air
Prevent runoff	Harvesting and disposal of biomass as hazardous waste may be required although generally not
Reduce dust emission.	
Reduce risk of exposure to soil	
Less destructive impact (applied in situ)	

2.7.5 Mechanisms of Metal Uptake from the Soil

Plant roots are responsible for transporting soil solutions from the soil to the shoots and constitute about 20–50% of plant biomass. Most work done on the mechanisms of root and plant cell uptake

has focused on the study of N, P, S, Ca, K and possibly Cu (Marschner, 1995). While some information have been gained from these studies of essential mineral elements, little is known about the mechanisms of mobilisation, uptake and transport of most environmentally hazardous heavy metals, such as Pb, Cd, Cu, Zn, U, Cr, Sr, and Cs. It is important to note that a large proportion of these metals remain in solid soil constituents. In order to acquire these 'soil-bound' metals, phytoextracting plants need to mobilise them into the soil solution. Plant roots possess the ability to solubilise soil-bound toxic metals by a process of acidification of soil environment using protons extruded from the roots. This is a mechanism that has been observed for Fe mobilisation in some Fe-deficient dicotyledonous plants. The mechanisms of uptake and translocation of Cr in plants differ with the lapse of time (Barcelo & Poschenrieder, 1997). Previous studies with wheat revealed that only Cr (VI) is taken up by plants (Bourque, 1967). Later studies using rice suggested that Cr (VI) is reduced to Cr (III) before penetrating plant roots (Myttenaere & Mousny, 1974). Both Cr (VI) and Cr (III) are believed to be taken up by plants. However, the two ions do not have a common uptake mechanism (Zayed & Terry, 2003). Uptake of Cr (III) seems to be passive, while that of Cr (VI) is considered to be active (Barceló & Poschenrieder, 1997). The uptake of Cr (VI) is mediated by the sulfate carrier but with lower affinity (Skeffington et al., 1976), Cr (III) tightly binds to carboxyl groups of amino acid in proteins forming binuclear complexes (Schlosser, 1991). It was reported that, following uptake, Cr (VI) is immediately reduced in cells to Cr (III). Once inside the cell, Cr (III) is located in the cytosol (Sayato, 1980; Yamamoto, 1981).

Plants have evolved two strategies to take up Fe from the soil. Non-grasses activate a reduction-based strategy I when starved for Fe whereas the grasses activate a chelation-based strategy II. The first strategy is the reduction-based Strategy of proton release. Under Fe-deficiency, Strategy

I, plants extrude protons into the rhizosphere, lowering the pH of the soil solution and increasing the solubility of Fe^{3+} (Olsen et al., 1981). The second strategy is done with proton leakage and revival material by plant in which trivalent iron (Fe^{3+}) (with very low solubility) is converted to divalent iron (Fe^{2+}) (with more solubility). This method exists in other monocotyledon and dicotyledon plants. In higher plants the ability to convert the extracellular Fe^{3+} to Fe^{2+} is due to physiological and morphological changes; that is done in accordance with intracellular iron levels. This structural change will determine the efficiency of iron uptake by the plants species (Pandey, 2003).

2.7.6 Heavy Metal Toxicity to Plants

Chromium is known to cause a decrease in enzyme activity and plant growth and causes membrane and root damage. Cr is observed to be toxic to higher plants at 100 ppm starting concentration (Davies et al., 2002). Effects of high iron concentrations on plants have been investigated and a number of visible effects associated with high iron concentrations were commonly observed (Cook, 1990). Symptoms which were observed include growth retardation, reduction in leaf size, deepening of green leaf colour (particularly in the youngest leaves), reddening or purpling of stems and older leaves; wilting of shoots; yellowing of oldest leaves, especially from the tips or margins; brown or black speckles or larger necrotic patches on leaves; blackening of leaf tips and stem bases; stiffening of stems, root stunting (particularly of adventitious roots), lack of root branching; and root flaccidity (Cook, 1990).

2.7.7 Factors Affecting Heavy Metal Uptake Mechanisms

A good knowledge of the various important factors that affect the uptake of metals by plants will enhance plant performance in the process of phytoextraction. Brief discussions of some notable factors include:

2.7.7.1 The Plant Species: Screening, identification and selection of plants species with superior remediation properties is important (Prasad and Freitasl., 2003). The uptake of contaminants is affected by plant species characteristic (Burken, 1996). The success of the phytoextraction technique depends on the identification and selection of appropriate plant species with hyperaccumulation capacities for heavy metals and production of large amounts of biomass using established crop production and management practices (Rodriguez et al., 2005).

2.7.7.2 Properties of Medium: Development and adoption of good agricultural practice (pH adjustment, addition of chelators if needed, fertilisers and composting) to enhance remediation of heavy metal contaminated soil is of utmost importance (Prasad and Freitas, 2003).

2.7.7.3 Root Zone: Absorption, storage and in a few cases metabolism of contaminants take place inside the root zone, in other words inside the plant tissue. Degradation of the absorbed contaminants in the soil by plant enzymes (in cases of organics) exuded from the roots is another phytoremediation mechanism. A morphological adaptation to drought stress is an increase in root diameter and reduced root elongation. These are responses to the lessened permeability of the dried soil (Merkl et al., 2005).

2.7.7.4 Vegetative Uptake: Environmental conditions affect vegetative uptake (Burken et al., 1996). Temperature has been found to affect root length considering that root structure under field conditions differs from that under greenhouse condition (Merkl, 2005). Fayiga (2004) opines that the success of phytoremediation, especially phytoextraction, depends on having a contaminant-specific hyperaccumulator. Mwegoha (2008) showed that understanding mass balance analyses and the metabolic fate of pollutants in plants are the keys to a successful phytoremediation. Availability of the metals may be affected by plants being able to lower the pH and oxygenate the

sediment (Fritiof, 2003). To this end, it may be necessary to increase the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients (Van Ginneken et al., 2007).

2.8 Regulatory Guidelines for Some Heavy Metals

There are over 35 metals that are of great concern because of human exposure to them in occupational or residential situations; 23 of these are heavy metals: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze, 1996).

Operations on a site influence the specific type of metal contaminant to be found in such an area. Contaminant concentrations, the physical and chemical forms of contaminants will also depend on the activities and disposal patterns for contaminated waste on the operating site. Other factors that may influence the form, concentration and distribution of metal contaminants include soil and ground water chemistry and local transport mechanisms (GWRTAC).

Riley (1992) and NJDEP (1996) both reported soil concentration ranges and regulatory guidelines for some heavy metals (Table 3).

Table 3: Regulatory guidelines for some heavy metals

Metal	Soil concentration range (mgkg−1)	Regulatory limits (mgkg−1)
Pb	1.00–69 000	600
Cd	0.10–345	100
Cr	0.05–3 950	100
Hg	<0.01–1 800	270
Zn	150–5 000	1 500

Serious contamination arises when concentrations of metals are above the regulatory limits. Regulatory limits gives an indication of the quality of soil required for sustainability or are expressed in terms of remedial policy, the soil quality required to correct the malfunctionality to support human, animal, and plant life. The regulatory values generally indicate the ultimately desired soil quality levels.

2.9 Metabolic Fate of Heavy Metal Pollutants in Plants

A successful phytoextraction process is one in which the heavy metal pollutants are accumulated in the harvestable parts of the plant. From the roots to stems and plant leaves, metals have been found to accumulate in various parts of the plants. The fern is capable of taking up a range of arsenic species including arsenate and arsenite, with up to 93% of the arsenic concentrated in the fronds (Zhang, 2002, Nandita, 2011). As arsenate is known to enter the plant root through the phosphate uptake system and to limit the toxicity, there is need for chemical reduction of As (V) to As (III) in the roots by the plants. In the case of Indian mustard, a large portion of the arsenic is transported to the shoots; however the addition of water soluble As-chelators can increase this fraction (Salt, 2002). The study by Salt (2002) reported that in most plants, a greater amount of Cd remains in the plant root and only smaller portion is translocated to the shoots. It was proven by experiments conducted by Mikus (2005), that zinc and cadmium concentrations in the shoots of plants are of linear correlation to total soil zinc and cadmium. It was also revealed that 80% of the accumulated lead is immobilised in the roots.

2.9.1 Utilisation of Phytoremediation by-products

Utilisation of by-products is based on hyperaccumulative plants and the mechanism of phytoremediation. Hyperaccumulation is defined as concentration of metal in the harvestable above ground tissues of the plant, with levels in the range of 0.1-1% of the dry weight of the

plant. Phytomining and diet enrichment of trace metals in the edible parts of plants were mentioned for utilisation of the by-products (Suresh & Ravi shankar, 2004; Raskin & Ensley, 2000). Phytomining is a green technology involving the use of hyperaccumulative plants to grow and concentrate a metal in the plant tissue. Phytomining when combined with biomass generation and its commercial utilisation as an energy source is seen as a product of the overall phytoremediation process. This is because it can be turned into a profit-making operation and the remaining ash can be used as bio-ore. An approach to the post-phytoremediation strategy would be to incineration of biomass, resulting in ashing which has high metal content, and non-atmospheric emission (Ghosh & Singh, 2005; Anderson, 1999). Also worthy of mention is diet enrichment by trace elements such as zinc, iron and selenium in the edible parts of plants for feed supplements or bio-fertiliser. Over 450 taxa are known to hyperaccumulate heavy metals, ranging from annual herbs to perennial shrubs and trees. However, the chemical forms of the heavy metals accumulated in these plants have to be clearly understood before application as a supplement can be achieved (Suresh & Ravishankar, 2004; Chantiratikul, 2008; Ensley, 2001).

2.9.2 Selection of Phytoremediation Plants

A key feature of an ideal plant for phytoremediation is the ability for a high accumulation rate of contaminants. Therefore plant selection should be considered based on issues of plant tolerance to pollutants, evapotranspiration rates of plants, climatic issues and weather (e.g. flood, drought) effects on the plants. Other factors may include plant growing season, root depth, and disease and pest resistance. Secondly, accumulation of high contaminant concentration in the plant body is necessary. Thirdly, the simultaneous polymetal-accumulation ability is important; the plant should be a fast-growing, high biomass plant and then possess strong resistance to metal toxicity (LvWeili, et al., 2004).

2.9.3 General characteristics of *Psoralea Pinnata*

Psoralea pinnata is a small tree species, which can form dense thickets, shading out species of the lower strata, crowd out shrub species and impede the regeneration of over storey species (Muyt 2001; Weber 2003). A characteristic feature of a phytoextractive material is the ability of plant to produce enough biomass (Reeves, 2003.). *Psoralea pinnata* is a South African shrub with excellent production of biomass, also having an array of characteristics that makes it promising for phytoremediation application. *Psoralea pinnata* is very fast growing (Blood, 2001) and can grow rapidly to 1.5 m in a year, reaching up to 2.4 to 3.0m when fully grown. It can also fix nitrogen which can change the soil fertility and affect species' persistence in the long term (Muyt 2001). It needs full sunlight, well-drained soil and little water as it grows thick and bushy. At a distance, it appears to be covered with dainty blue moths. Its flower structure is described as *papillonaceous,* similar to that of a sweet pea. Though it is native to stream sides, it survives occasional drought. It is best propagated by seeds (Gobalt, 2000) as seeds take about 2-3weeks to germinate.

CHAPTER THREE

3. Materials and methods

3.1 Soil collection

Potting soil was obtained from Plantland, a commercial gardening supplier in Pretoria, air-dried and designated as PS. A second soil was obtained from a grass field in UNISA main campus (Muckleneuk, Pretoria) by digging up to 30 cm below the soil surface and designated as US. The use of the two soils was to enable the comparison of a soil with known characteristics, which have been tested for plant growth and an unknown soil. The soils were homogenized by removing pebbles, stones and gravels with hand and then air-drying before storing in cellophane bags at 4^0C before use. All equipments used for soil sample collection were cleaned to avoid contamination. This is according to EPA Appendix B, Standard cleaning procedures, prior to use (Beiergrohslein, 1998).

3.1.1 Soil Characterisation

The two soils were characterised by the use of near-infrared reflectance spectroscopy (NIRS). The soil qualities analysed include organic matter, cation exchange capacity CEC, available nitrogen, available phosphorus and texture. Soil characterisation was done at the chemistry laboratories of the University of South Africa (Muckleneuk campus). Soil pH and heavy metal content of both soils were also analysed at the chemistry laboratories of the University of South Africa. Composition and nutritional state of the two soils used in this study are clearly stated in table 3.1 below. Soil pH was measured in double distilled water using a solid: liquid ratio of 1: 2.5 equilibration for 2 hours (Ramesh, 1998). Heavy metals were analysed using a Perkins Elmer 300^{TM} spectrophotometer (equipped with Quartz touch, automated sampler and skimmer cones,

and a peristaltic pump maintaining a 1ml min^{-1} sample uptake rate), a cross flow type pneumatic nebulizer and a double pass Scott-type spray chamber. Other operating conditions are summarised in Table 4.

Table 4: Composition and nutritional state of the two soils used in this study

Charecteristics	UNISA Soil (US)/	Potting Soil (PS)
pH-H_2O	7.41±0.25	6.43±0.49
CEC(meq/100g soil)	11.2	21.8
Organic carbon (% wt)	12.12	0.87
N_{tot} (% wt)	0.02	0.05
P_{tot} (% wt)	4.4	9.1
K (ppm)	3.2±0.29	14.8±0.52
Sand (%)	63.9	8.9
Silt (% wt)	15.3	18.0
Gravel (% wt)	≤ 5	N/A
Clay (% wt)	19.0	69.8
Ca (ppm)	61.5±0.39	82.8±0.53
Mg_{tot} (ppm)	1.5±0.79	8.5±0.82
Mn (ppm)	9.7±0.89	75.6±0.64
Na(ppm)	147±0.03	44.0±0.61
Fe_{tot}(ppm)	57.2±0.61	4.6±0.45
Cr_{tot} (ppm)	78.0±0.27	10.2±0.31

The results presented in the table above suggest that the soil samples used in this study are different from each other.

Table 5: FLAA-operating conditions

RF power (W)	1000
Plasma argon ($Lmin^{1}$)	600
Plasma nitrogen ($Lmin^{-1}$)	400
Nebulizer flow ($Lmin^{1}$)	2.5
Nebulizer	Cross-flow
Data acquisition	Peak hop transit
Resolution	Normal
Delay time (mins)	30
time (secs)	10
Number of replicates	3
Standards (ppm)	10, 25, 50, 75, 100, 200

The method used was ISO 11466 (Aqua regia) involving leaching out of the metals from the soil with 3:1 ratio HCl to HNO_3 and analysing the metals with FLAA.

3.2 Plant and Soil Preparation.

Psoralea pinnata seeds were collected from Silverhill Seeds and Books, Cape Town. All seeds were taken to the Department of Agriculture, Hamilton Street, Pretoria, South Africa, for validation. A nursery bed was prepared by tilling and watering soil for one week before the seeds were planted. This was done to help the plants in absorption of both nutrients and water. *Psoralea*

pinnata seeds were planted in the nursery bed and allowed to grow for 2 weeks before being transplanted into PVC pot (210mm× 230mm) containing contaminated soil samples of different concentration of Fe and Cr of two kinds of soil, in the greenhouse at the University of South Africa. Some plants, which were not transplanted into contaminated soil, were kept aside for metal analysis. Fifty-four (54) PVC pots were used for each type of the two kinds of soil besides the control. The experiments were watered (250ml of water) twice daily, with just enough water to keep the soil wet and avoid water logging and leaching (a method also adopted during the main phytoextraction experiment). The experiments were set up in triplicates. The soil was mixed with animal (horse) manure that was obtained from the Department of Veterinary Science, University of Pretoria, Onderstepoort at a ratio of 1:5 manure to soil. Animal manure (compost) was used because the recommended fertiliser was lethal to the plants at the recommended and varied dose. No planting hormone was added. The plants in the soil bed were allowed to grow for 1month and were then used for the subsequent experimentation. The bed was watered manually using a watering can to maintain moisture. Control experiments were set up with uncontaminated soil and planted with seeds.

3.3 Chemicals and reagents used in the study

All chemicals and reagents used in this study were of analytical grade. Working standard solutions were obtained from Merck South, Africa. Calculation and preparations of the required concentrations were based on the dilution equation:

$C_1V_1 = C_2V_2$

where:

C_1 = Molar concentration of stock solution

C_2 = Required molar concentration

V_1 = Volume of stock solution

V_2 = Volume required

3.3.1 Chemicals used for digestion of Samples

The chemicals used in this experiment include:

- 98% (pure) Nitric acid.

- 65.3(pure) Hydrochloric acid. Both chemicals were supplied by Merck , South Africa.

- Deionized water was used in making up to required volume, digested and extracted samples. Deionized water was prepared from Milli-Q instrument (Millipore, Bedford USA).

3.3.2 Digestion of Sample

All soil and plant samples were dissolved using acids. The acids were mixed concentrations of nitric acid and hydrochloric acid in a ratio of 1:3. The solution was assisted by gently heating, using a hot plate at 100^{o}C for 1 hour. The resulting solution was allowed to cool and was filtered with filter paper, into test tubes before analysis.

3.4 Method Development

3.4.1 Determination of Optimum pH for Growth of *Psoralea Pinnata*

pH experiment was conducted to determine the best pH concentration range plants record optimum growth. Results of the experiments were used in designing the phytoextraction experiments. The two kinds of soil (potting soil and UNISA soil), in the pots were adjusted to pH levels 2, 4, 5, 6, 8, and 10 with 3M HCl and 3M NaOH as required. This was done by amending soil with different acid and base concentrations and air-dried before pH measurement. This experiment was done in six treatments and in triplicates. Soil was left in plastic containers with cover to avoid loss of water, to age for two weeks. Plants were watered with 250 ml of tap water

to replace water lost through evapo-transpiration and leaching. *Psoralea pinnata* seedlings were used in all experiement. Plants were harvested after 4 weeks, dried at 100^{o}C for four days and weighed. The final pH of the soils from which plants were harvested was also determined.

3.4.3 Determination of Optimum Fertilizer usage for *Psoralea Pinnata*

NPK Slow Release fertilizer [3:1:5 (26)] recommended for the family *fabaceae*, to which *Psoralea pinnata* belongs was used in the experiment. Quantities from 5g to 20g were added in solution away from the root zone *Psoralea pinnata* plants growing inside PVC pots. This was done two weeks after transplanting 4-weeks old *Psoralea pinnata* into the PVC pots, to make sure that plants were already adapting and growing in the new environment. Again, because the set up was done under a greenhouse, the fertiliser had to be dissolved in water before application and the quantities were informed by not enough information for quantity of fertilizer dependence of *Psoralea pinnata* fertilizer manufacturer. This experiment had four treatments and was carried out in triplicates. The parameters to be measured include number of leaves and shoot height.

3.4.4 Determination of toxicity of Cr and Fe to *Psoralea pinnata*.

Toxicity tests were done to determine the effects of chromium and iron salts on *Psoralea pinnata*. Soil was amended with Cr and Fe to give final concentrations of 40, 80, 120,160, 320 and 480 ppm. Amended soil was made to undergo a wet and dry cycle for 2 weeks to attain partial ageing. This was achieved by repeatedly water-logging the soil and allowing it to dry over time without allowing leaching. Each salt concentration was in triplicates. Uncontaminated soil was used as a control and was designated C1, C2 and C3. PS and US indicate potting soil and UNISA soil, respectively. This experiment was done to establish what concentrations the plants could survive. Physical observation of plant response was the basis of establishing how the main phytoextraction

experiment would be designed. At soil pH of about 6, Cr (VI) becomes more soluble than Cr (III). Cr (VI) is the predominant form of chromium at this pH value and it is also recorded as the most easily transported in plant. This is the reason why Cr (VI) was chosen as the study species treatment. The temperature and light in the greenhouse took the form of 16/8 day light and night. Two uncontaminated soil types (potting soil and UNISA soil) were used as controls. They were analysed to determine their metal concentrations.

3.4.5 Control experiments

1. Potting soil without any amendment was used as the first control and designated C-PS
2. UNISA soil without any amendments of either iron or chromium was used as the second control and designated C-US

3.5 Experimental Design

One hundred and fifty-six (156) PVC pots were used for the experiment, each filled with 500g of experimental soil. The pots were divided into two sets of 78 PVC pots, one set for iron treatments and the other for chromium treatments. Each set was further subdivided into two subsets of 39 PVC pots. The first subset was used for potting soil samples and the other subset was used for UNISA soil samples. The control experiments were set up using both types of soil without any iron or chromium amendment. All experiments were set up in triplicates.

Four weeks old *Psoralea pinnata* plants were transplanted into the $Fe(NO_3)_3.9H_2O$(Ferric Nitrate) and $KCrO_4$ (Potassium Chromate) treated soil and allowed to stand in the greenhouse for 12 weeks. The plants were watered manually with 250 ml tap water using a watering can twice daily to maintain moisture at 70% at field capacity (Atagana, 2011) and avoid water logging and

leaching of metals. The greenhouse was ventilated through an air vent. Unwanted weeds were manually removed from all the experimental set up to avoid un-accounted for removal of metals from the soil. Biometric data example shoot height and number of leaves were measured twice a month with the exception of the measurement of the root length. Root length was measured before plant transplant and after plant harvest. Shoot height and root length were measured using a ruler. Differences in biometric data manifested in increase in size or number of leaves and length of shoot. Animal manure was used in the ratio of 1:5 manure to soil because the inorganic fertilizer [NPK = 3:1:5(26) SR] used prior to the experiment was lethal to *Psoralea pinnata*. Each treatment of plants in contaminated soil was separated from the other within the greenhouse to avoid cross-contamination of samples. The control samples/untreated controls were kept at a distance from the contaminated samples. Below is a figure to illustrate the design of the experiment.

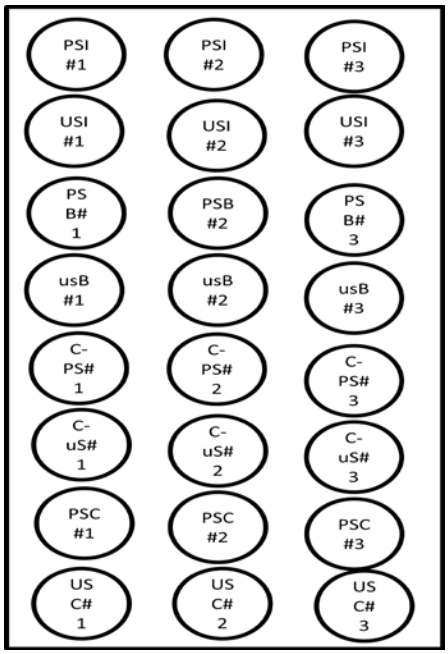


Fig. 3.1: Experimental design describing the arrangement of samples.

The figure above is a pictorial representation of the experimental design. The PVC pots were arranged in three rows. The uncontaminated control pots are designated as C-US and C-PS. The rest including USI, USC, USB, PSI, PSC and PSB represent the samples containing metal amendments.

3.5.1 Treatments

The following treatments were used in the experiment before *Psoralea pinnata* was transplanted into the pots containing the treatment samples.

Table 6: Treatments and sample concentrations

Treatments	Description	Concentration (ppm)
PSI	potting soil contaminated with iron	80, 130, 180, 230, 280, 330, 380, 430, 480
PSC	Potting soil contaminated with chromium	80, 130, 180, 230, 280, 330, 380, 430, 480
USI	UNISA soil contaminated with iron	80, 130, 180, 230, 280, 330, 380, 430, 480
USC	UNISA soil contaminated with chromium	80, 130, 180, 230, 280, 330, 380, 430, 480
PSB	Potting soil contaminated with both iron and chromium metals	40, 80, 120, 160, 200, 240, 280, 320
USB	UNISA soil with both iron and chromium metals	40, 80, 120, 160, 200, 240, 280, 320

3.6 Sampling and Data Collection

Measurement of plant parameters was done and recorded before plants were transplanted into contaminated soil. The length of the shoots was measured every two weeks using a ruler to determine the change in shoot height. At the end of the experiments the plants were removed from the PVC pots, washed free of soil with tap water, rinsed with distilled water and air-dried. Plants were separated into shoots and roots and weighed using Mettler Toledo balance model PB1502 (MICROSEP, Switzerland). Soil samples were collected from each experiment after harvest for analysis. Plant samples were kept in Whirlpak^{TM} bags (NASCO, South Africa) in the refrigerator until analysis.

The data collected include shoot length on the day of transplanting *P. pinnata* and every two weeks after, until the day of harvest. This was to determine change in the height of plant. Root length was also noted on the day of transplant and on the day of harvest of plant using a ruler to determine change in root length. During the time of experiment, numbers of plant leaves were recorded on the day of transplant and every two weeks after using a manual count. All data were collected manually. Wet shoot and dry weights, wet root and dry weights were also recorded before and after oven-drying. Plant biomass was weighed and recorded using a weighing balance.

3.7 Phytoextraction Experiment

Solutions of $KCrO_4$ and $Fe(NO_3)_3.9H_2O$ were prepared and added to the soil to give final concentrations of 80, 130, 180, 230, 280, 330, 380, 430, 480 and 500 ppm. The pH of the two soil types was adjusted to 5.5 by amending with 3M NaOH and HCl. Both $KCrO_4$ and $Fe(NO_3)_3.9H_2O$ was added to soil samples which were left to undergo a wet and dry cycle for 2 weeks to attain partial ageing.

In a separate simultaneous experiment, a different phytoextraction based experiment was set up to find out the effect of co-contamination of these two metals (iron and chromium) on *P. pinnata* and possible phytoextraction by the plant in a complex multimetal system. This experiment was done at a ratio of 3:2, chromium to iron as the possible composition of ferrochrome. Ferrochrome is an alloy containing both metals that pollute the environment in smelting processes.

3.8 Digestion and Analysis of Plant materials for Chromium and Iron.

The plant samples were sealed in aluminium foil, oven-dried for one week and kept in the refrigerator before metal analysis. Dried samples were homogenized using a mill. The samples were digested using Aqua Regia digestion process. This process involves an acidic mixture of concentrated HNO_3: HCl (APHA-1992) at the ratio of 1:3 with samples. The solution was assisted by gently heating using a hot plate at 100^oC for 1 hour. The resulting solution was filtered using Whatman #40 filter paper. Iron and chromium analyses were done in triplicate using Flame Atomic Absorption (FAAS) Spectroscopy (S10: AA 800). Elemental chromium and iron were determined by atomic absorption at the wavelengths of each element. Transportation index (Ti) gives the shoot/root chromium and iron concentration and depicts the ability of the plant to translocate the metal species from roots to shoots at different concentrations. It was calculated as:

$$Ti = \frac{\text{Chromium content in shoots}}{\text{Chromium content in roots}} \times 100$$

Metal uptake, also described as Metal Accumulation Factor (MAF), provides an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Mellem, 2009). It was calculated as follows:

Metal Accumulation Factor (MAF) = Average absorbed metal conc. in the plant tissue

Total chromium in soil

Comparison of the uptake of a plant species has limited application if one wishes to compare it under different treatments. Since change in MAF is related to the individual plant biomass and soil elemental concentration, the efficiency of MAF is better understood when comparisons are made between different harvests of plant species or elements.

3.8.1 Analysis of Chromium and Iron by using Flame Atomic Absorption (FAAS) Spectroscopy.

The concentration of chromium (Cr) and iron (Fe) in soil and plant tissues were determined with a spectrophotometer Perkins Elmer 300. Calibration of the spectrophotometer was done using standards of Cr and Fe prepared from metal grade standards. Standards were supplied by Merck (Pty) Ltd. The concentration of calibration standards were set (Table 3) based on the characteristic concentration check (mg/L) to get a linear correlation. The flame was calibrated for the respective heavy metals. For all calibrations, standards of each metal were prepared from metal grade standards. The filtered samples, after digestion, were diluted with distilled water to the appropriate concentration so that there were no problems of clogging the spectrophotometer tubes with saturation of the spectrophotometer.

Determination of Soil pH

The pH of the soil was determined by adding 10 gram of air dried soil to 15 ml of distilled water and stirring vigorously for a few minutes and then allowed to stand for 30 minutes. The process of stirring and allowing stand was repeated twice, after which the solution was allowed to finally stand. The pH of the solution was measured using a calibrated SympHonySB20 pH meter (Germany). Calibration of the pH meter was done using two buffer solutions with pH's 4 and 7.

Determination of Soil Organic Carbon

The percentage of organic matter in the soil samples was determined from the percentage carbon based on the relation OM % = C % × 1.732 (Zhang, 2004). OM% represents the percentage organic matter in the soil and C% is the percentage carbon in the soil. This is according to a previous study by Beiergrohslein (1998).

Determination of Cation Exchange Capacity.

The cation exchange capacity (CEC) was determined according to previous experiment and a method adopted by Ann Mary (2005). To determine the CEC, a plastic centrifuge tube was weighed; 20 ml of 0.1 M $BaCl_2$ saturating solution was added to 5 g of air dried soil centrifuge tube (plastic) and vigorously shaken at room temperature for 1 hour in a thermolyne shaker at 350 rpm. The resulting solution was centrifuged in a Marathon 3200R centrifuge at 1000 rpm for 30 minutes. 20 ml 0.002 M $BaCl_2$ was added to the soil in increments and then shaken in a thermolyne shaker at 350 rpm for 45 minutes. The solution was then centrifuged, using a Marathon 3200R, at 1000rpm for 20 minutes. The supernatant was then discarded. The centrifuge tube plus soil and entrained 0.002 M $BaCl_2$ of solution was weighed and 10 ml of 0.005 M $MgSO_4$ reactant solution was added to the soil. Solution was gently shaken at 200 rpm for 1 hour in thermolyne shaker. The exchange capacity of the suspension was then measured and using distilled water, the exchange capacity was adjusted to the exchange capacity of 0.0015M $MgSO_4$ ionic strength reference solution by measuring the conductivity. The centrifuge tubes plus contents were weighed to determine the volume of water that needed to be added for adjusting the conductivity. This was followed by centrifuging at 1000 rpm for 30 minutes and decanting the supernatant that was retained for analysis. The solution was analysed for magnesium using a

Perkins Elmer AAnalyst 300 Atomic Absorption Spectrometer (Germany) and the pH was also measured using SympHony SB20 pH meter. The CEC was calculated from the equation below:

CEC in meq/100 g = $100(0.01 - C1V_2)$/ (oven dry weight soil sample in g) (2)

where C1 is the concentration of Mg in the supernatant and V_2 is the volume of final supernatant solution (milliequivalents/milliliter). The experiment was done twice.

3.8.2 Statistical analysis

General Linear model of analysis of variance was used for describing percentage of heavy metal (iron and chromium) removal from the soil.

CHAPTER FOUR

4 Results

4.1 Determination of optimum pH for the growth of *Psoralea pinnata* for potting soil

The result from the determination of optimum pH for the growth of *P. pinnata* is presented in the table below:

Table 7: Result of pH experiment after 4weeks for potting soil. Values are means of three Replicates

Initial soil pH	Final soil pH after 4 weeks	Plant dry weight (g)	SD
2.0	2.8	0.0	0.17
4.0	4.5	3.8	0.32
5.0	5.3	12.8	0.88
6.0	6.1	3.5	0.14
8.0	7.7	2.9	0.51
10.0	9.2	0.0	0.05

The result of the experiment to determine optimal pH for growth of *P. pinnata* showed that the best growth was observed at initial pH 5.0. This was recorded when biomass production for *P. pinnata* at a dry weight of 12.8g (see table above). At pH 2 and 10, the plants died before the end of the four week growth period so there was no harvest. At pH 4, 6 and 8, *P. pinnata* survived however there was low production of biomass as the dry weight values were 3.8g, 3.5g and 2.9g. The experiments were conducted using potting soil and the result represents an average value for triplicate sampling.

4.2: Determination of optimum pH for the growth of *P. pinnata* in US Soil. Values are means of three replicates

The result from the determination of optimum pH for the growth of *P. pinnata* of UNISA soil is presented in the table below:

Table 8: Result of optimum pH growth dependence of P. pinnata in US Soil.

Initial soil pH	Final soil pH after 4 weeks	Plant dry weight (g)	SD
2.0	2.5	0.0	0.48
4.0	4.7	5.3	0.20
5.0	5.7	11.7	0.45
6.0	5.8	6.5	0.37
8.0	8.3	2.5	0.67
10.0	9.6	0.0	0.32

The above result of the experiment to determine optimal pH for growth of *Psoralea pinnata* in the UNISA soil showed that at initial pH 5.0, *Psoralea pinnata* recorded the highest growth and biomass production. Dry weight value at initial pH of 5.0 was 11.7g. At pH 2 and 10, the plants died before the end of the four week growth period so there was no harvest. At pH 4, 6 and 8, low production of biomass was recorded as the dry weight values were 5.3g, 6.5g and 2.5g.

4.3 Determination of optimum fertilizer application for the growth of *Psoralea pinnata*

Despite using the recommended fertilizer (NPK = 3:1:5(26) SR), for *P. pinnata* at low concentration of 5g/1L of water, all test plants did not survive.

4.4 Toxicity test result

The table below shows the survival of *P. pinnata* in a test of toxicity to iron and chromium after a 4-week growth period.

Table 9: The survival of *Psoralea pinnata* under Fe and Cr additions after a 4-week growth period.

Concentration of Iron and Cr (ppm)	Number of replicates plants that survived	Number of replicates plants that died
40	3	0
80	3	0
120	3	0
160	3	0
320	3	0
400	3	0
480	2	1
500	0	3
Control (C)	3	0

Results from the toxicity test in both soil types (US and PS) showed that *P. pinnata* survived during the 4-week growth period in the soil contaminated with the following concentrations of Fe and Cr: 40, 80, 120, 160, 320, 400, and 480 ppm. There was however, dead *P. pinnata* plants observed in one of the Cr treatment at 480ppm. At 500 ppm concentration for both Fe and Cr, all plants were dead at the end of 4weeks. Plants in the control experiments, which did not contain Fe

and Cr amendments, survived the 4-week growth period. These recorded results were for both soil types. Note that each replicate contains an average of four *P. Pinnata* seedlings

4.4 Phytoextraction experimentation

4.4.1 Measurement of growth of *Psoralea pinnata* in chromium and iron contaminated soil.

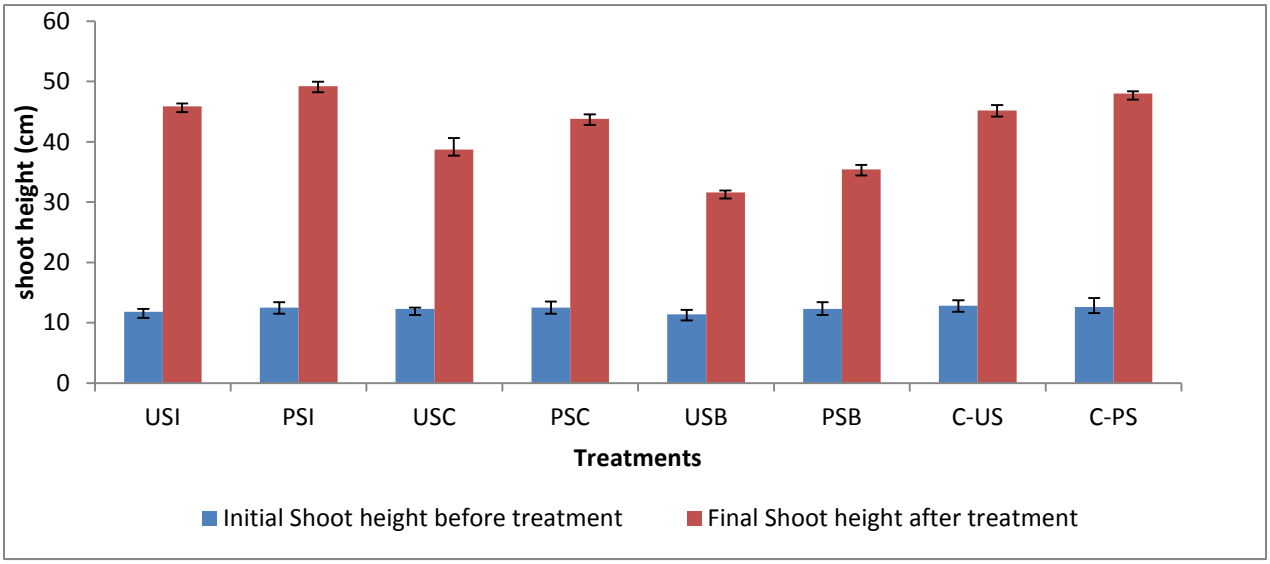
There was no uniformity in initial height of the plants used in the treatments at the time of transplanting into metal-contaminated soils at the beginning of the experiment. The changes in height were however measured relative to the initial heights of the individual plants.

4.4.1.1 Height of *Psoralea pinnata* in US soil amended with different concentrations Cr and Fe.

To show the result of the Height of *P. pinnata* in US soil amended with different concentrations Cr and Fe, the result of each concentration is presented as follows:

4.4.1.1.1 Height of *Psoralea pinnata* in US soil at 80 ppm of Fe and Cr treatment

The height of plants in soil US at 80 ppm Fe and Cr is presented in Figure 4.4.1 below.



Legend: USI=UNISA soil with addition of iron,

USC=UNISA soil with addition of chromium,

PSI=Potting soil with addition of iron,

PSC=Potting soil with chromium added,

USB=UNISA soil with both iron and chromium added,

PSB= potting soil with addition of both (iron and chromium) metals,

USC= UNISA soil with chromium added,

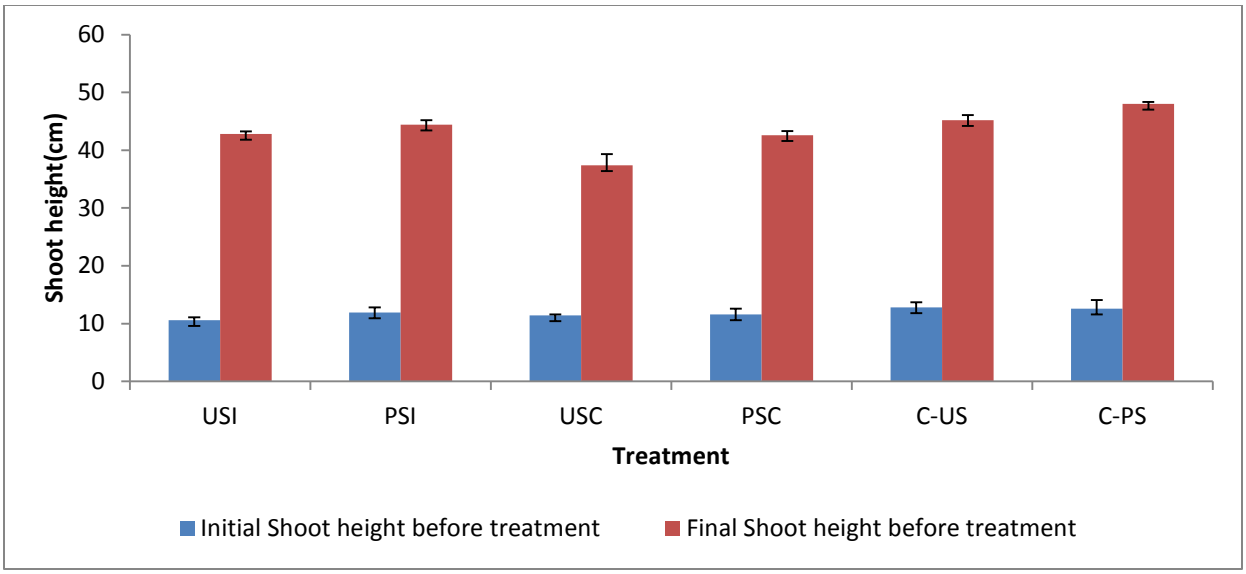
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.1: Height of plant shoot at 80 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

At 80 ppm concentration of iron and chromium, *P. pinnata* shoots of treated samples recorded more growth than the control plants. The treatment (PSI) showed the highest shoot growth (49.2 cm), a little above that of the control (C-PS) (48.0). The treatment (USI) also had more growth than the control. A sharp increase above the control A (C-US) was also recorded among the treated plants. The treatments with chromium all resulted in more growth. Their growths were less than the recorded growth in the cases of treatments with iron. The treatments (USB) and (PSB) (i.e. the treatments containing both metals), at this concentration, resulted in the least growth, especially the treatment (USB) (32.4 cm).

4.4.1.1.2 Shoot height of plants under different treatments, at 130ppm of Fe and Cr treatment after 12 weeks.

The height of plants in soil US at 130ppm Fe and Cr is presented in Figure 4.4.2 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

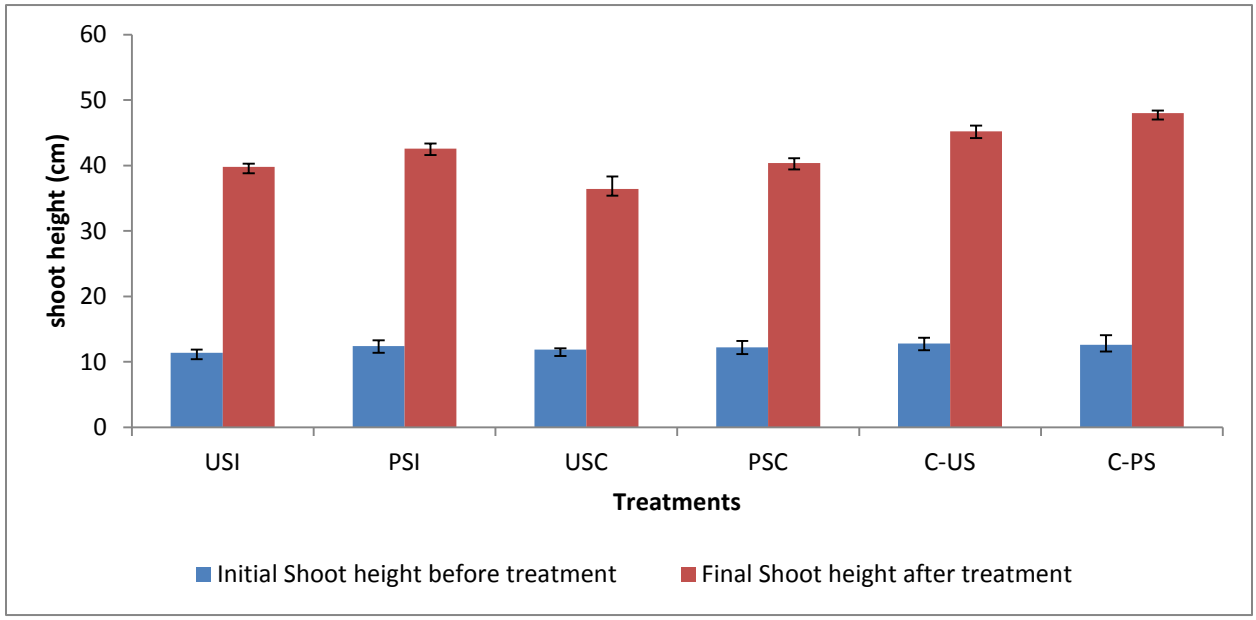
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.2: Height of plant shoot at 130 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

At concentration 130ppm, *P. pinnata* showed better shoot growth in Control B (C-PS) at 48.0 cm compared to the rest of the treatments. The least growth value in plant shoots was recorded in the treatment with chromium especially in soil US. There was a decrease in shoot height with an increase in metal contamination (from 80 ppm to 130 ppm). It was observed that growth of *Psoralea pinnata* was better in Soil PS than in Soil US.

4.4.1.1.3 Shoot Height of Plants under different treatments, at 180ppm of Fe and Cr treatment after 12 weeks.

The shoot heights of plants in both types of soil sample at 180ppm of Fe and Cr are represented in the figure below:



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

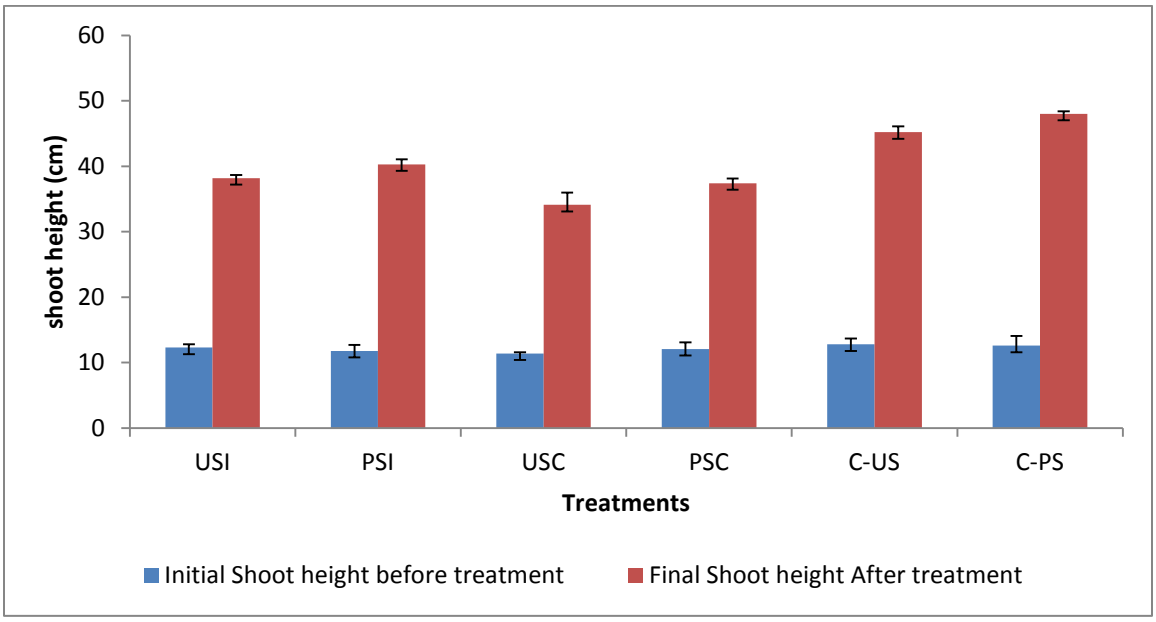
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.3: Height of plant shoot at 180 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

Results from the control experiments with potting soil showed that the control (C-PS) recorded the highest growth. This can be seen from Fig. 4.4.3. This growth was followed by the second control (C-US). The treatments with chromium (USC and PSC) showed considerably less growth in shoot height with increased metal concentration. It was recorded that the treatments with iron (USI and PSI) also had reduced growth in shoot height (39.8cm and 42.6cm respectively) as compared to the results in Fig. 4.4.1 (45.9cm and 49.2cm) and in Fig. 4.4.2 (42.8cm and 44.4cm respectively). With increased metal concentration, there was decrease in plant shoot growth as observed in the figure above.

4.4.1.1.4 Shoot height of plants under different treatments, at 230ppm of Fe and Cr treatment after 12 weeks.

The height of plants at 230ppm Fe and Cr is presented in Figure 4.4.4 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

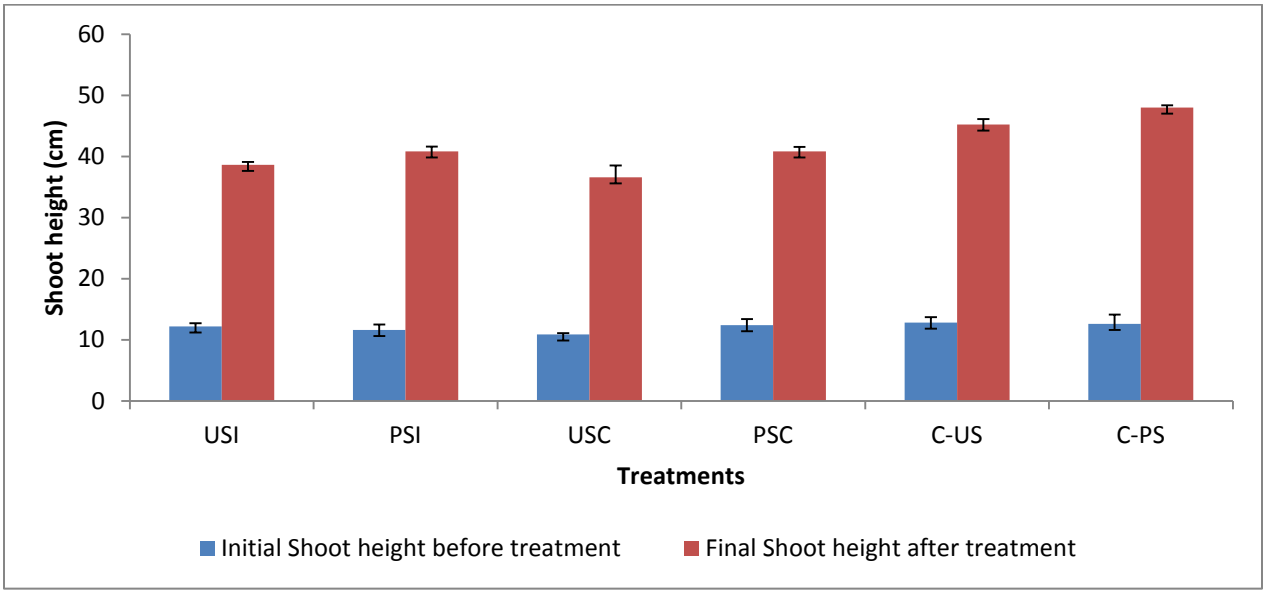
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.4: Height of plant shoot at 230 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

The Controls (C-PS and C-US) (48cm and 45.2cm) respectively had a better growth than the treatments. Growth of plant shoots was higher in the treated (PSI) (40.3cm) than in the treatment (USI) (38.2cm). This may be attributed to the type of soil on which treatment is done. The treatment USC has the least growth in shoot height at (34.1) cm and (PSC) (37.4 cm).

4.4.1.1.5 Shoot height of plants under different treatments, at 280ppm Fe and Cr treatment after 12 weeks of growth.

The height of plants at 280 ppm Fe and Cr is presented in Figure 4.4.5 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

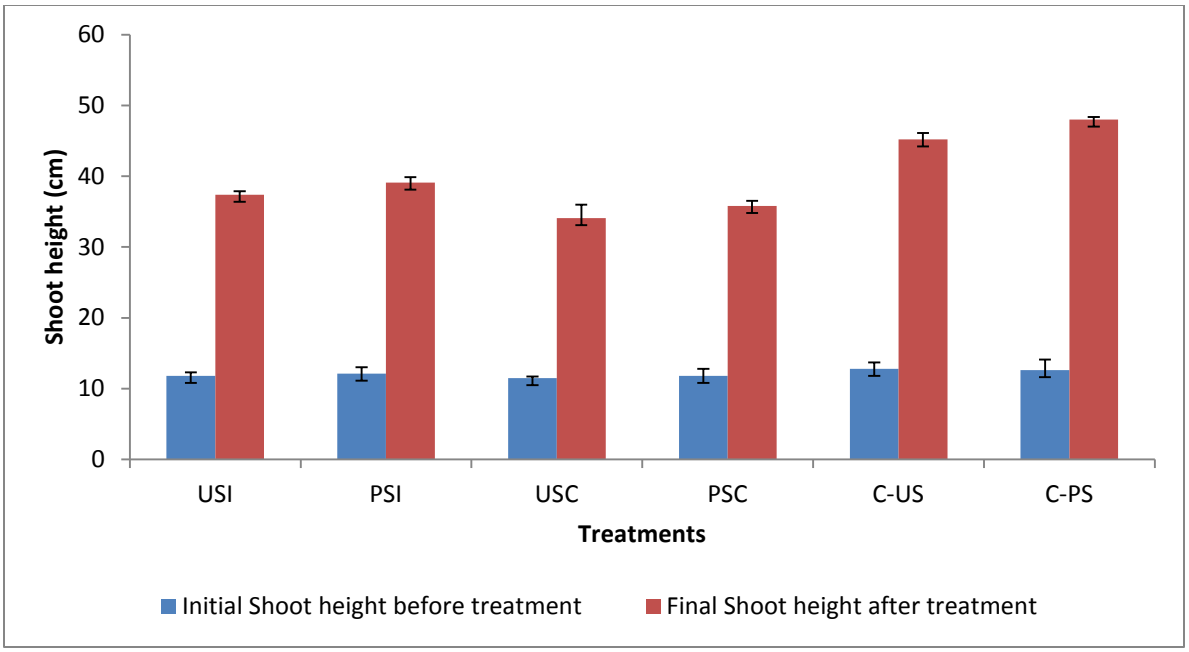
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.5: Height of plant shoot at 280 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

Values from the results of the control experiment, indicates that (C-PS) and (C-US) continued to maintain the highest values in final shoot height. Besides the controls, at 280 ppm, treatments USC and (PSC), recorded shoot height of 36.6cm and 40.8cm respectively. These values were more than the recorded growth values from the results in Fig. 4.4.4. The shoot heights of treatments PSC and PSI were 40.8 cm.

4.4.1.1.6 Shoot height of plants under different treatments, at 330ppm of Fe and Cr treatment after 12 weeks of growth.

The height of plants at 330 ppm Fe and Cr is presented in Figure 4.4.6 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

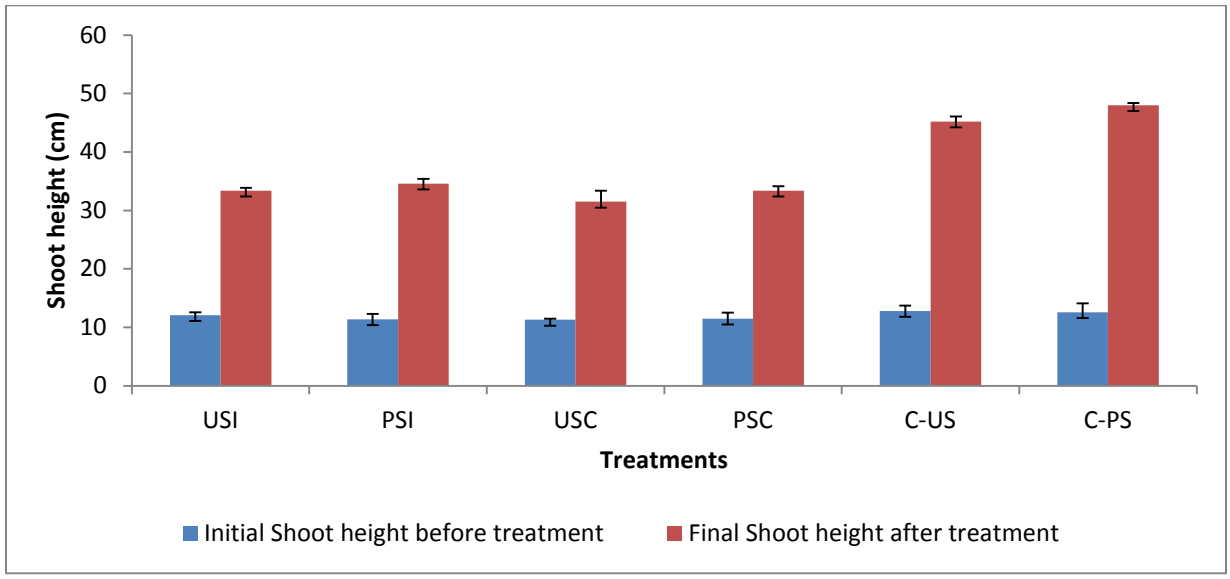
Figure 4.4.6: Height of plant shoots at 330 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

Results from the treatment of the *P. pinnata* plants at 330ppm showed that growth in plant shoots (shoot height) began to decrease especially in the treatments with chromium, an observation most noticeable in the treatment (USC) (34.1cm). Of all the treatments, none had a growth in shoot height equal to those of the controls. The highest shoot growth measurement of plant shoot in all

treatments was recorded in the treatment (PSI) (39.1 cm) followed by treatment (USI) (37.4 cm) and (PSC) (35.8cm).

4.4.1.1.7 Shoot Height of Plants under different treatments, at 380ppm of Fe and Cr treatment after 12 weeks.

The height of plants at 380 ppm Fe and Cr is presented in Figure 4.4.7 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

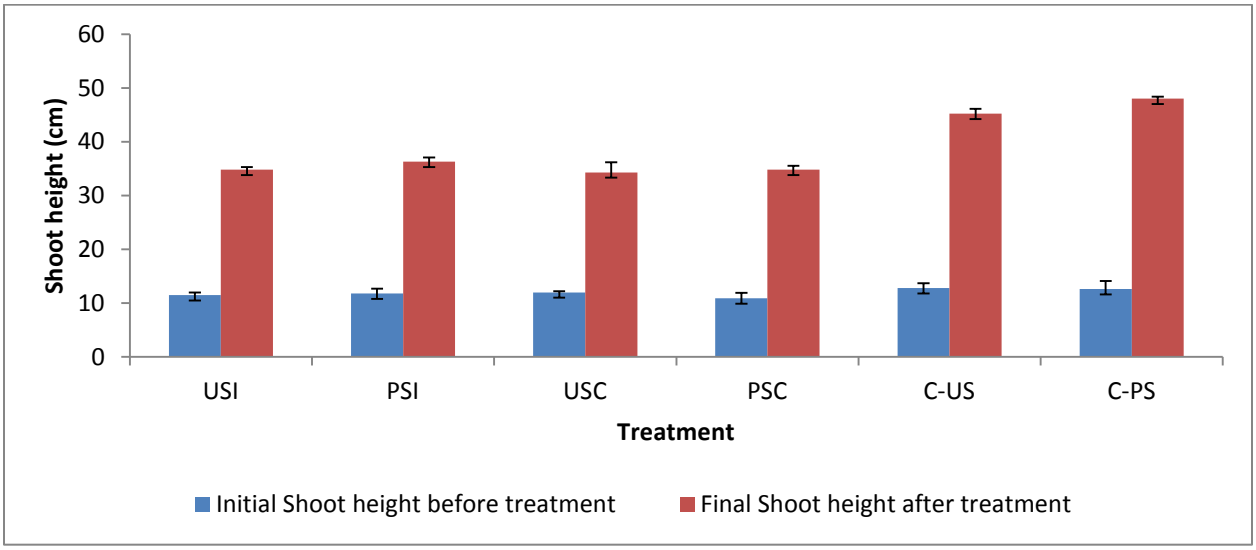
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.7: Height of plant shoot at 380 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

At concentration of 380ppm, results show that the Controls (C-US) and (C-PS) against the treatments, grew more. The plants treated with iron and chromium continued to decrease in shoot height. This was seen from the values of (USI) and (PSI) which were 33.4cm and 34.6cm respectively while (USC) and (PSC) were 31.5cm and 33.4cm respectively. The treatments (USI) and (PSC) both had the same value for growth in shoot height. This could strengthen earlier speculations of decrease in shoot length as a result of increased metal contamination.

4.4.1.1.8 Shoot height of plants under different treatments, at 430ppm of Fe and Cr treatment after 12 weeks of growth.

The height of plants at 430 ppm Fe and Cr is presented in Figure 4.4.8 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

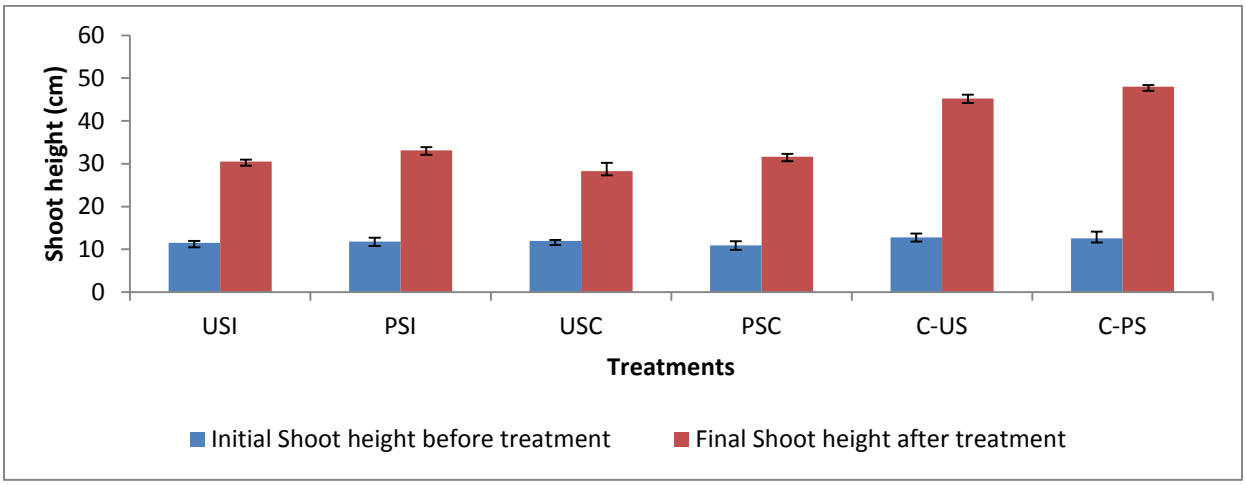
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.8: Height of plant shoot at 430 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

At 430ppm, treatment (PSI) had the highest value in shoot growth (36.3 cm) (Fig.4.4.8). The rest of the treatments showed less growth in shoot lengths. In decreasing order, they include (USI) which had almost same growth increase as the treatment (PSC) plants at 34.8 cm and the treatment with the least value for shoot height was the (USC) treatment, at 34.3 cm. None of the treatments with either iron or chromium metal contaminants caused growth to the same height as the control plants. Differences in growth of shoots within treatments were significant at $P \leq 0.05$.

4.4.1.1.9 Shoot height of plants under different treatments, at 480ppm Fe and Cr treatment after 12 weeks.

The height of plants at 480 ppm Fe and Cr is presented in Figure 4.4.9 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

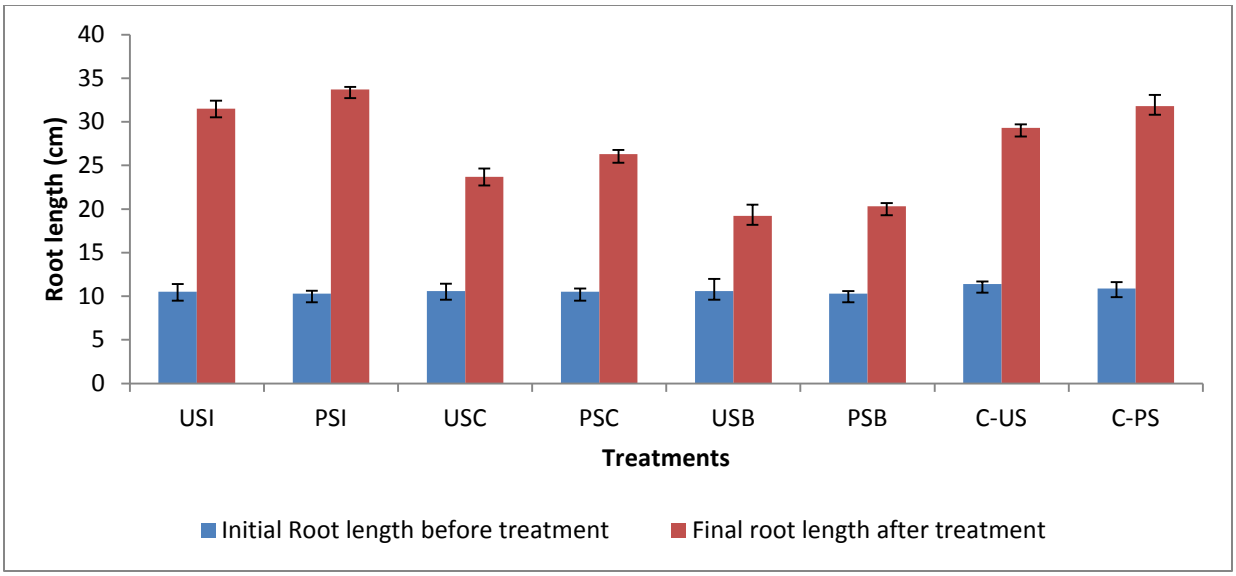
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.4.9: Height of plant shoots at 480 ppm of Fe and Cr treatments in soil after 12 weeks (Error bars indicate standard error of the mean).

Results from treatment of samples at 480 ppm iron and chromium contamination showed that the lowest values for shoot growth of *Psoralea pinnata* were recorded in all additions of iron and chromium at this concentration when compared to all previous treatments with lower iron and chromium concentrations. While the lowest values of growths in plant shoot were recorded, it was observed that stems were thickest under this concentration. It is also noteworthy that none of the treatments grew as much as that of the Control. The treatment with the highest value in shoot height was (PSI) at 33.1 cm. Other treatments in decreasing order of shoot height were (PSC), (USI) and (USC) with values: 31.6 cm, 30.5 cm and 28.3 cm respectively.

4.4.1.2.0. Root length of plants under different treatments, at 80ppm Fe and Cr treatment after 12 weeks.

Results of the root length of plant at 80 ppm Fe and Cr is presented in Figure 4.5.0 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

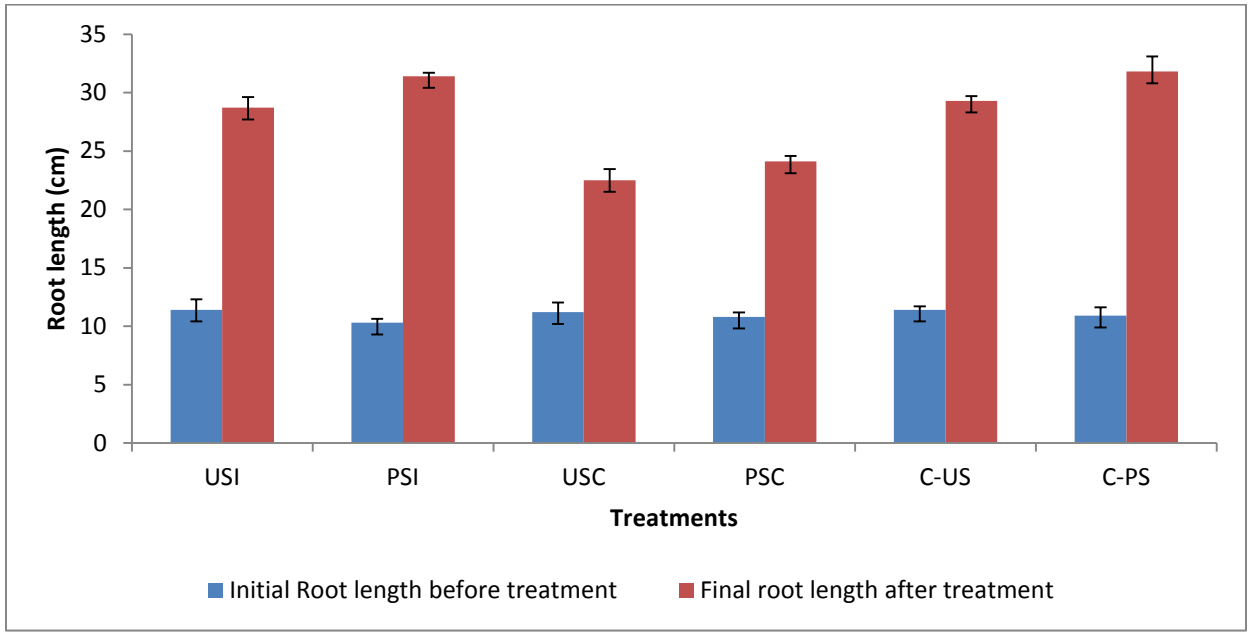
Figure 4.5.0: Length of plant roots at 80 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

Results show that root length was highest in the treatment (PSI) (33.7cm), above that of the controls. It was also recorded that the values for root length were higher in treatments set up in Soil B (PS) and in treatments with iron addition. In the soil with both metal additions (USB) and (PSB), root length was recorded to have been less as compared to treatments with one metal addition. The treatment (USI) had the second highest value for root length (31.5 cm), with the

treatment (PSC) coming next in value at 26.3 cm. Apart from the treatments having mixed contamination of iron and chromium, of the singly contaminated treatments, the treatment with the least value for root length was (USC) at 23.7 cm.

4.4.1.2.1 Root length of plants under different treatments, at 130ppm after 12 weeks

Results of the root length of plant at 1300 ppm Fe and Cr is presented in Figure 4.5.1 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

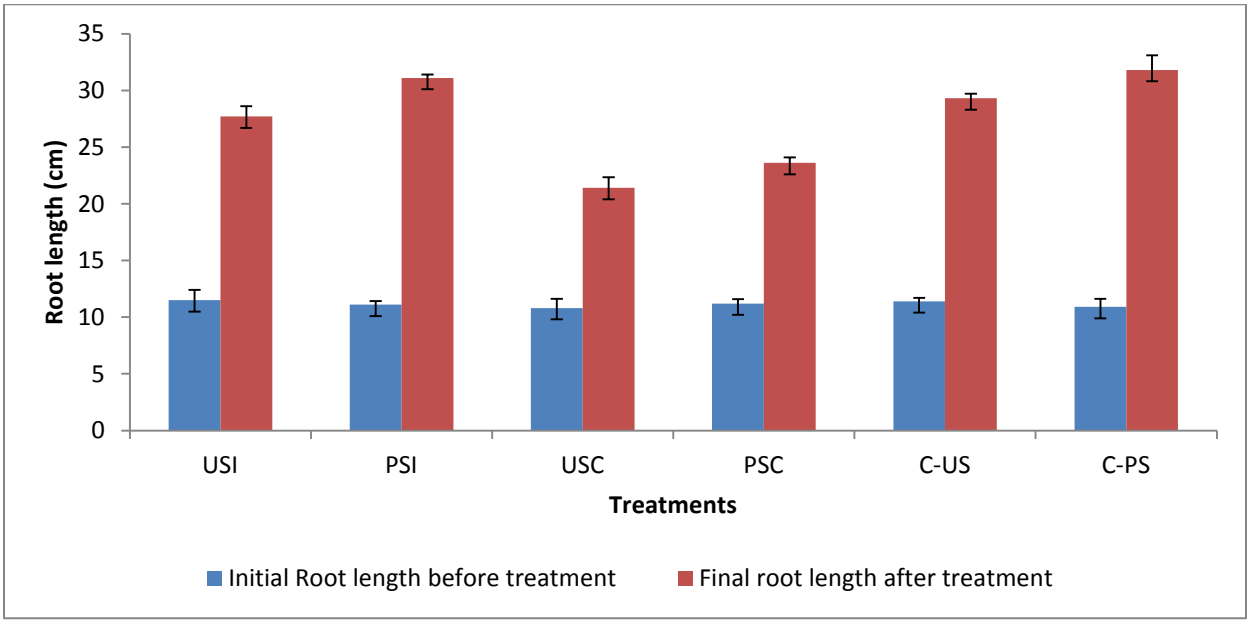
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.1: Length of plant roots at 130 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

Results for root length showed that the highest value was recorded for the treatment (PSI) at 31.8 cm from initial length 10.3 cm closest to the treatment (PSI), was the control (C-PS) at 31.8 cm from 10.9 cm initial root length. Least values in root length were recorded in the treatments with chromium addition (USC) and (PSC): 22.5 and 24.1 respectively.

4.4.1.2.2 Root length of plants under different treatments, at 180ppm Fe and Cr treatment after 12 weeks

Results of the root length of plant at 180 ppm Fe and Cr is presented in Figure 4.5.2 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

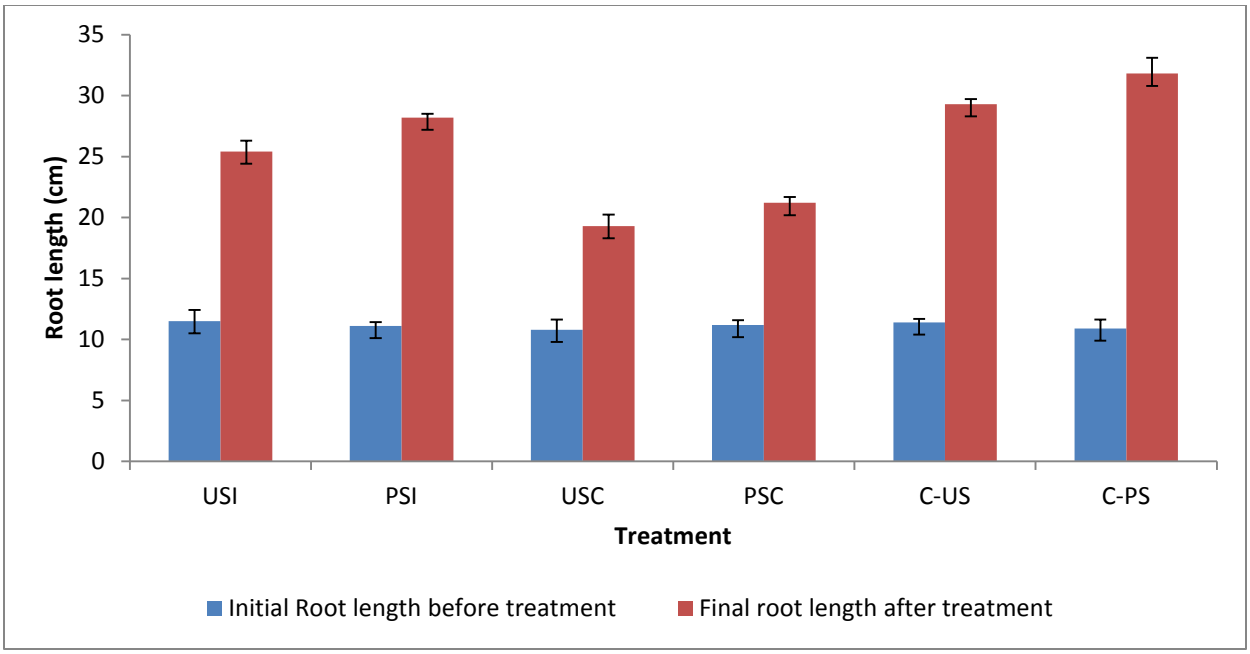
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.2: Length of plant roots at 180 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

The highest recorded value for root length was from the control (C-PS) (31.8cm) (Fig. 4.5.2). Apart from the controls, treatments recorded some decrease in root length. The treatment with the lowest value for root length was (USC) (21.4cm). However, among the treatments, the highest value for root length was recorded in the treatment (PS1) (31.1cm). The values for root length of other treatments were (USI) 27.7 cm and (PSC) 23.6 cm. Subsequently, plant roots length decreased with increased metal contamination at this concentration.

4.4.1.2.3 Root length of plants under different treatments, at 230ppm Fe and Cr treatment after 12 weeks

The results of the root length of plant at 230 ppm Fe and Cr is presented in Figure 4.5.3 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

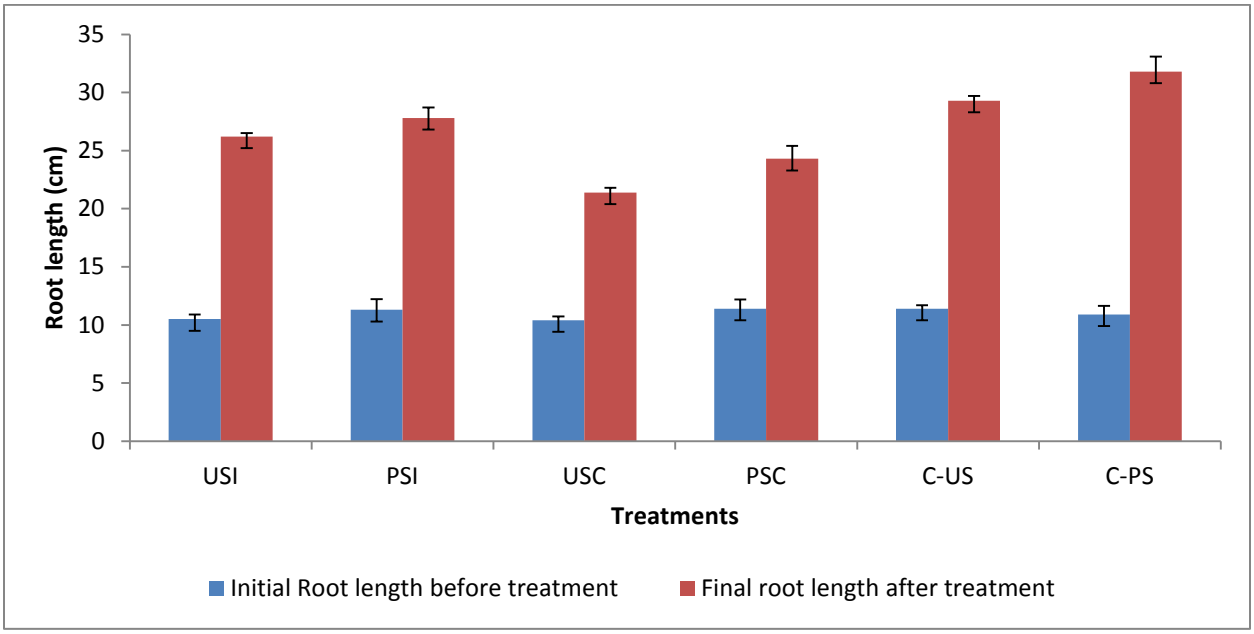
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.3: Length of plant roots at 230 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

Results from both controls (C-US) and (C-PS) (and (31.8cm) recorded the highest values for root length. With increased metal concentration, the treatments recorded significant decrease in the length of roots of *Psoralea pinnata.* This was observed in all the treatments (USI), (PSI), (USC) and (PSC) (25.4 cm, 28.2 cm, 19.3 cm and 21.2 cm respectively.)

4.4.1.2.4 Root length of plants at 280ppm Fe and Cr treatment after 12 weeks of growth.

The results of the root length of plant at 280 ppm Fe and Cr is presented in Figure 4.5.4 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

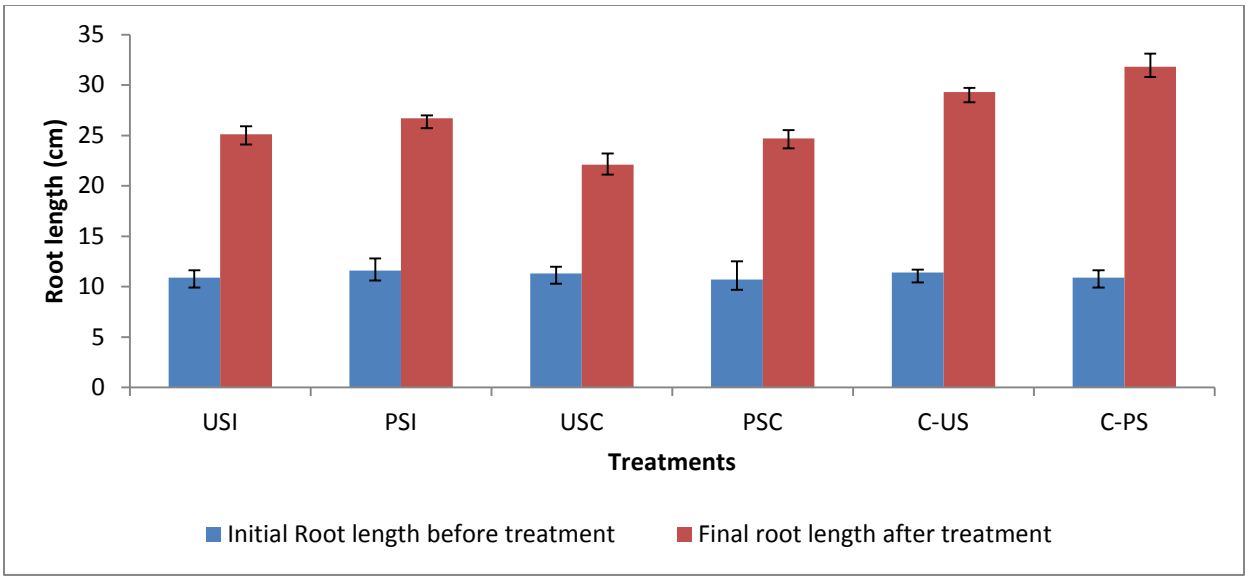
Figure 4.5.4: Length of plant roots at 280 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

Compared to the root length values from Fig. 4.5.4, under 280 ppm, better growth values were recorded with these treatments (USI, PSI, USC and PSC: 26.2 cm, 27.8 cm, 21.4 cm 24.3 cm) as

the values indicate. However, the highest growth rates in root lengths were recorded in the controls C-US and C-PS.

4.4.1.2.5 Plant root length at 330ppm of Fe and Cr treatment after 12 weeks.

The results of the root length of plant at 330 ppm Fe and Cr is presented in Figure 4.5.5 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

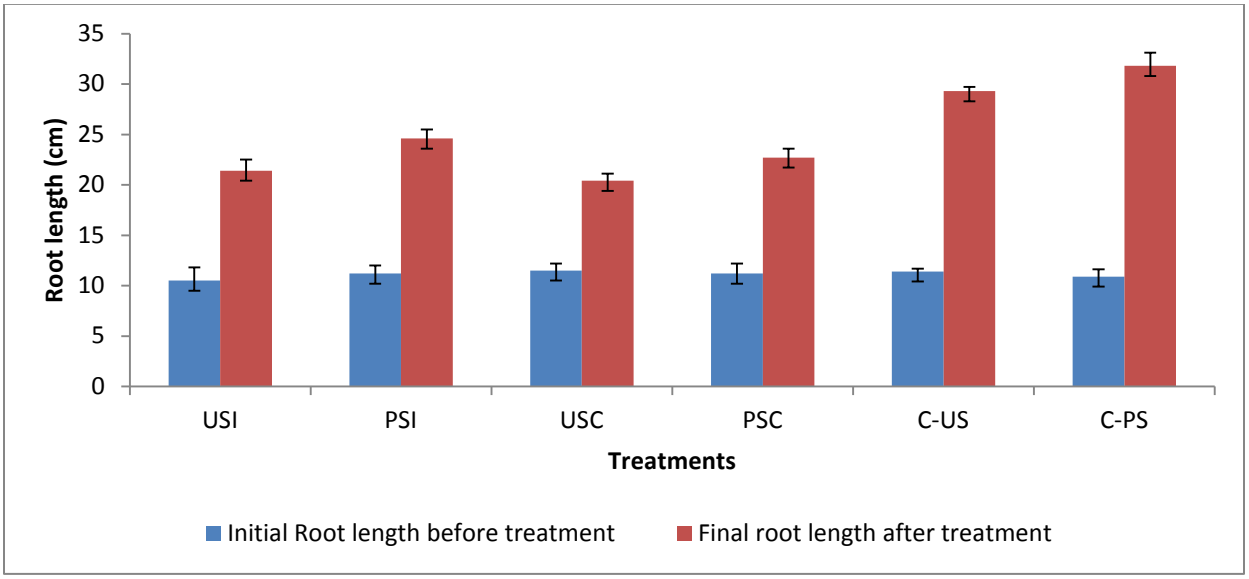
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.5: Length of plant roots at 330 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

Result from the experiment using 330ppm concentration showed that slight changes occurred in that root length. They were shorter (as seen in Fig. 4.5.5) than root lengths in Fig. 4.4.2.4. Root length values in decreasing order, apart from the control for the treatments (PSI), (USI), (PSC) and (USC), were 26.7 cm, 25.1 cm, 24.7 cm and 22.1 cm respectively.

4.4.1.2.6 Plant root length at 380ppm of Fe and Cr treatment after 12 weeks

The results of the root length of plant at 380 ppm Fe and Cr is presented in Figure 4.5.6 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

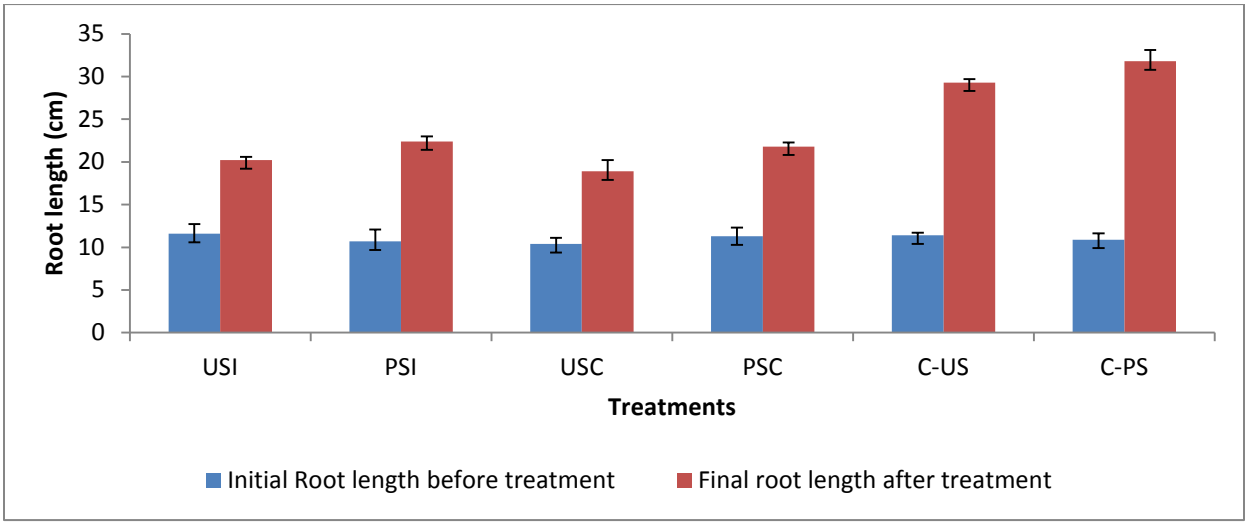
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.6: Length of plant roots at 380 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

At 380 ppm of metal salt concentration, Fig. 4.5.6 shows that values for root length further decreased when compared to previous figures. The lowest value for root length was recorded in the treatment (USC) (20.4 cm).

4.4.1.2.7 Plant root length at 430ppm of Fe and Cr treatment after 12 weeks

The results of the root length of plant at 430 ppm Fe and Cr is presented in Figure 4.5.7 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

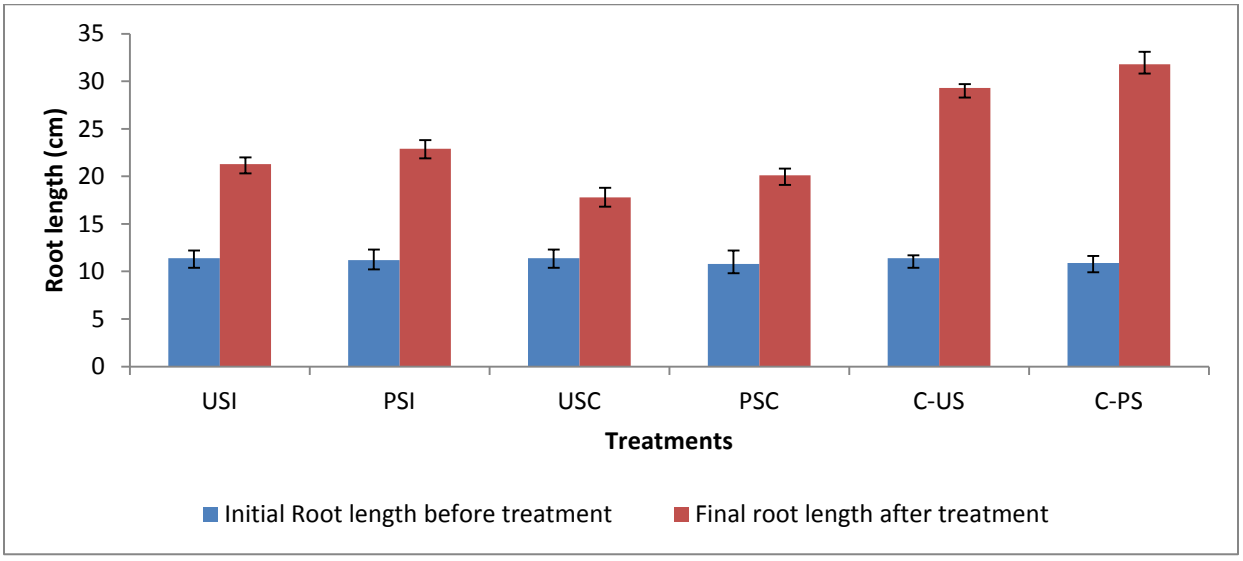
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.7: Length of plant roots at 430 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean).

There were further decreases of length of roots under this concentration and this resulted from the increased metal salt concentration. (USC) is the treatment with the least root length (18.9 cm) and the treatment with the highest value for root length is (PSI) (22.4 cm). Figure 4.5.7 also clearly shows that the control experiment completely dominated in the root length values of *Psoralea pinnata*. Other treatments and their values are (PSC) 21.8 cm and (USI) at 20.2 cm.

4.4.1.2.8 Root length of plants at 480ppm Fe and Cr treatment after 12 weeks

The results of the root length of plant at 480 ppm Fe and Cr is presented in Figure 4.5.8 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.8: Length of plant roots at 480 ppm of Fe and Cr treatment in soil after 12 weeks (Error bars indicate standard error of the mean)

Figure 4.5.8 above shows the lowest recordings for root length. While there was a clear dominance of the values of the controls in root length, the lowest recorded value of root length was in treatment (USC) at 17.8 cm and the highest was 20.1 cm in treatment (PSC).

Table 10: Percentage of shoot growth rate of *Psoralea pinnata* at different concentrations of Cr and Fe in both soil types

Treatments (ppm)	T80	T130	T180	T230	T280	T330	T380	T430	T480
USI	288.98	303.77	249.00	210.57	224.59	216.95	176.03	202.61	165.22
PSI	293.60	273.12	243.55	241.53	251.72	223.14	176.03	207.63	180.51
USC	214.63	228.07	205.88	199.12	235.78	196.51	178.76	185.83	135.83
PSC	250.4	267.24	231.15	126.45	229.03	203.39	190.43	219.26	189.91
PSB	187.80	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
USB	175.44	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C-US	253.13	253.13	253.13	253.13	253.13	253.13	253.13	253.13	253.13
C-PS	280.95	280.95	280.95	280.95	280.95	280.95	280.95	280.95	280.95

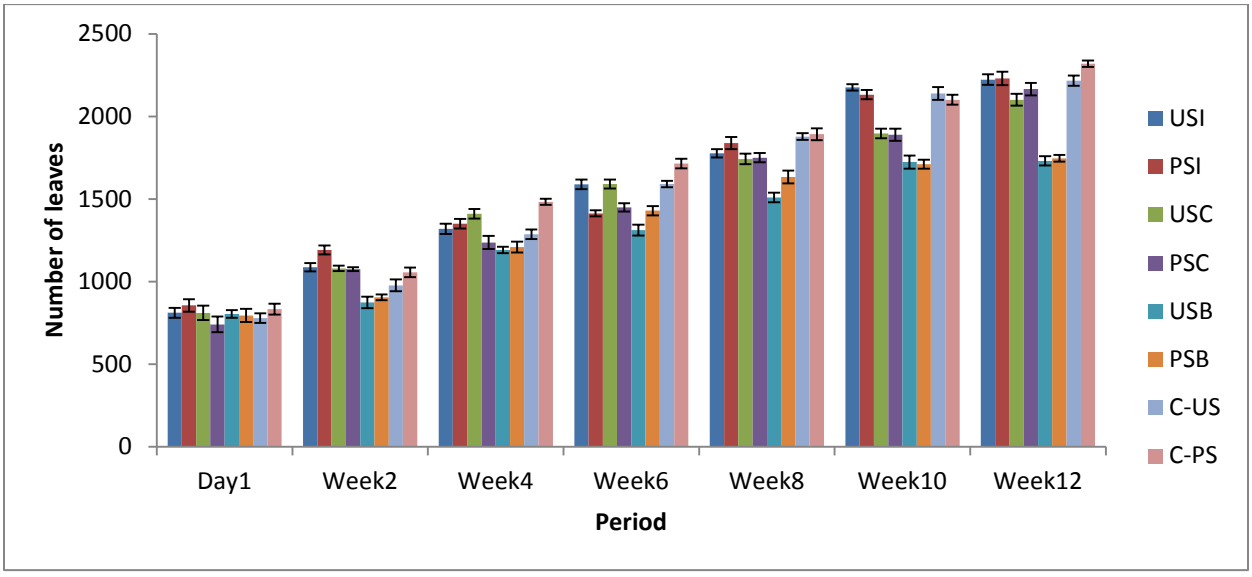
There were differences in the percentage of shoot growth rate of *P. pinnata* in different concentrations of treatments. These differences are shown in table 10; values of percentage shoot growth of plants show a decreasing trend from treatments with lower contaminant concentrations, to treatments of higher contaminant concentrations. This shows that shoot growth was decreased by increased contaminant concentration.

4.5 Leaves per plant (LPP) grown in Fe and Cr treated soil within 12weeks of the study

Plant leaves per plant were counted bi-monthly to check any relationship between the number of plant leaves, the change in height of the plants and metal retention ability.

4.5.1 LPP at 80ppm of Fe and Cr in both study soil samples: UNISA soil (US) and Potting Soil (PS).

The results of the number of leaves per plant of plant at 80 ppm Fe and Cr is presented in Figure 4.5.9 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

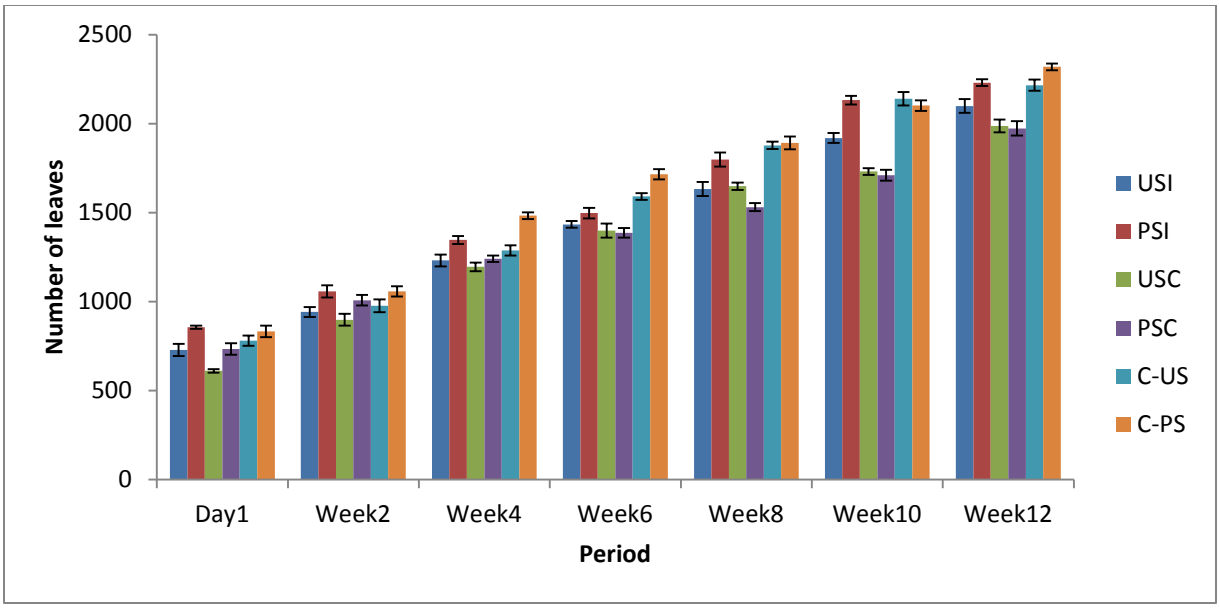
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.5.9: Number of leaves per plant at 80 ppm of Iron and Chromium in both soil samples (Error bars indicate standard error of the mean).

Results showed an increase in the number of leaves on *P. pinnata* over the 12 week period of the experiment. *Psoralea pinnata* has numerous tiny leaves. On day one the average number of leaves was 804 but two weeks later, the treatments began showing some differences with the plants in iron-contaminated soil PS having produced more leaves (1197). Gradually, production of leaves in samples with treated soil began to decrease, especially in the case of chromium contamination. The controls (C-US) and (C-PS) produced more leaves than all treatments. In the 12th week, the control plants had almost the same number of leaves as the plants in soils (US) and (PS) with iron contamination.

4.5.2 The number of LPP at 130 ppm of Fe and Cr in both soil samples

The results of the number of leaves per plant of plant at 130 ppm Fe and Cr is presented in Figure 4.6.0 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

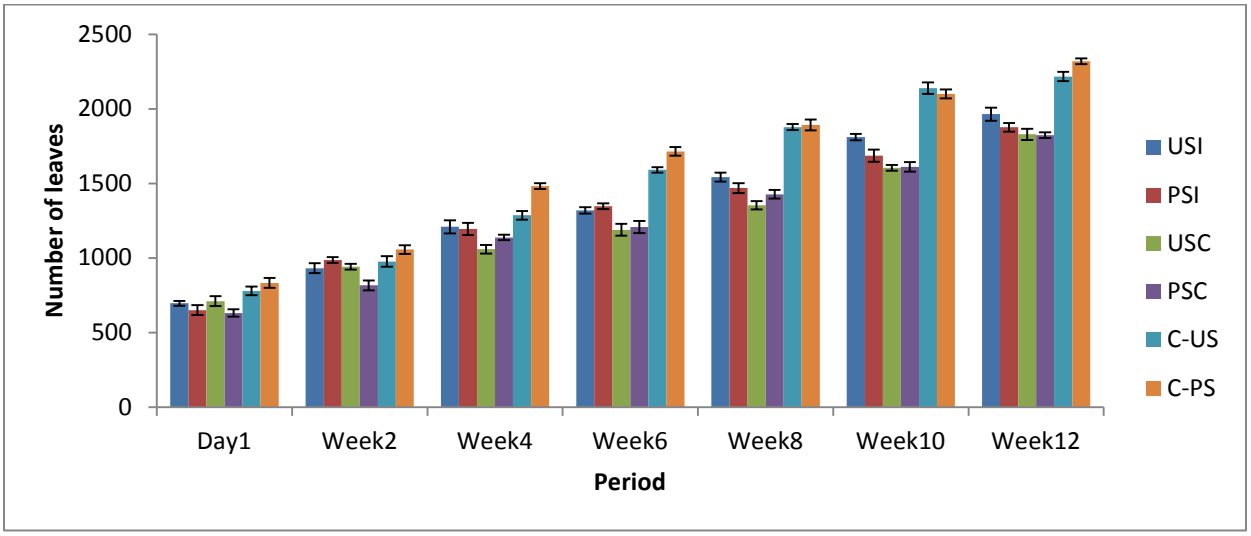
Figure 4.6.0: Number of leaves per plant at 130 ppm of $KCrO_4$ and Fe $(NO_3)_3.9H_2O$ in both soil samples (Error bars indicate standard error of the mean).

Results show the increase in the number of leaves at 130 ppm of iron and chromium contamination. The initial average number of leaves before transplant was 757. In week 2, both

the controls and the treatments showed a steady production of leaves. In week 4, the controls were better in the production of leaves. At the end of week 12, the control plant completely dominated in the production of tiny, healthy leaves, and the treatment plants (PSI) also produced the same number of leaves as the controls.

4.5.3 The number of LPP at 180 ppm of Fe and Cr in both soil samples

The results of the number of leaves per plant of plant at 180 ppm Fe and Cr is presented in Figure 4.6.1 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

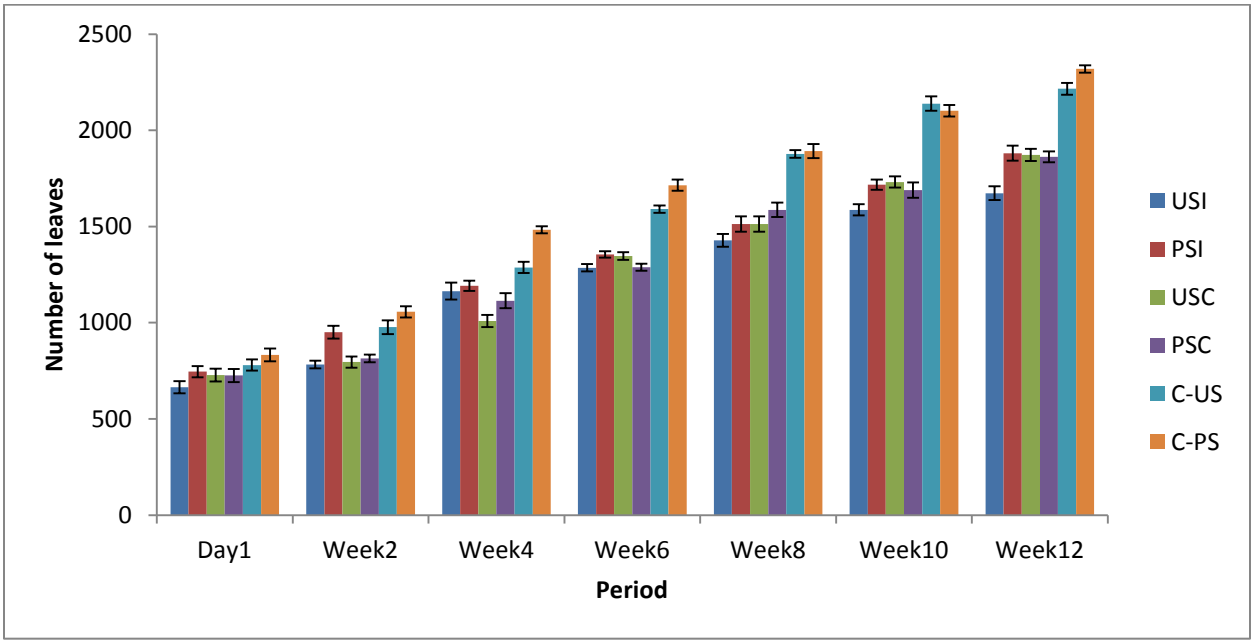
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.6.1: Number of leaves per plant at 180 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

The initial average number of leaves before transplant was 717. In the second week, the treatment and control plants produced tiny leaves almost at equal numbers. Week 4 saw the controls producing more leaves than the rest of the treated plants (Fig. 4.6.1).

4.5.4 The number of LPP at 230 ppm of Fe and Cr in both soil samples

The results of the number of leaves per plant of plant at 230 ppm Fe and Cr is presented in Figure 4.6.2 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

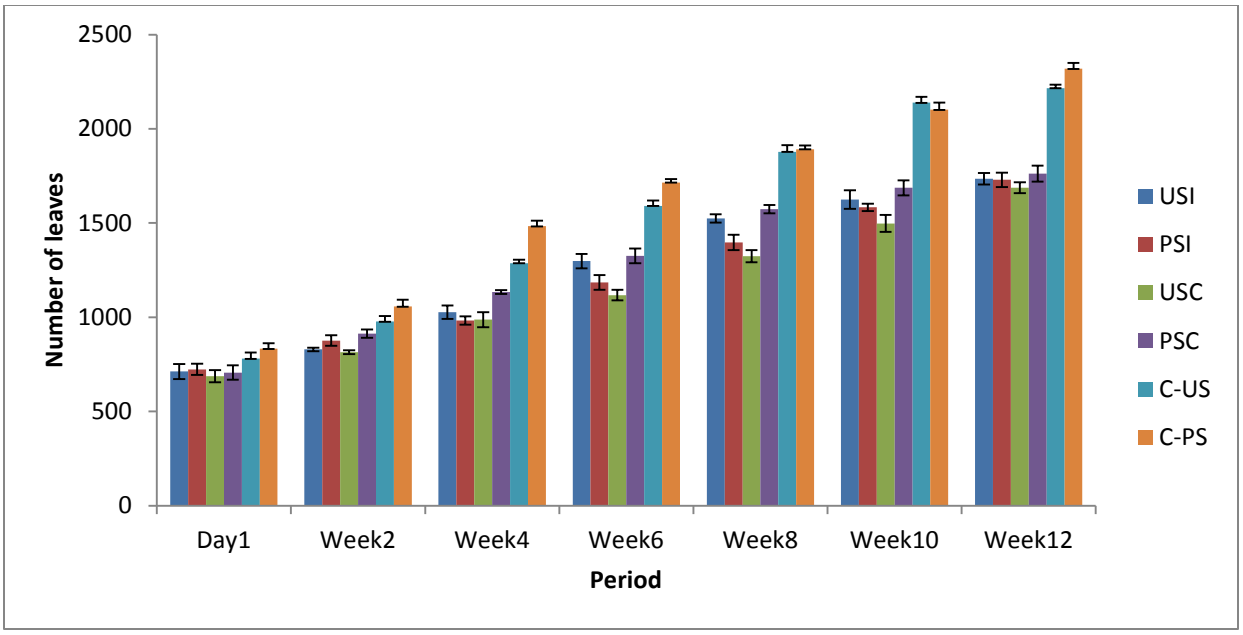
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.6.2: Number of leaves per plant at 230 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

Results (Fig. 4.6.2) show the growth pattern of the number of leaves and the difference between the treatments and the controls. The initial average number of leaves transplant was 746. In week 2, the treatment (PSI) produced leaves equaling the controls and they all began to show marked signs of differences in the production of leaves, but in week 4 and 6, the controls producing more leaves. At the end of week 12, all treated samples produced less number of leaves and a marked discoloration of leaves was observed.

4.5.5 The number of LPP at 280 ppm of Fe and Cr in both soils

The results of the number of leaves per plant of plant at 280 ppm Fe and Cr is presented in Figure 4.6.3 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

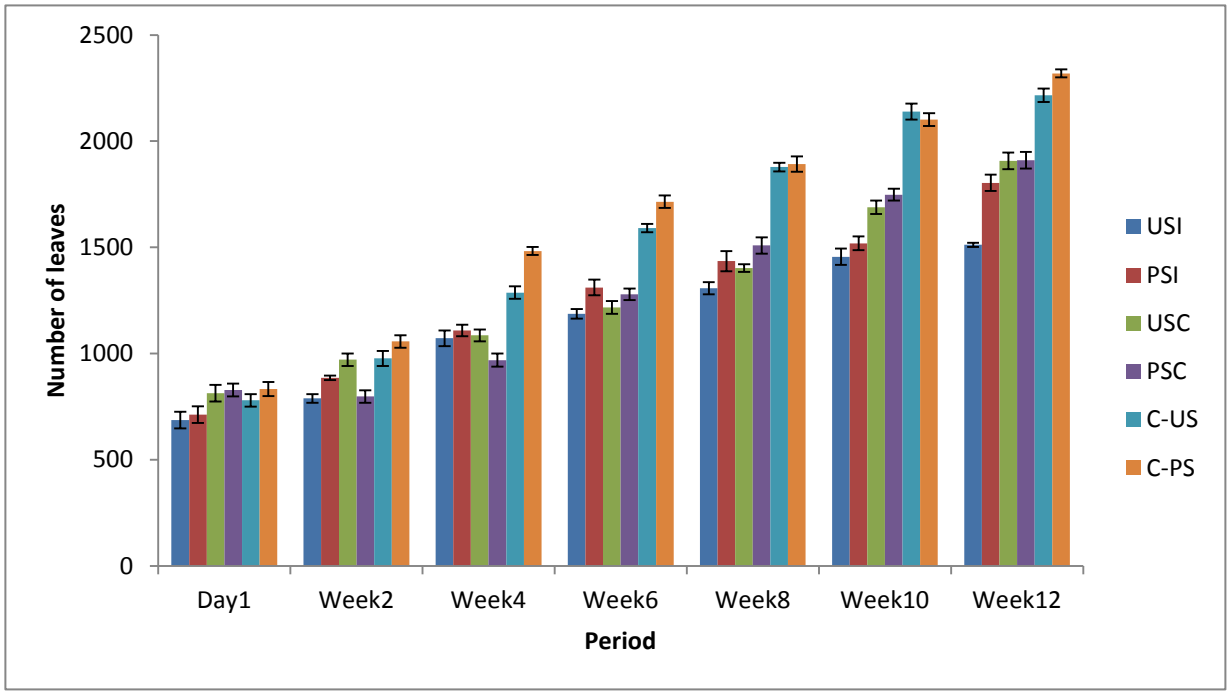
Figure 4.6.3 Number of leaves per plant at 280 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

Results (Fig. 4.6.3) show the production pattern of leaves at 280 ppm Fe and Cr treatments in comparison with the controls. The average number of leaves on the day immediately before transplant was 740. The treatments produced less leaves than the untreated control plants. By the end of Week 8 the controls had clearly produced more leaves. It was however observed that the stems of the treatments had grown a bit thicker than was observed in the control plants.

4.5.6 Number of LPP at 380 ppm of Fe and Cr in both soil samples

The results of the number of leaves per plant of plant at 380 ppm Fe and Cr is presented in

Figure 4.6.4 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

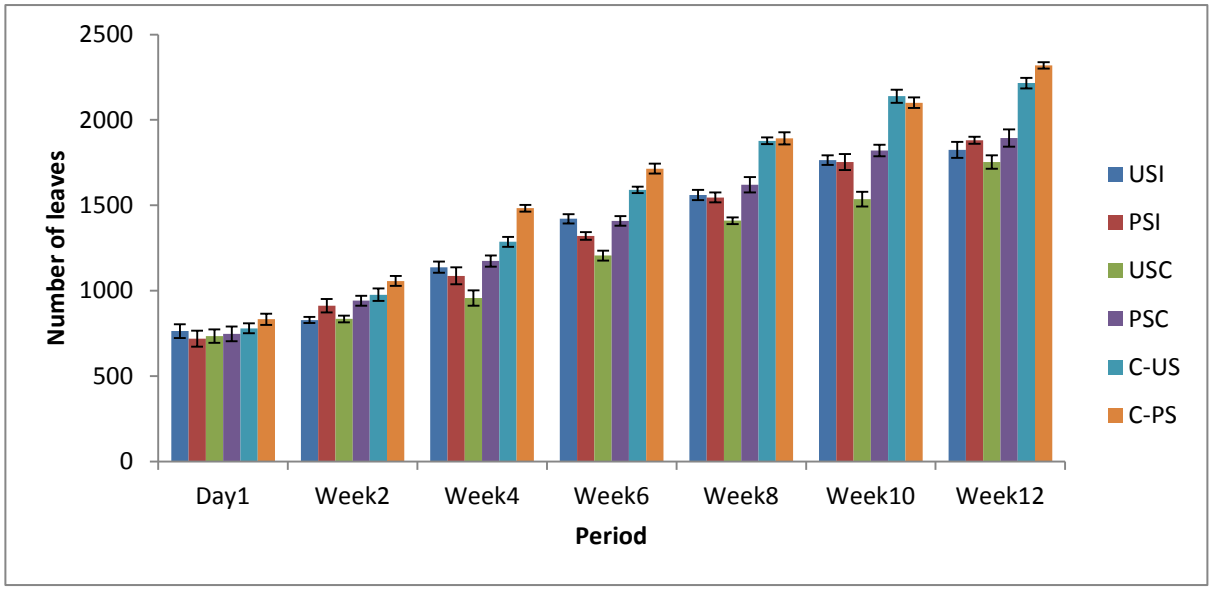
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.6.4: Number of leaves per plant at 380 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

The initial average number of leaves before transplant was 775. The control plants produced more leaves from Week 4 to Week 12. There was far less production of leaves in all treatments.

4.5.7 Number of LPP at 430 ppm of iron in both soil samples

The results of the number of leaves per plant of plant at 430 ppm Fe and Cr is presented in Figure 4.6.5 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

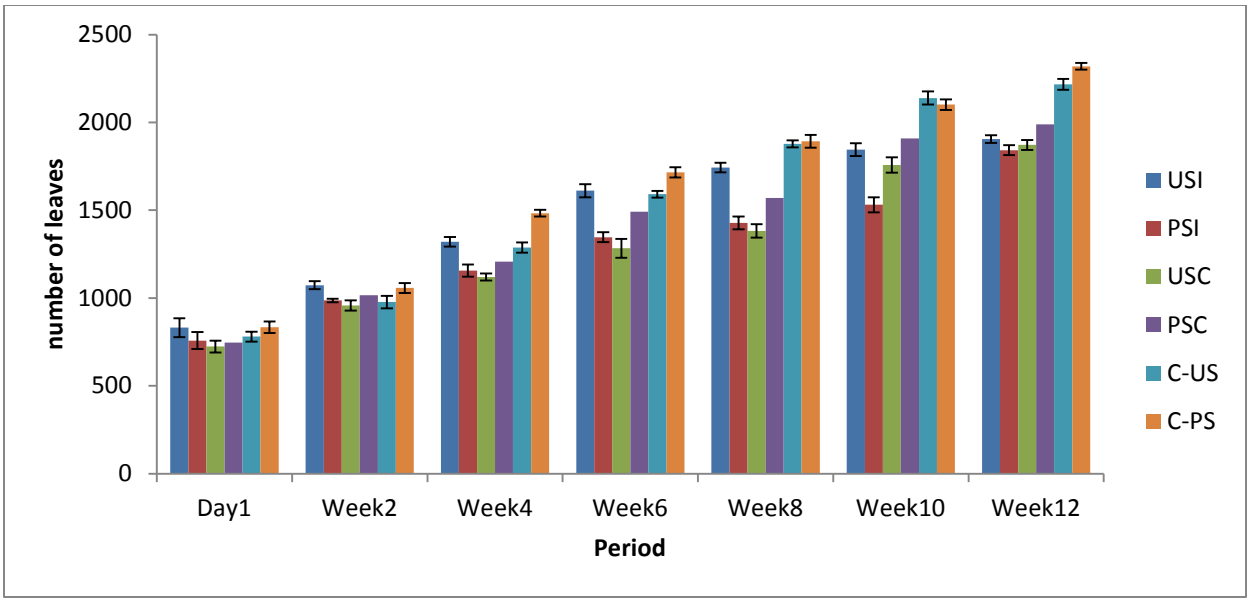
C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.6.5: Number of leaves per plant at 430 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

The initial average number of leaves before transplant was 762. With the planting of *P. pinnata* in more concentrated iron and chromium soil, almost all treatments with iron contamination produced more leaves than treatments of chromium concentration when compared at the same level of concentration. Of all treatments, the only treatment that produced more leaves compared to the controls after the twelfth week was the treatment (PSC).

4.5.8 Number of LPP at 480 ppm of Fe and Cr in both soil samples

The results of the number of leaves per plant of plant at 480 ppm Fe and Cr is presented in Figure 4.6.6 below



Legend: USI=UNISA soil with addition of iron

USC=UNISA soil with addition of chromium

PSI=Potting soil with addition of iron

PSC=Potting soil with chromium added

USB=UNISA soil with both iron and chromium added

PSB= potting soil with addition of both (iron and chromium) metals

USC= UNISA soil with chromium added

C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil

Figure 4.6.6: Number of leaves per plant at 480 ppm of Fe and Cr in both soil samples (Error bars indicate standard error of the mean).

The average number of leaves on the day immediately before transplant was 778. While the controls (C-US) and (C-PS) had produced more than 2000 number of leaves at the end of the twelfth week, none of the treatments produced a number of leaves up to two thousand. In Week

Two the plants in (USI) treated soil had produced more leaves (1073) than the other treatments, the controls included, but by Week Six, the controls had produced more leaves than all the treatments especially from Week Ten.

4.5.9 Percentage leaves growth rate of *Psoralea pinnata* at different concentrations of Fe and Cr added to both soil types

The table below shows the percentage leave growth of *P. pinnata* at different concentrations of Fe and Cr.

Table 11: Percentage leaves growth of *Psoralea pinnata* at different concentrations of Fe and Cr added to both soil types

Treatments (ppm)	T80	T130	T180	T230	T280	T330	T380	T430	T480
USI	173.77	188.32	181.18	151.58	155.18	143.68	120.09	138.74	129.24
PSI	160.51	160.51	188.17	152.14	157.91	139.14	153.02	161.61	143.01
USC	159.06	164.58	157.24	157.14	149.38	145.56	134.28	138.96	154.72
PSC	192.17	168.80	149.18	156.47	150.59	149.58	130.68	153.55	166.27
PSB	115.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
USB	119.62	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C-US	253.13	253.13	253.13	253.13	253.13	253.13	253.13	253.13	253.13
C-PS	280.95	280.95	280.95	280.95	280.95	280.95	280.95	280.95	280.95

The values in the table shows that leave formation in plants exposed to a higher concentration of contaminants were lower than those with a lesser exposure. An example is the treatment (USI). When 80 ppm iron was added to the soil, the percentage increase in leaf numbers of *P. pinnata* was 173.77, but with 480 ppm iron added to soil, the percentage of the growth rate of leaves was

measured at 129.24. So generally, the percentage increase in leaf numbers was greatly reduced by the increased contaminant concentration.

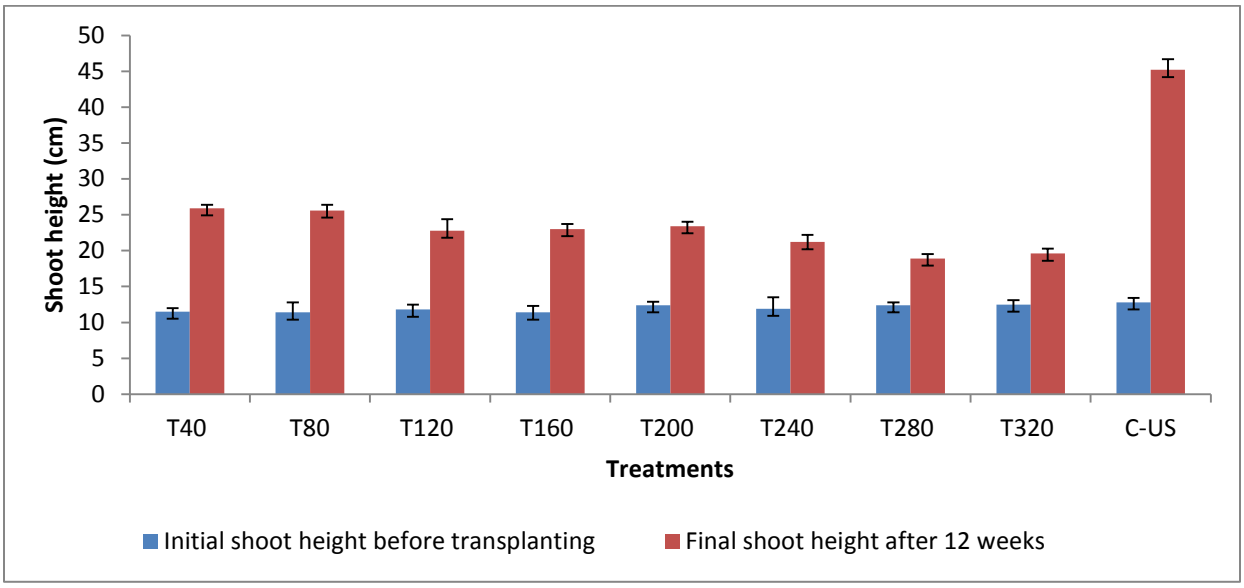
4.6 Measurement of growth of *Psoralea pinnata* in different concentrations of Fe and Cr contaminated soil samples

4.6.1 Measurement of the length of *Psoralea pinnata in* different concentrations of mixed Fe and Cr contaminated soil samples before and after 12 weeks of growth

The figures below show the growth pattern of *Psoralea pinnata* during the 12 weeks duration of the experiment.

4.6.1.1 Shoot height of plants in different concentrations of Fe and Cr contaminated soil US after 12 weeks

The results of height of plants in different concentrations of Fe and Cr contaminated soil US Figure 4.6.7 below



Legend: T40 = (24Cr &16Fe), T80= (48Cr & 32Fe), T120 = (72Cr & 48Fe), T160 = (96 Cr & 64Fe), T200= (120 Cr & 80Fe), T240 = (144 Cr & 96Fe), T280 = (168 Cr & 112Fe), T320 = (192

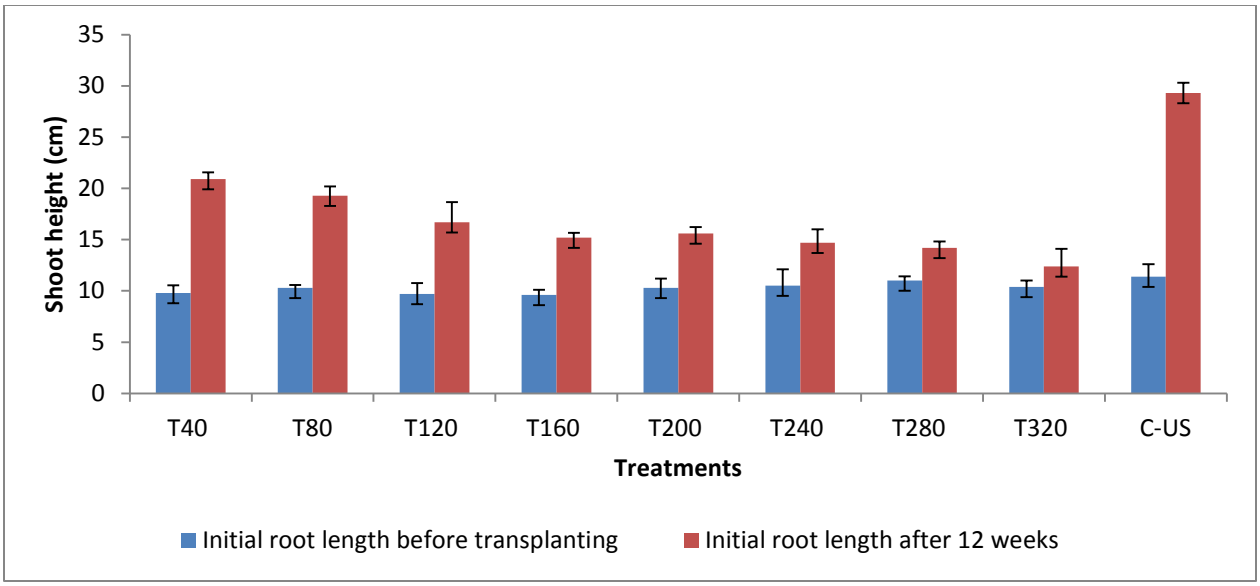
Cr & 128 Fe), C-US= control (A) on UNISA soil and C-PS= control (B) on potting soil.

Fig 4.6.7: Height of plant shoots in mixed of Fe and Cr treatments in soil US; (Error bars indicate standard error of the mean).

Results from the experimentation (Figure 4.6.7) of mixed concentration of Fe and Cr, shows the height of *Psoralea pinnata* plants in different individual concentrations in Soil US. When values for the treatments were compared with the control (C-US), all treatments had less growth of shoot and the Control had more. The growth in shoot height followed a steady decrease as the concentration of contaminants slightly increased. The highest recorded growth value (28.4 cm) was in the treatment (T80) which was a concentration of 48ppm Cr and 32 ppm of Fe.

4.6.1.2 Root length of plants in different concentrations of Fe and Cr in Soil US after 12 weeks

The results of Root length of plants in different concentrations of Fe and Cr contaminated soil US Figure 4.6.8 below



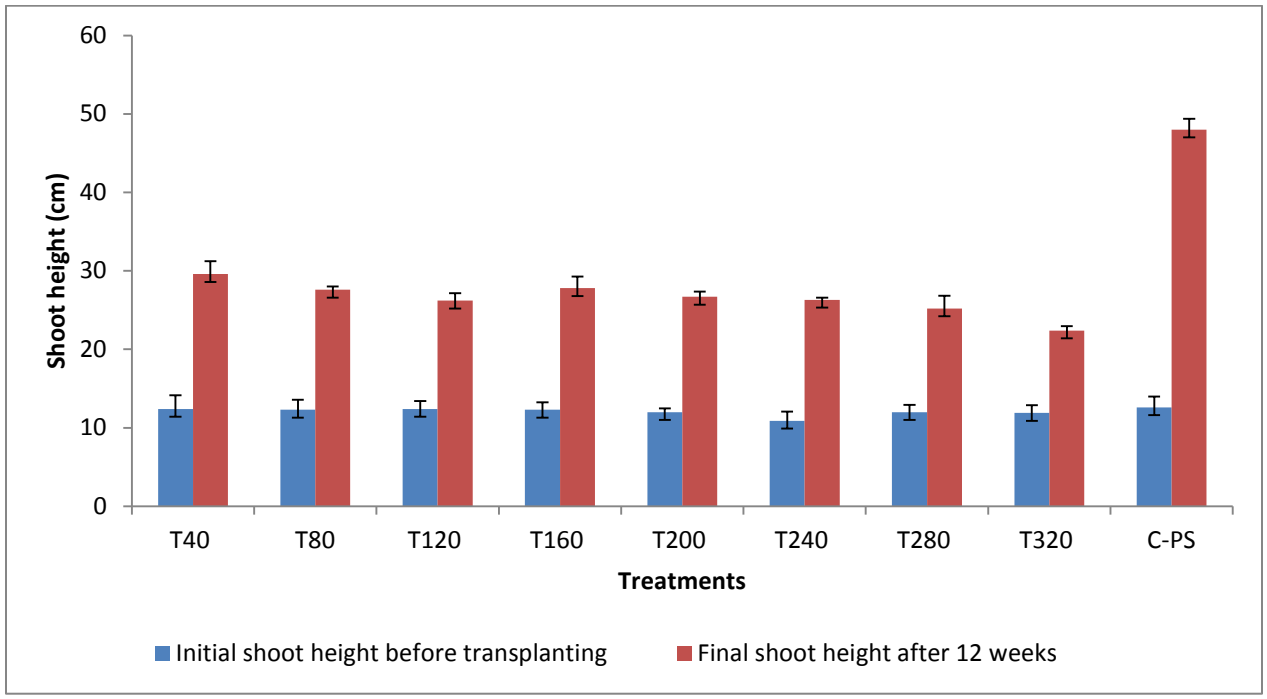
Legend: T40 = (24Cr &16Fe), T80= (48Cr & 32Fe), T120 = (72Cr & 48Fe), T160 = (96 Cr & 64Fe), T200= (120 Cr & 80Fe), T240 = (144 Cr & 96Fe), T280 = (168 Cr & 112Fe), T320 = (192 Cr & 128 Fe) C-US= control (A) on UNISA soil.

Fig. 4.6.8: Length of plant roots mixed of Fe and Cr treatments in soil US; (Error bars indicate standard error of the mean).

Results of values for root length (Fig. 4.6.8) above shows the root length of *Psoralea pinnata* after 12 weeks of growing in soil US treated with different concentrations of Fe and Cr. Roots were shortened and they showed a steadily decreasing pattern with increased concentration of the mixture of metals. The control clearly showed that there was a relationship between plant root length and metal concentration.

4.6.1.3 Shoot height of plants in different concentrations of Fe and Cr in soil PS after 12 Weeks.

The results Shoot height of plants in different concentrations of Fe and Cr in soil B (PS) is presented in the figure 4.6.9 below



Legend: T40 = (24Cr &16Fe), T80= (48Cr & 32Fe), T120 = (72Cr & 48Fe), T160 = (96 Cr & 64Fe), T200= (120 Cr & 80Fe), T240 = (144 Cr & 96Fe), T280 = (168 Cr & 112Fe), T320 = (192 Cr & 128 Fe)**,** C-PS= control (B) on potting soil.

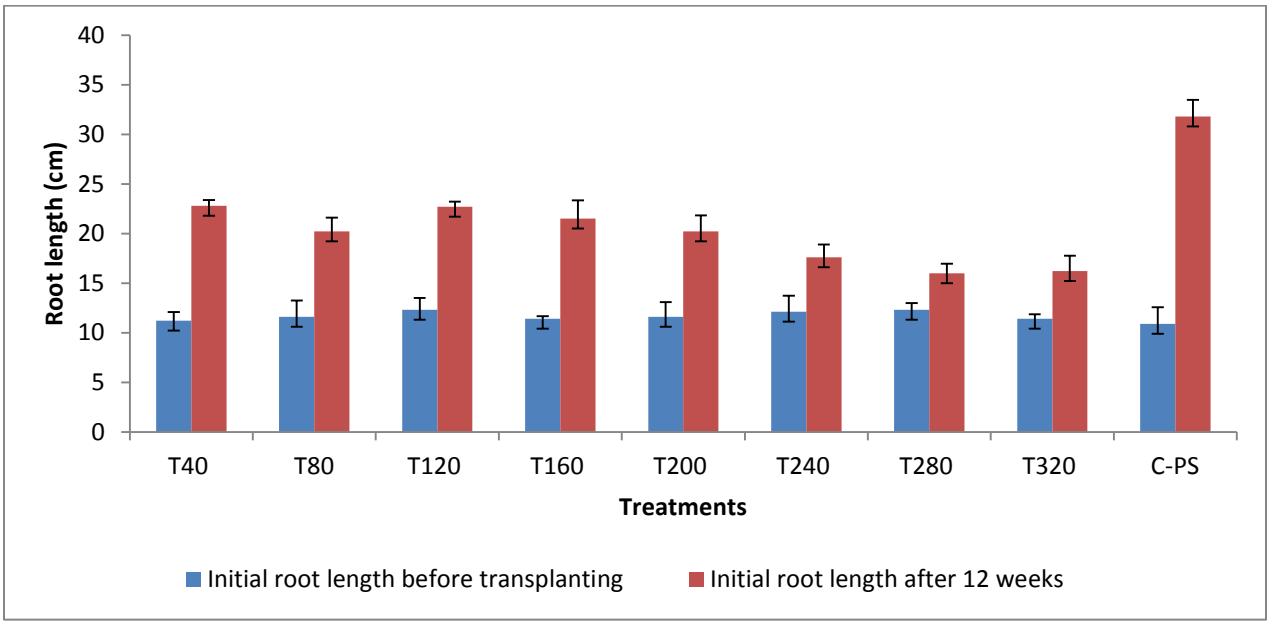
Fig. 4.6.9: Height of plant shoots in soil PS containing Fe and Cr additions (Error bars indicate standard error of the mean).

Results showing the values for plant shoots height (Figure 4.6.9) indicated the height of *Psoralea pinnata* plants in Soil PS which was treated with different concentrations of Fe and Cr during the

mixed contamination experiment. When compared with the control (C-PS), none of the treatments had growth in shoot height more than the control. The growth in shoot height showed a steady decrease as the concentration of contaminants increased. The highest recorded value of shoot growth (33.4 cm) was by treatment (T80) which was a concentration of 48 ppm Cr with 32 ppm of iron.

4.6.1.4 Root length of plants in different concentrations of Fe and Cr added to Soil PS after 12 weeks

Root length result of plants in different concentrations of Fe and Cr added to Soil PS is presented in figure 4.7.0



Legend: T40 = (24Cr &16Fe), T80= (48Cr & 32Fe), T120 = (72Cr & 48Fe), T160 = (96 Cr & 64Fe), T200= (120 Cr & 80Fe), T240 = (144 Cr & 96Fe), T280 = (168 Cr & 112Fe), T320 = (192 Cr & 128 Fe), C-PS= control (B) on potting soil.

Fig. 4.7.0: Length of plant roots in soil PS containing different concentrations of Fe and Cr treatments (Error bars indicate standard error of the mean).

From the growth of root length in the control, C-PS, decrease in root length was recorded starting from the treatment (T160) from where the values of root length began to decrease steadily. The mixed concentration of Fe and Cr in the Soil PS affected the plants roots.

4.7 Total Metal concentration and Analysis:

4.7.1 Iron (Fe) recovery results from samples in Soil US.

The table below show the result of Iron (Fe) recovery from samples in Soil US:

Table 12: Iron (Fe) recovery from samples in Soil US

Fe(tot) conc. Added to soil US (ppm)	Final Fe conc. In soil (ppm)	Fe (tot) in roots (ppm)	Fe (tot) in shoots (ppm)	Mean total Fe in plant tissue (ppm)	% Fe absorbed	Metal (Fe) accumulation factor.
T80	98.27	6.42	16.50	22.92	16.71	0.17
T130	142.34	6.44	19.00	25.44	13.60	0.14
T180	182.26	10.52	30.52	41.04	17.30	0.17
T230	227.01	11.61	30.40	42.01	14.63	0.15
T280	291.22	9.23	31.33	40.56	12.03	0.12
T330	329.05	10.14	32.09	42.23	10.91	0.11
T380	369.39	12.52	34.72	47.24	10.81	0.11
T430	420.74	14.05	32.62	46.67	9.58	0.10
T480	481.09	13.66	36.36	50.02	9.31	0.09
C-US	34.95	0.27	11.21	11.48	20.07	0.20

Results from the analysis of samples represented in the table above shows that *P. pinnata* was able to absorb Fe. From the table above, as the contaminant concentration increases, the total amount of Fe absorbed by the plant also increases. This is the case for the treatment T80 to T480 with the control plant absorbing the least amount of Fe. The results of the final soil concentration of Fe were measured in ppm to be 98.27, 142.34, 182.26, 227.01, 291.22, 329.05, 369.39, 420.74, and 481.09 corresponding to the treatments (T80), (T130), (T180), (T230), (T280), (T330), (T380), (T430) and (T480). This showed that there was reduction in concentration of Fe present in the soil after the plants have been harvested. It should be noted that before the treatments of soil US and soil PS, both soils were analysed to contain Fe and Cr, results which had been reported in table above.The percentage of absorbed Fe was highest in the treatment (T180) and later decreased with increasing metal contamination. The metal accumulation factor among the treatments, was highest in two treatments (T80) and (T180) at 0.17.

4.7.2 Iron (Fe) recovery results from samples in Soil PS.

The table below show the result of Iron (Fe) recovery from samples in Soil PS:

Table 13: Iron (Fe) recovery results (soil PS) final soil Fe concentration, total Fe concentration, percentage Fe absorbed, percentage change in Fe and Fe concentration factor

Fe (tot) conc. added to Soil PS. (ppm)	Final Fe conc. (ppm)	Fe in roots (ppm)	Fe in shoots (ppm)	Fe (tot) content in plant tissue.	% Fe absorbed	Metal (Fe) accumulation factor.
T80	47.18	8.02	21.60	29.62	35.01	0.35
T130	90.83	12.70	23.80	36.50	27.11	0.27
T180	132.17	8.53	31.82	40.35	21.86	0.22

T230	189.52	8.05	29.92	37.97	16.18	0.16
T280	226.08	11.64	32.58	44.22	15.54	0.15
T330	274.43	11.72	35.26	46.98	14.04	0.14
T380	322.75	12.04	43.68	55.72	14.49	0.14
T430	366.78	13.88	43.41	57.29	13.18	0.13
T480	415.64	15.46	45.11	60.57	12.50	0.12

When samples of soil PS were analysed, the results differed from the results obtained from the analysis of samples from Soil US and its samples. The results show that the percentages of Fe absorbed by *P. pinnata* in soil PS were greater that the percentages of Fe absorbed by the same plants in soil US. Result also show that with increased metal contamination the percentage of absorbed Fe decreased gradually. The percentage of plant-absorbed Fe was highest in the treatment T80 which also has the highest metal accumulating factor and later both parameters decreased with increasing metal contamination.

4.7.3 Chromium (Cr) recovery results from samples in Soil US.

The table below show the result of Chromium (Cr) recovery from samples in Soil US:

Table 14: Chromium (Cr) recovery results (soil US): final soil Cr concentration, total Cr concentration, percentage Cr absorbed, percentage change in Cr and Cr concentration factor

Cr(tot) conc. added to soil A.(ppm)	FinalCr conc in soil (ppm)	Cr(tot) in roots (ppm)	Cr(tot) in shoots (ppm)	Cr(tot) plant tissue content	% Cr absorbed	Metal (Cr) accumulation factor.
T80	133.71	3.93	13.80	17.83	11.28	0.11

T130	179.43	3.85	14.86	18.71	9.00	0.09
T180	138.24	4.19	16.14	20.33	7.88	0.08
T230	265.16	9.02	18.79	27.81	9.03	0.09
T280	310.78	6.36	24.54	30.90	9.94	0.10
T330	362.67	7.02	20.56	27.58	6.76	0.07
T380	314.54	7.89	23.08	30.98	6.76	0.07
T430	460.41	7.51	28.10	35.61	7.01	0.07
T480	443.85	7.36	25.02	32.38	5.80	0.06

Soil sample US and accompanying plant materials were analysed for Cr, using the flame atomic absorption spectrophotometer, showed the mean total Cr concentration in plant tissue as represented in the above table. A decrease of the values of the percentage absorbed Cr concentration in plants showed that with increased Cr concentration in treatments, the plants had absorbed a lower percentage concentration of Cr. This decreasing trend from T80 to T480 was also observed in the metal accumulation factor as indicated in the table above.

4.7.4 Chromium (Cr) recovery results from samples in Soil PS.

The table below show the result of Chromium (Cr) recovery from samples in Soil PS:

Table 15: Chromium (Cr) recovery results (soil PS)

Cr (tot) conc. added to soil B. (ppm)	Final Cr conc. in soil (ppm)	Cr (tot) in roots (ppm)	Cr (tot) in shoots (ppm)	Cr (tot) content in plant tissue.	% Cr absorbed by plant.	Metal (Cr) accumulation factor.
T80	61.54	5.24	13.8	19.04	21.11	0.21
T130	107.48	5.37	16.16	21.53	15.36	0.15

T180	152.57	6.29	17.02	23.31	12.26	0.12
T230	189.87	7.22	17.69	24.91	10.37	0.10
T280	239.71	7.98	21.92	29.90	10.30	0.10
T330	294.87	8.30	21.06	29.36	8.63	0.09
T380	342.15	7.28	23.21	30.49	7.81	0.08
T430	389.87	10.04	23.40	33.44	7.60	0.08
T480	439.93	11.81	26.53	38.34	7.82	0.08

The flame atomic absorption spectrophotometer analysis for Cr concentration in soil sample PS and accompanying plant materials show the mean total Cr concentration in these samples as recorded in the above table. The table show a decreasing trend in the percentage concentration of Cr with increased Cr contamination from low concentration treatment to high concentration treatment (i.e from T80 to T480). However, the percentage Cr concentrations of plants in soil PS were higher than the result for soil US. The treatment T80 has the highest percentage plant-absorbed Cr (21.11%). There was an almost steady decrease in the percentage Cr absorbed by the plant with the treatment T430 having the least value (7.60%).

4.8 The results of analysis of Fe and Cr in mixed concentrations using flame atomic absorption spectrophotometer

Below are presentations of the result of the analysis of Fe and Cr in mixed concentration using FLAAS.

4.8.1. Percentage of plant-absorbed Cr in mixed concentrations of Fe and Cr in soil US.

The result of the percentage of plant-absorbed Cr in mixed concentration of Fe and Cr in soil US is presented below

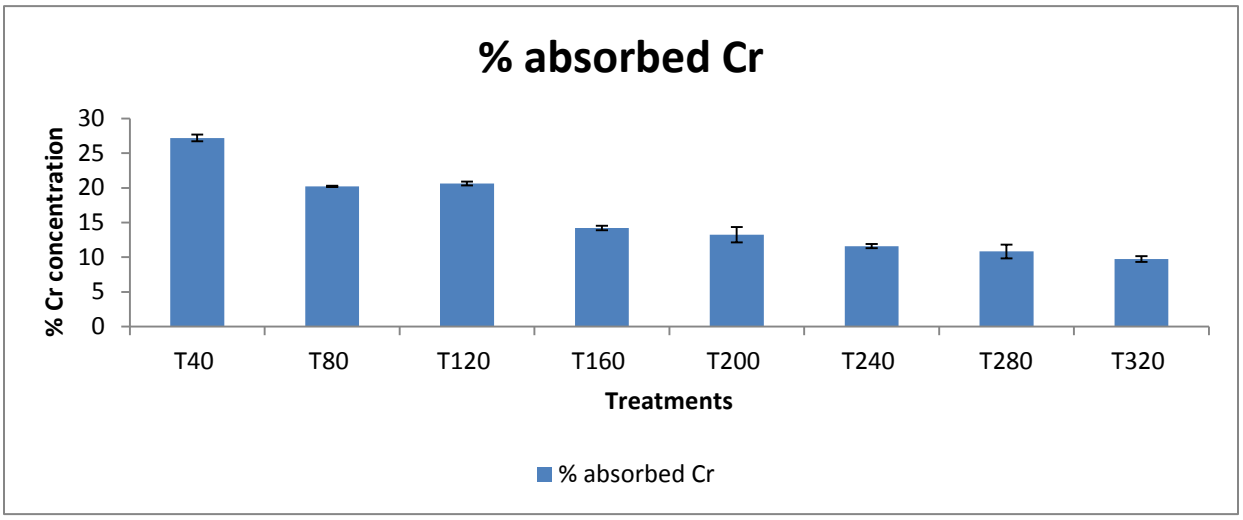


Fig. 4.7.1: Percentage of plant-absorbed Cr in mixed concentrations of iron and chromium in soil US.

The result of Cr analysis from the treatments with mixed concentrations of Fe and Cr in soil US was shown in the graph above (Fig. 4.7.1). The percentage concentration of absorbed Cr by *Psoralea pinnata*, the treatment (T40) (which has 24 ppm Cr and 16 ppm Fe) had the highest value. Apart from treatment (T120), the values of percentage of plant-absorbed Cr gradually reduced as the concentrations of both contaminants increased in the treatments.

4.8.2. Percentage of plant-absorbed Fe in mixed contamination of iron and chromium in soil US

The result of the percentage of plant-absorbed Cr in mixed concentration of Fe and Cr in soil US is presented below:

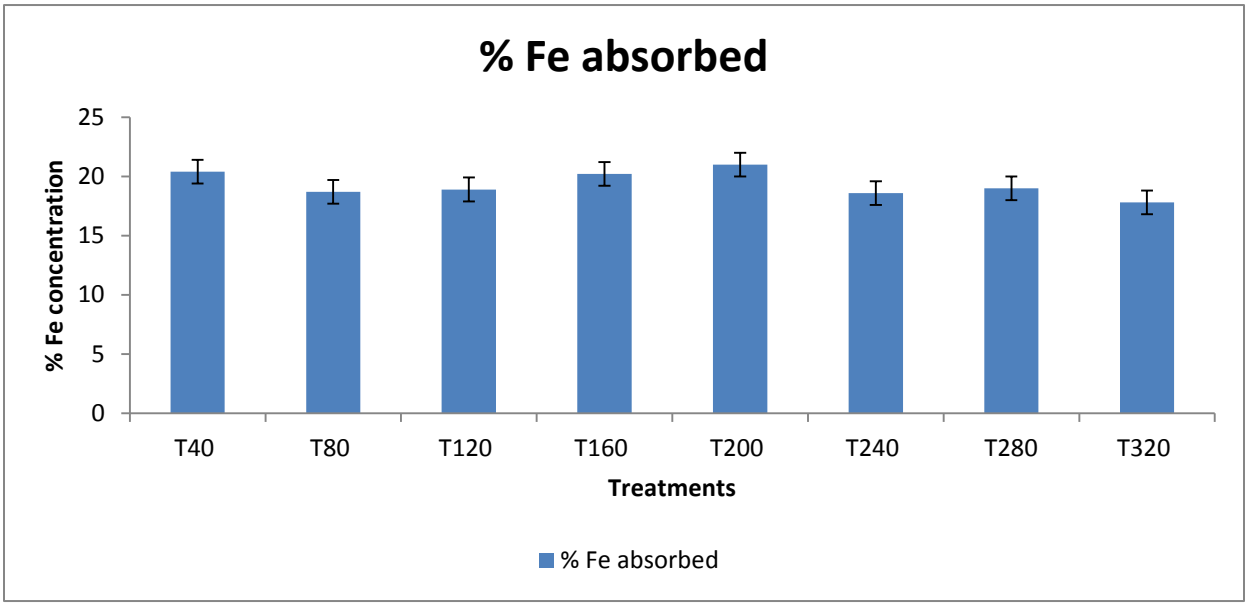


Fig. 4.7.2: Percentage of plant-absorbed Fe in mixed contamination of Fe and Cr in soil US

Values (represented in Fig. 4.7.2) showed the percentage concentration of absorbed Fe by *P. pinnata* in a mixed treatment of Fe and Cr in Soil US. The figure shows an increase in the percentage concentration of Fe starting from treatment (T40) which then reached a maximum at treatment (T200) with about 21% of Fe absorbed from the soil. As concentration of contaminants increased, there was decrease in the percentage of plant-absorbed Fe. The treatment with the least value was T320, 17.90% Fe absorbed.

4.9 Relationship between percentage absorption of Fe and Cr by *Psoralea pinnata* in soil US

The Relationship between percentage absorption of Fe and Cr by Psoralea pinnata in soil US is shown in the graph below:

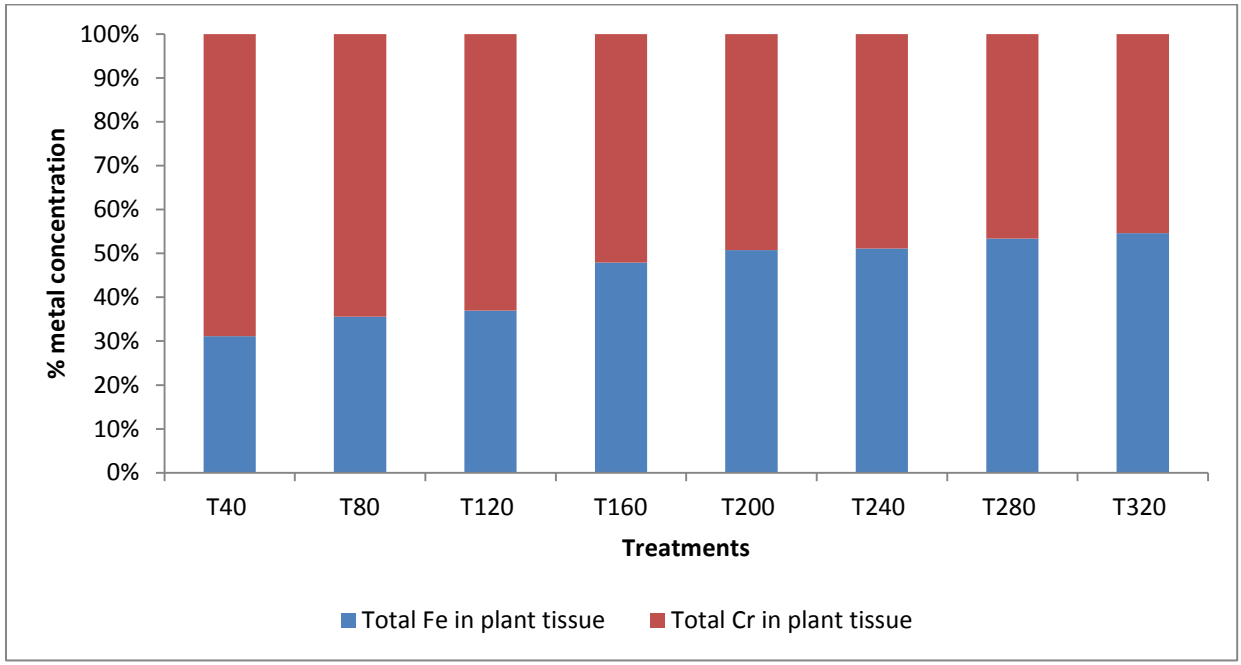


Fig. 4.7.3: Relationship between percentage absorption of Fe and Cr by *Psoralea pinnata* in soil US.

The percentage composition of each metal absorbed by *P. pinnata* (from individual treatments involving the combination of both metal contaminants) in soil US was established and represented in the above (figure 4.7.3). From treatment (T40) to (T120), Cr was the dominant metal absorbed by *P. pinnata* in preference to Fe. In the treatment (T200), Cr and Fe were almost equally absorbed with the rest of the treatments. From the treatment (T320), Cr had lost the competition to Fe. The treatment with the most iron absorption in relation to chromium absorption is (T320).

4.9.1 Percentage of plant-absorbed Cr in mixed contamination of Fe and Cr in soil PS

The graph below shows percentage of plant-absorbed Cr in mixed contamination of Fe and Cr in soil PS:

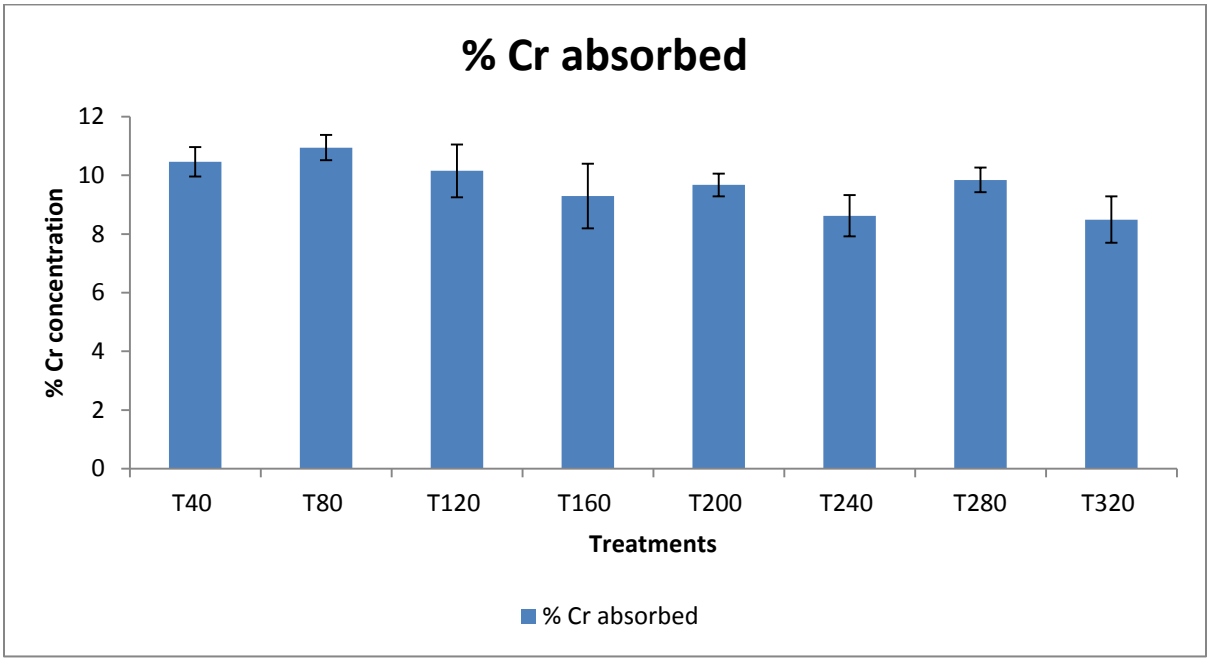


Fig. 4.7.4: Percentage of plant-absorbed Cr in mixed contamination of Fe and Cr in soil PS (Error bars indicate standard error of the mean).

Above is a description of how much Cr (in percentage) *P. pinnata* was able to absorb from the soil in a mixed treatment of iron and chromium or co-contamination of soil US with Fe and Cr. Treatment (T80) had the highest absorption with a 10.94% of Cr from the soil. Treatment (T40) had absorption of 10.46%. There was no sequence in the concentration of Cr absorbed or extracted from the soil by *P. pinnata* though the least was treatment (T320) with 8.49% Cr absorbed.

4.9.2. Percentage of plant-absorbed Fe in mixed contamination of Fe and Cr in soil PS

The graph below shows percentage of plant-absorbed Fe in mixed contamination of Fe and Cr in soil PS:

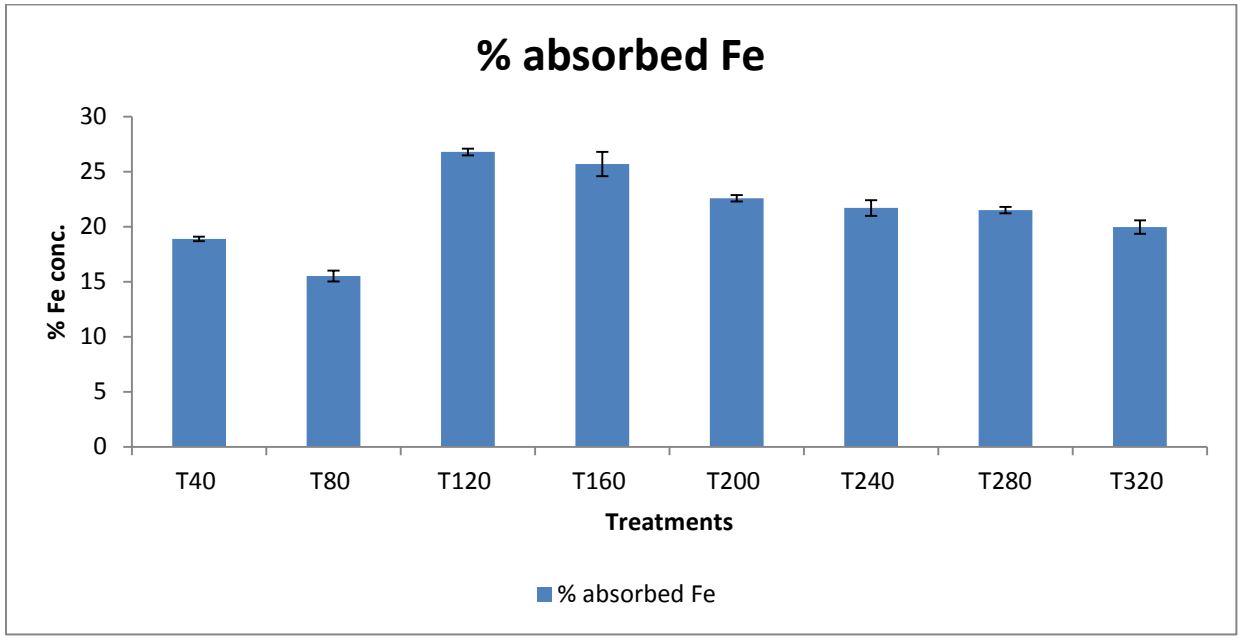


Fig. 4.7.5: Percentage of plant-absorbed Fe in mixed contamination of iron and chromium in soil PS (Error bars indicate standard error of the mean).

Values showed the percentage absorption of Fe in *P. pinnata* in a mixed treatment of soil PS with Fe and Cr. The highest percentage of plant-absorbed Fe was recorded by treatment (T120) (72 ppm Cr & 48 ppm Fe) at 27.05% and then a decrease in the values of the percentage of absorbed Fe as the concentrations of both metals increased to treatment (T320).

4.9.3 Relationship between the percentage absorption of Fe and Cr by *Psoralea pinnata* in soil PS (potting soil)

The graph below shows the relationship between the percentage absorption of Fe and Cr by *P. pinnata* in Soil PS.

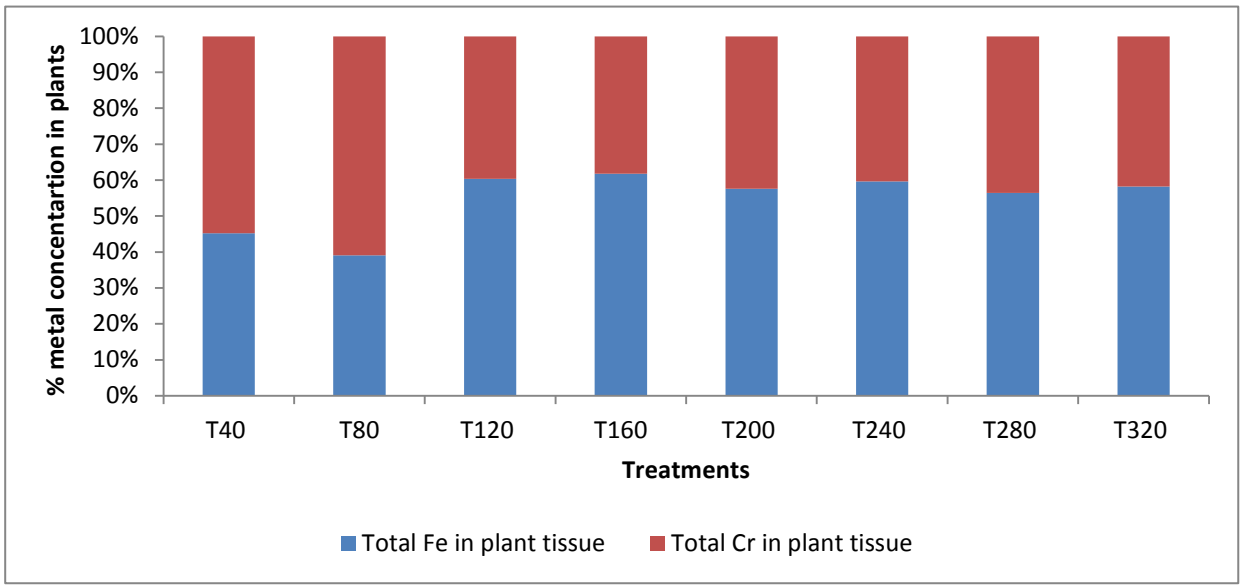


Fig. 4.7.6: Relationship between the percentage absorption of Fe and Cr by *Psoralea pinnata* in soil PS.

The graph above clearly shows that extraction of metals from soil by *P. pinnata* in Soil PS differed from those from soil US. Here, Cr had gained a competitive advantage in two treatments: (T40) and (T80). It was observed that the treatment (T80) resulted in greater absorption of Cr compared to Fe. The treatment with the most Fe absorption in relation to Cr was (T160).

4.9.4 Metal analysis of control plants

The results of metal analysis of control plants are presented in the table below:

Table 16: Results of metal analysis of control plants.

Plant	Chromium			Iron		
	Shoots	Roots	Total	shoots	Roots	Total
Control A (C-US)	5.68	0.66	6.34	3.21	0.27	3.48
Control B (C-PS)	1.26	0.12	1.38	2.80	0.31	3.11

The table above shows the result of metal analysis of the control plants used in the experiment. Control A (C-US) is the control on UNISA soil (soil US). *Psoralea pinnata* was planted in UNISA soil without adding any metal salt and control B (C-PS) is the control on potting (*Psoralea pinnata* planted on potting soil, (PS) without adding any metal salt). This was to enable comparison of all treatments with corresponding soil mediums.

4.9.5 Relationship between initial and final soil iron concentrations in soil US.

The relationship between the initial and final soil Fe concentrations of treatments in Soil US is presented in Figure 4.9.5 below

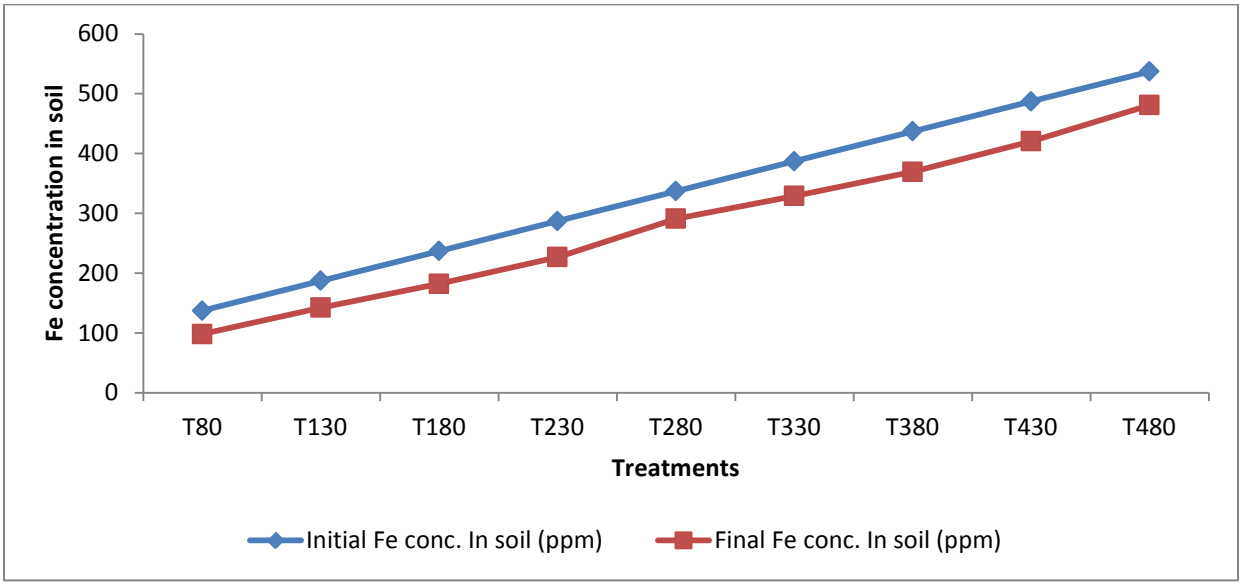


Fig. 4.7.7: Relationship between initial and final soil iron concentrations in soil US.

The relationship between the total soil concentration of iron before the introduction of *Psoralea pinnata*, designated as initial concentration and the total concentration of chromium after the in the introduction of *Psoralea pinnata*, is represented above. Twelve weeks after introducing *Psoralea pinnata*, there was reduction in the total concentration of Fe in the soil as the red bar represents in the graph.

4.9.6 Relationship between initial and final chromium concentrations in soil US.

The relationship between the initial and final Cr concentrations of treatments in Soil US is presented in Figure 4.7.8 below

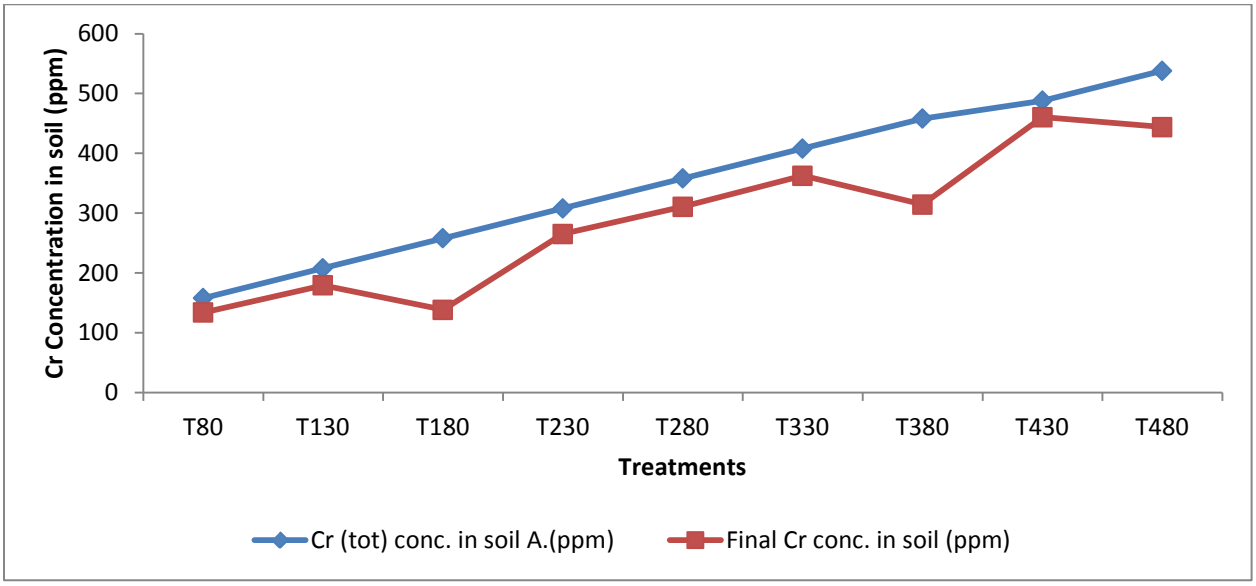


Fig. 4.7.8: Relationship between initial and final soil Cr concentrations in soil US.

There was a marked difference between the initial total concentrations of chromium in the soil (ie concentration of chromium in the soil before the introduction of *P. pinnata*) and the final concentration of chromium in the soil (ie concentration of chromium twelve weeks after the

introduction of *Psoralea pinnata*). It was observed that there was reduction in the concentration of chromium after twelve weeks of experiment.

4.9.7 Relationship between initial and final soil Cr concentrations in soil PS.

The relationship between the initial and final soil Cr concentrations of treatments in Soil PS is presented in Figure 4.7.9 below

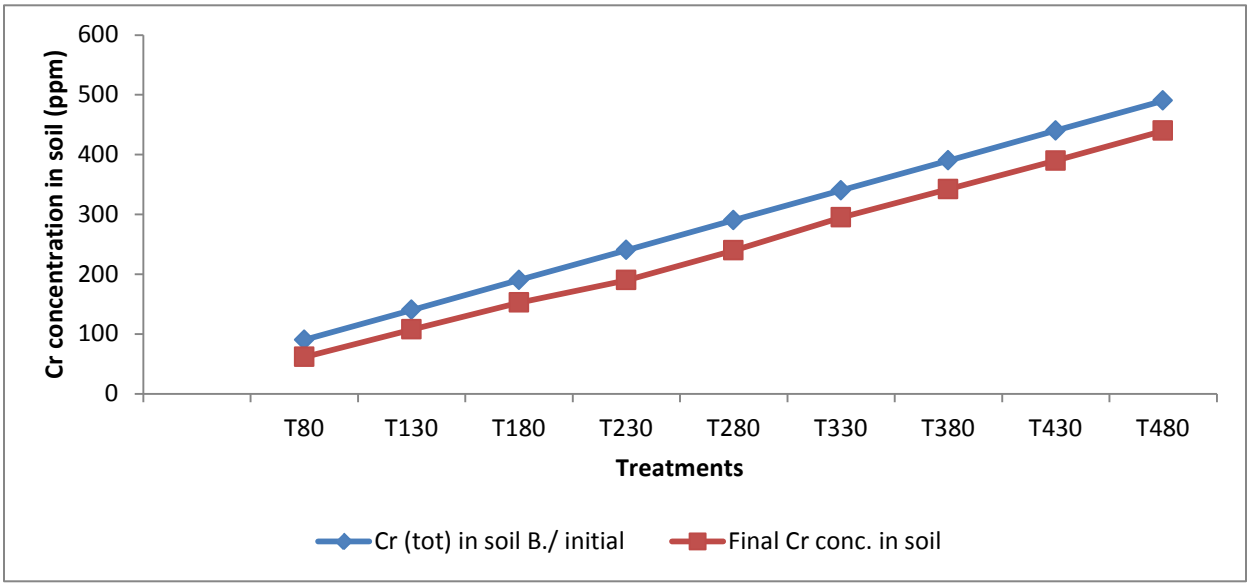


Figure 4.7.9: Relationship between initial and final soil Cr concentrations in soil PS.

The reduction in the Fe concentration in soil from the initial total soil chromium concentration to the final total soil chromium twelve weeks after *P. pinnata* was introduced is represented in the above figure (Fig. 4.7.9). The maximum difference (50.46 ppm) was found at the fifth treatment at treatment T280 while the minimum was at the first treatment, (T80) (28.66 ppm). Statistically, the difference was significant at $P \leq 0.005$.

4.9.8 Relationship between initial and final Fe concentrations in soil PS.

The relationship between the initial and final soil Fe concentrations of treatments in Soil PS is presented in Figure 4.8.0 below

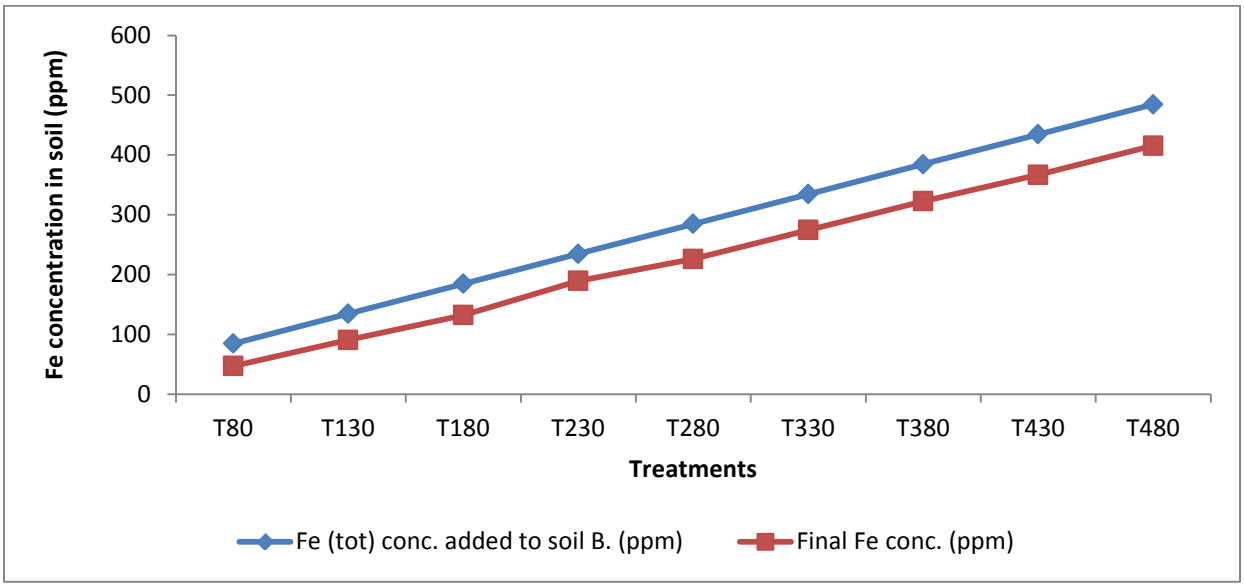


Fig. 4.8.0: Relationship between the initial and final Fe concentrations in soil PS.

The reduction in the iron concentration in soil from the initial total soil iron concentration after twelve weeks of *P. pinnata* introduction from one treatment to the next is indicated in the above figure (Fig. 4.8.0). The reduction (difference between the initial soil iron concentration and the final soil iron concentration after twelve weeks) showed to have followed an increasing order (37.42 ppm, 47.33 ppm, 52.43 ppm, 45.08 ppm, 58.52 ppm, 60.17 ppm, 61.85 ppm, 67.82 ppm, 68.96 ppm) but in percentage reduction values showed that it was decreasing as the contaminant concentration was increased from 80 ppm to 480 ppm.

4.9.9 Relationship between the percentages of absorbed Fe in soil PS and soil US.

The relationship between the percentage of absorbed Fe in *Psoralea pinnata* plant in UNISA soil (Soil US) and potting soil (soil PS) is presented in figure below.

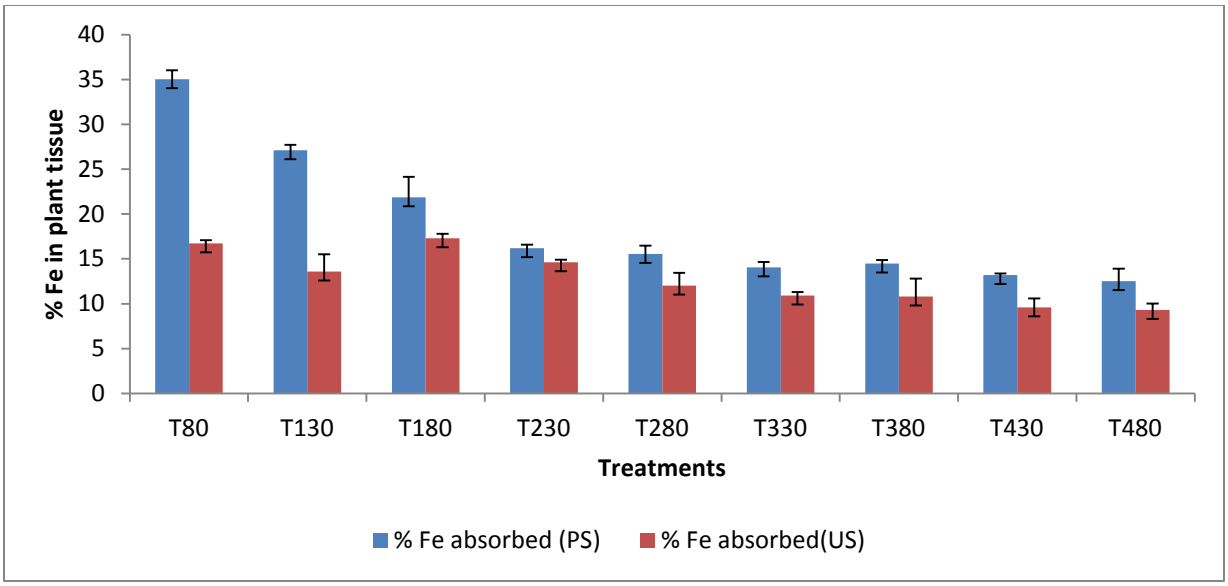


Fig. 4.8.1: The relationship between the percentages of absorbed Fe in *Psoralea pinnata* plants in UNISA soil (soil US) and potting soil (soil PS)

A comparison of the percentages of iron concentration that was extracted by *Psoralea pinnata* from soil US and soil PS is represented by Fig. 4.8.1. The graph shows that the percentages of Fe concentration extracted by *P. pinnata* from soil PS were greater than those from soil US. The most difference is seen at treatment (T80) (18.30 ppm) and the least in treatment (T230) (1.55 ppm).

4.9.9.1 The relationship between the percentages of absorbed Cr in *Psoralea pinnata* plants in potting soil (Soil PS) and UNISA soil (Soil US)

The graph below shows the relationship between the percentages of absorbed Cr in *P.pinnata* comparing Soil US and Soil PS.

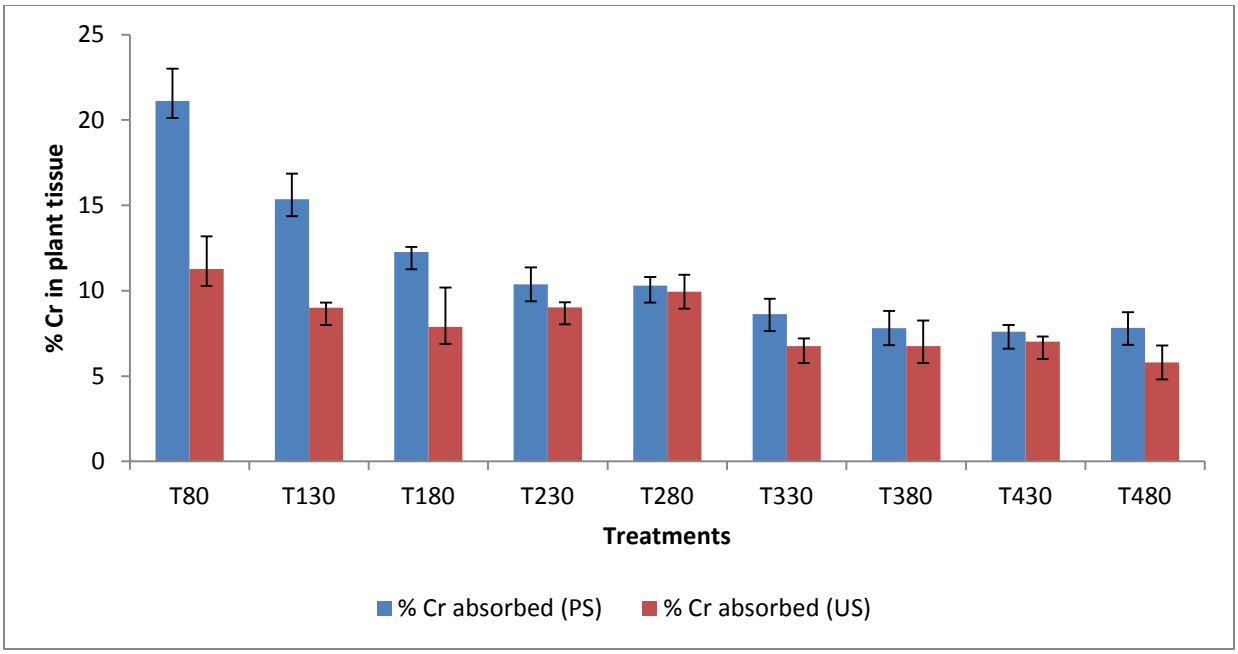


Fig 4.8.2: The relationship between the percentages of absorbed Cr in *Psoralea pinnata* plant in UNISA soil (Soil US) and potting soil (Soil PS).

Comparison of the percentages of chromium concentration that was extracted by *Psoralea pinnata* from soil US and soil PS were made. Treatments (T80), (T130) and (T180) showed the greatest differences in the percentages of chromium extracted by *P. pinnata* plants from the different soil types (Fig. 4.8.2). The graph showed that the percentages of Cr concentration extracted by *P. pinnata* from soil PS were greater than those from soil US.

Change in plant biomass among different treatment concentrations was evaluated in percentage by measuring the wet and dry weight of plants after harvesting. This was done so that the effect of the metal contaminants used in this study could be explained with respect to the water retention ability of the plant used in the study and the phytoextraction coefficients of *P. pinnata* can be established. Excess Cr decreased the water potential and transpiration rates and increased

diffusive resistance and relative water content in leaves of cauliflower (Chatterjee and Chatterjee, 2000).

4.9.9.2: Effects of different concentrations of Fe treatment on water retention ability of *Psoralea pinnata* in soil US.

The result of the effect of Fe concentration on water retention ability of *P. Pinnata* in Soil US is represented in the table below:

Table 17: concentration on water retention ability of *P. Pinnata* in Soil US:

US-Fe-treatments/ (ppm).	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T80	14.88	6.91	7.97	53.56
T130	15.60	5.97	9.63	61.73
T180	13.22	7.54	5.68	42.97
T230	13.83	8.45	5.38	38.90
T280	17.27	6.36	10.91	63.17
T330	12.85	6.78	6.07	47.24
T380	13.15	7.51	5.64	42.89
T430	13.08	7.04	6.04	46.18
T480	14.06	8.35	5.71	40.61
C-US	21.29	7.35	13.94	65.48

Values (Table 17) showed the change in biomass of *P. pinnata*. The control plant has the highest value at 65.48%., among the treatments the percentage of changes in biomass was highest in the

treatment (T280) (63.17%) but apart from (T280), gradually decreased from treatment (T280) (63.17) to T480 (40.61).

4.9.9.3 Effect of different concentrations of Fe treatment on water retention ability of *Psoralea pinnata* in soil PS.

The effect of Fe concentration on water retention ability of *P. Pinnata* in Soil PS is represented in the table below:

Table 18: Fe concentration on water retention ability of *P. Pinnata* in Soil PS

PS-FeTreatments/ (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T80	16.23	8.35	7.88	48.55
T130	16.88	7.45	9.43	55.86
T180	18.41	8.94	9.43	51.22
T230	17.83	9.12	8.71	48.85
T280	18.17	10.46	7.71	42.43
T330	16.98	7.94	9.04	53.24
T380	17.15	9.47	7.68	44.78
T430	16.80	10.09	6.71	39.94
T480	17.29	8.14	9.15	52.92
C-PS	23.08	7.16	15.92	68.98

Values (Table 18) above indicated the percentage change in biomass of *P. pinnata* in Soil PS under different concentrations of Fe. The control showed higher values than the treatments. However, among the treatments, treatment (T130) had the highest values (55.86%) and the

treatment with the least value was (T430) (39.94%) but generally, treatments in soil PS showed a greater percentage change in biomass than treatments in soil US.

CHAPTER FIVE

Discussion

5.1 Effects of pH and the use of fertilizer on growth of *Psoralea pinnata*

Results obtained from the determination of optimum pH for the growth of *Psoralea pinnata* showed that the *Psoralea pinnata* plant had more growth in soils with pH between 5 and 5.7. This was a recorded result, similar in both soils US and PS during the determination of optimum pH for the growth of *Psoralea pinnata.*. According to Vincent, et al (2012), *P. pinnata* as a shrub from the cape of South Africa, thrives better in soils with optimum pH of 5.5. The application of the recommended fertiliser (NPK = 3:1:5 (26) SR) to enhance the growth of plants in the family of *Fanabacea* to which *Psoralea pinnata* belongs to, was lethal to the plant at all the various concentration used. The death of plant was regardless of whether the fertilizer application was in solution or applied some distance away from the root zones of plants. Compost was therefore used.

5.2 Effects of different concentrations of Fe and Cr on growth of *Psoralea pinnata*

Fe and Cr concentrations affected the growth of *Psoralea pinnata*. At low concentration (80 ppm) of iron especially in the soil PS, shoots and roots of plants grew more than the rest of the treatments. Toxicity of Fe and Cr on *Psoralea pinnata* was observed in percentage reduction of shoot and root length and total biomass of the plants with increasing contaminant concentrations (from 80 ppm to 480 ppm). Although *Psoralea pinnata* survived in the 12 weeks period of experimentation, the percentage increase in plant height in the treatments involving soil (PS) were generally higher than the percentage increases in the plant height in soil (US). Adverse effects of Cr on plant height and shoot growth have been reported (Rout et al., 1997). In a study on the

effect of Cr (III) and Cr (VI) on spinach, Singh (2001) reported that Cr applied at 60 $mgkg^{-1}$ of soil and higher levels reduced the leaf size, caused burning of leaf tips or margins and slowed percentage leaf growth. Jain (2000) observed the yellowing of leaves at 40 ppm Cr that turned to necrosis at 80 ppm Cr. In a study with several heavy metals, Pedreno et al. (1997) found that Cr had a pronounced effect on leaf growth and preferentially affected young leaves, the most obvious in tomato plants by reducing the leaf sizes and the plants' heights. In this study, reduction in plant height of plants was observed at concentrations of 130 ppm and above of Fe and Cr in comparison to control. Singh (2001) concluded that Cr (VI) seems to act principally on plant roots, resulting in intense growth inhibition; this was evident in the form of percentage reduction in biomass. *Psoralea pinnata* exhibited sensitivity to iron and Cr and a high reduction in dry biomass of all plants was observed. Cr was added as hexavalent form, it was expected that both the forms are simultaneously present in soil. At the end of 12 weeks, total Cr was analysed and maximum Cr accumulation was in shoots rather than in roots in almost all treatments.

As concentrations of iron and chromium increased accordingly in the different treatments, the growth in plant shoots and roots accordingly decreased in each of the soils. It however may have occurred that Fe helped *Psoralea pinnata* produce more biomass at 80 ppm (especially in soil PS). An observation in both soil types showed that the plants with treatments containing Cr grew but not to the extent of plants with Fe amendments. It is evident that while Cr at low concentration may have contributed in reducing the growth of *Psoralea pinnata*, Fe may have caused better growth increase of shoots and roots in both soils.

Information resulting from the data in this study showed that *P. pinnata* demonstrated the ability to accumulate heavy metals. Accumulation, according to Pilon (2005), is an important characteristic needed for a plant to be used in phytoremediation of contaminated soils. *Psoralea*

pinnata in soil US, accumulated more of the total iron (50.02 ppm) from the soil, than chromium (32.38ppm). There was a significant difference between the ability of *Psoralea pinnata* to accumulate metals in different soil types. In soil (PS) *Psoralea pinnata* accumulated more of Fe (60.57 ppm) than Cr (38.34 ppm). There was generally better accumulation of metals by the plant in soil PS.

During the experiment involving soil US with mixed concentrations of Fe and Cr, there were preferences for accumulation of metals by *Psoralea pinnata.* Results show that in soil US (40 ppm mixed concentration of Cr and Fe), chromium was initially mostly accumulated by *Psoralea pinnata* (up to 68%). As the concentration of contaminants increased, at high concentrations (320 ppm mixed concentration of Cr and Fe), more iron was recorded to have been most accumulated in *Psoralea pinnata* (up to 55%). This result is supported by previous studies where it was observed that there is competition between Cr and other elements for binding. According to Sharma and Pant (1994), in maize the effects of Fe and Cr concentration varied with plant organ and Cr level. Sharma and Pant (1994) observed that Mn, Fe and Cu concentrations generally decreased with increasing Cr level. In a study on Cr (III)–Fe interaction, Bonet et al, (1991) reported that Cr enhanced growth of Fe-deficient plants. However, Cr concentration was correlated neither to changes of Mn, P or Fe tissue concentration nor to Cr-induced alterations of the Fe/Mn and P/Fe ratios. The reduction in the uptake of the element Fe could be mainly due to the chemical similarity of Fe and Cr ions in solution. Hence, the competitive binding to common carriers by Cr (VI) could have reduced the uptake of many nutrients. One of the reasons for the decreased uptake of most of the nutrients in Cr-stressed plants could have been because of the inhibition of the activity of plasma membrane H^+ ATPase (Shanker, 2003). Cr treatment also

markedly inhibited the incorporation of P, K, Ca, Mg, Fe, Mn, Zn and Cu in different plants (Biddappa and Bopaiah, 1989). Khan et al. (2001) observed that threshold values of the concentrations of N, P and K in dry weight of rice plants showed significant decrease caused by decrease in the concentration of Fe and affected the translocation of P, S, Mn, Zn and Cu from roots to leaves (Chatterjee and Chatterjee, 2000; Gupta et al., 2000). Cr is actively taken up and is a metabolically driven process in contrast to other metals which are passively taken up and retained by cation exchange sites of the cell wall (Shanker et al., 2004). This in part explains the higher accumulation of Cr by the plants. In addition, it is known that P and Cr are competitive for surface sites and Fe, S and Mn are also known to compete with Cr for transport binding. Hence, it is possible that Cr effectively competed with Fe in this study to gain rapid entry into the plant system. Poor translocation of Cr to the shoots could be due to sequestration of most of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant. It must be noted that Cr is a toxic and non-essential element to plants, and hence, the plants may not possess any specific mechanism of transport of Cr.

5.3 Conclusion

• Higher concentrations of Fe and Cr in soil resulted in poor quality and growth of the *Psoralea pinnata*.

• The impact of both Fe and Cr at higher and medium concentrations resulted in reduced shoot length, root length, and number of leaves; however, at lower concentrations, such impacts were minimal.

• The impact of Cr on growth of *P. pinnata* at concentration above 280ppm was more pronounced than that of Fe.

• There was difference between the accumulation of Cr in plant tissues and the accumulation of Fe in plant tissues in both soil types.

• The metal accumulation factor of *P. pinnata* for Fe was comparatively higher than for Cr. However, going by the fact that the higher the MAF value of plants, the more suitable the plant is for phytoextraction (Blaylock et al., 1997) and that if the MAF value is greater than 2, the plants are regarded as having high values (Mellem et al., 2009), *Psoralea pinnata* may not be an ideal hyperaccumulator for Fe and Cr considering the values (ranging from 0.06 to 0.35) obtained in this study.

• *Psoralea pinnata* has the potential of remediating soils with above required amounts of Fe (180ppm) more than those of chromium but may not yet be classified as a hyperaccumulator.

5.4. Recommendation

• *Psoralea pinnata* should be further studied for phytoremediation of Cr, Fe and other metal contaminated sites at field level considering that conditions may be different from a laboratory demonstration, since this is the first study using this plant in any phytoextraction demonstration.

• *Psoralea pinnata* by its own nature and characteristics is endemic and indigenous to South Afrcia as well as fast growing. It is also easily propagated and established; however it is being underutilized. Stakeholders may need to look into the potential of this plant for the purpose of cleaning up the environment especially for other metals and other associated species within the family *Fanabacea.*

• There is need for species screening and field adaptation trials to be conducted in the future for further verification of already established results.

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